



Microbial Evaluation and Proximate Composition of Kunu zaki, an Indigenous Fermented Food Drink Consumed Predominantly in Northern Nigeria

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Abstract

Microbiological and Proximate analyses of kunun zaki samples from Maiduguri metropolis were analyzed for the presence of some microorganisms frequently implicated in foodborne disease outbreaks and spoilages including *E. coli*, *S. aureus*, *Shigella* and *Salmonella sp.* The samples had high microbial content in varying percentages. Out of the 25 samples analyzed from each market, *S. aureus* showed the highest % (68 – 100%) followed by *E. coli*. (44-72%); *Salmonella* (28-40%) and *Shigella* (28-44%) had lower % of occurrence. The mean count of *S. aureus* was higher than counts of other organisms with mean values between $19.8 \times 10^2 - 36.52 \times 10^2$ CFUml⁻¹ followed by the mean *E. coli*. *Salmonella* and *Shigella* were low in most of the markets. The overall mean of Total Viable Counts (TVC) was 56.536×10^2 CFUml⁻¹. There was statistically significant ($p \leq 0.05$) difference between the means of the microbial groups at 95% confidence interval. The pH values ranged from 3.80 - 4.08 with samples from Monday market having the highest and Mairi market the lowest pH. The overall pH value was acidic. The proximate composition of the kunun-zaki from the different locations ranged from 89.8-85.4% moisture content, 4.9- 2.8% protein, 14.0-6.5% fat, 15.0-5.0% fibre, 14.7-12.0% dry matter and 2.0-1.0% total ash content. Recommendations were made on improvement of the product process-line and hygiene during sales and consumption.

Keywords: kunu-zaki, microbial count, proximate composition, Maiduguri.

Introduction

'Kunun-zaki' (Hausa) is a traditional fermented non-alcoholic beverage consumed by large population of people in northern Nigeria. Preparation protocol varies amongst people and can generally be produced from either the following substrates; millet (*Pennisetum typhoideum*), maize (*Zea mays*) or sorghum (*Sorghum bicolor*), but millet is the most common substrates (Akoma *et al.* 2006). Spices such as ginger, black pepper, red pepper, cloves and sugar are commonly added as flavor and taste improver (Ahmed *et al.* 2003).

It plays a vital role in the dietary pattern of the people in developing countries like Nigeria (Adeyemi and Umar 1994), and has been shown to contain carbohydrate, vitamin and minerals but low in protein (Ayo and Okaka 1998).

Sorghum made kunun-zaki contains starch, protein, fat, fibre and ash along with a wide array of amino acids. It is believed to be of immense social, economic, nutritional and medicinal importance to consumers (Efiuvwevwere and Akoma 1995).

The traditional production of kunu is still of village technology level and protocol not standardized. Generally, the process of manufacture involves wet milling of cereal grains with spices, wet sieving, and partial gelatinization of the slurry, sugar addition, bottling and sales. The level of ingredients such as spices and sweetener are not quantified (Adejuyitan *et al.* 2008). Tastes and cultural habits make for the variation in the method of preparation and partly explain the lack of consistency in product quality (Adeyemi and Umar 1994).

Kunu-zaki is liable to microbial spoilage if not adequately stored and could act as important medium for the transmission of pathogenic microorganisms. Many organisms can use the carbohydrate content for

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fermentation processes producing undesirable changes in them. The sugar used as sweetening agent could also contribute to these changes. Microbiological safety of foods is not to be overlooked considering the health risks. Our present study was designed to investigate the microbiological and proximate composition of kunu-zaki consumed in Maiduguri which to our knowledge had limited or no reports on its health safety evaluation.

Materials and Methods

A study on microbial evaluation and proximate composition of kunu zaki, an indigenous fermented food drink consumed predominantly in northern Nigeria was carried out in the Department of Microbiology laboratory of the University of Maiduguri, Nigeria.

The samples

Kunun-Zaki samples were collected from Mairi (MA), Tashun Bama (TB), Post Office (PO) and Monday Market (MM), all within Maiduguri Metropolis in sterile corked plastic tubes packed in iced container

Microbiological analyses

Kunu samples approximately 40ml each contained in pre-autoclaved containers were used for the isolation and enumeration of the microorganisms. In each isolation protocol, the sample was shaken and 10ml aseptically introduced into 90ml of sterile normal saline and was homogenized by shaking followed by further decimal dilutions to up to 10^{-6} concentrations. A 0.1ml quantity of appropriately diluted sample was used to inoculate freshly prepared media by spread-plating method. Media employed for the isolation and enumeration of the organisms included: Baird Parker Medium (BPM) (Lab M. Ltd, Bury Lancashire, United Kingdom) for *S. aureus*; Eosine Methylene Blue Agar (EMBA) (Himedia Laboratories Pvt Ltd, India) for *E. coli*; and Desoxycholate Citrate Agar (DCA) (Park Scientific Limited, Moulton Park, Northampton) for *Salmonella* and *Shigella spp.* Nutrient Agar (NA) (Biotech Lab. Ipswich, UK) was used for total viable count. Media were sterilized by autoclaving at 121°C for 15min except DCA which involved only boiling over gauze. In all cases of colony counts, the resulting colonies following inoculation and incubation were counted using digital colony counter (Labtech, New Delhi, India).

Isolation and enumeration of *E. coli*

EMBA plates were inoculated as described previously and incubated at 37°C for 48h after which typical colonies with greenish metallic sheen were subjected to biochemical tests for *E. coli*.

Isolation and enumeration of *S. aureus*

BPM were inoculated as above and incubated at 37°C for 48h. Greyish-black or black colonies with or without a halo were presumptively identified as Staphylococci as recommended by Macfaddin (1977) and coagulase test was carried out to further characterize *S. aureus*.

Isolation and enumeration of *Salmonella* and *Shigella sp*

One millilitre quantity of each of the kunu-zaki sample was inoculated into 9ml of pre-enrichment broths tetrathionate and selenite cysteine and incubated at 37°C as recommended by Macfaddin (1977). DCA plates were inoculated with 0.1ml of the pre-enrichment broths' 24h growth and incubated at 37°C overnight. Typical colonies with black centres were identified as *Salmonella spp* on DCA according to Macfaddin (1977). Pinkish colonies were identified presumptively as *Shigella* on DCA and subjected to further biochemical testing.

Total Viable Count

Nutrient Agar (NA) (Biotech Lab. Ipswich, UK) was inoculated with a 0.1ml of appropriately diluted kunu-zaki by spread-plating technique and incubated at 37°C for 24 h. Colonies were counted and multiplied by the dilution factor.

Biochemical identification of the isolates

The biochemical tests for the identification of the isolates were the citrate utilization, indole, methyl-red, Voges-proskauer, triple sugar iron (TSI), urease, oxidase, coagulase and catalase tests. Cowan and Steel (1965) and Cheesbrough (2004) s' procedures were used for these biochemical tests. The indole production test involved the inoculation of sterile peptone water with the test organism followed by incubation at 37°C for 48h. Kovacs' reagent (0.5ml) was thereafter added and the culture shaken for 1min. A red colour in the reagent layer indicated the presence of indole. Methyl red and Voges-Proskauer were also demonstrated according to Cowan and Steel (1965) with red colouration for positive methyl red and yellow colour for negative. A positive reaction for Voges-Proskauer was indicated by a strong red colour. The citrate utilization test involved heavy inoculation of tryptose-citrate medium from a 10-12h culture. The inoculated tubes were placed in 40°C water bath. After 90 minutes, 1 drop of 0.05% aq. bromothymol blue was added to each tube. A positive test was shown by blue colouration, whereas a green colour showed a negative result. The catalase test was conducted by immersing the cultured isolate on a clean oil-free glass slide, followed by the addition of few drops of hydrogen peroxide solution. An immediate effervescence showed positive test, whereas negative test was shown by no visible reaction. A yellow butt with a pinkish slant region and a trace of black precipitate indicating H₂S production was positive for TSI test. Colours for positive oxidase and urease were dark purple on a paper within 10s and reddish colouration respectively.

Chemical analyses

The pH of various samples of kunun-zaki was determined using a pH meter (WPA pH Meter, India) after standardization with pH 4, 10 and 7 buffers (BDH England) according to Akoma *et al.* (2006) procedure. PH measurements were taken 24 hourly starting from the time of sample collection. Crude protein of the samples was determined with macro-Kjeldahl procedure (AOAC 1990). Crude fat of 10ml of samples determined with soxhlet

procedure and total ash obtained by igniting 10ml sample at 600°C using muffle furnace (Laboratory electric furnace, typOH-857R, England) were determined according to the protocol of Pearson 1976. Crude fibre was determined according to the procedure of (AOAC 1990). Moisture content was determined by weighing and drying to constant weight.

Statistical analysis

Data were analyzed by Multiple-Sample Comparison using STATGRAPHICS Centurion XVI Version 16.1.05 (32-bit). When the F-test in the ANOVA was significantly ($p \leq 0.05$) different between the means, Multiple Range Tests were conducted to tell which means were significantly different from others.

Results

The mean count of different organisms from the markets is presented in Table 1. *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp* and *Shigella spp* were isolated in varying degrees. The mean count of *S. aureus* was higher than counts of other organisms with mean values between $19.8 \times 10^2 - 36.52 \times 10^2$ CFUml⁻¹. The mean *E. coli* count was second to *S. aureus* and the mean counts of *Salmonella* and *Shigella* were low in most of the markets. The overall mean of Total Viable Counts (TVC) was 56.536×10^2 CFUml⁻¹. There was statistically significant ($p \leq 0.05$) difference between the means of the microbial groups at 95% confidence interval as indicated with the asterisks. There is statistically significant difference ($P < 0.05$)

between *S. aureus* and the other bacteria; and organisms in columns 1, 3 and 4 were however statistically homogeneous in distribution.

The range of microbial counts of organisms isolated from the different markets is presented in Table 2. The overall highest range of the organisms was 14000 CFUml⁻¹ achieved in TVC. Most of the samples showed no detectable growth and were recognized as 0 count or minimum. With respect to the individual organisms, *E. coli* from the Post Office market had the highest range (9000 CFUml⁻¹) and *Shigella spp* had relatively low range with value not exceeding 6000 CFUml⁻¹.

Out of the 25 samples analysed from each market, *S. aureus* showed the highest % (68 – 100%) followed by *E. coli*. *Salmonella* and *Shigella* had lower % of occurrence (Table 3). The % was however calculated as number of sample that assayed positive for the respective organism.

The pH values ranged from 3.80 - 4.08 with samples from Monday market having the highest and Mairi market the lowest pH (Table 4). The overall pH value was acidic. The samples had high moisture content of between 87.10 – 89.82%, low protein and ash content of values between 2.80 - 4.90% and 1.0 – 2.0% respectively. There was marked variation in the crude fibre content from the different markets with a range of 3.56 – 15% (Table 4).

Values are mean of triplicate samples. There is statistically significant difference ($P < 0.05$) between the moisture content and protein, fat, crude fibre and ash contents of the samples. Values in the same columns are however statistically homogeneous in distribution.

Table 1. Mean counts ($\times 10^2$ CFUml⁻¹) of different microorganisms isolated from kunu-zaki from different markets in Maiduguri.

Market	<i>E. coli</i>	<i>S. aureus</i>	<i>Salmonella spp.</i>	<i>Shigella spp.</i>	TVC
Mairi	5.560	*36.520	3.280	2.200	*60.360
Tashan Bama	10.000	*19.800	6.360	5.732	*50.22 4
Post Office	9.080	*25.780	6.940	3.492	*56.160
Monday Market	11.720	*21.980	8.800	8.600	*59.400
Overall means	9.090	*26.020	6.345	5.006	*56.536

TVC = Total Viable Count. Figures indicated with the asterisks means that there is statistically significant difference ($P < 0.05$) between then and the non- asterisked column.

Table 2. Minimum and maximum values of count of microorganism isolated from different markets in Maiduguri (counts were expressed as CFUml⁻¹)

Market	<i>E. coli</i>	<i>S. aureus</i>	<i>Salmonella spp.</i>	<i>Shigella spp.</i>	TVC
Mairi	0.0-4100.0 (4100.0)	500.0-7000.0 (6500.0)	0.0-24000 (24000.0)	0.0-2000.0 (2000.0)	1500.0-13200.0 (11700.0)
Tashan Bama	0.0-8000.0 (8000.0)	0.0-7000.0 (7000.0)	0.0-5200.0 (5200.0)	0.0-3000.0 (3000.0)	10.0-11000.0 (10990.0)
Post Office	0.0-9000.0 (9000.0)	0.0-7500.0 (7500.0)	0.0-4800.0 (4800.0)	0.0-3000.0 (3000.0)	600.0-14000.0 (13400.0)
Monday Market	0.0-8000.0 (8000.0)	0.0-8800.0 (8800.0)	0.0-7000.0 (7000.0)	0.0-6000.0 (6000.0)	1200.0-13000.0 (11800.0)

Figures in parenthesis are range of counts.

Table 3. Occurrence of different microorganisms isolated from kunu-zaki samples

Market	<i>E. coli</i>	<i>S. aureus</i>	<i>Salmonella spp.</i>	<i>Shigella spp.</i>
Mairi	14 (56)	25 (100)	9 (36)	7 (28)
Tashan Bama	18 (72)	24 (96)	7 (28)	10 (40)
Post Office	11 (44)	23 (92)	10 (40)	11 (44)
Monday Market	18 (72)	17 (68)	10 (40)	8 (32)

Figures in parenthesis are % occurrence. In each market, 25 kunu-zaki samples were analyzed (n = 25 in each market).

Table 4: PH and Proximate composition of kunun-zaki samples collected from four different locations in Maiduguri.

SAMPLE	pH	%Moisture content	% Protein	% Fat	% Crude Fibre	% Ash
Mairi	3.80	87.90	3.76	14.00	3.56	2.00
Tashan Bama	4.08	88.35	4.90	11.5	5.00	1.00
Post Office	3.99	87.10	2.88	8.50	12.00	1.00
Monday Market	4.02	89.82	2.80	6.50	15.00	1.00

Discussion

High populations of microorganisms are present in the different kunu-zaki samples collected from Maiduguri metropolis to attract food safety attention (Tables 1 - 3). *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp* and *Shigella spp* were isolated in varying degrees. These organisms are known to be important as foodborne disease agents.

Staphylococcus aureus had the highest percentage of occurrence, highest mean and range second to *E. coli*. The range of *S. aureus* of 0 - 8.8 x 10³ CFUml⁻¹ fell within 10² – 10⁴ cells/g reported by Oranusi *et al.* (2003). Counts of 10⁶ cells/g of enterotoxigenic *S. aureus* (Bergdoll 1979) are however required to produce intoxication risk. *S. aureus* are found on the skin as normal flora of humans. Therefore in situations where there is relax in hygiene, their high load on products handled by man is not surprising. Jablonski and Bohach (1997) noted that dissemination of *S. aureus* from humans to food can occur by direct contact, indirectly by skin fragments, or through respiratory tract droplet nuclei.

Escherichia coli had between 44 - 72% (Table 3) of occurrence in the samples analyzed. *E. coli* range of 0 – 9.0x10³ CFUml⁻¹ was obtained from our study as opposed to on detectable growth of any coliform by Adejuyitan *et al.* (2008) and is similar to coliform range of 10¹ – 10³ cells/g reported by Oranusi *et al.* (2003). Presence of *E. coli* is not surprising since other authors had reported post processing contamination from producers, water, utensils and animal in the environment (Oranusi *et al.* 2003). Presence of these organisms depicts a problem in hygiene as they are indices of faecal contamination.

Compared to *Escherichia coli* and *Staphylococcus aureus*, *Salmonella* and *Shigella spp* were isolated in low numbers. *Salmonella spp* had between 28 - 40% occurrence and *Shigella spp* 28 - 44%. *Shigella spp* are needed in small dose to be able to present pathologic disorders. Both *Shigella* and *Salmonella* were not detectable in the product

by other authors or were not assayed for. *Shigella* and *Salmonella* could be product of contamination from the process line or during marketing since the two pathogens are not normal inhabitants of any of the materials used in its preparation.

The highest total viable counts (TVC) of 6.036 x 10³ cfuml⁻¹ was found in sample from Mairi, followed by Monday Market with count of 5.944 x 10³ CFUml⁻¹. Tashan Bama market had the lowest count of 5.02224 x 10³ CFUml⁻¹. TVC obtained from our study was lower 7.33 x 10⁶ - 81.67 x 10⁸ obtained by Amusa and Ashaye (2009), but however fell within the range of 1.0 x 10⁴ – 1.6 x 10⁴ obtained by Adejuyitan *et al.* (2008).

Therefore, unclean water used in the preparation of the drink could lead to contamination, also the use of improperly washed hands after going to the toilet in the preparation of the drink could also be a source of contamination.

The pH range of the kunu-zaki samples was between 3.95 and 4.08. The values were lower than 4.70-5.75 obtained by Akoma *et al.* (2006); 4.12-5.10 by Adejuyitan *et al.* (2008) and 5.25-5.65 obtained by Amusa and Ashaye (2009). The acidity of “kunun-zaki” beverage has been noted to be a result of lactic acid production by some bacteria during fermentation (Ashiru *et al.* 2003) and thus, the drink becomes sour to taste and organoleptically unacceptable with time. The result obtained for the moisture content of kunu-zaki fell within the range of 87.10- 89.82% which was higher than 85.42-86.62% reported by Adejuyitan *et al.* (2008) and 85.30-86.38 obtained by Amusa and Ashaye (2009). Moisture content contributes significantly to the microbial flora of food samples (Prescott *et al.* 2002). The % protein content of the samples between 2.80-4.90 agreed with 4.20 of wet-milled kunu of Adejuyitan *et al.* (2008) and 3.63 of kunu-zaki from unsieved kunu flour Amusa and Ashaye (2009) but disagreed significantly (P < 0.05) with 0.54% obtained by Akoma *et al.* (2006). The % crude fat between 6.50 and 14.00 disagreed significantly

with 0.45% obtained by Akoma *et al.* (2006) and 3.55-3.64 by Amusa and Ashaye (2009). There were also variations within the markets. The % crude fibre reported by Adejuyitan *et al.*, (2008) (0.25%) was significantly ($P < 0.05$) lower than our result (Table 4). The ash content of 1-2% agreed with that reported by Amusa and Ashaye (2009) and disagreed slightly with 2.1-3.30% reported by Adejuyitan *et al.* (2008). Where there is disparity between the proximate analyses results from the different markets (Table 4) and from other authors, the reason could be from the general non-standardized preparation operations of the beverage.

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