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Original Research Article

Hazard Analysis Critical Control Points for the Production of Kunun zaki from Millet.

ABSTRACT

Aim: To study the preparation of Kunun zaki in three locations in Port Harcourt namely; Bori-camp, Rumuodomaya, and Mgbuogba, so as to find out the critical control points of contamination associated with each preparation stage. A laboratory-prepared Kunun zaki was compared with the above three-mentioned preparations to find out if the shelf-life and safety of the products could be improved upon.

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 kununzaki 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 kununzaki were stored at 5^0C for three days for analysis on effect of storage.

Results: The microorganisms associated with freshly prepared kununzaki 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. Heterotrophic 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.

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

1. INTRODUCTION

Kunun zaki is one of the commonest traditional non-alcoholic fermented beverage made mainly from millet, sorghum, maize, and guinea corn [1 and 2]. It is a very nutritious locally-made beverage, common in northern Nigeria. It has low viscosity, sweet-sour taste with a pH of 4.60 to 5.00 and milky cream appearance, and contains about 1% protein,

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6%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 or commercial processing of kunun zaki. According to Olanrewaju *et al.,* [3], the traditional or commercial processing of kunun zaki 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 by the addition of hot water, with constant stirring to homogenize till it becomes gelatinized, while the other (smaller) portion 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 below.

Figure 1: Flow diagram of traditional/commercial kunun zaki production process

Due to the involvement of several people in the production of kunun zaki, and the use of unsterilized containers and milling machine, the final product is contaminated and may be microbiologically unsafe for consumption. Various microbial genera 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. *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.

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Saccharomyces cerevisiae compared most favourably with the commercially-prepared Kunun zaki from millet [5].This research work was conducted to study the preparation of kunun zaki in three locations in Port Harcourt namely; Bori-camp, Rumuodomaya, and Mgbuogba, so as to find out the critical control points of contamination associated with each preparation stage. A laboratory-prepared kunun zaki was compared with the above three-mentioned preparations to find out if the shelf-life and safety of the products could be improved upon. This work also did not only compare the microbiological, physico-chemical, and nutritional qualities of hawked and laboratory-prepared Kunun drinks(the final product) as done by [9] and [13], but also the microbiological qualities of the different stages involved in 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. 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 before milling. Milling of millet, spices, and sweet potato was done with Panasonic electric blender, model (MX-J110P), rinsed out with hot 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 100^0C was carefully added to portion A, compared to that at 60^0C added to portion A of commercial preparation. This was allowed to cool to a temperature of 30^0C, and then covered to allow fermentation to take place overnight (for 12 hours). And then carefully filtered using a sterile Cheesecloth. The final product was bottled and sealed in sterile screw-capped 50cl bottles.

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2.3 Enumeration of microorganisms associated with the different production stages of Kunun zaki

Each sample from the four different preparations were enumerated using ten-fold serial dilution technique. The diluent was 1% peptone water, and 0.1ml of the diluted samples were spread-plated in dupicates on Nutrient agar (NA) for total heterotrophic bacterial count, 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.

Nutrient agar and MacConkey agar plates were incubated for 24 hours at 37^0C, Mannitol salt agar and De Mann Rogosa and Sharpe agar plates were incubated for 48 hours at 37^0C, while 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].

2.5 Physico-chemical Analysis

pH, total titratable acidity, total solids, and turbidity were determined as described by AOAC [8].

2.6 Proximate Analysis

Crude protein, carbohydrate content, moisture content, ash, and minerals were also determined by AOAC [8].

Analyses of the nutritional contents, essential, toxic, and trace elements in kunun zaki were carried out based on location and storage time.

3. RESULTS AND DISCUSSION

3.1 Microbial Isolates

Nine bacterial isolates were isolated and identified from the different production stages of kunun zaki of the four preparations. They were identified 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 [9].

3.2 Microbial counts

The total heterotrophic counts, total coliform counts, total staphylococci counts, lactic acid bacteria counts, and total fungal counts are shown in Tables 2 to 5.

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3.3 Total Heterotrophic counts

Sample A (Water sample prior to steeping) of the four preparations had their counts between the range of 10^2 to 10^3/ml. Laboratory preparation had the least count of 3.2 x 10^2cfu/ml, while Bori-camp had the highest count of 1.44 x 10^3cfu/ml.

Sample B (Steeped millet water) of the four preparations had their counts between 10^3 to 10^4/ml. Counts in the four preparations increased by one log cycle.

Sample H (Freshly prepared kunun zaki) of the four preparations had their counts between 10^2 to 10^4/ml, while that carried out by [9] recorded 7.8 x 10^7cfu/ml as the total heterotrophic bacterial counts of freshly prepared Kunun zaki. Laboratory preparation counts dropped by one log cycle, while counts in the other three preparations were stable.

On refrigerated storage for three days at 5^0C, Laboratory preparation had the least count of 9.0 x 10^3cfu/ml, while Bori-camp had the highest count of 1.56 x 10^5cfu/ml.

3.4 Total coliform counts

Sample A (Water sample prior to steeping) of the four preparations had their counts between 10 to 10^3/ml. Laboratory preparation had one cycle lower than Mgbuogba, and two log cycles lower than Rumuodomaya and Bori-camp preparations.

Sample B (Steeped millet water) of the four preparations had their counts between 10^3 to 10^5/ml. Laboratory preparation had one log cycle lower than Mgbuogba and Rumuodomaya preparations, and two log cycles lower than Bori-camp preparation.

Sample H (Freshly prepared kunun zaki) of Bori-camp, Rumuodomaya, and Mgbuogba preparations were within the range of 10^3/ml, while [9] recorded the range of 2.2 x 10^6 to 5.2 x 10^6cfu/ml. No coliform was detected in Laboratory preparation.

On refrigerated storage for three days at 5^0C, Bori-camp count increased by two log cycles, where as Rumuodomaya and Mgbuogba increased by one log cycle. No coliform was detected in the Laboratory preparation.

3.5 Total staphylococci counts

Sample A (Water sample prior to steeping) of three preparations Bori-camp, Rumuodomaya, and Laboratory had counts between 10 to 10^2/ml. No staphylococci were detected in Mgbuogba preparation.

Sample B (Steeped millet water) of the four preparations ranged from 10^2 to 10^4/ml. Counts from Rumuodomaya and Laboratory preparations increased by one log cycle, and Mgbuogba had counts of 1.0 x 10^2cfu/ml.

Sample H (Freshly prepared kunun zaki) of three preparations Bori-camp, Rumuodomaya, and Mgbuogba had their counts within 10^3/ml. Counts in Bori-camp and Rumuodomaya stabilized, Mgbuogba increased by one log cycle, and none was detected in Laboratory preparation.

On refrigerated storage for three days at 5^0C, counts were within the range of 10^3/ml in Bori-camp, Rumuodomaya, and Mgbuogba preparations, while none was also detected in Laboratory preparation.

3.6 Lactic acid bacterial counts

Sample A (Water sample prior to steeping) of the four preparations were not plated out

Sample B (Steeped millet water) of the four preparations were between 10^4 to 10^5/ml. Laboratory preparation had the least counts of 2.85 x 10^4cfu/ml, while Bori-camp had the highest counts of 1.35 x 10^5cfu/ml.

Sample H (Freshly prepared kunun zaki) of the four preparations were within the range of 10^4/ml.

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On refrigerated storage for three days at 5^0C, counts in the four preparations were between 10^4 to 10^5/ml. Counts in Bori-camp preparation increased by one log cycle, while counts in the other three preparations stabilized.

3.7 Total fungal counts

Sample A (Water sample prior to steeping) of three preparations Bori-camp, Rumuodomaya, and Mgbuogba had their counts between the range of 10 to 10^2/ml. Rumuodomaya preparation had the least count of 9.0 x10^1cfu/ml, while the highest counts of 4.6 x 10^2cfu/ml were found in Bori-camp preparation. Laboratory preparation had no count.

Sample B (Steeped millet water) of the four preparations were between 10^3 to 10^5/ml. Laboratory preparation had the least counts of 1.7 x 10^3cfu/ml, whereas Bori-camp had the highest count of 1.95 x 10^5cfu/ml.

Sample H (Freshly prepared kununzaki) of the four preparations had their counts between 10^2 to 10^5/ml. Mgbuogba preparation had the least count of 1.0 x 10^4cfu/ml, while Bori-camp had the highest count of 1.36 x 10^5cfu/ml.

On refrigerated storage for three days at 5^0C, counts in Rumuodomaya and Laboratory preparations dropped by two log cycles, Bori-camp dropped by one log cycle, and Mgbuogba preparation stabilized.

The microbial load and types of organisms at various stages of kunun zaki preparation of the four locations are shown in Tables 2 to 5 below.

Table 2: Microbial load and types of organisms at various stages of kunun zaki preparation at Bori-camp, Port Harcourt.

Production Stages	Total Heterotrophs	Total Coliforms	Staphylococci	Lactic acid Bacteria	Fungi
		(cfu/g or ml)			
A	$1.44X10^3$	$1.8X10^3$	$1.52X10^2$	NP	$4.6X10^2$
B	$8.63X10^4$	$1.47X10^5$	$3.0X10^4$	$1.32X10^5$	$1.97X10^5$
C	$3.23X10^4$	$8.16X10^4$	$2.27X10^4$	$6.17X10^4$	$5.9X10^4$
D	$1.73X10^5$	$1.35X10^5$	$5.7X10^3$	$5.8X10^4$	$2.43X10^5$
E	$1.12X10^5$	$7.0X10^4$	$2.5X10^3$	$1.98X10^5$	$2.20X10^5$
F	$1.15X10^5$	$6.7X10^4$	$6.0X10^2$	$2.62X10^5$	$1.03X10^5$
G	$6.43X10^4$	$1.48X10^5$	$2.7X10^3$	$3.0X10^5$	$1.95X10^5$
H	$1.53X10^4$	$8.0X10^3$	8.3X103	$2.33X10^4$	$1.36X10^5$
I	$1.56X10^5$	$1.21X10^5$	$2.0X10^3$	$2.85X10^5$	$4.75X10^4$

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

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Table 3: Microbial load and types of organisms at various stages of kunun zaki preparation at Rumuodomaya, Port Harcourt.

Production Stages	Total Heterotrophs	Total Coliforms	Staphylococci (cfu/g or ml)	Lactic acid Bacteria	Fungi
A	$1.37X10^3$	$1.1X10^3$	$3.8X10^2$	NP	$9.0X10^1$
B	$2.78X10^4$	$3.9X10^4$	$9.9X10^3$	$3.0X10^4$	$2.2X10^3$
C	$3.0X10^4$	$3.0X10^4$	$5.25X10^3$	$1.1X10^4$	$2.15X10^3$
D	$2.51X10^4$	$1.80X10^4$	$5.4X10^3$	$2.98X10^4$	$3.1X10^3$
E	$1.28X10^4$	$2.3X10^3$	$3.0X10^2$	$9.0X10^3$	$1.03X10^4$
F	$9.0X10^3$	$1.0X10^4$	$6.0X10^2$	$3.0X10^4$	$9.0X10^2$
G	$1.45X10^4$	$1.01X10^4$	$7.25X10^3$	$1.51X10^4$	$2.0X10^2$
H	$1.29X10^4$	$8.75X10^3$	$1.3X10^3$	$2.97X10^4$	$2.5X10^2$
I	$1.30X10^4$	$1.82X10^4$	$7.65X10^3$	$2.2X10^4$	$1.2X10^4$

KEY

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

Table 4: Microbial load and types of organisms at various stages of kunun zaki preparation at Mgbuogba, Port Harcourt.

Production Stages	Total Heterotrophs	Total Coliforms	Staphylococci (cfu/g or ml)	Lactic acid Bacteria	Fungi
A	$1.19X10^3$	$8.0X10^2$	NIL	NP	$3.0X10^2$
B	$3.0X10^4$	$1.05X10^4$	$1.0X10^2$	$3.0X10^4$	$3.0X10^3$
E	$7.7X10^3$	$4.5X10^3$	NIL	$1.59X10^4$	$6.0X10^3$
F	$2.03X10^4$	$7.5X10^3$	NIL	$3.0X10^4$	$1.0X10^2$
G	$2.91X10^4$	$3.3X10^3$	$1.0X10^2$	$2.85X10^4$	$1.2X10^4$
H	$2.5X10^4$	$3.0X10^3$	$1.5X10^3$	$2.75X10^4$	$1.0X10^4$
I	$3.0X10^4$	$2.3X10^4$	$3.0X10^3$	$3.0X10^4$	$2.9X10^4$

KEY

A =Water sample prior to steeping
B =Steeped millet water
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

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Table 5: Microbial load and types of organisms at various stages of kunun zaki preparation from the Laboratory.

Production Stages	Total Heterotrophs	Total Coliforms	Staphylococci	Lactic acid Bacteria	Fungi
		(cfu/g or ml)			
A	$3.2X10^2$	$6.0X10^1$	$2.0X10^1$	NP	NIL
B	$4.3X10^3$	$3.0X10^3$	$3.0X10^2$	$2.85X10^4$	$1.7X10^3$
C	$9.6X10^3$	NIL	NIL	$1.5X10^1$	$6.0X10^2$
D	$8.0X10^3$	$3.1X10^3$	$3.5X10^2$	$1.0X10^4$	$2.1X10^4$
E	$5.7X10^3$	NIL	NIL	$8.8X10^3$	$2.15X10^4$
F	$3.0X10^2$	NIL	$1.0X10^2$	$1.0X10^4$	$1.0X10^2$
G	$7.7X10^3$	NIL	NIL	$2.3X10^4$	$1.0X10^2$
H	$6.0X10^2$	NIL	NIL	$2.0X10^4$	$5.6X10^2$
I	$9.0X10^3$	NIL	NIL	$3.0X10^4$	$1.94X10^4$

KEY

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

3.8 Physico-chemical Analysis

The results of the physico-chemical analysis as shown in Table 6, showed that the mean pH of freshly prepared kunun zaki of the four preparations was 4.95 ± 0.41, while that recorded by [1] was 5.00 ± 0.04. Upon storage at 5^0C for three days, the pH of the samples rose to 5.08 ± 0.39, hence confirming the work done by [1], who had a pronounced increase in pH at a lower storage temperature.

Total titratable acidity was found to be 0.33 ± 0.56g, while [1] recorded 0.20 ± 0.01g. Total acidity decreased to 0.28 ± 0.77g after stored at 5^0C for three days. There was no significant difference (*P* = .05) between total solids of freshly prepared samples and their storage at 5^0C for three days. Turbidity was 19.04 ± 1.2 NTU, which decreased to 18.23 ± 1.0 NTU after stored at 5^0C for three days.

Table 6: Effect of storage condition on the proximate and physico-chemical parameters of kunun zaki.

Condition	pH	Total acidity (g)	Total solids (%)	Protein (%)	Carbohydrates (%)	Ash (%)	Moisture (%)	Turbidity (NTU)
Fresh kunun	4.95 ± 0.41^b	0.33 ± 0.56^a	9.11 ± 0.12^{NS}	0.65 ± 0.31^a	12.31 ± 0.40^a	0.53 ± 0.25^b	89.74 ± 2.05^b	19.04 ± 1.20^b
Kunun at 5^0C (for 3 days)	5.08 ± 0.39^a	0.28 ± 0.77^b	9.58 ± 2.67^{NS}	0.39 ± 0.22^b	12.00 ± 0.54^b	0.22 ± 0.17^b	92.03 ± 1.73^a	18.23 ± 1.00^a

KEY

a, b, c, d = Means significantly (*P*= .05) different from each other.
a = Highest mean NS = Not significant

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d = Lowest mean NTU= Number transfer unit.

3.9 Proximate Analysis

The results of the proximate analysis as shown in Table 6 above showed that the mean protein content of freshly prepared kunun zaki of the four preparations was 0.65 ± 0.31%, which decreased to 0.39 ± 0.22% after stored at 5°C for three days. The carbohydrate content also decreased from 12.31 ± 0.40 to 12.00 ± 0.54%, ash content from 0.53 ± 0.25% to 0.22 ± 0.17%. Moisture content increased from 89.74 ± 2.05 to 92.03 ± 1.73% after stored at 5°C for three days.

Based on storage condition as shown in Table 7, the essential, trace, and toxic elements (calcium, copper, zinc, manganese, and lead contents) in agreement with [1], decreased after the products were stored at 5°C for three days.

Table 7: Effect of storage condition on the essential, trace, and toxic elements of kunun zaki.

Condition	Lead (ppm)	Copper (ppm)	Zinc (ppm)	Calcium (ppm)	Manganese (ppm)
Fresh kunun	0.07±0.25[a]	0.36±0.26[a]	1.36±0.81[a]	1.35±0.94[a]	0.73±055[a]
Kunun at 5°C (for 3 days)	0.02±0.20[a]	0.28±0.22[b]	1.25±0.78[b]	1.24±0.90[b]	059±0.45[b]

KEY

a, b, c, d = Means significantly (*P* = .05) different from each other.
a = Highest mean
d = Lowest mean
NS = Not significant

Based on location as shown in Table 8, Bori-camp preparation had lead content to be 0.44 ± 0.13ppm, this toxic element was negligible in Rumuodomaya, Mgbuogba, and Laboratory preparations. Results showed that Laboratory preparation had the highest calcium content of 2.60 ± 0.08ppm, while the least was found to be 0.14 ± 0.03ppm in Bori-camp preparation. Mgbuogba had the highest copper content of 0.57 ± 0.13ppm, while the least was found to be -0.01 ± 0.02ppm in Bori-camp.

Table 8: Effect of location on the essential, trace, and toxic elements of kunun zaki.

Location	Lead (ppm)	Copper (ppm)	Zinc (ppm)	Calcium (ppm)	Manganese (ppm)
Bori-camp	0.44±0.13[a]	-0.01±0.02[d]	0.42±0.05[d]	0.14±0.03[d]	0.27±0.76[d]
Rumuodomaya	-0.04±0.46[b]	0.48±0.06[b]	2.51±0.09[a]	1.42±0.79[b]	1.48±0.17[a]
Mgbuogba	-0.04±0.05[b]	0.57±0.13[a]	1.08±0.15[c]	1.02±0.06[c]	0.42±0.06[c]
Laboratory	-0.03±0.04[b]	0.25±0.04[c]	1.22±0.51[b]	2.60±0.08[a]	0.47±0.69[b]

KEY

a, b, c, d = Means significantly (*P* = .05) different from each other.
a = Highest mean
d =Lowest mean

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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 the flow diagram below;

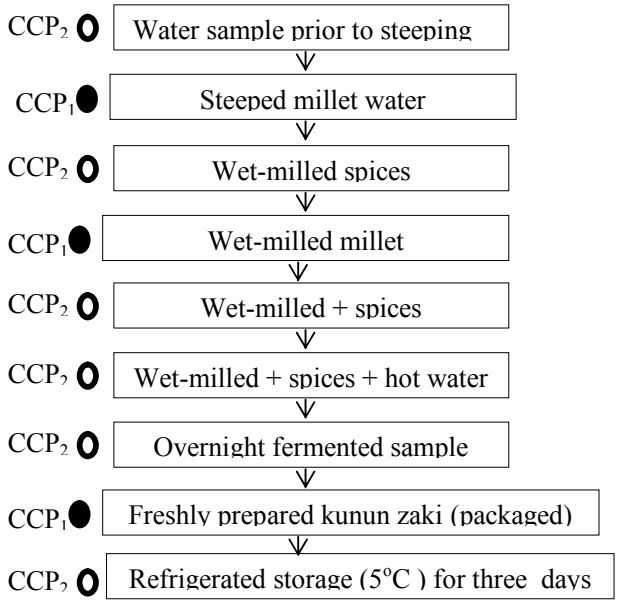

Figure 3: Flow diagram of critical control points in kunun zaki preparation (Modified from [10]).

KEY

O : Indicates a point of minor contamination.

● : Indicates a point of major contamination.

CCP1: Effective critical control point.

CCP2: Not absolute.

3.10 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) that might be associated with the final product as seen in the commercially-prepared products, but were not associated with laboratory-prepared product.

3.11 Steeped millet water

This is a point of major contamination in kunun zaki production as shown as 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.

3.12 Wet-milled spices

This is another point of minor contamination, since they spices 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 [7], and therefore should be

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thoroughly washed to remove accumulated soil or dust particles from farms that could introduce contaminants in the course of the preparation.

3.13 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.

3.14 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.

3.15 Wet-milled millet + Spices + Hot water

This is another point of minor contamination in kunun zaki production. The hot water added 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 hot water could be made hotter, and the levels of contaminants were at minimal level, the hot water could as well eliminate the contaminants. This was evidenced in Laboratory preparation where hot water was effective in eliminating the growth of coliforms and staphylococci, but was not effective in Bori-camp, Rumuodomaya, and Mgbuogba preparations. This could be attributed to high level of contaminants inherent in the mash, and also the hot water not being hot enough.

3.16 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 30^0C 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 [11]. 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.

3.17 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.

3.18 Refrigerated storage (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.

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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.

COMPETING INTERESTS

Authors have declared that no competing interest exist.

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