Journal of Scientific Research & Reports

17(4): 1-8, 2017; Article no.JSRR.38124
ISSN: 2320-0227

Organic Metabolites, Alcohol Content, and Microbial Contaminants in Sorghum-based Native Beers Consumed in Barkin Ladi Local Government Area, Nigeria

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Authors' contributions

This work was carried out in collaboration between two authors. Author SYG conceptualised and designed the study, edited the manuscript, effected all the corrections as indicated by the reviewers/editor. Author YD managed the analyses (bench work) of the study, the literature searches and wrote the protocol and first draft of the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JSRR/2017/38124
Editor(s):
(1) Christian Scheckhuber, Cinvestav Monterrey, Apodaca, Mexico.
Reviewers:
(1) Salauddin Al Azad, Khulna University, Bangladesh.
(2) Surajbhan Sevda, Indian Institute of Technology, India.
(3) Selma Gomes Ferreira Leite, Federal University of Rio de Janeiro, Brasil.
Complete Peer review History: http://www.sciencedomain.org/review-history/22757

Original Research Article

Received 13th October 2017
Accepted 8th January 2018
Published 16th January 2018

ABSTRACT

Aim: To determine the alcohol content, organic metabolites generated as well as microorganisms present in native beers.
Study Design: The work is descriptive.
Place and Duration of Study: Department of Biochemistry, University of Jos: May 2017- June 2017.
Methodology: Alcohol content, metabolites produced and microbes present in samples by applying gravity, mass spectrometric and microbial methods respectively.
Results: Results obtained indicated the presence of hexadecanoic acid, oleic acid, sulfurous acid, ethyl docosanoate, and 9 – octadecanone in both *Burukutu* and *Pito* samples. However they differed in some metabolites thus: in *Burukutu*, trifluoroacetic acid, 2-methyl nonadecane, 2-tetradecen-1-ol, pentanoic acid, 1-heptacosanol, cyclotetradecene, docosanoic acid and

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chloroformate. In *Pito*, dichloroacetic, pentadecanoic acid,1-hexadecanol,1-tetracosanol, methyl tetradecanoate, cyclobutan carboxylic acid, 3-heptanoic acid, cyclobutane, butyl ester, 2-decen-1-ol. Results also showed the mean alcohol content of *Burukutu* and *Pito* at 4.88% (v/v) and 3.35% (v/v) respectively. Microbial analysis of the samples indicated the presence of *Saccharomyces cerevisiae*, *Staphylococcus aureus, Streptococcus pyogenes* and *Escherichia coli.*

Conclusion: Native beers contain ethyl alcohol, undesirable metabolites which might contribute to alcohol toxicity and also presence of pathogenic microbes which predisposedrinkers to their infections.

Keywords: Burukutu; pito; metabolites; contaminants; beverages; drinkers; microbial.

1. INTRODUCTION

Burukutu and *Pito* are some of the indigenous alcoholic beverages. Both are produced mainly from grains of guinea corn (*Sorghum vulgare* and *Sorghum bicolor*). Sorghum is one of the cereals cultivated in the tropical regions of Arica and is about the largest cultivated in Northern Guinea savannah area of Nigeria [1]. It serves as a staple food of many of the world´s poorest and least privilege people [2]. *Burukutu* and *Pito* are prepared routinely by local native beer makers in several communities including Barkin Ladi (study area) which is famous for mining activities: mining ponds therefore abound in the environment. The underprivileged and poor natives, mostly women, use water from these mining ponds and wells for domestic and routine preparation of native beers. They naturally patronise tinker shades and potters for cheap and substandard brewing containers. Native beers are relatively cheaper than conventional bottled beers; hence, sellers enjoy consistent patronage of consumers who drink them as alternatives to factory-based lager beers. Since the quality of water and that of containers is poor, there is high chance of contamination of native beers prepared for human consumption and hence the basis of this work.

Objectives In order to achieve the aim of this work,techniques were applied thus:

- Gravimetric method to determine alcohol content of samples.
- Mass spectrometry to analyse metabolites present in samples.
- Microbial methods to identify the microbes in the samples.

2. MATERIALS AND METHODS

2.1 Reagents

Absolute alcohol, crystal violet, iodine, acetone, neutral red, peptone water, selenite-F broth (selective medium for Salmonella species), cystine lactose electrolyte deficient (CLED) agar, *Salmonella Shigella agar* (SSA), petroleum ether.

2.2 Equipment

Incubator (incubator bacteriological, 37°C), autoclave, triple beam balance, (MB-2510, 2610 g capacity), centrifuge (MSE minor), mass spectrometer, pH meter (corning 35) model, microscope, distillation assembly, and other assorted glass wares.

2.3 Sample Collection

Twenty samples of freshly prepared *Burukutu* and *Pito* were purchased at different drinking joints in Barkin Ladi area of Plateau State, Nigeria. The samples were collected in sterile plastic bottles and were centrifuged at 2600 revolution for 15 minutes. Thereafter, the supernatant was separated from the debris (pellets) by careful decantation. The resultant supernatant was doubly filtered using Whatman filter paper. The filtrate was used to determine the alcohol content as well as the organic metabolites. For the microbial aspect of the work, crude samples and pellets were used for isolation and enumeration of bacterial isolates.

2.4 Characterisation of Samples

The consistency and colour of the samples were observed and noted. The pH of each sample was determined.

2.5 Nutrient Agar Preparation

14 g *Salmonella Shigella agar* (SSA), 16 g Mackonkey agar and 12 g of Nutrient agar were weighed separately in 1000 ml flask after which 500 ml of distilled water was added. The bottles were made airtight and autoclaved at 121°C for 15 minutes. After the period, they were removed

and kept at room temperature to cool. The molten agars were then poured into ready-to-use sterile Petri dishes and allowed to solidify. The petri dishes containing the agars were subjected to sterility check by incubating them un-inoculated in an upside down position in an incubator at 37°C for 24 hours. No growth was observed the following day and hence not contaminated. The plates were then inoculated with the native beer samples using sterile wire loop and incubated at 37°C for 24 hours in oven.

2.6 Isolation of Bacteria in the Native Beer Samples

This was carried out using the spread plate method. The samples were serially diluted up to 10^{-4} and 1 ml of each diluted sample was introduced onto dry agar medium. Nutrient and Mac Conkey agar were used for this selective microbiology technique. A sterile glass spreader was used to streak the suspension onto the surface of the agar medium so that individual bacterial colony can be isolated. The plates were then incubated at 37°C for 18-48 hours.

2.6.1 Identification of organism

The bacterial isolates were identified following standard microbial procedure as described by [3]. The colony morphology of the isolates on different media was observed.

2.6.2 Gram staining

Native beer samples were stained both with primary stain (Gram stain) and with secondary stain (safranin). Using immersion oil, slides were viewed under microscope (at X100 magnification).

2.6.3 Catalase test

A drop of 3% H_2O_2 was placed on a glass slide. A portion of bacteria isolate was collected from the medium using a sterile wire loop and the growth was emulsified in drop of H_2O_2

2.6.4 Coagulase test

The slide method test was used for this study. A drop of saline on two separate spot was placed on the grease free slide, speck of growth of the test organism was picked using a sterile wire loop and emulsified in both spots, to one spot, a drop of plasma was added and to the other, a drop of saline was added. Both mixtures were swirled thoroughly by rocking. Thereafter, coagulation was observed at the spot where the growth was smeared on the plasma. The presence of clotting or coagulation indicate positive for *Staphylococcus aureus*.

2.6.5 Motility

A single colony of each of the organism was isolated into labelled test tubes containing peptone water and the tubes incubated at 37°c overnight. A drop of the organism was mixed in peptone water and incubated overnight. Thereafter, a portion was placed on a slide which was then gently turned. This preparation was then observed under the microscope for motile bacteria under X100 objectives [3].

2.7 Determination of Alcohol Content

Native beer samples were distilled using a simple distillation apparatus. Native beer samples were added to a distilling pot and heated to the boiling point. Alcohol vaporised first owing to its lower boiling point of 78.5°C. The resulting vapour got condensed in the distillation set up and converted to liquid and was then collected in a receiving flask leaving the aqueous component in the distilling pot thereby achieving separation. The alcohol content of the samples was then determined thus:

W1=Weight of sample before distillation
W2=Weight of sample after distillation
W3=Weight of ethyl alcohol.

$$\text{Percentage alcohol content} = \frac{W_1-W_2}{W_2-W_3} \times 100$$

2.8 Organic Metabolites Determination

The samples were introduced into the mass spectrometer through the injection point or sample inlet, on reaching the ion source; gaseous ions were produced from the native beer samples which were resolved into fragmented into their characteristics mass components according to their mass-to-charge ratio.

2.8.1 Operating conditions (GC-MS) machine

Mass Sectrometer:
Start time: 3.00 minutes; end time: 38.00 minutes; ACO mode: scan; Event time: 0.50 seconds; scan speed: 767; start m/z: 30.00; end m/z: 400.00; sample inlet unit: GC.

3. RESULTS AND DISCUSION

Biochemical and microbial analysis revealed that native beer are contaminated with both organic metabolites and pathogenic microbes.

3.1 Discussion

This work sought to determine the organic metabolites, alcohol content and microbial load of native beers consumed in Barkin Ladi metropolis of Plateau State, Nigeria.

The major physiologically active component of most alcoholic beverage is ethyl alcohol (ethanol); the remaining fractions are called congeners. Congeners are biologically active chemicals. They are produced in the process of fermentation when organicsin the alcoholic beverage breakdown [1]. They may also be added during the production process to improve the taste, aroma and appearance of alcoholic beverage [4].

The results in Table 1 showed that the native beers analysed contained undesirable organic metabolites. Hexadecanoic acid was found to be significantly present in both *burukutu* and *pito* (native beers) [5]. [6] reported that factory-based lager beers contained 13.4% hexadecanoic acid which is higher compared to native beers. However, according to World Health Organisation (WHO), consumption of palmitic acid (hexadecanoic acid) increases the risk of developing cardiovascular diseases. This was based on studies indicating that it may increase LDL levels in the blood [7]. Rats fed a diet of 20% palmitic acid for an extended period showed alterations in central nervous system control of insulin secretion and suppression of body's natural appetite – suppressing signal from leptin and insulin, hormones involved in weight loss [8]. Hexadecanoic acid is a saturated fatty acid whose catabolism will lead to the synthesis of ketone bodies when there is excessive acetyl CoA. When the rate of synthesis of ketone bodies exceeds the rate of their utilsation, their levels in blood rise which lead to ketonemia. This is followed by ketonuria; excretion of ketone bodies in the urine. Ketonemia and ketonuria are referred to as ketosis [9].

Acetoacetate and β-hydroxybutyrate are acids. If levels of ketone bodies are elevated, the pH of blood drops, resulting in ketoacidosis, a complication of untreated type 1 diabetes mellitus and sometimes in end stage II diabetes mellitus [10]. Palmitic acid can induce steatosis separately as well as in a mixture in hepatocyte primary cultures or in hepatoma cell lines [11]. Rising concentrations of saturated palmitic acid can attenuate the synthesis of triglyceride in goose hepatocytes [12] by an effect on diglyceride acyl transferase from the group of enzymes involved in synthesis of triglyceride. Oxidative stress is a stimulus able to trigger the apoptotic cascade in cells. The exposure to palmitic acid can lead to generation of reactive oxygen species (ROS) [12].

Table 1. Main organic compounds detected in *Burukutu*

Organic metabolites	Molecular formula
Hexadecanoic acid	$C_{16}H_{32}O_6$
Oleic acid	$C_{18}H_{34}O_2$
Fluoro acetic acid	$C_2H_3FO_2$
2-methyl nonadecane	$C_{20}H_{42}$
Sulfurous acid	H_2SO_3
2-Tetradecen-1-ol	$C_{14}H_{24}O$
Ethyl docosanoate	$C_{24}H_{48}O_2$
Pentanoic acid	$C_5H_{10}O_2$
9-octadecanone	$C_{18}H_{36}O_2$
1-Heptacosanol	$C_{27}H_{56}O$
Cyclotetradecene	$C_{14}H_{28}$
Docosanoic acid	$C_{22}H_{44}O_2$
di-2-ethylhexyl phosphoric acid	$C_{16}H_{35}O_4P$
Ethylchloroformate	$C_3H_5ClO_2$

Mono-unsaturated fats in human diet, (eg oleic acid) are associated with postmenopausal breast cancer [13], decreased low density lipoprotein (HDL) cholesterol [14]. Oleic acid in the membrane of red blood cells has been associated with increased risk of breast cancer [15]. Persistent oxidative stress, often involving enhanced peroxidation of polyunsaturated fatty acid in the cell membrane by intracellularly produced O- and N-central free radicals, alters cellular redox potential, activation of protein kinase and subsequent change in transcription factors. This enhances the development of breast cancer [16]. Thus, the carcinogenic process could be initiated or accelerated by lipid peroxidation –induced DNA and protein damage [17].

Native beers have their unique flavour due to the presence of ethyl docosanoate which is a volatile ester that constitute an important group of aromatic compounds. [18] reported that volatile esters are only trace compounds in fermented

beverages such as beers, and are extremely important for flavour profile. The result of this work showed that the esters in native beers are of higher molecular mass compared to factory based lager beer. [19] reported that the concentration of esters decreased during aging and that large molecular weight esters are hydrolysed to a high degree during storage. Since decrease in ester concentration during storage is largely attributed to esterase activity, pasteurization of beers is required to inactivate esterases that hydrolyse esters, thereby preventing decrease in ester concentration during storage. This lack of pasteurization of native beers might be responsible for its short shelf life; as compared to factory based lager beers.

The level at which sulfurous acid appears in alcoholic drinks is largely determined by the brewing process. However, [20] reported that not all sulfuryl compounds are formed during the normal brewing process; they can also result from bacterial contamination or poor handling. Sulfurous acidoccurs naturally in a number of foods and beverages as a result of fermentation, such as occur in beer and wine [21]. It is an important factor in delaying flavour staling and prolonging the shelf life of beers. With such a history of widespread application, sulfurous acid has been generally regarded as safe (GRAS) by the federal Department (FD); however, it is suspected that a low percentage of the population is sensitive to it. The manifestations of sulphurous acid sensitivity include a large array of dermatological, pulmonary, gastrointestinal, and cardiovascular symptoms. Asthmatics who are steroid-dependent or have a great degree of airway hyper reactivity, may be at an increased risk of having a reaction to a sulphurous acid-containing food [22]. As a result, they are not considered GRAS for use in foods recognised as a major source of vitamin B1 (thiamine), or “fruits or vegetables intended to be served raw to consumers or to be presented to consumers as fresh” [22]. Sulfurous acid can function as mild oxidizing agents; however, reactions in which this compound react as reducing agents (and are oxidized to the sulfate ion, SO_4^{2-}) are more numerous and more important [21]. It is therefore thought to have a significant effect in malting and brewing as inhibitors of oxidative damage [23].

Fluoroacetate, 2-methyl nonadecane, pentadecanoic acid, and cyclotetradecene, among others, are metabolites specific to *burukutu* whereas butyl esters, cyclobutane, 1-hexadecanol among others, are specific to *pito*. These metabolites could aggravate the toxicity of alcohol in drinkers.

The result in Table 3 shows that *burukutu* and *pito* contained mean alcoholic percentage of 4.99% (v/v) and 2.79% (v/v) respectively. These native beers contained 2-6% (v/v) of alcohol [24]. This result is similar to that of this investigation. [24] reported that the permissible level of alcohol in all alcoholic beer be between 4-5% (v/v). However, at these levels, they might still be toxic to cells since they have exceeded the blood alcohol concentration (BAC) of 0.03% (v/v) as reported by the European Union. Furthermore, alcohol toxicity might also be due to secondary metabolites such as acetaldehyde, malondialdehyde, 4-hydroxy-2-mononel, superoxide, hydroxyl radicals, hydrogen peroxide generated during its metabolism. Ethanal (acetaldehyde) in native beer samples puts drinkers at risk of its toxic effect [25]. It is a metabolite of ethyl alcohol oxidation which is toxic and could cause damage to, especially the brain including behavioural abnormalities, impaired memory and sedative effect; it is also a carcinogen. Acetaldehyde, the major alcohol-reactive metabolite, has been detected in the intestine of Wistar rats after alcohol exposure [26]. 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 [25]. However, [27] reported that acetaldehyde modulates rather than mediates some of ethyl alcohol toxic effects whereas [28] 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 in 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. Chronic alcohol consumption has been reported to increase the CYP2E1 activity in the liver, resulting in increased ROS formation and eventually oxidative stress. This increase may enhance ROS generation (superoxide anion, hydroxyl radicals, hydrogen peroxide, and hydroxyethyl radicals), which could lead to lipid peroxidation, oxidative protein damage, and DNA oxidation [29]. ROS production and oxidative stress is central to alcohol liver disease [29]. During this condition, the activities of the antioxidant enzymes (SOD, CAT, GSH-Px, GSH-Red) defence arsenal are sometimes overwhelmed [30].

Table 2. Main organic compounds detected in Pito

Organic metabolites	Molecular formula
Hexadecanoic acid	$C_{16}H_{32}O_6$
Oleic acid	$C_{18}H_{34}O_2$
Dichloroacetic acid	$C_2H_2Cl_2O_2$
Sulfurous acid	H_2SO_3
Pentadecanoic acid	$C_{15}H_{30}O_2$
Ethyl docosanoate	$C_{24}H_{48}O_2$
1-Hexadecanol	$C_{16}H_{34}O$
1-tetracosanol	$C_{24}H_{50}O$
9-octadecanone	$C_{18}H_{36}O$
Methyl tetradecanoate	$C_{15}H_{30}O_2$
Cyclobutan carboxylic acid	$C_5H_8O_2$
3-Heptanoic acid	$C_7H_{14}O_2$
Cyclobutane	C_4H_8
Butyl ester	$C_6H_{12}O_2$
2-decen-1-ol	$C_{10}H_{20}O$

Yeast cells and *Staphylococcus aureus*, were also isolated from the native beers. The presence of *Staphylococcus aureus* in the samples may be attributed to handling during production. *Staphylococcus aureus* is a normal flora of the skin and mucus membrane and a common etiological agent of septic arthritic [31], [32]. *Staphylococcus aureus* is the major cause of staphylococcal food poisoning characterised by diarrhoea and vomiting [33]. *Streptococci* are pathogenic Gram positive species which are implicated in meningitis, pneumonia, tonsillitis, septic arthritis, otitis media and scarlet fever [34]. They also cause colonisation of mucosal surfaces of the host naso-pharynx and upper airways [34]. 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 [34]. *Escherichia coli* are important members of the coliform group. It is part of the normal flora of the intestine of humans and vertebrates. Some strains of *Escherichia coli* can cause gastroenteritis and urinary tract infection as well as diarrhoea in infants [35].

Table 3. Mean percent alcohol content of Burukutu and Pito using simple distillation technique

Burukutu percentalcohol content (v/v)	Pito percentalcohol content (v/v)
5.72	2.72
6.18	2.68
5.36	2.70
3.57	4.00
3.50	3.20
4.80	2.20
5.80	3.20
4.70	2.80
5.50	2.50
4.80	1.90
4.99±0.91	2.79±0.58

Table 4. Microbial profile of native beer samples as determined by selective microbiology technique

Isolates	*Burukutu* samples	*Pito* samples
Staphylococous aureus	+	+
Escherichia coli	+	+
Streptococcus spp	+	+
Aspergilus niger	-	-
Aspergilus flavor	-	-
Saccharomyces cerevisiae	+	+
Salmonella Shigella	-	-

KEY: + = Present
- = Absent.

The presence of fungi may be attributed to the acidic nature of the sample since it has been observed that yeasts and moulds are capable of utilizing organic acids. Also, the presence of fungi in the beverage may lead to poisoning (by secondary metabolites such as aflatoxins); fungal contamination results in the production of undesirable odour, colour changes and even the taste of the sample [36]. Many of the fungal isolates produce toxin. The fungi of the genera *Aspergillus* and yeast are predominant in the production of toxins called mycotoxins, a disease

condition known as mycotoxicosis which develops when food containing mycotoxins are eaten [36]. Aflatoxins produced by *Aspergillus flavus* have been reported to be potent carcinogens to man [36]. The yeast cells isolated from the samples like *Saccharomyces cerevisiae* is associated with fermentation.

4. CONCLUSION

This study has proved that native beers contain ethyl alcohol and some undesirable metabolites, as well as *Staphylococcus*, *Streptococcus* and *Eschericia coli*. It is concluded therefore, that ethyl alcohol may not necessarily be the sole causal factor of ailment among the drinkers of *burukutu* and *pito* since the metabolites present in the natives beers are themselves toxic to the body cells.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/22757