CHEMICAL, MICROBIOLOGICAL AND SENSORY PROPERTIES OF DAIRY, NON-DAIRY YOGHURTS AND CHEESE PRESERVED WITH AFRICAN CARDAMOM (*Aframomum danielli* K. Schum.)ANDTURMERIC (*Curcuma longa*L.)

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ABSTRACT

Yoghurt and cheese are the common dairy products consumed in Nigeria, but they are highly susceptible to deterioration during storage. Natural preservatives are generally preferred to synthetic ones which are likely to be hazardous to human health. Literature is sparse on utilisation of African cardamom and turmeric in preservation of yoghurt and cheese. This study was designed to evaluate the potentials of African cardamom and turmeric in yoghurt and cheese preservation.

Milk was produced from soursop fruit using standard methods. Milk samples were collected from three-year old Sokoto Gudali (cow) and Red Sokoto (goat) at 7.00–8.00am daily and the samples were maintained at low temperature (-4^{o}C) using ice-cubes. Yoghurts and cheese were produced from the milks using established procedures. Lactic Acid Bacteria (LAB) from soursop, cow and goat milk yoghurts and cheese were isolated, identified and characterised using 16S rDNA molecular procedure. The LAB isolates were used as single or multiple-strain starter culture in yoghurt production. Yoghurt and cheese samples were preserved by equal proportion mixtures of aqeous extract (10g of spices dissolved in 100ml distilled water and centrifuged) of *Aframomum danielli* and *Curcuma longa* at 1.0%, 1.5%, 2.0% and 2.5% concentrations, and control for 12 weeks at refrigeration temperature (4^{o}C) and analysed biweekly for chemical (soluble solids, pH, total titratable acidity), proximate, mineral and microbiological (total plate and yeast counts) properties. Sensory attributes of the samples were determined using 9-point hedonic scale. Data were analysed using ANOVA at $\alpha_{0.05}$.

Five *Lactobacillus* spp were isolated and identified as *Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus licheniformis*, *Bacillus subtilis*and L*actobacillus rhamnosus.* The mixture of *Lactobacillus fermentum* and *Lactobacillus licheniformis* produce best quality yoghurt in terms of chemical characteristics. The yoghurts contained protein (0.92-8.75%),

fat (0.02-9.90%), ash (0.55-1.23%), moisture (73.43-91.87%) and carbohydrate contents (5.18-6.61%). Cheese had protein (13.04±0.02), fat (16.01±0.01), ash (1.60±0.02), moisture (55.81±0.04) and carbohydrate contents (13.54±0.02). Major mineral contents in yoghurt samples were calcium, magnesium, potassium, sodium, iron and zinc ranged from 40.07-640.75, 25.75-31.95, 869.50-2454.75, 60.00-85.00, 7.85-15.35 and 0.48-0.61 mg/100mL, respectively. Mineral contents in cheese were calcium, magnesium, potassium, sodium, iron and zinc ranged from 223.00±0.07, 37.00±0.01, 260.00±0.03, 17.00±0.00, 1.20±0.06 and 1.90±0.05 mg/100mL, respectively. Soluble solids and pH decreased, while total titratable acidity increased at 2.0 and 2.5% concentrations in the soursop, cowmilk, goatmilk yoghurt and cheese samples as storage period increased. The control counts after two weeks were $3.6x10^7$, $8.7x10^7$ and $1.7x10^6$ cfu/mL while the extracts preserved the samples for eight weeks, after which total plate counts were $1.4x10^7$, $5.3x10^5$ and $0.8x10^6$ cfu/mL for soursop, cowmilk and goatmilk yoghurts respectively. The rate of multiplication of yeast and mould counts were retarded in treated samples but conversely in control. There were no significant differences between the yoghurts made from soursop, cow milk and goat milk in terms of overall acceptability. However, overall acceptability of cheese from cowmilk was significantly ($p<0.05$) higher than others.

Mixture of aqeous extract of *Aframomum danielli* and *Curcuma longa* preserved refrigerated yoghurt and cheese for eight weeks. Both spices at 2.5% concentration performed best as preservatives.

Keywords: Non-dairy yoghurt, Cheese, Soursop fruit, African cardamom and turmeric

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CERTIFICATION

This is to certify that this work was carried out by Abiona, Stella Olusola in the Department of Food Technology, University of Ibadan, Ibadan. Nigeria.

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DEDICATION

This project is dedicated to my children Abiola, Eniola and Oluwadamilola Abiona for their endurance during the course of my studies.

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`LIST OF ABBREVIATIONS

ALP	Alkaline Phosphatase
ALT	Alanine AminoTransferase
AST	Aspartate AminoTransferase
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
Ca	Calcium
Cfu/ml	colony forming unit
CODEX	Alimenterous
^{0}Brix	degree brix
DNA	Deoxy ribonucleic acid
FAO	Food and Agricultural Organization of the United Nations
g	Gram
g/l	Grams/litre
GRAS	Generally Regarded As Safe
hr	Hour
Fe	Iron
IDF	International Dairy Federation
LAB	Lactic Acid Bacteria
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Value
Mg	Magnessium

µl	microlitre
mg/L	milligram per Litre
mg/kg	milligram per kilogram
MRS	Man, Rogosa and Sharpe
Na	Sodium
PCV	Packed Cell Volume
%	Percentage
pH	power of hydrogen
PCR	Polymerase Chain Reaction
K	Potassium
PDA	Potassium Dextrose Agar
RBC	Red Blood Cell
RDI	Recommended Daily Intake
SNI	Standard National Indonesia
TPC	Total Plate Count
TSS	Total Soluble Solids
TTA	Total Titratable Acidity
wt	Weight
WBC	White Blood Cell

CHAPTER ONE

1.0 INTRODUCTION

Foods that are fermented provide, improve and add nutrients to the diets of humans as they give different types of foods. The different types of fermented foods which exist in the world are over 4000(Anon, 2003). Fermented foods have been in existence since humans have been created on earth and fermented milks are the most important of all the fermented foods. Yoghurt is the first, popular and ancient fermented food because of its distinct flavour, viscous, creamy and well-known food with healthful benefit(Domagla, 2005). Fresh milk is the nature's single most nearly complete food in the world because of its high nutrients but it is liable to speedy microbial spoilage. Unhygienic milking, inefficient storage practice allow higher microbial growths in raw milk (Nooruddin *et. al.,* 2006). Therefore, preservation of raw milk is mandatory in processing it into diverse staple forms such as yoghurts and cheese through the process of fermentation.With the development of processing technologies, the growing competition in the food market, the urge to provide nutritious food with appealing natural preservatives and flavouring properties attracted attention of researchers. Currently, the use of chemical preservatives in foods is a great problem worldwide because of cancer related diseases(Halliwell *et. al.,* 1995).

In recent time, there is new area of interest inusing natural extract of spices and herbs as antimicrobials in foods generally. Diversification of food processing using fruit juice is still limited. The diversity of products is needed to give alternative choice of products to consumers. The processing of soursop fruit to yoghurts could exploit potential market for the fruits and the processing of fresh goat milk into yoghurts is still at cottage level. In the struggle to have different types of products in the market, the research and development came aboutadding carrot juice to get yoghurt to a value added product (Simova *et. al*., 2004). Ejechi *et. al.,* (1998), described the usuage of ginger spice to maintain microbial stability of pasterurised mango juice. Ogunwolu and Adio (2003) showed that extracts of *Aframomum danielli* preserved quality attributes of cashew juice.

Yoghurt is a basic and essential food of people in the world (Asia, Europe, India and Africa) and highly rich in nutrients and minerals (Yale-New Haven, 2013). The nutrients in the yoghurts were greater than milk because it is probiotics (Elaine, 2015). *Wara* is a fresh pasteurised cow milk, moist curd with drained off whey. The activity of microorganisms at the point of milking was much and numbers up to severalthousand per millilitre (IDF,

1981).Probiotics have been eaten and taken as cultured food for example yoghurt and kefir (Troller, 1973) and in accordance to (FAO/WHO, 2001) probiotics are living organisms but lives as parasite that benefit the host health, when eaten in sufficient quantity. Probiotics may be recommended for peoplehaving irritable bowel symptoms (Saudy, 2008). Recently some state governments in Nigeria have launched a school feeding programme "eat an egg a day", where pupils were given an egg to increase their protein nutritional intake. Also, some other states have included milk in their feeding programme, making it necessary to increase production and processing of milk products into more shelf stable forms,yoghurts or milk powder (Atta 2014).

Also, food scientists have attempted to develop new technologies that improve the quality and quantity of products, while consumers have also become more critical on the use of synthetic additives to preserve food or enhance characteristics such as colour and flavour (Corbo *et. al.,* 2009). Study has demonstrated that there are various types of raucous fruits and vegetables in abundance in Nigeria (IFST, 2007). The availableness of fruits is shortbecause it is seasonal and perishable in nature (Thompson, 2003). An example of short lived fruit is *Annona muricata*(soursop). This fruit is underutilised and has the ability to prevent diseases. Soursop (*Annona muricata* L.) fruits are important sources of vitamins and minerals and supply flavour, aroma and texture to the delight of consumers and are assume to have anticancer and antioxidant abilities (Luzia and Jorge 2012). Oberlies *et. al.,* (1997) and Cassileth (2008) stated that soursop fruits which have been tested during research works effectively on cancer cells. Hartati and Eka (2010) worked on determining the best stabiliser for soursop juice yoghurt while Abena *et. al.,* (2014) examined the vitamins, ascorbic acid and minerals on tropical underutilised fruits.

Research have been carried out on yoghurts and soft cheese, for example, preparation and nutritional assessment of garlic based yoghurt (Qureshi *et. al.,* 2011), mineral analysis of warankasi (Lawal and Adedeji, 2013) and African yam beans yoghurt supplemented with cow milk (Aderinola and Olarenwaju, 2014). A lot of attempts have been made to elongate yoghurt life. The cheese processed in West Africa is a unique class of cheese having a storage life of three days when submerge in whey. The short storage life of soft cheese is generally due to moulds growth and leads to losses by discolouration and bad flavour during the period of cold storage.

Attempts have been made on the keeping quality of probiotics yoghurts and soft cheese. However, Belewu *et. al*. (2005) preserved soft cheese with the use of biological and chemical preservativesfor a 15-day period. A few occurring substances have antimicrobial

effects in foods which are documented in literature (Belewu *et al*., 2011 and Adetunji, 2011). Thus, there is the need to combine the effects of some local spices like *Aframomum danielli* and curcumin at refrigerated temperature which serve as food preservatives, good sources of phenolic compounds and antioxidants. Studies have been conducted on preservation and flavouring of yoghurts and local cheese(Qureshi *et. al.,* 2011, Ashaye *et. al*., 2006; Adegoke *et*. *al*., 2013), but not much has been done on the combination of soursop fruits (non-dairy source), in addition with goat milk, cow milk and local cheese; and this has attracted the present study. Thus, this research examined processing and preservation of yoghurts from soursop fruit, cow milk, goat milk and soft cheese and proferred solutions that can be of help in harnessing the nutritional benefits like the high protein, fats and minerals, which can encourage consumption of yoghurt.

Objectives

Major objectives of this research were to critically examine result of *Aframomum danielli* and *Curcuma longa* on quality attributes as well as storage stability of yoghurts produced from soursop, cowmilk, goatmilk and soft-cheese.

This was achieved using the following specific objectives:

(1) to determine physical, chemical, microbiological qualities of yoghurt processed from soursop, cowmilk, goatmilk and soft-cheese.
(2) to ascertainthe result of *Aframomum danielli* and turmeric onthe chemical and microbiological characteristics of yoghurts and soft-cheese during storage.
(3) to segregate and characterise the lactic acid bacteria in soursop, cow milk, goat milk yoghurts and soft-cheese.
(4) to evaluate the nutritional quality, with particular reference to protein quality, of soft-cheese using rat feeding studies.
(5) to evaluate the sensory qualities of the yoghurt samples.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Yoghurt History

"Jugurt" was formed from Turkish word meaning yoghurt (Younus *et. al.,* 2002). It is possible that the processing of yoghurt begins since 9000BC from Middle East when there was the action of taming wild animals or breeding plants. IlyaIlyich Mechnikov, who is a biologist from Russian, reported an unproven observation that steady eating of acidic milk can give guard against enteric infections and help in a physiological age and normal death (Schmalstieg and Goldman, 2008). Codex Alimentarius definition (FAO, 1992) states that fermented,coagulated milk product called "yoghurt" is grown synergically using*Streptococcus thermophilus* (new name: *Streptococcus salivarius* subsp. *thermophilus*) and *Lactobacillus bulgaricus* (new name: *Lactobacillus delbrueckii* subsp. *bulgaricus*). Even though cheese and yoghurt were initially developed to elongate its shelf or storage life of milk which eventually leads to a product with distinct flavours and good health benefit attributes.

There has been global growth and improvement in the production of yoghurt. A lot of factors can lead to the accomplishment of yoghurt because it is a normal drink and it has high-quality properties (Roissart and Luquet, 1994). Yoghurt is a classic example of functional food which has health-promoting components further than customary nutriments. Yoghurts are also known as nutraceutical, super, medi and theurapeutic foods and the word'yoghurt' refers to foods that have been restructured to be 'functional'. Yoghurt, kefir and other dairy products can also function as nutraceuticals in treating different types of illness situation (Katz, 2001). Yoghurt and dairy products are used as commonmeans of expressing probiotics. The meaning of probiotics in accordance to FAO/WHO (2001) which was agreed by International Research Bodies (2003) "living microorganism, given in sufficient quantities bestow a healthy advantage or aid on the receiver". Probiotics is a recent word which means "for life", which is used in naming microorganisms connected with the beneficial special effects for man and animals. The microorganisms impart to gastrointestinal microbial stability. Consumption of probiotics yoghurt and indeed probiotic-containing foods is known to confer benefits like anti-hypertension properties (Lye *et. al.,* 2009), reduction of LDL-cholesterol levels (Sindhu and Khetarpane, 2013) and disruption of pathogenesis of hepatic encephalopathy (Solga, 2003). The probiotics microorganisms comprise majorly some yeast, the genera *Lactobacillus,Bacillus, Pediococcus* and*Bifidobacterium*.Probiotics

can be discovered in animal milk and plant milk products as well as being eaten as antibiotic therapy.

Yoghurt's nutritional information has a parallel component to the original source milk. Minerals as well as vitamins are often additionally mixed with products administer to children. Yoghurts either bottled or cups are regarded as additional supplements for children and can be refers to as medical, dietary, functional and foods (Katz, 2001).

2.2. Different Types of Yoghurt

Yoghurts may be groupedin accordance to their fermentation process, manufacturing process, flavouring andchemical component (Shah, 2000). Agreeing to the methods ofprocessing yoghurt like stirred and set type. The main difference between them is that set yoghurt is more or less semi-solid with the coagulum remaining intact and is usually packaged in cup-like packages. In its production, the milk is inoculated, put in packages and sealed before fermentation. Stirred yoghurt, on the other hand, is more liquid product obtained by fermenting the liquid milk base in tanks and when the curd is ruptured by stirring, the product chilled and packaged in bottles (Lee and Lucey, 2010).

Yoghurts can be further divided into different category; such as plain yoghurt, fruit yoghurts and flavoured yoghurt. Yoghurts exist in numerous varieties e.g. plain, flavoured, mixed and whole. Fluid yoghurt drinks, soft in different flavours and aroma and frozen yoghurt (Lutchmedial *et. al.*, 2004). Dried yoghurt prepared by freeze-drying or spray-drying, is also available in some areas and has been used by desert (Tamime and Robinson, 1999a).

2.3 Warankasi

Ogundiwin (1978a; 1978b), Uzogara *et. al.* (1990), Fashakin and Unokiwedi (1992) defined soft cheese as manufactured from milk by hand drawn the milk from the animal udder after adding juice from Sodom apple plant.Currently, the practice still exists among the Fulani that keeps cattles and process fresh milk (Ogundiwin and Oke, 1983). The terminology with which this product is called has seen some variations; it is also called Woagachi (O'Connor, 1993). Warankasi is an extremely delicate product, reported by Ashaye *et. al*. (2006) and its shelf life was within 3 days, its chemical properties changes after two days storage time under ambient temperature. The moisture content reduces by causing proteolysis and hardeningwhich leads to imparting a rancid aroma. (Appiah, 2000) stated that the differences in the component of warankasi brought variations in the tastingattributes of the processed foods.

2.4 Changes in Cheese Composition during Ripening

During storage of cheese, biochemical changes such as glycolysis, proteolysis and lypolysis take place changing the composition of the cheese and these changes impart characteristic features especially the texture and flavour.

2.4.1 Proteolysis

Proteolysis is a very important process in the growth of texture and flavour in cheese during ripening. In milk, cathepsin D and plasmin are the major enzymes that cause proteolysis (Barrett, 1972), while plasmin is active at 53ºC-55ºC. In causing textural changes to cheese matrix proteolysis leads to the collapse of protein structure and enlarge in pH. As a result, cheese flavour is affected and these changes if not controlled during ripening may lead to the production of a defective cheese. An example of a defect in cheese is the development of off-flavour, specifically bitterness. Sourness in cheese is often resulting to the production and increament of hydrophobic proteins by the activity of coagulant and starter proteinases. The accumulation of these peptides to excessive concentration may be due to either over production or shortage degradation by microbes.

2.4.2 Lypolysis

Lypolysis is the hydrolysis of triglycerides to yield liberated fatty acids. Lipase action is high in raw milk compared to pasteurized milk. According to Vlaemynck (1992), pasteurisation of milk partially inactivates milk lipase; Driessen (1989) stated that heating milk (pasteurization) at 72^0C for 10secs completely inactivates milk lipase. High salt concentration also is inhibitory to milk lipase. This indigenous milk enzyme has optimal activity of pH value of above 8 and temperature of above 35^oC.

2.4.3 The Free fatty acids

This Free fatty acids (FFAs) impart on cheese flavour serve as prerequisite meant for components likealdehydes, lactones, esters and alcohol. These were among the main significant ones that affect growth of different attributeslike savour in various types of cheese, for example soft and hard cheeses (Fenelon and Guinee, 2000).

2.4.4 pH

Reduction in the pH of cheese during ripening may contribute to continue the lactic acid manufacture by live cells of LAB (Korkeala and Bjorkroth, 1997) and the release of some amino acids (e.g. glutamic and aspartic acids) during proteolysis (Sallami *et. al*., 2004). Lypolysis can bestow improved acidity of a cheese system becauseof production of free fatty acids. Reduction in pH of a cheese system causes syneresis (loss of moisture) in the cheese. The given off of alkaline products from the breakdown of protein contributes to pH increase of cheese (Webb *et. al*., 1983).

2.5 Probiotics as a Functional Food

Foods that are refers to as 'functional' are processed food having substances that promote explicit bodily functions to being nourishing (Lourens-Hattingh and Viljoen, 2001). Functional foods are developed specifically to encourage health. Functional foods include foods having definite vitamins, minerals and fatty acids. Also, it is biologically active compounds for example plants-chemicals and those which can support beneficial microbial cultures of interest are within this category (Ndife and Abbo, 2009). Varieties of food for example various yoghurt, types of cheese, creams, baby foods, butter, mayonnaise, powder products or capsules. Messina *et. al.,* (1994a, b) endorsed the mixture of soy foods into foods for theraupectic treatment.

The word probiotics has developed in recent years anddefined as "live microorganisms given in quantities that absolutely influence the wellbeing of the host" according to (FAO/WHO, 2002).*Lactobacillus acidophilus* hinders the growth of C*andida albicans*, fungus which led to the occurrence of *Vulvoviginal candidiasis*. *Lactobacillus bulgaricus* produces acetaldehyde that flavours yoghurt and also produces lactic acid, which helps preserve the milk. This functional food have great impart on the microorganisms.Probiotics measures and controldiarrhoea as well as severe diarrhoea have been shown to have certain healing outcome (Cakır 2003).The probiotic concept is open to lots of different applications in a large variety of fields relevant for human and animal health.

2.6 Nondairy Probiotics Products and their Importance.

Nondairy probiotics foods have a global significance owing to the growing populations of vegetarians and lofty popularity of people who do not digest lactose. National Institute of Kidney Disease and Diabetes (USA), attached to American Institute of Health, lactose intolerance range are lower than 5% in Britain, European, Asian countries and some

tribes in Nigeria. Alm (2002) reported that less than 10% people in the Northern part of Europe do not digest lactose while more than 80% in tropical countries, and above 25% of USA population. Probioticsbacteria cannot supply lactase in adequate quantity, however yoghurt starter provide sufficient quantity and consequently (Ouwehand *et. al.,* 2003) reported that cheeses and yoghurt can be consumed by lactose-intolerant persons.

Table 2.1 shows the proportion of people who do not digest lactose in European and Asiatic countries. The expansion of novel products for people that makes lactose easily digestible is indispensable as milk free products are fitting into the markets with increasing popularity of people who cannot digest lactose.

Table 2.1: Prevalence of lactose deficiency, on percentage, of adult population in the world

Country	Adult population percentage
Finland	15 to 20
Sweden	<5
Ethiopia	80 to 90
Germany	15 to 20
Russia	20 to 30
France	30 to 40
Asian	60 to 100
Nigeria	80 to 90
Uruguay	60 to 65
USA	10 to 70
China	90 to 100
Greece	70 to 80

Source: Modified from Alm (2002).

2.7 Nutritional Benefits of Yoghurt

Milk and milk products for example yoghurts are excellent sources of some minerals as they are the best dietary supply of calcium and have a calcium-to-phosphorus ratio conducive for optimal skeletal growth. The presence and amount of vitamin D in yoghurts give them excellent calcium bioavailability (Katz, 2001).

There are reports on the use of cultured dairy products including yoghurts in the treatment of several ailments and disorders and it has been suggested that such products may have hypocholesterolemic effect (Eichholzer and Stahelin, 1993); prophylaxis used in the healing of gastrointestinal infection as well as potential impediment in colon chronic disease (Kampman *et. al*., 1994). Furthermore, cultured milk products have been employed in treating antibiotic-associated colitis (Colombel *et. al*., 1987).

2.8 Processing Steps in Yoghurt Manufacture

The major manufacturing steps in the two types of yoghurt processing are shown in (Figure 2.1)

2.8.1 Milk standardisation

This milk received frequently added tocreams in other to standardise theoily substances in the required product. Non-fat dry food, whey protein concentrates and milk powders are mixed along side to form slurry. Stabilisers likepectinase and pectin esters were frequently mixed to improve yoghurt properties for exampleappearance, texture, uniformityand mouthfeel which effect draining of whey (Tamime and Robinson, 1999a). Stabilisers help to provide evenness and uniformity. Over-stabilisation leads to “gelatinous” in yoghurt andprevents whey draining which can leads to lower-stabilisation (Vedamuthu, 1991). Some European countries, for exampleFrance as well as Netherlands did not permit stabilisers for some type of yoghurt (Tamime and Deeth, 1980).

2.8.2 Homogenisation

Homogenisation is an essential manufacturing stage for yoghurts having fat. Homogenised milkuses pressures of 10 to above 20MPa for the two phase pressures and the temperature-time series above 65°C. Homogenisation process is whenthe fat in milk is broken into simpler unitsby forcing them to pass through tiny whole while the whole powder homogenised fatty particleincreases significantly. Homogenisation did not allow oily cluster

throughout fermentation or storage, lowers whey draining, improves the colour to developthe uniformity of yoghurts (Vedamuthu, 1991). Whey protein and caseins form the new surface layer of fat globules, when milk is homogenised and raises the number of likely structure-building components in yoghurt (Walstra, 1998).

2.8.3 Heat treatment

Heat treatment is a very significant processing procedure foryoghurt preparation because it affects its microstructure and physical properties (Lucey *et. al.,* 1998a,b,c). This milk is subjected to heat before mixing starter culture during yoghurt processing, temperature-time series for dairy product are 85°C for about 30min or at least 95°C for at about 5min (Tamime and Robinson, 1999a,b). Notably, (Sodini *et. al*., 2004) reported increased pasteurisation time of up to 130°C for 16secs and UHT of up to 140°C and above for at least 16secs for milk sample. Milkheat treatment destroys unwanted microorganism, which provides less competition for the starter culture. Yoghurt starter cultures are sensitive to oxygen so heat treatment helps remove dissolved oxygen assisting the growth of starter cultures.

2.8.4 Fermentation

Milk mixtures are allowed to cool until when ready to add the microorganisms used in incubation. The most favourable temperatures for the starter cultureare above 35°C.And this processchanges lactose to lactic acid, this lowers the pH of milk. The increase in sourness of milk decreases the acidity or alkalinity from 7.0 to at least 4.6.

2.8.5 Cooling

Yoghurts reach the required pH of 4.6; they are partly cooled prior to fruits and flavourings mixture. Products from yoghurtare blast-chilled to over 10°C in the cold storage (4^0C) to decrease sourness enlargement (Tamime and Robinson, 1999a). During the manufacturing of yoghurt e.g. (set), yoghurts are openly moved to a cold store. And in stirred yoghurts, it is allowed to cool down in a vessel while the yoghurt is pumped into different packages

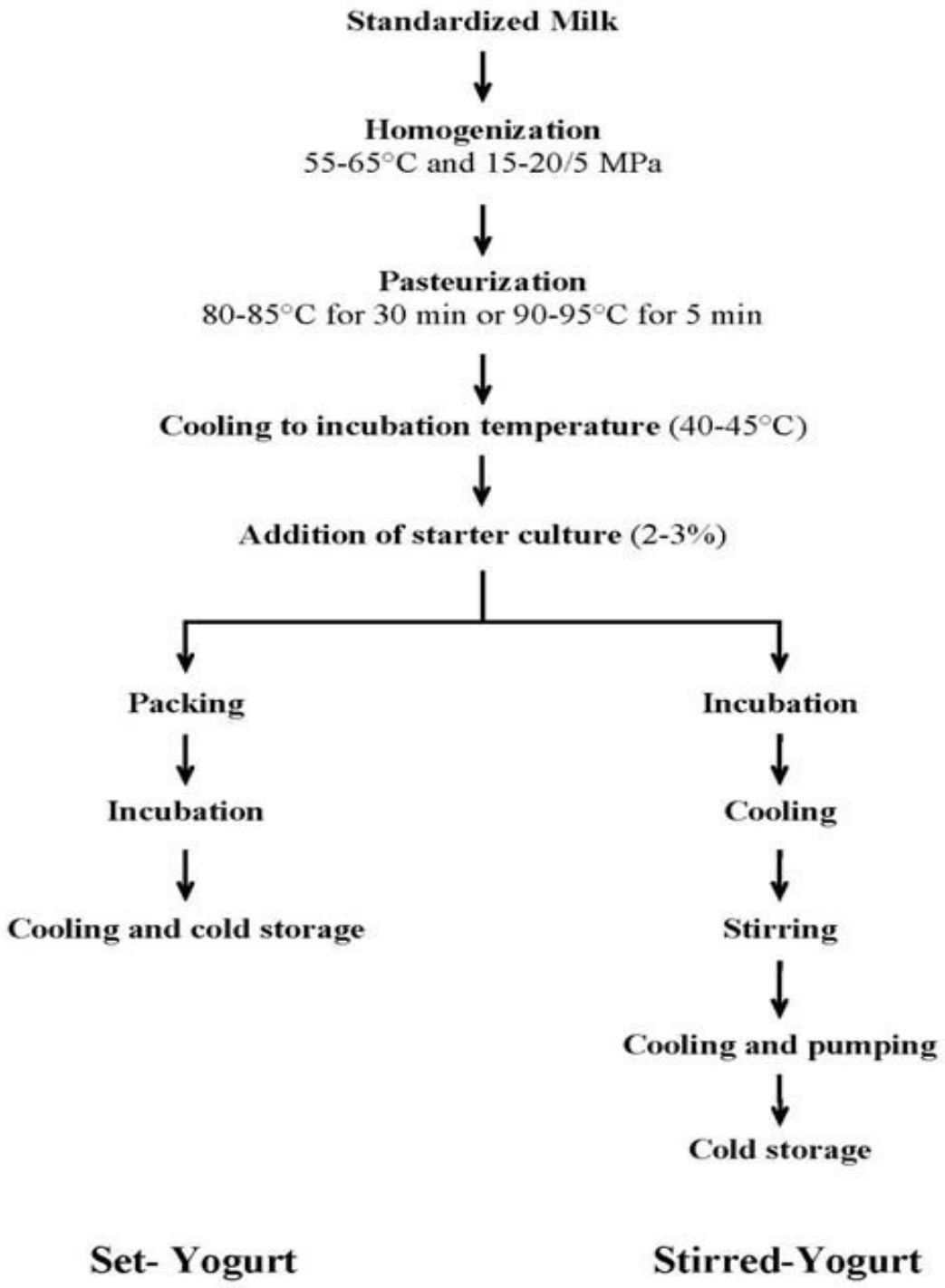

Figure 2.1: Theprocessing steps inset and stirred yoghurt manufacture

Source: Lee and Lucey (2010)

2.9 Yoghurt Starter Culture

The most necessary starter cultures are *Lactobacillusbulgaricus* and *Streptococcus thermophilus* for processing yoghurt(Robinson, 2002). Yoghurtstarter microorganisms grow collectively in dairy products, increasing the sournessand coagulating the caseins.Some countries have legal requirement for *Lactobacillus delbrueckii* subsp.*bulgaricus* by adding it todairy starter, since the typical aroma sole rely on the starter culture. The dairy starter is the vital constituent in the processingof premium dairy products. Dairy starters are express as safe, dynamic, high-quality desirable culture that bestow pleasing flavour and consistency dairy products. They are either inoculated directly or subculture prior to usage. Variousmicroorganisms in starter culture may not be constant in the processingof products to products. Fermentation rate is quite different daily, causing variations in processing plans. During batch processing, where specific plans are necessary and constant high-quality product is expected, undefined culture can be avoided. One of the disadvantages of undefined culture is that they give up being items of contradictory feature. Durso and Hutkins, (2003) reported that mixed culture are predominant, having physical, biochemical characterised strains, either using as single or mixed cultures.

2.9.1 Streptococcus thermophilus

*Streptococcus thermophilus*new name (*Streptococcus salivarius* subsp. *thermophilus*) is the single starter milk streptococcus in Streptococcus genus. DNA-DNA homology and fatty acid membrane profile research studies shows that,*Streptococcus thermophilus* can be regrouped and refers to as*Streptococcussalivarius* ssp. *thermophilus.Streptococcus thermophilus* and *Streptococcus salivarius* were not mentioned as they pose physiological differences.According to (Zirnstein and Hutkins, 1999), *streptococcus thermophilus* was recovered to species level, homology studies and huge phenotypic differences. The general characteristics of *Streptococcus thermophiles*are non-motile coccus, gram positive, homofermentative, facultative anaerobes and catalase negative.

2.9.2*Lactobacillus delbrueckii* subsp. *bulgaricus*

Member of *Lactobacillus delbrueckii* group is *Lactobacillus delbrueckii* subsp. *bulgaricus.* The group consist four different species having comparable genes:*Lactobacillus bulgaricus, Lactobacillus delbrueckii, Lactobacillus lactis* and*Lactobacillus leichmanii.* The DNA alignment over 80% causes the organisms to be regrouped as solitary specie with three different subspecies. *Lactobacillus delbrueckii* subsp. *lactis*have the former species

Lactobacillus lactis with*Lactobacillus leichmaniispp*. The outstanding two different subspecies are *Lactobacillus delbrueckii* ssp. *delbrueckii* as well as*Lactobacillus delbrueckii* subsp. *bulgaricus* as stated by (Limsowtin *et. al*., 2002). The general characteristics of these organisms include non-motile rods, gram positive, rod-like shape, single and short chains, long chains and often arranged in palisades.

2.9.3 *Lactobacillus acidophilus*

Lactobacillus acidophilus is used along with *Streptococcus thermophilus and Lactobacillus bulgaricus* inyoghurt production. This bacterium survives in more acidic environments thanmost microorganisms and grows best at 45^0C. *Lactobacillus acidophilus* occurs in nature in different foods for example meat and fish, animal milk, grains and human milk, intestines and mouths. *Lactobacillus acidophilus* absorbs lactose and metabolises it into lactic acid.

2.10 Health Benefits of Cow Milk

Cow milk is a complete food having balanced nutrient which is pricey due to its cost. The expansion of non-dairy product has cheaper alternative than normal dairy products (Pinthong *et. al.,* 1980). Dairy products approximate to about 83% of worldwide foods manufacture in year 2010 according to (FAOSTAT, 2012) consistsof additional minerals particularly calcium and phosphorus than human milk. Eight essential amino acids in raw milk are in different amounts, accordingto the stage of lactation. Well over70% of amino acids easily digest milk caseins and are heat stable. Thus, other 20% are grouped as whey proteins, and are easy to digest, but very heat-sensitive. The primary carbohydrate in cow milk is the milk sugar, made from one molecule glucose and galactose which isdisaccharide.

2.11 Health Benefits of Goat Milk

Goat milk has many benefits on human health, even more than cow milk.It plays more significant part in man nourishment due to its easier rate of digestion and fewer sensitive to reactions. Its health benefits also accredited to biofunctional parts like medium-chain triglycerides, polyunsaturated fatty acids and some serum proteins (Park, 1994b; Rampilli and Cortellino, 2004). Some documentation shows goat milk bioavailability of minerals (Campos*et. al.,* 2004; Haenlein, 2004).

The unique features regarding the component of goat's milk means that its dietary utilisation is clearly superior to cow's milk. Therefore, the long chain of amino is more easy

to digest (López-Aliaga *et. al.,* 2003; Haenlein, 2004), and less allergenic. Likewise, the high oil content in the goat milk can also digest easily (Alférez *et. al.,* 2001; Haenlein, 1996).Moreover, goat milk has useful effects likenutrition of children and elderly people, physiological functions andhealth maintenance.

2.12 Means of Preserving Milk: Heat Treatment

The storage period of non-sterile milk products, for example some types of yoghurt,pasteurised milk, cottage cheeseare usually restricted to within 3 weeks (Salvador and Fiszman 2004), which depends on raw ingredients value, after processing handling and processing condition. Spoilage is as a result of growth from organisms which survives post processing, microbial contamination. Cottage cheese and pasteurised milkvary in initial quality and affects the overall storage life. Therefore, in other to lower variability in milk products, there is a concern in extending storage life.

2.12.1 Pasteurisation

The most important process raw milk undergoes is pasteurisation which destroy any microorganism present in the milk. The process was named after a scientist Louis Pasteur who finds the spoilage-causing bacteria.Pasteurisation is therefore the most important operation in milk processing whereby the milk is heated to about 72^{o}C and cooling without allowing recontamination. Pasteurisation is carried out for these reasons:

i. to destroybacteria harmful to the health and ensuring milk products are safe for human consumption.

ii. to inactivate some unwanted enzymes and spoilage bacteria,to develop the keep value of the milk

iii. substantially reducesthe whole bacteria load

There are two methods of milk pasteurisation and dairy products:

i. Batch or Holding Time: 63^{0}C for 30minutes

ii. High Temperature Short Time or Continous Method: 71^{0}C for 15seconds

2.12.2 Homogenisation

Homogenisation is the breaking down of butterfat into into minute particlesby forcing the milk through nozzles at very high pressure. (Vedamuthu, 1991) reported that homogenisation reduces, increases whiteness, improves consistency of yoghurts, prevent fat parting throughout fermentation including storage and whey draining.

2.12.3 UHT (ultra-high temperature) treatment

This is the process of applying heat to intermittently flowing product with the use of high temperatures. This process makes the product commercially sterile. Combination of aseptic packaging with UHT, results in a commercially sterile product. Holding time and milk flow are importantprocess proceeding to operation. The UHT heating-time is less than 10 secs at 135–150°C (Montilla, *et. al*., 2005).

2.12.4 Commercial sterilisation

This is the application of heat at high temperatures for a time sufficient to render milk or milk products commercially sterile, thus resulting in products that are safe and microbiological stable at room temperature. The typical condition for sterilizing milk is by heating at 110–140 °C for 20–30 minutes (Montilla*et. al*., 2005).

2.13 History of Soursop Fruit

2.13.1 Description of soursop fruit

Soursop (*Annona muricata* L.) belongs to Annonaceae family. It is native to humid climate in American countries.Soursop fruits are prone to spoilage, mushy, generally sold in local markets(Abbo *et. al.,* 2006). Soursop fruit is a big heart-shaped safe to eat fruit with different shapes, yellow green in colour having white flesh (NAS, 1978).

Soursopjuice is diuretic, have anticancerous,antibacterial,sedative andastringentproperties (Asprey and Thornton, 1995).Plate 2.1 shows a picture of a soursop fruit. The skin of immature fruit is dark-green and eventually become yellowish-green when mature. Soursop fruits serve as raw materials for fruit products for example jellies, jam puree, power fruit bars, flakes wine, juice and beverages, (Abbo *et. al*., 2006). The inner surface of soursop is cream-coloured, fibrous and juicy.

2.13.2 Health benefits of soursop

The seeds, fruit, and leaves of soursop have been used traditionally in the management of diarrheal and diabetes-related diseases, as well as for sedative, antimicrobial, and insecticidal properties(Luna *et. al.,* 2006). Soursop is very rich in vitamin C content; its natural and potent antioxidants enhance the immune system and slow the aging processing.Soursop is rich in fibre; prevent osteoporosis, essential for bone mass formation and useful for strong bones, it is beneficial for the prevention of hypertension and soursop is rich in fibre. The fibrous content of soursop is delightful and nutritious and it helps with

bowel regularity and lowering cholesterol, a natural source to supplement daily iron needs and helps prevent anemia. The fruit is also high in vitamins B1 and B2.

2.13.3 Soursop uses and pharmacology

Soursop fruit is highly rich in carbohydrates for example glucose, lactose and galactose and contains vitamins B_1, and B_2 in big quantities. (Lutchmedial *et. al*., 2004) noted that calcium, magnesium, zinc, potassium, and phosphorous are in the fruit.Soursop is a delicious and healthy fruit; it is used medicinally to treat illness ranging from stomach ailments to worm infestation. (Lannuzel *et. al.,* 2006), suggested a relationship between soursop consumption and using it to treat Parkinson's disease because of very high concentration of annonacin. Also, (Jaramilo *et. al.,* 2000) verified in an in vitro experiment the organic acids extracted from the peel of fruits which is responsible for antileishmanial activity and (Osorio *et. al.*, 2007) also demonstrated the activity of the leaf extract against some *Leishmania* species and *Trypanosomia cruzi.*Ethanolic leaf extracts of *Annona muricata* show molluscicidal activity thought to be, at least in part, due to the annonacin acetogenins (Luna *et. al.,* 2006). Studies identifying the specific acetogenin compounds in the seeds, leaves, root and stem bark have explored the potent cytotoxicity of these compounds. Activity against certain human cancer cell lines has been demonstrated in vitro activity (Kim *et. al.,* 1998).

Plate: 2.1 Soursop (*Annona muricata*) fruit

Source: Warrington (2003).

2.14 Product Development and Potentials

Understanding consumer needs and preferences are critical to successful marketing and enhancing marketing value of a product. Nutritionally improved foods with at least one nutritional improvement over the conventional counterparts have been successful in the marketplace (Duncan, 1998). In addition to basic technologies, modern processes lead to milk fermentation under predictable, controllable and precise conditions to yield hygienic fermented dairy products of high nutritive value (Tamine and Robinson 1999b). Cultured dairy products are an excellent medium to generate an array of products that fit into the current consumer demands for health-driven foods.

Several technologies associated with culture addition, fermentation, or both are available for creating an assortment of flavours and textures in milk products (Robinson and Tamine, 1995). It appears that accentuating the positive attributes of inherent milk constituents, incorporating health-promoting cultures, and offering a variety of flavours and textures to the consumer could enhance fermented milk consumption (Rudrello, 2004). Product modification strategies include removal or reduction of fat, cholesterol, sodium, and calories and fortification with vitamins, calcium, fiber, active cultures and other physiologically active ingredients to align with health perceptions of consumers (Chandan, 1999).

2.15 Bio-preservation of Yoghurt

Cultured milk products are usually perishable products and have enhance their popularity due to the introduction of stabilisers, flavours, colourants and the use of starter culture into the product and have been transformed into various identities and appeals (Salih *et. al.,* 1990). Thus, new techniques have enlarged processing control demands, requiring more stringent manufacturing practices.Use of biopreservatives is one important alternative technology that could be used to extend the shelf life of ready-to- consume fermented milks and can preserve the freshness, flavour, texture and nutrient value of these products (Kroll, 1995).The antimicrobial system possessed by lactic acid bacteria offers scope for the development of an effective natural preservation process. The low molecular weight compounds elaborated by lactic acid bacteria (LAB), capable of exhibiting antagonism are termed as bacteriocins (Dodd *et. al.,* 1990). These LABs have inhibitory effect over spoilage organisms in yoghurt, cheese and other fermented foods and they not only inhibit growth of spoilage organisms, which as a result increase shelf life of a product, but also add therapeutic value to fermented foods. Different organic compounds for example hydrogen peroxide,

diacetyl,organic acids, and bacteriocins are formed for the period of fermentations of lactic acid (Zhennai, 2000). The bacteriocins according to Oyetaya *et. al.,* (2003) produced by LAB are considered as safe (GRAS) and have given rise to novel approach to control pathogens in foodstuffs (Savadogo *et. al.,* 2004). The potential application of bacteriocins as consumer-friendly biopreservatives either in the form of protective cultures or as additives is significant. Besides being less potentially toxic or carcinogenic than current antimicrobial agents, lactic acid bacteria and their by products have been shown to be more effective and flexible in several applications (Brink *et. al.,* 1994).

2.16 Biochemistry of Yoghurt Production

Streptococcus thermophilus in fermentation grows faster than *lactobacillus bulgaricus,*it increases the acidity of the milk and produces anaerobic conditions so that the milk becomes more suitable for the rapid growth of the latter. The *Streptococcus thermophilus* is responsible for initial acidification of the milk and together the two lactic acid bacteria (LAB) can produce more acid than when either is used alone. Once the lactobacilli have started growing the acidity increases further and beneficial substances are produced for the continued growth of the streptococci. The LAB ferment about 35% of the milk sugar through hydrolysis to glucose and galactose. Furthermore, glucose is changed into lactic acid through metabolism while the galactose moiety is released mainly by the coccus into the extracellular environment. *Streptococcus* is capable of producing *Lactobacillus* about 0.6-0.8% (pH of 4.2 to 4.5) and up to 0.5% lactic acid,pH of the product may reduce to 3.5 while lactic acid increases to up to 2%. The lactic acid production on milk protein result on the yoghurt texture. This is an important stage of yoghurt formation, which when not properly executed may cause a deformation in the gelation, and an eventual poor mouth feel of the final product. Robinson (1981) reported that slow acidification of milk to form yoghurt causes development of 'grains' in yoghurt. As fermentation progresses and pH continues to reduce, there is rearrangement of the gel network owing to collection of casein particles by attaining their iso-electric point (pH 4.6) which eventually leads to casein-casein interactions dominating the gel network (Lucey *et. al*., 1998a). The LAB involved in fermentation are also partly responsible for flavour development in yoghurt as*Streptococcus thermophilus* yield some diacetyl, which is responsible for yoghurt its creamy flavour, in contrast*Lactobacillus bulgaricus* also yield acetaldehyde, which gives yoghurt its typical quick flavour (Lutchmedial *et. al*., 2004). pH values are decreased to a range of 4.25 to 4.50, when fermentation comes to an end. Bacterial action is stopped by rapid cooling at thepH right level. Incorrect pH levels or acidification can lead to excess or insufficient tartness. Excess acidity may lead to flavour defects such as shrinkage of curd and wheying-off (Mistry, 2001).

2.17 Microbiology of Yoghurt

Early research of yoghurt microorganisms were carried out by ancient scientists.(Tamime and Robinson, 1985) discoveredsome biochemical characteristics for bacteria, yeasts and moulds inside yoghurt. Much of the credit for the study of yoghurt bacteria was attributed to Orla-Jensen. Yoghurt bacteria are now characterized as lactic acid bacteria which belong to the Lactobacillaceae and Streptococcaceaegenera. Some other strains such as *Lactobacillus helveticus, Lactobacillus jugurti, Lactobacillus acidophilus* and *Bifidobacterium* spp. are also sometimes used as adjuncts.*Lactobacillusbulgaricus* may ferment carbohydrates sugar to lactic acid. It is very sensitive to antibiotics and can grow in the presence of bile salts. The organisms in the yoghurt are stable and the normal size of *Lactobacillus delbrueckii* subsp. *bulgaricus* is 0.8 to 1.0×4 to 6μm, and*Streptococcus thermophilus* is above 0.9μmin in breadth (Rasic and Kurmann, 1978).

When a single culture like*Lactobacillus delbrueckii* subsp. *bulgaricus* or *Streptococcus thermophilus* is used, the end product is lower comparedin a mixed culture (Rasic and Kurmann, 1978). Two stages are concerned in yoghurt processsing and the one is when *Lactobacillus delbrueckii* subsp. *bulgaricus* stimulate the development of *Streptococcus thermophilus* by giving out vital protein end product from casein by proteolytic activity. *Lactobacillus delbrueckii* subsp. *bulgaricus*grow gradually since it is microaerophilic organism. Growth of *Streptococcus thermophilus* is gradual when the first stage ends, because of the elevated lactic acid application. The next level starts,when *Streptococcus thermophilus* produce sufficient organic acid (formic acid), which encourages increase of *Lactobacillus delbrueckii* subsp. *bulgaricus*. *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* are proteolytic bacteria with lactobacillus having higher proteolytic activity.

2.18 Microbial quality of yoghurt

One of the major concerns of consumers, manufacturers and food regulatory bodies is the microbial quality; and it is vital to the overall safety of the food product. Apart from yoghurt culture bacteria and any other probiotic bacteria, it is required that yoghurt contains no other microorganisms. The Codex Alimentarius standards for yoghurt permit a minimum of 10^7cfu/g in the finished product and this standard permits no yeast or moulds or any other microorganism that is not part of the specified starter culture for the product (Codex Standard for Fermented Milks, 2003). Sources of microbial contamination during yoghurt production

includes contaminated starters, poorly cleaned filters, contaminated cups and lids, overall hygiene in the manufacturing process, contaminated flavouring material and air quality in packaging areas (Vedamuthu, 1992). Inadequate pasteurization of milk before fermentation and overall poor sanitation practices during manufacturing may also result in contamination of the final product.

2.19 Textural Properties of Yoghurt

Viscosity, firmness and syneresis are one of the textural properties of yoghurt. Measuring the viscosity of yoghurt is difficult since it is viscosity changes and non-newtonian as shear stress changes. One has to be accurately specifying the measurement conditions used, to report on the apparent viscosity of yoghurt.The consistency of yoghurt is highly influenced by the type of starter cultures,milk composition, heat treatment and stabiliser. Viscosity and firmness increases,as the total solids increases (Becker and Puhan, 1989). The mixed culture of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* have been examined and use to manufacture even and gelatinous yoghurts. The microorganisms areusuallyrefers to as slime-producing bacteria which produce exopolysaccharides, that helps to enlarge viscosity.

Becker and Puhan (1989) remarked that yoghurt fortified with nondry matter and evaporated milk had lower viscosity compared to yoghurt fortified with ultrafiltered milk with similar composition. The vulnerability of bond breaking of 5.0% protein and 2.0% fat, yoghurts fortified with different milk ingredients for example non-dry matter; biogenic methane potential (BMP), sodium caseinate and whey ptotein concentrates (WPC) and reported that BMP yoghurt yielded the least syneresis.

2.20 Aroma Components of Yoghurt

The odour and sourness of milk produce are characterised by many unstable bacterial metabolisms produced as by-product of lactic acid fermentation or by other reactions. Lactic acid is considered to be one of the prime compounds significantly imparting yoghurt and its flavour (Beshkova*et. al.,*1998). The organic acid by-products mostly results to the taste and aromaof yoghurt.Chaves *et. al*., (2002) reported elevated application of acetaldehyde which is required to process a desired yoghurt flavour, which help to convert acetaldehyde into ethanol and lead to a small utilisation of acetaldehyde. (Guerra-Hernandez *et. al.,* 1995) stated that one of the major fragrance components of yoghurt is diacetyl and Nilsson (2008) reported

that citrate and lactoseare diacetl precursors. More than 90 compounds have impart on the final aroma of yoghurt, and these are benzaldehyde, and 2, 3-butanedio. Ott *et. al.,* (1997) examined more than fiveorganic compounds (2-methyltetrahydrothiophen-3-one, methional, 2-E-nonenal, guaiacol,1-nonen-3-one and 1-octen-3-one) which have strong flavour on yoghurt. Also, one of thecontributors to yoghurt aroma is acetaldehyde which has the net fragrant effect on the outcome of the mutual effects of all the aromatics in the product.

2.21 Molecular Characterisation Methods

A broad understanding exist that strains with related phenotypes do not have closely related genotypes, while the phenotypic techniques have been confirmed to be helpful. These methods also have unclear, poor reproducibility and discriminatory power. Milliere *et. al*., 1996 classified wild type strains as “atypical” and isolated from ordinary habitats which show phenotypic variability.

Genotypic techniques are based on the various level of bias from species level to individual strain level.Using designed primers as a result of amplification of targeted DNA fragments under controlled reaction conditions are basic forpolymerase chain reaction (PCR). The largely prevailing and broadly used phylogenetic marker codes are the 16S ribosomal RNA and the genes. Therefore, there is more measure of protection in the tRNA genes’ sequences. Morata *et. al.,* 1999 reported that about 12,000 sequences of 16S rDNA which are accessible for prokaryotic strains in gene banks.

The main generally used process to isolate and recognize intra-species level is the Restriction Fragment Length Polymorphism. An example of PCRfingerprinting methods is Randomly Amplified Polymorphic DNA (RAPD). RAPD use small random primers and low-stringency situation to magnify DNA indiscriminately. To generate a fingerprint, the fragments obtained are detached electrophoretically. Also, the great flexibility in primer choice is required to make a distinction of LAB at diverse classification levels from genus to intra-specific stage.

2.21.1 Importance of Molecular Biology

The molecular biology examines cells, their characteristics, chemical processes, part, cell's activities and growth metabolism. It is also the study of life, atoms and molecules level. Proteins are complex molecules made up of smaller units known as amino acids. It plays a host of roles in cells metabolism. Furthermore, proteins are constructed complex cells stored in molecules called deoxyribonucleic acid (DNA) in the cell nucleus. Nucleotides are the long chains of unitsjoined to each other end-to-end are deoxyribonucleic acid molecules. The importance of Molecular biology has provided new and completely diverse way of studying living organisms. (Rasko and Downes 1995) explained this technique as applications that work on man infection or molecular medication which has expand on the technical advances of biotechnology. The first discovery is how to isolate DNA, using restriction endonucleases by cutting it into pieces of various sizes, which means enzymes secluded from different bacteria that will piece DNA at known sequences of nucleotide bases.

2.22 Spices and their Uses

Spice arebark or vegetable substance, dried seed, fruit orroot mainly used for preserving, colouring and flavouring of food. They are spicy and fragrant, coloured, phenolic or having strong odour and they originated from diverse parts of precise plants like the barks, leaves and flowers (Dziezak, 1989). They preserve foods and even treat some diseases of man and animals, examples include: chile pepper, garlic, ginger, African cardamom, nutmeg, clove and turmeric. Spices are food supplements or products which have been used not only as flavouring and colouring agents but also as natural or artificial that preserves food and herbs in local medicine many several decades in Asia, Africa as well as some parts of the world (Srinivasan, 2005).

Spiceswere commonly used in warmer climate because of their antimicrobial properties and well-known in meat especially when prone to spoilage. Herbs and spices are used as food and to treat ailments by humans. Thus, spices for example saffron, they are food colourant, coloured spices and flax seed which contain some components which provide important shield against cancer related diseases (Lai and Roy, 2004). Other spices uses include medicinal, cosmestics, religious ritual or for perfume production. A variety of spices and oils are known to have preservation attributes (Karapinar, 1985). Thus, the preservation characteristics of spices are because of oleoresins and impulsive oils present in the spices (Pruthi, 1980). Spices also have economic importance nationally. Spice trade started around 2000 BCE in East Asia with herbs and pepper and also inSouth Asia and Middle Eastwith

cinnamon and pepper. Egyptians used exotic herbs to stimulate world trade and herbs for embalmment.

2.22.1 African cardamom (*Aframomum danielli)*

The plants which provide alligator pepper are herbaceous perennials of the Zingiberaceae family of flowering plants that are native to swampy habitat along the West African coast. Thus, this spice*Aframomum danielli* possesses preservation attributes (Adegoke *et. al.,* 2002),highly enrich in vitamins, minerals and subastances that slow or prevent oxidation is far superior than butylated hydroxyl anisole (BHA) and synthetic antioxidants like butylated hydroxyl toluene (BHT) (Adegoke and Skura, 1994; Adegoke and Gopalakrishna, 1998). *Aframomum danielli* when used in addition to hydrostatic pressure have potent synergistic inhibitory effects on food spoilage yeasts (Adegoke *et. al.,* 1997). Preservation characteristics of *Aframomum danielli* fine particle is linked with phytochemical components known as alkaloids. (Adegoke and Skura, 1994 and Fasoyiro *et. al*., 2001) stated the tendency and capability of destroying microbes of crude extracts of *Aframomum danielli.*

The nutrientsthat are present in the spice *Aframomum danielli* have been reported. The spice consists of 10.5% moisture content, 8.2% protein content, calorific value of 469.7Kcal/100g and it contains different quantities of minerals and vitamins like phosphorus, calcium, zinc, copper, sodium and manganese. Thus, concentrations of amino acids in *A. danielli* include Valine, Glutamic, Leucine, Lysine, Threonine, Serine, and Proline.*Aframomum danielli* has inhibitory effect on food spoilage organisms like *Salmonella enteriditics, Pseudomonas fragi, Psedomonas flourescens, Aspergillus flavus and Aspergillus niger* (Adegoke and Skura 1994).

2.22.2 Turmeric (*Curcuma longa L.*)

Turmeric is a derivative of the plant, *Curcuma longa* and of ginger family. Tumeric is the most common spice in regions of Mid-East and Asian countries for herbal remedies. Most important demethoxycurcumin, curcuminoids, bisdemethoxycurcumin and curcumin occur naturally in Curcuma species. Curcumin, a yellow pigment extracted from the root part of the plant species, is a active component of turmeric (Ireson *et. al.,* 2002). It has many biological effects like hypolipidemic (Babu and Srinivasan, 1997),antioxidant (Sharma *et. al.,* 2004) anti-inflammatory (Chainani-Wu, 2003 and Sharma *et. al.,* 2004) activities. Documentation in journal stated that its chemopreventive agent in treating several cancer diseases (Garcea *et.*

*a*l., 2005). Also, curcumin contribute to lowering pace of colorectal cancer in countries like China comparing to other countries because of very high usuage of curcumin in the country.

Usually, turmeric is commonly used in different cuisines for flavour and as colourants for foods like yoghurt, chicken and rice. Also, turmeric can be used in addition with other spices or alone by itself. One of the main substances in turmeric is curry fine particles. Soil factors, for example nutrientsthe genus diversity and level of acidity, affect the curcumin content and the source of turmeric (Hossain and Ishimine, 2005; Sasikumar, 2005).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

Soursop fruits were obtained from Oje, Ibadan, Oyo State. Cow milk was also obtained fromDairy Teaching and Study Unit, University of Ibadan, while milk from goat was purchased from Fulani settlement near Odeda village, along Iseyin road, Ibadan. Fresh milks were transported in ice-cubes from the source to the Food Chemistry laboratory of the University of Ibadan. Analyses were carried out inthe Nutrition Department, Biochemistry Department, University of Ibadan and International Institute of Tropical Agriculture (IITA), Ibadan. African cardamom spices and Turmeric rhizomes used were purchased from Bodija market, while yoghurt starter culture was procured from a store in Oojo, Ibadan.

3.2 Methods

3.2.1 Soursop juice preparation

Matured andfresh soursop fruits were washed with clean water, hand-peeled and deseeded (removal of the seeds). One hundred (100g) grammes of the pulp was blended with 1000ml of distill water using electric blender (Oster, UL-564A, Mexico) several times. The pulp was filtered using muslin cloth to obtain soursop juice (Nwachukwu and Ezeigbo, 2013).

3.2.2 Preparation of soursop yoghurt

The soursop juice was pasteurised at 90^{o}C for 30minutes, cooled to 43^{o}C. Five (5g)grammes of yoghurt starter culture with (*Streptococcus thermophillus, Lactobacillus bulgaricus* and *Lactobacillus acidophillus*) was mixed with a litre of pasteurised juice, thoroughly mixed and incubated at 43^{o}C for 5hours, cooled to 4^{o}C to produce soursop yoghurt (Hartati and Eka, 2010).

3.2.3 Preparation of cow milk yoghurt

Fresh cow milk obtained was sieved, in other to remove any foreign matter like hair and dirts during milking; pasteurized at 73^{o}C for 20 minutes (with the use of water bath), cooled to 43^{o}C. The yoghurt culture *(Lactobacillus bulgaricus*, *Streptococcus thermophillus*and *Lactobacillus accidophillus*) 5g was mixed to a litre of milk pasteurised and incubated at 43^{o}C for 11hrs. Thus, yoghurt was allowed to cool to 4^{o}C to form cow milk yoghurt (Dirar, 1993).

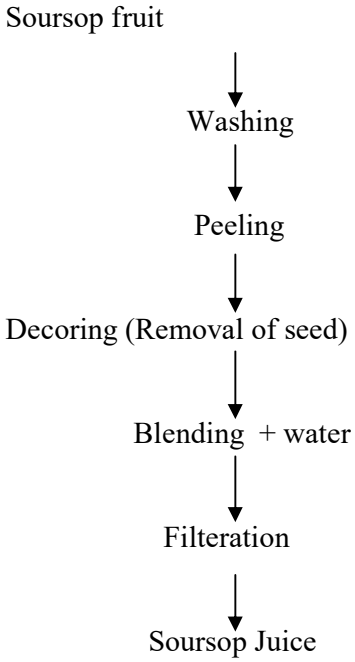

Figure 3.1: Processing of soursop juice

Source: Nwachukwu and Ezeigbo (2013)

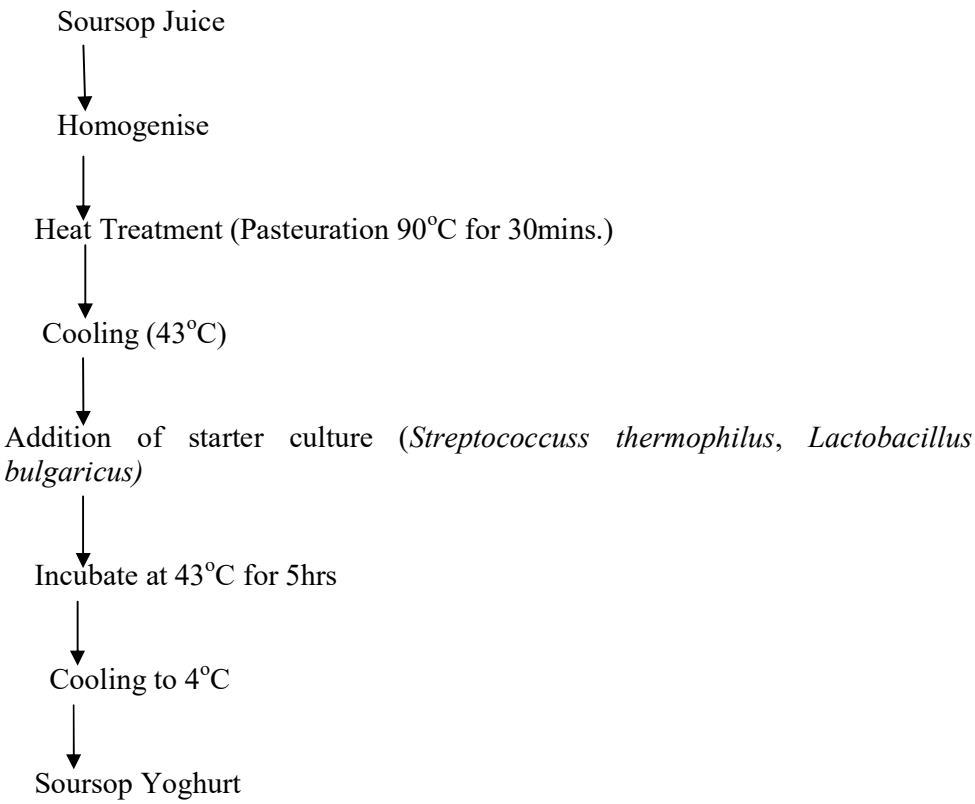

Figure 3.2: Processing of soursop yoghurt

Source: Hartati and Eka (2010)

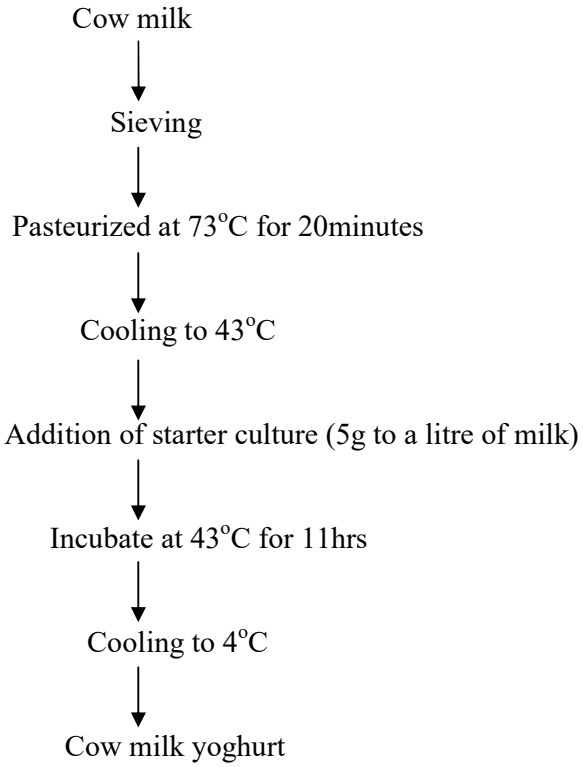

Figure 3.3: Processing of cow milk yoghurt

Source: Dirar, (1993)

3.2.4 Preparation of goat milk yoghurt

Fresh goat milks were sieved to remove any foreign matter like hair or stone during milking.It was pasteurised at 73^{o}C for 20 minutes; cooled to 43^{o}C. Yoghurt starter culture *(Lactobacillus bulgaricus, Streptococcus thermophillus*and *Lactobacillus acidophillus*) 5g, was mixed to a litre of pasteurised goat milk and incubated at 43^{o}C for 11hrs. The yoghurt was allowed to cool to 4^{o}C to form goat milk yoghurt (Wanda, 2005).

3.2.5 Cheese preparation

The fresh milk was sieved to eliminate irrelevant matter and pasteurised the milk at 72^{o}C for 20 minutes heated slowly in a water bath till the temperature reaches 72^{o}C. It was then lower to 40^{o}C to boost the action of proteinase enzyme in sodom apple. The sodom apple stem was crushed and the extracted juice adds to a small amount of warm milk. Thiscombination was subjected to heat at 70^{o}C for 20mins. Thus, removal of the scum and the curd facilitate the whey expulsion for about two to three minutes (Ashaye *et. al*., 2006).

3.2.6 Aqueous extract of *Aframomum danielli*

The removal of *Aframomum danielli* seeds from the pods and sorted by picking, washed and then air-dried for 10hrs at 60^{o}C to reduce the moisture content, milled into powder (Hammer mill of Phillip model H252K) and sieved to obtain fine powder (250µm) and kept in airtight container. Ten grams of *Aframomum danielli*powder was weighed and added to 100ml of distilled water and mixed thoroughly. The combination was placed in a refrigerator at 4^{o}C for four days and then centrifuged using 10,000rpm for 10mins and the mixtures obtained were used as *Aframomum danielli* extract. The supernatant was later stored at 4^{o}C in a refrigerator until required. Concentrations of*Aframomum danielli* 1.0%, 1.5%, 2.0% and 2.5% were prepared as described by Adegoke and Skura (1994).

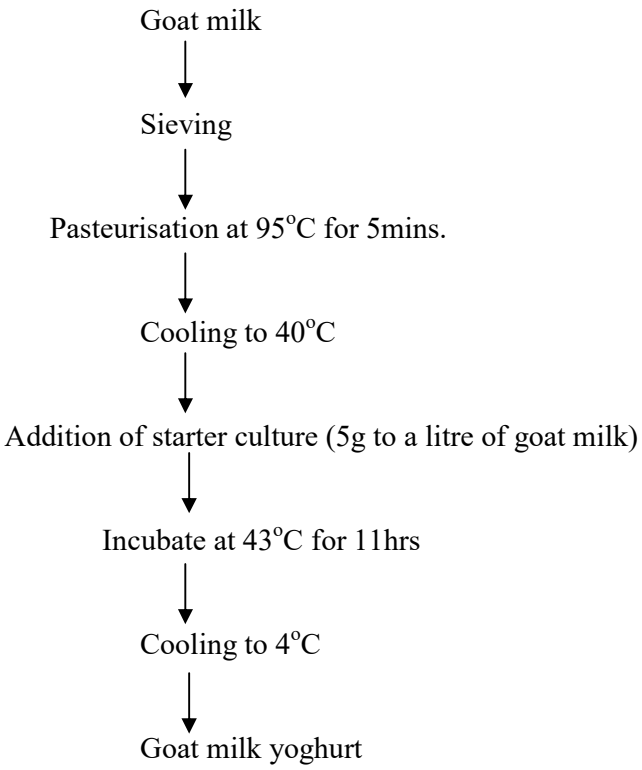

Figure 3.4: Processing of goat milk yoghurt

Source: Wanda, (2005)

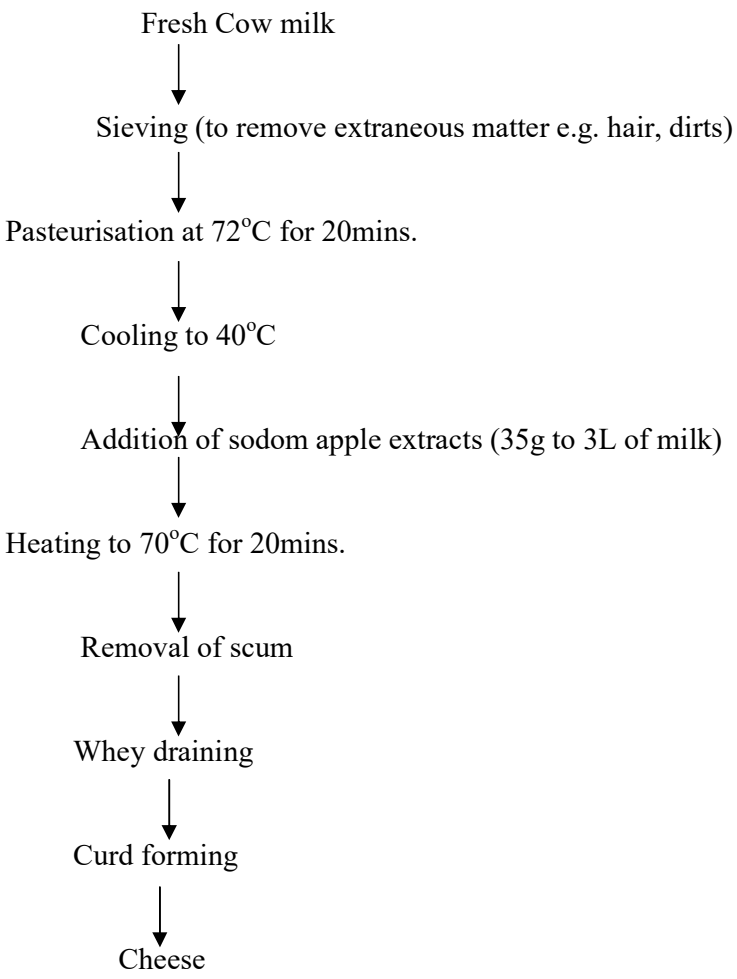

Figure 3.5: Processing of cheese from cow milk

Source: Ashaye *et. al*., (2006)

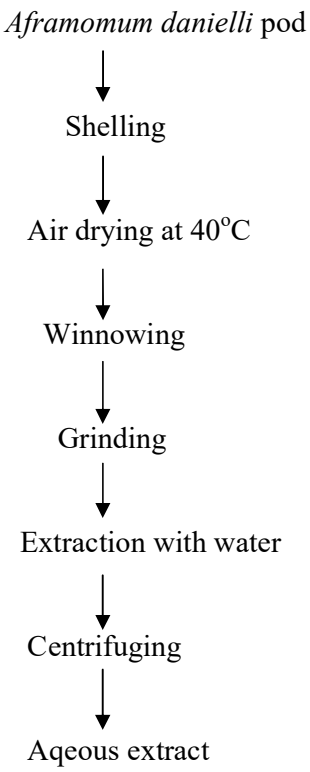

Fig. 3.6: Production of aqeous extract of *Aframomum danielli*

Source: Adegoke and Skura (1994).

3.2.7 Preparation of turmeric extract

The turmeric rhizomes were cleaned in water to eliminate soil lumps, peeled, re-washed.Ten grammes (10g) of turmeric rhizomes was measured into a 100ml beaker, milled using electric blender (Oster, UL-564A, Mexico) and soaked for two days in a refrigerator for proper extraction. The milled and soaked turmeric was filtered using muslin cloth to remove the residue, pasteurized at 72^{o}C for 20minutes and allowed to cool, bottled and stored in a refrigerated temperature until ready for use.

3.2.8 Starter culture

Freeze-dried yoghurt starter culture (ingredients: skim milk powder, sucrose, ascorbic acid, lactic bacteria *Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus)*was used per litre of milk. Each of the milk samples was heated to a prescribed pasteurisation temperature, allowed to cool to 42-44^{o}C. Starter culture (5g) was dissolved in a little amount of distilled water in a cup, poured into a litre of pasteurised milk, incubated for 4hrs, until yoghurt had reached the desired firmness.

3.3 Chemical Analysis of Yoghurts and Cheese Samples

3.3.1 Moisture content

Moisture content determination as described by AOAC (2005) was used. Freshly produced yoghurt (2g) was weighed into each of three previously dried and weighed glass crucibles. The crucibles with the samples were placed in a thermostatically controlled oven at 105^{o}C till a constant weight of solid material was obtained after five hours. The crucibles were then removed, cooled inside desiccators and re-weighed. The moisture content was calculated by difference in weights and expressed as percentage moisture.

$$\% \text{ Moisture} = \frac{\text{weight of moisture}}{\text{weight of sample}} \times 100$$

3.3.2 Protein content

Protein content was carried out using AOAC (2003) method. Samples (2ml) was measured to a kjeldahl flask, 4 tablets of kjeldahl catalysts sodium sulphate (each tablet contains 1gm of $Na_2SO_{4)}$, 1gm of copper sulphate and a tablet of kjeldahl catalyst selenium (each tablet contains 1gm of Na_2SO_4 + 0.05gm selenium) was added. 25mL concentrated sulphuric acid and 5 glass beads were added into the kjeldahl flask to prevent bumping during heating. The fume cupboard was heated lightly until solution assumes green colour. The solution was then allowed to cool and washed down the black particles. Reheat gently at first and then turn the burner full and heat until the green colour disappeared. It was cooled and transferred the digest with numerous washings to a 250mL flask.

Calculation:

$$\% \text{ Total Nitrogen} = \frac{(\text{sample titre} - \text{blank titre}) \times 0.1 \times 1.4007 \times 6.38}{10}$$

Blank = 0.39ml

% Protein (crude) = % Total Nitrogen x Conversion factor (6.25)

3.3.3 Fat content

Fat content was analysed with the method of AOAC (2003).Round-bottom flask(250 ml) was dried clean in an oven between 105-110^{o}C for at least 30 minutes and transferred into desiccators to lower the temperature (w2).Yoghurt sample (2mls) was accurately measured to the marked thimbles. About 300cm^{3}petroleum ether was filled into boiling flask at the boiling point of 40^{o}- 60^{o}C. The apparatus for the analysis was arranged and heated for at least 6 hours. And the container was disconnected while petrol ether on top of container was removed. The flask was free of washed chemical and dried at 105-110^{o}C for 1hr. Thus, flask was taken into a desiccator to cool and re-weighed (w3).

Calculation:

$$\% \text{ Fat} = \frac{(w_3 - w_1)}{w_2} \times 100$$

Where

Flask weight with extracted oil = w_3

Empty flask weight = w_2

Sample weight = w_1

3.3.4 Crude fibre

The method of Joslyn (1970) was used where one gram of yoghurt sample was measured to a 500ml conical flask and the digestion reagent added was 100ml; and washed all the areas of the flask which was used in boiling for 40minutes. The jacketed water condenser was used to avoid water losses and sieves using 15cm whatman filter paper and removed the residue using spoon and taken with the fibre to a desicator. It was driedovernight at 105^{o}C, taken into a porcelain dish, weighed (A) and ashed at 600^{o}C for 5 hours in a muffle furnace, allow to cool in asilica dish and weighed (B). % fibre content calculated as:

Calculation:

$$\% \text{ Fibre} = \frac{\text{(weight of A) - (weight B)}}{\text{sample weight}} \times 100$$

3.3.5 Ash content

This was carried out using method 945.46 by AOAC (2005).Homogenised yoghurt sample (2g) was measured to a crucible which had been previously ignited and weighed. The crucibles with their contents were taken to a muffle furnace maintained at 600^{o}C for 2hrs until its content was completely ashed. The crucible was taken directly to a silica dish to cool while the ash content was expressed as a percentage ash.

Calculation:

$$\text{Ash content (\%)} = \frac{(w_2 - w_1)}{w_s} \times 100$$

where

w_2 = crucible weight plus ash

w_1 = crucible weight

w_s = sample weight

3.3.6 Carbohydrate content

The crude protein, fibre, moisture and ash content carried out using standard methods (AOAC 2010). The total carbohydrate calculated by difference:

% Carbohydrate = 100 - [% protein+% fat+% Ash+% Crude fiber].

3.4 Mineral Composition of Yoghurt and Cheese Samples

3.4.1 Sample preparation:

Mineral content of each sample was carried out by AOAC method (1980) where 5g of yoghurt product was measured to a 250mL Erlenmeyer flask; 25mL Hydrochloric acid (HCL) solution was added and was brought to heating and cooled, transferred to 50ml flask. The mixture was sieved using Whatman filter while the solution after sieving was used for mineral determination using corresponding standards and blanks. The filtrate of each sample was used for Atomic Absorption Spectrophotometric analysis.

3.4.2 Minerals

The minerals determined are zinc, magnesium, potassium, calcium, sodium and ironcontent in the yoghurts and cheese samples were carried out using BUCK Scientific; Atomic Absorption Spectrophotometer (Model 210/211VGP). The spectrophotometer was connected to the source of electric power and ignition was turned on. Buck cathode lamb was used for each element with the machine set at wavelength for the respective elements: Ca – 239.9nm, Cu – 327.4nm, Fe-372nm, Pb-283.3nm, Mg-285.2nm, K – 262.8nm, Na – 330.2nm and Zn-307.6nm respectively.

3.5 Proximate Analysis of Soursop Seed and Pulp

3.5.1 Sample preparation

The matured fruits were picked, washed and weighed. Average weight was 156g for the soursop fruit. The mature, ripe fruit was peeled, while the pulp was collected into a clean basin and the seeds collected separately for proximate analysis. The seeds were milled using Thomas Scientific mini-miller; (Model 3383-L70) bagged and stored in zip-locked pouches until ready for use.

3.6. Physical Analysis of Yoghurts and Cheese Samples

3.6.1. pH

pH of yoghurt samples prepared were determined with a 50ml beaker at 20^0C using a pH meter (Melter Delta 340) after standardization at pH of 4.0 and 7.0

3.6.2 Viscosity

One hundred(100mL)metal beaker the viscometer was filledwith yoghurt at 5^oC and the rotor was immersed in it. Cole-Parmer Viscometer; (Model Wu-98965-40) wasswitched on and the resistance of the mixture against the applied velocity was calculated in decipoise (dPs). The reading was taken after about 20 seconds when the dial remained constant.

3.7 Chemical Analysis of Yoghurts and Cheese Samples

3.7.1. Soluble solids (^{o}BRIX)

Total solid was measured according to AOAC (2003) method. Brix was measured with a refractometer (ERMA INC.; Model RHB-10 (ATC) Japan). The prism in front of the refractometer was cleaned with soft tissue and observed to read zero sugar. The sugar level of each sample was thus taken by dropping two drops of yoghurt on the prism of the equipment and reading was taken in triplicates.

3.7.2 Titratable acidity

Total titratable acidity was measured usingAOAC (2003) method: Yoghurt sample (10ml) measured into a 250ml flasks and 1ml Rosaline solution B added to the conical flask. The solution was stirred and used as colour control; 1ml of phenolphthalein indicator was also added to another conical flask containing 10ml of yoghurt samples and titrated against 0.1M NaOH. The mixture was stirred continuously and titration terminated when the colour of the mixture matches the pink colour of the control. The titratable acidity i.e. (% lactic acid) was calculated from the formula:

Total titratable acidity as % lactic acid was calculated

Calculation:

$$\text{TTA (\% lactic acid)} = \frac{\text{Titre X Molarity X 90 X100}}{\text{1000 X Vol. of Sample}}$$

3.7.3 Vitamin C (as ascorbic acid)

The method of titration using dye solution described by Rangana (1987) and modified by AOAC (2001) was used. The working standard solution of ascorbic acid was prepared using 100mg Ascorbic acid in 100ml of 4% oxalic acid solution in a standard flask i.e.1mg/mL: The working solution (5mL) was pipetted into 100ml conical flask and 10ml of 4% oxalic acid was added and titrated against dye solution (V_1mL), containing2-6 dichlorophenol indophenol which oxidizes ascorbic acid and the show of pink colour gives the end point. The amount of dye used is equal to the amount of ascorbic acid.Thereafter, 5g of yoghurt was added to 100mL of oxalic acid and centrifuged at 100 rpm; 5mL of supernatant was taken and 10mL of 4% oxalic acid was added and titrated against the dye solution to obtain titre V_2mL.

Calculation:

$$\text{Ascorbic acid (mg/100g)} = \frac{0.5\text{mg}}{V_1\text{mL}} \times \frac{V_2}{5\text{mL}} \times \frac{100\text{mL}}{5\text{ (wt of sample)}} \times 100$$

3.8 Storage Studies of Yoghurt and Cheese Samples

Processed yoghurts from soursop, cow milk and goat milk as well as soft-cheese were preserved with (African cardamom and turmeric) extracts at various concentrations varying from 0%, 1.0%, 1.5%, 2.0%, and 2.5% and stored at refrigerated temperature (4^{o}C) for three months at Bioscience laboratory at IITA.

3.9 Microbiological Analysis

3.9.1 Serial dilutions of Yoghurt and Cheese Samples and Culture Methods

The samples (yoghurt and cheese) were mixed by shaking the bottle many times and the first(10^{-1}) dilution prepared by pipetting 1ml of the sample into a test tube containing 9ml sterilized distilled water using automatic micropipette according to the method of Dave and Shah (1996). This was done without allowing the tip of the pipette to touch the diluents, and the solution was thoroughly mixed. The second dilution, 10^{-2}, was done by pipetting 1ml aliquot of the first diluted (10^{-1}) solution into a test tube containing 9ml sterilized distilled water. Dilutions of 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were also prepared by repeating the same process.

3.9.1.1 Selective Media and Growth Conditions

One ml aliquot of the samples and dilutions were plated into MRS (Man, Rogasa and Sharpe) agar. The plates were incubated at 37^{o}C for three days under anaerobic conditions, (using this medium aimed at isolating and enumerating lactobacilli). After incubation, individual colonies were selected and transferred into sterile broth mediums. The following step is purifying the selected colonies with streak plate technique.

3.9.2 Total plate count

This was enumerated by diluting the samples decimally and spread plating 1ml aliquots on nutrient agar and incubating at 30^{o}C for 48hrs after which the colonies on each plate were counted using a colony counter (FDA, 1998). The colony counter was used for the enumeration of total bacterial count.

3.9.3 Yeast and mould count

This was carried out by plating 1ml of the aliquots on potato dextrose agar with (0.01% chloramphenicol incorporated to prevent bacteria growth) at 30^{o}C for 48hrs (FDA 1998).

3.10 Isolation, Identification and Molecular Characterization of Lactic Acid Bacteria from Yoghurt and Cheese Samples.

The isolates were examined according to their colony morphology, catalase reaction and gram staining.

3.10.1 Biochemical identification:

Gram staining procedure:

Gram staining procedure was done using crystal violet stain for one minute. The excess stain was removed under running water. Again, it was stained with gram iodine as mordant for one minute and washed under running water. The washed gram iodine mordant was fixed with 5% alcohol for 15secs and counter-stained with safrainine for 30 seconds, washed under tap water and dried with cotton towel gently (Dave and Shah 1996).

3.10.2 Catalase test

Catalase enzymes break down hydrogen peroxide into oxygen and water molecules ($2H_2O_2 \rightarrow 2H_2O + O_2$)

and oxygen production was noticed by the generation of O_2 bubbles. Catalase test was carried out by adding few drops of 3% hydrogen peroxide to a test –tube containing 24hr-old culture of each isolate (Dave and Shah 1996).

3.10.3 Gas production from glucose

The production of carbon dioxide gas from glucose is the test for determining homofermentative nature of isolate. MRS broths having inverted Durham tubes were utilised to test for CO_2 production. Five microlitres of overnight activated cultures were inoculated into 8ml MRS containing inverted tubes and incubated for five days at 42^{o} C.

3.10.4 Growth at different temperatures

Growth at 15^{o}C and 45^{o}C are frequently used for the classification of Bacilli (Hammes and Vogel, 1995). To determine the growth at given temperatures, the modified MRS media were used. Bromocresol purple was used to determine the colour change in acidity from purple to yellow, indicating lactic acid production and cell growth. Five microliters of overnight activated cultures were inoculated into 5ml test media, incubated at 45^{o}C and observed for seven days for colour and growth.

3.10.5. Molecular characterisation

Genomic DNA isolation was done using the method of Thottappily *et. al.,* (2003); molecular analysis carried out in Bioscience laboratory, IITA. The isolates grown overnight were transferred to eppendorf tube and spunned at 14,000revolutionsper minutes for 2mins. The supernatant was eliminated and 600µl of 2X CTAB buffer was added to the pellet and incubated at 65^{o}C for 20minutes. The sample was removed from the incubator, allowed to cool to room temperature 37^{o}C and chloroform 750µLadded. The sample was mixed by gentle inversion of the tube several times. The sample was spun at 14,000rpm mins and the supernatant was transferred into a new eppendorf tube and(750µL)of cold isopropanol was added to precipitate the DNA. The sample was retained in the freezer for 1hr, spun at 14,000rpm for 10mins; the supernatant was discarded and the pellet was washed with 70% ethanol.The sample was later air-dried for 30 minutes on the bench. Lastly, the pellet was re-suspended in 100µL of sterile distilled water and DNA concentration of the samples was measured on Thermo-Scientific^{TM} NanoDrop Model ND-2000c spectrophotometer at 260nm and 280nm while the genomic purity was determined between 1.8 and 2.0ng.

3.10.6 The amplification of 16S-IT rDNA region by polymerase chain reaction

For the amplification of 16S-IT rDNA region by polymerase chain reaction, EGE1 and L1 were used as primers. Forward primer was complementary to the 5'end of 16S rDNA and the reverse primer was complementary to the 3' end of ITS region. Forward primer uses EGE1:5' while reverse primer uses L1: 5'. The reaction was determined out in Eppendorf Master Cycler. The PCR conditions for the PCR machine programme were as follows:
Step 1: 94^{o}C for 3minutes (denaturation), Step 2: 37^{o}C for 0.4seconds (annealing) and Step 3: 72^{o}C for 1.0seconds (extension). All the PCR mixture was thoroughly mixed together in the PCR tubes and was made up to 25µL. The recipe of the PCR mixture is presented in Appendix 32.

3.10.7 Separation of amplification products

The procedure includes three essential steps: preparation of agarose gel, loading of the gel and the electrophoresis of the products.

3.10.7.1 Preparation of agarose gel

The PCR products were analysed using molten 1% of agarose gel(dissolved in 100µl 1x TAE buffer by boiling), cooled to 45^{o}C, 15µl ethidium bromide solution0.01% (10mg/ml) added and stirred. The agarose gel was poured into the gel casting stand where combs were placed.

3.10.7.2 Loading of agarose gel

Three microlitres of PCR products were mixed with 3µl gel loading dye. The samples were loaded into the wells, starting from the second well on the gel as DNA size- marker Thermo Fisher Scientific;(Model SM0312) was loaded into the fist well.

3.10.7.3 Electrophoresis of products

Polymerase chain reaction products were electrophoresed at 120V for 1hr. Amplification products were visualized in a gel documentation system Vilber-Lourmat; (Model DP VX5-P). The presence of DNA fragments with the size of 1500-2000bp indicated that amplification was achieved.

3.10.8 Purification of PCR products

The pelleted DNA was washed with 500 µl of 70% ethanol, centrifuged at 5000 rpm for 10minutes and ethanol was removed after the pellets were dried at 37^{o}C for 10minutes. The DNA was dissolved in 50 µl 1x TE solution and stored at -20^{o}C for sequencing.

3.10.9 Sequencing the DNA

Sequencing of DNA was carried by ABI PRISM 310 Genetic Analyzer and chain information procured was placed to NCBI database with BLAST analysis for molecular classification of the organisms.

3.10 The Use of LAB Isolates as Single and Mixed-Strain Starter Cultures in Yoghurt Production

3.10.1 Sample preparation and method:

Fresh cowmilk was sieved to remove extraneous materials like hair and dirts during milking process. Cowmilk was prepared by heating the milk to 73^{o}C for 20mins. The pasteurised milk was lowered to 43^{o}C and the yoghurt culture was mixed with the heated milk separately. The milk was measured and divided into two equal parts, for experimental treatments:

A= commercial freezed-dried yoghurt starter culture (yogourmet) and *Lactobacillus fermentus* isolate (1:1)

B= *Lactobacillus fermentus* isolate and *Lactobacillus licheniformis* isolate (1:1)

Two separate freshly prepared yoghurts were incubated at 43^{o}C for 11hrs and stored at 4^{o}C for further analysis. Different physical and chemical analysis like ash, fats, total solids and moisture and protein analysis were carried out on the yoghurt samples.Alkalinity and acidity was determined using phenolphthalein as marker by titration of 0.1 N NaOH while pH was determined by using pH meter (Melter Delta 340) after standardisation at pH of 4.0 and 7.0.

3.11 Animal Studies

3.11.1 Experimental animals

Twelve white female albino rats weighing 120-160g were purchasedfrom the Department of Veterinary Physiology, Faculty of Veterinary Medicine, and University of Ibadan. Rats were placed in metallic cages where food as well as water was given *ad libitum* for at least28 days.

3.11.2 Experimental design

The purchased rats were grouped into three categories inside the metallic enclosure made of bars and were fed with normal feed for 5 days. Group A (control) was fed with casein diet, Group B was fed with experimental diet (soft cheese) and Group C was fed with basal (nitrogen-free) diet. The test and control diets were arranged by mixing the control and experimental diets [soft cheese]. The test and control diet were given to the animals for a 28 days time duration. The time duration is significantly adequate for analytical changes in the test animal (Egounlety *et. al.,* 2002). The animals feaeces were air dried at 105^{o}C for 24h. The rats were slaughter with chloroform, at the end of the test period.Organs for example liver, spleen, kidney and heart were separated and weighed.

3.11.3 Proximate composition of control and experimental diets

Proximate compositions of yoghurt and cheese samples for examplefat, protein**,** moisture contents, fibre as well as ash were carried out using methods of AOAC (2005).

3.11.4 Mean weights of animals (mg/kg body wt)

The initial weight, final weight and the percentage weight gain of the experimental animals were recorded and calculated.

3.11.5 Nutritional evaluation of the experimental diet

Thenet protein utilization (NPU), true digestibility (TD),biological value (BV), andprotein efficiency ratio (PER)were determined as descibed by Egounlety *et. al*. (2002).

3.11.6 Biochemical parameters

Blood Counts

Frozen serum was used for complete blood count in accordance to method described by Davies *et. al.,*(1985).The Mean Corpuscular Hemoglobin (MCH), Corpuscular Volume (MCV),Hemoglobin, Corpuscular Hemoglobin Concentration (MCHC), Neutrophils, Lymphocytes, White Blood Cell (WBC), Red Blood Cell, Packed Cell Volume (PCV) and Platelets were determined on the grouped animals.

3.11.7 Analysis of serum of experimental animals.

Enzymes and biochemical parameters analysis carried out were: the alanine amino transferase (ALT), total protein, aspartate amino transferase (AST),alkaline phosphatase (ALP), creatinine, albumin, urea, with the procedure of Davies *et. al.,*(1985), Osundahunsi and Aworh (2003) and Randox (2008).

3.12 Sensory Evaluation

The degree of likeness of the three yoghurts samples (soursop, cow milk and goat milk)was determined using sensory evaluation according to the methods of Watts *et. al.,* (1989). Twenty semi-trained panelists evaluated the yoghurt samples based upon seven point hedonic scale. Panelists consist of students and staff of the Food Technology Department, University of Ibadan.Yoghurt samples were served cold and panelists were required to taste one product at a time and rinse their mouths with purified drinking water which they had been provided with. They were provided with a piece of sensory form and were asked to score the products on: thickness, mouth feel, colour, aroma, sourness and sweetness on the questionaire where 1 means dislike extremely, 2 means dislike very much, 3 means dislike moderately, 4 means dislike slightly, 5 means neither like nor dislike, 6 means like slightly, 7 means like moderately, 8 means like very much and 9 means like extremely.

3.13 Statistical Analysis

The analyses were determined in three copies and average figures were taken as numbers which were analysed using analysis of variance at $p \leq 0.05$ and average were detached with the use of Tukey's test in SARS statistical package.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 Proximate Compositions and Vitamin C (ascorbic acid) of Soursop Juice, Cow and Goat milks

The vitamin C contentand proximate composition of soursop juice, cow and goat milks the basic raw materials used are in Table 4.1. The results showed the ascorbic acid values of the samples and were considerably varied from one another. Average vitamin C content of soursop juice was 61.8mg/100ml. The result is similar with Worrell *et. al.* 1994, while Abbo *et. al.,* (2006) reported value of 70mg/100g for ascorbic acid content of soursop fruit obtained from the southern parts of Nigeria. Boakye (2013) also reported ascorbic acid content of 62.5mg/100 g for soursop pulp from Ghana. Another reports recount vitamin Ccontent of soursop at 29.6mg/100 g (Waston and Preedy, 2009), 22.6 mg/100 g (Badrie and Schauss, 2009). High value of vitamin C content in soursop fruit signifies the possible usage of this plant food as a high-quality antioxidant.Ascorbic acid content recommended for daily ingestion (RDI) for adults is 30 mg/day and children 17 mg/day (NRC, 1989). Okiei *et. al.,* (2009) reported (36.13mg/100g) for sweetsop ascorbic acid.

The differences in the ascorbic acid results can be accredited to variety of differences and before harvest environmental factors. Lee and Kadar (2000) compared the ascorbic acid content of readily available fruits,watermelon (8.0 mg/100g)and banana (15.3 mg/100g). The underutilised fruits have high ability to complement the everyday Vitamin C needs of consumers when incorporated to foods. Vitamin C has been linked with healing power like defence of teeth and skin,maintenance and the prevention of scurvy. Meanwhile, soursop fruit has ascorbic acid comparable to fruits much-admired as more vitamin C source (Lee and Kadar, 2000). Soursop and soursop products do not have oily glands as recorded by Onyechi *et. al*., (2012) result and this is in line with the findings in this study for soursop juice (Table 4.1) which is indicating that both juice and drink had small danger of rancidity and can give dietarysettlement.

Soursop juice protein value of (5.78%) has been found to be greater than the report for African bush mango juice (0.52%) and *Vitex doniana* (0.8%) (Onimawo, 2002) and the differences in protein values could be attributed to the variations in the fruitsand their botanical make-ups. Abena *et. al.,* (2014) reported that protein content for fruits had values ranging between 2.63% for African mango pulp to 6.71% for African mango.

Proximate compositions of fresh dairy milk are shown in Table 4.1. Results showed that moisture contents of cowmilk, goatmilk and soursop juice were appreciably diverse from one another. Soursop juice moisture content was significantly ($P<0.05$) higher than other

samples. Guler, (2007) reported higher values for goatmilk in pH, total acidity, viscosity, protein, fat and ash compared to cow milk, which are in agreement with the results of this study. The processing of yoghurt somewhat changed the value of protein, fat, ash, total solids and moisture for the dairy products, suggestive of the effect of the native microflora on such constituents. The pH of the fresh milk reduced from 5.98 to 5.71 for goat yoghurt and from 6.31 to 5.60 for cow milk yoghurt.Guler, (2007) stated that the processing method, whey draining, and containers used during processing affects yoghurts components. Tziboula-Clarke, (2003) reported that the major deviation between goats' and cows' milks are associated to the quantity of various types of milk protein, also their distinct structures and sizes of fat particles and protein colloidal aggregates. These variations could cause the milk behaving in a different way during processing which influences the ultimate value of goat's milk dairy products (Vargas *et. al.,* 2008). The unique attributes affect the make up of goat milk leading to its nutrition utilisation elevated than than cow milk. Protein in goat milk is more digestible (Haenlein, 2004) and less allergenic (Park, 1994a).

Protein in cow milk is of high-quality containing a balance diet of all the necessary protein end products. Lots of man staple foods are lacking in certain necessary amino acids, like wheat and maize-based diets having 57% and 58% of mandatory levels of lysine and cassava-based diets are lacking in leucine, valine and isoleucine having 79% of vital levels (WHO. FAO and UNU. 2007). Morand-Fehr *et. al.,* (2004), FAOSTAT 2009 and Orman *et. al.,*(2011) stated that there was a broad increase in goat milk quatity produced. The modern customers consider that a goat dairy product have a high-quality natural representation, does not have fat, are easily digestible, healthy for many gastrointestinal diseases and does not react like cow milk. As a result, goat milk and goat milk products have genuine prospect and profitable attributes.

Table 4.1: Means Value of Proximate Composition and Vitamin C of Fresh Soursop Juice, Cow and Goat milk Samples

Sample	Moisture content %	Protein %	Fat%	Ash%	CHO%	Vitamin C mg/100ml
Soursop Juice	88.55[a]	5.78[b]	0.00[c]	0.34[c]	5.35[c]	61.80[a]
Cow milk	86.36[b]	5.11[c]	2.03[b]	0.72[b]	5.79[b]	0.03[b]
Goat milk	73.32[c]	8.94[a]	9.70[a]	1.02[a]	7.02[a]	0.02[b]

Means in acolumn with the same superscripts are not significantly different from one another ($P<0.05$)

CHO: Carbohydrate

4.2 Proximate Analysis of Yoghurt and Soft Cheese

The proximate compositions of yoghurt and cheese samples are in Table 4.2. Yoghurts samples moisture content varied between55.81% to 91.89% and was significantly ($p<0.05$) different from one another. Moisture values of soursop yoghurt were more than cow milk and goat milk yoghurt samples. Higher percentage of moisture content in the soursop yoghurt could be owing to the high percentage in the moisture content of the soursop juice, (Table 4.1) its ripening stage and the method used in extracting the pulp for juice. However, Boakye *et. al*., (2014) reported that elevated moisture value noticed in the *Annona* species mixture and African mango shows the limited storage life of these perishable fruits and thus, the need for value addition of the fruits to increase their storage-life. The fruits can thus be used for industrial manufacture of jam, jellies and juices. Furthermore, moisture content of cow milk and goat milk yoghurts were relatively higher than cheese's sample because cheese processing involves curd forming and whey removal which allow water removal from the curd, and by that lowering the moisture content of cheese sample.

Crude protein of the yoghurts and cheese samples varied from 0.92% to 13.04% and were appreciably diverse from one another. Thus, protein content of cheese from this study which was 13.04% was lower than 16.94% to 21.84% reported by Tohibu *et. al.,*(2013). Crude protein value of cheese sample was 13.04% when compared to those of goat yoghurt 8.75% and cow yoghurt 5.55% while soursop yoghurt had the least protein content of 0.92% due to the fact that it was from non-dairy source. The high protein content in*Calotropis procera* might have been transferred into the cheese. Cheese samples had the highest fat and carbohydrate contents of all the dairy products examined in this study. Fat content of cheese has been reported by other researchers to have real and actual influence on the physical and sensory characteristics (Marinescu and Pop, 2009) and negative impact on the shelf stability of yoghurts (Saint-Eve, 2008; Farinde *et.al*, 2009). The cells of organs and glands uses fat to supply energy and in the production of some of their glands metabolism (Kathleen *et. al.,* 1996). Dietary value of cheese shows it is of appreciable nutritional status especially with respect to the protein and fat contents (Table 4.2). Cheese and yoghurts are good sources of protein, fat and carbohydrates (The Dairy Council, 2010). The average protein content of probiotic yoghurt from cow milk was 5.5% (Table 4.2). The results were similar to Janhoj *et. al.,* (2006) who stated that protein contents of low – fat stirred yoghurt ranges between 3.4 to 6.0%. The yoghurt preparation to some extent transformed the protein, fat, ash, total solids and moisture levels for goat and cow milk products, suggestive of the effects of native

microflora on these components. Soursop yoghurt increasesugar valueis due to its non-dairy sources. Values reached in this study for crude protein, moisture content, ash and carbohydrates are within the specified range reached by Osundahunsi *et. al*., (2007) for soy-yoghurts. The result of moisture, protein, fat, ash and carbohydrate content of soursop, cowmilk, goatmilk yoghurts and cheese were significantly different ($p<0.05$) from one another.

Soursop yoghurt had Vitamin C content of 16.4mg/100ml while cow and goat yoghurt had 0% Vitamin C. These decreases in Vitamin C contents could be possibly owing to the lipolytic activity of microorganisms on Vitamin C leading in a leakage of some Vitamin C. Viscosities of the yoghurts and cheese samples ranged from 0.18dPs to 1.62dPs respectively. Cow milk yoghurt had the highest viscosity of 1.62dPs, followed by goat milk yoghurt having 1.56dps, cheese having 1.49dPs and soursop yoghurt had the least viscosity of 0.18dPs. The viscosities correlated with the carbohydrate content and total solids of the yoghurts; and these findings is similar to the report of Mahdian and Tehrari (2007) who noted that higher total solids of milk-base improve viscosity of yoghurts.

Table 4.2: Means Values of Proximate Composition of Fresh Yoghurt and Soft Cheese Samples

Samples	Moisture Content%	Crude Protein%	Fat%	Fibre%	Ash%	CHO%	Vit.C (mg/100ml)	Viscosity(dps)
Soursop Yoghurt	91.87[a]	0.92[d]	0.02[d]	0.19[a]	0.55[d]	6.61[c]	16.4[a]	0.18[d]
Cow milk Yoghurt	86.38[b]	5.55[c]	2.06[c]	0.00[b]	0.82[c]	5.18[d]	0[b]	1.62[a]
Goat milk Yoghurt	73.43[c]	8.75[b]	9.90[b]	0.00[b]	1.23[b]	6.69[b]	0[b]	1.56[b]
Soft Cheese	55.81[d]	13.04[a]	16.01[a]	0.00[b]	1.60[a]	13.54[a]	0[b]	1.49[c]

Means with the same superscripts on the same column are not significantly different from one another ($p<0.05$)

4.3 Mineral Compositions of Soursop Juice, Cowmilk and Goatmilk

Table 4.3 shows the mineral compositions of soursop juice, cowmilk and goatmilk samples. Soursop juice comprises a significant amount of important minerals like potassium (840.87mg/100g) being the highest and followed in descending order by magnesium (102.84mg/100g), calcium (67.03mg/100g), sodium (42.30mg/100g), iron (37.37mg/100g) and zinc (20.73mg/100g). Results showed that the mineral compositions of the yoghurts and cheese samples were significantly different from one another ($p<0.05$). Soursop pulp and the seed had ash contents of 0.74% and 1.02% respectively (Table 4.5) which showed that they are high-quality sources of minerals and so can be used to make up for a deficiency to improve the mineral quality of diets. A comparison was reported by Onyechi *et. al*., (2012) betweenthe ash values of soursop pulp (0.92%) and drink (0.95%).

The calcium content of the cow milk (3950.26mg/100g) was significantly,($p<0.05$) different from that of goat milk (3844.98mg/100g). While goatmilk contained significantly, ($p<0.05$) higher amounts of magnesium, potassium, sodium, iron and zinc than cow milk and soursop. Khan *et.al.*, (2006) establish important influence of season and breed on the concentration of almost all the mineral elements in sheep milk. Thus, content of major elements in milk is different significantly from the content in the blood. Magnesium and calcium play a major function in photosynthesis, chemical reactions that occur in carbohydrate, nucleic acids and binding agents of cell walls. Calcium helps in teeth development. Magnesium is ancrucial mineral for enzyme activity and like calcium and chloride; magnesium also plays a role in balancing the acid-alkaline in the body. Phosphorus is needed for bone growth, kidney function and cell growth and plays a role in maintaining the body's acid-alkaline balance (Fallon and Enig, 2001). Minerals contribute to the structures of essential enzymes and the regulation of the activities of some enzymes. Potassium is a necessary nutrient and has an essential part in the synthesis of amino acids and proteins (Malik and Srivastava, 1982).

Sodium is the main cation in the extracellular fluids and is an important control device of osmotic pressure, acid-base balance and cellular membrane potential and it is also important for the active transportation of substances through the cellular membrane and cow milk contribution to daily sodium intake in human nutrition is quite low, but cheese and some dairy products contain additional quantities of salt which can supply considerable sources of sodium.

Cashman, (2002a) stated thatcalcium is accountable for a lot of regulatory functions, for example blood clotting, ordinary cardiac rhythm maintenance, muscle contraction, enzyme activation and hormone secretion). Dairy products like (cheese and yoghurt) are the only means of obtaining calcium. The mainstream of dietary calcium about 70% is from dairy products in milk, casein micelles comprise the natural carrier of calcium (Canabady-Rochellea and Mellemab, 2010). The recommended daily allowance for calcium is not easy to reach without consuming milk and dairy products. Special attention was given to the bioavailability of calcium from milk in the past. The bioavailability of calcium from cheese and yoghurt is equals to the one from the milk and the average calcium absorption from cow milk varies between 21% and 45%. Zinc is very important for sexual development, growth, healing of wounds as well as other physiological processes and normal functioning of the immune system. Zinc is a part of the hormone insulin, assists in the functioning of several other hormones that are important for reproduction and synthesis of DNA, RNA and proteins. Zinc is also a co-factor of many enzymes that are included in most of metabolic processes. Dairy products like cheese, yoghurt and cheese are very essential in human nutrition, but an insufficient source of zinc. The contribution of dairy products in western countries is estimated as the total zinc intake which ranges from 19 to 31 % (Cashman, 2002b).

Table 4.3: Mineral composition of fresh Soursop Juice, Cowmilk and Goatmilk

Samples	Calcium (mg/100g)	Magnesium (mg/100g)	Potassium (mg/100g)	Sodium (mg/100g)	Iron (mg/100g)	Zinc (mg/100g)
Soursop Juice	67.03[c]	102.84[c]	840.87[c]	42.30[c]	37.37[c]	20.73[c]
Cowmilk	3950.26[a]	1822.30[a]	4582.66[b]	5415.88[b]	6.85[b]	26.84[b]
Goatmilk	3844.98[b]	584.23[b]	5103.82[a]	6269.98[a]	11.34[a]	66.75[a]

Means with the different superscripts on the same column are significantly different from one another ($p<0.05$)

4.4 Mineral Compositions of Yoghurts and Soft Cheese

Results showed that the mineral compositions of yoghurt and cheese samples were significantly,($p<0.05$)different from one another. From the study, it can be reported that fermentation causes decrease in the mineral contents (Ca, Mg, K, Na, Fe and Zn) of all the yoghurts and cheese samples whencomparing Table 4.3 and Table 4.4. This was in agreement with the observation of Achinewhu (1983a) who reported that fermentation was found to cause reduction in its mineralcontents (Ca, Mg, K and P) during 'ugba' processing and fermentation however improved the quality of protein and nutritive value of 'ugba'(Achinewhu, 1983b). Miller *et. al*., (2000) stated thatessential mineralsare present in milk products in different levels depending on technological methods in processing the products, the milk-base type used and the method of analysis. In this study, high concentrations of most minerals, especially macro-elements sodium, potassium and calcium were found whereas most micro-elements concentrations have very low as in cheese and yoghurt samples. The calcium and zinc concentrations of cheese sample from this study were 223mg/100g and 1.90mg/100g respectively. Values obtained for calcium and zinc are lower than the values stated by Ahmed (2010) for cheese, whose value varied between 398±16 to 521±13.279mg/100g for calcium and 5.39 to 7.19mg/100g for zinc respectively.However, iron and potassium levels were higher when compared to those reported by Ahmed (2010) for white cheese, whose value ranges between 0.38±0.5 to 0.77±0.4mg/100g for iron and 49.33±1.8mg/100g to 79.00±4.01mg/100g respectively. Generally, the concentrations of micro-elements were very low in most of the cheese samples. The variations of most of the macro-and micro-elements could be attributed to contamination following milking, manufacturing process, animal species and environmental conditions.

The levels of calcium, iron, magnesium, potassium and sodium in the yoghurt were higher in cow, goat and soursop samples; which showed that cow milk, goat milk and soursop fruit are rich in mineral composition. Calcium an abundant mineral in milk and its products and is essential in bone and tooth mineralization, blood clotting, hormone secretion and nerve transmission. Goat milk yoghurt sample has the highest calcium with 640.75mg/100g and soursop yoghurt with the least with 40.05mg/100g (Table 4.4). Goat milk has many benefits than cow milkespecially on human health. Goat milk and goat milk products like cheese and yoghurt have a significant function in human nutrition for the reason that it has higher digestibility and less allergic reactions. Goat milk benefits are accredited to some serum proteins and bio-functional components for example medium-chain triglycerides,

polyunsaturated fatty acids(Haenlein, 2004; Rampilli and Cortellino, 2004). The importance of goat milk and its beneficial effects on bioavailability of copper, zinc, selenium and iron has been documented in journals by (Alferez *et. al.* 2003; Barrionuevo *et. al.,* 2003; Campos *et. al.,* 2004; Haenlein, 2004).

The levels of magnesium (in soursop, cow and goat milk yoghurt) recorded in this study ranged from 25.75 to 31.95mg/100g. The values were all lower than values obtained for all yoghurts analyzed by De la Fuente *et. al*., (2003) which ranged from 101±1-144±7mg/g. Magnesium, a required cofactor for over 300 enzyme systems in the body, is related to calcium and phosphorus in function. Sodium (Na) salt is essential in the body for blood pressure, extracellular fluid volume controland for nutrients transportation in and out of cells. Potassium and sodium are important in essential minerals in human nutrition, although their deficiencies are uncommon since their intake (especially that of sodium) is usually more than recommend values (Miller *et. al*., 2000). Soursop yoghurt had the highest sodium level of 85mg/100g and cheese sample the least with 17mg/100g Table 4.4.

The values of iron (Fe) and (Zn) contents of yoghurts and cheese from this study ranged from 1.2mg/100g to 15.35mg/100g and 0.48mg/100g to 1.9mg/100g respectively. The most abundant minerals in soursop yoghurt are potassium and sodium followed by cow yoghurt and goat yoghurt. Soursop yoghurt (Table 4.4) contains more potassium content of 2457.75mg/100g than values reported by Emma (1995) with 278 mg of potassium and 3.3g of ash in every 100g fruit pulp. Potassium was also found as one of the abundant minerals in some selected vegetables (Rumeza *et. al*., 2006). Potassium content(soursop, cow milk and goat milk yoghurt) was higher than the mean amount of potassium in cow milk yoghurts reported by Guler and Hasan (2008). The minerals (potassium and sodium) in soursop yoghurt were more than those of some commonly consumed plant foods such ascultivated and wild yams (Akindahunsi and Oboh 1999), cassava products (Akindahunsi and Oboh, 2003), edible wild seeds (Oboh and Ekperigin, 2004), and green leafy vegetables (Akindahunsi and Oboh, 1999; Oboh *et. al*., 2005). The potassium quantity shows great inconsistency with the type of the animal, having more values in cow's cheeses and smallest in ewe's cheeses. The ripening of cheese as wellas reduction in potassium could be as a result of whey draining as stated by Cichoscki *et. al.,*(2002).

Table 4.4: Means Values of Mineral Compositions of Fresh Yoghurt and Soft Cheese Samples

Sample	Calcium (mg/100g)	Magnesium (mg/100g)	Potassium (mg/100g)	Sodium (mg/100g)	Iron (mg/100g)	Zinc (mg/100g)
Soursop Yoghurt	40.07^{d}	31.95^{b}	2454.75^{a}	85.00^{a}	12.78^{b}	0.61^{b}
Cow milk Yoghurt	299.63^{b}	25.75^{d}	1344.25^{b}	70.00^{b}	15.35^{a}	0.51^{c}
Goat milk Yoghurt	640.75^{a}	26.40^{c}	869.50^{c}	60.00^{c}	7.85^{c}	0.48^{d}
Soft Cheese	223.00^{c}	37.00^{a}	260.00^{d}	17.00^{d}	1.20^{d}	1.90^{a}

Means with different superscripts on the same column are significantly different from one another ($p<0.05$)

4.5 Proximate Compositions of Soursop Seed and Pulp of Soursop Fruit

The moisture content of the pulp of soursop fruit indicated that the fruit had very high moisture content (87.23%) when compared with the seed (5.61%). The moisture content of soursop fruit compared with the results for the pulp of soursop fruit reported by Ifeoma *et. al*., (2004)who reportedthe moisture content of fruit pulp as 75%. The pulp of *Annona* species (soursop and sweetsop) high moisture contents, reflect the partial storage life of these climacteric fruits and there is need to add value to the fruits in other toincrease their shelf-life. Appiah *et. al.,*(2011) stated that moisture content of foods gives an indication of the available dry matter and plays a main role in determining the propensity of the food to spoil.

Crude protein contents of soursop seed and pulp was 7.07% and 2.40% respectively. The protein content (2.40%) of soursop pulp from this study is similar with the findings of Ifeoma*et. al.,*(2004) who reported a protein content of 2.9% for ripe soursop fruit pulp and more than the result of Ajiboye *et. al.,* (2014), who reported 1.43% for protein content of fresh soursop fruit. Though, fruits are usually not regarded as protein sources, these suggest the prospective for the fruit studied to contribute to protein needs of consumers, especially the rural-poor dwellers. Leakay *et. al.,* (2005) and Ainge and Brown (2001) reported that plant fats make up unsaturated fatty acids and usually lack the much trans-fats. Therefore, the considerate fat contents obtained in the fruits can not necessarily cause any health threats to consumers but rather serve as healthy fat sources for optimum health.The ash content for soursop seed and pulp was 1.02% and 0.74% respectively andthese values are however similar to the 0.5% for African bush mango (Okigbo,2001) but different fromthe ash content 13.6% for theseed of soursop fruits reported by Onimawo(2002). The crude ash gives an appropriate measure of the total mineral composition of foods, thus, the relatively high ash values of fruits examined in this studycan contributenutrients useful for the total health of consumers.

Table 4.5: The Proximate Analysis of Seed and Pulp of Soursop Fruit (g/100g)

Samples	Moisture Content%	Crude Protein%	Fat%	Fibre%	Ash%	Carbohydrate%
Seed	5.61[b]	7.07[a]	7.71[a]	18.41[a]	1.02[a]	60.33[a]
Pulp	87.23[a]	2.40[b]	2.52[b]	3.94[b]	0.74[b]	3.17[b]

Means in acolumn with the same superscripts are not significantly different from one another ($P<0.05$)

4.6 Microbiological Analysis of Soursop, Cow milk, Goat milk Yoghurts and Soft Cheese

Table 4.6 shows the results of total plate and yeast counts of yoghurts and soft cheese samples. The initial total plate count (TPC) of microorganisms in the soursop yoghurt was $3.0x10^7$cfu/ml and later decreased to $2.1x10^7$cfu/ml at 14^{th} day storage at (4^0C). There was a slight decrease in microbial count in the soursop yoghurt while the storage time increases owing to the storage environment which inhibited the activity of the microorganisms to survive in the yoghurt sample. Tamine and Robinson (1985) reported that decrease in total plate count can be accredited to the decline in viability of the LAB. Also, Oberman (1985) found 2.0 x 10^8cfu/g as the total count of lactic acid bacteria which contributed to good quality of yoghurt. The Codex alimentarius standard for yoghurt is a minimum of 10^7cfu/ml (LAB) in the finished product; and values for yoghurt obtained in this present study (Table 4.6) are in agreement with Codex Stardard.Hartati and Eka (2010) produced soursop yoghurt (probiotics fermented milk) using various stabilisers and reported 3.08 to 5.5 x 10^9cfu/g as the total plate count.

In this study, the total plate counts of cow milk yoghurt decreased from $5.2x10^5$cfu/ml to $4.2x10^5$ cfu/ml, while there were few yeasts (less than 100 organisms) on the14^{th} day storage (Table 4.6). Obi *et. al., (*2010) reported no growth of yeasts, moulds and coliform in skim and whole milk probiotic yoghurt during 35^{th} day storage period, which can be accredited to the elevated sterile conditions obtainable in the laboratory that prohibited contamination after production. Also, total plate count of goat milk yoghurt decreased from $4.5x10^6$cfu/ml to $6.2x10^5$cfu/ml and the total plate count of cheese decreased from $4.6x10^7$cfu/ml to $4.2x10^6$cfu/ml. Decreases in total plate counts can be credited to the reduction in viability of microorganism in the samples. According to Tamime (2005) as cited in Seelee *et. al.,* (2009) for the period of fermentation, *Streptococcuss thermophilus* produces formic acid and lactic acid which turn on the development of *Lactobacillus bulgaricus*producing diacetyl and acetaldehyde which are responsible for the typical yoghurt flavour.

Table 4.6: Microbiological Analysis of Soursop, Cow milk, Goat milk Yoghurts and Soft Cheese during 14 days Storage Period at (4^0C).

Product	Storage (days)	Total Plate Count (cfu/ml)	Yeast Count (cfu/ml)
Soursop Yoghurt	0	$3.0x10^7$	$3.2x10^4$
	7	$2.6x10^7$	$3.6x10^3$
	14	$2.1x10^7$	$2.7x10^3$
Cow milk Yoghurt	0	$5.2x10^5$	nil
	7	$4.8x10^5$	$<1x10^2$
	14	$4.2x10^5$	$<1x10^2$
Goat milk Yoghurt	0	$4.5x10^6$	$4.9x10^2$
	7	$3.8x10^6$	$4.2x10^2$
	14	$6.2x10^5$	$3.6x10^2$
Soft Cheese	0	$4.6x10^7$	$3.8x10^4$
	7	$4.7x10^6$	$4.2x10^3$
	14	$4.2x10^6$	$3.7x10^3$

Means of duplicate determinations

4.7 Storage Studies

Physico-chemical and Microbial Characteristics

The pH values of stored yoghurt decreased as the period of storage increased.pH of soursop yoghurt varied significantly ($p<0.05$) with one another with storage time. pH of soursop yoghurt treated with *Aframomum danielli* ranged between 4.32 and 3.23. There was no significant ($p<0.05$) changes in pH values of soursop yoghurt treated with *Aframomum danielli* at 2.0% and 2.5% with storage at refrigerated temperature (4^0C). The decrease in the pH of samples of probiotic yoghurt could be attributed to the metabolic activitiesof the lactic acid bacteria in the yoghurt culture and this is in line withthe findings of Shah (2000) who stated similar decrease in pH values throughout the storage of commercial yoghurts having*Lactobacillus acidophilus* and *Bifidobacterium bifidum.*Analysis showed that the pH of soursop yoghurt was considerablyaffected by different types, concentrations of the plant materials and the interactions between them (Table 4.7a). Turkey's B test which determines the level of significance showed that treatment from *Aframomum danielli* with concentrations 1.0% and 1.5% were not significantly($p<0.05$) different from concentrations 1.0%, 1.5%, 2.0% and 2.5% turmeric.

There were no major changes in total soluble solids of yoghurt samples treated with *Aframomumdanielli* at 1.0%, 1.5% and 2.0% concentrations but the treatments were significantly different ($p<0.05$) from one another (Table 4.7a).These activities may be due to the preservative effects of *Aframomum danielli* which had been reported with cashew juice by Ogunwolu and Adio (2003) and in the maintenance of quality cut of apple slices by Adegoke *et. al.,*(2002) and all these properties could be credited to maintenance of soluble solids and pH in samples treated with *Aframomum danielli*.There were no major changes in the total soluble sugar content of the yoghurts treated with turmeric at 1.0% and 1.5%, while total soluble solids of the treated samples were significantly($p<0.05$) different fromthe control sample.

There was major increase in total titratable acidity of soursop yoghurt with increase in storage time (Table 4.8a). This result is similar with findings of Tarakci and Erdogan (2003) that the acidity of soursop yoghurt improved over the storage period. The total titratable acidity of the soursop yoghurt sample treated with *Aframomum danielli* increased from 0.54% to 1.13% while the sample treated with turmeric increased from 0.54% to 1.10% respectively. The lactic acid content of soursop yoghurt ranges between 0.53% to 1.13%, meaning it was in line with the desired levels of lactic acid content in yoghurt. According to yoghurts standard, lactic acid content specification ranges between 0.5% - 2% (SNI 1992). Statistical analysis showed that the soursop yoghurt treated with *Aframomum danielli* at 1.5% and 2.0% were significantly different varies ($p<0.05$) from concentrations 1.5% and 2.0% of yoghurt treated with turmeric.

Table 4.7a shows the effects of the treatment concentrations on the proximate compositions of soursop yoghurt. The concentrationsof *Aframomum danielli* and turmeric gave protein contents of soursop yoghurt which ranged from 0.66% to 0.80% and 0.66% to 0.78%. The results showed that protein content of soursop yoghurt slightly decreased with storage time but significantly decreased ($p>0.05$) in the control sample. However, the protein value in soursop juice which was initially 5.78% was reduced to 0.92% after processing into yoghurts and was further reduced with storage time. These changes may be due to propagation of bacteria with the resident lactic acid bacteria which might lead to extreme utilisation of proteolytic products more than they were produced, therefore the reduction in nitrogen-containing compounds in the products(Abdalla and Mohamed, 2009). Analysis showed that the protein contents of soursop yoghurt were considerably affected by different concentrations in plant materials and the interactions between them. Turkey's test to further resolve the level of importance showed that the protein content of soursop yoghurt treated with *Aframomum danielli* at 1.5%, 2.0% and 2.5% respectively were statistically the same and there was no significance difference with other turmeric concentrations.

The fat contents of soursop yoghurt of all the different treatments were not significantly different ($p<0.05$) from one another (Table 4.7a). Fat stands for the original fat and other materials like phospholipids, sterols, essential oils and fat soluble pigments in foods. Normally, fruits have small amount of fat,thus they do not contain more plant sugar for energy. However, the fat content of soursop yoghurt from this study was very small which later significantly decreased with storage time. Leakay *et. al*. (2005) and Ainge and Brown

(2001) noted that plant fats primarily consist of unsaturated fatty acids and usually deficientthe good deal of trans-fats. Kathleen *et. al.,*(1996) also stated that fats utilise by organs and glands cells give larger energy in the cell metabolism. The low level of fat in the fruits means that fruits are not good sources of energy (Boakye, 2013) and can be recommended for losing weight or weight maintenance, to supply nutrients and lowers blood pressure (Basu and Panugonda, 2009; Asgary *et. al.,* 2014). Statistical analysis revealed that with soursop yoghurt, the concentrations of the treatments and their interactions were not significantly different from one another ($p<0.05$).

Table 4.8a shows the effect of *A.danielli* and turmeric on vitamin c content (mg/100ml) of soursop yoghurt for the period of storage. This study has shown that soursop fruit is rich in vitamin C (61.8 mg/100ml) and this figure is close to Abbo *et. al*., (2006) who stated 70mg/100ml for ascorbic acid while Boakye (2013) found that soursop fruit had ascorbic acid content of 63.67mg/100ml and sweetsop 20.33mg/100ml respectively. Different treatments reduced ascorbic acid content of soursop yoghurt from 9.65mg/100ml to 5.77mg/100ml with storage time and these values were significantly different ($p<0.05$) from one another. While the control sample vitamin C reduced significantly ($p<0.05$) with storage period, *Aframomum danielli* had a preservative action on vitamin C which caused a slight reduction in values of yoghurt sample with storage time (Table 4.8a). Vitamin C is used as an indication of high-quality of fruit and vegetable, since the nutrient is very responsive to various modes of deprivation in food processing and following storage (Ozkan *et. al.,* 2004). Soursop fruit can be used in treating vitamin C deficiency-relating ailments because of its high vitamin C content. With soursop yoghurt, concentrations of treatment and interactions between them appreciably ($p<0.05$) affected the vitamin C contents of yoghurt.

The total plate counts of soursop yoghurt treated with *Aframomum danielli* ranges from 5.1×10^8 to 1.4×10^7 (cfu/ml). This observation may be due to the antimicrobial broad spectrum of the spice *Aframomum danielli* (Adegoke and Skura 1994) on spoilage organisms. Samples treated with turmeric also had count reductions of $5.1x10^8$ to $1.7x10^7$ (cfu/ml) (Table 4.7b). Turmeric, ayellow-pigmented compound, is widely used to colour manyfoods,cereal foods, and meat and fish products as well as has antibacterial activity (Rai *et. al*., 2008).

The decrease in total plate count towards the end of the storage period could be attributed to the reduction in viability of lactic acid bacteria (Tamine and Robinson, 1985). Though, there were major increases in microbial counts in all the control samples in different

treatments. In samples treated with*Aframomum danielli* and turmeric, yeast counts reduced as storage time increased while yeast counts increased in the control sample as shown in Table 4.8b. Soursop fruit is susceptible to insect attack when overripe andthis may predispose it to microbial infection resulting in high yeast counts. Decrease in amount of yeasts in *Aframomum danielli* treated soursop yoghurt found in this research can be accredited to the antimicrobial property of the spice as reported by Adegoke and Skura (1994); Fasoyiro *et. al*., 2001 and Ogunwolu and Adio, (2003).

Table 4.7a: Effects of Concentrations of *A. danielli* and Turmeric on the Chemical Composition of Soursop Yoghurt

Soursop yoghurt	Treatment	Conc.	pH	Total Solube Solids (^{0}brix)	Total Titratable Acidity (%lactic acid)	Protein (%)	Fat (%)	Vitamin C (mg/100g)
	Aframomm danielli	0 %	3.2^{h}	3.03^{h}	0.98^{a}	0.66^{a}	0.02^{a}	5.98^{g}
		1.0%	3.7^{b}	3.90^{b}	0.96^{b}	0.80^{a}	0.02^{a}	7.09^{c}
		1.5 %	3.7^{b}	3.91^{b}	0.94^{c}	0.79^{a}	0.02^{b}	7.08^{d}
		2.0%	3.8^{a}	3.93^{b}	0.94^{c}	0.79^{a}	0.02^{a}	7.11^{a}
		2.5%	3.8^{a}	3.97^{a}	0.89^{g}	0.79^{a}	0.02^{ab}	7.11^{a}
	Turmeric	0 %	3.2^{h}	3.03^{h}	0.98^{a}	0.66^{a}	0.02^{a}	5.98^{g}
		1.0%	3.7^{b}	3.57^{e}	0.93^{de}	0.77^{a}	0.02^{a}	7.09^{c}
		1.5%	3.7^{b}	3.56^{e}	0.93^{d}	0.78^{a}	0.02^{a}	7.09^{c}
		2.0%	3.7^{b}	3.66^{c}	0.93^{de}	0.78^{a}	0.02^{a}	7.09^{c}
		2.5%	3.7^{b}	3.61^{c}	0.89^{f}	0.78^{a}	0.02^{ab}	7.10^{b}

Means in acolumn with the same superscripts are not significantly different from one another ($P<0.05$)

Table 4.7b: Microbiological Effects of Concentrations of *Aframomum danielli* and Turmeric on Soursop Yoghurt

Treatment	Conc.	Total Plate Count (cfu/ml)	Yeast Count (cfu/ml)
Aframomm danielli	0 %	$5.1x10^{8a}$	$4.5x10^{7a}$
	1.0%	$1.6x10^{7de}$	$2.3x10^{4cd}$
	1.5 %	$1.7x10^{7d}$	$2.3x10^{4cd}$
	2.0%	$1.5x10^{7f}$	$2.2x10^{4e}$
	2.5%	$1.5x10^{7f}$	$2.2x10^{4e}$
Turmeric	0 %	$5.1x10^{8a}$	$4.5x10^{7a}$
	1.0%	$1.6x10^{7de}$	$2.4x10^{4c}$
	1.5%	$1.6x10^{7de}$	$2.4x10^{4c}$
	2.0%	$1.7x10^{7d}$	$2.4x10^{4c}$
	2.5%	$1.7x10^{7d}$	$2.3x10^{4cd}$

Means with the same superscripts on the same column are not significantly different from one another ($p<0.05$)

Table 4.8a: Effects of*A. danielli* and Turmeric on the Chemical Compositions of Soursop Yoghurt with Storage Time

Week	P^H	Total Soluble Solids (^{0}brix)	Total Titratable Acidity (%lactic acid)	Protein (%)	Fat (%)	Vit. C (mg/100g)
0	4.3^a	4.17^a	0.53^g	0.92^a	0.02^a	9.65^a
2	4.1^b	3.69^b	0.74^f	0.89^a	0.02^a	7.48^b
4	3.8^c	3.58^c	0.91^e	0.78^a	0.02^a	6.89^c
6	3.6^d	3.43^d	0.98^d	0.75^a	0.02^a	6.61^d
8	3.3^e	3.39^e	1.03^c	0.70^a	0.02^a	6.20^e
10	3.1^f	3.32^f	1.07^b	0.62^a	0.02^a	5.75^f
12	3.1^g	3.27^g	1.10^a	0.53^a	0.02^a	5.47^g

Means in the same column with the same superscripts are not significantly different from one another ($p<0.05$).

Table 4.8b: Microbiological Effects of*A. danielli* and Turmeric on Soursop Yoghurt with Storage Time

Week	Total Plate Count (cfu/ml)	Yeast Count (cfu/ml)
0	$3.8x10^{7a}$	$3.2x10^{6g}$
2	$3.6x10^{7a}$	$3.0x10^{6f}$
4	$3.6x10^{7b}$	$2.4x10^{5e}$
6	$1.8x10^{7c}$	$3.0x10^{5c}$
8	$1.4x10^{7d}$	$2.9x10^{5d}$
10	$1.4x10^{7d}$	$2.7x10^{4b}$
12	$1.4x10^{7d}$	$2.5x10^{4a}$

Means in the same column with the same superscripts are not appreciably diverse from one another ($p<0.05$).

pHvalues of cow milk yoghurts treated with *Aframomum danielli* and turmeric extract Table 4.9a. The decreases in the pH values of yoghurt samples could be attributed to the metabolic activities of lactic acid bacteria in the yoghurt culture and this is similar with the findings of Shah (2000) which stated similar reduction in pH values during the commercial yoghurts storage period containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum.*Also, Younus *et. al.,*(2002) stated pH values of 4.35± 0.03 and 4.57± 0.03, and El Bakri and El Zubeir, (2009) noted to have a mean pH value of 4.62. Statistical analysis showed the variations in the pHvalues of cowmilk yoghurt, the concentrations in the treatments and the interactions between them. It also showed that the groups were different appreciably from one another. There was important difference in total soluble sugar of cow milk yoghurt treated with *Aframomum danielli* and turmeric which decreased with storage time (Table 4.10a). Values for soluble solids of cow milk yoghurts treated with *Aframomum danielli* and turmeric ranged between 14.64 and 14.47^0brix while the control sample was 11.10^0brix which showed thatthere were substrates available for yeast fermentation and eventual spoilage of the product in the event of improper storage.The titratable acidity (TTA) of cow milk yoghurts with treatments shows a significant increase ($p<0.5$) (Table 4.10a). Titratable acidity values for *Aframomum danielli*-treated yoghurt and turmeric-treated yoghurt are presentrd on (Table 4.9a). The increase in TTA can be credited to the activity of lactic acid bacteria which converts lactose to lactic acid. The acidity of yoghurts also affects the overall flavour of the products as reported (Barnes *et. al.,*1991). Kurman and Rasic (1991) recommended an activity level of (0.78 to 0.85%) lactic acid for yoghurt, Tarakci and Erdogan (2003) reported a high TTA value of 1.27-1.36% for yoghurt sample. The protein contents in cow milk yoghurt ranged from 5.55 to 4.33% with respect to storage period (Table 4.10a). Istikhar *et. al.,* (2009) stated mean protein content of 5.4% and 5.5% for probiotic and natural yoghurts respectively. The protein level in this study agrees with the findings of (Janhoj *et. al.,* 2006) which noted that protein values of low-fat stirred yoghurt ranges between 3.4 to 6.0%. Adolfsson *et. al*., (2004) reported that the protein values of commercial yoghurt is generally higher than that of fresh milk due to the addition of non fat dry milk. Law and Haandrikman (1995) reported that increase in protein content of yoghurt depends on the proteolytic activity of lactic acid bacteria which hydrolyses proteins into peptides and amino acids, thus the proteolytic system of lactic acid bacteria is essential for their growth in milk.

Possibly, the result of hydrolysis of protein under the influence of proteolytic enzymes is because of the free amino acid present in yoghurt. Therefore, these free amino acids combined to form the peptide bonds that will transform into protein during the storage period.

There was no important disparity between fat contents of cowmilk yoghurt treated with *Aframomum danielli* and turmeric as average fat contents of cow milk yoghurt obtained in this study ranged from 2.06 to 1.79% (Table 4.10a). This agrees with the result of Janhoj *et. al.,* (2006) that fat contents for low-fat stirred yoghurt ranges between 0.3 to 3.5%. Mutlu *et. al.,* (2005) stated 3.1% for fat content of bio-yoghurt made from goat's milk. El Bakri and El Zubier (2009) reported 2.75 to 3.82% values for fat content while Younus *et. al.,* (2002) results ranged from 2.9 to 3.50% for fat content of stirred yoghurt samples.

Total plate counts of cow milk yoghurt preserved with *Aframomum danielli* and Turmeric are in Table 4.10b. Initial total viable count ofmicroorganisms was between 5.0 to $5.2x10^5$. There was a significant reduction in total count of yoghurt treated with *A.danielli* with storage time but there was increase in total count of microorganisms in the control sample. Total plate counts for samples during the initial 14 days of storage were within acceptable standard of $<1x\ 10^6$cfu/ml Lourens-Hattingh and Viljoen, (2001); El Bakri and Zubeir, (2009) reported $<1x10^6$ cfu/ml as the yoghurt acceptable microbial standard for yoghurt Microbial count increased with increase in storage time in all control samples. While yeast counts reduce in the treated samples, control sample supported the propagation of yeast cells (Table 4.10). The industrial exploitation of LAB has also attracted considerable attention owing to the potential for production of lactic acid and bacteriocins, which can act as natural food preservatives against spoilage and pathogenic micro-organisms. *Lactobacillus acidophilus* is used mainly for yoghurt because it possesses dominant *lactobacillus* in the intestine. However, a variety of *Lactobacillus spp* have been used in probiotics preparations for example *Lactobacillus delbreuckii subsp. bulgaricus, Lactobacillus lactis, Lactobacillus fermentus, Lactobacillus plantarum, and Lactobacillus reuteri*(Vinderola *et. al.,* 2002).

(Vinderola *et al*., 2000; Sodini *et. al*., 2002 and Li *et., al.,* 2006) reported *Lactobacillus acidophilus* for laboratory scale studies. *Lactobacillus acidophilus* in addition with starter cultures incorporated into milk before fermentation allows propagation of*Lactobacillus. acidophilus*to some extent in milk which improves the initial number after processing and assists its adaptation to the product environment which will help its survivability during storage (Tamime, 2005). The relative viability of the organisms being used depends on the storage conditions of each bacterial type (Mattila-Sandholm *et. al*., 2002). The symbiotic relationship between yoghurt starter cultures *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* has been well established: *Lactobacillus delbrueckii* subsp. *bulgaricus* possesses proteolytic enzymes, oligopeptides and free amino acids which can be used as a nitrogen source for *Streptococcus thermophilus* during fermentation (Shihata, 2000). Conversely,*Streptococcus thermophilus* produces substances which stimulate the growth of *Lactobacillus delbrueckii* subsp. *bulgaricus*, including formic acid, pyruvate and CO_2 (Tamime and Robinson, 1999b). In addition, yoghurt manufacture by these two bacteria has good texture quality and flavour development (FDA, 2005).

Table 4.9a: Effects of Concentrations of *A. danielli* and Turmeric on the Chemical Composition of Cowmilk Yoghurt

Treatment	Conc.	pH	Total Soluble Solids (^{0}brix)	Total Titratable Acidity (% lactic)	Protein (%)	Fat (%)
Aframomum danielli	0%	3.9^{f}	11.10^{e}	1.02^{a}	5.57^{b}	1.96^{b}
	1.0%	4.2^{c}	14.64^{a}	1.00^{d}	5.58^{a}	1.98^{a}
	1.5%	4.2^{c}	14.61^{a}	1.00^{cd}	5.58^{a}	1.98^{a}
	2.0%	4.2^{c}	14.50^{bcd}	1.00^{c}	5.58^{a}	1.98^{a}
	2.5%	4.2^{c}	14.51^{bc}	1.01^{b}	5.58^{a}	1.98^{a}
Turmeric	0%	3.9^{f}	11.10^{e}	1.02^{a}	5.57^{b}	1.96^{b}
	1.0%	4.3^{a}	14.53^{b}	0.99^{e}	5.58^{a}	1.98^{a}
	1.5%	4.3^{a}	14.49^{cd}	0.99^{e}	5.58^{a}	1.98^{a}
	2.0%	4.3^{ab}	14.49^{cd}	1.00^{cd}	5.58^{a}	1.98^{a}
	2.5%	4.3^{ab}	14.47^{d}	1.00^{c}	5.58^{a}	1.98^{a}

Means with the same superscripts in the same column are not significantly different from one another ($p<0.05$)

Table 4.9b: Microbiological Effects of Concentrations of *A. danielli* and Turmeric on Cow milk Yoghurt

Treatment	Conc.	Total Plate Count (cfu/ml)	Yeast Count (cfu/ml)
Aframomum danielli	0%	$3.9x10^{7a}$	$1.0x10^{6a}$
	1.0%	$2.3x10^{6d}$	$8.8x10^{2b}$
	1.5%	$2.3x10^{6d}$	$8.0x10^{2d}$
	2.0%	$2.3x10^{6d}$	$7.9x10^{2de}$
	2.5%	$2.3x10^{6d}$	$7.9x10^{2de}$
Turmeric	0%	$3.9x10^{7a}$	$1.0x10^{6a}$
	1.0%	$2.3x10^{6d}$	$8.2x10^{2c}$
	1.5%	$2.3x10^{6d}$	$8.2x10^{2c}$
	2.0%	$2.3x10^{6d}$	$8.2x10^{2c}$

Means with the same superscripts on the same column are not significantly different from one another ($p<0.05$)

Table 4.10a: Effects of*A. danielli* and Turmeric on the Chemical Composition of Cowmilk Yoghurt with Storage Time

Week	pH	Total Soluble Solids (^{0}brix)	Total Titratable Acidity (% lactic)	Protein (%)	Fat (%)
0	4.30^a	14.83^a	0.64^g	5.55^f	2.06^a
2	4.22^b	13.30^b	0.74^f	5.56^e	2.06^a
4	4.19^c	12.44^c	0.92^e	5.57^d	2.05^b
6	4.14^d	12.04^d	1.01^d	5.58^c	1.98^c
8	4.02^e	11.86^e	1.07^c	5.58^c	1.92^d
10	3.91^f	11.75^f	1.22^b	5.60^b	1.89^e
12	3.80^g	11.49^g	1.28^a	5.61^a	1.84^f

Means with the same superscripts on the same column are not significantly different from one another ($p<0.05$)

Table 4.10b: Microbiological Effect of *A. danielli* and Turmeric on Cowmilk Yoghurt with Storage Time

Week	Total Plate Count $X10^3$ (cfu/ml)	Yeast Count (cfu/ml)
0	$9.6x10^{7f}$	$5.0x10^{5g}$
2	$8.7x10^{7e}$	$4.1x10^{5f}$
4	$5.7x10^{5d}$	$3.9x10^{5e}$
6	$5.5x10^{5c}$	$4.6x10^{4d}$
8	$5.3x10^{5b}$	$3.8x10^{4c}$
10	$5.0x10^{5a}$	$1.5x10^{4b}$
12	$4.9x10^{5b}$	$1.4x10^{4a}$

Means with the same superscripts on the same column are not appreciably different from one another ($p<0.05$)

The results in the changes of pH during storage period in goat milk yoghurts treated with *Aframomum danielli* and turmeric are in Table 4.11a. The decrease in pH of the control sample was more drastic due to increased acidification of the product by more prolific development of yoghurt culture bacteria. A reduction of pH can be seen all through the storage period and these could be owing to the increase of bacteria that converted milk sugar to lactic acids and decrease in the pH levels reflected in increased acidity of the products which resulted in the sour taste of yoghurt. Seelee *et. al.,* (2009) stated that goat milk mixed with 3% skim milk powder had a slight reduction in pH at 4^0C within three weeks storage time. Bozanic *et. al.,* (1998) described quicker acidification and lesser pH values in goat milk yoghurt. The enrichment of the microbial growth, acidity advancement and peptidase action of lactic acid bacteria in goats' milk are the variousbehaviour explained by Tamine and Robinson, (1999a).There was no major disparity ($p>0.05$) total soluble solids in goat milk yoghurt preserved with *Aframomum danielli* and Turmeric but was appreciably varied ($p<0.05$) from the control sample with storage time Table 4.11a. The soluble solids of goat milk yoghurtdecreased from 13.10 to 10.24^0brix with storage time (Table 4.12a). The soluble solids (^{0}brix) decreased in all samples but the control sample significantly showed lower readings than the treated samples at the closing stage of storage duration. With respect to the total soluble solids of goat milk yoghurt, statistical results revealed that there was no important difference between samples treated with *Aframomum danielli* and turmeric (Table 4.11).

Titratable acidity increased in all samples of goat milk yoghurt all through the storage duration. The TTA value ranges between 0.81 to 1.09% (Table 4.12a). When the storage period terminated, allyoghurt samples including control sample had titratable acidity within the range of a good finish product (0.85-0.90%) as reported by Jay (2000). There was no noteworthy variation ($p>0.05$) in the total titratable acidity of goat milk yoghurt preserved with *Aframomum danielli* and turmeric but was notably changes ($p<0.05$) from the control sample through storage time. Tarakci and Kucukoner (2003) reported a high TTA value for 1.27% - 1.36% for yoghurt.

There was no important variation ($p>0.05$) in the protein content of goat milk yoghurt treated with *Aframomum danelli* and turmeric. The protein content of goat milk yoghurt ranged from 8.75% starting from the day oneto 8.81% until storage period terminated Table 4.12a. Thomas and Mills, (1981) reported that the increament in protein content in yoghurt rely on the

proteolytic activity of lactic acid bacteria, which hydrolyses proteins (caseins) into peptides and amino acids. Janhoj *et. al.,* (2006) showed that low fat stirred yoghurt protein contents ranges from 3.4 to 5.6% for cow milk yoghurt.

Jenness (1980) reported that the fat content of goat milk (4.1%) changes in agreement to the animal type and there was a higher rate of digestion of goat milk compared to cow milk owing to the smaller size of the fat globules, which have a huge surface area and lipase in the gut which is liable to attack lipids more rapidly. Increase in fat levelof goat milk was due to acidic pH. The fat levels of goat milk yoghurt were similar with the outcome of Mutlu *et. al.,* (2005) who stated that bio-yoghurtfat contents ranges from 3.1 to 4.5% throughout storage for cow milk yoghurt. However, Abrahamsen and Holmen (1981), Tamime and Muir (1998) showed that goat milk was specific by high variables in its proportion during lactation period. Analysis showed that with different concentrations, goatmilk yoghurt treated with *Aframomum danielli* was not considerably different ($p>0.05$) from turmeric and use of *Aframomum danielli* at 1.5%, 2.0% and 2.5% were not appreciably different ($p>0.05$) from one another (Table 4.11a).

Results of total plate count (TPC) of goat milk yoghurt during the storage period are accessible in Table 4.12b. Initial total plate count of viable organisms was $4.5x10^6$cfu/ml. There was a reduction in total count of yoghurt treated with *A. danielli* and turmeric spice during storage period. This may be as a result of retarding of growth of microorganisms due to acid production. The microbial counts increased with increasing storage time in all control samples (Table 4.11b).

Yeasts cause major deterioration in dairy and fermented milks where low pH gives a careful condition for their growth. The conditions required by which to make yeast a natural part of the microbiota of dairy products are the nutritional requirement of yeast, the rate to grow at lower temperature, lower pH, lower moisture content, and higher salt or sugar concentrations together with their enzymatic activity(Hansen and Jakobsen, 2004).

Mould and yeast counts reduce as the storage time increased in treated sample (Table 4.12b). The initial total yeast count of $5.3x10^4$cfu/ml was obtained in *A. danielli* and turmeric spice. There significance increase in the yeast counts in the control yoghurt sample throughout the storage period. In this research, elevated proportion of preservatives lowered yeast counts with increase storage time as in roselle juice treated *A. danielli*which could be credited to

antimicrobial effect of *A. danielli* as reported by Fasoyiro *et al.,*(2001) and Ogunwolu and Adio (2003). Shisodia *et. al.,* (2005) reported that curcumin is a yellow colour extract from the root of turmeric. The ability or tendency of destroying microbes applied by the LAB is mostly owing to its actions in producing compounds of organic acids (Daeschel, 1989). Hutt *et. al*., (2006) had also stated that the *Lactobacillus* strains have middle effectiveness for out-competing cystitis-causing *Escherichia coli* from the large intestine. Nevertheless, *Lactobacillus acidophilus* shows a tough (26mm zone of inhibition) ability against bacteria*E. coli*-E6, and articulated a region of growth inhibition of 25mm against *E. coli* -E5. Goderska and Czarnecki (2007) recounted that *Lactobacillus acidophilus* yield a growth inhibition zone of 26mm against *Helicobacter pylori* and *Esherichia coli*. whileMillette *et. al*., (2007) described no retardation against gram negative bacteria following 8 h of fermentation by a combination of *Lactobacillus acidophilus* and *Lactobacillus casei.*

Table 4.11a: Effectsof Concentrations of *A. danielli* and Turmeric on the Chemical Composition of Goat milk Yoghurt

Treatment	Conc.	pH	Total Soluble Solids (^{0}brix)	Total Titratable Acidity (% lactic acid)	Protein (%)	Fat (%)
Aframomum	0%	3.6^{c}	10.17^{c}	0.85^{f}	8.77^{b}	11.23^{a}
danielli	1.0%	3.9^{a}	12.83^{b}	0.98^{ab}	8.78^{a}	11.00^{a}
	1.5%	3.9^{a}	12.90^{a}	0.98^{ab}	8.78^{a}	11.43^{a}
	2.0%	3.9^{a}	12.90^{a}	0.98^{a}	8.78^{a}	11.43^{a}
	2.5%	3.9^{a}	12.90^{a}	0.98^{a}	8.78^{a}	11.43^{a}
Turmeric	0%	3.6^{c}	10.17^{c}	0.85^{f}	8.77^{b}	11.23^{a}
	1.0%	3.9^{a}	12.90^{a}	0.97^{b}	8.78^{a}	11.00^{a}
	1.5%	3.9^{a}	12.89^{a}	0.97^{b}	8.78^{a}	11.00^{a}
	2.0%	3.9^{a}	12.89^{a}	0.97^{b}	8.78^{a}	11.00^{a}
	2.5%	3.9^{a}	12.89^{a}	0.97^{b}	8.78^{a}	11.00^{a}

Means with the same superscripts on the same column are not considerably vary from one another ($p<0.05$)

Table 4.11b: Microbiological Effects of Concentrations of *A. danielli* and Turmeric on Goat milk Yoghurt

Treatment	Conc.	Total Plate Count (cfu/ml)	Yeast Count (cfu/ml)
Aframomum	0 conc.	$1.5x10^{6a}$	$5.5x10^{4a}$
danielli	1.0 conc.	$1.4x10^{6b}$	$3.3x10^{2c}$
	1.5 conc.	$1.3x10^{6c}$	$3.2x10^{2c}$
	2.0 conc.	$1.3x10^{6c}$	$3.2x10^{2c}$
	2.5 conc.	$1.3x10^{6c}$	$3.2x10^{2c}$
Turmeric	0 conc.	$1.5x10^{6a}$	$5.5x10^{4a}$
	1.0 conc.	$1.4x10^{6b}$	$5.3x10^{2b}$
	1.5 conc.	$1.4x10^{6b}$	$5.3x10^{2b}$
	2.0 conc.	$1.4x10^{6b}$	$5.3x10^{2b}$
	2.5 conc.	$1.4x10^{6b}$	$5.3x10^{2b}$

Means with the same superscripts on the same column are not considerably vary from one another ($p<0.05$)

Table 4.12a: Effects of*A. danielli* and Turmeric on Chemical Composition of Goat milk Yoghurt with Storage Time

Week	pH	Total Soluble Solids (brix)	Total Titratable Acidity (% lactic)	Protein (%)	Fat (%)
0	4.35^{a}	13.10^{a}	0.81^{g}	8.75^{g}	8.1^{d}
2	4.19^{b}	11.99^{b}	0.83^{f}	8.76^{f}	10.21^{c}
4	3.85^{c}	11.07^{c}	0.89^{e}	8.77^{e}	10.75^{c}
6	3.71^{d}	10.73^{d}	0.93^{d}	8.78^{d}	11.61^{b}
8	3.63^{e}	10.65^{e}	0.99^{c}	8.79^{c}	12.05^{b}
10	3.52^{f}	10.52^{f}	1.05^{b}	8.80^{b}	12.46^{a}
12	3.31^{g}	10.24^{g}	1.09^{a}	8.81^{a}	12.66^{a}

Means with the same superscripts on the same column are not considerably vary from one another ($p<0.05$)

Table 4.12b: Microbiological Effects of *A. danielli* and Turmeric on Goat milk Yoghurt with Storage Time

Week	Total Plate Count (cfu/ml)	Yeast Count (cfu/ml)
0	$4.5x10^{6g}$	$5.3x10^{4a}$
2	$1.7x10^{6f}$	$5.3x10^{3f}$
4	$1.5x10^{6e}$	$4.8x10^{3d}$
6	$1.3x10^{6d}$	$4.4x10^{3c}$
8	$0.8x10^{6c}$	$3.7x10^{3e}$
10	$0.7x10^{6b}$	$3.4x10^{3b}$
12	$0.6x10^{6a}$	$3.1x10^{3b}$

Means with the same superscripts on the same column are not considerably vary from one another ($p<0.05$)

The results in the changes of pH in treated soft cheese samples with *Aframomum danielli* and Turmeric were presented in Table 4.13a. There was no significance difference($p>0.05$) in the pH of soft cheese treated with *Aframomum danielli* and Turmeric but significantly different ($p<0.05$) from the control sample. The pH decreases with storage time and agrees with the findings of Dermiki *et. al*., (2007) who observed a decrease in the pH of stored vacuum packaged cheese. The decrease in the pH of Wagashie (soft cheese) samples can be attributed to the production of acids (Korkeala and Bjorkroth, 1997) and specifically, acidic amino acids and free fatty acids (Dermiki *et. al*., 2007) by the activities of the proliferating microorganisms.

In this study, total soluble solids of soft cheese samples ranged from 8.60 – 5.08^0brix and which was lower than cheese made from goatmilk having 38.5 and 34.7^0brix obtained by (Srbinouska *et al.,* 2001). These results are however important as they indicate available substrates for yeast fermentation and eventual spoilage of the product in the event of improper storage. Turkey's test level of significance showed that 2.0% and 2.5% concentrations for turmeric and *Aframomum danielli* were not significantly different from one another.

The results of total titratable acidity of the soft cheese samples are shown in (Table 4.14a). The values increased from 0.58 to 0.92 as acidity increased over storage period, the value (0.58% lactic acid) was close to the value obtained by El Owni and Osman 2009, who stated 0.59% in mozzarella cheese. But the small level of acidity can be owing to changes in the lactic acid produced during ripening and these are often transformed to other flavorings compounds throughout the formation of cheese curds (Fashakin and Unokiwedi, 1992), Singh and Mittal, 1994). Turkey's test showed that the results from the concentrations of *Aframomum danielli* treatment was not appreciably different ($p<0.05$) from samples treated with turmeric.

Protein content obtained during this study for soft cheese sample (13.04%) was higher than the value obtained for the yoghurt samples. The result of this finding is similar to Oladipo and Jadesimi (2013) who described increase in the protein content of cheese samples subjected to garlic and ginger extracts. Aworh and Egounlety, (1985) stated that the chemically treated cheese sample, the decrease in the protein content can possibly be owing to breakdown of protein by proteolytic organisms in the extracts.

The non-reducing of the protein contents of the garlic and ginger extracts treated samples may probably owing to the ability of the extracts (ginger and garlic) to prevent oxidation. In the

production of cheese using vegetable coagulant from cardoon, *Cynara cardunculus* Galan *et. al.* (2008) reported protein content ranged between 20.99-26.94% while Adetunji (2008) reported the protein content of cheese ranged from 31.60-33.84% in the production of cheese using *Carica papaya* (leaf) and *Calotropis procera*. Proteins are considered as detailed nitrogenous organic substances that form a significant part of living tissues. Gaman and Sherrington, (1998) recounted FAO recommendation of average protein daily intake of 0.6g/kg of body weight per day. Also, the high levels of protein in cheese stated in this study thus provide a suggestion that cheese can comply with the protein requirement of the body. Therefore, cheese having high protein content can be recommended for children to alleviate poor growth and kwashiorkor.

The fat contents of soft cheese samples in the treatments used in this study are presented (Table 4.14a) and the values decreased significantly with storage period and were considerably different ($p<0.05$) from one another.El Owni and Osman (2009) reported the fat content of 16.5%±2.3 for cow milk cheese; this value is in agreement with 16.01% obtained in this study. The storage temperature of cheese favoured microbial propagation ensuing in the release of lypolytic enzymes which caused breakdown of fat.Suitable selecting of natural cheese is vital to attain a process cheese with the required chemical and functional characteristics (Zehren and Nusbaum, 2000). According to Acharya and Mistry (2005), who stated that the significant for process cheese manufacturers is to choose a base cheese with the needed degree of proteolysis.

The initial total plate count of organisms in the cheese examined was $4.2x10^4$cfu/ml and got reduced to $1.7x10^4$cfu/ml by the end of eighth week's storage in treated samples of *Aframomum danielli* and turmeric. However, control samples had microbial counts which increased with storage time.Initial yeast and mould counts were $1.02\text{-}1.03x10^2$ cfu/ml but the counts decreased with storage time in the soft cheese treated with *Aframomum danielli* and turmeric (Table 4.14b). There were increases in yeast and mould counts in control sample as storage time increased.

Table 4.13a: Effects of Concentrations of *A. danielli* and Turmeric on Chemical Composition of Soft Cheese

Treatment	Conc.	P^H	Total Soluble Solids (^{0}brix)	Total Titratable Acidity (% lactic acid)	Protein (%)	Fat (%)
Aframomum	0 conc.	5.0^{d}	6.24^{d}	0.76^{a}	13.28^{a}	14.81^{de}
danielli	1.0 conc.	5.8^{a}	6.94^{c}	0.64^{b}	13.28^{a}	14.84^{c}
	1.5 conc.	5.8^{a}	6.98^{b}	0.64^{b}	13.28^{a}	14.85^{b}
	2.0 conc.	5.8^{a}	7.10^{a}	0.64^{b}	13.28^{a}	14.85^{b}
	2.5 conc.	5.8^{a}	7.10^{a}	0.64^{b}	13.28^{a}	14.86^{a}
Turmeric	0 conc.	5.0^{d}	6.24^{d}	0.76^{a}	13.28^{a}	14.81^{de}
	1.0 conc.	5.8^{ab}	6.16^{e}	0.64^{b}	13.27^{b}	14.84^{c}
	1.5 conc.	5.8^{b}	6.16^{e}	0.64^{b}	13.27^{b}	14.84^{c}
	2.0 conc.	5.8^{b}	6.24^{d}	0.64^{b}	13.27^{b}	14.85^{b}
	2.5 conc.	5.8^{ab}	6.24^{d}	0.64^{b}	13.28^{a}	14.85^{b}

Means with the same superscripts on the same column are not significantly different from one another ($p<0.05$)

Table 4.13b: Microbiological Effects of Concentrations of *A. danielli* and Turmeric on Soft Cheese

Treatment	Conc.	Total Plate Count (cfu/ml)	Yeast Count (cfu/ml)
Aframomum danielli	0 conc.	$2.8x10^{6a}$	$1.0x10^{6a}$
	1.0 conc.	$7.5x10^{4e}$	$0.9x10^{6a}$
	1.5 conc.	$7.5x10^{4e}$	$0.9x10^{6a}$
	2.0 conc.	$7.3x10^{4e}$	$0.9x10^{6a}$
	2.5 conc.	$7.3x10^{4e}$	$0.9x10^{6a}$
Turmeric	0 conc.	$2.8x10^{6a}$	$1.0x10^{6a}$
	1.0 conc.	$9.6x10^{5d}$	$0.9x10^{6a}$
	1.5 conc.	$9.8x10^{5d}$	$0.9x10^{6a}$
	2.0 conc.	$9.8x10^{5cd}$	$0.9x10^{6a}$

Means with the same superscripts on the same column are not significantly different from one another ($p<0.05$)

Table 4.14a: Effect of *A. danielli* and Turmeric on the Chemical Composition of Soft Cheese with Storage Time

Week	P^H	Total Soluble Solids (^{0}brix)	Total Titrtable Acidity (% lactic acid)	Protein (%)	Fat (%)
0	6.8^a	8.60^a	0.58^e	13.04^c	16.01^c
2	6.1^b	6.24^b	0.62^d	14.11^a	17.61^a
4	5.4^c	5.53^c	0.66^c	13.04^c	16.40^b
6	4.8^d	5.08^d	0.71^b	13.10^b	14.32^d
8	4.4^e	5.08^d	0.73^a	13.10^b	9.80^e

Means with the same superscripts on the same column are not significantly different from one another ($p<0.05$)

Table 4.14b: Microbiological Effect of*A. danielli* and Turmeric on Soft Cheese with Storage Time

Week	Total Plate Count (cfu/ml)	Yeast Count (cfu/ml)
0	$4.2x10^{4c}$	$1.0x10^{6a}$
2	$3.7x10^{4d}$	$1.0x10^{6a}$
4	$3.6x10^{4d}$	$1.0x10^{6a}$
6	$4.3x10^{6a}$	$0.9x10^{6b}$
8	$4.1x10^{6b}$	$0.9x10^{6b}$

Means with the same superscripts on the same column are not significantly different from one another ($p<0.05$)

Increasing knowledge regarding the impact of diet on regulation at the genetic and molecular levels is altering the role of nutrition, resulting in new dietary planning.Diet not onlyprovides adequate nutrients to meet daily metabolic requirement but can also contribute to the improvement of human health. Consequently, extracts of plants or single compounds thereof believed to benefit human health and developed the food market to match a balanced diet. Bioactive nutraceuticals or probiotics foods are regarded foods or fraction of foods which provides medical or health benefits, including prevention,treatment of a disease, and broadly regarded to be vital for human health. Innovation of new health effects of bioactive compounds gives the scientific basis for upcoming efforts to use biotechnology to alter/fortify foods compositions as a way to develop public health. Oskoueian *et. al.* (2011) described that extract of root and latex of *J. curcas* plant to contain phenolics, flavonoid and saponins and revealed antioxidant, anticancer and anti-inflammatory activities and the components recounted to be concerned in the biological activities of the plant (Balasundram *et. al.,* 2004). Also, important bioactive compounds in peppers like flavonoids, capsaicinoids and capsinoids are connected biochemical and pharmacological effects for example ability to prevent oxidation and inflammatory qualities. Capsaicinoids give the strong feeling in hot peppers while capsinoids give mild compounds in sweet peppers. Capsinoids prevent inflammatory activity and to endorse energy utilization, to lower fat accumulation and raise body temperature in humans and these qualities make them useful for possible ways in food and pharmacology (Jayaprakasha *et. al*., 2012). Usually, bioactive compounds in aromatic plants have the ability to defend the body from damagecaused by free radicals induced oxidative stress by quenching singlet oxygen and inducing cytochrome or other enzymes (Li, 2006). Spices and herbscould prevent oxidative rancidity and defer the growth of off-flavor in a number of products (Sherman, 2001) and they are capable of destroying microbes which add to the reduction of microbial growth on foods and particularly snack foods and meat products (Li, 2006).

(LAB) could generate several bioactive compounds and LAB proteolytic system is effective in giving outencrypted molecules from many proteins in various food matrices. Alpha and beta–caseins, albumin and globulin obtained in milk and dairy products, rubisco gotten from spinach, beta–conglycinin obtained from soy and gluten derived from cereals make up high-quality source of significant bioactive compound. Diets having additional biologically active

compounds like plants foods and the ones which support useful microbial cultures are also important (Ndife and Abbo, 2009).

Fermented dairy products are receiving attention nowadays. Functional foods are stated as living microbes in food ingredients which benefically influence the host animal by ameliorating its intestinal microbial stability (Lourens-Hattingh and Viljoen, 2001). Yoghurt is an example of fermented food and is regarded as a probiotic carrier which is rich in all nutrients, vitamins and minerals (Lourens-Hattingh and Viljoen, 2001). Yoghurt has more nutrients than milk since lactose intolerance people are able to digest it ones the lactose in the milk are initially changed to lactic acid by the bacterial culture (Vesa *et. al*., 2000). Fermentation of soymilk with probiotic bacteriato shorten thefermentation time, of soymilk using BCT mixed culture (*Bifidobacterium* spp.,*Lactobacillus casei,* and *Streptococcus thermophilus*) either with or without glucose addition has been done (Bozanic *et. al.,* 2008b). The fermentationof soymilk with BCT mixed culture shorten up to 6 to 7hrs but the living probiotic cells count (*Lactobacillus casei*and bifidobacteria) did not appreciably increase and within probiotic specification during the storage period(Božanić *et. al.* 2008b); and was also constant within the 28 storage days to meet probiotic products requirement (Tamime *et al.* 2005).

*Bifidobacterium*species and LAB, particularly*Lactobacillus* strains, are generally use in food processing, fermentation of vegetables and milk, and also in fruit-based and vegetable-based products, like carrot, beet and celery (Karovicova *et.al*. 2002), garlic (Castro *et.al*.1998), green olives (Sanchez *et.al.,* 2000), green cucumber juice (Lu *et. al.,* 2001), onions and peas (Karovicova *et.al*., 1993), alfafa, clover, and galega (Shurkhna *et. al*., 2006), and cereals (Angelov *et. al.,* 2006).

4.8 Isolation and Characterization of Lactic Acid Bacteria

MRS agar is commonly used for isolation of *Lactobacillus bulgaricus*. Forty colonies were selected (whereby 10 colonies were used from each sample). While only 16 isolates were chosen for further characterization. The strains were all gram-positive rods, while the isolates from MRS plates were all bacilli with long and rounded ends and they appeared as a chain of 3-4 cells. Isolates were all catalase negative and they did not turn out gas from glucose (Table 4.15). They were able to grow at different temperatures of 45^0 and 15^0C. The main valuable analysis for determining strain differences is carbohydrates fermentation. Twelve distinct carbohydrates were used for the classification reason but only four gave clear and distinct results, which were glucose, fructose maltose and sucrose. The isolates were stored on MRS broth culture in refrigerated temperature of -80 ^{0}C for further molecular tests.

Lactobacillus species isolated on MRS agar comprised 82% of entire lactic acid bacteria isolates (Figure 4.5). El-Hadi-Sulieman *et. al.,* (2006) stated that the mainstream of lactic acid bacteria (substances separated from the pure form) from Garris (Sudanese traditional fermented camel milk) belonged to the genus *Lactobacillus* (74%). Comparable notes in the make up and variety of lactic acid bacteria isolated from traditional fermented milk of cows and ewes were recounted by Gonfa *et. al.,* (1999) came from Ethiopia, from Burkina Faso Savadogo *et. al.,* (2004), Ayad *et. al.,* (2004) Egypt and Abdelgadir *et. al.,* (2001) Sudan and all these isolates were mainly lactobacilli and lactococci.

Table 4.15 Biochemical Test Results ofIsolates from MRS plates

Sample	Gram staining	Cell Morphology	Catalase	Growth at 45^0C	Growth at 15^0C	Gas from glucose	Glucose	Fructose	Maltose	Sucrose
Isolates from soursop sample	G+	ROD	-	+	-	-	+	+	+	+
Isolates from Cowmilk Yoghurt	G+	ROD	-	+	-	-	+	+	+	+
Isolates from Goatmilk Yoghurt	G+	ROD	-	+	-	-	+	+	+	+
Isolates fromSoft Cheese	G+	ROD	-	+	-	-	+	+	+	+

Key: G- gram positive; – negative; + positive;

4.8.1 Molecular characterisation of lactic acid bacteria from soursop, cowmilk and goatmilk yoghurts, and soft cheese samples: DNA quantification

In order to assess the amount and value of the DNA, the DNAs were chosen as representatives of the cheese and yoghurts samples. The DNA secluded from samples of yoghurts and cheese were verified with 1% agarose gel electrophoresis in 1XTAE buffer, and the DNA concentration was quantified in nanodrop spectrophotometer at 260 nm. The whole amount of DNA isolated from the cheese and yoghurts samples rely on the number of bacteria in the samples and the efficiency of lysis. Genomic DNA solutions from lactobacilli by DNA isolation method was of adequate purity and yield for PCR applications. Genomic DNAs were seen as a spread of various molecular weights DNAs under ultraviolet light and genomic DNA patterns of isolates are shown in Figure 4.5.

DNA Quantification

Concentration (ng/µl)	**Purity**
1. 260.1	1.88
2. 180.6	1.92
3. 260.0	1.83
4. 150.6	2.0
5. 168.3	2.13
6. 172.2	1.90
7. 160.3	1.83
8. 148.0	1.78
9. 157.3	1.91
10. 192.1	1.72
11. 200.4	1.79
12. 198.0	1.88
13. 162.3	2.11
14. 150.3	2.18
15. 148.9	1.70
16. 267.1	1.91

The DNA for PCR is between 30 to 50ng

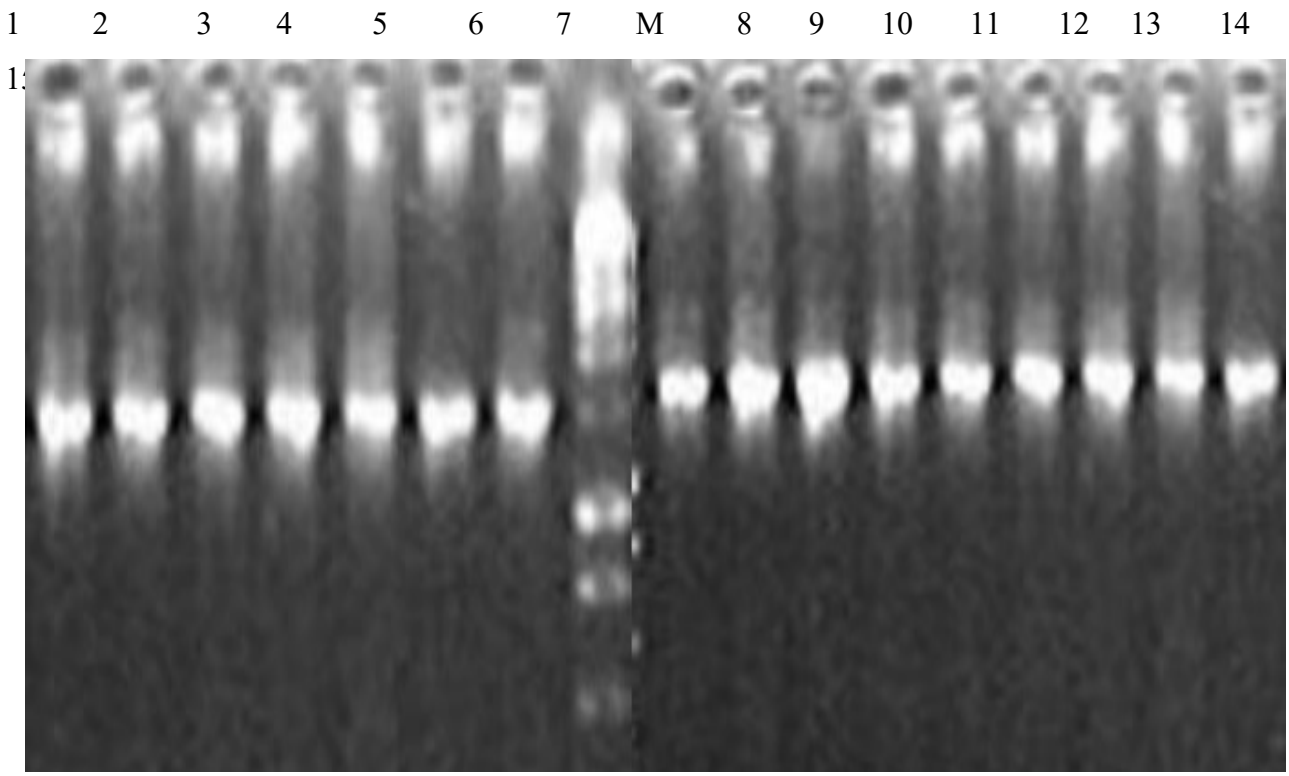

Figure 4.5: Representation genomic DNAs of yoghurt and cheese isolates

M - Molecular marker;

1=*Lactobacillus paracasei*;

2=*Bacillus subtilis;*

3 = *Lactobacillus plantarium-2;*

4 = *Lactobacillus licheniformis-3;*

5 = *Lactobacillus rhaminous;*

6 = *Lactobacillus fermentum;*

7 = *Lactobacillus paracasei;*

8 = *Lactobacillus licheniformis-2;*

9 = *Lactobacillus fermentum-3;*

10 = *Lactobacillus licheniformis;*

11 = Lactobacillus rhaminous;

12 = *Lactobacillus licheniformis;*

13 = Lactobacillus plantarium;

14 = *Lactobacillus fermentum-2*;

15 = *Bacillus subtilis-2;*

16 = Bacillus subtilis-

The 16S primer was capable to magnify DNA isolated from *Lactobacillus* species. Thus, agarose gel electrophoresis of the PCR products was distinctive. Sequencing results of 16rDNA were exported to the database and checked for homology alignment. The sequences blasted on the NCBI gene bank showed 100% homology (Figure 4.6). Based on the alignment results, cheese sample contained (*Lactobacillus paracasei*; *Bacillus subtilis;Lactobacillus plantarium-2; Lactobacillus licheniformis-3)*, cow milk yoghurt had (*Lactobacillus rhaminous;Lactobacillus fermentum;Lactobacillus paracasei; Lactobacillus licheniformis-2),* Goat milk yoghurt also had (*Lactobacillus fermentum-3; Lactobacillus licheniformis; Lactobacillus rhaminous;Lactobacillus licheniformis*) and soursop yoghurt had (*Lactobacillus planetarium*; *Lactobacillus fermentum-2*; *Bacillus substilis-2; Bacillus substilis-3*).

Figure 4.7 showing the phylogenetic tree based on 16SrRNA sequences of representative strains of each group. The alignment gave the interrelationship in the nucleotide planning of the organisms. The claude on the phylogenetic tree also established the correlation of the species organisms (Figure 4.12). The LAB species recognized in this research are more different when compared to those reported by El-Hadi-Sulieman *et. al.,* (2006) that isolated only two Lactobacillus species *(Lactobacillus plantarum and Lactobacillus paracasei)* from fermented camel milk. These variations in the variety of lactic acid bacteria noticed between cow milk, goat milk and soursop juice can be accredited to the intrinsic quality of the fermented milks used and the specific fermentation condition followed. As suggested by Hassaïne *et. al.,* (2007), fast acidifying strains of lactic acid bacteria could be potential candidates in the fermentation process as primary starter organisms whereas the poor acidifiers can be used as adjunct cultures, depending on their desirable properties such as proteolytic and autolytic activities. The proteolytic activity of dairy lactic acid bacteria is essential for the development of flavour compounds in different fermented milk products (Hassaïne *et. al.,* 2007). The production of good quality fermented dairy products is dependent on proteolytic properties of the starter bacteria, since peptidases and amino acids formed during fermentation have a direct impact on flavour development or serve as flavour precursors in dairy products.

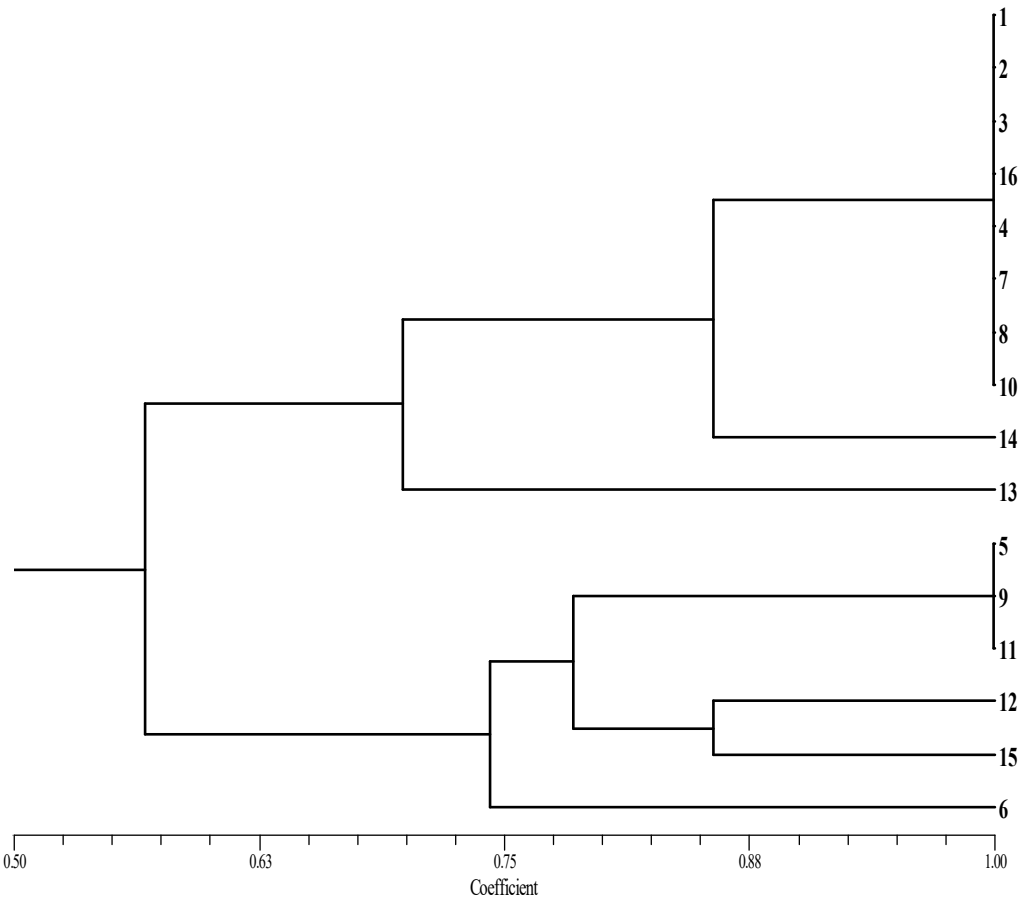

Figure 4.6: Dendogram of *Taq* 1 digests of representative LAB isolates from yoghurts and soft cheese.

4.8.2 Sequencing of Soft Cheese Isolates

Sequences with forward and reverse primers were matched and compared with primer walking and the gaps were completed. The sequences of 16S rDNA product of cheese isolate are shown in Figures 4.7 to 4.10. The BLAST analysis at the NCBI gene bank gave 100% homology to *Lactobacillus paracasei,* 100% homology to *Bacillus subtilis*, 100% homology to *Lactobacillus Plantarium*, and 100% homology to *Lactobacillus licheniformis*.

The quality of cheese and other fermented food products is dependent on the ability of flavour and aroma production of microorganisms which include starter culture. A number of different LAB has been evaluated for their ability to degrade amino acids to aroma compounds. *Lactobacillus lactis* subsp. *lactis* and *Lactobacillus lactis* subsp. *cremoris*, *Lactobacillius lactis*, *Lactobacillius helveticus*, *Lactobacillus bulgaricus*, *Lactobacillus casei* are capable of degrading methionine to methonethiol, dimethyledisulphide and dimethyltrisulphide (Yvon and Rijnen,2001). The phenotype analysis of the cheese strain indicated its relationship to *Lactobacillus plantarum*and this strain belongs to the group of the mesophilic lactobacilli; commonly met in the later phase of the maturing of cheese, together with *Lactobacillus casei, Lactobacillus brevis and Lactobacillus buchneri* (Hammes *et. al.,* 1999).

Proteolysis is also an important process that occurs during cheese ripening as lactic acid bacteria uses polypeptides generated by milk clotting enzymes and by bacterial cell-wall proteins. Rennet is the milk clotting enzyme which is responsible for casein degradation and because of casein degradation peptides are produced which are transported into the cell. In the cell, peptidases continue degradation to produce smaller peptides and amino acids. It has been known that amino acid composition plays an essential role in the aroma of cheese (Wouters *et. al.* 2002). Olarte *et. al.,* (2000) noted that the presence of *Lactobacillus plantarum*in cheese produced from Cameros goat's milk decreased the number of the enterobacteria and faecal coliforms in the final product.

CTCGCAATCTGATTTTGGCTTCAATTTTAGTTTGGGCAAACCGCATGACTTGTATGTC
TTTGAGTCACCAATTGACGCGTTGAGTTACTGGACACAACACCCAGAGCTAACTAAT
TGCATGATTACCTCAGTTGATGGCACCAAGGTTGAGAGTGTCGTGAATATGGCGGTC
AATATGTATAAAACAAAAGGGCAACTGCCAGCCACTATTTATGTTGGCTCCGACAAT
GACCCAGCTGGTCATCAGCTTTATGACAAGCTGCAAGAATCCATTTTAGCCAATACC
GAAGCCGAGGTAACCACCTTTAAGCCGCTCTTTCCACAGGACAATGCGATCACCCA
GGCAACTTATCAAAGGTTGAAACAGGCTGCGGATCAAGCGGGCATTGACTGGCGTG
AGTTGGCAGCAGCAGTTAAAGCAACGACTAACCTTTCAACGAACTTCCAGCAATCTT
TTCCGCTGCCACAAGCCATTGCCAAAAGTAGCGTGCTTATTGACCCCAACCCTGGAA
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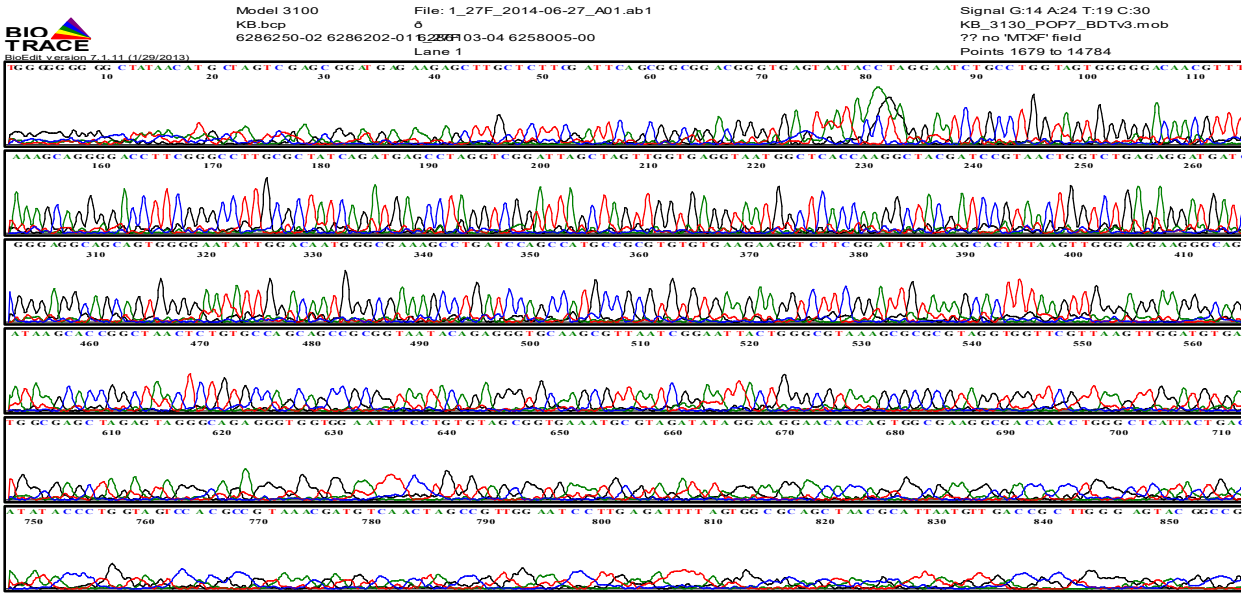

Figure 4.7: Sequencing of *Bacillus substilis* isolates from soft cheese.

TGCCATCCTTTACTCGCTTACTACAGGGTGTCAATGGCGTGAATTACCGCACGATTTC
CCGGAATGGCACACTGTCTACCGCTATTACGATATGTGGCGAGATAAACCAGACCC
GACAGCTGATTCGCTATTAGAAAGGCTTTTAAAAAAACTGTCGCTTCCTATCGTTTT
GCACAGGGCCGATCGGCCCGAACGTCGTTTGTGATTGTTGATGCTCAAAGTGTTAAA
ACCACTGATTTAACGAAAAATAGTGGCTAAGATGGCGGCAAAAAGATTTCAGGGAT
TAAGCGTCATATGGCGGTTGATATTAACGGTTTACCACAAGCCATTCTCGTGACACG
AGCTAATGTATCAGATCGTTCAGGTGCATTGGCTATGTTTAGTTTGGCTAGCCAAAA
TTTAGAGCTGGTTCAGCATGTCATGGTTGATGGTGGCTACACTGGCAATGACTTTGC
GGATCAGGTGAAGCTCATTTTGAATGCTAAGACGACGGTAGCTAAACGCAACGAGT
TGCATATGTTCACGGTGTTACCGCAACGATGGATCGTTGAACGTTCATGGAGTTGGC
TAGACAAATGTCGGCGACTTTGGAAAAACTGTGAACGTGCCCTTAACAGCAGTCTTC
AAATGGTTGTATTGGCCTTCCTGAAGATAGTTCTTAAAAGATACTAGACAGGTTCTA
AGACGACGGTAGCTAAACGCAACGAGTTGCATACGTTCACGGTGTTACCGCAACGA
TGGATCATTGA

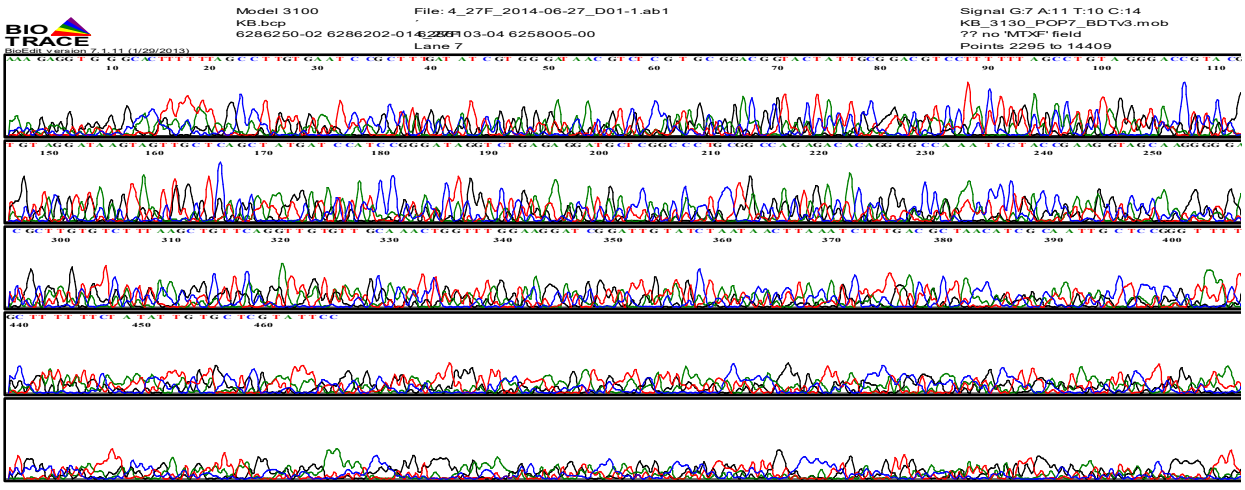

Figure 4.8: Sequencing of *Lactobacillus licheniformis* from soft cheese

ACTATCAACAACTGTGCCGCTAACTGTTGCCTTTTTGTTGTCACTGCTGTAGGAAACA
TTGTCAATCTGAACCTTTTTTTGCTGAAGTTGCGCTTTAGTTAACAGCCCTAACTTGC
GTTTGAGATCTAGAATGCGGAAAACGTGTTCATTGATTTCTGATTCCTTAATAGTGC
CGCTAGTTACTGCTGCATGAATCTGTGGAATAGCGGTTTCATAATCATTATTCATGAT
GCAATCATTTCCTGCTTCAACAGCAAGAACATCAACAGGAACTTGATCATGTTCTTG
AGCAAATTGCTTGATAGCTCCCATTTCCAATGCATCGGTAATGATTAAGCCATTATA
ACCAAGTTCATTACGCAACAAGTCTGTAACGACCTTTCGTGATAATGAAGCTGGGTA
TACTGGGTCAACAGCTTGCATTACGATATGTGCAATCATTACTGAATCTGCTCCTGCT
GTAATACCGGATTTAAAGGGAAGCAAATCCTCTTTTTCAAAATCCGCCTTAGATTTT
GTAACAACTGCAAAATCCGTATGCGTATCAATCGCTGATCCGTAACCCGGAAAATGT
TTAAGCGTTGCTGCAACACCAGCATTCTGCCATGCAGGGATCACATTCGTAATATAG
TTTGCAGTTGCCAAGTAATCTTGACCAAAAGTTCTGCCATAAATAAAACTAGAAGTA
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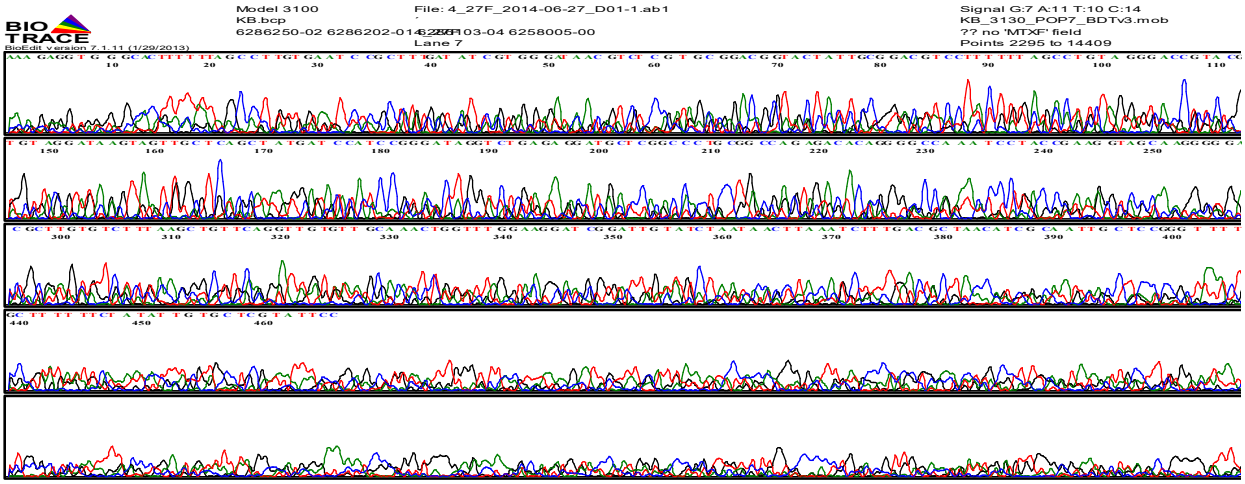

Figure 4.9: Sequencing of *Lactobacillus paracasei* from soft cheese

CAACAAATTGTCGGTTACGACGTTAAACTAGCCCACTTAATCGCCAAAAACATCGGG
GTCAAGAAGGTTAGGTTCGTCAACATTGCCTTTCCATCCCTAATTAGTGAGCTGCAA
AACAAGAAGTTCGACATGGTGATGGCCGGAATGGTTTGGACCAAGGAACGGGCCAA
GGCGGTTAGCTTCTCGAGTACCTACCACCACGGGGGTCAAGTTCTGTTGGTTTCTAA
GGCTAACGAAAACAAGTACAGCGGCATCAGCGCCTTGAAGGGAGCCACGTTGGGTG
CCCAACAATCCTCCGAACAAGAAACGATCGGGAAGTCCCTTTCCGGGGTAAAGTTG
GTCACCGAAAGTTCAATCACGACCCTGTCCCAAGAGGTGAAGGCGGGGACTTTGGA
TGGGTTGATCTTAGCCCAGACCTCGGCGGACGCCTTTGTGGCTGAACACCCTAATGA
TTACGCCATCGCCAAGAACATTACCTTCAAGATTAGCGCCAAAACTTCTGACCCGCG
GGTCGTGGTTCGCAAGTCCGATAAGGCCCTCTTAAAGGTCGTCAACAAGACAATCA
AGGACGCCAAGAAGTCCGGCGAATTAGACAAGCTCTTCAAAGAAGCCCAAAAGCTA
CAATACAGCAACAACCAATAAACGATCGATAGAATTAA

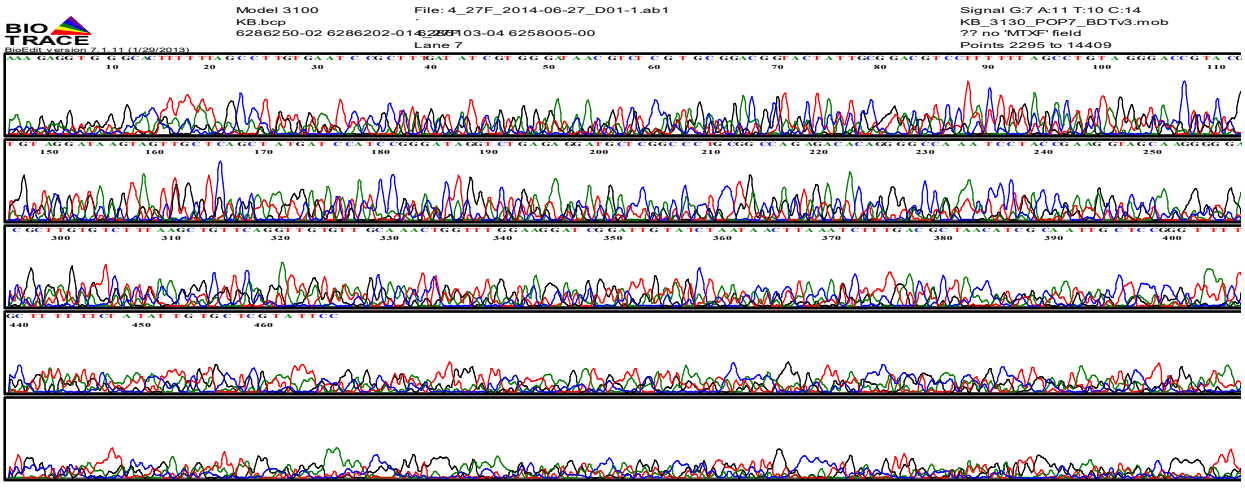

Figure 4.10: Sequencing of *Lactobacillus plantarium* from soft cheese

Figure 4.11: Alignments of *lactobacilli* from soft cheese

Figure 4.12: Phylogenetic trees of lactic bacteria acid isolated from soft cheese

4.8.3 Sequencing of Cowmilk Yoghurt Isolate

For the identification of LAB isolates with 16S-ITrDNA region, EGE1 and L1 primers were used. The primers were effective for the identification of LAB isolates as affirmed by Bulut *et. al.,* (2003). The result of 16S-ITrDNA was also verified by species specific PCR to increase detection sensitivity. The species-specific PCR amplification procedure has been developed and primers have been used for the amplification of an intragenic fragment of 968bp within the *lacZ* gene sequence of *Streptococcus thermophilus* (Lick *et. al*., 1995).

Sequences with forward and reverse primers were matched and compared with primer walking and the gaps were completed. On the basis of these key characters, subgroup A1 isolates were identified as *Lactobacillus rhaminous-2* and *Lactobacillus fermentum* while subgroup A2 isolates were identified as *Lactobacillus paracasei-2* and *Lactobacillus licheniformis-2*. The sequences of 16S rDNA product of cow milk yoghurt isolates are shown in Figures 4.13 to 4.16. The BLAST analysis at the NCBI gene bank gave 100% homology to *Lactobacillus paracasei-2,* 100% homology to *Lactobacillus fermentum*, 100% homology to *Lactobacillus rhaminous-2*, and 100% homology *Lactobacillus licheniformis-2*. Subgroup A1 and A2 were rod shaped isolates, catalase negative; growth at 45 ^{0}C, produced carbon dioxide from glucose and both isolates utilised carbohydrates like lactose and maltose. Similar characters for *Lactobacillus fermentus* have been observed by Chantaraporn and Somboon (2006). Lactobacilli are regarded as a major group of probiotic bacteria (Collins *et. al*., 1998; Metchnikoff, 1908; Tannock, 1998). Salminen *et. al*., (1999) proposed that probiotics are microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being of the host. Several lactobacilli, lactococci and bifidobacteria are held to be health-benefiting bacteria (Rolfe, 2000; Tuohy *et., al.,* 2003). The health-promoting effects of LAB are strain specific and result in different mechanisms to produce beneficial health impacts. *Lactobacillus rhamnosus* GG relieves lactose intolerance symptoms by hydrolyzing lactose into glucose and galactose and forming the physical appearance of milk into a thick substance, such as yoghurt, that passes through the gastrointestinal tract slowly, reducing the lactose pulse in the colon (Hove *et. al*., 1999; Drouault and Corthier, 2001).

GGGGGATTTTCATCGTCGCGCAGGCGTAGTTAACTAGCACCGACGGGATGATGGGG
ACCATGTACCCCACTGTCAACCCTAGGGCGGGATGGCGGTAGTGGGTTAAGCTGTCC
AAAATCTTGTTTTGCTTAAACTTATGGGAGAGTTTGACGTGCGATAACAGGGCGGCG
ACGGCACAATTGCCCAAAATATTCCCTGCCCAGTTAACCAAAAGACCGATCCACGG
GCCGTAACAGAGACCGACAAAAAACGCAAATAACGGCGTTGGAGAGGCCCGGAAT
CGCGTTCATTACCCCCACTAGGAGTAACATGAAGATCAGGTCGCGAATACCGTTGCT
GCGAATCATGGTTAAGAGCCCGGCCCGGTGAGCGGGGGTCAGGTCCATTAGGAGGC
GAATTTCAGGCATGAAGTCGTGCACGAGGGCCAACACCAAGAGGATGATGGCGATC
ACGCCCGGCCCAATTAGCCAGTACTTATTTAAACGGTGGTTTTCCATGAACTATTTCC
TTTCTAAGTTGCTGGCGTAGATGGTGGCCAGGGTGTGGTTGAGCAGGGGGTCGTGGG
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TCAGACGTTCCTGGTAGTGCTTAACATCTGCTGGGT

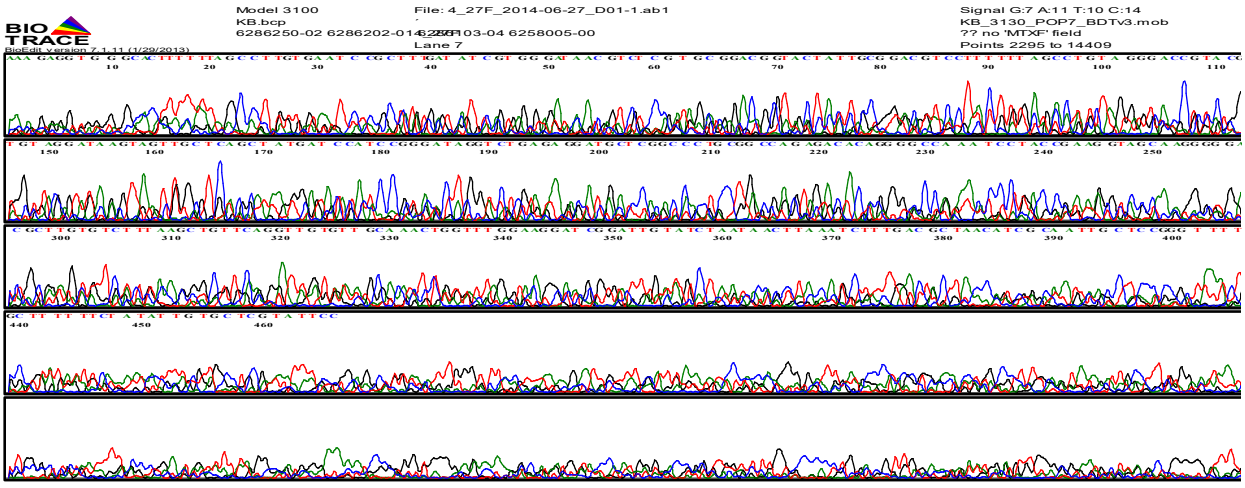

Figure 4.13: Sequencing of *Lactobacillus rhaminosus* from cow milk yoghurt

CGAGTGGGCCAATTTAAGCGTCGTCAGTTACTACAAGCTTTCCGCCACTCTCTACGC
CCTCGGGGTCATCAGCTTAGTGACCATTTGGTGGGTGGTGAACCAATTTGGCCAGTG
GCGGGGGAACCTGCGGATTATGCACGGGGTGGCAACGTATGCCTACCGCGCTTACC
TAGGCAATGTCTTTTGGCAGACCTTGCTTTGGGATTGGTGGGGTCGTCAATTAGCCA
CCACGCACCCATGGTTAGCGTTGGCGCTCCTCTGGCCGGCTACTTGGTTGTTAGCGTT
TGGTTTTGCCTACCTGTTACACCTGATATGGGGGCGCCGGCCGGTTAAACAAAAATG
ATTCAAGAACCACTAATTGATTGAAAGCGTTTAATTATCTGGTTTGAAAGGAAATAA
TTAAAGTAGACCACTTGACGAATCGACCAAAGACCGTTATGGTGAGGGTAGTTTAGT
TGCCTAGCCAGAATCGTTGGAGGGATTATGCTCAATCTTAATACAACTGCCGCCCAG
GTTCCCCAAGAAGTGGCCCGCTTAGACGCCACCACCCAGCGCCAGCTAAACGCCAA
CGCCGCGGTGCTCGTGCGGGGGCTGCGCCAGGACCTGGACATGACCACGGGAGAAT
TTGCGACATACGTAGGCTTAACGCCAACTTTAATTTCGTCCATTGAAGAGGTTCAGA
TTAACGTCTCCTACGCCCTGGTGGCTGACATCGCACACCGGGCGGGAAAACGGCTTA
ACATTGAGTATCGGTGATTTAAGAGAGTGATAGCAAGGGACTGGGAAAAGAGCTGT
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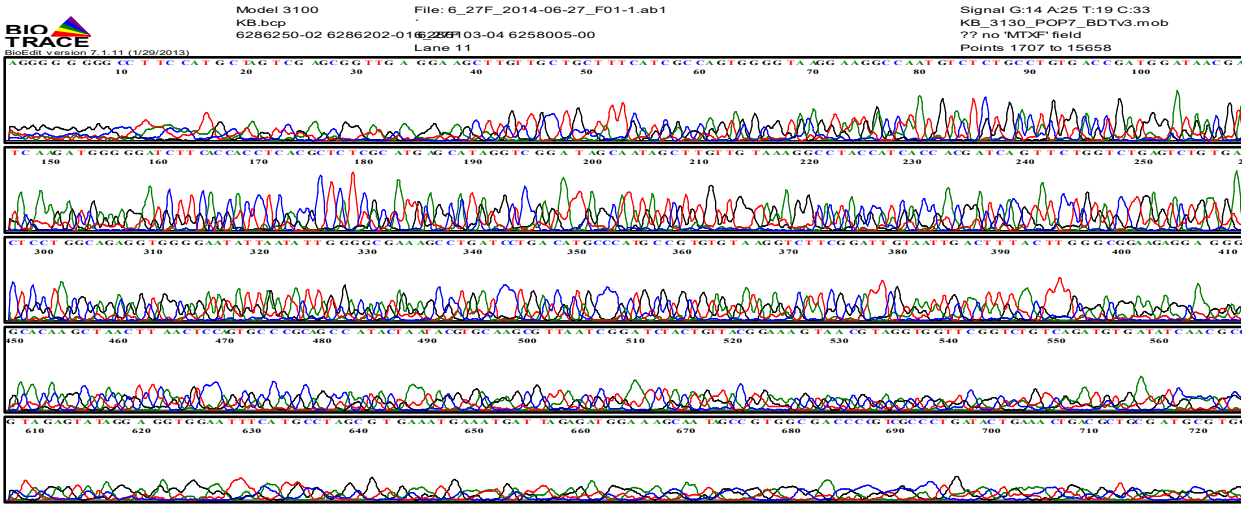

Figure 4.14: Sequencing of *Lactobacillus fermentum* from cow milk yoghurt

TGATTGATCAACTGCAGCCCGGCGCTTTGAGAGAACTGGCAAAACACCAAGAATAT
CTACTGACGCATTAAACTCGTCGATTAAGGTCTTCTGAAGGTAGGCAATAAAGTTCT
CTGCACCTGTTAGTGCTCTTTCCTGTGTCTGAAGGACAATAACAATTTGATCCGTAGC
AAAAAACGCCGAATCATTTTGCAAGCTCATCGTCGGTGGCACATCGATAAAGATGA
AATCATAATCTGATCGAAGTGGATCAACCAGTTGCTTAAAGAAGCCAACCTTTTCCG
CTTCGCCTTCAAATTGTCCGTCCAGGAATCGTGGATATTGGGCAAAGTCTACCCCAT
CCGGTAATAAGTCCAAATTTGGCGCTATATTAATTCTAATATCTTTTAATGGCTGATT
AGTAATGATTGCTGCCATTAGTGAAGTCTTTACTGTGATGATATTTTCTTCGGTCGAT
CCAGTCTTTAAAAGTAACTCTGTTAAATTGGCCTGAGGATCAAAGTCAATTGCTAAA
ACTTTAAAGCCCTTCTTTGCCAATGCCCAGCTCAGTAATGCAGTACTACTCGTTTTAC
CAACGCCACCCTTAAAATTTGCCATACTCAATACAGTTGCCATAAAGTACCGCCCCC
TTTTTCGTTAAGCCTACAATGGTATTAACACAATGTCAATAATTAAATAAATGCCGA
AAATCAGTAATTCGCACTATTGTGTTAAATGTATAATGTTATTAACATAGTGGTAAA
TGAATTTAACATTATGCATTATATATAGTGGTAAATTCATAATGTTATTAACATAGTG
GTAAATGGTAAGTGGTAAATGTTTGAGTTTACGTCAGAGCTTGCCGAAACGGCGCCA
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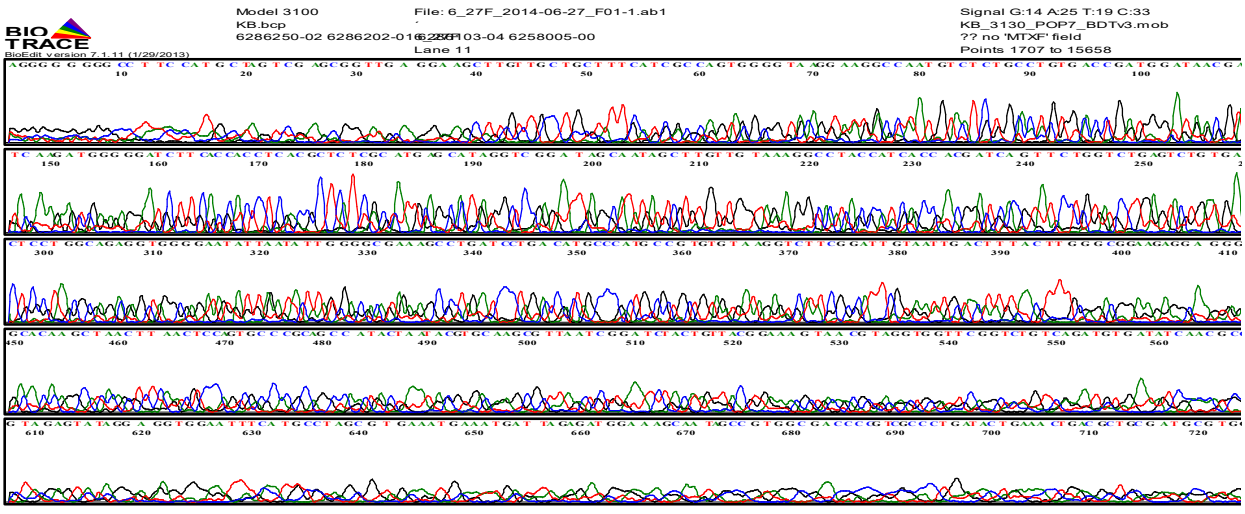

Figure 4.15: Sequencingof *Lactobacillus paracasei* from cow milk yoghurt

TTAGGCGAGACCACAAATCGCAGTGCTGATCGCAGCGCGTGATTTGTGGTTTTTTCT
TTGCACTGGGAGGTGATGACAACTTGCCAAAAATAGCTCAGATTTTTAACAACAACG
TGGCCTTGGTTGATCTAGACAACCGCGGCCAAGCCGTTGTAAGGGGACGTGGCATC
GCTTTTCAGAAGAGGCGAGGAGATGTTATTCCGACAAAGCAGATAGAGAAGATCTT
TTATCTAGCGAACGAGACTTCCCGACAAAATTTGTACTTTCTCTTAAAAAATATTCC
GATTGACGTGGTGACGACTACCTATGAAATTATTGATGTTGCCCAGAAACAATATCG
ACTGAAAGTGCTTGATTATATCTACATTACCTTGAGTGATCATATTTACGAGGCATA
TAAACGCTATCAGGCAGGGACTTATCAAGAAACAATGGTACCAGATTTTCATATTCA
ATATCCGGCCGAATATGCGGTGGCTAAACAGGCACTGCAAATCATTGCCACGAACC
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CTTCCGGCGAAGACGA

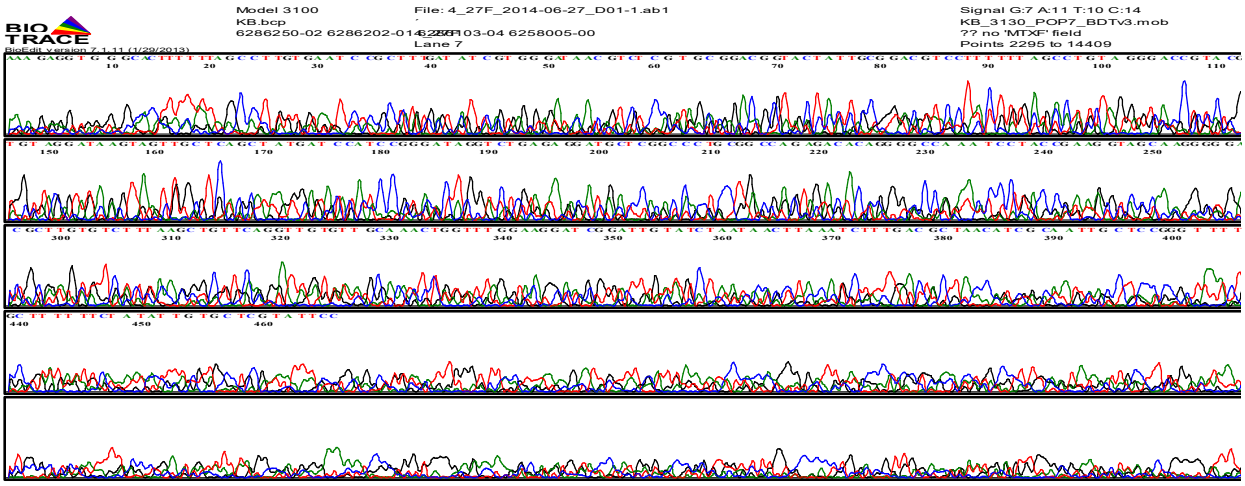

Figure 4.16: Sequencing of *Lactobacillus licheniformis from* cow milk yoghurt

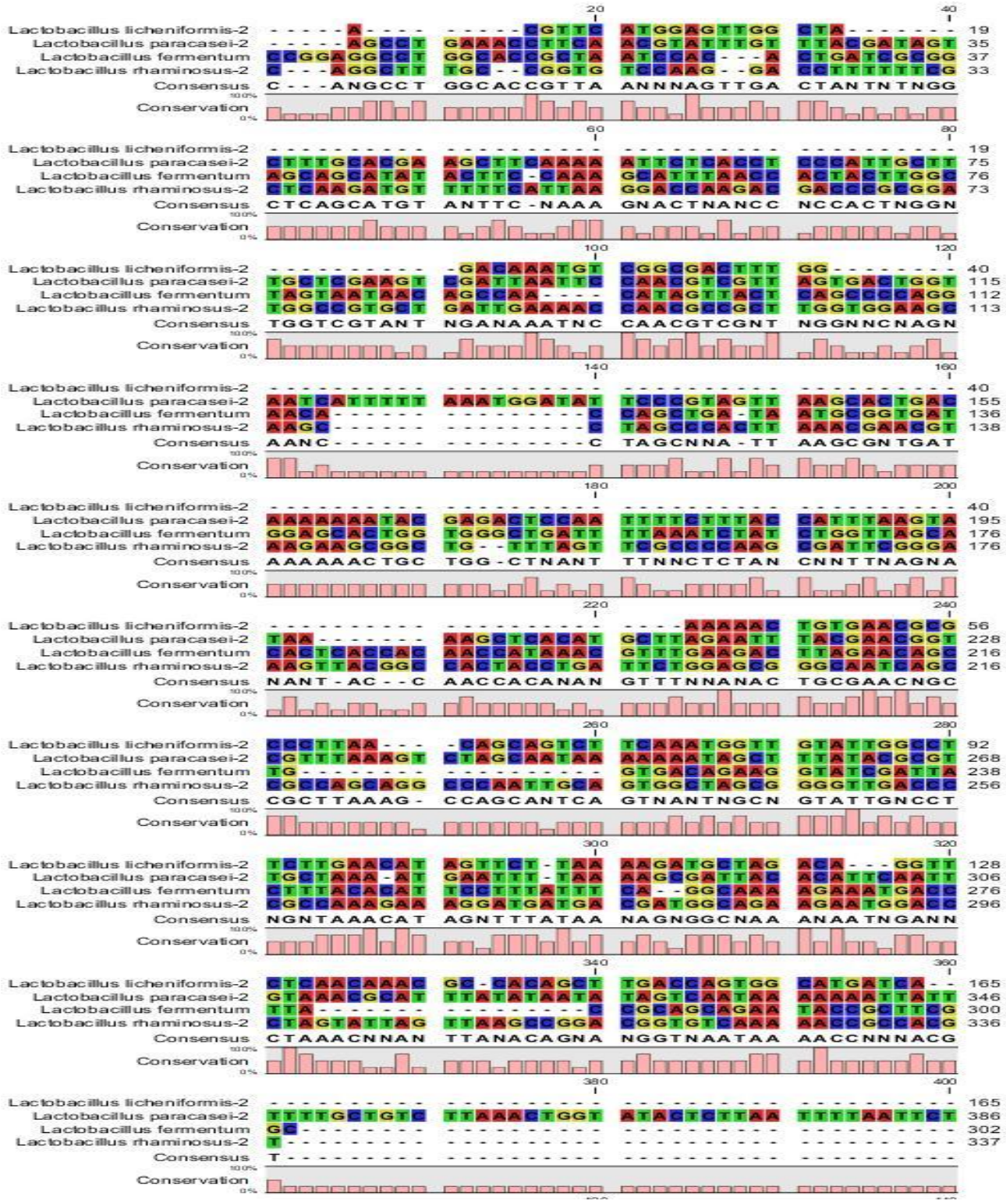

Figure 4.17: Alignment of *lactobacilli* from cow milk yoghurt

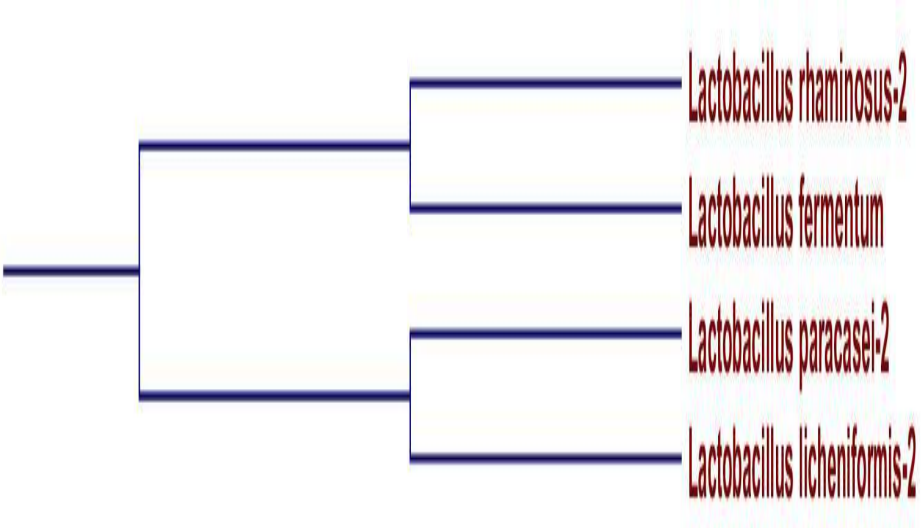

Figure 4.18: Phylogenetic tree of the lactic acid bacteria in cow milk yoghurt

4.8.4 Sequencing of Goatmilk Yoghurt Isolate

Molecular methods are important for bacterial identification (Greetham *et. al.,* 2002; Heilig *et. al.,* 2002) and possibly more accurate for LAB than are conventional phenotypic methods. In this study, a 1500-2900 bp segment of the 16S rRNA gene of the LAB isolates was sequenced and the sequence compared to strains in the National Center for Biotechnology Information (NCBI) Blast Library (Washington University). Sequencing results revealed that four out of the 10 isolates obtained from the goat milk samples were *Lactococcus species* (Figure 4.24).Sequences with forward and reverse primers were matched and compared with primer walking and the gaps were completed. Based on the alignment results, subgroup A1 was found as *Lactobacillus fermentus-3,* subgroup A2 as *Lactobacillus licheniformis-4* while subgroup B1 and B2 as *Lactobacillus licheniformis* and *Lactobacillus rhaminosus*. The sequences of 16S rDNA product of goatmilk yoghurt isolates are shown in Figures 4.19 to 4.22. The BLAST analysis at the NCBI gene bank gave 100% homology to *Lactobacillus fermentum-3,* 100% homology to *Lactobacillus licheniformis*, 100% homology to *Lactobacillus rhaminous*, and 100% homology *Lactobacillus licheniformis-4*.

Lactic acid bacteria are widely distributed in nature and are representatives of the genera*Lactobacillus, Lactococcus, Pediococcus* and *Leuconostoc*. They can be isolated from soils, waters, plants, silages, waste products, from the intestinal tract of animals and humans and they possess stable fermentation characteristics and are resistant to bacteriophages (Lee, 1996). They have potentials for establishing new, so-called "functional foods" (Reid, 1999 and Holzapfel *et. al*., 2001). The selection of health-promoting bacteria must rest on the documented impacts of selected probiotic strains on the microbial community harboured within the digestive tract (Tannock, 1998). According to Caplice and Fitzgerald, (1999), LAB produces small organic compounds that give the aroma and flavour to the fermented product: lactic acid bacteria of human and animal origin may serve as bacteria potentially promoting host-specific health and antimicrobial effect exerted by LAB is mainly due to acid production, hydrogen peroxide, fatty acids, aldehydes and other compounds (Daeschel, 1989). Hutt *et al*. (2006) also reported that*Lactobacillus* strains possess intermediate potency for out-competing cystitis-causing *E. coli* from the large intestine. Goderska and Czarnecki (2007) reported that *L. acidophilus* gave a growth inhibition zone of 26mm against *H. pylori* and *E. coli*.

ACGTTCATGGAGTTGGCTAGACAAATGTCGGCGACTTTGGAAAAACTGTGAACGCG
CCCTTAACAGCAGTCTTCAAATGGTTGTATTGGCCTTCTTGAACATAGTTCTTAAAAG
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AAAGTTTCGCGCAGCACCGATGCCACGATGGTCATTAAAACCGGTCGCGTGAAAAT
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CGTCGATGAGCCAAAGTTCAAAATCAATTGTTTGTTGCTGGATACTTTTTAATGTCTC
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AAATAAAGTGATTCGTTTGTAGAGACATGACTAGCACTGATCATTAAAGAACAAAA
GGCAACACAGATAATTGTCCTAAAAAACTGACGATGGCTTGCTGGATGGACACTGTT
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TGTGACTATTTAA

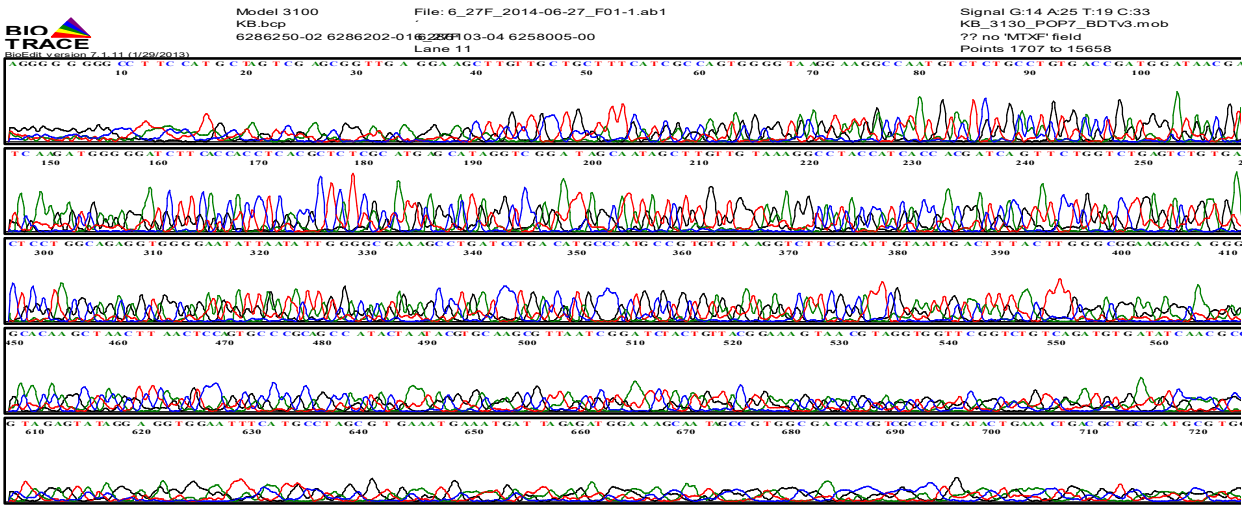

Figure 4.19: Sequencing of *Lactobacillus licheniformis* from goat milk yoghurt

TGGCGAGCAGGTTTTTGGTAAAAGCAATGAAGCCTCACTTAGTCAACTTGTACAAGA
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ATTTATGATTCATCTCCAGTATCTCATCGACCGACTGCAGCGTGTTGATACATATGCC
GTTACCATTGTCCCTGAGGTTGCCACTGAACTTAAGCAAAACTATCCGCAGTCTTAC
AAGATTGCCTCAGAAATTTTCAATGAAATTAAGGATCAACTCTATCGCAGTATGAGT
GAGGACGAACGACTTTACTTCATCATCCACATTCAGCGATTGATAAACGAAGCACCA
GCCCAGAATCATTCACAAAACGATTCATTATAACGCGCTCGCAGTCGTAGAAGCCTA
CACATAAGGGCTTTGAAGCAATCTACCAAAGATTGGGCCAGTTTGCTTCAAGCACGC
TTATGCTTTGGCTTCCAAGCGCTCAGGAGGAAAAGACTCATGAATAAGGTTTTTGAT
AAATTAAAACCGGTTTTTGAAGCCATCGCTGCTAACAAATATATTTCCGCGATTCGT
GATGGCTTTATCGCATGTATGCCGATCATCATCTTCTCAAGTATCTTTATGATGGTTG
CTTATGTTC

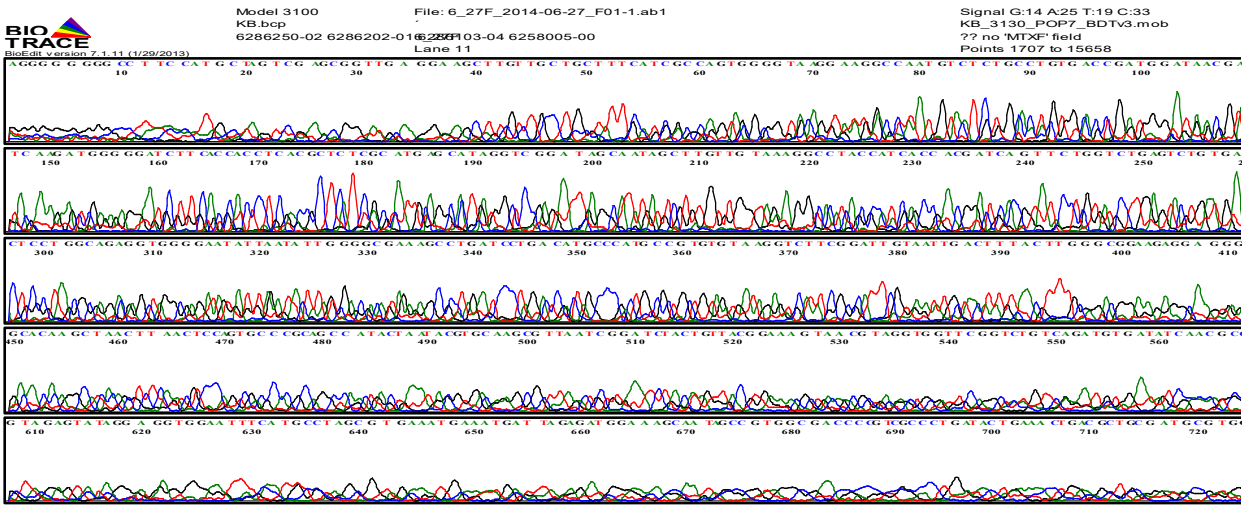

Figure 4.20: Sequencing of *Lactobacillus licheniformis*fromgoat milk yoghurt

CCGGAGGCCTGGCACCGCTAATCCACACTGATCGCGGAGCAGCATATACTTCCAAA
GCATTTAACCACTACTTGGCTAGTAATAACAGCCAACATAGTTACTCAGCCCCAGGA
ACACCAGCTGATAATGCGGTGATGGAGCACTGGTGGGCTGATTTTAAATCTATCTGG
TTAGCACACTCACCACAACCATAAACGTTTGAAGACTTAGAACAGCTGGTGACAGA
AGGTATCGATTACTTTACACATTCCTTTATTTCAGGCAAAAGAAATGACCTTACCGC
AGCAGAATACCGCTTCGGCAAGGCCAACTAGTTTTTATTATTTAATGTGTCAACTTG
ACAGGGTACAGTACCGGGCGTTTTAATTTTCCGTGTTTAGACCGGTGTAGCGACCAT
GGCCTCCGCCAAGCGCTGGATGCCGACCTCAATCTGGTCAGCCGGGGTGTTAGAGA
AGTTCAAGCGGAACTTGCCGGCGGGCGCGTCCTTCGGGTAGAAGGGCTCGCCCGGC
ACGAAGGCAACGTGATTAGCGATGCACTCGTTAAACAGGGCTTGGGTGTCCACCCC
ACCCGGAACCTCGACCCAGAGGAACATCCCCCCGGTGGGCCGGGAGAATTTGACGT
CGGCCGGGAAGTACTTTTCCATCGCCGCGATCATGGCGTCCTTGCGGCTGCGGTAAA
GGTCGGTGATTTGCCGGACGTGTTCATCGACGTCGTTTTGCTCAAAGAACTTGGCGA
TCGTGTACTGGGTGAAATTATCGGTGTGCAGGTCGACGGTTTGCTTGAGCATCGTGA
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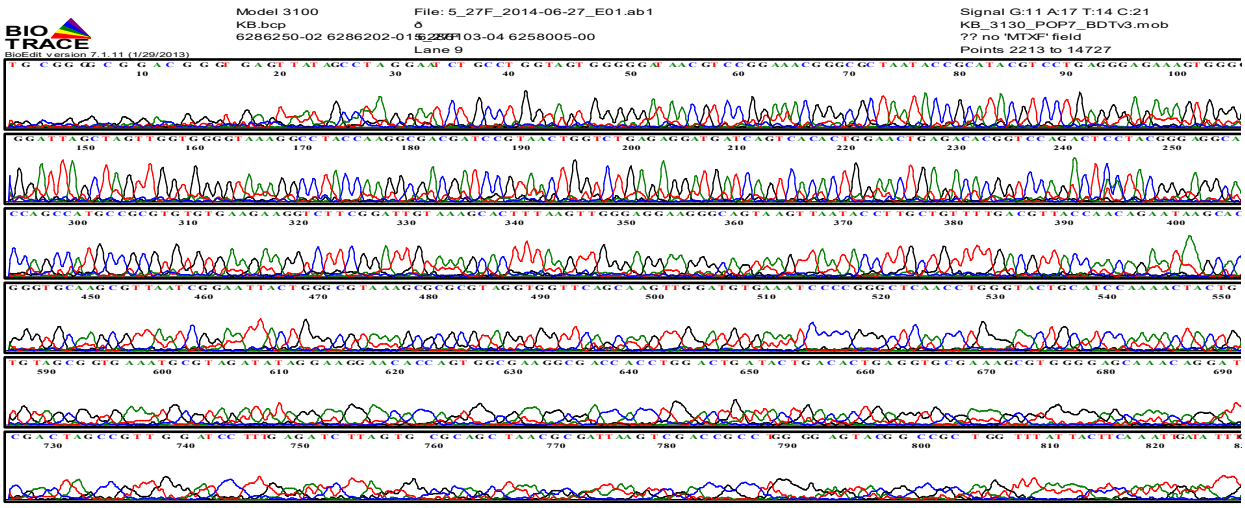

Figure 4.21: Sequencing of *Lactobacillus fermentum*fromgoat milk yoghurt

GGACTTTGTTCCAAACGAGCACAAGCTCAACGGGACGATCTTCTTTCCCAAGTGGGT
CCAAGAGCACCCGGGAATCACGGTTTGTTTCCAAAACGAGCACATTGATTTAATGCA
CGAAAGCCGGGAAAAGTACGCGACCTACCCGAAACTGGTGGTGCCAGAATTTGCCA
CCATCACTTACATGGGGGATGCGGGCCAGGATAATGATGAAGTCATCAACGAAGCC
CCGTACGAGGGGATGACCGACGACATCCGTAACGGTAACTTCTTTGACCAAAACTA
CCGTCGTCAAAAATAGCAAAATAAGAGGAGGTCAGGGAAAAAACGATTTTTCCCGG
CCTCCTCTTTGGTATAATGTCGTTAATTAACGGGGAAGGGAAGTGATGGGGGTGCCC
GCCAAACGAATTTTACCCTACGTCATCTTAGGGATCATTGAAGAACATGGCCAGCTA
TCCGGCAAGGATATCACCAAGGAATTTACGACCGACATTGGGGAGTTTTGGAAGTC
CTCGCACAGCCAAATCTATCCGGAACTGCGGCGGATGACCACCGACCATTGGCTGG
CCGTGGTCCCGGACCCGGATAACGATAAGGAAATTCACTACCAACTAACTACGGAG
GGGCGCCGGATTTTAAACCAGTGGTTACAAACGCCGAAT

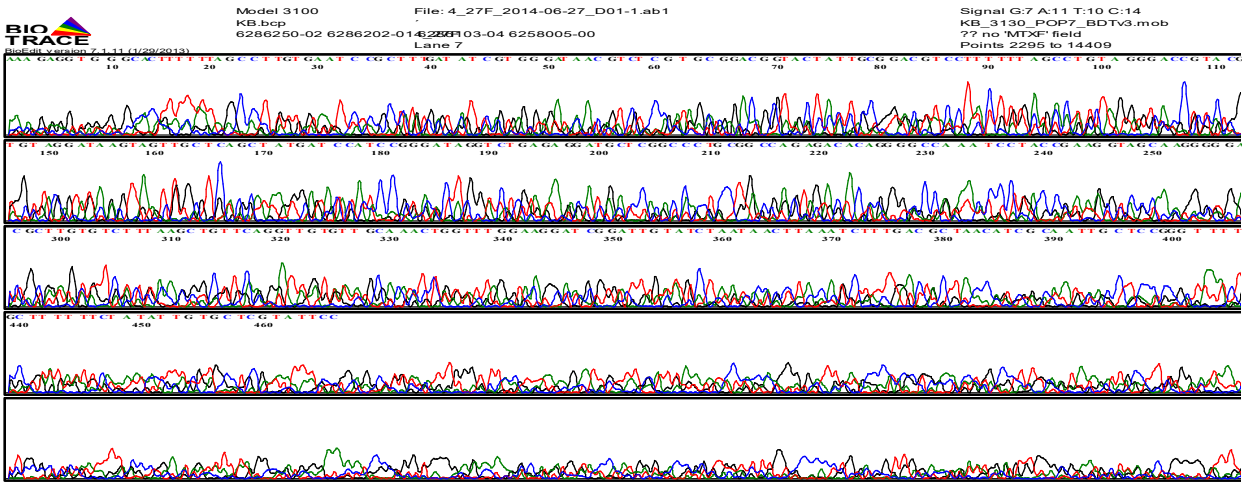

Figure 4.22: Sequencing of *Lactobacillus fermentum* from goat milk yoghurt

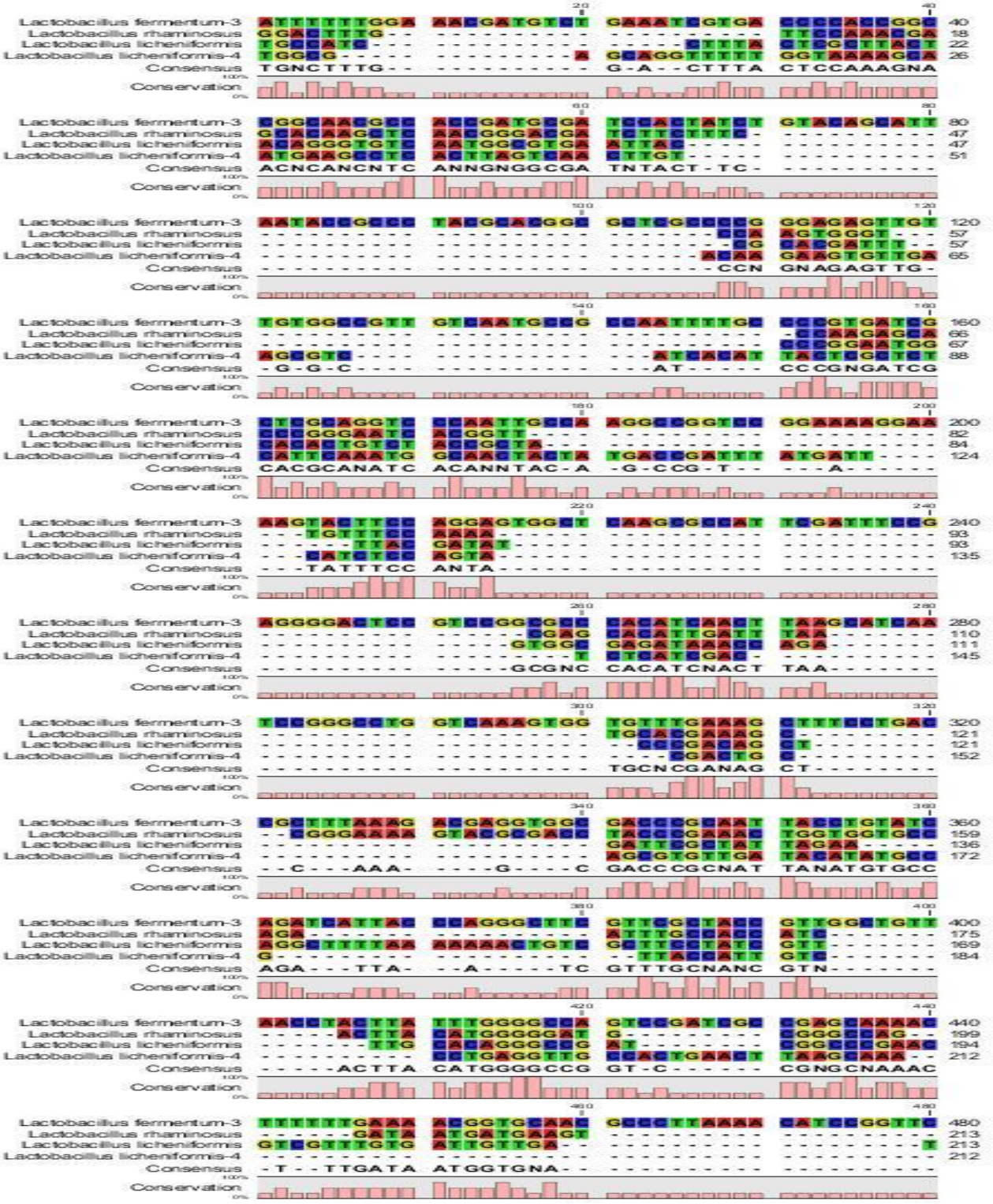

Figure 4.23: Alignment of *lactobacilli* from goat milk yoghurt

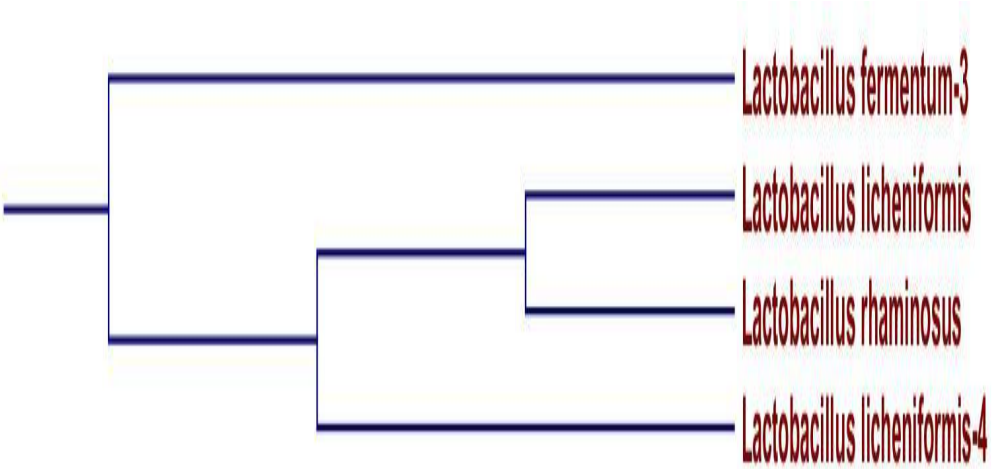

Figure 4.24: Phylogenetic tree of the lactic acid bacteria in goat milk yoghurt

4.8.5 Sequencing of Soursop Yoghurt Isolate

Subgroup A1 isolatesof lactic acid bacteria isolated from soursop yoghurt contained *Lactobacillus plantarum*, subgroup A2 had*Bacillus subtilis-3* while subgroup B2 contained *Lactobacillus fermentus-2 and Bacillus subtilis-2.Lactobacillus planetarum*was the only isolate in subgroup A1 category, showing it was abundant in the strain. In the case of tomato fruits, *Lactobacillus plantarum* was the most abundant species of LAB found (Di cagno *et. al.,* 2009). Species such as *Weissella cibaria, Lactobacillus plantarum* and *Lactobacillus lactis* subsp. *lactis* have been frequently found in environments associated with plants (Kostinek*et. al*., 2007, Escalante-Minakata *et. al.,* 2008 and Trias *et. al*., 2008). Isolations of lactic acid bacteria (LAB) from fruits and vegetables have frequently been reported (Nyanga *et. al*., 2007,Duangjitcha-roen*et. al.,* 2008 and Trias *et. al.,* 2008).Bacteriocins produced by LAB have attracted special interest as potential alternative safe commercial food preservatives and LAB have been used as food and feed preservatives for centuries.Bacteriocin-producing LAB can replace chemical preservatives for the prevention of bacterial spoilage and the outgrowth of pathogenic bacteria in food products (Di Cagno *et. al.,* 2009).

Sequences with forward and reverse primers were matched and compared with primer walking and the gaps were completed in this study. The sequences of 16S rDNA product of soursop yoghurt isolate are shown in Figures 4.25 to 4.28. The BLAST Analysis at the NCBI gene bank gave 100% homology to *Lactobacillus plantarum,* 100% homology to *Lactobacillus fermentum-2*, 100% homology to *Bacillus subtilis*-2, and 100% homology *Bacillus subtilis-3*.Introduction of *Lactobacillus acidophilus* in milk makes it more effective in lowering body cholestrol level (Sarkar, 2003). In fermented foods, LAB displays numerous antimicrobial activities mainly due to the production of organic acids and other compounds, such as bacteriocins and antifungal peptides (Simova *et. al*., 2004). *Lactobacillus acidophilus* which survives lowest pH ranges and tolerates the bile too has been proved to be very successful in preparation of yoghurt harbouring effective probiotic and these characteristics has made it most effective tool against lactose malabsorption and intolerance (Mustapha *et al*., 1997; de-Vrese *et al*., 2001).

CAACAAATTGTCGGTTACGACGTTAAACTAGCCCACTTAATCGCCAAAAACATCGGG
GTCAAGAAGGTTAGGTTCGTCAACATTGCCTTTCCATCCCTAATTAGTGAGCTGCAA
AACAAGAAGTTCGACATGGTGATGGCCGGAATGGTTTGGACCAAGGAACGGGCCAA
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GTCACCGAAAGTTCAATCACGACCCTGTCCCAAGAGGTGAAGGCGGGGACTTTGGA
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TTACGCCATCGCCAAGAACATTACCTTCAAGATTAGCGCCAAAACTTCTGACCCGCG
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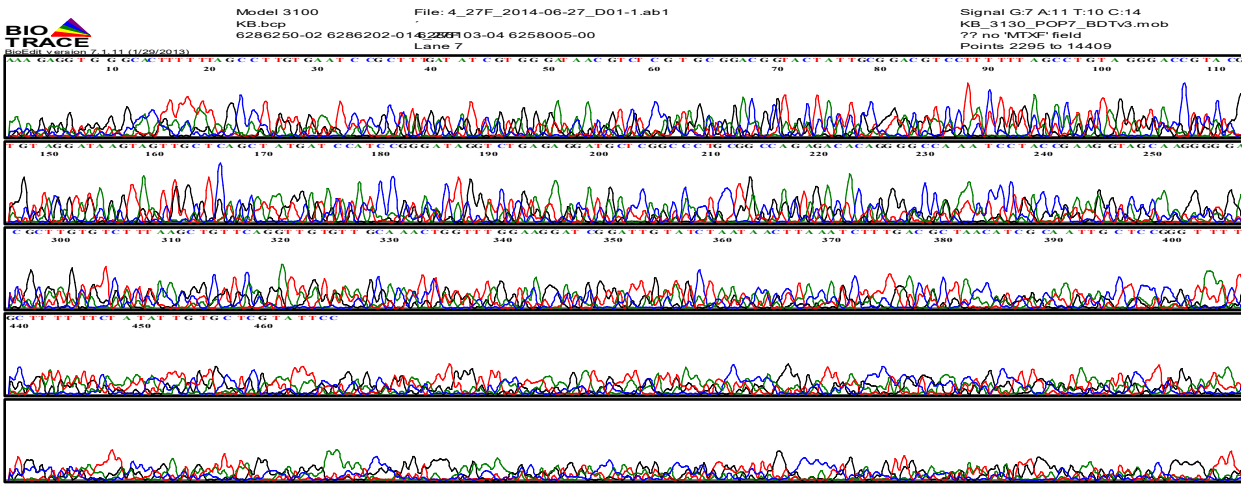

Figure 4.25 Sequencing of*Lactobacillus fermentum* fromsoursop yoghurt

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CCTCAACAGTGCGCCTATTTGATTAAGCAGTATGGCTACAACGCTGTAGACAAAGAA
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TCGAGCTGGCGACGAGAACGGCTGGGTTTTGCCGTTCCGTTGCATCATGTTGCCGAG
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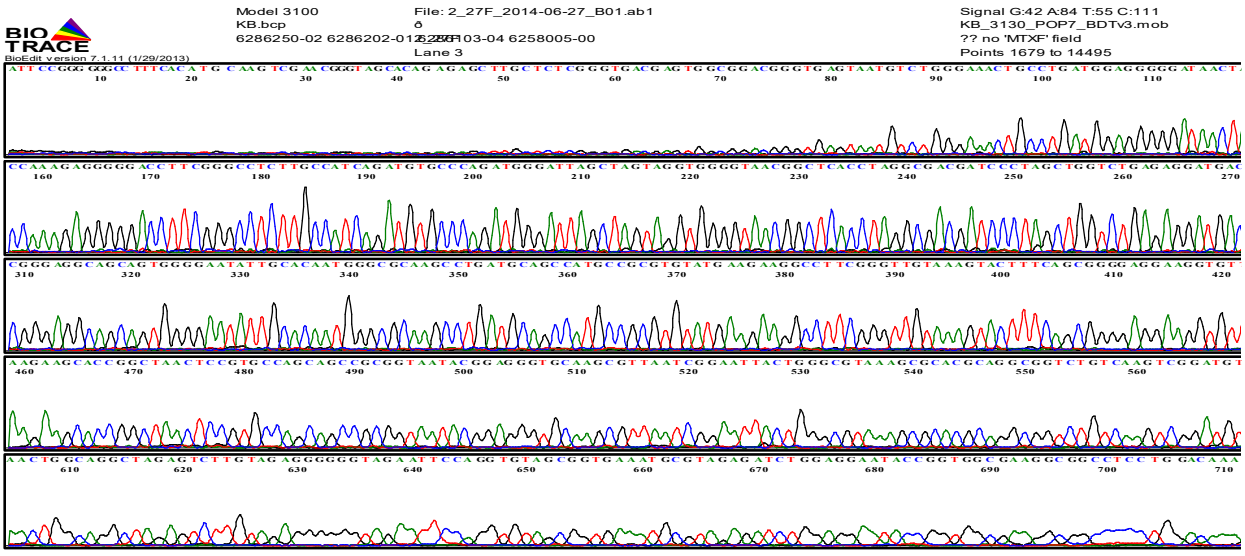

Figure 4.26 Sequencing of Bacillus *subtilis* from soursop yoghurt.

CATTGGCGTACGCTTCCGCCATACCGACCAACCGCCAGAAGACTGGGCCGGTTGTTT
CGTGACTGAAGCTAAACGCTATGTCTTCATCAGCAAGTGCTCGTGTTGTTTTAGCAA
AGCGAAAGCCTAGCACAAGAGCATTACCTAGTTGTGGGTCGTCGTGCAACTGGACA
AGTTCAGCACTCACGTAGTTGCCGGCTTCTCTCGTTTCCTTTATCCAAGCTTCCAGAA
CATTAGGTTTGTAGGTATTTCTAATATCAATCATGGTAGCCTCCTATTTGATAACCTG
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CGTCAGCTTGCTATCGCCTAGCATCAGTGCTGTCACTAACAGCTTCTTTTCATCGTGA
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TTGGCATAAAAGGCTAGGCGT

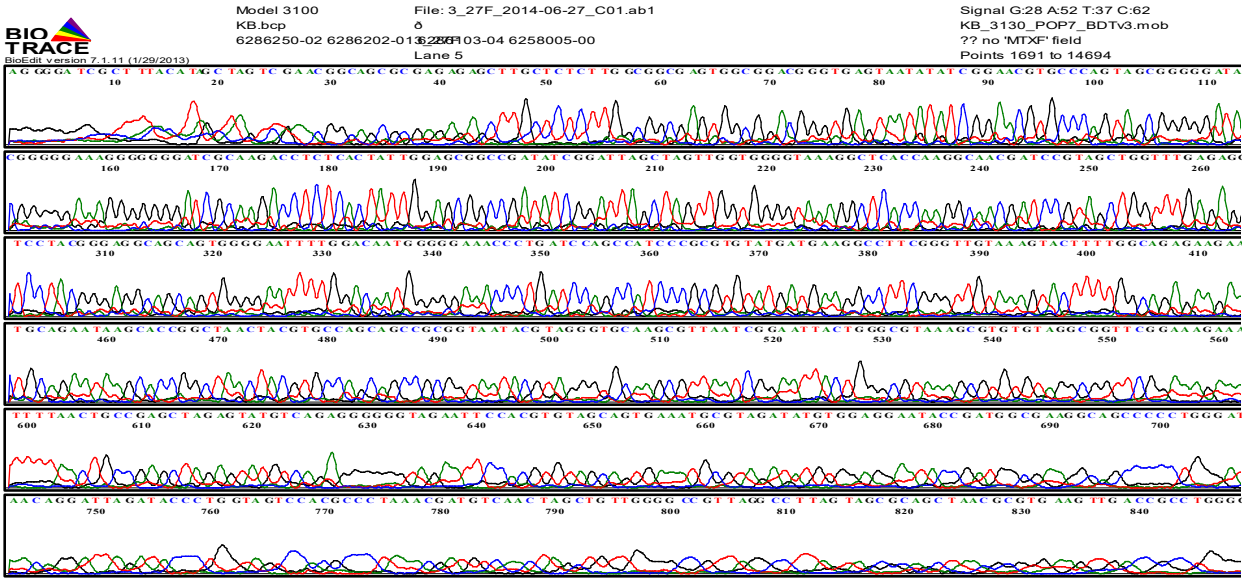

Figure 4.27 Sequencingof *Lactobacillus fermentum* from soursop yoghurt

ATTTTTTGGAAACGATGTCTGAAATCGTGACCCCACCGGCCGGCAACGCCACCGATG
CGATCCACTATCTGTACAGCATTAATACCGCCCTACGCACGGCGCTCGCCCCGGGAG
AGTTGTTGTGGCCGTTGTCAATGCCGCCAATTTTGCCCCGTGATCGCTCGCAGGTCCC
AATTGCCAAGGCCGGTCCGGAAAAGGAAAAGTACTTCCAGGAGTGGCTCAAGCGCC
ATTCGATTTCCGAGGGGACTCCGTCCGGCGCCCACATCAACTTAAGCATCAATCCGG
GCCTGGTCAAAGTGGTGTTTGAAAGCTTTCCTGACCGCTTTAAAGACGAGGTGGCGA
CCCGCAATTACCTGTATCAGATCATTACCCAGGGCTTCGTTCGCTACCGTTGGCTGTT
AACCTACTTATTTGGGGCCAGTCCGATCGCCGAGCAAAACTTTTTTGAAAACGGTGC
AACGCCCTTAAAACATCCGGTTCGCTGCATCCGCCAGTCCCGCCAGTACGGGTTTGG
CACCAAGTTTAGCGGCGATTACAGCTCAATTGATTCCTACGTCGAAACGATTTTAGC
GGCCGTTGAAAGCGGCGAACTACTGGCGCCGTCTGAATTTCACGGCCCAGTTCGGTT
TAAGGGCGCCGCGGATTTAAAGACGATGACCAAAGAGGGAGTTGAATACATCGAAC
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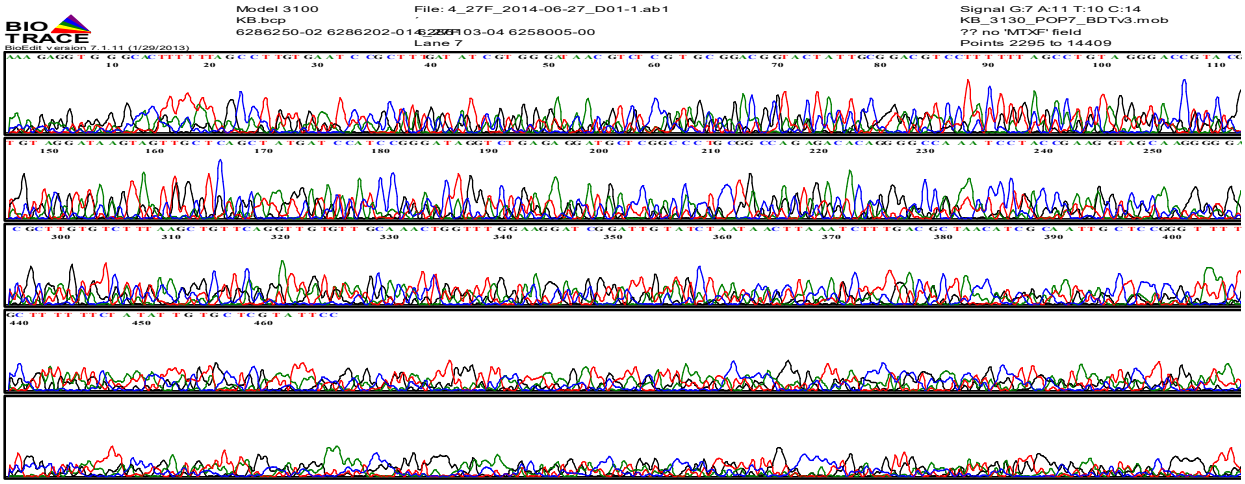

Figure 4.28 Sequencing of*Lactobacillus plantarium* from soursop yoghurt

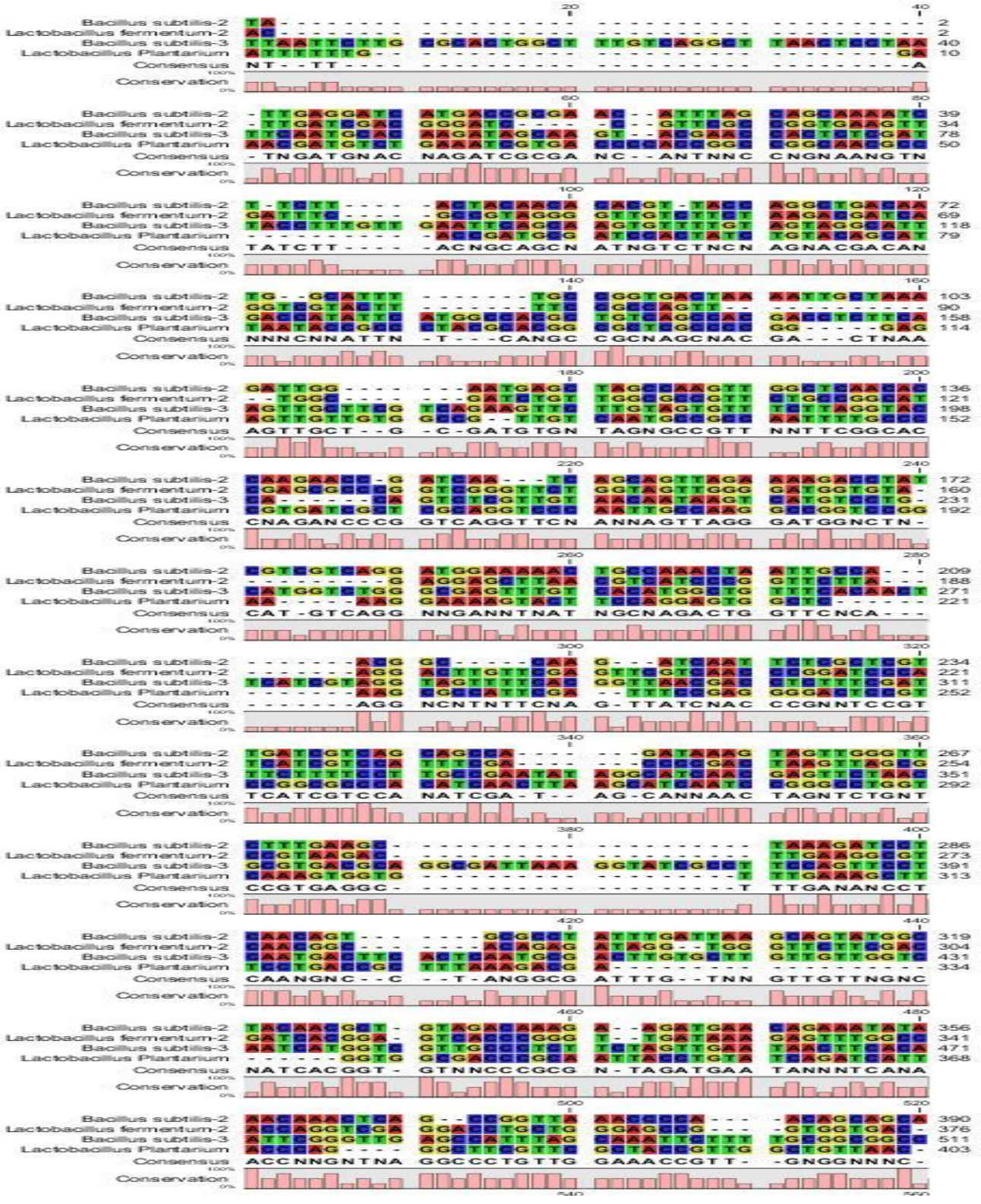

Figure 4.29 Alignments of lactobacilli from soursop yoghurt

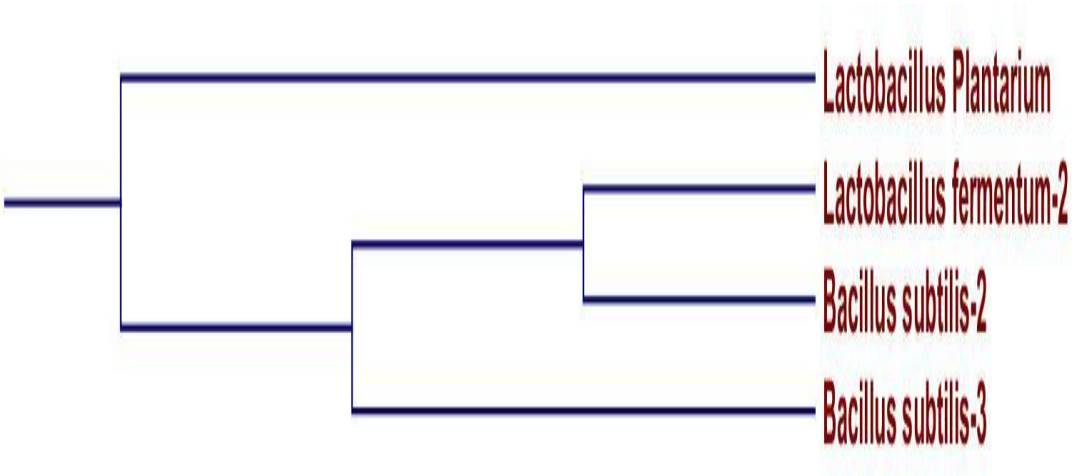

Figure 4.30: Phylogenetic trees of the lactic bacreria acid in soursop yoghurt

4.9 Specific roles of the Identified Non-Starter Lactic Acid Bacteria

In discussing the specific roles of *Lactobacillus* species in fermented foods one needs to consider their importance as probiotics and probiotics have been defined as live microorganisms which when administered in adequate amounts, confer a health benefit on the host (FAO/WHO 2002). *Lactobacilli* were among the first organisms used by man for processing foodstuffs (Konigs, 2000) and for preserving food by inhibiting invasion by other microorganisms that cause foodborne illness or food spoilage (Adams, 1999). Consumption of probiotic yoghurt and indeed probiotic-containing foods is known to confer benefits like anti hypertension properties (Lye *et. al.,* 2009), reduction of LDL-cholesterol levels (Sindhu and Khetarpane, 2013) and disruption of pathogenesis of hepatic encephalopathy (Solga, 2003).

Lactobacilli are gram-positive, non spore-forming rods or coccibacilli with a GC (Guanine-Cytosine) content generally in the 33–55% range (Coenye and Vandamme, 2003) and they are strictly fermentative, aerotolerant or anaerobic, aciduric or acidophilic, and have complex nutritional requirements (carbohydrates, amino acids, peptides, fatty acid esters, salts, nucleic acid derivatives, vitamins). Some lactobacilli are present as natural microflora of milk and are naturally present in fermented products and are thus included among the non-starter lactic acid bacteria. Non-starter lactic acid bacteria contribute to the flavour of fermented dairy foods (Beresford *et. al.,* 2001; Wouters, 2002). Lactobacillimay also be deliberately added as starters for fermented dairy foods, for technological reasons or to improve texture, flavour, taste or aroma. Thus,*Lactobacillus plantarum* and *Lactobacillius fermentus* are important bacteria used in food fermentations. Selection criteria for LAB depend on the type and the desired characteristics of the final product, the desired metabolic activities, the characteristics of the raw materials and the applied technology. The group of Lactic Acid Bacteria (LAB) occupies a central role in these processes and has a long and safe history of application and consumption in the production of fermented and beverages (Caplice and Fitzgerald, 1999). Lactic acid bacteria cause rapid acidification of raw materials through the production organic acids, mainly lactic acid.The production of acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharides and several enzymes by lactic acid bacteria is of importance because of shelf life enhancement, microbial safety, texture improvement and contribution to pleasant sensory profile of end products.

Bacillus licheniformis is a spore-forming probiotic which is often used in conjunction with other probiotic*Bacillus* species, like *Bacillus subtilis*.*Bacillus licheniformis* is the source material from which the antibiotic bacitracin is produced. As a probiotic, *Bacillus licheniformis* perform the same function as it does in bacitracin to prevent the growth of harmful bacteria. Moreover, *Lactobacillus rhamnosus, Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus brevis, Lactobacillus buchneri, Lactobacillus curvatus, Lactobacillus acidophilus* and*Lactobacillus pentosus* naturally present in unpasteurized milk (dairy products) (Wouters 2002). *Lactobacillus plantarum* is present in wine (non-dairy products) (Spano 2005). *Lactobacillus plantarum, Lactobacillus paraplantarum, Lactobacillus paracasei* subsp. *tolerans, Lactobacillus sake, Lactobacillus curvatus* and *Lactobacillus pentosus* are present in Batzos, a traditional Greek cheese from unpasteurized goat's milk (Psoni 2003). Much indigenous cereal fermentations involve the combined action of bacteria and yeasts. *Lactobacillus fermentum and Lactobacillusamylovorus* have been suggested to be the predominating microorganismsformed during the fermentation of sorghum dough in Sudanese Kisra (Asmahan and Muna, 2009). The *Lactobacillus*starter in sorghum flour fermentation was found to have decreased the traditional fermentation time from 19hrs to 6hrs, and this have contributed, in no small way, in encouraging the production and development of more sorghum based products (Asmahan and Muna, 2009).

In agriculture, lactobacilli have been used to preserve grass or maize in the form of silage (Adesogan and Salawu 2002); and these lactobacilli accelerate decrease in pH, conserve plant carbohydrates through homofermentation and conserve plant proteins by deamination and by decreasing proteolysis (Holzer *et. al*. 2003). Biomedical research on lactobacilli currently offers new prospects for applications and this may have far-reaching results in the future if the international community takes up the suggestion of Reid *et. al.,*(2005) with respect to the potential benefits of lactic acid bacteria probiotic and food or dietary supplements (lactobacilli included) in slowing morbidity and mortality associated with HIV/AIDS and gastroenteritis. Thus, this approachcan be of great potential value for remote communities with limited access to health care (Reid *et. al.* 2005).

4.10 Proximate Compositions of Experimental and Control Diets

The proximate compositions of experimental (TT) and control (casein) diets (CD) are shown in Table 4.16 and the composition of the nitrogen-free diet is shown in appendix 30. There were significant difference ($p<0.05$) in the moisture, protein, fat, ash, crude fibre and carbohydrate contents between experimental and control diets respectively. The control diet had the lowest moisture content of 9.81% while the experimental diet had the highest moisture content of 57.04%. The result of the moisture content of experimental diet is similar to the report of Belewu *et. al.,* (2005) who reported 60.25% to 64.23% moisture content for cheese treated with chemical and biological preservatives. The moisture content observed in experimental diet (57.04%) was similar to the values (63% to 64%) reported by Lawal and Adedeji (2013) for warankasi samples obtained from various locations (Oshodi, Mushin and Iyana Oba). Adetunji (2008)reported moisture content values of 61.70 to 62.50% with different coagulants. The high moisture content observed from the sample may probably be as a result of the moisture content of the milk used for processing and subsequent formation of thick curd. The results of moisture content of cheese in this study agree with the observations made by previous research on warankasi by Ogundiwin and Oke, (1983), Fasakin and Unokiwedi, (1992) and Aworh and Akinniyi (1999) reported that moisture content accounts for the textural property of the product sample. Therefore, moisture content is a measure of the water content in a product sample.

The protein content for the experimental diet was 16.10%, which was lower than the values of 31.60-33.84% reported by Adetunji *et. al*. (2008) in the production of cheese using *Carica papaya* (leaf) and *Calotropis procera* and 20.6-21.09% reported by Lawal and Adedeji (2013) for protein content of cheese recorded at various locations in Lagos Metropolis. The quality of protein in raw milk may be affected by many factors such as nutrition, specie and biological value of the protein in the milk. The increase in the protein content of samples may be due to the presence of some micro-organisms and/or their enzymes which aid in the synthesis of nitrogenous substances. The high level of protein of warankasi thus gives an indication that it can meet up with the protein requirements of the body. Unfortunately, these products are not eaten as staples in developing countries but as snacks (Metwalli *et. al*., 1992). Functionally, proteins are important in foods as they help in the growth and development of the body (Kathleen *et. al*, 1996).

Table 4.16: Means Values of Proximate Compositions of Experimental and Control Diets

Diet Sample	Moisture Content (%)	Protein Content (%)	Fat Content (%)	Ash Content (%)	Crude Fibre (%)	Carbohydrate Content (%)
CD	9.81[b]	8.77[b]	4.50[b]	8.50[a]	5.10[a]	45.32[a]
TT	57.04[a]	16.10[a]	10.24[a]	1.10[b]	1.35[b]	14.17[b]

Means in a column with the same letter are not significantly different from one another ($p<0.05$).

CD - control diet

TT – experimental diet

4.10.1 Protein evaluation quality of experimental and control diets

The results of nutritional evaluation of protein quality of experimental, control and nitrogen-free diets are shown in Table 4.17. Average final weight of the animals fed with experimental diets (TT) was the highest (160.55g) and this was significantly different ($P<0.05$) from those fed with nitrogen-free and control diets. The mean weight gain was highest for rats fed soft-cheese (experimental diet) with 35.47% followed by rat fed with control diets (21.20%) while rats fed with nitrogen-free diet had the lowest weight gain of 17.14%. Weight gain is known to be the most important criterion for measuring rat responses to experimental diets and a very reliable indicator of growth (Lovell 1989), thus the results in this study agree with the findings of Imaizumi *et. al.,* (1991) who found that probiotics diet caused body weight gain in diabetic rats. There were significant differences ($p<0.05$) in protein efficiency ratio (PER), net protein utilization (NPU), true digestibility (TD) and biological value (BV) between rats fed with experimental and control diets (Table 4.18). The results of PER values obtained from this study were 2.42 and 2.20 for control and experimental diets respectively. These values are in line with the findings of Osundahunsi and Aworh (2003) for rats fed maize based complementary foods enriched with soyabean and cowpea tempeh which ranged from 2.30 in ogi-soy tempeh to 2.42 ogi-cowpea tempeh respectively. The results of PER values are comparable with that reported by Oluwamukomi *et. al*., (2003) for rats fed sorghum-cowpea based weaning foods. The NPU which ranged from 69.46% for control diet to 76.35% for experimental diets were comparable to the values reported by Egounlety *et. al.,* (2002) for tempeh fortified maize based weaning foods-65.02% for ogi-cowpea tempeh to 72.42% for maize-cowpea tempeh diets respectively.The results of digestibility of the control and experimental diets are shown in Table 4.18. The true digestibility ranged from 63.44% in experimental diet to 41.55% in control diet. Digestibility of protein is related to the quality of protein in the feed. The higher ratio of total essential amino acid to total amino acid contents in samples resulted in rapid growth of rats. The high ratio of essential amino acids to total amino acids in meat products explain their superiority in protein quality when used as new protein sources for rats (Hoffman and Falvo, 2004). The nutritional quality of any protein relates to its amino acid composition, digestibility, and ability to supply the essential amino acids in the amounts required by the species consuming the protein (Endres, 2001). Fibre is able to alter the digestion rate by various mechanisms, which depend on the chemical composition of the fibre and its physical properties, such as viscosity (Stasse-Wolthuis,

1981; Topping *et. al*., 1988). Dietary fibre can decrease casein hydrolysis by different mechanisms, including a direct effect, reducing the enzyme-substrate binding (Schneeman and Gallahar, 1985), by altering the enzymatic conformation (Roehrig, 1988) or in the case of non-purified fibre sources, by interaction with enzyme inhibitors (Dunaif and Schneeman, 1981). Viscosity can reduce digestibility by modulation of the movement and contact of digestion and enzymes (Shah *et. al*., 1986). Lamghari El Kossori *et. al*., (2000) thought that the decrease in nitrogen release caused by soluble dietary fibre was due to the interaction of the fibre with enzymes or protein rather than to the viscosity.Dietary fibre, in varying degrees, appears to reduceor delay the absorption of protein, fat and carbohydrates,as well as certain minerals and vitamins.The digestion and absorption of nutrients in the smallintestine are influenced by the consumption ofdietary fibre and different sources of dietary fibrehave different effects on small intestine nutrientabsorption. It has been demonstrated that solubledietary fibre increases the time of intestinal transit(Brow *et al*., 1988), delays gastric emptying(Rainbird and Low, 1986) and glucose absorption(Todd *et al*., 1990) and can alter lipid assimilation(Pasquier *et. al*., 1996). Insoluble dietary fibredecreases intestinal transit time and increases faecalmass (Schneeman, 1990).

The results of the weights of internal organs excised from the rats fed with experimental (soft cheese) TT, nitrogen-free (BH) and control (CD) diets are shown in Table 4.19. Kidney weight of rats that were fed with BH was significantly lower (0.64) ($p<0.05$) than those fed with TT and CD control diets. There were no significant difference ($p>0.05$) in the weights of the liver, spleen and heart of all the animals. The results of organ weights suggest development of muscularized liver organ in order to handle some extraneous components of the diets (Ijarotimiro and Kehinro, 2012). The liver has been considered to be the nutritional indicator of the body of which dietary and toxic factors in the food consumed may interfere with its function. However, the body weights of experimental animals have influence on liver weight. The group of rats fed casein and experimental diets had higher weight gain and highest liver weights when compared with the rats fed with nitrogen-free diets. Relatively, low liver lipid and high liver weight have been reported in rats fed casein diet (Pore and Mager, 1978).

Table 4.17: Effects of feed treatment on weight gain

Samples	Initial Weight	Final Weight	Weight Gain (%)
CD	116.12[b]	140.74[b]	21.20[b]
TT	118.51[a]	160.55[a]	35.47[a]
BH	113.80[b]	133.30[b]	17.14[b]

Means with the same superscripts on the same column are not significantly different from one another ($p<0.05$).

CD - control diet

TT - experimental diet

BH – nitrogen-free diet

Table 4.18: Effect of feed treatment on protein quality

Samples	Protein Efficiency Ratio	Net Protein Utilization (%)	True Digestibility (%)	Biological Value (%)
CD	2.42[a]	69.46[b]	41.55[b]	95.96[a]
TT	2.20[b]	76.35[a]	63.44[a]	86.95[b]

Means with the same superscripts on the same column are not significantly different from one another ($p<0.05$).

CD - control diet

TT - experimental diet

Table 4.19: Selected organs relative to body weight of rats after feeding duration (28 days)

Sample	Kidney	Liver	Spleen	Heart
CD	0.849[a]	4.425[a]	0.932[a]	0.486[a]
TT	0.768[a]	4.344[a]	0.662[a]	0.502[a]
BD	0.643[b]	3.725[a]	0.506[a]	0.500[a]

Means in a column with the same letter are not significantly different from one another ($p<0.05$)

CD - control diet

TT - experimental diet

BH – nitrogen-free diet

4.10.2 Haematological parameters of rats fed with experimental (soft cheese), protein-free and control diets

The results of haematological parameters are shown in Table 4.20. Packed cell volume of rats fed with experimental TT (soft cheese), nitrogen-free (BH) and Control (CD) diets varied from 37.75% to 40.00%. There was no significance difference ($p>0.05$) between the PCV of all the animals. The packed cell volume (PCV) values (%) obtained were within the normal range for rats (37.6- 50.6%) reported by Johnson-Delaney (1996). Oyawoye and Ogunkule (1998) reported that normal values observed in the concentration of packed cell volume (PCV) in the blood suggest absence of a toxic factor (haemagglutamin) which could have adverse effect on blood formation. The haemoglobin concentration of rats fed experimental (soft cheese) diets ranged from 14.30g/100ml to 13.90g/100ml in control diet while that of rats fed nitrogen-free diet was 13.70g/100ml. The haemoglobin concentrations were in the same range with that reported by Johnson-Delaney (1996). Low PCV has been associated with protein deficiency Pkesatcha *et. al.,* (2012). Blood is of high physiological significances in the animal body and inadequate intake of dietary protein can affect blood synthesis of which any abnormal variation will affect its primary function.

Red blood cell (RBC) counts varied from 6.56 in nitrogen-free diet to 7.17 in experimental (soft cheese) diet. There were no significant difference ($p>0.05$) between the RBC counts of rats fed nitrogen-free, experimental diets and the control diet. The result of this study is in range with the report of Kumar *et. al.* (2011) for rats administered with *Lantana aculeate* weed extract with values which ranged from 7.89 in control to 8.37 in rats administered 100mg/kg body weight the RBC counts were higher than the report of Osundahunsi and Aworh (2003), 5.77 in maize cowpea tempeh to 5.96 in ogi-cowpea tempeh. There was no significant differences ($p>0.05$) in the values of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), for rats fed experimental, nitrogen-free and the control diets. The MCV values which ranged from 55.79fl to 58.59fl in the diets and the values obtained were comparable with that reported by Kolawole and Alemika (1996) for rats administered with halofantrine. In this study, mean corpuscular haemoglobin (MCH) ranged from 19.43pg in control diet to 21.09pg in nitrogen-free diets. The mean corpuscular haemoglobin concentration (MCHC) values of rats fed with the experimental diets and skimmed milk diet were not significantly different from one another and the value

ranged from 34.77% to 36.58% in all the rat diets respectively and these values were comparable with that reported by Edem (2009) for rats fed with diets containing palm oil. There were significant differences ($p<0.05$) between the white blood cell of the rats fed with the experimental diets and with those fed nitrogen-free (basal) control and diet.

Table 4.20: Means values of some hematological parameters of rats fed experimental and control diet

Diet Sample	PCV (%)	Hb (g/100ml)	RBC ($X10^3$/ µl)	MCV (fl)	MCH (pg)	MCHC (%)	WBC ($X10^3$/µl)
CD	40.00[a]	13.90[a]	7.16[a]	55.89[a]	19.43[a]	34.77[a]	7.53[a]
TT	40.00[a]	14.30[a]	7.17[a]	55.79[a]	19.96[a]	35.99[a]	6.63[c]
BH	37.75[a]	13.70[a]	6.56[a]	58.59[a]	21.09[a]	36.58[a]	7.23[b]

Means in a column with the same letter are not significantly different from one another ($p<0.05$)

CD - control diet

TT - experimental diet

BH – nitrogen-free diet

4.10.3 Biochemical parameters of rats fed with experimental (soft cheese), protein-free and control diets

The alkaline phosphatase (ALP), aspartate amino transferase (AST) and alanine amino transferase (ALT) values for all the diets were significantly different ($p<0.05$) from one another (Table 4.21) and the values ranged between (55.25-60.75 µ/L) for ALP, (45.00-47.50 µ/L) for AST and (17.25-20.50 µ/L) for ALT respectively. These values were found within specified rangesfor safety: ALP (56.8-128µ/L), AST (45.7-80.8 µ/L) and ALT (17.5-30.3 µ/L)(Johnson-Delaney 1996). The different diets used as experimental, control and protein-free diets did not alter the plasma enzyme levels. Lee (2009) reported that liver function tests are groups of blood test that give information about the state of a patient's liver. Benjamin (1978) also reported that increased values of liver enzymes are associated with higher activity and may likely indicate damage or hyperplasia of liver cells. Liver transferases (AST and ALT) are useful biomarkers of liver injury in a patient with some degree of intact liver function (Johnson-De1999).

The total protein for the control and protein-free diets were significantly different ($p<0.05$) from the experimental diet but all the diets were within the specified standard for total protein of between 5.6-7.6g/l. There was no significant difference in albumin values of the control and experimental dietsand they were within specifications Johnson-Delaney (1996). Fluctuations in the levels of total protein and albumin in the rats' blood serum are reflections of likely deviation from the normal liver function (Ahmed *et. al*. 1992). Lee (2009) reported that several biochemical tests are useful in the evaluation and management of patients with hepatic dysfunction and these tests can be used to detect the presence of liver disease, distinguish among different types of liver disorders, gauge the extent of known liver damage and follow the response to treatment.

Table 4.21: Means values of differential white blood cell count of rats fed experimental and control diets

Diet Sample	ALP (μ/L)	ALT (μ/L)	AST (μ/L)	Total Protein (g/dL)	Albumin (g/dL)	Globulin
CD	60.75[a]	20.50[a]	47.50[a]	6.93[b]	4.23[a]	2.90[a]
TT	57.00[b]	19.00[b]	46.00[b]	7.25[a]	4.45[a]	2.98[a]
BH	55.25[c]	17.25[c]	45.00[b]	6.60[b]	3.75[b]	2.83[a]

Means in a column with the same letter are not significantly different ($p<0.05$)

ALP; Alkaline phosphatase, ALT; Alanine amino transferase,

AST; Aspartate amino transferase

CD - control diet

TT - experimental diet

BH – nitrogen-free diet

4.11 Use of LAB Isolates as Single and Mixed-Strain Starter Culture in Yoghurt Production

The results obtained from the use of LAB isolates as single and mixed-strain starter culture in yoghurt processing are shown in Table 4.22. Statistical analysis revealed that treatments A and B were significantly different from one another ($p<0.05$). Treatment A was the combination of the commercialized starter culture (yogourmet) with one of the LAB isolate (*Lactobacillusfermentus*) and Treatment B was combination of two different LAB isolates (*Lactobacillus fermentus* and *Lactobacilluslicheniformis*) as starter culture. The average total titratable acidity of treatment A (Starter culture + LAB isolates) was 1.40% while the average total titratable acidity (TTA) of treatment B (LAB isolates) was 1.28%. The average TTA of natural yoghurt was 1.44%.The result of treatment A with respect to findings of Tarakci and Erdogan (2003) in which acidity increased over storage period. Guler and Mutlu (2005) also observed an increase in TTA during storage period. The pH values of treatments A and B are shown in Table 4.22. Treatment B had a lower pH value of 3.46, when compared with the pH value 4.12 of treatment A. Sutherland and Varman (1994) reported yoghurt's pH as 4.50 as lactic acid bacteria produce lactic acid during fermentation of milk- lactose, thus lowering its pH (Eke *et. al,* 2013). Food Standard Code requires that the pH of yoghurt should be a maximum of 4.50 in order to prevent the growth of any pathogenic organism (Donkor *et. al*., 2006). Gelation and acidification processes of yoghurt are affected by starter culture characteristics. Selection criteria for lactic acid bacteria include acidification rate, aroma, flavour and texture characteristics (Bouzar *et. al* 1997). Several studies refer to the effect of lactic acid bacteria producing exopolysaccharides on the rheological properties of yoghurt, which are often used to increase the viscosity of yoghurt products (Duboc and Mollet 2001; Laws and Marshall 2001).

The average protein content of treatment A was 3.5 while the average protein content of treatment B was 2.7 and the result for treatment A was in line with the findings of Janhoj *et. al*., (2006) who found that protein contents of low-fat stirred yoghurt ranged from 3.4% to 6.0%. The total soluble sugar (TSS) in treatments A and B were 14.6^0 brix and 9.4^0 brix respectively.The decrease in TSS for B may be attributed to yeast utilization of sugars in metabolic processes for energy production. Decreases in total soluble solids in yoghurts from 7.33% to 6.83% and 15.33% to 14.93% for corn milk and cow milk yoghurts respectively, have also been reported

and the reductions have been attributed to the utilization of sugar by the starter cultures (Vasiljevic and Jelen, 2002; Wang *et. al.*, 2002).

The average fat contents of treatments A and B which were 0.30% and 0.06% respectively were in line with findings of Janhoj *et. al*., (2006), who reported that fat contents for low-fat stirred yoghurt ranged from 0.3 to 3.5%. Fat content has been reported to have positive influence on the physical and sensory characteristics (Bille and Keya, 2002; Marinescu and Pop, 2009) and negative impacts on the shelf stability of yoghurts (Saint-Eve, 2008; Farinde *et. al*., 2009). Using LAB isolates as single or mixed strain starter culture, treatment A had better attributes for use as starter culture as its results met with yoghurt recommended standards.

Table 4.22: Means of Physical and Chemical Analysis of Single and Mixed Strain Starter Cultures

Treatments	Total Titratable Acidity (% lactic acid)	pH	Moisture Content (%)	Protein Content (%)	Fat Content (%)	Total Solids (^{0}brix)
A	1.40[a]	4.12[a]	87.65[b]	3.5[a]	0.30[a]	14.6[a]
B	1.34[b]	3.46[b]	91.74[a]	2.7[b]	0.06[b]	9.4[b]

Means in a column with the same letter are not significantly different ($p<0.05$)

Treatment A (Starter culture + LAB isolates)

Treatment B (LAB isolates + LAB isolates)

4.12 Sensory Evaluation of Soursop, Cow Milk and Goat Milk Yoghurts.

The mean sensory scores of organoleptic evaluation and acceptability of the different yoghurts samples are shown in Table 4.23. The statistical analysis revealed that there were no significant difference ($p>0.05$) among the yoghurt samples in sensory attributes. The mean scores for flavour ranged from 5.30±1.56 to 7.00±1.38. A higher score for flavour of cow milk yoghurt was observed indicating a higher acceptability due to its familiarity among panelists.Goatmilk and cowmilk yoghurts have the highest mean scores for appearance of all the samples evaluated because of the high fat content in the samples. The mean scores for thickness of yoghurt samples ranged from 5.70±1.30 to 7.50±1.39 and the level of likeness was in increasing orders of cow milk yoghurt, goat milk yoghurt and soursop yoghurt. Soursop yoghurt had the least value possibility because of extraction process from a fruit; which reduced its viscosity. Cowmilk yoghurt had the highest scores of 6.90 and 7.00 for both taste and flavour while soursop yohurt had the lowest scores of 5.15 and 5.30 for taste and aroma respectively. Most of the panelists appreciated cowmilk flavour which was attributed to its fat content. Saint-Eve *et. al*., (2008) reported that fat content had a considerable influence on the sensory and instrumental characteristics of yoghurt, because the oil acts as an aroma solvent and has better rheology compared to low fat and skimmed yoghurts. Sanful (2009), reported that flavour and aroma scored higher rating for increased coconut-milk input in the production of yoghurt.Average overall acceptability scores ranged from 4.85±1.53 to 7.35±0.87 among the yoghurt samples. The results showed that the overall acceptability of cow milk yoghurt was not significantly different ($p>0.05$) from goat milk yoghurt but cow milk yoghurt had higher values for overall acceptability, colour, flavour, taste and thickness. Yoghurt from cow milk was most preferred among the samples presented for sensory evaluation and Barnes *et. al.,* (1991) reported that mouth feel, flavour, sweetness, sourness, and the balance between these factors have been shown to affect the overall preference for yoghurt.

Table 4.23: Sensory Evaluations of Soursop, Cow Milk and Goat Milk Yoghurts.

Attributes	Soursop Yoghurt	Cow milk Yoghurt	Goatmilk Yoghurt
Appearance	4.80±1.44[a]	7.80±0.77[a]	7.30±0.98[a]
Colour	5.25±1.12[a]	7.75±1.07[a]	7.35±1.09[a]
Flavour	5.30±1.56[a]	7.00±1.38[a]	6.25±1.62[a]
Thickness	5.70±1.30[a]	7.50±1.39[a]	6.70±1.03[a]
Taste	5.15±1.53[a]	6.90±1.52[a]	6.55±1.39[a]
Overall Acceptance	4.85±1.53[a]	7.35±0.87[a]	6.65±1.42[a]

Means in a row with same superscript are not significantly different from one another ($p<0.05$).

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study showed that goat milk yoghurt and soft cheese samples had high protein, fat and carbohydrate contents when compared with soursop and cowmilk yoghurts. Further analysis revealed that soursop yoghurt had more minerals (potassium and sodium) than cow and goatmilk yoghurts while soft cheese had more zinc and magnesium than other yoghurts' samples.

Lactobacillus plantarium,Lactobacillus fermentum-2, *Bacillus subtilis*-2, *Bacillus subtilis-3*, *Lactobacillus fermentum-3,Lactobacillus licheniformis*, *Lactobacillus rhaminous* and *Lactobacillus licheniformis-4* were the predominant probiotic LAB isolates from yoghurts and soft-cheese sample.

The results from this study revealed that yoghurts' samples from soursop fruits, cow milk and goat milk treated with *Aframomum danielli* and turmeric extracts at 2.0% and 2.5% reduced microbial activities and improved storage stability of yoghurt and soft cheese samples for eight weeeks at refrigerated condition while control samples got spoilt within 3 days.

Animals fed with experimental diet (soft cheese) showed significant weight gain (35.47%), compared with the control and nitrogen-free diet (21.20% and 17.14%). Moreover, the results showed that the overall acceptability of cow milk yoghurt was not significantly different from goat milk yoghurt although cow milk yoghurt had a higher overall acceptability, colour, flavour, taste and thickness compared to other samples.

5.2 Recommendations

From this study, it has been realised that soursop fruit is a rich source of dietary fibre, ascorbic acid and can have significant impact on the health of consumers, therefore dairy and non-dairy fermented products (from soursop) can be recommended for children and the elderly for protection from diarrhea. As yoghurts and cheese are good sources of protein, minerals and vitamins (ascorbic acid), further studies should be carried out using soursop fruits for the production of jams, jellies and mamalades which can be included in a number of different diets or for commercial purposes to reduce the rate at which these fruits spoil during the harvesting period. The LABS identified in this study should be purified for commercial production of probiotics for possible use instead of importing starter cultures for yoghurts.

5.3 Contributions to knowledge

- Combine effect of *Aframomum danielli* and tumeric at refrigerated temperature improved storage stability of soursop, cow and goat milk yoghurts and soft cheese samples for 8 weeks while control samples got spoilt within 3 days.
- *Lactobacillus plantarum,Lactobacillus fermentum*, *Bacillus subtilis*, *Lactobacillus fermentum,Lactobacillus licheniformis* and *Lactobacillus rhaminosus* were identified in yoghurts and cheese samples using molecular methods.
- These *Lactobacillus* isolates were used successfully as starter culture when tested as multiple-strain starter culture in yoghurt production.
- Lactic acid bacteria isolates identified in soursop yoghurt were similar to LAB isolates in soft-cheese (*Lactobacillus plantarum, Bacillus subtilis)* and the *Lactobacillus plantarum* strain belongs to the group of mesophilic lactobacilli; commonly met in the later phase of the maturing cheese.

REFERENCES

Abbo, E.S., Olurin, T.O. and Odeyemi, G. 2006. Studies on the storage stability of soursop (*Annona muriatic* L.)juice. *African Journal Biotechnology* 5.19: 108–112.

Abdalla, M.O. and Mohammed, S.N. 2009. Effect of storage period on chemical composition and sensory characteristics of vacuum packaged white soft cheese. *Pakistan Journal Nut*rition 8.2:145-147.

Abdelgadir, W.S., Hamada, S.H., Muller, P.L. and Jakobsen, M. 2001. Characterization of the dominant microbiota of Sudanese fermented milk. *International Dairy Journal* 11:63-70

Abena, A.B., Faustina, D.W., Jacob, K.A. and Ibok, O. 2014. Dietary fibre, ascorbic acid and proximate composition of tropical underutilized fruits. *African Journal Food Science* 8.6: 305-310.

Abrahamsen, R. K. and Holmen, T. B. 1981. Goat milk yoghurt made from non- homogenized and homogenised milks, concentrated by different methods. *Journal Dairy Research* 48: 457-463.

Acharya, M.R. and Mistry, V.V. 2005. Effect of vacuum condensed or ultra filtered milk on pasteurized process cheese. *Journal of Dairy Science* 88: 3037–3043.

Achinewhu, S. C. 1983a. Chemical and nutrient composition of fermented products from plant foods. *Nigeria Food Journal* 1: 115-16.

Achinewhu, S. C. 1983b. Protein quality of African oil bean seed *(Pentactethra macrophylla). Journal Food Science 48: 1374-5.*

Adams, M.R. 1999. Safety of industrial lactic acid bacteria.*Journal Biotechnology* 68: 171– 178

Adegoke, G. O., Musenbi, S., Wachira, S., Muigai, C., M uigai, F., Ngumba, E., Karanja, P., Onyango, C. and Adoko, M. 2013. Production of probiotic yoghurt flavoured with the spice, *Aframomumdanielli*, strawberry and vanilla. *Food and Public Health* 3.2: 92-96.

Adegoke, G.O., Gbadamosi, R., Evwoerhurhoma, F., Uzo-Peters, P.I, Falade, K., Hiola, O., Moody, O. and Skura, B. 2002.Protection of maize (Zeamays) and soybean (*Glycine max*) using *Aframomum danielli.European Food Research Technol*ogy 214: 408-411.

Adegoke, G.O. and Gopalakrishna, A.G. 1998. Extraction and identification of antioxidants from the spice *Aframomum danielli. Journal Amazon Oil Chemicals Society* 75: 1047-1052.

Adegoke, G.O., Iwahashi, H. and Komatsu, Y.1997. Inhibition of *Saccharomycescerevisae* by combination of hydrostatic pressure and monoterpenes. *Journal Food Science* 62: 404 - 405.

Adegoke, G.O. and Skura, B.J. 1994. Nutritional profile and antimicrobial spectrum of the spice *Aframomum danielli. Plant Food Human Nutr*ition 45: 175-182.

Aderinola, T.A. and Olarenwaju, Z.Z. 2014. Quality and microbiology evaluation of African yam beans yoghurt supplemented with cow milk. *Journal of Microbiological Biotechnology and Food Science* 4: 70-77.

Adesogan, A. T. and S. B. Salawu. 2002. The effect of different additives on the fermentation quality, aerobic stability and in vitro digestibility of pea/wheat bi-crop silages containing contrasting pea to wheat ratios. *Grass Forage Science* 57:25-32.

Adetunji, V.O. 2008. Comparative assessment of the effect of crude extracts of *Carica Papaya* and*Terminalia cattapa* and a bacteriocin on vacuum packed West African soft cheese "Wara". *African Journal of Microbiological Res*earch 2: 272-276.

Adetunji, V.O. 2011. Effects of packaging, treatments and storage conditions on the survivability of aerobes and anaerobes in vacuum packaged 'Wara' a soft white cheese. *AdvanceJournal of Food Science and Technology*3.4: 289-293.

Adolfsson, O., Meydani, S.N. and Russell, R.M. 2004. Yoghurt and gut function. *American Journal of Clinical Nutrition* 80.2: 245-256.

Ahmed, M. Saheed, M.A., Alam, H. and Asgar, Z. 1992. Biological studies of indigenous medicinal plants II: Effects of *Applotaxis lappa* Dcne on various parameters of liver metabolism in rabbits. *Journal Islamic Academy Sciences* 5:51-56.

Ahmed, Z.S.A. 2010. Chemical and microbiological characteristics of *Jibna- Beida* (white cheese) produced in Sudan. Ph.D Thesis, University of Gezira, Sudan.

Ainge, L. and Brown, N. 2001.*Irvingiag wonbolu*. A state of knowledge report undertaken for the central african regional program for the environment-II, Task Order No. AID-623to-10-00008.

Ajiboye, A. E., Ajuwon, I. B. and Adebayo, M.R. 2014. Physico-chemical profile and microflora associated with spoilage of soursop fruit (*Annona muricata*). *Advances in Biotechnology Research* 1.1: 1-9.

Akindahunsi, A.A. and Oboh, G. 1999. Effect of some post- harvest treatments on the bioavailabilty of zinc from some selected tropical vegetables. *La Rivista Italiana Delle Grasse* 76: 885-887.

Akindahunsi, A.A. and Oboh, G. 2003. Effect of fungi fermentation on organoleptic properties, energy content and i*n vitro* multienzyme digestibility of cassava products (flour and gari) *Nutrition and Health* 17: 131-138.

Alferez, M. J. M., Barrionuevo, M., Lopez-Aliaga, I., Sanz Sampelayo, M. R., Lisbona, F., Robles, J. C. and Campos, M.S. 2001 Digestive utilization of goat and cow milk fat inmalabsorption syndrome. *Journal of Dairy Research* 68: 451–461.

Alferez, M.J., Lopez-Aliaga, I., Barrionuevo, M. and Campos, M.S. 2003. Effects of dietary inclusion of goat milk on the bioavailability of zinc and selenium in rats. *Journal Dairy Research70*: 181–187.

Alm, L. 2002.Lactose intolerance. In: *Encyclopediaof Dairy Sciences*. Roginsky H, Fuquay and Fox Eds. London, U.K.: Academic Press. pp 1533–1537.

Angelov, A., Gotcheva, V., Kuncheva, R. and Hrstozova, T. 2006. Development of a new oat-based probiotic drink. *International Journal Food Microbiology* 112:75–80.

Anon 2003. It's a tiny world (online). European food informationcouncil online.*Food Today*, 16: 3.Available:http://www.eufic.org/gb/food/pag/ food16/food163.htm.

AOAC 1980.Official methods of analysis.. Association of Official Analytical Chemists, 13th ed. Washington D.C.

AOAC 2001.Association of Official Analytical Chemists' Official Methods of Analysis – 17th ed. Washington D.C.

AOAC 2003.Official methods of analysis. Association of Official Analytical Chemists, Washington D.C.

AOAC 2005. 'Association of Official Analytical Chemists' Official Methods of Analysis – 18th ed. Washington D.C.

AOAC 2010.Official methods of analysis. Association of Official Analytical Chemists, 18th ed. Revision 3.Washington D.C.

Appiah, F. 2000. Studies on traditional cheese (Woagashie) processing in the Ashanti region of Ghana. M.Sc. thesis submitted to Food Science Department of Kwame Nkrumah University of Science and Technology Kumasi. Ghana.

Appiah, F., Oduro, I. and Ellis, W.O. 2011. Proximate, mineral composition of *Artocarpus altilis* pulp flour as affected by fermentation. *Pakistan Journal Nutr*ition 10.7: 653-657.

Asgary, S., Sahebkar, A., Afshani, M.R., Keshvari, M., Haghjooyjavanmard, S. and Rafieian-Kopaei, M. 2014. Clinical evaluation of blood pressure lowering, endothelial function improving, hypolipidemic and anti-Inflammatory.Effects of pomegranate juice in hypertensive subjects.*Phytotherapy Research* 28: 193 – 199.

Ashaye, O.A., Taiwo, O.O. and Adegoke, G.O. 2006. Effect of local preservative *Aframomum*

danielli on the chemical and sensory properties of stored warankasi. *African Journal of Agricultural Research* 1.1: 010-016.

Asmahan, A.A. and Muna, M. 2009. Use of starter cultures of lactic acid bacteria and yeasts in the preparation of kisra, a Sudanese fermented food. *Pakistan Journal Nutr*ition 8: 1349-1353.

Asprey, G.F. and Thornton, P. 1995. Medicinal plants of Jamaica. *Journal West Indian Medical Plants.*4: 69–92.

Atta, A. 2014. Kano state school feeding programme: A case study. London: PCD, cited in EPRI (2016) School feeding in Nigeria.Rapid Assessment. Economic Policy Research Institute and UNICEF, p.30.

Aworh, O.C. and Akinniyi, O.A. 1999. Consumer acceptability and microbial quality of "Soy – warankasi". A Nigerian soft cheese made from a mixture of soy milk and cow milk. *Nigeria Food Journal* 7:50-56.

Aworh, O.C. and Egounlety, M. 1985. Preservation of West African soft cheese by chemical treatment. *Journal Dairy Research* 52: 189-195.

Ayad, E.H.E., Nashat, S., Esadek, N., Metwaly, H. and Ei-Soda, M. 2004. Selection of wild lactic acid bacteria from traditional Egypt dairy products according to production and technology criteria. *Food Microbiology* 21: 715-725.

Babu, P.S. and Srinivasan, K. 1997. Hypolipidemic action of curcumin: the active principle of turmeric (*Curcuma longa*) in streptozotocin-induced diabetic rats. *Molecular Cellular Biochemistry* 166: 169-175.

Badrie, N. and Schauss, A.G. 2009. Composition, nutritional value, medicinal uses, and toxicology, In: Bioactive foods in promoting health, R.R.Waston and V.R. Preedy (Eds.), Academic Press, UK. pp 621– 641.

Balasundram, N., Sundram, K. and Samman, S. 2004. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses.

Food Chemistry 99: 191–203.

Barnes, D.L., Harper, S.J., Bodyfelt, F. W. and McDaniel, M.R. 1991. Correlation of descriptive and consumer panel flavour ratings for commercial prestirred strawberry and lemon yoghurts. *Journal of Dairy Science* 74: 2089-2099.

Barrett, A.J. 1972. Lysosomal enzymes. In: J.T. Dingle (Ed.), Lysosomes, a Laboratory Handbook. North-Holland Publishing Company. Amsterdam. pp. 46–135.

Barrionuevo, M., Aliaga, I.L., Alferez, M.J.M., Mesa, E., Nestares, T. and Campos, M.S. 2003.

Beneficial effect of goat milk on bioavailability of copper, zinc and selenium in rats.*Journal Physiology Biochemistry 59*: 111–118.

Basu, A. and Panugonda, K. 2009. Pomegranate juice: a heart-healthy fruit juice. *Nutrition Rev*iew 67:49–56.

Becker, T., and Puhan, Z. 1989. Effect of different process to increase the milk solids non-fat content on the rheological properties of yoghurt. *Milchwissenschaft* 44: 626-629.

Belewu, M.A., Belewu, K.Y. and Nkwunonwo, C.C. 2005. Effect of biological and chemical preservatives on the shelf life of West African soft cheese. *African Journal of Biotechnology* 4.10:1076-1079.

Belewu, M.A., Belewu, K.Y. and Ganiyu, R.O. 2011. Evaluation of lemon grass *Cymbopogon citrates*) oil and pure natural honey on the shelf life of cheese. *Electronic Journal of Environmental Agricultural and Food Chemistry (EJEAFChe)*10.4: 2129-2134

Beresford, T.P., Fitzsimons, N.A., Brennan, N.L. and Cogan, T.M. 2001. Recent advances in cheese microbiology. *International Dairy Journal* 11:259-274.

Bille, P. and Keya, E. 2002. A comparison of some properties of vat-heated and dry skim milk powder fortified set yoghurts. *Journal of Food Technology in Africa*, Vol. 7: 21-23.

Boakye, A.A. 2013. Assessment of some health beneficial constituents of edible portions of four underutilised fruits. M.Sc. (Food Sci. Technol.) Thesis.Univ. of Ghana.

Boakye, A., Wireko-Manu, D., Agbenorhevi, K. and Oduro, I. 2014. Dietary fibre, ascorbic acid and proximate composition of tropical fruits. *African Journal of Food Science*. 8.6: 305-310.

Beshkova, D., Simova, E., Frengova, G. and Simov, Z. 1998. Production of flavour compounds by yoghurt starter cultures. *Journal Index Microbiology Biotechnology* 20:180–186.

Bouzar, F., Cerning, J and Desmazeaud, M. 1997. Exopolysaccharide production and texture-promoting abilities of mixed-strain starter cultures in yoghurt production.*Journal of Dairy Science* 80:2310-2317.

Bozanic R, Tratnik L and Maric O 1998The influence of goat milk on the viscosity and microbiological quality of yogurt during storage. Mljekarstvo 48: 63–74.

Božanić, R., Letikapić, g. and Lovković, S. 2008b: Influence of temperature and glucose addition on growth and survival of BCT culture in soymilk. *Mljekarstvo*58: 171–179.

Brink, B., Minekns, M.J., Vander Vossen, M.B.M., Leer, R.J. and Huis Veld, J.H.J. 1994. Anti microbial activity of lactobacilli.*Journal Applied Bacteriol*ogy 77: 140-148.

Brow, N. J., Worldeng, J., Rumsey, R. D., Read, N.W. 1988. The effect of guar gum on the

distribution ofa radiolabelled meal in the gastrointestinal tract of the rat. *Britain JournalNutrition* 59: 223-231.

Bulut, C., Gunes, H., Okuklu, B., Harsa, S., Kilic, S., Coban, H.S. and Yenidunya, A.F. 2003. Homofermentative lactic acid bacteria of a traditional cheese, Comlek peyniri from Cappadocia region.*Journal of Dairy Research* 71: 1.

Cakir, I. 2003. Determination of some probiotic properties on *Lactobacilli* and *Bifidobacteria. Ankara University Ph.D.Thesis,Turkey, Ankara.*

Campos, M.S., Alferez, M.J.M. and Lopez-Aliaga, I. 2004. Beneficial effects of goat milk on the nutritional utilisation of iron and copper in malabsorption syndrome. The future of the sheep and goat dairy sectors.International Symposium Session 5. Zaragoza, Spain.

Canabady-Rochellea, L.S. and Mellemab, M. 2010. Physical- chemical comparison of cow's milk proteins versus soy proteins in their calcium-binding capacities. *Colloidsand Surfaces A: Physicochemical Engineering Aspects 366*: 110- 112.

Caplice, E. and Fitzgerald, G.L. 1999. Food fermentation: role of microorganisms in food production and preservation. *International Journal Food Microbiol*ogy 50: 131-149.

Cashman, K.D. 2002a. Macroelements, nutritional significance. In: Roginski, H., Fuquay, J.W., Fox, P.F. (eds.) Encyclopedia of Dairy Sciences, London: Academic Press, 2051-2058.

Cashman, K.D. 2002b, Trace elements, Nutritional Significance. U: Roginski, H., Fuquay, J.W., Fox, P.F. (eds.) Encyclopedia of Dairy Sciences, No 3, London: Academic Press, 2059-2065.

Cassileth, B. 2008.Complementary therapies, herbs and other OTC agents. (Williston Park) Cambridge, New York.*Oncology*22.10: 1202.

Castro A, Montano A, Sanchez AH, Rejano L. 1998. Lactic acid fermentation and storage of blanched garlic. *International Journal Food Microbiology* 39:205–11

Chainani-Wu, N. 2003. Safety and anti-inflammatory activity of curcumin: A component of turmeric (*Curcuma longa*). *Journal Altern Complement Medicine 9*: 161-168.

Chandan, R.C.1999. Enhancing market value of milk by adding cultures.*Journal Dairy Sci*ence 82:2245-2256.

Chantaraporn, P. and Somboon, T. 2006.Characterisation of lactic acid bacteria from traditional Thai fermented sausages. *Journal Culture Collect* 5:46-47.

Chaves, A. C. S. D., Fernandez, M., Lerayer, L. S., Mierau, I., Kleerebezem, M. and

Hugenholz, J. 2002. Metabolic engineering of a acetaldehyde production by *streptococcus thermophilus*. *Applied Environment Microbiology* 68: 5656-5662.

Cichoscki, A., Valduga, E., Valduga, A., Tornadijo, M. and Fresno, J. 2002. Characterisation of prato cheese, a Brazilian semi-hard cow variety: evolution of physico-chemical parameters and mineral composition during ripening. *Food Control*13: 329–336.

Codex Standard for Fermented Milks. 2003.Codex Standard 243. Queensland. New Zealand.

Coenye, T. and Vandamme, P. 2003. Extracting phylogenetic information from whole-genome sequencing projects: the lactic acid bacteria as a test case. *Microbiology*149: 3507– 3517.

Collins, J.K., Thornton, G. and Sullivan, G.O. 1998. Selection of probiotic strains for human applications. *International Dairy Journal* 8: 487-490.

Colombel, J.F., Cortot, A., Neu, C. and Romand, C. 1987. Yoghurt with *Bifidobacterium fongurn*reduces erythromycin induced gastrointestinal effects. *Lancet,* ii: 43.

Corbo, M.R., Bevilacqua, A., Campaniello, D, D'Amato, D. Speranza, B. and Sinigaglia, M. 2009. Prolonging microbial shelf life of foods through the use of natural compounds and non-thermal. Approaches- A review.*International Journal of Food Science and Technology* 44: 223-241.

Daeschel, M. A. 1989. Antimicrobial substances from lactic acid bacteria for use as food preservatives. *Food Technology*43: 164.

Dave, R.I and Shah, N.P. 1996. Evaluation of media for selective enumeration of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp *bulgaricus*, *Lactobacillus acidophilus* and bifidobacteria. *Journal Dairy Sci*ence 79.9:1529–1536.

Davies, E. T., Benson, J. A., Bicknel, S. R. and Gray, D. E. 1985. Ministry of Agriculture and Food Manual of Veterinary Investigation (MAFI) Laboratory Techniques. Vol. 2: pp 33-34, 49-50, 72-75, 86-88, 88-90.

Davis, J. G. 1975. The microbiology of yoghurt: In *Lactic acid bacteria in beverages and food.* J. G. Carr, C. V. Cutting and G.C. Whitting, Eds. New York: Academic Press: 245–266.

Dermiki, M., Ntzimani, A., Badeka, A., Savvaidis, I. N. and Kontominas, M.G. 2007. Shelf-life extension and quality attributes of the whey cheese Myzithra Kalathaki using modified atmosphere packaging. *LWT*. 41, 284–294.

De La Fuente, M.A., Montes, F., Guerrero, G. and Juarez, M. 2003.Total and soluble contents of calcium, magnesium, phosphorus and zinc in yoghurts. *Food Chemistry* 80: 573-578.

de Vrese, Stegelmann, M., Richter, A, Fenselau, S., Laue, C. and Schrezenmeir, J. 2001.

Probiotics-compensation lactase insufficiency.*Amazon Journal Clinical Nutrition* 73: 421S-429S.

Di-Cagno, R., Surico, R.F., Paradiso, A., De Angelis, M., Salmon, J.C., Buchin, S., De Gara, L. and Gobbetti, M. 2009. Effect of autochthonous lactic acid bacteria starters on health-promoting and sensory properties of tomato juices.*International Journal Food Microbiology* 128: 473-483.

Dirar, H.A. 1993. The Indigenous Fermented Foods of the Sudan: A study in African Food and Nutrition.CABS International, Wallingford, U.K. pp. 303-344.

Dodd, H.M., Horn, N. and Gassen, M.J. 1990. Analysis of genetic determinants for production of the peptide antibiotic nisin. *Journal General Microbiol*ogy 136: 444.

Domagla, J. 2005. Texture of yoghurt and bio-yoghurts from goat's milk depending on starter culture type. *Milchwissenschaft* 60.3: 289-292.

Donkor, N. O, Henriksson, A., Vasiljevic T, and Shah, N. 2006. Effect of acidification on the activity of probiotics in yoghurt during cold storage. *International Dairy Journal* 16: 1181–1189.

Driessen, F.M. 1989. Heat-induced changes in milk. Inactivation of lipases and proteinases (indigenous and bacterial). International Dairy Federation Bulletin 238, 71–90.

Drouault, S. and Corthier, G. 2001. Effets des bactéries lactiques ingérées avec des laits fermentés sur la santé. *Veterinary Research* 32: 101-117.

Duangjitcha-roen, Y., Kantachote, D., Ongsakul, M., Poosaran, N. and Chaiyasut, C. 2008. Selection of probiotic lactic acid bacteria isolated from fermented plant beverages. *Pakistan Journal Biological Science*11: 652-655.

Duboc, P. and Mollet, B. 2001.Applications of exopolysaccharides in the dairy industry. *International Dairy Journal* 11: 759-768.

Duncan, S.E. 1998. Dairy products: the next generation altering the image of dairy products through technology. *Journal Dairy Sci*ence 81: 877- 883.

Durso, L. and Hutkins, R. 2003. “Starter Cultures”, in *Encyclopedia of Food Science and Nutrition,* B. Caballero, L.Trugo and P. Finglas Eds, Academic Press, United Kingdom pp. 5583-5593.

Dziezak, J.D. 1989.Spices.*Food Technology* 43: 102 – 116.

Edem, D.O. 2009. Haematological and histological alterations induced in rats by palm oil-containing diets. *European Journal of Science Research* 32.3: 405-418.

Egounlety, M., Aworh, O. C., Akingbala, J.O., Houben, J.H. and Nago, M.C. 2002. Nutritional and sensory evaluation of tempe-fortified maize-based weaning foods. *International Journal Food Science Nutr*ition 53.1: 15-27.

Eichholzer, M. and Stahelin, H. 1993. Is there a hypocholesterolemic factor in milk and milk products*International Journal Vitamin Nutrition Res*earch 63: 159-167.

Eke, M.O, Olaitan, N.I and Sule, H.I (2013). Nutritional evaluation of yoghurt-like product from baobab (Adansonia digitata) fruit pulp emulsion and the micronutrient content of baobab leaves. *Advance Journal of Food Science and Technology* 5.10: 1266-1270.

Elaine, M. 2015. What's tasty, easy and has lots of health benefits? Yoghurt! Medicinenet.Retrieved 22 July 2015.

El-Bakri, J.M. and El Zubeir, I.E.M. 2009. Chemical and microbiological evaluation of plain and fruit yoghurt in Khartoum State, Sudan. *International Journal of Dairy Science* 4.1: 1-7.

El-Hadi Suleiman, A.M., Ilayan, A.A. and El-Faki, A.E. 2006. Chemical and microbiological quality of Harris, sudanesse fermented camel's milk product. *International Journal Food Science Technology* 41: 321-328.

EI Owni, O.A.O. and Osman, S.E. 2009. Evaluation of chemical composition and yield of Mozzarella cheese using two different methods of processing.*Pakistan Journal of Nutrition* 8.5:684 – 687.

Ejechi, B.O., Souzey, J.A., Akpomedaye, D.E. 1998. Microbial stability of mango (*Mangifera indica* L.) juice preserved by combined application of mild heat and extracts of two tropical spices. *Journal Food Protection* 61:725–727.

Emma, S. W. 1995. *Buah dan sayur untuk Terapi*, Penebar Swadaya, Jakarta.Hal.49-50.

Endres, J.G. 2001. Soy protein products: Characteristics, nutritional aspects and utilization, AOAC Press, Champaign, chap. 5, 6. pp. 10-14.

Escalante-Minakata, P., Blaschek, H.P., Barba de la Rosa, A.P., Santos, L. and De León-Rodríguez, A. 2008.Identification of yeast and bacteria involved in the mezcal fermentation of *Agave salmiana*.*Letter Applied Microbiology* 46: 626-630.

Fallon, S. and Enig, M.G. 2001.Nourishing traditions. The Cookbook that Challenges Politically Correct Nutrition and the Diet Dictocrats.National Book Networkpp: 40-45.

FAO. 1992. Codex Standard for fermented milks- CODEX STAN 243-2003. Rome: FAO/OMS.

FAOSTAT. 2009. Agricultural statistics (online: http://www.fao.org). FAO, Rome, Italy.

FAOSTAT. 2012. FAO statistical database. Accessed on: http://faostat.fao.org/.Accessed 21 September 2012.

FAO/WHO. 2001. Food and Agriculture Organization of the United Nations, World Health Organization.Evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Cordoba, Argentina.P.34.

FAO / WHO. 2002. *Human vitamin and mineral requirements*. Report of a Joint FAO and WHO expert consultation. Rome. Available at: http://www.fao.org/ DOCREP/004/Y2809E/y2809e00.htm. Accessed 17 September 2012.

Farinde, O.E., Adesetan, T., Obatolu, V. and Oladapo, M. 2009. Chemical and microbial properties of yoghurt processed from cow's milk and soymilk. *Journal of Food Processing and Preservation* 33: 245–254.

Fashakin, J.B. and Unokiwedi, C.C. 1992. Chemical analysis of warankasi prepared from cow milk partially substituted with melon milk. *Nigeria Food Journal* 10: 103–110.

Fasoyiro, S.B., Adegoke, G.O., Obatolu, V.A., Ashaye, O. and Aroyeun, S.O. 2001. The antioxidant property of *Aframomum danielli* in oils. *Journal Food Technology Africa* 6: 135-137.

F.D.A. 1998.Bacteriological Analytical Manual.US. Food and Drug Administration Center for Food Safety and Applied Nutrition. Gaithersburg: AOAC. 8th edn.Revision A, Chapter 4, 5, 10, 12 and 14.

F.D.A. 2005.Bacteriological Analytical Manual.US. Food and Drug Administration Center for Food Safety and Applied Nutrition. Gaithersburg: National Technical Information Service Publication. PB2005-102200.

Galan, E., Prados, F., Pino, A., Tejada, L and Fernandez-salguero, I. 2008. Influence of different amounts of vegetable coagulant from Cardoon *Cynara cardunculus* and calf rennet on the proteolysis and sensory characteristics of cheese made with sheep milk: *International Journal* 18: 93-98.

Gaman, P.M. and Sherrington, K.B. 1998. The Science of Food: An introduction to Food Science Nutrition and Microbiology. Pergamon Press, Oxford. 196-215.

Garcea, G., Berry, D.P., Jones, D.J., Singh, R., Dennison, A.R., Farmer, P.B., Sharma, R.A., Steward, W.P. and Gescher, A.J. 2005. Consumption of the putative chemopreventive agent curcumin by cancer patients: assessment of curcumin levels in the colorectum and their pharmacodynamic consequences. *Cancer Epidemiology Biomarker14*: 120-125.

Gibson, G.R., and Roberfroid, M.B. 1995. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics.*Journal Nutrition* 125: 1401-1412.

Goderska, K. and Czarnecki, Z. 2007. Characterisation of selected strains from *Lactobacillus acidophilus and Bifidobacterium bifidum*. *African Journal Microbiology Research*1: 065-078.

Gonfa, A., Fite, A., Urga, K. and Abegaz, B. 1999. Microbiological aspects of Ergo (*Itutu*) fermentation.*SINET*: *Ethiopia Journal Science 22.2: 283-290.*

Greetham, H.L., Giffard, C., Hutson, R.A. Collins, M.D. and Gibson, G.R. 2002. Bacteriology of the Labrador dog gut: a cultural and genotypic approach. *Journal Applied Microbiology* 93: 640-646.

Guerra-Hernandez, E.J., Estepa, R.G. and Rivas, I.R. 1995. Analysis of diacetyl in yoghurt by two new spectrophotometric and fluorometric methods. *Food Chem*istry 53:315–319.

Guler, Z. 2007. Level of 24 minerals in local goat mils its strained yoghurt and salted yoghurt. *Small Ruminant Research* 71: 130-137.

Guler, Z. and Hasan, S. 2008. The essential mineral concentration of Torba yoghurts and their wheys compared with yoghurt made with cows', ewes' and goats' milks. *International Journal of Food Sciences and Nutrition* 60: 2 153-164.

Guler, A. and Mutlu, B. 2005. The effects of different incubation temperatures on the acetaldehyde content and viable bacteria counts of bio-yoghurt made from ewe's milk. *International Journal DairyTech*nology 58: 174-179

Haenlein, G.F.W. 1996. Nutritional value of dairy products of ewe and goat milk. In production and utilization of ewe and goatmilk. Proceedings on the IDF/Greek national committee of IDF/Brussel, Belgium. Int. Dairy Fed. pp 159-177

Haenlein, G.F.W. 2004.Goat milk in human nutrition.*Small Ruminant Research* 51: 155–163.

Halliwell, B., Murcia, M., Chirico, S. and Aruoma, O.I. 1995. Free radicals and antioxidants in food and *in vivo*: what they do and how they work? *Critical Review Food Science Nutrition*35:7-20.

Hammes, W., Weiss, N. and Holzapfel, W. 1999.The Prokaryotes. New York: Springer-Verlag, pp 102-103.

Hammes, W.P. and Vogel, R.F. 1995. "The genus *Lactobacillus*" in the genera of lactic acid bacteria, edited by B.J.B. Wood (Blackie Academic and Professional, United Kingdom), pp. 19-49.

Hansen, T.K. and Jakobsen, M. 2004. Yeast in the dairy industry. http:/www.drugswell.com/winow/%20new9/Fungal_Biotechnology_in_Agricultural_Food_and_Environmental_Applications/DKE214_CH24pdf. Visited on August 03, 2013.

Hartati, C. and Eka, W. 2010. Production of soursop juice (*Anona muricata Linn*) yoghurt through lactic acid fermentation. Proceedings of the Third International Conference on Mathematics and Natural Sciences (ICMNS 2010). Nov. 23-25, 2010. Bandung, Indonesia. Editors-Siti Khodijah Chaerun, Ihsanawati. Coordinator-Roberd Saragih ISBN: 978-979-17090-3-3

Hassaïne, O., Zadi-Karam, H. and Karam, N. 2007. Technologically important properties of lactic acid bacteria isolated from raw milks of three breeds of Algerian dromedary (*Camelus dromedarius*) *African Journal Biotechnol*ogy 6: 1720-1727.

Heilig, H.G.H.J., Zoetendal, E.G., Vaughan, E.E., Marteau, P., Akkermans, A.D.L. and de Vos, W.M. 2002. Molecular diversity of *Lactobacillus* ssp. and other lactic acid bacteria in the human intestine as determined by specific amplification of 16S ribosomal DNA. *Applied Environmental Microbiol*ogy 68: 114-123.

Hoffman, J.R. and Falvo, M. J. 2004. Protein – Which is best? *Journal of Sports Science and Medicine* 3: 118-130.

Holzapfel, W., Habere, P., Geisen, R., Bjorkroth, J. and Schillinger, U. 2001. Taxonomy and important features probiotic microorganisms in food and nutrition. *Amazon Journal Clinical Nutr*ition 73:365-373.

Holzer, M., Mayrhuber, E., Danner, H. and Braun, R. 2003. The role of *Lactobacillus buchneri* in forage preservation. *Trends Biotechnol*ogy 21: 282–287.

Hossain, M.A. and Ishimine, Y. 2005. Growth, yield and quality of turmeric (*Curcumim longa l.*) cultivated on dark-red soil, gray soil and red soil in Okinawa. Japan. *Plant Production Sci*ence 8: 482-486.

Hove, H., Norgaard, H. and Brobech, M. P. 1999. Lactic acid bacteria and the human gastrointestinal tract. *European Journal Clinical Nutrition* 53: 339-350.

Hutt, P., Shchepetova, J., Loivukene, K., Kullisaar, T. and Mikelsaar, M. 2006. Antagonistic activity of probiotic lactobacilli and bifidobacteria against entero- and uropathogens. *Journal Applied Microbiol*ogy 100: 1324–1332.

International Dairy Federation 1981.New Monograph on UHT Milk.Doc. 133, IDF, Brussels, Belgium.

Ifeoma, B., Josephine, O., Timothy, E. and Charles, O. 2004. The biochemical analysis of soursop (*Annona muricata*) and sweetsop (*A.squamosa*) and their potential use as oral rehydration therapy. *Journal Food Agriculture Environ*ment 2.1: 39-43.

IFST. 2007 Products made of fruits. Available from www.ifst.com/:products.Vol.21. Accessed 5 November 2007.

Ijarotimiro O.S and Kehinro, 0.0. 2012. Formulation and nutritional quality of infant formula

produced from geminated pop corn, Bambara groundnut and African locust beans flour. *Journal of Microbiology Biotechnology and Food Sciences.*

Imaizumi, S., Nakatsu, Y., Sato, M., Sedarnawati, Y. and Sugano, M. 1991. Effects of xylooligosaccharide on blood glucose, serum and liver lipids and cecum short-chain fatty acids. *Agricultural Biology Chemistry*, 55:199-205.

Ireson, C.R., Jones, D.J., Orr, S., Coughtrie, M.W., Boocock, D.J., Williams, M.L., Farmer, P.B., Steward, W.P. and Gescher, A.J. 2002. Metabolism of the cancer chemopreventive agent curcumin in human and rat intestine.*Cancer Epidemiology Biomarker Prevention* 11: 105-111.

Istikhar, H., Attiq-ur- Rahman and Nigel, A. 2009. Quality comparison of probiotic and natural yoghurt. *Paskitan Journal of Nutrition* 8: 9-12.

Janhoj, B., Charlotte, F. and Michael, B. 2006. Sensory and rheological characterization of low fat stirred yoghurt. *Journal Texture Studies* 37: 276-299.

Jaramilo, M., Arango, G., Gonzalez, M., Robledo, S. and Velez, I. D. 2000. Cytotoxity and antileishmanial activity of *Annona muricata* pericarp. *Fitoterapia*. 71: 183-186.

Jay, J.M. 2000.Modern Food Microbiology*6th edition*. Aspen Publishers Inc., Maryland-USA : 121, 391.

Jayaprakasha, G. K., Bae, H., Crosby, K.J.L., Jifon, J.L. and Patil, B. S. 2012. Bioactive compounds in pepper and their antioxidants potentials. In: Tunick M. H., Gonzalez de Mejia E. (Eds.) Hispanic foods: Chemistry and bioactive compounds. American Chemistry Society.pp.43-56.

Jenness, R. 1980. Composition and characteristic of goat milk: review. *Journal Dairy Science* 64: 1605-1630.

Johnson-Delaney, C. 1996.*Exotic Animal Companion Medicine Handbook for Veterinarians*, Zoological Education Network.Kirkland Washington.Updated by Rat Fan Club 2014.

Johnson-De 1999. Special considerations in interpreting liver function tests. *Am. Fam. Physician*. 59.8: 2223-2230. PMID 1022 1307

Joslyn, M. A. 1970. Methods in Food Analysis. Physical, chemical and instrumental methods of analysis. 2nd Ed. Academic Press. New York. pp 49-66.

Kampman, E., Goldbolm, R. A., Van Den Brant, P. A. and Van't Veer, P. 1994. Fermented dairy products, calcium, and colorectal cancer in Netherlands- cohort study. *Cancer Research* 54: 3186-3190.

Karapinar, M. 1985. The effect of citrus oils and some spices on growth and aflatoxin

production by *Aspergillus parasticus* NRRL 2999. *International Journal Food Microbiology* 2: 239–245.

Karovicova J, Polonsky J, Drdak M, Simko P, Vollek V. 1993. Capillary isotachophoresis of organic acids produced by selected microorganisms during acid lactic fermentation. *Journal Chromatography* 638:241–246.

Karovicova J, Kohajdovaz Z, Hybenova E, Drdak M. 2002. Using of multivariate analysis for evaluation of lactic acid fermented cabbage juices. *Chemistry Papers* 56:267–274.

Kathleen, J., Wilson, W. and Anne, W. 1996.Anatomy and Physiology in Health and Illness. 8th Edition.Longman, Asia Limited; Hong Kong.p 274.

Katz, F. 2001. Active cultures add function to yoghurt and other foods. *Food Technology* 55 3: 46-49.

Khan, Z.I., Ashraf, M., Hussain, A., McDowell, L.R., Ashraf, M.Y. 2006. Concentrations of minerals in milko of sheep and goats grazing similar pastures in semiarid region of Pakistan. *Small Ruminant Research* 65: 274-278.

Kim, G.S., Zeng, L. and Alali, F. 1998. Muricoreacin and murihexocin C, mono-tetrahydrofuran acetogenins, from the leaves of *Annona muricata* . *Phytochemistry* 49.2:565-571.

Kolawole, J.A and Alemika, T.O.E., 1996. Effect of halofantrin on haematological parameters in rats. *West African Journal Biology Science* 5: 179-183.

Konigs, W.N., Kok, J., Kuipers, O.P. and Poolman, B. 2000. Lactic acid bacteria: the bug of the new millennium. *Current Opinion Microbiology* 3: 276–282.

Korkeala, H. and Bjorkroth, K.J. 1997. Microbiological spoilage and contamination of vacuum-packaged cooked sausages: A review. *Journal of Food Protection* 60: 724–734.

Kostinek, M., Specht, I., Edward, V.A., Pinto, C., Egounlety, M., Sossa, C., Mbugua, S., Dortu, C., Thonart, P., Taljaard, L., Mengu, M., Franz, C.M. and Holzapfel, W.H. 2007.Characterisation and biochemical properties of predominant lactic acid bacteria from fermenting cassava for selection as starter cultures.*International Journal Food Microbiology* 114: 342-351.

Kroll, D. 1995. Shelf life technology for processed foods. Business communication company, Inc. Available from: www.ga 088 shelf life technology for processed.

Kumar, P. and Mishra, H.N. 2004. Mango soy fortified set yoghurt: effect of stabiliser addition on physicochemical, sensory and textural properties. *Food Chemistry* 87: 501-507.

Kumar, K.V., Sharief, S.D., Rajkumar, R., Ilango, B. and Sukumar, E. 2011. 'Influence of

Lantana aculeate stem extract on haematological parameters in rats' *Advance in Bioresearch*2.1: 79-81

Kurman, J.A. and Rasic, J.L. 1991. The health potential of probiotics containing *bifidobacteria.* In: Theraupetic properties of fermented milks (R.K.Robinson). London: Elsevier Applied Food Science pp.117-158.

Lai, P.K. and Roy, J. 2004. Antimicrobial and chemo-preventive properties of herbs and spices. *Current Medical Chemistry* 11: 1451-1460.

Lamghari El Kossori, R., Sanchez, C., El Boustani, ES, Maucourt, N., Sauvaire, Y, Méjean, L. and Villaume, C. 2000. Comparison of effect of prickly pear (*Opunziaficus indica sp.*) fruit, arabic gum and citrus pectin on viscosity and *in vitro* digestibility of casein. *Journal Science Food Agric*ulture 80: 359-364.

Law, J. and Haandrikman, A. 1995.Proteolytic enzymes of lactic acid bacteria. *International Dairy Journal* 7:1-11

Lawal, A.K. and Adedeji, O.M. 2013. Nutritional and elemental analysis of warankasi (fermented milk product) sold in Lagos metropolis. *International Research Journal of Biotechnology* (ISSN: 2141-5153) 4.6: 112-116.

Laws, A.P. and Marrshall, V.M. 2001. The relevance of exopolysaccharides to the rheological properties of milk fermented with ropy strains of lactic acid bacteria. *International Dairy Journal* 11: 709-721.

Leakay, R.B., Greenwell, P., Hall, M.N., Atangana, A.R., Usoro, C., Anegbeh, P.O., Fondon, J.M. and Tchoundjeu, Z. 2005. Domestication of *Irvingiag abonensis:* Tree-to-tree variation in food thickening properties, in fat and protein contents of dika nut. *Food Chem*istry 90.3: 365-378.

Lee, B. 1996.Bacteria-based processes and products. In: *Fundamentals of Food Biotechnology* VEH, B. Lee (Ed), New York: 219-290.

Lee, M. 2009. Basic skills in interpreting laboratory data.ASHP.ISBN 978-1-58528-180-0. pp 259. Retrived 5 August 2011.

Lee, S.K. and Kader, A.A. 2000. Preharvest and postharvest factors influencing Vitamin C content of horticultural crops. *Postharvest Biological Technol*ogy 20: 207-220.

Lee, W.J. and Lucey, J.A. 2010. Formation and physical properties of yoghurt.*Asian-Aust. Journal Animal Science* 23.9: 1127 – 1136.

Li, T. 2006. The range of medicinal herbs and spices. In *Handbook of Herbs and Spices*; Peter, K.V., Ed.; Woodhead Publishing Limited: Cambridge, UK, Volume 3, pp. 113–125.

Li, S., Gokavi, S. and Guo, M.R. 2006. Selective enumeration of different strains of *Lactobacillus acidophilus* in goats' milk yoghurt beverage.*Journal Dairy Sci*ence 89 (Suppl. 1.): 182.

Lick, S., Keller, M., Bockelmann, W. and Heler, K.J. 1995. Rapid identification of *Streptococcus thermophilus* by primer-specific PCR amplification based on its *lacZ* gene, *Systematic Applied Microbiology*19: 74.

Limsowtin, G.K.Y., Broome, M.C. and Powell, I.B. 2002. Lactic acid bacteria, taxomomy In: H. Roginski H (Ed.): Encylopedia of Dairy Science, Elsevier Science Ltd, 1470-1478.

López-Aliaga, I. Alférez, M.J.M., Barrionuevo, M., Nestares, T. and Sanz Sampelayo, M.R. 2003. Malabsorption syndrome. *Journal of Dairy Research* 68: 451–461.

Lourens-Hattingh, A. and Viljoen, B.C. 2001.Yoghurt as probiotic carrier food.*International Dairy Journal* 11:1–17.

Lovell, R.T. 1989. Nutrition and feeding of fish. Van.Nostrand Reinhold Co. Inc.New York,

pp 318

Lu, Z, Fleming H.P and Feetrees R. F. 2001. Differential glucose and fructose utilization during cucumber juice fermentation. *Journal Food Science* 66:162–6

Lucey, J. A., Munro, P.A. and Singh, H. 1998a. Whey separation in acid skim milk gels made with glucono-δ-lactone: Effects of heat treatment and gelation temperature. *Journal Texture Studies* 29: 413-426.

Lucey, J.A. and Singh, H. 1998a. Formation and physical properties of acid milk gels: A review. *Food Research International* 30.7: 529–542.

Lucey, J. A., Teo, C.T., Munro, P.A. and Singh, H. 1998b. Microstructure, permeability and appearance of acid gels made from heated skim milk. *Food Hydrocoll*oids 12:159-165.

Lucey, J. A., Tamehana, M., Singh, H. and Munro, P.A. 1998c. Effect of interactions between denatured whey proteins and casein micelles on the formation and rheological properties of acid skim milk gels. *Journal Dairy Res*earch 65:555-567.

Luna, J.de S., De Carvalho, J.M. and De Lima, M.R. 2006.Acetogenins in *Annona muricata L.* (*Annonaceae*) leaves are potent molluscicides. *National Product Res*earch 20.3: 253-257.

Lutchmedial, M., Ramlal, R., Badrie, N. and Chang-Yen, I. 2004. Nutritional and sensory quality of stirred soursop (*Annona muricata L.*) yoghurt. *International Journal Food Science Nutr*ition 55.5:407-414.

Luzia, D.M.M. and Jorge, N. 2012. Soursop (*Annona muricata* L.) and sugar apple (*Annona*

*squamosa*L.):Antioxidant activity, fatty acids profile and determination oftocopherols. *Nutrition Food Sci*ence 42.6: 434 – 441.

Lye, H.S., Kuan, C.Y., Ewe, J.A., Fung, W.Y. and Liong, M.T. 2009. The improvement of hypertension by probiotics: Effects on cholesterol, diabetes, renin and phytoestrogens, *International Journal Molecular Science 27:* 3755–3775.

Mahdian, E. and Tehrani, M. M. 2007. Evaluating the effect of milk total solids on the relationship between growth and activity of starter cultures and quality of concentrated yoghurt. *American-Eurasian Journal Agriculture and Environment Sci*ence 2.5: 587-592.

Malik C.P. and Srivastava, A.K. 1982. Textbook of plant physiology. New Delhi: Ludhiana Nzikou, J.M., M. Mvoula-Tsieri, L. Matos, E. Matouba, A.C Ngakegni, M. Linder and S. Desobry, 2007. *Solanumnigrum* L. seeds as an alternative source of edible lipids and nutriment in congo Brazzaville. *Journal Applied Science* 7: 1107-1115.

Marinescu, A. and Pop, F. 2009. Variation in physicochemical parameters of probiotic yoghurt during refrigeration storage. *Carpathian Journal of Food Science and Technology*1.2:18-26.

Mattila-Sandholm, T., Myllarinen, P., Crittenden, R., Mogensen, G., Fonden, R. and Saarela, M. 2002. Technological challenges for future probiotic foods. *International Dairy Journal* 12: 173-182.

Messina, M.V., Messina, K.D.R. and Setchell, K.D.R. 1994a. The simple soybean and your health. Avery Pub. Group. New York.

Messina, M.J., Persky, V., Setchell, K.D.R. and Barness, S. 1994b. Soy intake and Cancer Risk: A review of the *in vitro* and *in vivo* data.*Nutrition Cancer* 21:112-131.

Metchnikoff, E. 1908. Prolongation of life: Optimistic studies, pp. 161-183. WilliamHeinemann, London.

Metwalli, N.H., Shalabi, S.I., zahran, A.S., El-Dameradash, O. 1992b. The use of soy-bean milk in soft cheese making II: Organoleptic and chemical properties of Domiati cheese made from a mixture of soybean milk and whole milk. *Journal Food Technology*, 17: 297-305.

Meydani, S.N. and Ha, W.K. 2000. Immunologic effect of yoghurt. *Amazon Journal Clinical Nutrition* 71:861–872.

Miller, G.D., Jarvis, J.K. and McBean, L.D. 2000.Handbook of dairy foods and nutrition. 2nd ed. In: The essential mineral concentration of Torba yoghurts and their wheys compared with yoghurt made with cows', ewes' and goats' milks, (eds.). CRC Press LLC. Boca Raton. Florida. pp 17-41.

Millette, M., Luquet, F. M. and Lacroix, M. 2007. *In vitro* growth control of selected pathogens

by*Lactobacillus acidophilus* and *Lactobacillus casei*fermented milk. *Letter Application Microbiology* 44: 314-319.

Milliere, J., Abidi, F., and Lefebre, G. 1996. Taxonomic characterization of *Lactobacillus delbrueckii subspecies bulgaricus* isoalated from Cameroonian zebu's fermented raw milk. *Journal of Applied Microbiology* 80: 583-588.

Mistry, V.V. 2001. Fermented milks and cream. In: *Applied Dairy Microbiology*. Marth, E.H. and Steele, J.L. (Eds.). Marcel Dekker, Inc., USA. p311.

Montilla, A., Moreno, F.J. and Olano, A. 2005. A reliable gas capillary chromatographic determination of lactulose in dairy samples. *Chromatographia* 62.6: 311–314.

Morand-Fehr, P., Boutonnet, J. P., Devendra, C., Dubeuf, J. P., Haenlein, G. F. W., Holst, P., Mowlem, L. and Capote, J. 2004. Strategy for goat farming in the 21st century.*Small Ruminants Research* 51: 175-183.

Morata, V.A., Gusils, C.H. and Gonzalez, S.N. 1999. Classification of the bacteria-traditional in *Enyclopedia of Food Microbiology,* C. Batt, P. Patel, R. Robinson, Academic Press, United Kingdom. pp. 173-183.

Mustapha, A., Jiang, T. and Savaiamo, D.A. 1997. Improvement of lactose digestion by humans following ingestion of unfermented acidophilus milk: influence of bile sensitivity, lactose transport and acid tolerance of Lactose acidophilus. *Journal Dairy Sci*ence 8:1537-1545.

Mutlu, B. G. and Serdar, A. 2005. Effect of cysteine and different incubation temperature on the microflora, chemical composition and sensory characteristics of bio-yoghurt made from goat's milk.*Food Chemistry* 100.2: 788-793.

National Academy of Science 1978. Under exploited tropical plant with promising economic value. Washington, DC. pp 64-67

Ndife, J. and Abbo, E. 2009. Functional Foods: Prospects and Challenges in Nigeria. *Journal of Science and Technology*1.5: 1-6.

Nilsson, D. 2008. Metabolically engineered lactic acid bacteria and their use. Patent number U.S. 7,465,575 B2.

Nooruddin, G.M., Chanda, G.C., Deb, A., Chowdhurt and Bilkis, T. 2006. Microbiological and hygienic quality of the market milk (cow milk) producedby the private dairy farms in Chittagong metropolitan area of Bangladesh. *Journal Agricultural Educational Technol*ogy 9: 131-136.

NRC. 1989. Recommended dietary allowances. National Research Council. National Academy Press, Washington D.C. USA.

Nwachukwu, E. and Ezeigbo, C. G. 2013. Changes in the microbial population of pasteurized soursop juice treated with benzoate and lime during storage. *African Journal of Microbiology Research* 7.31: 3992-3995.

Nyanga, L.K., Nout, M.J., Gadaga, T.H., Theelen, B., Boekhout, T. and Zwietering, M.H. 2007. Yeasts and lactic acid bacteria microbiota from masau (*Ziziphus mauritiana*) fruits and their fermented fruit pulp in Zimbabwe. *International Journal Food Microbiology* 120: 159-166.

Oberlies, N.H., Chsang, C.J. and McLaughlin, J.L. 1997. Structure-activity relationships of diverse annonaceous acetogenins against multidrug resistant human mammary adenocarcinoma (MCF-7/Adr) cells. *Journal Medical Chem*istry 40.13: 2102–2106.

Oberman, H. 1985. Fermented milks. In: Microobiology of fermented foods, J.B. Woods Ed. Vol.1. Elsevier Appplied Science Publishers, New York. 167- 190.

Obi, G., Ekperigin, M.M. and Kazeem, M.I. 2010. Nutritional and haemolytic property of egg plant (*Solanum macrocarpon*) leaves. Journal of Food Composition and Analysis –In press

Oboh, G. and Ekperigin, M.M. 2004. Nutritional evaluation of some Nigerian wild seeds. *Nahrung/Food* 48: 85-87.

O'Connor, C.B. 1993. Traditional cheese-making manual.ILCA Publishing. Addis Ababa. pp 24.

Ogundiwin, J. 0. 1978a. A study of milk clotting by the vegetable renin from the leaves of sodom apple *(Calotropisprocera).* In *Proc. of the 2nd Ann. Conf.' of NIFST,* Lagos, 7-9 September 1978, pp. 89-98.

Ogundiwin, J. 0. 1978b. A study of the traditional processing and chemical composition of 'warankasi' – Nigeria white soft cheese. In *Proc. of the 2nd Ann. Conf. of NIFST,* Lagos, 7-9 September 1978, pp. 99-105.

Ogundiwin, J. O. and Oke, O. L. 1983. Factors affecting the processing of Wara - a Nigerian white cheese. *Food Chemistry* 11: 1-13.

Ogunwolu, S.O. and Adio, S.O. 2003. Preservative effect of *A. melegueta* and *A. danielli* on the storage stability of cashew juice. Proceedings of the 27th Annual Conference of Nigerian Institute of Food Science and Technology (NIFST).13- 17th Oct. Kano, Nigeria. Uzochukwu, E. (Eds).

Okiei, W. M., Ogunlesi, A. L., Obakachi, V. and Osunsanmi, M. and Nkenchor, G. 2009. The voltametric and titrimetric determination of ascorbic acid levels in tropical fruit samples. *International Journal Electronchemical Sci*ence 4: 276-287.

Okigbo, R.N. 2001.Mycoflora within black plum(*Vitex doniana* sweet) fruit.*Fruits* 56 2:85-92.

Oladipo, I.C. and Jadesimi, P.D. 2013. Microbiological Analysis and Nutritional Evaluation of West African soft cheese (*wara*) produced with different preservatives. *American Journal of Food and Nutrition* Print: ISSN 2157-0167.

Olarte, C., Sanz, S., Gonzaiez, E. and Torre, P. 2000. Identification of lactic acid bacteria isolated from katyk goat. *Journal Applied Microbiol*ogy 88.9: 421- 429.

Oluwamukomi, M.O, Eleyinmi, A.F, Enujiugha, V.N and Atofarati, S.O. 2003. 'Nutritional, physico-chemical and sensory evaluation of sorghum and cowpea-based weaning formulation' *Nigeria Food Journal* 21: 11-17.

Onimawo, I.A. 2002. Proximate composition and selected physicochemical properties of the seed, pulp and oil of sour sop (*Annona muricata*). *Plant Foods for Human Nutr*ition 57: 165-171.

Onyechi, U., Ibeanu, U., Nkiruka, V., Eme, E. P. and Madubike, K. 2012. Nutrient phytochemical composition and sensory evaluation of soursop *(Annona muricata)* pulp and drink in South Eastern Nigeria. *International Journal of Basic and Applied Sciences* 12.6: 53 - 57.

Orman, A., Günay, A., Balcı, F. and Koyuncu, M. 2011. Monitoring of somatic cell count variations during lactation in primiparous and multiparous Turkish Saanen goats (*Caprahircus*). *Turkey Journal Veterinary Animal Science* 35.3: 169-175.

Oskoueian, E., Abdullah, N., Saad, W.Z., Omar, A.R., Ahmad, S., Kuan, W.B., Zolkifli, N.A., Hendra, R. and Ho, Y.W. 2011. Antioxidant, anti-inflammatory and anticancer activities of methanolic extracts from *Jatropha curcas* Linn. *Journal Medical Plants Research 5*: 49–57.

Osorio, E., Arango, G.J., Jimenez, N., Alzata, F., Ruiz, G., Gutierrez, D., Paco, M.A., Gimenez, A. and Robledo, S. 2007. Antiprotozoal and cytotoxic activities in vitro of Colombian Annonaceae. *Journal Ethnopharmacology* 111: 630-635.

Osundahunsi, O., Amosu, D. and Ifesan, B. 2007. Quality evaluation and acceptability of soy-yoghurt with different colours and fruit flavours. *American Journal of Food Technology*. 2.4: 273-280.

Osundahunsi, O.F. and Aworh, O.C. 2003. Nutritional evaluation with emphasis on protein quality of maize – based complimentary foods and enriched with soya beans cowpea-tempe. *International Journal of Food Science and Technology* 38:809-813.

Ott, A., Fay, L.B. and Chaintreau, A. 1997. Determination and origin of the aroma impact compounds of yoghurt flavour. *Journal Agriculture Food Chem*istry 45:850–858.

Ouwehand, A.C., Salvadori, B.B., Fonden, R., Mogensen, G., Salminen, S. and Sellars, R. 2003. Health effects of probiotics and culture-containing dairy products in humans. International Dairy Fed. (IDF), *Brussels* 380:4–19.

Oyawoye E.O. and Ogunkule, M. 1998. Physiological Biochemical of Raw Jack Beans on Broilers.*Proceeding of Annual Conference of Nigeria.Society of Animal Production.*pp 141-142.

Oyetaya, V.O., Adetuyi, F.C. and Akinyosoye, F.A. 2003. Safety and protective effect of *Lactobacillus acidophilus* and *Lactobacillus casei* used as probiotic agent *in vivo*. *African Journal Biotechnol*ogy 2: 448-452.

Ozer, B.H., Bell, A.E., Grandison, A.S. and Robinson, R.K. 1998. Rheological properties of concentrated yoghurt Labneh. *J. Texture Studies*. In: Evaluating the effect of milk total solids on the relationship between growth and activity of starter cultures and quality of concentrated yoghurt (Eds.) Mahdian, E. and MazaheriTehrani, M. 2007. *American-Eurasian Journal Agricultural and Environmental Sci*ence 2.5: 587-592.

Ozkan, M., Kirea, A. and Cameroglu, B. 2004. Effect of hydrogen peroxide on the stability of ascorbic acid during storage in various fruit juices. *Food Chemistry*88: 591-597.

Park, Y.W. 1994b. Nutrient and mineral composition of commercial US goat milk yoghurts. *Small Ruminant Research* 13: 63–70.

Pasquier, B., Armand, M., Guillon, F., Castelain, C., Borel, R., Barry, J. L., Pieroni, G. and Lairon,D. 1996.Viscous solubile dietary fibres alter emulsification andlipolysis of triacylglycerols in duodenal medium in vitro. *Journal Nutrition Biochemistry* 7: 293-302.

Pinthong, R. Macrae, R. and Rothwell J. 1980.The development of soya-based yoghurt.*Journal Food Technology*. 15: 647-667.

Pkesatcha, T., Tukkeerees, D. and Rohrer, J. 2012. Determination of phytic and in soybeans and black sesame seeds. Thermo Fisher Scientific Incorporated.USA/Canada. pp 1-4.

Pore, M.S. and Magar, N.G. 1978. Effects of feeding raji on serum and liver lipids.*Indian Journal Biotechnology Chemistry Biology Phys*ics 13: 180-190.

Pruthi, J.S. 1980. Spices and condiments. Chemistry Microbiology, Technology. *Advance Food Res*ponse 4: 16-31.

Psoni, L., Tzanetakis, N. and Litopoulou-Tzanetaki, E. 2003. Microbial characteristics of Batzos, a traditional Greek cheese from raw goat's milk. *Food Microbiology* 20: 575– 582

Qureshi, A.M., Hassan, S.Y., Sulariya, A.M., and Rashid, A. A. 2011. Preparation and nutritional evaluation of garlic based yoghurt. *Science Int*ernational (*Lahore*) 23.1: 59-62.

Ragana, S. 1987. Handbook of analysis and quality control for fruit and vegetable products. Tata Mcgraw Hill Publication, New Delhi. India.

Rai, D., Singh, J. K., Roy, N. and Panda, D. 2008. Curcumin inhibits FtsZ assembly: an attractive mechanism for its antibacterial activity. *Biochemistry Journal* 410:147-55.

Rainbird, A. L., Low, A. G. 1986. Effect of varioustypes of dietary fibre on gastric emptying in growing pigs. *Britain Journal Nutrition* 55: 111-121.

Rampilli, M. and Cortellino, G. 2004. Evaluation of bio-functional proteins in goat milk and cheeses. The future of the sheep and goat dairy sectors. International Symposium: Session 5-01, Zaragoza, Spain.

Rasic, J.L. and Kurmann, J.A. 1978. Yoghurt: Scientific grounds, technology, manufacture and preparation. Technical Dairy Publishing House, Berne, Switzerland.

Rasko, I. and Downes, C.S. 1995. *Molecular Biology and Human Genetic Disorders. Genes in Medicine.*London: Chapman and Hall,

Reid, G. 1999. The scientific basis for probiotic strains of *Lactobacillus*. *Applied Environment Microbiol*ogy 65.9: 3763-3766.

Reid, G., Anand, S., Bingham, M.O., Mbugua, G., Wadstrom, T., Fuller, R., Anukam, K. and Katsivo, A. 2005. Probiotics for the developing world. *Journal Clinical Gastroenterol*ogy 39: 485–488.

Robinson, R.K. 1981. Yoghurt manufacture- some considerations of quality.*Dairy Industries International* 46.3: 31–35.

Robinson, R.K., 2002. Yoghurt, role of starter cultures, in *Encyclopedia of dairy science,* H. Roginski, J. Fuquay, and P. Fox, Eds. Academic Press, United Kingdompp. 1059-1063.

Robinson, R.K. and Tamime A.Y. 1995.Microbiology of fermented milks. In: Dairy microbiology, Robinson R.K. 2nd ed, Vol. 2. London, Elsevier Applied Science. 291 - 343.

Roehrig, K. 1988. The physiological effects of dietary fibre: A review. *Food Hydrocolloids* 2:1-18.

Roissart, H., and Luquet, F. M. 1994. BacteÂriesLactiques: Aspect fondamentauxettechnologiques (Vol. 2). Grenoble, France: Lorica. In: Viability of lactic acid microflora in different types of yoghurt (Eds.)

Rolfe, R.D. 2000. The role of probiotic cultures in the control of gastrointestinal health. *Journal Nutrition* 130: 396S-402S.

Rudrello, F. 2004. Health trends shape innovation for dairy products (online). Euromonitor international archive; http://www.euromonitor.com/article.asp. Accessed Oct 5. 2011.

Rumeza, H., Zafar, I., Mudassa, I., Sheheena, H. and Masooma, R. 2006. Uses of vegetables as nutritional food: Role in human health. *Journal Agriculture Biology Sci*ence 1.1: 18-22.

Saint-Eve, A., Levy, C., Le Moigne, M., Ducruet, V. and Souchon, I. 2008. Quality changes in yoghurt during storage in different packaging materials. *Food Chemistry* 110: 285–293.

Salih, M.A., Sandine, W.E and Ayres, J.W.1990. Inhibitory effects of microgardTM on yoghurt and cottage cheese spoilage organisms. *Journal Dairy Science* 73: 887-893.

Sallami, L., Kheadr, E. E., Fliss, I. and Vuillemard, C.J. 2004. Impact of autolytic, proteolytic, and nisin-producing adjunct cultures on biochemical and textural properties of Cheddar cheese. *Journal of Dairy Science* 87: 1585–1594.

Salminen, S., Ouwehand, A., Benno, Y. and Lee, Y.K. 1999. Probiotics: how should they be defined? *Trends Food Science Technology* 10: 107-110.

Salvador, A. and Fiszman, S.M. 2004. Textural and sensory characteristics of whole and skimmed flavored set-type yogurt during long storage. *Journal Dairy Science* 87:4033–41.

Sanchez AH, De Castro A, Rejano L and Montano A. 2000. Comparative study on chemical changes in olive juice and brine during green olive fermentation. *Journal Agricultural Food Chemistry* 48:5975–80

Sanful, R. 2009.Promotion of coconut in the production of yoghurt.*African Journal of Food Science*3.5: 147-149.

Sarkar, S. 2003. Potential of acidophilus milk to lower cholesterol.*Nutrition and Food Science* 33 (6): 273-277.

Sasikumar, B. 2005.Genetics resources of curcuma, diversity, characterisation and utilisation. Plant genetics.*Resources Characterisation Utilisation*3: 230-251.

Saudy, E. 2008. Probiotics in yoghurt may aid gut health. Digestive diseases and sciences. Maryvale hospital medical centre.*Phoenix* 48.10: 2085 – 2091.

Savadogo, A., Cheik, A.T.I, Ouattara, B.H.N. and Traore, A.S. 2004. Antimicrobial activities of lactic acid bacteria strains isolated from Burkina Faso fermented milk. *Pakistan Journal Nutrition* 3: 174-179.

Schneeman, B.O. 1990. Carbohydrates: significance for energy balance and gastrointestinal function. *Journal Nutrition* 124, 1747-1753.

Schneeman, B. O. and Gallaher, D. 1985. Effect of dietary fibre on digestive enzyme activity and bile acids in the small intestine. *Proceedings Society Experimental Biology Medicine* 180: 409-414.

Schmalstieg, F.C. and Goldman, A.S. 2008. IlyaIlich Metchnikoff (1845-1915) and Paul Ehrlich (1854-1915): the centennial of the 1908 nobel prize in physiology or medicine". *Journal of Medical Biography* 16.2: 96–103.

Seelee, W., Tungjaroenchai, W. and Natvaratat, M. 2009. Development of low fat set-type probiotic yoghurt from goat milk. *Asian Journal of Food and Agro-Industry.* 2.04: 771-779.

Shah, N., Mahoney, R. R., Pellett, P. L. 1986. Effect of guar gum, lignin and pectin on the proteolytic enzyme level in the gastrointestinal tract in the rat: a time based study. *Journal Nutrition* 166: 786-794.

Shah, N.P. 2000. Probiotics bacteria: selective enumeration and survival in dairy foods. *Journal DairyScience* 83: 894-907.

Shah, S.P., Swartw, S.E. and Ouyang, C. (1995). Fracture mechanics of concrete. Wiley, New York.

Sharma, R.A., Euden, S.A., Platton, S.L., Cooke, D.N., Shafayat, A., Hewitt, H.R., Marczylo, T.H., Morgan, B., Hemingway, D. and Plummer, S.M. 2004.Phase I clinical trial of oral curcumin: Biomarkers of systemic activity and compliance. *Clinical Cancer Research* 10: 6847-6854.

Sherman, P.W. and Flaxman, S.M. 2001. Protecting ourselves from food.*Amazon Science* 89: 142–151.

Shihata, A. and Shah, N.P. 2000. Proteolytic profiles of yogurt and probiotic bacteria. *International Dairy J*ournal 10.5-6: 401-408.

Shishodia, S., Sethi, G, and Aggarwal, B. B. 2005.Curcumin: getting back to theroots. *Ann New York Academic Science,* 1056: 206–217.

Shurkhna, R.A, Validov, S.Z., Boronin, A.M. and Naumova, R.P. 2006. Modeling of lactic acid fermentation of leguminous plant juices. *Applied Biochemistry Microbiol*ogy 42:204–9.

Simova, E.D., Frengova, G.T. and Beshkova D.M. 2004. Synthesis of carotenoids by *Rhodotorula rubia* cultured with yoghurt starter whey ultra filterate. *Journal Society Dairy Tech*nology 31: 115-121.

Sindhu, S.C. and Khetarpaul, N. 2013. Effect of feeding probiotics fermented indigenous food mixture on serum cholesterol levels in mice. *Nutrition Research* 23: 1071-1080.

Singh, S. and Mittal, S.K. 1994. Development of soy cheese spread. *Journal Food Science Technology Mysore* 21.4: 205-211.

Sodini, I., Lucas, A. Oliveria, M. N., Remeuf, F. and Codrrieu, G. 2002. Effect of milk base and starter culture on acidification, texture and probiotic cell counts in fermented milk processing. *Journal of Dairy Science* 85 (10): 2479-2488.

Sodini, I.F., Remeuf, S., Haddad, A. and Codrrieu, G. 2004. The relative effect of milk base, starter and process on yoghurt texture: a review. *Crit. Rev*iews *Food Science Nutrition* 44:113-137.

Solga, S.F.2003. Probiotics can treat hepatic encephalopathy. *Medical Hypotheses* 61: 307-313

Spano, G., Beneduce, L., Perrotta, C. and Massa, S. 2005. Cloning and characterization of the hsp 18.55 gene, a new member of the small heat shock gene family isolated from wine *Lactobacillus plantarum*. *Research Microbiol*ogy156: 219– 224.

Srinivasan, K. 2005. Role of spices beyond food flavouring: nutraceuticals with multiple health effects. *Food Review International* 21: 167-188.

Srbinovski, K.S., Cizbanovski, T., Dzabirski, V., Andonov, S. and Palasevski, B. 2001. Dynamics of salt diffusion and yield of three types of goat's milk cheese. *Mljekarstov* 51.1: 15 – 26.

Staffolo, M.D., Bertola, N., Martino, M. and Bevilacgua, Y.A.2004. Influence of dietary fiber addition on sensory andrheological properties of yoghurt. *Int. Dairy J.* 14, 263-268.

Stasse-Wolthuis, M. 1981. Influence of dietary fibre on cholesterol metabolism and colonic function in healthy subjects. *World Revolution Nutrition Diet* 36: 100-140.

Sutherland, J.P. and Varnam, A.H. 1994. Milk and milk products technology. Chemistry and Microbiology. London: Chapman and Hall p8.

Tamime, A.Y. and Deeth, H. 1980. Yoghurt: Technology and biochemistry. *Journal Food Protection* 43:939-977.

Tamime, A.Y. and Muir, D.D. 1998. Strategies for modifying the structure of fermented milks, in texture of fermented milk products and dairy dessert. *IDF Special Issue* 2: 186-196.

Tamime, A.Y. and Robinson, R.K. 1985. Yoghurt: Science and Technology.Pergamon Press Ltd. Oxford, England. pp 1-5.

Tamime, A.Y. and Robinson, R.K. 1999a. Developments in yoghurt production and related products. In: Yoghurt: Science and Technology. Cambridge, UK: Woodhead Publishing Limited p. 349.

Tamime, A.Y. and Robinson, R.K. 1999b. Yoghurt: Science and Technology. 2nd edn. CRC

Press, Boca Raton, FL.
Tamime, A.Y. 2005. Probiotic Dairy Products, Oxford, UK: Blackwell Publishing Ltd. p.41

Tannock, G.W. 1998. Studies of the intestinal microbiota: a prerequisite for the development of probiotic. *International Dairy Journal* 8: 527-533.

Tarakci, Z. and Erdogan, K. 2003. Physical, chemical, microbiological and sensory characteristics of some fruit-flavoured yoghurt. *Journal Food Prot*ection 59: 402-406.

Thottappilly, G., Thresh, J.M., Calvert, L.A and Winter, S. 2003. Cassava: In virus and virus-like diseases of major crops in developing countries, G. Loebenstein and G. Thottappilly (eds), Pages 107-165, Kluwer Academic publishers p. 800

Thomas, T.D. and Mills, O.E. 1981. Proteolytic enzymes of starter bacteria.*Netherlands Milk and Dairy Journal*35.3-4: 255-273.

Thompson, A.K. 2003. Fruits and vegetables: Harvesting and storage, 2nd edition pp 115-369.

Todd, P. A., Benfield, P., Goa, K. L. 1990. Guar gum;a review of its pharmacological properties, and uses asa dietary adjunct in hypercholesterolaemia. *Drugs* 39: 917-928.

Tohibu, A. Amankwah, E. and Oduro, I. 2013. Chemical stability of vacuum packaged West African cheese (wagashie).*Scientific Research and Essays*. 8.26: 1212-1218.

Topping, D. L., Oakenfull, D., Trimble, R. P. and Illman, J. R. 1988. A viscous fibre (methycellulose) lowers glucose and plasma triacylglycerols and increases liver glycogen independently of volatile fatty acid production in the rat. *Britain Journal Nutrition* 59: 21-30.

Trias, R., Bañeras, L., Montesinos, E. and Badosa, E. 2008. Lactic acid bacteria from fresh fruit and vegetables as biocontrol agents of phytopathogenic bacteria and fungi. *International Microbiology* 11: 231-236.

Troller J. 1973.Yoghurt cultures.*Journal Society Dairy Technology* 26:16

Tuohy, K.M., Probert, H.M., Smejkal, C.W. and Gibson, G.R. 2003. Review: Using probiotics and probiotics to improve gut health. *Drug Discov*ery *Today* 8: 692-700.

Tziboula-Clarke, A. 2003.Goat milk. In: Encyclopedia of dairy sciences H Roginnski, J W Fuquay, P F Fox Eds. Vol. 2 London, UK: Academic UK: Woodhead Publishing Ltd. pp. 1270–1279.

Uzogara, S. G., Agu, L. N. and Uzogara, E. 0. 1990. A review of traditional fermented foods, condiments and beverages in Nigeria: Their benefits and possible problems. *Ecology Food Nutrition* 24: 267-278.

Vargas, M., Chafer, M., Albors, A., Chiralt, A. and Gonzalez-Martinez, C. 2008. Physicochemical and sensory characteristics of yogurt produced from mixtures of cows' and goats' milk. *International Dairy Journal* 18: 1146–1152.

Vasiljevic, T. and Jelen, P. 2002. Lactose hydrolysis in milk as affected by neutralizers used for the preparation of crude β-galactosidase extracts from *Lactobacillus bulgaricus* 11842. *Innovative Food Science and Emerging Technologies* 3: 175-184.

Vedamuthu, E.R. 1982. Fermented milks.In economic microbiology of fermented foods. Rose, A. H. (Ed.) Academic Press. London.

Vedamuthu, E.R. 1991. The yoghurt story-past, present and future.*Dairy Food Environmental* 20:156-159.

Vesa, T.H., Marteau, J. and Korpela, R. 2000. Lactose intolerance.*Journal Amazon College Nutr*ition 129:165-175.

Vinderola, C. G., Prosello, W., Ghiberto, D. and Reinheimer, J.A. 2000. Viability of probiotic (Bifidobacteium, Lactobaciluus acidophilus and Lactobacillus casei) and non-probiotic microflora in Argentinian fresco cheese.*Journal Dairy Science*83: 1905-1911.

Vlaemynck, G. 1992. Study of lypolytic activity of the lipoprotein lipase in lunch cheese of the Gouda type.*Milchwissenschaft* 47: 164–167.

Wall, M.M. 2006.Ascorbic acid, vitamin A, and mineral composition of banana (*Musa* spp.) and papaya (*Caricapapaya*) cultivars grown in Hawaii. *Journal Food Composition Anal*ysis 19: 434 – 445.

Walstra, P. 1998. Relation between structure and texture of cultured milk products. In: Texture of fermented milk products and dairy desserts. *Special Issue* 9802. International Dairy Federation, Brussels. pp. 9-15. *Sanitation* 7: 371-374.

Wanda, 2005. Changes in acidity of fermented milk products during their storage as exemplified by natural bio-yoghurt. *Milchwissenschaft* 60.3: 294-296.

Wang, Y. C., Yu, R. C. and Chou. C. C. 2002. Growth and survival of bifidobacteria and lactic acid bacteria during the fermentation and storage of cultured soymilk drinks. *FoodMicrobiology* 19: 501-508.

Warrington, I. J. 2003. "Annonaceae".*Apples: Botany, Production and Uses*.CABI Publishing. ISBN 0851995926. http://books.google.com/books.Retrieved 2008-04-20.

Waston, R.R. and Preedy, V.R. 2009. Bioactive foods in promoting health: Fruits and vegetables. Academic Press, UK. pp 628 – 629.

Watts, B.M., Ylimaki, G.L., Jeffrey-L, E. and Elias, L.G. 1989. Basic sensory methods for food

evaluation. The international develpoment research centre, Ottawa, Canada.
Webb, B.H., Johnson, A.H. and Alford, J.A. 1983. Fundamentals of Dairy Chemistry (2nd ed.). Westport, CT: AVI Publishing Co. Inc. p17.

Wouters, J.T.M., Ayad, E.H.E., Hugenholtz, J. and Smit, G. 2002. Microbes from raw milk for fermented dairy products. *International Dairy J*ournal 12: 91– 109.

Worrell, D.B., Carrington, C.M.S. and Huber, D.J. 1994. Growth, maturation, and ripening of soursop (*Annona muricata* L.) fruit. *Scientia Horticulturae* 57: 7–15.

Yale-New Haven 2013. Hospital nutrition advisor – Understanding yoghurt at the wayback Machine.ynhh.com. Retrived on 9 April 2013

Younus, S., Masud, T. and Aziz, T. 2002. Quality evaluation of market yoghurt/Dahi.*Pakistan Journal of Nutrition* 1.5: 226-230.

Yvon, M. and Rijnen, L. 2001.Cheese flavor formation by amino acid catabolism.*International Dairy Journal*11: 185-201.

Zehren, V.L. and Nusbaum, D.D. 2000. Processed cheese: Scientific and technological aspects-A review.2nd ed. Cheese ReporterPublishing Co. Inc., Madison, WI. pp27

Zirnstein, G. and Hutkins, R. 1999. *Streptococcus thermophiles*. In: Enyclopedia of Food Microbiology, C. Batt, P. Patel, and R. Robinson (Eds.) Academic Press, UnitedKingdom pp. 2127-2133.

Appendix 1: Analysis of Variance of *A. Danielli* and Curcumin on pH of Soursop Yoghurt Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	6	68.01393714	11.33565619	118629	<.0001
Preservatives	2	0.58143302	0.29071651	3042.38	<.0001
Concen(Preservatives)	12	11.01738095	0.91811508	9608.18	<.0001
Weeks*Concen(Preservat	84	4.57557714	0.05447116	570.05	<.0001
Error	210	0.02006667	0.00009556		
Corrected Total	314	84.20839492			

*Significant at 5% and above level of probability

Appendix 2: Analysis of Variance of *A. danielli* and Curcumin (^{0}Brix) Sugar of Soursop Yoghurt Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	6	25.53542857	4.25590476	1538.81	<.0001
Preservatives	2	6.42742857	3.21371429	1161.98	<.0001
Concen(Preservatives)	12	23.47714286	1.95642857	707.39	<.0001
Weeks*Concen(Preservat	84	16.14742857	0.19223129	69.51	<.0001
Error	210	0.58080000	0.00276571		
Corrected Total	314	72.16822857			

*Significant at 5% and above level of probability

Appendix 3: Analysis of Variance of *A. danielli* and Curcumin on Total Titratable Acidity (% lactic acid) of Soursop Yoghurt Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	6	11.15526222	1.85921037	32717.9	<.0001
Preservatives	2	0.55695683	0.27847841	4900.60	<.0001
Concen(Preservatives)	12	0.62890667	0.05240889	922.28	<.0001
Weeks*Concen(Preservat	84	0.81818540	0.00974030	171.41	<.0001
Error	210	0.01193333	0.00005683		
Corrected Total	314	13.17124444			

*Significant at 5% and above level of probability

Appendix 4: Analysis of Variance of *A. danielli*and Curcumin on Crude Protein (%) of Soursop Yoghurt Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	6	123.130714	20.521786	0.72	0.6326
Preservatives	2	26.825951	13.412976	0.47	0.6246
Concen(Preservatives)	12	345.891615	28.824301	1.01	0.4374
Weeks*Concen(Preservat	84	2418.957771	28.797116	1.01	0.4621
Error	210	5971.525467	28.435836		
Corrected Total	314	8886.331519			

*Significant at 5% and above level of probability

Appendix 5: Analysis of Variance of *A. danielli*and Curcumin on Fat (%) of Soursop Yoghurt Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	3	0.00001175	0.00000392	2.30	0.0804
Preservatives	2	0.00001810	0.00000905	5.32	0.0061
Concen(Preservatives)	12	0.00004260	0.00000355	2.09	0.0225
Weeks*Concen(Preservat	42	0.00006250	0.00000149	0.88	0.6832
Error	120	0.00020400	0.00000170		
Corrected Total	179	0.00033895			

*Significant at 5% and above level of probability

Appendix 6: Analysis of Variance of *A. danielli*and Curcumin on Vitamin C (mg/100ml) of Soursop Yoghurt Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	6	533.0249238	88.8374873	2884929	<.0001
Preservatives	2	0.0452006	0.0226003	733.93	<.0001
Concen(Preservatives)	12	61.9559295	5.1629941	167664	<.0001
Weeks*Concen(Preservat	84	20.0044476	0.2381482	7733.68	<.0001
Error	210	0.0064667	0.0000308		
Corrected Total	314	615.0369683			

*Significant at 5% and above level of probability

Appendix 7: Analysis of Variance of *A. danielli*and Curcumin on Total Plate Count (cfu/ml) of Soursop Yoghurt Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	6	3.8108301E16	6.3513835E15	22.37	<.0001
Preservatives	2	5.5295397E14	2.7647698E14	0.97	0.3793
Concen(Preservatives)	12	4.0816184E16	3.4013487E15	11.98	<.0001
Weeks*Concen(Preservat	84	7.1129671E16	8.4678179E14	2.98	<.0001
Error	210	5.9622807E16	2.8391813E14		
Corrected Total	314	2.1022992E17			

*Significant at 5% and above level of probability

Appendix 8: Analysis of Variance of *A. danielli*and Curcumin on Yeast Count of Soursop Yoghurt Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	6	5.2373029E14	8.7288382E13	Infty	<.0001
Preservatives	2	9.4634954E14	4.7317477E14	Infty	<.0001
Concen(Preservatives)	12	4.0355769E13	3.3629807E12	Infty	<.0001
Weeks*Concen(Preservat	84	1.0969782E15	1.3059265E13	Infty	<.0001
Error	210	0	0		
Corrected Total	314	2.6074138E15			

*Significant at 5% and above level of probability

Appendix 9: Analysis of Variance of *A. danielli*and Curcumin on pH of Cow milk Yoghurt Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	6	8.89722667	1.48287111	37669.7	<.0001
Preservatives	2	3.38508635	1.69254317	42996.1	<.0001
Concen(Preservatives)	12	5.00118667	0.41676556	10587.2	<.0001
Weeks*Concen(Preservat	84	3.76554476	0.04482791	1138.77	<.0001
Error	210	0.00826667	0.00003937		
Corrected Total	314	21.05731111			

*Significant at 5% and above level of probability

Appendix 10: Analysis of Variance of *A. danielli*and Curcumin on Total Soluble Sugar (^{0}brix) of Cow milk Yoghurt Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	6	371.580000	61.930000	24693.6	<.0001
Preservatives	2	1090.258349	545.129175	217362	<.0001
Concen(Preservatives)	12	433.733524	36.144460	14412.0	<.0001
Weeks*Concen(Preservat	84	336.848571	4.010102	1598.96	<.0001
Error	210	0.526667	0.002508		
Corrected Total	314	2232.947111			

*Significant at 5% and above level of probability

Appendix 11: Analysis of Variance of *A. danielli*and Curcumin on Total Titratable Acidity (cfu/ml) of Cow milk Yoghurt Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	6	15.13677714	2.52279619	115171	<.0001
Preservatives	2	0.27718857	0.13859429	6327.13	<.0001
Concen(Preservatives)	12	0.20756571	0.01729714	789.65	<.0001
Weeks*Concen(Preservat	84	0.33816571	0.00402578	183.79	<.0001
Error	210	0.00460000	0.00002190		
Corrected Total	314	15.96429714			

*Significant at 5% and above level of probability

Appendix 12: Analysis of Variance of *A. danielli*and Curcumin on Crude Protein of Cow milk Yoghurt Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	6	0.12522857	0.02087143	996.14	<.0001
Preservatives	2	0.00000000	0.00000000	0.00	1.0000
Concen(Preservatives)	12	0.00604571	0.00050381	24.05	<.0001
Weeks*Concen(Preservat	84	0.00171429	0.00002041	0.97	0.5468
Error	210	0.00440000	0.00002095		
Corrected Total	314	0.13738857			

*Significant at 5% and above level of probability

Appendix 13: Analysis of Variance of *A. danielli*and Curcumin on Fat of Cow milk Yoghurt Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	6	2.21355429	0.36892571	19368.6	<.0001
Preservatives	2	0.05376571	0.02688286	1411.35	<.0001
Concen(Preservatives)	12	0.01707429	0.00142286	74.70	<.0001
Weeks*Concen(Preservat	84	0.07656000	0.00091143	47.85	<.0001
Error	210	0.00400000	0.00001905		
Corrected Total	314	2.36495429			

*Significant at 5% and above level of probability

Appendix 14: Analysis of Variance of *A. danielli*and Curcumin on Total Plate Count (cfu/ml) of Cow milk Yoghurt Sample during Storage.

Source	Degreeof Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	6	4.5496758E17	7.582793E16	68636.9	<.0001
Preservatives	2	2.6471641E17	1.3235821E17	119806	<.0001
Concen(Preservatives)	12	2.268801E18	1.8906675E17	171137	<.0001
Weeks*Concen(Preservat	84	1.8819191E18	2.2403798E16	20279.2	<.0001
Error	210	2.320016E14	1.1047695E12		
Corrected Total	314	4.8706361E18			

*Significant at 5% and above level of probability

Appendix 15: Analysis of Variance of *A. danielli*and Curcumin on Yeast Count (cfu/ml) of Cow milk Yoghurt Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	6	7.3612561E13	1.226876E13	4236861	<.0001
Preservatives	2	14005570523	7002785261.7	2418.32	<.0001
Concen(Preservatives)	12	5.8335719E13	4.8613099E12	1678792	<.0001
Weeks*Concen(Preservat	84	2.8515668E14	3.3947224E12	1172324	<.0001
Error	210	608100999.75	2895719.0464		
Corrected Total	314	4.1711957E14			

*Significant at 5% and above level of probability

Appendix 16: Analysis of Variance of *A. danielli* and Curcumin on pH of Goat milk Yoghurt Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	6	36.47709714	6.07951619	182385	<.0001
Preservatives	2	2.97618857	1.48809429	44642.8	<.0001
Concen(Preservatives)	12	4.27457143	0.35621429	10686.4	<.0001
Weeks*Concen(Preservat	84	4.32676000	0.05150905	1545.27	<.0001
Error	210	0.00700000	0.00003333		
Corrected Total	314	48.06161714			

*Significant at 5% and above level of probability

Appendix 17: Analysis of Variance of *A. danielli*and Curcumin on Total Soluble Solids (^{0} brix) of Goat milk Yoghurt Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	6	277.8007619	46.3001270	5833.82	<.0001
Preservatives	2	845.1802540	422.5901270	53246.4	<.0001
Concen(Preservatives)	12	292.4472381	24.3706032	3070.70	<.0001
Weeks*Concen(Preservat	84	271.6849524	3.2343447	407.53	<.0001
Error	210	1.666667	0.007937		
Corrected Total	314	1688.779873			

*Significant at 5% and above level of probability

Appendix 18: Analysis of Variance of *A. danielli*and Curcumin on Total Titratable Acidity (% lactic acid) of Goat milk Yoghurt Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	6	3.02457714	0.50409619	15567.7	<.0001
Preservatives	2	0.03684571	0.01842286	568.94	<.0001
Concen(Preservatives)	12	0.74134286	0.06177857	1907.87	<.0001
Weeks*Concen(Preservat	84	0.16785143	0.00199823	61.71	<.0001
Error	210	0.00680000	0.00003238		
Corrected Total	314	3.97741714			

*Significant at 5% and above level of probability

Appendix 19: Analysis of Variance of *A. danielli*and Curcumin on Protein Content (%) of Goat milk Yoghurt Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	6	0.11761714	0.01960286	588.09	<.0001
Preservatives	2	0.00000000	0.00000000	0.00	1.0000
Concen(Preservatives)	12	0.00833143	0.00069429	20.83	<.0001
Weeks*Concen(Preservat	84	0.00246857	0.00002939	0.88	0.7442
Error	210	0.00700000	0.00003333		
Corrected Total	314	0.13541714			

*Significant at 5% and above level of probability

Appendix 20: Analysis of Variance of *A. danielli*and Curcumin on Fat Content (%) of Goat milk Yoghurt Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	6	691.9040000	115.3173333	51.51	<.0001
Preservatives	2	5.3280000	2.6640000	1.19	0.3063
Concen(Preservatives)	12	5.1788571	0.4315714	0.19	0.9986
Weeks*Concen(Preservat	84	89.4331429	1.0646803	0.48	0.9999
Error	210	470.100000	2.238571		
Corrected Total	314	1261.944000			

*Significant at 5% and above level of probability

Appendix 21: Analysis of Variance of *A. danielli*and Curcumin on Total Plate Count (cfu/ml) of Goat milk Yoghurt Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	6	12174.95634	2029.15939	6.456E7	<.0001
Preservatives	2	60.18007	30.09004	957410	<.0001
Concen(Preservatives)	12	92.87645	7.73970	246263	<.0001
Weeks*Concen(Preservat	84	65.65943	0.78166	24871.0	<.0001
Error	210	0.00660	0.00003		
Corrected Total	314	12393.67890			

*Significant at 5% and above level of probability

Appendix 22: Analysis of Variance of *A. danielli*and Curcumin on Yeast Count (cfu/ml) of Goat milk Yoghurt Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	6	5196886189	866147698	43.39	<.0001
Preservatives	2	14748280703	7374140351	369.41	<.0001
Concen(Preservatives)	12	4839031011	403252584	20.20	<.0001
Weeks*Concen(Preservat	84	16375921126	194951442	9.77	<.0001
Error	210	4191962200	19961725		
Corrected Total	314	45352081229			

*Significant at 5% and above level of probability

Appendix 23: Analysis of Variance of *A. danielli*and Curcumin on pH of Cheese Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	4	166.3064400	41.5766100	4454637	<.0001
Preservatives	2	13.7499840	6.8749920	736606	<.0001
Concen(Preservatives)	12	17.7260160	1.4771680	158268	<.0001
Weeks*Concen(Preservat	56	14.0373600	0.2506671	26857.2	<.0001
Error	150	0.0014000	0.0000093		
Corrected Total	224	211.8212000			

*Significant at 5% and above level of probability

Appendix 24: Analysis of Variance of *A. danielli*and Curcumin on Total soluble Solids (^{0}brix) of Cheese Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	4	390.2080000	97.5520000	52260.0	<.0001
Preservatives	2	101.0336000	50.5168000	27062.6	<.0001
Concen(Preservatives)	12	27.1464000	2.2622000	1211.89	<.0001
Weeks*Concen(Preservat	56	65.6720000	1.1727143	628.24	<.0001
Error	150	0.2800000	0.0018667		
Corrected Total	224	584.3400000			

*Significant at 5% and above level of probability

Appendix 25: Analysis of Variance of *A. danielli*and Curcumin on Total Titratable Acidity (cfu/ml) of Cheese Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	4	0.66185600	0.16546400	11281.6	<.0001
Preservatives	2	0.00012800	0.00006400	4.36	0.0144
Concen(Preservatives)	12	0.54172800	0.04514400	3078.00	<.0001
Weeks*Concen(Preservat	56	0.30198400	0.00539257	367.68	<.0001
Error	150	0.00220000	0.00001467		
Corrected Total	224	1.50789600			

*Significant at 5% and above level of probability

Appendix 26: Analysis of Variance of *A. danielli*and Curcumin on Protein Content (%) of Cheese Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	4	38.99401600	9.74850400	149212	<.0001
Preservatives	2	0.01164800	0.00582400	89.14	<.0001
Concen(Preservatives)	12	0.00808800	0.00067400	10.32	<.0001
Weeks*Concen(Preservat	56	0.01702400	0.00030400	4.65	<.0001
Error	150	0.00980000	0.00006533		
Corrected Total	224	39.04057600			

*Significant at 5% and above level of probability

Appendix 27: Analysis of Variance of *A. danielli*and Curcumin on Fat Content (%) of Cheese Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	4	1674.807136	418.701784	1.495E7	<.0001
Preservatives	2	0.063128	0.031564	1127.29	<.0001
Concen(Preservatives)	12	0.046968	0.003914	139.79	<.0001
Weeks*Concen(Preservat	56	0.060824	0.001086	38.79	<.0001
Error	150	0.004200	0.000028		
Corrected Total	224	1674.982256			

*Significant at 5% and above level of probability

Appendix 28: Analysis of Variance of *A. danielli*and Curcumin on Total Plate Count (cfu/ml) of Cheese Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	4	9.253994E14	2.3134985E14	102055	<.0001
Preservatives	2	8.8604786E13	4.4302393E13	19543.1	<.0001
Concen(Preservatives)	12	1.0313741E14	8.5947844E12	3791.42	<.0001
Weeks*Concen(Preservat	56	3.1702323E14	5.6611291E12	2497.29	<.0001
Error	150	340036000000	2266906666.7		
Corrected Total	224	1.4345049E15			

*Significant at 5% and above level of probability

Appendix 29: Analysis of Variance of *A. danielli*and Curcumin on Yeast Count (cfu/ml) of Cheese Sample during Storage.

Source	Degree of Freedom	Anova SS	Mean Square	F Value	Pr > F
Weeks	4	0.55772000	0.13943000	6971.50	<.0001
Preservatives	2	0.02535200	0.01267600	633.80	<.0001
Concen(Preservatives)	12	0.03616800	0.00301400	150.70	<.0001
Weeks*Concen(Preservat	56	0.02476000	0.00044214	22.11	<.0001
Error	150	0.00300000	0.00002000		
Corrected Total	224	0.64700000			

*Significant at 5% and above level of probability

Appendix 30: Formulated Composition of Nitrogen-Free Diet

Corn starch	65%
Glucose	5%
Sucrose	10%
Cellulose	5%
Vegetable oil	10%
Premix	2%
Oyster shell	1%
Bone meal	2%

Appendix 31: Nutient Agar Composition

Formulation	grams/litre
Peptone	5.0
Beef Extract	3.0
Sodium Chloride	8.0
Agar No 2	12.0
p^H	7.3± 0.2

It is used for the cultivation of bacteria and for the enumeration of organisms in water, sewage, faeces. Etc It is called a general purpose medium. Weigh 28g of powder, dissole in 1 liter of deionised water, allow to soak for 10 mins. Swirl to mix then sterile by autoclaving for 15mins at 121^0C. Cool to 47^0C, mix well then pour plates.

MRS de Man, Rogosa and Sharpe Agar

Formulation	grams/litre
Mixed Peptone	10.0
Yeast Extract	5.0
Beef Extract	10.0
Glucose	20.0
Dipotassium phosphate	2.0
Sodium acetate	5.0
Triammonium citrate	2.0
Magnesium sulphate	0.2
Manganase sulphate	0.05

Tween 80	1.08
Agar p^H	6.4±0

Potato Dextrose Agar Composition, p^H EUR-USP formula in g/l:

Formulation	grams/litre
Potato Extract(solid)	4
Dextrose	20
Agar	15
p^H	5,6 (approx.)

It is useful for the identification, cultivation and enumeration of yeasts and mould.Suspend 39g in 1litre of distilled water and boil to dissolve the medium completely. Sterile by autoclave at 115^0 for 10mins.

Appendix 32: PCR Recipe

PCR Mixture

DNA (5ng/µl)	5µl
50mM $MgCl_2$ (Bioline)	0.3 µl
10X Buffer (Bioline)	2.5 µl
Taq polymerase buffer	0.2 µl
dNTPS 2.5 mM	0.8 µl
Tween 20	2.5 µl
Primer 1	2.0 µl
Sterile deionised water (SdH_20)	4.2 µl

dNTP

Twenty microlitre of each 100mM dATP, dCTP, dGTP and dTTP are taken and mixed in an eppendof tube. 920µl of sterile deionised water was added to final concentration of 2mMsolution was mixed gently and stored at -20^0C.

PRIMER 1 of EGE1

590 µl primer EGE1 was dissolved in 295 µl of sterile deionised water to obtain 2 µg/ µl solution. 5 µl of stock solution were taken and mixed with 95 µl sterile deionised water. The resulting solution had 100 µl, 10 picomole / µl concentration. Stock and working solutions were stored at -20^0C.

PRIMER of L1

350 µl primer L1 was dissolved in 175 µl of sterile deionized water to obtain2 µg / µ stock solution. 5 µl of stock solution were then taken and mixed with 95 µl sterile deionized water. The resulting solution had 100 µl, 10 picomole/ µl concentration. Stock and working solutions were stored at -20^0C.

Appendix 33: Raw Cow and Goat milk collected from Teaching and Research Dairy Farm University of Ibadan

Appendix 34: Water bath equipment in Food Technology Department

Appendix 35: Individual Metallic cages used in Animal House