European Journal of Food Science and Technology
Vol.2,No.2, pp.13-22, September 2014
Published by European Centre for Research Training and Development UK (www.eajournals.org)

MICROBIOLOGICAL AND PHYSICOCHEMICAL STUDIES OF TWO NIGERIAN FERMENTED ALCOHOLIC DRINKS (PALMWINE AND BURUKUTU) IN EKITI STATE, NIGERIA.

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ABSTRACT: *Palm wine samples were collected from five (5) different towns in Ekiti State while burukutu were also collected from three different towns in Ekiti State. The pH of the palm wine ranged from 4.49 in sample PWB (Are- Ekiti) to 5.23 in PWD (Ikere-Ekiti). In the chemical analysis, total alkalinity ranged from 0.03% in PWA (Ado-Ekiti), PWD and PWE (Ikole-Ekiti) to 0.06 in PWB. Total solids ranged from 10.67^0 Brix in PWA to 16.57 in sample PWC (Ifaki-Ekiti). Total reducing sugar ranged from 10.81% in sample PWA to 18.94% in PWC while protein ranged from 0.31mg/l in PWE to 0.34mg/l in PWB and PWC. In the total bacteria count (TBC), it ranged from 0.9 X 10^5 cfu/ml in PWC to 2.3 X 10^5 cfu/ml in PWA. TBC in burukutu has the highest value of 8 in sample BK (Ado -Ekiti) and lowest of 4.9 in sample BM (Ikere-Ekiti). Total yeast count, ranged from 1 in PWB to 6 in PWD. For the burukutu sample, it ranged from 4 in sample BL (Ikole-Ekiti) to 88 in BK. In all, 2.9 x 10 microorganisms were isolated from the palm wine samples with 1.2 x 10 yeast cells and 1.7 x 10 bacteria cells. From the microbial load on the samples, 5 bacteria genera were isolated. Consumption of these alcoholic beverages are therefore not safe, as contaminants have been observed in the drinks and these contaminants and pathogens are dangerous as consumption can result to health hazard .*

KEYWORDS: Palmwine, Burukutu, Bacteria, Yeast and Alcoholic Beverages.

INTRODUCTION

Palm wine is a whitish, effervescent alcoholic beverage produced by the spontaneous fermentation of the sap of tree pals (family: Palmae). It is consumed in tropical and sub-tropical countries where palm grows. These include South America, Asia and Africa (1). It is refer to as toddy in Asian countries and tuba in South America. The type of palm which the fermented sap is consumed varies in different parts of the world according to the type of palm wine obtained from oil palm (*Elaeis Guineensis*), and raphia palm (*Raphia vinifera*) and Palmyra palm (*Borassus flaellifer*). Palm wine is a general name for alcoholic beverages produced from the sap of palm trees. It is different from the grape wines in that it is opaque. It is consumed all over the tropical world in Africa, Asia and South America. Palm wine is usually whitish and effervescent liquid both properties were derive from the fact that organisms are numerous and alive when the beverage is consumed (2).

Palm wine differs from conventional beers and table wines produced in the modern brewery and winery in three ways. First, the media fermented for such beers are usually grains and for the wines, grapes and fruit juice are used in wine. The basic principle is however, is the same: a sugar solution is fermented, essentially by yeast (*Saccharomyces spp.*). Secondly, whereas there is control of fermentation during the production

of modern beers and wine. Fermentation of palm wine is not controlled. Thirdly, the European beer and wines are usually clarified by removing microbial cells and other suspended material. Palm wine on the other hand is consumed without such clarifications. In essence, the basic differences between the true wines and the palm wine are a matter of technological differences between wine-making techniques and palm wine production methods. The methods of producing palm wine are likely to continue to change with enhanced technological advancement of the consuming countries (3).

During fermentation the yeast *Saccharomyces cerevisiae* produces a broad range of aroma-active substances, which are vital for the complex flavor of fermented beverages such as beer, wine and sake (4). Flavour active substance produced by fermenting yeast cells can be divided into six groups, sulfur-containing molecules, phenolic compounds and volatile esters (5). Although volatile esters are only trace compounds in fermented beverages, they comprise the most important set of derived aroma-active compounds. Volatile esters are of major industrial interest because they are responsible for the highly desired fruity, candy and perfume like aroma character of beer, wine, and sake (5). Flavour in the most important distinguishing characteristics of most fermented foods, flavour is usually classified according to the source of different compound contributing to it. This include flavour contributed by the substrate pre-fermentative flavour (compound formed during extraction and conditioning of substrate), fermentative flavour (produced by yeast and bacteria during alcoholic and malolactic fermentation) and past, fermentative flavour (compound that appear during the storage period) (6). Post fermentative flavour is usually a result of enzymatic. Is a complex phenomenon involving a number of factors in particular, it depends on the nature and concentration of the compounds initially present in the substrate, the capacity of the yeast to transform them and the fermentation condition employed.

Yeasts are eukaryotic microorganisms classified as fungi, with over 1,000 species currently known and described. It is believed that the species described so far represent only about 1% of all 1.5 million yeast species believed to exist on earth (7). Yeasts are unicellular fungus that reproduces either asexually by budding and transverse division (binary fission) or sexually through spore formation (8). Although most yeast are unicellular, some species with yeast forms may become multicellular through the formation of a string of connected budding cells known as pseudohyphae, or false hyphae, as seen in moulds. Yeast size can vary greatly depending on the species, typically measuring 3-4µm in diameter, although some yeast can reach over 40µm. The yeast species *Saccharomyces cerevisiae* has been used in baking and fermenting alcoholic beverages for thousand years (9).

Burukutu

This is a popular alcoholic beverage of vinegar-like flavour, consumed in Northern Guinea Savannah region of Nigeria, in the republic of Benin and Ghana. The preparation of burukutu involves steeping sorghum grains in water over night, following which excess water drained (10). The grains are then spread out onto a mat or tray, covered with banana leave and allowed to germinate. During the germination processes, the grains were watered on alternate days and turned over at intervals. Germination continues for four to five days until the plumule attain a certain length. The malted grains are spread out in the sun to dry for one to two days, following which the dried malt is ground to powder. Garri (a farinaecious fermented cassava product) is added to the mixture of the ground malt and six parts of water. The resulting mixture is allowed to ferment for two days, following which it is boiled for two days. The resulting product is cloudy alcoholic

beverage (11). The pH of the fermenting mixture decreases from about 6.4 to 4.2 within 24 hours of fermentation and decreases further to 3.7 after 48 hours. At the termination of the 2-days maturing period, *Acetobacter species and Candida species* are dominant microorganisms (12). The consumption of indigeneous alcoholic drinks (Palm wine and Burukutu) in Ekiti-State and even in Nigeria is of high rate, and there is need to investigate the microbial contamination of the drinks. Contamination occurs if the environment where the drinks have been prepared or sold or the handlers were of poor hygiene. These microbial contaminants can cause infection because of the toxins produces by these organisms. Therefore this research work was aimed to determine the microbiology and physicochemical characteristics of two Nigerian fermented alcoholic drinks (palm wine and burukutu).

Process flowchart for production of burukutu

Maize and sorghum.
↓
Soak for two days
↓
Malt(germinate) for five days.
↓
Grind or sun- dry and hold until used.
↓
Adjunct (gari) is added.
↓
Mix mash with cold water and boil for six to twelve hours
↓
Filter through a fine marsh.
↓
Cool filter.
↓
Ferment overnight (mixed natural inoculum).
↓
Boil for twelve hours.
↓
Cool concentrate and add starter (sediment from previous brew).
↓
Ferment for twelve to twenty four hours.
↓
Burukutu (11)

MATERIALS AND METHODS

Sample collection

Five samples of Palm wine was randomly collected from tappers in the Ekiti-State, in the southern-western region of Nigeria. The palm wine was harvested by the tappers using natural wood during tapping process using bamboo tube. After that sterile bottles were use to collect the palm wine and was kept in an icebox (4^0C) during transportation (30mins) to the laboratory. On the other hands, 3 burukutu samples were

collected. The physical and chemical property of each of the palm wine sample was determined within a day. The samples were aseptically filtered (with sterile Watman filter paper) and kept at 4^0C until analyses were carried out. Also, the microbiological analyses of the palmwine and burukutu samples were determined.

Preparation of samples

Samples of palm wine and burukutu were serially diluted. Dilutions 10^{-3} were used to inoculate plates of nutrient agar for total viable count, MacConkey agar for the total coliforms count and blood agar.

Physicochemical properties

This involved visual examination of the palm wine and burukutu samples. Color measurements of samples were carried out using a hunter lab clorflex colorimeter. The turbidity of the palm wine was estimated by measuring the transmittance at 650nm using a spectrophotometer as described by Taipaiboon (13). The taste and odour of the palm wine was also determined. The pH value was measured at ambient temperature with pH meter which was calibrated with pH 4.0 and 7.0. The total acidity was determined by titration with NaOH and phenolphytalin was used as an indicator which was calculated in term of lactic acid. The total soluble solids of palm wine sugar syrup were determined as degree Brix using hand refractometer. Total sugar and reducing sugar were quantified by titration with Fehling reagents. The results were expressed as gram of glucose per 100 gram of sample.

Bacteriological analysis

Bacterial plate count was carried out using the pour plate method. 20ml of molten nutrient Agar cooled at 41^0C was poured into each petri-dish containing 1ml of the sample. The medium and the inoculums were mixed which was then allowed to set after which they were incubated in inverted position at 37^0C. After 24 hours of incubation, the plates were counted and colonies were inoculated to the slant. The isolated organisms were identifying based on the morphological and biochemical observation.

Identification of organisms in palm-wine and burukutu

Isolation and identification of the organisms were done using standard morphological and physiological test. The test used in the identification of bacteria including morphological, gram reaction, mobility, aerobic growth, anaerobic growth, catalase, oxidation and fermentation of simple sugars. Standard pour plate comb were used to determine bacteria load, while spread plate was used to determine yeast load.

RESULTS AND DISCUSSION

Palm wine is produced by natural fermentations from the clear, sugary saps of various palm trees (14). After collection, the sap turns white from the growth of bacteria and yeast which are contaminants from the air, tapping utensils and normal flora of the tree. The wine usually turns sour within a short period due to the acid produced by the microorganisms (15).

From the table 1, it was shown that the physical properties were significantly different among samples, except for the taste which all have sweet taste. The pH of the palm wine samples also ranged from 4.49 in sample PWB to 5.23 in sample PWD and temperature ranged from 31.8 in sample PWC to 34.1 in sample

PWE. In the chemical analysis, the total alkalinity of the sample ranged from 0.03% in sample PWA, PWD, and PWE to 0.06 in sample PWB. The total solids ranged from 10.67^0 Brix in sample PWA to 16.57 in sample PWC. Total reducing sugar shows that sample PWD has the lowest value of 0.88% and the value ranged from 10.81% in sample PWA to 18.94% in sample PWC while the protein ranged from 0.31mg/l in sample PWE to 0.34mg/l in sample PWB and PWC as was presented in table 2. It is generally known that the primary sources of invertase are from yeast such as *saccharomyces cerevisiae, saccharomyces carlsbergensis* and fungi such as *Aspergillus oryzae* and *Aspergillus niger* (16). Moreover, an increase in total acidity and decrease in pH are also responsible for the inversion reaction. The inversion reaction occurs when the glucosidic linkage of disaccharide is hydrolyzed, releasing the monosaccharide units. In the chemical quality parameters analyzed such as pH, total acidity, total soluble solids, reducing sugar, it was determined that the pH and the total acidity of all palm wine samples were significantly different among the samples, since lactic acid is the main organic acid present in palm wine samples which leads to the differences. Microorganisms, mainly lactic acid bacteria have produced organic acid (Lactic acid), which then increase in total acidity and decrease in pH value. Normally, natural palm wine showed neutral pH approximately 7 as reported by Jitbunjerdkul (17) and Leaskan *et al.,* (18). Hence, a high percentage of total acidity and low pH indicates the initial fermentation step of palm wine, for example, during collection time. In the protein content which also showed variation in all the samples, the variation may be due to the different source of the palm wine samples. In addition, microorganisms may use protein as a carbon source or as a nitrogen source for their metabolism and genetic material as earlier reported by Adams and Moss, (19)

In the total plate count, observed in palm-wine sample analyzed, the total bacteria count ranged from 0.9 X 10^5cfu/ml in sample PWC to 2.3 X10^5cfu/ml in sample PWA while the mean values is 1.8X10^5cfu/ml. The total bacteria count in burukutu has the highest value of 8 in sample BK and the lowest value of 4.9 in sample BM with the mean value of 6.5. For the total yeast count, the value ranged from 1.0 in PWB to 6.0 in PWD, with the mean value of 3.2. In the burukutu sample, it ranged from 4.0 in sample BL to 88.0 in sample BK with the mean value of 6.3

Burukutu is a beverage produced by the fermentation of malted or germinated cereals. The commonest cereals used as sorghum and millet. The organisms which are responsible for producing the beverage are lactic acid bacteria and yeast (20). For the burukutu sample, the value ranged from 4 in sample BL to 8.0 in sample BA with the mean value of 6.3 as was shown in table 3. Table 4 also shows the biochemical characteristics of the Bacteria isolated according to Holt et al. (21). In all, 2.90x10 microorganisms were isolated from the palm wine samples with 12.0 yeast cells and 17.0 bacteria cells. Uzochukwu *et al.,* (22) suggested that the microorganisms are important for the fermentation of palm sap were mainly *saccharomyces* yeast and lactic acid bacteria. Table 5 shows the frequency and the percentage distribution of bacterial and the yeast isolated from the palm wine and burukutu samples, five bacterial genera were isolated and the most occurrences bacterial was *Lactobacillus spp.* with 35.3% distribution. *Acetobacter spp.* with 23.5% distribution, *Leuconostoc spp.* and *Corynebacterium spp* with 17.6% distribution respectively. *Listeria spp*. is the least among the bacteria isolated with percentage distribution of 5.9.In the yeast isolated, 12.0 *saccharomyces spp.* were isolated from the palm wine and burukutu with *saccharomyces cerevisiae* and *saccharomyces carlbsbergensis* has the percentage distribution of 50 % respectively.

The presence of *Listeria spp*. in burukutu is potentially dangerous as a source of human disease, either directly upon consumption of the alcoholic drink, or indirectly through secondary contamination of utensils, processing equipment, or other foods. A further risk arises through introduction of the carrier state in food handlers. 'Cross-contamination' of foods within food-processing establishment is also a significant hazard, the inocula usually being derived from incoming untreated foods or from air-borne or surface-borne contact with food ingredients (2).

According to Moy (23) it was reported that *Listeria* spp frequently cause food-borne infections. To improve the situation, firstly the deplorable hygienic conditions which lead to food contamination must be changed, or probably hygienic conditions were not observed at all. In ensuring the microbiological safety of food, proper hygienic conditions must be observed (24).

The lactic acid bacteria have been shown to be responsible for the consistency and soluble white coloration of palm wine through their production of gum likely dextrans in the early stage of fermentation in the beverages, which change the consistency and the color from transparent to whitish. In addition, a heavy suspension of yeast and bacteria also gave a milky-white appearance (18). This phenomenon was also contributed to the increase in turbidity of palm sap. Fermentation of palm wine can lead to the production of ethanol which is a volatile flavour compounds. During the collecting process, it is highly susceptible to spontaneous yeast lactic fermentation of the sugar sap. This process is reported to be rapid under sunlight. Sources of fermenting microorganisms are tapping implement (Knife and Bamboo tube) and air. Additionally, Sanarajeewa *et al.*, (25) reported that palm-wine undergoes spontaneous two stages of fermentation. The first is lactic fermentation and subsequently fermented by yeasts to produce ethanol.

Microbial analysis at different stages of palm wine fermentation was done by Uzochukwu et al. (22). They reported that the organisms found in palm wine in the early stage of fermentation (sugar 12%, pH 7-7.2) are mostly entirely *Leuconostocs* and *Lactobacilli* as well as a small proportion of incompletely identified fructans-producing bacteria which produced no acid in pure culture in sterile palm wine.

Since palm wine and burukutu are produced locally in most part of the country, proper cleaning of the rudimentary utensils used must be observed and must be kept in a clean place under sanitized conditions. Cracks and holes in these utensils should be prevented as these could harbour microorganisms and could duly contaminate the final product. Microbiological safety of fermented alcoholic drinks must be ensured by observing proper hygienic conditions during the preparation or processing of the drinks. Handlers of the drinks must be of good health and must obey all rules of personal hygiene, they must be free of any disease and undergo regular routine medical checkup. During preparation of the drinks or during the serving of the drinks to the consumers, they must wear good and protective clothing to prevent contamination of the drink by their body flora.

It must be ensured that the handlers, whenever in the processing or serving premises, are always on neat caps to prevent hairs falls thereby preventing contamination of the drinks. Proper contamination of the environment must be observed in other to ensure the microbiological safety of the produced drinks. Equipment and utensils used in the production of the drinks must be adequately sanitized. Also, the raw materials used in the production of these drinks especially burukutu must be adequately inspected and must

be certified appropriate for the production. Adherence to this practices will definitely prevent contamination of the products (i.e the fermented drinks) or will bring to a minimum safety level, the microbial load which might occur in the final product. Consumers of the local palm wine should be watchful of the environment of the sellers shop before buying the palm-wine.

Table 1: The physical properties of the palm-wine sample

Samples	pH	Temperature	Taste
PWA	5.10	32.4	Sweet
PWB	4.49	33.1	Sweet
PWC	4.60	33.8	Sweet
PWD	5.23	32.9	Sweet
PWE	4.87	34.1	Sweet

Table 2: Chemical quality of palm wine samples

Sample	Total Alkalinity(%)	Total solids % (Brix)	Reducing sugar (%)	Total sugar (%)	Protein (Mg/L)
PWA	0.03	10.67	0.99	10.81	0.33
PWB	0.06	15.93	1.85	14.32	0.34
PWC	0.05	16.57	1.74	18.94	0.34
PWD	0.03	12.07	0.88	11.72	0.32
PWE	0.03	12.40	1.15	12.60	0.31

Table 3: Total plate count and the total yeast count in the palm wine samples

Samples	Total plate count (TPC) 10^5cfu/ml	Total yeast count 10^4cfu/ml
PWA	2.3	2
PWB	1.4	1
PWC	0.9	4
PWD	2.1	6
PWE	1.8	3
Mean	1.7	3.2
BK	8	8
BL	6.6	4
BM	4.9	7
Mean	6.5	6.3

Table 4: Biochemical characteristics of the Bacteria isolates from Burukutu and Palmwine.

1solates	Spore	Indole	Ucrease	Voges proskauer	Methyl red	Maltose	Glucose	Fructose	Sucrose	Lactose	Galatose	Xylose	Probable Bacteria
PWA2	-	-	+	+	-	+	-	-	+	-	-	-	*Lactobacillus spp*
PWA4	-	-	+	+	-	+	-	-	+	-	-	-	*Lactobacillus spp*
PWB1	-	-	-	-	+	-	+	+	-	-	-	-	*Acetobacter spp*
PWB3	-	-	+	+	-	+	-	-	+	-	-	-	*Lactobacillus spp*
PWB5	-	-	+	+	-	+	-	-	+	-	-	-	*Lactobacillus spp*
PWC1	-	-	-	-	+	-	+	+	-	-	-	-	*Acetobacter spp*
PWC2	-	+	-	-	-	-	-	-	+	+	-	+	*Leuconostoc spp*
PWC3	-	-	+	+	-	+	-	-	+	-	-	-	*Lactobacillus spp*
PWC4	-	+	+	-	-	-	-	-	+	+	-	+	*Leuconostoc spp*
PWD4	-	-	+	+	-	+	-	-	-	-	-	-	*Lactobacillus spp*
PWD5	-	-	-	-	+	-	+	+	-	-	-	-	*Acetobacter spp*
PWE2	-	-	-	-	+	-	+	+	-	-	-	-	*Acetobacter spp*
PWE3	-	+	+	-	-	-	-	-	+	+	-	+	*Leuconostoc spp*
BK						-	-			+			*Listeria spp*
BL						-	-			-			*Corynebacterium* spp
BM						-	-			-			*Corynebacterium* spp

KEY

PWA- Isolates from Palmwine sample collected at Ado- Ekiti
PWB- Isolates from Palmwine sample collected at Are- Ekiti
PWC- Isolates from Palmwine sample collected at Ifaki- Ekiti
PWD- Isolates from Palmwine sample collected at Ikere- Ekiti
PWE- Isolates from Palmwine sample collected at Ikole- Ekiti
BK- Isolates from Burukutu sample collected at Ikere Ekiti
BL- Isolates from Burukutu sample collected at Ikole Ekiti \
BM- Isolates from Burukutu sample collected at Ado Ekiti
- (Negative test reaction) + (Positive test reaction)

Table 5: Frequency and the percentage distribution of bacterial and the yeast isolated from the palm wine and burukutu sample

Microorganisms/ Bacteria	Frequency	Percentage distribution (%)
Leuconostoc spp.	3	17.6
Lactobacillus spp.	6	35,3
Acetobacter spp.	4	23.5
Corynebacterium spp.	3	17.6
Listeria spp.	1	5.9
Yeast		
Saccharomyces cerevisiae	6	50
Saccharomyces carlbsbergensis	6	50

CONCLUSION

According to the microbiological safety of the palm-wine and burukutu observed in this research work, the palm-wine and burukutu samples were not up to the standard required. Consumption of palm-wine and burukutu is therefore not safe, as contaminants have been observed in the drinks and these contaminants are dangerous as they can cause diseases. When consumed e.g *Salmonellosis* / thyhoid fever caused by *Salmonella* spp, *Listeriosis* caused by *Listeria* spp. It is therefore safer not to consume palm wine and burukutu if it is observed by the consumer that the premises where the beverages were being prepared or sold is not clean and if the handlers are of poor personal hygiene.

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