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Production of Sorghum Based *Kunun Zaki* Using Selected Starter Cultures

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Abstract: In view of the need for better hygiene in *kununn-zaki* production, this study investigated the use of selected starter cultures for production of *kununn-zaki* using sorghum grains. *Lactobacillus fermentum* (L1), *Lactobacillus plantarum* (L2), and *Lactobacillus acidophilus* (L3) were isolated from naturally fermented *kununn-zaki* and then used in four different combinations as starter cultures for the production of *kununn-zaki*. The starter cultures which included L1 + L2, L1 + L3, L2 + L3, and L1 + L2 + L3 were used to produce four different *kununn-zaki* (K1, K2, K3, and K4) respectively. A control (*kununn-zaki* produced by spontaneous fermentation) was also prepared. Titratable acidity (TA) and pH were determined every 2 hours during a 6-hour fermentation period. Crude protein contents of the *kununn-zaki* products were determined. The organoleptic qualities of the produced *kununn-zaki* were judged by a 7-man panel based on appearance, aroma, taste, and overall acceptability using a 5-point hedonic scale. As fermentation proceeded, there were decreases in pH values, with accompanying increases in TA for all the *kununn-zaki* samples. K1 and K3 had the highest score (4.57± 0.53) in overall acceptability, and thus the most preferred among the *kununn-zaki* products. K3 was found to have the highest crude protein content (1.45%) followed by K1 (1.38%.). It is concluded that carefully selected starter cultures could be used to produce *kununn-zaki* with improved protein content and acceptable organoleptic qualities under more hygienic conditions.

Keywords: Fermentation, Lactic Acid Bacteria, *Kununn-Zaki*, Cereal Beverage, Sensory Quality

Introduction

Kununn-zaki is a refreshing non alcoholic cereal based beverage widely consumed in Northern Nigeria. Production of *kununn-zaki* remains a home-based industry. Currently, there is no large-scale factory production of the beverage. Equipment used in traditional production of *kununn-zaki* are rudimentary. The fermentation process is dependent on chance inoculation. Consequently, *kunun* products tend to vary in terms of quality attributes. Also, sanitary conditions during *kunun* production are poor giving rise to products of short shelf-lives (Elmahmood and Doughari, 2007).

Starter cultures have been exploited as a means of overcoming the challenges of traditional production of fermented foods. A lot of research is being done to isolate and characterize microorganisms associated with production of indigenous fermented foods with a view to exploiting their industrial potentials. Use of starters in food fermentations has been reported to improve the fermentation process by bringing about reduction in fermentation time (Halm *et al*., 1996; Hounhouigan *et al*., 1999; Mugula *et al*., 2003), enhanced predictability of the process, improved safety and reduced hygienic risks (Kimaryo *et al*., 2000). Starters also contribute to desirable organoleptic attributes (Annan *et al*., 2003).

Lactic acid bacteria have been shown to be important starter organisms in bacterial food fermentations (Anderson, 1988). Agarry *et al*. (2010) citing Efiuvwevwere and Akoma stated that *Lactobacillus fermentum* and *Lactobacillus leichmannii* were the dominant microorganisms at the end of *kunun-zaki* fermentation. The same authors found *Lactobacillius plantarum*, *Lactobacillus fermentum* and *Lactococcus lactis* to be the dominant lactic acid bacteria in *kununn-zaki* production. The also reported that production of *kununn-zaki* from combinations of malted millet and rice grains using a combination of *Lactobacillius plantarum*, *L. fermentum* and *Lactococcus lactis* as starter culture led to a *kununn-zaki* products with improved sensory and nutritional qualities.

Though a number of researches has been carried out on the use of starter cultures for improving the

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production of *kununn-zaki* using various cereal grains, research on the use of starter cultures for production of sorghum based *kununn-zaki* needs to be further explored. The aim of this study was to investigate the possibility of using selected indigenous starter cultures to produce sorghum based *kununn-zaki* with acceptable nutritional and sensory qualities under hygienic conditions.

Materials And Methods

Isolation of lactic acid bacteria

Kununn-zaki was prepared using the traditional method described by Gaffa and Ayo (2002). Ten fold serial dilutions of the *kununn-zaki* was carried out using sterile normal saline up to the 10^{-6} dilution. A volume of 0.1 ml of appropriate dilution was spread plated on De Man, Rogosa and Sharpe (MRS) agar. The plates were incubated in an anaerobic jar at 30℃ for 24 -48 hours. After incubation discrete colonies with typical lactic acid bacteria morphological characteristics were randomly picked and sub-cultured on MRS agar by streaking. Three successive sub-cultures were carried out to obtain presumably pure cultures.

Identification of lactic acid bacteria (LAB) isolates

Identification of LAB was done based on morphological, physiological, and biochemical characteristics (Kimaryo *et al*., 2000). Bacterial isolates that were Gram-positive, cocci or rod shaped, non-motile, and catalase-negative were considered to be lactic acid bacteria. The isolates were further tested for growth at 15°C only, 45°C only, and at both 15°C and 45°C, acid and gas production from 1% glucose in MRS broth without beef extract, production of ammonia from arginine and for their sugar fermentation abilities. Sugars used in the sugar fermentation test included arabinose, cellobiose, lactose, melezitose, melebiose, mannitol, mannose, raffinose, rhamnose, ribose, salicin, sorbitol, trehalose and xylose in MRS broth devoid of glucose and beef extract with phenol red as indicator. Identification up to the species level was carried out using an identification scheme (Nair and Surendran, 2005; Mithun *et al*., 2015) developed based on biochemical tests given in Bergeys Manual of Determinative Bacteriology.

Selection of lactic acid bacteria with potentials for use as starter culture

Five hundred grams of cereal starch paste in a previously sterilized plastic bowl was gelatinized by addition of 5000 ml of boiling water. The gelatinized cereal starch was then hydrolysed immediately by adding 200g of ground malted sorghum grains and stirred until the colour of the mixture changed from beige to light brown. The hydrolyzed cereal starch (HSS broth) was sterilized by autoclaving at 121°C for 15 minutes at 1kg/cm and then cooled to room temperature. A dense suspension of each lactic acid bacterial isolate was prepared in 1 ml of sterile saline. The turbidity was adjusted to be equivalent to that of No. 2 McFarland turbidity standard. After that, 1 ml of each standardized lactic acid bacterial isolate was used to separately inoculate 10ml volumes of HSS broth which were then incubated at room temperature for 24 hours for fermentation to take place. The potential of an organism for use as starter culture was established, using ability to produce good (buttery) aroma as quality index (Agarry *et al*., 2010). Based on good aroma, three most promising lactic acid bacteria were pre-selected as organisms to be used in preparation of starter culture.

Preparation of starter cultures used as inocula

Four different starter cultures were prepared by making combinations of the three selected lactic acid bacteria (*Lactobacillus plantarum, Lactobacillus fermentum*, and *Lactobacillus acidophilus*). Each selected lactic acid bacterium had previously been standardized using a No.2 Mcfarland standard. The starter cultures were prepared in 3 ml volumes as follows: 1. *Lactobacillus plantarum* (1.5 ml) + *Lactobacillus fermentum* (1.5 ml) 2. *Lactobacillus plantarum* (1.5 ml) + *Lactobacillus acidophilus* (1.5 ml) 3. *Lactobacillus fermentum* (1.5 ml) + *Lactobacillus acidophilus* (1.5 ml) 4. *Lactobacillus plantarum* (1 ml) + *Lactobacillus fermentum* (1 ml) + *Lactobacillus acidophilus* (1 ml). The starter cultures were coded S1, S2, S3, and S4 respectively.

Development of starter culture

The starter cultures were developed by separate inoculations of 1 ml volumes of the starter cultures (S1, S2, S3 and S4) into 50 ml volumes of hydrolyzed sorghum starch (HSS) broth and incubation at room temperature (26±2°C) for 12 hours. The different 12-hour fermented 50ml volumes of HSS broth were then transferred into separate 200 ml volumes of HSS broth and incubated at room temperature for 12 hours.

Production of *Kununn-zaki* using developed starter cultures (controlled fermentation)

Preparation of ground malted grain paste

Sorghum grains (500g) were washed with and steeped in 1000 ml of tap water (1:2 w/v) in a plastic container for 12 hours at room temperature (26±2°C). The steeped grains were drained in a wicker basket. The wet grains were then covered in the wicker basket with a moist cloth and left to germinate in a cupboard over a period of 4 days. The germinated grains were dried under the sun for 3days. The dried grains were surface sterilized by steeping in 1% sodium metabisulphite solution for 5 minutes after

which the sodium metabisulphite was washed off with cooled boiled water. The surface-sterilized grains were ground to paste using a domestic blender previously sterilized with 1 % sodium metabisulphite solution and rinsed with cooled boiled water.

Pre-fermentation processing of sorghum grains

One kilogram of sorghum grains (*Sorghum bicolor*) was washed in 1% sodium metabisulphite. The grains were steeped in 2000ml of tap water (1:2 w/v) in a plastic container for 24hours at ambient temperature. After 24 hours the steeped grains were rewashed with clean tap water, drained and steeped together with dry sweet potato (*Ipomoea batatas*; 150g), and spices - ginger (*Zingiber officinale;* 30g), clove (*Eugenia caryophyllata*; 10g) and black pepper (*Piper* sp.; 10g) - in 2000 ml of 1 % sodium metabisulphate solution for 5 minutes. After this the grains and spices were rinsed with cooled boiled water and then ground to paste using a electric blender. The blender was previously sterilized with 5% sodium metabisulphite for 5 minutes and rinsed with sterile cool boiled water.

Hydrolysis of sorghum starch

Two kilograms of unmalted sorghum grains paste was divided into two unequal portions (1:3 v/v). The larger portion was gelatinised by adding boiling water (1:1 v/v) in a plastic container, it was stirred vigorously for about 3 minutes at which point the beige colour of the paste turned to light brown which is the colour of *kunun.* A 200 g weight of malted sorghum grain paste was added and the mixture was stirred vigorously for 4 minutes to aid liquefaction and saccharificaton of the gelatinized starch. The hydrolyzed cereal starch was then allowed to cool to about 45^{o}C.

Starter Culture fermentation of hydrolyzed sorghum starch

One hundred gram weights of uncooked sorghum paste (The sorghum grains used to make the paste had previously been surface-sterilized) were inoculated with 150ml of the different developed starter cultures. The inoculum-uncooked paste mixtures were separately added to 200g weights of hydrolyzed sorghum starch. The mixtures were thoroughly and aseptically mixed for one minute and then incubated for 6 hours at room temperature (26$\pm$2°C). After the incubation, the different fermented products were sieved with muslin cloth previously sterilized with 5% sodium metabisulphite for 5 minutes. Four starter culture based *kununn-zaki* were produced: 1. *kununn-zaki* produced using *Lactobacillus plantarum* + *Lactobacillus fermentum* (k1) 2. *kununn-zaki* produced using *Lactobacillus plantarum* + *Lactobacillus acidophilus* (k2) 3. *kununn-zaki* produced using *Lactobacillus fermentum* + *Lactobacillus acidophilus* (k3) 4. *kununn-zaki* produced using *Lactobacillus plantarum* + *Lactobacillus fermentum* + *Lactobacillus acidophilus* (k4)

Production of *Kununn-zaki* using the traditional method (Natural fermentation)

Kununn-zaki was produced using a slight modification of the method described by Gaffa and Ayo (2002). The slight adjustment was in the reduction of fermentation time to six hours. Fermentation of hydrolysed sorghum starch was based on chance inoculation. The naturally fermented *kununn-zaki* was coded NFK.

pH and titratable acidity determinations

The pH of all the prepared *kununn zaki* were determined in triplicates at the end of the 6-hour fermentation using a pH meter (Horiba, M-8) that had been standardized with pH 4 and pH 7 buffers. Titratable acidity was also determined in triplicates for all the *kununn zaki* at the end of the production. For the determination of titratable acidity, 10ml of sample was measured into a conical flask and four drops of phenolphthalein indicator was added. This was titrated with standard 0.1N sodium hydroxide to distinct faint pink point. The titrable acidity, expressed as % lactic acid, was calculated for each sample as described by Amoa-Awua *et al*. (2006).

Proximate and mineral analysis

Moisture, crude protein, and ash contents of the three *kununn-zaki* products (K1, K3, and NFK) which were eventually adjudged to be the best were determined in triplicates using the method of AOAC (1990). The calcium, iron, magnesium, potassium, and phosphorus contents of the three *kununn-zaki* products were determined using the method described by AOAC (2006).

Sensory analysis

Sensory analysis was conducted on the different *kununn zaki* produced in this study. The sensory analysis was based on appearance, taste, aroma, and overall acceptability. This was done by A total of seven panelists among which were University of Jos students. All the panelists were quite familiar with the product. The samples were coded and served in transparent plastic cups. Water was provided for mouth rinsing before and after assessment of each *kununn-zaki* sample. The samples were assessed on a five point hedonic scale where 1 = dislike very much, 2 = dislike slightly, 3 = indifferent, 4 = like slightly, and 5 = like very much, a slight modification of the scale described by Larmond (1977).

Statistical analysis
Results from pH, titratable acidity, proximate, mineral and sensory analyses of the different *kununn-zaki* produced were subjected to manual analysis of variance (ANOVA). Means with significant differences ($P < .05$) were separated using least significant difference (LSD).

Results
Lactic acid bacterial isolates and their potentials for use in *kununn-zaki* production
A total of 7 lactic acid bacteria comprising six lactobacillus species and one cocci were isolated from the fermenting *kununn-zaki*. The lactic acid bacterial isolates included *Lactobacillus fermentum, Lactobacillus acidophilus, Lactobacillus plantarum,* three other Lactobacilli and one unidentified cocci. Characteristic features of the identified lactic acid bacterial isolates are presented in Table 1. When the isolates were inoculated in hydrolysed sorghum starch (HSS) to test for their potential for use in *kununn-zaki* production, *Lactobacillus fermentum, Lactobacillus acidophilus,* and *Lactobacillus plantarum* were found to have higher potentials. They produced the best aroma while fermenting HSS.

Changes in pH and titratable acidity of *kununn-zaki* during production
The pH of all the *kununn zaki* were found to have dropped by the end of the fermentation while the titratable acidity increased. Final pH of the *kununn-zaki* products ranged from 3.30-3.77 while titratable acidity (% lactic acid) ranged from 0.595 – 0.680. K3 had the lowest pH and highest titratable acidity values while K4 had the highest pH and lowest titratable acidity values. Differences in the final pH values of the *kunun* products were significant ($P< .05$) while the differences in titratable acidity were not. Details of changes in the pH and titratable acidity of the various *kununn zaki* produced are shown in Table 2.

Proximate and mineral compositions
The percentage crude protein, ash and moisture contents of the analysed *kununn-zaki* products (K1 [*kununn-zaki* produced with *L. fermentum* + *L. plantarum*], K3 [*kununn-zaki* produced with *L. fermentum* + *L. acidophilus*], and NFK [naturally fermented *kununn-zaki*]) ranged from (1.36 – 1.45), (3.84 – 3.90), and (91.95 - 92.05) respectively. K3 had the highest crude protein content of 1.45%. The differences in the crude protein contents of the three products were not significant ($P > .05$). The iron, calcium, magnesium, potassium, and phosphorus contents (ppm) of K1, K3, and NFK ranged from (0.39-0.48), (0.24-0.28), (0.38-0.45), (0.11-0.15) and (0.13-0.19) respectively. K3 had the highest contents of calcium, magnesium, and phosphorus. There were significant differences ($P < .05$) in the magnesium and phosphorus contents of the products. Details of the proximate and mineral contents of the analysed *kununn-zaki* are shown in Table 3.

Sensory quality of *kununn-zaki* products
The sensory quality attributes of the evaluated *kununn-zaki* products were in the following ranges: appearance (4.14-4.43), taste (4.00-4.71), aroma (4.14–4.42), and overall acceptability (4.14-4.57). K3 and K1 had the highest scores in terms of overall acceptability. Details of the sensory quality attributes of the products are shown in Table 4.

Discussion
The study investigated the possibility of using selected indigenous starter cultures to produce sorghum based *kununn-zaki* with acceptable nutritional and sensory qualities under hygienic conditions. The lactic acid bacteria, *L. plantarum, L. fermentum, and L. acidophilus*, isolated and used in this study as starter cultures have been reported to be associated with *kunun-zaki.* They were among the thirteen lactic acid bacteria isolated from *kunun-zaki* samples by Egbere *et al*. (2017). Efiuvwevwere and Akoma (1995) reported that *Lactobacillus fermentum* occurred frequently during the fermentation process of *kunun-zaki* production. Agarry *et al*. (2010) reported that *Lactobacillus plantarum* and *L. fermentum* were among the dominant lactic acid bacteria isolated from *kunun-zaki.*

The pH of the *kunun-zaki* products ranged from 3.30-3.77. This finding is comparable to that of Akoma *et al*. (2014) who reported a pH range of 3.25-4.20 and Efiuvwevwere and Akoma (1995) who reported a range of 3.5-3.71. The continues drop in the pH and accompanying increases in the acidity of the samples during fermentation could be attributed to metabolic activities of the lactic acid bacteria which normally would result in conversion of sugar to lactic acid, thereby increasing the acid content and lowering the pH. *Kununn-zaki* produced with *L. fermentum* + *L. acidophilus (*K3*)* had the lowest pH and highest titratable acidity values. The high acidity of K3 and K1 could have been responsible for their enhanced organoleptic quality characteristics which eventually made them the most preferred of the products.

The crude protein content of K3 and K1 were found to be higher than that of the control product even though the differences were not statistically significant ($p > .05$). This implies that using the selected starter cultures for *kunun-zaki* production would in no way deplete the already low protein content of *kunun-zaki*. The higher protein content values recorded for K3 and K1 could imply that use

of the starter cultures in *kunun-zaki* production may enhance the protein content of the product, thus enhancing its nutritional value.

Slight differences were observed in the mineral contents of the products. K3 had higher contents of calcium, magnesium and phosphorus compared to K1 and the naturally fermented control sample (NFK). While K1 had the highest content of potassium, NFK had the highest content of iron. In most cases however these differences were not statistically significant. The implication of this finding is that the mineral content of *kunun-zaki* produced using the starter cultures remains comparable to that of the control. There is therefore no nutritional quality loss in terms of mineral composition. The contents of calcium, potassium, and magnesium of the starter culture products were within the range reported by Akoma *et al*. (2014) for different *kunun-zaki* products from Bida.

The results of the sensory analyses showed that, though all the products, including the control, were acceptable to the panel of judges, K3 and K1 were the most preferred. Agarry (2010) similarly reported that *kunun-zaki* produced using starter cultures (*Lactobacillus plantarum*, *L. fermentum*, and *Lactococcus lactis*) had better sensory quality attributes than the traditionally prepared product.

Conclusion

Selected indigenous starter cultures could be used to produce sorghum based *kununn-zaki* with acceptable nutritional and sensory qualities under hygienic conditions. Use of the selected starter cultures in *kunun-zaki* production is likely to reduce some of the problems of the traditional method of producing *kunun-zaki* in which the fermentation process is dependent on chance inoculation. Such chance inoculations result in products that vary in terms of quality attributes and also in products with short shelf-lives due to high counts of spoilage organisms in them.

Conflict of Interest

The authors declare no conflict of interest

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Morphology	Growth at 15°C only	Growth at 45°C only	Growth at 15°C and 45°C	Acid and gas from glucose	NH_3 from arginine	Sugar Fermentation: Arabinose	Cellobiose	Mannitol	Mannose	Melebiolse	Raffinose	Ribose	Salicin	Lactose	Melezitose	Rhamnose	sorbitol	Xylose	Trehalose	Lactic acid bacteria
NR*	+	-	+	-	-		+	+	+	+	+	+	+							*L. plantarum*
NR			+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	*L. fermentum*
NR	-	-				+	+	+	+	-	-	-	+	+	+	-	-	+	+	*L. acidophilus*

Table 1: Characteristics of identified lactic acid bacterial isolates

*NR = Gram negative rod

Table 2: pH and titratable acidity of the *kununn-zaki* products

	Products[1,2,3]				
Analyses	K1	K2	K3	K4	NFK
Ph					
0h	4.68± 0.02[a]	4.70± 0.01[a]	4.65±0.02[a]	4.75± 0.02[a]	4.77±0.02[a]
6h	3.37± 0.04[ab]	3.59± 0.01[c]	3.30± 0.01[a]	3.77± 0.01[c]	3.55±0.01[b]
Titratable acidity					
0h	0.275±0.02[a]	0.268± 0.01[a]	0.271±0.02[a]	0.261± 0.01[a]	0.263±0.02[a]
6h	0.670±0.01[a]	0.601± 0.02[a]	0.680± 0.01[a]	0.595±0.02[a]	0.653±0.01[a]

[1]Values are Mean±SD of triplicate determinations.
[2]Values on the same row with different superscripts are significantly different (P < .05)
[3]K1=*kununn-zaki* produced with *L. fermentum* + *L. plantarum*, K2 = *kununn-zaki* produced with *L. plantarum* + *L. acidophilus*, K3 = *kununn-zaki* produced with *L. fermentum* + *L. acidophilus*, K4 = *kununn-zaki* produced with *Lactobacillus plantarum* + *Lactobacillus fermentum* + *Lactobacillus acidophilus*, and NFK = naturally fermented *kununn-zaki*

Table 3: Proximate and mineral compositions of the *kununn-zaki* products

	Products[1,2,3]		
Analyses	K1	K3	NFK
Proximate (%)			
Moisture	92.05±0.05[a]	92.01±0.03[a]	91.95±0.01[a]
Crude Protein	1.38±0.02[a]	1.45±0.01[a]	1.36±0.02[a]
Ash	3.88±0.05[a]	3.90±0.09[a]	3.84±0.06[a]
Mineral (ppm)			
Calcium	0.24±0.01[a]	0.28±0.03[a]	0.25±0.03[a]
Iron	0.42±0.02[a]	0.39±0.0 [a]	0.48±0.01[a]
Magnesium	0.38±0.0 [b]	0.45±0.04[a]	0.42±0.01[ab]
Potassium	0.15±0.02 [a]	0.13±0.02[a]	0.11±0.02[a]
Phosphorus	0.13±0.01[b]	0.19±0.01 [a]	0.15±0.01 [ab]

[1]Values are Mean±SD of triplicate determinations.
[2]Values on the same row with different superscripts are significantly different (P < .05)
[3]K1=*kununn-zaki* produced with *L. fermentum* + *L. plantarum*, K3 = *kununn-zaki* produced with *L. fermentum* + *L. acidophilus*, and NFK = naturally fermented *kununn-zaki*

Table 4: Sensory quality attributes of the *kununn-zaki* products

Quality Attributes	**Products**[1,2,3]				
	K1	K2	K3	K4	NFK
Appearance	4.43±0.53[a]	4.14±0.38[a]	4.29±0.49[a]	4.29±0.53[a]	4.42±0.53[a]
Aroma	4.29±0.49[a]	4.29±0.49[a]	4.14±0.38[a]	4.14±0.38[a]	4.42±0.53[a]
Taste	4.00±0.00[a]	4.71±0.49[a]	4.42±0.25[a]	4.42±0.53[a]	4.71±0.25[a]
Overall Acceptability	4.57±0.53[a]	4.43±0.53[a]	4.57±0.53[a]	4.14±0.38[a]	4.43±0.79[a]

[1]Values are Mean±SD of triplicate determinations.
[2]Values on the same row with same superscripts are not significantly different (P > .05)
[3]K1=*kununn-zaki* produced with *L. fermentum* + *L. plantarum*, K3 = *kununn-zaki* produced with *L. fermentum* + *L. acidophilus*, and NFK = naturally fermented *kununn-zaki*