Original Research Article

Physico-chemical Quality Characteristics and Hazard Analysis Critical Control Points for the Production of millet-based Kunun zaki obtained from three production locations in Port Harcourt, Nigeria.

C. N. Elele[1*], S. N. Ibe[1] and G. O. Abu[1]

[1]*Department of Microbiology, University of Port Harcourt, P.M.B. 5323, Choba, Rivers State,Nigeria.*

Authors' contributions

This work was carried out in collaboration between all authors. Authors CNE and SNI designed the study and wrote the first manuscript. Authors SNI and GOA managed the analyses of the study, helped in paper write up. All authors read and approved the final manuscript

ABSTRACT

Aim: To study the commercial preparation of Kunun zaki in three locations in Port Harcourt namely; Bori-camp, Rumuodomaya, and Mgbuogba, to compare the microbiological, physico-chemical, and nutritional qualities of commercial and laboratory-prepared Kunun drinks, and to establish the critical control points at various points of the production process.

Place and Duration of study: Sample collection areas were Bori-camp, Rumuodomaya, Mgbuogba, and the Laboratory, between August and December 2012. Sample analysis was done in the Food and Industrial Microbiology Laboratory of University of Port Harcourt.

Methodology: At selected stages of preparation of kunun zaki from Bori-camp, Rumuodomaya, Mgbuogba, and the Laboratory, nine samples each were collected into sterile screw-capped 50cl bottles for analysis at the Food and Industrial Microbiology laboratory of University of Port Harcourt. Analyses carried out were to determine the microbial contaminants, proximate and physico-chemical parameters. Samples of fresh kunun zaki were stored at 5^0C for three days for analysis on effect of storage.

Results: The microorganisms associated with freshly prepared kunun zaki and that stored at refrigeration temperature (5^0C) for three days were *Lactobacillus* spp, *Bacillus* spp, *Leuconostoc* spp, *Streptococcus* spp, *Micrococcus* spp, *Staphylococcus* spp, *Pseudomonas* spp, *Escherichia coli, Enterobacter* spp, *Penicillium* spp, *Mucor* spp, *Aspergillus* spp, *Rhizopus* spp, *Candida* spp,and *Saccharomyces* spp. Bori-camp preparation had the highest coliform and staphylococci counts of $7.80x10^5$cfu/ml and $7.47x10^4$cfu/ml respectively, while Laboratory preparation had the least counts of $6.2x10^3$cfu/ml and $7.7x10^2$cfu/ml for coliforms and staphylococci respectively. Total viable counts for commercially-prepared product ranged from $1.29x10^4$ to $2.5x10^4$cfu/ml, while Laboratory preparation was $6.0x10^2$cfu/ml. Fungal counts for commercially-prepared drink ranged from $1.0x10^4$ to $1.36x10^5$cfu/ml, while Laboratory-prepared drink had fungal counts of $5.6x10^2$cfu/ml. Yeasts were the main spoilage organisms which persisted at storage temperature of 5^0C for three days. The protein and carbohydrate contents, as well as calcium, zinc, copper, and manganese decreased after being stored for three days at 5^0C.

Conclusion: Therefore good manufacturing practices for Kunun zaki production implies the use of fresh non-moldy grains and spices, cooled boiled water for grain washing and steeping, sterile stainless steel containers, steam-sterilized grinder, and sterile screw-capped bottles for packaging be applied at all stages of the production process. Educating the producers on the hazards, critical control points (steeping,milling, and packaging), and monitoring procedures (pH and temperature maintenance) for Kunun zaki preparation are important.

Key words: *Kunun zaki, Commercially-prepared and laboratory-prepared kunun drinks.*

1. INTRODUCTION

Kunun zaki is one of the most common non-alcoholic fermented beverage made mainly from millet, sorghum, maize, and guinea corn [1 and 2]. It is a nutritious locally-made beverage, common in northern Nigeria. It has low viscosity, sweet-sour taste with a pH ranging from 4.60 to 5.00 and milky cream appearance, and contains about 1.2% protein, 67% carbohydrate, and minerals (copper, zinc, calcium, and manganese) 0.55 to 3.30ppm [1]. During the preparation of kunun zaki from millet, the ingredients needed are ginger (*Zingiber officinale),* cloves *(Syzygium aromaticum),* dried red pepper *(Capsicum cultivars*), sweet potato *(Ipomoea batatas*), millet *(Pennisetum typhoideum*), sugar and water. There are variations in the traditional preparation of kunun zaki. According to Olanrewaju *et al.*, [3], the traditional preparation of Kunun zaki generally adopted by commercial producers, involves steeping of the millet in local household utensils such as buckets, drums, or calabashes for 24 hours. The steeped grains are washed along with the spices, wet-milled into paste and divided into two portions. The first (larger) portion is subjected to heat treatment by the addition of hot water, with constant stirring to homogenize till it becomes gelatinized, while the other smaller portion (the inoculum) is added and stirred to homogenize. The mixture is then allowed to cool, left overnight to allow fermentation to occur and filtered. The final product can be sweetened with sugar and packaged in small non-sterilized bottles and bags for sale. The fermentation which occurs during steeping (before milling) and after milling involves mainly lactic acid bacteria and yeasts [4]. The flow diagram of traditional kunun zaki production process as described by Amakoromo [2] is shown in Figure 1.

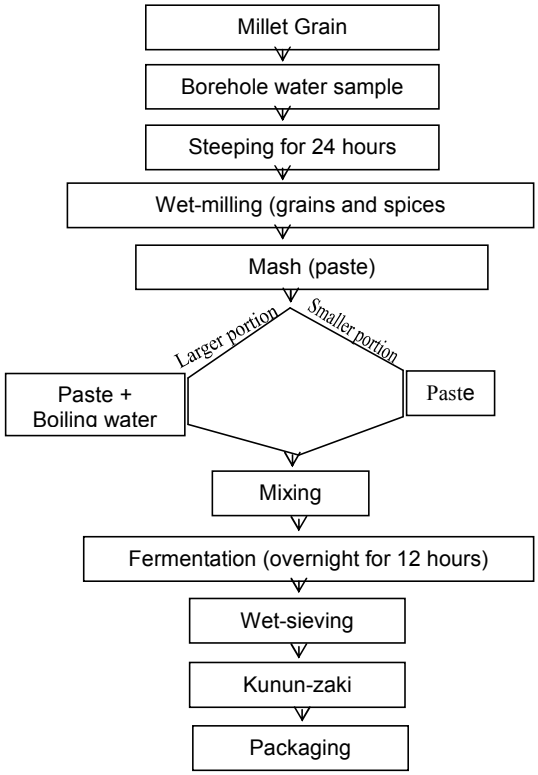

Figure 1: Flow diagram of commercial Kunun zaki production process.

Due to excessive handling by different people which increases the chances of cross-contamination, the use of unsterilized containers, and milling machine, the final product is contaminated and may be microbiologically unsafe for consumption. Various microbial genera (fermenting, spoilage and pathogenic organisms) recorded include; *Lactobacillus* spp, *Bacillus* spp, *Leuconostoc* spp, *Streptococcus* spp, *Micrococcus* spp, *Staphylococcus* spp, *Pseudomonas* spp, *Escherichia coli, Penicillium* spp, *Mucor* spp, *Aspergillus* spp, *Rhizopus* spp, *Candida* spp, and *Saccharomyces* spp [5]. *Lactobacillus* spp, *Streptococcus lactis*, and *Saccharomyces cerevisiae* isolates have been used as starter cultures singly and in pairs to produce Kunun zaki in the laboratory from millet [6].This research work was conducted to study the

Comment [OA1]: Delete line 64-94

commercial preparation of kunun zaki in three locations in Port Harcourt namely; Bori-camp, Rumuodomaya, and Mgbuogba, to compare the microbiological, physico-chemical, and nutritional qualities of commercial and laboratory-prepared Kunun drinks, and to establish the critical control points at various points of the production process.

2. MATERIALS AND METHODS

2.1Sampling

At selected stages of preparation of kunun zaki from Bori-camp, Rumuodomaya, Mgbuogba, and the Laboratory, nine samples each, as shown in Table 1 were collected into sterile screw-capped 50cl bottles for analysis at the Food and Industrial Microbiology laboratory of University of Port Harcourt, Nigeria. Analyses carried out were to determine the microbial contaminants, proximate and physico-chemical parameters. Samples of fresh kunun zaki were stored at 5^0C for three days for analysis on effect of storage.

Table 1: Nine sampling stages during Kunun zaki preparation.

Sample	Stage description
A	Water sample prior to steeping
B	Steeped millet water
C	Wet-milled spices
D	Wet-milled millet
E	Wet-milled millet + spices
F	Wet-milled millet + spices + Hot water
G	Overnight fermented sample (Unsieved)
H	Freshly prepared kunun zaki (Sieved and packaged)
I	3-day refrigerated kunun zaki.

2.2 Laboratory production of Kunun zaki:

The laboratory production varied from the commercial process by thorough washing of the grains with cooled boiled water, thereafter the washed grains were steeped for 12 hours in a cooled boiled water in a sterile stainless basin. The spices (ginger, red pepper, and cloves), and sweet potato were thoroughly washed respectively. The steeped millet grains were again washed with cooled boiled water before milling. Milling of millet, spices, and sweet potato was done with Panasonic electric blender, model (MX-J110P), washed and rinsed out with hot sterile water. The milled millet was mixed with ~~the~~ milled spices, and carefully stirred with a sterile stainless spoon in the sterile basin. Thereafter, the mixture was divided into two portions namely: A (larger portion) and B (smaller portion). Hot water for sample F at a temperature of 100^oC was carefully added to portion A. Portion B (the inoculum) was carefully added to portion A at a temperature of 55^oC, then allowed to cool to a temperature of 30^oC, covered to allow fermentation to take place overnight (for 12 hours); and then carefully filtered using a sterile Cheesecloth (sterilized by foiling and autoclaving at 121^oC for 15 minutes). The final product was bottled and sealed in sterile screw-capped 50cl bottles.

2.3 Enumeration of microorganisms at various stages of Kunun zaki production.

Microorganisms from each sample of the four preparations were enumerated using ten-fold serial dilution technique. The diluent was 0.1% sterile peptone water. 0.1ml of the diluted samples were spread-plated in dupicates on Nutrient agar (NA) for total viable counts, MacConkey agar (MacC) for coliforms, Mannitol salt agar (MSA) for staphylococci count, De Mann Rogosa and Sharpe agar (MRS) for lactic acid bacterial count, while for fungal counts (molds and yeasts) sabouraud dextrose agar adjusted to pH 3.5 with lactic acid was used.

Samples on Nutrient agar and MacConkey agar plates were incubated for 24 hours at 37^0C, those on Mannitol salt agar and De Mann Rogosa and Sharpe agar plates were incubated for 48 hours at 37^0C, while those on Sabouraud dextrose agar plates were incubated for 5 days at 26^0C. Digital illuminated colony counter was used to count the colonies. Pure cultures for identification of each isolate was made by streaking the individual colony on suitable medium and incubating appropriately, and then maintained in an agar slant contained in MacCartney bottles.

2.4 Identification of the microbial isolates

Bacterial isolates were sub-cultured from their respective stock cultures to an appropriate sterile medium and were incubated at 37^0C for 24 hours. Identification of the isolates were determined after carrying out biochemical tests and by comparison with the keys in Bergey's manual of determinative bacteriology [6]. Fungal isolates were identified with reference to [7].

Comment [OA2]: Cross-check your referencing numbering

2.5 Physico-chemical and Proximate Analyses

pH, total titratable acidity, total solids, and turbidity were determined as described by AOAC [9].

2.6 Proximate Analysis

Crude protein, carbohydrate content, moisture content, ash, and minerals were also determined by AOAC [9] based on location and storage time.

3. RESULTS AND DISCUSSION

3.1 Isolation and identification of microbial Isolates

Nine bacterial genera were isolated and characterized from the different production stages of kunun zaki of the four preparations. They were characterized as *Bacillus* spp, *Pseudomonas* spp, *Staphylococcus* spp, *Escherichia coli*, *Enterobacter* spp, *Micrococcus* spp, *Streptococcus* spp, *Lactobacillus* spp, and *Leuconostoc* spp. Mold and yeast isolates were four and two respectively, and they were identified as *Penicillium* spp, *Mucor* spp, *Aspergillus* spp, and *Rhizopus* spp, and *Saccharomyces* spp, and *Candida* spp. These organisms isolated confirmed the research carried out by [5].

Comment [OA3]: Did not see this data in any of this tables presented.

The presence of Lactic acid bacteria (*Lactobacillus* spp, *Leuconostoc* spp, *Streptococcus* spp, and *Bacillus* spp) are not surprising as most of them thrive in medium rich in fermentable substrates like sugars, which often led to the production of acids after fermentation [5].

Comment [OA4]: Bacillus as LABs????, cross check your facts

Coliforms like *Escherichia coli* in water and other samples indicates faecal contamination, and they are known to be causative agents of food borne gastroentiritis and bacterial diarrhoea diseases [10]. Their presence also could be attributed to the use of dirty containers, as well as dirty environment where the Kunun samples were processed [11].

Staphylococcus species were possible contaminants from handlers and utensils used during and after the preparation [5].

Pseudomonas spp and *Micrococcus* spp isolated in the four preparations have been implicated in food spoilage as they used the carbohydrate content of the food for undesirable fermentation process [12].

The presence of molds (*Penicillium* spp, *Mucor* spp, *Aspergillus* spp, and *Rhizopus* spp) could be attributed to unhygienic way the drink was prepared since their spores which could have emanated from the air have high rate of survival, and also their predominance on the grains and spices [13].

The yeasts (*Saccharomyces* spp, and *Candida* spp), isolated in the four preparations may be attributed to the acidic nature of the samples, since it has been observed that yeasts are capable of utilizing organic acids from cereals [1].

3.2 Determination of microbial counts

The total heterotrophic counts, total coliform counts, total staphylococci counts, lactic acid bacteria counts, and total fungal counts are shown in Table 2.

Table 2: Microbial load and types of organisms at various stages of Kunun zaki preparation obtained from the locations of study.

Production stages	Locations of study	Total Viable counts	Total coliforms (cfu/g or ml)	Staphylococci	Lactic acid bacteria	Fungi
A	Bori-camp	$1.44x10^3$	$1.8x10^3$	$1.52X10^2$	NP	$4.6X10^2$
	Rumuodomaya	$1.37x10^3$	$1.1X10^3$	$3.8X10^2$	NP	9.0X10
	Mgbuogba	$1.19x10^3$	$8.0X10^2$	NIL	NP	$3.0X10^2$
	Laboratory	$3.2x10^2$	6.0X10	2.0X10	NP	NIL
B	Bori-camp	$8.63x10^4$	$1.47x10^5$	$3.0X10^4$	$1.32X10^5$	$1.97X10^5$
	Rumuodomaya	$2.78x10^4$	$3.9X10^4$	$9.9X10^3$	$3.0X10^4$	$2.2X10^3$
	Mgbuogba	$3.0x10^4$	$1.05X10^4$	$1.0X10^2$	$3.0X10^4$	$3.0X10^3$
	Laboratory	$4.3x10^3$	$3.0X10^3$	$3.0X10^2$	$2.85X10^4$	$1.7X10^3$
C	Bori-camp	$3.23x10^4$	$8.16x10^4$	$2.27X10^4$	$6.17X10^4$	$5.9X10^4$
	Rumuodomaya	$3.0x10^4$	$3.0X10^4$	$5.25X10^3$	$1.1X10^4$	$2.15X10^3$
	Laboratory	$9.6x10^3$	NIL	NIL	1.5X10	$6.0X10^2$
D	Bori-camp	$1.73x10^5$	$1.35x10^5$	$5.7X10^3$	$5.8X10^4$	$2.43X10^5$
	Rumuodomaya	$2.51x10^4$	$1.80X10^4$	$5.4X10^3$	$2.98X10^4$	$3.1X10^3$
	Laboratory	$8.0x10^3$	$3.1X10^3$	$3.5X10^2$	$1.0X10^4$	$2.1X10^4$
E	Bori-camp	$1.12x10^5$	$7.0x10^4$	$2.5X10^3$	$1.98X10^5$	$2.20X10^5$
	Rumuodomaya	$1.28x10^4$	$2.3X10^3$	$3.0X10^2$	$9.0X10^3$	$1.03X10^4$
	Mgbuogba	$7.7x10^3$	$4.5X10^3$	NIL	$1.59X10^4$	$6.0X10^3$
	Laboratory	$5.7x10^3$	NIL	NIL	$8.8X10^3$	$2.15X10^4$
F	Bori-camp	$1.15x10^5$	$6.7x10^4$	$6.0X10^2$	$2.62X10^5$	$1.03X10^5$
	Rumuodomaya	$9.0x10^3$	$1.0X10^4$	$6.0X10^2$	$3.0X10^4$	$9.0X10^2$
	Mgbuogba	$2.03x10^4$	$7.5X10^3$	NIL	$3.0X10^4$	$1.0X10^2$
	Laboratory	$3.0x10^2$	NIL	$1.0X10^2$	$1.0X10^4$	$1.0X10^2$
G	Bori-camp	$6.43x10^4$	$1.48x10^5$	$2.7X10^3$	$3.0X10^5$	$1.95X10^5$
	Rumuodomaya	$1.45x10^4$	$1.01X10^4$	$7.25X10^3$	$1.51X10^4$	$2.0X10^2$
	Mgbuogba	$2.91x10^4$	$3.3X10^3$	$1.0X10^2$	$2.85X10^4$	$1.2X10^4$
	Laboratory	$7.7x10^3$	NIL	NIL	$2.3X10^4$	$1.0X10^2$
H	Bori-camp	$1.53x10^4$	$8.0x10^3$	$8.3X10^3$	$2.33X10^4$	$1.36X10^5$
	Rumuodomaya	$1.29x10^4$	$8.75X10^3$	$1.3X10^3$	$2.97X10^4$	$2.5X10^2$
	Mgbuogba	$2.5x10^4$	$3.0X10^3$	$1.5X10^3$	$2.75X10^4$	$1.0X10^4$
	Laboratory	$6.0x10^2$	NIL	NIL	$2.0X10^4$	$5.6X10^2$
I	Bori-camp	$1.56x10^5$	$1.21x10^5$	$2.0X10^3$	$2.85X10^5$	$4.75X10^4$
	Rumuodomaya	$1.30x10^4$	$1.82X10^4$	$7.65X10^3$	$2.2X10^4$	$1.2X10^4$
	Mgbuogba	$3.0x10^4$	$2.3X10^4$	$3.0X10^3$	$3.0X10^4$	$2.9X10^4$
	Laboratory	$9.0x10^3$	NIL	NIL	$3.0X10^4$	$1.94X10^4$

Comment [OA5]: The presence of coliforms and staph in water prior to steeping of grains is alarming and doubtful; authors should verify this facts Also, their presence in the finished products is questionable if actually the fermentation process was driven by lactic acid bacteria.

KEY

A = Water sample prior to steeping
B = Steeped millet water
C = Wet-milled spices
D = Wet-milled millet
E = Wet-milled millet + spices
F= Wet-milled millet + spices + hot water
G = Overnight fermented sample (Unsieved)
H = Freshly prepared kunun zaki (packaged)
I= 3-day refrigerated kunun zaki
NP= Not plated.

Apart from water sample (A) that was not plated for Lactic acid bacteria in the four locations of study, other organisms were isolated from sample A. This indicates that the water used to steep the millet was not sterilized. Boiled water sample used in Laboratory preparation still harboured some contaminants, though at minimal level when compared with those of commercial preparations.
When the grains were steeped in the water sample (A), a gradual increase and appearance of Lactic acid bacteria though plated was observed. The grains introduced more organisms (desirable and undesirable) into sample A.

Studies have shown that the method of preparation of Kunun zaki varies from different localities owing to preference in consumer taste, texture, or appearance [3], samples C and D were mixed to produce F by Mgbuogba's producer, and hence were not individually collected. The spices harboured undesirable organisms in two preparations (Bori-camp and Rumuodomaya),implying poor harvesting procedures and improper washing of the spices by the farmers and producers respectively. Laboratory preparation harboured no coliforms and staphylococci. In Laboratory preparation, the spices were thoroughly washed, hence no coliforms and staphylococci were isolated.

Spices used in Kunun zaki preparation are usually not peeled before milling. Thus, this necessitates them being thoroughly washed prior to milling. They are also known to be antimicrobials if devoid of dirts (accumulated soil or dust particles), and was observed in the Laboratory preparation where they eliminated coliforms and staphylococci in sample E.

Hot water added to portion A of sample E in the course of Kunun zaki preparation was not necessarily for sterilization purpose, but for flavor extraction from the spices, and gelatinization of the mash. If the sample (F) were plated immediately, the hot water added (although not hot enough as in the case of Commercial preparation), would have eliminated all organisms in the four preparations, but because the steeping and milling processes were carried out unhygienically in commercial preparation, water added was not hot enough, and samples were kept in filthy environment for 12 hours (overnight) before samples G and H were collected and then plated alongside with the previous samples collected, undesirable microorganisms were still isolated from F. Hot water added to portion A of Laboratory preparation eliminated the coliforms and staphylococci as evidenced in sample G, harboured the desirable microorganisms (Lactic acid bacteria) and yeasts after being allowed to cool and ferment overnight (12 hours). These fermenters emanated from the inoculum (portion B) added to portion A at a temperature of 55°C.

Coliforms and staphylococci still re-occurred in samples H and I of commercially-prepared Kunun zaki, while Laboratory-prepared Kunun zaki harboured none.
Spoilage organisms (*Pseudomonas* spp and *Micrococcus* spp) were isolated from I in the four preparations, implying a monitored and constant temperature could be employed.

SIR/MADAM, PLEASE NOTE THAT PHYSICO-CHEMICAL AND PROXIMATE ANALYSIS OF KUNUN ZAKI WERE CARRIED OUT ONLY ON FRESHLY-PREPARED KUNUN ZAKI (SAMPLE H) AND KUNUN ZAKI STORED AT 5°C FOR THREE DAYS (SAMPLE I).

TABLE 3: THE PHYSICO-CHEMICAL AND PROXIMATE CHARACTERISTICS OF FRESH AND STORED (AT 5°C FOR THREE DAYS) KUNUN ZAKI OBTAINED FROM THE LOCATIONS OF STUDY.

PParameters	A		B		C		D	
	Fresh Kunun	Kunun at 3	Fresh Kunun	Kunun at 3	Fresh Kunun	Kunun at 3	Fresh Kunun	Kunun at 3
pH	4.93±0.41^{b}	5.16±0.39^{a}	4.92±0.17^{a}	5.12±0.12^{b}	4.93±0.01^{b}	5.00±0.11^{a}	4.61±0.40^{a}	5.15±0.20^{b}
TTA(g)	0.36±0.04^{a}	0.31±0.01^{b}	0.37±0.56^{b}	0.28±0.77^{a}	0.37±0.20^{b}	0.30±0.22^{a}	0.40±0.11^{a}	0.31±0.09^{b}
TS(%)	6.30±2.12^{b}	6.13±2.67^{a}	6.00±1.89^{b}	5.82±1.95^{a}	9.00±1.97^{b}	8.30±1.99^{a}	7.00±2.02^{b}	6.20±2.11^{a}
CHONs(%)	2.50±0.21^{a}	2.12±0.19^{b}	3.14±0.30^{b}	2.70±0.33^{a}	2.50±0.22^{a}	2.39±0.18^{b}	2.44±0.23^{a}	2.35±0.20^{b}
CHOs(%)	82.32±0.40^{b}	82.01±0.54^{a}	81.73±0.42^{b}	81.19±0.50^{a}	84.55±0.39^{b}	84.49±0.44^{a}	82.65±0.36^{b}	82.34±0.43^{a}
ASH(%)	0.65±0.25^{a}	0.21±0.17^{b}	0.72±0.20^{a}	0.48±0.14^{b}	0.15±0.30^{a}	0.03±0.24^{b}	0.60±0.28^{a}	0.36±0.21^{b}
MC(%)	82.37±2.05^{a}	83.74±1.73^{b}	81.99±1.41^{b}	82.23±0.20^{a}	86.05±2.73^{b}	88.40±1.78^{a}	84.55±1.69^{b}	86.01±0.78^{a}
Pb(ppm)	0.47±0.13^{a}	0.40±0.29^{b}	-0.08±0.03^{NS}	ND	-0.01±0.01^{NS}	ND	-0.06±0.01^{NS}	ND
Cu(ppm)	0.01±0.03^{NS}	-0.03±0.03^{NS}	0.53±0.06^{a}	0.42±0.01^{b}	0.60±0.03^{a}	0.53±0.01^{b}	0.28±0.01^{a}	0.21±0.04^{b}
Zn(ppm)	0.45±0.55^{a}	0.39±0.45^{b}	2.58±0.81^{a}	2.43±0.78^{b}	1.15±0.05^{a}	1.00±0.09^{b}	1.25±0.15^{a}	1.18±0.51^{b}
Ca(ppm)	0.17±0.94^{a}	0.11±0.90^{b}	1.48±0.79^{a}	1.35±0.56^{b}	1.07±0.88^{NS}	0.97±0.64^{NS}	2.67±0.58^{a}	2.52±0.45^{b}
n(ppm)	0.30±0.45^{a}	0.24±0.35^{b}	1.63±0.17^{a}	1.33±0.20^{b}	0.47±0.32^{a}	0.36±0.23^{b}	0.53±0.21^{a}	0.41±0.11^{b}

KEY

A = Bori-camp Preparation **C = Mgbuogba Preparation**
B = Rumuodomaya Preparation **D = Laboratory Preparation**
TTA = Total titratable acidity **TS = Total solids**
CHONs = Proteins **CHOs = Carbohydrates**
MC = Moisture content **Kunun at 3 = Kunun zaki stored at 5°C for three days.**
Pb = Lead **Cu = Copper**
Zn = Zinc **Ca = Calcium**
Mn = Manganese **ppm = Parts per million**
NS = Not significant **a, and b = Means significantly (*P*= .05) different from each other.**
a = Highest mean **b = Lowest mean.**

Comment [OA6]: This table is poorly typed, Present the data for freshly produce kunun-zaki on a separate table and the ref produced on another

Comment [OA7]: There is nowhere in this report that the authors stated that statistical analysis was done. Please include this in materials and methods

3.3 Physico-chemical and proximate analyses

The pH of freshly-prepared Kunun zaki of the four preparations as shown in Table 3 were found to be acidic. The acidity of these samples could be attributed to the fermentative nature of Lactic acid bacteria (especially Lactobacilli) as reported by [4] for being responsible for lactic acid production, thus bringing about a gradual decrease of pH. Upon storage at 5°C for three days, a pronounced increase was found in the four preparations. This is not surprising, since the lower the storage temperature, the lower rate of metabolism of sugar and hence the lower the rate of acid production by the relevant microorganisms [1]. This observation is in agreement with [1] who reported the production of acid from sugar metabolism by Lactic acid bacteria.

Total titratable acidity of freshly-prepared Kunun zaki of the four locations varies from 0.36±0.04 to 0.40±0.11g. Upon storage at 5°C for three days, there was a reduction in total titratable acidity values of the four preparations. Hence, it was observed that while pH of the samples decreased, total titratable acidity increased.

The total solids of freshly-prepared Kunun zaki was found to be in the range of 6.00±1.89 to 7.00±2.02g in Bori-camp, Rumuodomaya, and Laboratory preparations. This may be due to the same procedures adopted during the production process, and also the addition of wet-milled sweet potato which led to the liquefaction of the samples, thus lower total solids and moisture content when compared with Mgbuogba's who did not add sweet potato in the course of the preparation process, hence a more viscous and higher total solids of 9.00±1.97g and moisture content (86.05±2.73%). A gradual decrease in total solids was found in the four preparations when stored at 5°C for three days.

Protein content was found to be higher in the three commercially-prepared Kunun zaki than the Laboratory-prepared Kunun zaki. This could be attributed to the addition of additives to the processed Kunun drinks [9]. Proteins in cereals are usually located in their testa and germs [14], and they might have been sifted off during processing (steeping, milling, and sieving), hence very low protein contents. A pronounced decrease of protein content was found in the four preparations, indicating that proteins were utilized by the relevant organisms upon storage at 5°C.

The carbohydrate contents of the three commercially-prepared Kunun zaki (fresh) ranges from81.73±0.42 to84.55±0.39%, while that of the Laboratory (fresh) was 82.65±0.36%. Upon storage at 5°C three days, a gradual decrease was found observed in the four preparations, indicating that carbohydrates were catabolised by the relevant organisms.

The percentage ash varies from0.15±0.30 to 0.72±0.20% in the commercially-prepared Kunun zaki (fresh), while Laboratory preparation was found to be 0.60±0.28%. Ash gives an idea of the quantity of mineral elements in the samples[1].

The mineral contents (Lead, Copper, Zinc, Calcium, and Manganese) as shown in Table 3 revealed that Bori-camp had lead concentration of 0.47±0.13ppm and was significantly not different from Rumuodomaya, Mgbuogba, and Laboratory preparations. Lead ingestion may constitute a serious risk to public health, since it may slow cognitive development, impair intellectual performance in children and increase blood pressure and cardiovascular diseases in adults [15]. The detection of lead in this location (Bori-camp) could be attributed to pesticides used by the farmer which may contain lead, or cosmetics used by the producer which might have been introduced unknowingly into the samples in the course of the preparation. Calcium which is required for bone development and strong teeth [1], was found to be high (2.67±0.58ppm) from Laboratory preparation, while the least was found to be0.17±0.94ppm from Bori-camp preparation, though significantly not different from Mgbuogba preparation. Zinc aids in food digestion, had the highest concentration of 2.58±0.81ppm from Rumuodomaya preparation, followed by 1.25±0.15ppm of Laboratory preparation, and the least was found to be 0.45±0.55ppm from Bori-cap preparation. Manganese and copper were relatively moderate because they become poisonous when taken in high concentration.Upon storage at 5°C three days, a gradual decrease was found observed in the four preparations, indicating that the relevant organisms were utilizing the minerals.

Comment [OA8]: Any statistical analysis???

3.4 Hazard Analysis Critical Control Points Analysis of Kunun zaki

From the results obtained, the stages of production were grouped into two levels of contamination; minor and major. The points are shown and discussed in Figure 2.

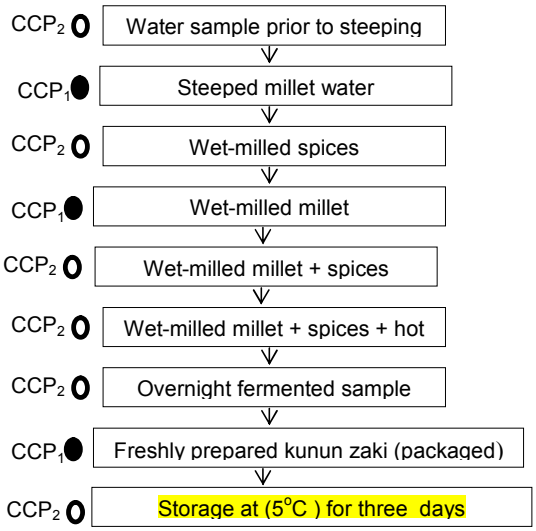

Figure 2: Flow diagram of critical control points of kunun zaki preparation Modified from Jay [16].

KEY

O: Indicates a point of minor contamination.

●: Indicates a point of major contamination.

CCP_1: Effective critical control point.

CCP_2: Not absolute.

3.4.1 Water sample prior to steeping

This is a point of minor contamination, especially when potable water is not used. Boiled potable water should be used to steep the grains so as to reduce microbial contaminants (coliforms, staphylococci, and fungi) [5], that might be associated with the final product as seen in the commercially-prepared products, but were not associated with laboratory-prepared product.

3.4.2 Steeped millet water

This is a point of major contamination because there was an increase in the total heterotrophic counts, staphylococci, coliforms, and fungal counts in the four preparations, thus indicating that most of the contaminants emanated from the raw materials (grains). Manual harvesting exposes the grains to microbial contaminants, especially from the hands of the harvesters and the soil. Therefore, the use of mechanized system of harvesting, transport in jute bags not baskets to processing units should be advocated to prevent this point of major contamination. Storage of grains must be under an atmosphere of low humidity to prevent fungal growth, and selection of good quality grains, that will yield safe products.

3.4.3 Wet-milled spices

This is another point of minor contamination, since spices have contaminants at a lower level when compared with the grains. Spices are known to be antimicrobial agents in the prevention of growth of undesirable organisms in food [8], and therefore should be thoroughly washed to remove accumulated soil or dust particles from farms that could introduce contaminants in the course of the preparation.

3.4.4 Wet-milled millet

Wet-milled millet is a point of major contamination, since the milling machine is a major source of contamination in this production stage. Milling machine made of stainless steel is recommended for easy sterilization. Cleaning-in-place processes like steam-washing and swabbing before and after milling should be done.

3.4.5 Wet-milled millet + Spices

This is another point of minor contamination. Well-washed grains and spices should always be used in the preparation of kunun zaki to ensure safety and wholesomeness of the product. Thus, clean spices known to be antimicrobials eliminated coliforms and staphylococci growth when wet-milled millet were mixed together in the laboratory preparation.

3.4.6 Wet-milled millet + Spices + Hot water

This is another point of minor contamination in kunun zaki production. The hot water added to portion A of sample E was not necessarily for sterilization purpose, but for the extraction of flavor from the spices (flavor enhancement) and for proper gelatinization of the mash. If the sample from this production stage were plated immediately the hot water was added, all the organisms including the fermenters would have been eliminated. Since portion B of sample E (the inoculum) was added to portion A at the temperature of 55°C,and then allowed to ferment for 12 hours (overnight) at 30°C, Lactic acid bacteria and yeasts (the fermenters) only were isolated from Laboratory-prepared Kunun zaki. Coliforms, staphylococci and along side with Lactic acid bacteria were isolated from the commercially-prepared Kunun zaki. The presence of these contaminants could be attributed to unclean water used in the preparation of the drink, the use of improperly washed hands after using the toilet, use of unsterilized containers, and dirty environment [17].

Comment [OA9]: What of bacterial spores???

3.4.7 Overnight fermented sample

This is a point of minor contamination in the production process of kunun zaki. Overnight fermentation process in kunun zaki production is best achieved at a minimum temperature of 30^{0}C for 12 hours in a trough or bucket. If this trough or bucket is not properly covered during the overnight fermentation process, insects and rodents could bring in further contaminants by transferring filth from contaminated areas to food through their waste products, and during regurgitation of filth onto clean food during consumption [18]. Thus, contamination from these pests could be prevented by proper covering of trough or bucket with a sterile cover, and the processing areas should be protected against their entry by sound sanitary practices.

3.4.8 Freshly prepared kunun zaki (packaged)

This is a point of major contamination in the production process of kunun zaki. Sterility of packaging materials (plastic bottles) must be ensured to prevent contamination by using sterile plastic bottles with screw

caps. Clean bottles were used in the laboratory preparation where no coliforms, staphylococci, and molds were associated with freshly prepared kunun zaki.

3.4.9 Storage at (5^0C) for three days

This is a point of minor contamination when the product is not stored at a stable refrigeration temperature. Storage of Kunun zaki at refrigeration temperature of 5^0C for three days, increased lactic acid bacterial counts to 10^5cfu/ml, and led to the isolation of spoilage organisms including *Pseudomonas* spp, *Micrococcus* spp and fungi in the four preparations, implying that a lower temperature could be used depending on the outcome of sensory tests.

4 CONCLUSION

It could be concluded that good manufacturing practices for Kunun zaki production implies the use of fresh non-moldy grains and spices, cooled boiled water for grain washing and steeping, sterile stainless steel containers, steam-washed grinder, and sterile screw-capped bottles for packaging be applied at all stages of the production process. Educating the producers on the hazards, critical control points (steeping,milling, and packaging) and monitoring procedures (pH and temperature maintenance) for Kunun zaki preparation are important.

ACKNOWLEDGEMENT

Authors acknowledge all the lecturers and technical staff of the Department of Microbiology for theiradvice and support during this work, Mrs Gloria, Mrs Hadiza and Mrs Hassanatu of Bori-camp, Rumuodomaya and Mgbuogba samples respectively, and Mr.Japhet Onwuegbu of Plant Science and Biotechnology Department for their assistance during the course of this work.

COMPETING INTERESTS

Authors have declared that no competing interest exist.

REFERENCES

1. Adebayo GB,Otunola GA, Ajao TA. Physicochemical, Microbiological and Sensory characteristics of kunun zaki from millet stored at selected temperatures. Advanced Journal of Food Science and Technology.(2010); 2:41-46.
2. Amakoromo ER. Fermented non-alcoholic cereal products. In: Indigenous fermented foods of Nigeria, University of Port Harcourt press, 1st edition. (2011); pp:66-67.
3. Olanrewaju OO,Victor OO,Adebolu TT. Safety of small-scale food fermentations developing countries. (2009); 11:29-34.
4. Odunfa SA, Adeyeye S. Microbiological changes during the traditional production of Ogi-baba, a West African fermented sorghum. Journal of Cereal Science. *(1985);*3:173-180.
5. Amusa NA, Odunbaku OA. Microbiological and nuritional quality of hawked Kunun (a sorghum-based non-alcoholic beverage) widely consumed in Nigeria. Pakistan Journal of Nutrition.(2009);1:20-25.
6. Ayo JA, Onuoha G, Ikumola DS,Esan YO, Ayo VA, Obetta. Production and evaluation of starter culture for Kunun zaki: a millet- based beverage drink. Nigerian Journal of Microbiology 25. (2011); 2463.
7. Bergey DH. Bergey's Manual of Determinative Bacteriology 9th edition, Baltimore, Williams and Wilkins Co.(1994).
8. Frazier WC,Westhoff DC. Food microbiology 3rd edition. Tata McGrawHill publishing company, Limited, New Delhi. (2008); pp:17-34.
9. Association of Official Analytical Chemists – AOAC. Official methods of analysis 15th edition, AOAC Arlington, U.S.A.(1990).
10. Onuorah ST, AdesiyunA A, Adekeye JO.Occurrence of staphylococci and coliform on kunun zaki and food utensils in its preparation Samaru, Zaria. Agricultural Food Journal.(1987);*1:31-34.*

11. Essien EC, Monago, Edor EA. Evaluation of the nutritional and microbiological quality of kunun (A cereal-based non-alcoholic beverage) in Rivers State, Nigeria. *The* Internet Journal of nutrition and Wellness. *(2011);* 10:2-10.
12. Amusa NA, Ashaye OA, Aiyegbayo AA, Oladopo MO, Oni MO, Afolabi OO. Microbiological and nutritional quality of hawked zobo drinks widely consumed in Nigeria. Journal of Food Agricultural Environment.(2005);3:47-50.
13. Rhodes A, Fletcher DL. Principles of Industrial Microbiology. Pergaman Press Oxford.(1966); pp:119.
14. Hamids AM, Fields ML. Evaluation of protein quality and available lysine of germinated and fermented cereal. Journal of Food Science.(1979);44:546.
15. Coutate TP.Food, the chemistry of its component 2nd edition, Cambridge. Royal Society of Chemistry.(1992);pp:265.
16. Jay MJ. Microbiological safety of foods. In: Modern Food Microbiology 4th edition, CBS Publishers and Distributors Pvt.Ltd. New Delhi. (2005);pp: 641-650.
17. Ogbonna IO, Opobiyi MY, Katuka B, Waba JT. Microbial evaluation and proximate composition of Kunun zaki, an indigenous fermented food drink consumed predominantly in Northern Nigeria. Internet Journal of food safety.(2011);13:93-97.
18. Vasconcellos JA. Quality Assurance for the Food Industry; a practical approach. (2003);pp: 205.
19. Adeyemi IA, Umar S. Effect of method of manufacture on quality characteristics of Kunun zaki, a millet-based beverage. Nigerian Food Journal. *(1994);*12:34-42.
20. Gaffa T, Gaffa AT. Microbial succession during Kunun zaki production. World Journal of Microbiology and Biotechnology. *(2004);*20:449-453.

Water sample prior to steeping