INVESTIGATION OF THE EFFECTS OF SOME PRESERVATIVES ON THE SHELF LIFE OF A DAIRY PRODUCT

(A CASE STUDY OF MAIZUBE FARM YOGHURT)

By

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(2005/21708EH)

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FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA

NIGERIA

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A PROJECT SUBMITTED TO THE

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DECLARATION

I declare that the work in the project report (thesis) entitled "Investigation of the effects of some preservatives on the shelf life of a dairy product" has been performed (Carried out) by me under the supervision of Engr. D. O. Agbajelola. No part of this project report (thesis) was presented for another degree or diploma elsewhere at any institution to the best of my knowledge.

MAD A IBRAHIM		22/11/2010
Name of Student	Signature	Date

CERTIFICATION

This is to certify that this Project Report (thesis) entitled "INVESTIGATION OF THE EFFECTS OF SOME PRESERVATIVES ON THE SHELF LIFE OF A DAIRY PRODUCT" by Ahmad A. Ibrahim meets the Requirements for the Partial fulfillment of the award of Bachelor of Engineering (B.Eng) degree in Chemical Engineering, Federal University of Technology, Minna.

.... 22/11/2010

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ABSTRACT

This project was aimed at investigating the effects of some preservatives on the Shelf life of a Diary Product a case study of Maizube Farm yoghurt. Sodium Benzoate, Potassium Sorbate and Chloroform were the preservatives used at 0.2 %, 0.2 % and 0.15 % concentrations, respectively, per weight of the product and storage was done both at room temperature and in a refrigerator. The shelf life of yoghurt preserved with Sodium Benzoate at room temperature was 13 days before spoilage while the one stored in a refrigerator was 36 days before spoilage. The shelf life of yoghurt preserved with Potassium Sorbate at room temperature was 11 days before spoilage while the one stored in a refrigerator was 33 days before spoilage. The shelf life of yoghurt preserved with Chloroform at room temperature was 10 days before spoilage while the one stored in a refrigerator was 32 days before spoilage. This shows that Sodium Benzoate is the best and its inhibitive ability can be enhanced with low temperature storage, thus the shelf life of Milk product is highly dependent on the storage temperature as well as the amount of preservative used.

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1.0 INTRODUCTION

Preservatives are chemical agents that inhibit the growth of micro-organisms in creams and cosmetic products. They are also substances that are added to food to prevent spoilage which responsible for food decaying. The term preservative is define further as natural or synthetic chemical that are added to products such as food, pharmaceutical, paints, biological samples etc. to retard spoilage whether from microbial growth or not (Website Dictionary/Encyclopedia).

There are thousand and one preservatives used in food industry but the preservatives of interest in this research work are Sodium Benzoate, Potassium Sorbate and Chloroform (English-*Wikipedia,* 2010).

Milk and its Products (yoghurt, cheese, etc.) are a perfect medium for microbial growth and cannot be stored at room temperature for 24 hours without spoilage occurring i.e. it needs to be stored at a temperature of 0-4 ^{0}C constantly so as to increase its shelf life to at most 21 days after production. In other words, for this temperature 0-4 ^{0}C to be maintained there is need for constant power supply but it is obvious to us Nigerians that power supply is very unstable. Maizube Farms Nigeria Ltd, which is the case study of this project, has production capacity of up to 3000 litres of processed milk per day at maximum production and its marketing environments are Abuja, Suleja, Minna, Makwa, Bida, Lagos etc. thus, the need for the use of preservatives to extend the shelf life of the milk product outside refrigeration cannot be over-emphasized. This will ensure proper profit maximization and loss reduction between the establishment and the affected customers/distributors in their different marketing environments (Macrae, 1997).

Furthermore, the focus of this project is to investigate and to propose the use of chemical means of food preservation in addition to the existing physical means which is thermal and refrigeration method of preservation particular to Maizube Farms establishment. With the use of preservatives especially Sodium Benzoate, most micro-organisms like yeast, mould and bacteria (thermoduric bacteria, psycrotrophic bacteria) including their enzymes that can survive pasteurization and still grow during refrigeration will be effectively killed or inhibited. Note, the storage temperature after heat treatment of milk product ideally should be 1-2 ^{0}C so as to minimize growth of psycrotrophic bacteria but 1-2 ^{0}C is practically impossible in production condition, so 4-7 ^{0}C is recommended and at this temperature bacteria still grow thus reducing the time required spoilage, so preservatives are the only solution to curb this occurrence (Macrae, 1997).

1.2 Aim and Objectives

The main aim of this project is to investigate the effect of Sodium Benzoate, Potassium Sorbate and Chloroform as preservatives on the shelf life of Maizube farm yoghurt at certain chosen concentrations and temperature. This aim will be accomplished through the following objectives.

- To collect raw yoghurt from Maizube Farms Nigeria Limited.
- To investigate the shelf life of the yoghurt at room temperature and in a Refrigerator.
- To investigate the effects of the preservatives on the shelf life of the yoghurt.
- To determine the change in pH per Time.
- To optimize the production process i.e. profit maximization as a result of longer shelf life and minimization of losses in terms of capital (money) investment.
- Finally, to propose the use of chemical method of preventing food spoilage to Maizube Farm Nigeria Limited.

1.1 Justification

In this particular research work, the preservatives employed for the investigation of Maizube yoghurt among the available preservatives are Sodium Benzoate, Potassium Sorbate and Chloroform. The reason behind this choice is that they have little or no documented possible adverse effect (PAE) when used at the prescribed concentrations and they have been proved to be most effective as a result of their high inhibitive ability (Krebs, 1983).

Chapter Two

2.0 LITERATURE REVIEW

2.1 Preservatives Used In Food Industry

Preservatives are natural or synthetic chemical that are added to products such as food, pharmaceuticals, paints, biological samples etc. to retard spoilage whether from microbial growth or not (www.answers.com).

In pharmaceutical industries, the common preservatives used for the production of drugs include citric acid, sodium Metabisulphite and Sodium Benzoate for the production of vitamin C syrup, cough syrup, mindol tablets etc. (Baka'i Pharmaceuticals Limited, Minna).

But in foods industries, the most common preservatives are Ascorbic acid, Sodium Nitrate, Potassium Nitrate, Sodium Nitrite, Calcium Nitrate and Nitrites are added particularly to foods as meats and cheese. Also, Sodium Benzoate is mainly used for milk product such as yoghurt produced from soya beans, animal milk or powered milk and soft drinks (www.answers.com).

Preservatives are added to different products specifically to increase their shelf life. Besides, combination of different preservatives has been proved to have a longer shelf life effect on products but dangerous to human health, e.g. combination of Ascorbic acid (vitamin C) and Sodium Benzoate leads to the release of benzene which is a known carcinogen (Christian Woman Mirror, June 2007).

The table below consists of some additives, mainly preservatives used in food industries with their respective English name, code of Federal Regular (CFR), Merek Index (MI), and Chemical Abstract Service (CAS). Possible Adverse Effect (PAE) and European Economic Community code (EEC) (www.answers.com).

Table 2.1: Various Preservatives and their EC, CFR, MI, CAS, PAE, EEC Identifications

EC	ENGLISH NAME	CFR	M.I	CAS	PAF
200	Ascorbic Acid	-	8495	110-44-1	-
201	Sodium Sorbic	-	8495	-	-
202	Potassium Sorbic	-	7459	2463-61-5	-
203	Calcium Sorbic	-	-	-	-
210	Benzoic Acid, Phenilormic acid	-	1100	65-85-0	Allergy?
211	Sodium benzoate	-	8326	532-32-1	-
212	potassium benzoate	-	1100	-	-
213	Calcium benzoate	-	1100	-	-
214	Ethyl-p-hydroxy-benzoate (PHB)	-	3777	-	-
215	Ethyl-p-hydroxy-benzoate sodium salt	-	-	-	-
216	PHB n- propylester	-	-	-	-
217	PHB sodium n- propylester	-	-	-	-
218	Methyl-p-hydroxy-benzoate	-	-	-	-
219	Methyl-p-hydroxy-benzoate sodium salt	-	-	-	-
220	Sulphur Dioxide	-	8677	7446-09-5	Irritation
221	Sodium sulfite	-	8451	7757-83-7	-
222	Sodium-hydrogen sulfite	-	8332	-	-
223	Sodium-metabisulfite	-	8396	7681-57-4	Allergy?
224	Potassium metabisulphite	-	7428	16731-55-8	Allergy?
225	Calcium-sulfite	-	-	-	-
226	Calcium sulfite	-	1642	-	PAE
227	Calcium hydrogen sulfite	-	-	-	PAE
228	Potassium hydrogen sulfite	-	-	-	PAE
230	Diphenyl, Bephnely	-	3322	92-52-4	-
231	Orthophenyl-phenol, orthercernol	-	7110	-	-
232	Sodium orthophenol phenol	-	7110	-	-
233	Thiabendazole	-	9017	148-79-8	-
234	Nisine	-	6383	1414-45-5	-
235	Natamycine, Pimarican	172.155	7230	7681-93-8	Protein
236	Formic acid	-	4098	68-18-6	Uria?

(www.physics.kee.hu/e_code/tartosit.html).

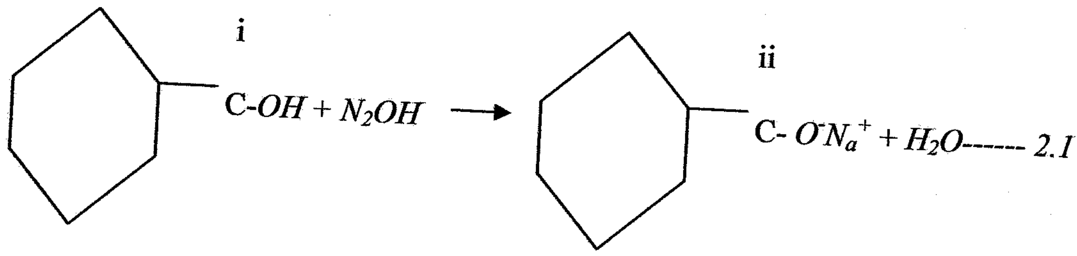
2.1.1. Sodium Benzoate as a Preservative

Sodium benzoate is an organic chemical compound also called Benzoate of soda. It is soluble in water and alcohol; it is a colourless white amorphous crystal aromatic compound with a sweetish taste (Sybil, 1986).

As a preservative, it is used as food addition, as an antiseptic, as intermediate in dye manufacture and in the production of pharmaceuticals. Sodium benzoate is one of the most effective food preservative since it kills most yeast, bacteria and fungi that survive thermal treatment of food such carbonate drink, fruit juice, milk product (yoghurt) etc. if function best only in acidic condition of $pH < 3.6$ which makes it most prevalent in the above mentioned foods and also found in alcohol-based mouth wash and silver polish (www.answers.com/sodium benzoate).

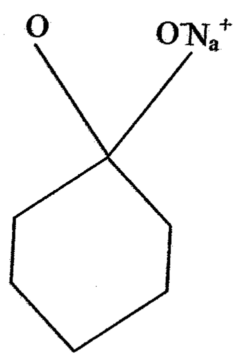
The taste of sodium benzoate cannot be detected by around 25 percent of the population but for those who can taste the chemical it tends to be perceived as sweet, salty or sometimes bitter. It is found naturally in Cranberries, Prunes, greengage plums, cinnamon, ripe cloves and apples. Its concentration is limited by the food, Drugs and Administration in the United States to 0.1 % by weight though organically grown cranberries and prunes can conceivably contain level exceeding this limit. The international programme on chemical safety found no adverse effects on human at doses of 647-825 mg/kg of body weight per day. Also combination of sodium or potassium benzoate with Ascorbic acid (Vitamin C, E300) may form benzene a known carcinogen. Heat, light and shelf life can affect the rate at which benzene is formed. The FDA is currently as of March 2006 performing tests but the Environmental Working Group is calling FDA to publicly release all tests and use their authority to force companies to reformulate to avoid the benzene forming combination (Clark, 1972).

Sodium Benzoate is produced by reacting sodium hydroxide with benzoic acid. In other word the reaction is a neutralization of benzoic acid in solution of the strong base. Sodium Hydroxide (NaOH).



Benzoic Acid | Sodium Hydroxide | Sodium Benzoate | Water

Structurally, Sodium Benzoate is represented as:-



(Denniston, 2004).

Table 2.2: Properties of Sodium Benzoate

Property	**Value**
Chemical Name	Sodium Benzoate
Other Names	E211. Benzoate of soda
Chemical Formula	$NaC_6H_5CO_2$
Molecular Mass	144.1053 $gmol^{-1}$
CAS Number	(532-32-1)
Density	1.44 gcm^{-3}
Melting Point	300 ^0C
Boiling Point	N/A

2.1.2 Potassium Sorbate

Potassium Sorbate is the potassium salt of Sorbic Acid. Its primary use is as a food preservative (E number 202). Potassium Sorbate is effective in a variety of applications including food, wine, and personal care, The molecular formula of potassium sorbate is $C_6H_7O_2K$ and its systematic name is potassium (*E,E*)-hexa-2,4-dienoate. It has a molecular weight of 150.22 g/mol. It is very soluble in water (58.2 % at 20 °C). It is prepared by the reaction of sorbic acid with potassium hydroxide (English-*Wikipedia,* 2010).

Potassium sorbate is used to inhibit molds and yeasts in many foods, such as cheese, wine, yogurt, dried meats, apple cider and baked goods. It can also be found in the ingredients list of many dried fruit products. In addition, herbal dietary supplement products generally contain potassium sorbate, which acts to prevent mold and microbes and to increase shelf life, and is used in quantities at which there are no known adverse health effects. Labeling of this preservative reads as "potassium sorbate" on the ingredient statement. Also, it is used in many personal care products to inhibit the development of microorganisms for shelf stability. Some manufacturers are using this preservative as a replacement for parabens (English-*Wikipedia,* 2010).

Also known as "wine stabilizer", potassium sorbate produces sorbic acid when added to wine. It serves two purposes. When active fermentation has ceased and the wine is racked for the final time after clearing, potassium sorbate will render any surviving yeast incapable of multiplying. Yeast living at that moment can continue fermenting any residual sugar into CO_2 and alcohol, but when they die no new yeast will be present to cause future fermentation. When a wine is sweetened before bottling, potassium sorbate is used to prevent refermentation when used in conjunction with potassium metabisulfite. It is primarily used with sweet wines, sparkling wines, and some hard ciders but may be added to table wines which exhibit difficulty in maintaining clarity after fining (English-*Wikipedia,* 2010).

Some molds (notably some *Trichoderma* and *Penicillium* strains) and yeasts are able to detoxify sorbates by decarboxylation, producing 1,3-pentadiene. The pentadiene manifests as a typical odor of kerosene or petroleum (English-*Wikipedia,* 2010).

Table 2.3: Properties of Potassium Sorbate

Property	**Value**
Molecular formula	$C_6H_7KO_2$
Molar mass	150.22 g/mol
Density	1.363 g/cm^3
Melting point	270 °C (decomposition)
Solubility in water	58.2 % at 20 °C
Solubility	soluble in ethanol, propylene glycol slightly soluble in acetone very slightly soluble in chloroform, corn oil, ether insoluble in benzene

2.1.3 Chloroform

Chloroform is the organic compound with formula $CHCl_3$. The colorless, sweet-smelling, dense liquid is a trihalomethane, and is considered somewhat hazardous. Several million tons are produced annually as a precursor to Teflon and refrigerants, but its use for refrigerants is being phased out. $CHCl_3$ has a multitude of natural sources, both biogenic and abiotic. It is estimated that greater than 90 % of atmospheric $CHCl_3$ is of natural origin (English-*Wikipedia,* 2010).

Chloroform was reported in 1831 by the French chemist Eugène Soubeiran, who prepared it from acetone (2-propanone) as well as ethanol through the action of chlorine bleach powder (calcium hypochlorite). The American physician Samuel Guthrie prepared gallons of the material and described its "deliciousness of flavor." Independently, Justus von Liebig also described the same compound. All early preparations used variations of the haloform reaction. Chloroform was named and chemically characterized in 1834 by Jean-Baptiste Dumas. In industry, chloroform is produced by heating a mixture of chlorine and either Chloromethane or Methane At 400–500 °C, a free radical halogenation occurs, converting these precursors to progressively more chlorinated compounds (English-*Wikipedia,* 2010).:

$CH_4 + Cl_2 \rightarrow CH_3Cl + HCl$ 2.2

$CH_3Cl + Cl_2 \rightarrow CH_2Cl_2 + HCl$ 2.3

$CH_2Cl_2 + Cl_2 \rightarrow CHCl_3 + HCl$ 2.4

Chloroform undergoes further chlorination to give CCl_4:

$CHCl_3 + Cl_2 \rightarrow CCl_4 + HCl$ 2.5

The output of this process is a mixture of the four chloromethanes, chloromethane, dichloromethane, chloroform, and carbon tetrachloride, which are then separated by distillation. The major use of chloroform today is in the production of the chlorodifluoromethane (R-22), a major precursor to tetrafluoroethylene (English-*Wikipedia,* 2010).:

$CHCl_3 + 2\ HF \rightarrow CHClF_2 + 2\ HCl$ 2.6

The reaction is conducted in the presence of a catalytic amount of antimony pentafluoride. Chlorodifluoromethane is then converted into tetrafluoroethylene, the main precursor to Teflon. Before the Montreal Protocol, chlorodifluoromethane (R22) was also popular refrigerant (English-*Wikipedia,* 2010).

Table 2.4: Properties of Chloroform

Property	Value
Molecular formula	$CHCl_3$
Molar mass	119.38 g/mol
Appearance	Colorless liquid
Density	1.483 g/cm^3
Melting point	-63.5 °C
Boiling point	61.2 °C
Solubility in water	0.8 g/100 ml (20 °C)
Refractive index (n_D)	1.4459

2.2 Milk and Its Composition

Milk is complex biological fluid consisting of fats, protein, minerals, vitamins, enzymes and sugar. The composition varies according to the breed of the animal for example, Friesan Holstein breed animal (Cow) are more likely to produce larger quantities of milk with low percentage of fat compared to Jersey breed and other Animals. The former breeds are found in Maizube Farms Nigeria limited. The greatest changes in composition occur during lactation. Colostrums. The first milk after calving has a high concentration of fat and protein especially immunoglubulins. Over the first weeks of lactation the fat and protein level decrease while the lactose level increases. The type of feed consumed by the cows affect the milk composition as well as the volume of milk produced (Macrae, 1997).

These components in milk are of great nutritional importance in the processing of milk into various foods such as cheese, yoghurt and cream. On the average, the milk contains about 3.5 % protein, 3.6 % fat, 4.9 % lactose, 0.7 % mineral and 87 % water (Macrae, 1997).

Table 2.4: Composition of Bovine Milk, Concentrations of the Major Components.

Constituents	Concentration(gl^{-1})
***Caseins**	24-48
α S_1: A,B,C,D	12-15
α S_2: A,B,C,D	3-4
β: A^1,A^2,A^3,B,C,D,E	9-11
K: A,B	3-4
Casein fractions	
µ1,µ2,µ3 from β-casein	1-2
* Whey protein	5-7
β- lactoglobulins (A,B,C,D)	2-4
α- Lactoalbumin A,B	1-1.5
Serum Albumin	0.1-0.4
Immunoglobulins	0.6-1
Lipids	
Triacylglycerols	32-46
Diacylglycerols	0.1-0.17
Monoacylglycerol	0.009-0.013
Cholesterol	0.1-0.15
Cholesterol esters	Trace
Free fatty acids	0.009-0.013
Phospholipids	0.2-0.3
Lactose	
Salt	
Total	
Ca	1.4(0.5)
Mg	0.1(0.08)
Inorganic P	0.96(0.36)
Citrate	1.6(1.4)
Sodium(Na)	0.59
K	1.1
Cl	1.1

2.2.1. Chemical Composition of Milk

The chemical compositions of milk include proteins (caseins, whey protein), lipids, enzymes, salts, lactose, trace element, vitamins etc. (Macrae, 1997).

2.2.1.1 *The Proteins*

The proteins may be divided into the caseins and soluble proteins which include enzymes. The caseins form about 80 % of the total protein in milk and are almost entirely organized into macro molecular units called MICELLES. The main casein are designated α s_1-caseins, α s_2- caseins, β- caseins, and K- caseins with their respective variants i.e. A,B,C,D as the case may be as shown in Table 2.4. Other minor caseins are derived from proteolysis and glycosylation. The soluble proteins (whey protein) are called so because they separate into whey during cheese making consisting of β- lactoglobulins, α- Lactalbulmins, immunoglobulin and serum albumin in decreasing amount. All the proteins are subjected to genetic variation (Macrae, 1997).

2.2.1.2 *Lipids*

Lipids in milk are secreted from the mammary gland in the form of globules surrounded by a membrane which is derived from the plasma membrane of the gland itself. The fat within the membrane consist mainly of triacylglcerols with small amount of di-and mono-aclyglycerols, free cholesterol and cholesterol esters, free fatty acid and phospholipids (Macrae, 1997).

2.2.1.3 *Enzymes*

Approximately 60 enzymes have been identified in milk. They are important because they are responsible for certain flavour and stability problems, may help protect the neonate against bacteria infection and can be used to test the efficiency of pasteurization. Proteolytic and lipolytic enzymes cause degradation of milk components when milk is stored under less than ideal condition. Enzyme plasmin found in milk fat Globule Membrane (MFGM) degrades caseins even at low temperature. Homogenization of milk release lipid from the MFGM which can lead to the degradation of fat into fatty acid, giving rancid flavour to the milk (Macrae, 1997).

2.2.1.4 *Salts*

Milk contains many salts all of which vary with stage of lactation, seasoned, diet and health of the animal. The main ones may be considered into three group (1) Colloidal calcium, magnesium, inorganic phosphate and citrate are associated with the casein content of the milk of the concentration of diffusible calcium and magnesium are closely related to soluble citrate concentration, the concentration of Ca^{2+} is inversely proportional to HPO_4^{2-} concentration and highly pH dependent (3) The potassium, sodium and chloride concentration (with lactose) in milk ensure that it is iso-osmotic with the blood (Macrae, 1997).

2.2.1.5 *Lactose*

Lactose in milk exist in two anomeric form, α – D – lactose and β – lactose in an equilibrium of about 37.3 % and to 62.7 % β at 20 ^{0}C. it is in inverse relationship with the chloride in milk, making it iso-osmotic with blood. It may be that evolution has favoured lactose over, say glucose because a given weight lactose exert only half of the osmotic pressure of the monosaccharide and therefore twice the energy value for a given osmotic pressure (Macrae, 1997).

2.2.1.6 *Trace Elements*

There are a large number of elements in milk at concentration of less than 1 mg^{-1}. They come from the foods eaten by the animal and some are essential for health. These trace elements in milk includes chromium (glucose tolerance), cobalt (in vitamin β_{12}), copper (enzyme cofactor, haemoglobin formation), zinc (insulin, enzyme cofactor) etc. (Macrae, 1997).

2.2.1.7 *Vitamins*

Bovine milk contains 13 vitamins that are important in human nutrition. Their concentrations vary with stage of lactation, season, diet and health of the animal. Vitamin A, B_1, B_2, panthothenic acid and B_{12} each supply more than 19 % of the dietary vitamin content of western diet (Macrae, 1997).

2.2.2. Physical Properties of Milk

The physical properties of milk include the following:-

1. **Viscosity**: - The viscosity of milk is dependent on shear rate and it behaves as a Newtonian lipid provided that the shear rate is > 10 s^{-1} and the fat globules are not aggregated (Macrae, 1997).

Under these conditions the viscosity of the milk increases according to Eiler's equation which state that:

$$\eta = \eta\theta \left(\begin{matrix} ** \ 1.25 \\ 0/0 \ max \end{matrix} \right) \quad \text{.......} \quad 2.7$$

Where: η = overall viscosity, $\eta\theta$ = viscosity of continuous phase,

θ = volume fraction of the spherical particles (fat globules + casein micelles + protein molecules + lactose) and θ max = hypothetical volume fraction at maximum packing (Macrae, 1997).

2. **Density**: - The density of milk is a result of the density of its constituent and will thus depend on factors that changes the proportion of those components in the milk and on the temperature which will change their densities. The specific gravity usually falls within the range of 1.023 – 1.032 at 20 °C specifically, the density of cow milk normally varies between 1.028 – 1.038 g/cm^3 (Macrae, 1997).

3. **Creaming**: - The fat in milk has a lower density than the surrounding medium so that the globules slowly separated and rise to the top under the influence of gravity. This creaming is accelerated under increase gravitation field such as in a centrifugal or cream separator (Macrae, 1997).

4. **Freezing point**: - The freezing point depends on the concentration of component in the milk. Measurement of it may be used as a means of determining contamination of milk by water. It is usually in the range of 0.530 to -0.570 °C with a mean of -0.540 °C. The lowering of the freezing point below that of water is due mainly to the lactose and chloride content of the milk (Macrae, 1997).

2.3. Processing of Liquid Milk

Major types of liquid milk ranges from skim or non fat milk, reduce or low-fat milk, high-fat milk to reduced fat high protein products. But traditionally the principal products sold as liquid milk was whole milk, heat treated and packed in a returnable containers. Typically Table 2.1 shows the chemical composition for different milk products. All figures in the table are in grams per 100 g of product (Macrae, 1997).

Table 2.5: The Chemical Composition of Different Milk Products.

Product	Fat	Solid-non fat	Total solid
Whole Milk	3.8-4.0	8.5	>12.3
Protein Milk	0.1	8.5	>8.6
High fat milk	5.0-6.9	8.5	>13.5
Reduced fat milk	1.0-2.0	8.5	9.5
Reduced fat	-	-	-
High protein milk	1.0-2.0	11.3	12.3-13.1

Enumerated below are the processes involved in liquid milk processing:-

2.3.1 Milking:

Historically, milk has been extracted from the udder by manual stimulation and expression of milk from the teats. Recently, milking has been mechanized through automated milking machines with references to Maizube farm milking process. The milk in this process is extracted from the teat and udder by a pulsating vacuum in rubber liner called teat cup fitted round the teat. The milk is carried from the cow through pipelines to the milk storage tank vessels (Macrae, 1997).

Milking is majority done twice a day i.e. morning and evening. But research work has shown the overall volume of milk can be increased by more frequent milking such as three times a day, but this is not a common practice amongst dairy farmers (Macrae, 1997).

2.3.2 Heat Treatment:

Milk is heat-treated to kill many of the micro organisms present in raw milk. The heat treatment makes the milk safe for drinking and extends the potential shelf life. Several heat treatment used include pasteurization UHT (Ultra High Temperature) and sterilization (Macrae, 1997).

2.3.3 Pasteurization:

The term "pasteurization" commemorates LOUIS PASTEUR, who in the middle of the 19 th century made his fundamental studies of the lethal effect of heat on micro-organisms and the use of heat treatment as a preservative technique. The pasteurization of milk is a special type of heat treatment that secures certain destruction of tubercle bacillus (T.B) and other pathogens without markedly affecting the physical and chemical proportion of the milk. There are two types of pasteurization, batch pasteurization and High Temperature Short Time (HTST) Pasteurization.

For Batch pasteurization, the temperature-time combination is 62-63 °C for 30 – 35 mins while HTST is 72 °C for 15 mins. N.B: The temperature-time combination may be increased depending on the milk products (Tetra, 2004).

UHT – The Ultra High Temperature heat treatment was developed to minimize damage to milk components caused by sterilization. UHT processing is increasingly used to heat treating milk as the shelf life is extended from days to months and can be stored well as transported without a refrigerator since all micro-organism are been killed or activated (Tetra, 2004).

The taste and appearance of UHT treated milk is different from pasteurization milk. The milk may taste cooked or burnt and has a sulphurous flavour/smell resulting from dissipation on storage. The milk appears to have a slight brown colour. Typically, the temperature-time condition for UHT is 130-150 °C for 1-35 mins (Tetra, 2004).

2.3.4 Sterilization:

It is a term used to describe milk that has been subjected to temperature in excess of 100 °C and packed in air tight container. Besides, the introduction of UHT processing and improved storage/distribution for pasteurized milk has declined the consumption of sterilized milk (Tetra, 2004).

2.3.5 Homogenization:

This is the breaking down of largest at globules to small globules which do not aggregate and rise to the surface. Homogenization the pressure for first stage ranges from 14000-20000 kpa and 3500 kpa for the second stage. The effect of homogenization on the physical structure of milk has many advantages which include; smaller fat globules leading to less cream line formation, whiter and more appetizing colour, reduced sensitivity to fat oxidation, better stability of cultured milk products etc. The disadvantages include; the milk might be less suitable for production of semi-hard or hard cheeses because the coagulum will be too soft and difficult to dewater (Tetra, 2004).

Table 2.6: Shows the Main Categories of Heat Treatment in the Dairy Industry.

PROCESS	TEMPERATURE	TIME
Pasteurization	63-65	15 s
UHT Pasteurization of milk	63	30 min
HTST Pasteurization of milk	72-75	15-20 s
UHT Pasteurization of cream	80	1-5 s
Pasteurization	125-138	2-4 s
(Flow sterilization)	135-140	a few seconds
Normally in container	115-120	20-30 min

(Tetra, 2004).

2.4. Preservation of Food (Milk Product)

Food preservation is a measure that makes food keeps well over a reasonable period of time. The objectives are to minimize damage or ideally to avoid damage altogether. Food preservation ensures that the consumer is provided with enjoyable, good tasting food which as far possible retains its full nutritional value. Above all, food preservation prevent health hazard caused by contamination of food with pathogenic organism as well as reduction of losses in terms of capital (money) to the establishment (Tetra, 2004).

2.4.1. Food Spoilage

A change food stuff that appreciably reduced its value, especially its nutritive valve, its sensory quality, palatability or usability is referred to as spoilage. Types of food spoilage include chemical, enzymatic, physical and microbiological changes. Chemical changes; important chemical are undesired oxidation, non-enzymatic browning reaction and chemically governed degradation reaction of food ingredient which begins during processing and continue during storage. The main ingredient affected by such reaction is protein, lipids, vitamins aroma compound and colourant. Enzymatic reaction/changes: - the most important ones are reaction caused by hydrolase like lipase, protease and oxidoreductase. It occurs primarily after the comminution of foods; because of the communition process enable the enzymes and substrate to come into direct contact (Tetra, 2004).

Physical changes: - The most important changes in physical properties are sign deposit, destabilization of emulsion and foams, undesired water absorption and undesired loss of water.

Microbiological food spoilage: - This is by far the most important type. It is caused by bacteria, yeast and mould. Micro-organism needs to be present on the food for spoilage to occur, even if they have been in activated by thermal treatment, their enzymes which has migrated into the food may still cause spoilage is manifested by a reduction in the aroma and flavor, texture and nutritive value of food studs. In addition to the economic loss/damage caused, it is well established that micro organisms release toxic substances that cause damage to health (Tetra, 2004).

2.4.2. Method of Preventing Chemical, Enzymatic and Physical Food Spoilage

i. Oxidation phenomena can be restricted by withholding or inactivating the oxygen that is in contract with the food through using oxygen impermeable packaging material.

ii. Chemical changes in food can be delayed by cold storage because chemical reactions are slowed down by lowering temperature.

iii. In the case of enzymatic reaction, blanching by heat is the preferred method for keeping spoilage under control i.e. once inactivated, the enzyme can no longer cause spoilage. Low water content limit enzymes activities and certain additives like citric acid or sulphur oxide inactive enzyme chemically.

iv. Physical changes, typified by precipitation reaction and destabilization are prevented by additives such as emulsifies and thickeners. But for water losses, the use of water-impermeable packaging is employed

N.B:- Emulsifiers are substances (additives) used to enhance proper mixing (Macrae, 1997).

2.4.3. Method of Preventing Microbiology Food Spoilage

The micro-organism that causes food spoilage can be combined in two essentially different ways e.g. physical and chemical ways. The physical method involves the use of heat, cold, drying and irradiation. The chemical method involve adding some kinds of preservatives to the food which gives long term protection ability but not unlimited (Tetra, 2004).

Preservation by heat involves killing all or certain micro-organism. The method in which micro-organisms are killed depend on the temperature and the duration of its action, also on the pH valve, water content pressure or choice of certain food ingredient. Unlike heat treatment, refrigeration does not kill microorganisms but merely inhibits their growth i.e. inhibit the effect. Note, ingredients of food are less damage by refrigerator compare with heat treatment. Dehydration or drying is probably the oldest food preservation method of all. They may not be killed (microorganism) but merely inhibited in their growth because they lack free water necessary. This method is used to preserve cereals, vegetable and liquid such as milk or coffee. Irradiation method is based on the fact that certain electromagnetic radiation kill microorganism e.g. ultra violet light, x-rays or γ -rays the effect of the irradiation depends on his amount of energy absorbed. Specifically, this research work is focused on the chemical method of preventing microbial food spoilage among others (Macrae, 1997).

2.4.4. Factors influencing Shelf Life of Food (milk product).

The shelf life of milk product is defined as the period between manufacture or processing and when the product is considered unsuitable for use by the consumer. The product may be considered to be unsuitable for consumption due to the presence of flavour defects and changes in physical appearance. The factors affecting the shelf life of pasteurized milk include:-

1. The presence of thermo uric bacteria in raw milk which are able to grow under refrigeration e.g. Bacillus cereus and B. Circulars. Thermoduric bacteria are bacteria that survive pasteurization, but not grow at pasteurization temperature. So processor that uses extended shelf life packaging techniques often encounter problems with shelf life due to the presence of low number of Bacillus Species in the raw milk.
2. The presence of Psychrotrophic bacteria in the plant after heat treatment step. These organisms grow under refrigeration and eventually cause spoilage as a result of biochemical reaction.
3. The temperature of the product after heat treatment. Ideally, milk should be stored between 1.2 ^{0}C to minimize growth of Plychrophic bacteria presence in the milk. But 1-2 ^{0}C is practically impossible in production condition, so 4-7 ^{0}C is recommended and at

this temperature bacterium still grows thus reducing the time required for spoilage to occur.

4. Other factors are presence of heat stable enzymes, action of lipase on damage fat globules on raw milk etc. (Macrae, 1997).

2.4.5. Need for Preservatives and Factors affecting Choice of Preservatives.

The use of preservatives to extend the life of foods and improve their safety has been recognized for thousands of years ago. One cause of instability might be that the food has been formulated in such away that it has little or no natural protection against micro-organism e.g. low-fat spread (food) contains significantly larger water droplet than butter and can readily support microbial growth, preservative is particularly useful in such product. Regardless of the chemical form of a preservative when it is added to food, its ionic state is determined largely by the pH of the food and the pKa of the acid. But benzoic acid is most suitable for foods at pH 2.5-4.

Factors affecting choice of preservatives used in food include:-

1. General consideration:- The type of preservative applicable to a particular food is determine by the composition of the food, the type of microbial spoilage that will take place and the desire shelf life. Important compositional variable include the pH of the food, since the efficiency of a given preservative decrease with increase in pH. The maximum pH at which a preservative is useful is often quoted and noted.
2. Preservatives are chosen on their specific physical properties such as solubility in particular food and case of handling, e.g. Calcium sorbate is sparingly soluble in water (1.2 g per 100 g) and is soluble for surface treatment of food such as cheese as it does it does not quickly dissolve in surface moisture or migrate into the cheese (Macrae, 1997).

2.5 Micro Organisms in Milk

Milk is sterile at secretion in the udder but is contaminated by bacteria even before it leaves the udder. Except in the case of Mantises, the bacterial at this point are harmless and few in number. Further infection of the milk by micro-organism can take place during milking, handling storage and other processing activities. The cattle health is of primary important since milk drawn aseptically from a health animal contains small number of bacteria. These are saprophytic types and of little significance as long as their growth are controlled. But milk from a cow with an infected udder is likely to contain large number of bacteria and these are pathogenic organisms. Therefore periodic infections of the herd to ascertain the health of each animal is necessary (Elliker, 1949).

2.5.1 Lactic Acid Bacteria:

This group of bacteria is able to ferment lactose to lactic acid. They are normally present in the milk and are also used as starter culture in the production of cultured dairy products such as yoghurt. Note lactic acid bacteria have recently been reclassified; the older names will appear in brackets as you will find the older names used for convenience sake in a lot of literature some examples in milk are:

- ***Lactococci***

  L. delbrueekii subsp Lactis (Streptococcus lactis)

  Lactococcus lactus subsp Cremoris (streptococcus cremoris)

- ***Lactobacilli***

  Lactobacillus casei

  L. delbrueekii subsp Lactis (L. Lactus)

  L. delbrueekii subsp. Bulgaricus (Lactobacillus bulgarieus)

- ***Leuconostoc***

Coliforms: Coliforms are facultative anaerobes with an optimum growth at 37 °C. Coliforms are indicator organisms; they are closely associated with the presence of pathogens but not necessarily pathogenic themselves. They also ferment lactose with the production of acid and gas is able to degrade milk protein. They are killed by HTST treatment; therefore their presence after treatment is indicative of contamination. *Escherichia coli, Enterobacter, aerogenes etc* are examples belonging to this group.

N.B: (1) Types of acid producers' micro-organism in milk include *streptococci, lactobacilli, coliforms, microbacteria etc.* They ferment lactose to lactic acid and other products such as acetic acid, ethyl alcohol and carbon dioxide. They can be homo-fermentative or hetero-fermentative depending on their products. (2) Types of gas producers micro-organisms in milk include coliforms, clostriedium, butyrium etc. lactose is fermented with accumulation of gas the gas may be mixture as in the case of yeast fermentation (Whiting, 1975).

2.5.2 Significance of Micro-Organisms in Milk

1. Information on the microbial content of milk can be used to judge its sanitary and the conditions of production.
2. If permitted to multiply, bacteria in milk can cause spoilage of the product.
3. Milk is potentially susceptible to contamination with pathogenic micro organisms. Precautions must be taken to minimize this possibility and destroy pathogens that may gain entrance.
4. Certain micro-organisms produce chemical changes that use desirable in the production of during products, such as cheese, yoghurt, etc. (www.university of Guelph/daily science and technology).

2.5.3 Spoilage Micro-organisms in Milk

The microbial quality of raw milk is crucial for the production of quality diary foods. Spoilage is a term used to describe the deterioration of a food texture, colour, odour of flavor to the point where it is unappetizing or unsuitable for human consumption. Microbial spoilage of food often involves the degradation of protein, carbohydrates and fats by the micro-organisms or their enzymes. In milk the micro-organisms that are principally involve in spoilage is psychrotophic organism. Most psychrotophs are destroyed by pasteurization temperature, however, some like *pseudomonas fluorescents, pseudomonas fragi* can produce proteolytic (organisms that degrade casein to peptides) and lipolytic (organisms that hydrolyze milk fat to glycerol and fatty acids) extra cellular enzymes which are stable and capable causing spoilage. Some species and stains of *Bacillus, Clostriedium, Cornebacterium, Arthrobacter, Lactobacillus, Microbacterium, Micrococcus, and Streptococus* can survive pasteurization and grow at refrigeration temperatures which can cause spoilage problems (Tetra, 2004).

2.5.4 Pathogenic Micro Organisms in Milk

Hygienic milk production practices, proper handling and storage of milk and mandatory pasteurization has decrease the threat of milk-borne diseases such as tuberculosis, brucellosis and typhoid fever. There have a number of food borne illnesses resulting from the ingestion of raw milk or dairy product made with milk that was not properly pasteurized or was poorly handled causing post-processing contamination. The following bacteria pathogens are still of concern today in raw milk and other diary products.

- *Bacillus cerus*
- *Listeria Monocytohenes*
- *Yersinia enterocolitica*
- *Salnmonella spp.*
- *Escherichia coli 0157:117*
- *Campylobacter jejuni* (Tetra, 2004).

It should also be noted that mould, mainly of species of *Aspegillus, Fusarium and Pericullium* can grow in milk and diary products. If the conditions permit, these moulds may produce mycotoxins which can be health hazard (Tetra, 2004).

2.5.5 Hazard Analysis and Critical Control Point (HACCP)

Raw and end products may be tested for the presence, level or absence of microorganisms. Traditionally these practices were used to reduce manufacturing defects in diary products and ensure compliance with specification and regulation. However, they have many draw backs:

i. Destructive and time consuming
ii. Slow response
iii. Small sample size
iv. Delays in the release of the food.

In the 1960s, the Pillsbury Company, the U.S. Army, and NASA introduced a system for assuring pathogens free food for the space program. This system, called Hazard Analysis and Critical Control Point (HACCP), is a focus on critical food safety area as part of total quality programs. It involves critical examination of the entire food manufacturing process to determine every step where there is a possibility of physical, chemical or microbiological contamination of the food which render it unsafe or unacceptable for human consumption (Tetra, 2004).

These identified points are the critical control point (CCP). There are seven principles to HACCP:-

1. Analyze hazards
2. Determine CCP's
3. Establish monitoring procedures
4. Establish deviation procedures
5. Establish critical limits
6. Establish verification procedures
7. Establish records keeping procedures (Tetra, 2004).

2.5.6 Starter Cultures

Starter cultures are those micro-organisms that are used in the production of cultured dairy products such as yoghurt and cheese. The natural micro flora of the milk is inefficient, uncontrollable and unpredictable or is destroyed altogether by heat treatments given to the milk. A starter culture can provide particular characteristics in a more controlled and predictable fermentation. The primary function of lactic starters is the production of lactic acid from lactose which has a preservative effect on the production and at the same time the nutritional value/digestibility are improved, resulting to drop in pH. Other functions of starter cultures may include the following:-

- Flavour, aroma and alcohol production
- Proteolytic and lipolytic activities
- Inhibition of undesirable organisms.

There are two groups of lactic starter cultures;

1. Simple or defined:- The single strain, or more than one in which the number is known
2. Mixed or compound:- More than one strain each providing its own specific characteristics.

Starter cultures may be categorized as mesophilic (organism that grows at moderate temperature i.e between 10-47 °C) and thermophiclic (organisms that grow at high temperature i.e. 40-80 °C) (Tetra, 2004).

Mesophilic

- *Lactococcus latis subsp. Cremoris*
- *L. delbrueckii subsp. Lactis*
- *L. Lactis subsp. Latis biovor diacetylactis*
- *Leuconostoc mesenteroides subsp. Crempris*

Thermophilic

- *Streptococcus salivarius subsp. Thermophilus (S. thermophilus)*
- *L. delbrueckii subsp. Lactis*
- *Lactobacillus delbrueckii subsp. Bulgarnis*
- *L. casei*
- *L. helveticus*
- *L. planetarium.*

Mixture of mesophilic and thermophilic micro-organism can also be used as in production of some cheese. N.B: The starter culture used for the production of Maizube yoghurt is a mixture of *Lactobacillus bulgaricus* which grow best at 41 °C and *Streptococcus thermophilus* which grow best at 45 °C (Tetra, 2004).

Starter culture is prepared commercially in lyophilized (freeze dryer), frozen or spray-dried forms, the diary product manufacturer need to inoculate the culture into milk or other suitable substrate. There are a number of steps necessary for the propagation of starter culture ready for production.

- Commercial culture
- Mother culture- first inoculation; all culture originate from this preparation
- Intermediate culture; in preparation of larger volumes of prepared starter.
- Bulk starter culture; this stage is used in diary product production (www.universityofguelph/diaryscienceandtechnology).

Chapter Three

3.0 EXPERIMENTAL METHODOLOGY

3.1 Instrumental and Experimental Procedures

The list below consists of equipment/materials and reagents (chemicals) used in the production of yoghurt from animal fresh milk as well as the investigation processes.

Equipments/Apparatus Used

I. Storage tank
II. Bulk (mixing) tank
III. Pasteurizer (heat exchanger)
IV. Incubation/fermentor
V. Milk filter
VI. Pumping machine (air pressure pump)
VII. pH meter
VIII. Digital weighing balance
IX. Medical SANYO refrigerator
X. Hot (SH3D)
XI. Volumetric flask
XII. Pipette (1 ml and 10 ml)
XIII. Beakers(40 ml)
XIV. Test tubes
XV. Burette(50 ml)
XVI. Disposable petri dish(20 pieces)
XVII. Packaging plastic bottles (1 little and 300 ml) etc

Reagents/Chemical used:

i. Nutrient agar
ii. Sodium hydroxide (0.45 % m/v)

iii. Phenolphthalein

iv. Bacterial culture (starter)

v. Preservatives (sodium benzoate)

vi. Distil water

vii. Buffer solution (4.0, 7.0, 10.0) etc. Note m/v means mass per volume.

3.2 Procedures for Yoghurt Production

The following procedures are necessary in yoghurt production (Maizube Farms Nigeria Ltd).

Milking: This was done through automated method i.e. from the cow udder to the storage tank with help of pulsating vacuum in rubber liner called teat cup fitted around the teat in the cow udder (Macrae, 1997).

Pumping: This was the next step which involves the transportation of the raw milk from the storage tank to the mixing or bulk tank with the help of the inbuilt air pressure pumping machine attached to the storage tank. Filtration process was carried out simultaneously with pumping via the filter connected in between the storage tank and the mixing tank. Note: addition of flavour and starter culture is followed by adequate stirring (mixing) (Macrae, 1997).

Pasteurization: This is a special type of heat treatment which involves destruction of micro-organism like *tubercle baccilus* etc. (T.B). *Phosphate enzymes, coliform* etc. present in the raw milk without markedly affecting the physical or chemical properties of the milk. Pasteurization process also involves combination of temperature and holding time (Macrae, 1997).

Homogenization: This process was done to stabilizer the emulsion against gravity separation with the help of a homogenizer. It primarily causes disruption of large fat globules into much smaller ones. It was operated at pressure between 10-25 Mpa. (100-250 bars) (Macrae, 1997).

Inoculation/culturing: Bacteria culture (starter) was added to the pasteurizer milk in the incubator at temperature of 41-46 ^{0}C the bacterial mixture was a mixture of *lactobacillus bulgartens* which grow best at 41 ^{0}C and *streptococcus thermophillus* which grows best at 45 ^{0}C.

Fermentation/Ripening: This process commence immediately the starter culture has been added and allowed to grow at room temperature for 4-5 hours or more depending on the quantity of the starter added. N.B: the bacteria produce substances which give the cultured product its characteristics properties such as acidity (pH), flavour, aroma and consistency. Also there will be drop in PH, as a result of the bacteria fermenting lactose to lactic acid. Other processes carried out during the yoghurt production include flavouring, packaging of the product etc (Macrae, 1997).

3.3 Procedure for Addition of Preservatives

Three 2 litre size plastic cups with covers were provided and labeled Sodium Benzoate, Potassium Sorbate and Chloroform. And one litre of the produced yoghurt was measured and poured into each cup. Then 0.2 g Sodium Benzoate was measured and poured into the cup labeled A, also, 0.2 g potassium Sorbate was measured and poured into the cup labeled B, and finally 0.015 g of chloroform was measured and poured into the cup labeled C. Adequate mixing was employed to ensure a homogenous mixture in all the cups.

Then six of Maizube plastic bottles labeled Sodium Benzoate (Refrigerator), Potassium Sorbate (Refrigerator) and Chloroform (Refrigerator), Sodium Benzoate (Room Temperature), Potassium Sorbate (Room Temperature), Chloroform (Room Temperature) were brought and the mixture prepared in each cup mentioned above were appropriately poured into these newly brought Maizube plastic bottles with regards to their respective names making six samples altogether. Then the first three samples were put in a refrigerator and the last three were stored at room temperature and beyond the reach of any other person.

Finally, experience test like pH, Odour, Colour, Taste and Temperature were carried out, there on, on all the samples at intervals as tabulated on the experimental results page(s).

3.4 Procedure for pH Determination

The pH meter was used for the determination of the Hydrogen ion concentration in each sample. Firstly, the electrode was rinsed with distilled water and later wiped gently with tissue paper. The pH meter was water proof double junction. Standardization of the pH meter was done using buffer solution of known pH value i.e. 4.7 and 10.0. The pH meter electrode was rinsed again with distilled water and dried.

Furthermore, the six samples were into different beakers and the pH meter was dipped into each sample sequentially followed by rinsing of the electrode with distilled water after each sample reading has been taken. N.B: during standardization of the pH meter using the buffer solution, the calibration button was pressed while the meter was inside the particular buffer solution e.g. 4.0 and was allowed to calibrate until a stable value corresponding to the buffer solution in question was obtained.

The pH reading was taking on a two days interval until spoilage occurs.

Chapter Four

4.0 RESULTS AND DISCUSSIONS

4.1 Experimental Results

Tabulated below are the results obtained from the samples (of Maizube Farm Yoghurt) experimental with Sodium Benzoate at 2 mg/g (0.2 %), Potassium Sorbate at 2 mg/g (0.2 %) and Chloroform at 1.5 mg/g (1.5 %).

Table 4.1: Effect of Sodium Benzoate Preservative (2 mg/g), on Shelf Life of Yoghurt Sample A stored at Room Temperature.

Duration of Storage (Days)	PARAMETERS			
	Odour	Colour	Taste	pH
11/08/2010	Pleasant	White	Sweet	4.08
13/08/2010	Pleasant	White	Sweet	4.09
15/08/2010	Pleasant	White	Sweet	3.92
17/08/2010	Pleasant	White	Sweet	3.81
19/08/2010	Pleasant	White	Sweet	3.79
21/08/2010	Pleasant	White	Sweet	3.74
23/08/2010	Slightly Unpleasant	Brownish White	Slightly Sour	3.62
25/08/2010	Slightly Unpleasant	Brownish White	Slightly Sour	3.60
27/08/2010	Completely Unpleasant	Brownish White	Sour	3.58

Table 4.2: Effect of Sodium Benzoate Preservative (2 mg/g), on Shelf Life of Yoghurt Sample A stored in a Refrigerator.

Duration of Storage (Days)	PARAMETERS				
	Odour	Colour	Taste	pH	Temperature (^0C)
11/08/2010	Pleasant	White	Sweet	4.08	4.00
13/08/2010	Pleasant	White	Sweet	4.23	4.00
15/08/2010	Pleasant	White	Sweet	4.30	4.00
17/08/2010	Pleasant	White	Sweet	4.33	4.00
19/08/2010	Pleasant	White	Sweet	4.30	4.00
21/08/2010	Pleasant	White	Sweet	4.23	4.00
23/08/2010	Pleasant	White	Sweet	4.22	4.00
25/08/2010	Pleasant	White	Sweet	4.18	7.00
27/08/2010	Pleasant	White	Sweet	4.18	6.00
29/08/2010	Pleasant	White	Sweet	4.15	7.00
31/08/2010	Pleasant	White	Sweet	4.00	4.00
2/08/2010	Pleasant	White	Sweet	3.87	4.00
4/08/2010	Slightly Unpleasant	Brownish White	Slightly Sour	3.75	5.00
6/08/2010	Slightly Unpleasant	Brownish White	Slightly Sour	3.65	6.00
8/08/2010	Slightly Unpleasant	Brownish White	Sour	3.55	6.00

Table 4.3: Effect of Potassium Sorbate Preservative (2 mg/g), on Shelf Life of Yoghurt Sample B Stored at Room Temperature.

Duration of Storage (Days)	PARAMETERS			
	Odour	Colour	Taste	pH
11/08/2010	Pleasant	White	Sweet	4.08
13/08/2010	Pleasant	White	Sweet	4.11
15/08/2010	Pleasant	White	Sweet	4.18
17/08/2010	Pleasant	White	Sweet	4.20
19/08/2010	Pleasant	White	Sweet	4.09
21/08/2010	Slightly Unpleasant	Brownish White	Slightly Sour	3.90
23/08/2010	Slightly Unpleasant	Brownish White	Slightly Sour	3.78
25/08/2010	Completely Unpleasant	Brownish White	Slightly Sour	3.69

Table 4.4: Effect of Potassium Sorbate Preservative (2 mg/g), on Shelf Life of Yoghurt Sample B Stored in a Refrigerator.

Duration of Storage (Days)	PARAMETERS				
	Odour	Colour	Taste	pH	Temperature (^0C)
11/08/2010	Pleasant	White	Sweet	4.08	4.00
13/08/2010	Pleasant	White	Sweet	4.14	4.00
15/08/2010	Pleasant	White	Sweet	4.14	4.00
17/08/2010	Pleasant	White	Sweet	4.17	4.00
19/08/2010	Pleasant	White	Sweet	4.20	4.00
21/08/2010	Pleasant	White	Sweet	4.20	4.00
23/08/2010	Pleasant	White	Sweet	4.23	4.00
25/08/2010	Pleasant	White	Sweet	4.11	7.00
27/08/2010	Pleasant	White	Sweet	4.07	6.00
29/08/2010	Pleasant	White	Sweet	4.00	7.00
31/08/2010	Pleasant	White	Sweet	3.80	4.00
2/08/2010	Slightly Unpleasant	Brownish White	Slightly Sour	3.70	4.00
4/08/2010	Slightly Unpleasant	Brownish White	Slightly Sour	3.58	5.00
6/08/2010	Slightly Unpleasant	Brownish White	Slightly Sour	3.55	6.00

Table 4.5: Effect of Chloroform Preservative (1.5 mg/g), on Shelf Life of Yoghurt Sample C Stored at Room Temperature.

Duration of Storage (Days)	PARAMETERS			
	Odour	**Colour**	**Taste**	**pH**
11/08/2010	Pleasant	White	Sweet	4.08
13/08/2010	Pleasant	White	Sweet	4.10
15/08/2010	Pleasant	White	Sweet	4.15
17/08/2010	Pleasant	White	Sweet	4.02
19/08/2010	Pleasant	White	Sweet	3.90
21/08/2010	Slightly Unpleasant	Brownish White	Slightly Sour	3.69
23/08/2010	Slightly Unpleasant	Brownish White	Slightly Sour	3.60
25/08/2010	Completely Unpleasant	Brownish White	Slightly Sour	3.35

Table 4.6: Effect of Chloroform Preservative (1.5 mg/g), on Shelf Life of Yoghurt Sample C Stored in a Refrigerator.

Duration of Storage (Days)	PARAMETERS				
	Odour	Colour	Taste	pH	Temperature (^{0}C)
11/08/2010	Pleasant	White	Sweet	4.08	4.00
13/08/2010	Pleasant	White	Sweet	4.14	4.00
15/08/2010	Pleasant	White	Sweet	4.14	4.00
17/08/2010	Pleasant	White	Sweet	4.17	4.00
19/08/2010	Pleasant	White	Sweet	4.20	4.00
21/08/2010	Pleasant	White	Sweet	4.20	4.00
23/08/2010	Pleasant	White	Sweet	4.23	4.00
25/08/2010	Pleasant	White	Sweet	4.11	7.00
27/08/2010	Pleasant	White	Sweet	4.07	6.00
29/08/2010	Pleasant	White	Sweet	4.00	7.00
31/08/2010	Slightly Unpleasant	Brownish White	Slightly Sour	3.80	4.00
2/08/2010	Slightly Unpleasant	Brownish White	Slightly Sour	3.70	4.00
4/08/2010	Unpleasant	Brownish White	Sour	3.58	5.00

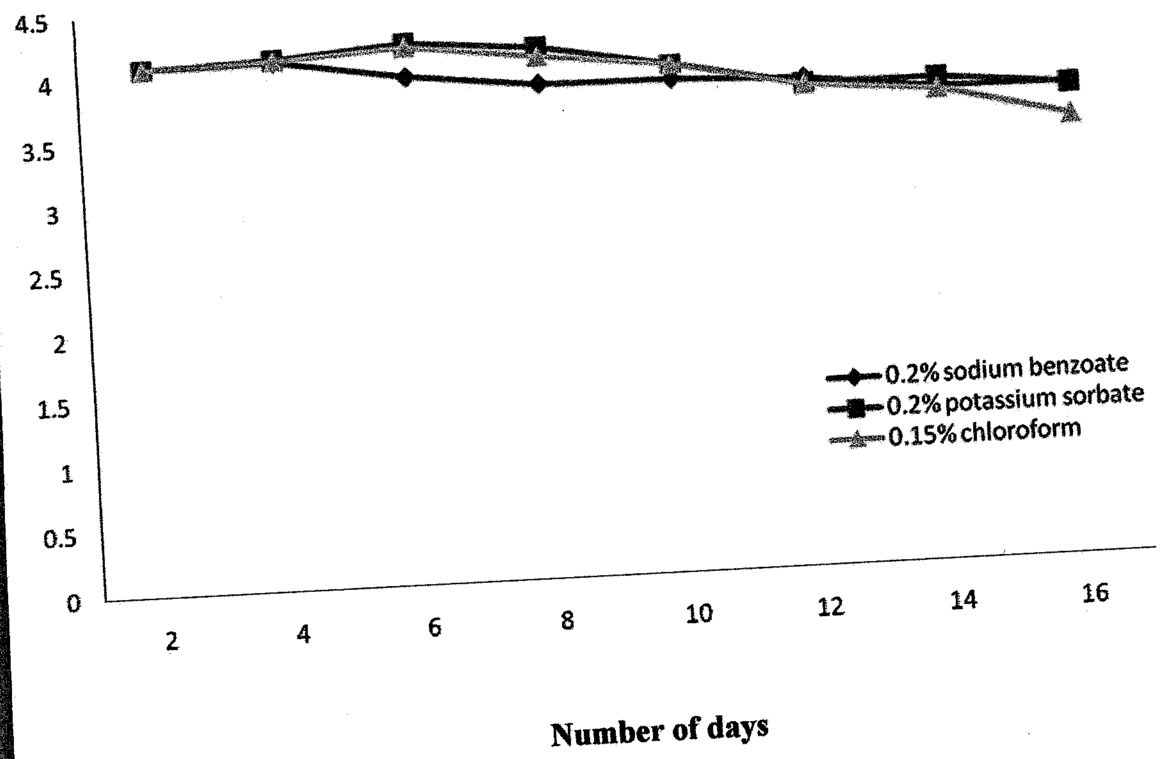


Figure 4.1. The Variation of PH with Time of Storage for Samples stored at Room Temperature.

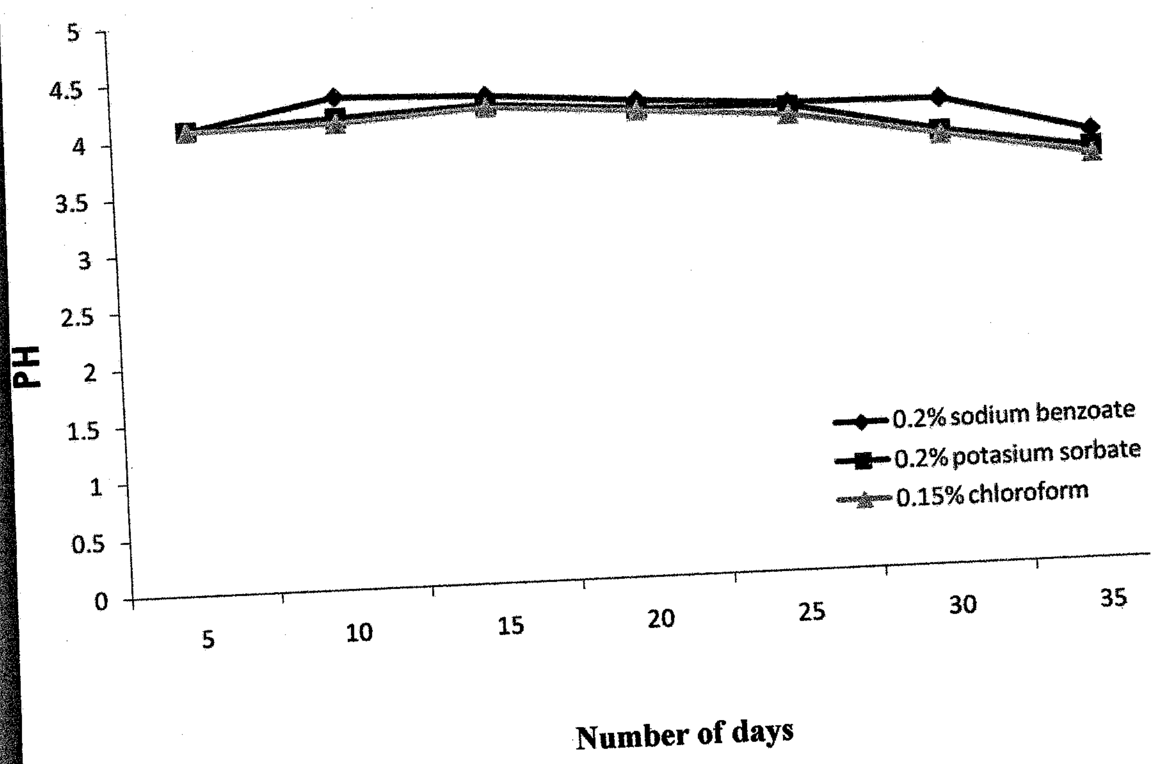


Figure 4.2 The Variation of PH with Time of Storage for Samples stored in a Refrigerator.

4.2 Discussion of Results

The effects of different Preservatives on the shelf life of yoghurt were undertaken in this work and observations were made as follows:-

Table 4.1 presents the effect of Sodium Benzoate (2 mg/g) as a preservative on sample A stored at room temperature. It can be observed that the sample maintained its colour, odour and taste within the first thirteen days i.e. from 11/08/2010 to 23/08/2010, with gradual decrease in pH. However after the thirteenth day, there were changes in colour, odour as well as taste confirming spoilage of the product. Table 4.2 presents the effect of Sodium Benzoate (2 mg/g) as a preservative on sample A stored in a refrigerator, the sample maintained its colour, odour and taste for not less than 36 days before spoilage was observed. However, with more inhibited decrease in pH.

Table 4.3 presents the effect of Potassium Sorbate (2 mg/g) as a preservative on sample B stored at room temperature. It can be observed that the sample maintained its colour, odour and taste within the first eleven days i.e. from 11/08/2010 to 21/08/2010, with gradual decrease in pH. However after the eleventh day, there were changes in colour, odour as well as taste confirming spoilage of the product. Table 4.4 presents the effect of Potassium Sorbate (2 mg/g) as a preservative on sample B stored in a refrigerator, the sample maintained its colour, odour and taste for not less than 33 days before spoilage was observed. However, with more inhibited decrease in pH.

Table 4.5 presents the effect of Chloroform (1.5 mg/g) as a preservative on sample C stored at room temperature. It can be observed that the sample maintained its colour, odour and taste within the first ten days i.e. from 11/08/2010 to 20/08/2010, with gradual decrease in pH. However after the tenth day, there were changes in colour, odour as well as taste confirming spoilage of the product. Table 4.6 presents the effect of Chloroform (1.5 mg/g) as a preservative on sample C stored in a refrigerator, the sample maintained its colour, odour and taste for not less than 32 days before spoilage was observed. However, also, with more inhibited decrease in pH.

Generally, from the above observation, as regards colour, odour and taste changes, the commonest type of change for all the samples was undesired oxidation/non-enzymatic browning reaction (chemical changes) and water absorption (physical change) which was due to frequent exposure of the samples to ambient air during the experimental days. The rate of browning (colour change) and undesired oxidation was increased in all the samples storedat room temperature as a result of the effect of storage temperature (room temperature) thus leading to greater microbial activities. But, the rate was slowed down for all the samples stored in refrigerator due to lowered temperature i.e. refrigeration storage, though there were variations in refrigerator temperature due power fluctuation as in 5 ^{0}C, 6 ^{0}C and 7 ^{0}C instead of 4 ^{0}C as recommended for milk products. These variations gave rise to the gradual browning reaction and undesired oxidation for these samples almost at the same time which ought not to be as a result of the percentage of the preservatives present in the samples. At this point, it is good to know that there was a decrease in the pH value of all the samples stored at room temperature despite the inhibiting power of the preservatives and storage temperature was responsible for that occurrence showing that at higher temperature these preservatives have little effect on the rate of fermentation. Also, for all the samples stored in the refrigerator an initial increase in the pH value in all the samples and afterwards a gradual decrease was obtained, thus showing that the inhibiting ability was enhanced by lowered storage temperature leading to reduced rate of fermentation at the onset until their effects were weaken.

Simply put, the antimicrobial activity were more pronounced at room temperature storage and less at the refrigerator storage, but in terms of inhibiting abilities of the preservatives Sodium Benzoate has more inhibiting ability than Potassium Sorbate and Chloroform (all at their prescribed standard value (Threshold limit value)).

Chapter Five

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Based on the experiment performed and the results obtained, the following conclusion can be made:-

Among all the preservatives used in this project, Sodium Benzoate was found to be the most effective; its inhibitive abilities can be enhanced with lower Temperature Storage. The normal acceptable range of the preservative used in this project work (i.e. > 0.2 % Sodium Benzoate, > 0.2 % Potassium Sorbate and > 1.5 % Chloroform) will guaranty safety as well as increase the shelf life of the product whether stored at room temperature or in a refrigerator. Therefore, the use of 0.2 % Sodium Benzoate should be strictly allowed to by diary companies since it produce a shelf life of > 35 days at refrigeration condition and 14 days at room temperature which ordinarily cannot survive 48 hours outside refrigerator without a preservative. Also, the chemical/physical changes undergone by the samples during storage like browning reaction and oxidation leading to colour, odour as well as taste changes will be taken care of by the use of oxygen impermeable packaging materials. Meanwhile the microbiological changes will be handled by addition of preservatives and through other physical treatments like thermal treatment and refrigeration.

Finally, the decrease in the pH value is normal to milk product since fermentation cannot be totally hindered but the rate can be controlled.

5.2 Recommendations

- ✓ I recommend that a research project on isolation of spoilage organizing and the determination of the percentage of Sodium Benzoate present in different milk product should be carried out in as much as manufacturers hardly adhere to 0.2 % Sodium Benzoate, 0.2 % Potassium Sorbate and 0.15 % Chloroform approved by FOA (Food and Drugs Administration, USA) and NAFDAC.

- ✓ For profit maximization and losses reduction for the establishment and distributors/customers, the use of chemical means of food preservation should be coupled with the common physical method i.e. heat treatment and refrigeration. This will greatly increase the shelf life of milk product.
- ✓ Each of the preservatives used in this project work should be added at the terminal stage of process since they are all thermally stable.
- ✓ That the pH of milk product should be monitored during room temperature culturing/fermentation process so as to control the level of lactic acid and hydrogen ion at storage condition.
- ✓ Finally, I recommend that good manufacturing practices should be adopted especially in the case where animal raw milk is used for the production of the yoghurt because raw milk is a very perfect medium for microbial growth. Examples of these practices include premises hygiene, cleaning and sanitizing facilities before and after production so as to reduce the microbial load etc.

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APPENDIX

Estimation of Total Capital Investment

The total cost and capital investment of purchasing Sodium Benzoate and Potassium Sorbate (Chloroform was collected for free from Departmental Laboratory) used as a preservatives are enumerated below.

500 g of Sodium Benzoate costs N 3000

1 g will cost $\frac{N3000}{500}$

= N 6.0

500 g of Potassium Sorbate costs $\frac{N3000}{500}$

= N 6.0

Recalling that the quantity used for both chemicals is 0.2 %

Therefore, the quantity of yoghurt used is 1000g, thus the quantity of Sodium Benzoate and Potassium Sorbate to be added equals

= 0.2 % x 1000 g

=0.002 x 1000 g = 2 g

So cost of adding 2 g of both Sodium Benzoate and Potassium Sorbate into the bottled yoghurt will be as follows:

1 g of both costs N 6

4 g will cost = 4 x 6 = N23

N.B: The cost can be lesser if the preservative is purchased in Bulk.

Also, Transport Money to Maizube Farms Nigeria Limited cost approximately N 4500.

So, I conclude by advising the management of Maizube Farms Nigeria Limited to venture into this viable project of the use of chemical means of food preservation. This is because the capital involvement is minimal as compare to the optimum profit maximization as a result of extended shelf life.