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Microbial Hazard and Critical Control Points Identification in Processing Two Traditional Fermented Alcoholic Cereal Beverages regularly consumed in Northern Nigeria

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ABSTRACT: *Burukutu* and *pito* are two major fermented alcoholic cereal beverages traditionally produced and universally consumed in the West African region. Knowing that traditional processing methods are easily amenable to contamination, hence the objective of this paper was to investigate the microbial hazards and identify the critical control points in the processing of *burukutu* and *pito* regularly consumed in Northern Nigeria using appropriate standard methods. Data obtained from this study show that during the processing of *burukutu* and *pito*, microbial contamination was brought about by milling operations, processing water, poor hygiene, and sanitary conditions. Bacterial and fungal populations were relatively similar in both beverages though slightly higher in *burukutu* than *pito*. Similar trend was also observed for coliform count, however, the values obtained were above the Codex Alimentarius standard limit for fermented foods and allied products. pH values varied between 3.40 and 3.75 for *burukutu*, and between 3.42 and 3.78 for *pito*, while total titratable acid (TTA) ranged from 1.22 to 1.94 g/ml and 1.22 to 1.94 g/ml respectively. Coliforms, *Staphylococcus aureus*, *Bacillus cereus*, *Cyberlindnera fabianii*, *Candida orthopsilosis*, *Candida parapsilosis*, *Candida haemulonis* were pathogens identified during processing and post-processing of these beverages. The detection of these pathogens of public health importance, implies that training of processors on personal hygiene, environmental sanitation, identified hazards and proper monitoring of critical control points as well as potential use of starter cultures for fermentation stages is strongly recommended. Such trainings are feasible strategies to ensure food safety and hence, enhance consumer acceptability of these alcoholic beverages.

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Pito and *burukutu* are two important traditionally produced alcoholic cereal beverages popularly consumed in some parts of West Africa including Ghana, Nigeria, Burkina Faso, and Republic of Benin (Kolawole *et al*., 2007; Oguntoyinbo and Franz, 2016). It is mostly served as a local entertainment drink (sorghum beer), while the incompletely fermented product is used as infant and children food among locals. They are produced mainly from the malting of red or white grain variants of guinea corn (*Sorghum bicolor* or *Sorghum vulgare*), a major source of energy and protein for people in Asia and Africa (Blandino *et al*., 2003), although they can also be produced from maize and millet (Sawadogo-Lingani *et al*., 2007). Their production is at cottage level and marketing chiefly remain women's activities

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from which they derive a substantial income. Production of *pito* and *burukutu* traditionally is a batch process carried out on small scale about two times a week, with important production steps being malting, milling, fermentation and maturation (Kolawole *et al*., 2007). The malting process has been reported by Konfo *et al*. (2015), to cause reduction in phytic acid, flavonoids and proanthocyanidin content which leads to an increase in the availability of minerals including iron and zinc, and essential amino acids mainly lysine, tyrosine and methionine and vitamins. The end product of the production process is a cream-coloured supernatant called *pito* and a brown-coloured suspension called *burukutu*, both having a vinegar-like flavor, low pH between 3.3 and 4.0 and are consumed while still fermenting (Odunfa *et al*., 1998). *Pito* and *burukutu* are rich in calories, β-group vitamins including thiamine, folic acid, riboflavin, and essential amino acids such as lysine, leucine, methionine (Lyumugabe *et al*., 2012). The production of *pito* and *burukutu* is associated with natural fermentation process, poor hygiene of producers, poor sanitary measures, unhygienic environmental conditions, unsatisfactory conservation of traditional processing methods with the use of rudimentary equipment without consideration for Good Manufacturing Practice (GMP), Good Hygiene Practices (GHP) and Good Housekeeping (GHK) (Oguntoyinbo, 2012). All these lapses have led to the presence of undesirable microbiological hazards which include pathogenic and spoilage microorganisms such as *Alcaligenes*, *Flavobacterium*, *Staphylococcus aureus*, *Aspergillus flavus*, *Aspergillus niger* and species belonging to the family Enterobacteriaceae in these alcoholic cereal beverages (Sanni *et al.,* 1999; Fadahunsi *et al*., 2013). The presence of these safety issues shows the need to develop strategies which will involve production process optimization to improve safety and overall quality of these two important alcoholic beverages in order to guarantee product acceptability, consistency and in turn more patronage and income. The Hazard Analysis Critical Control Point (HACCP) has been recognized as an effective food quality control strategy for assuring food safety from primary production through final consumption, using a 'farm to fork' approach (Singh *et al.,* 2018). It places emphases on prevention and control of potential hazards at every production step rather than the conventional end-product sampling practices (Nahemiah *et al.,* 2014). This strategy has been adopted across the food industry, especially in large food companies where it is usually part of their food safety management systems, as recommended by The Codex Alimentarius Commission, the International Food Standard-setting body overseen by the United Nation (UN) agencies, the Food and Agricultural Organization (FAO), and World Health Organization (WHO) (Nehemiah *et al.,* 2014). Although, Hazard Analysis Critical Control Point (HACCP) is not difficult to adopt for small scale processors like the local *pito* and *burukutu* producers, producers in developing countries encounter challenges such as limited resources and poor implementation of policies (Oguntoyinbo, 2012, Panisello *et al.,* 2001). However, Oguntoyinbo (2012), Obadina *et al.* (2008) and Nahemiah *et al*. (2014) amongst other researchers have identified and developed Hazard Analysis Critical Control Point (HACCP) strategy recognized during traditional fermented food production in West Africa with focus on production of legume-based condiments, wet cassava fermentation and soy-*kununzaki*, a traditional cereal-based fermented beverage. These strategies can also be adopted for enhancement of safety of other traditional fermented foods especially fermented alcoholic cereal beverages such as *pito* and *burukutu* in Nigeria. Hence, the need to research into a reliable and sustainable safety management approach and microbiological hazard control technique for the production these local beverages.

In this study, therefore, the objective is to investigate the microbial hazard and identify critical control points in processing *burukutu* and *pito* regularly consumed in Northern Nigeria.

MATERIALS AND METHODS

Selection of production sites: Different producers of *burukutu* and *pito* were identified in Lagos, Ogun, Plateau and Kano States in Nigeria and selected based on their consistency, processing and environmental conditions, consumers' acceptability and preference across selected geographical regions. Four local producers were selected based on interest in participation.

The sampling sites are:
Kano (K) – Sabongeri Market, Kano (8° 32' 23.64", 12° 0' 55.69");
Lagos (L) – Maami Market, Ikeja Cantonment, Ikeja (3° 21' 8.08", 6° 33' 53.77");
Ogun (O) – Maami Market, 174 Battalion barracks, Ita-Oluwo (3° 30' 10.67", 6° 37' 19.17") and
Plateau (P) – Farrigada Market, Jos (8° 53' 37.24", 9° 55' 13.08").

On-site observation and documentation of processing techniques was carried out. Sorghum grains, in-process samples as well as finished products were collected from the participating production sites. For in-process samples, at each production site, samples were collected at the steeping stage, after the milling stage, after the boiling and sieving stage, after primary

fermentation stage and after the maturation stage. At each sampling stage, at each production site, four random samples were collected and homogenised for chemical and microbial analysis. All samples were collected aseptically, labelled appropriately and immediately transported to the laboratory in an ice box at 4^0C.

Chemical analyses: pH values were measured according to the method described by Nahemiah *et al*. (2014). The pH of the beverage samples was determined using a reference pH meter (Mettler Toledo, Germany) after standardization with pH 4 and 7 buffers. *Burukutu and pito* samples (10 ml) were transferred to 15 ml Corning tubes, the meter electrode was first dipped into a buffer solution before dipping into sample for analysis and the pH was read automatically.

Titratable acidity was determined using the method described by Nahemiah *et al*. (2014). Briefly, 10 ml of the beverage samples were transferred into a volumetric flask and three drops of phenolphthalein indicator were added. The flask was shaken thoroughly and subsequently titrated against 0.1 N NaOH to a pink colour endpoint. Total titratable acidity was expressed as lactic acid, which is the most prominent acid in alcoholic beverages that gives the characteristic sourness in these beverages.

$$\mathrm{TTA} = \mathrm{TV(ml)} \times 0.009\ \mathrm{LA} \quad (1)$$

Where TTA = total titratable acid, TV = titre value; LA = lactic acid

Microbial enumeration: Sorghum grains, in-process and final samples were collected and analysed for microbial composition. One millilitre of each collected sample was homogenized in 9 ml of sterile distilled water and serial diluted. Aliquots (0.1 ml) of appropriate dilutions were plated in triplicates for total bacterial, fungal and coliform counts. Plate Count agar (Oxoid, UK) was used for microbial isolation and enumeration of total aerobic bacteria, MacConkey agar (Oxoid, UK) for Gram negative bacteria and coliforms while Rose Bengal Chloramphenicol agar (Oxoid, UK) was used for yeasts and moulds counts. Plates were incubated at 37 ^{o}C for 48 h and 28 ^{o}C for 48 h for bacteria and fungi respectively.

Identification of isolates: Pelleted cells of overnight cultures of bacteria and fungi were processed for 16S rRNA and D1/D2 region of the 26S rRNA PCR amplification respectively using an adapted protocol according to Dashti *et al.* (2009). The pellets were washed in 10 µl colony wash buffer twice, resuspended in 10 µl ultra-purified water and boiled at 95 °C for 5 min using a thermocycler. One microlitre of the boiled samples was used as template with primers AMBf (AMP_F 5'-GAG AGT TTG ATY CTG GCT CAG) and AMBr (AMP_R 5'-AAG GAG GTG ATC CAR CCG CA) for 16S rRNA gene amplification and NL4 (AMP_F D1/D2-NL4 – 5'-GGTCCGTGTTTCAAGACGG) and NL1 (AMP_R D1/D2-NL1 – 5'-GCATATCAATAAGCGGAGGAAAAG) for 26S rRNA gene amplification. Amplicons were quantified using Nanodrop and sent to Eurofin genomics (Eurofin Labs, Wolverhampton, UK) for sequencing. The DNA sequence data obtained was aligned on Seqman and consensus sequences were compared to redundant 16S rDNA sequence data available in Ribosomal Database Project- II (https://rdp.cme.msu.edu/) and the GenBank database using the BLAST algorithm. The 26S rDNA sequence data available on FASTA SEARCH (http://www.ebi.ac.uk/Tools/sss/fasta/nucleotide.html) to determine their closest phylogenetic relative.

Hazard analysis: The hazard analysis method of Nahemiah *et al*. (2014) was adopted to observe *pito* and *burukutu* production. Hazard analysis was conducted on *pito* and *burukutu* production process from four processors from the four states selected. Possible sources of contamination were identified and samples were collected at each stage of the production process in this regard. The processors' manufacturing, housekeeping, sanitary, personal and environmental hygiene practices as well as handling of final products were evaluated to ascertain potential points and sources of all forms of hazards. Flow diagram for *burukutu* and *pito* production was developed based on the methods employed by processors as observed during traditional production and different CCPs as well as operational prerequisites programmes were identified with the use of the CCP decision tree as developed by the Codex Alimentarius Committee on Food Hygiene (CODEX, 1993).

Statistical analysis: All the experiments were performed in triplicates, and the results were expressed as the mean standard deviations of three independent replicates. The gathered data were analysed using the Prism version 9.01 (GraphPad Software, San Diego, CA USA).

RESULTS AND DISCUSSION

Production of *burukutu* and *pito* were observed to involve the same processing steps in all the production sites selected. *Pito* and *burukutu* were produced concurrently by fermenting malted sorghum grains. Traditional production of both beverages involved a

batch process carried out on small scale about two times a week. The production process involved malting, milling, fermentation and maturation. The malting step is an essential part of the beverage making process which involves steeping of the sorghum grains in water overnight followed by draining and spreading on sacs for germination for about 2 days. After the malting, the grains were dried naturally, dry milled and mixed with water. This was followed by cooking for few hours to form slurry followed by cooling and fermenting for about 2 days at room temperature (28 ± 2 ºC). After this, water was added to the fermented mixture and cooked for few hours and allowed to cool. The cooled wort was left to ferment at room temperature overnight. The fermented product separated into two products; cream coloured supernatant called *pito* and a brown-coloured suspension called *burukutu*. Fig. 1 represents the flow diagram of the production process deduced from the local producers.

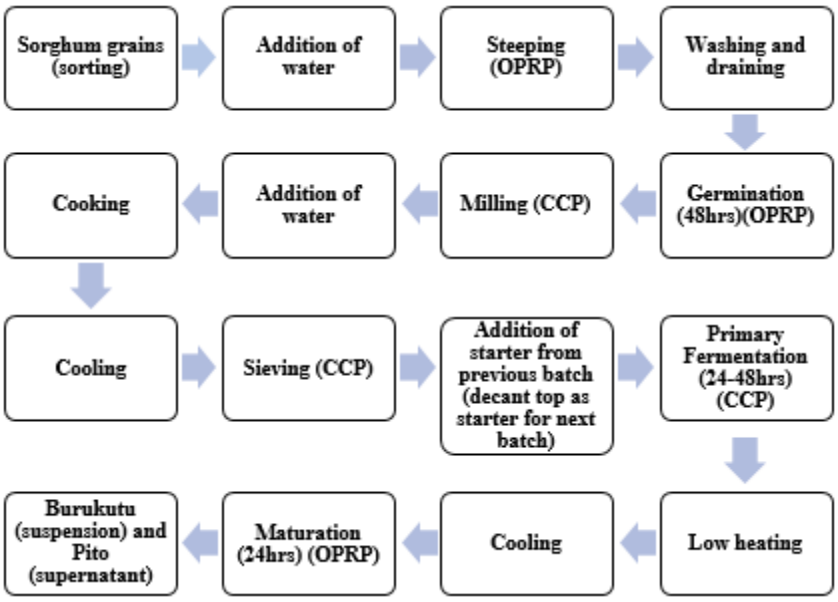

Fig. 1: Flow chart of *pito* and *burukutu* production process showing the critical control points (CCP) and operational prerequisites (OPRP). *Sources*: Oguntoyinbo and Franz (2016), Lyumugabe *et al.* (2012) with slight modifications as observed in this study.

Local productions sites of *burukutu* and *pito* were observed to be homes of processors in Ogun and Plateau States while it was backyard of local bars in markets for Lagos and Kano states. All sites observed lacked basic facilities to support good manufacturing, housekeeping and hygiene practices. Poor sanitary conditions were practised with raw materials and processing equipment left in open spaces uncovered with interference from pets, domestic animals and little children. Raw materials such as sorghum grains were sourced from local sellers and farmers, water was obtained from municipal pipes in barracks, while wells and stream water stored in plastic drums were used by home and local bar producers. Women were involved in the processing, mostly with the support of their children. Milling was outsourced to local dry millers by home producers while those in barracks had their dry milling machines. Final products were served in small calabashes.

Steeped sorghum samples had pH above 6.6, close to neutral pH. Acidity decreased after malting and milling to pH of about 5.7 and further drop in pH levels to about 5.0 was observed in sieved samples. After fermentation, the pH reduced significantly to below 4.0 which remained steady till the finished products. On the other hand, the TTA varied across the in-process samples and finished products, however, the highest TTA (>0.7g/ml) were recorded in the fermentation and maturation samples (Figure 2). Microbial counts at different stages of production of these beverages is shown in Figure 3. The beverages had total aerobic bacterial counts ranging from 10^6 to 10^9 cfu/ml and fungal counts ranging from 10^7 to 10^9 cfu/ml. Similar counts were recorded in the in-process samples collected across the four states. Lactic acid bacteria such as *Lactobacillus* dominated the bacterial population, other genera such as *Acetobacter, Streptococcus, Lactococcus, Leuconostoc* were also observed in all samples as well as yeasts such as *Saccharomyces cerevisiae, Pichia kudriavzevii, Kluyveromyces marxianus, Meyerozyma guillermondii*. The pathogens identified were *Staphylococcus aureus, Bacillus cereus, Bacillus*

anthracis, Enterococcus faecium, Shigella flexneri, Comomonas acidovorans, Micrococcus luteus, Candida orthopsilosis, Candida parapsilosis, Candida haemulonis, Rhodotorula mucilaginosa, Cyberlidnera fabianii, all detected in samples collected at different processing stages and in the final products (Table 1). Clinical relevance and public health importance of these pathogens are documented in Table 2.

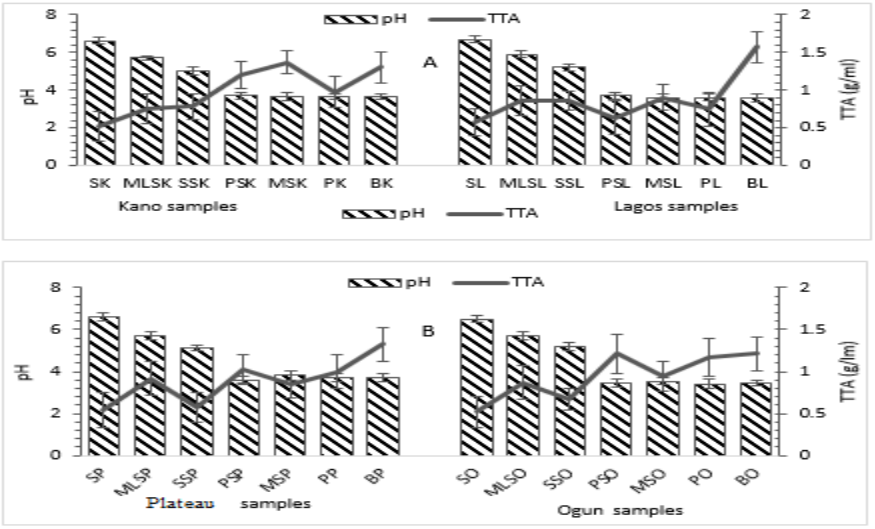

Fig. 2: pH and TTA (titrable acidity) changes during local production of *pito* and *burukutu* in Nigeria. Data presented are means and ± standard deviations of triplicate determinations. S, Sorghum; MLS, milled sample; SS, sieved sample; MS, maturation sample; P, *Pito*; B, *Burukutu*. The last letter accompanying the sample represents originating States as indicated in the materials and methods.

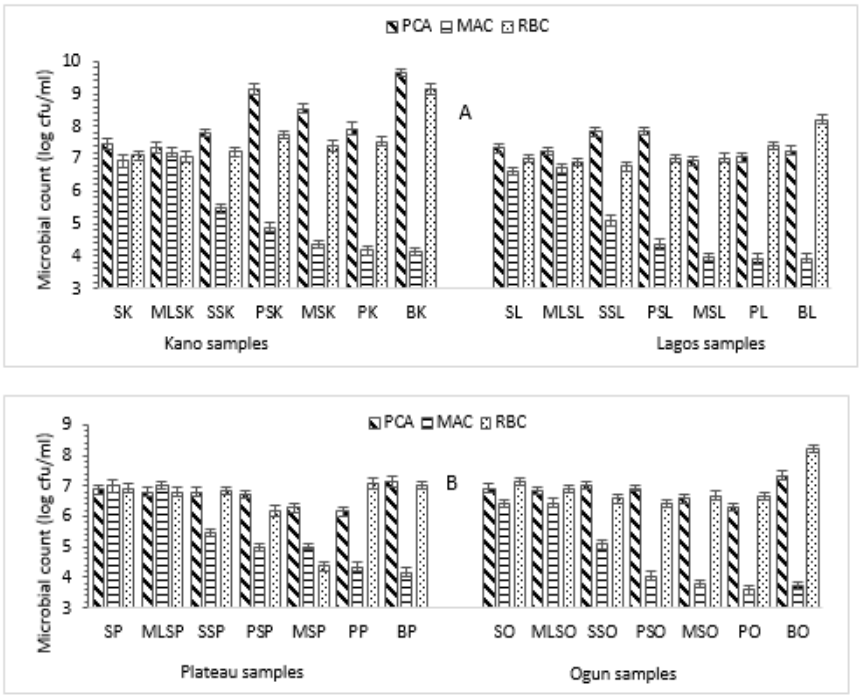

Fig. 3: Population counts of all bacteria (PCA), Gram-negative bacteria and coliform (MAC) and yeasts and molds (RBC) during traditional production of *pito* and *burukutu* in Nigeria. Data presented are means and ± standard deviations of triplicate determinations. S, Sorghum; MLS, milled sample; SS, sieved sample; MS, maturation sample; P, *Pito*; B, *Burukutu*. The last letter accompanying the sample represents originating States as indicated in the materials and methods. PCA, Plate count agar; MAC, MacConkey agar; RBC, Rose Bengal chloramphenicol agar.

Table 1: Microorganisms of safety concerns identified at different processing stages during *burukutu* and *pito* production.

Microorganism identified	Sorghum grains	Milled samples	Sieved samples	Primary fermentation samples (24hrs fermentation)	Maturation sample (12 hrs. into maturation)	*Pito*	*Burukutu*
Staphylococcus aureus	+	+	+	+	+	+	+
Bacillus cereus	+	+	+	+	+	+	-
Bacillus anthracis	-	-	-	-	-	-	+
Shigella flexneri	-	-	-	+	+	+	+
Enterococcus faecalis	-	-	+	+	-	+	+
Escherichia coli	+	+	+	+	-	+	+
Micrococcus luteus	-	-	-	+	+	+	+
Pseudomonas aeuruginosa	+	+	+	-	-	-	+
Comamonas acidovurans	-	-	-	+	-	-	+
Acetobacter aceti	-	-	-	-	+	+	+
Acinetobacter baumannii	-	-	-	+	+	-	+
Cyberlindnera fabianii	+	+	-	-	-	-	-
Meyerozyma guilliermondii	+	+	-	-	-	-	-
Candida orthopsilosis	+	+	+	+	+	-	+
Aspergillus spp	+	+	+	+	-	-	-
Rhodotorula mucilaginosa	-	-	-	+	-	-	+
C. parapsilosis	-	-	-	+	-	-	+
C. haemulonis	-	-	-	+	-	-	+

a). + represents present; b). - represents absent

Table 2: Clinical relevance of some pathogens isolated from *pito* and *burukutu*

Microorganism	Brief description of pathogenicity	Possible sources of contamination
Bacteria		
Bacillus cereus	Incriminated with food poisoning, severe eye infections, anthrax-like progressive pneumonia, mostly in neonates and immunocompromised (Bottone, 2010).	Environment
Micrococcus luteus	Found associated with pneumonia, bacteremia, endocardiatis (Von Eiff *et al*., 1996).	Human, animals, infected objects
Acinetobacter baumannii	Nosocomial infections, skin, bloodstream, urinary tract and other soft tissues (Lee *et al*., 2017)	Soil, water, wounds
Escherichia coli	Some strains have been associated with intestinal and extra intestinal diseases in infants and immunocompromised patients (Kaper *et al*., 2004).	Humans, faeces
Comamonas acidovorans	Found associated with endocarditis, bacteremia, ocular infection, urinary tract infection (Ojeda-Varga *et al.*, 1999).	Soil, water
Staphylococcus aureus	Food intoxication, causes skin and soft tissue infections such as abscesses and cellulitis, associated with methicillin resistant Staph. aureus infections (Kobayashi *et al.*, 2015).	Humans, infected droplets, skin, nose, armpit.
Shigella flexneri	Highly infectious, causes shigellosis, acute mucosal infections, tissue damage, ulcerations (Jennison and Verma, 2004).	Water, humans, faeces
Fungi		
Candida parapsilosis	Causes nosocomial infections, infection of neonates and infants, bloodstream infections (Van Asbeck *et al*., 2009).	Humans, abscesses
Rhodotorula mucilaginosa	Opportunistic pathogen, colonizes and infects susceptible patients (Wirth and Goldani, 2012).	Environment
Candida haemulonii var vulnera	Causes bloodstream and other invasive infections, opportunistic pathogen with resistance to some antifungals (Kumar *et al*., 2016).	Humans, nosocomial.
Candida orthopsillosis	Causes candidaemia, blood stream infections, known for antifungal resistance (Bonfietti, 2012).	Humans
Meyerozyma guilliermondii	Human infections, cutaneous origin. Opportunistic human pathogen, affects majorly immunocompromised patients (Papon *et al*., 2013).	Skin surface, sea water, animal faeces.
Cyberlindnera fabianii	Rarely associated with invasive infections (Desai *et al.*, 2019).	Soil, humans.

High coliform and Gram-negative bacterial counts were observed in the steeping and milling samples with reduction observed in samples collected after boiling and sieving and further drop in these counts were recorded in fermentation and maturation samples as well as finished products. This trend was observed in samples collected from the four local production sites. Statistically-significant difference was found in the total aerobic bacterial counts across the four states ($F= 12.51$, $p < 0.001$). A Turkey-Kramer post-hoc test revealed significant pair wise differences in the total aerobic bacterial counts between Lagos and Kano processors ($p < 0.05$), Plateau and Kano processors ($p < 0.001$) and Ogun and Kano processors ($p < 0.001$). In addition, statistically-significant difference was found in the fungal counts across the four states ($F= 3.125$, $p < 0.05$). A Turkey-Kramer post-hoc test revealed significant pair wise differences between Kano and Plateau processors ($p < 0.05$). Results from the processing stages revealed statistically-significant differences in Gram negative bacterial count across the different processing stages ($F= 48.1$, $p < 0.00001$). Primary fermentation samples (PS), maturation sample (MS) and finished products differed significantly from the early stages of processing samples; Steeped sample (S), milled sample (MS) and sieving samples (SS) in Gram negative bacterial counts ($p < 0.01$).

The identified hazards during *burukutu* and *pito* production are summarized in Table 3. The investigation revealed that sorghum grains contained different forms of hazards: physical i.e., stones, metal pieces, glass pieces and biological hazards like insects, larvae, microorganisms. The likely sources of these hazards were observed to be the suppliers, farms, soil, processors and handlers. Potential chemical hazards such as pesticide and fertilizer residues were also mentioned due to unsafe agricultural practices such as heavy use of pesticides and fertilizers by local farmers and retailers.

Table 3: Identified hazards during processing of sorghum grains for *burukutu* and *pito* production by fermentation.

Processing step	Type and description of Hazards	Possible sources	Possible control measures
Sorghum grains	Physical hazards - stones, metal pieces, glass pieces etc.	Farm, packaging, supplier, soil	Inspection
	Biological hazards -insects, larvae	Processor, handler	SQA
	Microbiological hazards- pathogens, spoilage organisms.	Grains, Farm, supplier, packaging, soil	GHP/ SQA, Inspection
	Chemical hazards -pesticides and fertilizer residues.	Farm, packaging, soil	Inspection and SQA
Steeping and germination (OPRP)	Microbiological hazard- vegetative pathogens and spores.	handlers, water, sacs, drums	GHP, GMP, SQA
	Biological hazards- larvae, insects,	Water, grains, sacs	SQA
	Chemical hazard- chloride, heavy metals	Water, sacs	SQA
	Physical hazard - sand, stone	Grains, water, drums, sacs	Inspection, SQA
Milling (CCP)	Physical hazards - stones, sand, metal, glass pieces	Miller, environment	Inspection, GMP
	Biological hazards -insects, larvae	Miller, grains, soil	GMP
	Microbiological hazards- *Bacillus cereus, E. coli, Staph. aureus, Aspergillus spp, Cyberlindnera fabianii, Candida orthopsillosis*	Miller, grains, processor, soil	GHP
	Chemical hazards – heavy metal residues, paint	Miller, environment	GMP
Boiling	Microbiological hazards- thermotolerant pathogens, spore forming bacteria like *B. cereus.*	Water, local pots, processor, environment	GHP, SQA, GMP
Sieving (CCP)	Microbiological hazards- pathogens, spore formers, spoilage microbes, thermotolerant pathogens.	Cloth sieve, food handler	GHP
Primary fermentation (CCP)	Microbiological hazards- pathogens, spore formers (*B. cereus*), spoilage microbes (*Rhodotorula mucilaginosa*), thermotolerant pathogens.	Food handler, environment, chance fermentation, back slopping samples	GMP, GHP, SQA, use of starter cultures
Boiling	Microbiological hazards- thermotolerant pathogens, spore forming bacteria like *B. cereus.*	Water, local pots, processor	GHP, SQA, GMP
Maturation (OPRP)	Microbiological hazards- pathogens, spore formers (*B. cereus*), spoilage microbes (*R. mucilaginosa*), thermotolerant pathogens	Food handler, environment	GHP, SQA, GMP
Packaging/ serving (CCP)	Microbiological hazard- coliforms, gram negative bacteria, pathogens, and spoilage microbes.	Processors, food handlers, serving utensils, environment	GHP, GMP, SQA

Key: SQA- Safety and Quality Assurance; GHP- Good Hygiene Practices; GMP- Good Manufacturing Practices; Chemical hazards except for total titratable acid and pH, were not analysed in this study as well as modes of contamination, however, due to agricultural, industrial, and other human practices, these potential hazards, sources, and modes of contamination are common.

Possible introduction of microbiological hazards i.e. pathogenic and spoilage microorganisms, endospore forming bacteria as well as pathogenic yeasts into the beverages was observed across all processing stages probably due to poor hygiene of processors, the use of unclean and untreated water for processing, use of dirty drums for steeping process, use of dirty cloth sieves for sieving, unhygienic milling conditions as well as spontaneous fermentation or back slopping methods of fermentation. Also, heating and boiling temperatures were not monitored increasing the chances of survival of pathogenic microbes in the finished products. Serving of the beverages in small calabashes and packaging in plastics can also lead to microbial contamination. Consequently, steeping, cooking and maturation (secondary fermentation) have been identified as the operational prerequisites programs in the production process that require monitoring to prevent significant hazards while sieving, milling, primary fermentation and packaging (serving) are the critical control points (CCP) which must be controlled and optimized to prevent microbial contamination. The three major forms of hazards: physical, chemical, and biological hazards were identified during the processing of *pito* and *burukutu*. Sorghum grains, which is the substrate for the beverage production, contained physical hazards like stones, sands, metal piece, glass and sac pieces which may cause mechanical damage to the grain itself and cuts, injury, bleeding to humans, hence careful sorting will be needed to for their removal. Unsafe agricultural practices and poor post-harvest storage conditions can also lead to the contamination of these grains by chemicals such as pesticides and fertilizers. Consumption of these chemical residues in food has been associated with negative health effects which include endocrine, carcinogenic, neurological, respiratory effects amongst others (Nicolopoulou-Stamati *et al*., 2016). In addition, biological hazards such as dead insects which may impact off flavour and affect the taste of the finished product if not removed as well as pathogens like coliforms and endospore-forming bacteria which found their way to the finished products were present in the sorghum grains. Unfortunately, the presence of these pathogens may have been caused by contamination from farmlands, poor handling of the grains by the suppliers, food processors and unhygienic storage and packaging conditions of the grains. Good Manufacturing Practices like storing the grains in a pest-free and dry environment as well as regular safety inspection to screen out mouldy and infected grains are important in ensuring food safety as presence of mycotoxins in cereals especially aflatoxins produced by fungal species such as *Aspergillus flavus* and *A. niger* has been reported by Blandino *et al*. (2003), Raji and Igbokwe (2005), Lyumugabe *et al*. (2012) and Bala *et al.* (2017). Also, GHP by both farmer/supplier and the beverage producer are required to prevent, eliminate or reduce these hazards and ensure food safety as some of the pathogenic microorganisms including *Bacillus cereus* found their way to the final products. According to the report of Nahemiah *et al*. (2014), thorough sorting and cleaning operations are critical in the preparation of cereal-based products to guarantee the safety of the final product. Water used for steeping of the sorghum grains is another possible source of microbial contamination. This step has been identified as an operational prerequisite programme as it requires proper monitoring and loss of control at this point could lead to introduction of significant biological hazards. Due to human unsafe practices such as dumping of domestic and industrial wastes in open waters, streams, rivers, pollution with heavy metals is a potential chemical hazard. Bioaccumulation of these toxic metals in the body of humans causes serious health hazards and may induce cancer and other risks (Jamshaid *et al*., 2018). As previously recommended by Oguntoyinbo (2012), source of water should be inspected to ensure clean, odourless, tasteless and colourless water as well as the possibility of water treatment with acceptable levels of chlorine, is used for the steeping process. Water from streams, open ponds, rivers and dirty tanks should be completely avoided. In this study, sacs used for the germination process by the local producers were observed to be dirty, used continuously without cleaning. In addition, the environments were untidy rooms exposed to dust, dirt, human and animal interference, all these constitute some risks to the safety of the beverages. The final product could also be impacted by the sanitary conditions of the milling environment. Milling machines used by the local beverage producers were either not washed or thoroughly cleaned before and after use; and some even contained residues of foods that were milled the previous day. Hence, milling has been considered a CCP as build-up of microorganisms from previous milling is a possible source of microbial contamination to the milled grains with probable impact on the final product. Therefore, proper cleaning of the milling machines and personalised use of milling machines by the local producers would limit the possibility of contamination and contributes greatly to the safety of the beverages. Sieving is another critical step in the production of *pito* and *burukutu.* The cloth sieves used to filter out the physical contaminants from the milled grains are themselves a likely source of microbial contamination. Observation from this study showed that they were used unhygienically and continuously without cleaning. Poor personal hygiene of these producers can also cause microbial contamination as faecal

coliforms like *Enterococcus faecalis, Escherichia coli, Shigella flexneri* were isolated from samples collected after sieving. Also, other contaminating microorganisms carry out unhealthy competition with the fermenting microbiota, leading to the production of toxic by-products that compromise the safety of the resulting beverages.

Samples collected after primary fermentation were dominated by lactic acid bacteria such as *Lactobacillus, Streptococcus, Lactococcus* responsible for homolactic fermentation and *Leuconostoc,* for heterolactic fermentation. Some *Bacillus* spp. which may also be involved in the fermentation process as reported previously by Jideani and Osume (2001) as well as the yeast, *Saccharomyces cerevisiae* carrying out alcoholic fermentation were also encountered. However, some pathogenic and spoilage fungi such as *Candida orthopsilosis, C. parapsilosis, Cyberlidnera fabianii, Rhodotorula mucilaginosa* were isolated from the samples in addition to spore forming pathogenic bacterium, *Bacillus cereus*. The presence of these pathogens and spoilage organisms may be due to chance or spontaneous fermentation and/or back slopping methods used in the primary fermentation process. The spontaneous and back slopping fermentation process leads to safety challenge which is in part due to the broad diversity of microorganisms in the beverages which causes unhealthy competition with the fermenting microbiota, leading to the production of toxic by-products that compromise the safety of these beverages (Evera *et al*., 2019). The primary fermentation step is identified as a CCP as some of the microorganisms introduced at this stage were observed in the final products. Safety and quality assurance (SQA), GMPs and the use of starter cultures with known functional properties that can impart desirable properties during traditional fermentation as previously suggested by Holzapfel (2002), Oguntoyinbo *et al.* (2007), Oguntoyinbo (2012) and Dike and Sanni (2012), Sanni (1993) will be required to reduce or eliminate the risk of microbial contamination.

The process of holding the beverage at ambient temperature for long hours during the maturation stage allows for microbial proliferation and possible recontamination from the environment (Nahemiah *et al*. 2014). Even though the pH of the final products were below 4.2, contamination with some Gram-negative bacteria like *E. coli, E. faecalis, P. aeruginosa, A. baumanii* were observed in the final products collected from Kano and Plateau state. These acid tolerant pathogenic strains may have originated from the producers, water used in washing the serving utensils, personal hygiene of handlers, poor sanitary conditions and unhygienic environment. Hence, packaging and serving is a CCP as the beverages are consumed immediately after. This is consistent with the findings of Bala *et al*. (2017) and Anaukwu *et al*. (2015). Moreover, the mechanism of acid tolerance among foodborne pathogen has also been documented (Arnod and Kapser, 1995). Some of the microorganisms isolated from the substrate, in-process and finished products were similar to those reported by Faparusi (1970), Raji and Igbokwe (2006), Kolawole *et al*. (2007) and Fadahunsi *et al.* (2013). *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus* are bacteria of significance in food borne diseases as they cause food intoxication, intestinal diseases and food poisoning respectively. *Staphylococcus* food intoxication is one of the most common food-borne illnesses with toxin that causes nausea, vomiting, abdominal cramping, prostration, and diarrhoea (Kobayashi *et al.,* 2015). The most important sources of these pathogens are humans, therefore, good personal hygiene and sanitation by food handlers is considered critical. Also, some species of *Staphylococcus* are considered lipolytic organisms, capable of causing hydrolytic and oxidative deterioration of the fat contained in the beverages (Nahemiah *et al.,* 2014). Pathogenic yeasts of clinical relevance such as *Candida parapsilosis, Rhodotorula mucilaginosa, C. haemulonii var vulnera, C. orthopsillosis, Meyerozyma guilliermondii, Cyberlindnera fabianii* encountered in *burukutu* and *pito*, mostly likely originated from humans, soil and the environment. They have been characterized as opportunistic pathogens causing nosocomial infections, infant and neonates' infection, candidaemia amongst other illnesses (Bonfietti, 2012, Wirth and Coldani, 2012, Desai *et al*., 2019). The presence of all these pathogens was regarded as a public health concern as *burukutu* and *pito* are consumed without any preservation and at room temperature (about 30^0C) for about two days. This can lead to rapid proliferation of these pathogens up to their infective dosages (10^2 to 10^5 per ml) and/or preformation of toxins as in the case of *Bacillus cereus, S. aureus,* enterococci and some coliforms.

High bacterial and coliform densities observed in the sorghum grains, in-process samples and final products collected from Kano and Plateau States compared to those obtained from Ogun and Lagos States could be a function of varying environmental conditions in terms of personal hygiene of the local producers, sanitation of the processing environment under which the beverages were prepared and the type of organisms, each individual producer might be harbouring, as producers from these States used their

homes and backyard of local bars as production sites. In addition, there was a lot of interference with children, pets and domestic animals with little consideration for cleanliness.

Conclusion: Contamination of traditional fermented alcoholic cereal beverages might have originated from the unhygienic processing environment, contaminated water used for production, unhygienic milling conditions, poor personal hygiene and sanitary conditions. Hazards identified from this study are of great significance and showed that food safety management approach, Hazard Analysis Critical Control Point (HACCP) will go a long way to prevent, reduce or eliminate such hazards in the production of these alcoholic beverages.

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Data Availability Statement: Data are available upon request from the first author or corresponding author.

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