Role of Organic Spices in the Preservation of Traditionally Fermented Kunun-zaki

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Kunun-zaki, produced by submerged fermentation of a combination of millet and sorghum, is a popular beverage in Northern Nigeria. Owing to the nature of the process involved in its production, kunun-zaki is highly susceptible to contamination by food spoilage microorganisms, leading to inconsistent quality and short shelf-life. In this study, we investigated various food spices, including cinnamon, garlic, and nutmeg, as potential preservatives that could be used to extend kunun-zaki shelf-life. Kunun-zaki varieties were fermented with each of these spices mentioned above and subjected to bacterial, nutritional, sensory, and quality maintenance assessments (using a twelve-member sensory panel to evaluate the organoleptic properties of kunun-zaki). Bacterial counts in the final products ranged between 10^{5-7} CFU/ml. We identified two bacterial genera, *Weissella* and *Enterococcus*, based on partial 16S rRNA gene amplicon sequencing. Three amino acids, namely leucine, aspartate, and glutamate, were abundant in all kunun-zaki varieties, while the total essential amino acid content was above 39%, suggesting that kunun-zaki could potentially be considered as a protein-rich food source both for infants and adults. The kunun-zaki products were also rich in carbohydrates, crude proteins, ash, crude fiber, and fat, with contents estimated as 81-84, 8-11, 0.8-4.0, 2.9-3.58, and 5.1-6.3%, respectively. However, this nutritional content depreciated rapidly after 24 h of storage, except for kunun-zaki fermented with garlic, which its crude protein and fat content was maintained for up to 48 h. Our results revealed that organic spices increased the nutritional content of the kunun-zaki varieties and could be potentially be used as natural preservatives for enhancing the kunun-zaki shelf-life. However, garlic might be considered a better alternative based on our preliminary investigation. The presence of the isolated microorganisms in the analyzed kunun-zaki samples should be highlighted to raise awareness on the possible health hazards that could arise from poor handling and processing techniques.

Keywords: Kunun-zaki, food spices, shelf-life

Introduction

Kunun-zaki is a popular beverage in northern Nigeria, and is produced by submerged fermentation of millet (*Pennisetum glaucum*), sorghum (*Sorghum bicolor*) or maize (*Zea mays*), in decreasing order of preference or a combination of at least two grain substrates [1–3]. Ingredients also included in kunun-zaki are sweeteners (honey or sugar), sweet potatoes and spices such as

ginger, cloves or black pepper [4, 5]. Kunun-zaki is an important beverage because it is an inexpensive source of vitamins, minerals, carbohydrates and proteins, for large population in Nigeria [6–9]. There has been a report that production methods of kunun-zaki are crude, with ingredients neither quantified nor standardized, leading to products of varying quality and consistency [1]. Most importantly, lack of control over the entire production processes makes kunun-zaki highly susceptible to contamination with pathogenic and food spoilage bacteria [10].

Lactic acid bacteria (LAB) have been reported as the dominant species isolated from kunun-zaki and there-

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fore, its fermentation is considered as the major contributor to the beneficial characteristics observed in kunun-zaki [11–13]. There has been report of microbial contamination at various stages of production [8] as processing is usually carried out under unhygienic conditions, thus, predisposing the final kunun-zaki to microbial pathogens [9, 14]. Noted that the spices used for making kunun-zaki microbial contamination while [9] reported that locally prepared beverages may serve as vehicles for zoonotic and food-borne diseases such as Staphylococcosis, salmonellosis, brucellosis, tuberculosis, Listeriosis, and shigellosis.

Organic additives as spices are generally used as flavouring, colouring, and preservation agents in food or beverages [15]. Many of these spices possess antimicrobial effects, and are derived from plant essential (volatile) oils which disrupt microbial cell membrane integrity, by negatively affecting the pH homeostasis and inorganic ions [16, 17]. This phenomenon has been associated with the volatile oils of black pepper, cloves, granium, nutmeg, oregano and thyme in their antimicrobial activities against *Enterococcus faecalis*, *Escherichia coli*, *Salmonella pullorum*, *Staphylococcus aureus* and *Yersinia enterocolitica* [18]. In addition, cinnamon volatile oils and their active compounds (cinamaldehyde and eugenol) have also shown antimicrobial activities against *E. coli*, *Pseudomonas aeruginosa*, *E. faecalis*, *S. aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Salmonella* sp. and *Vibrio parahaemolyticus* [19]. *In vitro* antimicrobial effects have also been attributed to many other volatile oils, including garlic, black cumin seeds, lemon grass, onions and laurel [20].

The current study assessed the impact of three readily available and affordable organic spices; garlic (*Allium sativum*), cinnamon (*Cinnamomum verum*) and nutmeg (*Myristica fragrans*), on the shelf-life and nutritional profile of kunun-zaki products, as potential preservatives in Kunun-zaki products.

Materials and Methods

Ethical clearance

Ethical clearance was obtained from Babcock University Health Research Ethics Committee (BUHREC) with ref number NHREC/24/01/2018.

Preparation of Kunun-zaki varieties under laboratory conditions

Kunun-zaki ingredients, including sorghum, millet, sweet potatoes (*Ipomea batatas*) and organic spices, including ginger (*Zingiber officinale*), cloves (*Syzygium aromaticum*), black pepper (*Piper nigrum*), garlic, cinnamon, and nutmeg were all purchased from the central market in Ilishan-Remo, Nigeria. Kunun-zaki was prepared in the laboratory according to the traditional fermentation method, described by [21] with slight modification. Briefly, 250 g of a mixture of millet and sorghum (ratio 4:1) was cleaned by manually removing stones and plant debris. The grains were then washed thoroughly with sterile distilled water, followed by steeping in 500 ml sterile distilled water (1: 2 w/v) for 3 h in a sterile 1000 ml beaker. The water was decanted and the grains washed with 500 ml sterile distilled water. The soaked grains were separated from the water and then wet milled into paste using an electric blender (NL-B1218 NULEK, New life, Japan). The paste was divided into two unequal parts in ratio 1:3 (v/v) and 500 ml boiling hot water was then added to the larger portion to gelatinize, while the smaller portion was mixed with blended 5.5 g sweet potato, 1 g black pepper, 1 g cloves, 1 g ginger and 5 g of either garlic, nutmeg or cinnamon. The smaller paste portion was added to the larger portion when the latter's was tempered to about 25°C. The mixture was stirred using a sterile glass rod and allowed to ferment for 12 h at room temperature. Subsequently, the samples were filtered using a sterile muslin cloth and the filtrate was the Kunun-zaki product. Three replicas of this fermentation was carried out.

Bacteriological assessment of kunun-zaki varieties

The bacterial composition of each Kunun-zaki variety at two critical control points (CCPs) was assessed by culture-dependent approach. The CCPs assessed included the grain-washing steps and the finished kunun-zaki products. One milliliter of sample was diluted in 9 ml sterile peptone water up to 10⁻⁵ dilution and pour plated on nutrient agar (NA) (Oxoid Basingstoke, UK) and De Man, Rogosa Sharpe (MRS) Agar (Hi Media, India). The plates were incubated at 37°C for 24 h, and colonies were recorded as colony forming units per ml (cfu/ml) of

sample. Distinct colonies on MRS agar were selected and re-streaked to obtain pure cultures, and were preserved in 15% glycerol.

The identity of bacterial isolates was determined by 16S rRNA gene sequencing. Genomic DNA of isolates was extracted Qick-DNA™ miniprep plus kit (Zymo research, Biolab, USA) following the manufacturer's instruction. The DNA was subsequently amplified by PCR using the primers 341F (5'-CCTACGGGAGG-CAGCAG3') and R806 (5'-GGACTACHVGG-GTWTCTAAT3-3'). Each PCR was 25 µl, containing; 12 µl PCR Master Mix (New England Biolabs), 1 µl template DNA, 10 µl sterilized distilled water and 1 µl of each primer. Thermo-cycling conditions were initial denaturation at 95°C/5 min, followed by 30 cycles of denaturation at 94°C/30 s, annealing at 50°C/30 s and extension at 68°C/5 min, followed by a final extension step at 65°C/5 min. The amplicons were resolved in 1.5% agarose gel stained with 0.1 µg/ml ethidium bromide. The amplicons were then submitted for sequencing at Inqaba Biotechnical Industrial (Pty) Ltd, South Africa. The DNA sequences obtained were compared to the NCBI 16S rRNA gene database to determine close relatives, using the BLAST search algorithm. DNA sequence data were deposited in the GenBank under the accession numbers MT023719-MT023727.

Nutritional evaluation of kunun-zaki varieties

The physicochemical properties of the final kunun-zaki products were determined by measuring pH, titratable acidity (TTA), and contents of moisture, ash, crude fiber, lipid, proteins, amino acids and carbohydrates, using the methods of [22].

Sensory and shelf-life assessment of kunun-zaki varieties

Kunun-zaki samples were either refrigerated or stored at ambient room temperature for 5 days. An informal sensory panel comprising male (8) and female (4) within the age range of 21–35 years was used to assess the quality attributes of the products; appearance, aroma, colour, taste, and overall acceptability at 12 h intervals. The pH of kunun-zaki samples was also determined intermittently with HI 2215 pH/ORP Meter (HANNA Instruments, Romania). For taste evaluation, panelists were requested to rinse their mouth with clean water between samples tasting; 15 ml of the samples were

served in clean transparent cups. The panelists' responses were recorded on a 7-points hedonic scale as recommended [23].

Statistical analysis

The effect of garlic, cinnamon and nutmeg on the pH of Kunun-zaki before and after fermentation, titratable acidity, and sensory quality were analyzed statistically with the aid of SPSS software version 21.0 using One-Way Analysis of Variance (ANOVA).

Results and Discussion

Physicochemical properties of kunun-zaki varieties

The pH of kunun-zaki before and after fermentation is presented in Fig. 1. Expectedly, pH reduced in all samples from 5.3–6.3 before fermentation to 4.1–5.2 after overnight fermentation. This may be attributed to acidification brought about by the formation of lactic acid, as previously observed by [24]. Titratable acidity, which measured the percentage of lactic acid in the kunun-zaki products, ranged between 0.0049 g/l to 0.0063 g/l signifying low TTA with poor production of lactic acid. This poor production invariably led to higher pH values (Table 1). These values were similar to those reported previously [25, 26]. However, it appeared that the samples containing cinnamon, garlic and nutmeg had a higher pH value and the control sample lacking these spices had the lowest pH; indicating the presence

Fig. 1. pH values of kunun-zaki varieties recorded before and after substrate fermentation. Error bars indicate standard deviation of the mean of data obtained from duplicate independent experiments.

Table 1. Titratable acidity and sensory qualities of different varieties of kunun-zaki fermented with organic spices.

Quality attributes	Kunun-zaki varieties			
	K1	K2	K3	K4
Titrateable acidity	0.0055 ± 0.00042	0.0057 ± 0.00031	0.0063 ± 0.00057	0.0049 ± 0.00027
Taste	1.8 ± 0.17 ^a	3.3 ± 0.37 ^b	3.83 ± 0.58 ^c	3.08 ± 0.45 ^d
Appearance	1.33 ± 0.14 ^a	3.25 ± 0.37 ^b	3.17 ± 0.51 ^c	2.42 ± 0.29 ^d
Aroma	1.50 ± 0.19 ^a	2.9 ± 0.38 ^b	3.50 ± 0.59 ^c	2.08 ± 0.38 ^d
Overall acceptability	1.42 ± 0.15 ^a	3.25 ± 0.37 ^b	3.8 ± 0.58 ^c	2.42 ± 0.38 ^d

¹ ± standard deviation of 12-member taste panellist (7-point hedonic scale: 1-like extremely, 2-like very much, 3-like slightly, 4-n either like nor dislike, 5-dislike slightly, 6-dislike very much, 7-dislike extremely).

² Different letters within the same row are significantly different ($p < 0.05$).

K1- Kunun without any spice; K2- Kunun with cinnamon; K3- Kunun with garlic; K4- Kunun with nutmeg.

Fig. 2. Bacterial counts (Log₁₀ CFU/mL) obtained after fermentation of different varieties of Kunun-zaki. Error bars indicate standard deviation of the mean of data obtained from triplicate independent experiments.

of these spices could have altered the pH of the final kunun-zaki products.

Bacterial quality of kunun-zaki varieties

The bacterial counts obtained from Kunun-zaki lacking the test spices, (control sample) had the lowest bacterial population (10^5 CFU/ml) compared to the other kunun-zaki varieties fermented with garlic, cinnamon or nutmeg which had higher microbial load (Fig. 2). Since the organic additives were adequately washed before adding them to substrates prior to fermentation, it is possible that these additives had their own normal flora which might have constituted additional nutritional substrates that further enhanced microbial growth in Kunun-zaki by 10–100 folds. High microbial populations in food products generally indicate low food quality and possible reduction in product shelf-life [27]. Kunun-zaki is a highly nutritious food substrate that may readily support the growth of active microorganisms; thus, accounting for the observed high microbial load in the samples.

DNA sequencing of the 16S rRNA gene amplicons

Table 2. 16S rRNA gene sequence identities of bacterial isolates obtained from kunun-zaki varieties.

Isolate	Source	Sequence length	Closest relative	% Similarity	GenBank accession
NOK19	Kunun ^a	395	<i>Weissella cibaria</i>	99.75	MT023719
NOK21	Kunun ^a	387	<i>Enterococcus faecium</i>	98.44	MT023720
NOK23	Kunun ^a	379	<i>Enterococcus faecium</i>	90.5	MT023721
GK29	Kunun with garlic	370	<i>Enterococcus faecalis</i>	99.19	MT023722
GK30	Kunun with garlic	363	<i>Enterococcus faecalis</i>	99.44	MT023723
GK31	Kunun with garlic	386	<i>Enterococcus</i> sp.	100	MT023724
NUTK42	Kunun with Nutmeg	371	<i>Enterococcus</i> sp.	100	MT023725
NUTK43	Kunun with nutmeg	373	<i>Enterococcus faecalis</i>	99.46	MT023726
NUTK50	Kunun with nutmeg	370	<i>Enterococcus faecalis</i>	99.46	MT023727

^aKunun-zaki without cinnamon, garlic or nutmeg.

identified bacterial isolates from the kunun-zaki samples as *Weissella cibaria*, *Enterococcus faecium*, *Enterococcus faecalis* and *Enterococcus* sp. (Table 2). *Enterococcus* and *Weissella* species belong to the lactic acid bacteria (LAB) group, and are frequently associated with food spoilage, particularly in raw milk, fermented cereals and vegetables [28]. *Weissella cibaria* has been reported to be a rare cause of nonfatal human infections and is often considered a contaminant when found in food products [29]. The bacterium *E. faecalis* is an opportunistic pathogen implicated in sepsis, endocarditis, meningitis, headaches, vomiting, increased blood pressure and even allergic reactions [30]. Therefore, the occurrence of these bacteria in the kunun-zaki products analyzed in the present study indicates potential health risk to consumers.

Sensory quality of kunun-zaki varieties

Sensory assessments of kunun-zaki varieties based on seven-points Hedonic Scale showed that Kunun-zaki (control) had mean value ranging from 1.3 to 1.8 for all sensory attributes, including taste, aroma, appearance and overall acceptability (Table 1). There was significantly different ($p < 0.05$) between the control and test varieties, with the former gaining overall acceptability while Kunun-zaki containing garlic was the least favorite of panelists. However, all varieties fell within the acceptable limit on the Hedonic scale (Table 1), suggesting that the inclusion of the garlic, cinnamon or nutmeg in Kunun-zaki may not lead to consumer rejection of product.

Proximate and amino acid composition of kunun-zaki varieties

The ash contents of finished products immediately

after fermentation ranged between 0.80–4.00% and 0.7–0.8 after 96 h; crude protein content between 8.1–11.2% and 4.9–7.4 after 96 h; fat content between 2.90–3.61% and 1.1–3.7 after 96 h; crude fibre between 5.10–6.30% and 0.9–2.6 after 96 h, moisture content between 93.3–94.8% and 90.0–91.8% after 96 h ; and total carbohydrate between 80.8–84.3% and 23.7–25.3% after 96 h (Table 3). The highest crude protein content was found in kunun-zaki with Nutmeg compared to kunun-zaki with cinnamon and kunun-zaki with garlic. Reasons for this might have been as a result of the additives added and probably because most of it might have been lost during processing [4] much of the proteins in cereals and other crops are usually located in the testa and germ, which are usually sieved off during processing. The % crude fibre in this study shows that the continuous utilization of the reducing sugars and extractable fat may have led to the increase in the crude fibre content proportion of the kunun-zaki samples. High fibre percentage in this work implies high energy constituent of the kunun-zaki samples, which makes it a more energetic beverage drink. The carbohydrate content of the samples reveals that the kunun-zaki samples analysed was rich in carbohydrate. Carbohydrate is a good source of energy while dietary fiber is very important in human nutrition as it lowers the body cholesterol level; consequently reducing the risk of cardiovascular diseases. Being a thirst-quenching beverage, Kunun-zaki has high moisture content ranging between 55–98% [31, 32]. The high moisture content may imply greater susceptibility of kunun-zaki to microbial spoilage, as previously suggested by [33, 34]. The moisture was not significantly different among the four kunun-zaki varieties analyzed in this study; therefore, the garlic, nutmeg or cinnamon did not significantly reduce the moisture content of

Table 3. Proximate compositions of kunun-zaki varieties after zero and 96 h of storage under ambient room temperature.

Kunun-zaki variety	Proximate parameter (%)											
	Crude protein		Fat		Ash		Crude fiber		Moisture		Carbohydrate	
	0 h	96 h	0 h	96 h	0 h	96 h	0 h	96 h	0 h	96 h	0 h	96 h
K1	10.1	5.5	3.6	1.1	0.8	0.7	5.8	1.4	94.8	90.0	84.3	23.7
K2	10.8	7.0	2.9	3.7	3.4	0.9	5.1	0.9	94.1	91.2	83.0	25.3
K3	8.1	7.4	3.0	3.5	4.0	0.7	5.5	2.6	94.5	91.5	81.3	22.7
K4	11.2	4.9	3.0	3.2	3.2	0.8	6.3	2.5	93.3	91.8	80.8	24.6

K1- Kunun without garlic, cinnamon or garlic; K2- Kunun with cinnamon; K3- Kunun with garlic; K4- Kunun with nutmeg.

Table 4. Amino acids composition of different varieties of kunun-zaki fermented with organic spices.

Kunun-zaki variety	Amino acids (g/100 g Protein)																		TEAA (%)
	Ala-nine	Argi-nine	Aspar-tate	Cystine	Gluta-mate	Glycine	Histi-dine	Isoleu-cine	Leucine	Lysine	Methi-onine	Phenyl-alanine	Proline	Serine	Thre-onine	Trypto-phan	Tyro-sine	Valine	
K1	5.6	3.6	5.9	5.3	20.4	3.3	2.4	4.2	10.1	2.1	2.6	7.6	8.4	5.5	3.7	8.1	3.1	5.7	50.2
K2	6.3	3.8	6.4	6.8	18.8	3.3	2.4	4.4	9.7	2.4	2.3	6.3	8.8	5.3	3.9	8.7	3.0	5.4	49.3
K3	6.5	3.4	6.9	4.8	19.5	4.2	2.1	4.4	9.9	2.7	2.2	7.2	8.4	5.5	3.6	1.0	2.8	5.2	41.6
K4	6.6	3.8	6.7	4.4	19.5	3.6	3.2	4.8	9.6	2.5	2.2	7.4	8.2	4.8	3.4	9.4	2.9	5.1	51.3

K1- Kunun without garlic, cinnamon or garlic; K2- Kunun with cinnamon; K3- Kunun with garlic; K4- Kunun with nutmeg.

kunun-zaki. However the nutritional content depreciated rapidly after 24 h in all samples except in kunun-zaki fermented with garlic whose crude protein and fat content lasted up to 48 h (Fig. 5).

Three amino acids, namely leucine, proline and glutamate were most abundant in the kunun-zaki samples at concentrations ranging from 9.5 to 20.3 g/100 g protein (Table 4). Comparatively, Variety K3 had the highest value for total essential amino acid (TEAA) (51.3%), while variety K2 had the lowest (41.6%) [35]. Recommended that food materials having TEAA values of 11% and 26% were adequate for adults and infant protein supplementations respectively; suggesting that the kunun-zaki reported in this study is rich in protein,

regardless of the variety.

Shelf-life of kunun-zaki varieties

Evaluation of kunun-zaki varieties for 96 h (5 days) of storage under refrigeration or ambient room temperature showed that the pH values of the final products decreased significantly within the first 24 h in both refrigerated and unrefrigerated sample. Although the decline was slightly less steep in the refrigerated samples (Fig. 3). The proximate composition of the kunun-zaki

Fig. 3. pH values of kunun-zaki varieties stored under ambient room temperature for 96 hours.

Fig. 4. The four varieties of kunun-zaki at 0 hour, 24 hours and 48 hours stored under room temperature. K1- Normal kunun-zaki, K2- Kunun-zaki with Cinnamon, K3- Kunun-zaki with Garlic, K4- Kunun-zaki with Nutmeg.

Fig. 5. Proximate composition (Protein, ash, fat, crude fibre, moisture and carbohydrate) of Kunun-zaki varieties monitored over five days storage at room temperature. Variety 1 - Kunun-zaki without organic additives; Variety 2 - Kunun-zaki with nutmeg; Variety 3 - Kunun-zaki with cinnamon; Variety 4 - Kunun-zaki with garlic.

varieties were also measured throughout the storage period, but only under ambient room temperature, as this was the condition Kunun-zaki are actually marketed. As shown in Table 3, except for the moisture content, the values of all other parameters measured decreased significantly between 0 h and 96 h. The most pronounced decline was recorded in ash, crude fibre and carbohydrate contents. These results indicated that kunun-zaki stored under room temperature in tropical environment is most nutritionally beneficial when consumed within the first 24 h after production, regardless of spices used.

Subsequently, the appearance, colour and texture of

the variously treated kunun-zaki varieties declined with increase in shelf-life duration (Fig. 4). The color and consistency of kunun-zaki products were also assessed as indicators of quality and spoilage over the 48 h storage period, as indicated in Fig. 4. Colour deterioration of the samples could have resulted from possible accumulation of microbial metabolic products. The decline in texture of the kunun zaki samples could be attributed to, among other factors, possible increase in microbial load of the product over time.

The proximate composition decreased with increase in keeping time (Fig. 5). The protein content of the samples

decreased in duration of storage for a period of 98 h. The slight increase in protein content could be due to protein hydrolysis, which involves a consistently active proteinase activity resulting in rapid amino acid production during fermentation. A slight increase in the protein content (4.5%) was observed in fermented locust bean [36]. The ash content decreased after 24 h for all the samples except normal kunun whose ash content increased during storage (98 h). The reduction in the ash content could be as a result of its usage as metabolic nutrients for the growth of microorganisms [32]. The effect of the organic additives on the moisture content was very slight. There was a slight decrease in all samples at 24 h and 48 h and an increase again on all samples at 72 h and 98 h. This could be said to be non-significant and could be due to the relatively high level of moisture in the product. The fat content of the four different samples were low compared to the result obtained by [24] and there was an increase as fermentation time increased especially for kunun-zaki with garlic. Dehulling and milling of grains has been shown to reduce the crude fibre content of food products [37]. After storage for 96 h there was a rapid decrease in carbohydrate content.

Conclusion

The results revealed that organic spices increased the nutritional content of the kunun-zaki varieties and have potential for application as natural preservatives for enhancing the shelf-life of kunun-zaki. However, garlic may be considered better alternative based on preliminary investigation. The presence of the isolated organisms in kunun-zaki samples analysed could serve as the need to promote awareness about possible health hazards that could arise from poor handling and processing techniques.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

References

1. Aboh MI, Oladosu P. 2014. Microbiological assessment of kunun-zaki marketed in Abuja Municipal area council (AMAC) in the Federal Capital Territory (FCT), Nigeria. *Afr. J. Microbiol. Res.* **8**: 1633-1637.
2. Adejuyitan J, Adelakun O, Popoola F. 2008. Evaluating the quality characteristics of kunun produced by dry-milled sorghum. *Afr. J. Biotechnol.* **7**: 2244-2247.
3. Gaffa T, Jodeani I, Nkama J. 2002a. Traditional production, consumption and storage of kunun; A non-alcoholic cereal beverage. *Plant Foods. Hum. Nutr.* **57**: 73-81.
4. Akoma O, Jiya E, Mishilia E. 2006. Influence of malting on the nutritional characteristic of Kunun-zaki. *Afr. J. Biotechnol.* **5**: 996-1000.
5. Onuorah SL, Adesigun AA, Adeleke JO. 1987. Occurrence of *Staphylococci* and *E. coli* form in kunun-zaki and utensils used in its production. *J. Food. Agric.* **11**: 31-34.
6. Essien E, Monago C, Edor E. 2009. Evaluation of the nutritional and microbiology quality of kunun (A cereal based non-alcoholic beverage) in Rivers State, Nigeria. *Int. J. Nutr. Well.* **10**: 1-4.
7. Osuntoki A, Korie F. 2009. Antioxidant activity of whey from fermented milk, fermented with *Lactobacillus* species from Nigerian fermented foods. *J. Food Technol. Biotechnol.* **48**: 505-511.
8. Oyewole OA, Isah P. 2012. Locally fermented foods in Nigeria and their significance to national economy. *J. Rec. Adv. Agric.* **1**: 92-102.
9. Umaru GA, Tukur IS, Akensire UA, Adamu Z, Bello OA, Shawulu AHB, et al. 2014. Micro flora of kunun-zaki and zobo drinks in relation to public health in Jalingo metropolis, North-Eastern Nigeria. *Int. J. Food Res.* **1**: 16-21.
10. Maoura N, Mbaiguinam M, Nguyen HV, Gaillardin C, Pourquie J. 2005. Identification and typing of yeast strains isolated from bili bili, a traditional sorghum beer of Chad. *Afr. J. Biotechnol.* **4**: 646-656.
11. Chelule PK, Mokoena MP, Gqaleni N. 2010. Advantages of traditional lactic acid bacteria fermentation of food in Africa. In Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology (ed), pp. 1160-1167. A Méndez-Vilas.
12. Van der Aa Kühle A, Jesperen L, Glover RKL, Diawawr B, Jakobsen M. 2001. Identification and characterization of *Saccharomyces cerevisiae* strains isolated from West African sorghum. *Yeast* **18**: 1069-1079.
13. Naumova ES, Korshunova IV, Jespersen L, Gennadi I, Naumov GI. 2003. Molecular genetic identification of *Saccharomyces sensu stricto* strains from African sorghum beer. *FEMS Yeast Res.* **3**: 177-184.
14. Ayandele AA. 2015. Microbiological analyses of hawked kunun and sobo drinks within LAUTECH campus, Ogbomoso, Oyo State, Nigeria. *IOSR J. Environ. Sci. Toxicol. Food Tech.* **9**: 52-56.
15. Alcicek A, Bozkurt M, Cabuk M. 2003. The effect of an essential oil combination derived from selected herbs growing wild in Turkey performance. *S. Afr. J. Anim. Sci.* **33**: 89-94.

16. Halender IM, Alakomi HI, Latva-Kala K, Mattila-Sandholm T, Pol I, Smid EJ, *et al.* 1998. Characterization of the action of selected essential oil components on Gram-negative bacteria. *J. Agric. Food. Chem.* **46**: 3590-3595.
17. Lambert RJW, Skandamis PN, Coote PJ, Nychas GJE. 2001. A study of the minimum inhibitory and mode of action of oregano essential oils, thymol and carvacrol. *J. Appl. Microbiol.* **91**: 453-462.
18. Dorman HJD, Deans SG. 2000. Antimicrobial agent for plants: antibacterial activity of plants volatile oils. *J. Appl. Microbiol.* **88**: 308-316.
19. Chang ST, Chen PT, Chang SC. 2001. Antibacterial of leaf essential oils and their constituent from *Cinnamomum osmophleom*. *J. Ethnopharmacol.* **77**: 123-127.
20. Kraig R. 2013. Herbs and spices for improving the gastrointestinal health of the rabbit. In: Proceedings of the 25th Hungarian Conference on Rabbit production. pp. 53-68. Kaposvar, Hungary.
21. Gaffa TIA, Jideani I, Nkama I. 2002b. Nutrients and sensory qualities of kunun-zaki from different saccharifying agents. *Int. J. Food Sci. Nutr.* **53**: 109-115.
22. AOAC. 2006. Official Methods of Analysis of AOAC International. (18th Ed.). AOAC International, Gaithersburg, MD., USA, ISBN13: 978-0935584752.
23. Larmond E. 1977. Laboratory Methods for Sensory Evaluation of Food. Research Branch, Canada Department of Agriculture. **1637**: 19-63.
24. Nkama I, Agarry OO, Akoma O. 2010. Sensory and nutritional quality characteristics of powdered kunun-zaki; A Nigerian fermented cereal beverage. *Afr. J. Food Sci.* **4**: 364-370.
25. Obanewo OS, Zidon G. 2008. Production and evaluation of powered kunun-zaki using fluidized bed dryer. *Nig. Food. J.* **21**: 144-146.
26. Obadina AO, Oyewole OB, Awojobi TM. 2008. Effect of steeping time of milled grains on the quality of Kunun-Zaki (a Nigerian beverage). *Afr. J. Food Sci.* **2**: 033-036.
27. Seema R. 2015. Food spoilage: Microorganisms and their prevention. *Asian J. Plant Sci. Res.* **5**: 47-56.
28. Tannock GW, Cook G. 2002. *Enterococci* as members of the intestinal microflora of humans. In: *The Enterococci, Pathogenesis, Molecular Biology and Antibiotics Resistance* (Gilmore, M. S. ed), pp. 101-132. ASM, Washington, DC. USA.
29. Olano A, Chua J, Schroeder S, Minari A, La Salvia M, Hall G. 2001. *Weissella confusa* (basonym: *Lactobacillus confusus*) bacteremia: a case report. *J. Clin. Microbiol.* **39**: 1604-1607.
30. Giraffe G. 2002. *Enterococci* from foods. *FEMS Microbiol. Rev.* **26**: 163-171.
31. Giese G. 1995. Measuring physical properties of foods. *J. Food Technol.* **2**: 49-51.
32. Amusa NA, Ashaye OA. 2009. Effect of processing on nutritional, microbiological and sensory properties of kunun-zaki (a sorghum based non-alcoholic Beverage) Widely Consumed in Nigeria. *Pakistan J. Nutr.* **8**: 288-292.
33. Battey AS, Duffy S, Schaffner DW. 2002. Modelling Yeast spoilage in cold-filled ready-to- drink beverages with *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii* and *Candida lipolytica*. *Appl. Environ. Microbiol.* **68**: 1901-1906.
34. El-mahmood AM, Doughari JH. 2007. Microbial quality assessment of kunun-zaki beverage sold in Girei town of Adamawa State Nigeria. *Afr. J. Food Sci.* **1**: 11-15.
35. FAO/WHO/UNU. 1985. Energy and protein requirements. Technical report series. No. 275, Geneva, 204.
36. Odunfa SA. 1985. African fermented foods In: *Microbiology of Fermented Foods*. Vol.2. B.J.B. Wood (Ed.) pp. 155-191. London: Elsevier Applied Science.
37. Adeyemi IA. 1983. Dry milling of sorghum for ogi manufacture. *J. Cereals Sci.* **1**: 221-226.