STUDIES ON DEVELOPING A STARTER CULTURE FOR THE FERMENTATION OF BURUKUTU BEER

BY

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DEDICATION

This piece of work is dedicated to the almighty God whose steadfast love never ceases.

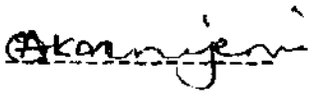
DECLARATION

This is to certify that I carried out the work reported in this thesis at the laboratories of the department of Microbiology, Ahmadu Bello University, Zaria, under the supervision of Dr. J.B. Ameh.

This thesis is an original work carried out by myself and is not a carry over or continuation of any particular work. No part of it has been presented elsewhere for a degree or a diploma. Any work of other investigators referred to is acknowledged in this presentation

A.B.U, Zaria

December, 1990.



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CERTIFICATION

This thesis entitled "Studies on Developing a starter culture for the fermentation of Burukutu beer" by Akinyemi Olubukola M. meets the regulation governing the award of the degree of master of science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

The microbiological profile during various stages of burukutu production was determined in 42 samples collected from six producers . The bacteria identified include many species of *Bacillus: B cereus, B subtilis B mycoides, Micrococcus luteus* and *M roseus, Enterobacter agglumerous* and *Serratia species*. A single species of genus Acetobacter which was responsible for the vinegary taste of the resulting burukutu fermentation. The bacteria were found to have no positive contribution to burukutu fermentation but were rather detrimental to spoilage.

The predominant molds observed are *Aspergillus spp, Mycelia sterilia, Rhizopus* species and *Fusarium* species. The presence of *Aspergillus* molds was found to be associated with the presence of aflatoxin in the beer.

Nine yeasts belonging to four general were obtained, they are three *Saccharomyces* species namely *Saccharomyces species* I, II, and III, two *Candida species* (I & II) a *Pichia species* and *Rhodotorula species*. Fermentation tests revealed that only the *Saccharomyces species* and *Candida species* have good fermentative ability.

Laboratory brewed burukutu was produced by using the various yeasts as starter cultures. Organoleptic and physiochemical studies (PH, titratable acidity and % ethanol) results showed that there is no difference in burukutu produced traditionally and those obtained from *Saccharomyces species* I and II. It is therefore proposed that *Saccharomyces species* I and II be adopted as starter culture for burukutu production.

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INTRODUCTION

Burukutu, an alcoholic beverage prepared from guinea corn (*Sorghum bicolor or S. vulgare*) remains to be the most widely consumed indigenous drink amongst the population group within the Savanna region of Nigeria. Traditionally, burukutu production involves the malting of sorghum grains, wort boiling, and pitching with "kumpa" (the foamy mass of old beer). The boiled wort is then separated, the supernatant is fermented into pito, while the colloidal suspension is further fermented to burukutu. Increased consumption of burukutu amongst the above mention population group and other Nigerians has been advocated by various investigators because of the high nutritional quality of burukutu compared to industrially produced lager beer. Ogbonna et al. (1983b) and Faparusi (1970) indicated that burukutu contains high content of vitamin C, B vitamins, protein and hexose sugars.

The additional advantages of locally available microbial strains and other raw materials, coupled with cheap price of burukutu after production over lager beer have led many workers to advocate the industrial production of the beer. The limitations to adopting traditional method of processing, however, are the inconsistency in taste and flavour of the burukutu beer prompted by uncontrolled fermentation. The short shelf life, the unhygienic condition associated with fermentation are also contributing factors.

Due to escalating cost of maintaining lager beer factories in Nigeria, considerable attention has been directed at producing indigenous beer from locally available cereals and food crops. Okafor (1975), Nwajifo and Bassir (1982), have successfully preserved and

bottled palm wine. Kaffir beer has also been produced industrially in South Africa using a strain of *Saccharomyces cerevisae* as starter culture with some lactic acid bacteria (Van der Walt, 1956).

Ogbonna et al. (1983 b) documented that the fermentation of burukutu is usually spontaneous by undefined microorganisms whose source could be the water used for mashing, aerial mycelia and some normal flora or the grain used for brewing. Microbiological studies conducted on Burukutu beer (Faparusi et al. (1973), Ogbonna et al. 1983 a, and 1983 b), indicated that these organisms varied from yeasts (which are *Saccharomyces cerevisae, Candida tropicalis, Candida tropicalis, Candida Cruzei, Hansenula anomala,* and *Kloekera apiculata*) to bacteria (which are *Lactobacillus species, Streptococcus species, Bacillus species*) and molds (*Mucor rouxi, Aspergillus species, Penicillum citirum,* and *Rhizopus species*. It is increasingly clear that very few of these microorganisms have any fermentative or any significant role in burukutu production. In Nigeria, starter, culture from microbial isolates which are considered necessary or potentially relevant to a given fermentation process are used singly or mixed as starters for producing desired foods or beverages (Okafor, 1981).

Collard and Levi (1959) used starter culture of mixed *Corynebacterium manihot* and *Geotrichum candidum* to achieve satisfactory products as opposed to the numerous organisms found in the traditional fermentation. Akinrele (1970) found that good quality Ogi (a corn-based food) could be produced with an inoculum consisting of equal level of *Lactobcillus plantarum, Acetobacter cloacae* and *Saccharomyces cerevisae* a temperature of 30°c to 32°c. Ekundayo (1969) found that an unidentified Candida species is of importance in alcoholic fermentation

of corn pito (a malted cereal beverage very similar to burukutu). He also claimed that the yield of alcohol could be increased by the addition of either *Geotrichum candidum* or *Lactobacillus* or both to the *Candida species*.

The adoption of a starter culture may be a very important tool that will aid the industrial production of burukutu beer. In spite of extensive studies done on burukutu, preliminary investigations have not been able to develop a starter culture for burukutu beer. This present study was therefore designed with the aim of developing a starter culture for burukutu production and include the following objectives:

(a) to quantify the isolates from burukutu taking note of organism of highest frequency.

(b) Isolation and identification of organisms associated with different stages of burukutu.

(c) Determination of some physiochemical characteristics (pH, titratable acidity, percentage ethanol, temperature) of both traditionally produced burukutu and laboratory brewed ones.

(d) Determination of fermentation ability of the selected strains for preliminary fermentation tests in the laboratory.

(e) Sensory evaluation of the traditional and laboratory brewed burukutu.

(f) Selection of the possible starter culture based on the results obtained from the above work, taking the following into consideration: occurrence of organisms (stages and frequency); fermentation ability and the organoleptic scores of the resulting beer.

CHAPTER TWO

2 REVIEW OF LITERATURE

2.1 Introduction:

Burukutu is one of the many African's indigenous alcoholic beverages. It is produced mainly form the grains of guinea corn (Sorghum vulgare and S. bicolor (Moench)). It remains to be the most widely consumed indigenous drink amongst the population group within the savanna region of Nigeria (personal contact). The survey conducted by Ogbonna et al. (1985a) about burukutu showed the origin of this beverage to be shrouded in mystery. Like many other African indigenous beverage, burukutu production involves basically fermentation of malted grains of guinea corn, which are then fermented naturally by normal flora of the grains (Faparusi et al., 1970). This art of burukutu production placed the product under brewing whose beginning cannot be precisely determined. Since the art of beer production involves malting of any grain for the production of fermented beverage, therefore, burukutu can therefore be classified under beer.

2.2 Brewing in Africa:

The preparation of traditional fermented alcoholic beverages from various cereals is wide spread in Africa. Platt (1955) reported that African alcoholic beverages can be classified into 5 major groups, namely: fermented honey water, fermented fruit and juices, fermented sap from various species of palm and bamboo, drink made from milk and 'the beer'. All of these groups of alcoholic beverages have more or less

familiar equivalent as mead, cider, fermented birch sap, fermented mares milk (Koumis) and old fashioned ales, all of the developed world (Platt, 1955). Alcoholic beverages from various cereals including burukutu fall under the 5th group which is 'the beer'.

2.3 Origin and socio-economic importance of alcoholic beverages in Africa

Although alcoholic beverages have been widely consumed in the world from prehistoric times dating from the days of biblical Noah, the consumption and production of these beverages in Africa can be traced to King Isis of Egypt who discovered the fermentation of wild wheat could leads to beer. The production of wine and beer from various fruits and cereals respectively widespread in all parts of the continent (Platt, 1955). Available evidence (Perderson, 1978) indicates that alcohol beverage consumption and types vary from one locality to another. Evidence in literature (Van der Walt, 1956; Nout, 1980; Ekundayo, 1969 Dirar, 1978; Faparusi et al., 1973; Ogbonna et al., 1983a) indicated that some documented forms of indigenous beer include Kaffir beer of South Africa; bussa, a Kenyan opaque maize beer; Merrisa, a Sudanese beer and Pito and Burukutu from Nigeria.

The economic importance of alcoholic beverages world wide has been documented (Platt, 1955; Pederson, 1978). In Africa, available evidence suggested increased consumption of beer and locally available alcoholic beverages among all population groups. This has been attributed to various factors. Platt (1955), for instance, indicated their consumption and the diverse role alcoholic beverages play in social gatherings. Socially, it is mandatory that alcoholic beverages be served if

marriages, burials, naming, and other social gatherings. Secondly alcoholic beverages form an important part of sacrifices offered to gods in many parts of Africa (Perderson, 1978). It is used for treatment against such ailment as common cold, and as a solvent for extraction of 'drugs' from tree bark and roots. This accounts for its increased consumption (Perderson, 1978).

The role of alcoholic beverages in many societies had been recognized decades ago. Richards (1939a) made an observation about the role of alcoholic drinks amongst the Bait society and summarized his finding thus "It is the people's only kind of entertainment, the chief break in monotony of their village life, and, as in most other Bait societies, the common and sometimes essential way of fulfilling social obligations. Beer is carried to chiefs as tribute used to reward labour, or given as an offering to spirits. Abundance of beer is the glory of a commoner's hospitality, or a chief's court. Without it tribal councils cannot be held and, marriage or initiation ceremonies do not take place." This society is probably a representative one in terms of roles of alcoholic beverages all over Africa.

Presently the production of alcoholic beverages is a flourishing enterprise, creating job opportunities for a considerable portion of Africans. The high nutritional content of these traditional alcoholic beverages relative to refined beer has also promoted its consumption (Nout, 1980; Aucamp et al., 196; Bryant, 1939).

2.4 Fermentation of grains

Perderson (1978) described beer as an alcoholic fermentation product of malted cereal. Fermentation, which is the backbone of any liquor

industry has been defined, and described by many workers. Okagbue (1989) described fermentation as a pron which microorganisms contribute to production of substances or materials which have well defined or potential commercial value. The processes are involved in the production of certain foods and beverages, chemicals and many other substances which may be of nutritional, agricultural or medical interest. Fermentation can be carried out by several organisms: bacteria, like lactic acid bacteria, yeasts and molds.

In beer fermentation, it involves the production of ethanol and carbon dioxide and wide range of other qualitatively minor but organoleptically important metabolic by-products of the yeast (Chukwura, 1989). The equation for conversion of sugar (which is usually the main substrate in wort) to ethanol by yeasts is given below:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

The end products re obtained after a series of chemical reactions via the Embden-mayerhorf-Parnas (E.M.P) pathway or glycolysis. Several enzymatic conversions re also involved. The EMP pathway (scheme) represents the alcoholic fermentation pathway for all yeast and this, has been adopted as the basis for the industrial fermentation of alcoholic products and beverages such as beer brewing, sake brewing, wine production, distilled spirits (Gin, Whiskey, etc) (Okafor, 1978).

Grain fermentation can be grouped into two: fermented food and fermented alcoholic beverages. Examples of fermented grains to food are very common with the oriental people. Some of them are soy source (toyo) made from soy bean and wheat, miso (also known as chiang) from soy bean and rice and so on. Some fermented grains food in Nigeria includes Ogi

(a cereal based food). Other fermented foods of Nigeria range from Gari (fermented, roasted cassava grits) 'foo-foo' (fermented paste from cassava) and many condiments from locust bean, called 'dawadawa' and soybean dawadawa.

2.5 Fermentation of burukutu and other similar beverages

Considerable attention has been focused on the production and evaluation of the indigenous beers from cereals. Ogbonna et al. (1993) especially such ones from maize, millet and sorghum. Such studies include the microbiological flora of such products and reflect on traditional method of production. The work of other researchers includes the biochemical or proximate analysis of these beers (Onwuliri et al., 1986).

2.5.1 Processing and Microbiological findings

Studies on microbiology of traditionally brewed beer include that of Nout (1980) on Bussa, a Kenya Opaque maize beer. Bussa is usually prepared from maize endosperm grits and finger millet malt (*Eleusine coracana*). Studies on this beer which contains a considerable amount of solid (maize and finger millet particles) shows that the microorganisms present during stage of uncooked grits are mainly: (a) Yeasts such as *Candida kruzei* and *Saccharomyces cerevisae*, (b) bacteria which are *Lactobacillus species, L. helviticus, L. salivarius, L. brevis, L. viridescens, L. planta* and *Pediococcus species. P. damnosus* and *P paralvus*. The finger millet used alongside contains a high microbial load (10^7 - $10^9 g^{-1}$ which is rather diverse. The following organisms were identified in the malt: *Corynebacterium species, Bacillus species,*

Listeria species Enterobacter cloacae, Klebsiella pneumoniae, Propioniobacterium species, Eubacterium species, Lactobacillus casei, (var Pedicoccus damnosus), Rhizopus species, Mucor species, Candida kruzei. The alcoholic fermentation of Bussa was dominated by *Saccharomyces cerevisae*, while the lactic fermentation was initiated by *hetero* and *homofermentative lactobacilli* such as *L. brevis, L. salivarius (var Salicinius), L. platarum, L. viridences, L. casei (var Salicinius), L. bucheri* and *L. plantarum* which predominated.

Dirar (1978) made a similar observation on Merissa beer whose fermentation processes are very complex. Merissa is prepared from Sorghum bicolor and its production processes consist of three distinct phases: Ajeen fermentation, a lactic souring of sorghum; deboba fermentation, a starter activating phase; and the actual fermentation which is the final phase leading to production of Merissa. The later two phases are alcoholic fermentation phases. Lactic acid bacteria and yeasts (*Saccharomyces species*) are the major microorganisms reported. Dirar (1978) failed to identify the importance of acids found during merissa fermentation.

The microbiology of Kaffir beer as reported by Van der Walt (1956) shows that the brewing of Kaffir beer involves two microbial conversions: the souring process which is done mainly by typical lactic acid flora, such as *Lactobacillus delbrickii, L. plantarum, L. brevis, L. fermenti, Pediococcus damnosus, Leuconostoc mesenteroides* and *Leuconostoc, dextranicum* and the alcoholic fermentation phase involving the activities of many yeasts which are over twenty different species. *Saccharomyces cerevisae, Candida cruzei* and *Kloekera apiculata*, were found to be the predominant species. The spoilage of the beer was

concluded to be due to excessive acetic acid formation which was found to be caused by the development of the common malt and beer *Acetobacter species*.

The work of Okafor (1975) on palm wine (an alcoholic beverage produced from the sap of palm trees) showed this drink to contain heavy suspension of yeasts and bacteria. This important alcoholic beverage in Nigeria is consumed also as much as burukutu. Several microorganisms are known to be present in palm wine. Okafor (1978) indicated seventeen (17) yeast isolates, 12 of which were *Candida species*, 3 of them belonging to *Endomycopsis species*. The bacterial isolates from palm wine include *Micrococcus*, *Streptococcus*, *Lactobacillus*, *Bacillus* and *Brevobacterium species*.

Pito is another alcoholic beverage readily prepared and consumed in Nigeria. It is very similar to burukutu except that, various types of grains are used to brew it, while sorghum is about the only grain used in burukutu production. In the studies of microflora of Pito, Ekundayo (1969) showed that the predominant microbes found during the production are: (a) molds (*Penicillum species*, *Rhizopus oryzae* and *Aspergillus species)* which were present on the grains. It was further noted that the first stage of fermentation souring of the extract from the malted grain is dependent upon microorganisms from the atmosphere while the second stage, after boiling to cancentrate, depends on the addition of a starter from a previous brew. *Candida species*, *Geotricum candidum* and *Lactobacillus species* appear to be important in both stages and resulting in the production of lactic acid.

Some amount of work has been done on Burukutu beer as related to its microflora. In a study of microbiology of burukutu beer, Faparusi et al.

(1973) found the microbial flora of both home and laboratory brewed beer to be similar. Their yeast isolates included. *Saccharomyces cerevisae*, *Candida tropicalis*, *C. kruzei*, *Hansenula anomala*, and *Kloekera species apiculata*. Their molds include *Mucor rouxii*, *Aspergillus flavus*, *A. oryzae*, *Penicillum citrinum* and *Aspor species*. Some species of *Lactobacillus* and *Streptococcus* were also identified as the bacterial components.

In a similar study by Ogbonna et al. (1983b) on Nigerian indigenous alcoholic beverages, they found the microbial flora of laboratory prepared burukutu to be the following: Fungal species were *Aspergillus flavus*, *A. fumigatus*, *A. oryzae*, *A. terreus*, *Cladosporium herbrus*, *Humicola grisae*, *Penicillum citrinum* and *P. stonifer*. The yeast isolates included: *Candida tropicalis*, *C. utilis*, *Hansenula anomala*, *Saccharomyces cerevisae*, *S. uvarum*, *Schizosaccharomyces pumbe*, while the bacterial isolates are: *Bacillus cereus*, *Lactobacillus brevis*, *L. plantarum*, *Leuconostoc mesenteroides*, *Streptococcus species*, *Staphylococcus species*, and the *Nematodes* found were *Ascaris lumbricoides* (ova), *Ancylostoma duodenum*, *Strongyloides stercoralis*, *Trichuris trichura*.

Ekundayo (1969) also reported the presence of *Candida*, *Myoderma*, *C. tropicalis*, *Hansenula anomala*, *Kloekera apiculata* and *Saccharomyces pastorianus*, and *Pediococcus cerevisae* in Burukutu undergoing maturation. Ekundayo (1969) had earlier indicated that microorganisms found in Pito as mentioned earlier were found to be similar to burukutu microflora.

2.5.2 Traditional Burukutu production

Various workers reported on the method of processing of burukutu based on the information collected from their immediate vicinity (Faparusi et al., 1973 and Ogbonna et al., 1983a). The basic method of preparation involves the use of Sorghum bicolor (Guinea corn grains). The grains are steeped, malted, ground and are mashed at various ratios of water and grits. The resulting wort is then boiled and allowed to ferment. From the method reported by these workers, a souring process after boiling is usually allowed for about 24 hours and before fermentation an adjunct (usually gari) is added to the fermenting wort at varying ratios. Personal communication with producers in Zaria area showed these two processes to be missing (i.e. souring and the use of adjunt). Below (Fig. 1) is a flow chat of traditional burukutu production in Zaria. All the steps found here are also present in other places, but with the addition of those two processes mentioned above.

Sheet 1

The flow chart of Burukutu and Pito processing in Zaria (A representative one).



2.5.3 Chemical characteristics

Available evidence indicates that biochemical analyses have been conducted on traditionally fermented Africana beer. Nout (1980) for instance, indicated that Bussa contains 0.5 - 1.0% lactic acid and 2-4% volume of ethanol. He noted that the beer becomes unacceptable after further fermentation and souring to about 2% lactic acid.

Aucamp et al. (1961) assessed the nutritional value of some Kaffir corn beer in the series of Kaffir corn malting and brewing, malted and unmalted, and indicated that Kaffir is rich in protein, fat, ash, fibre,

thiamine, riboflavin, and nicotinic acid indicated and is a rich source of vitamin C. They concluded that Kaffir beer which has always been regarded as alcoholic drink and food by Bantu, is a rich source of vitamin B and has a high nutritive value.

Comparative studies by Chevassus et al. (1961) and Aucamp et al. (1961) showed that the nutritional value of Opaque maize beer is superior to that of clear lager beer because of its higher crude protein content, thiamine and riboflavin.

Ekundayo (1969) in his studies on Pito determined the sugar content, amino acid, organic acids content of this drink. The ethanol content was determined alongside. In his report, percentage of alcohol in Pito was found to be 3. He also reported the presence of organic acids in this beer alongside with some amino acids.

The biochemical analysis carried out on burukutu by Ogbonna et al. (1983b) showed that the beverage has high nutritive value. It contains 52% sugar, 0.46% of ash, 0.52% of protein, 7.0µg/100ml vitamin C and (3%) alcohol. Faparusi (1970) in a similar work in southern Nigeria reported the presence of many sugars such as fructose (0.2%) maltose (0.05%), glucose (0.6%), galactose (0.36%), galacturonic acid (0.48%), sucrose (0.2%), maltose (0.12%), isomaltose (0.38%), stachyrose (0.43%) and maltotetrose (0.16%).

The comparative studies by Onwuliri et al. (1986) on Burukutu and Pito revealed that Burukutu is more acidic than Pito. The average percentage ethanol content of burukutu was 5.25 while that of pito was 3.62 respectively.

2.6 STARTER CULTURE

Starter cultures are usually developed from microflora associated with spontaneous fermentation of beverages and foods. In starter culture development microbial isolates which are considered relevant or potentially relevant to a given fermentation process are used as single or mixed inocula for producing desired foods or beverages (Okagbue, 1989). The organisms used for starter are usually well known and their function or advantage(s) would have been recognized. The role of starter cultures has been discussed by Genigeorge (1976). He discovered the starter culture of Lactobacillus and Pedicoccus for meats fermentation. He indicated that a starter culture should increase the safety, shelf stability, and nutritional value of food products; and that recommended starters are important in assuring desirable and reproducible flavour of fermented product. Although their main purpose is a rapid decrease of pH to a level at which growth of food borne pathogens are inhibited.

Furthermore, starter culture promotes controlled fermentation in any fermenting medium, while natural fermentation results in diverse metabolic products which is a sign of uncontrolled fermentation.

2.6.1 Uncontrolled fermentation (Natural)

Uncontrolled fermentation can be of two types: the fermentation can be carried out using some starters. These starters can be considered as indigenous starters since they are unrefined, and undefined organisms. The starters are either developed by allowing natural souring of some food materials, which are later added to the body of the main fermenting product, or left over of old product as in many cases of traditionally fermented beer. In some cases, this type of starter is not prepared nor

added to fermenting system. The fermenting system gets contaminated by natural flora of the atmosphere (aerial flora) directly.

Some of those foods and beverages such indigenous starter cultures (some of which are legume and cereal products from the Orient and some parts of Africa) are numerous. Some of the examples include soy sauce also known as toyo. Kanjang is another flavouring agent made from soybean and wheat, it uses a traditionally developed culture which comprises of *Aspergillus oryzae*, *Pediococcus talophillus* and *Lactobacillus delbruikei* when examined (Wang and Hesseltine, 1981). Miso, also known as chiang is a flavouring agent from whole soyabean and rice or barley. Yokotsuka (1985) identified Miso starter to contain *A. oryzae*, *Saccharomyces rouxii*, *Torulopsis hellsii*, and *Pediococcus halopilus*. Another example is sufu, a soyabean curd (fofu) used as cream cheese. It is common in China, hence it is called Chinese cheese. Its traditional starter consists of *Actinomucor elegans* and *Mucor dispersus* (Wang and Hesseltine, 1981).

The fermentation processes in many African beers are considered natural phenomena and early brewers were unable to understand the changes that occurred in the fermenting system. The following are some examples of local beers and wines which undergo uncontrolled fermentation, (but have a starter usually prepared for it by ignorant brewers who do not know anything about the composition of these starters). Sake, prepared by Japanese from steamed rice with Koji as the starter culture. Koji contains *Aspergillus oryzae* and *Saccharomyes species* as starter (Perderson, 1976).

Binuburain is another name of a beer prepared from rice. The sediment from the fermented products is pressed into cakes and used as

inoculum for the next batch of binuburain fermentation. The cake consists of yeast and bacteria of different species and genera (Perderson, 1978).

Kanji is a sour rice beverage prepared in India in which rice starch is hydrolysed by a strain of *Aspergillus species* and saccharification effected by species of 3 genera of molds (Pedersen, 1978).

Bakhars is the starter culture used for inoculation of the high alcoholic rice beer (Pachwai) made in India. The bakhars is prepared in small cakes. The beer is prepared by the addition of powdered bakhars into half boiled rice and allowing the beer to incubate for 24 hours. The whole mass is transferred to earthen jars where alcoholic fermentation proceeds. Strains of *Mucor* predominate in a mixture of other molds of genus *Rhizopus* and yeasts and bacteria (Perderson,1978). Other rice beers include sonti of India, Torani of India, Arak of East India, Arak of Ceylon, Rak of Thailand.

There are some corn beers from South America, Chica or Chicara. The fermentation is carried out by diastase enzymes, yeast and lactic acid bacteria. The inoculum starter is a chewed corn. Red corns are chewed by older women, collected from their mouth and introduced to fermenting mash. This is a common practice among primitive people, hence the source of these organisms is from the saliva of these women that chewed the corn (Perderson, 1978).

Kaffir beer is the most popular among sorghum beers. It is produced among the Bantus of South Africa. It is also called 'Mpoho' and 'Machawa' The fermentation is uncontrolled and an initial souring stage of the wort is allowed. The wort is then left to undergo second phase of fermentation which is an alcoholic fermentation.

Others are merissa of Sudan and bussa of Kenya. Aajen is the name of the inoculum (36 hours old fermented sourdough) added to 'Merrisa'. Fermentation of bussa traditional beer is spontaneous and is by mere contaminants and normal flora of the grains.

The production of burukutu in Nigeria is also in line with natural fermentation. The starter used traditionally is called 'Kumpa'. Kumpa is the brownish foamy mass of old beer or previously brewed beer which contains many types of organisms (yeast, bacteria and molds) (Faparusi et al., 1973; Ogbonna et al., 1983a and 1983b). Freshly boiled wort is then pitched with this foam and fermentation commences.

2.6.3 Controlled Fermentation

Controlled fermentations are mainly found in industrialized productions, and usually involve mass production of such products. Industrial starters produce reproducible flavour and can usually be grown on simple media They are capable of producing desired products and their end product do not include toxic and other undesirable materials especially if this product is for human and animal consumption (Okafor, 1987).

In the meat and dairy industries, there are quite a number of established starter cultures in use. Some of the examples are found in Europe where meat starter consists of eight specific strains of mold and yeast cultures. They are utilized for unique flavour development and prolonged shelf life (Eilberg and Liepe, 1977; Coretti, 1977).

In dairy products, the use of starter culture is not new. An example can be found in Yoghurt which is a fermented milk product. It has a starter culture which is made of *Lactobacillus bulgaricus* usually in

conjunction with *Streptococcus thermophilus*. Others are butter, with a mixed culture starter of *Streptococcus lactis*, *S. remoris* (*S. paractrovorus* and *Leuconostoc dextranicum* (*S. citrovorus*) (Perderson, 1978). There are other dairy products with monitored fermentation and specific starters and such example is cheese. Other products with established starter, under control fermentation are vegetables like olives saukraut and pickles.

In alcohol producing industries, there are quite a number of established products with specific starters. For instance, wine which is the product of an alcoholic fermentation by yeasts was generally fermented by normal flora of fruits like grapes in the past. Lately, in wine production, strains of yeast called *Saccharomyces cerevisae* (*var ellipsoideus*) are often used.

In beer production, pure cultures are used. In beer industries, where lager, stout and ale are produced, specific yeast strains are used as starter. In the history of beer production, Emil Christian Hansen in the middle of the nineteenth century introduced his pure culture of yeast at the Calsberg brewery. A system of fermentation control was set up that proved to be of inestimable value. Hansen had previously shown that brewery yeasts were a mixture of several species of yeast and that defects were caused by wild yeast cultures. The results of Hansen's pure yeast culture method were so successful in Calsberg brewery in Copenhagen that the method was adopted permanently in 1886. The use of industrial starter culture in the beer industry is something that is a custom now in every beer industry (Perderson, 1978). In the lager beer production, the yeast strains used as starter culture are bottom fermenting strains of *Saccharomyces uvarum* (formerly *S. calsbergensis*).

Several types of lager beer are now grouped based on their percent ethanol or level of hop. Among the local examples are '33', Harp, Gulder, Rock, and Star. Ale, which is a top-fermented beer, is brewed using strains of *Saccharomyces cerevisae*. Stout is a high-hops (concentrated) top fermented alcoholic beverage, so the organism used in its fermentation is the same as in ale production (Okafor, 1987).

Amongst Nigerian indigenous foods that usually undergo spontaneous fermentation, a lot of pioneer work had been done to establish starter cultures for them and quite a number of interesting and established findings are now available (Okagbue, 1989). These foods can now undergo controlled fermentation using the following starter cultures.

Collard and Levi (1959) carried out pioneer studies on the microbiology of 'gari' (cassava fermentation). He used a starter culture consisting of *Corynebacterium manihot* and *Geotrichum candidum* and satisfactory product was achieved.

Akinrele (1970) found that good quality 'Ogi' (a corn based fermented food) could be produced with an inoculum consisting of equal levels of *Lactobacillus plantarum*, *Acetobacter cloacae* and *Saccharomyces cerevisae* at a temperature of 30 to 32°C. Banigo et al. (1974) produced Ogi by fermenting millet corn with a mixture of *L. plantarum*, *S. rouxii*, and *Streptococcus latcis*.

An inoculum of an unidentified *Candida species* was found important in alcoholic fermentation of pito by Ekundayo (1969). He claimed that the use of *Geotrichum candidum*, or *Lactobacillus* or both with the *Candida species* will increase the alcohol yield.

Some organisms from burukutu system can also be selected and used as starter culture for burukutu production.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sources of Burukutu Samples

Representative samples of freshly prepared burukutu were collected from six chosen producers in Samaru based on organoleptic quality of their products. Samples were collected in sterile bottles.

Samples were collected at each stage of burukutu production. Seven batches of samples were (a) TS: which is the cooled, unpitched wort ready for pitching were also collected.collected from each producer. The stages of collection of samples were (b) the Pito stage: a clear supernatant of the brew which is usually light yellow to light brown in colour; (c) Burukutu at different ages after 2 hours, 12 hours, 18 hours, and 24 hours processing were collected. Burukutu is the darker liquid with colloidal suspension. (d) Kumpa which is the foamy mass on top of old beer, used in pitching freshly boiled wort

3.2 Isolation and enumeration of Microorganism

A procedure similar to that of Gilliland et al. (1976) was used to enumerate microorganisms from samples. Each sample was serially diluted in sterile normal saline and 0.1ml aliquots of appropriate dilutions (10^{-3}-, 10^{-4}, 10^{-5}, 10^{-6}) were spread on acidified malt extract agar (OXOID) for isolation of yeast; acidified, Potato dextrose agar (pH 4.5) (OXOID) for molds; slightly alkaline, Nutrient agar (OXOID) (pH 7.4) for bacteria, Tomato juice agar for *Lactobacillus species*, and Plate count agar for total aerobic plate count.

Inoculated nutrient agar plates (NA), plate count agar plate (PCA) and tomato juice agar (TJA) plates were incubated at 37°C for 24 hours. The potato dextrose agar plate (PDA) and malt extract agar plate (MEA) were both incubated 27°C for 24 hours. The PDA plates were left for further mold development at room temperature in a cupboard for a maximum of 10 days. Enumeration of yeasts and bacteria was done after 24 hours. Plates were reincubated for 24 hours in cases where colonies are not well developed. Enumeration of molds was done after the 5th day and plates were left for five more days for further mold development.

3.3 Purification and storage of Isolates:

Each distinct type of yeast, bacterial or mold colony was streaked on appropriate medium of enumeration mentioned above. In the case of yeast isolates, the method of Beech and Daverport (1971) was adopted. The yeasts were purified, by repetitive streaking on the acidified malt extract agar and incubated at 27°C for 24 hours until pure cultures were obtained. The isolates were stored on slants and kept a 4°C in a refrigerator.

The method recommended by Waksman as described by Skinner et al. (1961) was used to purified and stored molds. The same medium of enumeration mentioned above was used. The molds were then transferred several times and were left at incubating temperature (27°C) for seven7 days before finally stored on slants at 4°C.

Bacteria were purified by repetitive streaking on media of examination. All incubation were carried out at 37°C for 24 hours pure cultures from plates were stored on slants and kept between 4°C.

3.4 Characterization of yeast isolates:

The methods that were used in identification of yeast isolates were adapted from the methods of Beech and Davenport. (1971) and Okafor (1971).

3.4.1 Observation of colonial morphology:

The appearance of the colonies and their pigment formation were observed on 2.0% (w/v) MEA (DIFCO) reinforced with 0.05% (w/v) of ammonium ferric citrate (Beech and Davenport. 1971). The plates were examined, shapes, sizes and pigment formation was observed for up to seven days of incubation.

3.4.2 Vegetative morphology and Pellicle formation

These were observed in malt extract broth (DIFCO). The broth was prepared according to manufacturer's instructions and dispensed into universal bottles and sterilized at 121°C for 15 minutes. After cooling these were inoculated with yeast cultures and incubated for four days after which they were examined for shapes of the yeast (vegetative cells). Formation of film on the surface of the broth was taken as formation of pellicle. Examination involved microscopic observation of organism mounted on slides and stained with lactophenol in cotton blue.

3.4.3 Ascospore formation:

Ascopore formation by the yeast isolates was tested by growing on sodium acetate (0.5%) w/v agar. Spores formation were observed weekly for six weeks using the following procedure. A heat fixed smear of the yeast was flooded with 5% malachite green and steamed for three-five

minutes. It was decolorized in running water for 30 seconds, counterstained with 0.5% aqueous safranin for 10 seconds and was washed and examined. The spores which appeared blue in a red cell were looked for in many of the cases except in *Saccharomyces* which operates the exact reverse of the appearance of spores in yeast.

3.4.4 Observation of true mycelium Pseudomycelium:

These were observed on Dalman plates containing corn meal agar. Streaks were made across the agar surface (close to one side of the plate) with small inoculum using sterile loop. Two point inoculation were then made on either side of the streak at right angles to one another. One sterile coverslips was placed over one point inoculation and another one over the centre of the streaks. The plates were incubated for four days before examination. For observation, the slides are taken out of the petridish and the agar wiped off the back of the slide. The area of the inoculum under as well as outside the cover slip were studied using the high dry objective. The pseudomycelia are identified by their elongated, equal sized cells or elongated cells provided with blastospheres (round or oval budding cells) with a characteristic arrangement on the axis of Pseudomycelium. The true mycelia are differentiable from pseudomycelia by continuous growth of hypha tips followed by the formation of septa.

3.4.5 Fermentation of sugars:

Sugar fermentation test was carried out in test tubes containing inverted Durham tubes which contained the sugar medium which is made up of 0.45% (w/v) yeast extract, (DIFCO); 0.75% w/v peptone (DIFCO) and

bromothymol blue. The medium was distributed in 5.0ml aliquots in 150 x 15mm test tubes which were then sterilized at 121°C for 15 minutes. The carbon sources were filtered-sterilized separately and 6.0% w/v solutions of glucose, galactose, maltose, sucrose, lactose, innositol or 12.0 w/v of raffinose was used. Aliquots of each sugar solution (2ml) was then added asceptically to different fermentation tubes. The inocular which are test organisms, suspended in sterile distilled water after washing was added to each sugar in 0.1ml aliquots. The suspension of these cells contains an average of 10^6 cell per ml. The tubes were examined at two, three and seven days intervals for three weeks. The control was a basal medium in a test tube inoculated with test strains without the sugar.

3.4.6 Assimilation of carbon:

The liquid method for determination of sugar assimilation was used for the tests. The medium for the test contained 0.67% yeast nitrogen base (DIFCO) in distilled water. The broth was dispensed in 4.5ml portion into 18mm x 150mmtest tubes which were loosely screw capped and sterilized by autoclaving. The carbon sources used here are exactly the same as the ones used for sugar fermentation tests above. In this test, each carbon source was prepared at 5% stock (except raffinose - which is 10%) and were filtered sterilized. 0.5ml aliquots of the stock solution was then introduced asceptically into each yeast nitrogen based tube to give a final concentration of 2.0% (4% for raffinose) in a total of 5ml of the sugar YNB broth.

A young yeast culture (24 hours) of each test strain was suspended in sterile distilled water to give a suspension containing approximately

10^6 cells per ml. The yeast cells were washed twice with sterile distilled water and then left at room temperature for 24 hours to starve the cells to utilize any residual sugars on the cell surface. The tubes containing the various sugars were then inoculated with 0.05ml aliquots of the yeast suspension. Turbidity in the broth after a maximum of seven days incubation at room temperature was taken to indicate growth or assimilation of the carbon source tested.

3.4.7 Nitrate assimilation test:

The nitrogen assimilation test was carried out in liquid medium using yeast carbon base (YCB) (DIFCO). The preparation and inoculation of YNB medium was carried out as in the case of carbon assimilation above. The nitrogen sources tested were potassium nitrate and ammonium sulphate at a final concentration of 2%. The screw capped tubes with their content were inoculated with washed test strains and were incubated at room temperature for a maximum of seven days and examined for growth each day.

3.4.8 Acid production test:

This test was carried out using a medium containing 5.0% (w/v) glucose, 0.5% (w/v) precipitated calcium carbonate, 2.0% w/v yeast extract (DIFCO) 0.75% peptone (DIFCO) and 2.0% (w/v) agar). The medium was dispensed into 150 x 15mm screwed capped tubes and sterilized at 121°C for 15 minutes. The tubes were then allowed to cool down to about 45°C and was then shaken thoroughly and made into slants on a cold surface (in an open freezer). The yeast strains were then streaked down

the centre of the slants. Chalk dissolution is an indication of acid production.

3.4.9 Ability to utilize alcohol and Growth in ethanol:

The ability of the test strains to grow in 3% ethanol (w/v) was tested using the method of Skinner et al. (1961). This was achieved by sterilizing 97.0ml of distilled water at 121°C for 15 minutes. 3.0ml of absolute ethanol was then added to the sterile water after cooling. The 3% ethanol so obtained was dispensed in sterile tubes and yeast strains were inoculated into the tubes. 0.1ml of approximately 10^6 cells per ml suspension of 24 hours old washed yeast culture were used. They were incubated for three days at room temperature. Occurrence of turbidity or sediment was taken to indicate growth in 3% ethanol.

3.4.10 Growth in 50% (w/v) glucose concentration:

The test was carried out in a medium containing a final concentrations of 50% glucose, 1.0% yeast extract, 0.75% peptone (DIFCO), (DIFCO) and 2.0% (w/v) agar (OXOID). The plates were prepared inoculated after drying and incubated at room temperature and were examined for growth after four to seven days incubation.

3.4.11 Growth at 10% (w/v) sodium chloride

This was carried out using a medium containing 10% (w/v) sodium chloride, 1.0% w/v yeast extract (DIFCO) and 2.0% (w/v) agar (OXOID). The sterile medium was then dispensed into sterile plates and were allowed to solidify. Test strains of yeasts were streaked on the surface of the agar and examined for growth after 4-7 days of incubation.

3.4.12 Growth at different temperature

The ability of all the yeast cells to grow at different temperature was tested. Inoculated malt extract plates were incubated at different temperature (30°C, 37°C and 42°C). Growth was observed after 48 hours of incubation.

3.5 IDENTIFICATION OF MOLDS

The method recommended by Smith (1969) was adopted for mold identification.

3.5.1 Visual observation:

The appearance of the mycelia mass of molds were observed on malt extract agar after eight days of incubation. The following characteristics were noted: Colour of the colony (i.e. how compact and firm they looked, growth pattern and texture of the colony).

3.5.2 Microscopic observation:

The mycelia mass of the molds were teased out with pin on slides and stained with methylene blue to determine morphology of the vegetative cells under the microscope. Special attention was also given to observation of shape and sizes of spores (Smith, 1969).

3.5.3 Spot inoculation:

Aspergillus differential medium (ADM) of Bothast and Fennel (1974) inoculated with test organism and incubating for 5 days at room temperature was used to identify organisms suspected to be *Aspergilli*.

Production of orange pigment on the reverse of the colony confirmed the identity (Bothast and Fennel, 1974).

3.6 Identity of Bacteria

Gram stain technique: Heat fixed smears of the bacterial isolates were made from 24 old culture of the isolates and gram stained. The organisms were classified into gram negative rod, (for red stained rods), gram positive rod (for purple stained rods), gram positive cocci (for purple stained cocci), and gram negative cocci (for red-stained cocci).

3.6.1 Motility test:

Motility medium was used for motility test. The semi solid medium in tubes was then stab inoculated up to 5mm depth with a straight wire of 0.4mm in diameter. These were incubated at room temperature for 24-48 hours. Diffuse growth along the line of inoculation was recommended as positive motility test.

3.6.2 Spore observation:

A heat fixed smears of old culture (7 days) of the rod like isolates were made, spore stained using Ziehl Neelsen carbol fuchsen, steamed for three to five minutes, rinsed with water, developed with 95% ethanol for 30 seconds and then counter-stained with 1% methylene blue, rinsed and observed under the microscope. The appearance of red spore inside a blue bacterial cell indicate the presence of spores. The positioning, and shape of the spores were all noted.

3.6.3 Starch hydrolysis:

Starch agar was used to test for starch hydrolysis. The plates were spot inoculated with test organisms and incubated at 30°C for 24 hours. The plates were flooded with Lugols iodine solution. Formation of hallow at the spot of inoculation compared to the background colour change to blue - black was taken as hydrolysis.

3.6.4 Gelatin hydrolysis:

Gelatin hydrolysis was carried out exactly as in the case of starch hydrolysis above. Except that 5% gelatin was used in place of starch in nutrient agar. The plates were flooded with mercuric chloride solution and hallow formation resulted in plates where gelatin was hydrolysed after incubating the plates at 35°C for 24-48 hours.

3.6.5 Catalase test:

A drop of 3% of hydrogen peroxide was placed on a clean glass slide. A pinch of growth of the test organisms from freshly incubated cultures (24 hours) was placed with a nichrome wire loop into H_2O_2 on the slide. Positive results were indicated by bubbling or frothing was seen.

3.6.6 Growth on different media:

The bacterial isolates were grown on different media which include differential media such as MacConkey agar, Nutrient agar, Chocolate agar and blood agar. Note was taken of which organisms were able to grow well on MacConkey . The profuse nature of growth, colonial characteristics e.g. pigment and size of colonies were noted.

3.6.7 Haemolysis:

Haemolysis was noted on blood agar. Clearing zone or conversion of the opaque blood agar into a clear (greenish) translucent media was noted. Positive results were indicated by clear zone for alpha (α) haemolysis, clear greenish zone for βeta (β) haemolysis, and no colour change as Gamma (γ) haemolysis.

3.6.8 Lecithinase test:

Prepared egg yolk agar plates were inoculated with the Gram positive rods. The plates were then incubated for 24 hours. In the case of doubtful results, plates were incubated for additional 24 hours. The plates were examined for presence of an opaque zone in the immediate vicinity of the streak. The presence of an opaque zone indicated positive results while its absence indicated negative results.

3.6.9 Citrate Utilization:

Citrate agar slants in bijou bottles were inoculated with test organisms and were incubated for 24 hours. The development of deep blue colour in some cases was recorded for positive result and non colour development as negative results.

3.6.10 Methyl red-Voges Proskauer test (MR-VP):

About 5ml of MR-VP broth was inoculated and incubated for 48 hours at 35°C. The MR-VP test was carried out after the incubation period of 48 hours. 1ml of the incubated broth was put in a small serological tube. Two to three drops of methyl red indicator were added to the 1ml. A yellow colour on the addition of the indicator was taken for positive

methyl red test. To the rest of the broth in the original tube, five drops of 40% potassium hydroxide (KOH) were added. The mixture was shaken and loosely capped. The development of a red colour, starting from the liquid air interface within one hour (1hr) was taken for the positive and the rest as VP negative.

3.6.11 Aerobic and Anaerobic fermentation tests:

A semi solid medium similar to the motility medium and comprising of 1% peptone, 1% glucose and 0.2% agar was used. The indicator used is bromocrysol-purple. The media after preparation were stabbed with test strains from 24 hours old broth. Two tubes were used in each case. One was covered with paraffin after inoculation while the other one was left uncovered. Colour changes to yellow from purple, from top in the uncovered tubes or from the centre in the covered tubes and throughout the tube were noted.

3.6.12 Triple sugar Iron agar:

Gram negative rod isolates were subjected to this fermentation test. Slant of triple sugar iron agar, (OXOID) were prepared. Sterile needle was used in taking materials to be cultured from a solid medium and was streaked on the surface of the slanted medium; it was also stabbed into the butt three times. The tubes were loosely capped and were incubated at 35°C for 24 hours. Gas production was determined by appearance of bubbles or the pushing up of the butt from the broken part close to the bottom. Hydrogen sulphide (H_2S) formation was determined by the blackening of the butt at the slant-butt junction. Glucose fermentation

was also determined by colour change to yellow. When both butt and slant appear yellow, it was taken for fermentation of the sugars.

3.6.13 Deoxyribo nuclease test (DNAase)

A sector of DNA agar plate was inoculated and incubated. Plates were incubated at 37°C for 48 hours. 0.1M hydrochloric acid solution was used to precipitate the DNA around the streak. Clearing zone in the area of growth indicates positive DNase result.

3.7 Organoleptic test

3.7.1 Choice of panelists:

Twenty one interested patron all male drawn from Ahmadu Bello University served as panelists for evaluating organoleptic properties of the burukutu. The selection was based on interest, availability and familiarity with burukutu.

3.7.2 Organization for tasting:

Prior to the experiment, a form was prepared (Appendix 1). The form was designed based on the outlined proposed quality of good burukutu (personal investigation) from both consumers and producers. It contained seven attributes that denote characteristic of good and high quality burukutu. A four structured descriptive scale was used. A score of 4 is allocated to the best attribute while the least score which is 1 was allocated to the worst or poorest attribute. Each panelist was served 50ml of each sample was served to each panelist using equal sized calabash that is normally used to serve customers by the local

producers. The tasting was done twice from each producer on three different occasions.

3.7.3 Tasting procedures:

Collected burukutu samples were labelled A-F and the corresponding pots noted. The judges were allowed to sit randomly and prepared forms were given to each of them. They were allowed to fill in their names on the score sheets. The terminologies used in the forms were briefly explained and instructions on how to fill the forms were given. All samples were arranged in a row and they were asked to taste one at a time starting from A to F. The judges were also provided with warm water to rinse their mouth after tasting each sample. Spaces were provide for comments on the forms and judges were asked to comment freely (Appendix 1).

3.8 Fermentation characteristics

3.8.1 Temperature: The temperature of processing was noted for every beer production procedure using graded thermometer (range from 0°C - 200°C). The thermometer bulb was dipped in each case into the beer. Rise in temperature was read off directly from the thermometer as indicated by the rise in the mercury level.

3.8.2 pH:

The pH of the beer was read after thoroughly shaken 15mls of the beer in a test tube. The value was read using calibrated pye model 79 pH meter.

3.8.3 Titratable acidity (TA):

TA was done by titrating 10ml of sample with 0.1N sodium hydroxide (NaOH) solution to the phenolphthalein end point. Total acids was expressed as percentage lactic acid using the expression below:

$$\% \text{ TA} = \frac{\text{ml of base} \times \text{N of base} \times 0.09 \text{ equivalent of Lactic acid}}{\text{10g of sample}}$$

3.8.4 Percentage ethanol determination:

The percentage ethanol of both laboratory brewed and the traditionally prepared burukutu were determined by chemical oxidation method of Caputi and Wright (1969). This was carried out using micro kjeldahl apparatus. 1ml of beer sample was pipetted into the flask and distilled into acidified solution of potassium dichromate. The resulting solution was heated for 20min at 60°C to completer the oxidation of the ethanol to acetic acid. This was titrated with acidified ferrous ammonium sulphate solution using phenanthroline as indicator. Blank titration was performed. The result was expressed as percentage ethanol by volume using the expression below:

$$\% \text{ Ethanol by Vol} = 25.00 - \frac{(25 \times \text{sample titre})}{\text{blank titre.}}$$

3.8.5 Aflatoxin detection:

Presence of aflatoxin in locally produced beer was determined using thin layer chromatographic method. The solvent mixture used was Acetone

and chloroform at a ratio 5:95. Standard aflatoxin in solvent (chloroform) was used as control for the test. Another control used is water. Beer from various sources were spotted and run in the solvent along side with the controls. The resulting plates after running were dried and observed under ultra violet light (u.v).

3.9 Determination of the function of each group of organisms:

Pure culture of the selected organisms were prepared. These isolates (bacteria and yeasts) were used singly and in different combinations to pitch the boiled wort after cooling. Bacteria alone were used singly and in different combinations, yeast were used singly and in different combination, and bacteria and yeasts were used together in pairs and in different pair combinations. The resulting products were then assessed organoleptically.

3.10 Starter culture for Burukutu:

Many factors were considered during choice of starter culture. Factors such as the fermenting ability of each yeast during sugar fermentation test, the performance of each organism during trial fermentations, and their frequency of occurrence at the different fermentation stages. The stages of occurrence and the population of each isolate during enumeration was a factor. Organisms that performed very well during the trial test (for role determination) above were considered. Yeasts whose population was very scanty and did not occur in good number in the system were not chosen. The ones with higher population as determined by counts were considered. Non fermenting strains were not considered. In the final analysis, yeasts that produce

best beer as ranked by panel members were noted for use as possible starters.

3.11 Preparation of Inoculum for Burukutu:

Inoculum preparation for pitching of boiled wort was done based on Standard Industrial procedure (Okafor, 1987). The organisms of interest in case of yeast were introduced into 10ml of malt extract broth. These cultures after 24 hours of growth were transferred into a bigger flask containing 50ml of broth which were subsequently transferred into bigger flask (250ml) after 24 hours. The total volume of inoculum needed is about 10-20% of the total fermenting volume as recommended for industrial fermentation.

3.12 Burukutu preparation:

Burukutu was brewed in the laboratory from 500 grams of guinea corn *(Sorghum bicolor)* from which broken and damaged grains were removed, washed and steeped overnight. The grains were then drained for two hours in a basket. The grains were spread out on mat on the laboratory bench. The thickness was about 3-4cm and was covered with cheese cloth. It was watered on alternate days and turned regularly. The seedlings were allowed to grow for about 4 days at which the length of the plumule was about 1-1.5cm long (on the average). The germination was halted by packing the grains on top of one another and covered for about one to two days by which time, heat must have been generated within the hip of grains. The malted grains were sun dried for two days and was dry-milled into powder. The malted powder was then mashed at a ratio of 1:3 (Malt:Water). This was boiled for 3 hours. It was allowed to cool

overnight. On the following day, the water and malt slurry was heated for six hours. The boiled wort was then cooled to about 30°C, and was pitched with prepared inoculum (starter culture) and left to ferment for 18 hours after which consumption usually commences. The beer is usually consumed within 6 hours after production.

CHAPTER FOUR

RESULTS

Biochemical, physiological and microbiological data for characterization of microbial isolates obtained from several stages of burukutu production are presented in Tables 1 & 2. The microorganisms isolated included yeasts, bacteria and molds. Only *Rhodotorula* - a non fermenting yeast and three *Saccharomyces species* were identified as predominant yeasts in burukutu beer.

Only *Saccharomyces* III was however, evident in four stages of burukutu production which are pito, burukutu, TS, and 'Kumpa', stages; Other yeast genera such as *Candida, Pichia and Kloekera* were found.

Table 3 summarizes stages of occurrence of each yeast isolates. There are two types of *Candida species: Candida species* I and II; while only one species of isolate each *Pichia and Kloekera* were found. Spores were observed in all the yeasts isolates except *Kloekera, Candida* and *Rhodotorula species.*

From the biochemical characteristics (as shown in Table 1), none of the yeasts has good fermentative ability except the three *Saccharomyces species* and the two *Candida species.*

With the exception of *Saccharomyces species* II and *Bacillus mycoides*, all the microorganisms in the unfermented wort were destroyed by boiling. The population of yeast increased tremendously after 18 hours.

After active fermentation, the bacterial load of burukutu increased and *Acetobacter species* was the major bacterial isolates at this stage of fermentation. Nine distinct species of bacterial isolates were identified. The predominant ones fell into the *genus Bacillus* (Table 3)

and they are *B. cereus*, *B. mycoides*, *B. subtilis and B. species*. The stages of

Table: Morphological, Physiological and Biochemical Characteristics and Identity of yeast isolates

Code	MORPHOLOGY: COLONIAL APPEARANCE	MORPHOLOGY: CELLULAR MORPHOLOGY	BUDDING	SEXUAL	PELLICLE FORMATION	PSEUDOMYCELIA	TRUE MYCELIA	SPORULATION	PIGMENT	FERMENTATION OF SUGARS: GLUCOSE	GALACTOSE	MALTOSE	SUCROSE	LACTOSE	RAFFINOSE	INOSITOL	SUGAR ASSIMILATION: GLUCOSE	GALACTOSE	MALTOSE	SUCROSE	LACTOSE	RAFFINOSE	INOSITOL	NITRATE ASS: KNO_3	NH_3SO_4	ACID PROD	3% ETHANOL	50% GLUCOSE	GROWTH AT DIFFERENT TEMPERATURE: 30°C	35°C	37°C	42°C	GROWTH IN 10% NACL.	IDENTITY OF ORGANISMS
YB_1	SMOOTH	OVAL	+	-	-	+	-	-	+	-	-	-	-	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	+	+	+*	+*	-	Rhodotorula species
YK_1	SMOOTH	OVAL	+	-	-	+	-	-	+	-	-	-	-	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	+	+	+*	+*	-	Rhodotorula species
YB_2	SMOOTH	OVAL	+	-	+	+	-	+	-	+	-	+	-	+	-	-	+	±	+	+	-	-	-	+	-	+	+	+**	+	+	+	-	-	Saccharomyces species I
YK_2	SMOOTH	OVAL	+	-	+	+	-	+	-	+	-	+	-	+	-	-	+	±	+	+	-	-	-	+	-	+	+	+**	+	+	+	-	-	Saccharomyces species I
YP_2	SMOOTH	OVAL	+	-	+	+	-	+	-	+	-	+	-	+	-	-	+	±	+	+	-	-	-	+	-	+	+	+*	+	+	+	-	-	Saccharomyces species II
YB_3	SMOOTH	OVAL	+	-	+	+	-	+	-	+	-	+	-	+	-	-	+	+	+	+	-	-	-	+	-	+	+	+*	+	+	+	+*	-	Saccharomyces species II
YK_3	SMOOTH	OVAL	+	-	+	+	-	+	-	+	-	+	-	+	-	-	+	+	+	+	-	-	-	+	-	+	+	+*	+	+	+	+*	-	Saccharomyces species II
YP_3	SMOOTH	OVAL	+	-	+	+	-	+	-	+	-	+	-	+	-	-	+	+	+	+	-	-	-	+	-	+	+	+*	+	+	+	+*	-	Saccharomyces species II
YTS_3	ROUGH	OVAL	+	-	+	+	-	+	-	+	-	+	-	+	-	-	+	+	+	+	-	-	-	+	-	+	+	+*	-	+	+	+*	-	Saccharomyces species III
YB_4	ROUGH	OVAL	+	-	-	-	-	+	-	+	+	+	+	-	+	-	+	+	+	+	-	-	-	+	-	+	+	+*	+	+	+	+*	-	Saccharomyces species III
YK_4	SMOOTH	OVAL	+	-	-	-	-	+	-	+	+	+	+	-	+	-	+	+	+	+	-	-	-	+	-	+	+	+*	+	+	+	+*	-	Saccharomyces species III
YP_4	ROUGH	OVAL	+	-	-	-	-	+	-	+	+	+	+	-	+	-	+	+	+	+	-	-	-	+	-	+	+	+*	+	+	+	+*	-	Saccharomyces species III
YB_5	ROUGH	ROUND	+	-	+			+	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	+	+	+**	+	+	+	+**	-	Pichia species
YK_5	ROUGH	ROUND	+	-	+			+	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	+	+	+**	+	+	+	+**	-	Pichia species
YP_5	ROUGH	ROUND	+	-	+			+	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	+	+	+**	+	+	+	+**	-	Pichia species
YB_6	SMOOTH	ROUND	+	+	-		+	-	-	+	+	+	+	-	-	-	+	+	+		+		+	+	+	+	+	+*	+	+	+	+**	-	Candida species I
YK_6	SMOOTH	ROUND	+	+	-	+	+	-	-	+	+	+	+	-	-	-	+	+	+		+		+	+	+	-	+	+*	+	+	+	+**	-	Candida species I
YTS_6	SMOOTH	ROUND	+	+	-	+	+	-	-	+	+	+	+	-	-	-	+	+	+		+		+	+	+	-	+	+*	+	+	+	+*	-	Candida species II
YB_7	SMOOTH	ROUND	+	+	-	+	+	-	-	+	+	+	+	-	-	-	+	+	+		+		+	+	+	-	+	+*	+	+	+	+*	-	Candida species II
YP_7	SMOOTH	ROUND	+	+	-	+	+	-	-	+	+	+	+	-	-	-	+	+	+		+		+	+	+	-	+	+*	+	+	+	+*	-	Candida species II
YB_8	ROUGH	OVAL	+	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	+	+	+**	+	+	+	+*	-	Kloekera species
YP_8	SMOOTH	OVAL	+	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	+	+	+**	+	+	+	+*	-	Kloekera species

KEY: + = indicate growth/positive result
- = indicate no growth/negative result
+* = indicate scanty growth
± = indicate doubtful result

Table 2: Morphological, Physiological, Biochemical Characteristics and Identity of Bacterial Isolate

Code	GRAM REACTION		GROWTH ON MEDIA				CAT.	SPORES SHAPE	SWELL	NITRATE	CITRATE	PIGMENT ON N/A	LECITINASS	METYL RED	V.P	MOTILITY	FERMENT ANA	AERO	STARCH	GELATIN HYD	HAEMOLYSIS	IDENTITY
			NA	CH	BD	M/C																
TS_1	+ve	rod	+	+	+	-	+	CND	-	+	+	Milky	+	+	+	+	+	+	+	+	$+^{\beta}$	Bacillus cereus
BTS_2	+ve	rod	+	+	+	-	+	CND	-	+	+	Milky	-	+	+	+	-	+	+	+	$+^{\alpha}$	Bacillus subtilis
BK_1	+ve	rod	+	+	+	-	+	CND	-	+	+	Milky	+	+	+	+	+	+	+	+	$+^{\beta}$	Bacillus cereus
BK_2	+ve	rod	+	+	+	-	+	CND	-	+	+	Milky	-	+	+	+	-	+	+	+	$+^{\alpha}$	Bacillus subtilis
BK_3	+ve	rod	+	+	+	-	+	OVAL	Sli	+	+	Milky	+	+	+	+	+	+	+	+	$+^{\beta}$	Bacillus species
BK_4	+ve	rod	+	+	+	-	+	OVAL	TERM	+	+	Milky	+	-	-	+	+	+	+	+	$+^{\alpha}$	Bacillus species
BK_5	+ve	rod	+	+	+	-	+	CND	large	+	+	Milky	-	+	+	+	+	+	+	+	$+^{\beta}$	Bacillus species
BS_1	+ve	rod	+	+	+	-	+	CND	-	+	+	Milky	+	+	+	+	+	+	+	+	$+^{\beta}$	Bacillus species
BS_2	+ve	rod	+	+	+	-	+	OVAL	TERM	+	+	Milky	+	+	+	+	+	+	+	+	$+^{\alpha}$	Bacillus species
BS_3	+ve	rod	+	+	+	-	+	CND	-	+	+	Milky	-	+	+	+	-	+	+	+	$+^{\alpha}$	Bacillus subtilis
BS_4	+ve	rod	+	+	+	-	+	CND	-	+	+	Milky	+	+	+	+	+	+	+	+	$+^{\beta}$	Bacillus mycoides
BP_1	+ve	rod	+	+	+	-	+	CND	large	+	+	Milky	+	+	+	+	+	+	+	+	$+^{\alpha}$	Bacillus species
BP_2	+ve	rod	+	+	+	-	+	CND	-	+	+	Milky	+	+	+	+	+	+	+	+	$+^{\beta}$	Bacillus mycoides
BP_3	+ve	rod	+	+	+	-	+	CND	-	+	+	Milky	+	+	+	+	+	+	+	+	$+^{\beta}$	Bacillus cereus
BP_4	+ve	rod	+	+	+	-	+	CND	-	+	+	Milky	-	+	+	+	-	+	+	+	$+^{\alpha}$	Bacillus subtilis
BK_6	-ve	rod	+	+	+	+	+	-	-	+	+	Light yellow	-	+	+	+	+	+	+	+	$+^{\alpha}$	Enterobacter agglumerus
BP_8	-ve	rod	+	+	+	+	+	-	-	+	+	Sharp red	-	+	+	+	+	+	+	+	$+^{\alpha}$	Serratia species
BP_5	+ve	cocci	+	+	+	-	+	-	-	-	-	Yellow	-	-	-	-	-	+	+	-	$+^{\gamma}$	Micrococcus luteus
BP_6	+ve	cocci	+	+	+	-	+	-	-	-	-	Yellow	-	-	-	-	-	+	+	-	$+^{\gamma}$	Micrococcus luteus
BP_7	+ve	cocci	+	+	+	-	+	-	-	-	-	Pinkish	-	-	-	-	-	+	-	-	$+^{\gamma}$	Micrococcus roseus
BS_5	+ve	cocci	+	+	+	-	+	-	-	-	-	Yellow	-	-	-	-	-	+	+	-	$+^{\gamma}$	Micrococcus luteus
BS_6	+ve	cocci	+	+	+	-	+	-	-	-	-	Pinkish	-	-	-	-	-	+	-	-	$+^{\gamma}$	Micrococcus roseus
BS_7	+ve	rod	+	+	+	-	+	-	-	-	-	+ Milky	-	+	-	+	+	+	+	+	$+^{\alpha}$	Acetobacter agglumerus

KEY: NA = Nutrient agar
CH = Chocolate agar
BD = Blood agar
M/C = Mauconkey agar
+ = indicated growth/positive result
- = indicated no growth/negative result
$+^{\alpha}$ = indicated positive alpha haemolysis
$+^{\beta}$ = indicated positive beta haemolysis
$+^{\gamma}$ = indicated gamma haemolysis or no haemolysis

Table 4: Cultural, morphological characteristics and identification of Mold Isolates.

CODES OF ISOLATES	GROSS MORPHOLOGY: POTATO DEXTROSE(DA) Agar	Czapek's Agar CZA	IDENTITY
MTS_1	Whitish,colonies, grows moderately	Thick velvety colonies very tufted and whitish colonies, reverse not pigent.	Mycelia sterilia
MB_1	Whitish colonies, cotton like	Thick velvety,. Slow growth. light brown no pigment foraation.	Mycelia sterilia
MK_1	Whitish large Colony, cotton like	Tufted colony, dark at the centre, light brown to whitish at the edge. No pigmentation at the reverse of the plates.	Mycelia sterilia
MP_1	Light brown colonies fast spreading	Light brown colony, dark centred, velvety and tufted. No pigmentation at the reverse of the plates.	Mycelia sterilia
MP_2	Greenish white mold with velvet, appearance.	Colonies are velvety green with white patches and white margin, grows moderately well, with age colonies become dirty grey. Reverse greenish brown.	Aspergillus species
MTS_2	Black spoty molds	Colonies brownish yellow or ochraceous spreading gradually. Orange to brownish sclerotium present reverse colourless.	Aspergillus species
MK_2	Creaty white mold	Colonies spreading fast, yellowish brownish green with age, Reverse yellow.	Aspergillus species
MTS_3	whitish felted green mold	The aerial Mycelia of colonies are sooky, it is felted and reverse is colourless.	Aspergillus fumigatus
MTS_4	Dark green mold	The colours spread rapidly and aerial mycelium with older portion blackeny, Reverse yellowish.	Aspergillus flavus
MTS_5	Brownish cotton mold	Rapid spreading, colonies, early overgrown	Rhizopus species
MTS_6	Whitish (cotton like	Compact colonies, slow growing, reverse colourless	Phillantrophila species
MTS_7	Brownish and compact	Colonies spreading fast, brownish, No colour change observed.	Fusarium species

Table 3: Microbial Profile of different stages of Burukutu production:

Organism	Unboiled	boiled wort	12hrs	18hrs	24hrs	Kumpa
Molds						
Mycelium sterilia	+	-	+	+	+	+
Aspergillus species	+	-	+	+	+	+
Aspergillus fumigatus	+	-	+	-	+	-
Aspergillus flavus	+	-	+	+	+	+
Aspergillus niger	+	-	-	-	+	-
Fusarium species	+	-	+	+	+	+
Rhizopus species	+	-	-	-	+	-
Yeasts						
Rhodotorula species	+	-	+	+	+	+
Saccharomyces species I	+	+	+	+	+	+
Saccharomyces species II	+	-	+	+	+	+
Saccharomyces species III	-	-	+	+	+	+
Pichia species	-	+	+	+	+	+
Candida species	+	-	+	+	+	+
Kloekera species	-	-	+	+	+	+
Bacteria						
Bacillus cereus	+	-	+	+	+	+
Bacillus mycoides	-	+	+	+	+	+
Bacillus subtilis	-	-	+	+	+	+
Bacillus species	-	-	-	-	+	-
Micrococcus luteus	+	-	+	+	+	+
Micrococcus roseus	+	-	+	+	+	+
Serratia species	-	-	-	+	+	-
Acetobacter species	-	-	-	-	+	-
Enterobacter agglumerus	+	-	-	+	+	-

Table 5: Results of organoleptic assessment of Burukutu samples brewed with single or mixed inocula of isolates (starter)

Beer selected organisms for fermentation Test Starter	ATTRIBUTES							
	Sweetness	Sourness	Bitterness	Flavour	Colour	Odour	Consistency	Scores
1 Traditional brew-Control I	3.08±0.60	3.00±0.78	3.10±0.83	3.13±0.83	2.25±0.60	2.92±0.64	3.58±0.49	21.11*
2 Traditional brew-Control II	3.17±0.99	2.92±0.95	3.27±0.62	2.36±1.30	3.08±0.39	3.08±0.28	2.60±1.25	20.58
3 Saccharomyces species I	2.60±0.28	2.87±0.78	2.75±0.60	2.14±4.90	3.50±0.28	1.85±0.64	2.43±0.83	18.14
4 Saccharomyces species II	2.30±0.85	2.51±0.24	1.40±0.73	2.35±0.62	2.70±0.39	3.05±0.83	3.00±0.43	17.37
5 Saccharomyces spp I & II	3.16±0.90	3.37±0.24	2.87±0.63	2.71±0.42	2.87±0.36	2.35±0.28	3.70±0.30	21.56
6 Saccharomyces spp III	2.10±1.04	2.25±1.09	2.2 ±0.75	2.58±0.64	3.00±0.71	3.25±0.83	2.58±1.26	17.96
7 Saccharomyces spp II & III	3.33±0.62	2.75±1.09	2.36±0.77	2.50±1.30	3.67±0.47	2.67±1.25	1.92±0.86	19.20
8 Saccharomyces spp I and Candida spp I	2.67±1.11	3.05±1.28	3.00±0.74	2.55±0.89	2.55±0.89	3.17±0.90	2.67±1.37	19.96
9 Candida spp I	3.88±0.28	2.17±0.37	1.50±0.96	2.60±0.80	3.00±0.00	1.83±0.99	3.08±0.86	17.20
10 Pichia spp	1.50±0.83	2.60±0.20	2.17±0.37	3.23±0.42	2.8 ±0.22	1.35±0.50	2.80±0.83	16.45
11 Sacc. spp I & II + Ente. agglumeractis & Bacillus C.	2.07±0.60	2.30±0.23	2.75±0.30	2.54±0.38	3.00±0.0	3.00±0.30	1.40±0.20	17.80
12 Unpitched wort	1.00±0.20	2.00±0.00	1.98±0.40	1.00±0.00	1.20±0.20	1.8 ±0.40	1.00±0.00	10.55
13 All species of bacterial Isolates E agglumerous, Micrococcus luteus M. Roseus, Bacillus cereus, B subtilis, B. mycoides, Acetobacter spp	1.20±0.20	2.00±0.00	1.90±0.40	1.00±0.00	1.30±0.30	1.20±0.20	1.1 ±0,73	9.60
14 Saccharomyces spp I & II + Bacillus cereus Enterobacter agglumer Acetobacter spp	2.00±0.00	2.00±0.0	1.00±0.63	2.00±0.42	2.3 ±0.45	1.00±0.30	3.00±0.00	13.30

* Each value is an average of three different scores

occurrence of each species can be seen in Table 3. There are two species of *Micrococcus (M. luteus and M. variance).*

Two isolates of the member of family Enterobacteraceae were found and were identified as Enterobacter agglumerus and serratia species. A singly specie of Acetobacter was identified.

In development of starter culture, bacteria and yeast were utilized as sole microbiological flora for burukutu production. Molds were not considered. only *Saccharomyces species* I & and II and *Candida species* fermented sorghum wort from the production of burukutu beer. Tastes panel scores organoleptic test) showed that using these selected strains, they produced burukutu similar to traditional prepared brew (Table 5).

High acceptability of the beers as reflected by the scores of 21.56 out of total of 28 scores for *Saccharomyces spp.* I 7 and II is comparable with that of control I brew with a score of 21.11 for control I and control II (20.58). *Saccharomyces species* I on its own scores 18.14, while *Saccharomyces species* I coupled with *Candida species* I produced 19.96. *Pichia species* had a score of 16.45.

When bacterial isolates were used independently, no fermentation was evident. The panel rated the unfermented wort as 10.55. The score of bacteria pitched beer was 9.60 which is markedly different from control and that of yeast fermentation. The use of yeasts in combination with bacteria shows an intermediate result. *Saccharomyces species* I & II when used in combination with *Enterobacter agglumerus* and with *Bacillus cereus* scored 17.80 as opposed to 21.56 that was scored by the *Saccharomyces species* I and II alone.

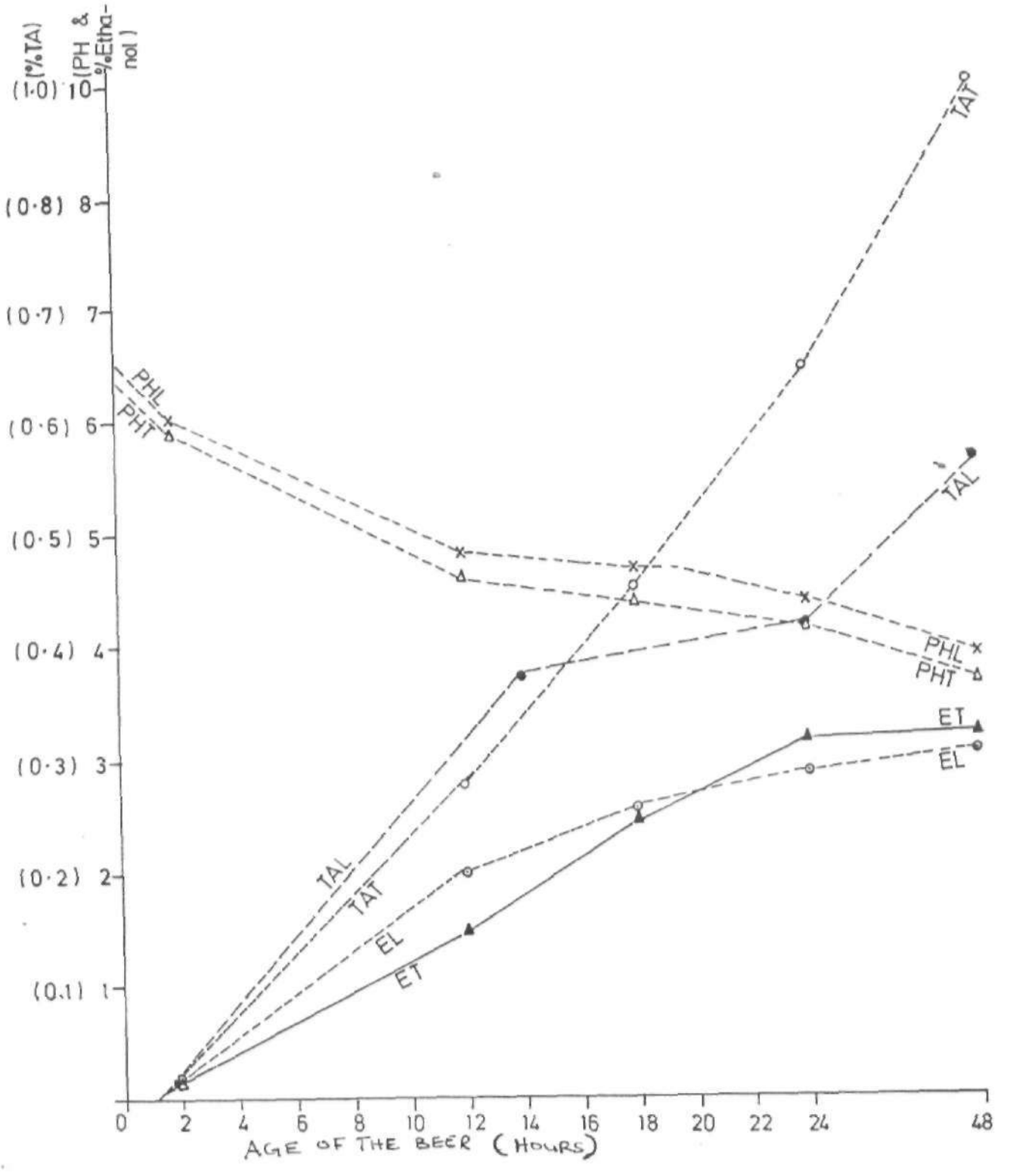


FIG.2 The changes in physiochemical characteristic of burukuu samples brewed in the laboratory compared with traditional bre

PHL= PH of Laboratory prepared burukutu

PHT= PH of Traditionally prepared burukutu

EL= % Ethanol of laboratory prepared burukutu

ET= % Ethanol of traditionally prepared burukutu

TAL= Titratable acid for laboratory prepared burukutu

TAT= Titratable acid for traditionally prepared burukutu.

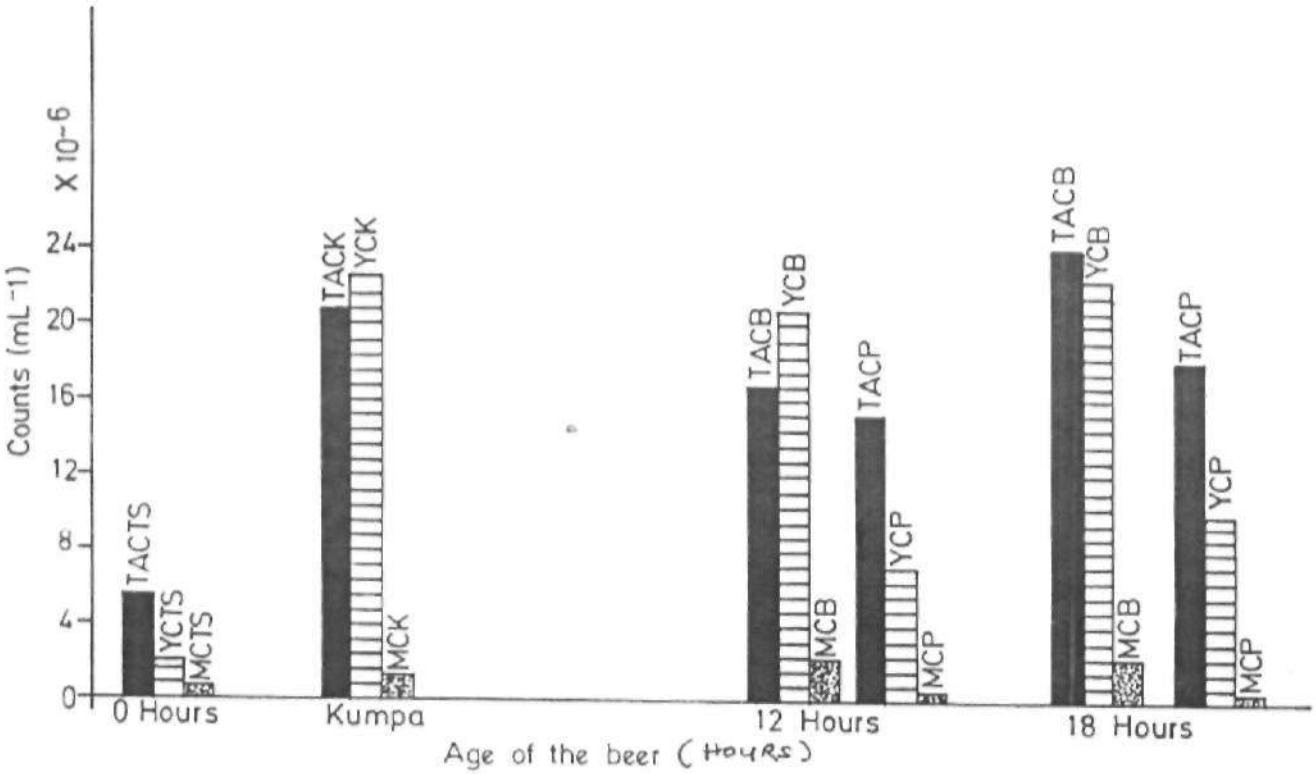


Fig. 3 Average counts of six different brew at different stages of fermentation using Histogram.

TACTS= Total aerobic bacterial count of boiled wort
YCTS= Yeast count of boiled wort
MCTS= Mold count of boiled wort
TACK= Total aerobic boiled count of kumpa
YCK= Yeast count of kumpa
MCK= Mold count of kumpa
TACB= Total aerobic count of burukutu
YCB= Yeast count of burukutu
MCB= Mold count of burukutu
TACP= Total aerobic bacterial count of pito
YCP= Yeast count of pito
MCP= Mold count of pito

The acid level of traditionally produced beer was slightly higher than the obtained value for laboratory brewed burukutu (fig. 2). There was no difference in the percentage ethanol content of both brews which ranged from 2.9 - 3.0.

The most predominant molds observed are *Aspergillus species: A. niger, A. fumigatus, A. flavus, Mycelia sterilia, Rhizopus species* and a Fusarium species (Table 4).

Aflatoxin was detected in all traditionally brewed beer and in laboratory brewed burukutu beer prepared from moldy grains. There was no aflatoxin in laboratory brewed beer whose grains were not moldy.

There was no difference in the types of microorganisms found in kumpa and burukutu (Table 3). Figure 2 represents changes in characteristics of burukutu samples brewed in the laboratory compared to traditionally brewed burukutu.

The histogram (Fig. 3) shows that the highest microbial count from locally produced brew is from kumpa followed by burukutu.

CHAPTER FIVE

DISCUSSION

The microbiological flora of different alcoholic beverages made from grains have been very well documented (Van der Watt, 1956; Faparusi et al, 1973; Ekundayo, 1969; Nout, 1980; Ogbonna et al., 1983a; Dirar, 1978). Data from present study showed a high concentration of diverse types of molds such as *Aspergillus species, Rhizopus species, Mycelia sterillia, and Fusarium species.* The presence of these molds may be attributed to their initial contamination of the sorghum grains used for malting. Faparusi et al. (1973) have documented that molds are capable of settling on sorghum grains especially under conditions of high relative humidity and warm temperature. Van der Watt (1956) had reported that molds have a beneficial effect on malting. Webb (1945) found that the diastatic activities of mold flora on maize and sorghum accounted for the bulk of sugar production required to attain high alcoholic content in Kaffir. They reported that this amylolytic ability of molds may be attributed to their ability to produce fungal amylase which in addition to amylase inherent in sorghum aids the malting process.

The disappearance of molds following boiling shows that boiling has a disinfecting effect during burukutu production and that only vegetative stages of these molds are produced. The reappearance of molds during fermentation and maturation may be due to their introduction into the process by the use of local starter culture (Kumpa) which has a significant load of molds (Table 3).

The present study shows that yeasts predominate in all stages but one of burukutu production (Table 3). This study also shows that yeasts

as reported by Van der Watt (1956); Ogbonna et al. (1983a) to be the major fermenting organisms of sorghum beers and thereby the most important group of microorganisms in burukutu production. Sugar fermentation tests of yeasts isolates have shown that only three yeast strains namely *Saccharomyces species* I and II and *Candida species* could be used for fermentation of burukutu since the other yeast isolates had little or no fermentative ability. These yeast strain mentioned above exhibited relative high fermentation rates of hexose sugars namely glucose, galactose, maltose, and sucrose. Reports from other studies (Faparusi, 1970) show that sorghum based wort consists of these sugars. Thus these yeasts can be used in the fermentation of burukutu beer.

Utilization of pure isolates of *Saccharomyces species I, Saccharomyces species II*, and *Candida species* produced burukutu beer which had good flavour and was highly accepted when compared to the control (traditionally produced brew). It can, therefore, be inferred from the present study that *Saccharomyces species* I and *Saccharomyces species* II (together) can be used as starter culture for Burukutu production. Other strains of yeast isolates like *Saccharomyces species* I and *Candida species* I can also be used. The use of *Saccharomyces species alone can be considered in the absence of other two starters (Table 5).*

The detection of aflatoxin by Thin layer chromatographic analysis might be due to the presence of Aspergillus species which are the major producers of this metabolite (Bassir, 1960). He also stated that there is a report from East Africa that Kenya beer may contain as much as 30µg/litre of aflatoxin. The possibility of using molds for fermentation or as starters in this present studies is ruled out because the molds could not ferment the brew, and based on the possible hazards posed by

the presence of metabolites formed by molds with regard to human health. Results from present studies also show a high load of Bacteria during different stages of burukutu production. The lack of lactic acid bacteria in the flora is at variance with previous studies (Faparusi et al., 1973; and Ogbonna et al., 1983a), which indicated the use of gari adjunct in production of burukutu in southern parts of Nigeria and some parts of the northern Nigeria. The lack of adjunct in production of burukutu in Zaria may account for the absence of lactic acid strains since lactic acid bacteria have been shown to be involved in Gari fermentation (Akinrele (1970); Akinrele, (1964), Ngaba and Lee (1974). The absence of a souring stage in the processing of burukutu in Zaria may also be an underlying reason for the absence of lactic acid bacteria. This is because during the souring stage, allowed by other burukutu producers in other parts of the country aid the proliferation of souring agents like Lactic acid Bacteria. Data from previous studies (Faparusi et al., 1973) have shown that the change in taste observed in some burukutu beer after 24 hours of fermentation, may be due to the presence of *Acetobacter species*. *Acetobacter* is known to possess oxidative ability with which it oxidises ethanol to acetic acid, thereby increasing the irregular flavour of the beer. Our finding also shows that the bacterial flora has only detrimental effect during fermentation as reflected by the taste panel with a score of 9.60 when used alone. Unpitched wort even had a higher score of 10.6 while pitched wort with yeasts gave similar results to those of the control (Table 5).

Although other strains of yeast isolates were investigated, none of them produced results comparable to *Saccharomyces species I & II. This proposed starter gave rise to a better product than other microbial*

isolates when matched against the accepted traditional brews: they are capable of fermenting available sugar in burukutu containing 3% ethanol which is similar to 2.9 - 3.2% found in traditionally brewed burukutu.

Tentatively, it can be recommended, from the present study, that Saccharomyces species I and II be utilized as starter cultures for burukutu production.

CHAPTER SIX

RECOMMENDATION AND CONCLUSION

It is recommended from the present studies that *Saccharomyces species* I and II be used as starter cultures instead of kumpa (local starter). This is because kumpa itself encourages microbial contamination including mold and bacteria which are detrimental to the production process.

Pasteurization of burukutu as a means of disinfecting the fermenting wort should be adopted since the organisms found in the system of the beer are quite heat sensitive.

Proper education should be given to local producer to aid the production of hygienically produced burukutu because the microbial load of traditionally brew is very high.

The consumption and industrial production of burukutu is advocated. This is because Burukutu is cheaper to produce because of the availability of raw materials and proposed starter to aid controlled fermentation. Given also that some other scientists (Faparusi 1970, Ogbonna et al. 1983b) have confirmed the higher nutritional value of burukutu vis a vis larger beer. It is recommended as a supplement for consumers. These will help in conserving foreign exchange.

APPENDIX I

Accesory Table: Organoleptic Test)

Mark the appropriate column in respect of A, B, C, D, E, F, G character

Attributes

Sweetness A	Very sweet 1	Moderately sweet 3	Slightly sweet 4	Not sweet 2
Sourness B	Very sour 2	Moderately sour 3	Slightly sour 4	Not sour 1
Bitterness C	Very bitter 1	Moderately bitter 3	Slightly bitter 4	Not bitter 2
Flavour D	Sour flavour 3	Slightly sour flavour 4	Sweet flavour 2	No flavour 1
Colour E	Dark brown 2	Brown 4	Light brown 3	Whitish 1
Consistency/ Concentrations F	Very thick 2	Thick 3	Slightly thick 4	Watery 1
Odour G	Sharp 'Kumpa'-like 3	Moderate 'Kumpa'-like 4	Slight 'Kumpa'-like 2	Rotten odour 1

Best quality of burukutu beer carried the highest scores while the worst attribute carried the least number.

1. Comment briefly on the beer.

2. How much can you pay for each bowl of beer? (N1.00, 70k, 50k, 30k).

3. If this quantity is reduced, can you still pay the same amount?

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