KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY

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MICROBIAL AND CHEMICAL PROCESSES ASSOCIATED WITH *BURUKUTU*, A GHANAIAN FERMENTED ALCOHOLIC BEVERAGE

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A Thesis Submitted to the Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology, Kumasi, in Partial Fulfilment of the Requirement for the Degree of Master of Philosophy in Microbiology.

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DECLARATION

I hereby declare that this submission is my own work towards the Master of Philosophy (Microbiology) and that, to the best of my knowledge; it contains no material(s) previously published by another person(s) or material(s), which has been accepted for the award of any other degree of the university, except where due acknowledgement has been made in the text.

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DEDICATION

This work is dedicated to the ALMIGHTY GOD, to my dear husband Mr. Isaac Atter, my sons Emmanuel and Ivan, and my late brother, Prince Odoom.

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My special thanks go to the Almighty God for his grace, mercy and favour to carry out this project work.

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To all those whose names were not mentioned, remember that there is virtue in silence.

ABSTRACT

To identify and enumerate the different microorganisms involved in the fermentation of *Burukutu*, a traditional fermented sorghum beverage and the chemical changes associated with the fermentation. Information on the different methods used in its production were also assessed and documented amongst the processes through a questionnaire survey and personal interviews. At each stage of the production process, microorganisms involved were enumerated, isolated and identified and also the chemical changes associated with the process. The keeping quality of *Burutuku* was also studied. Results showed that there were no differences in the processing procedures amongst the *Burukutu* producers within the Greater Accra region. Types of microorganisms involved in the fermentation were predominantly lactic acid bacteria; *Lactobacillus fermentum, L. plantarum, L. acidophilus, Lactococcus lactis* subsp.*lactis* and *Lactobacillus brevis,* which were mainly associated with the acidification process and the yeasts; *Saccharomyces cerevisiae* and *Candida krusei* that brought about the alcoholic fermentation. Of the LAB isolates, *Lactobacillus fermentum, L. plantarum* and *Lactococcus lactis subsp.lactis* showed amylolytic properties. LAB isolates had antimicrobial activities against *Staphylococcus aureus, Escherichia coli* and *Salmonella typhimurium*. During the fermentation, *Burukutu* microbial numbers (log_{10} CFU/ml) varied between 6.66 to 8.14 for lactic acid bacteria and 6.82 to 8.18 for yeast. Similarly, pH, titratable acid, soluble solids and alcohol level of *Burukutu* during the fermentation varied between 3.36 to 2.88, 0.54 to 0.82; 7.5 to 3.33 and 0.99 to 4.47, respectively. *Burutuku* keeps best for up to twelve weeks if glass bottled, pasteurized and stored in a climatic chamber.

This being the first major study of *Burukutu* in Ghana, it could be used to improve and upgrade the traditional production process.

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1.0 INTRODUCTION

Brewing of alcoholic beverages is a traditional process carried out in almost all African countries. These traditional beers are produced from sorghum, millet and maize and differ from European beers in that they are opaque and have a thick consistency because they are not filtered clear, are sour, no hops are added during processing hence do not have a bitter taste. They are consumed in an active state of fermentation since no attempt is made to arrest fermentation, making them effervescent. They are therefore generally called African opaque beers and have played an important part in the diet of the African people for thousands of years. Generally, the basic unit of operation include malting, mashing, straining, souring, boiling, and alcoholic fermentation, but procedures vary from place to place and even from producer to producer. The African opaque beers include *Gwalo* in Congo, *Bouza* in Ethiopia, *Busaa* in Kenya and Uganda, *Merissa* in Sudan, *Pombe* or *Bwalwa* in Tanzania, *Bojalwa* ja *Setswana* in Botswana, *Yalwa* or *joule* in Lesotho and South Africa, *Utshwala* or *Tswhala* in South Africa, *Zezuru* in Zimbabwe *Pito* and *Burukutu* in Nigeria and Ghana, *Chibuki* in Zimbabwe, *Dolo* in Burkina Faso, Cote d'Ivoire, Mali, Niger, Togo and *Tchapalo* in Cote d'Ivoire (Sefa-Dedeh *et al*., 1999; Faparusi *et al*., 1973; Togo *et al*., 2002; Sawadogo-Lingani *et al*., 2007; N'Da and Coulibaly, 1996).

In most African countries where sorghum is grown, a traditional fermented beverage drink is produced from it called sorghum beer (N'Guessan *et al*., 2009). These beverages bear different names depending on the country of origin. In Ghana, the two main sorghum beers are *pito* and *burukutu* with *pito* being the most common as its production and consumption is on a wider scale. *Burukutu* on the other hand is a lesser known alcoholic beverage but also enjoys a lot of patronage.

Burukutu, is a popular alcoholic beverage of a vinegar-like flavor prepared from sorghum grains and fermented guinea corn and consumed in the Northern Guinea Savanna region of Nigeria (Haard *et al.*, 1999). It is also prepared in the Benin Republic and Ghana (Odunfa, 1985). In the Southern regions of Ghana, it is one of the most popular alcoholic beverages in the poor urban neighborhoods where its consumption is limited to the low and middle income groups because it is more affordable than commercially brewed beer. It is often consumed as food because it is thick and heavy. The producers of *burukutu* are overwhelmingly women from the 'Loso' tribe in Togo with very few indigenous Ghanaians involved and this serves as a source of livelihood for them.

According to Banigo *et al.*, (1987), in Nigeria, *burukutu* is traditionally prepared by cleaning the sorghum grains prior to steeping in water at ambient temperatures in an earthenware pot for one day to soften the grains. This is followed by draining of the soaked grains in baskets for about 1 h. The steeping water is usually discarded. The grains are spread out on mats up to about 7.5-10 cm deep and then covered with mats. During this malting period, the grains are watered on alternative days and occasionally turned over. Germination, which begins within 24 h after steeping, is allowed to continue for a period of 3-4 days. The malt is then spread out in thin layers to dry in the sun for 1 or 2 days, and grinded into flour. The grinded sorghum malt is then mixed with water, cooked for about 4 h and blended with a mixture of 'kpokpo-gari' (large, coarse, starchy granules made from cassava tuber) and water. The mixture (gari-malt powder-water in the ratio 1:2:6) is left to ferment for one day in covered pot. It is then filtered through a fine cloth and ready for consumption. According to Faparusi (1970), the fermentation process mainly involves lactic acid bacteria and has an alcohol

content of 2-4% (v/v), the presence of yeast have also been confirmed (Oke and Ijeboor, 1997; Jideani and Osume, 2001). It is a nutritious beverage containing vitamins, iron,

manganese, magnesium, potassium, phosphorus, calcium, carbohydrate and protein (Egemba and Etuk, 2007; Eze *et al.*, 2011).

In Ghana, the process of production also involves malting, mashing, fermentation and maturation just as is reportedly done in Nigeria (Ekundayo, 1969). The fermentation process of this beverage just like many other traditional fermentation processes occur spontaneously and are difficult to control. The processing is however slightly different from what is done in Nigeria in that 'kpokpo-gari' (large, coarse, starchy granules made from cassava tuber) is not added and the malted grain is not spread out in thin layers to dry in the sun before grinding into flour. A number of detailed research have been carried out on *burukutu* produced in Nigeria (Ekundayo, 1969; Faparusi, 1970; Faparusi *et al.*, 1973; Banigo *et al.*, 1987; Obot, 2000; Kolawole *et al.*, 2007; Yabaya and Jatau, 2009; Eze *et al.*, 2011). In Ghana, extensive work has been done so far as microbial and chemical changes are concerned with the other sorghum based traditional alcoholic beverage *pito*, and there are publications in the international literature on this product (Glover *et al.*, 2005; Demuyakor and Ohta, 1991; Sefa-Dede *et al.*, 1999). However, the same cannot be said in the case of the lesser known *burukutu*. In view of this and the fact that slight differences exist in the methods which may affect the micro-flora responsible for the fermentation, there is the need to conduct a detailed study to investigate the process for producing *burukutu* in the Accra and Tema metropolis of the Greater Accra region as well as the microbiological and chemical changes that are associated with the fermentation process. This may help to optimize the production process and add to the end product. Extension of shelf life could also be achieved.

1.1 General Objective

This study was carried out to identify and enumerate the different microorganisms involved in the fermentation of *burukutu,* a traditional fermented sorghum beverage and the chemical changes associated with the fermentation.

1.2 Specific Objectives

- Evaluate the different processing methods used in the production of *burukutu*.
- To enumerate, isolate and identify the dominant microbial species involved in the *burukutu* fermentation including lactic acid bacteria and yeast.
- To investigate some technological properties involved in the fermentation of *burukutu.*
- To evaluate the amylolytic activity of the grain, malted grain and mash.
- To measure the variations in pH, titratable acidity, soluble solids, and percentage alcohol in *burukutu*.
- To explore various ways of prolonging the shelf life of *burukutu***.**

2.0 LITERETURE REVIEW

2.1 Sorghum

Sorghum (*Sorghum bicolor* (L.)Moench) is a cultivated tropical cereal grass and is generally, thought to have first been domesticated in North Africa (possibly in the Nile or Ethiopian regions) around 1000 BC. Sorghum makes up the genus *Sorghum* in the family *Poaceae* (or *Gramineae*). There are many subspecies of sorghum which are divided into four groups namely grain sorghum such as milo, grass sorghum used for pasture and as hay, sweet sorghum formerly called "Guinea corn", which develops a sweet juice in their stalks and are grown for syrup production, and broom corn for brooms and brushes (Kimber, 2000). According to Demuyakor and Ohta (1992), out of the many subspecies of sorghum, grain sorghum is the predominant one grown mainly for food in Ghana and Africa as a whole. Grain sorghums are classified as *Sorghum bicolour* (with many cultivars) and there is also *Sorghum vulgare* (Okorie and Oke, 2003). There are many varieties of *Sorghum bicolour*, ranging in colour from white through red to brown and mixed classes in the grain standards. (Rooney and Waniska, 2000).

As a world food grain, sorghum is ranked fifth (Pomeranz, 1987). The annual world production is over 60 million tonnes, of which Africa produces about 20 million tonnes (FAO, 2003). Sorghum is an important staple food crop serving over 400 million people in the semi-arid regions of the world (Andrew and Bramel-Cox, 1993). Due to its climatic conditions, it is cultivated mainly in Northern, Upper East and Upper West regions of Ghana as well as areas with low rainfall patterns. Several varieties of sorghum are available in Ghana with the most common varieties on the market being *Chere*, *Dorado*, *Latuor* and *Kapaala*. In many parts of the world including Africa, it is processed into a very wide variety of attractive and nutritious traditional foods, such as semi-leavened bread, cookies, cakes,

couscous, dumplings, fermented and non-fermented porridges. It is the grain of choice for brewing traditional African beers as well (Taylor, 2003; Taylor and Dewar; 2000, Carter and Capenter, 1981).

Starch is the major storage form of carbohydrate in sorghum. It consists of amylopectin, a branched-chain polymer of glucose, and amylose, a straight-chain polymer. With values ranging from 56 to 73%, the average starch content of sorghum is about 69.5% (Jambunathan and Subramanian, 1988). Sorghum is also a good source of lactobacilli, which is used in souring of foods, used in traditional fermented foods and drinks (USDA, 2005).

2.2 Traditional Alcoholic Beverages

The consumption of traditional beverages was widespread during the pre-colonial period and exhibited several unique features (Bryceson, 2002). These include alcoholic and non-alcoholic beverages, which are mainly cereal-based (Nout and Sarkar , 1999; Gqaleni *et al*., 1998; Katangole , 2008). First, the indigenous drinks were fermented, not distilled. Sorghum and millet beers and palm wine dominated, generally ranging between 2% and 4% of alcohol by volume (McCall, 1996). These traditional beverages are usually processed by women using local raw materials by methods inherited from past generations. These beverages play an important role in the nutritional, economic, daily, social, and cultural life of its people. Alcohol production and consumption was traditionally organized around traditional rituals, festival and other social activities which include marital arrangements, child-naming, settling of quarrels between individuals and between families and communities and also as expression of hospitality to visiting guests. Traditional alcoholic beverages feature prominently in many

traditional Ghanaian cultural groups. The main traditional alcoholic beverages in Ghana are: *pito, palm-wine, burukutu,* and *akpeteshie.*

Pito is a golden yellow to dark brown in colour with taste varying from slightly sweet to very sour. It contains lactic acid, sugars, amino acids, 2-3% alcohol and some vitamins and proteins. There are four types of *pito* in Ghana: *nandom, kokomba, togo* and *dagarti.* The peculiar characteristic of each *pito* lies in the differences in their wort extraction and fermentation method (Akyeampong, 1995; Sefa-Dedeh, 1999).

Palm-wine (*nsafufuo*) is widely consumed all over Ghana and is produced from sugary palm saps. The most frequently tapped palms are raffia palms and the oil palm. The sap of the palm tree according to Amoa-Awua *et al.,* (2006) is tapped and allowed to undergo spontaneous fermentation, which allows the proliferation of yeasts species to convert the sweet substrate into an alcoholic beverage.

Akpeteshie (local gin) is distilled from fermented palm wine or sugar-cane juice, and requiring a simple apparatus of two tins and copper tubing. The standardized alcohol strength of *akpeteshie* today is between 40-50% by volume (Akyeampong, 1996).

According to Akanidomo *et al.,* (2005) alcohol consumption has a long history in Nigeria for example, and its use is common in all the cultural groups in the country. It is one of the most available psychoactive substances in the country, and it is consumed in the form of traditional beverages (e.g., *burukutu, pito, emu funfun, ogogoro*). The percentage content of alcohol in different alcoholic beverages vary, ranging from 2-4% in *burukutu,* 2-8% in beer and palm-wine, and 30-60% in *ogogoro,* gin and brandy (Ababio, 1990). Every African country has a

traditional alcoholic beverage produce there. Some include cashew wine from Mozambique, the *t'ädj* which is a typical Ethiopian honey wine and *chang'aa,* the most predominant non-

commercial beverage among Kenya's poor and made from a variety of grains etc (ICAP, 2008).

2.3 African Opaque Beers

The history of beer making is as long as the history of humankind. All over Africa, different types of traditional beers are produced using cereals such as sorghum, millet and maize which are referred to as African opaque beers (Nanadoum and Pourquies, 2009). According to Dirar, (1993) the early Egyptian beer were probably similar to some of the traditional opaque beers found in various African countries today. *Merissa*, a traditional Ethiopian beer for example, has its consumption dating as far back as the 7th century BC. The brewing of these indigenous sorghum beers according to Pattison *et al.*, (1998), involves malting, souring, boiling, mashing, straining and alcoholic fermentation. According to Briggs *et al.*, (2004) the term `sorghum beers' is inexact as sorghum (raw grain or malt) is sometimes wholly or largely replaced with maize or millets or wheat or barley, and indeed in some cases bananas or manioc (cassava) serve as starchy adjuncts. Daiber and Taylor (1995) described opaque beer as a product of a lactic and alcoholic fermentation, which is sold in a microbiologically active state, with a shelf life of only 5-7 days. Other attributes include low alcohol content, acidic nature, suspended solids and a characteristic taste and colour (Chitsika and Mudimbu, 1992). Sorghum beer is characterised by its opacity due to the suspended particles of cereals, starch and yeast and it sourness (Dewar and Taylor, 1999). The ingredients and procedure for making these beers differ from place to place. For this reason, the beer characteristics such as

taste, colour and nutritional value vary. Some indigenous African beers made from malted sorghum include *burukutu, otika, borde, kaffir, bouza, pombe, shukutu, shakparo, amgba, dolo, tchapalo, kunun-zaki*, and *pito* (Egemba and Etuk, 2007). These beers provide their consumers with significant amount of valuable nutrients such as calories, proteins, minerals,

vitamins and others (Nanadoum and Pourquies, 2009). As a result of its low alcohol content and the large quantity of suspended solids, many consumers consider these sorghum beers to be more of food than just a beverage (Pattison *et al*., 1998).

2.3.1 Major differences between African and European Beers

African types of beers differ from European (lager) types in that lactic acid fermentation occurs during the processing, it is consumed while it is actively fermenting and contains large amounts of insoluble materials (Rooney and Serna-Saldivar, 1991). Other differences between the two include African beers are rarely or never flavored by herbs (in contrast to hopped European-style beers); complete starch conversion is avoided, and brewing does not produce an excess of yeast (Briggs *et al*., 2004). It is largely consumed by the poorest, and significantly contributes to the diets of millions of people and generates income for the women sellers who produce it at household level using the traditional technology. Other cereals such as millet or maize can be used as adjunct or as substitutes (Kayode *et al*., 2005). They are often less carbonated, have no hops and are consumed unrefined, including unfermented substrates and microorganisms (Haggblade and Holzapfel, 1993).

2.4 Traditional Method of *Burukutu* Processing in Ghana

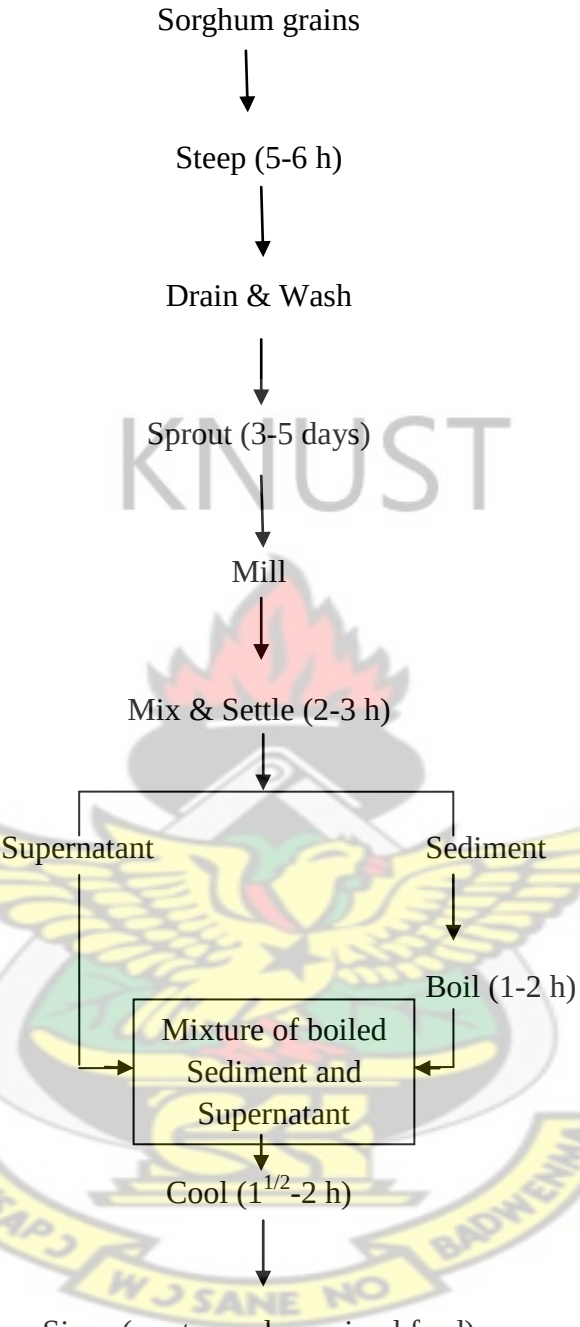
Burukutu is a traditional indigenous brown cloudy alcoholic beverage produced from sorghum grains and mostly produced at household level. It has an opaque colour due to

suspended solids. The production process is time consuming, complex and sometimes carried out under unhygienic conditions. The grains are steeped in water for 5-6 h. The grains are then poured in a basket to drain off the excess water without necessarily washing it. They are then spread on mats or in aluminum basins in a cool dry area to germinate. They are turned over intermittently to enhance the germination process. The germination last between 3-5

days depending on the humidity. The fresh malt is milled to obtain the flour. The flour is mixed with water in a ratio of about 1: 3 by stirring with the hand or a wooden ladle. The resulting mixture is allowed to settle for 2-3 h.

The supernatant is separated from the sediment and the latter cooked for 1-2 h after which it is mixed again with the supernatant to give the wort. The wort is allowed to cool, stirred and sieved with the waste used as animal feed. The sieved wort is left for 5-7 h for fermentation into sour wort or until it has assumed a slightly sour flavor. This is then cooked for 1-3 h to concentrate, sieved, cooled and then an old fermented batch of a previous brew is added to serve as an inoculum (back slopping). Back slopping is usually done to hasten the fermentation process. The mixture is allowed to ferment at room temperature for at least 2-8 h depending on the quantity of the inoculum added before selling. *Burukutu* has a very short shelf life and is expected to be consumed within 5 days after back slopping. It can however stay much longer (about a week or two) if not back slopped and tightly covered. Souring of the left over beverage after 5 days is usually overcome by mixing it with a freshly prepared one to make it fresh again for consumption, thus nothing goes waste. The unit operations in the traditional preparation of *burukutu* in Ghana are shown in the flow chart and pictorial presentation below.

FLOW CHART FOR *BURUKUTU* PROCESSING



Sieve

↓

Old brew added for fermentation (back slopping)

↓

Mature

Figure 1: Flow chart for the main steps involved in *Burukutu* processing

2.4.1 Pictorial Presentation of *Burukutu* Processing

Steeped sorghum grains

Draining of water from the grains

Malted sorghum grains

Milling the malted grains

Mixing the flour with water

Stirring with a wooden laddle

Separating the supernatant of the mash from the sediment after settling

Cooking the sediment of the mash

Fetching the cooked sediment

Addition of the cooked sediment to the supernatant

Sieving the lighter part of the mixture

Sieving the course part of the mixture

Mixture allowed to acidify

Cooked acidified mixture

Final sieving of *burukutu* *Burukutu* in pans

2.4.1 Reasons Why People Consume *Burukutu*

The production and consumption of this beverage is widespread among poor rural and urban population of the society. For this reason, the consumers are usually people of the lower socioeconomic brackets with low income and education. They consume this beverage because it is affordable; one does not feel intoxicated when the freshly prepared batch is consumed. However, there are cases where people place special requests for batches that have fermented for 72 h or more because of increase in the alcoholic content. It is heavy and so it is drank as a low-cost meal at any time of the day.This beverage is popular with people of all ages. Children, pregnant women and people presenting with malaria or fever symptoms are sometimes served with the unfermented beverage.

2.5 Fermentation of Foods

Jashbhai *et al*., (2008) described fermentation as a process, consisting of the transformation of simple raw materials into a range of value-added products by utilizing the phenomena of the growth of microorganisms and their activities on various substrates. This means that knowledge of microorganisms is essential to understand the process of fermentation. Fermented foods are defined by Campbell-Platt (1987), as 'those foods that have been subjected to the action of microorganisms or enzymes so that desirable biochemical changes cause significant modification in the food'. Fermentation depends on the activities of microorganisms to produce metabolites that can suppress the growth and survival of unwanted microflora in foodstuffs and allow desirable end products such as diacetyl,

acetaldehyde and vitamins to be produced (Ross *et al*., 2002). Fermentation is one of the oldest and most important methods of processing foods all over the world. In Ghana and most African countries, fermenting food as part of traditional food processing and preservation is extensively practiced. Generally, fermented foods constitute a substantial part of the basic

diet of many people and are also seen as a means of introducing variety into the consumption of staples such as cassava, maize and fish. Fermentation provides a natural way to reduce the volume of the material to be transported, to destroy undesirable components, to enhance the nutritive value and appearance of the food (Simango, 1997). Fermentation is known to soften food texture and alter its composition in such a way that it will require minimal energy both in cooking and preservation process (Nout and Motarjemi, 1997). It is also a means of improving sensory quality and acceptability of raw materials to such an extent that several foods are preferred in a fermented state (Steinkraus, 1997). It also leads to a general improvement in the shelf life, texture, taste and aroma of the final product. Traditional fermentations are usually labour-intensive, integrated into village life, familiar, utilize locally produced raw materials, inexpensive, have barter potential and the subtle variations resulting, add interest and tradition to local consumers. Small-scale food fermentation provides a form of employment for some people especially those that have unique local know-how (Wood, 1994). Traditionally, food fermentation in Africa is spontaneous and these spontaneous fermentations are difficult to control; are not predictable in terms of length of fermentation and quality of product; can produce unwanted products or products with a short shelf life and are sometimes not safe since they are liable to contamination by pathogens (Novellie and De Schaeprijver, 1986; Tamime, 1990; Nout, 1992). The microorganisms responsible for this type of fermentation may either be present naturally on the raw materials or as a contaminant. Most products such as cereals, legumes, root crops, fish and meat are fermented

spontaneously into a wide variety of foods consumed all over the continent. Substrates such as sorghum, maize and millet are fermented to produce a wide variety of foods such as *kenkey, banku, mahewu uji,injera* and *koko* in ways that differ from region to region (Odunfa, 1985). Fermented foods contribute to about one-third of the diet worldwide (Campbell- Platt, 1994). In view of the fact that indigenous fermented foods are widely consumed in Africa a

number of research has been carried out on them (Amoa-Awua *et al*., 2005; Sefa-Dedeh *et al*., 2004; Mugula *et al*., 2003; Omafuvbe *et al*., 2000). Although cereals according to Blandino *et al*., (2002), are deficient in some basic components (e.g. essential amino acids), fermentation may be the most simple and economical way of improving their nutritional value, sensory properties, and functional qualities. According to Odunfa (1985), African fermented foods can be grouped as follows:

- Fermented non-alcoholic starchy foods e.g. *Ogi, gari.*
- Alcoholic beverages e.g. *Burukutu*, kaffir beer, palm wine.
- Fermented vegetable proteins e.g. *Iru, ogiri.*
- Fermented animal proteins e.g. *Nono, momoni*.

2.6 Fermentation Process

According to Bibek (2004), Foods can be fermented in three different ways, based on the sources of the desirable microorganisms: natural fermentation, back slopping, and controlled fermentation.

1. Natural Fermentation

Many raw materials used in fermentation (usually not heat treated) contain both desirable and associated microorganisms. The conditions of incubation are set to favour rapid growth of the

desirable types and no or slow growth of the associated (many are undesirable) types. A product produced by natural fermentation can have some desirable aroma resulting from the metabolism of the associated flora. However, because the natural microbial flora in the raw materials may not always be the same, it is difficult to produce a product with consistent characteristics over a long period of time. Also, chances of product failure because of growth of undesirable flora and food borne diseases by the pathogens are high.

2. Back Slopping

In this method, some products from a successful fermentation are added to the starting materials, and conditions are set to facilitate the growth of the microorganisms coming from the previous product. This is still practiced in the production of many ethnic products in small volumes. Retention of product characteristics over a long period may be difficult because of changes in microbial types. Chances of product failure and food borne diseases are also high.

3. Controlled Fermentation

The starting materials (may be heat treated) are inoculated with a high population (10^6CFU/ml or more) of a pure culture of single or mixed strains or species of microorganisms known as a starter culture. Incubation conditions are set for the optimum growth of the starter cultures. Large volumes of products can be produced with consistent and predictable characteristics each day. Generally, there is less chance of product failure and food borne diseases. However, there may be no growth of desirable secondary flora. As a result, a product may not have some delicate flavour characteristics.

2.7 Alcoholic Fermentation

Fermentation products may be grouped into two general categories: alcoholic fermentation products and acidic fermentation products. Alcoholic fermentation occurs in yeast species that have metabolic pathways for converting pyruvic acid to ethanol (Talaro and Talaro, 1993). Alcoholic fermentation, the conversion of the principal sugars glucose and fructose, to ethanol and carbon dioxide is conducted by yeasts of the genus *Saccharomyces*, generally *S. cerevisiae* and *S. bayanus* (Boulton *et al.*, 1996). Alcoholic fermentation ecosystems are dominated by one genus of yeast: *Saccharomyces*. Beers are produced by fermenting maltose from malted grains whilst wines are produced by fermenting sugary fruit juices. Species of

Saccharomyces are ambient in the environment, and are present on the skins of fruits such as ripe grapes (Holloway *et al.*, 1990). Sugar concentration is reported to affect the yield of alcohol produced (Maiorella *et al.*, 1981). Usually the substrates for alcoholic fermentation include diluted honey, sugarcane juice, palm sap, fruit juices, germinated cereal grains or hydrolyzed starch all of which contain fermentable sugars that are rapidly converted to ethanol in natural fermentations by yeasts in the environment. Nearly equal weights of ethanol and carbon dioxide are produced and the carbon dioxide flushes out residual oxygen and maintains the fermentation anaerobic. The yeasts multiply and ferment rapidly and other microorganisms most of which are aerobic cannot compete. The ethanol is germicidal and, as long as the fermented product remains anaerobic, the product is reasonably stable and preserved (Steinkraus, 2002).

2.8 Malting

Malting involves germination of the grain until the food store (endosperm), which is available to support the development of the germ of the grain, has suffered some degradation from enzymes (O'Rourke, 2004). To commence malting the grains are steeped in water to increase

their moisture content to a level at which they are capable of germination (normally 42-46%). Too little water imparted to the grains at this stage produces weak embryo development and poor extract in the brewery. Too excessive hydration produces too much extract and even death of the embryo. Traditionally, germination would be carried out on floors with a bed grain depth of about 20 cm. As germination proceeds metabolic heat is produced and this is dissipated by regular (twice per day) raking of the beds with shovels. Raking also prevents matting of the rootlets and carbon dioxide build-up. Sorghum is particularly prone to fungal infection during malting and some of the natural surface flora fungi are aflatoxin-producing. It has also been shown that malted sorghum contains insufficient diastatic or amylolytic

power (Hornsey, 1999). During malting of sorghum grains, starch is hydrolyzed into fermentable sugars mainly by amylolytic organisms capable of hydrolysing starchy constituents (Michodjehoun-Mestres *et al.*, 2005; Achi, 1990). According to Briggs *et al.*, (2004), the main objectives of malting sorghum are to generate the enzymes needed for mashing and to supply sufficient soluble nitrogen to support the lactobacilli during souring and the yeast during the fermentation. The ideal malting temperature for sorghum is reported to be around 30°C and this was attributed to the tropical nature of sorghum (Agu and Palmer, 1996, 1997, 1998). It is this high malting temperature for sorghum that has been reported to facilitate the growth of aflatoxin producing *Aspergillus flavus*. Ogbonna, (2007) reported that enzyme activity is highest during the early stages of germination since the first 2-3 days coincide with the movement of the growth hormone (gibberellins). The germination process produces enzymes which hydrolyses starch and proteins to sugars and amino acids. Proteolytic enzymes improve amino acid availability, particularly lysine, tryptophan and methionine that are lacking in cereals. The process also contributes to the phytate level

reduction of the grain and improves iron and zinc levels (Elkhalil *et al*., 2000; Mahgoub and Elhag, 1998).

2.9 Kilning

Generally, most traditional alcoholic beverages undergo the process of sun drying after the malting stage ranging from 1-3 days depending on the weather. According to Bamforth (2003), the controlled drying involved in beer brewing which is known as kilning of green malt is able to arrest modification and render the malt stable for storage, ensure survival of enzymes for mashing; introduce desirable flavour and colour characteristics and eliminate undesirable flavours. This stage is however not employed in the processing of *burukutu* in Ghana even though this is done in Nigeria.

2.10 Milling

Grains, be it dry or malted must be milled before they can be extracted. Milling is carried out to reduce the size of the grain particles and to expose the endosperm to be attacked by enzymes during the mashing process, so that the greatest conversion of starch to fermentable sugars is achieved in the shortest possible time. The size of particle is important for the performance of the mash (Andrews, 2006). It is also to produce a distribution of particles that is best suited to the subsequent processes in brewing. In large part the grain should be converted to flour, with particles small enough to enable access of water. This will hydrate the particles and enable the enzymes in the grain to be activated. It will also "solvate" the substrate molecules (principally starch) that the enzymes are targeting (Bamforth, 2003).

2.11 Mashing

Mashing is the process whereby ground malt, or grist, is mixed with brewing water such that a fermentable extract is produced that will support yeast growth, with the subsequent production of beer (Hornsey, 1999). This according to Andrews (2006) is the enzymatic stage where the milled grain is mixed intimately with the water, which enables enzymes to start acting. The starch in the granules is very highly ordered, which tends to make the granules difficult to digest. In the case of barley for example, when the granules are heated, the molecular order is disrupted in a process called gelatinization. The starch molecules become susceptible to enzymic digestion. It is for these reasons that the mashing process involves heating. Several enzymes are required for the complete conversion of starch to glucose. α-Amylase, which is an endo enzyme, hydrolyses the α 1-4 bonds within amylose and amylopectin. β- Amylase, an exo enzyme, also hydrolyses α 1-4 bonds, but it approaches the substrate from the non-reducing ends, chopping off units of two glucoses. α-Amylase develops during the germination phase of malting (Bamforth, 2005).

2.12 Yeast

Yeasts are eukaryotic microorganisms classified in the kingdom fungi, with a total of 1,500 species currently described (Kurtzman and Fell, 2006) and this number is estimated to be only 1% of all yeast species (Kurtzman and Piškur , 2006). Most reproduce asexually by budding, although a few do so by binary fission. Yeast are unicellular, although some species with yeast forms may become multicellular through the formation of a string of connected budding cells known as pseudo hyphae, or false hyphae, as seen in most moulds (Kurtzman and Fell, 2005). The predominant yeast species seen is *Saccharomyces cerevisiae,* involved in basically three groups of indigenous fermented products: non-alcoholic starchy foods, alcoholic beverages and fermented milk. The functions of *Saccharomyces cerevisiae* are

mainly related to formation of alcohols and other aroma compounds, but stimulation of e.g. lactic acid bacteria, improvement of nutritional value, probiotic effects, inhibition of undesired microorganisms and production of tissue-degrading enzymes may also be observed (Jespersen, 2002). *Saccharomyces cerevisiae* which is the most frequently encountered yeast in fermented beverages and foods based on fruits and vegetables, an observation which is reflected in the existence of more than eighty synonyms and varieties for the species. All strains ferment glucose and many ferment other plant-associated carbohydrates such as sucrose, maltose and raffinose but none can ferment the animal sugar lactose (Adams and Moss, 2005). *S. cerevisiae* has been used in baking and in fermenting alcoholic beverages for thousands of years (Jean-Luc *et al*., 2007).

The involvements of yeast have been reported in several different types of indigenous African fermented foods and beverages (Torner *et al*., 1992; Halm and Olsen 1996; Hayford and Jespersen, 1999; Ezeronye and Okerentugba, 2000; Amoa-Awua *et al*., 2006). Jespersen (2003) reported an overview of their possible functions in African indigenous fermented foods and beverages. Hamad *et al.,* (1992) found that fermented sorghum with high number

of yeast has a more pleasant smell than dough with less yeast. The significance of yeast and moulds occurring in maize dough fermentation for *kenkey* production in Ghana has also been reported by Jespersen *et al.,* (1994) and Obiri-Danso (1994). Omemua *et al.,* (2007) also reiterated the significance of yeasts in the fermentation of maize for *ogi* production. Different strains of yeast belonging to 8 genera were identified from *pito* brewed in Ghana. They included *Saccharomyces cerevisiae, Candida tropicalis, Kloeckera apiculata, Hansenula anomala, Torulaspora delbrueckii, Schizosaccharomyces pombe* and *Kluvyeromyces africanus* (Sefa-Dede *et al*., 1999). Achi, (2005) isolated mainly *Saccharomyces cerevisiae* and *S. chavelieri* among the dominant microorganisms in a fermenting mixture of *burukutu*.

2.13 Lactic Acid Bacteria

Lactic acid bacteria (LAB) are a heterogeneous family of microorganisms that can ferment variety of nutrients (Poolman, 2002). LAB are Gram-positive, nonspore forming rods or cocci; most are aerotolerant anaerobes which lack cytochromes and porphyrins and are therefore catalase negative and oxidase negative. In lactic acid producing bacteria two major types of lactic acid fermentation occur. Homofermenters produce lactate as virtually a single product from the fermentation of glucose. Heterofermenters produce roughly equimolar amounts of lactate, ethanol/acetate, and carbon dioxide from glucose. Heterofermenters and homofermenters can be readily distinguished by the ability of heterofermenters to produce carbon dioxide in glucose-containing media (Adams and Moss, 2005). LAB are for the most part mesophilic, but some can grow at refrigerator temperature (4°C) and as high as 45°C. They prefer a pH in the range of 4.0-4.5, but certain strains can tolerate and grow at pH above 9.0 or as low as 3.2 (Bamforth, 2005). Generally, the LAB contains cocci (*Aerococcus, Streptococcus, Pediococcus, Lactococcus Leuconostoc, Enterococcus, Oenococcus, Tetragenococcus, Vagococcus and Weissella*) and rods (*Lactobacillus, Carnobacterium*). Occasionally, *Bifidobacterium, Microbacterium* and *Propionibacterium* are linked to the LAB; however they are not part of the LAB. They are ubiquitous in nature and can grow in foods with available carbohydrates, vitamins, minerals, amino acids, and low oxygen tension. The LAB group contains microorganisms that vary with regard to temperature of growth, heat resistance, nutrient requirements, water activity needed for growth, products produced, etc. LAB can normally be characterized by: microscopic appearance, catalase test, fermentation of carbohydrates, homo or hetero fermentation of glucose and need for nutritionally complex media (Cousin, 1994). LAB is also reported to have the ability to produce probiotics according to Temmerman *et al.,* (2002).

2.14 Lactic Acid Fermentation

Lactic acid fermentation is a process in which lactic acid is formed as a product of energy exchange which takes place during the metabolism of certain microorganisms (Anon., 2011). Lactic acid bacteria (LAB) is encounted in the production of fermented foods representing virtually all commodity groups responsible for the fermentation of several indigenous African foods. A number of studies have been published on the identification of LAB that are responsible for the fermentation of these foods and beverages, including (Amoa-Awua *et al.,* 2006; Johansson *et al.,* 1995; Hamad *et al.,* 1997; Olasupo *et al.,* 1997; Gassem 1999; Hayford *et al.,* 1999; Lei and Jakobsen 2004). Kolawole *et al.,* (2006) also isolated *Streptococcus* species from *burukutu* and *pito* beverages. *Weissella confusa*, *Lactobacillus brevis, Lactobacillus viridescens, Pediococcus pentosaceus* and *P. pentosaceus* subsp. *intermedius* were identified throughout *borde,* an Ethiopian spontaneously fermented, low or non-alcoholic cereal beverage fermentation as the dominant LAB by Abegaz, (2007). Mante *et al.,* (2003) also reported that fermented cassava dough was safe due to the inhibitory properties of LAB on enteric pathogens. Halm *et al.,* (1993) found *Lactobacillus fermentum* and *L. reuteri* as the dorminant LAB in fermented corn meal for *kenkey* production in Ghana.

Togo *et al.,* (2002) confirmed the presence of LAB in *chibuku,* (a Zimbabwean sorghum beer) in which five genera of LAB, *Lactobacillus, Lactococcus, Leuconostoc, Streptococcus and Enterococcus* were predominant. The nutritional value of food or feed fermented with lactic acid bacteria can be higher than that of the corresponding raw material. Maize for example, is deficient in vitamins A and $B_{12,}$ as well as the amino acids arginine and methionine, but when fermented there was a tremendous increase in their levels (Steinkraus, 1998). LAB fermentation irreversibly degrades mycotoxins without leaving any toxic residues. The detoxifying effect is believed to be through toxin binding effect (El-Nezami *et*

al., 2002; Haskard *et al.*, 2001; Turbic *et al*., 2002). Lactic acid fermentation of foods also confers a variety of important therapeutic benefits including anticarcinogenic activities (Hirayama, 1999). Chelule *et al.,* (2010) reiterated the fact that lactic acid fermentation improves the nutritional quality and reduced the levels of toxic or carcinogenic mycotoxins of *amahewu*, a traditional South African maize-based porridge. According to Kullisaar *et al.,* (2002) lactic acid fermentation is also considered to have several beneficial physiological effects such as antimicrobial activity which enhances the immune potency.

2.15 Production of Bacteriocins by LAB

Lactic acid bacteria (LAB) exert a strong antagonistic activity against many food-contaminating micro-organisms as a result of the production of organic acids, hydrogen peroxide, diacetyl, inhibitory enzymes and bacteriocins (Piard and Desmazeaud, 1992). According to Navarro *et al.,* (2000) bacteriocins are proteinaceous antimicrobial substances with a narrower spectrum of activity than antibiotics. They are secreted by some bacteria, which are thus adapted for competition against other micro-organisms growing in the same medium. Many LAB found in numerous fermented and non fermented foods produce a high diversity of different bacteriocins (Cleveland *et al*., 2001). Bacteriocins of LAB are considered natural bio-preservatives, as it is assumed that they are degraded by the proteases of the gastrointestinal tract (Cintas, 1995). Bacteriocins produced by LAB are of great interest to the food fermentation industry as natural preservatives because of their ability to inhibit the growth of many food spoilage and pathogenic bacteria, including *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium botulinum* (Stiles 1996; McAuliffe *et al.,* 1999; Bredholt *et al.,* 2001). The products formed after fermentation of foods for example lactic acid and bacteriocins by microorganisms in the fermenting

substrates make them safe for consumption. *Lactobacillus plantarum, Lactobacillus fermentum* and *Saccharomyces cerevisiae* which are probiotics were the microorganisms isolated from maize '*ogi*'. Some other microorganisms like *Clostridium bifermentans, Corynebaterium sp, Staphylococcus aureus, Aspergillus niger, Penicillium sp* and *Rhizopus stolonifer* were isolated during the first day of steeping but they were no longer isolated after 48 and 72 h of steeping (Ijabadeniyi, 2004). Several types of bacteriocins produced by LAB exist and these have been identified and characterized (Cleveland *et al.*, 2001; McAuliffe *et al.*, 2001). One of them is nisin, a bacteriocin which is produced by certain strains of *Lactococcus lactis.* Nisin is active against a wide range of Gram-positive bacteria. This bacteriocin has been the most extensively studied and is currently the only bacteriocin commercially available and marketed (Delves-Broughton, 1990). This bacteriocin has been used to control spoilage by undesired LAB in wine and beer (Delves-Broughton *et al.*, 1996) The inhibitory spectrum from bacteriocins produced by different species of lactobacilli varies greatly; most inhibit other lactobacilli or closely related Gram-positive bacteria (Delgado *et al.*, 2001; Yamato *et al.*, 2003; Chumchalová *et al.*, 2004), whereas others, are active against a wide spectrum of Gram-positive or Gram-negative bacteria (Vignolo *et al.*, 1993; Miteva *et al.*, 1998; Chin *et al.*, 2001). Currently, there is increasing interest in bacteriocins as natural food preservatives; moreover, there are strong evidences that these antimicrobials

may play an essential role in wine fermentation for example (Navarro *et al.*, 2000). The application of bacteriocins as food preservatives could be achieved either by using the bacteriocinogenic strain as a starter culture and/or a protective culture, or by using the bacteriocin as a food additive. Therefore, from a commercial point of view, an inexpensive method for large-scale production of cultures containing high levels of viable bacteriocin

producers, in a form suitable for product applications, is highly desirable (Gardiner *et al*., 2001).

2.16 Foodborne Pathogens

Foodborne infection occurs from the consumption of food and water contaminated with pathogenic enteric bacteria and viruses (Bibek, 2004). Many foodborne pathogens are ubiquitous in nature and generally found in soil, water, animals and plants. The numbers and types of microorganisms present in a finished food product are influenced by the original source of the food, its microbiological quality in the raw or unprocessed state, the sanitary conditions under which the product was handled or processed and the conditions for subsequent packaging, handling, storage, and distribution (Bhunia, 2008). Lactic acid fermentation is reported as effective in reducing or eliminating the growth of food borne pathogens. Generally, the addition of LAB to various foods including milk has been believed to be a biopreservation measure to inhibit and probably eliminate food spoilage and pathogenic microorganisms (Stiles, 1996), including some of today's major foodborne pathogens such as *Campylobacter jejuni*, *Yersinia enterocolitica*, *Escherichia coli* O157:H7, *Vibrio cholerae* among others (Roberts, 2001). The presence of pathogens in ready-to-eat (RTE) product is a serious concern since those products generally do not receive any further treatment before consumption.

2.17 Coliforms

Coliforms are members of the family Enterobacteriaceae. These bacteria make up approximately 10% of the intestinal microorganisms of humans and other animals. Coliforms are defined as facultatively anaerobic, Gram-negative, nonsporing, rod-shaped bacteria that

ferment lactose with gas formation within 48 h at 35^{o}C (Prescott, 1996). Faecal coliform bacteria are a group of bacterial that are passed through the faecal excrement of humans, live stock and wildlife. They aid in the digestion of food. A specific subgroup of this collection is the faecal coliform bacteria, *Escherichia coli*. These organisms may be separated from the total coliform group by their ability to grow at elevated temperatures (above 44^{o}C) and are associated only with the faecal material of warm-blooded animals. The coliforms include *Escherichia coli, Klebsiella, Enterobacter, Hafnia, Serratia,* and *Citrobacter* (Smith, 1985).

2.17.1 *Escherichia coli*

Escherichia coli is a Gram-negative, short rods (1–2 μm in length), aerobic, and generally motile organism. Some pathogenic strains are also acid resistant. Majority of *E. coli* strains are non pathogenic and exist harmlessly in the intestinal tract of humans and animals (Bhunia, 2008). This bacterium colonizes the gastrointestinal tract during the first hours of life. The function of *E. coli* as part of the intestinal flora has been linked to nutrition as a source of vitamin. Although regarded as part of the flora of the human intestinal tract, several highly adapted *E. coli* clones have evolved and developed the ability to cause disease in several areas of the human body (Eslava *et al*., 2003). Following ingestion of the pathogen and incubation period, symptoms appear as abdominal cramps, profuse diarrhoea, headache, chills, and fever. Symptoms can last for 7 to 12 days, but a person can remain a carrier and shed the pathogens in faeces for a long time (Bibek, 2004). Fermented foods are normally considered to be safe against foodborne diseases because of their low pH (Gadaga *et al*., 2004). However they have been isolated from a number of traditional beverages. *E. coli* and *Staphylococcus aureus* for example were isolated from *burukutu* and *pito* (Kolawole *et al.,* 2007). Faecal coliform bacteria were found in 48% market-vended beverages and *E. coli* in 17% street-vended beverages in Guatemala (Sobel *et al*., 1998).

2.18 Food Preservation

Since the earliest times humankind has searched for ways to make the food supply safer and to make food last longer. Without the use of some preservation technique, the natural microorganisms that are present everywhere in the environment will grow and multiply in foods. Preservation aims either to destroy or inhibit the growth of harmful microorganisms in food by making an environment unsuitable for them. A method of making food safer that is well-known and accepted today is pasteurization. Other familiar ones are drying, smoking, and salting which seek to reduce available water in a product. Modified Atmosphere Packaging (MAP), freezing foods, food irradiation, canning, making foods more acidic through fermentation and pickling are also done (Roberts, 2001).

2.18.1 Pasteurization

Pasteurization is a mild heat treatment used for milk, liquid egg, fruit juices, and beer. The main purpose of pasteurization is to achieve the following: destroy pathogens, reduce bacterial count, inactivate enzymes and extend shelf life (Vaclavik, and Christian, 2008). Pasteurization is typically carried out in the range of 60-80°C and applied for up to a few minutes. Where pasteurization is introduced to improve safety, its effect can be doubly beneficial. The process cannot discriminate between the target pathogen(s) and other organisms with similar heat sensitivity, so a pasteurization which destroys *Salmonella* for example, will also improve shelf-life. On its own, the contribution of pasteurization to

extension of shelf-life can be quite small, particularly if the pasteurized food lacks other contributing preservative factors such as low pH or a_w. Thermoduric organisms such as spore formers and some Gram positive vegetative species in the genera *Enterococcus*,

Microbacterium and *Arthrobacter* can survive pasteurization temperatures. They can also grow and spoil a product quite rapidly at ambient temperatures, so refrigerated storage is often an additional requirement for an acceptable shelf-life (Adams and Moss, 2005). The amount of time the product is heated depends on the temperature; higher temperatures require less time. The amount of bacteria killed with a heat method such as pasteurization depends on how high the temperature is, and how long the food is held at that temperature (Roberts, 2001). The advantages of using pasteurization are that minimal damage is done to the flavour, texture, and nutritional quality of the product (Vaclavik, and Christian, 2008).

3.0 MATERIALS AND METHODS

3.1 Sampling Sites

A total of four study areas were used for this work in the Accra and Tema metropolis. These were; Tema, geographically located on 5° 37' 0" North, 0° 10' 0" West; Zenu geographically located on 5° 43' 0" North, 0° 30' 0" West, Ashaiman, geographically located on 5° 42' 0" North, 0° 20' 0" West and Accra Newtown, geographically located on 5° 5' 0" North, 3° 5' 0" West all within the Greater Accra region of Ghana.

The production site at Ashaiman was the biggest of all the sites visited. It was made up of a wooden structure and roofed with an aluminium sheet. One part of the structure was partly opened and served as the initial preparation place for the beverage whilst the other parts were fully enclosed for the storage and retailing of the product. This site used six large size cooking pots and had about 8–10 workers who were all women. There was the availability of running pipe borne water. Prevailing sanitary conditions at the site was generally poor but comparatively better than the other sites.

The Tema site was located in a densely populated area with poor sanitary conditions. It was a small processing site made of wooden structures. Part of the facility served as a bedroom for the processor and her family and the other part was used as the production and retailing site. This site had two cooking pots with only two workers. Water for processing was bought from a lorry tanker.

The Accra New-Town site was small and located in a crowded compound house near a washroom and a very big open and chocked gutter. There were two separate aluminium roofed structures. One was for retailing the product and the other for processing. There were

two cooking pots and two workers. Water for processing was bought from lorry tankers. Sanitary conditions were deplorable with a lot of flies hovering around.

The Zenu site was made of concrete blocks. It used two cooking pots and had three workers. This processor also bought water from lorry water tankers.

3.2 Sampling

From each processor samples of *burukutu* were collected on three different occasions. Samples were collected at Tema, Ashaiman, Zenu, and Accra New-Town. Sampling was done from October 2010 to July 2011. Based on a scheduled invitation, samples were collected during processing directly into sterile stomacher bags. Samples were transported in ice chest with packs to the CSIR-Food Research Institute's microbiology laboratory for analyses. Samples collected from the processors on each visit included the dry sorghum grains (BM), malted sorghum grains (AM), supernatant of the mash (SU) and sediment of the mash (SE). The others were acidified mash after the initial boiling (M), the freshly prepared *burukutu* (F) and the old stock or batch of *burukutu* to be used for back slopping (I). The final product mixed with an old batch ready for sale was bought into a 50 litre gallon and transported to the laboratory for analyses at 0, 12, 24, 36, 48, 72, 96 and 120 h intervals. The samples were microbiologically and chemically analysed.

3.3 Microbiological analyses

3.3.1 Homogenization and Serial Dilution

Ten grams (10 g) of each sample was added to 90.0 ml sterile Salt Peptone Solution (SPS) containing 0.1% peptone and 0.85% NaCl, with pH adjusted to 7.2 and homogenized in a stomacher (Lad Blender, Model 4001, Seward Medical). After homogenizing for 30 s at normal speed, ten-fold dilutions were prepared. The homogenate was serially diluted (1:10) and 1 ml aliquots of each dilution were directly inoculated into Petri dishes and the appropriate isolation media added. All analyses were done in duplicate.

3.3.2 Enumeration of Aerobic Mesophiles

Aerobic mesophiles were enumerated by the pour plate method using Plate Count Agar (Oxiod CM325; Oxoid Ltd., Basingstoke, Hampshire, UK). Plates were incubated at 30°C for 72 h in accordance with the Nordic Committee on Foods Analysis Method (NMKL. No. 86, 2006).

3.3.3 Enumeration and Confirmation of Total Coliforms

Total coliforms were enumerated by the pour plate method using Trypton Soy Agar (Oxoid CM131), pH 7.3 overlaid with Violet Red Bile Agar (Oxoid CM107), pH 7.4 and incubated at 37°C for 24 h. Colonies were confirmed using Brilliant Green Bile Broth (Oxoid CM31), pH 7.4 incubated at 37°C for 24 h as described by (NMKL. No. 44, 2004).

3.3.3.1 Identification of Total Coliforms

All total coliform isolates which were Gram negative and oxidase negative were identified using API 20E (BioMérieux, Marcy-l'Etoile, France).

3.3.4 Enumeration and Confirmation of *Escherichia coli*

E. coli were enumerated by the pour plate method using Trypton Soy Agar (Oxoid CM131), pH 7.3 overlaid with Violet Red Bile Agar (Oxoid CM107), pH 7.4 and incubated at 44°C for 24 h. Colonies were confirmed using EC Broth (Oxoid CM853), pH 6.9, followed by Trypton Water (Oxoid CM87), pH 7.5, all incubated at 44°C for 24 h as described by (NMKL. No. 125, 2005).

3.3.5 Enumeration of Yeasts and Moulds

Yeasts and moulds were enumerated by the pour plate method using Oxytetracycline-Glucose Yeast Extract Agar (Oxoid CM545; Oxoid Ltd., Basingstoke, Hampshire, UK) to which OGYEA supplement was added to inhibit bacteria growth. The pH was adjusted to 7.0 and incubated at 25°C for 120 h in accordance with ISO 7954 (1987).

3.3.5.1 Isolation of Yeast

About 15 colonies were selected from a segment of the highest dilution or suitable plate of yeast colonies on OGYEA and examined by microscopy, purified by successive sub culturing in Malt Extract Broth (Oxoid CM57) and streaking on Malt Extract Agar (Oxoid CM59) pH 5.4 until pure colonies were obtained.

3.3.5.2 Macroscopic and Microscopic Examination of Yeast

Colonies on solid media were examined macroscopically for colonial morphology. Characteristic described included colour, surface, size, form, margin, and elevation. Cultures were also microscopically observed as wet mounts for cellular morphology.

3.3.5.3 Identification of Yeast Isolates

Isolates were identified by determining their pattern of fermentation and assimilation of various carbohydrates using ID 32 C galleries (BioMérieux, Marcy-l'Etoile, France).

3.3.6 Enumeration of Lactic Acid Bacteria

Lactic acid bacteria were enumerated by pour plate method using deMan, Rogosa and Sharpe (MRS, Oxoid CM361) agar (De Man *et al.*, 1960), pH 6.2 to which has been added 0.1% cycloheximide supplement to suppress yeast growth and Cystein HCL to achieve anaerobic conditions during incubation without having to use Anaerocult A. The plates were incubated anaerobically in an anaerobic jar at 30°C for 120 h.

3.3.6.1 Isolation of Lactic Acid Bacteria

About 15 colonies of lactic acid bacteria were selected from a segment of the highest dilution or suitable MRS agar plate. The colonies were sub-cultured into the corresponding broth medium and streaked repeatedly on agar until pure colonies were obtained.

3.3.6.2 Characterisation of Lactic Acid Bacteria Isolates by Gram Reaction

Gram reaction was determined using 3% freshly prepared potassium hydroxide solution as described by Gregersen (1978). The tip of a cover slip was used to pick a pure colony of LAB and added to a drop of potassium hydroxide solution on a slide. The colony was mixed thoroughly with the solution using the cover slip and drawn for the production of slime. Formation of a slime indicated Gram negative reaction and non-slimy reaction indicated Gram positive reaction.

3.3.6.3 Characterisation of Lactic Acid Bacteria Isolates by Catalase Reaction

A drop of 3% freshly prepared hydrogen peroxide solution was placed on a clean glass slide and a single colony of the pure culture picked and emulsified. This was then observed for bubbles or effervescence resulting from the liberation of free oxygen as gas bubbles. This indicated the presence of the enzyme catalase in the culture and vice versa.

3.3.6.4 Characterisation of Lactic Acid Bacteria Isolates by Oxidase Test

Oxidase test was done using Identification Sticks (Oxoid Ltd., Basingstoke, Hampshire, UK)

3.3.6.5 Microscopic Examination

Cell shape and arrangements were determined using the phase contrast microscope and the wet mount technique. A drop of sterile distilled water was placed on a clean slide and a small amount of the pure culture emulsified in it. A cover slip was placed on it and examined under the microscope using the X40 magnification and oil immersion using the X100.

3.3.6.6 Growth at Different Temperatures

Two tubes containing MRS broth (Oxoid CM359) were inoculated with pure colony mass of the test organism and incubated at 15°C and 45°C respectively for 72-96 h. Growth in the tubes were determined by visual turbidity after the incubation period. This was done for all the isolates.

3.3.6.7 Gas Production from Glucose

The ability to ferment and produce gas from glucose was assessed in MRS sugar basal medium. The medium was composed of peptone 10 g, yeast extract 5 g, tween 80 1ml, di-potassium hydrogen phosphate 2 g, sodium acetate 5 g, tri-ammonium citrate 2 g,

$MgSO_4.7H_2O$ 0.2g, $MnSO_4$. $4H_2O$ 0.05 g, 1 litre distilled water, pH 6.5, thus without glucose and meat extract. The basal medium was dispensed in 5 ml aliquot into test-tubes containing inverted Durham tubes and sterilized by autoclaving at 121°C for 15 minutes. The glucose was prepared as 10% solution and sterilized by filtration and added aseptically to the basal medium to give a final concentration of 2%. The inoculated tubes were examined for the production of gas after incubation at 30°C for 72 h.

3.3.6.8 Salt Tolerance Test

Salt tolerance test was done using MRS broth (Oxoid CM359) containing 6.5% and 18% (w/v) NaCl with incubation period of 4 days at 30°C. The tubes were then observed for growth of the inoculums.

3.3.6.9 Growth at Different pH

Growth in MRS broth (Oxoid CM359) with pH adjusted to 4.4 using concentrated HCl and 9.6 using 0.1 N NaOH were determined by visual turbidity after 72-96 h of incubation at 30°C.

3.3.6.10 Identification of Lactic Acid Bacteria

Isolates were tentatively identified by determining their pattern of carbohydrate fermentation using the API 50 CH (BioMérieux, Marcy-l'Etoile, France) and comparing them to the API database.

3.3.7 Antimicrobial Studies

The inhibitory potential of lactic acid bacteria cultures was investigated using the Agar Well Diffusion method as described by Schillinger and Lücke (1989) and Olsen *et al*., (1995). The appropriate agar was poured into Petri dishes and allowed to solidify and dry for 1-2 days.

Circular wells were made in the agar using sterile cork borers. Twelve cultures of lactic acid bacteria isolated at different stages of *burukutu* fermentation were each cultured in MRS broth (Oxoid CM359) at 30°C for 24 h. A volume of 0.1 ml of the cultures was transferred into wells and left to diffuse into the agar for approximately 4-5 h. The wells were overlaid with about 10 ml of the appropriate soft agar (0.7% agar) containing the indicator strains. The indicator lawns were prepared by adding 0.25 m1 of 10^{-1} dilution of an overnight culture of the indicator organism to 10 ml of MRS agar (MRS, Oxoid CM361), for lactic acid bacteria.

3.3.8 Survival of Enteric Pathogens in Fermenting *Burukutu* Sample

The ability of different enteric pathogens to survive in fermenting *burukutu* sample was studied by the method described by Mante *et al*., (2003). The enteric pathogens used were *Escherichia coli, Streptococcus faecalis, Staphylococcus aureus, and Salmonella typhimurium,* all obtained from the Food Research Institute, Microbiology laboratory. Pure cultures of each pathogen in nutrient broth at a concentration of 10^5 cfu/ml were each inoculated into a fermenting batch. For the different fermentation periods, 10 ml was collected at intervals and the population of surviving pathogens enumerated by spread plate incubated at the appropriate temperatures.

3.4 Enzymatic Analysis

3.4.1 Tests for Amylase Secretion by Lactic Acid Bacteria

Purified bacterial cultures were streaked on Nutrient agar (Oxiod CM3; Oxoid Ltd., Basingstoke, Hampshire, UK) containing 2% soluble starch (with pH adjusted to 7.2) and incubated in an anaerobic jar at 30°C. After 5 days of incubation, the plates were flooded

with iodine solution. Production of amylase was indicated by the formation of a clear zone around the colonies with the remaining parts of the plates staining blue-black as described by

Almeida *et al*., (2007). The extent of secretion was quantified by measuring the width of the clear zone around the colony.

3.4.2 Preparation of Enzyme Extract

Enzyme extract of the dry sorghum grains (BM), malted sorghum grains (AM), supernatant of the mash (SU) and sediment of the mash (SE) was prepared by grinding 5 g of sample in 50 ml of 0.1 M potassium hydrogen phosphate buffer, pH 6.5. After maceration, the suspension was washed with petroleum ether to extract the oil and centrifuged at 4000xg for 5 min. The supernatant constituting the crude enzyme extract was stored at -20°C as described by Terlabie *et al*., (2005).

3.4.3 Determination of α-amylase Activity

Alpha amylase activity was determined by the assay method of Bernfeld (1955). About 2 ml of the enzyme extract was mixed with 1 ml of 1% starch solution (VWR-Prolabo) and incubated for 1 h at 40°C. The reaction was stopped by the addition of 3 ml dinitrosalicylic acid reagent and heated for 5 min. It was allowed to cool and diluted with 18 ml distilled water and the optical density measured at 550 nm in a spectrophotometer (Cecil 1021 1000 Series). In a blank determination, the dinitrosalicylic acid reagent was added before the starch solution. The amount of reducing sugars formed was calculated from a standard curve prepared with known concentrations of maltose (Sigma).

3.5 Chemical Analyses

3.5.1 Determination of pH

The pH of solid samples and distilled water were weighed in a ratio of 1:1 and homogenized in a stomacher bag and the pH determined with a pH meter (Radiometer PHM 92; Radiometer Analytical A/S, Bagsvaerd, Denmark) after calibration using standard buffers. The pH of liquid samples was determined directly.

3.5.2 Determination of Titratable Acidity

The titratable acidity was determined by the method described by Amoa-Awua *et al*., (2006). For each sample (fermenting *burukutu*) 10 ml of sample was made up to 200 ml with distilled water and 80 ml titrated against 0.1 m NaOH using 1% freshly prepared phenolphthalein as indicator. One millilitre of 0.1 m NaOH was taken as equivalent to 0.009 g lactic acid.

3.5.3 Determination of Alcohol Content

The percentage of alcohol was determined by the method according to AOAC (1990). For each sample (fermenting *burukutu*) 100 ml was diluted with 50 ml of distilled water. The mixture was then distilled with about 100 ml of the distillate collected. The specific gravity of the distillate was determined by a ratio of the weight of 25 ml of the distillate by the weight of equal volume of water using the 25 ml specific gravity bottle at 20°C. The obtained value was then referred to a reference table AOAC (1990) for the percentage alcohol.

3.5.4 Determination of Soluble Solids

Soluble solids were determined by placing a drop of the sample on the lens of a hand held refractometer and the reading taken through the eye piece as Brix.

3.6 Bottling of *Burukutu*

Freshly prepared and back slopped *burukutu* sample purchased from one of the processors into a 12 gallon capacity container and transported to the Food Research Institute processing section was used. The sample was poured out into a heating vessel and pasteurized at 70°C for 15 min. It was then filled and cupped immediately into new pep plastic bottles and glass bottles of 300 ml capacity that had already been sterilized with a mixture of 1% sodium meta bisulphite and citric acid and rinsed with sterilized distilled water. The filled plastic bottles were immediately cooled under running pipe borne water. The filled glass bottles were further pasteurized in the bottle at 85°C for 30 min and allowed to cool to room temperature. Both glass and plastic bottled samples were kept under two different conditions i.e. room temperature and climatic chamber (4°C and humidity 32%rH)) and observed over a period of twelve weeks for shelf life studies.

3.7 Colour Measurement

The colour of both glass and plastic bottled *burukutu* samples were measured by pouring the thoroughly mixed sample into a Petri dish (to the brim) and the measuring head of the chromameter (CR310) firmly placed onto the covered Petri dish for the reading. Three replicates of each sample were done.

3.8 Sensory Analysis

Sensory analysis was conducted on the samples for acceptability based on appearance, colour, consistency, aroma, taste, mouth feel and overall acceptability. This was done by a total of ten untrained panelist selected from the CSIR- Food Research Institute who were familiar with the product. The coded samples were served in transparent plastic cups together with biscuit and water to rinse their mouths between each sample and assessed on a nine-point hedonic scale where 1 = dislike extremely and 9 = like extremely (the acceptability test sheet used can be found in Appendix 5). Data obtained was analysed using the mean and standard deviation to assess the level of consumer acceptability of the products as well as analysis of variance (ANOVA) to establish the significant difference among the various treatments.

4.0 RESULTS

4.1 General Information on the *Burukutu* Production from Processors

A total of ten *burukutu* processors who were all women were interviewed at various processing sites in the Accra and Tema metropolis of the Greater Accra region; Accra-Newtown, Ashaiman, Maamobi, Tema and Zenu. Majority (80%) of the processors were from the Loso tribe in Togo with only 20% from Ghana. According to the respondents, the main raw-material used in the preparation of *burukutu* is sorghum which is often obtained from the local market and are aware of only one process of making *burukutu*. The quantity of sorghum processed per day by each processor/brewer depends on the size of her trade. About 80% buy between 1-2 bags at a time and the remaining 20% buying 3-4 bags. However, they have problems with storage since most of them keep the sorghum bags on the bare floor at the production site. The sorghum waste generated after processing are often sold as animal feed to poultry farmers for additional income.

Brewing/processing of *burukutu* takes only a day (24 h) with over 90% starting at dawn and only 10% around mid-day. A day's production of *burukutu* took between 5-7 days for about 80% of processors to sell depending on the number of customers and whether or not the

processor was a wholesaler or retailer of the product. Tightly closed fresh *burukutu* that has not been back slopped could be stored for a week or two. However, a back slopped one can be stored up to a maximum of 5 days in plastic containers or barrels after which it becomes sour or acidic and unpalatable for most consumers. The left over fermenting batch was often kept and used in inoculating (starter culture) freshly prepared *burukutu* by over 80% of the processors.

Many of the brewers of *burukutu* intimated that comparably *burukutu* was much heavier than *pito* (another fermented alcoholic product in Ghana) because of the large quantity of suspended solids. Consumers thus consider *burukutu* to be more of food than just a beverage.

All the processors (100%) did confirm that although the processing of *burukutu* was laborious and tiresome, it was profitable and also preferable by most consumers of locally brewed alcoholic beverages. (Questionnaire at Appendix 1)

4.2 Microbial Numbers in *Burukutu*

Total mean microbial numbers in samples of *burukutu* is shown in Figure 2 (Table in Appendix 2). Initial average microbial counts on the dry sorghum grains before malting (BM) were 6.62 $\log_{10}$ CFU/ml for aerobic mesophiles, 5.23 $\log_{10}$ CFU/ml for yeasts and moulds, 1.37 $\log_{10}$ CFU/ml for lactic acid bacteria, 5.73 $\log_{10}$ CFU/ml for total coliforms and 3.36 $\log_{10}$ CFU/ml for *E. coli*. However, these microbial numbers (CFU/ml) increased after malting (AM) to 8.59 $\log_{10}$ CFU/ml for aerobic mesophiles (29.80% rise), 7.55 $\log_{10}$ CFU/ml for yeasts and moulds (44.40% rise), 7.75 $\log_{10}$ CFU/ml for lactic acid bacteria (465.70%

rise), 7.78 $\log_{10}$ CFU/ml for total coliforms (35.80% rise) and 5.54 $\log_{10}$ CFU/ml for *E. coli* (64.90% rise). These changes were statistically (p = 0.00) significant (Appendix 3).

Even though the supernatant (SU) and sediment (SE) samples recorded a further increase in the microflora these increases were not statistically (p = 0.00) significant. The mean microbial numbers (CFU/ml) were 8.88 and 9.17 $\log_{10}$ CFU/ml for aerobic mesophiles; 7.35 and 7.52 $\log_{10}$ CFU/ml for yeast and moulds; 8.11 and 8.34 $\log_{10}$ CFU/ml for LAB; 7.75 and 7.80 $\log_{10}$ CFU/ml for total coliforms, 6.19 and 6.34 $\log_{10}$ CFU/ml for *E. coli* in SU and SE respectively.

However, there were significant average reductions in microbial counts (34.30 to 64.20%) in the acidified mixture (M). There was a 1000 folds reduction in the aerobic mesophiles and yeast population, 100 folds reduction in LAB and a 10,000 folds reduction in total coliforms and *E. coli*.

Initial microbial numbers (CFU/ml) in freshly prepared *burukutu* (F) were low; 4.52 $\log_{10}$ CFU/ml for aerobic mesophiles; 3.46 $\log_{10}$ CFU/ml for yeasts and moulds and 3.61 $\log_{10}$ CFU/ml for LAB. Total coliforms and *E. coli* were absent in the freshly prepared product. It was however realised that mean microbial numbers in the old or previous product which was kept and used as a starter inoculum (I) for the freshly prepared *burukutu* were high; 8.14; 7.89; 7.62; 1.82 and 1.29 $\log_{10}$ CFU/ml for aerobic mesophiles, yeast and moulds, LAB, total coliforms and *E. coli* respectively.

Generally, during the period of fermentation, increases in microbial numbers were gradual. Microbial numbers increased by 10 fold after 12 h, decreased after 24 h and then increased

slightly again after 36, 48 up to 120 h. These increases were not statistically significant. All the total coliforms and *E. coli* died and could not be detected in the samples after 24 h.

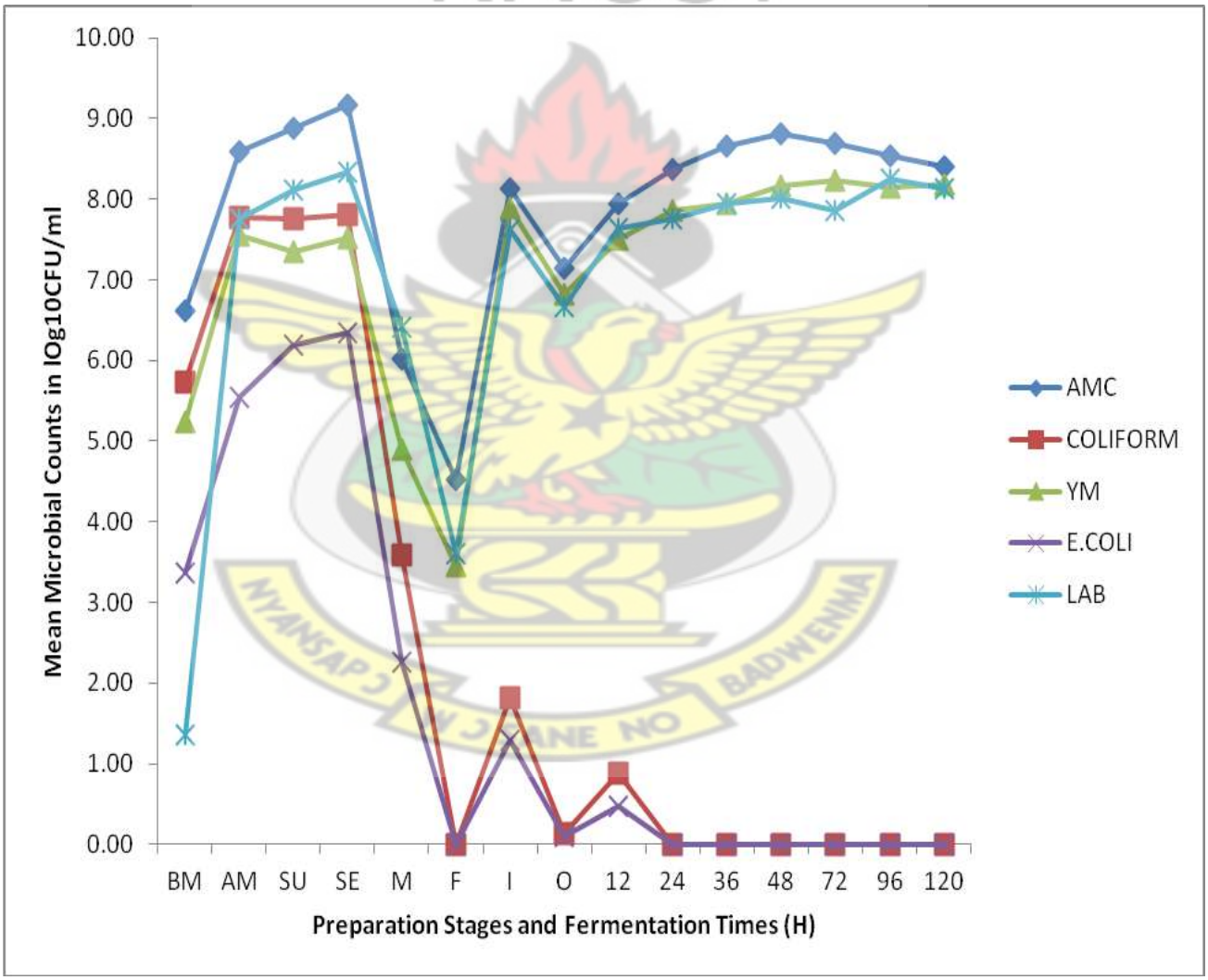


Fig 2: Mean microbial numbers in *burukutu* through the various stages of preparation

4.3 Identification of Total Coliforms Bacteria Isolates

A total of twelve representative coliforms isolates from *burukutu* were identified as *Enterobacter agglomerans* using the API-20E Identification System (Table 1).

Table 1: Biochemical Profile of Coliform Isolates from *Burukutu* Samples Using API-20E

Test	**Isolate Number**											
	1	2	3	4	5	6	7	8	9	10	11	12
Ortho – Nictrophenyl-B – Galactosidase	+	+	+	+	+	+	+	+	+	+	+	+
Arginine	-	-	-	-	-	-	-	-	-	-	-	-
Lysine – decarboxylase	-	-	-	-	-	-	-	-	-	-	-	-
Ornithine	-	-	-	-	-	-	-	-	-	-	-	-
Citrate	+	+	+	+	+	+	+	+	+	+	+	+
Hydrogen Sulfide production	-	-	-	-	-	-	-	-	-	-	-	-
Tryptophan deaminase	-	-	-	-	-	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-	-	-	-	-	-
Voges – Proskauer	+	+	+	+	+	+	+	+	+	+	+	+
Gelatin Hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-
Glucose fermentation	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+
Inositol	+	+	+	+	+	+	+	+	+	+	+	+
Sorbitol	+	+	+	+	+	+	+	+	+	+	+	+
Rhamnase	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	-	-	-	-	-	-	-	-	-	-	-	-
Melibiose	-	-	-	-	-	-	-	-	-	-	-	-

Amygdaline	+	+	+	+	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+	+	+	+	+
Species Identified	*EA*	*EA*	*EA*	*EA*	*EA*	*EA*	*EA*	*EA*	*EA*	*EA*	*EA*	*EA*

EA – Enterobacter agglomerans

4.4 Identification of Lactic Acid Bacteria Isolates

Out of the 180 lactic acid bacteria isolates from *burukutu* processors, 33.3% were *Lactobacillus fermentum*, 25% *Lactobacillus plantarum*, 16.7% *Lactobacillus acidophilus*, 16.7% *Lactococcus lactis* subsp. *lactis and* 8.3% *Lactobacillus brevis.*

These isolates were initially classified into a total of twelve groups. The dominant species were able to grow at 45°C but not at 15°C. They also grew in 6.5% NaCl and at pH 4.4 and 9.6 but not in 18% NaCl. In the API gallaries, they fermented galactose, D-glucose, D-fructose, D-mannose, ribose, melibiose, saccharose etc. The dominant lactic acid bacteria species were *Lactobacillus fermentum* (Table 2).

The second most dominant species grew at 15°C but not at 45°C nor in 18% NaCl and 6.5% NaCl. They also grew in both pHs 4.4 and 9.6 and were able to ferment L-arabinose, ribose, galactose, D-glucose, D-fructose, D-mannose, mannitol, sorbitol, α-methyl-D-mannoside, N-acetyl glucosamine, amygdaline, arbutin, esculin, salisin, cellobiose, maltose, lactose,

melibiose, saccharose, trehalose, melezitose,D-raffinose, β-gentiobiose, D-turanose and gluconate. The species was identified as *Lactobacillus plantarum.*

Another group of isolates identified were cocci and occurred in singles, pairs and short chains, grew at 15°C and not at 45°C. These isolates grew in both 6.5 and 18% NaCl and at pH 9.6 but not pH 4.4. This group fermented ribose, galactose, D-glucose, D-fructose, L-arabinose, amygdalin, cellobiose, salicin, lactose, maltose, trehalose and gentiobiose. The isolates were identified as *Lactococcus lactis* subsp. *lactis*.

Other species identified based on their biochemical characteristics and pattern of carbohydrate fermentation were *Lactobacillus acidophilus* and *Lactobacillus brevis*.

Table 2: Biochemical characteristics of lactic acid bacteria isolates from *Burukutu* Samples Using API-50 CH.

	Isolate Number											
Test	1	2	3	4	5	6	7	8	9	10	11	12
Tetrad formulation	-	-	-	-	-	-	-	-	-	-	-	-
Gram stain	+	+	+	+	+	+	+	+	+	+	+	+
Catalase test	-	-	-	-	-	-	-	-	-	-	-	-
Oxidase test	-	-	-	-	-	-	-	-	-	-	-	-
Anaerobic growth	+	+	+	+	+	+	+	+	+	+	+	+
CO_2 from glucose	+	-	-	-	-	+	-	+	+	+	-	-
Nutrient broth, pH 4.4	+	+	+	+	+	+	+	+	+	+	-	-
Nutrient broth, pH 9.6	+	+	+	+	+	+	+	+	+	+	+	+
Growth in 6.5% NaCl	+	-	+	-	+	+	+	+	+	+	+	+
Growth in 18% NaCl	-	-	-	-	-	-	-	-	-	-	+	+

Growth at 15°C	+	+	-	+	-	-	-	-	-	-	-	-
Growth at 45°C	-	-	+	-	+	+	+	+	+	+	-	-
Species Identified	*LB*	*LP*	*LA*	*LP*	*LA*	*LF*	*LA*	*LF*	*LF*	*LF*	*LL*	*LL*

NB:

LB – Lactobacillus brevis *LL – Lactococcus lactis subsp. lactis*

LP – Lactobacillus plantarum *LF – Lactobacillus fermentum*

LA – Lactobacillus acidophilus

4.5 Tests for Amylase Secretion by Lactic Acid Bacteria Isolates

Out of the total number of isolates, 58.30% were able to produce amylase (Table 3). All the *L. fermentum* isolates examined produced clear zones of inhibition ranging from 2.00 – 4.20 mm in diameter. Other isolates that showed clear zones of inhibition were *L. plantarum* (3.00 – 12.00 mm) and *L. lactis* (5.00 mm). The remaining lactic acid bacteria isolates did not secrete amylase.

Table 3: Amylase secretion by lactic acid bacteria isolates from *burukutu* samples

Isolates	LB_1	LP_1	LA_1	LP_2	LA_2	LF_1	LA_3	LF_2	LF_3	LF_4	LL_1	LL_2
Amylase zone(mm)	-	12.0	-	3.0	-	4.2	-	3.5	2.0	3.0	5.0	-

4.6 Tests for Antimicrobial Activity by Lactic Acid Bacteria Isolates

The result shows that *L. fermentum* isolates showed very strong antimicrobial activity against *Staphylococcus aureus* with inhibition zones ranging from 3.00 mm to greater than 5.00 mm in diameter (Table 4). However, antimicrobial activity against *Escherichia coli* and *Salmonella typhimurium* were low ranging from 2.00 – 4.00 mm for both pathogens. One of the *Lactobacillus plantarum* isolate also showed low antimicrobial activity against *E. coli* with no interactions observed amongst *Staphylococcus aureus* and *Salmonella typhimurium.* Similarly, *Lactobacillus acidophilus* isolates showed very low activity or no activity at all against the pathogenic indicator strains (Table 4).

Table 4: Antimicrobial activity between lactic acid bacteria isolates from *burukutu* samples and pathogenic indicator strains

Isolates	**Indicator Strains**		
	Escherichia coli	***Staphylococcus aureus***	***Salmonella typhimurium***

LB$_1$	-	+++	+
LP$_1$	++	-	-
LA$_1$	-	++	-
LP$_2$	-	+	-
LA$_2$	+	-	-
LF$_1$	++	+++	++
LA$_3$	-	++	-
LF$_2$	++	+++	++
LF$_3$	++	++++	++
LF$_4$	+	++	+
LL$_1$	++	+	-
LL$_2$	+	+	-

NB:

- : no inhibition
+ : 2-3 mm inhibition zone
++ : 3-4 mm inhibition zone
+++: 4-5 mm inhibition zone
++++: > 5 mm inhibition zone

4.7 Survival of Enteric Pathogens in Fermenting *Burukutu* Samples

The survival of four different enteric pathogens namely *Escherichia coli, Streptococcus faecalis, Staphylococcus aureus, and Salmonella typhimurium* at a concentration of about 10^5

in the fresh back slopped *burukutu* showed varying degree of survival time. *Salmonella typhimurium* was the first pathogen to die out from the fermenting sample after 16 h at a pH of 3.26. As the pH continued to drop to 3.24 and 3.13 *Escherichia coli* and *Staphylococcus aureus* died out after 20 and 36 h respectively. *Streptococcus faecalis* could not also be detected after 40 h at a pH of 3.06 (Table 5).

Table 5: pH changes and survival of enteric pathogens inoculated into a fermenting *burukutu* sample at room temperature

Fermenting	**pH**	**Microbial Counts (CFU/ml)**

Time (Hrs)					
		Escherichia coli	***Streptococcus faecalis***	***Staphylococcus aureus***	***Salmonella typhimurium***
0	3.38	5.0×10^5	4.7×10^6	6.8×10^5	3.5×10^4
4	3.36	9.8×10^4	9.8×10^5	7.1×10^4	2.2×10^2
8	3.31	7.1×10^2	7.3×10^5	3.8×10^3	6.4×10^1
12	3.27	5.9×10^1	6.2×10^4	6.5×10^2	1.0×10^0
16	3.26	7×10^0	5.5×10^2	4.4×10^1	nd
20	3.24	nd	6.7×10^1	1.2×10^1	
24	3.19		4.2×10^1	3.9×10^0	
28	3.17		2.6×10^1	3.0×10^0	
32	3.16		1.5×10^0	9×10^0	
36	3.13		1.0×10^0	nd	
40	3.06		nd		

nd: Not detected

4.8 Microscopic and Macroscopic Examination of Yeast Isolates from *Burukutu* Samples and their Identification

Preliminary identification of the 180 yeast isolates from *burukutu* was based on microscopic and macroscopic observations. The isolates were classified into seven main groups based on their cell and colony morphology as well as growth in liquid. The assimilation of carbon sources by the isolates and subsequent identification was performed using the ID 32 C

galleries. Despite the variations observed from all the seven groups, five of the isolates representing groups 1, 2, 4, 5 and 7 (Appendix 4) were identified as *Saccharomyces cerevisiae*. These isolates were able to assimilate glucose, galactose, sucrose, maltose, raffinose and trehalose but could not assimilate lactose. The remaining groups i.e. 3 and 6 were identified as *Candida krusei* as they were able to assimilate glucose, glycerol, glucosamine and lactic acid but not arabinose, raffinose, maltose and trehalose.

4.9 α-Amylase Activity

Amylase activity was detected in the sorghum grains before malting (BM), after malting (AM), the supernatant of the mash (SU) and the sediment of the mash (SE) as shown in Fig. 3. For all the four processors, a general trend was observed. The amylase activity in the dry grain ranged from 2.65 to 6.85μmol/ml. This increased drastically to 13.05 to 22.35μmol/ml after the malting period. There was however a decrease of the amylase content in the supernatant from 11.60 to 20.15μmol/ml. The amylase content of the sediments also ranged from 2.15 to 7.15μmol/ml.

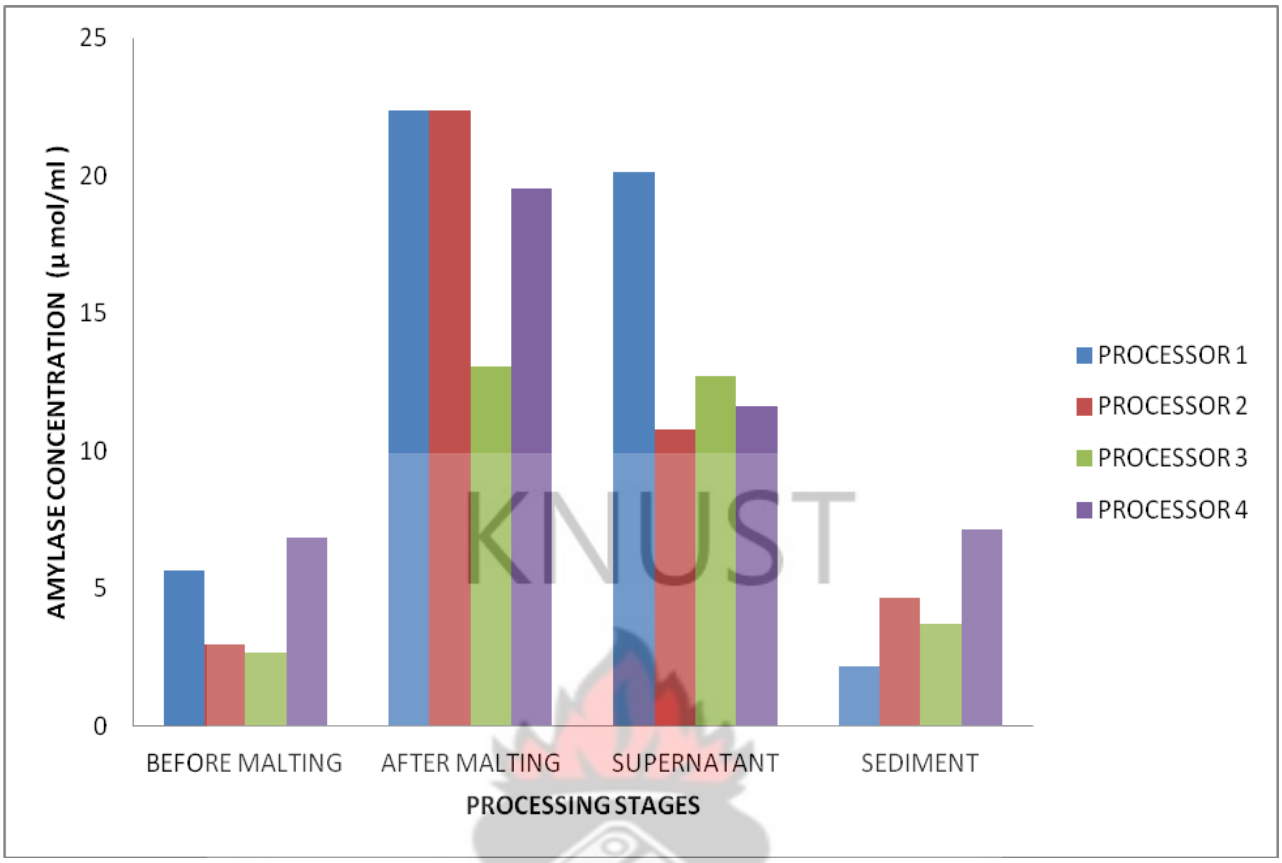


Fig. 3: α-Amylase activity of BM, AM, SU and SE samples from sorghum grains and mash

4.10 Chemical Analyses

4.10.1 pH and Titratable Acidity

The pH and titratable acidity of the fermenting *burukutu* from four processors are shown in Fig 4. There was a gradual decrease in average pH values from the initial 3.18 to 3.03 after 120 h of fermentation. This decrease well corresponded with a general increase in average titratable acidity from 0.37 to 0.97.

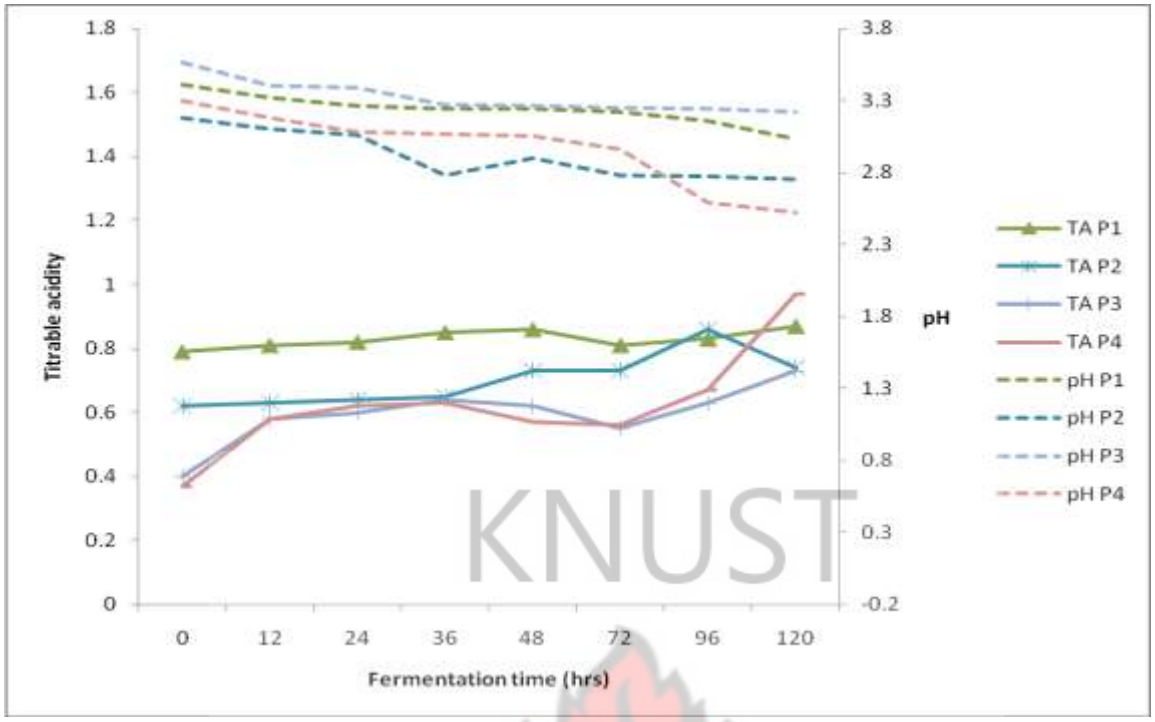


Fig 4: Mean pH and Titratable acidity changes during the fermentation of *burukutu* samples

4.10.2 Alcohol and Soluble Solids

Figure 5 shows that the alcoholic content of the *burukutu* samples increased with fermentation time whilst percentage total soluble solids decreased. Initial total soluble solids were high ranging from of 6.50 - 8.00% but decreased to 2.80 - 3.50% brix after 120 h of fermentation. These decreases however resulted in an increase in the alcohol level of the product from an initial value in a range of 0.71-1.20% to 3.19 - 5.43% at the end of 120 h fermentation.

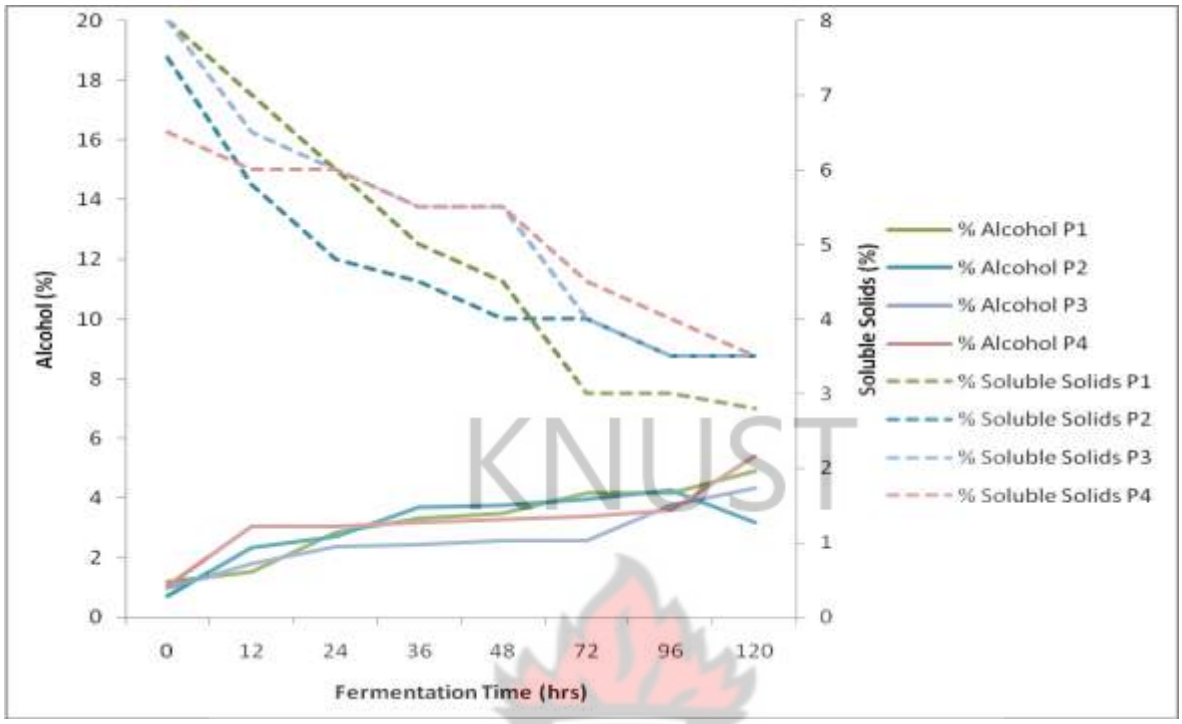


Fig 5: Mean percentage alcohol and soluble solid changes during the fermentation of *burukutu* samples.

4.11 Shelf Life Study

4.11.1 Microbial Quality

The result of the shelf life study carried out on the pasteurized and bottled *burukutu* over a three month period is presented in Table 6. The organisms monitored included, aerobic mesophiles, yeast and moulds, total coliforms and *E. coli*.

The microbial numbers (CFU/ml) in the *burukutu* sample before pasteurization (BP) were 4.60×10^6 for aerobic mesophils and 2.70×10^6 for yeast and moulds, total coliforms and *E. coli* were absent.

On pasteurization and packaging none of the microorganisms monitored were detected in the sample. However, the population of aerobic mesophiles in the products stored in plastic bottles at room temperature (RPB) increased by a 1000 fold on the 3rd day of storage. The

plastic bottles in which the products were stored had also blotted. This further increased to 10^4 CFU/ml on the 6th day and 10^5 CFU/ml on the 9th day. By the 17th day, the population of aerobic mesophiles again increased slightly. This same trend was observed in the yeast and moulds population.

However, products stored at room temperature and in glass bottles (RGB) had low populations of aerobic mesophiles as well as yeast and moulds with counts ranging from 10^1 CFU/ml on the 3rd day to 10^2 CFU/ml on the 6th and 9th day. The population of aerobic mesophiles however declined by the 17th day and again by the 31st day with no counts of yeast and moulds. Aerobic mesophiles and yeast and moulds could not be detected in the product over the remaining storage period.

The products packaged in both plastic and glass bottles and stored in a climatic chamber at a temperature of 4°C and a humidity of 32%rH (CPB and CGB) showed a different trend. The CPB had very low counts of 10^1 CFU/ml from the 3rd to the 9th day for both aerobic mesophiles and yeast and moulds. There was a decline to 10^0 CFU/ml by the 17th day, after which they all died out and could not be detected. The CGB products however had no microbial counts from the beginning to the end of the study. Total coliforms and *E. coli* were absent in all the four products.

Table 6: Microbial population of pasteurized and bottled *burukutu*

Day	Sample	AMC (CFU/ml)	Y&M (CFU/ml)	T. Coliforms (CFU/ml)	*E. coli* (CFU/ml)
1	BP	4.60×10^6	2.70×10^6	nd	nd
1	RPB	nd	nd	nd	nd
	RGB	nd	nd	nd	nd
	CPB	nd	nd	nd	nd
	CGB	nd	nd	nd	nd
3	RPB	7.40×10^3	2.10×10^3	nd	nd
	RGB	3.50×10^1	1.50×10^1	nd	nd
	CPB	2.30×10^1	1.60×10^1	nd	nd
	CGB	nd	nd	nd	nd
6	RPB	5.40×10^4	3.70×10^4	nd	nd
	RGB	2.10×10^2	1.90×10^2	nd	nd
	CPB	2.90×10^1	1.10×10^1	nd	nd
	CGB	nd	nd	nd	nd
9	RPB	5.60×10^5	3.80×10^5	nd	nd
	RGB	7.20×10^2	6.70×10^2	nd	nd
	CPB	5.10×10^1	4.40×10^1	nd	nd
	CGB	nd	nd	nd	nd
17	RPB	9.60×10^5	7.30×10^5	nd	nd
	RGB	6.00×10^1	2.00×10^1	nd	nd
	CPB	5.00×10^0	3.00×10^0	nd	nd
	CGB	nd	nd	nd	nd

31	RGB	2.80×10^1	nd	nd	nd
	CPB	nd	nd	nd	nd
	CGB	nd	nd	nd	nd
45 -101	RGB	nd	nd	nd	nd
	CPB	nd	nd	nd	nd
	CGB	nd	nd	nd	nd

NB:

RGB: Room Temperature Glass Bottle
RPB: Room Temperature Plastic Bottle
CPB: Climatic Chamber Plastic Bottle
CGB: Climatic Chamber Glass Bottle
BP: Before Pasteurization
nd: Not Detected

4.11.2 Physico-chemical Quality

The chemical parameters monitored included pH, titratable acidity, alcohol content, soluble solids and the physical parameter was colour measurement. The sample before pasteurization (BP) had an alcohol content of 0.41% but reduced to 0.23% in the plastic bottled samples and 0.14% for the glass bottled samples (Table 7). This also affected the soluble solids resulting in an increase from 8.00% in the sample before pasteurization (BP) to 9.00% in both the plastic and glass bottled samples. During the study, the pH of all the samples remained in the region of 3.00. The RPB sample recorded the lowest pH of 3.05 and the highest alcohol level of 3.80% on the 17th day. The total soluble solids also reduced to the lowest level of 5.00% on the 9th and 17th day with an increasing titratable acidity level.

The RGB and CPB samples recorded a pH level of 3.22 and 3.26, alcohol content of 1.29 and 0.52, titratable acidity level of 0.59% and 0.55%, a reduction in the soluble solids to 8.50% and 8.70% respectively on the last day. A more stable product was observed with the CGB sample. The pH ranged from a minimum of 3.15 on the 1st day to a maximum of 3.37 on the

73rd day. The alcohol and titratable acidity levels were very stable hovering around 0.14 to 0.16 and 0.27% to 0.33% respectively throughout the period and hence a marginal reduction in the soluble solids from 9.00% from the beginning to 8.90% by the end of the period.

The colour of the products was also monitored as it plays a very key role in the overall acceptability of the final product. On visual inspection, the colour of the final bottled samples

was described as dark brown just like what is known for *burukutu*. The colour of all the bottled samples did not change much compared to the original. The BP sample recorded a scale of L=52.88, a=+9.73, b=-0.95 but this reduced slightly to L=51.12, a=+9.36, b=-2.38 for the plastic bottled samples and further again to L=50.00, a=+9.50, b=-3.18 for the glass bottled samples. However due to the different storage conditions they further changed to L=50.24, a=+9.70, b=-1.49 for RPB by the 17th day. The sample RGB changed to L=50.38, a=+6.53, b=-3.31, L=50.50, a=+6.83, b=-2.66 for CPB and L=50.56, a=+6.41, b=-2.01 for CGB on the last day of storage.

Table 7: Some physical and chemical analysis of pasteurized and bottled *burukutu* sample

Day	**Sample**	**pH**	**Alcohol**	**TA**	**SS**	**Colour**
1	BP	3.05	0.41	0.45	8.0	L=52.88, a=+9.73, b=-0.95
1	RPB	3.20	0.23	0.34	9.0	L=51.12, a=+9.36, b=-2.38
	RGB	3.15	0.14	0.27	9.0	L=50.00, a=+9.50, b=-3.18
	CPB	3.20	0.23	0.34	9.0	L=51.12, a=+9.36, b=-2.38
	CGB	3.15	0.14	0.27	9.0	L=50.00, a=+9.50, b=-3.18
3	RPB	3.13	1.14	0.42	8.5	L=52.32, a=+9.15, b=-2.14
	RGB	3.25	0.54	0.41	9.0	L=48.81, a=+9.48, b=-2.81
	CPB	3.31	0.26	0.37	9.0	L=48.51, a=+9.62, b=-2.31
	CGB	3.34	0.15	0.29	9.0	L=48.92, a=+9.40, b=-2.22
6	RPB	3.10	2.64	0.50	8.1	L=49.75, a=+9.27, b=-2.49
	RGB	3.24	0.73	0.42	9.0	L=48.67, a=+9.31, b=-3.46
	CPB	3.30	0.33	0.38	9.0	L=48.94, a=+9.52, b=-2.30
	CGB	3.34	0.15	0.30	9.0	L=48.95, a=+9.39, b=-2.13
9	RPB	3.08	3.26	0.81	5.0	L=50.67, a=+8.91, b=-0.75
	RGB	3.23	0.74	0.51	9.0	L=48.34, a=+9.63, b=-2.85
	CPB	3.29	0.46	0.45	9.0	L=49.56, a=+9.37, b=-2.23
	CGB	3.35	0.14	0.30	9.0	L=48.97, a=+9.43, b=-2.05
17	RPB	3.05	3.80	0.86	5.0	L=50.24, a=+9.70, b=-1.49
	RGB	3.22	0.78	0.53	9.0	L=48.14, a=+9.89, b=-3.21
	CPB	3.27	0.53	0.48	9.0	L=49.31, a=+9.47, b=-2.19
	CGB	3.33	0.15	0.32	9.0	L=49.97, a=+9.57, b=-2.19
31	RGB	3.23	0.76	0.56	9.0	L=48.15, a=+9.72, b=-2.89
	CPB	3.30	0.51	0.54	9.0	L=49.72, a=+9.36, b=-2.22
	CGB	3.36	0.15	0.32	9.0	L=49.90, a=+9.37, b=-2.33
45	RGB	3.21	0.75	0.56	8.7	L=48.55, a=+9.11, b=-3.23
	CPB	3.29	0.50	0.53	8.9	L=49.38, a=+8.99, b=-2.12
	CGB	3.37	0.16	0.33	8.9	L=49.38, a=+9.12, b=-2.44
59	RGB	3.25	0.73	0.54	8.7	L=50.83, a=+8.22, b=-3.21
	CPB	3.28	0.49	0.52	8.9	L=50.91, a=+8.63, b=-2.21

	CGB	3.36	0.15	0.32	8.9	L=50.02, a=+8.71, b=-2.19
	RGB	3.26	0.69	0.54	8.7	L=50.30, a=+8.12, b=-3.20
73	CPB	3.25	0.52	0.52	8.9	L=50.99, a=+8.52, b=-2.22
	CGB	3.37	0.15	0.33	8.9	L=50.86, a=+8.73, b=-2.07
	RGB	3.24	0.93	0.56	8.5	L=50.72, a=+6.64, b=-3.22
87	CPB	3.25	0.52	0.53	8.7	L=50.55, a=+6.49, b=-2.21
	CGB	3.36	0.15	0.32	8.9	L=50.55, a=+6.44, b=-2.00
	RGB	3.22	1.29	0.59	8.5	L=50.38, a=+6.53, b=-3.31
101	CPB	3.26	0.52	0.55	8.7	L=50.50, a=+6.83, b=-2.66
	CGB	3.36	0.14	0.33	8.9	L=50.56, a=+6.41, b=-2.01

(L = luminosity, a = red /green component, b = yellow / blue component).

4.12 Sensory Analyses on Bottled *Burukutu*

The sensory evaluation was done to find out the level of consumer acceptability (Appendix 5) of three different packages of *burukutu* i.e. Room Glass Bottled (RGB), Climatic Plastic Bottled (CPB) and Climatic Glass Bottled (CGB). The Room Plastic Bottled (RPB) samples were not used for the sensory analysis because of the short life span.

The mean scores of appearance of RGB samples ranged from 7.9 to 8.1. and was liked very much, colour were 8.1, 8.2, 7.9 and 7.9 for two weeks, one month, two months and three months storage respectively (Table 8). The mean scores of consistency ranged from 7.8 to 8.2. This implies that the consistency of each RGB was liked very much. The aroma of the samples stored for 3 months had the highest mean score of 8.1 with RGB stored for two months having the lowest mean score of 7.6.

The taste of each RGB sample stored over the period was liked very much with RGB sample stored for three months having the highest mean score.

The mean scores of the mouth feel of RGB ranged from 7.6 to 8.0 with RGB stored for one month having the lowest mean score of 7.6 and were liked very much.

The overall acceptability of RGB sample stored for three months was liked very much with a mean score of 8.0 as compared to the other months and the product in general was liked very much based on each of the parameters evaluated.

Table 8: The mean and standard deviation score on the RGB samples.

Period of Time	Appearance	Colour	Consistency	Aroma	Taste	Mouth feel	Overall Acceptability
2 weeks	7.9 ± 0.57	8.1 ±0.32	8.1 ± 0.32	7.7 ± 0.67	7.8 ± 0.92	7.9 ± 0.57	7.7 ± 0.48
1 Month	8.1 ± 0.74	8.2 ± 0.63	8.0 ± 0.94	7.9 ± 0.74	7.5 ± 0.64	7.6 ± 0.26	7.5 ± 0.42
2 Month	7.9 ± 0.57	7.9 ± 0.57	7.8 ± 0.33	7.6 ± 0.64	7.7 ± 0.95	7.9 ± 0.74	7.9 ± 0.74
3 Month	8.1 ± 0.74	7.9 ± 0.74	8.2 ± 0.42	8.1 ± 0.88	8.0 ± 0.47	8.0 ± 0.78	8.0± 0.42

From the results (Table 9), it can be deduced that the CPB samples stored for two weeks was liked extremely based on each of the parameters evaluated and thus the most preferred. The samples were still liked very much from the first to the third month of storage.

Table 9: The mean and standard deviation score on the CPB samples.

Period of Time	Appearance	Colour	Consistency	Aroma	Taste	Mouth feel	Overall Acceptability
2 weeks	8.4 ± 0.52	8.7 ± 0.48	8.7 ± 0.48	8.5 ± 0.52	8.6 ± 0.52	8.6 ± 0.52	8.5 ± 0.53
1 Month	8.3 ± 0.82	8.2 ± 0.79	8.2 ± 0.63	7.9 ± 0.74	7.7 ± 0.95	7.6 ± 0.97	7.7 ± 0.59
2 Month	8.0 ± 0.67	7.8 ± 0.92	7.8 ± 0.63	7.9 ± 0.99	7.7 ± 0.59	7.8 ± 0.63	7.8 ± 0.72
3 Month	7.9 ± 0.82	7.9 ± 0.73	7.7 ± 0.82	7.7 ± 0.82	7.8 ± 0.79	7.8 ± 0.91	7.9 ± 0.56

The overall acceptability of the CGB samples stored for 2 weeks was liked extremely in each of the parameters evaluated except appearance which was liked very much (Table 10). From the first to the third month of storage, each of the parameters evaluated was liked very much.

Table 10: The mean and standard deviation score on the CGB samples.

Period of Time	Appearance	Colour	Consistency	Aroma	Taste	Mouth feel	Overall Acceptability

2 weeks	8.4 ± 0.52	8.8 ± 0.42	8.7 ± 0.48	8.8 ± 0.42	8.8 ± 0.42	8.6 ± 0.52	8.6 ± 0.74
1 Month	8.1 ± 0.99	8.2 ± 0.92	8.1 ± 0.99	7.5 ± 0.58	8.0 ± 0.54	8.0 ± 0.94	7.9 ± 0.99
2 Month	7.9 ± 0.74	7.8 ± 0.79	7.5 ± 0.84	7.8 ± 0.92	7.7 ± 0.59	7.9 ± 0.74	7.8 ± 0.63
3 Month	8.0 ± 0.82	8.1 ± 0.74	8.1 ± 0.74	8.0 ± 0.79	8.1 ± 0.94	8.0 ± 0.67	8.1 ± 0.87

Seventy percent of the panelist suggested that the samples be differentiated into two products i.e. CPB and CGB should be grouped into low alcohol content *burukutu* whilst RGB should be high alcohol content *burukutu.*

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

Production of *Burukutu*

The study of the manufacturing practices of ten *burukutu* producers in the Accra and Tema metropolis did not reveal any differences in the processing procedures used in producing the traditional alcoholic beverage. The same unit operations as already outlined in figure 1 were observed at all the production sites visited. This procedure, as practiced in Ghana, is different from the process reported from Nigeria for the production of *burukutu* (Banigo *et al.*, 1987). The major difference in the Nigerian process is the addition of lumps of gari (kpokpo-gari) as adjunct during mashing providing additional starch for hydrolysis into fermentable sugars. This was not observed in the Ghanaian process and it was therefore expected that the alcohol content reported for the Nigerian product would be higher due to the additional fermentable sugars for conversion into alcohol by yeasts. This was however difficult to verify because of the manner of handling of *burukutu* by the Ghanaian processors during marketing. Most

processors allow the product to ferment for about 8 h which yields an alcohol content of about 1.5 to 3.0%. However, during sale of the product, processors sometimes added unfermented brew which initially diluted the alcohol content but invigorated the fermentation and slowly built up the alcohol content again or do fresh back slopping. This is done more or less continually by most processors. This is not the practice in Nigeria. Comparably, the alcohol content in Nigerian *burukutu* is between 2-4% (Banigo *et al.*, 1987; Faparusi *et al.*, 1973). The study has shown that even though the process of *burukutu* production in Ghana is similar to that of *pito*, they differ in consistency as the ratio of flour to water is about 1:3 for

burukutu and 1:5 for *pito*, the malted grain is also dried before milling in the prepation of *pito* thereby making the latter lighter. In Nigeria, the thicker consistency of *burukutu* is obtained by the addition of gari lumps as an adjunct.

Of the various production sites visited during the study, the general sanitation and hygienic conditions were poor which may explain the high population of aerobic mesophiles (an indicator of the sanitary quality of foods) recorded at the various stages of *burukutu* preparation (Michodjehoun - Mestres *et al*., 2005). The processors neither wash the grains nor pick unwanted materials such as stones from them but only pour the grains from the sacks straight into the steeping water. Furthermore, the quality of the water used in processing may also be a contributory factor since out of the four sites, only one had access to running tap water with the rest buying water from lorry tankers which may be of varying microbiological quality. Finally, most of the processors use their bare hands or unwashed wooden laddles during the mixing and stirring process and the product was also left to stand uncovered making it susceptible to external contamination by air bourn spores.

Although *E. coli* was not isolated from the fresh *burukutu*, the previous product used as a starter inoculum (I), was found to contain *E. coli*. The bacteria could have come from poor handling, poor personal hygiene and lack of toilet facilities on the processing premises. Kolawole *et al.,* (2007) also attributed the presence of *E. coli* to improper sanitary condition during processing of the *burukutu* from the water supply, unsterilized utensils and contamination by flies in Nigeria.

Souring/Lactic Acid Fermentation of *Burukutu*

Lactic acid bacteria; *Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus acidophilus, Lactococcus lactis* subsp. *lactis* and *Lactobacillus brevis* were found to dominate the souring or acidification process. Population of these bacterial organisms were highest between 96 and 120 h of fermentation. Sawadogo-Lingani *et al.,* (2007) who studied *pito* production in northern Ghana and Burkina Faso, reported *Lactobacillus fermentum* as the dominant species during acidification of the product. This agrees with the findings of Marcellin *et al.,* (2009). Kayode *et al.,* (2006) also isolated *Lactobacillus fermentum,* among others from opaque sorghum beer in Nigeria and Glover *et al.,* (2009) used a double-strain combination of *Lactobacillus fermentum* and *Saccharomyces cerevisiae* as a starter culture in the production of *pito* that yielded a preferable taste and aroma comparable to the traditional product. Faparusi *et al.*, (1973) isolated *L. plantarum* and *L. brevis* among others in a 24 h fermented mash-gari mixture during *burukutu* production in Nigeria. Amoa-Awua *et al.,*

(2006) also found *L. plantarum* to be the dominant lactic acid bacteria in palm wine in Ghana. The presence of *L. acidophilus* and *Lactococcus lactis* subsp. *lactis*, has also been reported in other traditional beers such as *chibuku* and *tchapalo* (Togo *et al*., 2002; Marcellin *et al.*, 2009).

Alcoholic Fermentation of *Burukutu*

In the present study, *Saccharomyces cerevisiae* formed about 70% of the yeast isolates involved in the alcoholic fermentation of *burukutu* and thus may be key to its production. Faparusi *et al.*, (1973); Ogbonna *et al*., (1983); Oke and Ijebor (1997) and Achi (2005), isolated *Saccharomyces cerevisiae* as the dominant yeast in *burukutu* production In Nigeria. In Ghana, Glover *et al.*, (2005) found *S. cerevisiae* to be the dominant yeast in the alcoholic fermentation of *pito* within eight geographical regions of Ghana and at four production sites in Burkina Faso.

Numerous studies have also documented *S. cerevisiae* as predominant in alcoholic fermented beverages (Jespersen, 2003; Barnett *et al*., 2000; Battcock and Ali, 1993).

In addition to *Saccharomyces cerevisiae*, *Candida krusei* was also found in high numbers in *burukutu*. Faparusi *et al.*, (1973) again isolated *Candida krusei* as one of the dominant microorganisms in a three day malted grain for *burukutu* production. *Saccharomyces cerevisiae, Candida krusei* and lactic acid bacteria were the dominant microorganisms in this study as well as other studies (Nout, 1980; Zulu *et al.*, 1997; Amoa-Awua and Jacobsen, 1996; Halm and Olsen, 1996; Holzapfel, 1997; Hounhouigan *et al.*, 1999; Blanco *et al.*, 1999; Gadaga *et al.*, 2001). Halm *et al.*, (1996), have shown that *S. cerevisiae* and *C. krusei* can inhibit the growth of mycotoxin-producing moulds such as *Aspergillus flavus, Aspergillus*

parasiticus and Penicillium citrinum. In some other studies, *C. krusei* has been associated with the production of flavour and aroma (Oyewole. 2001).

Food Safety of *Burukutu*

One of the goals of this study was to explore the possibility of industrializing the production of *burukutu* as this has been achieved for other African traditional beverages. For example, shake-shake (*chibuku*), a traditional South African opaque beer, has been brewed by Accra Brewery and introduced on the Ghanaian market. The development of a suitable starter culture for the production of *burukutu* could help in its industrialization.

Candidate isolates from this study; *L. fermentum, L. plantarum* and *Lactococcus lactis* subsp. *lactis* which showed amylolytic properties as well as *Saccharomyces cerevisiae, Candida krusei* could be further studied in the development of a starter culture. Sawadogo-Lingani *et al*., (2007) again reported the amylolytic properties of *L. fermentum* in *pito*.

The five dominant LAB isolates; *L. fermentum, L. plantarum, L. acidophilus, L. brevis* and *Lactococcus lactis* subsp. *lactis* had antimicrobial activity against *Escherichia coli, Staphylococcus aureus* and *Salmonella typhimurium*.

Low pH levels (3.13-3.38) during the fermentation process of the challenge test could have contributed to the antimicrobial activity against the bacteria pathogens (Steinkraus, 2002; Tadesse *et al*., 2005a; Svanberg *et al*., 1992; Kalui *et al*., 2009; 2010). Other contributory factors to the antimicrobial activity may be the production of hydrogen peroxide, bacteriocins and other antimicrobial substances (Mbugua and Njenga, 1992; Kingamko *et al*., 1994; Tanasupawat and Komagata, 1995; Reinhiemer, *et al*., 1990; Hammes and Tichaczek, 1994).

The various interactions between the isolates from the fermenting product and the pathogens could add to the notion that fermented foods in general possess antimicrobial activity which makes them safe for consumption (Mensah *et al*., 1991; Simango and Rukure 1992; Halm *et al*., 1993; Olsen *et al*., 1995).

There was an increase in the amylase activity from the dry grain to the malted grain as it was during the malting stage that the starch in the sorghum grain is hydrolyzed into fermentable sugars by amylolytic organisms. It was also noted that α-amylase activity peaked on day 3 after malting (Mundy, 1982; Uvere *et al*., 2000). However, amylase content decreased during the settling period of the mash when the supernatant separates from the sediment. This is because a greater amount of the enzymes from the malting stage are found in the supernatant.

Chemical Analysis

The gradual decrease in pH values and increased titratable acidity may be as a result of microbial activity on the carbohydrates to produce organic acids such as lactic acid in the fermenting *burukutu*. This agrees with work done by Inyang and Idoko (2006) that a

decreased pH during the fermentation process could accelerate LAB growth (Halm *et al.*, 1993; Muyanja *et al.*, 2003; Mugula *et al*., 2003; Namugumya and Muyanja, 2009).

It was observed that there was a gradual increase in the alcoholic content of the *burukutu* samples with fermentation time which resulted in a decrease in the percentage of total soluble

solids. This may be due to the breakdown of fermentable sugars which serves as the substrate for the microorganisms (Tiisekwa *et al*., 2000). The increase in alcohol content and the decrease in total soluble solids can also be attributed to the alcoholic fermentation carried out by the yeasts (O'Rourke, 2000**)**.

Shelf Life Study of Bottled *Burukutu*

In extending the shelf life of the *burukutu* product, various options were considered; bottling in glass and plastic containers. Results showed that bottling in glass was the best option. This is because the product pH, alcohol level, titratable acidity, soluble solids, colour, appearance, consistency, aroma, taste, mouth feel and microbial population all did hold good for twelve weeks. However, the product in plastic containers at room temperature deteriorated after three weeks.

Although the product in the different containers were stored in a climatic chamber (4°C and a humidity of 32%rH) and at room temperature, the product in glass bottles in the climatic chamber remained best.

In a similar work carried out on *kunun-zaki*, an African fermented cereal - based food drink, refrigerated samples stored longer than at room temperature (Olasupo *et al*., 2000). According to Scott and Sullivan (2008), the process of refrigeration halts the fermentation process.

The reduction in the alcohol level of the *burukutu* sample after pasteurization may be a consequence of the evaporation of the alcohol from the sample during the heating process. This effect was more pronounced on the glass bottled samples as the pasteurization was done

twice. Some amount of water was also lost in the process resulting in an increased total soluble solid.

After pasteurization it was generally observed that more of the fermentable sugars were broken down, the total soluble solids reduced and alcohol levels increased. The pasteurized bottled samples remained microbiologically and chemically stable throughout the twelve weeks of storage.

Sensory Analysis

From the results, bottled pasteurized *burukutu* can be stored ideally in climatic chambers (4°C and 32%rH) as the ten member panelist rated that product the highest among the rest.

5.2 CONCLUSION

The production techniques of *burukutu* were similar among the processors. The lactic acid bacteria; *Lactobacillus fermentum, Lactobacillus acidophilus, Lactobacillus plantarum Lactococcus lactis* subsp. *lactis* and *Lactobacillus brevis* were the dominant organisms involved in the souring or acidification process. The LAB isolates also had antimicrobial activities against the pathogens *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimurium*. *Saccharomyces cerevisiae* was identified as the predominant yeast responsible for the alcoholic fermentation in a microflora which included *Candida krusei*.

Burutuku can keep for twelve weeks if pasteurized, glass bottled and stored in a climatic chamber (4°C and 32%rH).

5.3 RECOMMENDATION

Due to financial constrains, the lactic acid bacteria and yeast isolates were not identified by molecular methods and so future research should be conducted using molecular methods to develop strains with better and stable genetic properties for use as starter cultures to provide assured qualities for commercial production of *burukutu*.

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APPENDICES

Appendix 1: Survey questionnaire

- What is your name and occupation?
- Which ethnic group do you come from?

- How long have you been making *burukutu*?
- What raw materials do you use?
- Is there more than one process for making *burukutu*?
- If yes, are there variations in the process and the raw materials for making *burukutu*?
- Where do you get your raw materials from?
- What quantity do you buy at a time?
- How do you store them?
- How many bags do you process in a batch?
- What is the duration of your processing?
- Do you sometimes vary the process and raw materials?
- How long does it take you to sell a batch?
- How long can you keep a batch?
- What do you do with the left over?
- Where do you get your inoculums from?
- What do you use the waste generated after processing for?
- How do you sell the *burukutu*? Is it by bulk selling or retailing?
- What is the difference between *burukutu* and *pito*?
- Why do some people prefer *burukutu* to *pito*?
- Is it a profitable venture?

Appendix 2: Mean values of triplicates and standard deviations of microbial population in $\log_{10}$ CFU/mls of four processors.

SAMPLES	AEROBIC MESOPHILES	YEAST & MOULDS	LACTIC ACID BACTERIA	COLIFORM	*E.COLI*

BM	6.62 ± 0.82	5.23 ± 0.57	1.37 ± 1.66	5.73 ± 0.94	3.36 ± 2.64
AM	8.59 ± 0.93	7.55 ± 0.72	7.75 ± 0.76	7.78 ± 0.56	5.54 ± 1.09
SU	8.88 ± 0.57	7.35 ± 0.82	8.11 ± 0.59	7.75 ± 0.82	6.19 ± 0.95
SE	9.17 ± 0.47	7.52 ± 0.62	8.34 ± 0.43	7.80 ± 0.93	6.34 ± 1.05
M	6.03 ± 1.24	4.89 ± 0.65	6.40 ± 1.84	3.58 ± 1.16	2.27 ± 1.51
F	4.52 ± 0.46	3.46 ± 0.85	3.61 ± 0.50	0.00 ± 0.00	0.00 ± 0.00
I	8.14 ± 0.55	7.89 ± 0.49	7.62 ± 0.56	1.82 ± 3.64	1.29 ± 2.58
O HR	7.14 ± 0.34	6.82 ± 0.34	6.66 ± 0.36	0.88 ± 1.76	0.47 ± 0.94
12 HR	7.95 ± 0.45	7.49 ± 0.54	7.64 ± 0.60	0.13 ± 0.26	0.00 ± 0.00
24 HR	8.37 ± 0.41	7.86 ± 0.30	7.75 ± 0.28	nd	nd
36 HR	8.65 ± 0.38	7.94 ± 0.40	7.94 ± 0.44	nd	nd
48 HR	8.82 ± 0.43	8.16 ± 0.34	8.00 ± 0.42	nd	nd
72 HR	8.69 ± 0.06	8.23 ± 0.29	7.87 ± 0.40	nd	nd
96 HR	8.54 ± 0.21	8.15 ± 0.24	8.26 ± 0.35	nd	nd
120 HR	8.41 ± 0.38	8.18 ± 0.43	8.14 ± 0.41	nd	nd

nd = not detected

Appendix 2b: Mean values of pH, titatable acidity, soluble solids and alcohol level from four processors

Fermentation Time (Hrs)	Mean pH	Mean Titratable Acidity	Mean Soluble Solids	Mean Alcohol Level
0	3.36	0.54	7.50	0.99
12	3.25	0.65	6.33	2.18
24	3.19	0.67	5.70	2.75
36	3.09	0.69	5.13	3.15
48	3.11	0.69	4.88	3.27
72	3.05	0.66	3.88	3.53
96	2.94	0.74	3.50	3.95
120	2.88	0.82	3.33	4.47

Appendix 3: Analysis of Variance (ANOVA) of microbial population

```
ONEWAY amc coli ym ecoli lab BY nam_sam
 /STATISTICS DESCRIPTIVES
```

/MISSING ANALYSIS

/POSTHOC=TUKEY ALPHA(0.05).

Oneway

Notes

Output Created		17-Jan-2012 21:29:44
Comments		
Input	Active Dataset	DataSet0
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	60
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each analysis are based on cases with no missing data for any variable in the analysis.
Syntax		ONEWAY amc coli ym ecoli lab BY nam_sam /STATISTICS DESCRIPTIVES /MISSING ANALYSIS /POSTHOC=TUKEY ALPHA(0.05).
Resources	Processor Time	00:00:00.172
	Elapsed Time	00:00:00.172

[DataSet0]

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
AMC	Between Groups	92.609	14	6.615	19.382	.000
	Within Groups	15.358	45	.341		
	Total	107.968	59			
Coliform	Between Groups	589.160	14	42.083	30.813	.000
	Within Groups	61.458	45	1.366		
	Total	650.618	59			
YM	Between Groups	115.674	14	8.262	28.230	.000
	Within Groups	13.171	45	.293		
	Total	128.845	59			
E. Coli	Between Groups	336.146	14	24.010	17.964	.000
	Within Groups	60.147	45	1.337		
	Total	396.294	59			
LAB	Between Groups	216.641	14	15.474	25.245	.000
	Within Groups	27.583	45	.613		
	Total	244.225	59			

Post Hoc Tests

Homogeneous Subsets

AMC

Tukey HSD

Stage of processing	N	Subset for alpha = 0.05				
		1	2	3	4	5
F	4	4.5200				
M	4		6.0300			
BM	4		6.6250	6.6250		
0	4		7.1400	7.1400	7.1400	
12	4			7.9500	7.9500	7.9500
I	4				8.1400	8.1400
24	4				8.3700	8.3700
120	4				8.4075	8.4075
96	4				8.5375	8.5375
AM	4				8.5950	8.5950
36	4					8.6525
72	4					8.6875
48	4					8.8150
SU	4					8.8750
SE	4					9.1750
Sig.		1.000	.345	.124	.059	.207

AMC

Tukey HSD

Stage of processing	N	Subset for alpha = 0.05				
		1	2	3	4	5
F	4	4.5200				
M	4		6.0300			
BM	4		6.6250	6.6250		
0	4		7.1400	7.1400	7.1400	
12	4			7.9500	7.9500	7.9500
I	4				8.1400	8.1400
24	4				8.3700	8.3700
120	4				8.4075	8.4075
96	4				8.5375	8.5375
AM	4				8.5950	8.5950
36	4					8.6525
72	4					8.6875
48	4					8.8150
SU	4					8.8750
SE	4					9.1750
Sig.		1.000	.345	.124	.059	.207

Means for groups in homogeneous subsets are displayed.

Coliform

Tukey HSD

Stage of processing	N	Subset for alpha = 0.05			
		1	2	3	4
F	4	.0000			
24	4	.0000			
36	4	.0000			
48	4	.0000			
72	4	.0000			
96	4	.0000			
120	4	.0000			
0	4	.1308			
12	4	.8775	.8775		
I	4	1.8225	1.8225		
M	4		3.5825	3.5825	
BM	4			5.7300	5.7300
SU	4				7.7475
AM	4				7.7750
SE	4				7.8050
Sig.		.660	.107	.398	.453

Means for groups in homogeneous subsets are displayed.

YM

Tukey HSD

Stage of processing	N	Subset for alpha = 0.05			
		1	2	3	4
F	4	3.4550			
M	4		4.8900		
BM	4		5.2325		
0	4			6.8200	
SU	4			7.3525	7.3525
12	4			7.4925	7.4925
SE	4			7.5200	7.5200
AM	4			7.5500	7.5500
24	4			7.8600	7.8600
I	4			7.8875	7.8875
36	4			7.9400	7.9400
96	4			8.1500	8.1500
48	4			8.1600	8.1600
120	4			8.1850	8.1850
72	4				8.2325
Sig.		1.000	1.000	.052	.595

Means for groups in homogeneous subsets are displayed.

E. Coli

Tukey HSD

Stage of processing	N	Subset for alpha = 0.05			
		1	2	3	4
F	4	.0000			
24	4	.0000			
36	4	.0000			
48	4	.0000			
72	4	.0000			
96	4	.0000			
120	4	.0000			
0	4	.1000			
12	4	.4700	.4700		
I	4	1.2925	1.2925		
M	4	2.2668	2.2668		
BM	4		3.3575	3.3575	
AM	4			5.5425	5.5425
SU	4			6.1950	6.1950
SE	4				6.3400
Sig.		.298	.057	.067	1.000

Means for groups in homogeneous subsets are displayed.

LAB

Tukey HSD

Stage of processing	N	Subset for alpha = 0.05		
		1	2	3
BM	4	1.3650		
F	4		3.6100	
M	4			6.4075
0	4			6.6650
I	4			7.6225
12	4			7.6400
AM	4			7.7525
24	4			7.7525
72	4			7.8650
36	4			7.9425
48	4			8.0050
SU	4			8.1075
120	4			8.1400
96	4			8.2550
SE	4			8.3375
Sig.		1.000	1.000	.064

Means for groups in homogeneous subsets are displayed.

Appendix 4: Cell and colony characteristics of yeasts isolated from *burukutu*

Isolates	Cell Morphology	Colony Morphology	Growth in Liquid
1	Cylindrical, single cells, oval, mono budding	Colour : creamy Surface : hirsuit Measurement : 4-8 mm Form : filamentous Margin : filiform Elevation : raised	Pellicle between glass liquid interphase and sediment at the bottom
2	Single cells, cylindrical, oval mono budding	Colour : white Surface : smooth Measurement : 2-5 mm Form : circular Margin : entire Elevation : umbonate	Sediment at the bottom of tubes
3	Single cells, cylindrical, oval mono budding	Colour : off white Surface : smooth Measurement : 4-7mm Form : circular Margin : filiform Elevation : umbonate	Pellicle between glass liquid interphase and sediment at the bottom

4	Single cells, oval	Colour : brownish Surface : smooth and shiny Measurement : 2-7 mm Form : circular Margin : entire Elevation : umbonate	Sediment at the bottom of the tubes
5	Single cells, oval, mono budding	Colour : creamy Surface : smooth Measurement : 2-4 mm Form : circular Margin : entire Elevation : umbonate	Sediment at the bottom of tubes
6	Single cells, cylindrical, oval, few mono budding cells	Colour : off white Surface : hirsuit Measurement : 2-7 mm Form : filamentous Margin : filiform Elevation : convex	Pellicle between glass liquid interphase
7	Single cells, fission, oval, cylindrical	Colour : creamy Surface : smooth Measurement : 2-5 mm Form : circular Margin : entire	Sediment at the bottom of tubes

Elevation : umbonate

Appendix 5: Acceptability test form

Name…….. Date……..

Product……..

Sample Code…….

Please before you is a sample of *burukutu*. Please show your degree of likeness by using the scale provided below. Please use the biscuit and water provided to wash your mouth before moving on to the next sample. Thank you.

Scale	Interpretation	Appearance	Colour	Consistency	Aroma	Taste	Mouth feel	Overall acceptability
9	Like Extremely							
8	Like very much							
7	Like moderately							
6	Like slightly							
5	Neither like nor dislike							
4	Dislike slightly							
3	Dislike moderately							
2	Dislike very much							
1	Dislike extremely							

Comments: ……