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Biochemical and microbiological analyses of *Burukutu* (native beer) and a brand of factory-based lager beer (STAR)

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ABSTRACT: Consumption of *Burukutu* (native beer) among the people of the area of study is a norm. The techniques applied in its production are crude and non scientific. As a result, undesirable compounds, which may themselves be toxic, could be produced as components of the 'brew'. Also, microbes present may be pathogenic. Ten samples were aseptically collected. A composite of 2 ml portions of each of nine (9) *Burukutu* samples was prepared for microbial count and identification. In the case of organic metabolites and alcohol content, individual samples were analysed. Spectrophotometry was applied to determine the alcohol content; mass spectrometry method was used to identify the metabolites generated. Nutrient agar and indole test were applied for aerobic plate count and microbial identification. The results obtained revealed three isolates in all the samples thus: *Escherichia coli*, *Bacillus* species and *Saccharomyces cerevisiae*. The alcohol content ranged between 2.0-2.6% (v/v). Metabolites generated in both *Burukutu* and factory-based lager beer included glycerine, 1-pentanol, methanol, 1-glyceraldehyde, trimethylene oxide, 1-butanol, toluene, benzene acetic acid Propanoic acid, 2-hydroxy ethyl ester, Heptafluorobutyric acid, n-Pentyl ester, 4-butoxy-5,7-dinitrobenzo[1,2,5] Thiadiazole, Benzene acetic acid, 2 butyl ester, ethyl alcohol. Both *Burukutu* contain undesirable metabolites which may synergise with alcohol making it toxic to the system. Personal and environmental hygiene will help reduce levels of contaminants in alcoholic beverages otherwise pathogenic microbes could grow in the liquor being consumed.

Keywords: Agar, alcohol, beer, *Burukutu*, lager, metabolites, microorganisms.

INTRODUCTION

Brewing of alcoholic beverages is a traditional process carried out in most African countries. These traditional beers are produced using sorghum, millet and maize as substrates. They differ from European beers in being opaque and having a thick consistency since they are not filtered clear (Fadahunsi et al., 2013).

The African opaque beers include *Burukutu* and *Pito* in Nigeria. *Burukutu* has been described as a popular alcoholic beverage of a vinegar-like flavor prepared and consumed in the Northern Guinea Savanna region of Nigeria (Holzapfel, 1997). It has a sour taste resulting from the action of the lactic acid bacteria (*Lactobacillus spp*.) and opaque color because of suspended solid and yeast materials (Haard et al., 1999). But these drinks are prepared using unscientific techniques. This could result in metabolic shift from a precursor to undesirable and harmful metabolites, whose synergetic effect with alcohol could exert some level of toxicity to the body (Gazuwa and Denkok, 2017). The sanitary culture of the 'brewers' is suspect which favours contamination of the drinks.

Thus, this work was designed to quantify the alcohol content, and qualitatively analyze the organic metabolites generated in both native and factory based lager beer. Microbial load of the samples was also analysed.

MATERIALS AND METHODS

Equipment

Weighing balance (model No. RL.1014), Centrifuge machine,

Spectrophotometer (model no. t60 visible spectrometer), Autoclave, complete Soxhlet apparatus, Pressure pump, Incubator, Microwave, Refrigerator, GCMS machine.

Chemicals and reagents

Nutrient agar, peptone water, distilled water, Lugols iodine, Kovac's reagent, absolute ethanol, safranin, crystal violet, silver nitrate, potassium dichromate, sulfuric acid.

Experimental design

In all, 10 samples (9 *burukutu* and 1 factory-based beer) were analysed for different organic metabolites and alcohol content. Only *burukutu* samples were used for microbial screening; factory-based lager beer was not.

Collection of samples

Samples of *burukutu were* purchased from local drinking parlours located in Jenta Adamu, Angwan Rukuba, British junction, Farin gada and Busa Buji areas in Jos and kept refrigerated at 5°C until when needed for analysis.

Treatment of samples before analysis

The samples were spun in a centrifuge at 2000 rpm for 30 minutes. The supernatant was separated from the pellet by decantation.

Determination of alcohol content

This was achieved by using spectrophotometric technique. From the standard curve generated at 560 nm (Figure 1), concentration of alcohol in the samples was determined by extrapolation.

Preparation of samples for mass spectrometry

The samples were partially distilled to reduce the amount of water and to enable the GCMS sensitivity to capture the alcohol and the compounds present. 1 µl of the sample was injected into the equipment which passed through the injector to the separation column. The automated machine separated the compounds and ionised them.

Serial dilution and inoculation of sample for culturing

The first step in serial dilution was gentle swirling of the tube containing the sample, to ensure that the cells were evenly distributed in the tube. Inoculums were incubated at 37°C for 24 hours.

Procedure for isolation of microorganism

On pure culture, microorganism was fixed to a microscope slide using a flame sterilized inoculating loop by flaming the loop into pure culture medium and smeared onto microscope slide. The smear was allowed to air dry and heat fixed by carefully passing it through flame. The microscope was set at objective x100 oil immersion and microbes observed.

Indole test

The indole test is a biochemical test performed on bacterial species to determine the ability of the organism to convert tryptophan into indole. This is carried out to test for the presence of *Escherichia coli.* The pH of the media was 7.2 at 25°C and rich in tryptophan. The microorganisms were sub- cultured from the Petri dishes into five bottles containing broth, one for each sample containing growth of microorganisms and incubated at 37°C.

RESULTS AND DISCUSSION

The thrust of this project work was to determine the organic metabolites, alcohol content and microbial load of *burukutu* samples consumed in Angwan Rukuba, Busa Buji and Farin gada areas of Jos North Local Government Area of Plateau State. The investigation revealed the mean alcohol content at 2.6%, 2.0%, 2.1% (v/v) for Angwan Rukuba, Busa Buji and Farin gada respectively. The result of the investigation is not in tandem with that of (Egemba and Etuk, 2007). Ababio (1990) reported that the percentage alcohol content of *burukutu* ranged from 2.2 to 2.4%, which is within the range of results obtained from this work. Alcohol consumption is a common practice in both rural and urban societies in Jos metropolis (Gazuwa et al., 2008). Chronic alcohol consumption is a major risk factor for the development of liver fibrosis, alcohol liver diseases (ALD), and hepatocellular carcinoma (HCC) (Hassan et al., 2002). Alcohol-dependent induction of cytochrome P450 2E1 (CYP2E1) leads to formation of acetaldehyde (Purohit et al., 2005). CYP2E1-dependent alcohol metabolism leads to increased hepatic oxidative stress due to the generation of reactive oxygen species (ROS) including hydroxyethyl radicals (McKillop and Schrum, 2009). ROS production and oxidative stress are central to alcohol liver disease (Sergent et al., 2001). During this condition, the activities of the antioxidant enzymes (SOD, CAT, GSH-Px, and GSH-Red) defense arsenal are sometimes overwhelmed (Ajiboye, 2010). Oxidative damage to cellular protein is one of the

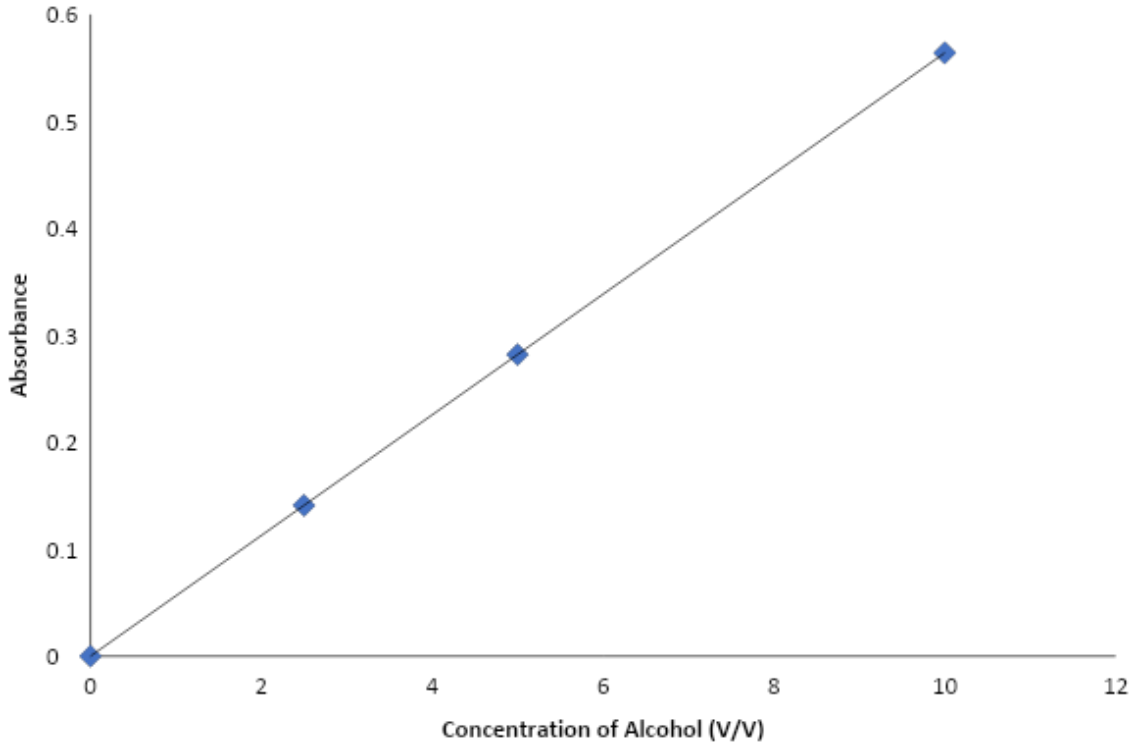

Figure 1. Standard curve showing the relationship between absorbance and concentration of alcohol.

Table 1. Alcohol content of burukutu samples.

Samples	Absorbance	Alcohol content
AngwanRukuba	0.153	2.6%
BusaBuji	0.106	2.0%
FarinGada	0.131	2.1%

The alcohol content of the samples was derived from the standard curve.

Table 2. Metabolites in samples of *burukutu* obtained from Angwan Rukuba area of Jos metropolis.

S/No.	Chemical formula of compound	Name of compound
1	$C_5H_{12}O$	1-Butanol, 3-methyl
2	C_5H_12O	1-Pentanol
3	$C_5H_{10}O_3$	Propanoic acid, 2-hydroxy ethyl ester
4	$C_3H_6O_2$	Acetaldehyde methoxy
5	$C_4H_5O_5$	Methyltartronic acid
6	$C_3H_8O_3$	Glycerine
7	$C_4H_{10}O_4$	1,2,3,4-butanetetrol
8	$C_3H_6O_3$	Glyceraldehyde

deleterious outcomes of chronic ethanol consumption (Abraham et al., 2002). Also, acute and chronic alcohol exposure has been shown to damage DNA in a variety of systems, cells, and species, including humans (Wu and Cederbaum, 2003). This could lead to irreversible loss of protein function and play a role in experimental ALD (Fataccioli et al., 1999).

Tables 1, 2, 3 show that *burukutu* produced from all the sites within the study area contains higher alcohol such as n-propanol, isobutanol, 2-methyl-1-butanol, 3-methyl-1-butanol. Regulation of higher alcohol biosynthesis is complex since they can either be produced as by-products

Table 3. Metabolites in sample of *burukutu* obtained from Faringada area of Jos metropolis.

S/No.	Chemical formula of compound	Name of compound
1	$C_2H_4O_2$	Acetic acid
2	$C_5H_{12}O$	1-Butanol, 3-methyl
3	$C_5H_{12}O$	1-Pentanol
4	$C_5H_{10}O_3$	Propanoic acid, 2-hydroxy ethyl ester
5	$C_4H_6O_5$	Methyl-tartronic acid
6	$C_3H_6O_3$	2-Hydroxy propanoic acid
7	$C_3H_8O_2$	1,3-Propanediol
8	C_3H_6O	Trimethylene oxide
9	C_3H_8O	Propylene oxide
10	$C_3H_8O_3$	Glycerine
11	$C_3H_8O_3$	Glyceraldehyde

Table 4. Metabolites in sample of *burukutu* obtained from Jenta Adamu area of Jos Metropolis.

Serial number	Chemical formula of compound	Name of compound
1	$C_2H_4O_2$	Acetic acid
2	$C_2H_7NO_2$	Ammonium acetate
3	$C_5H_{12}O$	1-butanol,3-methyl
4	$C_8H_{16}O_3$	Isoamyl lactate
5	$C_9H_{11}F_7O_2$	Heptafluorobutyric acid, n-Pentyl ester

of amino acid metabolism or via pyruvate and ethanol produced from carbohydrate metabolism (Stewart and Russell, 2000). Amyl alcohol is reported to be the most present and quantitatively significant flavor compound of higher alcohol groups. Active amyl and its isomer isoamyl alcohols are often times described as amyl alcohol. This compound affects palatability of the beer. Beer flavor is described by sensory analysis; when amyl alcohol content increases, it is considered heavy (Molina et al., 2007). Another higher alcohol that affects beer quality is isobutyl alcohol, and its undesirable effect can be perceived when its concentration in beer exceeds 20% of the total concentration of three other alcohols, including n-propanol, isobutyl, and amyl (Dack et al., 2017). There are two metabolic pathways for higher alcohol biosynthesis in *Saccharomyces cerevisiae*: one is through glycolysis and the other through amino acids metabolism. In both pathways, α-ketoacids are formed, decarboxylated to aldehydes, and dehydrogenated to produce the corresponding primary alcohol (Toh et al., 2018). Studies focused on the volatile compounds production by simultaneous fermentation with the yeast species, *Saccharomyces cerevisiae* and *Torulasporadel brueckii*, revealed that amongst the volatile compounds, alcohols account for more than 88% largest relative proportion (Malcorps and Dufour, 1992.The outcome of their findings is in tandem with this investigation. From Tables 4, 5 and 6, different metabolites were generated: ammonium acetate, isoamyl lactate, heptafluorobutyric acid for Table 4. In Table 5, they included 1-pentanol, propionoic acid-2-hydroxy ester, oxirene, methoxypropanal, glycerine, toluene and Phenylethyl alcohol. In Table 6, they included benzene alcohol, ethyl alcohol, acetate-2-butyl ester, hydroxyethyl ester and 1-pentanol. The common metabolite in samples in Tables 5 and 6 is toluene. Inhalation of toluene leads to a variety of neurologic manifestations such as tremor, ataxia, anosmia, sensorineural, dementia and epileptic seizures.

This study also reveals the generation of ester in samples from all the sites where samples were obtained. Esters are largely formed during the active phase of primary fermentation by the enzymatic condensation of organic acids with alcohols. Volatile esters in beer are divided into two major groups: the acetate esters and the medium-chain fatty acid (MCFA) ethyl esters. The acetate esters are synthesized from acetate with ethanol or a higher alcohol although dozens of different esters can be formed during the fermentation stage of any beer or spirit (Engan, 1981). The higher proportion of esters produced remains inside cells of lager yeasts (*Saccharomyces pastorianus*, *Saccharomyces carlsbergensis*, *Saccharomyces uvarum*), and in the fermenting medium for ale yeasts (*Saccharomyces cerevisiae*), which explains the higher complex flavor combination of ale beers (Nikulin et al., 2018). This investigation, as contained in Table 6, shows more esters in the factory based lager beer than in the locally prepared alcoholic beverage; which agrees with the findings of Nikulin et al. (2018). Among the carbonyl

Table 5. Metabolites in sample of burukutu obtained from BusaBuji area of Jos Metropolis.

S/No.	Chemical formula of compound	Name of compound
1	$C_4H_{12}O$	1-butanol, 3-methyl
2	$C_5H_{12}O$	1-pentanol
3	$C_5H_{10}O_3$	Propanoic, 2-hydroxy ester
4	$C_4H_6O_5$	Methyl tartaric acid
5	$C_3H_{16}O_3$	2-hydroxypropanoic acid
6	$C_4H_8O_2$	Oxirane
7	$C_4H_8O_2$	Methoxypropanal
8	$C_3H_8O_3$	Glycerine
9	$C_3H_6O_3$	1-glyceraldehyde
10	$C_8H_{10}O$	Phenyl ethyl alcohol
11	C_7H_8	Toluene

Table 6. Metabolites in factory based lager beer (STAR).

S/No.	Chemical formula of compound	Name of compound
1	$C_5H_{12}O$	1-butanol, 1,3-methyl
2	$C_5H_{12}O$	1-pentanol
3	$C_7H_{13}BrO_2$	Propanoic acid, 2-hydroxy ethyl ester
4	$C_{10}H_{10}N_4O_5$	4-butoxy-5,7-dinitrobenzo[1,2,5] Thiadiazole
5	$C_{10}H_{18}O_4$	Ethanedioxic acid dibutyl ester
6	C_6H_{14}	Pentane,3-methyl
7	C_7H_8	Toluene
8	$C_9H_{12}O$	Benzene ethanol, methyl
9	$C_{12}H_{16}O_2$	Tetracycloheptane
10	$C_{12}H_{16}O_2$	Benzene acetic acid, 2 butyl ester
11	C_2H_5OH	Ethyl alcohol

compounds, metabolites present in the samples include acetaldehyde and vicinal diketones, especially diacetyl. Carbonyl compounds exert a significant influence on beer flavour stability (Holt et al., 2018). High levels of carbonyl compounds cause stale flavour in beers (Bamforth, 2011). Mycotoxins contamination affects ester production which may occur at different stages of brewing. Many of them may be transferred from cereal grains to malt and then to beer due to their high-temperature resistance (aflatoxins, zearalenone, and deoxynivalenol) and water solubility (Rodrigo et al., 2015). The investigation of Pascari et al. (2018) is in tandem with the outcome of this investigation as there are a lot of microbial isolates from the *burukutu* samples.

Sulphur compounds are secondary metabolic products of yeast strains produced during wort metabolism and many such compounds make a significant contribution to beer and fresh spirit flavor. Although small amounts of sulphur compounds are components of alcoholic beverages, they can cause unpleasant off-flavors (Kuzdralinski et al., 2013). From this work, Sulphur containing compounds are not produced in both factory based lager beer and locally 'brewed' *burukutu,* which is at variance with the findings of Russell and Stewart (2014).

From Table 7, the high mean microbial count recorded indicates high level of contamination with bacteria and fungi. The report of Gazuwa and Denkok (2017) revealed that the substrates, container types and water constituted the sources of contamination. Microorganisms isolated in this work were *Bacilli species, Yeast, E. coli, bacillus species* and *S. cerevisiae.* This result is not in tandem with work done by Eze et al. (2011) when they worked on the proximate and microbial analysis of *burukutu* and *pito* consumed in Ilorin, Nigeria. Some of these organisms isolated are pathogenic to man. *Escherichia coli* is an important member of the coliform group. It is part of the normal flora of the intestine of humans and vertebrates. Kolawole et al. (2007) reported that some strains can cause gastroenteritis and urinary tract infection as well as diarrhea in infants and young children. Its presence is an indication of faecal contamination of the samples. This may be attributed to improper sanitary conditions during processing of the *burukutu,* water supply, low quality utensils and contamination by flies.

Table 7. Colony count and microbes isolated from composite of samples of *burukutu.*

Samples	Serial dilutionplates			Microorganisms
	10	10	10	
A	CG	240	120	*Bacilli species, Yeast*
B	CG	5	6	*S. cerevisiae*
C	152	68	140	*E. coli, S.cerevisiae*
D	CG	68	10	*E. coli, bacillus species*
E	CG	68	CG	*Bacilli, S.cerevisiae*

Key: CG **–** Conference growth, A – *AngwanRukuba,* B – *FarinGada*, C –*JentaAdamu*, D- *BusaBuji*, E – British.

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. *Bacillus cereus* and *Bacillus subtilis* are the most encountered in the group. *Bacillus cereus* when grown on food causes food poisoning by the production of an enterotoxin (Chikodili et al., 2015).

The yeast isolated from these samples such as *Saccharomyces cerevisiae* is associated with fermentation. The association of lactic acid bacteria and yeast has been observed in several cereal foods (Halm et al., 1993). Nout (1999) reported the development of lactic acid bacteria stimulated by yeast provide soluble nitrogen compounds and other growth factors. Yeast metabolites for example CO_2, pyruvate, propionate, acetate and succinate have been shown to stimulate *lactobacilli* in *kefir* (Leroi and Pidoux, 1999). It was also observed that the continuous growth of the yeast population at the end of fermentation led to products that are destabilized. Houhouigan et al. (1993) made a similar observation on naturally fermented alcoholic beverages.

Conclusion

The production of native beer involves the use of antiquated technique which did not put into consideration the regulation of physico-chemical parameters which could lead to the generation of other organic metabolites which may synergise with alcohol making it toxic to the system. Personal and environmental hygiene will help reduce levels of contaminants in alcoholic beverages otherwise pathogenic microbes will be present in the liquor being consumed.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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