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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

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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.

Original Research Article

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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

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Kunun zaki were stored at 5ºC for three days for analysis on effect of storage.
Results: The microorganisms associated with freshly prepared Kunun zaki and that stored at refrigeration temperature (5ºC) 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. Freshly-prepared Kunun zaki had the highest coliform and staphylococci counts of $8.0x10^4$ and $8.3x10^3$cfu/ml respectively in Bori-camp preparation, while Laboratory-prepared Kunun zaki harboured none of these organisms. Total viable counts of commercially-prepared (Bori-camp, Rumuodomaya, and Mgbuogba) products ranged from $2.50x10^4$ to $1.53x10^6$cfu/ml, while Laboratory-prepared product was $6.0x10^4$cfu/ml. Fungal counts of commercially-prepared Kunun zaki ranged from $2.5x10^3$ to $1.36x10^5$cfu/ml, while Laboratory-prepared Kunun zaki had fungal counts of $5.6x10^2$cfu/ml. Yeasts were the main spoilage organisms which persisted at storage temperature of 5ºC for three days. The protein and carbohydrate contents, as well as calcium, zinc, copper, and manganese decreased after being stored for three days at 5ºC.
Conclusion: Therefore Kunun zaki could be safely consumed after storage at 5ºC for three days, if good manufacturing practices like 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 the production stages. Educating the producers on the hazards, critical control points (steeping, milling and packaging), and temperature maintenance for Kunun zaki preparation are important.

Keywords: Kunun zaki; commercially-prepared and laboratory-prepared Kunun zaki.

1. INTRODUCTION

Kunun zaki is one of the most common non-alcoholic fermented beverage made mainly from millet, sorghum, maize, and guinea corn [1,2]. 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 Odunfa and Adeyeye [4]. 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 susceptible to contamination and may be microbiologically unsafe for consumption. Consequently, the adoption of Hazard

Analysis Critical Control Point concept into small-scale fermented foods like Kunun zaki in developing countries would encourage the production of microbiologically safe Kunun zaki [5].

In previous studies microbial genera (fermenting, spoilage and pathogenic organisms) recorded were; *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 [6]. *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 [7]. This research work was conducted 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.

2. MATERIALS AND METHODS

2.1 Sample Collection

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 5ºC 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 (unseived)
H	Freshly-prepared Kunun zaki (seived 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 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 100ºC was carefully added to portion A. Portion B (the inoculum) was carefully added to portion A at a temperature of 55ºC, then allowed to cool to a temperature of 30ºC, 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 121ºC 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 37ºC, those on Mannitol salt agar and De Mann Rogosa and Sharpe agar plates were incubated for 48 hours at 37ºC, while those on Sabouraud dextrose agar plates were incubated for 5 days at 26ºC. 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 37ºC 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 [8]. Fungal isolates were identified with reference to [9].

2.5 Physico-chemical and Proximate Analyses

pH, total titratable acidity, and total solids were determined as described by AOAC [10].

2.6 Proximate Analysis

Crude protein, carbohydrate content, moisture content, ash and minerals were also determined by AOAC [10] based on location and storage time.

2.7 Statistical Analysis

The physico-chemical parameters, proximate characteristics, and minerals of freshly-prepared and stored Kunun zaki (5ºC for three days) were determined statistically using analysis of variance.

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of Microbial Isolates

Different production stages of Kunun zaki obtained from the locations of study were found to habour a total of nine bacterial genera: *Lactobacillus* spp., *Streptococcus* spp., *Bacillus* spp., *Leuconostoc* spp., *Micrococcus* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Escherichia coli*, and *Enterobacter* spp., fungal genera were: *Aspergillus* spp., *Penicillium* spp., *Mucor* spp., *Rhizopus* spp., and yeasts genera: *Saccharomyces* spp., and *Candida* spp. These microorganisms are similar to those obtained by Amusa and Odunbaku [6] in their study of microbiological and nutritional quality of hawked kunun zaki. Table 2 shows the microbial load and types of organisms at various production stages of Kunun zaki. Bori-camp had the highest total viable count of $1.53x10^{12}$cfu/ml at production stage H (freshly-prepared drink), while the lowest count of $3.0x10^{4}$cfu/ml was obtained from Laboratory preparation of the same production stage. Similarly, same production stage H of Bori-camp preparation had the highest coliform count of $8.0x10^{4}$cfu/ml, while no coliform was isolated from Laboratory-prepared sample of same production stage. The desirable and undesirable microorganisms isolated are of particular interest because of their involvement in different activities.

The presence of Lactic acid bacteria (*Lactobacillus* spp., *Leuconostoc* spp., and *Streptococcus* 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 [6].

Coliforms like *Escherichia coli* in water and other samples indicate faecal contamination, and they are known to be causative agents of food borne gastroentiritis and bacterial diarrhea diseases [11]. Their presence also could be attributed to the use of dirty containers, as well as dirty environment where the Kunun samples were processed [12].

Staphylococcus species were possible contaminants from handlers and utensils used during and after the preparation [6]. They are normal flora of the skin of man and could have been introduced into the samples during and after the production process [6].

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 [13].

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 [14].

Bacillus species isolated were possible contaminants from environmental sources, they produce endospores which help them to survive heat treatment [15].

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 viable 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
	Bori-camp	$8.63x10^7$	$1.47x10^5$	$3.0X10^4$	$1.32X10^5$	$1.97X10^5$
B	Rumuodomaya	$2.78x10^5$	$3.9X10^4$	$9.9X10^3$	$3.0X10^4$	$2.2X10^3$
	Mgbuogba	$3.0x10^4$	$1.05X10^4$	$1.0X10^2$	$3.0X10^4$	$3.0X10^3$
	Laboratory	$4.3x10^3$	$3.0X10^3$	$3.0X10^2$	$2.85X10^4$	$1.7X10^3$
	Bori-camp	$3.23x10^6$	$8.16x10^4$	$2.27X10^4$	$6.17X10^4$	$5.9X10^4$
C	Rumuodomaya	$3.0x10^4$	$3.0X10^4$	$5.25X10^3$	$1.1X10^4$	$2.15X10^3$
	Laboratory	$9.6x10^3$	NIL	NIL	1.5X10	$6.0X10^2$
	Bori-camp	$1.73x10^6$	$1.35x10^5$	$5.7X10^4$	$5.8X10^4$	$2.43X10^5$
D	Rumuodomaya	$2.51x10^4$	$1.80X10^4$	$5.4X10^3$	$2.98X10^4$	$3.1X10^3$
	Laboratory	$8.0x10^3$	$3.1X10^3$	$3.5X10^2$	$1.0X10^4$	$2.1X10^4$
	Bori-camp	$1.12x10^5$	$7.0x10^4$	$2.5X10^3$	$1.98X10^5$	$2.20X10^5$
E	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	$8.8X10^3$	$2.15X10^4$
	Bori-camp	$1.15x10^5$	$6.7x10^4$	$6.0X10^2$	$2.62X10^5$	$1.03X10^5$
F	Rumuodomaya	$9.0x10^3$	$1.0X10^4$	$6.0X10^2$	$3.0X10^4$	$9.0X10^2$
	Mgbuogba	$2.03x10^3$	$7.5X10^3$	NIL	$3.0X10^4$	$1.0X10^2$
	Laboratory	$3.0x10^2$	NIL	$1.0X10^2$	$1.0X10^4$	$1.0X10^2$
	Bori-camp	$6.43x10^5$	$1.48x10^5$	$2.7X10^3$	$3.0X10^5$	$1.95X10^5$
G	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	$1.53x10^6$	$8.0x10^3$	$8.3X10^3$	$2.33X10^4$	$1.36X10^5$
	Rumuodomaya	$1.29x10^4$	$8.75X10^3$	$1.3X10^3$	$2.97X10^4$	$2.5X10^2$
	Mgbuogba Laboratory	$2.5x10^4$	$3.0X10^3$	$1.5X10^3$	$2.75X10^4$	$1.0X10^4$
		$6.0x10^2$	NIL	NIL	$2.0X10^4$	$5.6X10^2$
	Bori-camp	$1.56x10^8$	$1.21x10^5$	$2.0X10^3$	$2.85X10^5$	$4.75X10^4$
I	Rumuodomaya	$1.30x10^6$	$1.82X10^4$	$7.65X10^3$	$2.2X10^4$	$1.2X10^4$
	Mgbuogba	$3.0x10^4$	$2.3X10^4$	$3.0X10^3$	$3.0X10^4$	$2.9X10^4$
	Laboratory	$9.0x10^3$	NIL	NIL	$3.0X10^4$	$1.94X10^4$

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

Lactic acid bacteria in the four locations of study were not plated for at stage A of processing, however other organisms were isolated. This indicates that the water used to steep the millet was not sterilized. Boiled water sample used in Laboratory preparation still harbored some contaminants, though at minimal level when compared with those of commercial preparations.

When the grains were steeped in the water at stage A, a gradual increase and appearance of Lactic acid bacteria was observed across all samples. The grains introduced more organisms (desirable and undesirable) into stage (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], stages C and D were mixed to produce F by Mgbuogba's producer, and hence were not individually collected. The spices harbored 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 harbored 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 or stage E.

Hot water added to portion A of stage 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 (except bacterial spores that require moist heat to be killed) 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, which however harbored the desirable microorganisms (Lactic acid bacteria;$2.0x10^4$cfu/ml) 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 55ºC.

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.

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, and the Laboratory-prepared drink was significantly different from the commercially-prepared drinks. 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 5ºC 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 Adebayo et al. [1] who reported the production of acid from sugar metabolism by Lactic acid bacteria.

Table 3. The physico-chemical and Proximate characteristics of freshly- prepared Kunun zaki obtained from the locations of study

Parameters	A	B	C	D
PH	4.93 ± 0.41^{NS}	4.92 ± 0.17^{NS}	4.93 ± 0.01^{NS}	4.61 ± 0.40^{a}
TTA(g)	0.36 ± 0.04^{NS}	0.37 ± 0.56^{NS}	0.37 ± 0.20^{NS}	0.40 ± 0.11^{a}
TS(%)	6.30 ± 2.12^{c}	6.00 ± 1.89^{d}	9.00 ± 1.97^{a}	7.00 ± 2.02^{b}
CHONs(%)	2.50 ± 0.21^{NS}	3.14 ± 0.30^{a}	2.50 ± 0.22^{NS}	2.44 ± 0.23^{b}
CHOs(%)	82.32 ± 0.40^{NS}	81.73 ± 0.42^{c}	84.55 ± 0.39^{a}	82.65 ± 0.36^{NS}
ASH(%)	0.65 ± 0.25^{b}	0.72 ± 0.20^{a}	0.15 ± 0.30^{d}	0.60 ± 0.28^{c}
MC(%)	82.37 ± 2.05^{c}	81.99 ± 1.41^{d}	86.05 ± 2.73^{a}	84.55 ± 1.69^{b}
Pb(ppm)	0.47 ± 0.13^{a}	-0.08 ± 0.03^{NS}	-0.01 ± 0.01^{NS}	-0.06 ± 0.01^{NS}
Cu(ppm)	0.01 ± 0.03^{d}	0.53 ± 0.06^{b}	0.60 ± 0.03^{a}	0.28 ± 0.01^{c}
Zn(ppm)	0.45 ± 0.55^{d}	2.58 ± 0.81^{a}	1.15 ± 0.05^{b}	1.25 ± 0.15^{c}
Ca(ppm)	0.17 ± 0.94^{d}	1.48 ± 0.79^{b}	1.07 ± 0.88^{c}	2.65 ± 0.58^{a}
Mn(ppm)	0.30 ± 0.45^{d}	1.63 ± 0.17^{a}	0.47 ± 0.32^{c}	0.53 ± 0.21^{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 Cu = Copper,
Pb = Lead Ca = Calcium, Zn = Zinc ppm = Parts per million, Mn = Manganese
a, b, c and d = Means significantly (P= .05) different from each other.
NS = Not significant
a = Highest mean d = Lowest mean.

Similarly, total titratable acidity of freshly-prepared Kunun zaki from the Laboratory was significantly different from the commercially-prepared drinks. Upon storage at 5ºC 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.This would serve to lower total solids and moisture content when compared to the samples from Mgbuogba's that lacked sweet potatoes in 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 5ºC for three days.

Table 4. The physico-chemical and Proximate characteristics of stored Kunun zaki (5ºC for three days)obtained from the locations of study

Parameters	A	B	C	D
PH	5.16±0.39^{NS}	5.12±0.12^{a}	5.00±0.11^{b}	5.15±0.20^{NS}
TTA(g)	0.31±0.01^{a}	0.28±0.77^{b}	0.30±0.22^{NS}	0.31±0.09^{NS}
TS(%)	6.13±2.67^{c}	5.82±1.95^{d}	8.30±1.99^{a}	6.20±2.11^{b}
CHONs(%)	2.12±0.19^{d}	2.70±0.33^{a}	2.39±0.18^{c}	2.35±0.20^{b}
CHOs(%)	82.01±0.54^{NS}	81.19±0.50^{b}	84.49±0.44^{a}	82.34±0.43^{NS}
ASH(%)	0.21±0.17^{b}	0.48±0.14^{b}	0.03±0.24^{b}	0.36±0.21^{b}
MC(%)	83.74±1.73^{NS}	82.23±0.20^{NS}	88.40±1.78^{a}	86.01±0.78^{b}
Pb(ppm)	0.40±0.29^{a}	ND	ND	ND
Cu(ppm)	-0.03±0.03^{d}	0.42±0.01^{b}	0.53±0.01^{a}	0.21±0.04^{c}
Zn(ppm)	0.39±0.45^{d}	2.43±0.78^{a}	1.00±0.09^{c}	1.18±0.51^{b}
Ca(ppm)	0.11±0.90^{d}	1.35±0.56^{b}	0.97±0.64^{c}	2.52±0.45^{a}
Mn(ppm)	0.24±0.35^{d}	1.33±0.20^{a}	0.36±0.23^{c}	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 Cu = Copper
Pb = Lead Ca = Calcium
Zn = Zinc ppm = Parts per million
Mn = Manganese a, b, c, and d = Means significantly (P= .05) different from each other.
NS = Not significant a = Highest mean d = Lowest mean.

From the results obtained in Table 4, 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 [10]. Proteins in cereals are usually located in their testa and germs [16], 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 5ºC.

The carbohydrate contents of the three commercially-prepared Kunun zaki (fresh) ranged from 81.73±0.42 to 84.55±0.39%, while that of the Laboratory (fresh) was 82.65±0.36%. Upon storage at 5ºC three days, a gradual decrease was observed in the four preparations, indicating that carbohydrates were catabolised by the relevant organisms.

The percentage ash varies from 0.15±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 [17]. 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 5ºC three days, a gradual decrease was observed in the four preparations, indicating that the relevant organisms were utilizing the minerals.

3.4 Hazard Analysis Critical Control Points Analysis of Kunun zaki

From the results obtained, there were Control Points (CPs) and Critical Control Points (CCPs) that were examined to ensure safety and wholesomeness of Kunun zaki. The points were grouped into two levels of contamination; minor (the control points) and major (the critical control points) Jay [18] and discussed in the flow diagram.

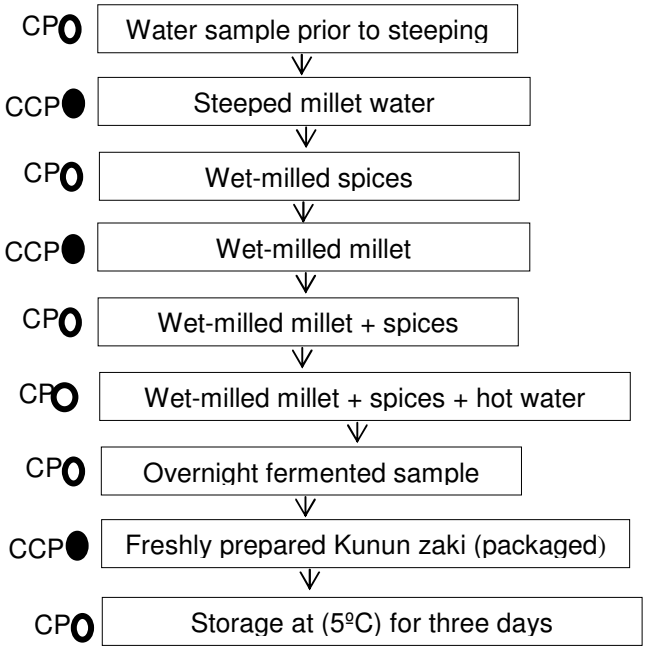

Diagram 1. Flow diagram of Control Points and Critical Control Points of Kunun zaki preparation Modified from Jay [18]

KEY:

O : Indicates a point of minor contamination.

● : Indicates a point of major contamination.

CP: Control Point

CCP: Critical Control Point

3.4.1 Water sample prior to steeping

This is a control point and a point of minor contamination in Kunun zaki production when potable water is not used. Cooled boiled potable water should be used to steep the grains so as to reduce microbial contaminants (coliforms, staphylococci, and fungi) [6], 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 critical control point and also a major point of contamination because of an increase in the total viable 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

Wet-milled spices is another control point and a point of minor contamination in Kunun zaki production, 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 [9], 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 critical control point and a major point of contamination in Kunun zaki production, 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 control point and a point of minor contamination in the production process. 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 control point and a 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 (except bacterial spores that require moist heat to be killed) would have been eliminated. Since portion B of sample E (the inoculum) was added to portion A at the temperature of 55ºC and then allowed to ferment for 12 hours (overnight) at 30ºC, Lactic acid bacteria and yeasts (the fermenters) only were isolated from Laboratory-prepared Kunun zaki. Coliforms, staphylococci and alongside 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 [19].

3.4.7 Overnight fermented sample

This is a control point and 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 30ºC 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 [20]. 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 critical control point and 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 (5ºC) for three days

This is a control point and a point of minor contamination when the product is not stored at a stable refrigeration temperature. Storage of Kunun zaki at refrigeration temperature of $5^{o}C$ for three days, increased lactic acid bacterial counts to 10^{5}cfu/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 Kunun zaki could be safely consumed after storage at 5ºC for three days, if good manufacturing practices like 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 the production stages. Educating the producers on the hazards, critical control points (steeping, milling, and packaging), and temperature maintenancein Kunun zaki preparation are important.

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COMPETING INTERESTS

Authors have declared that no competing interest exists.

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