

Original Research Article

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

Physico-chemical & microbiological quality characteristics of Kunun zaki obtained from three production sites in Port-Harcourt, Nigeria.

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

Results: The microorganisms associated with freshly prepared kununzaki and that stored at refrigeration temperature (5°C) 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 7.80×10^5 cfu/ml and 7.47×10^4 cfu/ml respectively, while Laboratory preparation had the least counts of 6.2×10^3 cfu/ml and 7.7×10^2 cfu/ml for coliforms and staphylococci respectively. Heterotrophic counts for commercially-prepared product ranged from 1.29×10^4 to 2.5×10^4 cfu/ml, while Laboratory preparation was 6.0×10^2 cfu/ml. Fungal counts for commercially-prepared drink ranged from 1.0×10^4 to 1.36×10^5 cfu/ml, while Laboratory-prepared drink had fungal counts of 5.6×10^2 cfu/ml. Yeasts were the main spoilage organisms which persisted at storage temperature of 5°C 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°C.

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,

incorporate the parameters - physico-chemical

5°C

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NDER PEER REVIEW

48 6% carbohydrate, and minerals (copper, zinc, calcium, and manganese) 0.55 to 3.30ppm[1].
 49 During the preparation of kunun zaki from millet, the ingredients needed are ginger (*Zingiber*
 50 *officinale*), cloves (*Syzygium aromaticum*), dried red pepper (*Capsicum cultivars*), sweet
 51 potato (*Ipomoea batatas*), millet (*Pennisetum typhoideum*), sugar and water. There are
 52 variations in the ~~traditional or commercial~~ processing of kunun zaki. According to Olanrewaju
 53 *et al.*, [3], the traditional or commercial processing of kunun zaki involves steeping of the
 54 millet in local household utensils such as buckets, drums, or calabashes for 24 hours. The
 55 steeped grains are washed along with the spices, wet-milled into paste and divided into two
 56 portions. The first (larger) portion is subjected to heat ^{treatment} by the addition of hot water, with
 57 constant stirring to homogenize till it becomes gelatinized, while the other (smaller) portion is
 58 added and stirred to homogenize. The mixture is then allowed to cool, left overnight to allow
 59 fermentation to occur and filtered. The final product can be sweetened with sugar and
 60 packaged in small non-sterilized bottles and bags for sale. The fermentation which occurs
 61 during steeping (before milling) and after milling involves mainly lactic acid bacteria and
 62 yeasts [4]. The flow diagram of traditional kunun zaki production process as described by
 63 Amakoromo [2] is shown in Figure 1 below.

6% carbohydrate?
 (doubtful)

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 but why
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 Production.
 Summarize
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 report

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

91 Due to the involvement of several people in the production of kunun zaki, and the use of
 92 unsterilized containers and milling machine, the final product is contaminated and may be
 93 microbiologically unsafe for consumption. Various microbial genera recorded include;
 94 *Lactobacillus* spp, *Bacillus* spp, *Leuconostoc* spp, *Streptococcus* spp, *Micrococcus* spp,
 95 *Staphylococcus* spp, *Pseudomonas* spp, *Escherichia coli*, *Penicillium* spp, *Mucor* spp,
 96 *Aspergillus* spp, *Rhizopus* spp, *Candida* spp, and *Saccharomyces* spp. *Lactobacillus* spp,
 97 *Streptococcus lactis*, and *Saccharomyces cerevisiae* isolates have been used as starter
 98 cultures singly and in pairs to produce Kunun zaki in the laboratory from millet.

what calibre of person. Specif.

I disagree.
 even if we
 see Agary
 et al 2010.
 and
 Ejiro
 1995.

The fermenting organisms involved in
 Kunun-zaki production has been
 extensively studied and their
 reports are on the net. → See the above
 ref for some of those.

NDER PEER REVIEW

99 *Saccharomyces cerevisiae* compared most favourably with the commercially-prepared
 100 Kunun zaki from millet [5]. This research work was conducted to study the preparation of
 101 kunun zaki in three locations in Port Harcourt namely; Bori-camp, Rumuodomaya, and
 102 Mgbuogba, ~~so as to find out the critical control points of contamination associated with each~~ } note
 103 ~~preparation stage. A laboratory prepared kunun zaki was compared with the above three-~~
 104 ~~mentioned preparations to find out if the shelf-life and safety of the products could be~~
 105 ~~improved upon. This work also did not only compare the microbiological, physico-chemical,~~
 106 ~~and nutritional qualities of hawked and laboratory-prepared Kunun drinks (the final product)~~
 107 ~~as done by [9] and [13], but also the microbiological qualities of the different stages involved~~
 108 ~~in the production process.~~ as to establish the CCPs at various points of the production process *

110 2. MATERIALS AND METHODS

112 2.1 Sampling

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

120 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.

135 2.2 Laboratory production of Kunun zaki:

136 The laboratory production varied from the commercial process by thorough washing of the
 137 grains with cooled boiled water, thereafter the washed grains were steeped for 12 hours in a
 138 cooled boiled water in a sterile stainless basin. The spices (ginger, red pepper, and cloves),
 139 and sweet potato were thoroughly washed respectively. The steeped millet grains were
 140 again washed before milling. Milling of millet, spices, and sweet potato was done with
 141 Panasonic electric blender, model (MX-J110P), rinsed out with hot water. The milled millet
 142 was mixed with the milled spices, and carefully stirred with a sterile stainless spoon in the
 143 sterile basin. Thereafter, the mixture was divided into two portions namely: A (larger portion)
 144 and B (smaller portion). Hot water for sample F at a temperature of 100°C was carefully
 145 added to portion A, ~~compared to that at 60°C added to portion A of commercial preparation.~~
 146 This was allowed to cool to a temperature of 30°C, and then covered to allow fermentation to
 147 take place overnight (for 12 hours), and then carefully filtered using a sterile Cheesecloth.
 148 The final product was bottled and sealed in sterile screw-capped 50cl bottles.

how do you
maintain sterility
does the basin
have a cover?
how was the
blender sterilized?
°C
how was the
cheesecloth
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UNDER PEER REVIEW of various stages of Kunun-zaki production

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 duplicates 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°C, Mannitol salt agar and De Mann Rogosa and Sharpe agar plates were incubated for 48 hours at 37°C, while Sabouraud dextrose agar plates were incubated for 5 days at 26°C. 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°C 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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202 3.3 Total Heterotrophic counts

203 Sample A (Water sample prior to steeping) of the four preparations had their counts between
204 the range of 10^2 to 10^3 /ml. Laboratory preparation had the least count of 3.2×10^2 cfu/ml,
205 while Bori-camp had the highest count of 1.44×10^3 cfu/ml.
206

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207 Sample B (Steeped millet water) of the four preparations had their counts between 10^3 to
208 10^4 /ml. Counts in the four preparations increased by one log cycle.
209

210 Sample H (Freshly prepared kunun zaki) of the four preparations had their counts between
211 10^2 to 10^4 /ml, while that carried out by [9] recorded 7.8×10^7 cfu/ml as the total heterotrophic
212 bacterial counts of freshly prepared Kunun zaki. Laboratory preparation counts dropped by
213 one log cycle, while counts in the other three preparations were stable.
214

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215 On refrigerated storage for three days at 5°C , Laboratory preparation had the least count of
216 9.0×10^3 cfu/ml, while Bori-camp had the highest count of 1.56×10^5 cfu/ml.
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Analyse Here
data
Statistical

218 3.4 Total coliform counts

219 Sample A (Water sample prior to steeping) of the four preparations had their counts between
220 10 to 10^3 /ml. Laboratory preparation had one cycle lower than Mgbuogba, and two log
221 cycles lower than Rumuodomaya and Bori-camp preparations.
222

223 Sample B (Steeped millet water) of the four preparations had their counts between 10^3 to
224 10^5 /ml. Laboratory preparation had one log cycle lower than Mgbuogba and Rumuodomaya
225 preparations, and two log cycles lower than Bori-camp preparation.
226

227 Sample H (Freshly prepared kunun zaki) of Bori-camp, Rumuodomaya, and Mgbuogba
228 preparations were within the range of 10^3 /ml, while [9] recorded the range of 2.2×10^6 to 5.2
229 $\times 10^6$ cfu/ml. No coliform was detected in Laboratory preparation.
230

231 On refrigerated storage for three days at 5°C , Bori-camp count increased by two log cycles,
232 where as Rumuodomaya and Mgbuogba increased by one log cycle. No coliform was
233 detected in the Laboratory preparation.
234

Hope you
know the
implication
of this result
What is the
source of
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235 3.5 Total staphylococci counts

236 Sample A (Water sample prior to steeping) of three preparations Bori-camp, Rumuodomaya,
237 and Laboratory had counts between 10 to 10^2 /ml. No staphylococci were detected in
238 Mgbuogba preparation.
239

240 Sample B (Steeped millet water) of the four preparations ranged from 10^2 to 10^4 /ml. Counts
241 from Rumuodomaya and Laboratory preparations increased by one log cycle, and
242 Mgbuogba had counts of 1.0×10^2 cfu/ml.
243

244 Sample H (Freshly prepared kunun zaki) of three preparations Bori-camp, Rumuodomaya,
245 and Mgbuogba had their counts within 10^3 /ml. Counts in Bori-camp and Rumuodomaya
246 stabilized, Mgbuogba increased by one log cycle, and none was detected in Laboratory
247 preparation.
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What is the
implication of
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Staph is water
prior to steeping?
This result is
clarifying if
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249 On refrigerated storage for three days at 5°C , counts were within the range of 10^3 /ml in Bori-
250 camp, Rumuodomaya, and Mgbuogba preparations, while none was also detected in
251 Laboratory preparation.

252 3.6 Lactic acid bacterial counts

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

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

257 Sample H (Freshly prepared kunun zaki) of the four preparations were within the range of
258 10^4 /ml.
259

UNDER PEER REVIEW

On refrigerated storage for three days at 5°C, counts in the four preparations were between 10⁴ to 10⁵/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 10²/ml. Rumuodomaya preparation had the least count of 9.0 x 10¹cfu/ml, while the highest counts of 4.6 x 10²cfu/ml were found in Bori-camp preparation. Laboratory preparation had no count.

Sample B (Steeped millet water) of the four preparations were between 10³ to 10⁵/ml. Laboratory preparation had the least counts of 1.7 x 10³cfu/ml, whereas Bori-camp had the highest count of 1.95 x 10⁵cfu/ml.

Sample H (Freshly prepared kununzaki) of the four preparations had their counts between 10² to 10⁵/ml. Mgbuogba preparation had the least count of 1.0 x 10⁴cfu/ml, while Bori-camp had the highest count of 1.36 x 10⁵cfu/ml.

On refrigerated storage for three days at 5°C, 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	8.3X10 ³	2.33X10 ⁴	1.36X10 ⁵
I	1.56X10 ⁵	1.21X10 ⁵	2.0X10 ³	2.85X10 ⁵	4.75X10 ⁴

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KEY

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

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* Please ~~summarize~~ ^{the} results in microbiology - one or two paragraphs. Also, merge tables 2 - 5. ~~make them~~ ^{interrelated} ~~to be more comprehensive~~ statistically analyze the data to justify your claim.

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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	1.37×10^3	1.1×10^3	3.8×10^2	NP	9.0×10^1
B	2.78×10^4	3.9×10^4	9.9×10^3	3.0×10^4	2.2×10^3
C	3.0×10^4	3.0×10^4	5.25×10^3	1.1×10^4	2.15×10^3
D	2.51×10^4	1.80×10^4	5.4×10^3	2.98×10^4	3.1×10^3
E	1.28×10^4	2.3×10^3	3.0×10^2	9.0×10^3	1.03×10^4
F	9.0×10^3	1.0×10^4	6.0×10^2	3.0×10^4	9.0×10^2
G	1.45×10^4	1.01×10^4	7.25×10^3	1.51×10^4	2.0×10^2
H	1.29×10^4	8.75×10^3	1.3×10^3	2.97×10^4	2.5×10^2
I	1.30×10^4	1.82×10^4	7.65×10^3	2.2×10^4	1.2×10^4

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KEY

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A = Water sample prior to steeping

F = Wet-milled millet + spices + hot water

305

B = Steeped millet water

G = Overnight fermented sample (Unsieved)

306

C = Wet-milled spices

H = Freshly prepared kunun zaki (packaged)

307

D = Wet-milled millet

I = 3-day refrigerated kunun zaki

308

E = Wet-milled millet + spices

309

NP = Not plated

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

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Production Stages	Total Heterotrophs	Total Coliforms	Staphylococci	Lactic acid Bacteria	Fungi
(cfu/g or ml)					
A	1.19×10^3	8.0×10^2	NIL	NP	3.0×10^2
B	3.0×10^4	1.05×10^4	1.0×10^2	3.0×10^4	3.0×10^3
E	7.7×10^3	4.5×10^3	NIL	1.59×10^4	6.0×10^3
F	2.03×10^4	7.5×10^3	NIL	3.0×10^4	1.0×10^2
G	2.91×10^4	3.3×10^3	1.0×10^2	2.85×10^4	1.2×10^4
H	2.5×10^4	3.0×10^3	1.5×10^3	2.75×10^4	1.0×10^4
I	3.0×10^4	2.3×10^4	3.0×10^3	3.0×10^4	2.9×10^4

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A = Water sample prior to steeping

G = Overnight fermented sample (Unsieved)

317

B = Steeped millet water

H = Freshly prepared kunun zaki (packaged)

318

E = Wet-milled millet + spices

I = 3-day refrigerated kunun zaki

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F = Wet-milled millet + spices + hot water

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	3.2×10^2	6.0×10^1	2.0×10^1	NP	NIL
B	4.3×10^3	3.0×10^3	3.0×10^2	2.85×10^4	1.7×10^3
C	9.6×10^3	NIL	NIL	1.5×10^1	6.0×10^2
D	8.0×10^3	3.1×10^3	3.5×10^2	1.0×10^4	2.1×10^4
E	5.7×10^3	NIL	NIL	8.8×10^3	2.15×10^4
F	3.0×10^2	NIL	1.0×10^2	1.0×10^4	1.0×10^2
G	7.7×10^3	NIL	NIL	2.3×10^4	1.0×10^2
H	6.0×10^2	NIL	NIL	2.0×10^4	5.6×10^2
I	9.0×10^3	NIL	NIL	3.0×10^4	1.94×10^4

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°C 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 $0.33 \pm 0.56\text{g}$, while [1] recorded $0.20 \pm 0.01\text{g}$. Total acidity decreased to $0.28 \pm 0.77\text{g}$ after stored at 5°C for three days. There was no significant difference ($P = .05$) between total solids of freshly prepared samples and their storage at 5°C for three days. Turbidity was 19.04 ± 1.2 NTU, which decreased to 18.23 ± 1.0 NTU after stored at 5°C 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°C (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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You should present the data for each treatment separately instead of taking their means.

did you do statistical analysis and check for normality?

Again, what is the implication of the results?

The results for each treatment are being given. Should be shown separately and not lumped together.

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3.9 Proximate Analysis

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

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

371 **Table 7: Effect of storage condition on the essential, trace, and toxic elements of**
372 **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 ± 0.55^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	0.59 ± 0.45^b

376

KEY

377 a, b, c, d = Means significantly ($P = .05$) different from each other.
378 a = Highest mean
379 d = Lowest mean
380 NS = Not significant

381

382 Based on location as shown in Table 8, Bori-camp preparation had lead content to be $0.44 \pm$
383 0.13ppm , this toxic element was negligible in Rumuodomaya, Mgbuogba, and Laboratory
384 preparations. Results showed that Laboratory preparation had the highest calcium content of
385 $2.60 \pm 0.08\text{ppm}$, while the least was found to be $0.14 \pm 0.03\text{ppm}$ in Bori-camp preparation.
386 Mgbuogba had the highest copper content of $0.57 \pm 0.13\text{ppm}$, while the least was found to
387 be $-0.01 \pm 0.02\text{ppm}$ in Bori-camp.

*(Expl
the
result)*

388

389 **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

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KEY

392 a, b, c, d = Means significantly ($P = .05$) different from each other.
393 a = Highest mean
394 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;

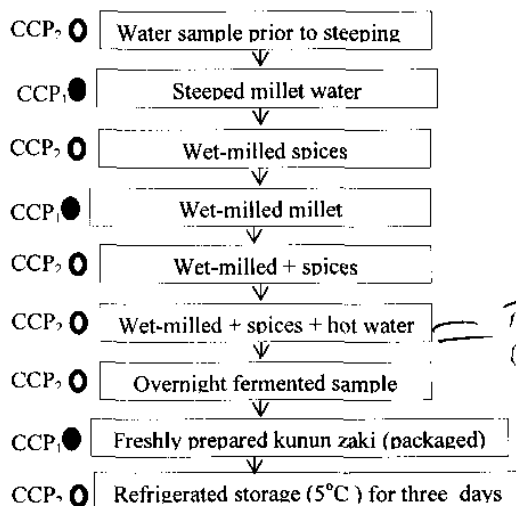


Figure 3: Flow diagram of critical control points in kunun zaki preparation (Modified from [10]).

KEY

○ : 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

in HACCP study, as usually, the potential hazards are established, then the parameters used to control the CCPs are established. PLS see. Oranusi et al (2003) & Akom et al (2012)

X-

This point is not agree with you write-up in that media.

This ref cited here appears to be incorrect.

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

455 3.13 Wet-milled millet

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

460 3.14 Wet-milled millet + Spices

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

466 3.15 Wet-milled millet + Spices + Hot water

467 This is another point of minor contamination in kunun zaki production. The hot water added
468 was not necessarily for sterilization purpose, but for the extraction of flavor from the spices
469 (flavor enhancement) and for proper gelatinization of the mash. If the hot water could be
470 made hotter, and the levels of contaminants were at minimal level, the hot water could as
471 well eliminate the contaminants. This was evidenced in Laboratory preparation where hot
472 water was effective in eliminating the growth of coliforms and staphylococci, but was not
473 effective in Bori-camp, Rumuodomaya, and Mgbuogba preparations. This could be attributed
474 to high level of contaminants inherent in the mash, and also the hot water not being hot
475 enough.

476 3.16 Overnight fermented sample

477 This is a point of minor contamination in the production process of kunun zaki. Overnight
478 fermentation process in kunun zaki production is best achieved at a minimum temperature of
479 30°C for 12 hours in a trough or bucket. If this trough or bucket is not properly covered
480 during the overnight fermentation process, insects and rodents could bring in further
481 contaminants by transferring filth from contaminated areas to food through their waste
482 products, and during regurgitation of filth onto clean food during consumption [11]. Thus,
483 contamination from these pests could be prevented by proper covering of trough or bucket
484 with a sterile cover, and the processing areas should be protected against their entry by
485 sound sanitary practices.

486 3.17 Freshly prepared kunun zaki (packaged)

487 This is a point of major contamination in the production process of kunun zaki. Sterility of
488 packaging materials (plastic bottles) must be ensured to prevent contamination by using
489 sterile plastic bottles with screw caps. Clean bottles were used in the laboratory preparation
490 where no coliforms, staphylococci, and molds were associated with freshly prepared kunun
491 zaki.

492 3.18 Refrigerated storage (5°C) for three days

493 This is a point of minor contamination when the product is not stored at a stable refrigeration
494 temperature. Storage of Kunun zaki at refrigeration temperature of 5°C for three days,
495 increased lactic acid bacterial counts to 10⁵cfu/ml, and led to the isolation of spoilage
496 organisms including *Pseudomonas* spp, *Micrococcus* spp and fungi in the four preparations,
497 implying that a lower temperature could be used depending on the outcome of sensory tests.

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Re call
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