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Organic Contaminants and Microbial Load of Native Beers Locally Prepared Within Jos Metropolis

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Authors' contributions

This work was carried out in collaboration between all authors. Author SYG designed the work and analyzed the samples. Authors KHJ and SGM collected and processed the samples. Each author proofread and edited the draft manuscript before submission for publication in this Journal.

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ABSTRACT

Introduction: Ethyl alcohol toxicity is implicated in impaired immune system, disease conditions such as hepatitis, cirrhosis, and cancer. The incidence of these ailments among drinkers of native beers: *burukutu*, *pito*, and *goskolo* in the area of study are high. *Goskolo* is banned lethal liquor secretly consumed by native beer drinkers.
Aim: This work was performed to ascertain the presence of some organic contaminants in native beers.
Study Design: The work is descriptive in nature.
Place and Duration of Study: Samples were obtained from vendors in Jos metropolis of Jos North Local Government Area of Plateau State, Nigeria from June 2015 to August 2015.
Materials and Methods: Thin layer chromatography (TLC), culture media, Gram staining, catalase and biochemical tests were applied to achieve the aim of the work.
Results: Results indicated the presence of not only ethyl alcohol but of ethanal, *Streptococci, Candida krusei, Candida pseudotropicalis, Candida tropicalis* and *Lactobacilli* in *burukutu* and *pito* samples. No microbial growth was observed for the control. There were no spots on TLC plates for *goskolo*. There were other spots on the TLC plates for *burukutu* and *pito* samples which did not match the R_F values of ethanol, acetate and ethanal standards implying the presence of other

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contaminants in the samples. *Candida* and *Streptococci* species are pathogenic, ethanal is toxic, and presence of other spots on TLC plates could be for other toxicants in the samples.
Conclusion: Contaminants in the native beer samples may contribute significantly to the observed complications of disease conditions associated with chronic consumption of native beers.

Keywords: Burukutu; Pito; Goskolo; contaminants; Streptococci; Saccharomyces; Lactobacilli.

1. INTRODUCTION

Foods, including native beers, become hazardous when contaminated by biological agents such as bacteria, fungi and their toxins. For example, they may contain mycotoxin contaminants such as zearolenone, citreoviridin, moniliformin and ochratoxins, which are secondary metabolites of *Aspergillus flavus, Fusarium graminaerum*, *Pennicillium citreo-viride*, *Fusarium moniliforme* and *Aspergillus ochraceus* respectively, or bacterial toxins, produced by *Staphylococcus aureus* and *Clostridium botilinum* respectively [1,2,3].

2. MATERIALS AND METHODS

2.1 Experimental Design

Five samples each of *burukutu* and *pito* were purchased fresh from the field randomly in Jos metropolis. Qualitative analysis of *Burukutu*, *Pito* and *Goskolo* (banned liquor in the area) samples for acetate, ethanal and ethyl alcohol presence was done against the respective standards by thin layer chromatography.

2.2 Microbial Profile of Samples of *Burukutu* and *Pito*

Five (5) samples each of *burukutu* and *pito* were analysed for the presence of microorganisms. The following were used as culture media and diluents: Universal Beer Agar, UBA, [4,5], Nutrient Agar (NA), blood agar (UBA ACUMEDIA Manufacturers INC, Michigan 48912.) and sterile distilled water.

2.3 Preparation and Inoculation of Culture Media

2.3.1 Nutrient agar medium

14 g was dissolved in 500 ml of distilled water in a 1000 ml bottle. The bottle was made airtight and autoclaved at 121℃/in^2 for 15 minutes. Thereafter, it was removed and kept at room temperature to cool. The molten agar was then poured into ready-to-use sterile Petri dishes and allowed to solidify. The plates were subjected to sterility check by incubating them un-inoculated in an upside down position in an incubator at 37℃ for 24 hours. They were deemed not contaminated if no growth was observed the following day. The plates were then inoculated with native beer samples using sterile wire loop and incubated at 37℃ for 24 hours in oven.

2.3.2 Universal beer agar

Beer samples were diluted as follows: 1 ml aliquot of a beer sample was pipetted into a sterile 20 ml sample bottle followed by addition of 9ml sterile distilled water which was aseptically transferred, mixed thoroughly and allowed to settle. 1 ml of the diluted sample was measured using a 2 ml sterile pipette and transferred into labeled sterile Petri dishes; subsequently, 15 ml of sterilised molten UBA medium was poured and mixed gently with the diluted sample and the agar medium to ensure homogeneity and then allowed to solidify. The prepared Petri dishes were then incubated in an incubator at 37℃ in inverted position for 72 hours.

2.4 Gram Staining

Beer samples were stained both with primary stain (Gram stain) and secondary stain (safranin). Using immersion oil, slides were viewed under microscope (at×100 magnification).

2.5 Catalase Test

This was carried out on colonies thus: 3 drops of hydrogen peroxide (H_2O_2) were added onto a sterilised glass slide. With the aid of a sterile glass rod, some colonies of the organism were picked from the grown culture in the Petri dish and added on to the H_2O_2 solution on the slide. Observations were made and results recorded.

Biochemical tests of samples were performed by inoculating them in glucose, xylose, sucrose, galactose, urea slant, raffinose, dolcitol and inositol; thereafter, they were incubated at 37℃ for 48 hours in an incubator.

2.6 Determination of Volatile Organic Compounds in *Burukutu* and *Pito*

This was done by distillation followed by thin layer chromatographic method. Filtered samples were distilled at 78℃ over a period of 4 hours. Resultant distillates and residues were kept in labeled capped test tubes and refrigerated at 0℃ until needed for TLC analysis.

For the TLC analysis, glass plates were used as inert supports to each of which silica gel slurry of 0.25 mm thickness was layered having been prepared by mixing 50 g of the silica powder in 95 mls of distilled water. Plates were activated at 105℃ for 1 hour. Samples were spotted on the TLC plates using capillary tubes. Mobile phase used contained n-hexane/petroleum ether in the ratio 50:70 v/v respectively. Spotted TLC plates were placed vertically in an air tight chromatographic tank saturated with the mobile phase with level just below the origin. The set up ran for 55 minutes after which the solvent front was marked off immediately using pencil. To identify the spots on the TLC plates, 50% H_2SO_4 in water (v/v), 10% H_2SO_4 in methanol (v/v) and iodine crystals were used.

3. RESULTS

3.1 Organic Metabolites

Tables 1 and 2 bear results for TLC analysis of ethanol metabolites in *burukutu, pito* and *goskolo* respectively. Numerous spots were obtained for both distillates and residues. Among these spots, the relative fronts (R_F) values of some matched those of acetate, ethanal and ethyl alcohol standards. *Burukutu* had more spots than *Pito*. No spots were observed for *Goskolo* samples.

The result for *pito* indicated spots which were fewer than in *burukutu*; these spots meant, in addition to ethyl alcohol, that there were other components in the samples; residue of *pito* gave rise to three spots whose R_F values were 0.7, 0.5, and 0.1 respectively.

The R_F value for ethyl alcohol standard and that of residue number two spot were the same---hence *pito* contains ethyl alcohol even though there were other spots on the plate that did not correspond with those of the standards used indicating the likely presence of other components whose standards may not have been used during the experiment.

Table 1. Thin layer chromatographic analysis of *Burukutu* samples against ethanol, ethanal and ethanoic acid standards

Sample	R_F value
Ethanol standard	0.5
Distillate - 1	0.3
Distillate - 2	0.5
Residue - 1	0.6
Residue - 2	0.2
Ethanal standard	0.6
Distillate - 1	0.6
Residue - 1	0.2
Residue - 2	0.1
Ethanoic acid standard	0.3
Residue	0.7
Distillate	0.1

Table 2. Thin layer chromatographic analysis of *Pito* samples against ethanol, ethanal and ethanoic acid standards

Sample	R_F value
Ethanol standard	0.5
Distillate	No spot
Residue – 1	0.7
Residue – 2	0.5
Residue – 3	0.1
Ethanal standard	0.6
Residue	0.2
Ethanoic acid standard	0.2
Distillate	0.2
Residue	0.4

Table 3 bears results for *goskolo* samples where no spots were obtained. Perhaps an appropriate or suitable mobile phase was not used and therefore the seeming ‘zero’ spot.

Table 3. Thin layer chromatographic analysis of *Goskolo* samples against ethanol standard

Sample	(R_F) value
Ethanol standard	0.5
Goskolo1	No spot
Goskolo 2	No spot
Goskolo 3	No spot
Goskolo 4	No spot
Goskolo 5	No spot

Table 4. Biochemical tests results for microbial load of *Burukutu* and *Pito* samples

Sample	Glucose	Xylose	Sucrose	Galactose	Urea slant	Raffinose	Dolcitol	Inositol	Inference
B1	+	-	+	+	-	+	+	-	**S. cerevisiae present*
B2	+	-	+	+	-	-	+	-	*S. cerevisiae present*
B3	+	-	-	-	-	-	-	-	**C. krusei present*
B4	+	-	-	-	-	-	-	-	*C. krusei present*
B5	+	-	-	+	-	-	-	-	*C. krusei present*
PT1	+	+	+	+	-	+	-	-	*C. pseudotropicalis*
PT2	+	-	+	+	-	-	-	-	*S. cerevisiae present*
PT3	+	-	-	-	-	-	-	-	*C. krusei present*
PT4	+	-	+	+	+	-	-	-	*C. tropicalis present*
PT5	+	-	-	+	-	+	+	-	*C. pseudotropicalis*

**s = saccharomyces, *c= candida. *b = burukutu sample 1, 2, 3, 4 5.*pt = pito sample 1, 2,3,4,5*

Table 5. Microbial profile of native beer samples as determined by catalase test and Gram staining

Sample	Catalase test	Gram staining	Inference
B1 + H_2O_2	White effervescence	Large *cocci* in pairs	Yeast cells present
B2 + H_2O_2	White effervescence	Large *cocci* in pairs	Yeast cells present
B3 + H_2O_2	White effervescence	Short rods in chain; large colonies	Lactobacillus
B4 + H_2O_2	White effervescence	Large *cocci* in pairs	Yeast cells present
B5 + H_2O_2	No effervescence	Long *cocci* in chains noticed (gram positive)	*Streptococci* presence suspected
PT1 + H_2O_2	White effervescence	Large *cocci* in pairs	Yeast cells
PT2 + H_2O_2	White effervescence	Large *cocci* in pairs	Yeast cells
PT3 + H_2O_2	White effervescence	Large *cocci* in pairs	yeast cells
PT4 + H_2O_2	White effervescence	Large *cocci* in pairs	Yeast cells
PT5 + H_2O_2	White effervescence	Large *cocci* in pairs	Yeast cells
Control+H_2O_2	No effervescence	Nothing was seen	-

**b = burukutu sample 1, 2, 3, 4 5.*pt = pito sample 1, 2,3,4,5*

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3.2 Microbial Profile

The results of the screening for microorganisms in the samples are summarised in Tables 4 and 5 where different species of microorganisms were isolated in *burukutu* and *pito* samples; the most prevalent microorganisms in the samples were *Saccharomyces cerevisiae*, *Candida krusei, Lactobacilli strains,* and *Streptococci strains* in *burukutu.* In *pito* samples, *Candida pseudotropicalis, Candida tropicalis* were the dominant. *Goskolo* was not subjected to microbial analysis.

Saccharomyces cerevisiae, Candida krusei, Streptococci species, and Candida tropicalis were isolated in all the test groups but control. The control used was star® (a brand of factory-based lager beer). They were confirmed to be present using biochemical tests. Observations were compared with standard references in literature. Gram staining native beer samples indicated the presence of *Streptococci species* (gram positive), yeast cells, and *Lactobacillus*.

4. DISCUSSION

The aim of this work was to analyse for the presence organic contaminants including microorganisms present in native beer samples. Contaminants present in native beers could themselves be toxic. [6] reported contamination of native beers by iron; also, [7] reported contamination of traditional alcoholic beverages by both zinc and manganese. Alcoholic solutions of salts of cadmium, lead, iron and zinc caused adverse effects on some hepatic and nephrotic parameters [8].

The results of TLC of *burukutu s*amples indicated several spots some of which had identical relative fronts (R_F) values compared with those of standards of acetate (ethanoic acid), ethyl alcohol and ethanal (acetaldehyde)-- hence acetate (ethanoic acid), ethyl alcohol and ethanal were present in *burukutu.* The presence of ethanal (acetaldehyde) in *burukutu* samples suggests that drinkers are at risks of its toxic effects. Acetaldehyde is a metabolite of ethyl alcohol oxidation which is toxic and could cause damage to, especially the brain inducing behavioural abnormalities, impaired memory, and sedative effects; it is also a carcinogen [9]. Acetaldehyde, the major alcohol-reactive metabolite, has been detected in the intestine of Wistar rats after alcohol exposure [10,11]. Therefore, when drinkers of *burukutu* exhibit these abnormal behaviours and signs, it may be the result of synergistic action between acetaldehyde and ethyl alcohol or that of acetaldehyde. However, [12] reported that acetaldehyde modulates rather than mediates some of ethyl alcohol toxic effects whereas [13] reported that acetaldehyde does not contribute at all to the pharmacological effects of ethyl alcohol contending that *in vivo* concentration of acetaldehyde in target organs are insufficient to induce significant pharmacological actions. It readily reacts with the amino moiety of polypeptides/proteins and amino acids forming adduct thereby causing mutation and hence impaired function of the protein. It induces the deficiency of vitamin B1 which is critical to the function of the brain; furthermore, acetaldehyde induces deficiency of NAD^+ and niacin, the consequences of which is necrotic! Another metabolite of ethyl alcohol oxidation present in *burukutu* samples is acetic acid generated due to the catalytic action of aldehyde dehydrogenase, the enzyme that catalyses the oxidation of acetaldehyde (ethanal) to acetate. By its catalytic action, aldehyde dehydrogenase generates acetic acid which is a slow process. NAD^+ is the coenzyme required to activate both alcohol dehydrogenase and aldehyde dehydrogenase. In the conversion of acetate to acetylCoA, the equilibrium favours non-formation of acetylCoA and therefore acetate appears in blood whose accumulation causes acidosis.

Plate 1. Control; no growth after 24 hours of incubation at 37℃

That there were other spots with their corresponding R_F values suggest that *burukutu* and *pito* contain components other than ethyl alcohol alone; drinkers therefore ingest ethyl alcohol along with other undesirable components. The extent of contamination was

higher in the residues than in the corresponding distillates. In reality, sellers stir the product well before serving and hence the crude, not the distillate, is the one actually ingested. Food spoilage microbes, food utensils, packaging materials, and domestic water are sources of potentially toxic chemicals in foods [14].

Plate 2. Grown colonies on nutrient, MRS and blood agar after 24 hours of inoculation

Plate 3. Grown colonies on nutrient and blood agar after 24 hours of inoculation

In most cases, the catalase tests results for samples were positive (white effervescence on the slides) following addition of hydrogen peroxide solution [15].

Streptococci are pathogenic gram positive species which are implicated in meningitis, pneumonia, tonsillitis, septic arthritis, otitis media, and scarlet fever [16]; also cause colonisation of mucosal surfaces of the host naso-pharynx and upper airways [17,18,19]. Therefore, such conditions in drinkers of native beers might be due to this microbe or in synergy with alcohol since alcohol is known to suppress immune system [20].

Plate 4. Microbial culture 48 hours after inoculation

Plate 5. Microbial catalase test results after inoculation; effervescence formation following addition of H_2O_2 solution

5. CONCLUSION

From the foregoing, organic toxicants such as ethanal as well as *Streptococci* and *Candida* species, which are pathogenic, are present in native beers. Hence, consuming native beers predisposes the drinker to these pathogens more so that the sanitary culture of the 'brewers' of the native beers is generally suspect which favours microbial growth.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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