KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COMPARATIVE STUDIES ON THE QUALITIES OF COMMERCIALIZED YOGHURT IN KUMASI AND THE EFFECT OF NATAMYCIN ON YOGHURT DURING STORAGE

BY

DZIGBORDI BAKAR BSc (Hons.)

A THESIS SUBMITTED TO THE DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY, COLLEGE OF SCIENCE, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (FOOD SCIENCE AND TECHNOLOGY)

MARCH 2011

DECLARATION

I hereby declare that this submission is my own work towards the M.Sc. and that to the best of my knowledge, it contains no material previously published by another person or material which has been accepted for the award of any other degree of the University except where due acknowledgment has been made in the text.

Dzigbordi Bakar	PG2820708		
……	……..…….	…….	………..
Student name	**Student number**	**Signature**	**Date**

Certified by supervisors:

Rev. J. Adubofuor …….. …….

Dr F. Dufie Wireko-Manu ……..….. ……..……

Signature **Date**

Certified by Head of Department:

Prof. Ibok Oduro

……. ……. ……..

Signature **Date**

ACKNOWLEDGEMENT

I am greatly indebted to my supervisors Rev. J. Adubofuor and Dr. F. Dufie Wireku-Manu. I will always remain extremely grateful for their invaluable guidelines, contributions and encouragement which have afforded me immeasurable experiences. My profound gratitude also goes to Dr Ray B. Voegborlo for his fatherly advice and encouragement and his continuous interest in my academic and professional development. I am also indebted to Mr William I. Ofosu and Mr William Apaw for their time, and expertise in helping me with the statistics and to Mr Emmanuel Achiampong for his expertise in the microbiological studies conducted in this research.

This study would not have been successful were it not for the cooperation and help provided by yoghurt manufacturers in the Kumasi metropolis. I owe them a debt of gratitude. I also wish to thank my sensory panelists and all those who took part in the survey on yoghurt, for their dedication to my project.

Furthermore, I would like to thank my family members and my close friends for their constant encouragement in all my endeavours. They were always there as my pillar when I needed support.

Above all, I thank God who was with me in the beginning and has seen me through to the end of this stage in my education.

ABSTRACT

Yoghurt is a fermented dairy product which is consumed as a dessert, snack or as a pro-biotic food drink and has been one of the dairy products patronized by consumers in the Kumasi metropolis. The study was undertaken to assess consumer preference for yoghurt and to compare the nutritional, microbial, physicochemical and sensory qualities of seven freshly prepared brands of vanilla-flavoured stirred yoghurts obtained from the manufacturers in Kumasi, Ghana. The preservative effect of natamycin on yoghurt during storage was also studied. These products were coded as Y1, Y2, Y3 up to Y7. 284 consumers of yoghurt in Kumasi gave many reasons for their preference of the various brands of yoghurt and 26.40% thought the most important nutrients obtainable from yoghurt were fats and proteins, and 26.10% believed vitamins were more readily available in this product. However 31.8% of consumers had no idea about which vitamin(s) were most available in the product. Some consumers indicated that an improvement of the shelf life of the products would be necessary and appreciated. Apart from the fat content which did not vary significantly, there were significant differences ($P<0.05$) in the nutritional composition and physicochemical properties of the seven commercialized brands of yoghurt. The protein content ranged from 2.08 ± 0% to 3.10 ± 0.19%, while fat contents ranged from 0.24 ± 0.19% to 0.59 ± 0.41% and energy values from 229 to 338KJ per100ml of yoghurt. Four out of seven brands had protein contents lower than the minimum permitted value (2.7%) set by the Codex Standards for such products. The total coliform (0 to $9.30\pm0 \times 10^2$ cfu/ml) and yeast counts ($8.40\pm0.35\times10^5$ to $14.00\pm0.39\times10^5$cfu/ml) did not meet Codex Allimentarius and Ghana standards for fermented milk products, which require that these microbial contaminants should not be present at all. In terms of consumer sensory preferences, Y3 was the least accepted product while Y6 was the most preferred product. Preservation of samples of yoghurt with 5 to 10 ppm of natamycin resulted in 8 ppm of natamycin being the most appropriate concentration for improving the keeping quality of the product. This concentration of the preservative gave the highest percentage decrease (69.36%) of yeast loads in yoghurt during storage and also resulted in relatively minimal changes in important physicochemical properties such as pH, titratable acidity and total soluble solids than lower concentrations of 5 to 7 ppm. There were significant differences ($P<0.05$) in pH, titratable acidity, total soluble sugars, total coliform and yeast counts of all commercialized yoghurts with and without natamycin throughout the 35 days of storage at 5 ± 1 degrees Celsius.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Yoghurt is a fermented dairy product obtained through anaerobic fermentation of lactose in milk by relevant microorganisms most of which are classified as pro-biotic (Tull, 1996). Lactose in evaporated whole milk, skimmed milk or fresh cow's milk is converted into lactic acid by a symbiotic bacterial culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* growing at temperatures in the range of 40–45^{o}C (Wood, 1985).

Since the 1960s there has been worldwide increase and development in the production of yoghurt. In 2001, more than 9 million tons of yoghurt were produced, mostly in Europe (6.6million tons) (IDF, 2002). However, it is becoming more popular in other parts of the world including Africa. Several factors account for the success of yoghurt: the fact that it is a natural drink, has good organoleptic characteristics (fresh, acidulated taste and characteristic flavour) and good nutritional value. It also has prophylactic and therapeutic properties (Roissart and Luquet, 1994). Many Ghanaians consume yoghurt as a dessert, snack or as a pro-biotic food drink to aid digestion and to re-establish a balance within the intestinal micro-flora (Sanful, 2009a).

Yoghurt is a preferred dairy product in areas where people are prone to lactose-intolerance. It is preferred over milk because it contains lactic acid which is readily

digested as compared to lactose in unfermented milk. Yoghurt is a good dietary source of calcium, magnesium, phosphorus and zinc which are important in physiological

processes and their contribution to total phosphorus intake has been reported as 30-45% in western countries (Flynn and Cashman, 1997). Essential minerals are present in dairy products at various levels depending on the type of milk used, the technological treatments during production of dairy products and the accuracy of analysis. Many researchers have advocated the consumption of some cultured dairy products such as yoghurt in the prevention and treatment of several diseases: prophylaxis against the treatment of gastrointestinal infection, management of lactose intolerance and of hypercholesterolaemia, the prevention of neoplastic disease (Fernandes *et al*., 1987; Fernandes and Shahani, 1990) and treatment of antibiotic associated colitis (Colombel *et al*., 1987). For these reasons probiotic organisms are increasingly incorporated into food as dietary adjuncts to help maintain a healthy microbial gastrointestinal balance and their availability in yoghurt has made it increasingly popular in many parts of the world.

Like any other food product, the quality of yoghurt is a key to its acceptability and marketability. One important aspect of the quality of yoghurt relates with the physical properties of the yoghurt gel which should possess a smooth textural character in the mouth during consumption, as well as a low tendency to serum separation during storage (Riener *et al*, 2010). In other words, higher viscosity and greater water-holding capacity of yoghurt are essential. The pH and titratable acidity of the product are also important quality factors since they are responsible for its characteristic taste. Finally,

the total nutritional value, sensory characteristics and microbial safety also determine acceptability of the product.

The pH and acidity of yoghurt are influenced by the activity of the microorganisms responsible in fermentation of the milk during yoghurt production. Most of the other quality factors mentioned are affected by the type of milk used in the manufacturing process, additives present in the product and manufacturing practices and conditions (Bonczar *et al*., 2002).

Although yeasts are not involved in the fermentation of yoghurt, they are frequently associated with the spoilage of the final product. Due to the inherent low pH of yoghurt, the product acts as a selective environment for the growth of yeasts (Suriyarachchi and Fleet, 1981). It is not uncommon to find yeast populations of 10^3 cells/g or more in retail samples of either plain or fruit yoghurts (Fleet, 1990), appearing as contaminants from the processing equipment and to a lesser effect, from the fruit, honey and sugar used as additives during production. Under normal storage conditions at low temperatures of about 5^oC, yoghurt has an expected shelf life of 30 days (Davis, 1970). However, when storage temperatures are abused, there is rapid growth of yeasts and spoilage is evident in excessive gas formation, off flavours and discolouration.

Though yoghurt is acknowledged as a product with quite a short shelf life, few attempts have been made to preserve it over a longer period. This is especially true with plain yoghurts. Pasteurized and chemically preserved yoghurts exist in some areas and have much longer shelf life periods. The use of natamycin as a preservative in dairy food and other products such as meats, juices and wines has been investigated (Food Standards

Australia New Zealand, 2004; Var *et al.*, 2004; El-Diasty *et al.*, 2009). This natural antibiotic has been found to have strong cidal activity towards susceptible microorganisms and is particularly effective against fungi, which may produce

mycotoxins. Its superiority over other preservatives has been attributed to its wide spectrum of activity at low concentrations and its effectiveness without changing organoleptic characteristics of the products (Food Standards Australia New Zealand, 2004).

1.2 PROBLEM STATEMENT

Theauthenticity and authentication of products are emerging topics within the food sector (Karoui *et al*., 2004) and are presently a major concern for producers, distributors and consumers (Fernandez *et al*., 2003). Correct labelling of food products is important to ensure that consumers make well informed choices when purchasing. This will also ensure that there is fair competition among manufacturers and that only good quality products are released into the market. It is also essential in ensuring that products conform to local and/or international standards and can achieve the specified shelflife. There are some yoghurts sold on the Ghanaian market which are inadequately labelled as far as the nutritional composition of products are concerned. Consequently, consumers of such products are not fully aware of the composition or nutritional value of these products.

Despite the ever growing popularity of yoghurt in the Kumasi metropolis, adequate information on the nutritional value and sensory quality of yoghurt products are not fully known. Storage stability of yoghurts sold in local markets has also occasionally

been a problem in the sense that the product has a short shelf life which needs to be addressed.

Irregular power supply and fluctuations have often been a problem to sellers which sometimes cause biochemical changes leading to spoilage of the product before the expiry date. Manufacturers of the brands of yoghurt sold within the Kumasi metropolis have specified refrigeration temperatures on the labels as the sole means of preservation of their products.

1.3 JUSTIFICATION

The documentation of the nutritional value of yoghurts will enhance its popularity among Ghanaians and enable them to make choices based on quality of the products. The database of information that will be provided by the results of this study will indicate whether the products meet the appropriate legal and labellingrequirements, and whether they are safe or not. The Ghana Standards Board, The Food and Drugs Board, and manufacturers of the yoghurt will have a basis for either encouraging production of yoghurt or standardizing yoghurt by improving manufacturing and quality assurance practices in this regard.The successful use of natamycin in improving the keeping quality of yoghurts may present a solution to the problem of short shelf life, expand the market reach and improve the economic benefits of yoghurt production in the Kumasi metropolis. Standardization of yoghurt and extension of shelf life could promote economic growth in Ghana by ensuring high quality products which can be exported because they meet international standards.

1.4 OBJECTIVE

The aim of this study is to evaluate the quality of yoghurts produced within the Kumasi metropolis and assess the effect of natamycin on yoghurt during storage.

Specific Objectives

The specific objectives of this work are:

- To determine consumer preferences of different brands of yoghurt in Kumasi.
- To determine the nutritional, physicochemical and sensory qualities of vanilla flavoured stirred yoghurt prepared and sold by seven (7) producers identified in Kumasi.
- To determine the appropriate concentration and point of application of natamycin required to improve the keeping quality of stirred vanilla flavoured yoghurt.
- To study the physicochemical properties of the seven products under refrigeration using natamycin as a preservative.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 ORIGIN OF YOGHURT

Yoghurt is derived from the Turkish word “Jugurt” used for any fermented food with acidic taste (Younus *et al*., 2002). It is likely that the origin of yoghurt was from Middle East after domestication of milk producing animals began around 9000 B.C. It is also reported that the Russian biologist IlyaIlyichMechnikov, co-winner of 1908 Nobel Prize in physiology, had an unproven hypothesis that regular consumption of sour milk could provide protection against entericinfections and their possible role to help attain a physiologicalold age and normal death (Schmalstieg and Goldman, 2008). Believing *Lactobacillus* to be essential for good health, Mechnikov worked to popularize yoghurt as a foodstuff throughout Europe.

Traditionally, different bacteria have been involved in the fermentation of milk but according to the Codex Alimentarius definition (FAO, 1992), the coagulated, fermented milk product can only be called “yoghurt’ if the bacteria synergically grown in the milk are *Streptococcus thermophilus* (new nomenclature: *Streptococcus salivarius* ssp. *thermophilus*) and *Lactobacillus bulgaricus* (new nomenclature: *Lactobacillus delbrueckii* ssp. *bulgaricus*).

2.2 PRODUCTION OF YOGHURT

Modern yoghurt production is a well-controlled process that utilizes milk, milk powder, sugar, fruit, flavour, colouring, emulsifiers, stabilizers, and specific cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (in a ratio of 1:1) for the fermentation (Robinson and Tamime, 1975). Though the term yoghurt is usually associated with acidification of cow milk, other raw materials have been successfully employed in the production of yoghurts. The use of goat milk, sheep milk, soybean milk, coconut milk, tiger nut milk, and combinations of some of these milk sources and types are reported by several researchers (Imele, 2001; Maria *et al*., 2002; Farinde *et al*., 2008; Sanful, 2009a; Sanful 2009b).

Yoghurt is usually prepared from normal whole milk although skim milk, full cream milk with added skim milk powder, or partially evaporated milk are also used. When whole cows' milk is used, its water content is usually reduced by about a quarter in a vacuum pan or by adding about 5% milk solids followed by water reduction. Whole milk is also sometimes fortified with dairy ingredients such as skim milk powder to increase the total solids and the concentration of protein .The milk is homogenized and pasteurized by heating at about 80-90^{o}C for 15 to 60 minutes, with higher temperatures requiring less time. This treatment kills vegetative bacteria and expels most of the oxygen and produces reducing substances which help to initiate and maintain anaerobic conditions in the milk suitable for the growth of the inoculum. After pasteurization the milk is cooled to 45-48^{o}C, care being taken to prevent the uptake of oxygen, and the inoculum is added aseptically at around 2%, by volume, with gentle mixing. Incubation is done in small bottles or cartons at about 42-45^{o}C for about 3 to 5 hours till

coagulation occurs, or until a pH of 4.5 is attained. At the end of the incubation period the fermented product is rapidly cooled to about 5^{o}C to stop lactic acid production (Jay, 2000). Apart from this general method for producing plain yoghurt, some additives such as fruit pieces, nectars, jams and honey could be added after the fermentation process to obtain different varieties of the product (Lutchmedial *et al.*, 2004).

2.2.1 Biochemistry of yoghurt production

In yoghurt manufacture, the high heat treatment of milk prior to fermentation leads to the interaction between whey protein and casein. Exposure of previously buried hydrophobic groups in the unfolded whey proteins promotes hydrophobic interaction which will later be crucial to gel formation during the fermentation process (Smits and Van Brouwershaven, 1980).

Fermentation begins with *Streptococcus thermophilus* which grows faster than *lactobacillus bulgaricus,* increasing the acidity of the milk and producing anaerobic conditions so that the milk becomes more suitable for the rapid growth of the latter. The *S. 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 substances are produced which are beneficial for the continued growth of the streptococci. These LAB ferment about 35% of the lactose in milk through hydrolysis to glucose and galactose. Only the glucose is changed into lactic acid, while the galactose moiety is released mainly by the coccus into the extracellular environment (Goodenough and Klein, 1976). The *Streptococcus* is capable of producing about 0.5% lactic acid and the Lactobacillus

about 0.6-0.8% (pH of 4.2-4.5). However, when incubation is prolonged, pH of the product can decrease to about 3.5 with lactic acid increasing to about 2%. At a pH of 4.2-4.5 there are equal numbers of both bacteria present in yoghurt, but at lower pH values and higher levels of acidity, the number of rods exceeds that of the cocci. This is because the *streptococci* tend to be inhibited at pH values of 4.2-4.4, whereas the lactobacilli can tolerate pH values of 3.5-3.8 (Jay, 2000). Yoghurt starter bacteria activity is found to be dependent on the composition of milk base, the amount of inoculum, milk temperature, incubation time and cooling time of milk. High total solids of the milk base has been reported to improve growth of yoghurt bacteria (Ozer and Robinson, 1999), decreasing fermentation time (Tamime *et al.*, 1989), and decreasing pH or increasing acidity (Yeganehzad *et al.*, 2007).

The lactic acid produced acts on milk protein to give yoghurt its texture. During acidification, the unfolded whey proteins (caused by heat treatment), which are either associated with casein micelles or free in the serum, interact with each other when the pH is close to their iso-electric point (pH 5.2–5.3), causing gel formation dominated by protein-protein interactions. 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 due to aggregation of casein particles as they reach their iso-electric point (pH 4.6). This eventually leads to casein-casein interactions dominating the gel network (Lucey *et al.*, 1997; Lucey *et al.*, 1998; Lucey and Singh, 1998).

The LAB involved in fermentation are also partly responsible for flavour development in yoghurt. *S. thermophilus* produces some diacetyl, which gives yoghurt its creamy or buttery flavour, whereas *L. bulgaricus* produces acetaldehyde, which helps to give yoghurt its characteristic sharp flavour (Lutchmedial *et al*., 2004). Sandine *et al.* (1972) reported that unflavoured yoghurt may have a weak flavour if desirable fermentation end-products, such as lactic acid, acetic acid, and acetaldehyde are not in optimal amounts. Harsh acid flavour occurs as a result of overproduction of acetaldehyde in relation to diacetyl when *L. bulgaricus*predominates the starter culture or when excessive culture is used (Crawford, 1962; Lindsay *et al.*, 1965).

During fermentation, vitamins B-12 and C are consumed and folic acid is produced while there are little differences in composition of other vitamins in milk and yoghurt (Meydani and Ha, 2000). The compositions of most other nutrients also remain unaltered.

By the end of the fermentation period pH values are decreased to a range of 4.25-4.5. Bacterial action is stopped by rapid cooling at the right lactic acid 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).

2.3 TYPES OF YOGHURT

The two main types of yoghurt are set and stirred yoghurt. 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 a

more liquid product obtained by fermenting the liquid milk base in large tanks after which the curd is broken by stirring and the product chilled and packaged in bottles (Lee and Lucey, 2010).

Yoghurts are available in many varieties including plain, flavoured, mixed with fruit purees and whole or sliced fruit. Fluid yoghurt drinks, soft or hard-frozen in various flavours and frozen yoghurt sticks are also commercially available (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 dwellers in the preparation of food dishes, soups and even consumed like biscuits with tea (Tamime and Robinson, 1999a). The type of yoghurt is an important consideration in monitoring the quality of the product. The presence of additives such as gelling, flavour-enhancing and stabilizing agents as well as fruits and fruit jams have an effect on sensory, nutritional, physicochemical and microbial quality of the fermented milk. For instance, it has been found that sweetened yoghurt and fruit yoghurts are more susceptible to microbial spoilage as they provide additional fermentable substrates for microbes such as yeast (Davis, 1970; Davis, 1975).

2.4 NUTRITIONAL AND HEALTH BENEFITS OF YOGHURT

Milk and milk products such as yoghurt are good sources of some minerals. They are the best dietary source of calcium and have a calcium-to-phosphorus ratio that is conducive for optimal skeletal growth. The presence and amount of vitamin D in these products give them excellent calcium bioavailability (Katz, 2001; Shermark *et al.*, 1995). Yoghurt is also nutritionally rich in protein and the B-vitamins (riboflavin, vitamin B6 and vitamin B12). People who are moderately lactose-intolerant can enjoy

yoghurt without ill effects due to the conversion of lactose to lactic acid during the fermentation of the product (Alm, 1982; Kolars *et al*., 1984).

Many researchers have reported the use of cultured dairy products including yoghurts in the treatment of several ailments and disorders. It has been suggested that such products may havehypocholesterolemic effect (Eichholzer and Stahelin, 1993), prophylaxis for the treatment of gastrointestinal infection, and potential prevention of colon cancer (Kampman *et al*., 1994). In addition, cultured dairy products have been successfully employed in the treatment of antibiotic associated colitis (Colombel *et al*., 1987). Studies carried out by Zemel *et al*., (2005) revealed that obese individuals who ate three servings of low fat yoghurt a day as part of a low calorie diet lost 22% more weight than the control group who only cut back on calories and did not have extra calcium.

2.5 IMPORTANT QUALITY PARAMETERS OF STIRRED YOGHURT

The quality of any food product can be defined against a wide range of criteria, including, the chemical, physical, microbiological and nutritional characteristics, or simply in relation to its overall appeal to potential consumers. To ensure that a product is of good quality it must:

1. be safe for human consumption with respect to chemical and microbial contamination;
2. conform to local or international regulations;
3. achieve a specified shelf life without spoilage;
4. have a high organoleptic standard (Tamime and Robinson, 1999b).

In general, the overall properties of yoghurt, such as acidity level, the production of aroma compounds (diacetyl, acetaldehyde, acetoin) as well as the sensory profile, and nutritional value, are important traits of the product (Kneifel *et al*., 1992). These properties are influenced by the chemical composition of the milk base, processing conditions, the additives included, and the activity of starter culture during the incubation period (Georgala *et al*., 1995; Kneifel *et al*., 1992; Ulberth and Kneifel, 1992).

2.5.1 Nutritional quality

Normally all fermented milk products have nutritional values corresponding to the composition of the milk from which they are made even though small differences in the concentration of chemical constituents could be present due to the manufacturing and fermentation processes as well as the effects of some ingredients used. The main differences that may occur are: (i) a considerable formation of lactic acid and a consequent decrease of lactose; (ii) an increased content of free molecules such as small peptides, amino acids and fatty acids (Gambelli *et al*., 1999). Slight increases in mineral composition may be due to leaching from some metal equipment and the type or source of water used for production. It is important that the final product maintains the desirable content of important minerals such as calcium and phosphorous, while limiting contamination by other minerals.

Apart from the final product having a nutritional value similar to that of the milk base, the Codex Alimentarius recommends standard permissible levels for some important nutrients. For instance a minimum of 2.7% (w/w) protein and a maximum of 15% (w/w)

fats are generally required for yoghurts (Codex Standard 243-2003). Whole milk yoghurts have been found to contain up to 5.7% (w/w) protein and 3.0% (w/w) fat while fat free yoghurt contains 5.4% (w/w) and 0.2% (w/w) protein and fat respectively. Drinking yoghurts have also been reported to have protein content up to 3.1% (w/w) and only traces of fat (The Dairy Council, 2008). Table 2.1 shows the nutritional composition of some common types of yoghurt. The Ghana Standards Board also has specifications for the fat content of yoghurt. The product should be designated 'skimmed yoghurt' if its fat content is below 0.5%, and 'partially skimmed yoghurt' if its fat content is between 0.5 and 3.0%. It requires that the product should be labeled yogurt only when its fat content is above 3.0% (Ghana Standard 337-2003).

Table 2.1: Nutritional composition of yoghurts

	Plain Whole Milk Yoghurt	Plain Low Fat Yoghurt	Virtually Fat Free (diet) Yoghurt	Drinking Yoghurt
Energy (kJ)	333	237	380	263
Carbohydrate (g)	7.8	7.4	8.2	13.1
Protein (g)	5.7	4.8	5.4	3.1
Fat (g)	3.0	1.0	0.2	Trace
Fiber (g)	None	None	None	None
Sodium (mg)	80	63	71	47
Potassium (mg)	280	228	247	130
Calcium (mg)	200	162	160	100
Magnesium (mg)	19	16	16	11
Phosphorus (mg)	170	143	151	81
Iron (mg)	0.10	0.10	0.10	0.10
Copper (mg)	Trace	0.03	0.03	0.03
Zinc (mg)	0.70	0.60	0.60	0.30
Chloride (mg)	170	235	252	75
Manganese (mg)	Trace	Trace	Trace	Trace

(Food Standards Agency, 2002**)**

2.5.2 Physicochemical and sensory quality of yoghurt

Milk supplements can affect the chemical and physical properties of yoghurt. This is because of their effects on fermentation time, starter culture metabolism and their interaction with milk proteins, which form the building blocks of the yoghurt gel network. Several researchers have reported products with greater viscosity and firmer texture, when the protein content is increased (Alvarez *et al.,*1998 *;*Magenis *et al.,*2006*;*Abd El-Khair, 2009). White (1995) reported that increased protein content in yoghurt resulted in an increase in the level of bound water which led to firm and viscous yoghurts. Saxena *et al.* (1994) also reported that yoghurt containing *Lactobacillus acidophilus* and made from milk enriched with fructose and casein hydrolysates had superior texture. Syneresis causes the separation of milk whey from the milk solids in

the yoghurt and is undesirable, as it changes texture and some sensory properties. This is prevented by the heat treatment of the milk or starting mix, which causes denaturation of whey proteins and increases their hydrophobic properties. The proteins, hereby, associate with k-casein to increase their water holding capacity and form a firmer gel network. Treatment of milk at 90^{O}C for 10 minutes produced more viscous yoghurt than heating at 80^{O}C for 20 minutes (Abd El-Khair, 2009). This was also the case with temperatures of 95^{O}C for 5 minutes as compared to 65^{O}C for 15 minutes (Thompoulus *et al*., 1993). Yoghurts prepared from milks with higher total solids have also been reported to have improved rheological properties with respect to viscosity and syneresis (Shaker *et al*., 2000; Mahdian and MazaheriTehrani, 2007). Properties such as the total solids of the milk are also important in fermentation Kinetics (Tamime *et al*., 1989; Ozer *et al*., 1998). Tamime *et al.,* (1989) showed that the incubation time required to reach a pH of 4.6 was shortened as the total solids of the milk base was increased. Chemical properties such as pH, titratable acidity and development of flavour compounds are also crucial to the acceptability of the final product and are mostly regulated through the control of fermentation by lactic acid bacteria (LAB) in the yoghurt starter culture.

Like all food products it is important that the yoghurt meant for consumption appeal to the senses that will guarantee its acceptability by consumers. Generally, plain yoghurt appears whitish unless the colour is altered by the addition of colouring and flavouring agents or fruits. It has its characteristic desirable sour taste which may vary depending on the total acidity (mainly lactic acid) permitted by the manufacturer. However, the Codex Standards for fermented milk (2003) requires that this should not be less than

0.6% lactic acid. All other sensory parameters such as mouthfeel, sweetness, viscosity and flavour notes are also important and are permitted to vary according to the disposition of the manufacturer. Though flavour of these products may vary from one product to the other, the main flavour compound present are: acetaldehyde (2 to 41 ppm), diacetyl (0.2 to 2.3 ppm), acetoine (2.2 to 28.2 ppm), ethanol (0.2 to 9.9 ppm), acetone (1.8 to 3.4 ppm) and butanone-2 (0.1 to 0.6 ppm), with acetaldehyde being the predominant (Kneifel *et al*., 1992; Xanthopoulos *et al*., 1994).

2.5.3 Microbial quality of yoghurt

The microbial quality of any food is one of the major concerns of consumers, manufacturers and food regulatory bodies. 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. 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). The Ghana standards for milk and milk products also require that no cells of colifarns, E. coli or Salmonella should be detected in the final product (Ghana Standard 337-2003).

Sources of microbial contamination during yoghurt production include 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, 1991). Inadequate pasteurization of milk before fermentation and

overall poor sanitation practices during manufacturing may also result in contamination of the final product.

2.6 SPOILAGE OF YOGHURTS

The shelflife of yoghurt has been found to be about 4 weeks at 5^{o}C and 3 days at 20^{o}C (Lourens-Hattingh and Viljoen, 2002). When storage periods exceed these times, the product is mainly spoiled by growth of yeasts or moulds (Lacroix and Lachance, 1990). Many researchers have discovered that though yeast cells are not involved in the fermentation process during yoghurt production, they are a major cause of spoilage of the product. When produced under "Good Manufacturing Practices", yoghurts should not contain more than 1 yeast cell per gram (Davis, 1970) and if refrigerated at 5^{o}C or less, it should not undergo spoilage by yeasts (Davis, 1975). The introduction of sugar and fruit into yoghurts makes yoghurts a less selective growth environment and such yoghurts are likely to support the growth of a wider variety of yeast species. Furthermore, the low pH of yoghurt and the ability of yeasts to utilize organic acids create a selective environment for yeast growth (Fleet and Mian, 1987). In some instances, its resistance to preservatives might be an added cause of its prevalence in the product (Green and Ibe, 1987).

When yeast population reaches 10^{5}–10^{6} cells per gram, spoilage becomes evident with an initial swelling of the yoghurt package due to gas production by yeast fermentation (Fleet, 1990). There is depletion in the total solids and total soluble sugar contents, and a consequent production of alcohol. Eventually, the package ruptures and the yoghurt acquires a yeast-like, fermentative flavour and odour, and a gassy appearance

(Suriyarachchi and Fleet, 1981). Occasionally, yeast colonies are seen on the bottom of the package.

Other spoilage organisms of concern in yoghurt are coliform bacteria which are almost always found in raw milk. These indicator organisms are capable of fermenting lactose with the production of acid and gas. Apart from the danger these bacteria pose to human health (may cause mastitis), they cause deterioration of the product by altering physicochemical and sensory qualities through increased acidity and reduced lactosecontent (Dairy foods science notes, 2010).

It has been discovered that milk and milk products have been destroyed by the secretion of extracellular enzymes by psychrotrophic bacteria during long periods of refrigerated storage (Cogan, 1977; Cousin, 1982). The most common psychrotrophs in these products are gram-negative rods which produce a variety of enzymes that cause chemical deterioration of milk resulting in off-flavours. Gram-negative psychrotrophs do not survive pasteurization, thus their occurrence in heat treated products are attributed to post-pasteurization contamination. Even though the bacteria are destroyed in pasteurization their enzymes are not inactivated, and may continue to degrade milk products (Champagne, *et al.*, 1994).

2.7 PHYSICAL AND CHEMICAL PRESERVATION OF YOGHURT

The shelf-life of cultured milk products such as yoghurt have been extended by adopting various techniques such as the use of bacteriocins, chemical preservatives, Lactoperoxidase system, high pressure treatment, post-production heattreatments, UV irradiation and carbonization (Sarkar, 2006).

2.7.1 Refrigeration and heat treatment of yoghurt

Traditionally refrigeration has been the main way of preserving. When kept under refrigeration at $5^{O}C$ or lower, it is expected that yoghurt maintains its original good organoleptic, physicochemical and microbial qualities for about 30 days and not undergo spoilage by yeast (Davis, 1970, Davis, 1975). Refrigeration of yoghurt commences immediately after fermentation and addition of sweeteners, fruits or flavour. It helps to arrest further fermentation by lactic acid bacteria (LAB) and hence stops acidification. During refrigeration yoghurt culture bacteria develop at a much reduced rate, while any coliform bacteria present may either develop very slowly or die out completely. Refrigeration temperatures and the continual growth of LAB, resulting in increased acidity of the product, create an unfavourable environment for coliform activity (Jay, 2000).

Yeasts, on the other hand, have been reported to have a competitive growth over mesophilic starter culture bacteria and contaminant psychrotrophic bacteria, and may continue to develop somewhat slowly during refrigeration. Their ability to assimilate sugars, lactose as well as lactic acid present in the product and to grow at low temperatures and pH grant them this advantage.Although the populations of contaminating yeasts remain relatively stable at low temperatures, the numbers quickly increase when the yoghurts are exposed to higher temperatures, and the shelf life of the product is substantially decreased (Viljoen, 2001). This is evident even at temperatures of $10^{O}C$, which is still quite low (Viljoen *et al.*, 2003). These researchers also revealed that at higher temperatures, not only a wider diversity of yeasts developed, but the yeast loads developed much earlier during the shelf life of the yoghurts. Despite the

competitive increase of yeast numbers, the population of starter cultures of lactic acid bacteria remains constant or continues to increase probably due to a symbiotic effect whereby both populations benefit from the interaction. This mutualistic effect may be attributed to the yeasts providing the necessary growth factors or vice versa (Fleet, 1990; Viljoen, 2001).

Thermization is a mild heat treatment or heat shock of the fermented product at a temperature below pasteurisation temperature. Neirinckx (1972) suggested a heat-treatment of 60–65^{O}C for thermization of cultured milk products with pH 4.2–4.5. This treatment was found to induce inactivation of yeasts and moulds in yoghurt and enhance the shelf-life to 6-8 weeks when stored at 12^{O}C (Neirinckx, 1972). Another way to ensure microbiological safety of yoghurts is through pasteurization, after which the product is proposed to be stable for up to 9 months (Rychlik *et al*., 2006). However, because pasteurization is more detrimental to dietetic properties of cultured milk products than thermization its application is not encouraged as a method of preservation (Sarkar, 2006). Other researchers have suggested microwave heating as an alternative way of prolonging shelf life of these fermented milk products.

2.7.2 The use of natamycin as preservative in food products and yoghurt

Natamycin, also known as pimaricin, is a natural polyene macrolide antibiotic which was first discovered in 1955 in culture filtrates of *Streptomyces natalensis* isolated from soil samples near the Natal Province of South Africa. It is produced by submerged aerobic fermentation of the *Streptomyces natalensis*. After several days the antibiotic is obtained either by broth extraction or by extraction of the mycelium. Dried natamycin

extracted from the fermentation broth is white to cream-coloured and has little or no odour or taste (Farid *et al*., 2000). This polyene has a molecular weight of 665.725g/mol and the molecular formulais $C_{33}H_{47}NO_{13}$. It consists of a large lactone ring consisting of 25 carbon atoms, with a rigid lipophilic chain containing four conjugated double bonds (also known as a tetraene) and a flexible hydrophilic portion bearing several hydroxyl groups (McGinnis and Rinaldi, 1985). Natamycin assumes a cylindrical structure due to the alignment of the hydroxyl groups of its amphipathic chain towards each other (Figure 2.1). The exterior of the cylinder is completely non-polar.

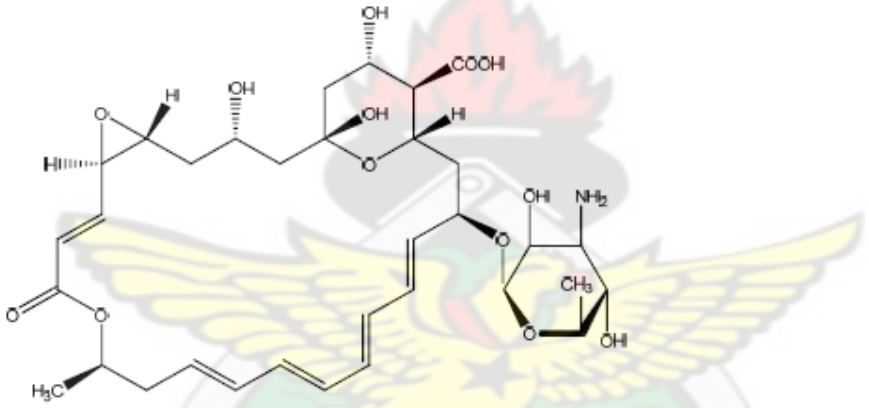


Figure 2.1: Chemical structure of natamycin(European Food Safety Authority 2009).

Natamycin can effectively inhibit the growth of most moulds and yeasts but not bacteria and viruses (Khoudokormoff and Petru, 1974). As compared to other antimicrobial agents such as sorbic acid, it effectively inhibits the growth of mould and yeast at amounts of 1-10mg/kg, while the application of sorbic acid requires an amount of 500mg/kg. Its antimicrobial mechanism is that it binds to and alters fungal cell membrane sterols (primarily ergosterol, the principal sterol in fungal membranes), so that vital structures inside the cell (required for survival of the fungal cell) pass through

the cell membrane and out of the cell (Hamilton-Miller, 1974; Norman *et al*., 1976; McGinnis and Rinaldi, 1985; Franklin and Snow, 1998). The polyene enters the cell membrane by complexing with the ergosterols via the hydrophobic face and forming a ring with the hydrophilic portions in the centre. In so doing they form a polar pore through which small ions like K^+ and H^+ can pass freely; disrupting the cell's ionic control (Griffin, 1994; Deacon, 1997). At low concentrations, selective changes in membrane permeability may occur. Leakage of potassium ions is the first detectable event, and, at high concentrations, leakage of amino acids and other metabolites occurs. Because bacteria do not possess membrane sterols, their lack of sensitivity to this antibiotic is thus explained. As a macrolide it also has the ability to affect enzymatic sequences involved in the synthesis of membrane constituents at the level of the early cyclic precursors in the ergosterol biosynthetic pathway (Mukhtar, *et al.*, 1994).

The use of natamycin as a preservative in the food industry has been ongoing for decades. The activity of natamycin against yeasts and moulds, but not bacteria, makes it convenient for use in foods that undergo a ripening period after processing. It is a hurdle to fungal growth in dairy products, meats and other foods such as juices and wines. Commercial preparations of natamycin, under the trade names of Natamax™ (Danisco) and Delvocide® (DSM) contain about 50% natamycin blended with lactose. It is approved for use in various applications in over 60 countries (Table 2.1) (Delves-Broughton *et al*., 2005). At present, South Africa permits the widest use of natamycin in foods including cheese and cheese products, yoghurts, processed meat products, fish products, wine and fruit wine, fruit juices, fruit pulp and some canned foods. The Codex GSFA (Codex standard for fermented milks, 2003) also permits its use in cured non-

heat treated processed meats and poultry (Food Standards Australia New Zealand, 2004).

Table 2.2 Worldwide authorization of natamycin

Country	Codes	Country	Codes
Algeria	A	Lithuania	A
Argentina	AB	Luxembourg	AR
Australia	AB	Mauritius	AB
Austria	AB	Mexico	A
Bahrain	A	Morocco	AB
Belgium	AB	Netherlands	AB
Brazil	AB	New Zealand	AR
Bulgaria	A	Norway	AB
Canada	A	Oman	P
Chile	A	Poland	AB
China	ABCD	Portugal	AB
Colombia	A	Paraguay	A
Croatia	AB	Qatar	P
Cyprus	A	Saudi Arabia	P
Czech Republic	AB	Singapore	A
Denmark	AB	Slovak Republic	AB
Ecuador	A	Slovenia	AB
Egypt	A	Spain	AB
Eire	AB	South Africa	ABDEFGHJK
Estonia	AB	Sweden	AR
Finland	AB	Switzerland	AB
France	AB	Syria	P
Germany	AB	Taiwan	AB
Greece	AB	Tunisia	AB
Hungary	AB	Turkey	AB
Iceland	A	Ukraine	A
India	A	U.A.E	P
Italy	AR	U.K.	AB
Jordan	P	U.S.A.	A
Kuwait	P	Uruguay	A
Latvia	AB	Venezuela	A
Lebanon	P	Yemen Republic	P

Note:A= surface treatment of specified cheese, cheese rind (shredded cheese in United States only); B=surface treatment of specified processed meats; C = surface treatment of certain baked goods; D=fruit juice; E=wine; F=fish products; G= yogurt; H= canned food; I=sour cream; I= cream cheese; K= cottage cheese: P=permitted additive (Delves-Broughton *et al*., 2005).

Several researchers have investigated the effectiveness of natamycin in preserving foods such as yoghurt. El-Diasty *et al.,* (2009) reported that natamycin proved to be a suitable and effective antifungal agent which increases the shelf life of yoghurt without changing its organoleptic characteristics. Var *et al.,* (2004) also reported that when the preservative was used, the population of yoghurt bacteria decreased approximately 3 log cycles in 30 days at 4 ± 1°C and that after 30 days of storage, no growth of moulds and yeasts was detected. It has been suggested that the dosage levels of natamycin for yoghurt preservation is in the range of 5ppm-10ppm (Thomas and Delves-Broughton, 2001).

Some benefits of the use of natamycin as a food additive are that it:

- enhances the quality of food products, and significantly extends the shelf life of foods by preventing yeast and mould spoilage;
- reduces products being recalled as a result of spoilage (and reduces manufacturing costs);
- replaces or partially replaces chemical preservatives and meets consumer demand for food preserved with natural ingredients;
- adds no adverse flavour to foods (unlike sorbic acid which can impart a bitter taste);
- has stronger inhibitory activity as compared to sorbic acid;
- prevents formation of potentially carcinogenic mycotoxins;
- covers a very broad spectrum of activity - most yeasts and moulds are sensitive to very low levels of the preservative (<1 - 40 ppm);

- does not act against bacteria - unlike sorbic acid : This makes it useful for food products in which bacteria are key to the ripening process;
- it has been proven to be a safe antimycotic agent. (http://www.penglaichem.com/OLDPAGE/Natamycin.htm).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. SOURCE OF RAW MATERIALS AND SAMPLES OF YOGHURT

Samples of freshly prepared yoghurts were collected directly from seven manufacturers in the Kumasi metropolis. Samples were kept in thermos coolers containing ice cubes and transported to the laboratory for analysis.

Natamycin was obtained from South Africa.

Spray dried whole milk powder (TMC Dairies (N.I) ltd., Northern Ireland, UK), sugar and flavouringagent were obtained from the Kejatia market in Kumasi (Appendix iv).

Plate 1: Natamycin being weighed into a beaker.

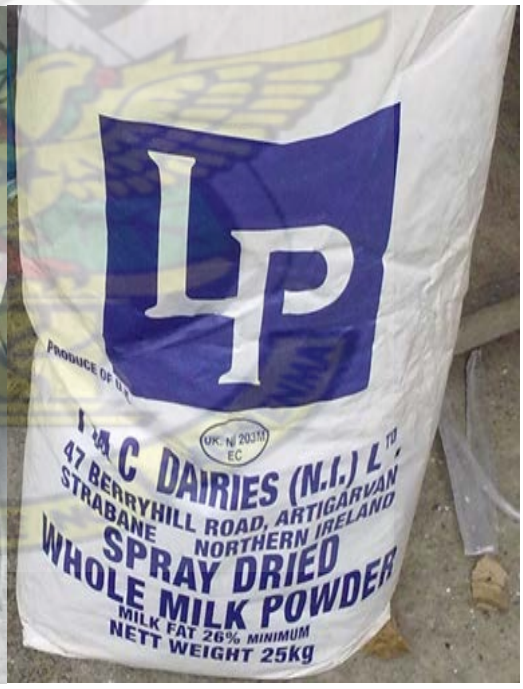


Plate 2: A bag of Spray dried whole milk powder.

3.2 SURVEY ON THE POPULARITY AND CONSUMER PREFERENCES OF VANILLA FLAVOURED STIRRED YOGHURT IN KUMASI

A survey was conducted to determine consumer preferences of yoghurt. After a study of important quality parameters and sensory attributes of the product a questionnaire was developed to find out the most preferred brand of yoghurt, reasons for the preference of the brands as well as suggestions to improve the products. Two hundred and eighty four (284) respondents took part in the survey and were randomly selected at yoghurt vending shops and their environs in the metropolis where vanilla flavoured stirred yoghurts were commonly sold. The questionnaire used for the survey is shown in appendix I.

3.3 ANALYSIS ON FRESH YOGHURT SAMPLES

The freshly manufactured yoghurt samples collected from seven (7) manufacturers within the Kumasi metropolis wereanalysed.

3.3.1 Proximate composition and mineral analyses

3.3.1.1 Determination of moisture content

Two grams (2 g) of freshly produced yoghurt was weighed into each of three previously dried and weighed glass crucibles. The crucibles with the samples were then placed in a thermostatically controlled oven at 105^{o}C till a constant weight of solid material was obtained after 5 hours. The crucibles were then removed and cooled in a dessicator and then weighed. The moisture content of the samples was calculated (Appendix II) by difference in weights and expressed as a percentage (AOAC method 990.20, 2005).

3.3.1.2 Determination of ash content

Two grams (2 g) of homogenized yoghurt sample was weighed into each of three previously dried and weighed porcelain crucibles and heated for about 20 minutes over a boiling water bath till they were visibly dry. The crucibles with their contents were then transferred into a muffle furnace at 600^{o}C and incinerated for 2 hours. The crucibles were removed, placed in a dessicator to cool then weighed and the ash content calculated (Appendix II) and expressed as a percentage (AOAC method 945.46, 2005).

3.3.1.3 Determination of crude protein content

Two grams (2g) of the sample was placed in a Kjeldahl digestion flask also containing a Selenium based catalyst and 25ml of concentrated H_2SO_4 added in a fume chamber. The flask was swirled gently to effect proper mixing and heated in a digestion chamber until digestion was complete after 5 hours. The digest was cooled and transferred into a 100 ml volumetric flask and made up to the mark with distilled water. 10 ml of the diluted digest was put in the steam distillation unit, which was previously flushed with distilled water. 18 ml of 40% NaOH was then added to the solution in the steam distiller. 25 ml of 2% boric acid was pipetted into a conical flask and two drops of bromocresol green-methyl red mixed indicator added.This mixture was placed under the condenser outlet of the distillation system, with the tip of the condenser completely immersed in it. The distillation was carried out until all the boric acid solution turned from pink to yellowish green. The solution in the conical flask was titrated against 0.1 N HClsolution and the end point recorded. The distillation and titration processes were done with triplicate samples of the diluted digest. A blank was also taken through the same procedure using

distilled water in place of the sample. The crude protein content was then calculated using a factor of 6.25 (Appendix II) (AOAC method 991.20, 2005).

3.3.1.4 Determination of crude fat content

About 100 g of yoghurt was poured into a previously weighed petri dish and dried over a water bath till most of the water had evaporated. The sample was then transferred to an oven and further dried at $105^{O}C$ till a constant weight was obtained. The weights of water lost and dried solids obtained were determined by subtraction and later used to calculate the total amount of fat on wet weight basis.

Two grams (2 g) of the dried sample was weighed into each of two paper thimbles. The thimbles were sealed and placed in soxhlet extractors. About 150 ml of petroleum ether was poured into each of two previously dried and weighed round-bottomed flasks attached to the extractors. Extraction was carried out for 16 hours. After this the petroleum ether was recovered from the soxhlet with only small amounts left in the flasks. The flasks were then removed and placed in an oven (with the door partially closed) for the ether to completely evaporate. The flasks were cooled in a dessicator, weighed and the fat content calculated (Appendix II) on wet weight basis using the water content determined after drying the wet sample (AOAC, 2005).

3.3.1.5 Determination of carbohydrate content

The percentage carbohydrate in yoghurt was determined by subtracting the percentage of moisture, ash, protein and fats obtained from 100 percent.

3.3.1.6 Determination of energy value of yoghurt

The energy contents of samples of yoghurt were determined by calculation based on the official conversion factor (Council Directive on Nutrition labeling of foodstuffs, 1990). The energy value was obtained in kJ/100g of yoghurt using factors of 17 kJ/g, 17 kJ/g and 37 kJ/g for protein, carbohydrate and fat respectively.

3.3.1.7 Determination of mineral composition of yoghurt

The ash obtained after the determination of ash content was first dissolved in 5ml concentrated HCl (11.8M) and filtered into a 50ml volumetric flask (modification of AOAC 985.35, 2005). The solution was made up to the 50ml mark with more distilled water and transferred into a plastic sample bottle with a lid. The concentrations of minerals Ca, P, Mg, Se, Zn and Fe in the samples were measured by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

This spectroscopic method uses inductively coupled Argon plasma as an emission source. In the emission source a strong high-frequency magnetic field is generated in a relatively small volume flushed with Argon. The Argon is hereby exposed to a high-voltage Tesla discharge which creates seed electrons and ions which accelerate and collide with Argon atoms to produce a large amount of heat. 4-5ml of the digested sample is introduced into the spectrophotometer through a pump which is connected to a nebulizer. When the nebulizer of the system injects a fine aerosol spray of the sample into this emission source, the high energy causes complete vaporisation and total breakdown of the analyte into free atoms which are available for excitation. The excited elements emit light of unique frequencies as they return to the ground state. The light

emitted by each element is proportional to the concentration of that element in the sample and is measured by an emission spectrometer. The spectrometer separates the unique frequencies into discrete wavelengths and quantifies the results. The concentration of each element given by the spectrometer was used to calculate the content of that element in the original sample of yoghurt (Appendix II). Ca, P, Mg, Zn and Fe were detected at wavelengths of 422.673, 213.618, 279.553, 213.857 and 259.940nm respectively.

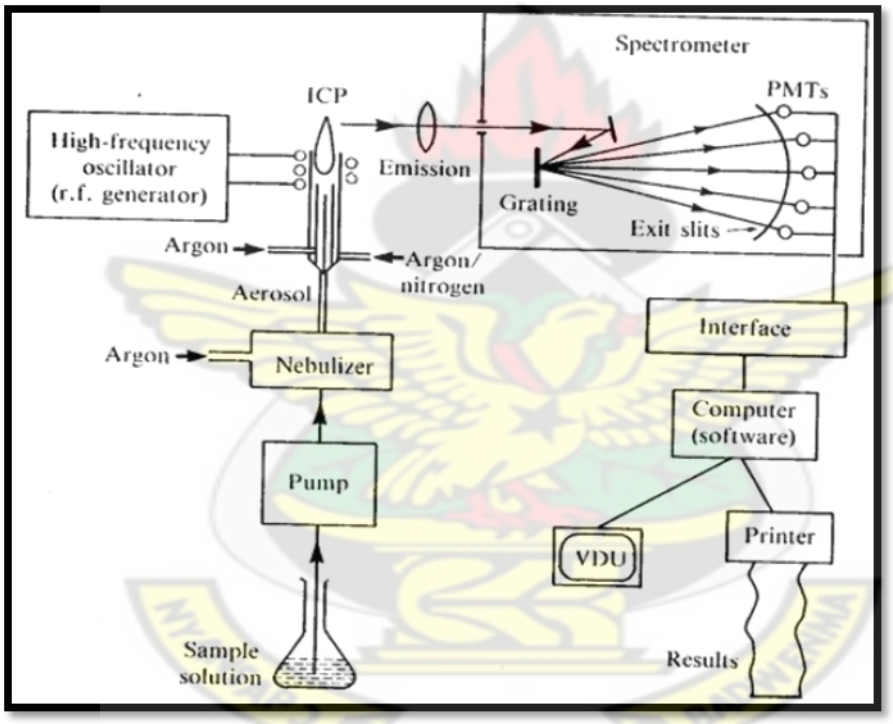


Figure 3.1: Instrumental setup of an ICP-OES.

Concentrations of Na and K in the samples were determined by Flame Photometry. This technique uses a flame that evaporates the analyte and also sublimes and atomizes the metals in it and then excites a valence electron to an upper energy state. Light is emitted at characteristic wavelengths for each metal as the electron returns to the ground state.

An optical filter selects the characteristic wavelength of the analyte of interest and sends it to a detector that converts it into an electric signal which is presented as the output. At the end of the analysis the output obtained were used to determine the concentrations of the analytes in the actual samples using a standard calibration curve plotted for each mineral. Standard solutions of 5, 10, 15, 20 and 30mg/L of Na were prepared from dry reagent grade NaCl and plotted against their output absorbances given by the photometer (Appendix II). In the determination of K, 2.5, 5, 7.5, 10 and 15mg/L of standard solutions were prepared from dry reagent grade KCl and used to obtain the calibration curve of absorbance against concentration. The preparation of standards was based on AOAC methods (AOAC method 969.23, 2005). The equations of the lines obtained from the graphs were used to determine the concentrations of each of the analytes in the sample.

3.3.2 Physicochemical analyses of fresh yoghurt

3.3.2.1 Determination of total solids

Two grams (2g) of yoghurt was weighed into each of three previously washed, dried and weighed glass crucibles. The crucibles with the samples were then placed in a thermostatically controlled oven at 105°C for 5 hours till a constant weight of solid material was obtained. The crucibles were then removed and cooled in a dessicator and then weighed, and the total solids of the samples calculated and expressed as a percentage (AOAC method 990.20, 2005).

3.3.2.2 Determination of total soluble sugars

The total soluble sugar content was determined by placing a few drops of yoghurt sample on the prism surface of a hand-held Brix refractometer (ATAGO Manual Refractometer) and reading the results on a total percentage scale. The results were adjusted based on tabulated values for the temperature correction from the temperature of the sample ($25^{O}C$) to the reference temperature ($20^{O}C$) of the refractometer.

3.3.2.3 Determination of pH

About 30 ml of yoghurt was poured into three 50ml beakers and an electric digital pH meter (BECKMAN Φ 340 pH/Temp. Meter) was used to determine the pH of the samples. The pH meter was dipped into the sample and the reading was taken after about 4 minutes when it was stable.

3.3.2.4 Determination of titratable acidity

0.1N Sodium hydroxide was prepared and standardized by first weighing 4g of NaOH pellets into a clean dry beaker and dissolving with distilled water in a 1000ml volumetric flask. The solution was titrated against 0.1M Oxalic acid (universal standard) with phenolphthalein to a pink end point colour. The exact concentration of NaOH was determined by calculation using the mole ratio of the acid and base (Appendix II).

Twenty milliliters (20ml) of fresh yoghurt sample was measured into each of three 250ml conical flasks and diluted with 20ml distilled water. The diluted yoghurt samples were then titrated with the standardized 0.1N NaOH using phenolphthalein indicator,

until a pink end point colour was observed. The titratable acidity was finally calculated (Appendix II) using the acid factor of lactic acid (0.009g) (AOAC method 990.20, 2005).

3.3.2.5 Determination of viscosity

The 100ml metal beaker of the viscometer was filled with the yoghurt at $5^{O}C$ and the rotor was immersed into it. The viscometer was then switched on and the resistance of the fluid against this applied speed was measured in decipoise (dPs). A reading was taken after about 20seconds when the dial remained at the same reading.

3.3.3 Microbiological analysis of fresh yoghurt

3.3.3.1 Preparation of serial dilutions

The sample was thoroughly mixed by shaking the bottle several times and the first (10^{-1}) dilution prepared by pipetting 1ml of the sample into a test tube containing 9ml sterilized distilled water by use of an automatic micropipette. This was done without allowing the tip of the pipette to touch the diluent, 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.

3.3.3.2 Total coliform count

Test tubes of MacConkey broth (CM005-OXOID Ltd.) were prepared and labelled. The MacConkey broth had the following composition:

Peptone	20.00g/l	
Lactose	10.00g/l	
Bile salts	5.00g/l	
Sodium chloride	5.00g/l	
Neutral red	0.075g/l	with pH: 7.4

It was prepared by dissolving 40g of powdered broth in 1L of distilled water. 5ml of liquid broth was measured into each test tube and the test tubes were covered. They were then sterilized by autoclaving at 120^{o}C for 15 minutes and allowed to cool to room temperature.

One millilitre(1ml) of each dilution 10^{-1},10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}dilution of the sample was pipetted into each set of three test tubes, by using fresh pipette tips for every dilution. The test inoculated broths were incubated (Gallenkamp Plus II Incubator) at 37^{o}C for about 48 hours after which the colour change in test tubes from red to yellow or cream were recorded and the total coliform counts determined by use of Most Probable Number (MPN) tables (Appendix II) (AOAC, 2005).

3.3.3.3 Total yeast count

Yeast Extract Agar (CM0019 –OXOID Ltd.) with the following composition was used for the plating:

Peptone	5.0g/l

Agar Agar 15.0g/l

Yeast Extract 3.0g/l with pH: 7.2

The culture medium was prepared by dissolving 23g of powdered agar in 1L distilled water and gently heating at 70^{o}C to effect complete dissolution. The agar was sterilized in an autoclave at 121^{o}C for 15 minutes and then allowed to cool to about 40^{o}C before pouring into the petri dishes.

Using fresh sterile pipette tips for each of the dilutions 10^{-1},10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}, 1ml aliquot of diluted sample was transferred into the initially sterilized and cooled petri dishes. About 5ml of yeast extract agar was poured into each plate and allowed to solidify before incubating at 37^{o}C for about 48 hours. The dishes were then removed from the incubator and the yeast colonies counted with a Colony counter (Stuart Scientific). Yeast colonies for plates inoculated with dilutions of 10^{-4} were reported.

3.3.4 Sensory evaluation of fresh yoghurt

Sensory evaluation was done to determine the degree of liking of the seven brands of yoghurt, 30 untrained panelists evaluated all the brands of yoghurt by affective testing based on a seven point hedonic scale. Panelist consisted of students and staff of the Food Science and Technology department of KNUST, Kumasi. Sensory evaluation was done in the conference room of the food science and Technology department. All panelists were familiar with yoghurt and consumed it from time to time. The same 30 panelists evaluated two products at the same time each day.

Yoghurts were served cold (5^{o}C). About 70 ml of each brand of vanilla flavoured stirred yoghurt was poured into each of 30 transparent plastic cups. The cups containing the

samples were coded, and served to the first 15 panelists in the order A followed by B while the other 15 panelist were presented with the two cups of yoghurt in the order B followed by A. Panelists were required to taste one product at a time and rinse their mouth with purified drinking water which they had been provided with. They were provided with score cards (Appendix III), and scored the products based on colour, Aroma, sourness, sweetness, thickness and mouthfeel on a seven-point hedonic scale with 1being dislike very much, 2 dislike moderately, 3 dislike slightly, 4 neither like nor dislike, 5 like slightly, 6 like moderately and 7 like very much.

3.4 DETERMINATION OF APPROPRIATE CONCENTRATION OF NATAMYCIN FOR PRESERVATION OF YOGHURT

3.4.1 Preparation of yoghurt

Stirred yoghurt was prepared based on procedures described by The Northeast Center for Food Entrepreneurship at the New York State Food Venture Center (Cornell University New York State Agricultural Experiment Station, 2010).1050g of whole milk powder was mixed with 7Litres of clean tap water, and the mixture homogenized by intense stirring in a saucepan. The milk was heated to a temperature of 90^{o}C and held at this temperature for 15minutes with continuous stirring, and then cooled to a temperature of 45^{o}C by allowing the saucepan to remain in a bowl of cold water (5^{o}C). The pasteurized milk was inoculated with 2% yoghurt starter culture and transferred into seven labeled plastic containers with each having a capacity of 1 Liter. The containers were covered with their lids and incubated at 45^{o}C (GallenkampPlus II Incubator, England) for four hours. The final pH was about 4.5. Each liter of set yoghurt

was stirred to obtain stirred yoghurt and 50g of sugar and 5ml of vanilla flavour were added.

3.4.2 Addition of different concentrations of natamycin to stirred yoghurts

Natamycin was added as preservative to each 1L of yoghurt at concentrations of 5ppm to 10ppm with the control containing no preservative. The white powdered antibiotic was weighed and dissolved in sterilized distilled water and then introduced into the yoghurt. To obtain a concentration of 5ppm (5mg/1000g) in 1000g of yoghurt, 0.05g of natamycin powder was dissolved in 100ml of sterilized water in a volumetric flask after which a 10ml aliquot was measured into 990g of yoghurt. This procedure was repeated using 0.06g, 0.07g, 0.08g, 0.09g and 0.10g of natamycin to obtain concentrations of 6ppm, 7ppm, 8ppm, 9ppm and 10ppm respectively. For the controls, 10ml of sterilized distilled water without natamycin was added to 990g of yoghurt and stirred. Each of the seven containers of yoghurt were distributed into five 200ml labeled (N5, N6, N7, N8, N9, N10 and N0 corresponding to natamycin at 5ppm, 6ppm, 7ppm, 8ppm, 9ppm, 10ppm and control respectively) bottles. The bottles of yoghurt were stored at 5 $\pm 1^{o}$C for further analysis. The procedures of preparation of yoghurt and addition of natamycin to yoghurt were repeated under exact conditions to obtain duplicate samples of yoghurt.

3.4.3 Monitoring the quality of natamycin-preserved yoghurt under refrigeration

Yeast counts were determined after the preparation of yoghurt samples. Titratable acidity, total soluble sugars as well as pH of yoghurts with different concentrations of natamycin were also determined starting from the day of preparation. Determination of

these quality parameters were repeated every week after preparation for four weeks. All measurements were done using duplicate bottles of each sample.

3.5 DETERMINATION OF POINT OF ADDITION OF NATAMYCIN TO YOGHURT

Based on the results obtained from the optimization of the concentration of natamycin in sections 3.4.1 to 3.4.3, a fresh set of stirred yoghurt was prepared with 8ppm of natamycin following the procedure previously described. To two 1L container of yoghurt natamycin was added before the four-hour incubation of milk base (NB); to another two containers of the product the preservative was added after incubation (NA). The controls (N0) contained no preservative. The samples were bottled and the same quality parameters were analyzed weekly for four weeks beginning on the day of manufacture as described in section 3.4.3.

3.6 PHYSICOCHEMICAL AND MICROBIAL ANALYSES ON THE SEVEN BRANDS OF YOGHURT WITH AND WITHOUT NATAMYCIN

8ppm of Natamycin was added to 1L of all seven brands of freshly prepared commercialized vanilla flavoured yoghurts. The yoghurts were packaged in six 150ml bottles with covers and refrigerated at $5\pm1^{O}C$ for further analysis. This was repeated to obtain duplicate samples, and controls for each brand of yoghurt contained no preservative. The samples were labeled YN1 to YN7 to represent seven brands of yoghurt preserved with natamycin, and C1 to C7 to represent their corresponding controls. Sampling was done on 7-day intervals starting from the day of manufacture

(day 0). Changes in total soluble sugars, total solids, titratable acidity, pH and yeast counts were monitored during refrigeration for 5 weeks.

3.7 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS OF DATA

Data obtained from the survey were analyzed by EPI INFO ™ 3.5.1. Frequency distributions were used as a form of descriptive statistics. Chi squared (X^2) analyses were performed on the data collected for the preference of the different brands of yoghurt by consumers. The following hypotheses were put forward:

Null hypothesis (H_O) = the preference of one brand of yoghurt or another is exclusively influenced by the reasons given.

Alternative hypothesis (H_A) = the preference of one brand of yoghurt or another is not exclusively influenced by the reasons given.

A Single Factor categorical design was used to determine significant differences and relations in quality parameters among the seven products. One Way Analysis of Variance and Fisher's least significant difference (LSD) procedure were performed for each of the quality parameters. Correlation was also determined between some of the factors during the shelf life studies of natamycin-preserved yoghurts. The STATGRAPHICS Centurion XV.I statistical program was used for this design and analysis.

A two factor complete block design was used to determine significant differences and relations in quality parameters of yoghurts preserved with different concentrations of natamycin, and yoghurts preserved with natamycin added at different stages of production.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 POPULARITY AND CONSUMER PREFERENCES OF STIRRED YOGHURT IN KUMASI

The survey involved 148 (52.1%) female respondents and 136 (47.9%) male respondents. For the sake of this study, a consumer was defined as any person above 16 years of age who had ever purchased and consumed bottled vanilla flavoured stirred yoghurt within the Kumasi metropolis of Ghana. The seven different brands of yoghurts were labeled Y1, Y2, Y3, Y4, Y5, Y6 and Y7.

Table 4.1 shows that a large percentage (58.5%) of respondents consumed vanilla flavoured stirred yoghurt for its sensory benefits, while 23.2% and 16.9% consumed it for its nutritional value and health benefits respectively. The appreciable number of consumers (40.10%) who drank yoghurt for its nutritional and health benefits is indicative of the growing awareness of the many benefits of yoghurt.

Table 4.1: Popularity of commercialized yoghurts on the Kumasi market

Question	Frequency of responses	Percentage frequencies
Reasons for consumption of yoghurt		
For its health benefits	48	16.9
For its nutritional value	66	23.2
For its sensory benefits	166	58.5
For other reasons	4	1.4
Most frequently purchased products		
Y1	31	10.9
Y2	10	3.5
Y3	29	10.2
Y4	**71**	**25.0**
Y5	59	20.8
Y6	43	15.1
Y7	41	14.4

The popularity of the commercialized brands of yoghurt in Kumasi was in a decreasing order as follows; Y4, Y5, Y6, Y7, Y1, Y3 and Y2 with frequencies of 25.0%, 20.8%, 15.1%, 14.4%, 10.9%, 10.2% and 3.5 % respectively. This trend could be associated with their general qualities and/ or the number of vending points for the individual product. It was observed, during the study that some products were distributed in many areas in the metropolis, while others such as Y2 were only sold near their points of production.

From Table 4.2 it can be observed that respondents gave many reasons for choosing the various brands of yoghurt as their most preferred. Many of the respondents (47) based their preference of a brand of yoghurt on its flavour. The most popular brand of yoghurt, Y4 was chosen because it was the most available and was also assessed as the one with the best quality. Some consumers also preferred products Y3 and Y4 because they trusted the authenticity of these products. These could be due to the popularity of the manufacturers with the consumers. From Chi squared analysis ($p < 0.05$) the hypothesis is rejected; the preference of one brand of yoghurt or another is not exclusively influenced by the reasons given.

Table 4.2: Reasons for consumers' preferences of different brands of yoghurt in Kumasi

Reasons given	Frequency of responses given for each brand						
	Y1	Y2	Y3	Y4	Y5	Y6	Y7
Best flavour and consistency				1	3		
Best overall sensory benefits		1	1	8	15	3	9
Best packaging							13
Best quality	2			11	3	3	1
Better value for money	1	1		4	5		
Consistent taste	6				1		1
For its attractive colour				4		1	
High viscosity and best taste				3	8	1	
High viscosity and is more filling				1	2	1	
I has the best flavour	5	2	2	9	5	17	7
The most advertised product		1					
The most available in the area	3	2	9	15	8		2
The most refreshing product	1			1			
No particular reason	11	2	7	8	6	7	5
Popularity with other consumers		1	4	4	1	2	
Most preferred volume of product	2				2		1
Trust of authenticity			5	1			
Well priced product			1	1		8	2
Total	31	10	29	71	59	43	41

Consumers' knowledge of the nutrients mostly obtained from drinking yoghurt is indicated in Table 4.3. A small number of consumers (8.8%) mentioned that carbohydrates were the most important nutrients obtained from yoghurts, whereas 15.5% stated that yoghurt was a good source of minerals. Out of those who indicated that minerals were the most important nutrients obtained from yoghurt, 68.2% named calcium as the primary mineral in the product, while the remaining 31.8% had no idea of the particular mineral present. The percentage of consumers who had no idea at all of the nutritional value of yoghurt was 23.2%. Out of those who indicated that it was a good source of vitamins, 27.00% did not know which particular vitamins were present. Furthermore, vitamin C is definitely very low in milk and yoghurt but more consumers

(14.90%) mentioned it as the vitamin most present compared to those who mentioned vitamin B (16.2%). This revealed a lack of knowledge about the nutritional value of the products, which could be a reflection on the lack of nutritional information on their labels.

Table 4.3: Consumers' awareness of nutritional quality of yoghurt

Assessment of consumer awareness	Frequency of response (%)
Nutrient(s) considered to be most important in yoghurt	
Carbohydrates	8.8
Fats and Proteins	26.4
Minerals	15.5
No idea	23.2
Vitamins	26.1
Minerals present in yoghurt	
Calcium	68.2
No idea	31.8
Vitamins present in yoghurt	
No idea	27.0
Vitamin A	12.2
Vitamin B	16.2
Vitamin C	14.9
Vitamin D	8.1
Vitamins D&E	21.6

Table 4.4 shows frequencies of the most important quality parameters considered by consumers before purchasing a brand of yoghurt. From the results, 27.10% of the respondents considered colour as the first most important quality of yoghurt which is an indication that the appearance of yoghurt is an important quality parameter in determining its acceptability by consumers. The second and third most important sensory attributes considered were aroma (21.2%) and texture (21.1%). Since aroma, colour and texture had higher responses than price, it can be inferred from the survey that the sensory qualities of yoghurt were very important to consumers in purchasing

the product. The results of this study reaffirm the fact that if a food product is not perceived to have a pleasant appearance, smell, texture or taste, it is unlikely to be purchased and consumed (Hetherington and Rolls, 1996). Furst *et al.* (1996) also reported that people consider sensory perception of food as a dominant factor which is less negotiable than other factors influencing their food choices in the supermarket or restaurant.

Table 4.4: Important parameters considered by consumers prior to the purchase of yoghurt

Important parameter considered	Frequency of responses (%)		
	First consideration	**Second consideration**	**Third consideration**
Aroma	10.9	**23.2**	9.5
Colour	**27.1**	12.0	4.2
Expiry date	2.8	1.8	3.9
Intact seal	1.4	0.7	1.1
Mouthfeel	4.6	10.2	17.6
Nutritional value	16.9	3.9	6.3
Price	12.7	6.7	5.6
Sourness	4.6	7.4	14.8
Sweetness	14.1	18.7	15.8
Texture	4.2	15.1	**21.1**
Volume of bottle	0.7	0.4	-

Concerning improvement and further development in yoghurt products (Table 4.5), most consumers (24.6%) stated that no further improvement or development was required in the brands of yoghurt. The percentage of consumers who indicated the necessity for an increase in shelf life of the products was 15.1%. 8.8% required that the nutritional information should be displayed on the labels. 7.4% of consumers indicated that yoghurts should be fortified with vitamins and/or minerals and 6.7% requested that the viscosity of yoghurts should be increased.

Table 4.5: Consumers' suggestions for further development of yoghurts

Suggestions	Frequency of responses (%)
A sugar free variety	3.9
Addition of fruits	2.8
Consistency in taste	4.6
Consistency in thickness	0.7
Fortification with vitamins and/or minerals	**7.4**
Improvement in colour	1.8
Improvement in labels	1.4
Improvement in nutritional value (with additives)	2.2
Improvement in packaging	1.4
Improvement in texture	0.7
Extended shelf life	**15.1**
Increase in sweetness	0.7
Increase in sweetness and shelf life; and reduction in sourness and aftertaste	2.1
Increased viscosity	**6.7**
introduction of set yoghurt	1.8
No development necessary	**24.6**
Probiotic yoghurt	1.1
Reduction in sourness	3.5
Reduction in sweetness	1.4
Reduction in viscosity	2.1
Showing nutritional information on the label	**8.8**
Stopping recycling of yoghurt bottles	0.4
Varying flavours and colours	4.9

4.2 COMPARATIVE STUDIES ON BRANDS OF YOGHURT

4.2.1 Nutritional quality of the seven brands of stirred yoghurt

Moisture

Moisture contents ranged from 80.68±0.08% to 86.09±0.13% and increased in the order Y5, Y6, Y7, Y2, Y1, Y4 and Y3 respectively (Table 4.6). The results showed significant differences ($P<0.05$) between the moisture contents of the brands of yoghurt.

Ash

From table 4.6 Y6 had the highest ash content (0.66±0.03%) and Y4 the lowest (0.41 ± 0.03%). No significant differences (P>0.05) existed between the yoghurt brands, with the exception of Y6. The ash contents were generally lower than 0.81 ± 0.29% and 0.661 ± 0.087% reported by El Zubeir *et al*., (2005) and El Bakri and El Zubeir (2009) for plain yoghurt samples. The variations in ash contents of yoghurts from different manufacturers could be attributed to the compositions of the milk bases used in manufacturing of the product. Mineral composition of fresh milk from dairy cattle can vary as a result of nutrition, lactation, season, handling of milk after pasteurisation and storage of milk (Wehr and Frank, 2004).

Table 4.6: Proximate composition of seven brands of yoghurt

Brand of yoghurt	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Energy (kJ/100g)
Y1	84.72 ± 0.16^{a}	0.50 ± 0.08^{a}	2.19 ± 0.07^{a}	0.44 ± 0.04^{ab}	11.54 ± 0.40^{a}	250
Y2	83.83 ± 0.08^{b}	0.42 ± 0.10^{a}	2.27 ± 0.35^{a}	0.27 ± 0.18^{a}	12.43 ± 0.16^{b}	260
Y3	86.09 ± 0.13^{c}	0.46 ± 0.03^{a}	3.10 ± 0.19^{c}	0.34 ± 0.01^{ab}	9.64 ± 0.14^{c}	229
Y4	85.14 ± 0.11^{d}	0.41 ± 0.03^{a}	2.77 ± 0.08^{b}	0.24 ± 0.19^{a}	10.87 ± 0.29^{d}	241
Y5	80.68 ± 0.08^{e}	0.44 ± 0.02^{a}	2.35 ± 0.08^{a}	0.34 ± 0.02^{ab}	16.79 ± 0.05^{e}	338
Y6	82.91 ± 0.07^{f}	0.66 ± 0.03^{b}	3.04 ± 0.08^{bc}	0.59 ± 0.41^{b}	13.45 ± 0.58^{f}	302
Y7	83.42 ± 0.08^{g}	0.46 ± 0.03^{a}	2.08 ± 0.0^{a}	0.44 ± 0.05^{ab}	13.34 ± 0.22^{f}	278

Means followed by the same superscript in a column denote values that are not significantly different (p>0.05)

Protein

The protein content of the brands of yoghurt differed significantly (P<0.05) from one brand of yoghurt to the other, and ranged from 2.08 (Y7) to 3.10 (Y3). Four of the products (Y1,Y2,Y5 and Y7) had protein contents lower than the minimum permitted amount (2.7%) set by the Codex Standards for yoghurts (Codex Standard 243-2003).

It is reported that the protein content of commercial yoghurt is generally higherthan that of fresh milk because of the addition of non fat dry milk during processing, and concentration, which increases the proteincontent of the final product (Adolfsson *et al*., 2004). Thus the generally low protein content of the yoghurt brands in this study could be attributed to the use of milk with low protein content for the production.

Fat

The fat content of the various yoghurt brands of vanilla flavoured stirred yoghurt increased from 0.24 ± 0.19% to 0.59 ± 0.41% in the order: Y4, Y2, Y5, Y3, Y1, Y7 and Y6. There was no significant difference ($p>0.05$) between the fat contents of all seven products and values were far lower than those reported for similar products by Younus *et al*., (2002) and El Bakri and El Zubier (2009). These researchers reported values in the ranges of 2.94-3.50% and 2.75-3.82% respectively. The fat contents of the brands were also below those generally recorded for low fat yoghurt (1%), but slightly higher than that of fat free yoghurt (0.2%) and drinking yoghurts **(**http://www.milk.co.uk/publications/default.aspx.).

In Ghana, local laws (Ghana Standard 337-2003) require that yoghurts with fat content below 0.5% be designated as *skimmed yoghurt* (and this generally places them in a category close to fat free yoghurts, and makes them a good choice of refreshment for individuals on low fat diets).

Carbohydrates

There were significant differences ($P<0.05$) between the carbohydrate contents of all the seven yoghurts ranging from 10.87 % in Y4 to 16.79% in Y5.

The carbohydrate contents of the products were comparable to the value of 13.1%, reported for drinking yoghurts (Food Standards Agency, 2002).

Apart from fat and protein, the other major nutrient in milk and milk products is carbohydrate which is mainly lactose. High carbohydrate content of yoghurt generally results in an increase in the total solids which consequently improves texture and viscosity while decreasing syneresis (Lorenzen *et al*., 2002). Considering the relatively low fat content of the products the high amount of carbohydrate is the main contributor to the products' energy value.

Energy

The energy values reported in Table 4.6 varied with respect to the high carbohydrate contents of the products. Product Y3, with the lowest carbohydrate content had the lowest energy value of 229kJ/100g, while Y5 with the highest carbohydrate, provided the greatest amount of energy (338kJ/100g).

These values are comparable to energy values of plain yoghurts and some other fermented milk products reported by Gambelli *et al*., (1999), which ranged from 262-402kJ/100g. Energy values in this study were also close to energy values of plain low fat (237kJ/100g) and fat free (230kJ/100g) yoghurts (Food Standards Agency, 2002).

Mineral contents of yoghurts

The contents of all minerals (Table 4.7) differed significantly between all brands of yoghurt.

Essential minerals are present in milk products in varying levels depending on technological treatments of the products, the type of milk base used and the accuracy of analysis (Miller *et al*., 2000). Calcium is the most predominant mineral in milk and milk products and it is essential in bone and tooth mineralization, blood clotting, hormone secretion and nerve transmission. Its content in commercialized drinking yoghurts in Kumasi were generally in the ranges of those reported for preserved commercialized yoghurts in local markets in Madrid, Spain (1090–2050 mg/L) (De la Fuente *et al.*, 2003). Brand Y3 had Ca content close to the value reported for cow milk yoghurt (1,145±96mg/kg) (Guler and Hasan, 2008).

Table 4.7: Mineral contents of seven brands of yoghurt (mg/kg)

Brand of yoghurt	Ca	P	Mg	Na	K	Fe	Zn
Y1	1486.39 ± 3.46^{a}	855.71 ± 4.05^{a}	208.86 ± 3.00^{a}	296.86 ± 1.14^{a}	294.50 ± 0.68^{a}	0.8734 ± 0.01^{a}	4.69 ± 0.30^{ab}
Y2	1320.85 ± 6.72^{b}	1019.19 ± 6.58^{b}	212.60 ± 3.47^{a}	416.46 ± 17.36^{b}	542.90 ± 2.45^{b}	0.9982 ± 0.01^{b}	5.40 ± 0.19^{e}
Y3	1122.58 ± 1.92^{c}	913.85 ± 6.50^{c}	219.45 ± 6.87^{b}	261.22 ± 6.08^{c}	339.14 ± 9.11^{c}	0.8449 ± 0.01^{a}	4.31 ± 0.25^{ac}
Y4	1443.81 ± 3.95^{d}	1039.86 ± 0.73^{d}	347.01 ± 3.05^{c}	295.47 ± 4.34^{a}	206.00 ± 4.32^{d}	1.0576 ± 0.01^{c}	3.57 ± 0.20^{d}
Y5	1381.14 ± 2.34^{e}	892.50 ± 5.56^{e}	213.76 ± 2.69^{ab}	312.66 ± 3.37^{d}	446.73 ± 4.22^{e}	0.7283 ± 0.01^{d}	5.13 ± 0.54^{be}
Y6	1266.79 ± 2.89^{f}	876.73 ± 4.79^{f}	188.16 ±3.44^{d}	358.57 ± 1.86^{e}	404.17 ± 2.10^{f}	0.9239 ± 0.02^{e}	4.07 ± 0.07^{cd}
Y7	1203.82 ± 7.84^{g}	1086.17 ± 4.64^{g}	174.56 ± 3.26^{e}	337.54 ± 3.76^{f}	439.56 ± 7.34^{e}	1.0035 ± 0.04^{b}	4.91 ± 0.32^{be}

Means followed by the same superscript in a column denote values that are not significantly different (p>0.05)

Phosphorus plays a vital role in the structure of cell membranes and virtually all metabolic processes (Miller, 2008). The contents of this important major mineral in some of the drinking yoghurts in Kumasi were lower than (878±15-1560±14mg/L) as indicated in most of the brands analyzed by De la Fuente *et al*., (2003). The Phosphorus content of three brands Y2,Y4 and Y7 were higher than (1009±48mg/kg) as reported by Guler and Hasan (2008) for cow milk yoghurts.

Levels of magnesium (Mg) recorded in this study ranged from 174.56±3.26 to 347.01±3.05mg/kg and were much lower than the mean value of 406±20mg/kg as reported by Guler and Hasan (2008).The values were all higher than values obtained for all yoghurts analyzed by De la Fuente *et al*., (2003) which ranged from 101±1-144±7mg/L. Magnesium, a required cofactor for over 300 enzyme systems in the body, is related to calcium and phosphorus in function (Flynn and Power 1985). Studies suggest a positive correlation between high magnesium intake in humans and increased bone density (Martini and Mayer 1999).

Sodium (Na) salt is necessary in the body for the control of extracellular fluid volume and blood pressure and for the transport of many nutrients into and out of cells. Both Na and K are essential minerals in human nutrition, but their deficiencies are rare since their intakes (especially that of Na) usually exceed the recommend values (Miller *et al*., 2000). In the present study, Na and K were recorded in amounts much lower than the mean amount in cow milk yoghurts reported by Guler and Hasan (2008) which are 460±41mg/kg (Na) and 1711±41mg/kg (K).

Iron (Fe) contents of yoghurts from Kumasi were higher while Zn contents were lower than values reported for cow milk yoghurts (0.729±0.14 and 7.289±0.69mg/kg respectively) manufactured by Guler and Hasan (2008). Zn contents were generally comparable to commercialized yoghurts in Madrid (4.0±1-7.3±0.3mg/L)(De la Fuente *et al*., 2003).

4.2.2 Physicochemical properties of the seven brands of yoghurt

pH

The pH of yoghurtsranged from 3.87 for Y5 to 4.45 for Y6 and all samples showed significant differences ($P<0.05$) at 95.0% confidence level.

The pH values of all yoghurts analyzed were lower than values reported by Younus *et al*., (2002), and El Bakri and El Zubeir (2009). Younus *et al*.,(2002) reported pH values of 4.35± 0.03 and 4.57± 0.03 in marketed yoghurts in Islamabad, Pakistan, while plain yoghurts in Khartoum state, Sudan were reported to have a mean pH value of 4.62 (El Bakri and El Zubeir, 2009). Products Y4 and Y6 had pH values in the range of (4.2-4.5) at the end of the normal fermentation period of 4-5 hours, while all other brands of yoghurt had lower pH values which were closer to that of yoghurts which have undergone a protracted fermentation period (pH3.5) (Jay, 2000).

Table 4.8: Physicochemical properties of the seven brands of yoghurt

Brand of yoghurt	pH	Titratable acidity (%)	Total Soluble Sugars (^{O}Brix)	Total Solids (%)	Viscosity (dPs)
Y1	4.10 ± 0.02^{a}	0.85 ± 0.05^{a}	11.36 ± 0.00^{a}	15.28 ± 0.16^{a}	1.63 ± 0.23^{a}
Y2	4.12 ± 0.00^{a}	0.93 ± 0.01^{b}	11.36 ± 0.00^{a}	16.17 ± 0.08^{b}	1.37 ± 0.32^{a}
Y3	3.92 ± 0.07^{bc}	1.23 ± 0.02^{c}	9.57 ± 0.00^{b}	13.91 ± 0.13^{c}	1.00 ± 0.00^{b}
Y4	4.39 ± 0.01^{d}	0.67 ± 0.01^{d}	10.57 ± 0.00^{c}	14.86 ± 0.11^{d}	1.53 ± 0.06^{a}
Y5	3.87 ± 0.03^{b}	1.25 ± 0.01^{c}	15.93 ± 0.00^{d}	19.32 ± 0.07^{e}	2.53 ± 0.30^{c}
Y6	4.45 ± 0.01^{e}	0.92 ± 0.04^{b}	12.97 ± 0.00^{e}	17.09 ± 0.07^{f}	1.67 ± 0.21^{a}
Y7	3.95 ± 0.02^{c}	1.06 ± 0.01^{e}	11.93 ± 0.00^{f}	16.58 ± 0.08^{g}	1.50 ± 0.00^{a}

Means followed by the same superscript in a column denote values that are not significantly different (p>0.05)

Titratable acidity

The titratable acidity of the seven products showed significant differences (P<0.05). The values ranged from 0.67 ± 0.01% in Y4 to 1.25 ± 0.01% in Y5.

The titratable acidity of all the products satisfied the minimum recommended value of 0.6%set by Codex Standards for yoghurt and related products.The values for Y1, Y2 andY6 were very close to values cited by Jay (2000) for a good finished product (0.85-0.90%). The titratable acidity of all the brands of vanilla flavoured yoghurts were similar to those reported by Younus *et al*., (2002). The latter study reported acidities in the range of 0.87±0.04% to 1.13±0.03%. The fact that the brand with the highest total solids (Y5) also had the highest percentage acidity confirmed reports that increase in total solids causes an increase in the rate of acidification or pH reduction during yoghurt production since this improved the growth of *lactobacillus bulgaricus* (Tamime *et al.*, 1989; Ozer *et al*., 1998; Ozer and Robinson, 1999; Yeganehzad *et al.*, 2007).

The acidity of the product determines the degree of survival of yoghurt bacteria in the course of storage and, later on, leads to changes in the yoghurt structure and viscosity as

well as its sensitivity to syneresis (Savello and Dargan, 1997; Vlahopoulou and Bell, 1990). The acidity of yoghurts also affects the overall flavour of the products as explained in the work of Barnes *et al.*,(1991a). They reported that in the US, the relatively high extent of sourness (mainly caused by lactic acid) along with the intensity of acetaldehyde (the key volatile compound of yoghurt) have resulted in low consumer acceptance, and the ratio of sweetness to sourness provides some insight into flavour balance. It is, therefore, important that acidity of retail yoghurts fall within a range that will give the most acceptable taste.

Total soluble sugars

The mean values of the total soluble sugars (TSS) of the brands of yoghurt ranged from 9.57 ± 0^{o}Brix (Y3) to 15.93 ± 0%^{o}Brix (Y5). There were significant differences between the TSS of the samples ($P<0.05$). 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.

Total solids

Values for total solids (TS) are presented in Table 4.8. The TS of the yoghurt brands varied significantly ($P<0.05$) from 13.80± 0.13% (Y3) to 19.32±0.07% (Y5). The TS of four of the product (Y2, Y5, Y6 and Y7) were higher than those reported for similar products in Pakistan (13.38±1.34%) (Younus *et al*., 2002), Sudan (9.3±2.52%) (El Zubeir *et al*., 2005), Khartoum state-Sudan (14.04±1.83%) (El Bakri and El Zubeir, 2009) and Turkey (15.89%) (Karagozlu *et al*., 2005).

The total solids of these finished products is an important indicator of aroma compound production (Mahdian and Tehrani, 2007), availability of fermentable substrates for live LAB (Ozer and Robinson, 1999) during storage of the product and a determinant of product thickness and tendency to reduce whey separation (Mahdian and Tehrani, 2007).

Viscosity

Viscosities of the yoghurts ranged from 1.00 ± 0.00dPs to 2.53 ± 0.30dPs for Y3 and Y5 respectively, and there were significant difference between the viscosities of all the brands of yoghurt ($P<0.05$) studied. The viscosities correlated with both the carbohydrate content and total solids of the yoghurts; as the carbohydrate and total solids contents increased from one brand of yoghurt to the next, the viscosities also increased. This agrees with reports that higher total solids of milk base improves viscosity of yoghurts (Mahdian and Tehrani, 2007). In the survey conducted on popularity of yoghurts some consumers (2.1%) called for reduction of the viscosity of some brands of yoghurt while others (6.7%) requested for an increase in product viscosity.

4.2.3 Microbial quality of the seven brands of yoghurt

Coliforms

The total coliform counts of the products are represented in Table 4.9 and the values indicate the products varied significantly ($P<0.05$) at 95.0% confidence level. Yoghurts

from manufacturer Y3 recorded no coliforms while Y2 recorded the highest counts (9.30×10 cfu/ml).

The coliform counts reported were lower than those reported by Younus *et al*., (2002) which were 0, 7.1 x 10^2 and 3.39 x 10^3cfu/ml for three brands of yoghurt analyzed. El Bakri and El Zubeir (2009) also presented a higher mean coliform content of 3.93±4.35 log cfu/ml. Codex standards and local standards require that yoghurts contain no coliforms at all. Hence, irrespective of the low coliform counts observed in the study, they were not indicative of products of acceptable microbial quality (Codex Standard 243-2003; Ghana Standard 337-2003). The differences in microbial loads could be due to differences in hygienic practices of manufacturers in the production process.

Table 4.9: Yeast and total coliform loads of the seven brands of yoghurt

Brand of yoghurt	Coliforms (×10cfu/ml)	Yeast (×10^5 cfu/ml)
Y1	3.80 ± 0.09^{bc}	12.50 ± 0.02^{ab}
Y2	6.00 ± 0.29^{cd}	13.30 ± 0.16^{ab}
Y3	0.00 ± 0.00^{a}	8.40 ± 0.35^{c}
Y4	4.50 ± 0.47^{bc}	10.00 ± 0.00^{ac}
Y5	1.50 ± 0.00^{ab}	13.70 ± 0.05^{b}
Y6	9.30 ± 0.00^{d}	14.00 ± 0.39^{b}
Y7	4.30 ± 0.00^{bc}	12.40 ± 0.00^{ab}

Means followed by the same superscript in a column denote values that are not significantly different (p>0.05)

Yeast

From Table 4.9 the yeast counts increased from 8.40×10^5 cfu/ml (Y3) to 14.00×10^5cfu/ml (Y6). There were significant differencesbetween the yeast counts of the brands of yoghurt (P<0.05).

The yeast counts for all the products were higher than counts reported by Suriyarachchi and Fleet (1981) and also higher than yeast and mould counts reported by El Bakri and El Zubeir (2009). The former reported 55% of samples with average yeast counts less than 10^3 cells/g and 45% of samples with counts in excess of 10^3 but up to 10^5, while the latter reported yeast and mould counts of 4.09 ± 4.57log cfu/ml. As in the case of coliform counts, the observed yeast counts in this study were indicative of poor microbial quality with reference to the codex standards (0cfu/g) for yoghurts. These microbial loads may be attributed to the production practices of the manufacturers; as to whether good manufacturing practices (GMPs) and good hygienic practices (GHPs) were observed at all production sites. Since yeasts are considered as natural contaminant of yoghurt, there are inconsistencies in acceptable threshold of yeast load in yoghurt. For instance under good manufacturing practices, the final product should contain not more than 1 yeast cfu/g at the time of production (Suriyarachchi and Fleet, 1981). Nevertheless, this is not adhered to in all areas, as in some studies the yeast loads were extended to less than or equal to 50 cfu/ml (Li and Li, 1998). Different surveys of retail yoghurts revealed that samples could contain counts more than 10^5cfu/g (Rohm *et al*., 1990; AL-Tahiri, 2005).

4.2.4 Sensory evaluation of the seven brands of yoghurt

30 untrained panelists evaluated each brand of yoghurt based on a seven point hedonic scale with 1 being “dislike very much” and 7 being “like very much”.

Aroma

From Table 4.10 the mean scores for aroma ranged from 5.00 ± 1.69 (Y3) to 6.58 ± 0.67 (Y6). This means that the aroma of all the products was well appreciated by the panelist. There was a significant difference between the mean scores for aroma of the seven products ($P<0.05$). Differences in aroma could be due to the addition of flavouring compounds or, in some cases, the extent of formation of flavour compounds during fermentation (Crawford, 1962; Lindsay *et al.*, 1965).

Table 4.10: Mean scores for sensory attributes of the seven brands of yoghurt

Brand of Yoghurt	Aroma	Colour	Sourness	Sweetness	Thickness	Mouthfeel
Y1	5.19 ± 1.19^{ab}	5.97 ± 0.84^{a}	5.00 ± 1.34^{ab}	5.36 ± 1.11^{ab}	5.23 ± 1.26^{a}	5.32 ± 1.11^{a}
Y2	5.68 ± 1.40^{bc}	6.32 ± 0.65^{abc}	5.52 ± 0.96^{ad}	5.84 ± 0.86^{bd}	5.48 ± 1.26^{a}	5.84 ± 0.86^{ab}
Y3	5.00 ± 1.69^{a}	6.16 ± 0.64^{ab}	4.32 ± 1.92^{c}	4.03 ± 1.56^{c}	4.48 ± 1.57^{b}	4.36 ± 1.85^{c}
Y4	6.13 ± 0.99^{cd}	6.61 ± 0.56^{cd}	4.52 ± 1.71^{bc}	5.13 ± 1.06^{a}	6.36 ± 0.66^{d}	6.13 ± 1.03^{bd}
Y5	6.16 ± 1.07^{cd}	6.52 ± 0.63^{bcd}	6.06 ± 0.85^{d}	5.94 ± 1.53^{d}	6.19 ± 1.11^{cd}	6.56 ± 0.72^{d}
Y6	6.58 ± 0.67^{d}	6.74 ± 0.45^{d}	5.94 ± 1.34^{d}	6.07 ± 0.77^{d}	6.32 ± 0.83^{d}	6.23 ± 0.88^{bd}
Y7	5.90 ± 1.35^{c}	6.32 ± 1.22^{abc}	6.13 ± 0.81^{d}	5.87 ± 0.92^{bd}	5.65 ± 1.08^{ac}	5.91 ± 1.14^{b}

Means followed by the same superscript in a column denote values that are not significantly different (p>0.05)

Colour

There was a significant difference ($P<0.05$) between the mean scores for colour of the products. The mean scores for colour ranged from 5.97 ±0.84 (Y1) to 6.16 ± 0.64 (Y3), indicating that the panelists moderately liked the colours of the products.

Sourness

The mean scores for sourness of all brands of yoghurt showed significant difference ($P<0.05$). Brand Y3 had the lowest mean score of 4.32 ± 1.92, and the highest score was recorded for Y7 (6.13 ± 0.81). With a score of 4 being interpreted as “neither like nor dislike”, 5 being “like slightly”, and 6 being “like moderately”, it is clear from the low

scores that the sourness of the products were not well appreciated by panelists. The relatively high titratable acidity (1.23%) and low TSS (9.57°Brix) recorded for Y3 could explain its high level of sourness which was not liked by the panelists. In the case of Y5 the titratable acidity of 1.25% was slightly higher than that of Y3. However, its relatively higher sugar content of 15°Brix made it more acceptable to consumers. The high sugar content might have masked the high acidity of the product, making the sourness more acceptable. In the case of Y4 which had the second lowest score for sourness, the titratable acidity of 0.67% was the lowest among all the products. Though this value was close to the minimum requirement set by the codex standards (0.60%), the panelists neither liked nor disliked the product.

Sweetness

The mean scores for sweetness of all products showed significant differences between the products ($P<0.05$). The lowest mean score of 4.03 ± 1.56 was recorded for Y3 while Y6 had the highest score of 6.07 ± 0.77. The excessive acidity of Y3 might have masked its sweetness level because the TSS was relatively low (9.57 °Brix). The sweetness of Y6 was the most appreciated by panelists though it did not have the highest total soluble sugars. AlthoughY5 had the highest soluble sugars its sweetness was slightly toned down by its high lactic acid content. Hence the level of acceptance of product Y6 was relatively higher than Y5.

Thickness

The mean scores for thickness of products ranged from 4.48 ± 1.57 to 6.37 ± 0.66 and the level of liking was in an increasing order of Y3, Y1, Y2, Y7, Y5, Y6 and Y4. This was not unexpected, since Y3 had the lowest viscosity (1.00 ± 0dPs). The viscosity or

thickness of yoghurt is influenced by its total solids and this is reflected in results obtained for Y3 as it had the lowest total solids and carbohydrate content. The yoghurt labeled Y5 had the highest viscosity but not the highest mean score for thickness, which could mean that its viscosity was beyond an acceptable level for panelists.

Mouthfeel

The mean scores for mouthfeel also showed significant differences ($P<0.05$) between all the products. Again Y3 had the lowest score for mouthfeel (4.36 ± 1.85). The highest score was recorded for Y5 (6.57 ± 0.72). The mouthfeel of yoghurt has been known to be affected by the fat and carbohydrate contents of the product. A product with too low fat contents coupled with too high carbohydrate content could result in a chalky taste while a product with too high fat content could also have an undesirable mouthfeel. Improper homogenization of the milk base during yoghurt manufacture sometimes results in a product with non uniform texture, as the milk fat may form small aggregates within the product. This is perceived as an unsmooth or grainy texture on the tongue. Graininess may also be as a result of slow fermentation causing casein-casein interactions to dominate the gel network as opposed to protein-protein interactions (Lucey *et al*., 1997; Lucey *et al*., 1998; Lucey and Singh, 1998).

Overall mean score for all sensory attributes of each product

In order to assess the quality of individual brands of yoghurt the overall mean score was calculated as the composite of all the sensory attributes evaluated. Figure 4.1 shows that the overall mean score for Y3 was the lowest (4.73 ± 0.92) Of all the sensory attributes evaluated. It also had the lowest mean scores among all the sensory attributes except colour. The highest overall mean score of 6.31 ± 0.47 was recorded for Y6 which also

had the highest mean scores for aroma, colour and sweetness. As in the case of all the individual sensory attributes, there was a significant difference ($p<0.05$) among overall mean scores of the seven brands of yoghurt. Mouthfeel, flavour, sweetness, sourness, and the balance between these factors have been shown to affect the overall preference for yogurt (Barnes *et al.*, 1991b). These characteristics, and many others, are important attributes for the acceptance of a stirred yogurt product.

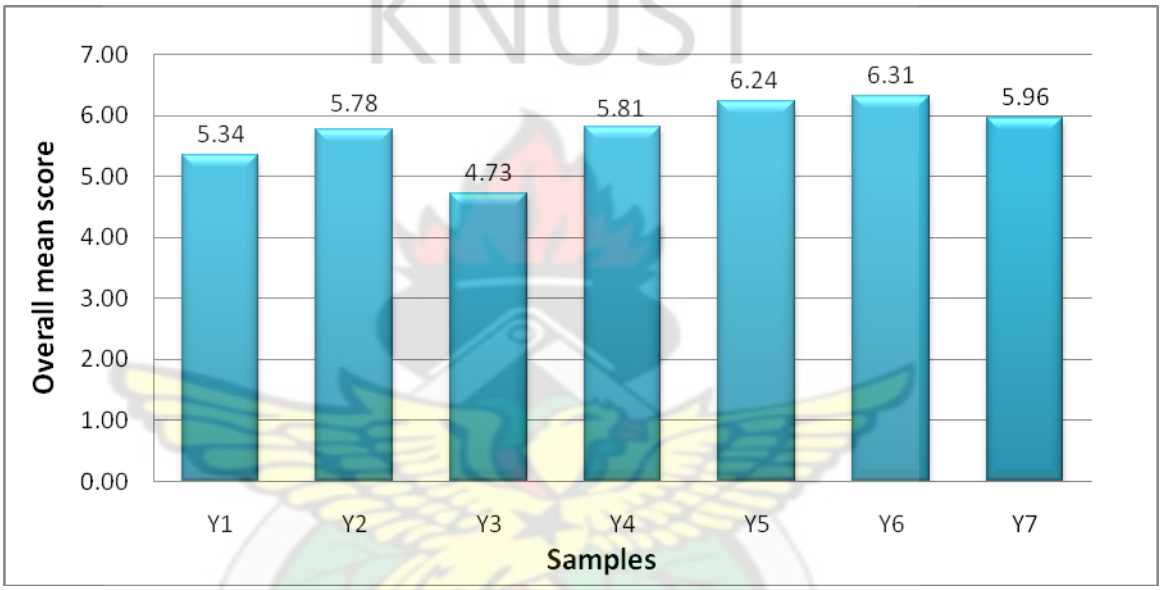


Figure 4.1: The overall mean scores of the sensory attributes of seven brands of yoghurt

4.3. QUALITY CHANGES OF NATAMYCIN-PRESERVED YOGHURT

Table 4.11 shows changes in pH of yoghurts with different concentrations of natamycin during the four week storage period at $5\pm1^{O}C$. There were no significant differences ($p>0.05$) in pH of the yoghurts with different concentrations on the day of manufacture to the 7^{th} day of storage. However, significant differences ($p<0.05$) were observed among yoghurts with different concentrations of natamycin on the 14^{th}, 21^{st} and final days of storage. The pH of yoghurts with 6 to 10ppm of natamycin also differed

significantly from week to week. Hence, both the concentration of natamycin and days of storage had a significant effect on the pH ($p<0.05$). As the number of days of storage increased, pH values decreased (correlation coefficient=-0.9829) (Appendix VIII).

Table 4.11: The pH of yoghurts preserved with different concentrations of natamycin during storage at $5^{O}C$

Concentration of natamycin	0 Day	7th Day	14th Day	21st Day	28th Day
N0	4.56 ± 0.01^{a}	4.55 ± 0.00^{a}	4.42 ± 0.00^{a}	4.22 ± 0.01^{a}	4.12 ± 0.01^{a}
N5	4.56 ± 0.01^{a}	4.55 ± 0.00^{a}	4.41 ± 0.00^{b}	4.23 ± 0.01^{a}	4.11 ± 0.01^{a}
N6	4.56 ± 0.01^{a}	4.55 ± 0.01^{a}	4.41 ± 0.00^{b}	4.22 ± 0.00^{a}	4.11 ± 0.00^{a}
N7	4.56 ± 0.01^{a}	4.55 ± 0.00^{a}	4.41 ± 0.00^{b}	4.24 ± 0.01^{ab}	4.15 ± 0.00^{b}
N8	4.56 ± 0.01^{a}	4.55 ± 0.01^{a}	4.42 ± 0.01^{ab}	4.26 ± 0.01^{c}	4.19 ± 0.01^{c}
N9	4.56 ± 0.01^{a}	4.55 ± 0.01^{a}	4.41 ± 0.00^{b}	4.25 ± 0.00^{cb}	4.19 ± 0.01^{c}
N10	4.56 ± 0.01^{a}	4.55 ± 0.00^{a}	4.41 ± 0.00^{b}	4.26 ± 0.01^{c}	4.18 ± 0.00^{c}

Means followed by the same superscript in a column denote values that are not significantly different ($p>0.05$)

As the yoghurts remain in storage, the viable lactic acid bacteria (LAB) cause decreases in pH values of yoghurts with different concentrations of natamycin throughout the storage period. During yoghurt production Lactose is metabolized into its monomers which are later metabolized by the LAB to produce lactic acid (Van Denmark and Batzing, 1987) which resulted in a decrease in the pH. The pH of yoghurts containing natamycin at concentrations of 8, 9 and 10 ppm did not reduce as much from the 14th day onwards as compared to those of yoghurts with lower concentrations of the preservative. Yoghurts with lower concentration of natamycin had pH values of up to 4.11 at the end of the storage period while those containing more preservative had pH values only as low as 4.18.

The extent of decrease in pH varies with the rate of growth of bacteria, interactions between yoghurt culture bacteria and other probiotic or spoilage microorganisms present in the product, additives present (e.g. fruits), storage temperatures and physicochemical properties such as TS and TSS which provide fermentable substrate for LAB and other spoilage bacteria such as coliforms. Several researchers have reported different degrees of decrease in pH under different storage conditions, as affected by the factors mentioned above (Yeganehzad *et al*., 2007; Akpan *et al*., 2007; Viljeon *et al*., 2003).Viljoen *et al*. (2003) reported decreasing pH values of 3.8–3.1 in fruit yoghurt and 3.9–3.0 in plain yoghurt starting right after manufacture and correlating with increasing yeast numbers at temperatures of 15 and 20^{O}C. At low temperature of 5 and 10^{O}C, pH values of both fruit and plain yoghurts only reached about 3.7 (Viljeon *et al*., 2003). In studies carried out by Akpan *et al*., (2007), preservation of soy yoghurts with 20mg/ml of sodium benzoate at ambient temperature resulted in a decrease in pH from 4.4 to 3.95 after 21 days, while the same concentration of sodium benzoate at 4^{O}C resulted in a pH drop only to 4.07. The same study also reported a pH value of 3.79 at the end of a 21-day refrigerated storage of yoghurt preserved with 10mg/ml Sodium benzoate, while the drop in pH was not that low (4.07) when higher concentrations (20mg/ml) of the same preservative were used. In the use of natamycin as a preservative in yoghurt, El-Diasty *et al.*, (2009) reported a reduction of pH from 4.56 to 4.50 when 10mg/kg and 20mg/kg of the preservative were used, while in the control, pH reduced from 4.56 to 4.14 in 28 days. In the present study pH reduced from 4.56 to 4.18 in yoghurts preserved with natamycin at 10ppm, and 4.56 to 4.12 in control samples.

Changes in TSS of yoghurts preserved with different concentrations of natamycin

Figure 4.2 shows the progressive decrease of TSS of the yoghurts from the day of preparation to the end of the storage period.The TSS of yoghurts containing different concentrations of natamycin reduced throughout the 28 days of storage. There were significant differences ($P<0.05$) in TSS of the yoghurts during the storage period (Appendix VIII). The results also indicated that both the concentrations of natamycin and the number of days of storage had significant effects on the TSS of yoghurts. Similarly to the pH, the number of days of storage had a negative correlation with TSS (correlation coefficient=-0.7646; $P<0.05$) (Appendix VIII). The concentration of natamycin had a positive and significant correlation with TSS (correlation coefficient=0.2640; $P<0.05$). Hence, as the number of days of storage increased, TSS of yoghurts decreased with yoghurts containing higher concentrations of natamycin decreasing less than those of lower concentrations.

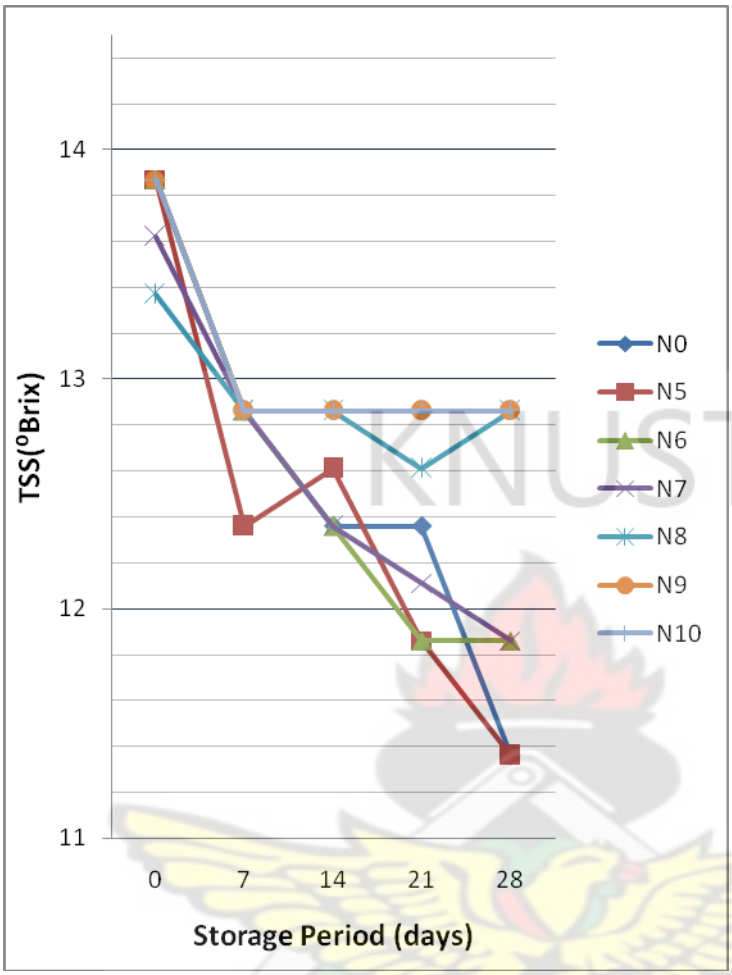


Figure 4.2: Effect of concentrations of natamycin on TSS of yoghurts under refrigeration

In samples containing 8, 9 and 10ppm, the decrease was from about 13.87 to 12.86 °Brix while in the control and samples with lower concentrations of the preservative, TSS decreased to much lower values.The lowest TSS value at the end of the storage period was (11.36 °Brix) in yoghurt with 5ppm of natamycin.

Changes in titratable acidity (TA) of yoghurts preserved with different concentrations of natamycin

Both the concentration of natamycin and number of days of storage had significant effects on the TA of yoghurts during storage. As the number of days of storage increased, TA increased (correlation coefficient = 0.7574; $P<0.05$) but as the concentration of natamycin in yoghurt increased, the increase in TA of yoghurts was less pronounced (correlation coefficient = -0.3454; $P<0.05$) (AppendixVIII) (Figure 4.3).

The major acid in yoghurt is lactic acid which is a product of lactose fermentation by yoghurt culture bacteria. The higher acid contents of samples N0 (1.05±0.08%), N5 (0.98±0%), N6 (1.07±0.09%) and N7 (0.93±0.08%) at the end of storage period indicate that these samples supported the proliferation of more LAB than samples N8, N9 and N10in which there were lower acid levels recorded at the end of the storage period (Figure 4.3).

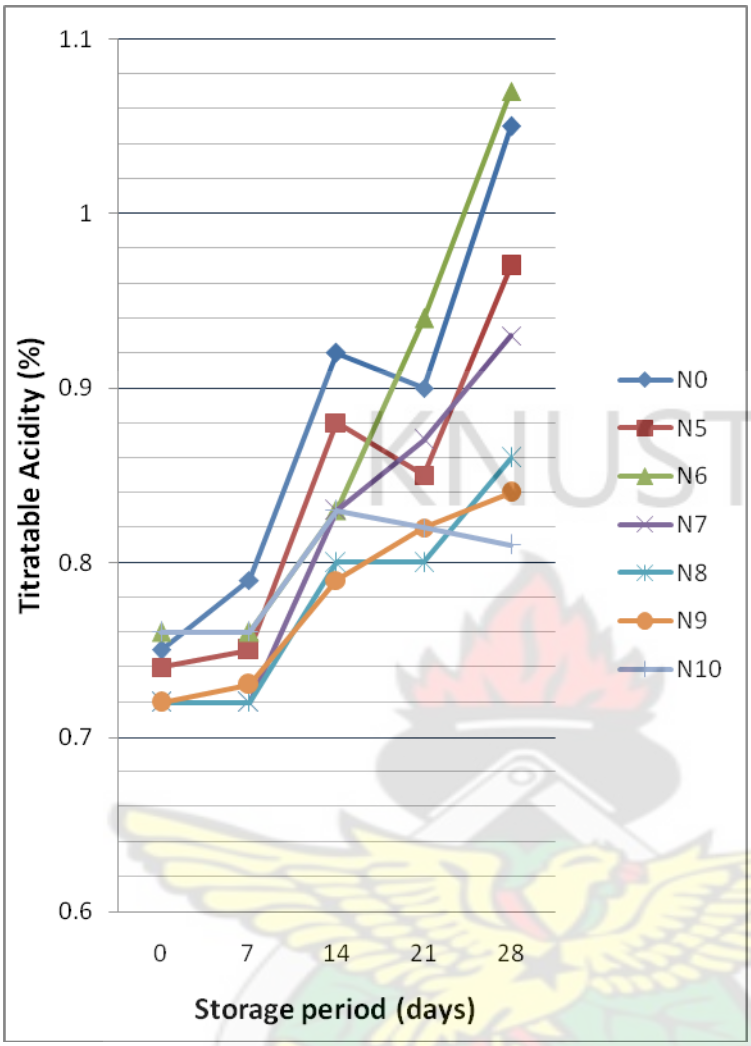


Figure 4.3: Effect of concentrations of natamycin on TA of yoghurts under refrigeration

The lower acid level observed in samples with higher concentrations of natamycin at the end of the storage period correlated with the less pronounced decrease in TSS and pH levels in these samples. Higher concentrations of natamycin (8, 9 and 10ppm) in yoghurts were better suited for preventing extreme changes in physicochemical properties than lower concentration of the preservative. It has been reported that the proliferation of contaminant yeast in yoghurt during storage may provide necessary growth factors for yoghurt bacteria (Viljoen, 2001). The symbiotic relationship between

LAB bacteria and yeast could explain the less pronounced increase in acidity and reduction in pH in yoghurts containing higher concentration of natamycin (N8, N9 and N10). Consequently the reduction of yeast cells in yoghurt samples containing higher concentration of natamycin caused a reduction in proliferation of the bacteria. This observation was also reported in the work of Var *et al.*, (2004) who reported that yoghurt bacteria reduced 3 log cycles in 30 days at 4±1^{O}C when 5, 10, 15 and 20ppm of natamycin were used.

At the end of storage samples N8, N9 and N10 had TA values of 0.87±0%, 0.84±0.01% and 0.80±0.01% respectively. The final acidities of all yoghurts at the end of the storage period were within the values reported by El-Diasty *et al.*, (2009). In their study, El-Diasty *et al.* (2009) reported titratable acidities ranging from 0.78 ± 0.01% to 0.89 ± 0.02% for yoghurt treated with 10 and 20ppm natamycin and for control samples the acidity ranged from 0.78 ± 0.01 to 0.92 ± 0.01%.

Changes in Total yeast counts during storage of yoghurts preserved with different concentrations of natamycin

The concentration of natamycin and number of days in storage had significant effect ($P<0.05$) on the total yeast count in the yoghurt samples. Both factors also had significant non-zero correlations with yeast counts of yoghurts (Appendix VIII). There was no significant difference ($P>0.05$) in yeast counts in all samples on the day of preparation. However, from the 7th to 28th day of storage, there were significant differences ($P<0.05$) in yeast counts between yoghurts containing different concentrations of natamycin.

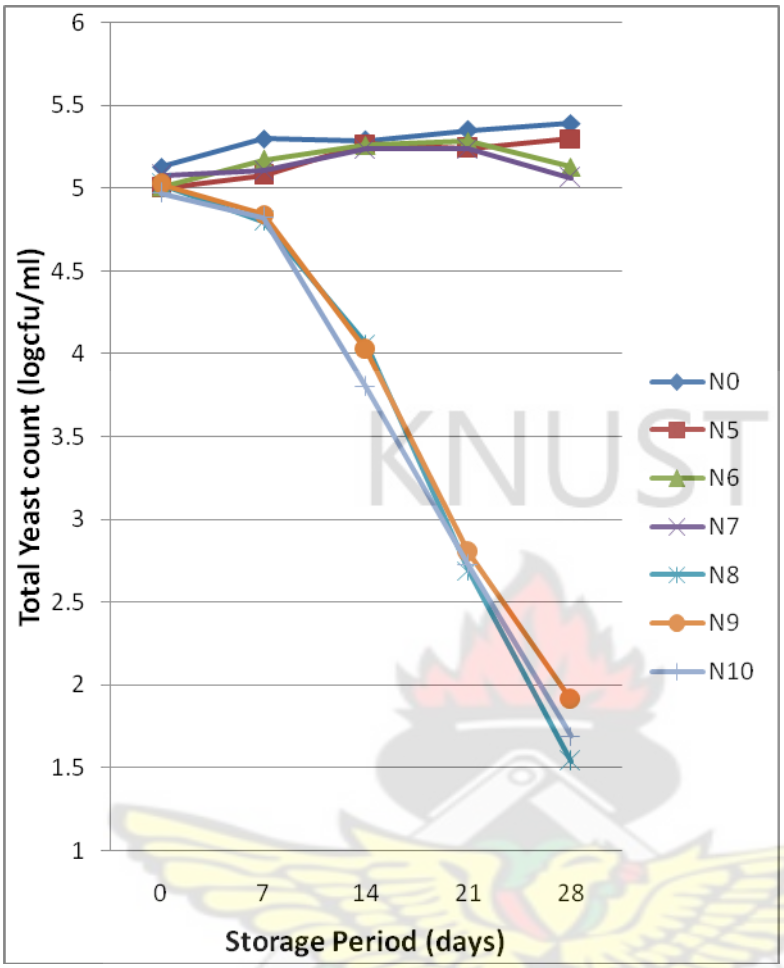


Figure 4.4: Effect of concentrations of natamycin on yeast counts in yoghurts under refrigeration.

From figure 4.4 yeast counts reduced gradually from 5.03±0.04, 5.03±0.04 and 4.90±0.08 to 1.54±0.09, 1.91±0.19 and 1.69±0.30logcfu/ml in samples containing 8, 9 and 10ppm of natamycin respectively, while in samples containing no natamycin and 5ppm, yeast counts continued to increase throughout the period. In these samples yeast cells increased from 5.13±0.07 and 5.0±0.0 on the day of preparation to 5.39±0.01 and 5.30±0.02logcfu/ml respectively on the final day of storage. Yoghurts containing 6 and 7ppm of natamycin showed very slight increases in yeast counts from the day of preparation till the 14th day of storage, after which the numbers of yeast began to slowly reduce. Destruction of yeast cells by natamycin occurs as the antibiotic enters the cell

membrane by complexing with its ergosterols resulting in the creation of polar pores. This causes a disruption of the integrity of the membrane so that vital structures pass through the cell membrane and out of the cell (Hamilton-Miller, 1974; Norman *et al*., 1976; McGinnis and Rinaldi, 1985; Franklin and Snow, 1998)

The results differ from those reported by Var *et al.*, (2004) and El-Diasty (2009). The former stated that no yeast and moulds were detected in yoghurts containing 5, 10, 15 and 20ppm of natamycin after storage at 4 ± 1 ºC for 30days, while the later reported no yeast in yoghurts with 10 and 20ppm of the preservative from the 3rd to 35th day of storage at 4 ± 1 ºC. This difference could be attributed to the higher concentrations of natamycin used in the previous studies, and the initial yeast loads in the samples before storage.

The effects of the point of addition of 8ppm natamycin during yoghurt production

In Figure 4.4, the study of the effect of different concentrations of natamycin on yoghurt during storage showed that 8ppm of natamycin resulted in the highest percentage decrease in the yeast counts in yoghurts. Yeast counts decreased by 69.36%, 61.99% and 65.99% in yoghurts with natamycin at 8, 9 and 10ppm respectively. Although yoghurts with natamycin at these concentrations yielded similar results in their physicochemical and microbial quality, the use of 8ppm of the preservative for further work would be more economical. 8ppm of natamycin was, therefore, added to yoghurts during preparation; before incubation of the milk base (NB) and after incubation/fermentation (NA). The controls (N0) contained no natamycin.

Table 4.12: Changes in pH, TA and TSS of yoghurts with 8ppm natamycin added before and after incubation of milk base.

	0 Days	7th Day	14th Day	21st Day	28th Day
pH					
N0	4.54 ±0.03^{a}	4.33 ± 0.02^{a}	4.24 ± 0.02^{a}	4.20 ± 0.01^{a}	4.00 ± 0.04^{a}
NB	4.53 ± 0.02^{a}	4.44 ± 0.01^{b}	4.31 ± 0.01^{b}	4.25 ± 0.01^{b}	3.97 ± 0.01^{a}
NA	4.55 ± 0.01^{a}	4.46 ± 0.01^{b}	4.29 ±0.02^{ab}	4.29 ± 0.00^{c}	4.01 ± 0.01^{a}
Titratable Acidity					
N0	0.71 ± 0.00^{a}	0.73 ± 0.00^{a}	0.98 ± 0.01^{a}	0.99 ± 0.01^{a}	1.01 ± 0.02^{a}
NB	0.72 ± 0.00^{a}	0.72 ± 0.01^{a}	0.86 ± 0.01^{b}	0.92 ± 0.04^{a}	0.95 ± 0.02^{a}
NA	0.72 ± 0.01^{a}	0.72 ± 0.01^{a}	0.90 ± 0.01^{c}	0.93 ± 0.00^{a}	0.95 ± 0.04^{a}
TSS					
N0	13.37 ± 0.00^{a}	13.11± 0.35^{a}	12.61 ± 0.35^{a}	2.36 ± 0.00^{a}	11.36 ± 0.00^{a}
NB	13.37 ± 0.00^{a}	12.86± 0.00^{a}	12.36 ± 0.00^{a}	12.60± 0.33^{a}	11.86 ± 0.00^{ab}
NA	13.62 ±0.35^{a}	12.61 ± 0.35^{a}	12.36 ± 0.00^{a}	12.36± 0.00^{a}	12.11 ± 0.35^{b}

Means followed by the same superscript in a column denote values that are not significantly different (p>0.05)

The Effect of Point of addition of natamycin on the pH, titratable acidity and total soluble sugars of yoghurt during refrigeration

There was no variation in pH, TA and TSS values between the yoghurts with natamycin added before fermentation and those with the preservative added after fermentation. pH values of 4.54±0.03, 4.53±0.2 and 4.55±0.1 were recorded for samples N0, NB and NA respectively immediately after preparation of yoghurts. Only the number of days of storage had a significant correlation (Correlation coefficient = -0.9518 with corresponding P<0.05, Appendix VIII) with the pH change during storage. As reported earlier on, the pH of all samples of yoghurt decreased with increasing number of days of storage. The pH of samples did not differ significantly on the day of manufacture and on the 28th day of storage pH values were 3.97±0.01 (NB), 4.01±0.01(NA) and 4.00±0.04 (Control).

The titratable acidity (TA) increased significantly ($P < 0.05$) while TSS decreased significantly ($P<0.05$) in all samples of yoghurt as storage progressed. TA increased in both treated and control samples, but the increase was higher for the controls at the end of the storage period. Storage began with titratable acidity of 0.71±0.00%, 0.72±0.00% and 0.72±0.01% for N0, NB and NA and ended with values of 1.01±0.02%, 0.95±0.02% and 0.95±0.04% respectively. There were no significant differences ($p>0.05$) in TSS of yoghurts from the day of preparation till the 21st day of storage, but on the final day there were significant differences ($P<0.05$) in the soluble sugar contents. The TSS decreased from 13.37±0.00, 13.37±0.00 and 13.62±0.35°Brix to 11.36±0.00, 11.86±0.00 and 12.11±0.35^{O}Brix in N0, NB, NA respectively.

The effect of point of addition of natamycin on the yeast counts in yoghurt during refrigeration

As shown in figure 4.5, yeast counts decreased in the treated samples, while the controls supported the proliferation of yeast cells. This reflected in the lower TSS in the control at the end of the storage period, and consequently in their higher levels of lactic acid as compared to the samples treated with natamycin. There were no significant differences ($p>0.05$) in total yeast counts between NB (samples treated with natamycin before fermentation of milk base) and NA (samples treated with natamycin after fermentation of milk base). The yeast loads increased from 4.81±0.29 log cfu/ml to 6.31±0.02 log cfu/ml in the control (N0), and decreased from 4.91±0.17logcfu/ml and 5.05±0.08 log cfu/ml to 1.48±0.0 log cfu/ml and1.72±0.17 log cfu/ml in NB and NA respectively.

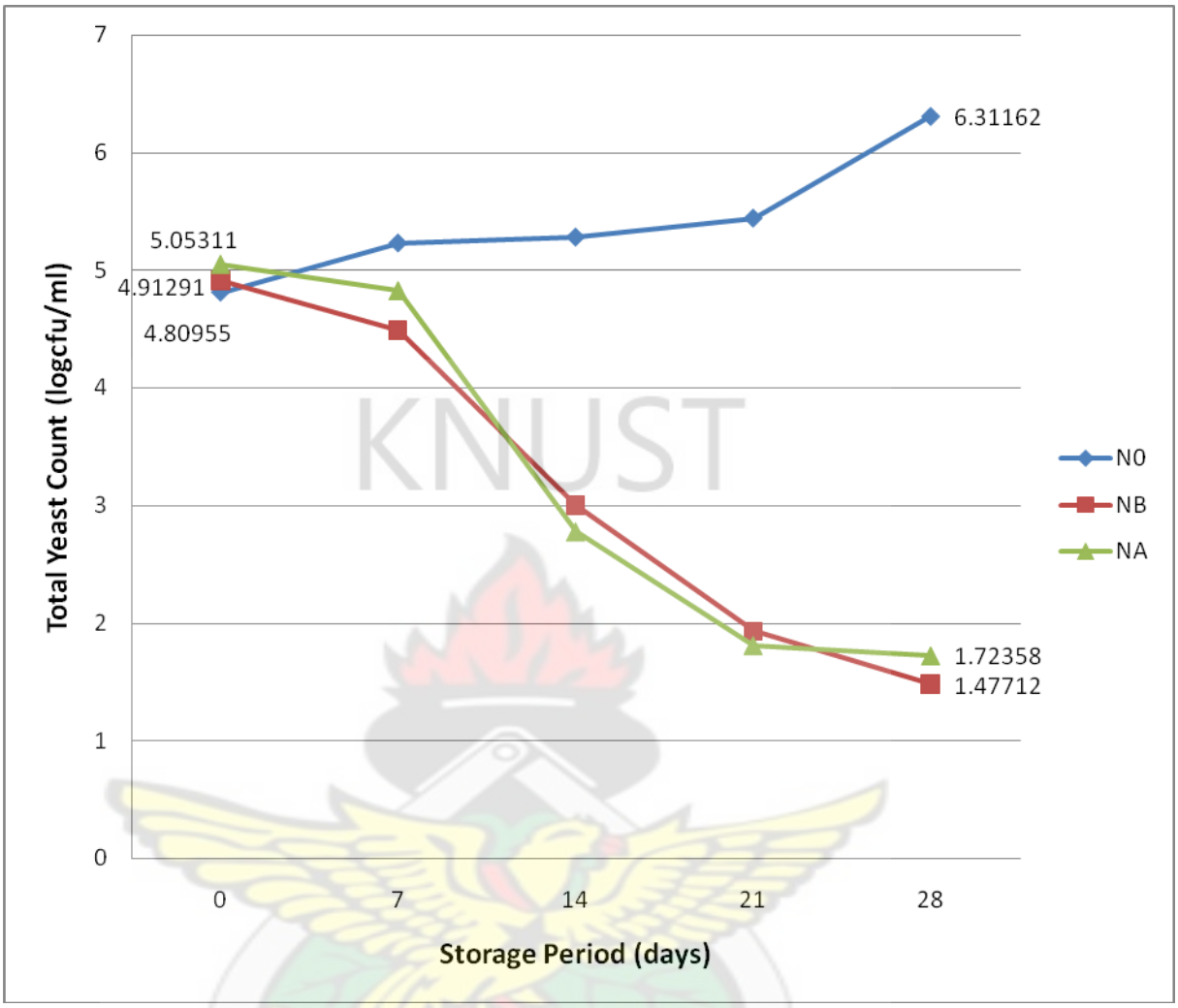


Figure 4.5: Relationship between point of addition of natamycin and yeast count in yoghurt during refrigerated storage.

4.4 PHYSICOCHEMICAL AND MICROBIAL CHANGES IN COMMERCIALIZED YOGHURTS PRESERVED WITH 8ppm OF NATAMYCIN

Changes in pH of yoghurts during Storage

All the samples with natamycin and their corresponding controls had pH values within the range of 3.99-4.46 on the day of manufacture, and there were no significant

differences between pH values of unpreserved yoghurts ($P>0.05$), while the samples containing natamycin showed significant differences at 95% confidence level ($P<0.05$). The graphs in Figures 4.6a and 4.6b show that the pH of all samples reduced gradually during the storage period with control samples showing lower pH values than their corresponding treated samples. The preserved product with the highest initial pH was Y6 with a value of 4.45. At the end of the storage period product Y7 had a higher pH value (3.98) than Y6 (3.82). These changes could be due to the levels of total solids which provided more fermentable substrate for LAB in Y6 than in Y7. The product Y3 hand the lowest pH value throughout the study, followed by product Y1.

In the control samples the decrease in pH was more drastic due to increased acidification of the product by more prolific development of yoghurt culture bacteria. The pH were 4.27, 4.33, 4.04, 3.89, 4.44, 4.46 and 4.43 for the seven brands respectively and decreased respectively to 3.28, 3.52, 3.26, 3.50, 3.44, 3.57 and 3.91 on the 35^{th} day. The decreased pH levels reflect an increase in acidity of the products which would be detected as a sour taste in the product.

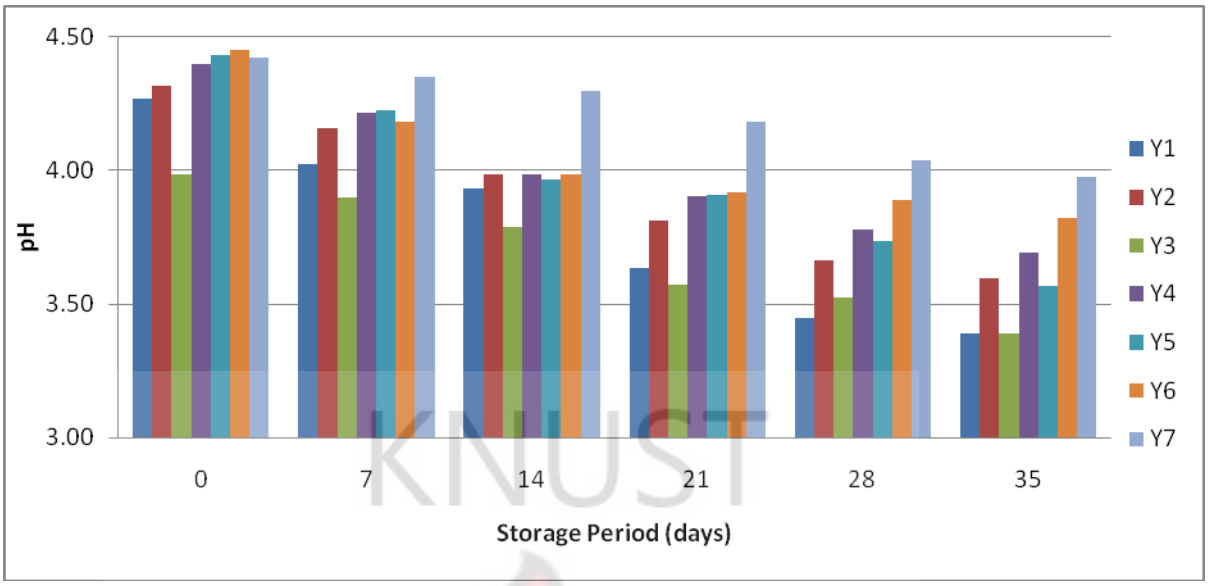


Figure 4.6a:Changes in pH of seven brands of commercialized yoghurts containing natamycin during 35 days of storage.

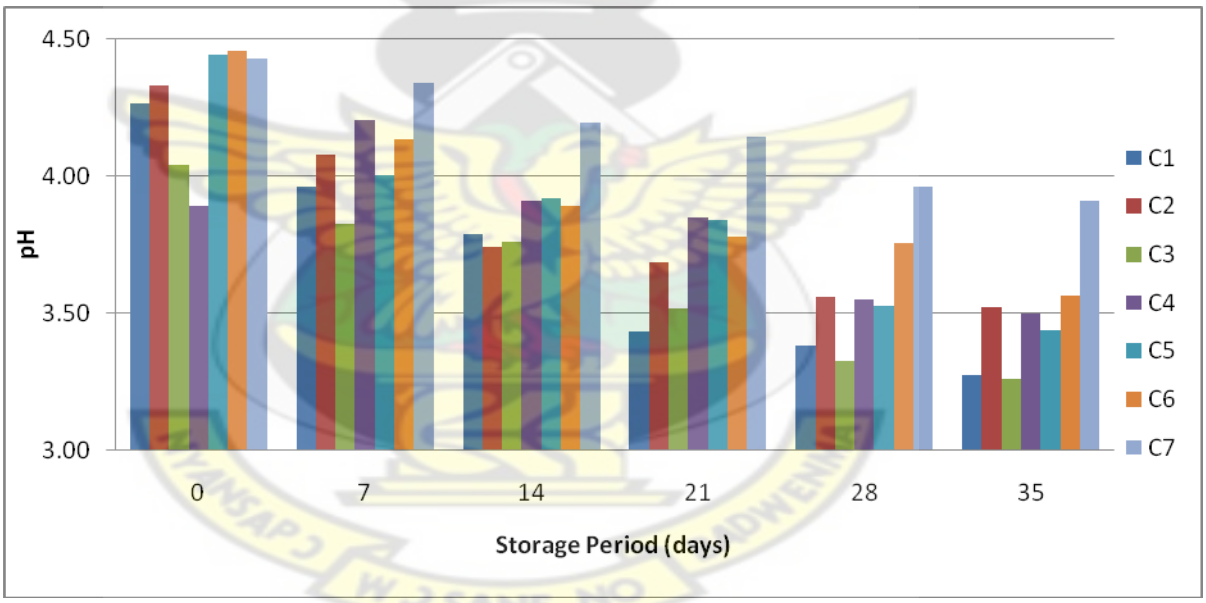


Figure 4.6b: Changes in pH of seven brands of commercialized yoghurts containing natamycin during 35 days of storage.

Changes in Titratable Acidity contents of yoghurts during Storage

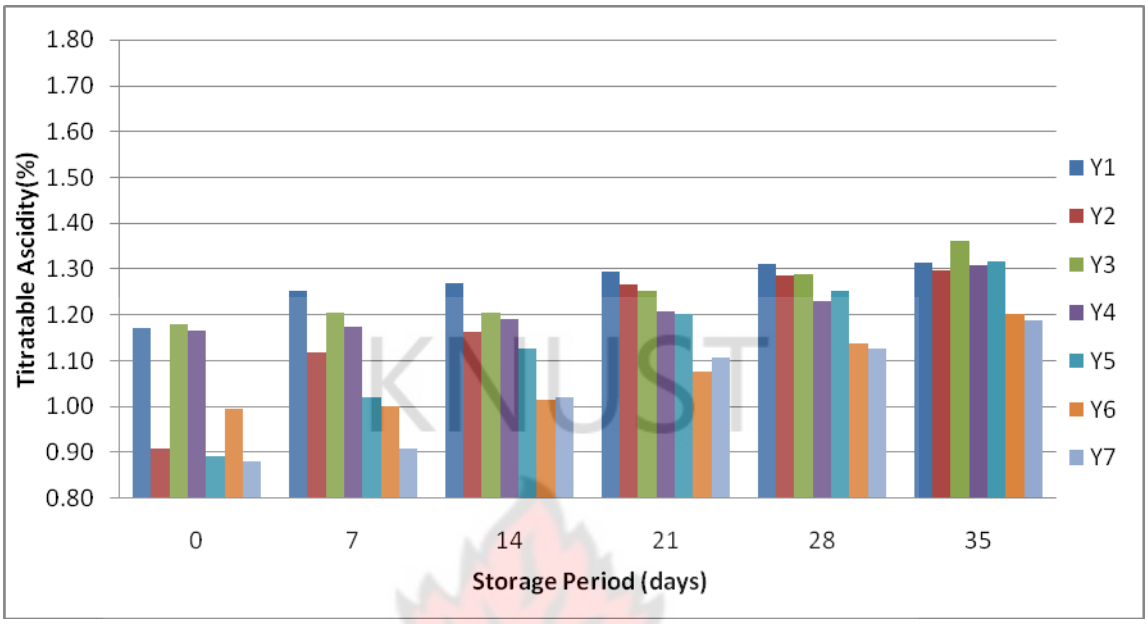


Figure 4.7a:Changes in titratable acidity of seven brands of commercialized yoghurts containing natamycin during 35 days of storage.

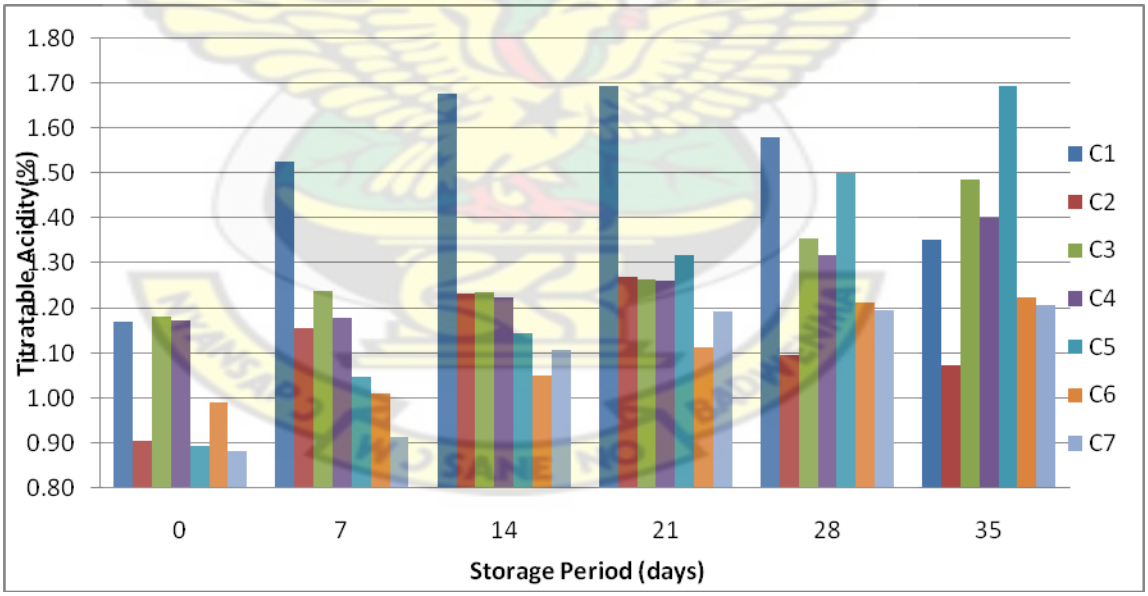


Figure 4.7b: Changes in titratable acidity of seven brands of commercialized yoghurts containing natamycin during 35 days of storage.

Titratable acidity (TA) increased in all samples throughout the storage period (Figures 4.7a and b). Statistical analysis (Appendix X) showed that there were significant

differences ($p<0.05$) in titratable acidities of all samples during each week of storage. Titratable acidity of preserved commercial brands of yoghurt did not increase as much as their corresponding control yoghurts which did not contain the preservative. TA of the seven brands of yoghurt (Y1-Y7) containing natamycin were 1.17, 0.91, 1.18, 1.17, 0.89, 1.00 and 0.88%respectively at the beginning of the study and increased to 1.31, 1.30, 1.36, 1.31, 1.32, 1.20 and 1.19% on the final day. At the end of the storage period none of the samples had TA within the range of a good finished product (0.85-0.90%) (Jay, 2000), even though on the day of manufacture samples Y5 and Y7 fell within this range.

The brand of yoghurt labelled C3 had the highest acidity (1.18±0.003%) on the day of manufacture but did not show the highest acidity at the end of the storage period (1.49±0.007%), even though the value increased during storage. This may be attributed to its low total soluble solids (10.36°Brix) at the beginning of storage. Greater reduction in TSS, could cause the yeast to resort to the assimilation of lactic acids in the sample (Fleet and Mian, 1987). The TA of unpreserved samples C1 and C2 increased from 1.17 and 0.90% respectively on the day of manufacture up to1.69 and 1.27% on the 21st day of storage after which it decreased slightly to 1.35 and 1.07% at the end of storage

Changes in total soluble solids (TSS) of yoghurts during storage

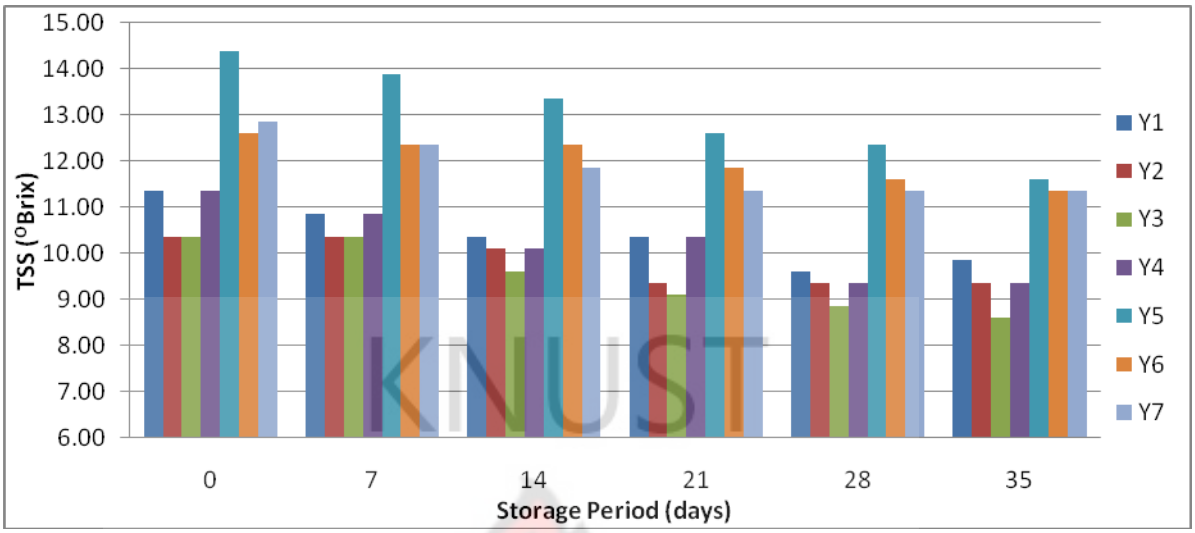


Figure 4.8a:Changes in total soluble solids (TSS) of seven brands of commercialized yoghurts containing natamycin during 35 days of storage.

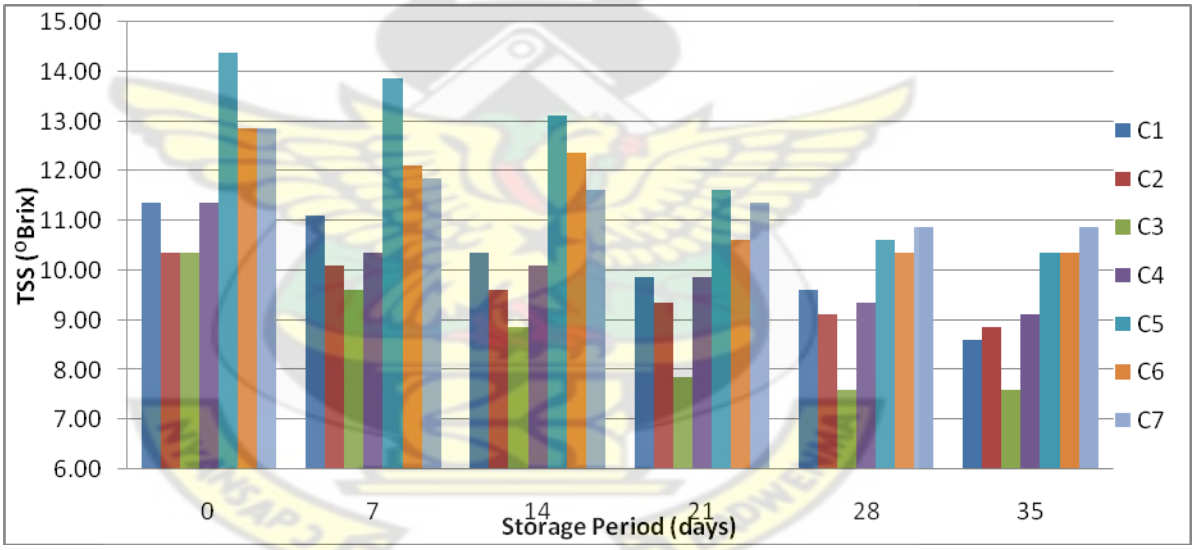


Figure 4.8b: Changes in total soluble solids of seven brands of commercialized yoghurts containing natamycin during 35 days of storage.

The TSS of all samples analysed differed significantly ($P<0.05$; Appendix X). The TSS decreased in all samples, but the unpreserved products showed lower values than their corresponding preserved samples at the end of storage (Figures 4.8a and b). TSS of the seven brands Y1 toY7 containing natamycin were 11.36, 10.36, 10.36, 11.36, 14.37,

12.61and 12.86°Brix respectively at the beginning of the study, and decreased to 9.86, 9.36, 8.61, 9.36, 11.61, 11.36and 11.36°Brix on the final day. In the unpreserved products TSS were 11.36, 10.36, 10.36, 11.36, 14.37, 12.86 and 12.86°Brix for brands 1 to 7 respectively on the day of production. On the 35th day of storage these values had decreased to 8.61, 8.86, 7.61, 9.11, 10.36, 10.36 and 10.86°Brix respectively.

Changes in yeast counts of yoghurts during storage

There were significant differences ($p<0.05$) in yeast counts of yoghurts preserved with natamycin on all days of storage except on the 28th day (Appendix X, Table Xg). From figure 4.9a the preserved brand labeled Y6 had the highest initial yeast count of 5.10±0.28 log cfu/ml, but did not result in the highest yeast load at the end of the storage period. The preserved sample containing the highest number of yeast cells at the end of storage was Y1 with 2.09 ± 0.12 log cfu/ml. This would imply that the yeast load of fresh Y1yoghurt does not determine the rate of reduction of yeast by natamycin. If this were so, the sample labeled Y1 should have recorded lower yeast counts than Y6 at the end of storage. It could be that the lower pH value of sample Y1 (4.27) as compared to Y6 (4.46) at the beginning of the study resulted in decreased activity of the preservative in this sample. Stark (2004) confirmed that the preservative is less stable in foods outside the pH range of 5 to 9. This could also account for the fact that yeast cells were still present in all preserved yoghurts after 35 days of storage.

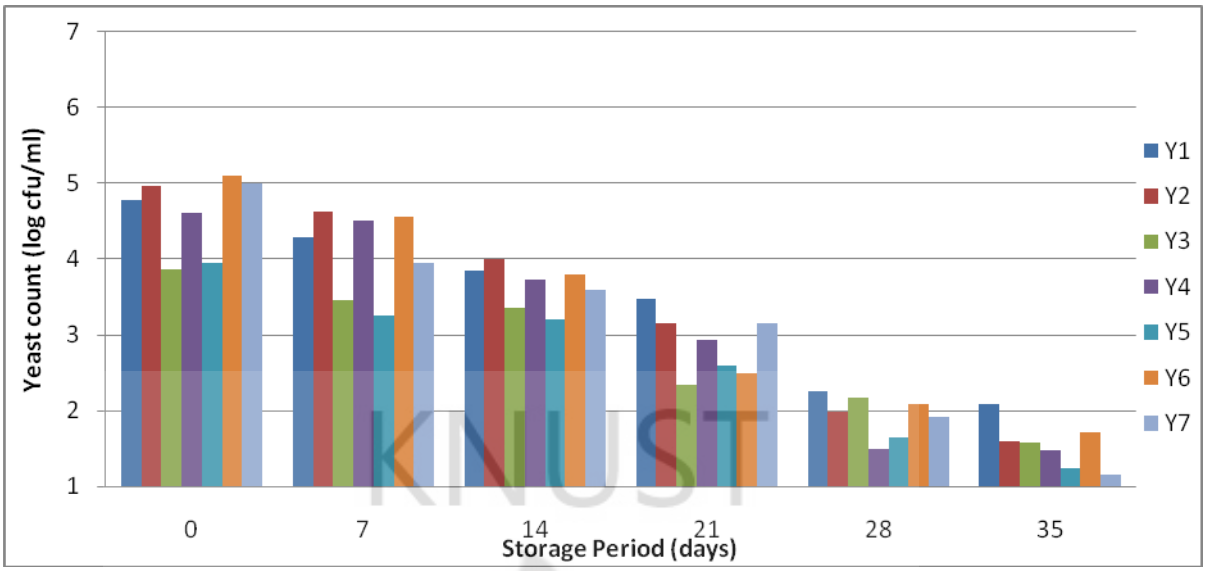


Figure 4.9a:Changes in yeast counts of seven brands of commercialized yoghurts containing natamycin during 35 days of storage.

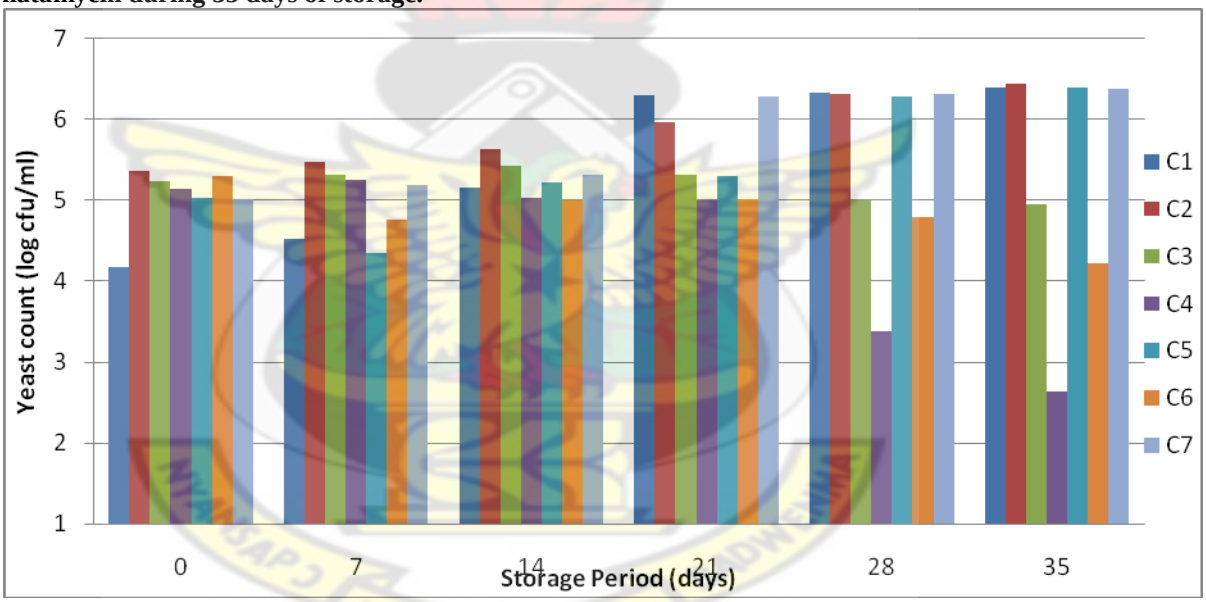


Figure 4.9b: Changes in yeast counts of seven brands of commercialized yoghurts containing natamycin during 35 days of storage.

In the case of unpreserved yoghurts (figure 4.9b) which were used as the controls yeast counts increased in C1, C2 and C7 from 4.17±0.05, 5.37±0.10 and 5.01 ± 0.02 log cfu/ml on the day of production to 6.39±0.01, 6.44±0.00 and 6.38±0.06 log cfu/ml respectively on the final day of storage. In sample C3 yeast counts increased from

5.24±0.04 log cfu/ml at the start of storage, to 5.43±0.02 log cfu/ml on the 14th day after which it gradually decreased throughout the rest of the period to a final value of 4.95±0.05 log cfu/ml. The decrease in yeast counts of C3 could be explained by its relatively low TSS content at the beginning of the storage period, indicating less fermentable substrate for the yeast cells. C4 showed a trend similar to C3, and yeast counts increased only till the 7th day after which they decreased till the end of storage. At the end of the study C2 had the highest count while C4 (2.63±0.46logcfu/ml) recorded the lowest. Generally there were significant variations in yeast counts of all unpreserved yoghurts throughout storage.

Changes in total coliform loads of yoghurts during storage

Total coliform counts in preserved yoghurts ranged from 4.42 ± 0.34 (Y4) to 2.49 ± 0.24 log cfu/ml (Y7) on the day of manufacture but no coliforms were detected on the last day of storage. In sample Y7 the decrease in coliforms was gradual, beginning on the day of manufacture (2.49±0.24logcfu/ml) till there was none detected on the last day of storage, while all other samples showed erratic changes in coliform count throughout the period. In samples Y1 and its control C1 and also C2, C5 and C7 coliforms were not detected on the 28th day of storage, while all the other samples were completely free from coliforms on the 35th day of storage.

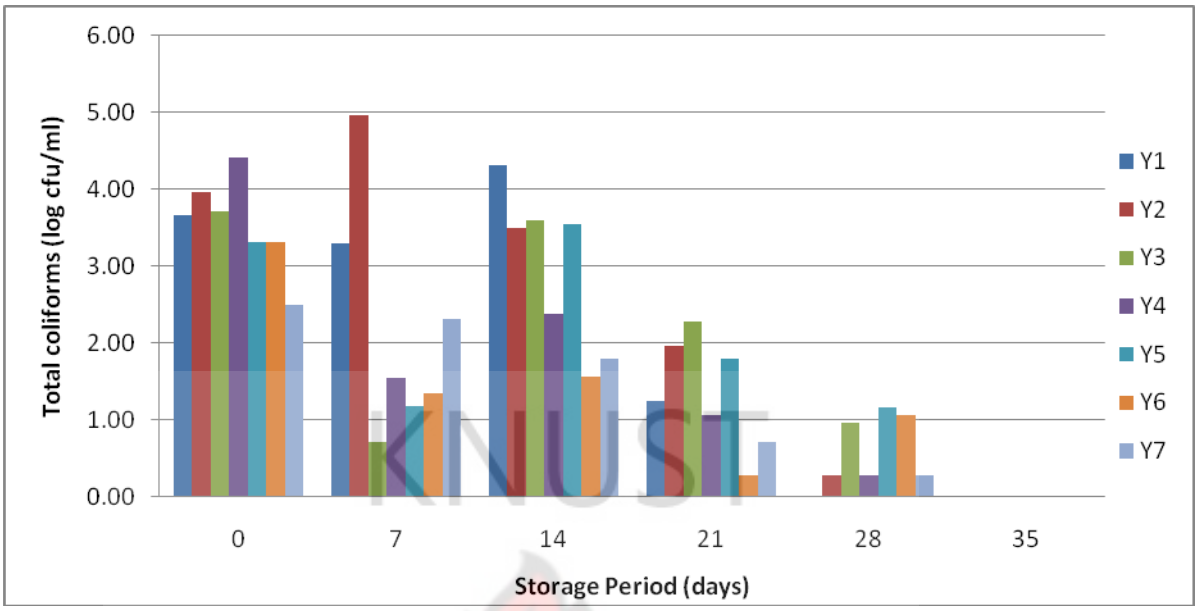


Figure 4.10a: Changes in total coliform loads of seven brands of commercialised yoghurts containing natamycin during 35 days of storage.

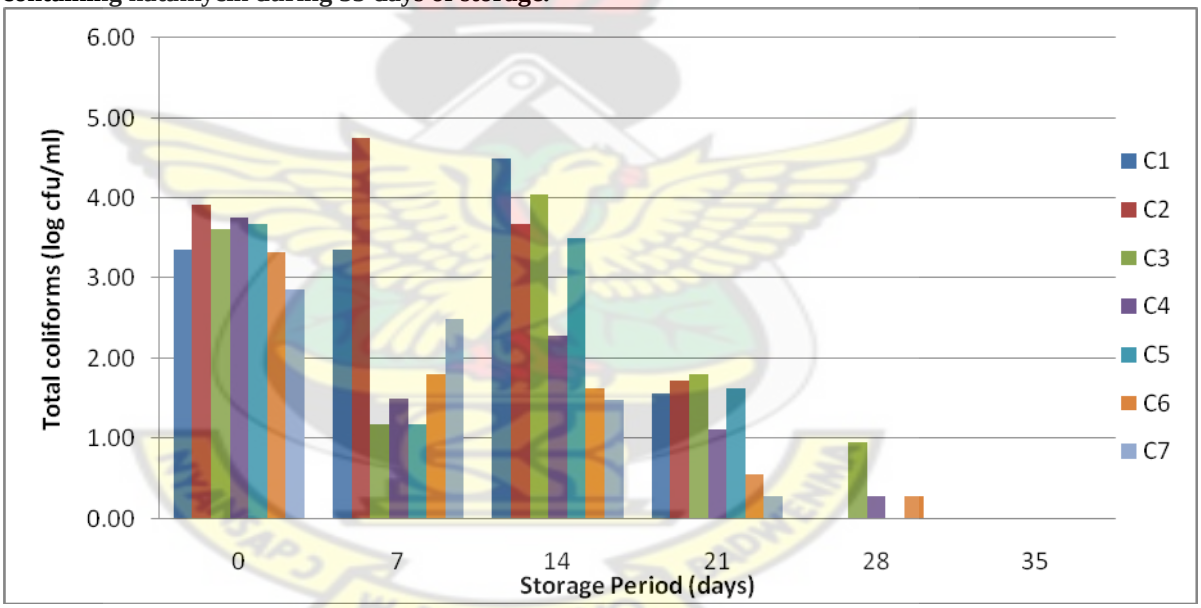


Figure 4.10b: Changes in total coliform loads of seven brands of commercialised yoghurts containing natamycin during 35 days of storage.

Though some researchers have reported the growth of coliforms at temperatures between 3-6^{O}C, their growth is generally poor or very slow at temperatures of 5^{O}C. And they have been reported to grow over a pH range of 4.4-9 (Jay, 2000). The reduction of coliform counts in different brands of yoghurt in this study agrees with results reported

by Vahedi *et al.*, (2008). They indicated that in yoghurts containing apple, coliforms were detected only until the third day of storage, while yoghurts containing strawberry supported the growth of coliforms until the 14th day of storage. Natamycin does not have any effect on coliforms ((Khoudokormoff and Petru, 1974)), hence the reduction in coliform counts is not due to the preservative. Coliform growth is known to be inhibited during refrigeration (Dairy Foods Science Notes, 2009).

CHAPTER FIVE

5.1 CONCLUSION

Many (23.20%) of the consumers of drinking yoghurts marketed in Kumasi had no idea of the nutritional quality of yoghurt and 27.1, 23.2 and 21.1% of consumers considered colour, aroma and texture as the three most important quality parameters compared to the nutritional value and the price. The survey revealed that Y2 was the least purchased brand of drinking yoghurt in Kumasi and Y4 was the most purchased. Comparison of nutritional, physicochemical, microbial and sensory qualities of seven commercialized vanilla flavoured drinking yoghurts in Kumasi generally showed significant differences ($p<0.05$) in these quality parameters among all the products. In the comparative study, product Y3 was the least acceptable product by the panelists whilst Y6was the most acceptable product.

Natamycin at concentrations of 8-10ppm in vanilla flavoured drinking yoghurt proved to be more effective against yeast growth in yoghurt as compared to lower concentrations of 5-7ppm. These higher concentrations of natamycin also resulted in relatively minimal changes in important physicochemical properties (pH, TA and TSS) than lower concentrations. Natamycin at 8ppm was considered the optimum concentration for the preservation because it gave the highest percentage decrease (69.36%) of yeast loads in yoghurt. Addition of natamycin before and after fermentation of the milk base did not have any significant effect ($p>0.05$) on quality parameters measured.

Commercialized vanilla flavoured drinking yoghurts preserved with 8ppm natamycin and stored at $5^{O}C$ for 35 days showed decreases in yeast counts from 5.10±0.28 log cfu/ml in Y6 to 1.15±0.21 log cfu/ml in Y7, while unpreserved yoghurts showed increased yeast counts from a minimum of 4.17±0.05logcfu/ml in C1 to a maximum of 6.44±0.00logcfu/ml in C2. Total soluble sugar contents and pH values decreased in all yoghurts while titratable acidity increased with storage time. At the end of the storage period all preserved yoghurts had titratable acidities higher than 1.00%.However, none of the products had titratable acidity within the range of a good finished product on the 35^{th} day. Yoghurts with incresed acidity, lowered pH and lowered total soluble sugars would loose some of their desirable physichochemical and organoleptic qualities. They would be percieved to have a more sour taste and also become less viscous. Preservation with natamycin resulted in less acidity of yoghurts as compared to none preserved samples. This implies that preservation of yoghurt with natamycin would result in a more organoleptically acceptable product since excessive acidity would be prevented.

5.2 RECOMMENDATIONS

It is recommended that:

- Nutritional composition of yoghurts should be displayed on packages to provide consumers with more knowledge on the product and enable them make better informed choices when purchasing products.

- Manufacturers of commercialized yoghurts in Kumasi should be encouraged to improve upon the microbial quality and ensure standard physicochemical properties by maintaining GMPs and GHPs.
- The use of natamycin as a preservative in yoghurt should be encouraged by the Food and Drugs board, and should be made available in the country by importers of food and chemical products.
- Research into the use of natamycin in other local foods such as; Asana, Sobolo (roselle drink), palm wine or other fermented drinks and kenkey should be encouraged.

REFERENCES

ABD EL-KHAIR, A.A., (2009).Production and evaluation of a high protein version of non-fat yogurt.*Research Journal of Agriculture and Biological Sciences*, 5(4): 310-316.

ADOLFSSON, O., Meydani, S.N. and Russell, R.M., (2004). Yoghurt and Gut Function. *American Journal of Clinical Nutrition*, 80 (2): 245-256.

AKPAN, U.G., Mohammed, A.D., Aminu, I., (2007). Effect of preservative on the shelf life of yoghurt produced from soya bean milk. *Leonardo Electronic Journal of Practices and Technologies*, 11: 131-142.

AL.OTAIBI, M. and El.Demerdash, H., (2008). Improvement of the quality and shelf life of concentrated yoghurt (labneh) by the addition of some essential oils.*African Journal of Microbiology Research* 2: 156-161.

ALM, L., (1982).Effect of fermentation on Lactose, glucose, and galactose content in milk and suitability of fermented milk products for lactose intolerant individuals.*J. Dairy Sci*., 65: 346-352.

AL-TAHIRI, R. (2005). A comparison on microbial conditions between traditional dairy products sold in Karak and Same products produced by modern dairies.*Pak. J. Nutr*., 4: 345-348.

ALVAREZ, F., Argüello, M., Cabero,M., Riera, F.A., Alvarez, R., Iglesias J.R. and Granda, J., (1998). Fermentation of concentrated skim-milk.Effects of different protein/lactose ratios obtained by ultrafiltration-diafiltration, *J. Sci. Food Agric*. In: Production and evaluation of a high protein version of non-fat yogurt, (ed.)Abd El-Khair, A.A., (2009). *Research Journal of Agriculture and Biological Sciences*, 5(4): 310-316.

AOAC (2005).Official Methods of Analysis, Association of Official Analytical Chemists, 15thEdition.Horwitz, W. and Latimer, G.W. (Ed).AOAC International, Mariland-USA.

BARNES, D.L., Harper, S.J., Bodyfelt, F.W. and McDaniel, M.R., (1991a). Prediction of Consumer Acceptability of Yogurt by Sensory and Analytical Measures of Sweetness and Sourness.*J Dairy Sci*, 74:3746-3754.

BARNES, D.L., S.J. Harper, F.W. Bodyfelt, and M.R. McDaniel.(1991b). Correlation of descriptive and consumer panel flavor ratings for commercial prestirred strawberry and lemon yogurts.*Journal of Dairy Science* 74:2089-2099.

BASIC DAIRY BACTERIOLOGY, Dairy Food Science Notes- Cornell University *foodscience.cornell.edu/cals/.../BACT-General-08-b-w-links.doc (accessed on 2nd November, 2010).*

BONCZAR, G., M. Wszolek and A. Siuta, (2002). The effects of certain factors on the properties of yoghurt made from ewe's milk. Food Chem., 79: 85-91.

CAIS-SOKOLIŃSKA, D., Michalski, M.M. and Pikul, J., (2004).Role of the Proportion of Yoghurt Bacterial Strains in Milk Souring and the Formation of Curd Qualitative Characteristics.*Bull Vet InstPulawy*48, 437-441.

CHAMPAGNE,C.P., Laing, R.F., Roy, D. and Mafu, A.A., (1994). Psychrotrophs in Dairy Products: Their Effects and Their Control. *Critical Reviews in Food Science and Nutrition*, 34(1): l-30

CODEX STANDARD FOR FERMENTED MILKS, 2003.Codex Standard 243

COGAN, T.M., (1977). A review of the heat stable lipases and proteinases and the quality of dairy products.*Irish Journal* of *Food Science and Technology*, 1: 95-105.

COLOMBEL, J.F., Cortot, A., Neu, C. and Romand, C., (1987). Yoghurt with *Bifidobucteriurnfongurn*reduces erythromycin induced gastrointestinal effects. *Lancet,* ii: 43.

CORNELL UNIVERSITY NEW YORK STATE AGRICULTURAL EXPERIMENT STATION, 2010. Yoghurt Production.http://necfe.foodscience.cornell.edu/ publication/pdf/FS_YoghurtProduction.pdf.

COUNCIL DIRECTIVE on Nutrition labeling of foodstuffs (1990, September 24) No. 90/496. *Official Journal of European Communities* No L276/40.

COUSIN, M.A. (1982).Presence and activity of psychrotrophic microorganisms in milk and dairy products: a review. *Journal of Food Protection,* 45: 172-207.

CRAWFORD, R.J.M., (1962). How to succeed with yogurt. *Dairy Eng*. In: Flavor Evaluation and Characterization of Yogurt as Affected by Ultra-High Temperature and Vat Processes, (eds.) Labropoulos, A.E., Palmer, J. K. and Tao, P., (1982).*J Dairy Sci* 65:191-196.

DAIRY FOODS SCIENCE NOTES, (2010).Basic Dairy Bacteriology www.cals.cornell.edu/cals/foodsci/extension/.../BACT-General-07-rev.doc *(accessed on 2nd November, 2010).*

DAIRY FOODS SCIENCE NOTES, (2009).Shelf Life of fluid milk Products-Microbial Spoilage www.cals.**cornell**.edu/cals/foodsci/extension/.../CU-DFScience-Notes-Bacteria-Milk-Shelf-Life-Evaluation-06-09.pdf *(accessed on 9th November, 2011).*

DAVIS, J. G. (1970). Laboratory control of yogurt. *Dairy Ind*., 35:139-144.

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

DE LA FUENTE, M.A., Montes, F., Guerrero, G., Juarez, M., (2003).Total and soluble contents of calcium, magnesium, phosphorus and zinc in yoghurts.*FoodChem* 80: 573-578.

DEACON, J.W., (1997).Prevention and control of fungal growth. In: *Modern Mycology*, 3rd Edition. Deacon, J.W. (ed.), Oxford: Blackwell Science, : 289–290.

DELVES-BROUGHTON, J., Thomas, L.V., Doan, C.H., Davidson, P.M., (2005).Natamycin. In: *Antimicrobials in Food*, Third Edition. Davidson, P.M., Sofos, J.N., Branen, A.L. (eds). CRC Press; Tailor and Francis Group, Baca-Raton-USA: 275-287.

DUBLIN-GREEN, M. and Ibe, S.N., (2005). Quality Evaluation of Yoghurts Produced Commercially in Lagos Nigeria. *African Journal of Applied Zoology and Environmental Biology*, 7:78-82.

EICHHOLZER, M., and Stahelin, H. (1993). Is there a hypocholesterolemicfactor in milk and milk products? *Int. J. Vit. Nutr. Res*, 63: 159-167.

EL-BAKRI, J.M. and ElZubeir**, 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-DIASTY, E.M., El-Kaseh, R.M., and Salem, R.M., (2009).The effect of natamycin on keeping quality and organoleptic characters of yoghurt.*Arab J. Biotech*., 12 (1): 41-48.

EL ZUBEIR, I. E. M., Abdalla W., M., and El Owni O. A. O., (2005).Chemical composition of fermented milk (roub and mish) in Sudan.*Journal of Food Control* 16 (7): 333- 337

EUROPEAN FOOD SAFETY AUTHORITY (EFSA) Panel on Food Additives and Nutrient Sources added to Food (ANS), (2009). Scientific Opinion on the use of natamycin (E 235) as a food additive. *EFSA Journal* 7(12):1412-1437.

FAO.(1992). Codex Standard for Fermented Milks- CODEX STAN 243-2003. Rome: FAO/OMS.

FARID, M.A., El Enshasy, H.A., El Diwany, A.I., El Sayed, E.A., (2000). Optimisation of the cultivation medium for natamycin production by *Streptomyces natalensis*. Journal of Basic Microbiology 40 (3), 157-166.

FARINDE, E.O., Obatolu, V.A., Fasoyiro, S.B., Adeniran, A.H. and Agboola, E.R, (2008). Use of alternative raw materials for yoghurt production. *African Journal of Biotechnology* 7 (18): 3339-3345.

FERNANDES, C.F. and Shahani, K.M., (1990). Anticarcinogenic and immunological properties of dietary lactobacilli.*J Food Protect*, 53: 704-10.

FERNANDES, C.F., Shahani, K.M.,andAmer, M.A., (1987). Therapeutic role of dietary *lactobacilli* and lactobacillic fermented dairyproducts. *E M S Microbiol Rev*, 46: 343-356.

FERNANDEZ, C., Astier, C., Rock, E., Coulon, J.B., and Berdague´, J.L., (2003). Characterization of milk by analysis of its terpenefractions. *International Journal of Food Science and Technology*. In: A review of the analytical methods coupled with chemometric tools for the determination of the quality and identity of dairy products, (eds.) Karoui, R. and De Baerdemaeker, J., (2007). *Food Chemistry*, 102: 621-640.

FLEET, G. H. and Mian, M. A., (1987).The occurrence and growth of yeasts in dairy products.*International Journal of Food Microbiology*. In: Survival of dairy associated yeasts in yoghurt and yoghurt-related products, (eds.) Lourens-Hattingh, A., and Viljoen, B. C., (2002). *Food Microbiology*, 19: 597–604.

FLEET, G. H., (1990).Yeasts in dairy products- A review. *Journal of Applied Bacteriology*, 68: 199–211.

FLYNN, A. and Cashman, K., (1997). Nutritional aspects of minerals in bovine and human milks. In: *Advanced dairy chemistry*Vol3., Fox P.F., (ed.). London: Chapman and Hall : 257-302.

FLYNN, A. and Power, P., (1985). Nutritional aspects of minerals in bovine and human milks.In:.*Developments in dairy chemistry*. Fox P.F. (editor). The essential mineral concentration of Torba yoghurts and their wheys compared with yoghurt made with cows', ewes' and goats' milks, (eds.) Güler, Z. and Hasan, S., (2008). *International Journal of Food Sciences and Nutrition* 60:2,153-164.

FOOD STANDARDS AGENCY, (2002).McCance and Widowson's The Composition of Foods,6th edition. In: The Nutritional composition of Dairy products, The Dairy council. http://www.milk.co.uk/publications/default.aspx. (accessed on 24th February, 2010).

FOOD STANDARDS AUSTRALIA NEW ZEALAND, (2004) .Natamycin –Extension of use as a food additive. Initial Assessment Report; Application A 542.

FURST, T., Connors, M., Bisogni, C. A., Sobal, J. and Winter Falk, L. (1996) Food choice: a conceptual model of the process.*Appetite*.In:Nutrition Education: linking research, theory and practice, (ed.) Contento I.R., (2010).*Jones and Bartlett Learning* : 34pp .

FRANKLIN, T.J. and Snow, G.A., (1998). Antiseptics, antibiotics and the cell membrane. In: *Biochemistry and Molecular Biology of Antimicrobial Drug Action*, 5th Edition. Kluwer Academic Publishers, Dordrecht, Holand: 55–56.

GAMBELLI , L., Manzi , P., Panfili , G., Vivanti , V. and Pizzoferrato, L., (1999). Constituents of nutritional relevance in fermented milk products commercialised in Italy. *Food Chemistry,* 66: 353-358.

GHANA STANDARD 337-2003.Milk and Milk Products Specification for yoghurt and sweetened yoghurt. Ghana Food and Drugs Board Library, Accra.

GEORGALA, A. I. K., Tsakalidou, E., Kandarakis, I. and Kalantzopoulos, G., (1995). Flavour production in ewe's milk and ewe's milk yogurt, by single strains and combinations of *Streptococcus thermophilus* and *Lactobacillus delbrueckii*subsp. *bulgaricus*, isolated from traditional Greek yoghurt. *Tech. Lait*, 75: 271–279.

GOODENOUGH, E. L., and Klein, D. H., (1976).Qualitative and quantitative changes in carbohydrates during the manufacture of yoghurt.*Journal of Dairy Science.* In: *Modern Food Microbiology sixth edition,* (ed.) Jay, J.M., (2000). Aspen Publishers Inc., Maryland- USA : 121, 391.

GREEN, M. D. and Ibe, S. N., (1987). Yeasts as primary contaminants in yoghurts produced commercially in Lagos, Nigeria. *J. Food Prot*. In: Temperature abuse initiating yeast growth in yoghurt (eds.) Viljoen, B.C., Lourens-Hattingh, A., Ikalafenga, B. and Peter, G., (2003).*Food Research International* 36: 193–197

GRIFFIN, D.A., (1994). Fungicides. In: *Fungal Physiology*, 2nd Ed., New York: Wiley-Liss, Inc. : 416–417.

GÜLER, 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.

HAMILTON-MILLER, J.M.T., (1974).Fungal sterols and the mode of action of the polyene antibiotics. In: *Advances in Applied Microbiology*. Perlman, D. (ed.), New York: Academic Press: 109–134.

HETHERINGTON, M. M. and Rolls, B. J. (1996) Sensory-specific satiety: theoretical frameworks and central characteristics. In Capaldi, E. D. (ed.), *Why We Eat what We Eat: The Psychology of Eating*. American Psychological Association, Washington, DC, pp. 267–290.

HELLER, C. L., (1957). Yoghurt and Other Cultured Milks. International *Journal of Food Sciences and Nutrition*,11 (4): 167-170.

IMELE, H., (2001). Preliminary study on the utilization of coconut in yoghurt production.*The Journal of Food Technology in Africa* 6(1): 11-12.

INTERNATIONAL DAIRY FOODS ASSOCIATION, (2002).*World Dairy Situation*, Bulletin of the International Dairy Federation No.378, IDF, Brussels.

JAY, J.M., (2000). *Modern Food Microbiology sixth edition*. Aspen Publishers Inc., Maryland- USA : 121, 391.

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 Res.*, 54: 3186-3190.

KARAGOZLU, C., Kilic, S., Uysal, H., Akbulut N. and Kesenkas, H., (2005).The quality of yoghurt on retail in Turkey.*Pak. J. Biol. Sci.* In: Chemical composition of fermented milk (roub and mish) in Sudan (eds.) El Zubeir, I. E. M., Abdalla W., M., and El Owni O. A. O., (2005). *Journal of Food Control* 16 (7): 333- 337.

KAROUI, R. and De Baerdemaeker, J., (2007). A review of the analytical methods coupled with chemometric tools for the determination of the quality and identity of dairy products. *Food Chemistry*, 102: 621-640.

KAROUI, R., Dufour, E., Pillonel, L., Picque, D., Cattenoz, T., and Bosset, J. O., (2004). Fluorescence and infrared spectroscopies: a tool for the determination of the geographic origin of Emmental cheeses manufactured during summer. *Le Lait*. In: A review of the analytical methods coupled with chemometric tools for the determination of the quality and identity of dairy products, (eds.) Karoui, R. and De Baerdemaeker, J., (2007). *Food Chemistry*, 102: 621-640.

KATZ, F., (2001). Active cultures add function to yogurt and other foods. *Food Technol*. 55(3): 46 -49.

KHAN, M.T., Hussain, M., Ajid, A.andRasool, S.A., (2008).Microbial Population Load and Enzyme Production of Indigenously Isolated Yeast.*Pak. J. Bot*., 40(5): 2225-2230.

KHOUDOKORMOFF, B. and Petru, M., (1974).On the possible development of antibiotic resistance amongst fungi with special reference to the use of pimaricin as a preservative in the food industry. Unpublished report submitted to WHO by Gist-Brocades NV, Delft.

KNEIFEL, W., Ulberth, F., Erhard, F., and Jaros, D., (1992). Aroma profiles and sensory properties of yoghurt and yoghurt-related products I. Screening of commercially available starter cultures. *Milchwissenschaft*, 47(6): 362–365.

KOLARS, J.C., Levitt, M.D., Aouji, M., Savaiano, D.A., (1984).Yogurt-anautodigesting source of lactose.*New England Journal of Medicine*. In: The Use of Tiger-Nut (Cyperusesculentus), Cow Milk and Their Composite as Substrates for Yoghurt Production (ed.) Sanful, R.E., (2009b). *Pakistan Journal of Nutrition* 8 (6): 755-758.

LABROPOULOS, A.E., Palmer, J. K. and Tao, P., (1982). Flavor Evaluation and Characterization of Yogurt as Affected by Ultra-High Temperature and Vat Processes. *J Dairy Sci* 65:191-196

LACROIX, C. and Lachance, O., (1990). Effect of various humectants and a_w on proteolysis- Yeast and mold growth and shelf-life during cold storage of yogurt.*Canadian Institute of Food Science and Technology Journal*, 23: 101–108.

LEE, W. J. andLucey, J.A., (2010).Formation and Physical Properties of Yogurt.*Asian-Aust. J. Anim. Sci.*, 23(9): 1127 – 1136.

LI, F. and Y. Li. (1998). Study on the contamination level and the tolerable limit of mould and yeast in yoghurt. *Wei Sheng Yan Jiu.*, 27: 257-258.

LINDSAY, R. C., Day, E.A. and Sandine, W.E., (1965). Green flavor defect in lactic starter cultures. *J. Dairy Sci*. In: Flavor Evaluation and Characterization of Yogurt as Affected by Ultra-High Temperature and Vat Processes, (eds.) Labropoulos, .E., Palmer, J. K. and Tao, P., (1982).*J Dairy Sci* 65:191-196

LOURENS-HATTINGH, A., and Viljoen, B. C. (2001). Review: Yoghurt as a probiotic carrier food. *International Dairy Journal*, 11: 1–17.

LOURENS-HATTINGH, A., and Viljoen, B. C., (2002).Survival of dairy associated yeasts in yoghurt and yoghurt-related products.*Food Microbiology*, 19: 597–604.

LORENZEN, P. C., Neve, H., Mautner, A., and Schlimme, E. (2002).Effect of enzymatic cross-linking of milk proteins on functional properties of set-style yoghurt. International Journal of Dairy Technology, 55, 152–157.

LUCEY, J. A., and Singh, H. (1998). Formation and physical properties of acid milk gels: A review. Food Research International, 30(7), 529–542.

LUCEY, J. A., Munro, P. A., and Singh, H. (1998). Whey separation in acid skim milk gels made with glucono-delta-lactone: Effects of heat treatment and gelation temperature. Journal of Texture Studies. In: Formation and physical properties of acid milk gels: A review (eds.) Lucey, J.A. and Singh, H., (1998). Food Research International, 30(7), 529–542.

LUCEY, J. A., TetTeo, C., Munro, P. A., and Singh, H. (1997). Rheological properties at small (dynamic) and large (yield) deformations of acid gels made from heated milk. Journal of Dairy Research. In: Formation and physical properties of acid milk gels: A review (eds.) Lucey, J.A. and Singh, H., (1998). Food Research International, 30(7), 529–542.

LUTCHMEDIAL, M., Ramlal, R., Badrie, N. and Chang-yen, I., (2004).Nutritional and sensory quality of stirred soursop (Annonamuricata L.) yoghurt.*International Journal of Food Sciences and Nutrition*, 55 (5): 407 – 414.

MAGENIS, R.B., Prudencio, E.S., Amboni, D.M.C., Junior, N.G.C., Oliveira, R.V.B., Soldi, V. and Benedet, H.D., (2006). Compositional and physical properties of yogurts manufactured from milk and whey cheese concentrated by ultrafiltration. *Int. J. Food Sci. and Technol*. In: Production and evaluation of a high protein version of non-fat yogurt, (ed.) Abd El-Khair, A.A., (2009). *Research Journal of Agriculture and Biological Sciences*, 5(4): 310-316.

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 J. Agric. and Environ. Sci*., 2 (5): 587-592.

MARIA, C. K., Leandros P. V. and Efthymia, K., (2002). Manufacture of yoghurt from stored frozen sheep's milk. *Food Chemistry* 77: 413–420.

MARTINI, L.A. and Mayer, J., (1999). Magnesium supplementation and bone turnover. *Nutr Rev* 57:227-229.

MATTIA, A., Cerniglia, C. and Baines, J. Safety evaluation of certain food additives and contaminants; natamycin (pimaricin).WHO Food Additives Series: 48. http://www.inchem.org/documents/jecfa/jecmono/v48je06.htm, (accessed on 19th November, 2009).

MCGINNIS, M.R. and Rinaldi, M.G., (1985). Antifungal drugs: Mechanisms of action, drug resistance, susceptibility testing, and assays of activity in biological fluids. In: *Antibiotics in Laboratory Medicine*, 2nd Edition. Lorian, V., (ed.). Williams and Wilkins, Baltimore, USA : 223–281.

MEYDANI, S.N. and Ha, W. K., (2000).Immunologic effect of yoghurt.*Am J.Clin.Nutr.*,71: 861–872.

MILLER, D.D., (2008). Minerals. In: Fennema's Food Cemistry, 4th edition. Damodaren, S., Parkin, K.L. and Fennema, O.R. (editors). CRC Press, Taylor and Francis group, Florida-USA : 531-545.

MILLER, GD, 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.) Güler, Z. and Hasan, S., (2008). *International Journal of Food Sciences and Nutrition* 60:2,153-164.

MISTRY, V.V., (2001).Fermented milks and Cream. In: *Applied Dairy Microbiology*. Marth, E.H. and Steele, J.L. (ed.). Marcel Dekker, Inc., USA :311

MUKHTAR, H., Hakkou, A. and Bonaly, R. (1994). Studies on the activity of *Kluyveromyceslactis* S-adenosylmethionine: 24-sterol methyltransferase in presence of polyenic antifungal agents. *Mycopathologia*, 126: 75–83.

NEIRINCKX, J., (1972), "Thermization of yoghurt and French cheese", *Revue Lait-fr*, 1972 : 299, 465, 467.

NORMAN, A.W., Spielvogel, A.M. and Wong, R.G. (1976).Polyene antibiotic-sterol interaction.*Adv. Lipid Res*., 14: 127–170.

O'RELL, K.R. and Chandan, R.C., (2006). Yoghurt: Fruit Preparations and Flavouring materials. In: *Manufarcturing Yoghurt and Fermented Milks*. Chandan, R.C. (ed.). Blackwell Publishing :151.

OZER, B.H. and Robinson, R.K., (1999). The Behaviour of starter cultures in concentrated yoghurt (Labneh) produced by different techniques. *Lebensm-Wiss-Technol*. In: Studying microbial, physiochemical and sensory properties of directly concentrated probiotic yoghurt. (eds.) Yeganehzad, S., Mazaheri-Tehrani, M. and Shahidi, F., (2007).*AfricanJournal of Agricultural Research*, 2 (8): 366-369.

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 J. Agric. and Environ. Sci*., 2 (5): 587-592.

RIENER, J., Noci, F., Cronin, D.A., Morgan, D.J., Lyng, J.G., (2010). A comparison of selected quality characteristics of yoghurts prepared from thermosonicated and conventionally heated milks. *Food Chemistry*, 119 (3): 1108-1113.

ROBINSON, D. K. (1981). Yoghurt manufacture- some considerations of quality. *Dairy Industries International*, 46(3), 31–35.

ROBINSON, R. K. and Tamime, A.Y., (1975).Yogurt-a review of the product and its manufacture.*J. Soc. Dairy Technol*. 28:149-163.

ROHM, H., F. Lechner and M. Lehner.(1990). Microflora of Austrian natural-set yoghurt.*J. Food Protect*. In: Microbial Population Load and Enzyme Production of Indigenously Isolated Yeast (eds.) Khan, M.T., Hussain, M., Ajid, A. andRasool, S.A., (2008). *Pak. J. Bot*., 40(5): 2225-2230.

ROISSART, H., and Luquet, F. M. (1994).BacteÂriesLactiques: Aspects fondamentauxettechnologiques (Vol. 2). Grenoble, France: Lorica. In: Viability of lactic acid microflora in different types of yoghurt, (eds.) Birollo, G.A., Reinheimer, J.A. and Vinderola, C.G. (2000). *Food Research International* 33 (2000) 799-805.

RYCHLIK, M., Sax, M. and Schieberle, P., (2006).On the role of short-chain free fatty acids for the development of a cheese-like off-note in pasteurized yoghurt.*LWT-Food Science and Technology* 39: 521–527.

SANDINE, W. E., Daly, C., Elliker, P.R. and Vedamuthy, E.R., (1972).Causes and control of culture-related flavor defects on cultured dairy products.*J. Dairy Sci*. In:

Flavor Evaluation and Characterization of Yogurt as Affected by Ultra-High Temperature and Vat Processes (eds.) Labropoulos, A.E., Palmer, J. K. and Tao, P., (1982). *J Dairy Sci* 65:191-196

SANFUL, R.E., (2009a).Promotion of Coconut in the Production of Yoghurt.*African Journal of Food Science* 3 (5): 147-149.

SANFUL, R.E., (2009b).The Use of Tiger-Nut (Cyperusesculentus), Cow Milk and Their Composite as Substrates for Yoghurt Production.*Pakistan Journal of Nutrition* 8 (6): 755-758.

SARKAR, S., (2006).Shelf-life extension of cultured milk products.*Nutrition and Food Science*, 36(1): 24-31.

SAVELLO, P.A., Dargan R.A., (1997). Reduced yogurt syneresis using ultrafiltration and very-high temperature heating.*Milchwissenschaft*. In: Role of the Proportion of Yoghurt Bacterial Strains in Milk Souring and the Formation of Curd Qualitative Characteristics (eds.) Cais-Sokolinska, D., Michalski, M.M. and Pikul, J., (2004). *Bull Vet InstPulawy*48, 437-441

SAXENA, S. N., Mital, B. K. and Garg, S. K., (1994).Effect of casitone and fructose on the growth of *Lactobacillus acidophilus* and its survival during storage.*International Journal of Food Microbiology*, 21(3): 271–272.

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 Biography16 (2): 96–103.

SHAKER, R.R., Jumah, R.Y.and Abu-Jdayil, B., (2000). Rheological properties of plain yoghurt during coagulation process: impact of fat content and preheat treatment of milk. *J. Food Eng*. 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 J. Agric. and Environ. Sci*., 2 (5): 587-592.

SHERMARK, M. A., Saavedra, J. M., Jackson, T. L., Huang, S. S., Bayless, T. M. and Perman, J. A., (1995). Effect of yogurt on symptoms and kinetics of hydrogen production in lactose-malabsorbingchildren.*Am. J. Clin. Nutr*., 62: 1003-1006.

SMITS, P., and van Brouwershaven, J. H. (1980).Heat-induced association between beta-lactoglobuline and casein micelles.*Journal of Dairy Research*, 47(3), 313–325.

STARK J, (2004).Permitted preservatives - natamycin.In*Encyclopaedia of Food Microbiology*. Robinson RK, Batt, CA and Patel P (eds.) Academic Press, New York :1776-1781.

SURIYARACHCHI, V. R. and Fleet, G. H., (1981).Occurrence and growth of yeasts in yoghurts.*Applied Environmental Microbiology*, 42: 574 -579.

TAMIME, A.Y., M. Kalab and G. Davies, (1989). Rheology and Microstructure of strained yoghurt (labneh) made from cow's milk by three different methods. *Food Microstructure*, 8: 125-135.

TAMIME, A.Y., Robinson, R. K., (1999a).Developmrnts in yoghurt production and related products. In Yoghurt: Science and Technology. Cambridge, UK: Woodhead Publishing Limited: 349.

TAMIME, A.Y., Robinson, R. K., (1999b). Quality control in yoghurt manfacture. In Yoghurt: Science and Technology. Cambridge, UK: Woodhead Publishing Limited: 535.

THE DAIRY COUNCIL, [no date].*The Nutritional Composition of Dairy Products:* 16-22 http://www.milk.co.uk/publications/default.aspx. (accessed on 24th February, 2010).

The Northeast Center for Food Entrepreneurship at the New York State Food Venture Center, Cornell University, (http://www,nysaes.cornell.edu/necfe/).Accessed on 10/12/2009.

THOMAS, L. V. and Delves-Broughton J. 2001.Applications of the natural food preservative natamycin.*Res. Adv. Food Sci*. 2:1-10.

THOMPOULUS, C., Tzia, C. and Milkas, D., (1993). Influence of processing of solids-fortified milk on coagulation time and quality properties of yoghurt.*Milchwisenssenschaft*, 48(8): 426-430.

TULL, A., (1996). Food and Nutrition, Oxford University Press: 109-111.

ULBERTH, F. and Kneifel, W. (1992).Aroma profiles and sensory properties of yoghurt and yogurt-related products. II. Classification of starter cultures by means of cluster analysis. *Milchwissenschaft*, 47(7): 432–435.

VAHEDI, N., Tehrani, M.M. and Shahidi, F., (2008).Optimizing of Fruit Yoghurt Formulation and Evaluating Its Quality During Storage. Amarican-Eurasioan Journal of Agricultural and Environmental Science 3(6) : 922-927.

VAN DENMARK, P. J. and Batzing, B. L. (1987). The microbes: an introduction to their nature and importance. Menlo Park, CA: Benjamin/ Cummings Publishing Company. In: Temperature abuse initiating yeast growth in yoghurt (eds.)Viljoen, B.C., Lourens-Hattingh, A., Ikalafenga, B. and Peter, G., (2003). *Food Research International* 36: 193–197

VAR, I., Sahan, N., Kabak, B., and Golge, O., (2004). The effects of natamycin on the shelf life of yogurt.*Archivfür Lebensmittelhygiene*,55 (1); 7-9.

VEDAMUTHU, E.R., (1991). The yoghurt story; past, present and future, VI.*Dairy, Food and Environmental sanitation* 9: 513–514.

VILJOEN, B.C., (2001). The interaction between yeasts and bacteria in dairy environments.*International Journal of Food Microbiology*69 :37-44.

VILJOEN, B.C., Lourens-Hattingh, A., Ikalafenga, B. and Peter, G., (2003).Temperature abuse initiating yeast growth in yoghurt.*Food Research International* 36: 193–197

VLAHOPOULOU, I., Bell A.E., (1990).The application of oscillatory testing to bovine and caprine yoghurt gel systems. In: *„Rheology of food, pharmaceutical and biological materials with general rheology"*. Carter, R.E (Ed). Elsevier, London :331-356.

WEHR, H.M. and Frank, J.F, (2004). Minerals and Food additives. In: *Standard Methods for the Examination of Dairy Products*. American Public Health Association, Wshignton DC-USA ; 441

WHITE, C.H., (1995). Manufacture of high quality yogurt. *Cult.Dairy Prod. J.*, 30(2): 18-19, 21, 23-24, 26.

WOOD, B. J. B. (1985). Microbiology of fermented foods Vol. 1 .London: Elsevier Science Publishers. In: Viability of lactic acid microflora in different types of yoghurt, (eds.) Birollo, G.A., Reinheimer, J.A. and Vinderola, C.G. (2000). *Food Research International* 33 (2000) 799-805.

XANTHOPOULOS, V., Picque, D. and Bassit, N. (1994), Methods for the determination of aroma compounds in dairy products: a comparative study *Journal of Dairy Research*, 61 :289-97.

YEGANEHZAD, S., Mazaheri-Tehrani, M. and Shahidi, F., (2007).Studying microbial, physiochemical and sensory properties of directly concentrated probiotic yoghurt.*AfricanJournal of Agricultural Research*, 2 (8): 366-369

YOUNUS, S., Masud, T. and Aziz, T., (2002).Quality Evaluation of Market Yoghurt /Dahi.*Pakistan Journal of Nutrition* 1 (5): 226-230.

ZEMEL, M.B., Richards, J., Mathis, S., Milstead, A., Gebhardt, L., and Silva, E., (2005). Dairy augmentation of total and central fat loss in obese subjects.*International Journal of Obesity*,29: 391–397.

APPENDIX I

QUESTIONNAIRE ON SURVEY CONDUCTED TO DETERMINE POPULARITY AND CONSUMER PREFERENCES IN VANILLA FLAVOURED STIRRED YOGHURT IN KUMASI

Male	
female	

Gender

1. Do you drink vanilla flavoured fresh yoghurt sold in

Yes	No

bottles?

2. Why do you drink bottled yoghurt?

For its nutritional value	
For its health benefits	
For its sensory benefits (ie: taste, flavour, etc.)	
For other reasons. Please state	

3(a). Which nutrients do you know are **most** important in yoghurt? (tick only one)

Fats and proteins	
Carbohydrates	
Minerals	
vitamins	
No idea	

(b). If you ticked minerals or vitamins, which particular one(s) are mostly obtained from drinking yoghurt?.....……..

4. Which brand of **bottled vanilla flavoured** yoghurt (on the Kumasi market) do you usually buy?.....……..

5. Why do you buy the brand you have indicated?……..

6.(a) Which of the following is the **1st most important** thing you consider when you purchase your **favorite brand** of bottled vanilla flavoured yoghurt?

Sweetness	
Sourness	
Aroma	
Mouthfeel	
Colour	
Texture	
Nutritional value	
Price	
Other reason	

(b) Which of the following is the **2nd most important** thing you consider when you purchase your favorite brand of bottled vanilla flavoured yoghurt?

Sweetness	
Sourness	
Aroma	
Mouthfeel	
Colour	
Texture	
Nutritional value	
Price	
Other reason	

(c) Which of the following is the **3rd most important** thing you consider when you purchase your favorite brand of bottled vanilla flavoured yoghurt?

Sweetness	
Sourness	
Aroma	
Mouthfeel	
Colour	
Texture	
Nutritional value	
Price	
Other reason	

7. What new development would you appreciate in your favorite brand of bottled yoghurt in Kumasi?

…….
.

APPENDIX II

MATERIALS AND EQUIPMENT

Reagents used

- Sulfuric acid (BDH Chemicals Ltd., Pool England)
- Sodium hydroxide pellets- AnalaR (BDH Chemicals Ltd., Pool-England)
- Boric acid (BDH Chemicals ltd., Pool England)
- Hydrochloric acid (Pharmacos Ltd., Essex-England)
- Petroleum ether (BDH Chemicals Ltd., Pool-England)
- MacConkey broth (CM005-OXOID Ltd., Basigstoke-England)
- Yeast Extract Agar (CM0019-OXOID Ltd.)

Instruments used

- Thermos coolers
- Oven (Gallenkamp Hotbox oven, England)
- Convection oven (GENLAB Ltd.)
- Muffle furnace (Gallenkamp)
- Soxhlet extractors

- Inductively Coupled Plasma Optical Emission Spectrophotometer (VARIAN-Vista PRO simultaneous ICPOES series)
- Flame Photometer (JENWAY PFP-7, UK)
- Hand-held Brix refractometer (ATAGO Manual Refractometer)
- Electric digital pH meter (BECKMAN Φ 340 pH/Temp. Meter)
- Viscometer (HAAKE Viscotester VT-02)
- Automatic micropipette
- Incubator (Gallenkamp Plus II Incubator)
- Electric Colony counter (Stuart Scientific).

FORMULAE USED IN DETERMINATION OF NUTRITIONAL, PHYSICOCHEMICAL AND MICROBIAL QUALITY OF YOGHURT

1. PERCENTAGE MOISTURE CONTENT

$$\%\text{Moisture} = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

M_1= initial weight of empty glass crucible

M_2=weight of glass crucible + wet sample

M_3= weight of glass crucible + dry sample

2. PERCENTAGE ASH CONTENT

$$\%\text{Ash} = \frac{M_3 - M_1}{M_2 - M_1} \times 100$$

M_1= initial weight of empty crucible

M_2=weight of glass crucible + wet sample

M_3= weight of glass crucible + ash

3. PERCENTAGE TOTAL PROTEIN

$$\%\text{Total Nitrogen} = \frac{(V_s = V_b) \times 1.4007 \times N_A \times 100}{M_s \times 10}$$

%Total Protein= %Total Nitrogen×6.38

V_s= Titre value of acid titration against digested sample solution

V_b= Titre value for acid titration against blank (distilled water taken through the same procedure)

N_A= Normality of acid (0.1N HCl)

M_s= Initial mass of sample = Density of sample × volume taken

6.38 = protein conversion factor for milk

4. PERCENTAGE FAT CONTENT

$$\% \text{ Fat in Yoghurt} = \frac{m_f \times W_D}{m_s \times W_T} \times 100$$

m_f = mass of fat extracted

m_s= mass of dried sample taken for extraction

W_D= mass of total dried sample

W_T= mass of wet sample originally taken and dried

5. PERCENTAGE CARBOHYDRATE

%Carbohydrate=100 - (M+A+P+F)

M = percentage moisture

A = percentage ash

P = percentage protein

F = percentage fat

6. ENERGY VALUE

Energy (kJ/100g) = (17×P)+(17×C)+(37×F)

P = percentage protein

C = percentage carbohydrate

F = percentage fat

7. CONCENTRATIONS OF MINERALS (Ca, P, Mg, Zn and Fe) BY ICP-OES

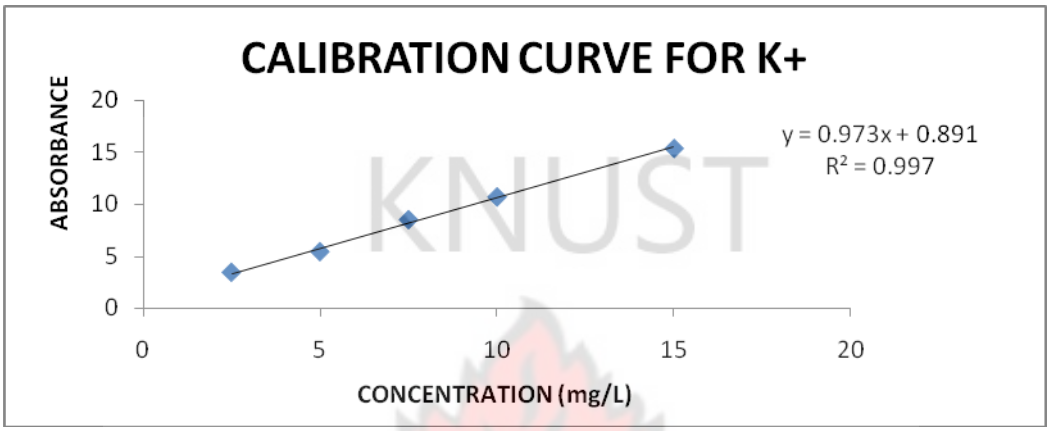
$$\text{Concentration of mineral} = \frac{C_A \times V}{m_s} \text{ mg/kg} \quad \text{or ppm}$$

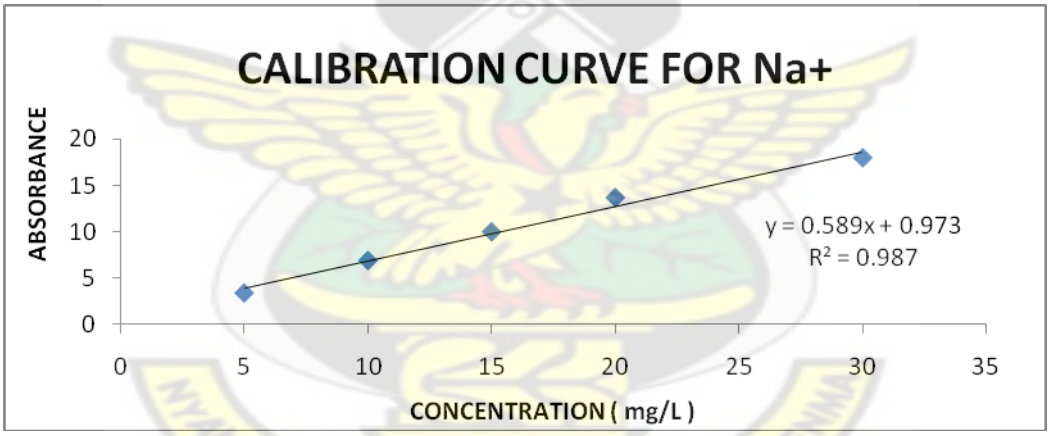
C_A= Concentration of analyte obtained from spectrophotometer; in mg/L

V= Total volume of solution made up after final dilution; in L

m_S= Mass of test portion taken; in Kg

8. CONENTRATION OF MINERALS (Na, K) BY FLAME PHOTOMETRY





Concentration of mineral = $\frac{X_A \times V}{m_s}$ mg/Kg or ppm

X_A= Concentration of analyte obtained from equation of graph; in mg/L

V= Total volume of solution made up after final dilution; in L

m_S= Mass of test portion taken; in Kg

9. THREE-TUBE MPN TABLE

No. of Tubes Positive in:			MPN in the inoculum of the middle set of tubes	No. of Tubes Positive in:			MPN in the inoculum of the middle set of tubes
First Set	Middle Set	Last Set		First Set	Middle Set	Last Set	
0	0	0	<0.03	2	0	0	0.091
0	0	1	0.03	2	0	1	0.14
0	0	2	0.06	2	0	2	0.20
0	0	3	0.09	2	0	3	0.26
0	1	0	0.03	2	1	0	0.15
0	1	1	0.061	2	1	1	0.20
0	1	2	0.092	2	1	2	0.27
0	1	3	0.12	2	1	3	0.34
0	2	0	0.062	2	2	0	0.21
0	2	1	0.093	2	2	1	0.28
0	2	2	0.12	2	2	2	0.35
0	2	3	0.16	2	2	3	0.42
0	3	0	0.094	2	3	0	0.29
0	3	1	0.13	2	3	1	0.36
0	3	2	0.16	2	3	2	0.44
0	3	3	0.19	2	3	3	0.53
1	0	0	0.036	3	0	0	0.23
1	0	1	0.072	3	0	1	0.39
1	0	2	0.11	3	0	2	0.64
1	0	3	0.15	3	0	3	0.95
1	1	0	0.073	3	1	0	0.43
1	1	1	0.11	3	1	1	0.75
1	1	2	0.15	3	1	2	1.2
1	1	3	0.19	3	1	3	1.6
1	2	0	0.11	3	2	0	0.93
1	2	1	0.15	3	2	1	1.5
1	2	2	0.20	3	2	2	2.1
1	2	3	0.24	3	2	3	2.9
1	3	0	0.16	3	3	0	2.4
1	3	1	0.20	3	3	1	4.6
1	3	2	0.24	3	3	2	11
1	3	3	0.29	3	3	3	>24

10. NORMALITY OF STANDARDIZED SODIUM HYDROXIDE

Normality of NaOH= $\frac{C_A \times V_A}{V_B} \times \frac{2}{1}$

C_A= Concentration of Oxalic Acid (0.1M)

V_A= Volume of acid measured (10ml)

V_B= Tire value/ volume of NaOH titrated

$\frac{2}{1}$ = mole ratio of NaOH to Oxalic acid (**2**NaOH + $C_2O_4H_2 \rightarrow Na_2C_2O_4$ + **2**H_2O)

11. PERCENTAGE TITRATABLE ACIDS

%Lactic Acid= $\frac{(V_t \times 0.0090)}{Ms} \times 100$

Vt= Titre value of NaOH

Ms= Initial mass of sample= Density × volume taken

0.0090= acid factor for Lactic acid

APPENDIX III

SENSORY EVALUATION SCORE CARD

Code…….
Date……..

ID number……
Phone number…….

- You are presented with two coded samples of yoghurt.
- Please observe, smell and taste each one. Rinse your mouth with water after drinking the contents of each cup.
- Indicate your perception for each parameter by using the number scales provided.

1 Aroma

Sample 401 ☐

Sample 402 ☐

Score	Scale
Dislike very much	1
Dislike moderately	2
Dislike slightly	3
Neither like nor dislike	4
Like slightly	5
Like moderately	6
Like very much	7

Colour, sweetness, sourness, thickness and mouthfeel were also assessed in a similar manner.

APENDIX IV

PICTURES OF PROJECT WORK

Plate 3: Determination of titratable acidity of yoghurt by titration to a pink endpoint.

Plate 4: Measurement of viscosity of yoghurt by a viscometer.

Plate 5: Heat treatment of milk at 90^{O}C for 15minutes.

Plate 6: Natamycin being weighed into a beaker, and a 100ml suspension of natamycin.

Plate 7: Addition of natamycin to1L of a brand of yoghurt.

Plate 8: Yoghurts in refrigeration.

Plate 9: Dishes showing yeast colonies after incubation on the 28th day of storage.

APPENDIX V

CHI SQUARED ANALYSIS OF REASONS FOR PREFFERENCES OF BRANDS OF YOGHURT

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	347.720	102	0.000
Likelihood Ratio	278.341	102	0.000
Linear-by-Linear Association	12.067	1	**0.001**
N of Valid Cases	284		

STATISTICS FOR CONSUMER PREFERENCES OF COMMERCIALIZED YOGHURTS

Table IVa: Consumers' suggestions for development of individual brands of yoghurt

Suggestions	Frequency of responses (%)for each brand						
	Y1	Y2	Y3	Y4	Y5	Y6	Y7
A sugar free variety	6.5	-	-	7.0	-	7.0	2.4
addition of fruits	-	10.0	3.7	5.6	-	-	4.9
Consistency in taste	-	-	-	15.5	3.4	-	-
consistency in thickness	-	-	-	-	1.7	-	2.4
fortification with vitamins and/or minerals	6.5	-	-	7.0	10.2	2.3	17.1
Improved colour	-	-	-	-	-	11.6	-
Improved labels	-	-	-	-	5.1	2.3	-
Improved nutritional value with additives	-	-	-	-	-	4.7	2.4
Improved packaging	-	10.0	-	-	1.7	2.3	2.4
Improved texture	-	-	-	1.4	-	2.3	-
Improving nutritional value with additives	-	-	-	1.4	1.7	-	2.4
Increased shelf life	48.4	30.0	-	11.3	16.9	4.7	9.8
Increased sweetness	-	-	3.7	-	1.7	-	-
increased sweetness and shelf life; and reduced sourness and aftertaste	3.2	-	14.8	-	-	-	2.4
Increased viscosity	6.5	10.0	33.3	4.2	-	-	9.8
introduction of set yoghurt	-	-	3.7	1.4	-	-	7.3
Nothing	12.9	30.0	11.1	25.4	28.8	34.9	22.0
Probiotic yoghurt	-	-	-	1.4	1.7	-	2.4
Reduced sourness	-	-	22.2	1.4	-	7.0	-
Reduced sweetness	-	10.0	-	-	-	4.7	2.4
Reduced viscosity	-	-	-	-	10.2	-	-
show nutritional information on the label	16.1	-	-	12.7	11.9	2.3	7.3
Stop recycling of yoghurt bottles	-	-	-	-	1.7	-	-
Varying flavours and colours	-	-	7.4	4.2	3.4	14.0	2.4

APPENDIX VI

STATISTICAL ANALYSIS OF DATA ON NUTRITIONAL, PHYSICOCHEMICAL AND MICROBIAL QUALITY OF COMMERCIALIZED YOGHURTS

Table VI a: ANOVA for nutritional composition of commercialized Yoghurts

	Moisture	Ash	Protein	Fat	Carbohydrate	Ca	P	Mg	Na	K	Fe	Zn
F-Ratio	956.87	7.53	20.34	1.29	165.31	2357.44	984.84	631.74	140.18	436.26	110.59	13.66
P-Value	0.0000	0.0009	0.0000	0.3230	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Table VI b: ANOVA for physicochemical and microbial quality of commecialised yoghurts

	pH	TA	TSS	TA	Viscosity	Yeast	Coliforms
F-Ratio	150.15	833.46	-	824.92	15.40	3.01	**6.19**
P-Value	0.0000	0.0000	-	0.0000	0.0000	0.0418	0.0024

APPENDIX VII

STATISTICAL ANALYSIS OF DATA ON SENSORY ACCEPTABILITY OF COMMERCIALIZED YOGHURT

Table VII a: ANOVA for sensory evaluation of commercialized Yoghurts

	Colour	Aroma	Sourness	sweetness	Thickness	mouthfeel	Mean score
F-Ratio	3.95	6.39	9.71	11.91	11.09	12.66	18.81
P-Value	0.0009	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Table VIIb: Overall mean score of sensory attributes of commercialized yoghurts

Yoghurt	Average ± SD
Y1	5.34 ± 0.82^{a}
Y2	5.78 ± 0.69^{b}
Y3	4.73 ± 0.92^{d}
Y4	5.81 ± 0.58^{b}
Y5	6.24 ± 0.63^{c}
Y6	6.31 ± 0.47^{c}
Y7	5.96 ± 0.73^{bc}

APPENDIX VIII

STATISTICAL ANALYSIS OF DATA ON THE KEEPING QUALITY OF YOGHURT PRESERVED WITH DIFFERENT CONCENTRATIONS OF NATAMYCIN

Table VIIIa: ANOVA for the effect of natamycin and period of storage on pH

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Concentration of Natamycin	0.00529714	6	0.000882857	4.21	0.0014
B:trying natamycin 2.Days of Storage	1.85634	4	0.464086	2214.02	0.0000
RESIDUAL	0.0123671	59	0.000209613		
TOTAL (CORRECTED)	1.87401	69			

Table VIIIb: Correlations between pH and concentrations of natamycin and between pH and days of storage

Factors	pH
Concentration of Natamycin	
Correlation coefficient	0.0421
Sample size	(70)
P value	0.7294
Days of Storage	
Correlation coefficient	-0.9829
Sample size	(70)
P value	0.0000

Table VIIIc: TSS of yoghurts with different concentrations of natamycin during storage period

Conc. Of natamycin	Sample size	Day of manufacture	7 Days in Storage	14 Days in Storage	21 Days in storage	28 Days in storage
N0	2	13.87 ± 0.0^{a}	12.86 ± 0.0^{a}	12.36 ± 0.0^{a}	12.36 ± 0.0^{ab}	11.36 ± 0.0^{a}
N5	2	13.87 ± 0.0^{a}	12.36 ± 0.0^{b}	12.61 ± 0.35^{ab}	11.86 ± 0.0^{c}	11.36 ± 0.0^{a}
N6	2	13.87 ± 0.0^{a}	12.86 ± 0.0^{a}	12.36 ± 0.0^{a}	11.86 ± 0.0^{c}	11.86 ± 0.0^{b}
N7	2	13.62 ± 0.35^{b}	12.86 ± 0.0^{a}	12.36 ± 0.0^{a}	12.11 ± 0.35^{ac}	11.86 ± 0.0^{b}
N8	2	13.37 ± 0.0^{b}	12.86 ± 0.0^{a}	12.86 ± 0.0^{b}	12.61 ± 0.35^{bd}	12.86 ± 0.0^{c}
N9	2	13.87 ± 0.0^{a}	12.86 ± 0.0^{a}	12.86 ± 0.0^{b}	12.86 ± 0.0^{d}	12.86 ± 0.0^{c}
N10	2	13.87 ± 0.0^{a}	12.86 ± 0.0^{a}	12.86 ± 0.0^{b}	12.86 ± 0.0^{d}	12.86 ± 0.0^{c}

Table VIIId: ANOVA for the effect of natamycin and period of storage on TSS

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Concentration of Natamycin	4.39286	6	0.732143	7.38	0.0000
B:trying natamycin 2.Days of Storage	21.88	4	5.46999	55.10	0.0000
RESIDUAL	5.85714	59	0.0992736		
TOTAL (CORRECTED)	32.13	69			

Table VIIIe: Correlations between TSS and concentrations of natamycin and between TSS and days of storage

Factors	TSS
Concentration of Natamycin	
Correlation coefficient	0.2640
Sample size	(70)
P value	0.0272
Days of Storage	
Correlation coefficient	-0.7646
Sample size	(70)
P value	0.0000

Table VIIIf: TA of yoghurts with different concentrations of natamycin during storage period

Sample	Sample size	**0 Days**	**7th Day**	**14th Day**	**21st Day**	**28th Day**
N0	2	0.75 ± 0.005^{ab}	0.79 ± 0.012^{ab}	0.92 ± 0.017^{a}	0.90 ± 0.040^{ab}	1.05 ± 0.084^{ab}
N5	2	0.74 ± 0.022^{ab}	0.75 ± 0.005^{b}	0.88 ± 0.036^{a}	0.85 ± 0.003^{ab}	0.97 ± 0.0^{abc}
N6	2	0.76 ± 0.0^{b}	0.76 ± 0.0^{b}	0.83 ± 0.008^{bc}	0.94 ± 0.133^{a}	1.07 ± 0.087^{a}
N7	2	0.72 ± 0.003^{c}	0.72 ± 0.012^{c}	0.83 ± 0.008^{bc}	0.87 ± 0.031^{ab}	0.93 ± 0.081^{bcd}
N8	2	0.72 ± 0.006^{ac}	0.72 ± 0.012^{c}	0.80 ± 0.006^{bc}	0.80 ± 0.005^{b}	0.86 ± 0.003^{cd}
N9	2	0.72 ± 0.003^{8c}	0.73 ± 0.017^{c}	0.79 ± 0.0^{b}	0.82 ± 0.031^{a}	0.84 ± 0.009^{d}
N10	2	0.76 ± 0.003^{8b}	0.76 ± 0.006^{a}	0.83 ± 0.002^{c}	0.82 ± 0.016^{ab}	0.81 ± 0.007^{d}

Table VIIIg: ANOVA for the effect of natamycin and period of storage on TA

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
MAIN EFFECTS					
A:Concentration of Natamycin	0.101519	6	0.0169198	7.40	0.0000
B:trying natamycin 2.Days of Storage	0.369469	4	0.0923671	40.41	0.0000
RESIDUAL	0.134863	59	0.00228581		
TOTAL (CORRECTED)	0.60585	69			

Table VIIIh: Correlations between TA and concentrations of natamycin and between TA and days of storage

Factors	TA
Concentration of Natamycin	
Correlation coefficient	-0.3454
Sample size	(70)
P value	0.0034
Days of Storage	
Correlation coefficient	0.7574
Sample size	(70)
P value	0.0000

Table VIIIi: Total yeast counts (log cfu/ml) of yoghurts with different concentrations of natamycin during storage period

Sample	Size	0 Days	7th Day	14th Day	21st Day	28th Day
N0	2	5.13±0.07^{a}	5.30±0.02 ^{a}	5.29±0.01^{a}	5.35±0.04^{a}	5.39±0.01^{a}
N5	2	5.00±0.0^{bc}	5.08±0.0^{b}	5.27±0.16^{a}	5.24±0.05^{a}	5.30±0.02^{a}
N6	2	5.01±0.01^{bc}	5.17±0.02^{c}	5.26±0.08^{a}	5.28±0.0^{a}	5.13±0.07^{a}
N7	2	5.08±0.0^{ab}	5.10±0.08^{bc}	5.24±0.01^{a}	5.24±0.01^{a}	5.06±0.02^{a}
N8	2	5.02±0.04^{abc}	4.80±0.01^{d}	4.06±0.03^{b}	2.69±0.30^{b}	1.54±0.09^{b}
N9	2	5.02±0.04^{abc}	4.84±0.02^{d}	4.02±0.17^{b}	2.80±0.28^{b}	1.91±0.19^{c}
N10	2	4.97±0.08^{c}	4.82±0.02^{d}	3.80±0.28^{b}	2.72±0.17^{b}	1.69±0.30^{bc}

Table VIIIj: ANOVA for the effect of natamycin and period of storage on total yeast count.

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Concentration of Natamycin	41.2673	6	6.87788	13.43	0.0000
B:trying natamycin 2.Days of Storage	18.1643	4	4.54107	8.87	0.0000
RESIDUAL	30.2174	59	0.512159		
TOTAL (CORRECTED)	89.6489	69			

Table VIIIk: Correlations between total yeast count and concentrations of natamycin and between total yeast count and days of storage

Factors	Total yeast count
Concentration of Natamycin	
Correlation coefficient	-0.5178
Sample size	(70)
P value	0.0000
Days of Storage	
Correlation coefficient	-0.4323
Sample size	(70)
P value	0.0002

Tables VIIIm: ANOVA for pH, TSS, TA and Yeast count on day of manufacture to 28th day of storage by concentrations of natamycin

Quality parameter		Storage Period (Days)				
		0	7	14	21	28
pH	*F-Ratio*	0.00	0.67	4.33	9.09	51.62
P-Value		1.0000	0.6813	0.0380	0.0051	0.0000
TSS	*F-Ratio*	4.33		7.00	10.50	
P-Value		0.0380		0.0108	0.0033	
TA	*F-Ratio*	7.35	10.80	16.01	1.66	6.90
P-Value		0.0094	0.0031	0.0009	0.2616	0.0112
Yeast	*F-Ratio*	2.93	67.85	48.80	126.36	356.51
P-Value		0.0926	0.0000	0.0000	0.0000	0.0000

APPENDIX IX

STATISTICAL ANALYSIS OF DATA ON YOGHURT PRESERVED WITH 8ppm NATAMYCIN ADDED AT TWO STAGES OF PRODUCTION

Table IXa ANOVA for pH against point of addition of natamycin and days of storage

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:B.Point of addition of natamycin	0.0178867	2	0.00894333	8.36	0.0019
B:Storage period	0.992453	4	0.248113	232.01	0.0000
RESIDUAL	0.0245967	23	0.00106942		
TOTAL (CORRECTED)	1.03494	29			

Table IXb: Correlations between pH and point of addition of natamycin and between pH and days of storage

Factors	pH
Point of addition of Natamycin	
Correlation coefficient	0.1297
Sample size	(30)
P value	0.4946
Days of Storage	
Correlation coefficient	-0.9518
Sample size	(30)
P value	0.0000

Table IXc: ANOVA for the effect of point of addition of natamycin and period of storage on TA

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:B.Point of addition of natamycin	0.0167256	2	0.00836279	12.28	0.0002
B:Storage period	0.379075	4	0.0947688	139.18	0.0000
RESIDUAL	0.0156612	23	0.000680922		
TOTAL (CORRECTED)	0.411462	29			

Table IXd: Correlations between TA and Point of addition of natamycin and between TA and Days of storage

Factors	TA
Point of addition of Natamycin	
Correlation coefficient	-0.1560
Sample size	(30)
P value	0.4103
Days of Storage	
Correlation coefficient	0.8947
Sample size	(30)
P value	0.0000

Table IXe: ANOVA for the effect of Point of addition of natamycin and period of storage onTSS

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:B.Point of addition of natamycin	0.0157267	2	0.00786333	0.10	0.9045
B:Storage period	9.14505	4	2.28626	29.32	0.0000
RESIDUAL	1.79336	23	0.077972		
TOTAL (CORRECTED)	10.9541	29			

Table IXf: Correlations between TSS and point of addition of natamycin and between TSS and days of storage

Factors	**TSS**
Point of addition of Natamycin	
Correlation coefficient	0.0338
Sample size	(30)
P value	0.8593
Days of Storage	
Correlation coefficient	-0.8835
Sample size	(30)
P value	0.0000

Table IXg: Yeast counts during storage of yoghurts with natamycin added before and after incubation of milk base.

Sample	**0 Days**	**7th Day**	**14th Day**	**21st Day**	**28th Day**
N0	4.81±0.29343[a]	5.23±0.05963[a]	5.28±0.02896[a]	5.44±0.00665[a]	6.31±0.01498[a]
NB	4.91±0.170435[a]	4.49±0.01982[b]	3.00±0.06162[b]	1.93±0.03617[b]	1.48±0.0[b]
NA	5.05±0.081285[a]	4.83±0.13568[c]	2.78±0.24903[b]	1.81±0.047338[c]	1.72±0.17185[b]

Table IXh: ANOVA for the effect of point of addition of natamycin and period of storage on Yeast

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:B.Point of addition of natamycin	32.7242	2	16.3621	16.73	0.0000
B:Storage period	19.3646	4	4.84115	4.95	0.0050
RESIDUAL	22.4918	23	0.977902		
TOTAL (CORRECTED)	74.5805	29			

Table IXi: Correlations between yeast counts and point of addition of natamycin and between total yeast count and days of storage

Factors	Yeast counts
Point of addition of Natamycin	
Correlation coefficient	-0.5634
Sample size	(30)
P value	0.0012
Days of Storage	
Correlation coefficient	-0.4752
Sample size	(30)
P value	0.0080

Table IXj: ANOVA for effect of point of addition of natamycin on pH, TSS, TA and Yeast count during storage

Quality parameter		Storage Period (Days)				
		0	**7**	**14**	**21**	**28**
pH	*F-Ratio*	0.50	44.21	8.59	30.50	1.05
P-Value		0.6495	0.0059	0.0573	0.0101	0.4515
TSS	*F-Ratio*	1.00	1.50	1.00	1.00	7.00
P-Value		0.4648	0.3536	0.4648	0.4648	0.0741
TA	*F-Ratio*	0.54	0.86	139.30	5.43	5.23
P-Value		0.6312	0.5065	0.0011	0.1007	0.1052
Yeast	*F-Ratio*	0.74	36.69	172.85	7108.25	1494.82
P-Value		0.5493	0.0078	0.0008	0.0000	0.0000

APPENDIX X

STATISTICAL ANALYSIS OF DATA ON THE KEEPING QUALITY OF COMMERCIALIZED YOGHURTS PRESERVED WITH AND WITHOUT NATAMYCIN

Table Xa: Changes in pH of commercialized yoghurts with and without natamycin

Sample	0 Days	7th Day	14th Day	21st Day	28th Day	35th Day
Y1	4.27 ± 0.00^{a}	4.03 ± 0.01^{a}	3.93 ± 0.01^{a}	3.64 ± 0.02^{a}	3.45 ± 0.01^{a}	3.39 ± 0.00^{a}
Y2	4.32 ± 0.02^{b}	4.16 ± 0.01^{c}	3.99 ± 0.01^{c}	3.81 ± 0.01^{b}	3.67 ± 0.01^{b}	3.60 ± 0.02^{b}
Y3	3.99 ± 0.01^{c}	3.90 ± 0.00^{b}	3.79 ± 0.01^{b}	3.57 ± 0.01^{c}	3.53 ± 0.04^{a}	3.39 ± 0.01^{a}
Y4	4.40 ± 0.01^{d}	4.22 ± 0.01^{b}	3.99 ± 0.01^{c}	3.91 ± 0.01^{d}	3.78 ± 0.03^{c}	3.69 ± 0.01^{c}
Y5	4.43 ± 0.03^{de}	4.23 ± 0.01^{d}	3.97 ± 0.01^{c}	3.91 ± 0.01^{d}	3.74 ± 0.02^{bc}	3.57 ± 0.01^{b}
Y6	4.45 ± 0.00^{e}	4.18 ± 0.00^{c}	3.99 ± 0.02^{c}	3.92 ± 0.03^{d}	3.89 ± 0.01^{d}	3.82 ± 0.03^{d}
Y7	4.42 ± 0.00^{de}	4.35 ± 0.03^{e}	4.30 ± 0.02^{d}	4.18 ± 0.00^{e}	4.04 ± 0.08^{e}	3.98 ± 0.02^{e}
C1	4.27 ± 0.01^{a}	3.96 ± 0.03^{b}	3.79 ± 0.01^{b}	3.43 ± 0.03^{a}	3.38 ± 0.01^{b}	3.28 ± 0.01^{a}
C2	4.33 ± 0.01^{a}	4.08 ± 0.02^{c}	3.74 ± 0.01^{a}	3.69 ± 0.02^{c}	3.56 ± 0.01^{c}	3.52 ± 0.01^{c}
C3	4.04 ± 0.08^{a}	3.83 ± 0.06^{a}	3.76 ± 0.00^{ab}	3.52 ± 0.02^{b}	3.33 ± 0.02^{a}	3.26 ± 0.01^{a}
C4	3.89 ± 0.71^{a}	4.21 ± 0.01^{d}	3.91 ± 0.01^{c}	3.85 ± 0.00^{e}	3.55 ± 0.01^{c}	3.50 ± 0.00^{c}
C5	4.44 ± 0.01^{a}	4.00 ± 0.03^{b}	3.92 ± 0.03^{c}	3.84 ± 0.03^{e}	3.53 ± 0.02^{c}	3.44 ± 0.02^{b}
C6	4.46 ± 0.02^{a}	4.14 ± 0.02^{cd}	3.89 ± 0.00^{c}	3.78 ± 0.03^{d}	3.76 ± 0.02^{d}	3.57 ± 0.04^{d}
C7	4.43 ± 0.02^{a}	4.34 ± 0.01^{e}	4.20 ± 0.01^{d}	4.14 ± 0.01^{f}	3.96 ± 0.00^{e}	3.91 ± 0.00^{e}

Table Xb: Changes in TA of commercialized yoghurts with and without natamycin

Sample	0 Days	7th Day	14th Day	21st Day	28th Day	35th Day
Y1	1.17 ± 0.002^{a}	1.25 ± 0.010^{a}	1.27 ± 0.005^{a}	1.30 ± 0.008^{a}	1.31 ± 0.007^{a}	1.31 ± 0.003^{a}
Y2	0.91 ± 0.015^{b}	1.12 ± 0.005^{b}	1.16 ± 0.007^{b}	1.27 ± 0.007^{b}	1.29 ± 0.006^{b}	1.30 ± 0.006^{b}
Y3	1.18 ± 0.003^{a}	1.20 ± 0.012^{c}	1.21 ± 0.014^{c}	1.25 ± 0.006^{c}	1.29 ± 0.007^{b}	1.36 ± 0.003^{c}
Y4	1.17 ± 0.002^{a}	1.17 ± 0.001^{d}	1.19 ± 0.002^{c}	1.21 ± 0.001^{d}	1.23 ± 0.003^{c}	1.31 ± 0.004^{ab}
Y5	0.89 ± 0.005^{d}	1.02 ± 0.007^{e}	1.13 ± 0.004^{d}	1.20 ± 0.004^{d}	1.25 ± 0.004^{d}	1.32 ± 0.004^{a}
Y6	1.00 ± 0.002^{c}	1.00 ± 0.000^{f}	1.01 ± 0.005^{e}	1.07 ± 0.003^{e}	1.14 ± 0.004^{e}	1.20 ± 0.007^{d}
Y7	0.88 ± 0.008^{d}	0.91 ± 0.003^{g}	1.02 ± 0.001^{e}	1.11 ± 0.001^{f}	1.13 ± 0.010^{e}	1.19 ± 0.006^{e}
C1	1.17 ± 0.000^{a}	1.52 ± 0.008^{a}	1.68 ±0.004^{a}	1.69 ± 0.004^{a}	1.58 ± 0.004^{a}	1.35 ± 0.008^{a}
C2	0.90 ± 0.008^{b}	1.16 ± 0.008^{b}	1.23 ±0.005^{b}	1.27 ± 0.011^{b}	1.09 ± 0.008^{b}	1.07 ± 0.003^{b}
C3	1.18 ± 0.003^{a}	1.24 ± 0.002^{c}	1.24 ±0.008^{b}	1.26 ± 0.006^{b}	1.35 ± 0.010^{c}	1.49 ± 0.007^{c}
C4	1.17 ± 0.005^{a}	1.18 ± 0.001^{d}	1.22 ±0.024^{b}	1.26 ± 0.006^{b}	1.32 ± 0.011^{d}	1.40 ± 0.007^{d}
C5	0.89 ± 0.003^{c}	1.05 ± 0.001^{e}	1.14 ±0.007^{c}	1.32 ± 0.006^{c}	1.50 ± 0.012^{e}	1.69 ± 0.017^{e}
C6	0.99 ± 0.002^{d}	1.01 ± 0.004^{f}	1.05 ±0.008^{d}	1.11 ± 0.004^{d}	1.21 ± 0.011^{f}	1.22 ± 0.006^{f}
C7	0.88 ± 0.002^{e}	0.91 ± 0.008^{g}	1.11 ±0.004^{e}	1.19 ± 0.003^{e}	1.20 ± 0.003^{f}	1.21 ± 0.010^{f}

Table Xc: Changes in TSS of commercialized yoghurts with and without natamycin

Sample	0 Days	7th Day	14th Day	21st Day	28th Day	35th Day
Y1	11.36 ±0.00^{a}	10.86 ±0.00^{a}	10.36 ±0.00^{a}	10.36 ±0.00^{a}	9.61 ±0.35^{a}	9.86 ±0.00^{a}
Y2	10.36 ±0.00^{b}	10.36 ±0.00^{a}	10.11 ±0.35^{a}	9.36 ±0.00^{b}	9.36 ±0.00^{a}	9.36 ±0.00^{b}
Y3	10.36 ±0.00^{b}	10.36 ±0.00^{a}	9.61 ±0.35^{a}	9.11 ±0.35^{b}	8.86 ±0.00^{b}	8.61 ±0.35^{c}
Y4	11.36 ±0.00^{a}	10.86 ±0.00^{a}	10.11 ±0.35^{a}	10.36 ±0.00^{a}	9.36 ±0.00^{a}	9.36 ±0.00^{b}
Y5	14.37 ±0.00^{d}	13.87 ±0.00^{b}	13.35 ±0.68^{b}	12.62 ±0.36^{c}	12.36 ±0.00^{c}	11.61 ±0.35^{d}
Y6	12.61 ±0.35^{c}	12.36 ±0.00^{c}	12.36 ±0.00^{c}	11.86 ±0.00^{d}	11.61 ±0.35^{d}	11.36 ±0.00^{d}
Y7	12.86 ±0.00^{c}	12.36 ±0.71^{c}	11.86 ±0.00^{c}	11.36 ±0.00^{e}	11.36 ±0.00^{d}	11.36 ±0.00^{d}
C1	11.36 ±0.00^{b}	11.11 ±0.35^{c}	10.36 ±0.00^{c}	9.86 ±0.00^{c}	9.61 ±0.35^{b}	8.61 ±0.35^{b}
C2	10.36 ±0.00^{a}	10.11 ±0.35^{ab}	9.61 ±0.35^{b}	9.36 ±0.00^{b}	9.11 ±0.35^{b}	8.86 ±0.00^{b}
C3	10.36 ±0.00^{a}	9.61 ±0.35^{a}	8.86 ±0.00^{a}	7.86 ±0.00^{a}	7.61 ±0.35^{a}	7.61 ±0.35^{a}
C4	11.36 ±0.00^{b}	10.36 ±0.00^{b}	10.11 ±.35^{bc}	9.86 ±0.00^{c}	9.36 ±0.00^{b}	9.11 ±0.35^{b}
C5	14.37 ±0.00^{d}	13.87 ±0.00^{e}	13.12 ±0.36^{d}	11.61 ±0.35^{e}	10.61 ±0.35^{c}	10.36 ±0.00^{c}
C6	12.86 ±0.00^{c}	12.11 ±0.35^{d}	12.36 ±0.00^{e}	10.61 ±0.35^{d}	10.36 ±0.00^{c}	10.36 ±0.00^{c}
C7	12.86 ±0.00^{c}	11.86 ±0.00^{d}	11.61 ±0.35^{f}	11.36 ±0.00^{e}	10.86 ±0.00^{c}	10.86 ±0.00^{c}

Table Xd: Changes in Yeast loads of commercialized yoghurts with and without natamycin

Sample	0 Days	7th Day	14th Day	21st Day	28th Day	35th Day
Y1	4.78 ± 0.01^{ac}	4.28 ± 0.03^{a}	3.85 ± 0.08^{a}	3.47 ± 0.24^{a}	2.26 ± 0.08^{a}	2.09 ± 0.12^{a}
Y2	4.96 ± 0.03^{ac}	4.62 ± 0.10^{b}	3.99 ± 0.01^{b}	3.16 ± 0.23^{ab}	1.99 ± 0.41^{abc}	1.59 ± 0.16^{bc}
Y3	3.87 ± 0.01^{b}	3.45 ± 0.03^{c}	3.36 ± 0.08^{c}	2.34 ± 0.08^{c}	2.18 ± 0.26^{ab}	1.58 ± 0.03^{bc}
Y4	4.61 ± 0.28^{c}	4.50 ± 0.28^{ab}	3.73 ± 0.02^{a}	2.93 ± 0.04^{abd}	1.50 ± 0.28^{c}	1.48 ± 0.00^{bcd}
Y5	3.94 ± 0.08^{b}	3.26 ± 0.12^{c}	3.21 ± 0.09^{d}	2.59 ± 0.11^{bcd}	1.65 ± 0.07^{bc}	1.24 ± 0.34^{cd}
Y6	5.10 ± 0.28^{a}	4.56 ± 0.06^{ab}	3.80 ± 0.01^{a}	2.49 ± 0.30^{cd}	2.09 ± 0.12^{abc}	1.72 ± 0.17^{b}
Y7	5.00 ± 0.01^{a}	3.94 ± 0.06^{d}	3.60 ± 0.02^{e}	3.16 ± 0.45^{ab}	1.92 ± 0.31^{abc}	1.15 ± 0.21^{ad}
C1	4.17 ± 0.05^{a}	4.52 ± 0.05^{a}	5.16 ± 0.09^{b}	6.30 ± 0.05^{d}	6.34 ± 0.06^{c}	6.39 ± 0.01^{d}
C2	5.37 ± 0.10^{e}	5.47 ± 0.03^{c}	5.64 ± 0.05^{d}	5.97 ± 0.02^{c}	6.32 ± 0.02^{c}	6.44 ± 0.00^{d}
C3	5.24 ± 0.04^{cd}	5.31 ± 0.02^{c}	5.43 ± 0.02^{d}	5.32 ± 0.00^{b}	5.00 ± 0.00^{b}	4.95 ± 0.05^{c}
C4	5.14 ± 0.03^{bc}	5.25 ± 0.05^{c}	5.04 ± 0.06^{a}	5.00 ± 0.00^{a}	3.39 ± 0.30^{a}	2.63 ± 0.46^{d}
C5	5.04 ± 0.06^{b}	4.35 ± 0.49^{a}	5.23 ± 0.04^{bc}	5.30 ± 0.00^{b}	6.29 ± 0.02^{c}	6.40 ± 0.02^{d}
C6	5.30 ± 0.05^{d}	4.77 ± 0.09^{ab}	5.00 ± 0.00^{a}	5.01 ± 0.05^{a}	4.79 ± 0.19^{b}	4.22 ± 0.11^{b}
C7	5.01 ± 0.02^{b}	5.19 ± 0.03^{bc}	5.31 ± 0.02^{c}	6.28 ± 0.03^{d}	6.32 ± 0.02^{c}	6.38 ± 0.06^{d}

Table Xe: Changes in Total coliform count of commercialized yoghurts with and without natamycin

Sample	0 Days	7th Day	14th Day	21st Day	28th Day	35th Day
Y1	3.66 ± 0.00^{ac}	3.30 ± 0.48^{a}	4.32 ± 0.00^{a}	1.25 ± 0.10^{a}	ND^{a}	ND^{a}
Y2	3.97 ± 0.01^{ab}	4.97 ± 0.00^{b}	3.50 ± 0.76^{a}	1.97 ± 0.00^{b}	0.28 ± 0.39^{a}	ND^{a}
Y3	3.71 ± 0.47^{ac}	0.71 ± 0.22^{c}	3.59 ± 0.63^{a}	2.28 ± 0.14^{b}	0.96 ± 0.00^{b}	ND^{a}
Y4	4.42 ± 0.34^{b}	1.55 ± 0.12^{d}	2.38 ± 0.00^{b}	1.07 ± 0.15^{ac}	0.28 ± 0.39^{a}	ND^{a}
Y5	3.32 ± 0.00^{c}	1.18 ± 0.00^{cd}	3.54 ± 0.00^{a}	1.80 ± 0.24^{b}	1.16 ± 0.28^{b}	ND^{a}
Y6	3.32 ± 0.00^{c}	1.34 ± 0.03^{d}	1.57 ± 0.09^{b}	0.28 ± 0.39^{d}	1.07 ± 0.15^{b}	ND^{a}
Y7	2.49 ± 0.24^{d}	2.32 ± 0.00^{e}	1.80 ± 0.24^{b}	0.71 ± 0.22^{cd}	0.28 ± 0.39^{a}	ND^{a}
C1	3.35 ± 0.04^{ab}	3.36 ± 0.00^{b}	4.49 ± 0.24^{e}	1.57 ± 0.03^{bc}	ND^{a}	ND^{a}
C2	3.92 ± 0.07^{b}	4.75 ± 0.60^{b}	3.67 ± 0.42^{cd}	1.72 ± 0.36^{c}	ND^{a}	ND^{a}
C3	3.61 ± 0.61^{db}	1.18 ± 0.00^{a}	4.04 ± 0.00^{de}	1.80 ± 0.24^{c}	0.96 ± 0.00^{b}	ND^{a}
C4	3.76 ± 0.45^{b}	1.50 ± 0.19^{a}	2.28 ± 0.14^{b}	1.11 ± 0.10^{bc}	0.28 ± 0.39^{a}	ND^{a}
C5	3.68 ± 0.51^{ab}	1.18 ± 0.00^{a}	3.50 ± 0.06^{c}	1.63 ± 0.00^{b}	ND^{a}	ND^{a}
C6	3.32 ± 0.00^{ab}	1.80 ± 0.24^{a}	1.63 ± 0.00^{a}	0.56 ± 0.00^{a}	0.28 ± 0.39^{a}	ND^{a}
C7	2.85 ± 0.27^{a}	2.49 ± 0.24^{b}	1.48 ± 0.22^{a}	0.28 ± 0.39^{a}	ND^{a}	ND^{a}

Table Xf: ANOVA for pH, TSS, TA and Yeast count on day of manufacture to 28th day of storage for unpreserved commercialized yoghurts

Quality parameter		Storage Period (Days)					
		0	**7**	**14**	**21**	**28**	**35**
pH	*F-Ratio*	1.32	59.03	259.58	221.96	340.27	309.30
P-Value		0.3590	0.0000	0.0000	0.0000	0.0000	0.0000
TSS	*F-Ratio*	-	59.57	65.94	91.50	34.67	50.89
P-Value		-	0.0000	0.0000	0.0000	0.0001	0.0000
TA	*F-Ratio*	2385.91	2662.62	756.31	1769.82	694.72	1019.06
P-Value		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Yeast	*F-Ratio*	104.44	10.01	43.50	722.42	139.08	133.80
P-Value		0.0000	0.0038	0.0000	0.0000	0.0000	0.0000
Coliforms	*F-Ratio*	1.96	47.88	67.04	14.79	5.63	1.32
P-Value		0.1992	0.0000	0.0000	0.0012	0.0195	0.3590

Table Xg: ANOVA for pH, TSS, TA and Yeast count on day of manufacture to 28th day of storage for all commercialized yoghurts with natamycin

Quality parameter		Storage Period (Days)					
		0	**7**	**14**	**21**	**28**	**35**
pH	*F-Ratio*	**248.92**	**261.68**	**220.98**	**302.83**	**58.70**	**303.16**
P-Value		**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**
TSS	*F-Ratio*	**239.92**	**48.88**	**33.38**	**91.50**	**106.17**	**81.50**
P-Value		**0.0000**	**0.0000**	**0.0001**	**0.0000**	**0.0000**	**0.0000**
TA	*F-Ratio*	**843.80**	**701.20**	**398.07**	**514.47**	**283.59**	**343.14**
P-Value		**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**
Yeast	*F-Ratio*	**23.14**	**38.52**	**47.51**	**5.79**	**2.46**	**5.93**
P-Value		**0.0003**	**0.0001**	**0.0000**	**0.0181**	**0.1317**	**0.0170**
Coliforms	*F-Ratio*	**12.97**	**107.11**	**14.59**	**23.07**	**5.45**	**-**
P-Value		**0.0018**	**0.0000**	**0.0012**	**0.0003**	**0.0213**	**-**

Table Xh: ANOVA for pH, TSS, TA and Yeast count on day of manufacture to 28th day of storage for all commercialized yoghurts with and without natamycin

Quality parameter		Storage Period (Days)					
		0	**7**	**14**	**21**	**28**	**35**
pH	*F-Ratio*	1.95	86.68	251.04	244.72	110.12	315.65
P-Value		0.1150	0.0000	0.0000	0.0000	0.0000	0.0000
TSS	*F-Ratio*	450.07	50.67	42.48	90.64	59.13	66.42
P-Value		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
TA	*F-Ratio*	1159.38	1415.05	666.64	1329.91	592.59	843.45
P-Value		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Yeast	*F-Ratio*	38.19	33.25	543.79	146.19	197.41	300.03
P-Value		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Coliforms	*F-Ratio*	4.90	64.03	24.42	17.37	6.19	**-**
P-Value		0.0028	0.0000	0.0000	0.0000	0.0009	**-**