DETERMINING THE POTENTIALS OF LACTIC ACID BACTERIA ISOLATED FROM 'NONO' FOR USE AS STARTER CULTURES IN YOGHURT PRODUCTION

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APRIL, 2016.

DECLARATION

I declare that the work in the thesis entitled **"Determining the Potentials of lactic acid bacteria isolated from 'nono' for use as starter cultures in yoghurt production"** was carried out by me in the Department of Microbiology, under the supervision of Professor J. B. Ameh and Professor S.E. Yakubu. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any institution.

Dungus, Falmata Bashir Maina

_____	_____	_____
Name of student	Signature	Date

CERTIFICATION

This thesis entitled **"DETERMINING THE POTENTIALS OF LACTIC ACID BACTERIA ISOLATED FROM 'NONO' FOR USE AS STARTER CULTURES IN YOGHURT PRODUCTION"** Dungus, Falmata Bashir Maina Meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

Prof. J. B. Ameh

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Dean, School of Postgraduates Studies	Signature	Date

DEDICATION

This thesis is dedicated to Allah the Beneficent the Merciful, my supportive husband, children and encouraging parents.

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ABSTRACT

This study was aimed at isolating, characterizing and optimizing lactic acid bacteria from 'nono' for use as starter culture in yoghurt production. The Lactic acid bacteria were grown on de Mann Rogosa and Sharpe medium agar (MRS agar, Oxoid) and β-disodium glycerophosphate agar (M17 agar, Oxoid). After several presumptive tests which included microscopy and conventional biochemical tests, the isolates were further characterized base on their different sugar fermentation profiles using Analytical profile index (API KIT, Biomereux, France). Six different strains were obtained and identified as *Lactococcus lactis* sub specie lactis, *Streptococcus thermophilus*, *Pediococcus pentasaceus*, *Lactobacillus fermentum*, *Lactobacillus brevis*, and *Lactobacillus pentosus*. These lactic acid bacteria were further subjected to tests for exopolysaccharide (EPS) production where *Streptococcus thermophilus* and *Lactococcus lactis* were discovered to be EPS producing strains which is a desirable quality in yoghurt production. The co-culture of these two strains was then termed the "local starter culture" and the specific growth rates, doubling times and acidification rates in cow, soy and goat milk fermentations were determined. Comparatively, findings from this study showed no variation ($p \geq 0.05$) in the acidification rates of the local starter culture with that of foreign starter cultures which further affirmed the potentials of the local starter culture in yoghurt production. The yoghurt samples produced with the local starter cultures were compared with their counterparts produced from foreign commercial starter culture and compared base on their physicochemical, proximate, microbiological, viscosity, sensory properties and shelf-life. Profile of the physicochemical analysis of the yoghurts produced with the local starter culture showed decrease in pH from 5.74 to 4.27 while titratable acidity increased from 0.38% to 1.32%

after 6 hours of fermentation with no significant difference ($p \geq 0.05$). Similarly, the local starter culture impacted no significant variation ($p \geq 0.05$) in the proximate composition of the yoghurt samples with moisture contents of 86.01% and total solids of 15.02%. Results from this study showed no significant difference ($p \geq 0.05$) in the general acceptability of all the yoghurt samples. The shelf-life study depicts the importance of keeping yoghurts at low temperature irrespective of the type of milk or starter culture used in order to slow down spoilage. Findings from this study demonstrate the potentials of local starter cultures to perform equally good like the exotic strains in yoghurt production.

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CHAPTER ONE

1.0 INTRODUCTION

Yoghurt derived its name from the Turkish word ‘Yoghurmak’ which means "to thicken" (Adams and Moss, 1995). It has been in the making since the dawn of history and is consumed throughout the world in various forms and varieties. Yoghurt is considered the first probiotic long recognized for the beneficial effects of live lactic acid bacteria and also its nutritional qualities (Guarner and Shaafsnna, 1998). Probiotics are live microorganisms which, when administered in adequate amounts, provide health benefits to the host (Araya *et al*., 2002). Lactic acid bacteria are the most common bacteria considered as potential probiotics (Osundahunsi *et al.,* 2007).

However, yoghurt is a fermented milk product that evolved empirically some centuries ago by allowing naturally contaminated milk to sour at a warm temperature, in the range of 40-50^{o}C (Ihekoronye and Ngoddy, 1985). Apart from fresh milk, fermented milk is the most common milk food in Africa (FAO, 1998).

The fermentation process lowers the pH of the milk which initially falls within the range of 6.4-6.8 to as low as 4.3-4.5 and therefore, yoghurt is preserved by its acidity (0.85-0.95% acidity) which in turn inhibits the growth of putrefactive or pathogenic bacteria (Stanier *et al.*, 1979).

The defining property of yoghurt is the presence of specific strains of live lactic acid bacteria (LAB) referred to as the “starter culture” (Guarner and Shaafsnna, 1998). In the United States to be named yoghurt, the product must contain two specific strains of

lactic acid bacteria which are *Lactobacillus bulgaricus* and *Streptococcus thermophilus.* Co-cultures of this two lactic acid bacteria form the "conventional starter culture" (FAO and WHO, 2001). Other lactic acid bacteria are also used in yoghurt production for additional health benefits or for taste such as strains of *Lacctobacillus acidophilus, Bifidobacterium bifidus/infantis* and *Lactococcus lactis/casei* (Fabian and Elmadfa, 2006).

Yoghurt is made by adding the bacterial cultures to milk, which causes the transformation of the milk's sugar, lactose, into lactic acid which in turn coagulates the milk protein giving yoghurt its unique pudding-like texture and tangy taste (Wood, 1992).

Virtually milk from any source can be used for yoghurt production but commercially, source from cow is most popular. The use of goat milk as an excellent raw material for yoghurt production is undeniable. It has beneficial effects on health maintenance, physiological functions, in the nutrition of children and elderly people, and according to some authors, can be consumed without negative effects of suffering from cow milk allergy. This highlights the market potential of goat milk (Ribeiro and Ribeiro, 2010).

In researchers' efforts to find alternative raw materials for yoghurt production from plant sources, different plant milks have been tested which included milk from rice, groundnut, almond, coconut and soybeans amongst which milk from soy beans gave the most promising result (Pinthong *et al.,* 1980). Soymilk is produced from the seed of the leguminous plant, soybean (*Glycine max).* Research has shown that soymilk has no cholesterol and no sugar with high isoflavone content and high quality protein (Pinthong *et al.,* 1980). In addition, soymilk yoghurt improves bone health, reduces menopausal

symptoms and risk of heart disease and certain cancers (Sipos, 1988). These immense benefits have stimulated a lot of researchers on incorporating soybean into indigenous diets (Sipos, 1988).

Irrespective of the source, milk is processed into different products among which yoghurt is one of the most widely consumed. Conventionally, not only is yoghurt a wonderful, quick, easy and nutritious snack, but also research evidence points to the fact that milk and yoghurt may actually add years to life as is found in some countries where fermented dairy products are a dietary staple (Bakalinsky *et al*., 1996). Further research showed that regular consumption of these products boosts the immune response, lowers low density lipoprotein, raises high density lipoprotein, lowers body fat (i.e. obesity), protects against ulcers, helps in curing mild gastrointestinal problems and boosts the body's ability to build bone (Hilton *et al*., 1992; Gill *et al*., 2001; Wang *et al*., 2004; Fabian and Elmadfa, 2006).

Lactic acid bacteria are mostly associated with the production of fermented milk products such as yoghurt. They play key roles in producing desirable flavour, aroma and good physical appearance in the fermented milk products (Tannock, 2004). Many Lactic acid bacteria produce exopolysaccharides (EPS), which are sugar polymers that provide viscosifying, stabilizing, and water-binding effects in yoghurt and other fermented milk products. The growing demand for all-natural, healthy food products, foods with low fat or sugar content and low levels of additives, as well as cost factors has increased the interest of food industry to use LAB polysaccharides (Patel *et al*., 2010). In several fermented dairy products, LAB contributes to the characteristic structure of the product by producing exo-polysaccharides (Talarico and Dobrogosz, 1989).

In Nigeria, locally fermented milk product referred to as ‘nono’ is commonly prepared by Hausa/ Fulani cattle rearers and it is mostly available in the northern part of the country (Yahuza, 2001; Bankole, 1990). ‘Nono’ is rich in protein, essential amino acids, phosphorus and vitamins (Nebedum and Obiakor, 2007). ‘Nono’ is produced by spontaneous fermentation of milk, and numerous attempts have been made to study microorganisms that are associated with the process (Akinyanju, 1989; Bankole, 1990; Okagbue and Bankole, 1992). Lactic acid bacteria are mostly associated with the production of ‘nono’. It is therefore necessary to monitor the growth of these lactic acid bacteria in pasteurized milk from both dairy and plant milks in order to optimize their potentials in yoghurt production.

1.1 Statement of the Research Problem

The popularity of yoghurt has led to the increased production of affordable bottled yoghurt on small scale level by individuals in recent times in Nigeria. This is partly due to the economy, with people trying to find a means of survival. As a result, poor quality milk, unhygienic practices associated with the process and the use of cheap foreign commercial starter cultures has given rise to yoghurt with highly variable quality and short shelf life. However, the existing Industrial starter cultures present in Nigerian markets lack the necessary characteristics for product diversification, and the commercial availability of new interesting local starter culture is limited.

Predominantly, ‘nono’ is being prepared and hawked by the nomadic Hausa/Fulani cattle herdsmen, who control over 80% of Nigeria’s Cattle production. ‘Nono’ is a staple in the diet of the Fulani/Hausa while most non- Fulani/Hausa see the preparation as

apparently unhygienic with poor shelf life (Obi and Ikenebomeh, 2007) and therefore resort to using the one produced under more hygienic and controlled conditions. This gesture has imposed neglect on the exploitation of local starter cultures which adapts better to our environment than the foreign starter organisms in yoghurt production. This is forcing the Nigerian government to expend over one billion Naira annually on the importation of starter cultures for yoghurt, cheese and pharmaceutical products. (Yahuza, 2001).

Earlier studies on Nigerian fermented milk products have concentrated on the microorganisms associated with the products (Adebesin *et al*., 2001; Obi and Ikenebomeh, 2007; Oyeleke, 2009), and antagonistic activities of the associated lactic acid bacteria against pathogenic organisms (Adeniyi *et al*., 2006) without studying the technological properties which form basic requirements for strain selection (Ayad *et al*., 2004). In developing countries and indeed in tropical regions of sub-Saharan Africa, the production of milk and milk products is limited, scarce and expensive (Fashakin and Unikiwedi, 1992).

The value of goat milk in human nutrition has so far received very little academic attention and few facts are available (Haenlein, 1984; Haenlein, 1988; Park, 1991; Haenlein, 1992). In spite of the nutritional merits of soymilk yoghurts, they have still not gained much popularity in many developing countries (Cheman *et al*., 1989; Hadjipanayioto, 2004).

1.2 Justification

Although fermented milk products such as yoghurt was 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 (De Vuyst, 2000).

Identification, characterization and selection of wild LAB from traditional dairy products made around the world in a natural way has been considered in the search for new industrially important cultures (Ayad *et al*., 2004). Often, wild strains are adapted to specialized niches, which have evolved with minor or major changes of the genotype and phenotype, in dairy industries. The use of such strains offers great potential for new applications or for existing processes (Fortina *et al*., 1998).

The lactic acid flora of African naturally fermented milk has been studied in various countries with the goal of using local LAB strains as starter cultures to produce fermented milk products from pasteurized milk, thereby preserving the characteristics of the local products (Mutukumira, 1996; Sserunjogi, 1999; Gadaga, 2000; Abdelgadir *et al*., 2001; Gonfa *et al*., 2001).

Studies directed to characterize traditional fermented milk products show that those made from raw milk harbour a diversity of LAB (Bernardeau *et al*., 2008) depending on geographical region, where a few may show particular interesting technological features that upon optimization may have industrial applications (Buckenhiiskes, 1993).

The isolation and optimization of wild-type strains from traditional products, to be used as starter cultures in yoghurt processing, is indeed a highly active field of research in Food and industrial microbiology today as scientific communities all over the world are

committed in search of lactic acid bacteria with desirable physiological properties (Steinkraus, 2002).

Fermented dairy products represent about 20% of the total economic value of fermented foods produced world-wide. The market share of such products continues to grow (McKay and Baldwin, 1990). Up to 93% of consumers believe certain foods have health benefits that may reduce the risk of disease (Clydesdale, 2004) and this has created a strong demand for functional foods which are foods containing specific ingredients that can reduce the risk of having certain diseases such as diarrhoea, cancer and cardiovascular conditions. Worldwide, the dairy sector, which is strongly linked to probiotics, is the largest functional food market, accounting for nearly 33% of the broad market (Leatherhead Food International, 2006). LAB bacteria are the main ‘‘bioactive ingredients” added to the dairy matrix in order to generate this health benefit. LAB containing drinks are the fastest-growing dairy product worldwide and data show that the global market for probiotic functional foods has grown by 19% in recent years and is expected to grow by 5% annually (Nutraingredients-USA, 2007).

Goat milk and its products have three-fold significance in human nutrition which includes feeding more starving and malnourished people in the developing world than from cow milk, treating people afflicted with cow milk allergies and gastro-intestinal disorders and filling the gastronomic needs of consumers (Haenlein, 2004). Perhaps the greatest benefit of goats’ milk, however, is that some people who cannot tolerate cows’ milk are able to drink goats’ milk without problems. It is easier to digest than cows’ milk and may have certain therapeutic values (Haenlein, 2004).

In order to meet the protein demands in developing countries, where animal protein is also grossly inadequate and relatively expensive for most people, research effort is geared towards finding alternative sources of protein from legume seeds (Nsofor and Maduako, 1992). This factor has made vegetable milk to become an alternative source of milk. In this regard, soymilk has been recognized as being nutritionally helpful. Murti *et al.,* (1992) stated that soy yoghurt (a product from soymilk) accords advantages in terms of nutrition and health, since it contains no cholesterol or lactose and only small quantities of saturated fatty acids, in addition to its advantage of low cost.

Soy yoghurt is an excellent candidate for such products (DeValdez and Giori, 1993). A first benefit of soy beverage fermentation is the reduction of its ‘‘beany” flavour (Stern *et al*., 1977; Desai *et al*., 2002; Blagden and Gilliand, 2005). Soy is also considered a good substrate for functional foods since fermentation by lactic acid bacteria has the potential to reduce the levels of some carbohydrates which can be responsible for gas production in the intestinal system and increase free isoflavone levels (Chien *et al*., 2006; Wei *et al*., 2007) and favour desirable changes in bacterial populations in the gastrointestinal tract (Benno *et al*., 1987; Bouhnik *et al*., 2004). Soy yoghurt also benefits bone health (Messina *et al.*, 2001), which is a concern for ageing people.

1.3 Aim and Objectives

1.3.1 Aim

The aim of this study was to determine the yoghurt producing potentials of lactic acid bacteria isolated from ‘nono’ for use as starter culture in producing yoghurts from cow, goat and soy milks.

1.3.2 Objectives

The objectives of this study were to:

1. Isolate and characterize lactic acid bacteria associated with ‘nono’ production.
2. Screen the isolates for exopolysaccharide (EPS) production.
3. Determine the growth parameters of the isolates in cow, soy and goat milk fermentations.
4. Produce and compare yoghurts with the local and foreign commercial starter cultures using cow, goat and soy milks base on their physicochemical, proximate, microbiological, viscosity and sensory properties.
5. Determine the shelf-life of the produced yoghurt samples.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History of Yoghurt

The introduction of fermented milk products such as yoghurts into the diet of man is thought to date back to the dawn of the civilization (Mckinley, 2005).

Yoghurt fermentation was invented probably by accident by Balkan tribes thousands of years ago. Until the 1900s, yoghurt was a staple in diets of people in the Russian empire (and especially central Asia and the Caucasus), Western Asia, South Eastern Europe and India (Tamine, 2002). In 1905, Stamen Grigorov (1878-1945), a Bulgarian student of medicine in Geneva, first examined the microflora of the Bulgarian yoghurt and he described it as consisting of spherical and rod-like lactic-acid bacteria. In 1907, the rod-like bacteria was called *Lactobacillus bulgaricus*. The Russian Nobel Laureate biologist, Iiya Iiyich Metchnikoff from the institute Pasteur in Paris was influenced by Grigorov's work and hypothesized that regular consumption of yoghurt was responsible for the unusual long life spans of Bulgarian peasants believing Lactobacillus to be essential for good health (Metchnikoff, 1907).

Metchnikoff worked to popularize yoghurt as a foodstuff throughout Europe and in 1919 Isaac Carasso industrialized the production of yoghurt. Carasso who was from Ottoman Salonika, started a small yoghurt business in Barcelona and named the business Danone (little Daniel) after his son. The brand later expanded to the United States under an Americanized version of the name: Dannon. Yoghurt was first introduced to the United States by American immigrants Sarkis and Rose colombosian, who started "Colombo and

sons creamery" in Andover, Massachusetts in 1929. Yoghurt's popularity in the United States was enhanced in the 1950s and 1960s when it was presented as a health food. By the late 20th century, yoghurt had become a common American food item.

Today, yoghurt is a common food item throughout the world, a nutritious food with unique health benefits. It is rich in protein, calcium, riboflavin, vitamin B_6 and vitamin B_{12} (Mckinley, 2005).

2.2 Nigerian Traditional Yoghurt 'Nono'

'Nono' is a general name used for locally fermented milk and it is widely consumed in many African countries, including Nigeria (Uzeh *et al*., 2006). It is an opaque white to milky coloured liquid food drink gotten from fermented raw milk. It is a healthy food whose consumption transverses the Saharan tribes of West African Sub-region extending to the inhabitants of the Mediterranean region and also the Middle East. In the Middle East, it is called 'dahi' or 'lassi' (Nahar *et al*., 2007). Predominantly, 'nono' is prepared and hawked by the nomadic Hausa/Fulani cattle herdsmen, who control over 80% of Nigerian's cattle production (Ogbonna, 2011). Consumers have a strong preference for this traditionally produced and processed milk products due to the satisfying nature and also its high protein content (Belewu and Aina, 2000). It could also be due to its affordability in comparison with imported, processed milk products whose prices are higher.

2.3 Milk (Base Material for Yoghurt Production).

Milk is generally defined as that fluid normally secreted by female mammals from their mammary glands for the nourishment of their young (Ihekoronye and Ngoddy, 1985). Milk is an essential first food for man, forming an important part of man's diet both for

infants and adults. The majority of milk consumed throughout the world is bovine milk, although in some regions, milk from goat, ewe or mare may be more commonly used. Milk contains carbohydrate in form of lactose, fat, vitamins and chloride, inorganic phosphate and citrate (Komorowski and Early, 1992).

Milk is an excellent source of nutrients such as vitamins, amino acids, fats, minerals, proteins and sugar, making it an excellent medium for microbial proliferation (Akinyele *et al*., 1999). Based on the above fact, milk is processed primarily into stable products with longer shelf life such as buttermilk, yoghurt etc. (Akinyele *et al*., 1999).

Yoghurt, a fermented milk product obtained by souring of milk using starter cultures (FAO, 1990), can be manufactured from cow milk, powdered milk, vegetable milk (soymilk), etc as base materials (FAO, 1990). The milk of small ruminants such as goats is of particular economic interest in the developing countries. The production of this type of milk has become a useful strategy to tackle the problems of under-nutrition (Haenlein, 2004). It is a very good source of calcium and the amino acid tryptophan. It is also a good source of protein, phosphorus, riboflavin (vitamin B_2) and potassium. Goat milk is an excellent raw material for yoghurt production since it compares favorably well with cow milk in terms of nutrient composition (Ohiokpehai, 2003; Obatolu *et al*., 2007). For the manufacture of fermented milk, starter culture containing the appropriate strains of lactic acid bacteria is added to previously pasteurized and cooled milk.

2.4 Goat Milk

Goat milk, as defined by US Food and Drug Administration (FDA), is the normal lacteal secretion, practically free of colostrum, obtained by the complete milking of one or

more healthy goats (FDA-CFR, 2010). Herding of goats is thought to have evolved about10, 000 years ago in the mountains of Iran, making goats one of the oldest domesticated animals (Haenlein, 2007). Goat milk, and the cheese made from it was venerated in ancient Egypt with some Pharaohs supposedly placing these foods among the other treasures in their burial tombs (Smith, 2006). Goat milk continued to play an important role in human nutrition in the area acknowledged as the cradle of modern civilisation (Hatziminaoglou and Boyazoglu, 2004). It is estimated that over 80% of the world's goat population is located in Asia and Africa (Morand-Fehr *et al*., 2004). By deduction, it is probable that more people in the world drink milk from goats than from any other animal. What makes goats so popular is their ability to provide high quality food under diverse climatic conditions and resilience to extreme and capricious environments (Silanikove, 2000). In regions such as Europe, Oceania and North and South America dairy production from goats has become a more commercialized operation, for example, cheese production is a significant industry in countries such as France and Italy, where goat cheese is regarded as a gourmet food and receives the highest prices among cheese varieties in the market. In addition, dairy goat and dairy sheep farming are a traditional and fundamental part of the national economy in many Mediterranean countries, such as Spain, Greece, Turkey and Morocco (Park, 2007). New markets and uses for goat milk are also being pursued, for example, as a basis for medicinal and infant foods. It is imperative that quality and safety of goat milk are optimized to ensure consumer confidence owing to the growing interest in existing and new goat dairy products worldwide Milk from goats (*Capra hircus*) accounted for 2.4 percent of global milk production in 2010 (FAOSTAT, 2012). Although the world production of goat milk has been relatively small compared to that of cow milk

(2.1% versus 84.6% of the total milk production, respectively), the worldwide goat population has reached 758 million heads with 55% increase during the last 20 years, and goat milk production has reached 12.2 million tonnes with 58% increase during the same period (Haenlein and Abdellatif, 2004).

India is the main producer of goat milk (30%), followed by Bangladesh (17%) and Sudan (11 %). Home consumption of goat milk is reported to be very high. Goats are the main suppliers of dairy and meat products for rural populations (Haenlein, 2004). Some goat breeds, such as the Bedouin goat, are able to survive under extreme environmental conditions on meagre fodder and water intake, which makes them particularly suited for surviving in regions with harsh climatic conditions. However, goat is not just associated with underdevelopment and poverty – dairy goat farming is also significant to the economies of some Mediterranean countries owing to the connoisseur interest in goat milk products such as cheeses and yoghurt (Haenlein, 2004). The proximate composition of goat milk is very similar to that of cow milk but in contrast to cow milk, the lactose content of goat milk can be increased by supplementing the diet with plant oil (Chilliard *et al*., 2005; Raynal-Ljutovac *et al*., 2008). The proportion of saturated fatty acids in goat milk (65–75 g/100 g total fatty acids) is comparable to that in cow milk. However, goat milk is rich in short- and medium-chain fatty acids with 6–10 carbon atoms, containing up to twice as much as cow milk (Sanz Sampelayo *et al*., 2007). For this reason, caproic (C6:0), caprylic (C8:0) and capric acids (C10:0) are named after goats. These fatty acids have a different metabolism to that of long-chain fatty acids and are a source of rapidly available energy, particularly relevant for people suffering from malnutrition or fat absorption syndrome and in the diets of preterm babies (feeding formulas for premature infants often contain

medium-chain triacylglycerols) and elderly people (Raynal-Ljutovac *et al*., 2008). Goat milk also contains branched-chain fatty acids with fewer than 11 carbon atoms, of which there are almost none in cow milk; this is thought to give goat milk its characteristic "goaty and muttony flavours" (Sanz Sampelayo *et al*., 2007). Although some reports suggest that goat milk contains less *trans*-C18:1 fatty acids than cow milk, other studies have shown that the *trans*- fatty acid content is similar in the two milks. The actual content depends on the feeding system, management regime and diet. Goat milk has a smaller fat globule size than cow milk which may make it more easily digestible (Raynal-Ljutovac *et al.*, 2008). Anecdotal evidence, stemming in part from cultural beliefs and in part from research studies (Haenlein, 2004; Ribeiro and Ribeiro, 2010), suggests that goat milk has lower allergenicity than cow milk. These studies report that although goat milk contains the same proteins (including β-lactoglobulin) as cow milk, some goat milk proteins differ in their genetic polymorphisms, resulting in lower allergenicity. The major fraction in goat casein is β-casein, which makes it similar to human milk (Martin *et al*., 2002). Milk from some goat breeds that lack αs1-casein altogether (which predominates in cow milk) has been shown to be less allergenic (El-Agamy, 2007).

Goat milk has been reported to contain four times as much of the oligosaccharidesialic acid as cow milk (about 23 mg/100 g vs 6 mg/100 g) (Puente *et al*., 1996).

The mineral content of goat milk varies from 0.70 to 0.85%. Compared to human and cow milk, goat milk contains more calcium, phosphorus and potassium. The vitamin content of goat milk is similar to that of cow and human milk.

In addition to contributing to the specific "goaty" flavour, the higher proportion of medium-chain fatty acids in goat milk are known to be anti-bacterial, antiviral, inhibit development and dissolve cholesterol deposits, and can be absorbed rapidly from the intestine (Shingfield *et al*., 2008). In relation to other types of milk, goat milk presents advantages such as smaller size fat globules, low allergenic properties (Martín-Diana *et al*., 2003), a balance of essential amino acids, high levels of calcium, selenium, phosphate and rich in vitamins A and B. However, goat milk is deficient in folic acid and vitamin D. Furthermore, a certain therapeutic value in human nutrition has been attributed to goat milk (Alférez *et al*., 2006).

Thus, these characteristic undoubtedly contributes to the specific health promoting properties of goat milk. Goat milk is reported to form a finer curd than cow milk following acidification, which mimics the conditions in the stomach, suggesting it would be more readily digested (Park, 2007).

The special characteristics concerning the composition of goat milk mean that its nutritional utilization is markedly higher than is the case with cows' milk. Thus, the protein of goat milk is more digestible (Park, 1994; López-Aliaga *et al*., 2003; Haenlein, 2004), and less allergenic (Lara-Villoslada *et al*., 2004). Similarly, the fat of goat milk is more digestible (Haenlein 1996; Alférez *et al*. 2001), and it may be considered an excellent source of energy for use in various metabolic processes and even for combating metabolic diseases (Sanz –Sampelayo *et al*., 2007).

The use of goat milk becomes an opportunity to diversify the dairy market since it allows the development of added value to the fermented products with particular

characteristics compared to cow's milk. The major differences between goats' and cows' milk are related to the different proportions of the different kinds of casein, and also to the different structures and sizes of fat globules and protein micelles (Tziboula-Clarke, 2003).

2.5 Soy Milk

Soybean is a rich source of protein and economical protein food. Soy milk, the water extract of soybeans, is typically produced by grinding the soaked soybeans with water. As an inexpensive and convenient source of high quality proteins, soy milk is one of the most important traditional beverages that are consumed widely in Asian countries, including China, Japan, Korea, Singapore and Thailand. Increasingly, low milk production in West Africa to meet the present population demand, high cost of milk, the protein demand in developing countries of the world, incidence of cardiovascular disease, lactose intolerance and other contributing factors have led to efforts in finding alternative sources of protein in legume seeds and production of "imitation milk". Production of yoghurt and other dairy products from soybeans should be of considerable economic interest/importance.

In recent decades, extensive evidence has indicated the strong relationships between soy food consumption and health-promoting effects (Murti *et al.,* 1992). Soy milk possesses a balanced nutrient combination, which is similar to cow's milk, but free of cholesterol, gluten and lactose, plus favorable phytochemical compounds linked to health (Murti *et al.,* 1992). Consequently, it has drastically spread its popularity to Western consumers in European countries, Australia and the United States, especially among vegetarians, milk allergy patients or people of lactose-intolerance, who use soy milk as a

dairy alternative (Chien *et al*., 2006). In response to a gradual increase in sales and consumption, various new products have been introduced into the soy milk market. Some basic changes are made to the flavour or the soybean source (Blagden and Gilliland, 2005). However, the most recent innovations are focusing on producing "functional soy milk".

Functional soy milk can be considered as soy milk that contains extra bioactive components and may help to enhance health or lower risk of diseases (Siddhuraju *et al*., 1996). Soybean is a good source of phenolic compounds with antioxidant properties and has an extraordinarily high amount of isoflavones, a group of phytoestrogens that have been reported to possibly lower the risk of hormonal and age-related diseases. However, the presence of natural anti-nutrients, such as trypsin inhibitors (TI), lectins, phytic acids and indigestible oligosaccharides, has limited its consumption. Thus, modifying the processing methods could be an effective way to improve the health-promoting bioactive components and/or reduce the undesired compounds originally present in soybeans, to support functional soy milk product development (Siddhuraju *et al*., 1996).

The heating process during conventional soy milk making considerably destroys most of the anti-nutritional factors in soy milk and improves the digestibility of soy protein, as well. However, compounds like phytic acid, which interferes with the availability of calcium, is not reduced effectively. At the same time, over-heating to eliminate trypsin inhibitor activity to a great extent can cause damage to amino acids, as well as loss in the overall nutritional value of soy milk. Lately, incorporating the fermentation process into soy milk production has become a popular method to improve the acceptability and health properties of soy milk. Research has shown that soy milk fermented with properly selected

microbial species had major advantages in decreasing indigestible oligosaccharides, like raffinose and stachyose, and beany flavour (Blagden and Gilliland, 2005).

Soy-based foods may have some health benefits to consumers due to their hypolipidemic, anticholesterolemic and antiatherogenic properties as well as due to reduced allergenicity (Siddhuraju *et al*., 1996). It also contains isoflavones, which can reduce the risk of most hormone-associated health disorders (Nsofor and Maduako, 1992). However, consumption of soymilk is undesired due to the presence of unpleasant off-flavors carried over from soy beans (Kanda *et al.,* 1976; Murti *et al*., 1992). The problems of soymilk can be improved by lactic fermentation, so production of fermented soymilks such as soymilk yoghurt is important. Fermented foods that have potential probiotic properties are produced worldwide from a variety of food substrates (Buono *et al.,* 1990).

In order to improve the organoleptic properties and nutritional profile of any product, fortification with soy milk is very much relied upon by yoghurt manufacturers (Tamime and Robinson, 2004; Sodini *et al*., 2005). The use of non-fat dried milk (NFDM) to fortify yoghurt may result in excess proteins which may impair the taste of yoghurt (Deeth and Tamime, 1980) and thus there is a need for an alternative ingredients like soy milk that can offer an opportunity to lower the lactose content of the yoghurt as Soy milk has good water holding capacity (Mian, 2006). This Multifunctional ingredient would offer a cost effective approach to yoghurt manufacture.

2.6 Lactic Acid Bacteria (LAB) in Fermentation

Lactic acid bacteria (LAB) are a group of gram-positive bacteria including the genera *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus,* and *Streptococcus.* The general description of the bacteria included in the group is gram-positive, non-spore forming, cocci or rods, which produce lactic acid as the major end product during the fermentation of carbohydrates. Lactic acid bacteria are nutritionally fastidious, requiring carbohydrates, amino acids, peptides, nucleic acids and vitamins. Recent taxonomic revisions of these genera suggest that the lactic acid bacteria comprise the following: *Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus,* and *Weissella* (Stiles and Holzapfel, 1997). The classification of lactic acid bacteria into different genera is largely based on morphology, mode of glucose fermentation, growth at different temperatures, and configuration of the lactic acid produced, ability to grow at high salt concentrations, and acid or alkaline tolerance. The lactic acid bacteria can be mainly divided into two groups based on the end-products formed during the fermentation of glucose (Figure 2.1). Homofermentative lactic acid bacteria such as *Pediococcus, Streptococcus, Lactococcus* and some lactobacilli produce lactic acid as the major or sole end-product of glucose fermentation. Homofermentative lactic acid bacteria use the Embden-Meyerhof-Parnas pathway to generate two moles of lactate per mole of glucose and derive approximately twice as much energy per mole of glucose as heterofermentative lactic acid bacteria. Heterofermentative lactic acid bacteria such as *Weissella* and *Leuconostoc* and some lactobacilli produce equimolar amounts of lactate, CO_2 and ethanol from glucose via the

hexose monophosphate or pentose pathway (De Vuyst and Vandamme, 1993; Axelsson, 1998).

2.6.1 Lactic acid bacteria and their uses in food

Lactic acid bacteria are industrially important organisms recognized for their fermentative ability as well as their health and nutritional benefits (Gilliand, 1990). Species used for food fermentations belong to the genera *Lactococcus, Streptococcus, Pediococcus, Leuconostoc, Lactobacillus,* and the newly recognized *Carnobacterium*. These organisms have been isolated from grains, green plants, dairy and meat products, fermenting vegetables, and the mucosal surfaces of animals (Lindgren and Dobrogosz, 1990).

Once used to retard spoilage and preserve foods through natural fermentations, they have found commercial applications as starter cultures in the dairy, baking, meat, vegetable and alcoholic beverages industries. They produce various compounds such as organic acids, diacetyl, hydrogen peroxide, and bacteriocins or bactericidal proteins during lactic fermentations (Lindgren and Dobrogosz, 1990). Not only are these components desirable for their effects on food taste, smell, colour and texture, but they also inhibit undesirable microflora. Hence, lactic acid bacteria and their products give fermented foods distinctive flavors, textures, and aromas while preventing spoilage, extending shelf-life, and inhibiting pathogenic organisms.

2.6.2 Antimicrobial compounds produced by lactic acid bacteria

The preservative action of starter culture in food and beverage systems is attributed to the combined action of a range of antimicrobial metabolites produced during the

fermentation process (Caplice and Fitzgerald, 1999). These include many organic acids such as lactic, acetic and propionic acids produced as end products which provide an acidic environment unfavourable for the growth of many pathogenic and spoilage microorganisms. Acids are generally thought to exert their antimicrobial effect by interfering with the maintenance of cell membrane potential, inhibiting active transport, reducing intracellular pH and inhibiting a variety of metabolic functions (Ross *et al*., 2002). They have a very broad mode of action and inhibit both gram-positive and gram-negative bacteria as well as yeast and moulds (Caplice and Fitzgerald, 1999). One good example is propionic acid produced by *Propionibacterium freudenreichii* subsp. *Shermanii* (propionic acid bacteria), which has formed the basis for some biopreservative products, given its antimicrobial action against microorganisms including yeast and moulds.

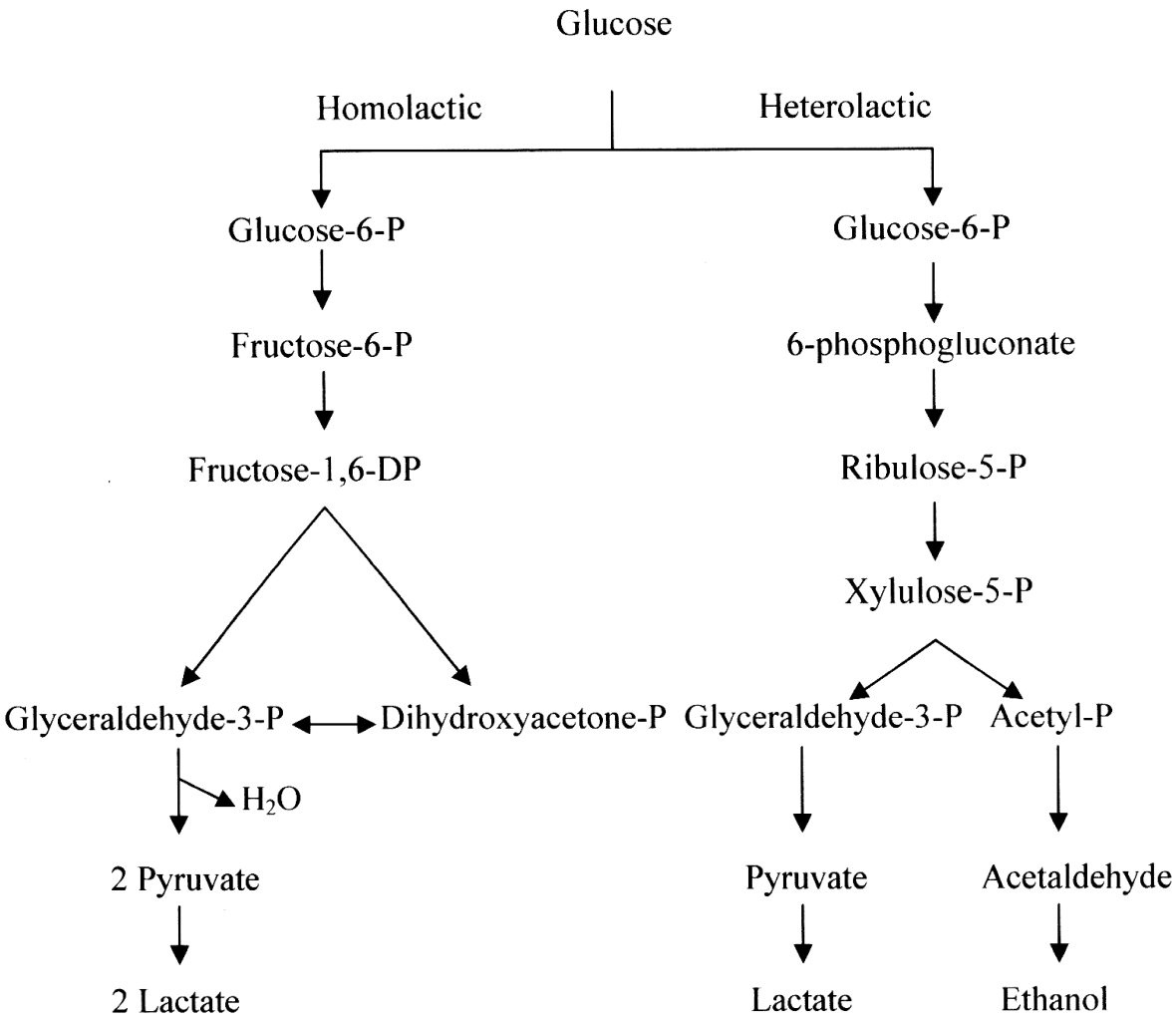

Figure 2.1: Generalized scheme for the fermentation of glucose in lactic acid bacteria (Caplice and Fitzgerald, 1999).

MicrogardÔ is a Food and Drug Administration (FAD)-approved fermentate produced by *Propionibacterium freudenreichii* subsp. *Shermanii* which contains propionic acid and is used in an estimated 30% of the cottage cheese manufactured in the United State (Daeschel, 1989). In addition to acids, starter strains can produce a range of other antimicrobial metabolites such as ethanol from the heterofermentative pathway, hydrogen peroxide produced during aerobic growth and diacetyl which is generated from excess pyruvate coming from citrate (Daeschel, 1989). In particular, hydrogen peroxide can have a strong oxidizing effect on membrane lipids and cellular proteins and is produced using such enzymes as the flavor-protein oxidoreductases NADH peroxidase, NADH oxidase and a-glycerophosphate oxidase (Codon, 1987). Obviously, each antimicrobial compound produced during fermentation provides an additional hurdle for pathogens and spoilage bacteria to overcome before they can survive and/or proliferate in a food or beverage, from time of manufacture to time of consumption. Since any microorganism may produce a number of inhibitory substances, its antimicrobial potential is defined by the collective action of its metabolic products on undesirable bacteria.

2.6.3 Bacteriocins from lactic acid bacteria

Bacteriocins are ribosomally synthesized antimicrobial compounds that are produced by many different bacterial species including many members of the lactic acid bacteria (Jack *et al.*, 1995). Some bacteriocins produced by lactic acid bacteria, such as nisin, inhibit not only closely related species but are also effective against food-borne pathogens and many other gram-positive spoilage microorganisms (Tagg *et al.*, 1976). For this reason, bacteriocins have attracted considerable interest for use as natural food

preservatives in recent years, which have led to the discovery of ever increasing potential sources of these protein inhibitors.

The developing interest in microorganisms found in food is primarily due to the biotechnological potential of new bacterial species and strains (Leisner *et al*., 1999). In biotechnological aspects, the wild strains of the LAB are prospective bacteriocins producers (Padmanabha-Reddy *et al*., 1994; Park *et al*., 2003) and probiotics (Rinkinen *et al*., 2003).

Since bacteriocins are isolated from foods such as meat and dairy products, which normally contain lactic acid bacteria, they have unknowingly been consumed for centuries. A study of 40 wild-type strains of *Lactococcus lactis* showed that 35 produced nisin (Hurst, 1981). Nisin is the only bacteriocin with GRAS (Generally Regarded as Safe) status for use in specific foods and this was awarded as a result of a history of 25 years of safe use in many European countries and was further supported by the accumulated data indicating its nontoxic, nonallergenic nature. Therefore, bacteriocinogenic starters, particularly if used in natural fermentations, will most likely afford the best opportunities for the application of bacteriocins in near future.

2.6.4 Exopolysaccharide (Eps) produced by lactic acid bacteria

Lactic acid bacteria (LAB) are used in many fermented foods particularly fermented dairy products such as cheese, buttermilk, and fermented milks. LAB produces lactic acid, carbon dioxide, and diacetyl/acetoin that contribute to the flavor, texture, and shelf life of fermented foods. Some LAB produce exopolysaccharide (EPS), and generally, EPS play a major role as natural texturizer in the industrial production of yoghurt, cheese

and milk based desserts. Recently, EPS produced by LAB have received increasing attention, mainly because of their health benefits. In particular, immune stimulation, antimutagenicity, and the antitumor activity of fermented dairy products prepared with EPS-producing LAB or EPS themselves have been investigated (Chabot *et al.*, 2001; Tsuda *et al.*, 2008).

EPS are polysaccharides secreted from the cell, or produced on the outer cell by extracellular enzymes. EPS from LAB are divided into two classes, homo-EPS and hetero-EPS. Homo-EPS are composed of one type of monosaccharide, whereas hetero-EPS consist of regular repeating units of 3-8 different carbohydrate moieties synthesized from intracellular sugar nucleotide precursors (Ganzle *et al.*, 2005). The biosynthesis of homo-EPS and hetero-EPS are different. Homo-EPS are made from sucrose using glucansucrase or levansucrase (Kralj *et al*., 2004; van Hijum *et al*., 2004), and the synthesis of hetero-EPS involves four major steps, which are sugar transportation, sugar nucleotide synthesis, repeating unit synthesis, and polymerization of the repeating units (de Vuyst *et al*., 2001). The major physiological function of EPS is believed to be biological defenses against various stresses such as phage attack, toxic metal ions, and desiccation (Ruas-Madiedo *et al*., 2002). Research has also shown that EPS+ LAB can enhance the functional properties of fermented dairy products. These properties show that EPS have wide technical potentials for development of novel and improved food products with enhanced texture, mouth-feel, taste perception and stability, representing potential sources for economic gains for the dairy industry. To avoid syneresis (separation of water), at present, yoghurt manufacturers still rely on pre-fermentation processing such as increasing milk solids through the addition of milk powder, whey powder, caseinate, etc. or the concentration of milk (by evaporation,

membrane filtration, etc.), heat treatment of the milk prior to inoculation, homogenisation, incubation conditions and handling of the ripened coagulum, and/or addition of stabilisers or viscosifiers (not allowed in all countries) such as chemically modified plant carbohydrates and gelatin. The application of lactic acid bacterium strains excreting heteropolysaccharides is a promising alternative (De Vuyst, and Degeest, 1999).

EPS also have the potential to be used as surface carriers of bacteriocins or bacteriocin producing LAB, and species such as *Leuconostoc mesenteroides*, *Streptococcus mutans* and several lactobacilli (*Lactobacillus brevis*, *Lactococcus lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *Lactobacillus casei*, *Lb. sake*, *Lb. rhamnosus*,) and thermophilic (*Lb. acidophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb.helveticus* and *S. thermophilus*) are known to produce EPS. The isolation and characterization of EPS from new wild LAB species, which are ubiquitous in traditional fermented dairy products, is a key strategy towards finding strains with optimized production of EPS.

2.6.5 Lactic acid bacteria as functional starter cultures

A starter culture can be defined as a microbial preparation of large numbers of cells of at least one microorganism to be added to a raw material to produce a fermented food by accelerating and steering its fermentation process. The group of lactic acid bacteria occupies a central role in these processes, and has a long and safe history of application and consumption in the production of fermented foods and beverages (Caplice and Fitzgerald, 1999). They cause rapid acidification of the raw material through the production of organic acids, mainly lactic acid. They also produce acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharides, and several enzymes. In this way, they enhance shelf-life

and microbial safety, improve texture, and contribute to the pleasant sensory profile of the end product.

The direct addition of selected starter cultures to raw materials has been a breakthrough in the processing of fermented foods, resulting in a high degree of control over the fermentation process and standardization of the end product. Strains with the proper physiological and metabolic features were isolated from natural habitats or from successfully fermented products (Oberman and Libudzisz, 1998). However, some disadvantages have to be considered. In general, the initial selection of commercial starter cultures did not occur in a rational way, but was based on rapid acidification and phage resistance. These starters are not very flexible with regard to the desired properties and functionality of the end product. Originally, industrial starter cultures were maintained by daily propagation. Later, they became available as frozen concentrates and dried or lyophilized preparations, produced on an industrial scale, some of them allowing direct vat inoculation (Sandine, 1996).

The earliest productions of fermented milk products were based on the spontaneous fermentation, resulting from the development of the microflora naturally present in the raw milk and its environment. The quality of the end product was a reflex of the microbial load and spectrum of the raw material. Spontaneous fermentation was later optimized through backslopping, i.e., inoculation of the raw material with a small quantity of whey from a previously performed successful fermentation, and the resulting product characteristics depended on the best-adapted strains dominance (Leroy and De Vuyst, 2004). The starter-culture applied in this, so-called, natural fermentation, is usually a poorly known micro

flora mix that although having a predominance of LAB, may also contain non- LAB microorganisms, and its microbial diversity and load are usually variable over time.

Because the original starter cultures were mixtures of several undefined microbes, the daily propagation probably led to shifts of the ecosystem resulting in the disappearance of certain strains. Moreover, some important metabolic traits in lactic acid bacteria are plasmid-encoded and there is a risk that they are lost during propagation. It is further likely that loss of genetic material occurred due to adaptation to the food matrix. The biodiversity of commercial starters has therefore become limited. This often leads to a loss of the uniqueness of the original product and the loss of the characteristics that have made the product popular (Caplice and Fitzgerald, 1999).

In contrast, the fermentation of traditional fermented foods is frequently caused by natural, wild-type lactic acid bacteria that originated from the raw material, the process apparatus, or the environment, and that initiate the fermentation process in the absence of an added commercial starter. Moreover, many traditional products obtain their flavour intensity from the non-starter lactic acid bacteria (NSLAB), which are not part of the normal starter flora but develop in the product, particularly during maturation, as a secondary flora (Beresford *et al.*, 2001). Pure cultures isolated from complex ecosystems of traditionally fermented foods exhibit a diversity of metabolic activities that diverge strongly from the ones of comparable strains used as industrial bulk starters. These include differences in growth rate and competitive growth behaviour in mixed cultures, adaptation to a particular substrate or raw material, antimicrobial properties, flavour, aroma, and quality attributes. Wild strains need to withstand the competition of other microorganisms

to survive in their hostile natural environment, so that they often produce antimicrobials such as bacteriocins. In addition, they are more dependent on their own biosynthetic capacity than industrial strains and harbour more amino acid converting enzymes that they play a key role in flavour formation. Such findings underline the importance of the Protected Designation of Origin (PDO) of many of these products, which is crucial from an economical point of view since they contribute to the survival of small-scale fermentation plants in a world of ongoing globalization. A recent trend exists in the isolation of wild-type strains from traditional products to be used as starter cultures in food fermentation (Beukes *et al*., 2001).

2.7 Classification of Starter Bacteria

The bacteria used in the manufacture of fermented dairy products are generally lactic acid bacteria (LAB). However, *Propionibacterium shermanii* and *Bifidobacterium* spp. which are not lactic acid bacteria, although *Bifidobacterium* species do produce lactic acid, are also used.

There are currently sixteen genera in the LAB species but only few are of importance in food fermentation, these include *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus* and *Tetragenococcus* genera.

2.7.1 *Enterococcus*

These organisms are Gram-positive, catalase negative cocci that tend to form chains of varying length. They are normal inhabitants of the intestinal tract of man and other animals and are often used in microbiology as indicators of faecal contamination. Some

species of the genus are pathogens. Apart from their ability to grow at 45°C, at pH 9.6, in high concentrations of salt, in high concentrations of bile salts, their general heat tolerance and their insensitivity to a range of antimicrobial agents they are superficially similar to lactococci. The biochemical identification key developed by Manero and Blanch (1999) is particularly helpful in identifing enterococci.

There are concerns about enterococci in foods, partly because some are pathogens. However, it is their ability to exchange antibiotic resistance genes, particularly for glycopeptide antibiotics (vancomycin and teicoplanin), that perhaps raises most concern. Prior to recent taxonomic research, the *Enterococcus* species used as starters were classified as faecal streptococci and Group D streptococci.

2.7.2 *Leuconostoc*

Leuconostoc species are important flavour producers in some fermented dairy products. There is general agreement that two species, *Leuconostoc mesenteroides* subsp. *cremoris* and *Leuconostoc lactis* are important in starter cultures. Unlike lactococci, leuconostocs grow on Rogosa agar (Mullan, 2001) and are hetrofermentative producing carbon dioxide from glucose and usually fructose.

On microscopic examination, leuconostocs generally appear as Gram positive cocci similar in size and shape (occur in pairs and in usually short chains) to lactococci. However, small rods can often be found and since leuconostocs grow on Rogosa agar, there can be a tendency to assume that these cultures are contaminated, with lactobacilli for example. Unlike lactococci, leuconostocs do not produce ammonia from arginine and

produce the D-isomer of lactic acid. With some exceptions leuconostocs only grow weakly in milk, and are not capable of reducing litmus before coagulation in litmus milk medium.

2.7.3 *Streptococcus*

Streptococcus thermophilus is the only species of this genus found in starter cultures. This streptococcus is classified as a thermophile growing at ≥45°C and is widely used in the manufacture of yoghurt (Tamime and Robinson, 1999). It is a component, along with lactococci, in some DVI/DVS cultures where it produces acid rapidly during scalding and may confer an additional measure of bacteriophage (phage) protection. Like lactococci and many leuconostocs, strains of *S.thermophilus* are catalase-negative; cocci shaped and occur in pairs and chains. Generally, most strains produce long chains. L-lactic acid only is produced and carbon dioxide is not produced from glucose. Some strains produce urease and have the potential to produce CO_2 from urea.

2.7.4 *Lactobacillus*

This genus consists of a large group of Gram positive, catalase negative, rod-shaped bacteria. Some species are homofermentative while others are hetrofermentative. While some species produce mainly L-lactate from glucose, others produce D-lactate. Since some strains exhibit significant racemase activity, a racemase is an isomerase enzyme, D/L lactic acid is also produced.

Lactobacilli are used as starters in the manufacture of yoghurt. They are also used to reduce the incidence of bitterness and as probiotics in yoghurt type products. *Lb.*

delbrueckii subsp. *bulgaricus* is widely used along with *S. thermophilus* as a starter in yoghurt manufacture. This subspecies is homofermentative, produces almost 2% w/v lactic acid in milk, has an optimum temperature of 42°C and grows at temperatures of 45°C and higher. It will not grow in low concentrations of salt and is sensitive to bile salts (Caplice and Fitzgerald, 1999).

Lb. acidophilus, which is normally present in the intestine, is generally not used as a starter; it is widely used as a probiotic. This bacterium, is homofermentative, producing high concentrations of D-lactic acid in milk, has an optimum temperature of 37°C, and is relatively tolerant of oxygen, compared with Bifidobacterium species that are frequently used in conjunction with this organism. Little growth occurs at temperatures less than 20°C and most strains show no growth at 15°C. Because *Lb. acidophilus* produces D-lactate there have been some concerns about its use in infant nutrition.

Lb. casei is also a normal inhabitant of the small intestine and is resistant to bile. It is used as a probiotic. L-lactate is the main isomer of lactose produced although some strains produce small concentrations of D-lactate due to weak racemase activity. Rogosa agar is widely used as a general isolation medium for lactobacilli.

Latobacillus helveticus is frequently used along with other thermophilic lactic acid bacteria in the manufacture of a range of fermented milk products and yoghurt. One advantage of including this species along with *Lb. delbrueckii* subsp. *bulgaricus* is that *Lb. helveticus* utilises galactose and this can be useful if products free of reducing sugars are required. The species is homofermentative and produces high concentrations of D/L lactic

acid in milk. Many strains grow at 45°C although lower temperatures 42-43°C generally give higher recoveries when enumerated using selective media such as Rogosa or modified MRS agars. Most strains show no or little growth at 15°C (some atypical strains may take several weeks to grow at 15°C or below).

2.7.5 *Lactococcus*

Originally, the bacteria in this group were classified as members of the genus *Streptococcus* and were designated as lactic streptococci. They were differentiated from other streptococci, some of which are pathogens, by their specific reaction with Group N antiserum and by their tolerance to temperature, salt and dyes. It is now known that serotyping LAB has limited value in species differentiation, strains of the same species may react with different sera and some strains may exhibit no group antigen (Kilpper-Balz and Schleifer, 1987).

2.8 Yoghurt Production

Yoghurt gels are formed by the fermentation of milk with thermophilic starter bacteria heated normally at high temperatures (e.g. 60°C for 30 min), which causes the denaturation of whey proteins. Denatured whey proteins interact and cross-link with κ-casein on the surface of casein micelles. There is increased casein-casein attraction as the pH of milk decreases from approximately 6.6 to 4.5 during yoghurt fermentation, which results in gelation as casein approaches their iso-electric point. Physical properties of yoghurt gels, including whey separation play an important role in quality and consumer

acceptance. An understanding of gelation process during fermentation is critical in manipulating physical properties of yoghurt (Lee and Lucey, 2004).

2.9 Benefits of Yoghurt

Consumption of fermented-milk products is associated with several types of human health benefits partly because of their content of lactic acid bacteria. Several experimental observations have indicated a potential effect of lactic acid bacteria (LAB) against the development of colon tumors (Wollowski *et al*., 2001). Epidemiological studies suggest that consumption of fermented milk and the dietary intake of dairy proteins are inversely related to the risk of hypertension, resulting from a blood pressure lowering effect of released bioactive peptides (Jaubiainen and Korpela, 2003).

Recently, the role of fermented milks containing lactic acid bacteria (LAB), such as *Lactobacillus, Bifidobacterium* and *Streptococcus thermophilus*, has been studied (Saikali *et al*., 2004). A wide range of other health benefits, including improved lactose digestion, diarrhea prevention, immune system modulation and serum cholesterol reduction, have been ascribed to fermented milk consumption. The use of yoghurt as a calcium source has made it one of the most rapidly growing dairy products, but presently it is more than just a calcium source. This versatility, together with their acceptance as a healthy and nutritious food, has led to their widespread popularity across all population subgroups (Mckinley, 2005). Yoghurt, 'Kefir', and similar fermented milk products are on the way to becoming major nutraceuticals aimed at treating a variety of disease conditions (Katz, 2001).

2.10 Types of Yoghurt

The market now offers a vast array of yoghurts to suit all palates and meal occasions. Yoghurts come in a variety of textures (e.g. liquid, set and stirred curd), fat contents (e.g. regular fat, low-fat and fat-free) and flavors (e.g. natural, fruit, cereal, chocolate), can be consumed as a snack or part of a meal, as a sweet or savory food (Tamime and Robinson, 1999).

2.11 Nutritional Profile of Yoghurt

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. Yoghurt is an excellent source of protein, calcium, phosphorus, riboflavin (vitamin B2), thiamin (vitamin B1) and Vitamin B12, and a valuable source of folate, niacin, magnesium and zinc. The protein it provides is of high biological value, and the vitamins and minerals found in milk and dairy foods including yoghurt are bioavailable. Yoghurt particularly the low-fat varieties, provide an array of important nutrients in significant amounts in relation to their energy and fat content, making them a nutrient-dense food. Eating dairy products, such as yoghurt, helps to improve the overall quality of the diet and increases the chances of achieving nutritional recommendations (Mckinley, 2005). Vitamins and minerals may be added and often are for products given to children. Yoghurts may be spoonable or drinkable, and may be considered dietary supplements for infant consumption. So they cross the line between dietary supplements, medical foods, and conventional foods (Katz, 2001).

2.12 Yoghurt Texture

The texture of a food can be classified as a flow, deformation, and/or disintegration of the food substance after the application of force according to Jaros and Rohm (2003). The study of the deformation or disintegration and flow of food substances is known as rheology (Rao, 1999).

Rheological characteristics of food materials, such as fermented dairy products, are imperative in the blueprint of flow procedures, the control of quality, storage, as well as processing and in foretelling the texture of food substances (Shaker *et al*., 2000). The texture of food products is a representation of all the traits discernible through tactile, mechanical and when appropriate, visual and acoustic receptors. The texture of food is a central consideration that contributes to the sensory characteristics of food.

Yoghurt may be categorized as a pseudo-plastic substance (a substance that has a yield stress that must be surpassed for flow to be set off). This may either be a visco-elastic fluid if the yoghurt in question is stirred or designed for drinking, or may be a visco-elastic solid if the yoghurt in question is set yoghurt. A visco-elastic property shows that the substance in question has a number of the elastic characteristics of an ideal solid as well as a number of the flow traits of an ideal (viscous) fluid. Yoghurt, in addition, shows time-bound shear thinning characteristics, although yoghurt is not an exact thixotropic substance because shear is not entirely reversible after the shear ends. Texture directly impacts the mouth feel of a food product, as mouth feel is defined by the discernment of feelings on the tongue as well as all through the mouth following the ingestion of a food material. The moving of food in the oral cavity, as well as the feelings perceived by the individual, play a

part in the general acceptance as well as liking of the product. Texture is made up of physical traits such as viscosity, density, as well as surface tension.

In regards to rheology, yoghurt is held to be a non-Newtonian food, implying that the rate of shear is not proportional to the corresponding stress. The textural traits of yoghurt can be generalized by viscosity, syneresis, and strength of the gel (Sodini *et al*., 2004).

Yoghurts are structured substances that act as extremely weak gels (Harte *et al*., 2007). According to (Jaros and Rohm, 2003) the examination of the rheological traits of dairy foods is critical in establishing the makeup of the said product. According to (Charm, 1971) and (Tunick, 2000) the textural characteristics of yoghurt gels are key factors in ascertaining consumer preference as well as production strategies for yoghurt. As described by (Jaros and Rohm, 2003) the most important factors that affect the rheological and textural properties of yoghurt include the makeup and considerations of milk base; homogenization, dry matter fortification, pre-heat treatments, incubation time-temperature, starter culture and after incubation treatments.

2.13 Quality and Shelf- Life of Yoghurt

Yoghurts shelf life is based on whether the products display any of the physical, chemical, microbiological or sensory characteristics that are unacceptable for consumption. Studies of changes in these quality characteristics during storage would be instrumental in predicting the shelf life of the product (Salvador and Fiszman, 2004).

Knowledge of the behavior of yoghurt during long storage is important, because its shelf life is based on whether the products display any of the physical, chemical or sensory characteristics that are unacceptable for consumption (Salvador and Fiszman 2004). Flavor and texture are the most pronounced factors that influence the quality and acceptance of yoghurt and related fermented milks (Labropoulos *et al.*, 1984). Textural attributes, including the desired oral viscosity, are important criteria for quality and consumer acceptance of yoghurt (Walstra 1998).

The shelf life of yoghurt can be adequately determined using flavor deterioration or yeast counts as failure indices. Flavor defects develop at a higher rate than textural changes during storage and seem to be the earliest manifestation of product failure. The shelf life of yoghurt is expected to range from 7 to 14 days at a storage temperature of 4°C. The end of shelf life of yoghurt is accompanied by an increase of 10 to 15% of free whey and 0.5 to 0.6% of lactic acid and drop of 0.3 to 0.4 pH units.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of Samples

'Nono' samples were purchased from Hausa/ Fulani women at Tudun Wada, Samaru and Sabon Gari local markets in Zaria, Nigeria.

Samples of fresh cow and goat milks were obtained from National Animal Production Research Institute (NAPRI) Zaria, Nigeria. The fresh milk samples were transferred into sterile containers and transported in ice bags to the laboratory within five minutes for processing. Soybean seeds (variety TGX-923-2E) were obtained from the Institute for Agricultural Research (IAR), Zaria. The commercial yoghurt starter culture was obtained from NAPRI, Zaria.

3.2 Isolation, Characterization and Identification of LAB from 'Nono'

3.2.1 Isolation of lactic acid bacteria from 'nono'

Lactic acid bacteria were isolated from the 'nono' samples by serial dilution in 0.1% peptone water. Selective media β-disodium glycerophosphate agar (M-17) and de Mann Ragosa and Sharpe media (MRS agar) were used for the isolation of wild strains of lactic acid bacteria. The samples were inoculated through streak plate method. After inoculation, plates were incubated at 37^{o}C for 24-48 hours. Representative strains of LAB were obtained and purified by successive sub- culturing on MRS and M17 agar, and then confirmed using the following test:

Catalase test - a drop of 6% hydrogen peroxide (H_2O_2) solution was placed on the slides and loops full of the bacterial isolates were placed in the solution. The solutions were stirred using glass rods and observed for reaction. Gas bubble production was recorded as positive result.

Gram stain – a thin smear of the bacterial isolates was made on glass slides and heat fixed. It was then covered with crystal violet and then washed with water without blotting and covered with Gram's iodine. The slides were decolourized for 10-30 seconds with gentle agitation in 30% acetone solution, washed with water and covered with safranin for 10-30 seconds. The slides were then washed and let dry prior to microscopic examination.

Endospore Test- Smears of the organisms were prepared on grease- free slides and fixed, the slides were placed over boiling water in a beaker resting it on the rim with the bacterial film uppermost for 40 seconds where large droplets of water was allowed to condense underneath the slide. The smears were then flooded with 5% aqueous solution of malachite green and allowed to stand for 2 minutes while the water continues to boil. The slides were removed from the boiling water, washed and air-dried. Endospores stained green while vegetative cells stained red on microscopic examination.

Motility test- Nutrient agar slants were prepared and a growth of the test organism were picked and stabbed on the agar slant at right angle to the bottom of the test tube. The growth patterns of the organisms were observed after 36 hours of incubation at 37°C. Motile organisms grew away from the line of stab while non-motile organisms grew along the line of stab.

Characteristics of the isolated strains, such as catalase reaction, Gram staining, morphology, and motility were studied. Strains that were catalase negative, Gram positive, non- motile and with bacillus or coccus shape were preliminarily identified as LAB.

Further characterisation was performed by using the following tests: growth at different temperatures (10, 40 and 45°C), Carbondioxide ($C0_2$) gas production as well as the ability to grow in different concentrations of Sodium chloride NaCl (4.0 and 6.5%) in MRS and M17 broth, as described by (Schlinger and Lucke ,1989) and(Dykes *et al*., 1994).

3.2.2 Identification of the lactic acid bacteria to the species level

The presumptive colonies were identified to the species level using API 50 CH kit (Biomerieux, Marcy l'Etoile France). Pure isolates of the LAB were grown on MRS and M17 agar and harvested after 48 hours. The bacterial cells were prepared in API 50 CHL medium and used to inoculate the test strips immediately. The strips were incubated anaerobically in their incubation trays at 30^oC and read after 24 and 48 hours. Interpretations of the fermentation profiles were facilitated by systematically comparing all results obtained for the isolates studied with information from the computer-aided database APILAB Plus V.3.2.2. in which the identification of a microorganism is accompanied by the following information: (i) The percentage of identification (%id) is an estimate of how closely the profile corresponds to the taxon relative to all the other taxa in the database. (ii) The T-index represents an estimate of how closely the profile corresponds to the most typical set of reactions for each taxon. Its value varies between 0 and 1, and is inversely proportional to the number of atypical tests. (iii) Comments on the quality of identification derived from the % id and the T-index of the selected taxon (excellent identification % id > 99.9 and T> 0.75).

3.3 Screening of Isolates for Exopolysaccharides (EPS) Production:

The isolates were screened for Exopolysaccharide production following the method described by (Guiraud, 1998).

Procedure: The LAB strains precultivated on MRS agar were streaked onto LTV agar (0.5% (w/v) tryptone, 1% (w/v) meat extract, 0.65% (w/v) NaCl, 0.8% (w/v) KNO3, 0.8% (w/v) sucrose, 0.1% (v/v) Tween 80 (Merck), 1.7% (w/v) agar, pH 7.1 ± 0.2,(Sawadogo- Lingani *et al*., 2007) and incubated at 30°C for 48 h.

The sticky aspect of the colonies was determined by testing them for slime formation using the inoculation loop method (Knoshaug *et al*., 2000). Positive results were confirmed by plating out on MRS-sucrose and M17-sucrose agar as described by (Pidoux *et al*., 1990).

3.4 Determination of the Growth Parameters of the Local Starter Culture during Cow, Soy and Goat Milk Fermentations:

3.4.1 Enumeration of the local starter bacteria during cow, soy and goat milk fermentations.

This was carried out hourly as described by (Mohammed *et al.,* 2007) and (APHA, 1992). De Mann Rogosa and sharpe (MRS) medium (Oxoid) was used for enumeration of *L.lactis* while M-17 agar (Oxoid) was used for *S. thermophilus.*

At 60 minutes intervals, 10 ml of the fermented milks were removed from the incubator and serially diluted in 0.1% sterile peptone water (lg peptone, 8g NaCl, 1L water) and then pour plated and incubated in anaerobic gas jars at 37°C for 48h.

3.4.2 Determination of maximal specific growth rates (μmax) of the local starter culture during cow, soy and goat milk fermentations:

Maximal specific growth rates (μmax) of the local starter culture during cow, soy and goat milk fermentations was calculated using the equation adopted by (Riazi and Ziar, 2008). :

$$\mu \text{ max} = \frac{\ln X_2 - \ln X_1}{t_2 - t_1}$$

Where X_2 and X_1 are the cell biomass at time t_2 and t_1 of exponential phase, respectively. Doubling time (td) was calculated as:

$$td = \frac{\ln 2}{\mu}$$

where μ is the specific growth rate.

3.4.3 Determination of maximal acidification rates σ (pH max/ σt) of the local starter culture during cow, soy and goat milk fermentations:

pH of the fermenting milks was taken and maximum values obtained at exponential phase were used to calculate maximal acidification rates **σ**(pH max/**σ**t).

$$\boldsymbol{\sigma} \text{ pH max/ t} = \frac{pH_1 - pH_2}{t_2 - t_1}$$

Where pH_2 and pH_1 are the pH values at exponential phase for time t_2 and t_1 respectively.

3.5 Preparation of Soymilk

Soy milk was produced by the method of (Lee *et al*., 1990) where 500g of Soybeans (*Glycine max)* was steeped in water for three hours to remove the soybean hulls and drained. The de-hulled soybeans was steeped in aqueous 0.05% $NaHCO_3$ for 20 minutes at 25 °C, boiled at 100 °C for five minutes and rinsed.

Soybeans

↓

Steeping (water, 3 hrs)

↓

Steeping in aqueous (0.05% $NaHCO_3$, 20 min)

↓

Blanching (100°C, 5 min)

↓

Milling (add water 7:1)

↓

Filtration (cheese cloth, 170- mesh sieve)

↓

Soy milk

Fig. 3.1: Flow Chart for the preparation of soy milk.

The blanched soybeans were milled with the addition of enough water to give a water to-dry-soybeans ratio of 7:1 on a weight basis. The resultant slurry soybeans was filtered through cheese cloth and re-filtered with 170-mesh sieve to obtain soymilk (Figure 3.1).

3.5.1 Preparation of bacterial inoculums size for fermentation

The bacterial inoculums size for the yoghurt fermentation was prepared using McFarland turbidity standard scale number 2.

3.5.2 Preparation of McFarland turbidity standards

The turbidity standards were prepared according to the method of microbio lab (2010). The method involved preparing 1% solution of anhydrous $BaCl_2$ and 1% solution of H_2SO_4 mixed in the proportion below, tightly sealed in sets of tubes and stored at room temperature in the dark.

3.6 Yoghurt Production

Duplicate samples of the three milk types were pasteurized at 60°C for 30min.The pasteurized milk were subsequently placed in a water bath to cool down to 40°C prior to inoculation with the starter culture as described by (Collins *et al.*, 1991) .The first portion of the cooled milk samples was inoculated with the commercial yoghurt starter (50:50 pure culture of mixed strains of *Lactobacillus bulgaricus* and *Streptococcus thermophillus*; 0.5 g of the culture was used to inoculate 1000 ml of the milk to initiate fermentation) as described by (Lee *et al*., 1990). A preculture of each of the two LAB isolates from 'nono' (*Streptococcus thermophilus* and *Lactococcus lactis*) was prepared; and the mixed culture inoculated into the second portion of cooled mixture of each of the milk samples at 5%

final volume as described by (Murti *et al*., 1992). All the inoculated milk samples were poured into plastic cups and incubated at 40°C to ferment for 4-6 hours. After incubation, the samples were cooled in an ice bath, placed in a cabinet at 6±2°C until evaluation within 12 hours as shown in the flow- chart (Figure 3.2).

3.7 Physicochemical Analysis of the Yoghurt Samples

The following physicochemical parameters were determined for the yoghurt samples:

3.7.1 Hydrogen ion concentration (pH):

In order to obtain the pH of the samples, 5 ml from each sample was measured and suspended in 10 ml of distilled water. The pH was determined using an electronic digital pH meter (Hanna Instrument 8021).

3.7.2 Titratable acidity

The acidity was measured as described by (Olubamiwa *et al*., 2007). An aliquot of 0.5 ml of a 1% solution of phenolphthalein in 95% alcohol was added to 10 ml of yoghurt sample. Acidity was measured by titrating the resulting mixture with 0.1N NaOH, expressed as g equivalent lactic acid/100g, (AOAC, 1990; O'Connor, 1995). All the determinations were carried out in triplicates and mean values were calculated.

3.8 Proximate Analysis of the Yoghurt Samples

Samples were analyzed for proximate composition using standard methods as described by (Egan *et al*., 1981) and (AOAC, 1990).

Table 3.1: McFarland turbidity standards result interpretation.

McFarland Standard	Amt of 1% $BaCl_2$(ml)	Amt of 1%H_2SO_4	Approximate Cell Count Density (x10^8 cells)
0.5	0.05	9.95	1.5
1.0	0.1	9.9	3.0
2.0	0.2	9.8	6.0
3.0	0.3	9.7	9.0
4.0	0.4	9.6	12.0

3.8.1 Determination of moisture content

The oven drying method was used as described by (AOAC, 2005) where the samples were heated and the loss of weight was used to calculate the moisture content of the samples. A clean platinum dish was dried in an oven and cooled in a desiccator. The cooled dish was weighed (W_1). From the yoghurt samples, 5g was weighed and spread on the dish, the dish containing the sample was weighed (W2). It was then transferred into the hot- air oven at 105^{o}C to dry for about 3h. A pair of tongs was used to transfer the dish into a desiccator, allowed to cool and weighed. The dish was returned into the oven for 30 min and again cooled in a desiccators and weighed (AOAC, 1980).The process was repeated until a constant weight (W3) was obtained and the calculation was done using the formula below:

The percentage moisture content was calculated thus:

$$\% \text{ Moisture content} = \frac{W2 - W3 \times 100}{W2 - W1}$$

3.8.2 Determination of ash content

Ash is defined as inorganic substance remaining in the residue after ignition. The ash content was calculated after burning and weighing as described by (AOAC, 2005). A porcelain crucible was dried in an oven at 100^{o}C for 10 minutes, cooled in a desiccator and weighed (W1). Two grams of the sample were placed into a previously weighed porcelain crucible and reweighed (W2). It was first ignited and then transferred into a furnace, which was then set at 550^{o}C. The sample was left in the furnace for 8 hours to ensure proper

ashing. The crucible containing the ash was then removed and cooled in the desiccator and weighed (W3).

The percentage ash content was calculated as:

$$\% \text{ Ash content} = \frac{W3 - W1}{W2 - W1} \times 100$$

3.8.3 Determination of nitrogen and crude protein content

The macro Kjeldah's method as described by (AOAC, 2005) was used to determine the crude protein content. Two grams of the samples was introduced into the digestion flask. Ten grams of copper sulphate and sodium sulphate in the ratio of 5:1 and 25ml of concentrated sulphuric acid was added to the digestion flask. The flask was placed into digestion block in fume cupboard and heated until frothing ceased given a clear and light blue colouration. The mixture was allowed to cool and was diluted with distilled water until it reached 25ml of volumetric flask. 10ml of the mixture was poured into the distillation apparatus and 10ml of 40% sodium hydroxide was added. The released ammonia by boric acid was allowed to continue until Ten millimeters of boric acid is treated with 0.02m of hydrochloric acid until the green colour change to purple. The nitrogen in the sample was then determined. The percentage nitrogen of the sample was calculated as;

$$\% \text{ Nitrogen} = \frac{\text{molar mass of N2} \times \text{acid conc. (0.02m)} \times \text{volume made} \times \text{titre value} \times 100}{10 \times 1000 \times \text{sample weight (2g)}}$$

$$\% \text{ Crude protein} = \% \text{ Nitrogen} \times 6.25$$

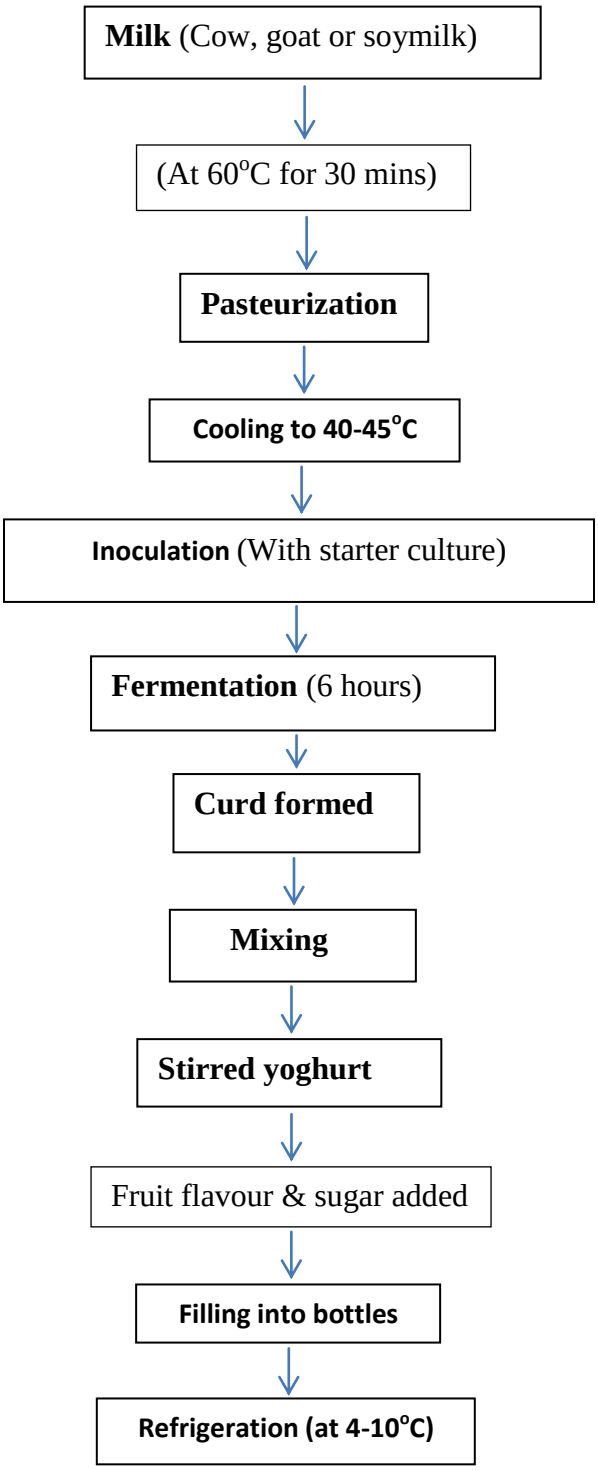

Fig. 3.2: Flow Chart for production of yoghurt.

3.8.4 Determination of total solids

Total solids (TS) represent the components that remain after the complete removal of water from milk measured in grams.

The total solids were determined as described by (AOAC, 2005). Three grams of the sample was weighed into a dry Petri dish of a known weight. The total portion was pre-dried for 25minutes on steam bath and then dried for 3hours at 100^{o}C in forced draft air oven. The total solid sample is the weight of the dried sample residue and was calculated as:

$$\% \text{ Total Solid} = \frac{W2 - W1 \times 100}{W1 - W}$$

Where:
W = Weight of the dish
W1 = Weight of dish and sample test portion
W2 = Weight of dish and dry sample

3.8.5 Determination of fat content

The soxhlet solvent extraction method as described by (AOAC, 2005) was used to determine the fat content. In this method 2g of the sample was weighed into a flat bottom flask of known weight with the extractor mounted on it. The thimble was held half way into the extractor and the weighed sample was carefully transferred into the thimble and the thimble was plugged with cotton wool.

The extraction was carried out at the temperature of 40 – 60^{o}C for 8hours. The solvent was removed by evaporation and then, the remaining part of the flask was dried in

the oven at 80^0C for 30minutes and was finally cooled in a desiccator. The flask was reweighed and the percentage fat was calculated as;

$$\% \text{ Fat} = \frac{\text{Weight of extracted fat} \times 100}{\text{Weight of sample used}}$$

3.9 Microbiological Analysis

Microbial counts of the fermented milk samples were determined using the method described by (Jimoh and Kolapo, 2007) using ten ml of each yoghurt samples and placed in 90 ml sterile 0.1% peptone water and shaken to prepare 10^{-1} dilution. Then decimal dilution series was prepared in 0.9% NaCl saline solution. 0.1 ml of the dilute sample was plated out on nutrient agar, manitol salt agar (MSA), Eosin methylene blue agar (EMB), *Salmonella-Shigella* agar (SSA), de Mann Ragosa and Sharpe medium (MRS) agar and Sabourad Dextrose Agar (SDA) using the spread plate method where a sterile bent glass rod was used to spread the innocula on the various agar media for determination of total viable count, *Staphylococcus* count, *E. coli* count, *Salmonella* count, LAB and Yeast and Mould counts respectively. The plates for Yeast and Mould count were incubated at 25^oC, *E. coli* at 44.5^oC, LAB at 37^oC and the rest at 35^oC for 48 hours and colony forming unit per ml of sample (cfu/ml) was enumerated.

3.10 Determination of Viscosity:

Viscosity of the yoghurt samples was measured in terms of relative and apparent viscosity i.e. consistency using an improvised consistometer, as described by the (Cole-Parmer, 2000). This meter works on the principle of measuring resistance to flow under gravitational force. A known quantity (15 grams) of uniformly and consistently stirred

sample was allowed to flow under its own weight for 15 seconds. The distance covered by moving edge of each sample was noted and compared with the standard yoghurt (prepared in the laboratory by addition of 0.5 percent gelatin and 2 percent starter culture in pasteurized milk and incubated at 42^{o}C. The ratio between the distance traveled by the sample and the standard yoghurt was reciprocated and then converted into percentage of viscosity by multiplying by 100.

3.11 Sensory Evaluation

Members of the 15-member panel were untrained panelists consisting of students and staff of Microbiology Department Ahmadu Bello University, Zaria. Sensory evaluation was done on a five point hedonic scale. Characteristics evaluated included texture, aroma, colour, taste and overall acceptability.The yoghurt samples were scored on a scale of 1–5 (1 = poor, 2 = fair, 3 = good, 4 = very good and 5 = excellent).

3.12 Shelf- Life Determinations

The shelf-life of the yoghurt samples was determined at ambient (27^{o}C±2^{o}C), refrigeration (4^{o}C ±2^{o}C) and freezing temperatures for 14 days. The samples were physically observed using visual appearance, wheying-off and smell characteristics to determine their wholesomeness followed by total viable counts, LAB counts and yeast and moulds counts during the storage period.

3.13 Statistical Analysis

Statistical analysis was conducted using ANOVA analysis (S). Comparisons were made using Duncan test for multiple comparisons, $P<0.05$ was considered statistically significant.

CHAPTER FOUR

4.0 RESULTS

Six different strains of lactic acid bacteria were isolated in this study namely: *Lactobacillus brevis, Streptococcus thermophilus, Pediococcus pentosaceus, Lactobacillus fermentum, Lactobacillus pentosus,* and *Lactococcus lactis* spp *lactis.*

Table 4.1 shows the colonial and physiological properties of the isolates. Although not all the strains belong to the same genus, they exhibit very similar cultural and physiological characteristics. The isolates presented as wet and milky colonies on plate and the majority of them were rod-shaped with few being cocci while all were non-motile, catalase negative, non-spore formers and gave positive reactions to Gram staining.

Table 4.2 shows the growth characteristics of the isolated strains with respect to their abilities to grow at various temperatures, salt concentrations and carbondioxide (CO_2) production. This result shows that all the isolates were able to grow at 40^0C, majority of them at 10^0C and some at 45^0C. Almost all the isolates were able to grow at salt concentrations of 4% (w/v) and 6.5% (w/v) with most of the isolates showing negative result to CO_2 production.

Table 4.3 shows the result of exopolysaccharide production by the isolated strains. From this result, it is evident that only two of the isolates produced exopolysaccharide which presented as slimy and opaque colonies on close observation and this result was used as one of the basic criteria for selection of the isolates to be used as local starter cultures for the rest of the study.

Table 4.1: Cultural and Physiological Properties of LAB Isolates

Code	Colonial Morphology	Vegetative morphology	Gram staining	Catalase Test	Spore formation	Motility	Identity
S1	Wet slimy and milky	Cocci	+	-	-	-	*L. lactis*
S2	Wet and milky	Rods	+	-	-	-	*L. fermentum*
S3	Wet and milky	Rods	+	-	-	-	*L. pentosus*
S4	Wet and milky	Rods	+	-	-	-	*L. brevis*
S5	Wet and milky	Rods	+	-	-	-	*P. pentosaceous*
S6	Wet slimy and milky	Cocci	+	-	-	-	*S. thermophilus*

S1-S6 = codes for isolates before characterization

+ = positive

- = negative

Table 4.2: Growth characteristics of isolated strains

Isolates	Growth at			Growth at NaCl		CO_2 Production
	10°C	40°C	45°C	4%	6.5%	
Lactococcus lactis	+	+	-	+	+	-
Lactobacillus fermentum	-	+	+	+	+	+
Lactobacillus pentosus	+	+	-	+	+	-
Lactobacillus brevis	+	+	-	+	+	+
Pedicoccus pentosaceous	+	+	+	+	-	-
Streptococcus thermophilus	-	+	+	-	-	-

+ = positive growth

- = no growth

Table 4.3: Exopolysaccharide (EPS) production by isolated strains

ISOLATES	EPS PRODUCTION
S. thermophiles	2^+
L. lactis	3^+
L. brevis	-
L. fermentum	-
L. pentosaceous	-
L. pentosus	-

Key:

2^+ = Slightly opaque

3^+ = Opaque

- = Nil

Table 4.4 shows result for the maximal specific growth rates and doubling times per minute of the lactic acid bacteria (*Streptococcus thermophilus and Lactococcus lactis*) chosen as local starter cultures in cow, soy and goat milk fermentations. The result shows that *Streptococcus thermophilus* showed a higher doubling time in cow and goat milk fermentations with shorter specific growth rates while *L. lactis* showed lower doubling times and higher specific growth rates in cow and goat milk fermentations as compared to soy milk fermentation.

Table 4.5 shows the Maximal Acidification rates of the local starter cultures during fermentation of cow, soy and goat milks. From the result it can be deduced that the acidification rate was higher in cow and goat milks compared to that of soy milk.

Fig 4.1 shows the relationship between the growths of the local starter culture (*Streptococcus thermophilus and Lactococcus lactis*) during soy milk fermentation. The result revealed that *Streptococcus thermophilus* showed higher growth as compared to *Lactococcus lactis*.

Fig 4.2 shows the relationship between the growths of the local starter culture (*Streptococcus thermophilus and Lactococcus lactis*) during cow milk fermentation. *Streptococcus thermophilus* showed a higher growth compared to *Lactococcus lactis*.

Fig 4.3 shows the relationship between the growths of the local starter culture (*Streptococcus thermophilus and Lactococcus lactis*) during goat milk fermentation. *Streptococcus thermophilus* showed a higher growth compared to *Lactococcus lactis*.

Table 4.4: Mean Maximal specific growth rates and doubling times of the local starter culture (*S. thermophilus* and *L. lactis*) in cow, soy and goat milk fermentations.

	Specific growth rate (μmax) per hour			**Doubling time (mins)**		
	Goat milk	Soy milk	Cow milk	Goat milk	Soy milk	Cow milk
S. thermophilus	0.111 ±0.006[b]	0.423 ±0.008[a]	0.123±0.004[b]	6.428 ±0.002[a]	1.664 ±0.004[b]	6.430±0.001[a]
L.lactis	0.289 ±0.003[b]	0.403 ±0.002[a]	0.301±0.002[b]	0.789 ±0.001[b]	1.728 ±0.006[a]	0.805±0.001[b]

Values are means and superscripts with different letters significantly varied ($p < 0.05$)

Table 4.5: Maximal Acidification rates during fermentation of cow, soy and goat milks by the local starter culture (*S. thermophilus* and *L. lactis*)

Milk source	**Acidifying rate h^{-1}** **Mean ± SEM**
Cow Milk	0.63 ± 0.006^{b}
Soy milk	0.195 ± 0.012^{a}
Goat milk	0.61 ± 0.008^{b}

Values are Means and superscripts with different letters varied significantly ($p < 0.05$).

Key: SEM= Standard Error of Mean

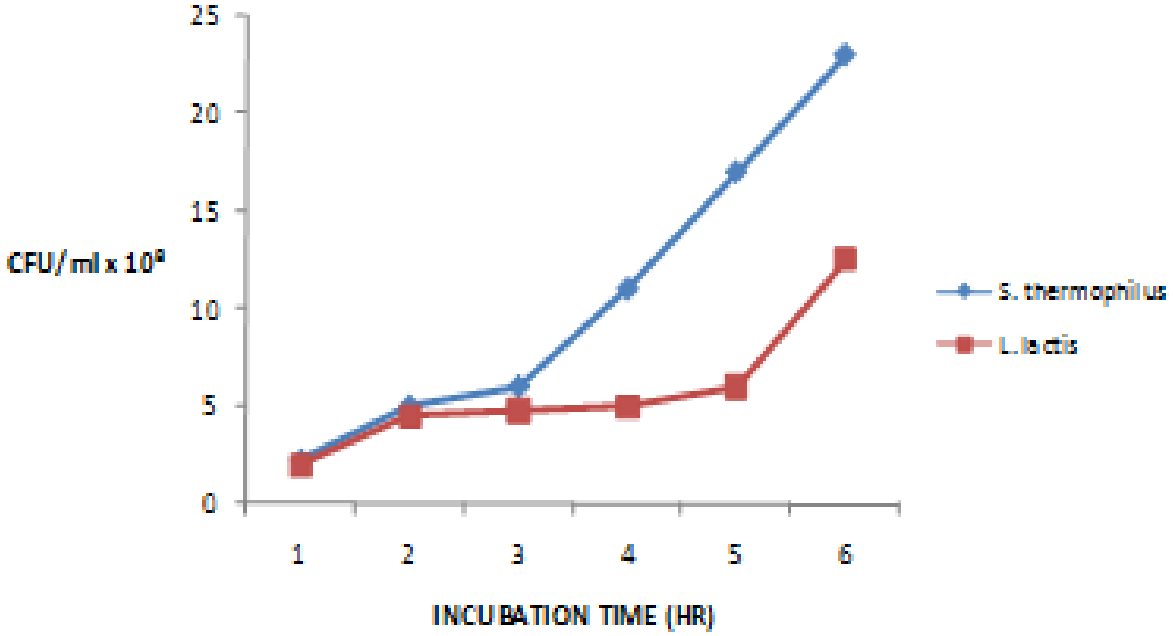

Fig 4.1: Relationship between growths of the local starter culture (*S. thermophilus* and *L. lactis*) during soy milk fermentation.

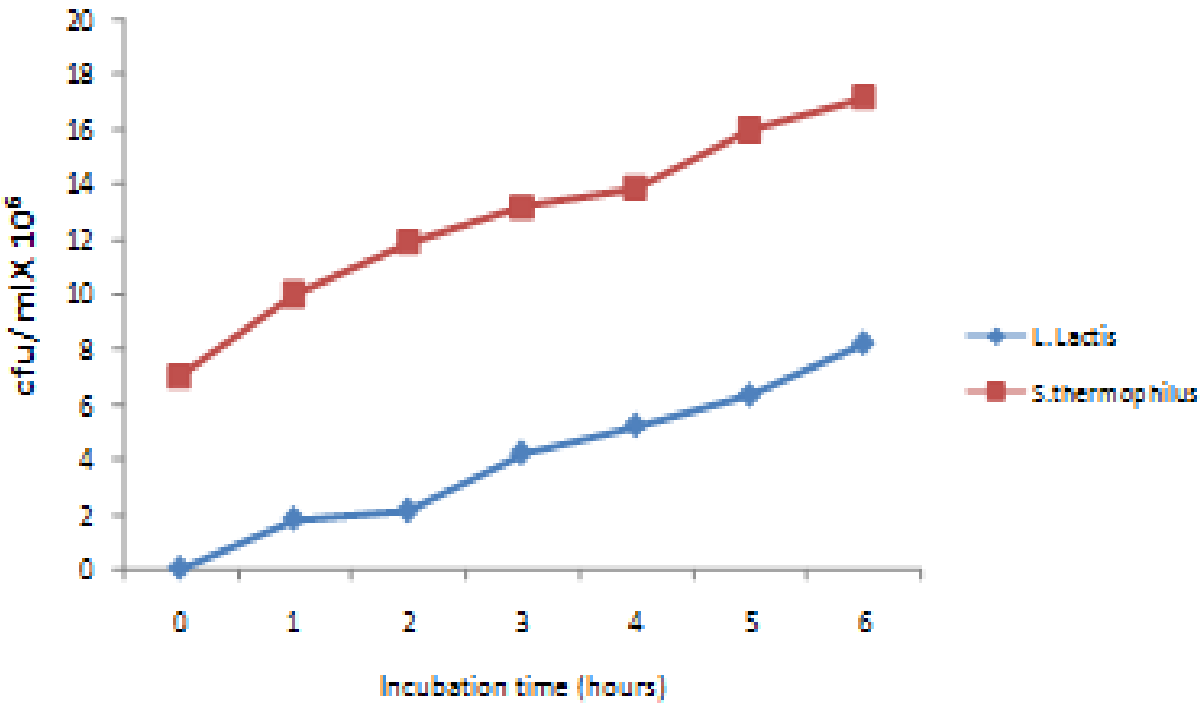

Fig 4.2: Relationship between growths of the local starter culture (*S. thermophilus* and *L. lactis*) during cow milk fermentation.

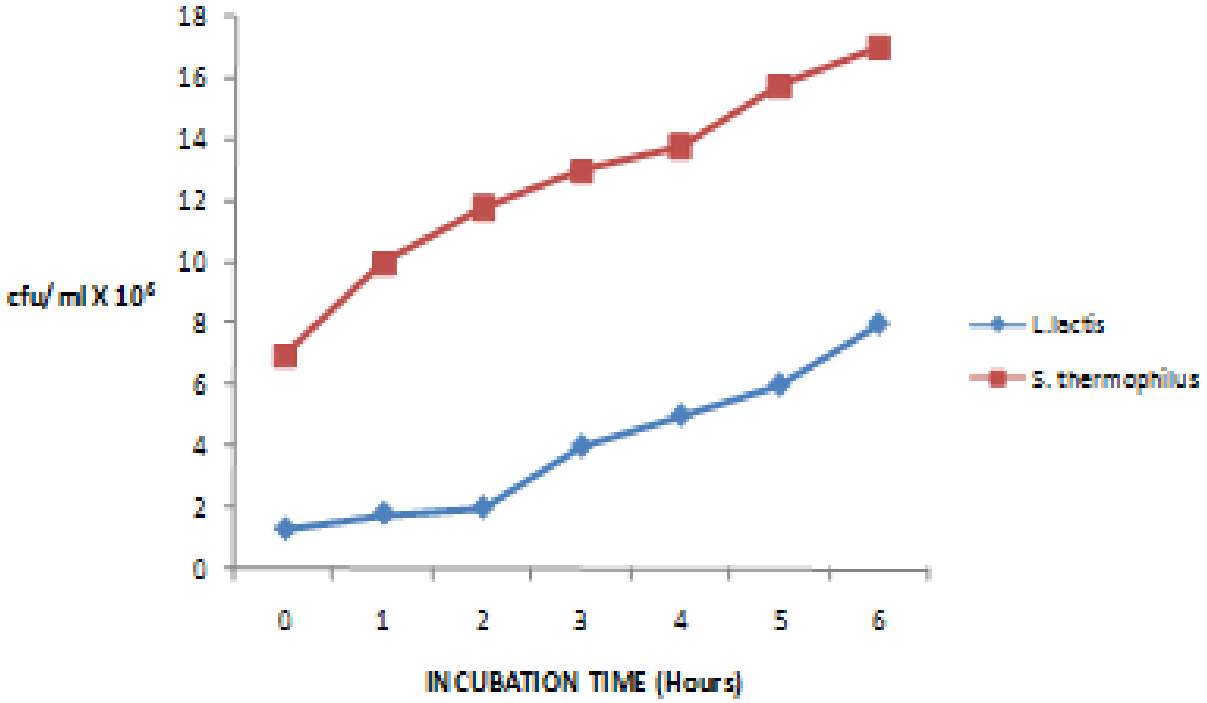

Fig 4.3: Relationship between growths of the local starter culture (*S. thermophilus* and *L. lactis*) during goat milk fermentation.

Fig 4.4 shows the percentage lactic acid produced by the local starter culture (*Streptococcus thermophilus and Lactococcus lactis*) during cow, soy and goat milk fermentations. The result shows that the percentage lactic acid produced in cow and goat milk fermentations were higher compared to that of soy milk.

Fig 4.5 shows the result for the physicochemical analysis of the produced yoghurt samples. The result shows the pH changes in the milk samples with respect to time during yoghurt production. The pH of all the milk samples decreased with increase in time where soy milk yoghurts with both local and commercial starter cultures had the highest pH at the end of fermentation.

Fig 4.6 shows the result for the titratable acidity of the produced yoghurt samples. The titratable acidity of all the milk samples increased with increase in time with cow milk yoghurts from both local and commercial starter cultures showing the highest titratable acidity at the end of fermentation.

Fig 4.7 shows the result for the proximate analysis of the produced yoghurt samples. There was no significant difference in the studied parameters among all the samples except in the ash and fat contents where goat milk yoghurts from both local and commercial starter cultures presented higher fat and ash contents.

Table 4.6 shows the result for the microbiological analysis of the produced yoghurt samples. The result showed that there was no contamination in all the yoghurt samples and the LAB counts were in conformity with the rules set by dairy food regulatory agencies.

Fig 4.8 shows the result for the viscosity of the yoghurt samples. Soy milk yoghurts from both local and commercial starter cultures showed higher viscosity.

Table 4.7 shows result for the sensory evaluation of the yoghurt samples. The result revealed that there was no statistically significant difference between all the yoghurt samples in terms of general acceptability.

The result for the shelf life study of the yoghurt samples following physical observation of whey separation, changes in flavour and appearance as indices of spoilage during the storage period of fourteen days at ambient, refrigerated and freezing temperatures (Appendix VII). Result showed spoilage after days two at ambient temperature for all yoghurt samples, day four at refrigerated temperature and from day ten at freezing temperatures. There was high level of deterioration in soy milk based yoghurts during the storage period.

Table 4.8 shows the total aerobic counts of the yoghurt samples during the storage periods. There was decrease in the total aerobic count with days and temperature of storage.

Table 4.9 shows the lactic acid bacteria count of the yoghurt samples where cow and goat milk based yoghurts with both the commercial and local starters showed higher counts compared to the soy based yoghurts. This count also decreased with days and temperature of storage.

Table 4.10 shows result for the yeast and mould counts during the storage period. There was decrease in the number of counts with storage.

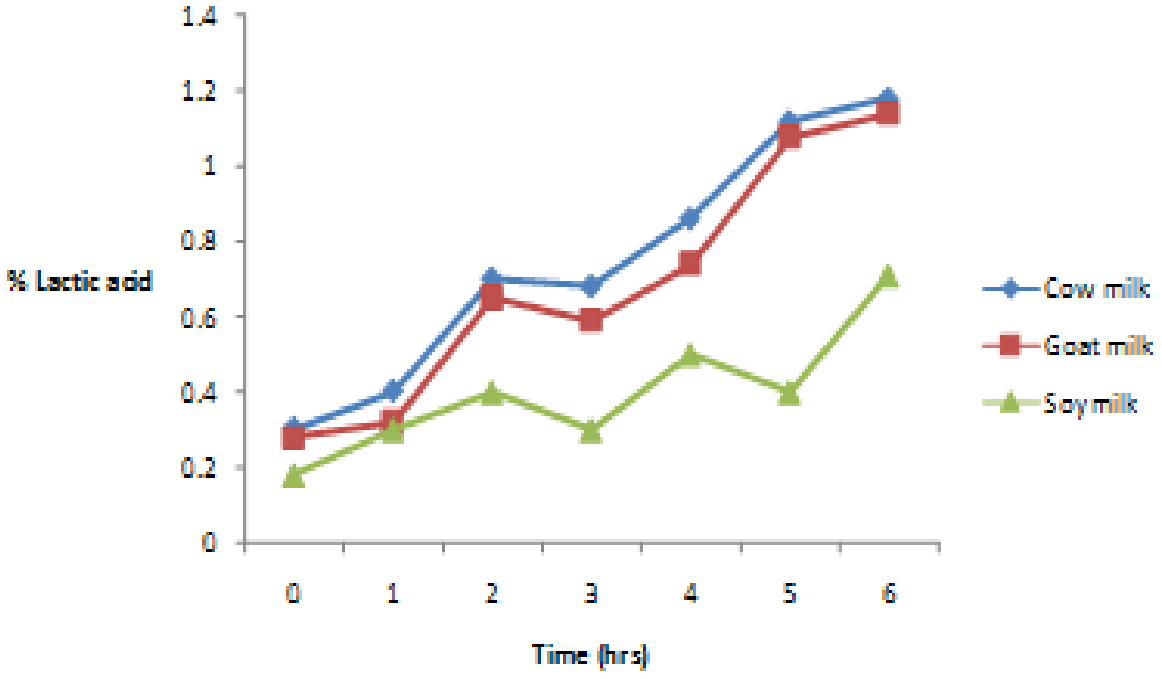

Fig 4.4: Percentage lactic acid produced by the local starter culture (*S. thermophilus* and *L. lactis*) during cow, soy and goat milk fermentations.

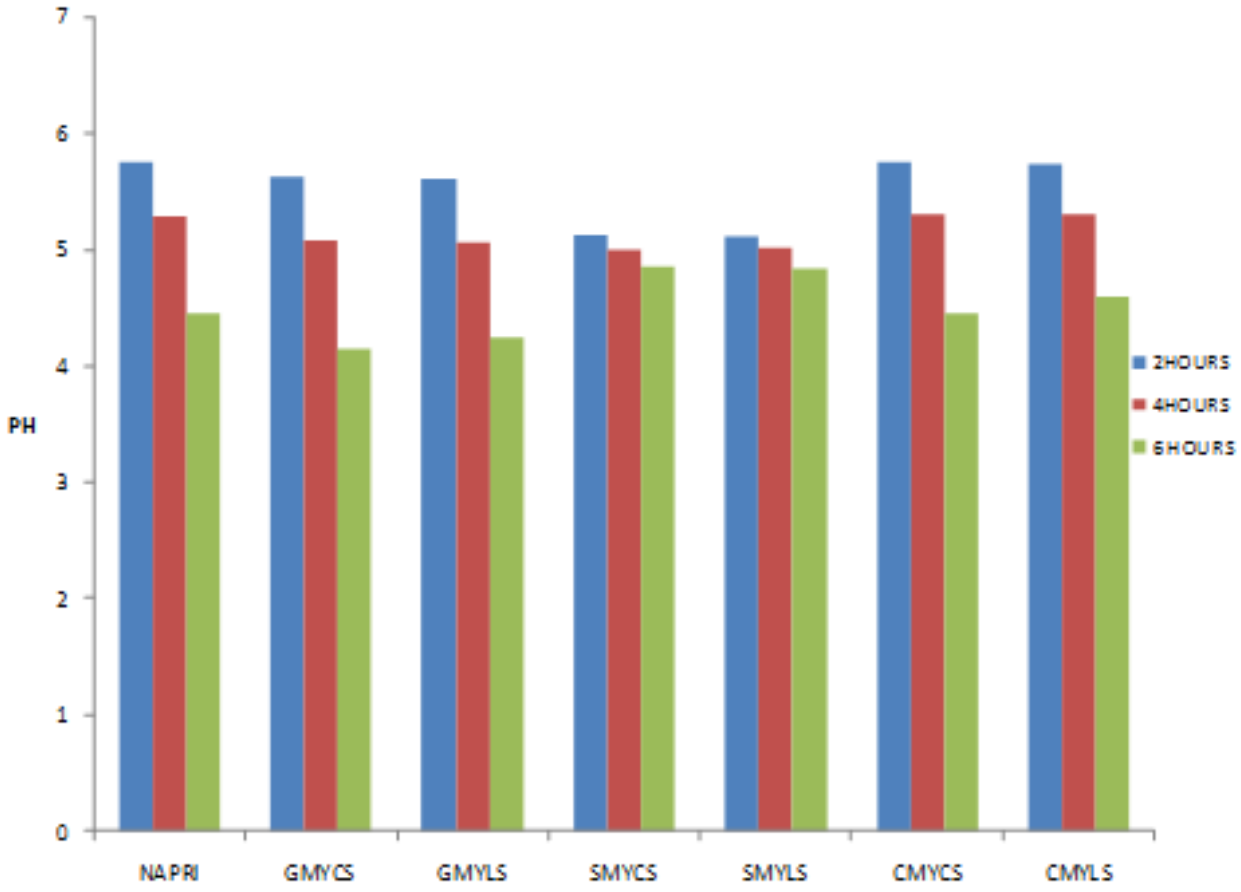

Fig 4.5: Effect of pH in relation to time of yoghurt production

Key: NAPRI- Control yoghurt

GMYCS- Goat milk yoghurt with commercial starter

GMYLS- Goat milk yoghurt with local starter

SMYCS- Soy milk yoghurt with commercial starter

SMYLS- Soy milk yoghurt with local starter

CMYCS- Cow milk yoghurt with commercial starter

CMYLS- Cow milk yoghurt with local starter

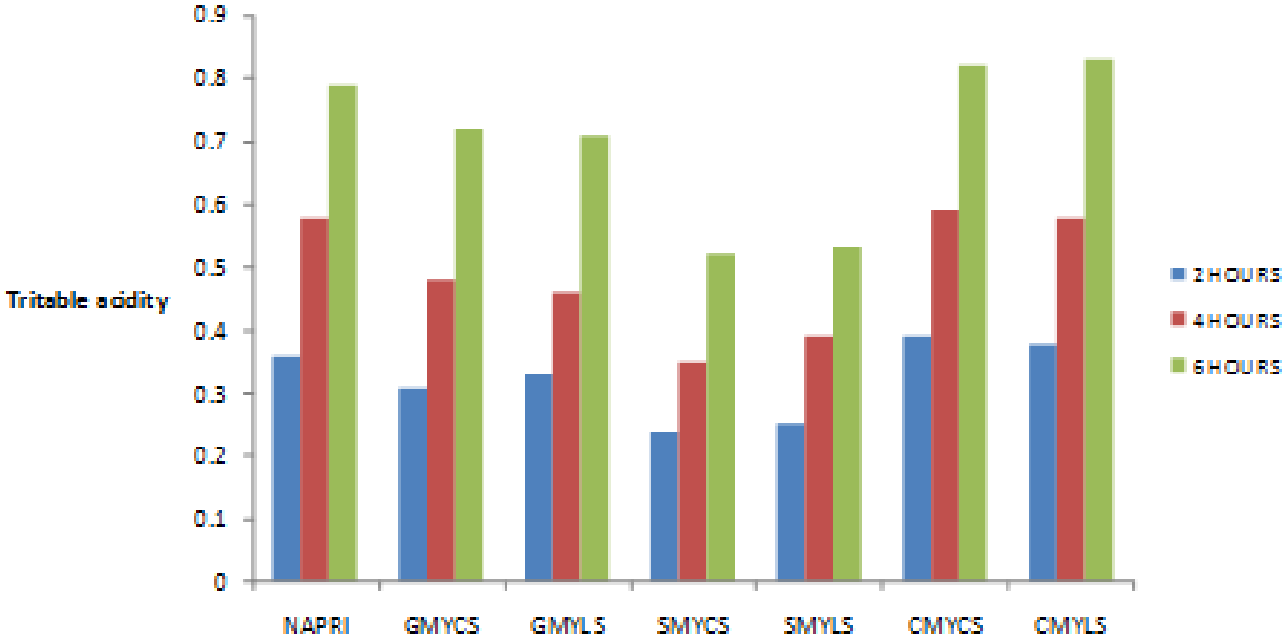

Fig 4.6: Effect of Titratable Acidity in Relation to Time of Yoghurt Production.

Key: NAPRI- Control yoghurt

GMYCS- Goat milk yoghurt with commercial starter

GMYLS- Goat milk yoghurt with local starter

SMYCS- Soy milk yoghurt with commercial starter

SMYLS- Soy milk yoghurt with local starter

CMYCS- Cow milk yoghurt with commercial starter

CMYLS- Cow milk yoghurt with local starter

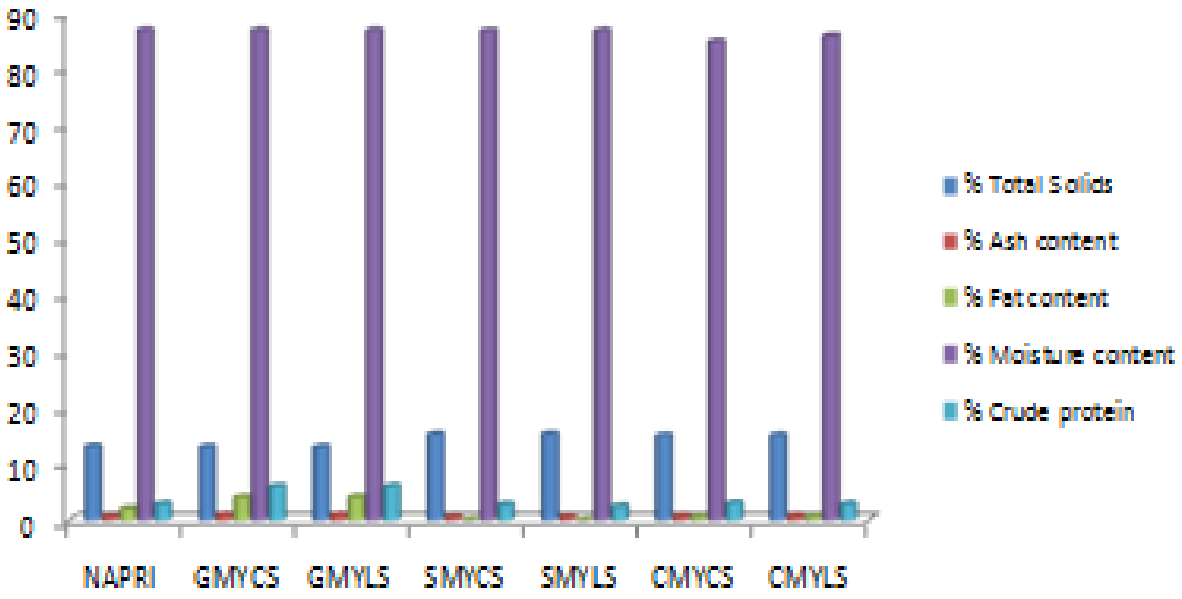

Fig 4.7: Proximate Analysis of the Yoghurt Samples

Key: NAPRI- Control yoghurt

GMYCS- Goat milk yoghurt with commercial starter

GMYLS- Goat milk yoghurt with local starter

SMYCS- Soy milk yoghurt with commercial starter

SMYLS- Soy milk yoghurt with local starter

CMYCS- Cow milk yoghurt with commercial starter

CMYLS- Cow milk yoghurt with local starter

Table 4.6: Microbiological Analysis of the Produced Yoghurt Samples (log cfu/ml)

	NAPRI (Control)	CMYCS	CMYLS	SMYCS	SMYLS	GMYCS	GMYLS
TVC	20.8±0.2	20.9±1.1	19.6±1.2	14.3±1.2	13.5±0.2	18.8±1.2	18.6±1.4
LAB count	26.3±0.1	26.8 ±0.3	25.5±0.1	16.1±1.2	15.0±1.2	20.9±0.1	19.2±0.2
Salmonella	ND	ND	ND	ND	ND	ND	ND
E. coli	ND	ND	ND	ND	ND	ND	ND
Staphylococcal count	ND	ND	ND	ND	ND	ND	ND
Yeast and mould	ND	ND	ND	ND	ND	ND	ND

Key

TVC = total viable bacterial counts

ND = not detected

NAPRI- Control yoghurt

GMYCS- Goat milk yoghurt with commercial starter

GMYLS- Goat milk yoghurt with local starter

SMYCS- Soy milk yoghurt with commercial starter

SMYLS- Soy milk yoghurt with local starter

CMYCS- Cow milk yoghurt with commercial starter

CMYLS- Cow milk yoghurt with local starter

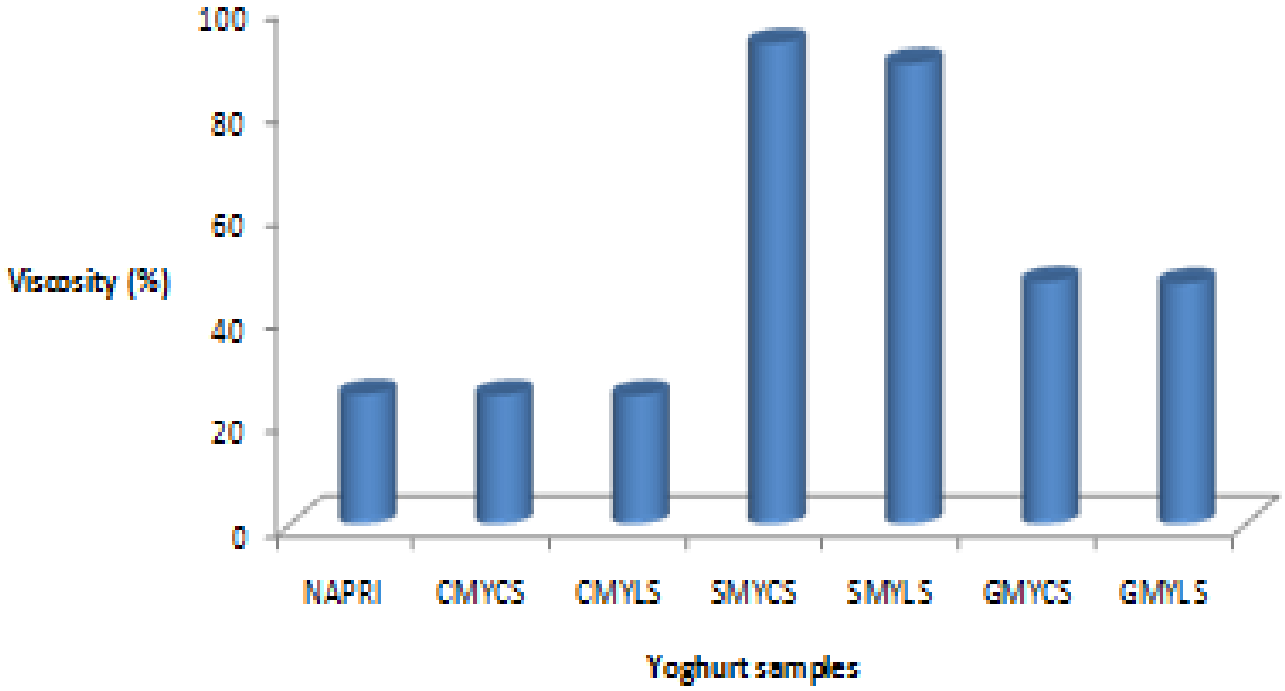

Fig 4.8 Viscosity of Yoghurt Samples

Key: NAPRI- Control yoghurt

GMYCS- Goat milk yoghurt with commercial starter

GMYLS- Goat milk yoghurt with local starter

SMYCS- Soy milk yoghurt with commercial starter

SMYLS- Soy milk yoghurt with local starter

CMYCS- Cow milk yoghurt with commercial starter

CMYLS- Cow milk yoghurt with local starter

Table 4.7: Sensory Evaluation of Yoghurt Samples

SAMPLE	Appearance	Texture	Aroma	Flavour	Colour	Taste	General acceptability
GMYLS	3.4 [a] (0.1)	3.4 [a b] (1.7)	3.6 [a] (0.2)	3.5 [ab] (0.2)	3.7 [bc] (0.2)	3.7 [bc] (0.2)	3.3 [a] (0.2)
CMYLS	3.1 [a] (0.1)	3.4 [ab] (0.1)	3.3 [a] (0.1)	3.3 [a] (0.2)	3.3 [ab] (0.2)	3.3 [ab] (0.2)	3.5 [a] (0.2)
SMYLS	3.1 [a] (0.1)	3.2 [ab] (0.2)	3.1 [a] (0.2)	3.1 [a] (0.2)	3.0 [a] (0.2)	3.3 [ab] (0.2)	3.7 [a] (0.2)
GMYCS	4.0 [b] (0.1)	3.4 [b] (0.2)	3.1 [a] (0.3)	2.9 [a] (0.2)	4.2 [c] (0.2)	2.9 [a] (0.2)	3.5 [a] (0.2)
SMYCS	2.9 [a] (0.3)	3.5 [b] (0.2)	3.5 [a] (0.3)	3.8 [b] (0.2)	2.8 [a] (0.2)	4.0 [c] (0.2)	3.6 [a] 0.3)
CMYCS	3.2 [a] (0.2)	3.0 [ab] (0.2)	3.7 [a] (0.3)	3.5 [ab] (0.2)	3.8 [b c] (0.2)	3.6 [ab] (0.2)	3.7 [a] (0.3)
NAPRI	3.0 [a] (0.2)	2.8 [a] (0.2)	3.3 [a] (0.2)	3.2 [ab] (0.2)	3.9 [b c] (0.2)	3.5 [abc] (0.3)	3.6 [a] (0.2)
P-value	0.001 [*]	0.145	0.095	0.068	0.000 [*]	0.032 [*]	0.878

Kruskal wallis Anova; Duncan test P-value≤ 0.05

Key: NAPRI- Control yoghurt

GMYCS- Goat milk yoghurt with commercial starter

GMYLS- Goat milk yoghurt with local starter

SMYCS- Soy milk yoghurt with commercial starter

SMYLS- Soy milk yoghurt with local starter

CMYCS- Cow milk yoghurt with commercial starter

CMYLS- Cow milk yoghurt with local starter

Table 4.8: Mean Total Aerobic Counts of Yoghurt Samples during Storage Expressed in log_{10} Cfu/ml (Shelf-Life Study).

Storage days	DAY 0	Day 4		Day 10		Day 14	
Storage temp.	Ambient	Refrigerated	Freezing	Refrigerated	Freezing	Refrigerated	Freezing
Control(NAPRI)	4.8±0.2a	4.6±1.4a	2.5±0.4a	4.4±0.2a	2.1±0.1a	2.5±0.2a	0.8±0.1a
CMYCS	4.8±1.1a	4.9±1.2a	2.5±0.2a	4.4±0.2a	2.1±0.2a	2.8±0.1a	0.6 ±0.2a
CMYLS	4.5±1.2a	4.2±0.4a	2.3±0.1a	4.3±0.4a	2.2±0.1a	2.3±0.2b	0.6±0.3a
GMYCS	4.4±1.2a	3.9±1.2b	2.1±0.2a	4.1±0.1a	2.5±0.3a	2.6±0.2a	0.5±0.1a
GMYLS	3.6±1.1b	3.8±0.1b	2.0±0.1a	3.9±0.3a	2.6±±0.1a	2.2±0.2b	0.4±0.2ab
SMYCS	3.1±1.2b	3.5±0.7b	1.7±0.2b	3.2±0.1b	2.3±0.1b	2.2±0.1b	0.3±0.2ab
SMYLS	2.9±1.1c	3.1±0.7b	1.7±0.1b	3.1±0.1b	1.8±0.2c	2.1±0.2b	0.2±0.1b
P-value	**0.004****	**0.03***	**0.001****	**0.01****	**0.002****	**0.0024****	**0.01****

Values are Means and superscripts with different letters varied significantly ($p < 0.05$).

Key- NAPRI- Control yoghurt

GMYCS- Goat milk yoghurt with commercial starter

GMYLS- Goat milk yoghurt with local starter

SMYCS- Soy milk yoghurt with commercial starter

SMYLS- Soy milk yoghurt with local starter

CMYCS- Cow milk yoghurt with commercial starter

CMYLS- Cow milk yoghurt with local starter

Table 4.9: Mean Lactic Acid Bacteria Counts of Yoghurt Samples during Storage Expressed in log_{10} Cfu/ml (Shelf-Life Study).

Lactic acid bacteria	DAY 0	DAY 4		DAY 10		DAY 14	
storage	Ambient	Refrigerated	Freezing	Refrigerated	Freezing	Refrigerated	Freezing
Control(NAPRI)	6.6±0.1a	4.9±0.5a	1.6±0.2a	4.2±0.2a	3.5±0.1a	4.5±0.2a	1.8±0.1
CMYCS	6.8±0.3a	4.5±0.7a	1.4±0.2a	4.0±0.2a	3.6±0.2a	4.8±0.1a	2.3 ±0.2a
CMYLS	5.7±0.1a	3.6±0.3c	1.1±0.1a	3.9±0.1a	3.7±0.1a	4.3±0.2a	1.4±0.3b
GMYCS	4.9±0.1b	4.6±0.3a	1.7±0.3a	4.2±0.2a	3.2±0.3b	3.6±0.2b	2.1±0.1a
GMYLS	4.4±1.4b	4.1±0.1b	1.1±0.1a	3.8±0.6a	3.2±0.1b	2.8±0.2c	2.0±0.2a
SMYCS	4.1±1.2c	4±0.7b	0.9±0.2b	3.3±0.2b	3.0±0.1b	2.5±0.1c	1.9±0.2ab
SMYLS	4.1±1.2c	3.8±1.1c	0.8±0.1b	2.7±0.1c	2.6±0.2c	2.1±0.2c	1.1±0.2b
Pvalue	0.0001**	0.03*	0.001**	0.01**	0.002**	0.0024**	0.01**

Values are Means and superscripts with different letters varied significantly ($p< 0.05$).

Key- NAPRI- Control yoghurt

GMYCS- Goat milk yoghurt with commercial starter

GMYLS- Goat milk yoghurt with local starter

SMYCS- Soy milk yoghurt with commercial starter

SMYLS- Soy milk yoghurt with local starter

CMYCS- Cow milk yoghurt with commercial starter

CMYLS- Cow milk yoghurt with local starter

Table 4.10: Mean Yeast and Moulds Counts of Yoghurt Samples during Storage Expressed in log_{10} Cfu/ml (Shelf-Life Study)

Storage Days	DAY 0	DAY 4	DAY 10	DAY 14
Control(NAPRI)	ND	4.9±0.6b	6.8±1.4a	0.6±0.2b
CMYCS	ND	4.1±0.4b	6.6±0.6a	0.7±0.2b
CMYLS	ND	3.8±0.1b	6.5±0.2a	0.7±0.4b
GMYCS	ND	2.5±1.2c	7.0±0.2a	0.7±0.1b
GMYLS	ND	2.5±0.1c	6.9±0.6a	0.5±0.3b
SMYCS	ND	6.7±1.3a	3.9±0.2b	2.6±0.1a
SMYLS	ND	6.0±0.7a	2.8±0.6b	2.4±0.1a
P-value	-	0.03*	0.001**	0.01**

Values are Means and superscripts with different letters varied significantly ($p < 0.05$).

Key- NAPRI- Control yoghurt

GMYCS- Goat milk yoghurt with commercial starter

GMYLS- Goat milk yoghurt with local starter

SMYCS- Soy milk yoghurt with commercial starter

SMYLS- Soy milk yoghurt with local starter

CMYCS- Cow milk yoghurt with commercial starter

CMYLS- Cow milk yoghurt with local starter

CHAPTER FIVE

5.0 DISCUSSION

Majority of the Lactic acid bacteria (LAB) isolated from 'nono' belong to the genus *Lactobacillus*. This is in close agreement with the report of (Abdel Moneim *et al*., 2006) who reported that *Lactobacillus* constituted 74% of the LAB associated with *garris* (a Sudanese fermented camel's milk product). This is not surprising as strains of these genera of LAB are known to contaminate raw milk during milking from various sources such as the exterior of the udder, dairy utensils, dust, grass, cattle dung and feedstuffs (Sharpe, 1981). Some strains of *Lactobacillus* reported in the present study are similar to those reported in many cultured African dairy products.

However, MRS and M17 media are the best suitable media for the isolation of lactic acid bacteria as reported earlier by (Ghoddusi, 2002).

Morphological and Biochemical characterization showed that all the isolates studied exhibited the features of lactic acid bacteria being rod or cocci shaped, Gram positive, catalase negative, non-motile, non-spore formers able to grow at different temperatures, ability to dissimilate different sugars either homo or heterofermentatively (Tables 4.1 and 4.2) with few being able to produce CO_2 as stated earlier by (Axelsson, 1998). Their growth at salt concentrations of 4.0% and 6.5% reflects their well known salt tolerance which is in comparison with the usual milk-fermenting strains of lactic acid bacteria (Stanley, 1998).

Exopolysaccharides from lactic acid bacteria have found their most valuable application in the improvement of the texture properties of yoghurts. Two out of the isolates in this study (*Strepcococcus thermophilus* and *Lactococcus lactis*) produced exopolysaccharides, (Table 4.3) which was however not a surprise since many strains of LAB have been reported to produce expolysaccharides (De Vuyst *et al*., 2001; Ayad *et al*., 2004 and Sawadogo *et al*., 2007). The formation of exopolysaccharide in food has been reported to function as viscosifying agents, stabilizers, emulsifiers, gelling agents or water binding agents (De Vuyst *et al*., 2001) and may play important role in the texture of the product (De Vuyst and Degeest, 1999). Exopolysaccharide production is therefore an important property of LAB in yoghurts.

The presence of *Lactococcus lactis* in this study is of great importance. (Koiskowski, 1982) reported that these organisms are used for the preparation of different types of cheese. However, they may be used for the preparation of yoghurt where multi-strains starter culture is used to produce the desired acidity. The role of these strains have not been yet characterized, however, they may play a role in the preparation of fermented milks (Koiskowski, 1982). Different studies reported that these strains have the ability to produce slime characteristics, which may be used to improve the quality of the final product.

Findings from this work reveal that the growth of the local starter cultures in soy milk compares favourably well with that of cow and goat milks in yoghurt production. The growth of the local starter bacteria in goat and cow milks markedly increased with a maximal specific growth rate of $0.2891h^{-1}$ and $0.301\ h^{-1}$ with doubling times (td) of

approximately 59.2 minutes for *L. lactis* (Table 4.4). Comparatively, *S. thermophilus* exhibited lower doubling time 53.6 minutes and 53.57 in goat and cow milks with a specific growth rate of 0.111 h^{-1} and 0.123 h^{-1} respectively. However in soy milk, *L. lactis* had a kinetic growth rate of 0.403h^{-1} and a doubling time of 58.3minutes. Therefore, the low lactic acid bacteria counts in the soy milk could be attributed to higher specific growth rate and doubling time compared to that observed in the goat and cow milks. The study also shows that *S. thermophilus* attained a lower maximal specific growth rate in the soy milk. Therefore, the shorter doubling times expressed for *S. thermophilus* in cow and goat milks, is consistent with earlier finding (Raizi and Ziar, 2008), that the biomass increase of *S. thermophilus* should be higher in a mixed culture.

The acidifying activity of *S. thermophilus* and *L. lactis* in soy, cow and goat milk fermentations is as shown in Table 4.5. After 6 hours of fermentation, the pH dropped as a result of a maximal acidification rate of 0.195h^{-1}, 0.61 h^{-1} and 0.65 h^{-1} respectively. Comparatively, this result showed significant variation ($P < 0.05$) in the level of acid production in goat and cow milks compared to that of soy milk. (Davis, 1981) recommended lactic acid of 0.1% in yoghurt. The value reported in this study is considered to be satisfactory. The lactic acid contents obtained in this study compared favourably with values (0.17-1.16%) reported in the earlier studies by (Jimoh and Kolapo, 2007).

The study showed significant difference ($P<0.05$) between the hourly survival of *L. lactis* and *S. thermophilus* in soy, cow and goat milk fermentations (Fig 4.1, 4.2 and 4.3). The growth of *S. thermophilus* confirmed its well-known high growth ability in the conventional milk (Tamime and Robinson, 1999). It is evident from the findings of this

work that growth and the proportion of the two bacteria strains in all the milk fermentation were not the same, with *S. thermophilus* having higher cell density than *L. lactis* at the end of the fermentation despite the fact that the milks were inoculated with same proportions of the two organisms. This variation in growth between *S. thermophilus and L. lactis* has been attributed to differences in growth response to compositions of the milk (Hickey *et al*., 1986). Reports have also shown that *S. thermophilus* only uses a few sugars with most strains preferring saccharose and lactose; on the other hand, glucose and fructose are more slowly fermented (Tamime and Robinson, 1999). This finding agrees with works of (Dorato *et al.,* 2004) and (Alham and Mostafa, 2007) who had also reported higher growth of *S. thermophilus* during fermentation of cow milk.

However, it was observed that the growth of both bacteria in soy milk was characterized by longer lag time that resulted in unsteady acidification of the milk (Fig 4.3). Comparatively, the starter cultures exhibited increased growth with relatively steady acidification activity in cow and goat milk fermentations (Fig 4.4).

There was no significant difference in the pH with respect to the type of starter culture used but rather in the type of milk used. The pH decreased with increase in time of fermentation (Fig 4.5). The PH at 6 hours ranged between 4.5- 4.79 which is slightly higher than the recommended range of 3.9-4.5 for yoghurts. The higher pH value observed for soy milk yoghurts in this study is a common characteristic attributed to most plant seed yoghurts which lacks the sugar lactose found in dairy milks as reported by (Trindade *et al.,* 2001).

The titratable acidity increased with increase in time (Fig 4.6). There was no significant difference in the titratable acidity with respect to the type of starter culture used but rather with the type of milk used. At 6 hours the titratable acidity ranged from 0.69 - 1.26. The titratable acidity reported in this study is slightly lower compared to the work of (Salvador and Fiszman, 2004) with titratable acidity values of 0.82 - 1.10 and 0.82 - 1.15 reported for whole and skimmed dairy yoghurt respectively. The decrease in pH and corresponding increase in titratable acidity with time during the milk fermentations is consistent with conventional findings of (Salwa *et al*., 2004; Hussain *et al*., 2009) that fermentation of milk sugars by LAB will result in progressive increase in titratable acidity, with pH decrease.

The proximate composition of the different yoghurt samples in (fig 4.7) shows that the ash content ranged between 0.57 and 0.98%. The ash content is a reflection of the mineral compositions of the milk samples and is quite comparable. This is an indication that all the samples investigated had micronutrients. However it is apparent that the values of Goat milk yoghurt with commercial starter (GMYCS) and Goat milk yoghurt with local starter (GMYLS) had higher ash contents than the other milk samples as reported by (Algnesh *et al.,* 2007) for cow milk yoghurts.

All the samples had high moisture contents which ranged between 85% - 87.01%. This could affect the stability and safety of food with respect to microbial growth and proliferation hence the products will require cold storage. The moisture content of the yoghurt samples studied was in conformity with the works of (Heaney and Weaver, 1990). The significance of moisture content in milk and milk products is that, high moisture

content implies high water activity which supports microbial growth consequently reducing the shelf life of the products (Ajai *et al.,* 2012).

The major contrast in the food proximate content was the values obtained for the fat contents. From previous studies, fat contents are usually higher in animal origin than plant origin. The findings in this study agree with earlier studies because Goat milk yoghurt with commercial starter (GMYCS) and Goat milk yoghurt with local starter (GMYLS) presented with highest fat contents of 4.07% and 4.08% respectively followed by NAPRI (Control) with 2.05%. Cow milk yoghurt with commercial starter (CMYCS) and Cow milk yoghurt with local starter (CMYLS) had a fat content of 0.6% and 0.65% respectively. Soy milk yoghurt with commercial starter (SMYCS) and Soy milk yoghurt with local starter (SMYLS) had the least value with 0%-0.01 which falls within the range reported by (Belewu *et al.,* 2010) for plant and dairy-based milks. Fat content of food is the major contributor to the energy value of food. This implies that goat milk are more energy yielding than soy and cow milk. According to (USDA, 2001) yoghurt with less than 0.5% fat content should be labeled as "non -fat yoghurt" those with fat content with range of 0.5-2.0% should be labeled as "low fat yoghurt while those with fat contents above 3.25% should be labeled " yoghurt"

The total solid content for the yoghurt samples fall within the range of 12.9%-19.2% similar to the work reported by (Dublin- Green and Ibe, 2005) who reported values for plain yoghurts ranging from 13.6%-18.8%.

The protein content of the milk samples ranged from 2.8% - 6.01%. The protein content is in agreement with the work of (Burrington, 2000). Although, most authors

recorded a very low values for the crude protein (Burrington, 2000.), it is believed that this might be due to the fact that the milk samples that were analyzed by most of these authors were dry milk samples. The high protein content of this product showed that its consumption will help eliminate protein deficiencies that have become the bane of poor nations. Soymilk also contain appreciable amount of protein sufficient for body growth and development.

The microbiological quality of the yoghurt samples from the result showed that there has been no contamination in all the yoghurt samples which shows the effectiveness in the pasteurization as well as the production processes and hence safe for consumption (Table 4.6). The LAB load observed in this study is in line with the standard of International Regulatory Body for dairy products which recommended a minimum of 10^6 cfu/g of viable lactic acid bacteria for effective probiotic functions in yoghurt throughout their shelf life (Adolfsson *et al*., 2004).

Viscosity of the yoghurts made from all the milk samples was shown in (Fig 4.8) from the result, the yoghurts made from soy milk showed higher viscosity when compared to the other samples. The rheological characteristics of yoghurt are governed by milk composition, temperature and time of heat pretreatments, type and quantity of starter culture employed to inoculate the milk, fermentation temperature and storage conditions of the final product. Several authors have studied the correlation among yoghurt rheology and structure, evaluating the effect of milk heat treatment, type of starter culture, incubation temperature, storage time, etc. (Parnell-Clunies *et al*., 1986).However, viscosity is affected by the state and concentration of fats, protein, temperature, pH and milk age (Park 2007).

The results of the sensory properties have revealed that the general acceptability was not significantly affected by the type of the milk. It has been observed that there was no significant difference in general acceptability between cow, soy and goat milk yoghurts (Table 4.7). Studies have demonstrated that a good acceptable yoghurt which is comparable to one made from cow milk can equally be made from goat and soy milk.

However, when each of the parameters studied was given due consideration, there were significant differences in terms of taste, colour and appearance. Goat milk yoghurt with commercial starter (GMYCS) was more preferred in terms of appearance while Soy milk yoghurt with commercial starter (SMYCS) was the least preferred. There was no significant difference between all the yoghurt samples in terms of texture, aroma and flavour. These findings are in agreement to the findings of (Bille, 2000) and (Amanze, 2011) who reported that soy milk yoghurt and dairy milk yoghurts are quite comparable and further reported that soy milk yoghurt can compete favourably with cow milk yoghurt and it can be a good substitute for commercial production. There might also be other reasons attributing to differences in acceptability scores such as preparation method and ingredients used as reported by (Trindade, *et al.,* 2001) who concluded that yoghurt with best sensory quality was obtained using homogenized soy milk (17MPa) with 2% sucrose addition and fermented for 6 hours.

The physical observations of all the yoghurt samples from cow, goat and soy milks showed spoilage after 48 hours of storage at ambient temperature (Appendix VII). This agrees with the work of (Dublin- Green and Ibe, 2005) who reported that yoghurts stored at room temperature was spoiled by yeast and became unacceptable after 3-4 days. Change

in colour from creamy to brownish (in soy based yoghurts) and change in flavour as well as whey separation in the yoghurt samples stored at refrigerated temperature were observed from Day 4 to Day 14. The reason for this might be due to the fact that no preservative was added. It has been reported by many authors that physical properties of yoghurts during storage are affected by the milk composition, processing condition, type of heat treatment applied, acidity, coagulum handling and presence of stabilizer (Parnell-Clunies *et al*., 1986). All the yoghurt samples retained their colour with no whey separation at freezing temperature till Day 10. This is in line with the finding of (Ashaye *et al*., 2001) in which the shelf- life of yoghurts in freezing temperature (-18°C) was longer than those stored at refrigerated and ambient temperatures. Higher levels of deterioration recorded for soy based yoghurts agree with the work of (Omueti and Ajomale, 2005) who reported that plant-based yoghurts have shorter shelf-life as compared to the conventional yoghurts made from animal milks.

The deterioration and changes in sensory quality are largely governed by the growth of yeasts and molds in the yoghurt samples (Table 4.10) and this is in agreement with the works of (Al-Kadamany *et al*., 2002).

Total aerobic count and LAB counts of all the stored yoghurt samples (table 4.8 and 4.9) decreased with days of storage and temperature of storage. The significant increase in both bacterial (Table 4.8 and Table 4.9) and fungal counts(Table 4.10) of stored yoghurt samples within the first four days of storage might be further affirming microbial activities in the early days of storage. However, the subsequent reduction in microbial count in the later days might be consequent upon a modified environment which may not be favourable

for subsequent microbial proliferation as reported by (Çon *et al*. 1996). Freezing drastically reduced the total aerobic count in all the stored yoghurt samples. The aerobic mesophilic bacteria count is significantly affected by the storage effects at different days under refrigerated and freezing conditions as reported by (Con *et al*. 1996). The highest total aerobic count and LAB count was recorded in cow milk yoghurt with commercial starter 20.9±1.1 and 26.8 ±0.3 cfu/ml stored at ambient temperatures respectively (Table 4.8 and 4.9) and this was reduced to 2.3±0.1 and 7.2±0.2 cfu/ml respectively on Day 14 at freezing temperature.

CHAPTER SIX

6.0 SUMMARY AND CONCLUSION

6.1 Summary

The local starter cultures (*Strepcococcus thermophilus* and *Lactococcus lactis*) isolated from 'nono' in this study has the potentials to perform equally good like the exotic strains in yoghurt production. Their ability to produce exopolysaccharide is another interesting perspective. Comparatively, findings from this study showed no variation ($p \geq 0.05$) in the acidification rates of the local starter culture with the foreign starters which further affirmed the potentials of the local starter to produce good yoghurt. Profile of the physicochemical analysis of the local starter culture showed decrease in pH from 5.74to 4.27 while titratable acidity increased from 0.38% to 1.32% after 6 hours of fermentation. Similarly, the local starter culture impacted no significant variation ($p \geq 0.05$) in the proximate composition of the yoghurt samples with a moisture content of 86.01%, total solids of 15.02% and viscosity of 24.80%. The local starter culture also conferred sensory properties such as taste, flavor, aroma and texture to the yoghurt samples which were acceptable to consumers. The shelf-life study revealed spoilage of all the yoghurt samples by yeasts and moulds after 48 hours at ambient temperature and days 4 at refrigerated temperature while only wheying off was observed in samples stored at freezing temperatures after days 10 which depicts the importance of preserving the products at low temperatures irrespective of the type of milk or starter culture used in order to slow down spoilage.

It is also evident from this study that the local starter cultures do well not only in cow milk but also in soy and goat milk yoghurt productions.

6.2 Conclusion

Six different strains of Lactic acid bacteria associated with "nono" were isolated and biochemically characterized.

Two out of the isolates (*Strepcococcus thermophilus* and *Lactococcus lactis*) were exopolysaccharide producing strains revealing their technological potentials in yoghurt production and hence termed the "local starter culture".

Results of the growth parameters of the local starter culture which included specific growth rates, doubling times and acidification rates in soy, cow and goat milk fermentations reveal that the co-culture of the two isolates (local starter culture) can work in synergism in yoghurt production.

Findings from this study demonstrates a novel approach to the technological potentials of the local starter culture in producing yoghurts with physicochemical, proximate, viscosity and sensory properties acceptable to consumers competitively with the commercial starter cultures.

Shelf-life study of the yoghurt samples from this work depicts the importance of storing yoghurts at low temperatures irrespective of the type of milk or starter culture used so as to slow down spoilage.

6.3 Recommendations

i. Exploitation of LAB from our environment should be given due consideration as such studies will provide the basis for the development of starter culture bank, which will be used for future investigations and their ultimate utilizations in our dairy industry.

ii. There is need for the development of more sophisticated system for the isolation, propagation and preservation of the best performing indigenous LAB strains

iii. Further work should include detailed molecular studies on the best performing strains, phage susceptibility and control as well as investigating their ability to withstand Lyophilization.

iv. There is the need to promote a more sedentary form of cattle and goat rearing for diary milk production in Nigeria to meet the potential large market in the country.

v. The utilization of soymilk in yoghurt production should be encouraged so as to solve the problem of protein-calorie malnutrition in Nigeria in particular, Africa and the world in general.

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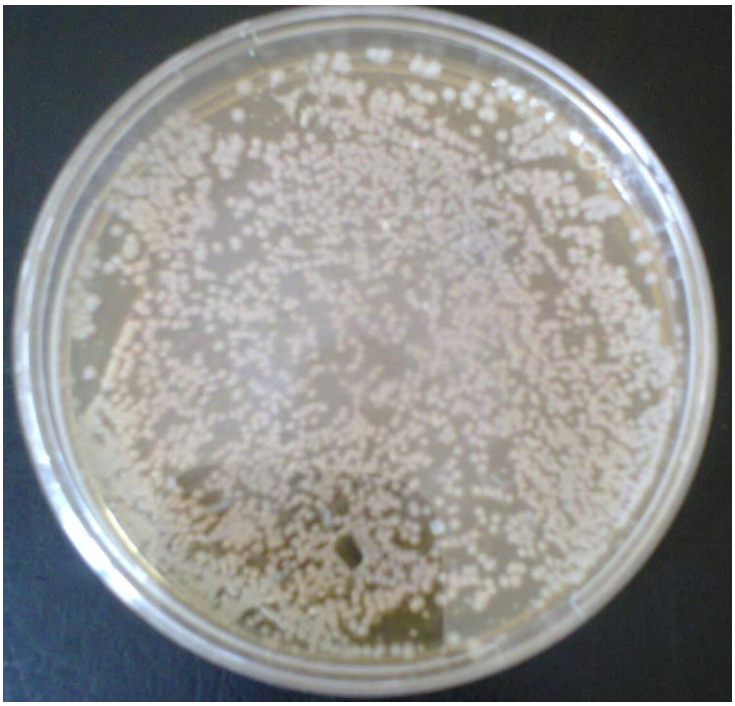
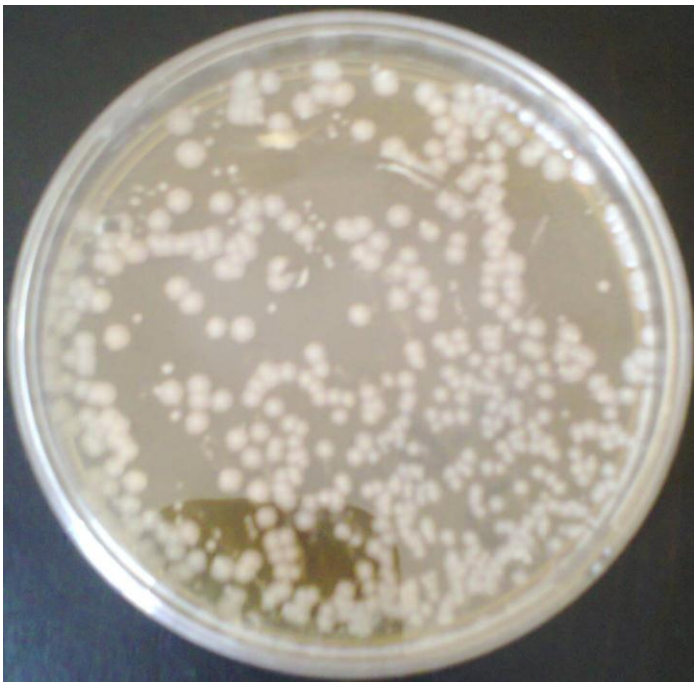
APPENDICES

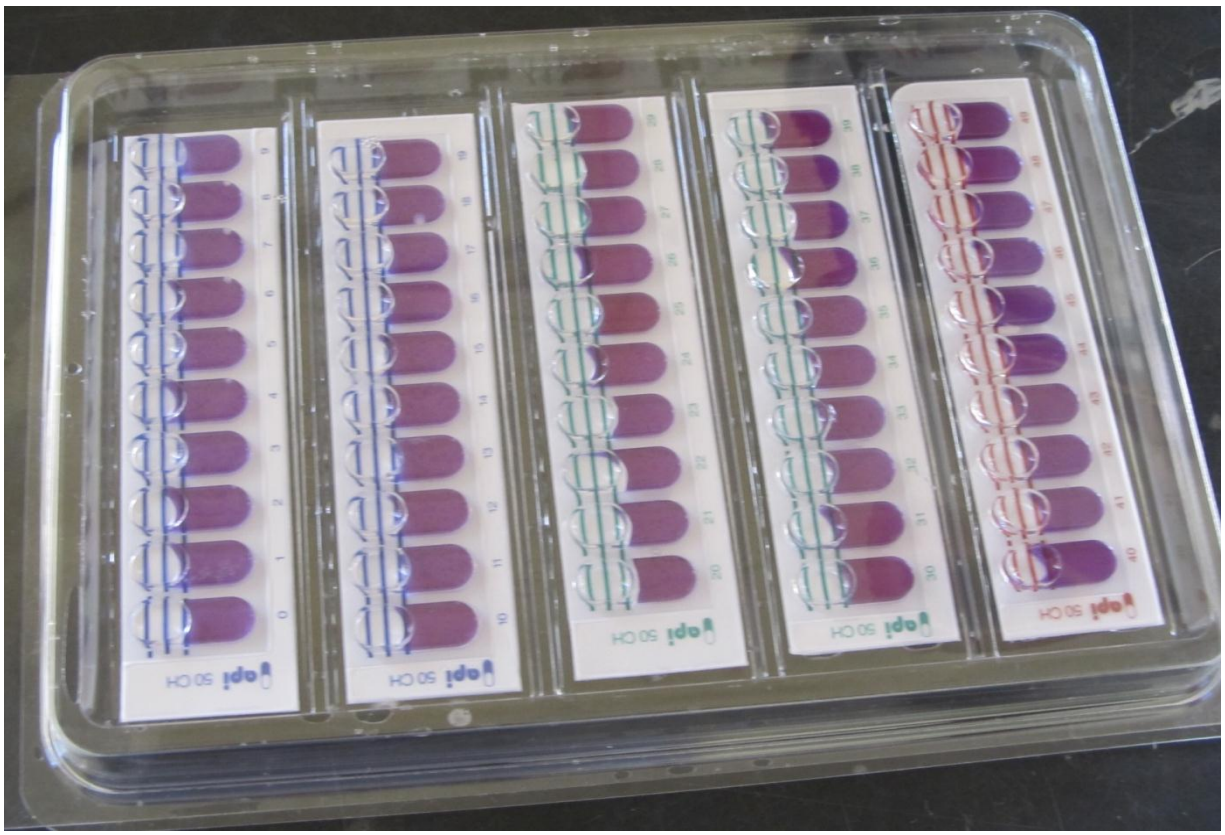
Appendix I: Plate Showing Lactic Acid Bacteria on M17 Agar



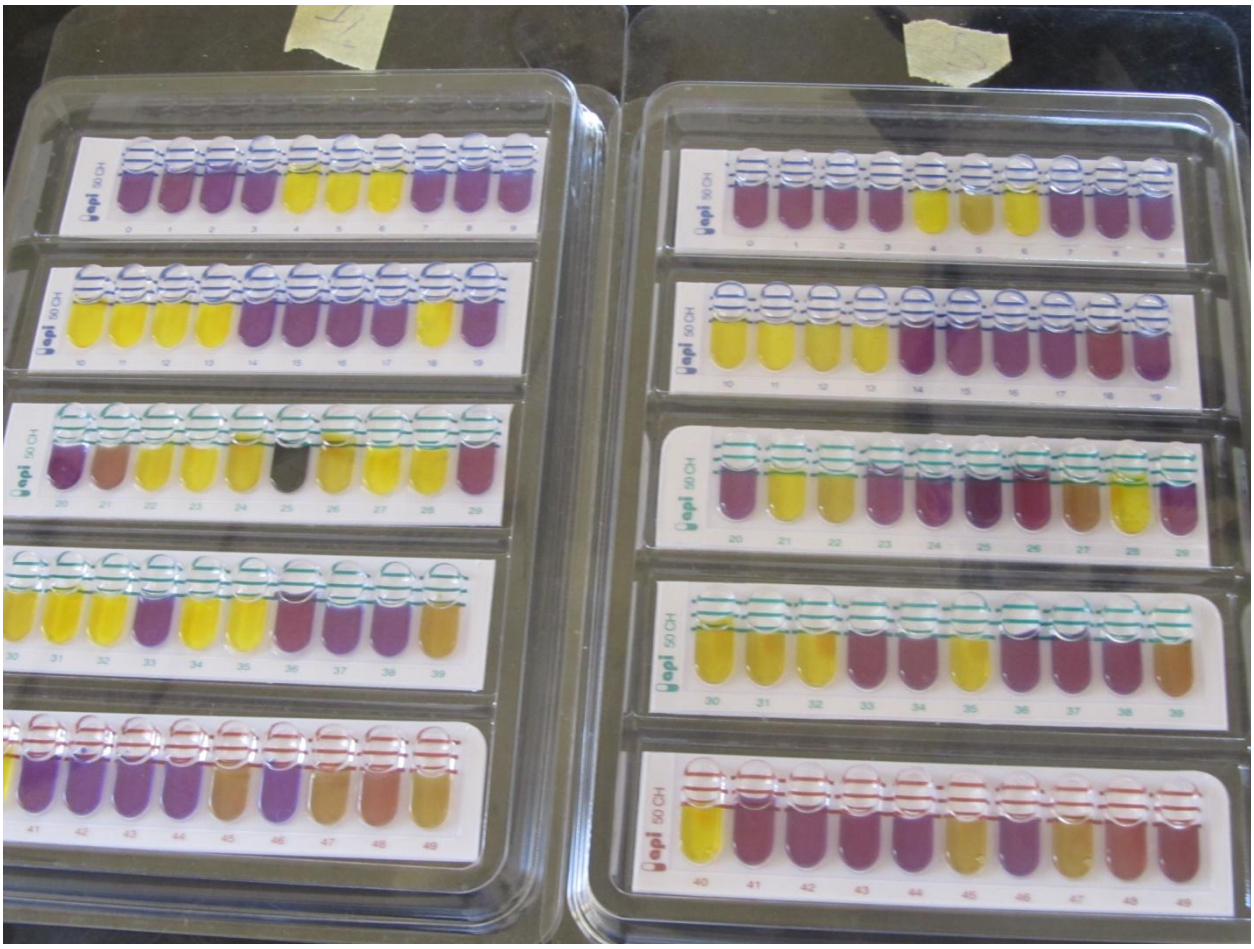
Appendix II: Plate Showing Lactic Acid Bacteria on MRS AGAR.



Appendix III: Plate showing LAB carbohydrate fermentation profile before fermentation (API-test).



Appendix IV: Plate showing LAB carbohydrate fermentation profile after 48 hours fermentation



Appendix V: Sample of Sensory Evaluation Questionnaire

YOGHURT SAMPLE

Tick appropriately

PARAMETERS	POOR (1)	FAIRLY GOOD (2)	GOOD (3)	VERY GOOD (4)	EXCELLENT (5)
Appearance					
Texture					
Aroma					
Flavour					
Colour					
Taste					
General acceptability					

RATING- Five point hedonic scale.

Comment _____

_____

_____

Name _____

Signature _____ Date _____

Appendix VI: **Carbohydrate fermentation profile of the lactic acid bacteria isolates**

Carbohydrate	*L. brevis*	*S. thermophilus*	*P. pentosaceus*	*L. fermentum*	*L. pentosus*	*Lactococcus lactis* ssp *lactis*
Glycerol	-	-	-	-	-	-
Erythritol	-	-	-	-	-	-
D-Arabinose	-	-	-	-	-	-
L-Arabinose	+	-	+	-	-	-
Ribose	+	-	+	+	+	-
D-Xylose	+	-	+	+	+	+
L-Xylose	-	-	-	+	+	-
Adonitol	-	-	-	-	-	-
B-Methyly-Xyloside	-	-	-	-	-	-
Galactose	+	-	+	-	-	-
D-Glucose	+	+	+	+	+	-
D-Fructose	+	-	+	+	+	+
D-Mannose	+	-	+	+	+	+
L-Sorbose	-	-	-	+	+	-
Rhamnose	-	-	-	-	-	-
Dulcitol	-	-	-	-	-	-
Inositol	-	-	-	-	-	-
Mannitol	+	-	+	-	-	-
Sorbitol	-	-	-	-	+	+
a-Methyly-D-mannoside	-	-	-	-	-	-
a-Methyly-D-glucoside	-	-	-	-	-	-
N-acetyl-glucosamine	+	-	+	-	-	-
Amygdalin	+	-	+	-	+	-
Arbutin	+	-	+	-	-	-
Esculin	+	-	+	+	+	-
Salicin	+	-	+	-	+	+
Cellobiose	+	-		-	+	-
Maltose	+	-	+	+	+	-
Lactose	+	+	+	+	+	+
Melibiose	+	-	+	+	+	+
Sucrose	+	+	+	-	+	-
Trehalose	+	-	+	-	+	+
Inulin	-	-	+	-	+	-
Melezitose	-	-	-	-	-	-
D-Raffinose	-	-	-	+	-	-
Starch	-	-	-	-	+	-

Glycogen	-	-		-	-	-
Xylitol	-	-	-	-	-	-
b-Gentobiose	+	-	-	-	-	-
D-Turanose	-	-	-	-	+	-
D-Lyxose	-	-	+	-	-	-
D-Tagatose	-	-	-	-	-	-
D-Fucose	-	-	-	-	-	-
D-Arabitol	-	-	-	-	-	-
L-Arabitol	-	-	-	-	-	-
Gluconate	-	-	-	+	-	-
2-keto gluconate	-	-	-	-	-	-
5-keto-gluconate	-	-	-	-	-	-

Key:

+ = Positive
-= Negative

Appendix VII: Physical Observation of the Yoghurt Samples during Storage (Shelf Life Study)

Storage days	**Day 0**	**Day 1**			**Day 2**		
Storage temperature	Ambient	Ambient	Refrigerated	Freezing	Ambient	Refrigerated	Freezing
Yoghurt samples							
Control (NAPRI)	Whitish and wholesome	Whitish, whey not separated	Whitish, whey not separated not spoilt	Whitish, whey not separated not spoilt	Whitish, whey not separated smell spoilt	Whitish, whey not separated not spoilt	Whitish, whey not separated not spoilt
CMYCS	Whitish and wholesome	Whitish, whey not separated	Whitish, whey not separated not spoilt	Whitish, whey not separated not spoilt	Whitish, whey not separated smell spoilt	Whitish, whey not separated not spoilt	Whitish, whey not separated not spoilt
CMYLS	Whitish and wholesome	Whitish, whey not separated	Whitish, whey not separated not spoilt	Whitish, whey not separated not spoilt	Whitish, whey not separated smell spoilt	Whitish, whey not separated not spoilt	Whitish, whey not separated not spoilt
GMYCS	Whitish and wholesome	Whitish, whey not separated	Whitish, whey not separated not spoilt	Whitish, whey not separated not spoilt	Whitish, whey not separated smell spoilt	Whitish, whey not separated not spoilt	Whitish, whey not separated not spoilt
GMYLS	Whitish and wholesome	Whitish, whey not separated	Whitish, whey not separated not spoilt	Whitish, whey not separated not spoilt	Whitish, whey not separated smell spoilt	Whitish, whey not separated not spoilt	Whitish, whey not separated not spoilt
SMYCS	Creamy and wholesome	Creamy, whey not separated, not spoilt	Creamy , whey not separated not spoilt	Creamy , whey not separated not spoilt	Creamy , whey not separated smell spoilt	Creamy , whey not separated not spoilt	Creamy , whey not separated not spoilt
SMYLS	Creamy and wholesome	Creamy, whey not separated, not spoilt	Creamy , whey not separated not spoilt	Whitish, whey not separated not spoilt, whey not separated not spoilt	Creamy , whey not separated smell spoilt	Creamy , whey not separated not spoilt	Creamy , whey not separated not spoilt

STORAGE DAYS	**DAY 3**		**DAY 4**		**DAY 10**		**DAY14**	
STORAGE TEMP	**Refrigerated**	**Freezing**	**Refrigerated**	**Freezing**	**Refrigerated**	**Freezing**	**Refrigerated**	**Freezing**
Control (NAPRI)	Creamy white, whey not separated or spoilt	Creamy white, whey not separated or spoilt	Creamy white, whey separated not spoilt	Creamy white whey separated not spoilt	Creamy white whey separated not spoilt	Creamy white whey separated not spoilt	Creamy white whey separated spoilt	Creamy white whey separated not spoilt
CMYCS	Creamy white, whey not separated or spoilt	Creamy white, whey not separated or spoilt	Creamy white, whey separated or spoilt	Creamy white whey separated not spoilt	Creamy white whey separated not spoilt	Creamy white whey separated not spoilt	Creamy white whey separated spoilt	Creamy white whey separated not spoilt
CMYLS	Creamy white, whey not separated or spoilt.	Creamy white, whey not separated or spoilt	Creamy white, whey separated not spoilt	Creamy white whey separated not spoilt	Creamy white whey separated not spoilt	Creamy white whey separated not spoilt	Creamy white whey separated spoilt	Creamy white whey separated not spoilt
GMYCS	Whitish not separated not spoilt.	Whitish not separated not spoilt.	Whitish whey separated not spoilt.	Whitish whey separated not spoilt.	Creamy white whey separated not spoilt	Creamy white whey separated not spoilt	Creamy white whey separated spoilt	Creamy white whey not separated not spoilt
GMYLS	Whitish not separated not spoilt.	Whitish not separated not spoilt.	Whitish whey separated not spoilt.	Whitish whey separated not spoilt.	Creamy white whey separated not spoilt	Creamy white whey separated not spoilt	Creamy white whey separated spoilt	Creamy white whey not separated not spoilt
SMYCS	Creamy whey not separated not spoilt	Creamy whey not separated not spoilt	Creamy whey not separated spoilt	Creamy whey separated not spoilt	Brownish whey separated spoilt	Brownish whey separated not spoilt	Brownish whey separated spoilt	Brownish whey separated not spoilt
SMYLS	Creamy whey not separated not spoilt	Creamy whey not separated not spoilt	Creamy whey separated not spoilt	Creamy whey separated not spoilt	Brownish whey separated spoilt	Brownish whey separated not spoilt	Brownish whey separated spoilt	Brownish whey separated not spoilt