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MICROBIOLOGICAL EXAMINATION AND NUTRITIONAL QUALITY ASSESSMENT OF BURUKUTU SOLD IN NGURU NSUKKA, ENUGU STATE, NIGERIA

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ABSTRACT

The microbiological and nutritional qualities of burukutu sold in Nguru Nsukka were carried out. Six samples of burukutu were examined for total plate count of miro-oranisms using pour plate technique and also for nutritional contents. The total plate count ranged from 8.7% to 21.7%. The bacterial isolated were *Staphylococcus specie, Streptococcus specie, Bacillus specie, Pseudomonas specie*, while the fungi isolated were *Aspergillus specie, saccharomyces specie* and *Fusarium specie*. The nutritional contenst were PH 3.34, Alcoholic content 0.98%, Moisture content 93.0%, Ash content 1.07%, Crude protein 2.75%, Crude fiber, 0.35% and Carbohydrate 1.92%.

Keywords: Burukutu, Microbiological, Nutritional, Micro-organisms and Nsukka.

INTRODUCTION

Burukutu is a popular indigenous alcoholic beverage drink consumed mainly in the Northern and Savanna region of Nigeria. It is a local brew of vinegar like flavour, a brown coloured suspension made from fermented sorghum and the other protein enriched grains (James, 2000). The age long drink, also known as “B.K.T” serves as a source of alcohol for those who lack the financial means to patronize refined brew like beer and other foreign or imported drinks. It is produced mainly from the grain guinea corn (*Sorghum vulgare* and *Sorghum bicolour*). The process of production of burukutu involves, malting, mashing, fermentation and maturation (Kolawole *et al*., 2007).

The production process of this indigenous alcoholic drink involves fermentation at its initial production stage and comes out as an alcoholic drink. The micro-organism associated with fermentation include: *Saccharomyces cerevisiae, Saccharomyces chavelieria and Leuconstoc Mesteroides* (Faparusi *et al*., 2007).

Sorghum is a large variable genus with many cultivars. It constitutes a major source of energy and protein for people in Asia and Africa and it serves as a staple food for many of the world's poorest and least privileged people (Hulse *et al*., 2002). It is the main source of carbohydrate and protein for millions of people, mostly in the southern and Northern Sahara. Various types of food are prepared from sorghum whole kernels, for instance, unfermented and fermented porridge and pastes, and alcoholic and non-alcoholic beverage in Africa generally (Chikunda and Faramahans, 2001). Like other products, sorghum products have poor nutritional value, this is due to their deficiency in Lysine, threonine, tryptophan and the presence of antinutritional factors such as tannins and phytates that interact with proteins, vitamins and minerals thus restricting their bio-availability (Salunke *et al.,* 1999). The above factor contributes to anaemia and other nutritional diseases in developing countries where the consumption of sorghum product is high.

Furthermore, sorghum proteins are less digestible and mineral available than those of the other cereals. Various sample techniques have been investigated to improve the protein digestibility and mineral availability of sorghum by reducing its tannin and phytate contents. These techniques include malting, fermentation and cooking (Kazanas and Field, 2001).
Recently many people in Nguru Nsukka especially the elderly have included burukutu as part of their diet because they clam it quenches hunger and thirst. There have been complains about high rate of weight loss, diarrhoea and drunkenness among them. This could be as a result of unhygienic environment in which this drink is prepared. This prompted the desire to examine the microbial and nutritional quality of burukutu in Nguru Nsukka, Enugu Nigeria.

AIMS AND OBJECTIVES

- To isolate microorganisms in burukutu.
- To identify and characterize the organisms presents.
- To know how to improve the quality of burukutu.
- To know the alcoholic content of burukutu.

MATERIALS AND METHODS

MATERIALS

Microscope, Autoclave, Hot air oven, Weighing balance, Incubator, Conical flask, Measuring cylinder, Slide, Petri dish, Wire loop, Filter paper, Volumetric flask, Beaker, Crucible, Hot plate, Burette, Gloves, Dessicator, pipette, Bunsen burner and Staining rack

REAGENTS

Mac Conkey agar, Nutrient, Sabourand dextrose agar, Acetone, Decolonzer, Lugols iodine, mordant, Crystal Violet, Primary stain, Safranin, Secondary stain, Distilled water, Peptone water, Kovac reagent, Glucose, Lactose, Fructose, Sucrose, Hydrogen peroxide, Sulphuric acid, Sodium hydroxide, Sodium sulphate, Copper sulphate, Boric acid, Zinc metal, Methyl red and Oil immersion.

SAMPLE COLLECTION

A total of 6 samples of burukutu were collected from 3 different beer parlours in Nguru Nsukka. They were collected using sterile bottle and were sent to the laboratory.

STERILIZATION OF GLASS WARES

All glass wares used for the analyses were washed with detergent and water and rinsed with distilled water or tap water and allowed to dry. After drying they were raped in a kraft paper and were sterilized in the hot air oven under the temperature of 180^{o}C for 2 h.

PREPARATION OF CULTURE MEDIA

All culture media used were all prepared according to manufacturer's instruction written on the side of the container.

Types of Media used were

1) Nutrient agar.
2) macConkey agar
3) subouraud dextrose agar.

METHOD OF PREPARATION

NUTRIENT AGAR

2.8g of the powdered agar was measured and dissolved in 100ml of distilled water, it was gently heated to dissolve the medium completely and was sterilized in autoclave at 121^{o}C for 15mins. The medium was later on dispensed in the Petri dishes as desired.

MACCONKEY AGAR

7.8g of the agar was suspended in 150ml of distilled water. It was boiled to dissolve completely and was sterilized by autoclaving at 121^{o}C for 15mins. The medium was poured into sterile Petri dish plates. The surface of the gel was dried before inoculation.

SABOURAUD DEXTROSE AGAR

6.5g of the agar was dissolved in 100ml of distilled water. It was sterilized by autoclaving at 121^{o}C for 15mins. The medium was poured out in sterile Petri dishes and allowed to solidify before inoculating.

PREPARATION OF SAMPLES

The pour plate method was used, 1ml of the sample was measured aseptically from each bottle using sterile pipette. Each of the 1ml sample was poured in the Petri dishes and the melted nutrient agar, macconkey, sabourand dextrose agar were also poured into Petri dishes containing the sample and was mixed well by carefully swirling each molten agar in the sterile plate clock wise and anti clock wise. Each plate was allowed to solidify and was incubated invertedly for 24 h at 37^{o}C for bacteria growth and for 72 h at 37^{o}C for fungi growth.

MICROSCOPIC EXAMINATION

GRAM STAINING

Gram staining is a staining technique used in differentiating between gram positive and gram negative bacteria's.

PREPARATION OF SMEAR

A drop of water was placed on a sterilized grease free microscopic slide. Inoculums were picked from the culture plate using a sterilized wire loop that was sterilized under a Bunsen burner flame until red hot. Then the inoculums were stirred with the drop of distilled water on the slide to make a smear with a definite round shape. Then the smear was allowed to air dry before staining.

STAINING TECHNIQUE

The smear was placed on a staining rack and was flooded with crystal violet and was allowed to stand for 1 minute.

After 1 minute it was washed with distilled water and allowed to stand for 1 minute after the smear was decolourised with 95% acetone. This was allowed to stand for 30 seconds and was washed off with distilled water. Then the smear was flooded with safranin and allowed to stand for 30 seconds, and was washed off with distilled water. Excess water was drained off the slide and allowed to air dry.

Then a drop of oil immersion was dropped on the smear and then viewed under the microscope using x100 objective.

CHARACTERIZATION AND IDENTIFICATION OF ISOLATES

Bacteria isolates were characterized and identified after studying the gram reaction as well as cell micro morphology. Other tests performed were, catalase and oxidase, indole, sugar fermentation and citrate utilization.

CATALASE TEST

This is one of the bio-chemical tests that help to determine certain bacteria that can produce enzyme catalase which have the ability to oxide hydrogen peroxide into water and oxygen.

PROCEDURE

Hydrogen peroxide was introduced into a test tube. A wire loop was sterilized under a Bunsen burner flame and was used to pick an inoculated sample into the hydrogen peroxide in the test tube. The reaction was observed and observations were made.

A vigorous reaction producing bubbles that last for more than a minute shows that the organisms contain enzyme catalase and it shows positive result. But in the case of no reaction it shows that organism does not contain enzyme catalase and the result is negative.

INDOLE TEST

This is a biochemical test that helps to determine the ability of certain bacteria to decompose amino tryptophan to indole. Indole is a crystalline organic compound that is contained in coaltar and feaces.

PROCEDURE

PREPARATION OF PEPTONE WATER

15g of media was dissolved in 1000ml of distilled water. Heat to boiling to dissolve the media completely, sterilize by autoclaving at 121^{o}C for 15minutes and distribute into tubes.

Because 15g is too much, 1.5g of the media was dissolved in 100ml of sterilized water to get the preferred quantity.

After preparing the peptone water, 5ml of peptone water was measured into a test tube placed in a test tube rack. A wire loop sterilized by flaming under Bunsen burner flame was used to pick inoculums from the plate of culture and was inoculated inside the peptone water in the test tube. It was covered with cotton wool and allowed to stay for 48 h. Then after 48 h, 3 drops of Kovac reagent was introduced into the test tube and stirred well then observation of dark pink ring indicated a positive result while yellow ring indicates negative result.

SUGAR FERMENTATION TEST

This test the ability of bacteria organism to ferment sugar to produce gas and acid.

PROCEDURE

The peptone water was prepared as before, three test tubes were placed in a test tube rack. 9ml of peptone water was introduced into the test tubes. Using a wire loop inoculums were picked from the culture plate and was inoculated inside the test tubes. 1g of sample sugar such as glucose, lactose, and fructose was measured and put inside the test tube containing the peptone water. It was covered with cotton wool and mixed well. Then 3 drops of methyl red was put inside each test tube and mixed well. Durham's tube was inserted in an inverted position inside the test tube and allowed to stay for 24 h. After 24 h the observation was made and result recorded. A colour change to yellow showed acid production and was recorded as positive fermentation.

DETERMINATION OF NUTRITIONAL CONTENT USING PROXIMATE ANALYSIS

PH

The PH of the sample was determined directly using a PH meter.

ALCOHOLIC CONTENT

The sample was distilled in a distillation apparatus to recover the alcohol-water. Thereafter the percentage alcohol was determined using the pycnometer (S.G bottle).

MOISTURE CONTENT

A Petri dish was weighed and the value recorded. Two grams of the sample was weighed out and poured into the weighed Petri dish and was reweighed. The Petri dish containing the sample was heated in an oven for 1 h at 100^{o}C. It was removed after 1 h and put in dessicator for cooling. Then after cooling, it was reweighed and the value recorded. Then the moisture content was obtained by calculating the weight of Petri-dish and sample before drying and weight of Petri-dish and sample after drying.

ASH CONTENT

A crucible was weighted and the value recorded. Then two grams of the sample was weighed into the weighed crucible and then reweighed. Crucibles with the sample were placed in the muffle furnace to ash at a temperature of 500^{o}C for 2 h. Then the ashed sample was removed and cooled in a dessicator and was weighed after cooling.

CRUDE PROTEIN

0.5g of the sample was weighed into Kjeldahl flask (distillation apparatus). 0.1g copper sulphate was added into the flask. Ten grams of sodium sulphate acid was added to the flask. Then the mixture was placed in a heating mantle to digest the sample until the solution turns to bluish green in colour. The solution was allowed to stand for 24 h in order to solidify. After cooling the digest sample turned into a white solid. 200ml of distilled water was used to dissolve the solidified digest and it was allowed to cool for some minutes. 60ml of 40% sodium hydroxide was added to the digested sample. 2 pieces of zinc metal was well connected to a distillation column for distillation to take place. 100ml of 4% boric acid was added to a separate 250ml conical flask. Two drops of screen methyl red was added to it as an indicator. On addition of the indicator the colour of the mixture in the flask turned light pink. The distillation was stopped when the distillate in the flask turns colourless at this point the protein was extracted. The distillate was titrated with 0.1m H_2SO_4, the end point was then observed immediately the colour changed to light pink.

CRUDE FIBRE

Two grams of the sample was weighed into a conical flask. 200ml of 1.25% sulphuric acid was added. The mixture was heated for 30 minutes on a hot plate for acid treatment. After 30 minutes it was brought down and allowed to cool and was filtered using what man filter paper. The filtrate was washed with distilled water and tested with PH paper for acidity.

The sample was resoaked in 200ml of 1.25% sodium hydroxide and then placed on the hot plate for another 30 minutes. The solution was filtered using a weighed filter paper. The filter paper was oven dried and weighed again.

The filter paper containing the residue was placed in a clean weighed crucible to ash. After ashing, the crucible was placed in a dessicator to cool and then reweighed.

CARBOHYDRATE CONTENT

This is the subtraction of the sum of other composite factors from 100%.

RESULT

Table 1: The Total Plate Count of Isolates

Sample	Total plate count
A	200
B	176
C	144
D	160
E	161
F	80

TABLE 2: STANDARD PLATE COUNT OF COLONIES FORMED

Samples	Colonies	Colonies	Colonies
	I	II	III
A	66	68	66
B	58	50	68
C	48	40	56
D	50	57	53
E	51	54	56
F	25	35	20

TABLE 3: PERCENTAGE DISTRIBUTION OF COLONIES

Colonies Code	Mean plate Count	Percentage distribution
I	298	32.4%
II	304	33.0%
III	319	34.6%

TABLE 4: MORPHOLOGICAL CHARACTERISTICS OF ISOLATES

Code	Morphological characteristics	Gram reaction	Presumptive bacteria
I	Blue and cream large round colonies ranging from 1mm-5mm on MacConkey agar	Gram positive	*Streptococcus Clostridium Bacillus Staphylococcus*
II	Yellow coloured colonies on Nutrient agar	Gram positive	*Leuconostoc Lactobacillus Micrococcus*
III	Red, Pink coloured colonies on sabouraud dextrose agar	Gram Negative	*Pseudomonas Enterobacter*

TABLE 5: MORPHOLOGICAL AND MICROSCOPIC CHARACTERISTICS OF FUNGI ISOLATES

Code	Morphological characteristics	Microscopic Examination	Presumptive bacteria
I	Candiosphere with swollen head	Green coloured candioshere bearing bottle shaped phidide	*Aspergillus spp*
II	Colonies with septae hypha	Elongated cells single and in chains	*Mucor fusarium*
III	Root like rhizoids	Brown coloured well	*Rhizopus*

		developed mycelium	
IV	Large colonies with bounded cell without hyphae	Cream round budding cell in cluster	*Yeast saccharomyces spp*

TABLE 6: BIOCHEMICAL TEST

Catalase	Indole	Oxidase	Citrate	Glucose	Sucrose	Lactose	Fructose	Oraganism isolated
+	+	-	+	AG	AG	AG	G	*Lactobacillus spp* *Staphylococcus spp*
+	-	+	-	AG	AG	G	AG	*Micrococcus* *Pseudomonas spp*
-	+	-	-	AG	AG	AG	AG	*Streptococcus spp* *Bacillus spp*

TABLE 7: PERCENTAGE VALUES OF THE NUTRITIONAL CHARACTERISTICS OF BURUKUTU SAMPLES

Parameter	**% Value**
PH	3.34%
Alcoholic content	0.98%
Moisture content	93%
Ash content	1.0%
Crude protein	2.75%
Crude fiber	0.35%
Carbohydrate	1.92%

DISCUSSION

The plate count of the burukutu samples shows that sample A had the highest growth while F had the least growth. Table 1 shows the total plate count of the microorganism isolated. Table 2 shows the standard plate count of different colonies. Table 3 shows the percentage

morphological characteristics of bacterial isolated. Table 4 shows the morphological and microscopic characteristics of fungi isolated. Table 5 shows the biochemical test on isolates. Table 6 shows the nutritional value percentage of burukutu. The micro-organism isolated from these samples were *Bacillus species, Streptococcus species, Pseudomonas species, Aspergillus species, fusarium species, saccharomyces species.* The result is in line with the work of Kolawole *et al*., (2007), when they worked on the proximate and microbial analysis of burukutu produced in Ilorin, Nigeria.

The presence of *Staphylococcus* in the sample may be attributed to handing during production.

Bacillus species which are gram positive aerobic spore formers were also present. Most members of the genus are saprophytic organisms prevalent in soil water, air and on vegetation.

The yeast isolated from these samples like *Saccharomyces* is associated with fermentation. The association of lactic acid bacteria and yeast has been observed in several cereal foods (Akinrele, 2002). Nout (1990) reported that the development of lactic acid bacteria stimulated by yeast provide soluble nitrogen compounds and other growth factors. It was also observed that the continuous growth of the yeast population at the end of fermentation indicated that the products were yet stabilized.

The proximate analyses of the locally vended burukutu sample were also recorded. Ababio content of burukutu ranges from 1.4%. The result obtained from the work was within this range. The PH value of 3.34 was obtained from the study and was in line with the result recorded by Lgyor *et al*., (2006).

CONCLUSION

The high microbial counts recorded shows exposure of the samples to different genera of bacteria and fungi leading to their contamination. This may be attributed to high moisture and protein contents as shown in the proximate analysis of the samples. This high counts could also be as a result of large number of people that visit beer parlor, resulting to an increased microbial numbers. The main sources of contamination include humans, sewage, utensils, processing equipment and environment, handling and storage condition and rodents (Eze *et al*., 2008)

This research has shown that burukutu contains some nutrients that are essential for body building as well as pathogenic micro-organisms that could be harmful to man and pose a serious public health hazard to the consumers.

RECOMMENDATION

We hereby recommend that since some of the microorganisms isolated were as a result of poor hygiene, the producers and vendors should practice good manufacturing and hygienic practises. They should make sure that the processing equipment such as the milling machine, their environment, handling and storage condition will not be to favour the growth of this micro-organism. The water used in the production should also be clean water free from germs.

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