STUDIES ON THE EFFECT OF HEAT TREATMENT AND CHEMICAL PRESERVATIVES ON THE KEEPING QUALITY OF BURUKUTU BEER

BY

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DEDICATION

This work is dedicated to My Father, Mallam M. J. Yusuf and Brother Zubairu Salawu for their inumerable sacrifices towards the success of this programme

DIGITIZED

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 has not been presented elsewhere for a degree or a diploma. Any work of other investigators referred to is acknowledged in this presentation.

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CERTIFICATION

This thesis entitled "Studies on the Effect of Heat Treament and Chemical Preservatives on the keeping Quality of Burukutu Beer" by Momoh Jimoh Abubakar meets the regulation governning 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

A field study of the actual process involved in the making of burukutu beer and laboratory investigation of the variations in the different microbial population and fermentation products during the various stages of fermentation was carried out as a background study for the preservation of the product .

Two main stages were designated in the production of burukutu beer; viz - alcoholic fermentation stage where the activities of yeasts lead to the production of burukutu beer that remain consummable for a period of 24 hours and acetic acid fermentation stage where the activities of acetic acid bacteria lead to the spoilage of the product after 24 hours of alcoholic fermentation.

Preservation experiments were carried out on fresh burukutu beer using heat treatment and chemical preservatives, and a combination of heat treatment and chemical preservatives. Heat treatment at 60°C for 30 minutes preserved fresh burukutu beer for a period of 2 weeks while 1% concentration of the chemical preservatives used (sorbic acid, benzoic acid and sodium metabisulfite) preserved fresh burukutu beer for a period of 3,4 and 6 weeks respectively. Combined heat treatment plus 0.25% concentration of the chemical preservatives preserved burukutu beer for periods ranging from 7-11 weeks.

Organoleptic tests and determination of physiochemical characteristics (% ethanol, % titratable acidity, % volatile acid and PH) carried out on preserved burukutu beer were very much comparable to those offresh burukutu beer.

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CHAPTER ONE

INTRODUCTION

Burukutu, one of the African beers produced from cereal grains, is an alcoholic beverage brewed from guinea corn grains (Sorghum vulgare or Sorghum bicolor), and forms the staple beverage drink in the savanna region of Nigeria. Traditionally, preparation methods involve a series of stages which include malting of the Sorghum grain and mashing of the malted grains. After mashing, the resultant wort is separated into a supernatant liquor and a colloidal suspension. The latter is boiled after which it is mixed with the former. The mixture now undergoes a second boiling stage. The boiled wort is then pitched with 'kumpa' (the foamy mass of previous brew).

Fermentation of burukutu beer is spontaneous in nature and is brought about by undefined microorganisms whose source could be the water used for mashing, aerial mycelia and some normal flora on the grain used for brewing (Ogbonna et al, 1983). Various workers including Faparusi et al. (1973), Ogbonna et al. (1983) and Akinyemi (1990), reported various species of bacteria, yeasts and moulds as the microorganisms being encountered in the production of burukutu beer. However, only

very few of these organisms play any fermentative or significant role in burukutu beer production. Unlike in lager beer, these microorganisms are alive and form a heavy suspension in the finished product and hence constitute spoilage hazards in the burukutu beer. This, coupled with the spontaneous and uncontrolled nature of burukutu fermentation which results in a product of variable quality, has affected the popularity of the product. Also, the traditional burukutu beer brewer employs very poor equipment, lacks storage facilities resulting in a beer of very poor shelf-life. The unhygienic manufacturing techniques further compound the waning popularity of the product, especially among populations outside the native region and the higher income groups.

The nutritional value of burukutu beer is considered superior to that of clear lager beer due to higher contents of crude proteins, thiamine, riboflavin, Vitamin C and hexose sugar (Faparusi, 1970). In view of this, the increased consumption of the beer has been advocated by various workers not only by the local consumers but also Nigerians in general. The additional advantages of local availability of microbial strains and other raw materials, and cheap price of burukutu beer have made it necessary to concentrate more efforts on its production over that of lager beer

whose production is becoming increasingly unattractive due to escalating cost of maintenance. Hence considerable efforts have been directed towards producing *indigeneous beer from locally available cereals and* food crops.(Akinyemi, 1990).

However, to achieve this, there is the need to improve the image of the product by developing adequate hygienic manufacturing techniques that will lead to ahygienic product of high standard quality and acceptable shelf-life. Consequently, adequate preservative techniques need be developed to give the product a longer shelflife. As part of efforts to render the product spoilage-free and better keeping quality, development of starter culture has been attempted. This entails using only microbial isolates which are considered necessary or potentially relevant to a given fermentation process, singly or mixed as starters for producing desired food or beverages (Okafor, 1981). Akinyemi (1990) was able to develop a mixed starter culture of Saccharomyces species I and II for the production of burukutu beer which had excellent organoleptic quality as that of traditionally produced, but with better keeping quality.

Preservative measures have been taken since earlier *times to overcome food deterioration. Food has been* preserved in wines (alcohol), in vinegar (acetic acid)

and in solutions of common salt or sugar. Food has also been protected from atmospheric contamination by various forms of covering. The drying of fish and meat either in the sun or over fire, the use of spices are also some local methods of food preservation since antiquity [Hobbs and Christian (1973), FAO/WHO(1967)) Anonymous.

Works on the preservation of African alcoholic beverages are scanty. Despite the myriads of researches carried out on burukutu beer, no preliminary investigation has been directed towards its preservation. Preservation of burukutu beer may be a veritable tool that will facilitate the future large-scale industrial production of the product. This work is an attempt towards the ultimate realization of the production of burukutu beer of high standard quality and acceptable shelf- life and seeks to achieve the following specific objectives:

i. A field study of the actual process involved in the making of burukutu beer and laboratory investigation of the variations in the different microbial populations and fermentation products during the various stages of production. This will help provide information regarding pattern of spoilage.

ii. Preservation of burukutu using both heat and chemical treatments.

iii. Determination of the appropriate concentration of the preservatives that will yield best results.

iv. Selection of the best preservative treatment.

v. Comparative sensory evaluation of fresh burukutu beer and the preserved one.

C H A P T E R T W O

LITERATURE REVIEW

2.1 Brewing in Indigenous African Communities

The traditional production of alcoholic beverages in various African Communities is a common art and a process of considerable antiquity. There are five main groups of African alcoholic beverages (Platt, 1955). This includes: fermented honey water; fermented fruits and juices; fermented saps from various species of palm and bamboo, drinks made from milk and the beers. All of these have more or less a familiar equivalent in mead, cider, fermented birch sap, fermented mare's milk (Koumiss) and old fashioned ales. Burukutu and other alcoholic beverages from various cereals are classified under beer.

The best studied of the indigenous alcoholic beverages is the Kaffir beer of South Africa where production is now on an industrial scale. Vander Walt (1956) reported that the brewing of Kaffir beer involves two stages of fermentation viz., the lactic acid fermentation stage and the alcoholic fermentation stage. The lactic acid stage, which is the souring stage is carried out by the following typical lactic acid flora: Lactobacillus delbrickii, L Plantarum, L brevis, L fermenti, Pediococcus damnosus, Leuconostoc mesenteroides; Leuconostoc dextrinicum. The alcoholic

fermentation stage involves the activities of many yeasts which are over twenty species. The spoilage of the beer according to the study, was due to excessive acetic acid formation by the common malt and beer Acetobacter species.

Another indigenous African beer is the Merrisa brewing which was described as a fine art with unique characteristics probably not found in other African or non-African beer (Dirar, 1978). This assertion is not unconnected with the complex fermentation processes involved in the production of the beer. Merrisa brewed from 'Feterita' (Sorghum bicolor) consists of three distinct phases: Ajeen fermentation, a lactic acid bacterial souring of Sorghum; Deboba fermentation, a starter activating stage and the actual fermentation which is the final stage leading to the production of the merrisa beer. The latter two stages represent the alcoholic fermentation stages. The major microorganisms reported in the fermentations are the lactic acid bacteria and yeasts of the Saccharomyces species.

Nout (1980) carried out a study on the microbiology of Busaa, a Kenyan opaque maize beer. The beer is commonly prepared from maize endosperm grits and finger millets malt (Eleucine coracana). The product contains a considerable amount of solid matter (maize endosperm

and finger millet particles) which is kept in stable colloidal dispersion throughout the liquid. The product has undergone several stages of spontaneous lactic acid and alcoholic fermentation when it reaches the consumers. The following organisms are mainly present during the stage of uncooked grits: Yeasts such as Candida cruzei and Saccharomyces cereviseae; Bacteria such as Lactobacillus species; L helviticus; L salvarius L brevis; L viridensces L planta; Pediococcus species P damnosus and P paralus. The finger millet used alongside contains a high microbial load (10^7–$10^9 g^{-1}$) which is rather diverse. The following microorganisms were identified in the malt: Corynebacterium species; Bacillus species; Listeria species; Enterobacter species; Enterobacter cloacae; Klebsiella pneumoniae; Propanibacterium species; Eubacterium species; Lactobacillus casei (var Pediococeus damnosus): Rhizopus species; Mucor species, Candida cruzei. The alcoholic fermentation of busaa was dominated by Saccahromyces species while the lactic acid fermentation was initiated by hetero- and homofermentative Lactobacilli such as L brevis; L salivarius (var saccinus); L platarum; L viridensis; L casei. (var salicinus); L bruchei and L plantar um which predominated.

Okafor (1975) described palm wine as an alcoholic beverage in Nigeria produced from the saps of various

palms and which contains a heavy suspension of live yeasts and bacteria. The drink rates as high as burukutu in terms of popularity and consumption in Nigeria. A number of microorganisms are known to be present in palm wine. Okafor (1978) reported the presence of 17 yeast isolates, 12 of which were Candida species and 3 of them belonging to Endomycopsis species. The bacterial isolates include Micrococcus species; Streptococcus species; Lactobacillus species; Bacillus species and Brevibacterium species. The fermentation follows the typical lactoalcoholic fashion.

Pito is another alcoholic beverage of immense importance in Nigeria. It is very similar to burukutu, the difference being in the grains used for brewing. While Sorghum is about the only grain used in burukutu brewing, various types of grains are used to brew Pito. Ekundayo (1969) shows that the predominant microorganisms found during pito production are: Moulds such as Penicillium species, Rhizopus oryzae and Aspergillus species, which were present in 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 concentrate) 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.

Various workers have investigated the microflora of burukutu beer. Faparusi et al (1973) in a study of the microbiology of burukutu beer found the microflora of both home and laboratory brewed beer to be similar. Their yeast isolates included Saccharomyces cereviseae; Candida tropicalis; Candida kruzei; Hansenula anomala; Kloekera apiculata. The moulds isolated included Mucor rouxii, Aspergillus flavus; Aspergillus oryzae; Penicillium citrinum and Aespora species. Some species of Lactobacillus and Streptococcus were also identified as the bacterial isolates.

Ogbonna et al. (1983) also carried out an invetigation on Nigerian indigenous alcoholic beverages and found out that the microflora of laboratory prepared were as follows: Moulds such as Aspergillus flavus; A fumigatus; A oryzae; A terreus; Cladosporium herbus; Humicola grisae; Penicillium citrinum and Penicillium stonifer. The yeast isolates included Candida tropicalis; Candida utilis; Hansenula anomala, Saccharomyces cereviseae; S ovarum; Schizosaccharomyces pombe; while the bacterial isolates were Bacillus cereus ; Lactobacillus brevis; Lactobacillus plantarum,

Leuconostoc mesenteroides; Streptococcus species; staphylococcus species and the Nematodes found were Ascaris lumbricoides (ova); Ancylostoma duedenum; Strongyloides sterocolaris and Trichuri trichura.

Ekundayo (1969) also reported the presence of Candida mycoderma; Candida tropicalis: Hensenula anomala Kloekera apiculata Saccharomyces pastorianum and Pediococcus cereviseae in burukutu undergoing maturation. Ekundayo 1969) also indicated that microorganism found in pito as mentioned above were similar to those found in burukutu.

In a very recent work, Akinyemi (1990) attempted the development of starter culture for the production of burukutu beer. The following organisms were isolated in the work: Bacteria, including Bacillus cereus B subtillis; Bacillus mycoides; Micrococcus luteus and Micrococcus roseus; Enterobacter agglumerous; Serratia species and a single species of Acetobacter. Moulds which include Aspergillus species; Mycelic sterilla; Rhizopus species and Fusarium species. Yeasts including Saccharomyces species; Candida species; Pichia species and Rhodotorula species. Saccharomyces species was proposed for use as starter culture for burukutu production in the work. The linkage of the presence of Acetobacter species and Aspergillus species with the vinegary tastes of burukutu beer after 24 hours,

and the presence of aflatoxin, respectively in the work and underscores the necessity for a sound method of preservation of the product.

2.2 Origin and Socio-economic Importance of Alcoholic Beverages in Africa

Fermented honey drinks may have been the earliest alcoholic beverages known to man and the discovery of them has been attributed to the Hermites. (Seyffert 1930). Also alcoholic beverages have been widely consumed in the world from prehistoric time dating back from the days of biblical Noah. Consumption and production of these beverages in Africa is traceable to the King Osiris of Egypt who discovered that the fermentation of wild wheat and barley could lead to beer and later spread the art by engaging in tours of the rest of the continent and teaching mankind how to cultivate the vine and how to enjoy their fruit (Platt, 1955). The production of wine and beer from various fruits and cereals has since then become a widespread art in all parts of the continent with the beverage types and consumption varying from one locality to another (Pederson, 1978). Some documented forms of indigenous alcoholic beverages in literature include, as mentioned earlier Kaffir beer of South Africa, Merissa, a Sudanese beer, Busaa, a Kenyan Opaque maize beer and pito and burukutu from Nigeria (Van der Walt

1956, Dirar, 1978; Nout 1980; Ekundayo 1969; Faparusi et al, 1973).

The economic importance of alcoholic beverages world wide has been documented Platt (1955), Pederson (1978). In Africa, the consumption of beer and locally available alcoholic beverages among all population groups has been in the rise. This is attributable to various factors. Platt (1955) indicated their consumption and the diverse role alcoholic beverages play in social gathering. It is mandatory that alcoholic beverages be served in marriages, burial and naming ceremonies and other social gatherings. Also alcoholic beverages form an important part of sacrifices offered to gods in many parts of Africa (Pederson, 1978). It is used for treatment against such ailments as common cold and as a solvent for extraction of 'drug' from tree bark androots. This accounts for its increased consumption (Pederson, 1978).

Beer drinking in the words of an observer (Richards 1939) is 'the peoples' only kind of entertainment, the chief break in the monotony of their village life, and as in most other Bantu societies, the common and sometimes essential way of fulfilling social obligations.... it (beer) is carried to chief as tribute used to reward labour, or given as an offering to spirits.... Abundance of beer is the glory of

commoner's hospitality, or a chief's court. Without it, tribal councils cannot be held and marriages or initiation ceremonies do not take place'.

Huntingford (1950) also reported that among the Nandi tribe in Kenya, beer is a social necessity and not merely an enjoyment: 'if a Nandi cannot from time to time give a beer party, even a small party, he will be considered mean and will not be asked by his neighbours to part-take of beer. He will be unofficially but nontheless effectively pushed out of his rightful place in the society'. These instances clearly depict the role of alcoholic beverages all over Africa.

The superior nutritional value of these traditional alcoholic beverages over their refined counterparts has contirbuted immensely to their increased consumption (Nout, 1980; Bryant 1939).

2.3 Fermentation

Fermentation which is the backbone of any liquor industry has undergone numerous changes in meaning during the past hundred years (Doelle, 1969). From the derivation of the term, it signifies merely a gentle bubbling or boiling condition. The term was first applied when the only known reaction of this kind was the production of wine, the bubbling of course being

caused by the production of carbon dioxide. The meaning was changed to signify the breakdown of sugar into ethanol and carbon dioxide by Gay Lussac who studied the chemical aspect of the process (Prescott and Dun 1959). Pasteur finally marked the birth of chemical microbiology with his association of microbes with fermentation in 1857 (Doelle, 1969). He used the term 'cell' and 'ferment' interchangeably in referring to the microbe. The term fermentation thus become associated with the idea of cell, gas production and the production of organic products.

Fermentation processes are invovled in the production of certain food and beverages, chemicals and many other substances which may be of nutritional, agricultural or medical interest. Fermentation can be carried out by several organisms, for example, bacteria (e.g. Lactic acid bacteria), yeasts e.g. Saccharomyces careviseae and moulds e.g. Rhizopus nigricans.

Grains, when subjected to fermentaiton, could lead to either of two products, viz. fermented food and fermented alcoholic beverages. Fermented foods are common among the Oriental people and include Soy source (toyo) made from soy bean and wheat, Miso (Chiang) made from soy bean and rice. In Nigeria, the fermented grains foods are Ogi (a cereal based

food), Garri (fermented roasted Cassava grits), Foofoo (fermented paste from cassava) and many condiments from locust beans called dawadawa and soy bean dawadawa. Among fermented alcoholic beverages is the beer whose fermentation involves the production of ethanol and carbon dioxide, and a wide range of other qualitatively minor but organoleptically important metabolic by-products of the yeasts (Chukwura 1989). The yeasts accomplish this task via the Embdon-Mayerhof-Parnas (Emp) pathway or glycolysis, a complex chemical energy-yeilding reactions which requires organic compounds as electron acceptors (fermentation). The scheme has been adopted as a basis for the industrial fermentation of beer and other alcoholic beverages such as sake, wine, distilled spirit, etc. (Okafor, 1978). The equation for the conversion of sugar, the main substrate in wort, is usually written in a very simplified form thus:

$$C_6 H_{12} O_6 \rightarrow 2C_2 H_5 OH + 2CO_2$$

as against the complex and long equations of the Embdon-Mayerhof-Parnas (EMP) pathway.

2.4 Traditional Burukutu Production

The methods used in traditional burukutu production vary and depend on individual brewer and locality. Several investigators have reported different methods of producing the beer traditionally based on information

collected from individual brewer and localities. Basically, the method involves the steeping of sorghum grains in water, malting of the grains, mashing at various ratios of water and grits, wort-boiling and pitching. while some workers reported two stages of fermentation (Faparusi et al. 1973) which are the souring stage and the alcoholic stage, others reported only the alcoholic stage (Akinyemi, 1990). Overleaf is the flow sheet for traditional burukutu manufacture as prepared by a local brewer in Samaru, Zaria. (Personal Communication).

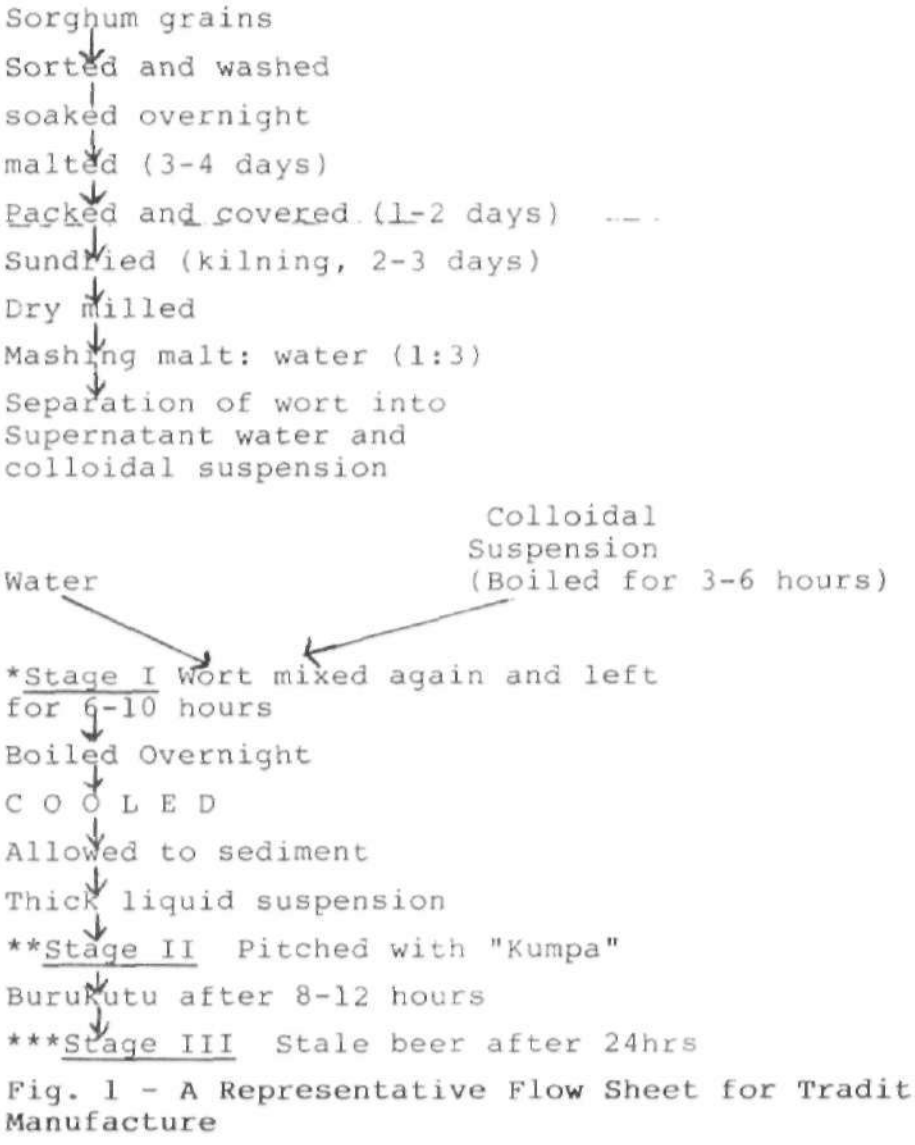


Fig. 1 - A Representative Flow Sheet for Traditional Burukutu Manufacture

2.5 Chemical Qualities of African Beer

Apart from the microbiological findings on African Traditional Alcoholic Beverages, considerable survey has also been directed towards unveiling their biochemical characteristics. Faparusi (1970) reported the presence of many sugars in burukutu beer such as Maltose (0.05%), fructose (0.2%), galactose (0.36%) galactoronic acid (0.48%) glucose (0.6%) and sucrose (0.2%).

Ogbonna et al (1983) in a chemical survey of burukutu beer reported the beverage to be of high nutritional value containing such chemicals as 52% sugar, 0.46% ash, 0.52% protein, 7.0µ/100 ml vitamin c and 3% alcohol.

The comparative studies by Onwuliri et al (1986) on burukutu revealed that burukutu is more acidic than pito with each averaging in percentage ethanol content of 5.25 and 3.62 respectively.

Ekundayo (1969) reported the presence of sugars, amino acids and organic acids in his chemical analysis of pito. He reported the percentage ethanol in Pito to be 3%.

Aucamp et al (1961) and Chevassus et al (1961) in a study of the chemical characteristic of opaque maize beer reported that the nutritional value of the opaque maize beer was supereior to that of clear lager beer due to high crude protein content, thiamine and riboflavin. In the same study, Aucamp et al. (1961) assessed the nutritional value of some Kaffir corn beer and reported that Kaffir beer is rich in protein, fat, fibre, thiamine, riboflavin, nicotinic acid and vitamin C. Also Nout (1980) reported that Busaa contains 0.5% - 1.0% lactic acid and 2-4% volumes of alcohol. These various chemical constituents found in the traditionally prepared alcoholic beverages in Africa confer on the products higher nutritional values over their lager beer counterparts and hence underscores their potential as sources of essential nutrients such as protein and vitamins.

2.6 Microbial Spoilage of Alcoholic Beverages

Spoilage of alcoholic beverages by micro-organisms has occurred since ancient times and has constituted a serious problem for mankind. However, it was not until about 1862 when the problem of spontaneous generation of life was at a heated debate that spoilage processes became linked with micro-organisms (Desrosier 1970). Incidentally, Louis Pasteur then became

interested in the problems of the great wine and beer industries of France which were threatened with ruin from disease and souring which were then believed to be due to the process of spontaneous generation of life in bottles and kegs (Desrosier, 1970). Pasteur later reported that he has found the cause of the disease of wine and beer to be a microscopic vegetation. Thus spoilage of wines and beer has been instrumental in the final nipping in the bud of the erroneous theory of spontanous generation of life.

In the traditional and modern brewing processes, several micro-organisms other than the desired ones required for fermentation frequently found their ways into beer and cause beer spoilage. In the lager beer industry, Gerherd (1975) reported the following microorganisms as the spoilage micro-organisms frequently encountered in the lager brewing industries: Lactobacilli, the wild yeasts (yeasts other than the desired fermentation yeasts); Acetic acid bacteria and Achromobacter anaerobium. He reported that the organisms may have entered the brewery from the air, water used in brewing, the dust of cereal, brewery workers, locations in the brewery which are hard to clean and contaminated pitching yeasts. Since these spoilage organisms can result in a great loss to the industry, it has become important for the

profit-oriented brewers to exercise preventive measures in the form of sanitary construction of brewery equipment, proper air and water treatment and treatment measures such as pasteurization, sterile filtration and radiation of beer to forestall any incidence of spoilage micro-organism (Gerherd, 1975).

In the traditional brewing processes, fermentation is uncontrolled and spontaneous; also the local brewers hardly observe any sanitary measure in the course of production. The high nutritional value of the traditional alcoholic beverages as earlier discussed makes the product a good medium for microbial growth. Hence, the presence of heavy suspension of micro-organisms in the final product (Okafor, 1975).

Nout (1980) reported that due to the use of rudimentary equipment and spontaneous fermentation method, the shelf life of busaa, a Kenyan opaque maize beer, cannot be extended beyond 24 hours at ambient temperature and consequently only small amounts of Busaa are brewed at a time and consumed the same day.

Okafor (1975) reported that the microorganisms in palm wine, an alcoholic beverage produced from the sap of various palms are alive and metabolize away the pleasant sugery taste of the fresh wine within 36-38 hours and produce various organic acids leading to the spoilage of the product.

In Kaffir beer, a traditional beverage of the Bantu people of South Africa, Van der Walt (1956) reported that within 1-8 days, the pleasant sour taste due to lactic acid becomes masked by a vinegary flavour due to acetic acid formation by acetic acid bacteria, rendering the beer unsaleable. He concluded that in breweries operating with primitive equipment, this spoilage assumes harzadous proportions.

Dirar (1983) reported that during the merissa stage in the fermentation of merissa, a Sudanese beer, there was an increase in the number of acetic acid bacteria, accompanied in the late stages of fermentation by an increase in the volatile acids. He presumed that the common spoilage of Merissa at the end of the day was due to the accumulation of volatile acids produced by acetic acid bacteria.

Akinyemi (1990) reported the presence of acetic acid bacteria in burukutu beer and associated the microorganism with the spoilage of the beer after 24 hours of its production. In the work, the presence of aflatoxin in the traditionally brewed burukutu beer was also reported.

It is clear from the foregoing that these spoilage hazards encountered in the traditional brewing processes necessitate the need for a sound preservative method for the beer

2.7 PRESERVATION

The ability to preserve food has been a useful art since the dawn of civilization. The preservation of food and beverages at times and locations of their abundance for later consumption throughout the world represents one of the most valuable contribution of science to mankind. Some of the methods of food preservation, however, date back to historical era, e.g. drying of fruit, meat etc. was commonly followed even by primitive man who hardly knew the cause of deterioration of the food. The real understanding of the role of microbes in food spoilage started with the demonstration of microbial putrification of organic matter by Pasteur in 1860 (Carl and Trout, 1968). Since the, several studies have been made on the role of microbes in the spoilage of preserved food material, the type of microorganism involved, the type of spoilage caused, method of improving the processing etc. The method of food preservation aims at keeping out microorganisms by maintaining unfavourable conditions for their growth either by the use of high temperature, low temperature, drying, irradiation or with chemical preservatives.

If left unpreserved, all foods will in no time go sour, rancid, putrid or in some other way, decompose and for some food such as milk and meat products the

time required for this to happen is short (Gordon 1966). Food contamination may arise through the processing of food under unhygienic condition, the use of poor quality raw materials or food handlers harbouring microorganisms. Also storage of processed food in poor packaging material, prolonged storage without good refrigeration facilities are some of the traditionally known sources of food spoilage.

To overcome food deterioation, measures have been taken since earlier times. Food had been preserved in wines (alcohol), in vinegar (acetic acid) and in solutions of common salt or sugar. It has also been protected from the atmospheric contamination by various forms of covering. The drying of fish and meat either in the sum or over fire, the use of spices are also some local methods of food preservation since antiquity (FAO/WHO 1967). Anonymous.

Omakwu (1980) studied the preservative effect of three spices, namely, ginger, tarmarid and black pepper in Kunun-tsamiya (a local cereal food drink) and found that the spices may have afflicted lethal or sub-lethal injury on the organisms by their constituent acids and oils, and that addition of the spices at the locally used concentration to known amount of Kunun tsamiya curtails if not eliminates any microbial health hazards.

Onuora et al (1987) determined the probable antimicrobial acitivities of four spices: Ginger, black pepper, red pepper and cloves, routinely added to kununzakin and found that cloves inhibit Staphylococcus aureus and Escherichia coli in Kunun-zaki.

Oruchie and Levi, (1957) made an attempt at preserving palm wine with a sulfite preservative. This attempt failed because no consideration was given to the effect of PH of the medium.

Faparusi (1969) treated palm wine with different concentrations of sulfite and found that the pH of the medium (palm wine) was about 4.0 to 5.0 when it is considered for drinking and that at this pH the concentration of sulfite required to suppress microbial activities would be excessive for human consumption.

Uwaifo and Basir(1982) preserved and bottled palm wine successfully with the chemical n-octyl-gallate.

Okafor et al (1982) preserved and bottled palm wine successfully using heat and chemical treatment methods. After a preliminary treatment of fresh palm wine samples with different concentrations of chemical preservatives and different intensities of heat, he found out that heat treatment of wine samples at 60°C for 30 minutes and addition of low concentrations of the various chemical preservatives (sorbic acid, sodium metabisulfite and diethyl pyrocarbonate) preserved thewine samples for months..

It must be stated that no attempt has been made anywhere before in literature to preserve burukutu beer.

2.7.1 Preservation by Heat Treatment

The heat treatment of food antedates recorded history. Since the discovery of fire, man used heat to prepare certain food, both to preserve them and enahance their flavour. Applying heat to liquid appears to be relatively recent practice. The Swedish scientist, Sheele in 1782 (Sommer, 1952) used heat in the preservation of Vinegar. Seventeen years prior to this (in 1765) Spallazani preserved meat extracts in sealed flasks by boiling for 1 hour (Sommer, 1952).

In 1824, William Deules, as Professor of Obstetrics at the University of Pennsylvania recommended heating milk to near boiling point and then cooling it for infant feeding (Carl and Trout 1968).

Nicholas Appert, a French man is credited with fathering the canning industry. In 1804 he published a treatise entitled 'The act of preserving animal and vegetable substances", a work that earned him a prize (Carl and Trout 1968).

Just over a century ago, Pasteur established that spoilage is caused by microorganisms and that heat will kill them. On that basis, he also showed that the shelf life of beer and vinegar could be extended

by heating them close to boiling temperatures. Today, pasteurization is used for a variety of reasons, (Carl and Trout 1968:

- to eliminate heat — sensitive pathogenic microorganisms
- to kill various organisms in order to clear the ground for an added starter organism.
- when a more severe heat treatment-sterilizzation would damage the product.
- to improve shelf-life when the surviving microorganisms can be controlled by some auxilliary procedures.

Thus the main object of pasteurization is the reduction and elimination of pathogenic and food spoilage organisms.

According to Douglas (1992), Pasteurization is a low-order, time- and temperature-dependent heating process that is designed to destroy all pathogens present in food, to reduce the bacterial load in the case of milk and egg, to reduce the yeast and mould count in the case of beer and wine and fruit juice, and to extend the shelf life. Heating is generally done below boiling point of water. The principles of Pasteurization were developed by and named after Louis Pasteur and his work on wine in 1860 in France.

There are two methods of Pasteurization, viz, the low temperature, long time method (LTLT) and the high temperature short time method (HTST) (Douglas 1992). In LTLT method, foods are pasteurized at low temperature for a longer time while in the high temperature short time (HTST) foods are exposed to heat treatment at higher temperature for a short time. These two methods find application in the milk industries where milk is pasteurised at 62.8°C for 30 minutes (low temperature long time method) and at 71.7°C for 15 seconds (High temperature short time method) (John and Robert, 1992).

In the lager beer industries, pasteurization has been developed by the brewer far more extensively than any other methods of biological stabilization. Except for some isolated cases, pasteurization is the only commercial method of beer preservation (Gerherd, 1975). The overwhelming majority of bottled and canned beer produced in most industries has been pasteurized. Pasteurization is normally done at 60°C for 15-20 minutes (Gerherd 1975).

2.7.2 Chemical Preservation

Chemical preservatives have been in use since historical times to preserve food (Douglas, 1992). Salt can be added to food for its contribution to the preservation of the food; for example, Fish and cured

meat products. The action of salt results from the osmotic pressure created in the aqueous environment surrounding the microbial cell, Pasmolysis, the partial dehydration of the cell results, and the viability of the microbe is thus destroyed. Smoke is also a type of chemical preservative that has been used since historical times to preserve foods especially meat and fish products (Douglas, 1992). The action of smoke results from the formation of small amounts of preservatives, such as phenols, formalin, cresols, etc, and the internal temperature and dehydration of the meat tissues associated with the hot-smoking process.

Chemical compounds identical with or different from those naturally occuring in food may be incorporated directly or indirectly during processing, packaging and storage of food (Markakis, 1970). Food additives are defined as the chemicals which are introduced into foods to improve nutritional value, enhance quality or consumer acceptability, improve keeping quality, make the food more readily available or facilitate its preparation for table use. Chemical preservatives find application in the improvement of keeping quality and is one of ways of protecting food from microbial deterioration. Besides, extending the periods of use of food, a chemical

preserative should be safe for human consumption, impart no undesirable organoleptic changes to the food, conceal not quality of the food, be economical and capable of being analysed (Markakis, 1970).

2.7.2.1 Sulfur dioxide

Sulfur dioxide (SO_2) is a colourless chemical with a characteristic choking smell and has a melting point of -75°C and a boiling point of -10°C (Faith et al, 1965).

The addition of sulfur dioxide or sulfurous acid salts to food and beverages for the purpose of preservation is known variously as sulfiting, sulfitization, sulfurization or sulfuring (Schroeter 1966).

Gaseous or liquified sulfur dioxide (SO_2), sulfites, bisulfites and metabisulfites are used to reduce or prevent spoilage by microorganisms and to selectively suppress the growth of undesirable organisms. (Markakis, 1970). According to food and drug administration (FDA) regulation, SO_2 and the sodium and potassium sulfites, bisulfites and metabisulfites are generally recognised as safe (GRAS) for use in food that are not major sources of thiamine since vitamin B_6 is destroyed by this compound.

Sulfur dioxide and its organic derivative finds extensive application in food processing and

preservation. Vegetable products and fruits have been preserved and pretreated by the use of sulphur dioxide and sulfite (Joslyn et al, 1954). Sodium metabisulfite has been described as the preferred source of sulfur dioxide for food processing application (Mansoto company 1964). Anonymous

Sulfur dioxide has found a wide application in the beer industry. Sulforous acid salts have been some of the chemicals normally used to reduce the dissolved oxygen content of beer which may cause oxidation of beer resulting in a variety of undesirable effects including significant changes in flavour (Schroeter 1966).

Schroeter (1966) proposed that sulphur dioxide (0.029%) should be added as an aqueous solution to malted mash before fermentation as beers brewed with malt containing sulfur dioxide appeared to give better foam stability. Addition of small amount of sulfurous acid salts to brewer's malt mash increase the solubility of malt proteins (Schroeter, 1966).

The acceptable daily intakes of sulfur dioxide for man is 0.35 mg/kg body weight (Mossel, 1971).

The inhibiting effect of sulfur dioxide may be due to its reaction with the Carbonyls of carbohydrate thus preventing their utilization as a source of energy or it may be due to the reduction of S-S linkages in

enzyme proteins, thus preventing the action of enzymes essential for cell metabolism (Joslyn and Braverman, 1954)

Rehm (1964) showed that Nicotinamide adenine dinucleatide (NAD)-dependent factors concerned with the metabolism of certain carbohydrates by some yeasts and bacteria are inhibited by sulfurous acid probably due to the formation of addition compounds between the two components. The antimicrobial action of sulfurous acid has been found to decrease by the formation of sulfonates with carbonyls in foods (Rehm, 1964). However, carbonyl addition compounds formed with acataldehyde, pyruvate, ketoglutarate and acetone have an inhibitory effect on yeasts.

2.7.2.2 Sorbic acid

Sorbic acid (CH_3-CH-CH-CH-CH-COOH), Trans-2,4hexadianoic acid, is a white crystalline solid with mildly acrid odour (Fenema, 1976). It has a melting point of 133°C and a molecular weight of 112.13 and its dissociation constant is $8.3x10^6$ (Fox and Cameron 1977)

Sorbic acid was first discovered in 1859 by A. W. Hofman from mountain Ash berries, the fruit of the rowanberry tree (Sorbus aucuparia) and the antimicrobial properties discovered in 1939 by Emuller and in 1940 by C.M. Gooding (Elmer, 1974).

sorbic acid is employed as food preservatives usually in form of its sodium, calcium or potassium

salts. These compounds are permitted in foods at levels not exceeding 0.2% (Smith et al, 1962). The acceptable daily intake of sobic acid for man is 12.5 mg/kg body weight (Mossel, 1971).

Sorbic acid acts primarily against fungi (yeasts and moulds) but the growth of certain bacteria such as Clostridium species are also inhibited. Against Lactobiacilli and Clostridia sorbic acid is rather inactive. Emard and Vaughn (1952). However, its potassium salt may inhibit the growth of catalase-positive bacteria.

Sorbic acid is used in a wide variety of food such as cheese, baked product, fruit juice and pickles. The method of application involves direct incorporation, surface coating or incorporation in wrapping materials (Fennema, 1976).

The antimicrobial activity of sorbic acid depends on pH because only the undissociated acid is fungistically active. Sorbic acid is effective up to a pH limit of 6.5 (Chiechester and Tanner 1968). The percentage of undissociated acid varies from 98% at pH 3 to 0.6% at pH 7. (FAO/WHO 1967).

Sorbic acid has been allowed to be used as food preservative on the basis that they are metabolized by the human in the same manner as fatty acids, the end products being carbon dioxide and water (Melnick et al, 1954).

It is reported that the antimicrobial action of sorbic acid is selective and that growth of undesirable organisms is suppressed without interferring with those that are beneficial. Example is in cheese production where mould growth is controlled without interferring with the microorganisms necessary for the maturing process (Fox et al, 1977)

Wine with residual content of sugar is becoming increasingly popular (Elmer 1974). To inhibit after fermentation, potassium sorbate can be added in concentrations of 200-400 ppm depending on the alcoholic content (Elmer, 1974).

Bell et al. (1959) studied the effect of sorbic acid on 66 species of moulds, 32 yeasts and 6 strains of lactic acid bacteria and reported that all grew in the presence of 0.1% sorbic acid at pH 7.0. The moulds and yeasts were inhibited when the pH was lowered to 3.5.

Fresce et al. (1973) reported that sorbic acid and its salts prevent microbial growth by inhibiting cellular uptake of substrate molecules such as amino acids, phosphates and organic acids.

Melnick et al, (1954) reported that sorbic acid inhibit moulds by suppression of the acitivty of dehydrogenase enzymes. Clostridia have an abundance of dehydrogenase enzymes and thus may be the reason

why these compounds are not effective in inhibiting this type of organisms (Melnick et al, 1954).

it is also stated (Melnich et al, 1954) that sorbic acid is not effective when added to materials containing high concentration of microorganisms. In microorganisms on which sorbic acid has an inhibitory effect, this compound has been found to allow germination, shedding of the spore wall and some elongation of the vegetative cell but prevents division of the vegetative cell (Gould, 1964)

2.7.2.3 Benzoic Acid

Benzoic Acid is a white crystalline solid which sublimes when heated and is slightly soluble in cold water and readily soluble in hot water and sometimes organic compounds such as ether, chloroform, alcohol, benzene, carbon bisulfite and turpentine (Holmy 1954). Denzoic acid melt at 122.38°C and boils at 249.2°C and it has molecular weight of 122.2 (Faith et al 1965).

Benzoic acid (C_6H_5 COOH) and its sodium salts, benzoate and soda have been widely employed as antimicrobial agents in foods. Benzoic acid occurs naturally in cranberries, prunes, cinnamon and cloves (Wyss 1948).

Benzoic acid has been found to cause no deleterious effect when used in small amounts. It is readily eliminated from the body via urine after conjugation

with glycine to form hyppuric acid (benzoyl glycine) (FAO/WHO 1967).Anonymous

The effectiveness of benzoic acid as a preservative is higher at lower pH levels indicating that the undissociated acid may be the most active form. Hence it is suitable for use in acid foods such as apple cider, fountain syrups, margarine and pickle relishes (Desrosier, 1970).

Benzoic acid is allowed in foods usually in concentrations ranging between 0.1% and 0.4%, except in some special cases where higher concentrations are permitted (Heiman, 1980). The acceptable maximum daily intake of benzoic acid is 5 mg/kg body weight (Mossel, 1971).

Bosund, (1962) indicated that the benzoates and related compounds inhibit the growth of microorganisms by in some way, interferring with the utilization of acetate required for the formation of energy-rich compounds which are necessary for cell metabolism.

The similarity in antimicrobial activity between benzoic acid and salycylic acid has been shown (Bosund 1962). Both compounds were taken up by respiring microbial cells and both compounds were found to block the oxidation of glucose and pyruvate to acetate in Proteus vulgaris. Also benzoic acid has been reported to cause an increase in the rate of glucose

oxidation in Proteus vulgaris (Bosund 1962).

Gould (1964) in an investiageion of the inhibitory effect of Sodium benzoate on spore-forming bacteria found that if the phases of growth of bacteria spores are considered to be: (1) germination (loss of refractility under phase microscope, increase in heat sensitivity etc.) (2) rupture and shedding of the spore wall; (3) enlargement and outgrowth into vegetative cell and (4) cell division or multiplication, benzoates allowed stages <1> and <2> to take place but prevented stages <3> and <4> or prevent elongation of the vegetative cells and multiplication.

Fresce et al (1973) showed that the benzoate, like propionate and sorbate act against microorganisms by inhibiting the cellular uptake of substrate molecules.

The toxicity of benzoic acid involves inhibition of pepsin digestion gastro intestinal disturbances and allergic responses (FAO/WHO 1967). Anonymous

CHAPTER THREE

MATERIALS AND METHODS

3.1 SAMPLING

With close participation, arrangements were made with an experienced burukutu beer brewer in Samaru, Zaria to prepare burukutu for this work. At the start of each stage of fermentation, about 1 litre of the samples were rushed within five to ten minutes to the laboratory in a sterilized 1 litre beaker covered with Aluminium foil. Samples were collected at the following stages:

Stage I where wort was mixed with supernatant after boiling and left for about 6-10 hours (*in Figure I)

Stage II where 'Kumpa' was added (** in Figure I)

Stage III after 24 hours of Kumpa addition.(***in Figure I

The beaker and its contents were incubated at room temperature. Samples were collected at 3 hour-intervals for Stage I and 4 hour-intervals for Stage II and introduced into small sterile bottles and kept in a refrigerator. Samples were analysed for their microflora, ethanol contents, PH, titratable acidity and volatile acid contents within 24 hours of sampling. As for the preservation experiments fresh burukutu

samples were collected and were immediately given various preservative treatments. The preserved samples were monitored weekly to detect physical and microfloral changes. At the end of the preservation, samples were analysed for their ethanol contents, PH, titratable acidity and volatile acid contents.according to Dirar (1978) and Okafor (1975)

3.2 MEDIA

Selective media were used to count the different microbial groups. Yeasts were counted on malt extract yeast extract agar acidified with lactic acid (PH4) Moulds were counted on potato dextrose/yeast extract agar acidified with lactic acid (PH4). Lactic acid bacteria were counted on a Sorghum extract/yeast extract agar acidified with lactic acid (pH 5.4) containing 2000 ppm sorbic acid and 25 ppm fungizone Acetic acid bacteria were counted on a sorghum malt extract/yeast extract agar acidified with lactic acid (pH 5.4) containing 3% ethyl alcohol and 25 ppm fungizone. Total aerobic counts were also determined by counting organisms on plate count agar (PCA). Counts were also made on 1% yeast extract, 1% glucose, 1% calcium carbonate agar (YGC) plates for determination of total anaerobic count. Rogosa agar was also used for the determination of lactic acid bacteria count. Plates for counting yeasts, moulds and acetic acid bacteria and aerobic bacteria were incubated aerobically

at room temperature, while plates for counting lactic acid bacteria and total anaerobic counts were incubated anaerobically using candle jars at room temperature.

Where needed, 2% agar was added to media. Media pH was adjusted (by addition of lactic acid) after autoclaving for 15 minutes at 15lb pressure. The spread plate method was used during inoculation whereby 0.1 ml of suitable dilutions of samples were aseptically spread on the surface of an agar plate. Counts were made 48hours after incubation. Where there were no growthsafter 48 hours, plates were incubated further. All counts were determined per millilitre of material. (Dirar, 1978; Nout, 1980).

Sorghum malt extract was prepared by steeping sorghum grains in water, allowing it to germinate and drying (kilning). The grains (malt) were then milled and the grits so formed extracted with warm liquor to produce wort. The wort was filtered and evaporated to a thick syrup. Sorghum extract was prepared in the same way except that there was no germination stage. (Personal communication).

3.3 PHYSICO-CHEMICAL ANALYSIS

3.3.1 Ethanol Determination - the percentage ethanol determinationwas done by chemical oxidation method of Caputi and Wright (1969). This was carried out using microkjeldahl apparatus. 1ml of burukutu beer sample was pipetted into the flask and distilled into acidified

solution of potassium dichromate. The resulting solution was heated for 20 minutes at 60°C to complete the oxidation of the ethanol to acetic acid. This was titrated with acidified ferrous ammonium sulfate solution using phenanthroline as indicator. Blank titration was performed. The result was expressed as percentage ethanol by volume using the following expression:

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

3.3.2 Titratable Acidity (TA) - Titratable acidity was determined by titrating 10ml of beer sample with 0.1N sodium hydroxide (NaOH) solution to the phenophtalein end-point. Total acid was expressed as percentage lactic acid by using the following expression:

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

Steam volatile acid was determined by titrating 20 ml of sample collected as distillate from 1g of sample and expressed as percentage acetic acid (Dirar, 1978).

3.3.3 PH - PH was determined by shaking 15ml of the beer sample in a beaker and reading off the pH using caliberated Pye model 79 PH metre.

3.4 Preservation

Three methods of preservation were used:

3.4.1 Pasteurization - Fresh burukutu samples were heated in a water bath in a sterile medical bottles. Three minutes were allowed at the initial stage enable the beer sample to attain the required temperature. Pasteurization was then carried out at 60°C for 30 minutes. After treatment, the bottles were kept at room temperature and the effectiveness of the preservation was monitored at weekly intervals by determining changes in colour, odour and micorbial load. At the end of the storage the physio-chemical characteristics (as in 3.3) of the preserved beer were determined according to Okafor, (1975)

3.4.2 Chemical Preservatives - The chemical preservatives used in this experiment were benzoic acid, sorbic acid and sodium metabisulfite.

3.4.2.1 Sorbic Acid - A stock solution of 1% sorbic acid was prepared by dissolving 1g of sorbic acid crystals in 100 ml of distilled water. This was then boiled in a water bath for 5 minutes for the crystals to dissolve completely after which it was sterilized by autoclaving at 121°C for 15 minutes at 15lb pressure. Different concentrations of sorbic acid (0.25%, 0.5% and 1%) were added to fresh burukutu samples in a sterile medical bottles and kept at room temperature for 24 hours for a preliminary experiment, after which the effectiveness of the preservation were then monitored weekly by detecting changes in colour, odour and microbial load. At the end of the storage, the physico-chemical

Prior to the tasting procedure, a form was prepared (Appendix I). The form was designed based on the outlined proposed quality of good burukutu beer (Personal Communication) from both consumers and producers. It contains seven attributes that denotes characteristics of good and high quality burukutu. A four-structure descriptive scale was used. A score of four was allocated to the best attribute while the least score which is one was allocated to the worst or poorest attribute. Each participant was served with 30ml of each preserved sample using equal sized calabash that is normally used to serve consumers by the local brewers.

Tasting Procedure - the preserved burukutu beer samples were labelled A-G and the corresponding bottles noted. The judges were allowed to sit randomly and the 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 instruction on how to fill the forms were given. All samples were arranged in a row and the judges asked to taste one at a time starting from A-G. The judges were also provided with warm water to rinse their mouth after tasting each sample. Spaces were provided for comments in the form.

C H A P T E R F O U R

RESULTS

The microbial population of supernatant (water removed from wort after mashing) is shown in Table I. The different groups of microorganisms counted were predominated by the moulds followed by aerobic bacteria, yeasts and anaerobic bacteria in that order. Lactic acid bacteria were not detected.

Table 2 shows variation in microbial population and physio chemical characteristis at the stage where boiled wort was mixed with the supernatant and left for about 6-10 hours. Mild microbial activities indicated by slight increase in microbial counts with corresponding decrease in pH and titratable acidity was observed. At this stage, there was a very low change in titratable acidity and hence lactic acid bacteria were not present at this stage.

High level of microbial activities were observed to take place at the stage of addition of "Kumpa" (Table 3). There was a high increase in the yeast population with corresponding increase in percent alcoholic content. After the 16th hour of fermentation, the population of yeasts started to decline but the percentage alcoholic content continued to rise culminating in 3.45 at the 36th hour. The population

TABLE I - MICROBIAL POPULATION OF SUPERNATANT

ORGANISM	VIABLE COUNT PER ML (AVERAGE)*
Yeasts	3.0×10^4
Moulds	7.2×10^5
Aerobic Bacterial	4.4×10^5
Anaerobic Bacteria	2.0×10^3

*Each Figure is an average of 3 different counts

TABLE 2: VARIATION IN MICROBIAL POPULATION AND PHYSIO CHEMICAL CHARACTERISTICS AFTER MIXING WORT WITH SUPERNATANT

Fermentation Time (Hours)	PH	% Titratable Acidity	Yeasts	Moulds	Bacteria Aerobic	Bacteria Anaerobic
3	5.75	0.06	$3.6x10^3$	$6.0x10^4$	$3.6x10^3$	$1.8x10^{3*}$
6	5.20	0.20	$5.8x10^3$	$7.1x10^4$	$5.3x10^3$	$2.2x10^3$
9	4.90	0.37	$6.7x10^4$	$9.0x10^4$	$6.9x10^3$	$3.4x10^3$
12	4.70	0.48	$9.2x10^3$	$2.8x10^5$	$7.2x10^3$	$5.2x10^3$
15	4.30	0.52	$2.1x10^4$	$3.7x10^5$	$1.1x10^4$	$7.8x10^3$

*Each figure is an average of 3 different readings

TABLE 4: EFFECTS OF VARIOUS CONCENTRATIONS OF HEAT TREATMENT AND CHEMICAL PRESERVATIVES ON FRESH BURUKUTU AFTER 24 HOURS

Treatment	% of Concentration	Yeasts	Moulds	Bacteria	
				Aerobic	Anaerobic
Untreated	-	$2.6x10^7$	$1.2x10^4$	$1.5x10^5$	$1.8x10^3$
S.A.	0.25	$1.8x10^4$	$0.5x10^2$	$2.8x10^3$	$1.2x10^3$
B.A.	"	$2.4x10^4$	$0.6x10^2$	$2.3x10^4$	$0.9x10^3$
S.M.	"	$0.2x10^2$	$0.9x10^2$	$2.6x10^3$	$0.5x10^3$
S.A.	0.5	$1.3x10^3$	$0.3x10^2$	$2.3x10^3$	$1.1x10^3$
B.A.	"	$1.8x10^3$	$0.5x10^2$	$2.4x10^3$	$0.6x10^3$
S.M.	"	0	$0.6x10^2$	$2.1x10^2$	$0.2x10^3$
S.A.	1.0	$1.2x10^2$	$0.1x10^2$	$1.5x10^2$	$0.2x10^3$
B.A.	"	$1.3x10^2$	$0.2.10^2$	$1.6x10^2$	$0.4x10^2$
S.M.	"	0	$0.4x10^2$	$1.3x10^2$	$0.5x10^2$
Heat	60^oC for 30min.	0	$1.8x10^2$	$1.1x10^2$	$1.1x10^2$

KEY: S.A = Sorbic Acid
B.A = Benzoic Acid
S.M = Sodium Metabisulfite

*Each figure is an average of 3 different readings

TABLE 3: VARIATION IN MICROBIAL POPULATION AND PHYSICOCHEMICAL CHARACTERISTICS OF FERMENTING BURUKUTU

Fermentation Time (hr)	PH	%TA	% of VA	% Alcoholic Content	Yeasts	Moulds	Bacteria Acetic Acid	Anaeto
4	4.95	0.07	0.02	1.5	3.5×10^{6}	0.2×10^{2}	0.05×10^{2}	2.5x10
8	4.62	0.10	0.05	1.65	7.2×10^{7}	0.6×10^{2}	0.09×10^{2}	2.8x1
12	4.32	0.25	0.09	1.75	7.6×10^{7}	3.2×10^{2}	1.1×10^{2}	5.5x1
16	3.91	0.39	0.22	2.50	1.6×10^{8}	5.2×10^{2}	1.6×10^{2}	1.5x1
20	3.82	0.52	0.38	2.98	2.8×10^{8}	9.5×10^{2}	1.6×10^{3}	2.6x1
24	3.73	0.91	0.41	3.2	4.0×10^{6}	2.7×10^{3}	1.8×10^{4}	3.5x1
36	3.51	1.02	0.65	3.45	9.6×10^{5}	1.3×10^{4}	2.3×10^{5}	2.1x1

KEY: TA = titratable acidity
VA = volatile acid

*Each figure is an average of 3 different readings

of moulds decreased as compared to Table 2 and later started to increase while fermentation lasted. The acetic acid bacterial population increased tremendously starting from the 20th hour also with a corresponding rise in percent volatile acid content. There was also an initial decrease in anaerobic bacteria followed by a gradual increase. The pH and titratable acidity at this stage gradually decreased and increased respectively.

Table 4 shows the effects of the preliminary heat treatment of fresh burukutu beer samples.

TABLE 5: ORGANOLEPTIC SCORES OF FRESH BURUKUTU, PRESERVED BY HEAT AND CHEMICAL PRESERVATION METHODS

	Sweetness	Sourness	Flavour	Colour	Bitterness	Odour	Consistency	Score
Fresh Burukutu	3.8	3.9	3.0	4.0	3.9	3.6	3.9	26.10
Untreated (Control)	0.9	2.3	0.9	1.1	2.3	1.5	0.8	9.90
1% Benzoic Acid (4 weeks)	2.5	3.8	2.9	3.5	3.8	2.1	3.5	21.80
1% Sorbic Acid (5 weeks)	2.6	3.8	2.8	3.4	3.8	2.3	3.6	22.00
% Sodium Metabisulfite (7 weeks)	2.5	3.8	2.9	4.0	3.8	2.3	3.6	22.90
Pasteurization at 60° for 30 minutes (2 weeks)	2.5	3.8	2.8	3.8	3.8	2.8	3.5	22.90
Pasteurization + 0.25% Benzoic Acid (8 weeks)	2.4	3.8	2.9	3.8	3.8	3.0	3.6	23.30
Pasteurization + 0.25% Sorbic Acid (10 weeks)	2.6	3.9	2.8	3.8	3.9	3.0	3.7	23.70
Pasteurization + 0.25% Sodium Metabisulfite (12 weeks)	3.4	3.9	2.9	4.0	3.9	3.0	3.8	24.80

*Each value is an average of 3 different scores

and chemical preservatives for 24 hours. The highest concentrations of the three chemical preservatives used yielded the best results in terms of reduction in the number of the various groups of microorganisms present with sodium metabisulfite being the best followed by sorbic acid and then benzoic acid. The low temperature long time heat treatment (60°C for 30 minutes) used reduced markedly the numbers of microorganisms present in the beer.

Figs 2-8 show the results of various treatment of fresh burukutu beer monitored at weekly intervals. Fig 2 shows that post pasteurization storage of fresh burukutu allows steady and gradual rise in microbial activities. All yeasts were inhibited while mould population steadily increased. Aerobic bacterial population decreased in the 1st week and later increased alongside anaerobic bacterial population. After the 3rd week of storage there was colour change from brown to light brown while the 'Kumpa'like odour gradually diminished indicating the beginning of spoilage.

Fig 3 shows 1% benzoic acid suppressed microbial activities up to 3 weeks after which microbial activities resumed at the 4th week indicating the beginning of spoilage.

Fig 4 depicts the inhibition of microbial growth of fresh burukutu beer treated with 1% sorbic

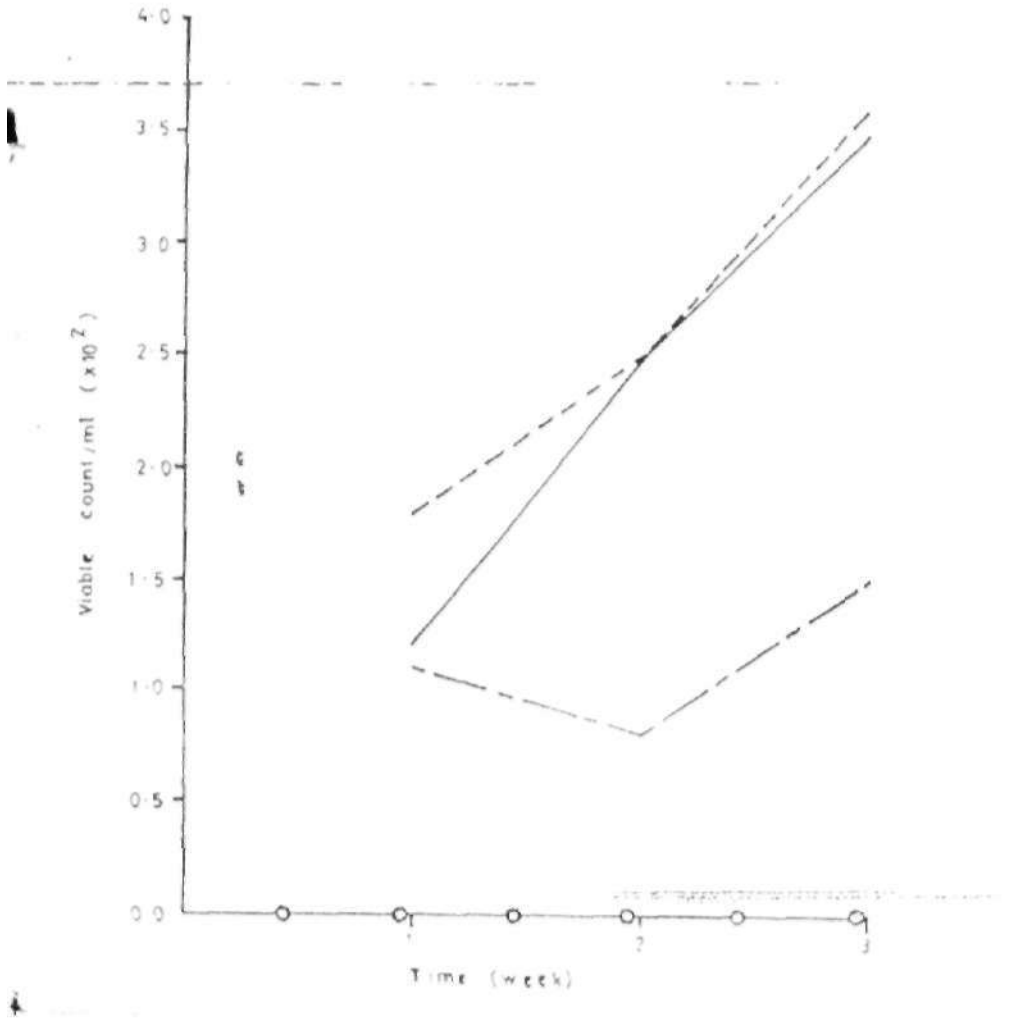


Fig. 2: Effect of Pasteurization of Burukutu Beer at 60°C for 30 Minutes (Store for 3 Weeks) on Microbial Counts

Key -------- moulds
— — — aerobic bacteria
_____ anaerobic bacteria

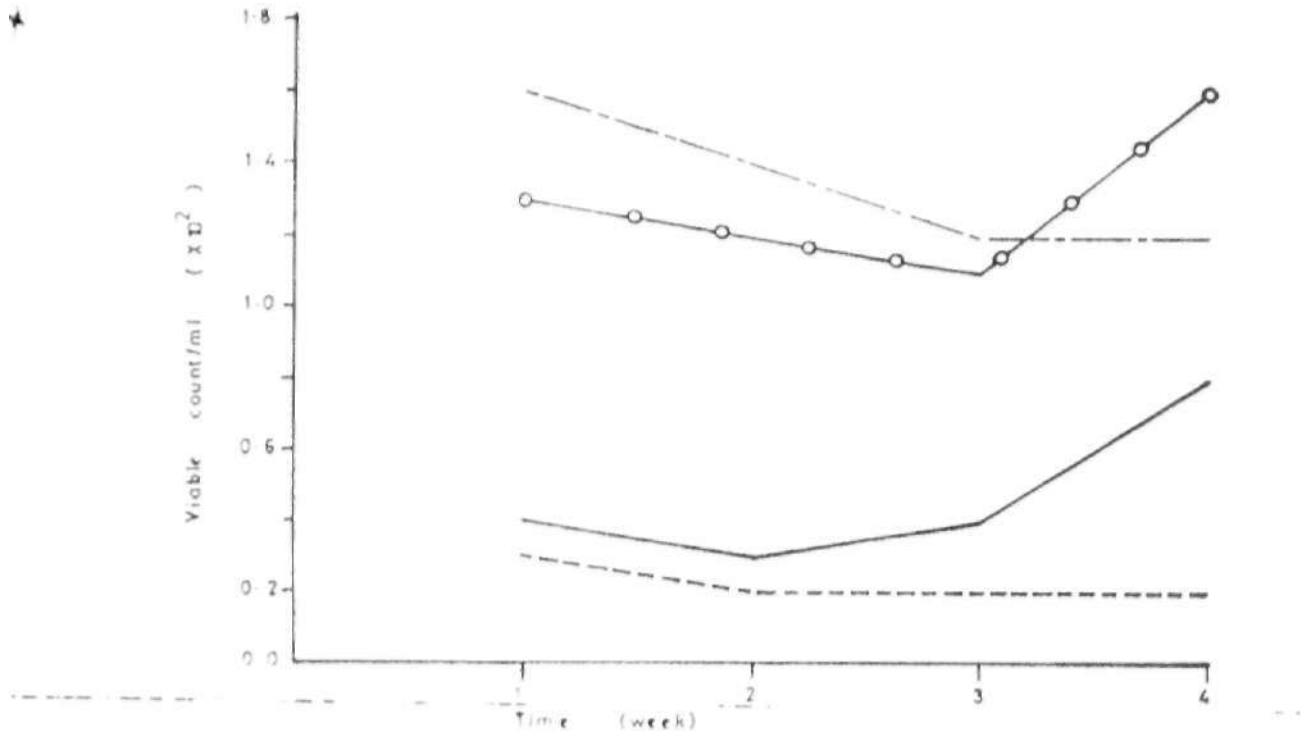


Fig. 3: Effects of 1% benzoic acid on microbial counts of burukutu stored for 4 weeks

Key o-o--o yeast
------ moulds
—— —— aerobic bacteria
———— anaerobic bacteria

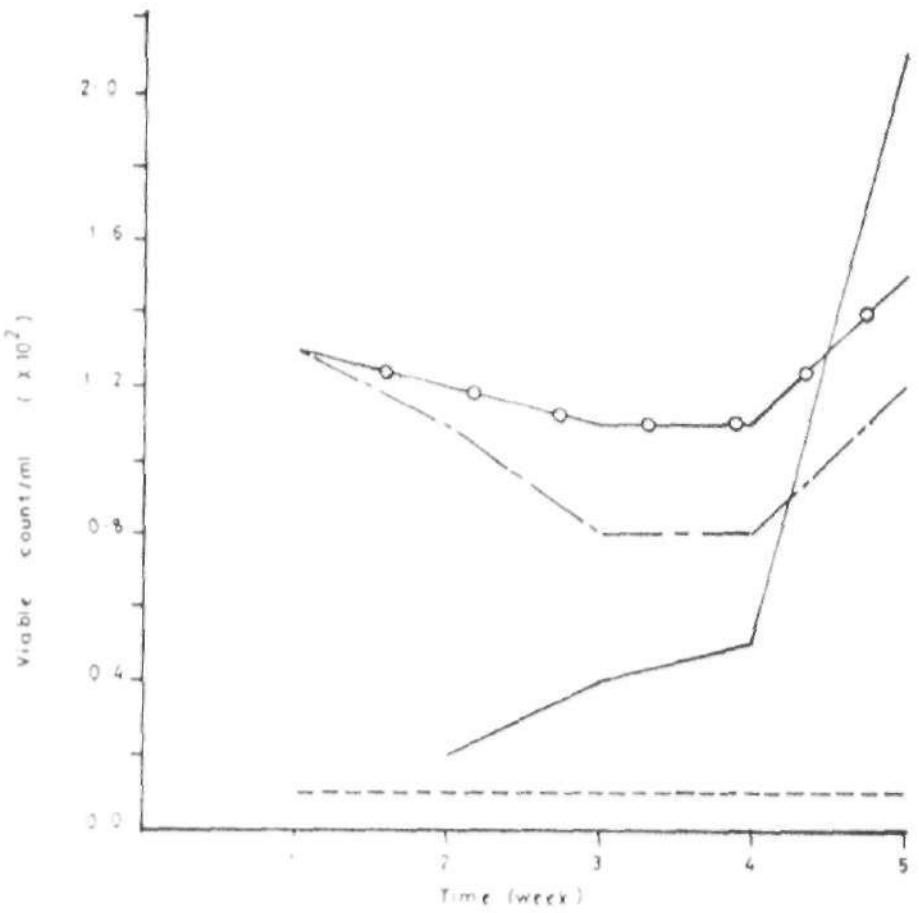


Fig 4: Effect of 1% sorbic acid on microbial counts of burukutu stored for 5 weeks

Key o-o-o-o- yeast
-------- moulds
___ _ ___ aerobic bacteria
_____ anaerobic bacteria

acid for 4 weeks. Microbial growth resumed on the 5th week as shown by increase in yeasts and anaerobic counts.

In Fig 5, 1% sodium metabisulfite completely inhibited yeasts population of burukutu and inhibited microbial activites for 6-weeks after which activities resumed indicating the beginning of spoilage.

Combined effects of heat plus the lowest concentration of each chemical preservatives [GRAS (p.31)] (0.25%) on the keeping quality of fresh burukutu are shown in Figs 6-8. Heat plus 0.25% benzoic acid inhibited microbial activites for 7 weeks (Fig.6) while heat plus 0.25% sorbic acid inhibted microbial activites for 9 weeks (Fig 7). Figure 8 shows the combined effect of pasteurization and 0.25% sodium metabisulfite on microbial counts of burukutu beer. Microbial activities were inhibited for 11 weeks with resumption of activities on the 12th week. In all the preservation experiments, colour, pH and odour changes were noted after the resumption of microbial activities.

The comparative organoleptic scores of preserved burukutu beer, fresh burukutu and untreated (control) ones are shown in Table 5. Fresh burukutu emerged with the highest score of 26.10 out of a maximum of 28 while untreated (control) sample emerged with the lowest score. Out of the treated burukutu samples, combined heat treatment plus 0.25% sodium metabisulfite

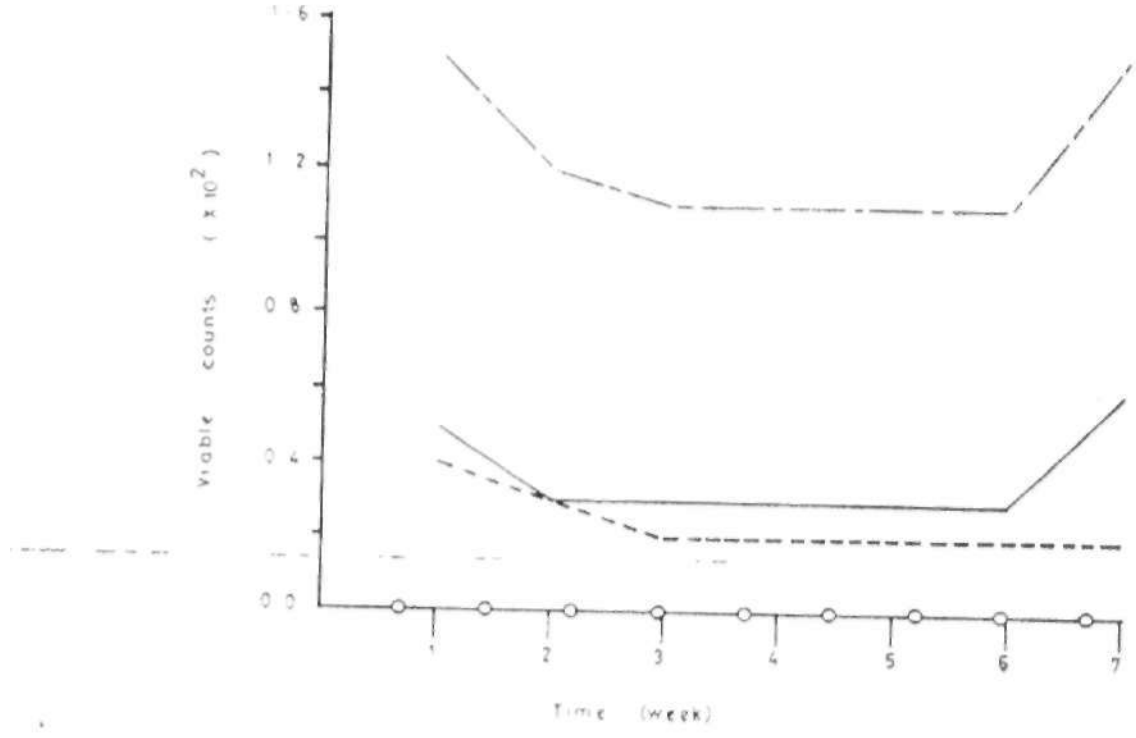


Fig. 5 : Effects of 1% sodium metabisulfite on microbial counts of burukutu stored for 7 weeks

Key
- o-o-o-o yeast
- ------- moulds
- —— – —— aerobic bacteria
- ———— anaerobic bacteria

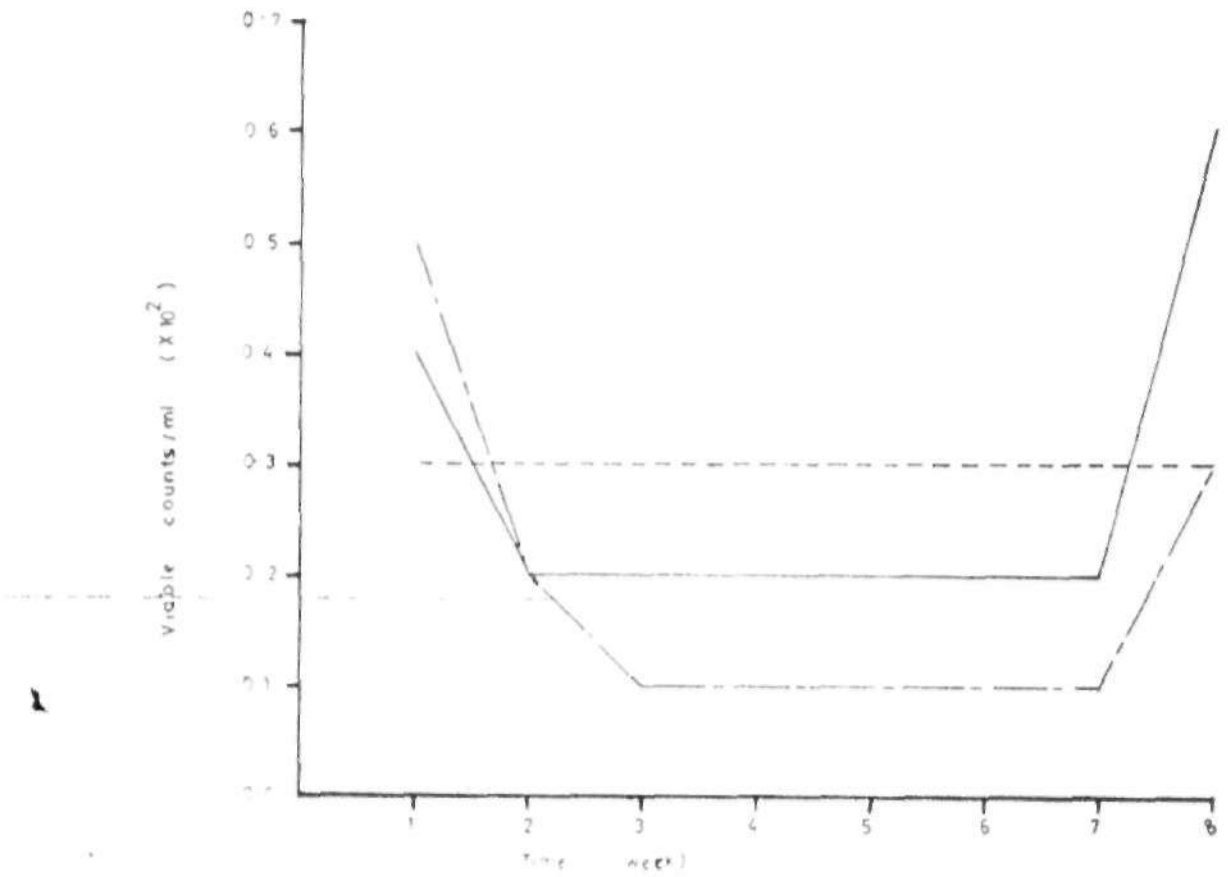


Fig 6: Combined effects of pasteurization (60°C. for 30 minutes) and 0.25 benzoic acid on microbial counts of burukutu stored for 8weeks.

Key ------- moulds
— - — aerobic bacteria
——— anaerobic bacteria

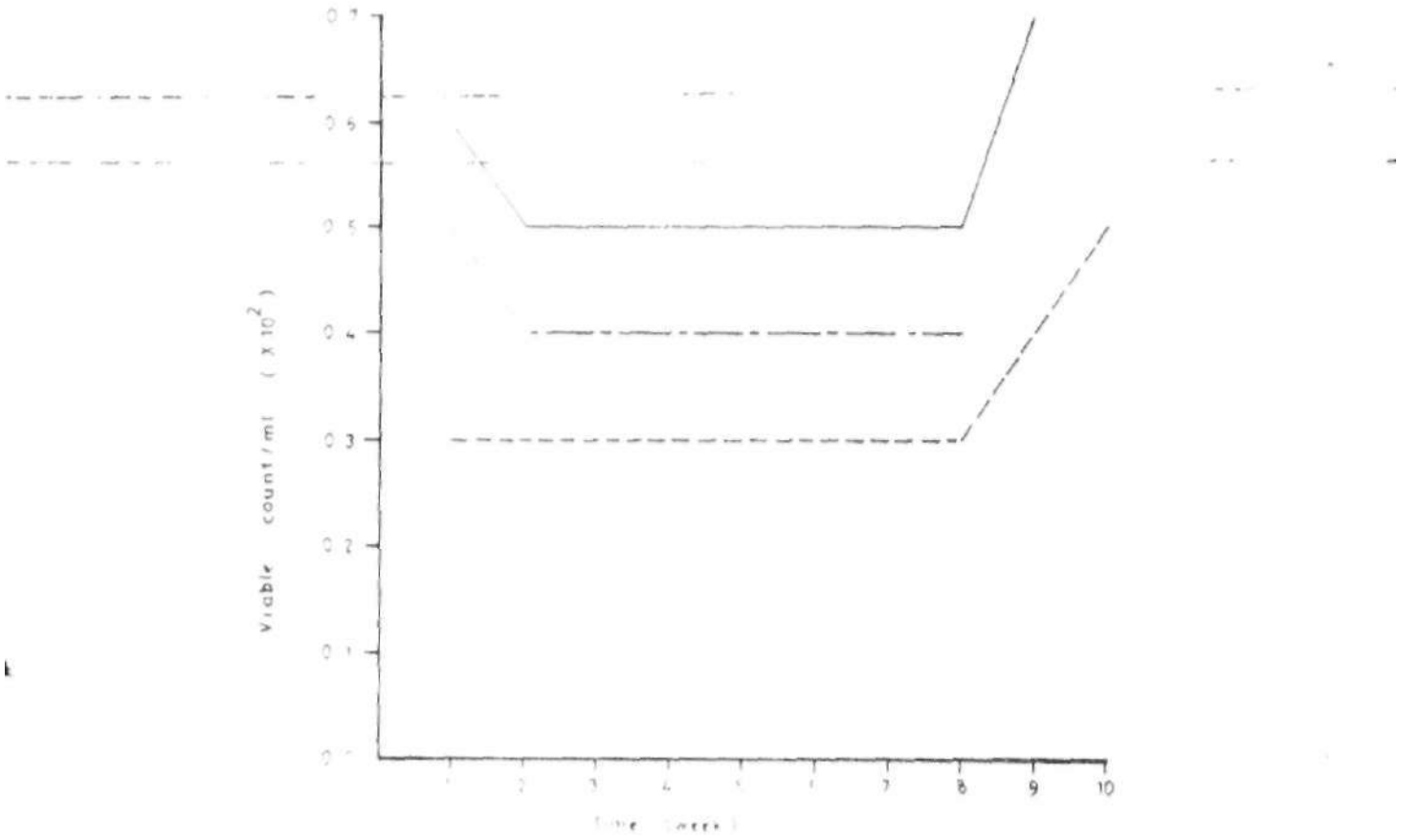


Fig. 7: Combined effect of pasteurization (60°C for 30 minutes) and 0.25% sorbic acid on microbial counts of burukutu stored for 10weeks

Key ------- moulds
— – — aerobic bacteria
———— anaerobic bacteria

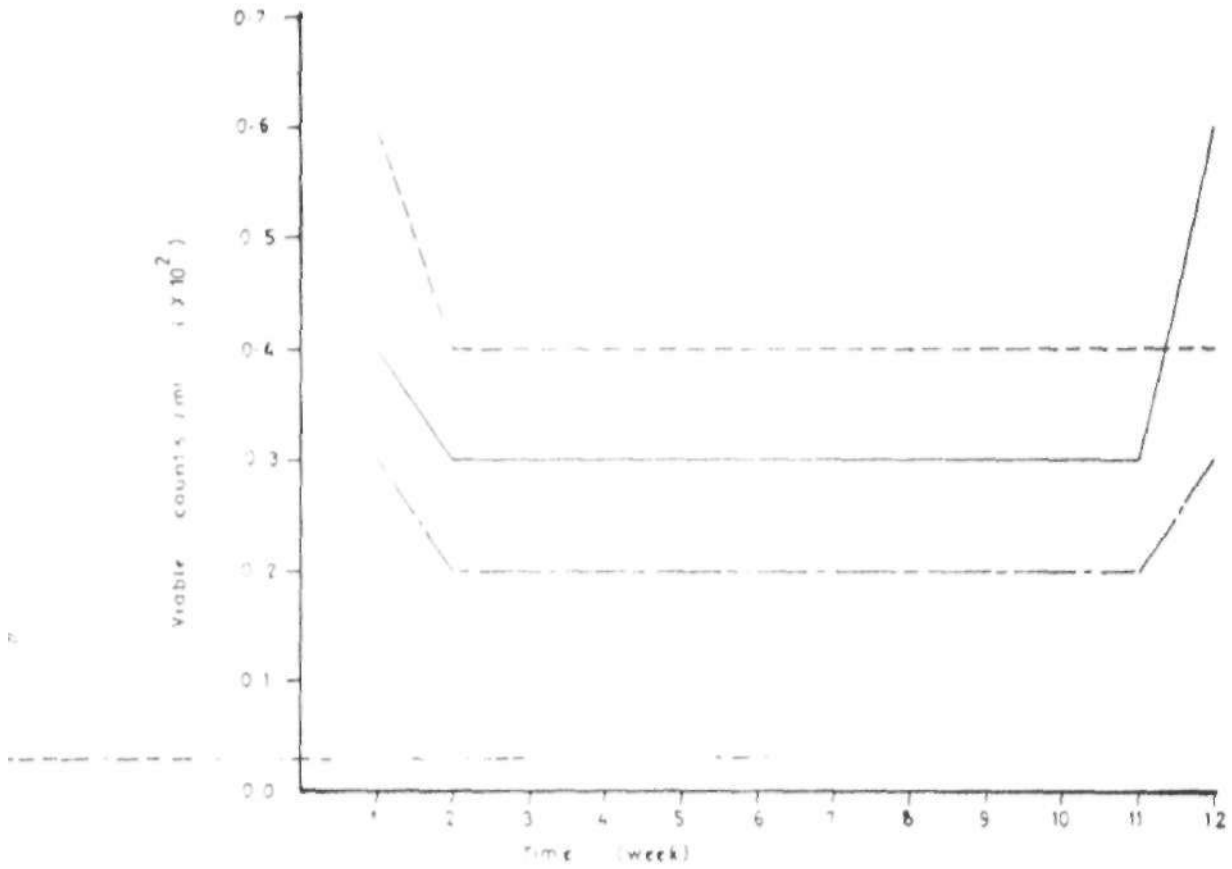


Fig. 8: Combined effect of pasteurization metabisulfate on microbial counts of burukutu stored for 12 weeks

Key ------- moulds
— - — aerobic bacteria
——— anaerobic bacteria

emerged with the highest scores followed by heat plus 0.25% sorbic acid and then heat + 0.25% benzoic acid. Treatments with 1% sodium metabisulfite, 1% sorbic acid, 1% benzoic acid and heat treatment at 60°C for 30 minutes followed respectively in that order. From the organoleptic tests, it is deduced that treated burukutu samples do not only keep longer than fresh ones, but compared favourably with fresh burukutu in terms of good quality attributes.

Table 6 shows the physicochemical characteristics of preserved burukutu beer. It shows that preserved burukutu beer still retained most of its chemical characteristics it possessed when fresh.

TABLE 6: PHYSIOCHEMICAL CHARACTERISTICS OF PRESERVED BURUKUTU BEER

Treatment	Period	% T.A.	%V.A.	% A/C	PH
1% Bensoic Acid	4wks	0.38	0.02	2.94	3.90
1% Sorbic Acid	5wks	0.39	0.03	2.97	3.7
1% Sodium Metabisulfite	7wks	0.38	0.03	2.98	3.8
0.25% Benzoic Acid + Heat (60°C for 30 Min)	8wks	0.40	0.05	2.98	3.8
0.25% Sorbic Acid Heat (60°C for 30 Min.)	10wks	0.42	0.02	2.96	3.7
0.25% Sodium Metabisulfite and Heat (60° for 30min.)	12wks	0.39	0.01	2.97	3.8
Heat (60°C for 30min)	2wks	0.36	0.02	2.94	3.9

KEY -TA - titratable acidity
VA - volatile acid
A/C -Alcoholic content

*Each value is an average of three different readings

C H A P T E R F I V E

DISCUSSION

The microflora of the supernatant is diversified: consisting of yeasts, moulds, aerobic and anaerobic bacteria (Table I). The source of these microorganisms could be the water used for mashing, aerial mycelia and some normal flora on the grains used for brewing (Ogbonna et al, 1983).

The supernatant microflora later forms the bulk of the microflora of the wort at the next stage when the supernatant is mixed with the boiled wort and left for about 6-10 hours. At this stage, there is low level of microbial activity. The yeast flora at this stage plays no active fermentative role and is more likely to constitute wild yeast spoilage of the burukutu beer. The mould flora also seems to have no positive function except to constitute spoilage and poisoning hazards (Akinyemi 1990). However, they (moulds) could have been partly responsible for the diastatic activities on grains thereby aiding in maliting processes since they are found to produce amylase (Akinyemi, 1990, Van der Walt, 1956). No lactic acid bacteria were encountered at this stage indicating that this stage is not a lactic acid fermentation stage. This is further indicated by the low level of titratable

acidity and hence lactic acid. Also the low count of yeasts shows that this stage is not an alcoholic fermentation stage. At this stage, the wort is very mildly sour and rather sweet in taste

The lack of lactic acid fermentation stage could be attributed to the absence of souring stage and the non-use of Garri adjunct for the production of burukutu in Zaria as against what obtains in Southern part of Nigeria where long souring stage and the use of garri adjunct occurs (Akinrele, 1970, Faparusi, 1973; Ogbonna et al, 1983 and Akinyemi, 1990).

At the stage when 'Kumpa' was added to the wort there was high level of microbial activities which shows that the yeast population of 'Kumpa' is quite active and forms the main inoculum . The high increase in the population of yeasts accompanied by the production of high amount of alcohol indicates that this stage is primarily an alcoholic fermentation stage. The fermented wort (now called burukutu) could be consumed within 24 hours of fermentation. However, if fermentation is allowed to continue after 24 hours, the beer becomes sour due to the increase in the volatile acid content occasioned by the increase in the population of acetic acid bacteria. Also the pH value of the beer reduced markedly to about 3.5 leading to the breakdown of the colloidal nature of the burukutu

beer. This leads to the sedimentation of the solid beer components. At this stage, the beer is considered spoilt and undrinkable. This has necessitated the practice whereby the local brewers only produce the amount of beer that will last just for the day.

In any preservative experiment, an essential aspect is the elimination or inactivation of the micro-organisms present and where complete elimination is not possible, those organisms whose growth brings about undesirable changes should be inhibited (Okafor, 1975). The method applied should neither introduce additives in amounts which are harmful to the consumers or impair flavour too seriously. The method should also achieve beer stabilization and meet any prevailing food standards (Okafor, 1975). These have been achieved to a large extent in this work.

After heat treatment, all yeasts were inhibited while the populations of moulds, aerobic bacteria and anaerobic bacteria stabilized for some times and later increased (Fig. 2). It will seem that treatment of burukutu with heat alone is inadequate as microbial activities resumed gradually within hours of treatment. Only yeasts population was completely inhibited leaving moulds and other microbial groups to rise in number. According to Akinyemi (1990) moulds found in burukutu beer around Zaria include toxigenic species like Aspergillus which could be responsible for the aflatoxin detected in the burukutu beer sample.

Sodium metabisulfite inhibted all yeasts at 1% concentration and also suppressed markedly the bacterial and mould population. It could be observed that sodium metabisulfite is the weakest of all the three chemical preservatives in terms of bacterial population reduction. This is in agreement with Mossel (1971) who reported that sodium metabisulfite is more active against gram negative bacteria than the gram positive one. According to Akinyemi (1990), the bulk of the bacterial population in burukutu are the gram positive ones.

Sorbic acidwas next to sodium metabisulfite in terms of effectiveness in yeasts reduction. However, sorbic acid reduces the population of moulds, and bacteria more than sodium metabisulfite. This is in agreement with Mossel (1971) who reported that sorbic acid has a wide spectrum of antimicrobial potency, including spore-formers. Sorbic acid can be tolerated by human body at higher concentration than either benzoic acid or sodium metabisulfite (Okafor 1975). The only disadvantage of sorbic acid as a preservative seems to be its low sulubility as observed in this work, which is in agreement with the findings of Okafor, (1971).

Benzoic acid is the least effective preservative in this studies in terms of reduction in population of yeasts, moulds and bacteria. It is, however, more

effective in reducing the anaerobic bacterial population than sorbic acid.

The chemical preservatives are more effective in checking fungal load than heat treatments. The observed pH all fell within acidic range indicating that all three chemical preservatives were active in acidic medium.

It will seem that the bulk of the surviving microorganisms in the treated burukutu samples are the anaerobic bacteria and in some cases yeasts. This could be so because of the existence of some degree of anaerobiosis occasioned by the large amount of carbondioxide produced by the yeasts action and the effect of bottling. This situation also explains the disappearance of acetic acid bacteria, a strict aerobe which is the main spoilage microorganism in burukutu beer. Also since the preservative treatments were done while the beer was fresh and when the population of acetic acid bacteria has not reached a deleterious level, not much of acetic acid bacteria were expected.

From the organoleptic scores of the preserved burukutu beer, sodium metabisulfite plus heat treatment which carries the highest scores seems to be the best preservative treatment for burukutu beer. The physicochemical characteristics of preserved burukutu beer still compares favourably with its fresh counterpart and also in consonance with organoleptic tasting.

In view of the foregoing and from the point of view of regulations governing acceptable preservation practices as enunciated above, the use of 0.25% sodium metabisulfite plus heat treatment at 60°C for 30 minutes in a 100-500 ml capacity bottle is tentatively recommended for the preservation of burukutu beer.

C H A P T E R S I X

RECOMMENDATION AND CONCLUSION

The development of starter cultures and proper education of the local burukutu brewers are recommended to aid in the production of burukutu beer of sound hygienic quality.

The advantages of burukutu beer over its lager beer counterpart in terms of higher nutritional value, availability of local strains and cheaper raw materials cannot be overemphasized. In view of this, it becomes pertinent that effective production and preservative techniques be established for the production of product of high hygienic quality and longer shelf-life.

The preservation of burukutu has been achieved to a large extent in this work where heat treatment and various chemical preservatives were used to preserve burukutu for periods ranging from 2-11 weeks. The most appropriate method of storing burukutu has been found to be that of combined heat treatment at $60^{o}C$ for 30 minutes and addition of 0.25% sodium metabisulfite. It is hoped that with the adoption of this preservative measure, a solid base would have been laid for the ultimate realization of the industrial production of the beer, an achievement that will surely redeem the dwindling popularity of the product.

APPENDIX I

ORGANOLEPTIC TEST

SCORE THE APPROPRIATE COLUMN IN RESPECT OF A, B, C, D. E, F, G CHARACTER

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 F	Very Thick 2	Thick 3	Slightly thick 4	Watery 1
Odour G	Sharp Kumpa-like 3	Moderate Kumpa-like 4	Slightly Kumpa-like 2	Rotten Odour 1

Best quality of Burukutu beer carried the highest scores while the worst attribute attracted the least scores.

(1) Comment briefly on the preserved burukutu
(2) Do you prefer drinking your beer always fresh or in a preserved and bottled form? Why?
(3) Would you pay more or less than the price of the fresh beer?

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