QUALITY ANALYSIS OF KINDIRIMO (LOCALLY FERMENTED MILK PRODUCT)

BY

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2000/9477EA

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FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA,
NIGER STATE.

NOVEMBER, 2006.

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BEING A FINAL YEAR PROJECT SUBMITTED IN FULFILMENT FOR THE AWARD OF BACHELOR OF ENGINEERING (B. ENG.) AGRICULTURAL ENGINEERING.
FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGER STATE.

NOVEMBER, 2006.

DECLARATION

I hereby declare that this project is a record of a research work that was undertaken and written by me. It has not been presented before any degree or diploma or certificate at any university or institution. Information derived from personal communications, published and unpublished works of others were duly referenced in the text.

.....
Abu Amos A.

15/11/2006
.....
Date

CERTIFICATION

This project entitled "Quality Analysis of Kindirimo (Locally Fermented Milk)" by Abu Amos A. meets the regulations governing the award of the degree of Bachelor of Engineering (B.ENG) of the Federal University of Technology, Minna and it is approved for its contribution to scientific knowledge and literary presentation.

Prof. E.S.A. Ajisegiri
Project Supervisor.

16/11/2006
Date

External Examiner

02/11/2006
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16/11/2006
Date

DEDICATION

I dedicate this project to the Lord God Almighty, the God of impossibilities, the creator of the universe, His son Jesus Christ, He that gives wisdom and understanding to all men and to believers all over the world.

My dedication goes to late Mrs. Sarah Ogundein (Mama Dayo) who was like a mother to me, giving me inspiration any time I am with her and to late David Baba Ndagi Nagya who was my best friend on campus.

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At his own time He made everything beautiful. In spite of all trouble, trial the Lord saw me through.

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ABSTRACT

The aim of this project was to determine the physical, chemical biological and microbiological properties of *Kindirimo*. The pH, colour, density, bacteria count tests were respectively carried out on the locally fermented milk product. The pH test reflected an acidity value of 4.95. Its density was found to be 1022kg/m^3. It has a dirty white colour with a sweet smell. The microbiological quality of fermented milk product (*Kindirimo*) was determined. The bacteria isolated include *Bacillus Subtilus, Klebsiella Spp and Escherichia Coli*. However, the range of microorganisms isolated from the product pose serious threat to food safety, and the need ensure the microbiological safety of the product can not be over emphasized.

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CHAPTER ONE

1.0 INTRODUCTION

Milk is often described as nature's most nearly perfect single food. It is the natural food of the new born mammals which provides the sole source of nourishment directly after birth. According to Oxford Advanced Learner's Dictionary, defines milk as "the white liquid produced by cows, goats and some other animals as food for their young and used as a drink by humans."

Milk is an exceptionally good source of proteins which is of high nutritional value in promoting the growth of children. It provides the best source of calcium in the diet and consequently supports sound bone and teeth development. It contains a useful miscellaneous of vitamins including vitamin A, Thiamin Riboflavin, Biotin, Niacin Panthothenic acid and vitamin D (Ihekoronye and Ngoddy,1985).

Fresh cow milk contains about 87 percent of water in which we have various dissolved salts, carbohydrates and proteins.

The density of milk varies between 1026Kg/m^3 and 1032Kg/m^3 at 20°C. The variation is due to difference in fat and solid contents. The density of cow milk increased as its temperature increases, until it get to the maximum density which is obtained after 12 hours of heating (Ihekoronye and Ngoddy, 1985). This increase in density of the cow milk is due mainly to the liberation of gases such as carbon dioxide and nitrogen which are present in freshly drawn milk to the extent of 4 – 5 percent.

Cow milk in particular is the most predominant raw material for manufactured dairy products such as liquid milk, whole milk powder, condensed milk and evaporated milk.

Kindirimo is a locally fermented milk product that is produced and widely consumed in many African countries, including Nigeria. The nature of fermented products is different from one region to another. Thus is depending on the local indigenous microflora, which in turn reflected the climatic conditions of the area. Thus traditional fermented milk in regions with a cold temperature climate contained mesophilic bacteria such as *Lactococcus* and *Leuconostoc spp*, whilst thermophilic bacteria, which include mostly *Lactobacillus* and *streptococcus*, prevailed in regions with a hot, subtropical or tropical climatic (Kurmann, 1994).

The Fulani people ferment their milk in calabashes and basins. These containers need to be seeded with a natural microbial inoculum's before it could be use for the production of fermented milk. Containers filled with milk fresh milk heated for sometime and are covered and placed in house. The milk are coagulated, the whey and protein were homogenized. These fermented milk contained important lactic acid bacteria (Salvadogo et al., 2001).

In Nigeria, cow is most commonly used as the dairy animal, while goat and sheep are kept for the production of meat hides and skins. Not all breed of cow are good milk producers, for example, the N'dama cattle, the Boron cattle and the Afrikander cattle are just good for beef production and they are used as draught animal (Mayhew and Penny, 1988).

This was so because of the protein content contained in the food by the dairy cattle which is of high importance in milk production..

The major characteristics of dairy cattle are body build and legs. A good dairy animal will be slim, well covered, but not too fat. The stomach should be large and dairy cows eat huge quantities of food.

The legs should be short and sturdy to support the body. Hind legs should be far apart to support the udder.

Examples of dairy cattle are White Fulani, Sudanese and Sokoto breeds.

a) White Fulani

This is a multi-purpose breed which fattens well on natural grassland and is well suited on the dry tropics. It can be crossed with European dairy animals to produce a good savannah milk cow.

b) Sudanese

There are a number of related breeds classed under this heading. Some examples are the Kenana or Blue Nile, the White Nile, the Bagagara and the Butana. The Kenana are probably the productive of African dairy breed. Most of these cattle have short coats and loose skin, with long ears. The male have a large hump; the females a very small one (Mayhew and Penny, 1988).

c) Sokoto

These are short horned Zebu cattle, both sexes having a hump and a pronounced dewlap. The Sokoto is a moderate milker under natural grazing conditions. It is docile, sturdy and

reliable and thus well suited to regular milking. Sokoto cattle are also used as draught animals but are rather slow (Mayhew and Penny, 1988).

The choice of breed is essentially a personal matter. Among the exotic breed, the Jersey is the most efficient producers of milk, it is clear that Jersey breed produces the highest percentage of fat (4.49 percent) and the highest percentage of crude protein 3.79 percent. Generally, the milk production efficiency of a breed is not based on average yield of milk but it based on fat and crude protein percentage, because this plays a major role in milking grading.

1.1 Objective

The objective of this project is determining the physical, chemical, biological and microbiological properties of Kindirimo (Locally fermented milk product).

1.2 Scope of Study

The scope of this project will determine the quality analysis of Kindirimo (Locally fermented milk product).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Milk as a Dairy Food Product

There are two key words that found here. These are "Dairy" and "food." By the New Lexicon Webster's Encyclopedic Dictionary of English language, "dairy" is defined as a "part of a farm given over" for the production of milk, cream, cheese etc. Similarly, food is defined as "any substance which, by process of metabolism, a living organism can convert into fresh tissue, energy etc.", (Isaac, 1974).

For the foregoing, milk is both a dairy product and food. Milk is not only an excellent food for young calves; it has become a valuable food for both human children and adults. It is an important source of animal protein and it is because of this importance of milk to human diet, that the Food and Agricultural Organization (FAO) stipulated a minimum per capital consumption per year of 40Kg for developing nations (Ajisegiri, 1992).

2.2 Constituents of Milk

The constitution of milk varies according to the source that may be affected by the breed, nature of its food and the season during which lactation is had. The most important constituents of milk are fat, protein (casein), lactose and the minerals, collectively referred to as ash. Water is a major constituent taking about 88 percent of milk content. It serves as the solvent for the colloidal system which milk is. Table 2.1 shows the composition of milk from the different sources:-

Table 2.1 Composition of Milk according to Source (%)

Source	Total Solid	Fat	Crude Protein	Casein	Lactose	Ash
Cow	12.57	3.80	3.35	2.78	4.75	0.70
Goat	13.18	4.24	3.70	2.80	4.51	0.78
Sheep	17.00	5.30	6.70	4.60	4.60	0.80
Water Buffalo	16.77	7.45	3.78	4.30	4.78	0.78
Woman	12.57	3.75	1.63	-	6.98	0.21

SOURCE: Jennes and Patten, (1959)

Table 2.2 gives the composition of fresh milk and its contribution to diet.

Table 2.2 Composition of milk and its contribution to diet

Component (nutrient)	Amount in 100g of milk	Percent contribution to nutrient. Allowances by 1 pint of milk daily	
		Man with daily Expenditure of 12.6MJ	Girl (3-4 years) with daily expenditure of 6.2MJ
Energy	272kJ	3	25
Protein	3.3g	22	34
Carbohydrate	4.7g	4	8
Fat	3.8g	6	12
Water	88g	-	-
Calcium	109mg	86	69
Iron	0.1Mg	1	2
Vitamin A	56mg	17	29
Thiamine	50mg	17	33
Niacin	90µg	4	9
Riboflavin	170µg	48	95
Ascorbic acid	1.5mg	55	75
Vitamin D	0.1µg	-	3(max)

SOURCE: Delia and Herbert, (1986)

Cow milk, which is the most widely used source of milk, has the following compositions as shown on Table 2.3.

Table 2.3 Composition of Cow Milk

Constituent	Content (%)
Water	87.1
Fat	3.9
Protein	3.3
Lactose	5.0
Ash (mineral)	0.7

SOURCE: Delia and Herbert, (1986)

From the table above, it could be seen that cow milk has a solid (non-fat) content of 9 percent, total solid of 12.9 percent. Milk, as can be seen from Table 2.2, is a major source of energy. It contains carbohydrate, fat and protein in the descending order of content.

The major constituents of milk are discussed below.

2.2.1 The Water of Milk

Water is the solvent of the colloidal solution. It gives bulk to the milk and holds the solid in solution or suspension (Artherton and Newlander, 1987).

2.2.2 The Carbohydrate of Milk

This consists of the disaccharide lactose. It is also called milk sugar. Lactose is the only sugar produced by mammals, but it lacks sweetness as may be compared to other

forms of sugars. Milk contains bacteria called lactic bacilli, which have enzymes that can breakdown lactose into lactic acid, as shown below:

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow{\text{lactic bacilli}} 4CH_3CH(OH)COOH$$

(Lactose) (Lactic acid)

2.2.3 The Fat of Milk

The fats in milk are oily droplets of minute sizes ranging from 5μm to 10μm. Due to this size oil droplets, a drop of milk contains million of fat droplets. The fat of milk is more digestible than other forms of fat. This is because of emulsified nature of the milk substrate. It is higher than the aqueous phase of the milk. Because of this, milk is allowed to stand, so that the fat droplets can rise to the top and coalesce to form larger droplets forming a larger area of cream over the liquid mass of milk. Where it is not desired in milk solution, the coalesced fat is removed from the top of the milk mass. The fat of milk may be broken down in sizes through a process called homogenization. With or without homogenization, milk is a stable emulsion. The fat-water interface is stabilized by adsorbed natural emulsifiers in the milk (Delia and Herbert, 1986).

2.2.4 The Protein in Milk

Proteins are high biological value. The proteins contained in milk are:

1. Casein (2.6%) which is precipitated under acid conditions.
2. Lactalbumin (0.12%)
3. Lactoglobulin (0.3%)

Lactalbumin and Lactoglobulin are called Whey Proteins. They remain in solution after acidification. Casein is a family of phosphorus containing proteins. It binds the calcium and other minerals present in the milk.

2.2.5 The Mineral Elements of Milk

These occur in form of salts or as constituents of organic nutrients. Some of the minerals are solutions. Others are in colloidal dispersion either as sole particles or combined with protein. The milk from a particular animal species contains all the mineral elements required by the young ones to grow. Some of the mineral elements present in milk include calcium, phosphorus, chlorine and iodine. Iron is present but in small quantities. This makes milk a deficient source of iron for the young animals. Some of the elements are found in combined forms, such as calcium phosphate (Banwart, 1989).

2.2.6 The Vitamins of Milk

Vitamins are present in milk either as fat globules or in aqueous solution. Vitamin A and D are found in milk fat. Vitamin A is found in a more appreciable quantity than vitamin D. the quantity of each depends on the season and nature of feed give to the animal. Vitamin A in milk is partly due to carotene, which gives milk its creamy color.

Other vitamins found in milk are Riboflavin, Thiamine, Ascorbic Acid and small quantities of niacin. The quantity of these vitamins in milk depends on the treatment given to it before it reaches the consumer (Ihekoronye and Ngoddy, 1985).

2.3 Types of Milk

Milk can be distinguished according to its water or fat content. By this, milk can either be fresh or dried; and whether fresh or dried, it can be whole, semi-skimmed or skimmed.

By fresh milk, it is implied that the water content of the milk is retained, while dried milk implies milk from which the water has been evaporated. Whole milk is complete milk. It contains all the essential nutrients of milk and by standard; it should contain not less than 3 percent of fat. Semi-skimmed milk is that from which a proportion of the fat has been removed. Semi-skimmed milk should contain 1.5-1.8 percent fat. Skimmed milk is that from which the fat is removed to a level that it contains 0.3 percent or less of fat. In practice, skimmed milk contains 0.1 percent fat. The nutritional value of skimmed milk is as good as that of whole milk, except for the loss of fat and fat-soluble vitamins A and D that are lost when the cream is skimmed off (Delia and Herbert, 1986).

Table 2.4 shows the average composition of whole and skimmed milk from dairy cattle.

Table 2.4: Average Composition of Whole and Skimmed Milk from Dairy Cattle.

Nutrient per 100g	Whole Milk (%)	Skimmed Milk (%)
Water	87.5	90.2
Protein	3.3	3.2
Fat	3.8	0.2
Carbohydrate	4.8	5.0
Mineral (Ash)	0.5	0.7
Energy value	65kcal = 272kJ	33kcal = 142kJ

SOURCE: Delia and Herbert, (1986)

2.4 Properties of milk

The properties of milk are discussed under four broad headings, namely: physical, chemical, biological and microbiological properties.

2.4.1 Physical properties of milk

Milk is a dilute emulsion, colloidal dispersion and a true solution (Paul, 1959). The followings are the physical properties of milk:

a) **Shape**

Milk is a fluid and as such deforms continuously under shear. It has the ability to flow and as such has no definite volume. As a fluid, it takes the shape of its container.

b) **Colour**

Milk has a bluish-whitish colour. The white or milky appearance is due to the colloidal dispersion of fat globules, Calcium caseinate and Calcium phosphate; and the principal substances that impart a yellowish color as a Carotene and Riboflavin. The colour of milk can be affected by the breed of animal and the nature of feed given to the animal that is producing the milk.

c) **Flavor**

It is not easy to discern the natural taste of milk. Milk has a pleasant taste and slightly sweet. It derives its sweetness from the Lactose contained in it, and its saltiness is from the mineral salts such as the presence of chloride.

2.4.2 Chemical Properties of Milk

Milk chemically consists most of triglycerides of fatty acids to about 98.3 percent. It is generally characterized by a relatively high proportion of short-chained fatty acids that are significant in flavors and off flavor of milk and milk products. Other chemical components of milk are diglycerides, monoglycerides, phospholipids, and sterol free fatty acids. Others include waxes, fat soluble vitamins, such as vitamin A, D and E. These in addition to the presence of mineral salts give milk specific chemical properties. They are:

1. **pH**

This specifies the acidity level of the milk. Milk is slightly acidic with an approximate pH of 6.6 to 6.7 at 25°C (Ajisegiri, 1992). The temperature at which pH is

measured is important because milk exhibits a great dependence on temperature. At the same temperature, for the pH value to alter, a great deal of bacterial souring must occur (Ihekoronye and Ngoddy, 1985).

When milk turns sour, the pH drops and when it reaches 5.2, the milk curdles and casein is precipitated in the form of flocculent curds. This should however not be confused with the clotting of milk, which is the formation of a tough mass of calcium caseinate.

2. **Oxidation-Reduction**

This property is a function by which if the oxygen level is increased, it is oxidation, but when lowered, it is reduction. The reduction of oxygen can be brought about by bacteria, which uses up the oxygen contained in the milk. The bacteria also produce some reducing substances into the milk during metabolism and this may affect the level of oxygen contained in the milk (Artherton and Newlander, 1987).

2.4.3 Biological Properties of Milk

This is an estimation of how milk will behave, with respect to the presence of microorganisms. Milk harbors a number of microorganisms, each of which has a specific demand for continual existence. Some of these organisms are pathogenic in nature, while others seek to bring milk to deterioration.

The main biological property of milk is:

Coagulation

Coagulation is clotting or curdling. It is brought about by enzymatic action or by the addition of acid to milk. The milking cow has enzyme, rennet, produced by the bacteria present in the milk, and which can cause coagulation of milk.

2.4.4 Microbiological Properties of Milk

The composition of any food product dictates the type of flora associated it. The study of the microbiology of food product comprises of the safety and spoilage of the food. In terms of milk and milk products, it deals with the safety and spoilage of fluid milk and milk products as well as the fermentation of milk into a plethora of cheese, yoghurt, and fermented milk.

Some microorganisms may beneficial in some foods, while they are not in others. This is because any food is a substrate and certain characteristics of the food dictate what can grow or not grow in its medium. The level of water content in food promotes the multiplicity of the organism. A microorganism present in a food substance can be aerobic, anaerobic or facultative. High oxidation-reduction potential favors anaerobes and permits facultative process.

Milk naturally contains antibacterial substance apt to stop contaminating flora form involving if it is stored at ambient temperature.

2.5 Milk Processing

Milk is a highly perishable food item. It is for this reason that milk processing started with the objective of converting perishable milk into concentrated and long shelf

life dairy products like butter, cheese, ghee and so on. Processing of food items involves treatment given to them in order to preserve them and prevent spoilage. Two major things are responsible for the spoilage of milk and these are the content and extent of content of microorganisms and the extent of chemical constituents and nature of milk. Therefore, to process milk is to seek to eliminate the microorganisms in milk or adjusting their conditions of presence that will be suitable for safe keeping. These are aimed at "inactivating the disease causing organisms and to prolong he shelf life of the milk product" (Ajisegiri 1992). The processing of milk involves the following:

2.5.1 Pasteurization of Milk

Pasteurization is the process by which bacteria are removed from milk by means of special heating process.

In this process, about 0.03-0.04% of hydrogen peroxide (H_2O_2) is added to the milk as soon as possible after milking before the heating process takes place. Another batch of hydrogen peroxide is added after 12-20 hours since the enzymes catalyses in the milk destroys the hydrogen peroxide.

After all these stages, the fat globules of milk rise to the upper surface to form a distinct cream line. The depth of which is often taken as an index of richness of milk quality. The objectives of pasteurization are:

1. To kill all the pathogens present in the milk.
2. To improve the keeping quality of the milk (Harper, 1976).

Where the milk is being pasteurized for making yoghurt, cheese or cream, another purpose of pasteurization is to destroy microorganisms that would interfere with the

activities of desirable organisms such as the starter culture. The methods used in pasteurization include the following:

a) **Holder Method**

This method involves heating milk to 62°C and holding the temperature for 30 minutes (Ajisegiri, 1992). This method is used in batch processing of milk. The pasteurized milk is cooled fast.

b) **High Temperature Short Time (HTST) Method**

In this type of chemical milk separation, milk is held for 15-16 seconds at temperatures between 71.7°C and 75°C through the use of a plate-type heat exchanger, a system in which close temperature control is of critical importance. The product is then cooled rapidly after heating it to prevent the growth of surviving bacteria in the milk.

This brings about 90-99% destruction of bacteria present in the milk, with a very little change in the Lactose and fat constituents. Vitamin C may be substantially destroyed along the process.

c) **Ultra High Temperature Very Short Time (UHTVST) Method**

This involves heating milk to 137.8°C for 1 second. This has, however, been modified to either heating milk to 93.4°C and held for 3 seconds at 149.5°C for 1 second. Milk pasteurized in this way is said to be "ultra pasteurized."

The efficiency of pasteurization depends on:

1. Pasteurization temperature
2. The holding time
3. Total number of bacteria

2.5.2 Homogenization of Milk

Homogenization of milk prevents this decreasing the diameter and size distribution of fat globules, causing the speed of rise to be similar for the majority of globules.

Homogenized milk has a creamier structure, brand flavor and whiter appearance. The processing given to milk may be specific depending on the intended use. For instance, if milk is to be evaporated or condensed, it is sterilized instead of being pasteurized.

2.5.3 Sterilization of Milk

Sterilization is the complete elimination of all microorganisms. The food industry uses the more realist term "commercial sterilization"; a product is not necessarily free of all microorganisms, but those that survive the sterilization process are unlikely to grow during storage and cause product spoilage.

Milk can be made commercially sterile by subjecting it to temperatures in excess of 100°C, and packaging it in air-tight containers. The milk may be packaged either before or after sterilization. The basis of ultra-high temperature is the sterilization of food packaging, then filling into pre-sterilized containers in a sterile atmosphere. Milk that is

processed in this way using temperatures exceeding 135°C permit a decrease in the necessary holding time (2-5 seconds) enabling a continuous flow operation.

2.5.4 Milk Fermentation

Fermented milk is a product prepared by controlled fermentation of milk to produce acidity and flavor to a desired level. The popular products are yoghurt, acidophilus milk, cultured buttermilk, and so on. Fermented milk is very popular throughout the world. Yoghurt is one of the most popular brands of fermented milk that originated centuries ago in Bulgaria. It is now produced and consumed in most parts of the world. Although the consistency, flavor and aroma may vary from one region to another, the basic ingredients and manufacturing are essentially consistent.

Yoghurt is made from milk of various animals, but in most cases, cow and buffalo milk are used. To make a good quality product, raw milk used must be of low bacterial count, free from antibiotics, sanitizing chemicals, mastitis milk and colostrums. The milk also should be free from contamination by bacteriophages

2.5.5 Milk Condensation

In this product, sugar is used to increase the shelf life of condensed milk. Sucrose, in the form of crystals or solution, increased the osmotic pressure of the liquid. This in turn prevents the growth of microorganisms. Milk is heated to 85-90°C for several seconds after it has been clarified and standardized. This treatment destroys osmophilic and thermophilic microorganisms, inactivating lipases and proteases, decreases fat separation and inhibits oxidative changes. This product is similar to the evaporation;

post-evaporation addition is recommended to avoid undesirable viscosity changes during storage. Sugar is added for a final concentration of not less than 45%.

2.5.6 Milk Dehydration

Dehydration refers to the nearly complete removal of water from foods to a level of less than 5%. Although there are many types of driers, spray driers are the most widely used type of air convection drier. It turns out more tonnage of dehydrated products than all other types of driers combined. It is limited to food that can be atomized, that is liquids, low viscosity pastes, and purees. Drying takes place within a matter of seconds at approximately 200°C. Evaporative cooling maintains low product temperatures; however, prompt removal of the product is still necessary.

2.5.7 Milk and Milk Product

a) Powdered Milk

This is milk from which almost all the moisture has been removed. Powdered milk is less expensive and easier to store than fresh milk, but has a disadvantage in that it never tastes quite like the real thing.

Milk is also processed by various drying processes into powders. Whole milk and skimmed-milk powders for human and animal consumption and buttermilk (the residue from butter making) powder is used for animal food. The main difference between production of powders for human or for animal consumption is the protection of the process and the product from contamination. Many people in the world today drink milk

reconstituted from powdered milk because milk is about 88% water, and it is much less expensive to transport.

b) Skimmed Milk

The product left after the cream is removed is called skim, or skimmed milk. Reacting skimmed milk with rennet or with an acid makes casein curds from the milk solids in skim milk, with whey as a residue. In some countries, a portion of cream is returned to the skim to make low fat milk for human consumption. By varying the amount of cream returned, producers can make a variety of low fat milks to suit their local market.

c) Cheese

Cheese making started as a way of preserving milk to a long life product. In a simple definition, cheese is the fresh or ripened product obtained after coagulation and whey separation of milk, cream or partly skimmed milk, buttermilk or a mixture of these. Principally, cheese making involves concentration, preservation and ripening. Milk is pasteurized and cooled t inoculation and setting temperature. An additive like salt is added. Started culture is inoculated using rennet, the curd is cooked, pressed and salted; and the cheese is ripened.

d) Butter

Butter is the fatty product exclusively derived either from cream, milk or milk by-products. Apart from milk fat, it contains some non-fat milk solids, water and, occasionally, additives. In contrast with milk and cream, where the fat globules are

dispersed in the water phase, properly worked butter consists of water dispersed in fat. The continuous phase consists of milk fat in which aqueous droplets, some fat globules and tiny air bubbles are evenly distributed. In more practical terms, butter is a yellowship fatty paste. It is soft and spread able at room temperature and harder if it is cold. It has a mild to slightly acid taste.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Assessment of Fermented Milk

The method for the determination of the fermented milk is into two groups, indirect and direct.

Direct methods are based on colony formation or on direct microscopic counting of the bacteria. Indirect methods include dye reduction tests, estimation of ATP or pyruvate, impedance and radiometry. These methods can be used to indicate milks with high numbers of bacteria.

3.1.1 Methods of Fermentation

Kindirimo is a traditional milk product fermented with *Lactobacillus* and may be prepared using whole or skimmed milk depending on the market demand. The product is well known and recognised for its therapeutic benefits in gastrointestinal tract. The milk heated to high temperature like 95°C for 1 hour or similar temperature-time combinations, basically to reduce the microbial load and also to favour the slow growing starter bacteria. Some milk has acidity as high as 1 percent lactic acid, but for therapeutic purpose, the acidity range of 0.6-0.7 percent is more common. The fermented milk may be sweetened, if demanded by the market.

3.1.2 Quality of Fermented Milk

The acceptability or non-acceptability of a product depends on its quality level, though the psychology of food acceptance also plays a vital role in the choice of food items. However this psychological effect can be played down by the quality attributes of the product (Frazier and Westhoff, 1978). Quality is a peculiar or essential character or

inherent feature, which makes a thing what it is properly. By this, quality can be likened to the degree of excellence, the superiority in kind which defines a social status of a product since it relates to the attribute of an elementary sensation of product that makes it unique. The quality attributes of fermented milk makes it acceptable or by which it induces acceptability include:-

a) Ingredients b) Colour c) Durability

3.2 Materials

1. 1 liters of Kindirimo Milk and Yoghurt (Maizube) drink each.
2. Distilled water
3. Nutrient Agar
4. MacConkey Agar
5. Aluminum foil
6. Cotton wool
7. Nutrient broth
8. Syringe and Needle
9. Masking tape
10. Glucose

3.3 Specific Test

The following tests were carried out in the laboratory for Kindirimo and Yoghurt drink.

3.3.1 pH Determination

i) A quantity of each sample was placed in a container

ii) The pH meter was dipped into a buffer solution and left for five minutes to cleanse the nipples

iii) The terminal nipple was placed in a separate sample of the substrate to be tested and left to be tested.

iv) The meter was then placed on the solution; and it was allowed to stabilize and the pH was measured. This test was carried out for Kindirimo and Yoghurt.

3.3.2 Density Determination

This was determined using the fundamental approach as follows:-

i) An empty density bottle was washed, rinsed and dried.

ii) The mass of empty density bottle

iii) The mass of empty density + sample

3.3.3 Colour Evaluation

This test was subjectively carried out.

3.3.4 Bacteria Count Test

i) 28.g of powdered Nutrient Agar (Oxoid brand) distilled water was sterilized using Autoclave at a temp of 121°C for 15 minutes and then cooled to 45°C.

ii) 56.0g of MacConkey Agar (Oxoid brand) was dissolved in distilled water and the process was repeated as above.

iii) 9.0ml of Normal Saline was dispersed in test tubes, cocked and sterilize at a temperature of 121°C for 15 minutes and was used for several dilutions of Kindirimo and Yoghurt.

iv) Several dilutions of Kindirimo and Yoghurt 1.0ml was plate out (inoculation) from 10^{-2} and 10^{-3} solution using pour plate method.

v) The sample was incubated in the incubator at 37^0C for 24 hours both aerobic and anaerobic incubation.

vi) After incubation, the counting was done.

vii) The glass was washed, dried and sterilized.

After incubation, the counting was done and further analysis was done as follows.

a) Gram Stain: -This is the first test done and this defines the evolutionary trend of bacteria.

i) A smear was made on a slide and it was flooded with crystal violet and allows to stay for 30 seconds.

ii) It was drain and decoloured with absolute ethyl alcohol for 1 minute and then Counter stain with safrananine for another 1 minute.

iii) It was finally washed with distilled water.

iv) It was blot dried with Whatman filter paper and then view at X100 (oil immersion objective) on microscope. Under the microscope, the colour of the cells, the shape and arrangements were noted (this will help in the identification of organisms).

- *Bacillus subtilis*: - Dark bluish colour (colour of crystal violet) and is positive. The shapes were cylindrical (rod) and arrangement scattered (cluster).
- *Echrichia Coli (E Coli)*:- This is reddish in colour, short cylindrical in shape (short rods) scattered (cluster) and is negative.
- *Klebscella spp*: - Reddish in colour as *E. Coli* only that they are longer than *E.Coli* in shape.

b) Catalyse Test: - 3% Hydrogen peroxide was used on clean glass slide and bubbles were observed when mixed with bacteria colony, presence of bubble indicates positive result that is the bacteria has ability to produce gas.

c) Coagulate: - The same procedure as above but blood serum was use to precipitate (coagulation) were observed. Presences of precipitate indicate a positive result.

d) Citrate Utilisation: - Simon's citrate Agar was prepared and inoculates with bacteria colony and incubated at 37°C for 4 days. The initial colour was green and it then changes to blue after 4 days. Incubation indicates positive result.

e) H_2S Production: - Triple sugar Iron Agar was prepared and incubated at 37°C for 24 hours. The initial colour of the medium was red, it then changes to black. This indicates the presence of H_2S.

f) Methyl Red (MR):- Dextrose Phosphate broth was prepared and inoculated and incubated at 37°C for 24 hours. After incubation, 2 drops of methyl red reagent was added to the culture floating. The reagent on growth culture indicates positive result. If the reagent was mixed, it indicates negative result.

g) Voges Prauster Test (VP):- 2 drops of dextrose Phosphate and 3.0 ml of alcohol α-napthol were added to the grown culture. It changes from dark purple colour to light pink colour.

h) Urease Test: - Kovac's reagent was used on a grown culture in Nutrient broth. It changes from dark brown to red which indicate positive result. No change in colour indicates negative result.

i) Oxidase Test: - Oxidase reagent was used. 2 drops of the reagent was placed on a Whatman Filter paper inside a clean pietredish, the bacteria colony was rubbed on the

position, a change in colour from purple to bluish purple colour indicate a positive result. No change in colour indicates negative result.

j) CHO Utilisation (Sugar Fermentation): - 1% of sugar was dissolved in Nutrient broth containing phenol red indicator. Sterilized at 121°C for 15 minutes and then used. This was done for each sugar separately. After preparation, they were inoculated and incubated at 37°C for 24 hours. Initial colour red, changes to yellow indicates positive result. No change in colour indicates negative result.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 Results

4.1.1 Result on pH Determination

The purpose of determining the pH is to confirm that fermented milk has been produced. Milk in its natural state is expected to have pH of 6.6 to 6.7, (Artherton and Newlander, 1987). Kindirimo has it pH as 4.95 while Yoghurt is 5.75.

4.1.2 Result on Density Determination

Determination of density for Kindirimo is as follows:

Capacity of the Density bottle = 50 cm^3

Mass of empty density bottle = 12.11g

Mass of empty density bottle + sample = 63.22g

Difference = 63.22 – 12.11

= 51.11g

Density = Mass/Volume

= 51.11/50

= 1.022g/cm^3

Density = 1022kg/m^3

Determination of density for Yoghurt is as follows:

Capacity of the Density bottle = 50 cm^3

Mass of empty density bottle = 12.11g

Mass of empty density bottle + sample = 68.21g

Difference = 68.21 – 12.11

= 56.10g

Density = Mass/Volume

= 56.10/50

= 1.122g/cm^3

Density = 1122kg/m^3

The change in densities of Kindirimo and Yoghurt processing could be linked with partial coagulation during incubation. More so, since the added starter culture is a collection of bacteria, they will grow in a substrate such as milk; especially under the incubating temperatures of 45°C. The growth in bacteria and the resultant massive growth in milk solid constituent will increase the mass per unit volume.

4.1.3 Colour Test

Colour test shows that Kindirimo is dirty white in colour. No much can be said about the observed colour. However, the change in colour from its expected bluish-white could, like other changes, be attributed to temperature change. As a result of the temperature change, certain contents are broken down chemically. This could alter the milk that is processed. The whitish colour in Yoghurt is due to homogenization effects.

4.1.4 Bacteria Count Test

Table 4.1 Total Viable Count (NA) or plate count

Sample	10^{-2}	10^{-3}	Av. CFU/ml
Kindirimo	763	592	5.9×10^6

Table 4.2 Coliform count (MCA)

Sample	10^{-2}	10^{-3}	Av. CFU/ml
Kindirimo	478	314	1.9×10^{6}

KEY:

10^{-2} and 10^{-3} are the dilution factors

NA = Nutrient Agar

MCA= MacConkey Agar

AV = Average

CFU = Colony Forming Unit = S.I unit of microbial counts.

ml = Milliliters

Table 4.3 Characterization and Identification

G. Stain	Catalyse	Coagulate	Urease	Citrate	MR	VP	H_2S	Indole	Oxidase	CHO Utilisation				Organism
										S	M	L	G	
+R	+	-	-	+	-	+	-	-	-	+	-	-	-	*Bacillus Subtilis*
- R	-	-	-	+	+	-	-	+	-	+	-	+	+	*Echrichia Coli*
- R	-	-	-	-	-	-	-	-	-	+	-	+	+	*Klebsiella Spp*

KEY:

+R = Gram Positive rod

-R = Gram Negative rod

+ = shows positive reaction with the test

- = shows negative reaction with the test

CHO = Carbohydrate Utilisation

S = Sucrose Sugar

L = Lactose Sugar

G = Glucose

M = Menthol Sugar

MR = Methyl red Test

VP = Voges Prausker Test

H_2S = Hydrogen Sulphide Production Test.

For Yoghurt, there was no microbial multiplication.

The Viable and Coliform count are very high. The organisms isolated are aerobic bacteria; it does not contain anaerobic bacteria and also fortunately does not contain Salmonella bacteria that cause typhoid fever (a killer disease) most common now among the middle belt.

The organisms isolated are:

i) *Echrichia Coli* (*E. Coli*): - It causes gastro entities, vomiting, nausea etc.

ii) *Klebsiella spp*: - Some of the species causes serious pneumonia and some species cause complication to ulcer patients.

iii) *Bacillus Subtilis*: - This organism is less pathogenic it is common every where in the air but can cause complication if the body immunity is depressed. The accumulation in the human body under a disease condition can be a problem especially during the activities of cell multiplication, Sporolation etc.

Table 4.4 Summary of Test on Kindirimo and Yoghurt

Test	Kindirimo	Yoghurt
pH	4.95	5.75
Density	$1022kgm^3$	$1122kgm^3$
Colour	Dirty-White	White
Bacteria Count Test	Bacteria found *(Bacillus Subtilis, Klebsiella spp, Echrichia Coli)*	Bacteria not found

4.2 Discussion of Result

Kindirimo is a by-product of fresh cow milk. A fresh cow milk is suppose to be fresh except otherwise if the cow as been depressed with some pathogens as a result of disease condition.

pH could be explained as due to time between collection and analysis which could result in decrease in the pH i.e. it makes it acidic. The temperature variation between the point of collection and of analysis could affect the pH of the milk.

The results obtained from the microbial analysis of Kindirimo and Yoghurt show that Kindirimo was contaminated with microorganisms of public health concern. The high total bacterial and coliform count in Kindirimo may be a consequence of the low level of hygiene maintained during the processing and sale of the products. This includes the handlers, quality of water used and the utensils. During the sale of Yoghurt, clean hands and other equipments were sterilized so as to avoid contamination of their final product from the production point to final consumers. The exposure of both Kindirimo while it is displayed for sale in bowls can serve as source of contamination. The detection

of *Bacillus Subtilis*, *Klebsiella,* and *Echrichia Coli* species in Kindirimo as the case may be, indicates possible faecal contamination. Being enteric bacteria, their presence indicates poor hygienic practices among handlers of Kindirimo. Due to the significance of the faecal-oral route transmission for many bacterial food-borne diseases, basic hygiene measures assume a decisive importance in food safety management

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The pH for the raw milk, in agreement with literature provisions, show slightly acidic state, for Kindirimo was highly acidic. This is also as expected. It was discovered that, since absolute control of temperature changes could not be achieved; the acidity of Kindirimo may go beyond expected values. It is therefore pertinent to have absolute control over temperature.

5.2 Recommendations

Use sterile, clean container for production of Kindirimo and it should be done under aseptic condition.

Fresh milk collected should be pasteurized before usage for anything.

Marketing of fresh bi-product like Kindirimo like Fulani should be done in a good sanitized condition.

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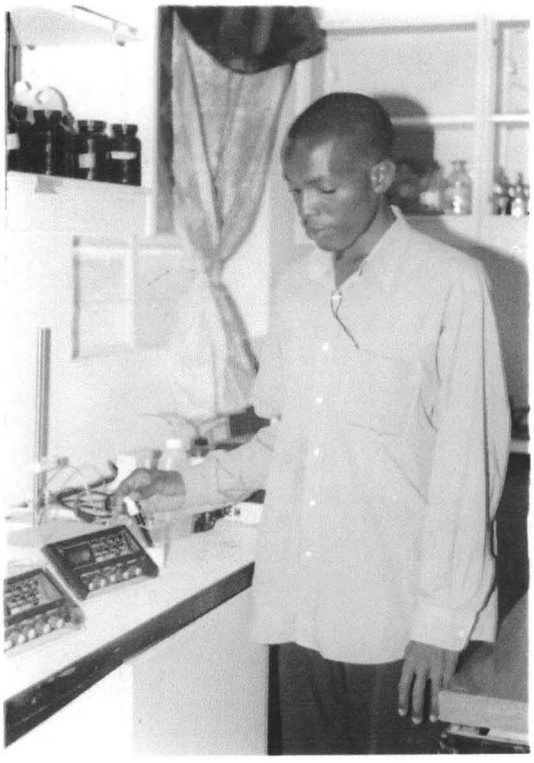
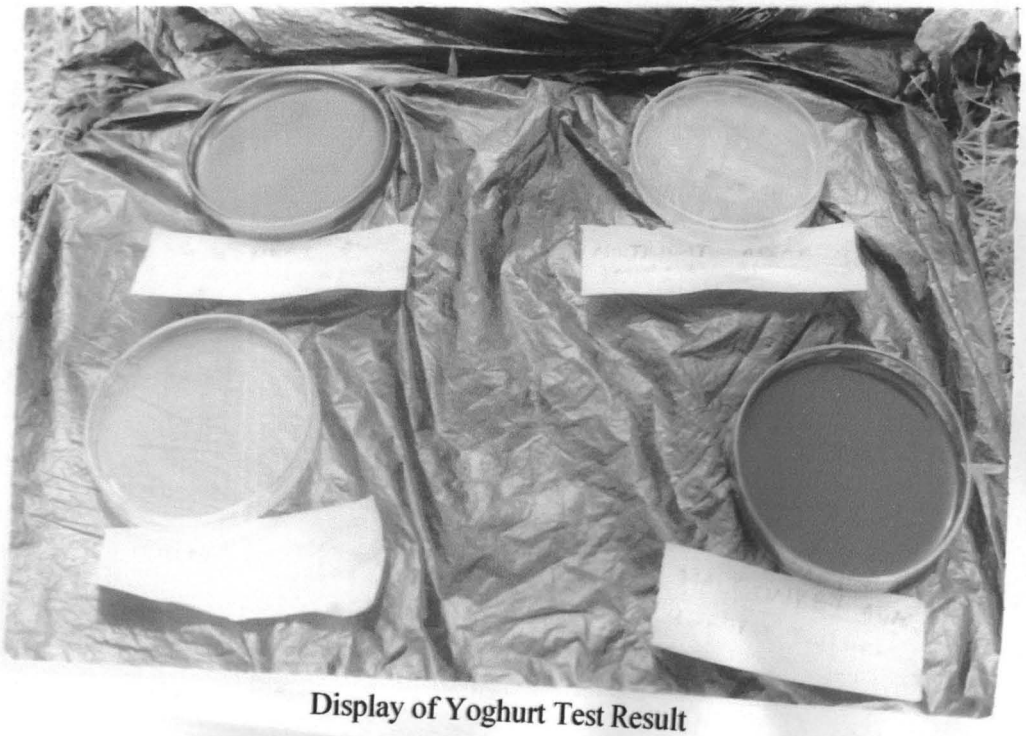
APPENDIX A

Fresh Kindirimo Milk

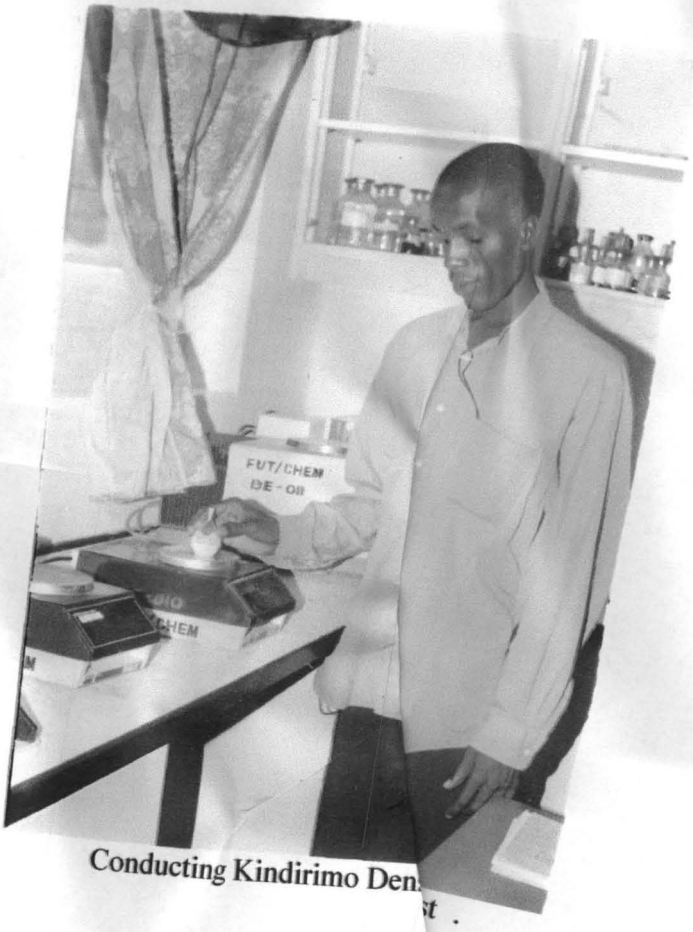


Conducting Kindirimo pH test.

APPENDIX B



Display of Yoghurt Test Result



Conducting Kindirimo Den st .