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SHELF LIFE EXTENSION IN *BURUKUTU*, A NIGERIAN ALCOHOLIC BEVERAGE USING ANTIMICROBIOLOGICALLY ACTIVE SPICES

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ABSTRACT

Common food preservation methods such as pasteurization or the use chemical preservatives render *burukutu*, a Nigerian alcoholic beverage unacceptable because they further gelatinize starch or eliminate the beer's characteristic effervescence through the killing of active yeasts. Spices are known to be selective in their inhibition of different types of microorganisms. The objective of this study was to select spices, which would inhibit the growth of spoilage organisms but allow the growth of active yeasts such that shelf life of *burukutu* is extended with retention of beer's characteristic effervescence. Lactic acid bacteria, acetic acid bacteria, yeasts and molds isolated during the fermentation/spoilage of *burukutu* were screened for susceptibility to ethanolic extracts of the rhizome of *Zingiber officinale*, leaves of *Cymbopogon citratus*, seeds of *Aframomum melegueta*, leaves of *Gongronema latifolium* and the tree bark of *Sacoglottis gabonensis*in using plate assays .*Burukutu* brewed with aqueous extracts of these spices were also tested for acceptability using the 9-point Hedonic scale and a panel of regular *burukutu* drinkers. No spice extracts showed absolute selective antimicrobial activity against any specific organisms. However, in the plate assays, extracts of *Zingiber officinale* showed a stronger inhibitory activity on the growth of molds and the acetic acid bacteria than it demonstrated against yeasts. Burukutu brewed with this spice showed a reduced rate of growth of all organisms particularly, the molds and the acetic acid bacteria and hence prolonged the shelf life for at least three days. *Burukutu* flavored with this spice was also acceptable; mean score by panelists was $7.2963^{a}\pm0.49$ compared with $7.7037^{a}\pm0.39$ for the control.

Key words: *Burukutu* shelf life extension, spices

INTRODUCTION

Burukutu is a thick, creamy or brown colored, sour and alcoholic beverage of vinegar-like flavor with a cloudy appearance drunk in West and Central Africa (Kolawole*et al*., 2007). Banigo *et al.* (1987) have described the details of the brewing of *burukutu* in Nigeria. This involves the malting of sorghum grains, which is ground into flour and after mixing with starchy adjuncts, is cooked and then fermented about 24hr. Fermentation is initiated using a back slop containing organisms from a previous brew and involves mainly lactic acid bacteria(LAB) and yeasts(Faparusi, 1970; Jideani and Osume, 2001; Atter *et al*., 2014). *Burukutu* has an alcohol content of 2-4% (v/v), and is a nutritious beverage containing in addition to carbohydrates and proteins, vitamins and minerals (Eze *et al.*, 2011). *Burukutu* is more affordable than lager or other forms of bottled beers and therefore very popular among the low and middle income men. However, with current interest in foods perceived as "natural" and the declining economies of many countries where this drink is produced, its consumer base is widening indicating the need for a scale up in production.

The limited shelf life of sorghum beers due to microbial spoilage has been reported as the major problem confronting commercial brewers in many countries where these beverages are produced (Lyumugabe *et al*., 2012).The shelf life of *burukutu* in most parts of tropical Africa does not usually exceed 3 days (Van der Walt, 1956). According to this author, the metabolic activities of mesophilic LAB are primarily responsible for the spoilage. These bacteria, along with other undesirable bacteria such as the acetic acid bacteria (AAB), usually *Acetobacter*, produce acetic acid, volatile off-flavors, fruity odors, and pellicles which render the taste, odor and texture of the beer unacceptable to consumers.

Various attempts have been made towards the preservation of *burukutu* beer. Ogbadu *et al*. (1997) reported that pasteurization at 60ºC for 30min in combination with 0.25% sodium metabisulphite stabilized *burukutu* for up to 11 weeks. However, Novellie and De Schaepdrijver (1986) had noted earlier, that pasteurization led to an unacceptable increase in beer viscosity through further gelatinization of starch. Pasteurization also caused elimination of amylolytic enzymes and elimination of the beer's characteristic effervescence through the killing of active yeasts. The use of pure chemical compounds would also have a similar effect on these organisms. Considering this peculiar characteristic, any treatments applied in the preservation of *burukutu* must selectively inhibit the growth of only spoilage microorganisms.

Spices and herbs are gaining importance in recent years as potential sources of natural and safe food preservatives (Edward and Ohaegbu, 2012; Gottardi *et*

al., 2016). The main preservative effect of spices is exerted by their essential oils (EOs), which are known to be antimicrobiologically active. The chemical nature of many spice EOs has been studied (Onyenekwe and Hashimoto, 1999; Ajaiyeoba and Ekundayo, 1999; Shaaban *et al*., 2013; Ezekwe *et al*., 2014; Tchouya *et al*., 2016).Their mechanisms of action is also becoming better understood and it has been demonstrated that different EOs affects different types of microorganisms to different extents. For example, *Foeniculum vulgare* Mill (fennel) seeds contain trans-anethole and estragole to which Gram positive and negative strains of bacteria showed different sensitivities (Diao *et al*., 2014). Similarly, *S. aureus* was more susceptible to cinnamon EO, which contained cinnamaldehyde than *E. coli* (Zhang *et al*., 2016).

From the foregoing, spices may therefore, provide compounds with selective antimicrobial activity, which may be suitable for the preservation of a peculiar beverage such as *burukutu*. Spices are also used to add flavors to foods, and with the increasing popularity, all over the world of flavored alcoholic beverages (Mosher and Johnson, 2005); the addition of spice flavors to *burukutu* may increase its acceptance to a wider range of consumers.

The objectives of this study are therefore to select spices, which are inhibitory to AAB and LAB but not to yeasts and to extend the shelf life and improve the flavor of *burukutu* by brewing with such spices.

MATERIALS AND METHODS

Spices and spice extracts

Five spices commonly used in food and medicine in many parts of the world were obtained locally and used in this work. They were: Ginger rhizomes (*Zingiber officinale*), Lemon grass leaves (*Cymbopogon citratus*), alligator pepper seeds (*Aframomum melegueta*), leaves of *Gongronema latifolium* and the tree bark of *Sacoglottis gabonensis.* The two last spices are locally known in South East Nigeria as *utazi* and *nche* respectively. Ethanolic extracts of spices were obtained by Soxhlet extraction of 20 g of each dried spice powder in 100 ml of 95% ethanol at 78°C using Soxhlet apparatus. The extracts were then concentrated to 20 ml on a water bath and dried at room temperature. Extracts were reconstituted in dimethyl sulfoxide (DMSO) before use. Water extraction was done by homogenizing 100 g each, of spice with 100 ml sterile distilled water using a laboratory blender. A sterile muslin cloth was used to sieve the fine mash and to obtain approx. 50% concentration of the extracts. Extracts were stored at 4 °C until needed.

Brewing of Burukutu

The initial steps, up to boiling of the "wort" in the brewing of the *burukutu* used in this study were done in a brewery near our laboratory in Awka, Nigeria (6° 12′ 37.9008″ N and 7° 4′ 20.1972″ E). Grain used was *Sorghum*

vulgare var., red sorghum and adjunct was *garri* (granular flour made from gelatinized fermented fresh cassava mash).The process in this brewery followed steps described by Banigo *et al.* (1987). *Burukutu* wort was collected immediately after boiling and transported aseptically to our laboratory in an ice box where it was inoculated with starter comprised of a 24 h old previous brew of *burukutu* to initiate fermentation. *Burukutu* was produced after a 24h fermentation period.

Flavored *burukutu* was brewed by the addition of water extracts of spices (50% concentration) to wort immediately after addition of starter culture. Each spice extract was added until spice flavor became just perceptible. About 100 ml, 120 ml and 300 ml, respectively of extracts of ginger, lemon grass and *nche* were required in 1 L of *burukutu* for this purpose. Aliquots of 70 ml and 80 ml were required for alligator pepper and *utazi*.

Isolation of microorganisms associated with burukutu fermentation

Various selective media were used to isolate the different microorganisms known to be involved in the fermentation and or spoilage of *burukutu*. Lactic acid bacteria were isolated on Man-Rogosa-Sharpe (MRS) agar with cycloheximide (De Man Rogosa and Sharpe, 1960). Malt Extract Agar (MEA) containing chloramphenicol was used to isolate yeasts and molds, which were further distinguished by their colony characteristics. Acetobacter agar (Glucose) from HiMedia Laboratories, Pvt. Ltd., Mumbai-400086, India was used to isolate acetic acid bacteria.

Burukutu brewed as already described was held at room temperature for four days, during which period it suffered spoilage. Various organisms listed above were isolated at 24 h intervals by taking10 ml samples, from which one ml aliquots of appropriate dilutions were plated on appropriate media. The predominant colony types on each of the microbiological media were purified by repeated streaking and partially identified by morphological and biochemical tests. The schemes of Bergey (Holt *et al.*, 1994), Kurtzman *et al*. (2011) and Alexopoulos *et al.* (1996) were used to identify the bacteria, yeasts and molds respectively. These isolates were also used as representative organisms for LAB, AAB and yeasts in determining spice antimicrobial activity against organisms associated with *burukutu*.

Screening of spice extracts for antimicrobial activity against microorganisms isolated from burukutu

Antimicrobial activity of the different spice extracts were tested against microorganisms described above using the agar well diffusion method (Perez *et al*., 1990). Ampicillin (0.5µg/ml) was used as a positive control for bacterial isolates and cycloheximide (0.5µg/ml) for fungal isolates, while 5% DMSO served as the negative control. The diameters of growth inhibition zones

around the wells were considered as direct measurement of activity/no activity of extract against particular test organisms.

Effect of addition of spices on the acceptability of burukutu

Acceptability of the spice flavored *burukutu* was tested by sensory evaluation using twenty seven panelists selected from frequent consumers of *burukutu* who frequently visited the collaborating brewery. Panelists were asked to compare the flavored *burukutu* samples relative to the unflavored control on a 9-point Hedonic scale as described in Watts *et al.* (1989). Results were analyzed using one way ANOVA and the Tukey HSD test (VassarStats Statistical computation Website; http://vassarstats.net/, Accessed 20 August. 2017).

Effect of addition of ginger on the growth of microorganisms associated with burukutu

Among the five spices tested, ginger was found to be the most inhibitory against spoilage and least inhibitory against fermentation microorganisms. *Burukutu* flavored with ginger was also the most acceptable of the flavored samples during sensory analyses. This spice was therefore used for further studies.

The effect of addition of ginger was determined by viable counts of the different microorganisms in *burukutu* brewed with and without this spice at 24 h intervals using selective media and sampling methods as described in the section "Isolation of microorganisms associated with *burukutu*". All determinations were done in triplicate.

Shelf life of burukutu

Samples of *burukutu* brewed with and without ginger added, were distributed in 500 ml quantities in sterile containers and stored at room temperature on the bench and shelf life determined by measurement of characteristics described below. Formation of pellicle was determined by visual inspection. pH was measured after homogenizing with an equal volume of distilled water using a pH meter (Hanna HI 991001). Titrable acidity (TA) was determined on samples clarified with muslin cloth according to the methods of AOAC (1990). Alcohol content was determined by the distillation-hydrometric method described by AOAC (1990). Acceptability of the samples following storage was performed daily using the panel and methods described in the section "Effect of addition of spices on the acceptability of *burukutu*". Tests were performed in triplicates and each container was discarded after one sampling for tests.

RESULTS AND DISCUSSION

Isolation of microorganisms associated with burukutu fermentation

Colony type considered dominant on MRS agar was cream to white under microaerophilic conditions. Microscopically they were Gram-positive nonspore-forming, rod shaped

and non-motile. Other characteristics were production of gas from glucose, negative catalase reaction, growth at 45°C, tolerance of 0.3 and 10% bile salts and inability to hydrolyze starch. This organism was presumptively described as *Lactobacillus* sp. Creamy white irregularly shaped colonies growing on Acetobacter agar were considered as acetic acid bacteria. Further study of this colony revealed Gram-negative, rod-shaped cells, which were catalase positive and survived in 10% NaCl.

Two types of colonies were observed on the malt extract agar plates; large mold colonies of different morphologies and a smaller smooth colony. Microscopic examination of the smaller colony type revealed ovoid budding cells suggesting it to be a yeast. The isolate fermented glucose and sucrose but failed to ferment lactose and raffinose. This yeast is probably *Saccharomyces* sp. The predominant mold was moderately rapid growing, green and showed a velvety texture with a yellowish reverse. Microcopically, hyphae were septate, hyaline and conidiophores branched. Phialides were grouped in brush-like clusters at the ends of the conidiophores, characteristics suggestive of *Penicillium* sp. These microorganisms were similar to those usually isolated from *burukutu* (Van der Walt, 1956; Faparusi, 1970; Jideani and Osume, 2001; Kolawole*et al*., 2007; Eze*et al*., 2011; Atter*et al*., 2014)

Screening of spice extracts for antimicrobial activity against microorganisms isolated from burukutu

Extracts of three spices, *utazi*, *nche* and lemon grass did not show antimicrobial activity against most of the test organisms comparing their zones with those produced by the antibiotic controls in plate assays. Extracts of ginger and alligator pepper in contrast were active, even though none demonstrated absolute selectivity against any specific test organisms. However, they demonstrated different degrees of activities against the different organisms tested, according to their inhibition zone diameters. Ginger was more strongly inhibitory to AAB and the molds and less strongly inhibitory to the LAB and yeasts. AAB and molds are known to play only spoilage roles in *burukutu*. Alligator pepper showed a similar pattern of activity but however, strongly inhibited yeast growth (Table 1).Yeast growth is responsible for the characteristic effervescence of *burukutu* and is therefore desirable. The differences in the inhibitory activity of spices used in this study against tested organisms may be related to their varying chemical compositions. The EOs of the more antimicrobially active spices, ginger, alligator pepper and lemon grass seem to be dominated by terpenoids. Ginger from the work of Onyenekwe and Hashimoto (1999) is composed of zingiberene (29.5%) and sesquiphellandrene (18.4%), while 82.6% of Alligator EO is composed of humulene and caryophyllene (Ajaiyeoba

Table 1: Antimicrobial activity (as diameter of zone of inhibition, mm) of spice extracts against microorganisms isolated during *burukutu* fermentation

	Spice Extract / Diam. Inhibition zone (mm)						
Test organism	**Ginger**	**Alligator pepper**	**'Utazi'**	**Lemon grass**	***Sacoglottisgabonensis***	**Ampicillin**	**Cycloheximide**
Lactobacillus	14±0.6	14±1.5	Nil	7±1.0	Nil	24±1.0	
Acetobacter	28±1.5	28±2.0	7±1.2	10±2.1	10±2.0	30±2.0	
Saccharomyces	16±1.0	30±2.0	Nil	14±2.0	Nil		28±1.5
Penicillum	24±2.0	18±1.2	8±1.7	Nil	6±2.0		26±2.0
DMSO [Control]	Nil	Nil	Nil	Nil	Nil		

and Ekundayo, 1999). Lemon grass EO contains mainly geranial (37.71%) and neral (33.17%) according to a study by Shaaban *et al*. (2013). On the other hand, among the least active spices; *utazi* is dominated by linear aliphatic compounds (27.06%) and unsaturated fatty acids (Adeleye *et al*., 2011; Ezekwe *et al*., 2014), while *nche* contains phenolic compounds, bergenin and gallic (Tchouya*et al*., 2016).

Observations from this study, suggest the potential of partially purified spice extracts as preservatives for many traditional foods fermented with mixed starters, in which there is a need to inhibit spoilage and pathogenic microorganisms while cultures imparting character or health benefits remain active. Such foods include palmwine, *kombucha* and *sauerkraut.*

Effect of addition of spices on the acceptability of burukutu

Mean sensory analyses scores from the panelists were; $7.7037^{a}\pm0.39>7.2963^{a}\pm0.49>6.1481^{b}\pm0.49>5.7778^{b}\pm0.50>4.6667^{b}\pm0.55$, respectively for Control>Ginger>Alligator pepper>Lemon grass >*Utazi* flavored *burukutu* samples. This result shows that the unflavored samplewas the most acceptable to the panelists used during this study. This panel was drawn from regular *burukutu* consumers and their decision may be associated with resistance to drinking habits. However, it is noteworthy that no statistical difference ($p<.05$) was recorded for this sample and the ginger flavored sample. *Nche* flavored samples were noticeably dilute and were not tested.

Effect of addition of ginger on the growth of microorganisms associated with burukutu

The addition of ginger during the brewing of *burukutu* reduced the growth rate of all groups of organisms studied, albeit to different extents. The LAB and the yeasts were less severely affected as expected from the results of the plate assays of antimicrobial activity. These organisms showed lower counts in the flavored *burukutu* than in the control during respective days of fermentation or spoilage. Both organisms also seemed to reach their death phases later in the flavored samples (Fig. 1). This enabled

the presence of active yeasts, desirable for the production of effervescence and vigor in the drink. Ginger showed a stronger inhibitory activity on the growth of molds and the AAB, organisms associated with only spoilage. Onset of mold growth was delayed for one day and AAB growth was delayed for as much as four days (Fig. 2). There were also lower counts of these organisms in the flavored sample in comparison to the control sample for respective days of storage. The reduction in the rate of microbial growth in the flavored sample was very likely to have contributed to its better shelf life.

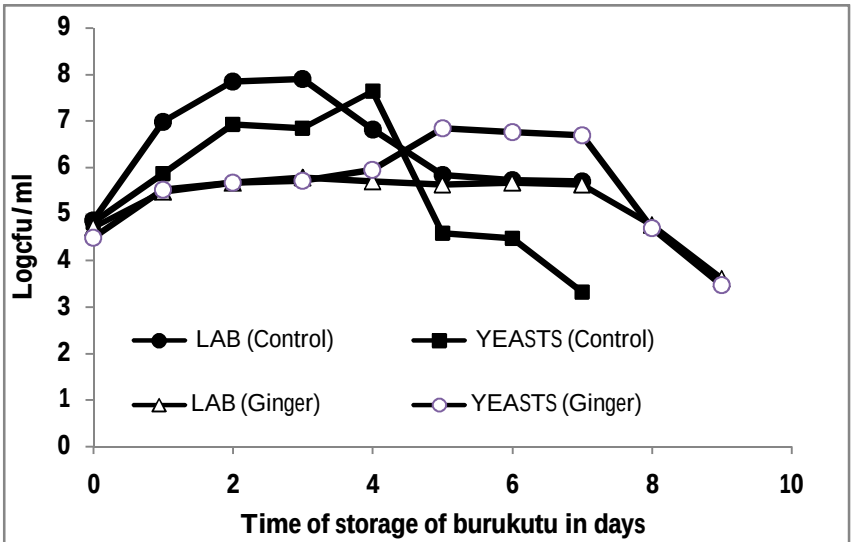

Fig. 1: Effect of addition of ginger on the growth of LAB and Yeasts during fermentation of *burukutu*

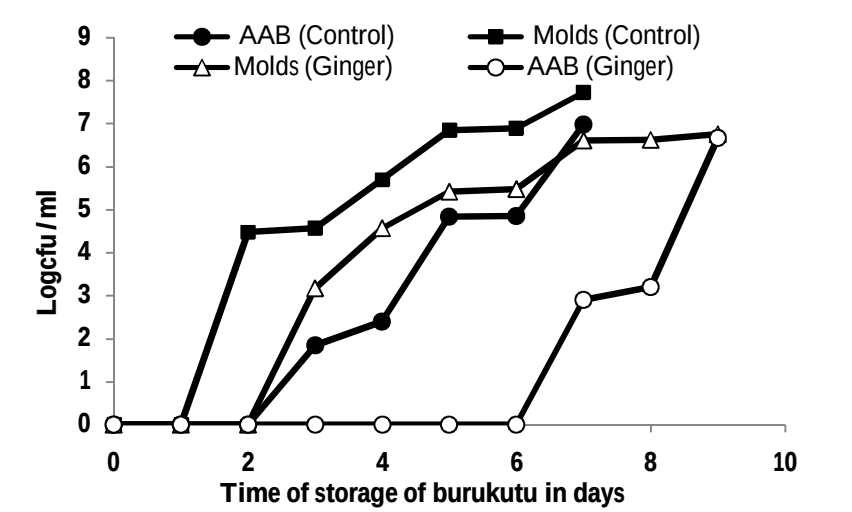

Fig. 2: Effect of addition of ginger on the growth of AAB and Molds during *burukutu* fermentation

Shelf life of burukutu

Mean score given by the panelists during sensory evaluation showed that *burukutu* brewed with ginger remained acceptable to them after four days of storage at room temperature, scoring at least 6/9 or "like slightly" on the Hedonic scale. The control sample scored at least 6/9 for only one day. On the basis of this test, ginger extended shelf life of *burukutu* for at least three days. This length of time would permit distribution of this product over a wider area and hence encourage possible scale up of the business of *burukutu* production. Other characteristics showed longer periods of shelf life extension. Pellicles were observed in the control samples within four days while the flavored samples showed this symptom of spoilage in nine days. Changes in pH, TA and ABV were slightly faster in the control than in the flavored sample of *burukutu* (Table 2).

Generally, the overall mechanism of preservation by the addition of ginger would most probably be related to the provision of an additional "hurdle" to those already existing from the presence of alcohol and a low pH in *burukutu* due to fermentation. Earlier reports of shelf life extension using spices include Edward and Ohaegbu (2012) who used ginger and garlic to extend shelf life of Kunun-zaki by approximately four (4) days.

CONCLUSION

This work has demonstrated that spices can serve as sources of preservatives, which can be used to extend the shelf life of foods such as *burukutu*. Further studies involving the extraction and purification of active components from these spices will provide substances, which will be useful in preservation of modern health foods in which spoilage and pathogenic microorganisms need to be inhibited while cultures imparting character or health benefits remain active.

Table 2: Effect of ginger extracts on pH, Titrable acidity (TA) and Alcohol content (ABV) of *Burukutu* during storage

		Storage time in Days									
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Flavored *burukutu* (Ginger extract)	pH	3.85± 0.12	3.81± 0.14	3.72± 0.11	3.70± 0.12	3.70± 0.08	3.68± 0.14	3.66± 0.10	3.65± 0.12	3.64± 0.09	3.62± 0.15
	TA (%)	0.51± 0.08	0.60± 0.10	0.64± 0.12	0.67± 0.10	0.71± 0.16	0.72± 0.15	0.73± 0.11	0.76± 0.14	0.77± 0.18	0.78± 0.11
	ABV (%)	2.05± 0.14	2.95± 0.11	3.46± 0.08	3.67± 0.14	3.80± 0.12	4.10± 0.16	4.00± 0.10	3.92± 0.10	3.91± 0.15	3.91± 0.16
Control (No extract)	pH	3.82± 0.09	3.71± 0.12	3.70± 0.16	3.66± 0.18	3.66± 0.20	3.65± 0.22	3.63± 0.13	3.60± 0.18	3.55± 0.08	3.52± 0.11
	TA (%)	0.60± 0.07	0.65± 0.09	0.71± 0.14	0.74± 0.09	0.76± 0.08	0.79± 0.10	0.80± 0.11	0.81± 0.13	0.81± 0.09	0.80± 0.11
	ABV (%)	2.05± 0.17	3.01± 0.24	3.86± 0.20	4.23± 0.17	4.20± 0.16	4.10± 0.22	3.91± 0.22	3.82± 0.17	3.71± 0.18	3.67± 0.22

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