Microbiological and nutritional assessment of *burukutu* and *pito* (indigenously fermented alcoholic beverages in West Africa) during storage

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Abstract: Spoilage of both fermented and unfermented food products during storage is caused by microorganisms and this is achieved with the aid of extracellular enzymes they produced, which breakdown the food product into new substances resulting into changes in their organoleptic properties. This renders it unacceptable and unsafe to the consumers hence causing economical loss to the producer. Studies were therefore conducted to investigate the microbiology, physicochemical properties, enzyme activities and proximate analysis of both fresh and stored samples of *Burukutu* and *Pito* for seven days at ambient temperature. The result obtained revealed that thirty nine isolates were obtained from both fresh and stored samples. The bacterial isolates were identified as S*taphylococcus aureus, Pseudomonas fragilis*, *Bacillus megaterium*, and *Lactobacillus brevis,* while the yeast and fungal isolates were identified as *Torulopsis* sp*, Saccharomyces cerevisiae, Candid krusei and Aspergillus niger* The pH decreased from 3.48 to 3.08 and from 3.09 to 2.99 at day 0 to day 7 in *Burukutu* and *Pito* samples respectively while the Total Titrable Acidity (TTA) increased from 1.55 to 1.87 and 1.35 to 1.63 in *Burukutu* and *Pito* respectively. The alcoholic content decreased from 2.3% to1.4% and from 0.9% to 0.5% in the fresh burukutu and pito samples respectively during storage. However, the proximate analysis revealed that parameters such as % crude fiber, ash, protein and carbohydrate decreased significantly ($P<0.05$) in the stored samples while the % moisture content increased from 97.35 to 98.02 and 98.56 to 97.66 from day 0 to 7 day for both *Burukutu* and *Pito* respectively. The enzyme activities showed that the amylase and proteinase enzymes increased from day 0 to day 3 and later decreased on day 7 for both *Burukutu* and *Pito* samples. The fresh samples of the alcoholic beverages were accorded better acceptability in all tested sensory parameters than the stored samples by the consumers.
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1. Introduction:

Burukutu and *Pito* are produced locally at the cottage level in some parts of West Africa. They possess attributes that include a sour taste as a result of lactic acid, a pH range of 3.3 to 3.5 and cloudy appearance because of inclusion of suspended solids and yeast. Nutritionally these alcoholic beverages comprise nutrients such as iron, manganese, magnesium, phosphorus, calcium, vitamins, 26.7g starch and 5.9g protein per liter (Egemba and Etuk, 2007). However, some of the naturally occurring microflora of sorghum have been previously reported to secrete toxic substances, such as mycotoxins (Isabel et al, 2005). Alais and Linden, (1999) submitted that with pasteurization of freshly prepared alcoholic beverages at 60^0C for 30 min will preserved for two weeks (Alais and Linden, 1999).

In Africa, cereal beers are regarded as food by consumers because they satisfy hunger, and also because of their high nutritional value and taste. Both beverages are produced by local women for sale to generate income for their families.

Burukutu and *Pito* are produced from millet, sorghum and maize. The method of production of the alcoholic beverages are similar except that certain additives like gari ground malt and water in a ratio of 1:2:6, gari: malt: water are introduce during the mashing process in the production of *burukutu* while during production of pito such addition is not involved (Faparusi et al., 1973: Ekundayo, 1969). Traditional *burukutu* and pito preparation is a batch process carried out on a small scale 2 or 3 times a week. The stages in their preparation are malting, mashing, fermentation and maturation. Sorghum grains are stepped in water overnight. This is followed by draining the water and spreading the dried grains on banana leaves to germinate. Watering of the grains was carried out on alternate days and mixed at intervals. Its period of germination ranges between four to five days. After malting of the grains they were spread in the sun to dry naturally followed by grinding. The grinded substance is normally allowed to ferment for 2 days. It is then boiled for four hours, cooked, and left to mature for two days (Faparusi, 1971: Faparusi et al., 1973). The microorganisms

involved in the fermenting mixture are *Saccharomyces cerevisiae* and *S.chavelieri, Leuconostoc mesenteroides* and *Lactobacillus* spp. At 48 hour of fermentation period, the pH droped from 6.4 to 3.7 while in the maturation process *Acetobacter* spp. and *Candida* spp are observed to show the highest occurrence causing improved souring of the beverage. The final product showed a cloudy liquid that have vinegar taste.

Food spoilage is a metabolic process that causes foods to be undesirable or unacceptable for human consumption due to changes in sensory characteristics. There are three types of microorganisms that cause food spoilage: yeasts, moulds and bacteria, but the organisms widely reported to associate with food spoilage are *Aspergillus species* and related moulds because they grow faster and are highly resistance to high temperature and low water activity. This paper investigated microbial dynamic and other factors occurring in the stored and fresh samples of *pito* and *burukutu* at room temperature for seven days.

2. Materials and Methods

Collection of Samples

Fresh burukutu and pito samples used for this study were collected in sterile bottles from the producers immediately after production in Agbowo and Ojo in Ibadan metropolis, Oyo state of Nigeria. The samples were transported to the laboratory for immediate use.

Isolation of microorganisms

The media used for the isolation of microorganisms from samples of burukutu and pito were De Mann Rogosa and Sharpe Agar (MRS), Nutrient Agar (NA), MacConkeyAgar, and Potato Dextrose Agar (PDA) (Oxoid). One ml of each sample was differently serially diluted and separately aseptically transferred into sterile Petri dishes containing the isolation media stated above. MRS agar plates were incubated anaerobically at 37°C for 24 hours using the anaerobic jar, Maconkey, and NA plates were incubated at 35°C for 24 hours, while PDA plates were incubated at 30^0C for 7days.

Identification procedure

The microorganisms isolated were repeatedly sub-cultured until pure cultures were obtained. These cultures were transferred differently to agar slants in a McCartney bottles and stored in the refrigerator at 4^0C. The identities of the bacterial cultures were confirmed based on their morphological, physiological and biochemical characteristics using Bergeys manual of systematic bacteriology as reference (Sneath, 1984). The identification procedure for the yeast and fungi isolates were carried out according to the methods described Sanni and Lonner (1993) and Bernett et al. (1960) respectively.

Physicochemical study

Measurement of pH:

The pH of both the fresh and stored samples was measured using a calibrated pH meter (Mettler Toledo, AG8603 Switzerland model).

Titratable Acidity:

This was determined according to the procedure described by Ashenafi and Busse (1991).

Determination of Alcholic content:

This was carried out using the Specific Gravity method described by (AOAC, 1990).

Enzymatic Activities in Fresh and Stored Samples

Amylase and Proteinase activities were monitored using the modified method of Bernett and Fergus (1971) and Kunitz (1946) respectively.

Proximate Analysis of Fresh and Stored Samples

The determinations of Crude protein, Carbohydrate, Moisture, Ash and Crude fibre contents, were carried out according to the methods of AOAC (2002).

Sensory Evaluation

The sensory evaluation panel consisted of ten students (from different parts of Nigeria) in the University of Ibadan, Nigeria who are consumers of *burukutu* and *pito*. The panelists were instructed to rate the samples for appearance, taste, aroma and acceptability. The ratings were presented on a nine point Hedonic scale rating ranging from; like extremely (9 points) like much (8) like moderately (7) like slightly (6) neither like nor dislike (5) dislike slightly (4) dislike moderately (3) dislike very much (2) to dislike extremely (1 point) as described by Larmond (1977). Each sample was evaluated three times by each panelist.

One-way ANOVA was used to analysed the results and means differences were sorted out based on Duncan Multiple Range Test (Duncan, 1955; Steel and Torrie, 1960).

3. RESULTS

Table 1 shows that a total number of thirty nine isolates were obtained from both fresh and stored samples of burukutu and pito. They were identified as *Saccharomyces cerevisiae, Candida krusei, Torulopsis spp., Lactobacillus brevis, Staphylococcus aureus, Pseudomonas fragilis and Aspergillus niger*. The microbial loads were observed to decrease in both stored samples of burukutu and pito when compared with both fresh samples of burukutu and pito. *S. cerevisiae* decreased from $3.2x10^8$cfu/ml in the fresh to $1.60\ x10^8$cfu/ml in stored burukutu sample while it also decreased from $1.39\ x10^8$ cfu/ml in fresh pito sample to$1.00\ x10^8$cfu/ml in stored sample. Similar trend was observed in occurrence of *Candida krusei* in

both fresh samples of burukutu and pito when compared with both stored samples of *burukutu* and *pito*. *Torulposis sp.* ($1.22x10^8$ cfu/ml) was detected at the 7th day in the stored *burukutu* sample but was absent in both fresh and stored samples of *pito*.

Also, *A.niger* was only detected on the 7th day showing$1.02x10^8$ cfu/ml while *Bacillus megateriun* occurred in fresh pito sample showing $2.30x10^8$ cfu/ml and disappeared during the storage period. However, the population of *Lactobacillus brevis* decreased in fresh samples of *burukutu* and *pito* when compared with both the stored samples. The population of *S. aureus* decreased from $4.80x10^8$ cfu/ml in the fresh to $2.70x10^8$cfu/ml in stored burukutu sample while its occurrence was detected in the fresh pito sample showing $1.20x10^8$ cfu/ml which disappeared during the storage period. Moreover, *P. fragilis* was present in fresh burukutu sample but was detected on day 3 recording $3.48x10^8$ which was not detected on day 7. Similar trend was observed in pito samples.

The Amylase assay showed that the amylase activity in the fresh *burukutu* and *pito* samples increased from 0.94mg/ml to 1.80mg/ml and from 0.98mg/ml to 1.10mg/ml in both stored *burukutu* and *pito* samples respectively while (Table 2) the proteinase activity increased from 0.66mg/ml and 0.60mg/ml in the fresh *burukutu* and *pito* sample respectively to 0.77mg/ml and 0.71mg/ml in the stored samples of burukutu and pito respectively. The pH recorded in the fresh samples of *burukutu* and *pito* decreased from 3.48 and 3.66 respectively to 2.75 and 3.12 in stored samples of *burukutu* and *pito* respectively. The Titratable acidity increased from 1.55m and 1.87 in fresh burukutu and pito sample respectively to 1.87M and 1.92M in the stored samples of *burukutu* and *pito* respectively. However, the alcoholic content decreased from 2.80% and 3.09% in the fresh *burukutu* and *pito* samples respectively to 1.4% and 20.0% in the stored samples of *burukutu* and *pito* respectively.

The results of the proximate analysis of both the fresh and stored *burukutu* and *pito* samples are presented on Table 3. It revealed that the ash content decreased from 0.035% in the fresh *burukutu* sample to 0.002% in the stored *burukutu* sample while in the fresh pito sample, it decreased from 0.001% to 0.00% in the stored sample. The crude fibre was observed to decrease from 2.49% in the fresh *burukutu* sample to 1.37% in the stored while it decreased from 1.05% in the fresh *pito* sample to 0.09% jn the stored sample. However, the moisture content increased in the fresh *burukutu* sample from 97.35% to 98.2% in the stored sample, the same trend was noticed in the fresh and stored samples of pito.

The sensory evaluation of both fresh and stored burukutu and pito samples is shown in Table 4. It was noticed that the stored samples of *burukutu* and *pito* scored lower in all the parameters tested for when compared with the fresh samples.

Table: 1 Microorganisms Isolated from Fresh and stored Samples of *Burukutu* and *Pito*

Microorganisms	Fresh	3 Days	7 Days
Burukutu	**Cfu/ml**		
S. cerevisiae	3.24×10^8	2.72×10^8	1.60×10^8
Candida albican	3.81×10^8	2.60×10^8	1.20×10^8
Torulopsis spp	ND	ND	1.22×10^8
Lactobacollus brevis	8.66×10^8	1.68×10^8	1.35×10^8
S. aureus	4.80×10^8	2.70×10^8	2.70×10^8
Pseudomonas fragilis	ND	3.48×10^8	ND
Pito			
S. cerevisiae	1.39×10^8	1.21×10^8	1.0×10^8
Candida albican	1.34×10^8	1.20×10^8	1.20×10^8
Aspergillus niger	ND	ND	1.02×10^8
Bacillus megaterium	2.30×10^8	ND	ND
Lactobacollus brevis	6.64×10^8	3.26×10^8	1.76×10^8
S. aureus	1.20×10^8	ND	ND
Pseudomonas fragilis	ND	1.18×10^8	**ND**

Table 2: Enzymatic and Physicochemical Studies

Sample	Day	Amylase activity mg/ml		Proteinase activity mg/ml	pH	Titratable activity (M)	Alcoholic Content (%)
Burukutu(fresh)	0	0.94 ±0.32a		0.66 ±0.56a	3.48±0.34c	1.55 ±0.25a	2.80 ±0.01c
	3	2.23 ±0.21c		0.95 ±0.73c	3.00±0.65b	1.68±0.89b	2.12±0.33b
	7	1.80 ±0-48b		0.77 ±0.33b	2.75±0.28a	1.87 ±0.06c	1.46 ±0.41a
Pito	0	0.98 ± 0.84a		0.60±0.44a	3.66±0.22c	1.65±0.11a	3.09±0.67c
	3	2.30 ±0.39c		0.85 ±0.25c	3.25±0.57b	1.78 ±0.30b	2.82±0.35b
	7	1.10±0.47b		0.75 ±0.63b	3.12±0.56a	1.92 ±0.42c	2.00 ±0.43a

Values are means of replicate determinations ± standard deviation; values in the same column followed by the same superscripts are not significantly different according to Duncan's Multiple Range Test (P<0.05).

Table 3: Changes in Proximate (%) analysis during storage

parameters	fresh burukutu	stored burukutu	fresh pito	stored pito
ash content	0.035 ±0.5b	0.0025±0.49a	0.001 ±0.45b	ND±0.40a
crude fiber	2.49 ±0.65b	1.379 ±0.60a	1.05±0.58b	0.90 ±0.0.55a
moisture	97.35 ±0.67a	98.20 ±0.51b	96.56 ±0.53a	97.66 ±0.65b
crude protein	5.80 ±0.47b	4.37±0.39a	5.42 ± 0.46b	4.56 ±0.65a
carbohydrate	82.85±0.63b	76.22 ±0.54a	80.16 ±0.68b	73.38 ±0.83a

Values are means of replicate determinations ± standard deviation; values in the same column followed by the same superscripts are not significantly different according to Duncan's Multiple Range Test (P<0.05).

Table 4: Sensory Evaluation of both Fresh and Stored Samples

Samples	Taste	Aroma	Appearance	Acceptability
Fresh Burukutu	82 ±0.77 b	50 ± 0.66 b	60 ± 0.11 b	65. ± 0.00 b
stored Burukutu	30 ± 0.36a	20 ± 0.12 a	35 ±0.88a	10 ± 0.93a
Fresh Pito	85 ±0.30b	60 ± 0.01 b	50 ±0-61b	70 ±0.24b
Stored Pito	40 ±0.22a	10 ±0.71a	20 ±0.55a	20 ± 0.08a

Values are means of replicate determinations ± standard deviation; values in the same column followed by the same alphabets are not significantly different according to Duncan's Multiple Range Test (P<0.05).

4. Discussion

The occurrence of microorganisms such *Bacillus megaterium, Staphylococcus aureus, Pseudomonas fragilis Lactobacillus brevis, Saccharomyces cerevisiae, Torulopsis sp, Candida krusei and Aspergillus niger during* storage of burukutu and pito samples had earlier been documented by Thomas (1994; Eze et al., 2008; Kolawole et al., 2010). This occurrence might be adduced to the biodegradeting potential of the microflora to convert diverse substrates by secretion of extracellular enzymes. In addition microorganisms are found associating in the habitats they are found. However, the detection of some pathogenic microorganisms such as *Staphylococcus aureus*, spore forming bacteria and *A. niger* in stored foods is highly

hazardous to the consumers as this could cause food poisoning due the production of enterotoxins and economic loss to the producer (Singleton, 1995, Frazier and Westhorf, 2004). Furthermore, non compliance with aseptic and hygiene rules during production and packing could be responsible for the contamination of these products by the microorganisms.

The occurrence and disappearance of some microorganism during the storage period might be due to death of some microflora which is caused by nutrient depletion and microbial succession resulting from the production of some metabolites which create a new environment favourable to the existence of some microorganisms and unfavourable to the survival of others (Sanni et al., 1999). The increased moisture content of the stored beverages ia an indication of an environment susceptible to microbial invasion due to the favourable water activity created (David and Verma, 1989; Uzochukwu et.al., 2000).

The decrease in pH of both burukutu and pito samples during storage might probably resulted from the production of some acidic metabolites by the microflora present during storage. Documented reports on the production of acidic metabolites by microbes during the utilization of glucose to liberate energy for growth and metabolism are available (Kolawole et al., 2007).

The observed increased in the amylase and proteinase activities during the early stages of storage were due to the microbial hydrolysis of the carbohydrate and protein content of the alcoholic beverages for growth and metabolic activities. The noticeable decrease in the amylolytic and proteolytic activities at the end of the storage period might be due to depletion of the carbohydrate and protein content of the beverages coupled with the death of these microorganisms resulting in the absence or low microbial load.

The proximate analysis showed a significant decrease in the levels of all the parameters investigated. Such occurrence could be due to the utilization of the nutrients present in the alcoholic beverages without replacement during the storage period. The low acceptability of the stored products recorded during sensory evaluation could be linked to the undesirable metabolites produced by the microorganisms.

There is the need for mass awareness among families, communities, producers, vendors and consumers of *Burukutu* and *Pito* on the health implication of consuming these beverages if it had stayed for more than 3days. However, it is advisable that burukutu and pito should be consumed fresh.

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