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Monitoring the Microbial Load at Chosen Critical Control Points in the Production of Kunun-zaki

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Abstract:*The microbial load at chosen Critical Control Points (CCPs) in the Production of Kunun-zaki was studied. Standard procedure was employed in the laboratory production of Kunun-zaki. Four critical control points were set. Established culture methods were employed in the isolation of microorganisms. The Total Heterotrophic Bacterial Count (THBC) varied significantly across the CCPs. THBC was highest at the Ground Millet point (1.36 x 10^7±0.0 cfu/g) and lowest at the Steeped Millet point (0.20 x 10^7±0.14 cfu/g). Similarly, there were significant differences in the Total Heterotrophic Fungal Count (THFC), Total Shigella Count (TSC) as well as Total Coliform Count (TCC). While THFC was highest at the third CCP (1.25 x 10^5±0.07 cfu/ml), TSC (2.95 x 10^5±0.21 cfu/ml) and TCC (0.15 x 10^2±0.07 cfu/ml) were highest at the first CCP. Five bacterial genera (Bacillus spp, Escherichia coli, Leuconostoc spp. Staphylococcus aureus, and Vibrio spp.) were present across the four CCPs with Staphylococcus aereus and Bacillus spp occurring at all CCPs. Also, four fungal genera (Aspergillus spp, Fusarium spp, Penicillium spp and Rhizopus spp) were isolated with only Rhizopus spp occurring at all CCPs. The presence of these microbes at detectable levels constitute a public health risk. Better production and fermentation practices could improve the shelf life as well as microbiological quality of Kunun-zaki.*

Keywords*: Kunun-zaki, Critical Control Points, microorganisms, public health, shelf life.*

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I. Introduction

Man continues to devise adaptive featuresnecessary for survival in their peculiar environments. The Northern Nigeria is exposed to very high temperatures with low humidity[1]. As expected, the people in this region make efforts to balance the effects of these weather conditions on their body systems. One of such adaptive measures is their choice of diet; such as using cold, refreshing drinks to ease off the scotching effect of the sun. Kunun-zaki is one of these drinks, indigenous but not restricted to Northern Nigeria presently. It is consumed based on its thirst-quenching attribute. This attribute is based on its high moisture content. Studies have shown that this beverage has moisture content ranging between 55-98%[2, 3, 4].Thus, this drink is consumed all year round but moreduring the dry season when thirst is worst.

Kunun-zaki is a non - alcoholic cereal beverage[5, 3]. Quite nutritious, Kunun consists mostly of carbohydrates, vitamins and proteins[5, 6]. It is the presence of reasonable levels of carbohydrate that accounts for its chance fermentation to produce lactic acid[7]. Kunun is produced in shanty houses in Northern Nigeria[8]and preferentially made from Millet, Sorghum, Guinea corn and Maize respectively[5, 3, 9]. Although this beverage could be made from all three cereals, it is commonly made from Sorghum and Millet in the ratio of 1:2 w/w[2]. Kunun-zaki production is a traditional procedure and most times, a family art. Production starts with steeping the cereals in household utensils like buckets, calabashes and other earthenware[5, 3]. The steeped cereals is then grinded into a mush, gelatinized, spiced and the drink sweetened with honey, sugar or sweet potatoes[3]. Spices used include clove, red or black pepper and ginger.

The methods of production of this important beverage does not give much attention to shelf life of the final product considering their wide distributio[5,3,10].This method of production as described above is crude, not standardized, with levels of ingredients not quantified and largely a family art. Thus, there are significant variations in the procedures depending on taste and cultural habits. This had led to differences in quality and stability. Studies have shown that some cultures prefer Kunun-zaki with much pepper or sweet taste and others prefer it with no pepper or sugar[8].This lack of consistency and standard in production procedures raises concerns on the hygiene and safety ofthis nutritious, thirst-quenching beverage.

Microorganisms such as bacteria and fungi are ubiquitous and easily contaminate food materials. This poses a public health risk andcould lead to food poisoning. The production processes for Kunun-zaki are rather clumsy, with poor hygiene eminent on the part of the local producers. This may be a leading introduction route for microorganisms. Microbial contamination of food has been linked to handling and some organisms isolated from food are notably normal flora of man[11]. Previous studies have reported the presence of microorganisms including pathogens in Kunun-zaki[9]. Introduction of microorganisms may be linked with the water used in the production of this beverage among other things[5]. The high water content and poor packaging conditions of Kunun-zaki have also been implicated for contamination[3].

Hazard Analysis Critical Control Points (HACCP) advocates a holistic approach in food production[12]. This principle is a preventive food administrative system. Every step in food production, storage and distribution is monitored for hazards. Kunun production is quite simple and pays no attention to HACCP. This beverage is a drink of choice for its high nutrient content as well as cost effectiveness. However, it may not be safe for consumption due to the traditional, uncontrolled methods of production. Further, the production processes may account for the very poor shelf life of this beverage.

This research was aimed at introducing HACCP concept in Kunun-zaki production in order to establish the hazards and make the drink safer for human consumption while improving its shelf life. The specific objectives for this study wereLaboratory Production of Kunun-zaki, determining the Critical Control Points and Isolation and identification of the microorganisms at each CCPs.

II. Materials And Methods

Sample Collection

Commercially available millet, ginger, black and red pepper as well as granulated sugar were procured from Mile 3 market, Diobu in Port Harcourt, Rivers State, Nigeria.

Kunun-Zaki Production

Traditional method as described by Adeyemi and Umar[8] was used. Five hundred grams of millet (*Pennisetumtyphoidem*) were cleaned and steeped in 1000 ml of tap water for 24 h. After 24 h, the water was decanted and the grains washed in fresh tap water and blended with 10 g of powdered ginger (*Zingiberofficinale*) in 2 vol. tap water. Then, the slurry was sieved using muslin cloth in abundance of tap water. Sedimentation was done by allowing the filtrate to stand for 5 h at ambient temperature. After sedimentation, the supernatant was discarded, leavinga pasty, milky sediment of about 800 g. This was divided into two portions of 400 g each. To one portion, boiling water was added and allowed to cool before mixing with the second 400 g portion. To the mix, 3 volumes tap water was added and allowed to ferment for 8 h. The resulting Kunun-zaki was sweetened and packaged in clean bottles.

Isolation Of Dominant Microorganisms At The Critical Control Points

Isolation of microorganisms associated with Kunun-zaki production was done using Nutrient Agar (NA), Mannitol Salt Agar (MSA), Mac Conkey Agar (MCA) and Potato Dextrose Agar (PDA). Diluent used was 0.1% peptone water[3]. All media were obtained commercially and prepared according to the manufacturer's instruction. For clarity, four CCPs were set. These were the Dry Millet Stage, the Steeped Millet Stage, the Milled Millet Stage and the Finished Kunun-zaki Stage.

At the 1st and 2nd CCPs, serial dilution was performed. Ten grams of dry or steepedmillet was diluted in with 90 ml peptone water and diluted down to 10^{-5} [13]. Then, 0.1 ml aliquot of the 10^{-3} to 10^{-5} dilutions were spread on the various media using the spread plate technique[14]. While the bacteria media plates (NA, MSA and MCA) were incubated at 37ºC for 24 h, the fungion PDA plates were incubated at 37ºC for 48 h. All samples were plated in triplicates according to Elmahmood and Doughari[3].

At the 3rd and last CCPs, 10 ml milled or finished Kunun-zaki was diluted with 90 ml peptone water and then diluted and plated as already described above. All discrete representative bacterial colonies were isolated at each of the four CCPs and sub cultured on NA plates at 37ºC for 24 h to obtain pure cultures. Similarly, discrete fungal spores were incubated at 37ºC for 48 h.

Microbial identification was done using standard morphological characteristics as well as biochemical tests as previously described[14, 13].

Data Analysis

Analysis of Variance was used to test all data for significance using SPSS. Significance was set at $p \leq 0.05$. Students Newman Kuel's test was used to separate means where differences occurred between the CCP's.

III. Results

Identification Of Pure Cultures.

The mean Total Heterotrophic Bacterial and Fungal counts were obtained and recorded for the four Critical Control Points studied. THBC showed significant difference across the CCPs studied (Table 1). Similarly, the THFC varied significantly across all control points in the production of Kunun. Also, the Total Shigella Count (TSC) as well as Total Coliform Counts varied across all points studied (Table 1). Most striking was the TCC which showed a clear pattern. TCC was highest as the Dry millet stage but reduced significantly and was least in the finished Kunun-zaki.

Table 1: Microorganisms associated with the chosen CCPs inLaboratory production of Kunun-zaki

Critical Control point	Microbiological parameters THBC ($x10^7$cfu/ml)	THFC ($x10^5$sfu/ml)	TSC ($x10^5$cfu/ml)	TCC ($x10^2$cfu/ml
Dry Millet	1.32 ± 0.08^{c}	0.35 ± 0.07^{a}	2.95 ± 0.21^{b}	0.15 ± 0.07^{b}
Steeped Millet	0.20 ± 0.14^{a}	0.70 ± 0.14^{b}	2.40 ± 0.57^{b}	$0.09\pm0.01^{a\,b}$
Ground Millet	1.36 ± 0.0^{c}	1.25 ± 0.07^{c}	1.15 ± 0.07^{a}	$0.09\pm0.01^{a\,b}$
Finished Kunun-zaki	1.07 ± 0.01^{b}	0.25 ± 0.07^{a}	0.60 ± 0.28^{a}	0.00 ± 0.00^{a}

*means with same superscript along the columns are not significantly different ($p\leq0.05$)
KEY: THBC= Total Heterotrophic Bacteria Count
THFC= Total Heterotrophic Fungal Count
TSC= Total Staphylococcal Count
TCC= Total Coliform Count

Table 2: Characterization of Bacterial Isolates

ISOLATE	**ColonialMorphology**								**BIOCHEMISTRY**			**SUGAR UTILIZATION**			**PROBABLE ORGANISM**
	Size (mm)	Outline	Elevation	Colour	Texture	Sporulation	Gram's reaction	Motility	Catalase	Oxidase	Coagulase	Glucose	Lactose	Sucrose	
ISO 1	0.3	Entire	Slightly raised	Creamy	Smooth	-	+	-	+	-	+	+	+	+/G	*Staphylococcus aureus*
ISO 2	0.1	Entire	Raised	Pinkish	Moist	-	-	+	-	-	-	+/G	+	+/AG	*Escherichia coli*
ISO 3	1	Entire	Slightly Raised	Bluish	Wrinkled	-	-	+	+	+	-	+	-	+	*Vibrio* spp
ISO 4	1	Entire	Flat	Reddish	Moist	-	+	-	-	-	-	+	+	+	*Leuconostoc* spp
ISO 5	1.5	Wavy	Flat	Grey	+	+	+	+	-	-	-	±	±	±	*Bacillus* spp

Key: ISO= Isolate; + = Positive; - = Negative; ± =delayed fermentation; +/G = Positive with gas production; +/AG= Positive with Acid and gas production

Five Bacterial genera were identified in the chosen CCPs in the Laboratory Production of Kunun-zaki (Table 2). Of these genera only *Staphylococcusaureus* and *Bacillus* spp. were present in all four CCPs (Table 4).

Table 3: Characterization of Fungal Isolates

ISOLATE	Morphology on PDA	Growth (37 °C)	Microscopy	Probable Organism
ISO 1	Fluffy, whitish, nipple-shaped, elevated and non-capsular	+	Sickle-shaped, multi-segmented spores	*Fusarium* spp.
ISO 2	Fluffy, white with a yellowish pigmentation.	+	Aseptate hyphae, dark green, irregular shape, conidia present.	*Aspergillusflavus*
ISO 3	Dark green and grainy	+	No definite shape, brush-like, spores and conidiophores present	*Penicillium* spp
ISO 4	Fluffy, white and grey colonies.	+	Ribbon-like, non-septate hyphae, haphazardly branched	*Rhizopus* spp
ISO 5	Fluffy, black colonies	+	Round head, septate hyphae with spores	*Aspergillusniger*

Table 4: Bacterial Isolates associated with the different Critical Control Points during Kunun-zaki production under laboratory condition

Critical Control points	Bacterial Isolates
	***Staphylococcus aureusE. coliVibrio* spp. *Leuconostoc* spp*Bacillus* spp.**

Dry Millet	+	+	-	+	-
Steeped Millet	+	+	+	+	+
Ground Millet	+	-	+	+	-
Finished Kunun-zaki	+	-	+	+	-

Similarly, four fungal genera were identified in the production of Kunun-zaki across the determined CCPs (Table 3). Only *Rhizopus* spp. showed presence in all stages while *Aspergillusniger* was present only in the finished product (Table 5).

Table 5: Fungal Isolates associated with the different Critical Control Points during Kunun-zaki production under laboratory condition

Critical Control point	**Fungal Isolates**				
	Aspergillus flavus	***Aspergillusniger***	***Fusarium* spp.**	***Penicillium* spp.**	***Rhizopus* spp**
Dry Millet	+	-	+	-	+
Steeped Millet	+	-	-	+	+
Ground Millet	+	-	-	+	+
Finished Kunun-zaki	-	+	-	-	+

IV. Discussion

The presentstudy reveals a challenging level of microorganisms in this very popular, thirst-quenching drink. All determined CCPs studied presentedwith significant levels of microorganisms. The THBC was highest at the dry millet stage and least at the steeped millet stage. This shows that the source of millet and other cereals used for Kunun-zaki production could be a principal source of bacterial contamination. Millet used in the current study was sourced commercially. It is known that bacteria are ubiquitous and may have been introduced variously. Organisms such as *S. aureus* can be introduced by handling this grain as has been reported in previous studies[5]. *E. coli* is a normal flora of the human intestine and has been successfully used as an indicator of fecal contamination. The millet available commercially are products of various preservation processes including drying. Drying in Nigeria is done in sometimes contaminated locations by sunning. This is a possible introduction route.

Similarly, THFC was quite high in the dry Millet CCP but highest in the steeped Millet CCP. This is in support of contamination from the point of purchase.The millet used for Kunun-zaki production may be contaminated. Steeping the already contaminated millet used in this study increased the THFC to the highest point in the entire production chain. This is expected for fungi. Fungi are known to thrive more in moisture and in fact adequate hydration is a condition for fungal cultivation[15, 16]. It is also important to ascertain the aseptic state of the earthenware and utensils used. These have been implicated as possible contamination routes[3, 17]. Further, the spices used in the production of Kunun have been identified as contamination routes [3], introducing spoilage and pathogenic microbes[18.]

Elmamood and Doughari[3] report that the pH of Kunun-zaki is too low to allow the proliferation of pathogenic microorganisms. However, the presence of *E. coli*, *S. aureus* and *Bacillus* spp. pose a public health hazard. *S. aureus* is a normal flora man's skin, nose, palms, hairs, etc. It is a known etiological agent of septic arthritis and other ailments[19]. *E. coli* is a coliform used to indicate fecal contamination. Certain*E. coli* strains are implicated for gastroenteritis, diarrhoea and urinary tract infections[20]. *Vibrio* and *E. coli* appeared only once across all CCPs. *Vibrio* spp. has been implicated to contaminate food, leading to change in both physical and nutritional qualit[21].

The presence of *S.aureus* calls for attention as they are known normal flora of man[5, 22, 3.] More attention should be paid to the production processes to make it more aseptic and reduce the incidence of this organism. It is possible that this organism may have been perpetually introduced in the production process by handlers[11].

Although some of these organisms occurred minimally, it is not acceptable as their presence in food renders it unsafe for human consumption[23]. Contamination by these pathogens may have occurred at the various CCPs. Apart from *S. aureus*, *Bacillus* spp. and *Rhizopus* spp. that were present at all four CCPs, others may have been introduced by various processes including sieving and packaging of the final product. For example, *A.*

niger was present only in the finished Kunun-zaki. This could be traced to handling at this stage[18]. The fungal species isolated are spoilage organisms and may account for the low shelf life of the beverage[24]. Also, the fungi isolated from Kunun drink produce mycotoxins. For example, *Penicillium*, *Fusarium* and *Aspergillus* species are known to produce mycotoxins in food products[25]. These toxins have been implicated in liver cancer, renal tumour and other ailments[25].

Kunun-zaki has a characteristic soured taste[5, 22, 7]. This could be due to the production of lactic acid following the fermentation aided by microbes present in this study. Previous studies by Efiuvwevwere and Akoma[26] reported that this acidity is due to fermentation by *Lactobacillusleichmanni* and *L. fermentatum*. *Lactobacillus* species have been organisms of choice in fermentation process. This is due to their ability to ferment and also improve the shelf life of food products[22]. Oranusi et al.[5] have reported poor shelf life of this beverage. This they blamed on the production processes. It is also notable that the shelf life varies from producer to producer since there are no adopted formulae for Kunun production presently[5, 8]. Agarry et al.[22] reported that use of starter cultures to ensure consistency in the fermentation will not only improve the nutritional value but also increase the shelf life of food products. Thus fermentation organisms may also vary as well as microbial load. This will depend on factors of production per person.

However, the present study did not isolate any *Lactobacillus* species but isolated *Leuconostoc* and *Rhizopus* spp in appreciable levels. *Leuconostoc* has also been reported present in Laboratory produced Kunun-zaki[22]. These organisms have been successfully deployed for fermentation. Wileyet al.[12] reported that *Rhizopus*spp has been used to ferment popular products like soybean (Tempeh) while sauerkraut and pickles are fermented with *Leuconostoc* spp. These organisms were present at all stages where fermentation could occur in this study.

The fermentation period in the present study was increased. This is due to report in previous studies that chance fermentation does not always occur in Kunun production due to a few factors including time[22, 3]. The extended fermentation time yielded appreciable fermentation. This improved fermentation would have led to higher volume of lactic acid and thus increase the acidity of the drink. This in turn may explain the microbial load. Among the bacteria, only *S. aureus* and *Bacillus* spp. were present at all CCPs. *Bacillus* spp. are spore-forming and may be able to withstand harsh conditions such as increased acidity.

Various reports have shown that Kunun-zaki produced using chance, indigenous fermentation process has high counts of spoilage and pathogenic microorganisms. This may be responsible for its short shelf-life[22, 27, 17]. It is possible that this problem could be reduced if starter cultures are employed in its fermentation process as done in the developed world.

V. Conclusion

Kunun-zaki is loaded with microorganisms. Isolated microorganisms are important either as pathogens, spoilage organisms or fermenters. The CCPs set in the present study offered details of these microbial contaminations. All studied CCPs had organisms. Introducing the HACCP concept in the production of Kunun-zaki is important. Pasteurization as a sterilization method for other food and dairy products will reduce the hazard associated with Kunun consumption. Proper storage and use of standard fermentation organisms in a controlled manner will improve the microbial quality as well as shelf life of Kunun-zaki. Further, Kunun production should be standardized and made more consistent for better results.

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