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Hazards and critical control points of kunun-zaki, a non-alcoholic beverage in Northern Nigeria

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Abstract

A hazard analysis of kunun-zaki preparation was carried out for three producers around Zaria, Nigeria. This analysis consisted of observing the raw materials and environment, watching all steps of the preparation and packing, recording temperatures during cooking and display and collecting samples of kunun-zaki for total viable bacterial count, coliform, staphylococcal and *Bacillus* counts and isolation of *Salmonella* and *Shigella*. Kunun-zaki usually attained a temperature of about 74°C immediately after gelling and is held at ambient temperature for sale. Leftovers were refrigerated. The total viable bacteria count increased with time from immediately after gelling to when the kunun-zaki was ready for consumption. Coliforms, *B. cereus* and *Staphylococcus aureus* were isolated, but had count less than 10^4 cells ml^{-1}. Though the level of counts appear safe, the presence of coliforms, *S. aureus* and *B. cereus*, preparation in a highly contaminated environment and holding at ambient temperature for sale could be risky. Education of producers on the hazards, critical control points and the importance of hygienic environment is imperative. The control measures and monitoring procedures for kunun-zaki preparation are suggested.

Keywords: Kunun-zaki; Contamination; Hazards; CCP; Control measures

1. Introduction

Kunun-zaki, an indigenous non-alcoholic beverage, is produced and widely consumed by adults and infants in the savannah region of Nigeria as a refreshing drink, an appetizer, a food complement and to quench thirst. It is also used as a substitute for or to complement soft drinks and wines at social gatherings.

Kunun-zaki is prepared from either guinea corn (*Sorghum bicolor*), millet (*Pennisetum typhoides*) maize (*Zea mays*), rice (*Oryza sativa*) or wheat (*Triticum aestivum*). Traditionally, the production involves steeping of whole grains for 6–24 h, wet milling with spices and sweet potato, gelling of about 3/4 of the mixture in hot water pitching with about 1/4 fresh (ungelled) part of the mixture and allowing for overnight fermentation. The supernatant is ready for consumption after sieving.

The short shelf-life of one day for kunun-zaki, the unhygienic condition of local production, pitching with mixed culture from the fresh wet milled mixture, and the resultant inconsistent taste and flavour from different producers are limitations to mass production of this product.

There are many concerns about the sanitation of street-vended kunun-zaki. For example, well water is the main source of water in many localities in Zaria; animals are commonly reared in households where kunun-zaki is prepared; spices and grains used are highly prone to contamination (Inabo et al., 2000), and production is done mainly by the local inhabitants living in suburbs with poor sanitary conditions.

To develop a better understanding of the microbiological problems associated with kunun-zaki production, it became extremely necessary to apply the hazard analysis critical control point (HACCP) strategy. HACCP strategy identifies hazards associated with different stages of preparation and handling, assesses the relative risk and identifies points where control measures would be effective (Bryan, 1988; Ehiri et al., 2001). This study therefore, assesses the hazards associated with consumption of kunun-zaki and identifies the critical control points (CCP). Measures that could ensure safety of kunun-zaki are emphasized.

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2. Materials and methods

2.1. Selection of participants

Preceding the hazard analysis was a survey study of 240 samples of polyvinyl chloride (PVC) packed, bottled and bulk-packaged kunun-zaki purchased from different producers/vendors around Zaria (Samaru, Sabon Gari and Zaria City). This study resulted in a close interaction with the producers. Based on the packaging method, location, type and number of consuming population and willingness to participate in the study, three producers were selected, one each from Samaru, Sabon Gari and Zaria City for hazard analysis.

2.2. Description of production sites, the producers and vending operations

Producer A lived in a two-bedroom flat close to Ahmadu Bello University, Samaru, Zaria. Water used for kunun-zaki preparation was fetched when the tap was running and stored in drums and large plastic containers well covered. The producer keeps poultry and has a family of five. Two of the children assist the mother in poultry keeping and kunun-zaki processing. The finished product is dispensed with a plastic cup into PVC packs, tied and sold to the University and Samaru communities with a population of about 50,000 people. Leftover kunun-zaki was refrigerated and sold the following day.

Producer B lived in a two-bedroom apartment in a large compound with eight other families. The house was close to a motor park and a busy market (Sabon Gari market). Water for processing was obtained from a tap in buckets and stored in open drums and covered clay pots. Chickens, goats and dogs were seen roaming in the compound. Finished product was dispensed into bottles and vended in Sabon Gari market and motor park by children. No leftover was observed on the 2 days of the hazard study.

Producer C lived in fenced compound with the normal Islamic setting. There were two wives and eight children in the household. Water for kunun-zaki preparation was obtained directly from a well at the centre of the compound. Goats, sheep and chicken were seen roaming around the compound. Part of the finished product was sent in bulk in a large container to a canteen (cafeteria) located in the market at a suburb of Zaria City, serving a population of about 20,000 people. Some were vended in bulk by the children. Leftover kunun-zaki was consumed by the members of the family.

2.3. Traditional kunun-zaki preparation

The method of preparation involves the use of millet (*P. typhoides*) or Guinea corn (*S. bicolor*), but other grains could be used based on availability. The grain is steeped for 12–24 h, wet milled with spices which include ginger (*Zingiber officinale*), red pepper (*Capsicum anunm*), cloves (*Eugenia calophylloides*) and black pepper (*Piper nigrum*). About 1/4 quantity of the wet-milled mixture is removed and mixed with wet-milled sweet potato (*Ipomoea batatas*) for pitching. The remaining 3/4 quantity is gelled with hot water, allowed to cool and pitched with the 1/4 mixture. The final mixture is allowed to ferment overnight before sieving. The supernatant fluid is diluted with water to required consistency and sweetened with sugar to taste, while the remnants are sun-dried and used as animal feed. The procedure is subject to modifications by different producers as observed in this study (Fig. 1). The final product is reported to contain 9.5% dry matter, 90.5% moisture, 0.3% protein, 1.0% fat, 1.5% ash, 12.2% carbohydrate and a pH range of 4.7–5.0 (Sopade and Kassum, 1992).

2.4. Hazard analysis

Hazard analysis and critical control points were conducted on three products (PVC packed, bottled and bulk-packaged kunun-zaki) from three different producers around Zaria. Producers were observed as they carried out the kunun-zaki preparation.

The hazard analysis consisted of observing the raw materials used, the environment, kunun-zaki preparation and the packaging practices to identify sources and modes of actual or potential contamination. Samples were taken at different stages of preparation and subsequently tested for total aerobic plate count and for certain pathogens. The analysis also included measuring temperature immediately after gelling, during holding and when ready for serving (consumption). Time and temperature exposure/variation were also recorded. The thermometer was washed, cleaned with 70% alcohol and dried before inserting into the product. The pH of the samples was determined using pH meter (Model 29MK2 PYE UNICAM).

Based on the observations and discussions made during the preparation, a detailed schematic diagram of kunun-zaki processing was prepared. Potential sources of contamination from raw materials, equipment, utensils and persons preparing the kunun-zaki were noted. The likelihood of microbial survival or destruction and likelihood of microbial multiplication were noted on the diagram as described by Ehiri et al. (2001). Areas that needed to be monitored were noted (Fig. 1).

2.5. Sample collection

One hundred millilitres of the samples were collected after each step of production and the finished product was for sale in sterile wide-mouth bottles. Samples were

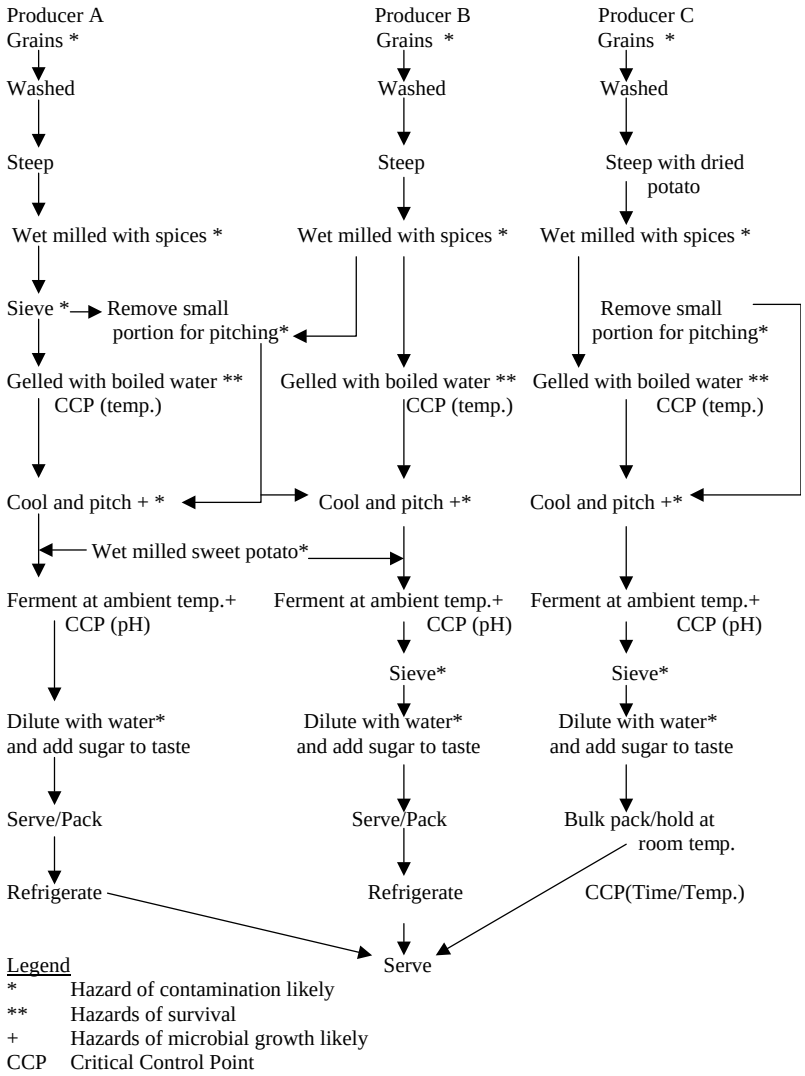

Fig. 1. Preparation of kunun-zaki by producers A–C. *, Hazard of contamination likely; **, hazards of survival; +, hazards of microbial growth likely; CCP, critical control point.

placed in sterile bottles using the commercial dispensing cup for sale. All samples collected were held in ice pack and taken to the laboratory within 2 h of collection for analysis. Two hundred and forty samples were also purchased from vendors for microbiological analysis.

2.6. Laboratory procedures

2.6.1. Enumeration of micro-organisms

Each PVC pack was cleaned externally with 70% ethanol to disinfect it. It was thoroughly shaken and punched using a sterile needle. An aliquot of 100 ml was evacuated into sterile universal bottles. Appropriate serial dilutions of all the samples were carried out and 0.2 ml of the selected dilution was spread on duplicate plates using sterile glass spreader. This technique was used for the enumeration of total aerobic viable count, coliform, bacillus and staphylococcal counts on nutrient agar (Difco), eosin methylene blue (EMB) agar (Oxoid), dextrose tryptone agar (Oxoid) and Baird Parker agar (Oxoid) supplemented with tellurite and egg yolk emulsion, respectively. All cultures were incubated at 37°C for 24 h except for coliform organism which was incubated at 37°C and 44°C for 24 h. Media used were prepared according to the manufacturers' instructions. Samples of water, raw materials and swabs of utensils used for processing were cultured for the presence of coliform organisms and *Bacillus* spp.

2.6.2. Characterization of isolates

Confirmation of coliform organisms were carried out by inoculating colonies into lactose broth with Durham tubes and incubating at 37°C and 44°C for 24 h and another 24 h in the absence of gas production (Speck, 1976). The presence of gas constituted a presumptive test and the broth was streaked out on EMB agar

incubated at 37°C for 42 h. Typical colonies on EMB plates appearing bluish black with greenish metallic sheen which are characteristics of *E. coli* or brownish colonies often convex and mucoid which are characteristics of *Enterobacter aerogenes* confirmed the presence of coliform organisms. Isolates were stored on nutrient agar slants at 4°C for further confirmatory tests which included IMVIC test, carbohydrate utilization, reaction on TSI, gelatin liquefaction, nitrate reduction, urease production and motility. Large, flat, irregular, wrinkled or smooth, ground-glass colonies, 4–6 mm in diameter were counted as *Bacillus*. Confirmation was as described by Yusuf et al. (1992). Confirmation of typical colonies of *S. aureus* on Baird–Parker agar was on the basis of the results of catalase, coagulase, phosphatase production, nitrate reduction and carbohydrate utilization (Umoh et al., 1999). For isolation and confirmation of *Salmonella* and *Shigella*, procedures recommended by Speck (1976) were followed. The preenriched samples in lactose broth were subcultured into selenite F broth for selective enrichment, and on *Salmonella–Shigella* agar (SSA). Typical colonies were Gram-stained and characterized (Speck, 1976).

2.6.3. Haemolysis of human and sheep red blood cells

Isolated *S. aureus* were inoculated on blood agar base (Oxoid) containing 10% sheep blood. The plates were incubated at 37°C for 24 h and a zone of haemolysis around colonies was observed and recorded as either alpha (α), beta (β), or gamma (γ) (Umoh et al., 1990).

2.6.4. Statistical analysis

One-way analysis of variance and least significance difference (LSD) were used to compare means of total aerobic, staphylococcal, coliform and bacillus counts for bulk, cellophane packaged and bottled kunun-zaki (Snedecor and Cochran, 1976).

3. Results

Flow charts showing hazards and CCP during preparation of kunun-zaki for the three different producers are presented in Fig. 1. The figure revealed that producer A sieved product before gelling, while B and C sieved products after gelling with hot water. Producer A and B added wet-milled sweet potato at the point of pitching, while producer C steeped dry potato along with grains and spices. All three producers held a small portion of the wet-milled mixture of grains and spices for pitching.

The time–temperature exposure of kunun-zaki for the different producers gave an appreciable drop within 1 h for producers B and C, having decreased from 74°C to 36°C for B and 70–47°C for C as compared to 72–64°C for A. However, the temperature of the three products finally decreased to about 30°C during fermentation.

Table 1 shows the bacterial count at different stages of kunun-zaki production. All the samples taken during preparation and when it was ready to serve had low count ranging from <1 to $4.36\log_{10}\text{cfu ml}^{-1}$ except mesophilic aerobic count which ranged from 3.63 to $6.54\log_{10}\text{cfu ml}^{-1}$ at point of consumption. Coliform and *S. aureus* appeared in all the samples after pitching and increased to $2.90\log_{10}\text{cfu ml}^{-1}$ at point of consumption, while bacillus count increased from 1.69 to $4.36\log_{10}\text{cfu ml}^{-1}$ (Table 1). Six of the 10 *S. aureus* isolates tested were of alpha haemolytic pattern and four produced beta haemolysis. Possible sources of contamination common to the three producers include the presence of animal in or near the house, contamination from handlers, water, water vessels, raw materials, utensils and environments. Neither *Salmonella* nor *Shigella* was isolated from products of the three producers at any stage of production.

Out of 240 samples of vended kunun-zaki purchased, 130 were PVC packaged, 90 were bottled and 20 were bulk packaged (Table 2). While the bulk-packaged samples had significantly ($P<0.05$) higher bacillus count, the PVC-packed and -bottled samples had higher coliform count. However, bottled samples had lower staphylococcal and total viable counts (Table 2).

4. Discussion

Heat treatment of food (e.g. cooking) not only improves the taste, smell, appearance and digestibility, it also reduces the number of microorganisms, improves keeping qualities by inhibiting moulds, yeast and bacteria that promote decay and infection. Thus, heat treatment is a practice aimed at improving the overall safety of food. This makes it a CCP.

Kunun-zaki prepared by the three producers attained a temperature of 74°C immediately after gelling and that temperature should be high enough to kill large numbers of vegetative cells, but not heat-resistant spores (Bryan, 1988). The total aerobic plate count of 3.63–$4.32\log_{10}\text{cfu ml}^{-1}$ recorded immediately after gelling from the three producers could be explained either by survival of spores which could have come initially from the grains, sweet potato, spices and sweetening agents or by reduction of, but not total elimination of, a very large number of vegetative cells that propagated during steeping. Studies in this environment also confirmed the presence of high levels of spores and vegetative cells in spices (Obuekwe and Ogbimi, 1989; Inabo et al., 2000). The practice of pitching with fresh portion of the wet-milled grains, spices and sweet potato, appears the likely point for the contamination with coliform, *Bacillus* and *Staphylococcus*. Gelling at 74°C,

Table 1
Bacterial counts, processing temperature and pH at various stages of kunun-zaki preparation for three producers

Procedure/period of sampling	Producer A mean			Producer B mean			Producer C mean		
	Count $\log_{10}$ cfu ml^{-1}	Temp °C	pH	Count $\log_{10}$ cfu ml^{-1}	Temp °C	pH	Count $\log_{10}$ cfu ml^{-1}	Temp °C	pH
After gelling									
TAPC	3.63	72	—	4.08	74	—	4.32	70	—
CC	<1			<1			1.32		
SC	<1			<1			<1		
BC	2.04			1.91			1.69		
After cooling and Pitching									
TAPC	4.62	45	—	4.79	38	—	5.54	44	—
CC	<1			1.18			2.91		
SC	1.00			1.86			<1		
BC	2.52			2.40			2.00		
Holding overnight									
TAPC	5.79	29	4.8	6.08	28	4.9	6.38	29	4.8
CC	1.90			2.00			2.23		
SC	1.98			2.11			1.90		
BC	3.00			4.34			3.60		
Ready to serve									
TAPC	5.81	29	4.8	6.30	28	4.8	6.54	29	4.6
CC	1.90			2.26			2.08		
SC	2.00			2.11			2.90		
BC	3.11			4.36			3.60		

Counts are means of duplicate samples: TAPC, total aerobic plate count; CC, coliform count; SC, staphylococcal count; BC, *Bacillus* count; — not determined.

Table 2
Mean and range of bacterial count of vended kunun-zaki sold in Zaria, Nigeria

Procedure	Samples/counts $\log_{10}$ cfu ml^{-1}		
	Bulk $n = 20$	PVC $n = 130$	Bottled $n = 90$
Total aerobic count			
Mean	6.40[a]	6.18[a]	5.99[b]
Range	4.18–8.79	4.53–6.62	4.65–6.51
Staphylococcal count			
Mean	2.86[a]	2.66[a]	1.83[b]
Range	1.90–3.40	1.78–3.00	1.60–2.30
Coliform count			
Mean	2.18[a]	3.56[b]	3.20[b]
Range	1.60–2.60	1.70–4.26	1.90–3.60
Bacillus count			
Mean	4.00[a]	3.72[b]	3.00[b]
Range	2.34–4.61	2.30–4.32	2.15–3.40

n = No. of samples tested.
Means in rows with the same superscript (a,b) are not significantly different.
LSD $\alpha = 0.05$.
PVC = polyvinylchloride.

fermentation overnight at ambient temperature and holding at ambient temperature for sale appear to be the major CCP of kunun-zaki. Increases in total aerobic plate count, coliform count, staphylococcal count and bacillus count were observed as fermentation progresses, indicating that these microrganisms participated in the fermentation of kunun-zaki (Table 1). During the interval of holding, spores that survived, gelling could germinate and injured vegetative cells could resuscitate. The high counts found at point of serving obtained for products from producers B and C could be associated with the processing method adopted since these producers sieved their products after gelling as opposed to producer A who sieved before gelling. Sieving before gelling could have reduced contamination by pathogenic bacteria that may reach the product during and after sieving.

The isolation of *E. coli* and *S. aureus* from kunun-zaki after gelling is attributed to post-processing contamination from producers, water used for dilution to obtain the required consistency, utensils and animals present in the environment. However, the low pH (4.6–4.9) recorded for the samples and the potential antimicrobial effect of some of the spices used, may have contributed to keeping the count of pathogens low, despite the ambient temperature of 28 ± 2°C and high water activity (a_w) which is optimum for growth of these pathogens. Similar effect of spices on pathogens was confirmed by Onuorah et al. (1987). The rapid drop in temperature observed in the product from producer B could be attributed to the fact that the product was left open after

gelling as opposed to producers A and C in which containers were covered immediately following gelling. However, the temperature of 38°C supported growth of pathogens in product B.

It is important to note that holding kunun-zaki at ambient temperature for sale could be risky. The Standard Organisation of Nigeria (SON) stated that coliform bacteria and pathogenic micro-organisms should not be present in beverages (SON, 1985). It is also reported that counts of 10^7 cells g^{-1} for *B. cereus*_(ICMSF, 1974), and 10^6 cells g^{-1} for enterotoxigenic *S. aureus* (Bergdoll, 1979) are required to present a risk of intoxication. The vended kunun-zaki had counts ranging from 10^1–10^3 cells g^{-1} for coliforms and *S. aureus* and 10^2–10^4 cells g^{-1} for *B. cereus*. Though fermentation could be used to improve the hygienic quality of food, inadequate application of the processes and faulty practices may negate its benefits (Ehiri et al., 2001). Such practices as adding wet-milled raw cereal as starter, sieving after gelling, diluting to the required consistency with water, and preparation in a contaminated environment may lead to post-processing contamination. Holding the product for sale at ambient temperature could encourage growth of these pathogens especially *Bacillus spp*. to hazardous levels. In the event of starter failure for a naturally fermented product like kunun-zaki and under appropriate temperature emetic and diarrhoeagenic toxin could be elaborated especially when the product is held at ambient temperature for sale (Bryan et al., 1981).

Therefore, the major hazards associated with kunun-zaki preparation are, the presence of spores of pathogenic strains which could germinate at ambient temperature after a heat shock from gelling. The 10^4 cells for *B. cereus* appear safe, but inadequate drop in pH and holding at ambient temperature for sale may encourage growth to hazardous levels. The presence of coliform and *S. aureus* and preparation in a contaminated environment could present a risk. More so, the *S. aureus* isolates were alpha haemolytic and likely to be human biotypes and more enterotoxigenic than animal biotypes which are often beta haemolytic (Bergdoll, 1979). A study of complimentary food preparation and handling in Eastern Nigeria also confirmed the presence of enteric pathogens and spores of pathogens (Ehiri et al., 2001).

In conclusion, the HACCP study has revealed that many factors contributed to the contamination of kunun-zaki including dirty environment and poor quality of water. The CCP for kunun-zaki are gelling temperature, pH after fermentation and holding product at ambient temperature for sale. There is need to educate the producers on the hazards and CCPs of kunun-zaki preparation. Such control measures and monitoring procedures as washing hands at intervals with soap during kunun-zaki preparation, checking indicator of heat treatment by use of colour change, washing raw ingredients before use, washing equipment and utensils thoroughly with soap before and after use, sieving before gelling, using boiled and cooled water to dilute product to the required consistency and preparing kunun-zaki in qualities that could be sold off the same day especially where there is no means of refrigeration are necessary for preparation of a safe product.

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