Production of Kunun-zaki (A Nigerian fermented cereal beverage) using starter culture

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The dominant lactic acid bacteria (*Lactobacillius plantarum*, *L. fermentum* and *Lactococcus lactis*) isolated from fermenting Kunun-zaki were characterized, developed and used as starter culture for the controlled production of Kunun-zaki. The Kunun-zaki produced in this study (millet, millet+wheat, millet+malted rice, millet+wheat+malted rice, millet+malted rice+starter culture, millet+wheat+malted rice+starter culture) were evaluated for its pH, titratable acidity (% lactic acid), proximate, mineral and sensory quality attributes. There was a steady drop in pH with corresponding increases in titratable acidity showing strong correlation (r) throughout the fermentation period in all the samples. Generally, the crude protein content (%) of all the products was low (1.02-1.14). However, the Kunun-zaki produced using the combination of millet+wheat+malted rice showed a higher iron, calcium, magnesium and potassium content and was significantly different ($p<0.05$) from the rest products. Furthermore, the Kunun-zaki produced with the addition of starter culture to either millet+malted rice or millet+wheat+malted rice was generally preferred in taste, aroma, appearance and overall acceptability and differed ($p<0.05$) from the other products. This study has shown that use of starter culture in the production of Kunun-zaki has affected the sensory and nutritional qualities of the product positively. Therefore, large-scale production of this popular indigenous cereal beverage could be possible.

Key word: Kunun-zaki, lactic acid bacteria, developed starter culture, fermentation, sensory quality**,** cereal.

INTRODUCTION

Kunun-zaki is a fermented non-alcoholic cereal beverage. It is a popular refreshing drink in northern Nigeria. Kunun-zaki production is essentially a home-based industry and at present, there is no large-scale factory production. Traditional production of Kunun-zaki is characterised by the following: the fermentation is dependence on chance inoculation and rudimentary equipments are used in its production process, as a result, products of varying quality attributes are produced; the sanitary quality of the product during production and sale is poor thereby giving rise to products of short shelf-life (Elmahmood and Doughari, 2007). Efiuvwevwere and Akoma (1995) studied the microbiology of the fermentation process of Kunun-zaki and reported that *Lactobacillus fermentum* and *Lactobacillus leichmannii* were dominant at the end of the fermentation period. Akoma et al. (2006) reported an elevated lymphocyte counts in the blood samples of albino rats fed with Kunun-zaki suggesting that Kunun-zaki has medicinal attributes. Lactic acid bacterial fermentation is used to improve sensory and nutritional properties of foods (Anderson, 1988) and to produce products of high and consistent qualities, the fermentation process has to be controlled using tailor-made starter culture (Cooke et al., 1987); this would improve the products' shelf-life quality thereby reducing microbial risks associated with traditionally food process.

Scientist have embarked on extensive studies to isolate and characterized microorganisms associated with production of indigenous fermented foods with the possibility of exploiting their industrial potentials (Leisner et al., 2001; Adnan and Tan, 2007). Banigo et al. (1974) developed a starter culture comprising of mixtures of *Lactobacillus plantarum, Streptococcus lactis* and *Saccharomyces rouxi* for the controlled production of ogi (a porridge prepared from fermented cereal). Olasupo et-

al. (1997) produced ogi using a bacteriocin-producing *Lactobacillus* strain as a starter culture. The ogi produced by these workers had extended shelf-life of 11 days as against the 7 days reported for the un-controlled fermentation (Olasupo et. al., 1997). Hydrolytic enzymes have been used by several workers to produce Kunun-zaki which increased the nutritional and sensory quality of the product (Ayo and Okaka, 1998; Gaffa and Ayo, 2002; Akoma et. al., 2002). There are no reports in the literature on the production of Kunun-zaki using tailor-made starter culture. The objective of this study is to produce Kunun-zaki using locally developed starter culture and evaluate its nutritional and sensory quality attributes.

MATERIALS AND METHODS

Isolation of dominant microorganisms associated with Kunun-zaki production using BHI-sodium azide enrichment technique

The dominant microorganisms associated with Kunun-zaki production were isolated using BHI-sodium azide enrichment technique described by Lindquist (1998). 10ml of Kunun-zaki produced as described by Akoma et al. (2002) was mixed with 100ml of sterile Brain Heart Infusion broth (BHI: Biotech Laboratories Ltd, Ipswich, UK) supplemented with 0.02% (w/v) sodium azide (Sigma, UK) and incubated at 30±2^{o}C for 24h. Sodium azide inhibits cytochrome activity therefore selectively enriched for lactic acid bacteria. Sub-cultures were made from the 24h enriched medium by streaking them on prepared MRS agar (LAB M, Lancashire, UK) plates; these were incubated for the 24h at 30±2^{o}C. Following incubation, discrete colonies (typical pin point) were randomly picked and purified on fresh MRS agar plates. Cultures of the isolates were considered to be pure after three successive subcultures on MRS agar plates; pure cultures of the microbial isolates were subsequently sub-cultured on MRS agar slants in Bijou bottles; these were covered with sterile mineral oil and kept in the refrigerator for further studies.

Identification of lactic acid bacteria (LAB) isolates using API 50 CHL kits

Each isolates was tested for catalase activity by placing a drop of 3% H_2O_2 on 24h slant culture grown on MRS agar, immediate formation of bubbles of gas indicates the presence of catalase (Collins *et. al*. 1987). Only catalase negative isolates were Gram stained and subjected to hot-loop test to differentiate between homolactics from heterolactics. Isolates that showed evidence of gas (CO_2) production in the hot-loop test were deemed to be heterolactics (Sperber and Swan, 1976). Based on the preliminary studies, 30 isolates obtained from three Kunun-zaki preparations were pruned down to 10 of which 5 were homolactics and the rest heterolactics. Pure cultures of the LABs isolates were grown on MRS agar plates anaerobically (Gas Pak, BBL, Cockeyville, USA) for 24h. Colonies were transferred to 5ml MRS broth overnight and incubated at 30^{o}C following which, the culture was centrifuged (3000rev/min for 5min), washed with sterile saline and re-suspended in saline. The turbidity of the suspension was determined by the McFarland's method according to the manufacturer's instructions. Aliquots of cell suspensions was pipetted into the API 50 CHL strip wells (bioMerieux SA, France) and coated with mineral oil following which the strips were incubated at 30^{o}C for 48h. Results were read after 48h. Fermentation of carbohydrate was indicated by a yellow colour except for esculine (dark brown) colour reactions were scored against the API 50 CHL chart as provided by the manufacturer.

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

Pure cultures of LAB isolates were tested (singly and or in various combinations) for their potential use as starter culture for Kunun-zaki production. Fresh cultures of the isolates grown on MRS agar plates were harvested with sterile cotton swabs and suspended (separately) in 1ml of sterile saline (0.85% NaCl) to prepare a dense suspension (equivalent to a no. 2 McFarland turbidity standard); this was used to inoculate singly or in various combinations into 10ml of sterile Hydrolyzed Cereal Starch Broth (HCS-broth: 500g gelatinized cereal starch was hydrolyzed with 200g ground malted rice to which 2g of soy bean flour was added and sterilized at 121^{o}C for 10min) and incubated at ambient temperature (30±2^{o}C) for 24h. Production of good aroma (buttery) was use as quality index to establish their potential use in inoculum development.

Development of starter culture

One millilitre each of the three species of LABs selected earlier (*Lactobacillus plantarum*, *L. fermentum* and *Lactococcus lactis*) in sterile saline suspension, were added to 50ml of HCS-broth and incubated for 12h following which it was transferred to 200ml HCS-broth and subsequently incubated for 12h.

Production of Kunun-zaki using developed starter culture (controlled fermentation)

Preparation of ground malted rice paste

500g of paddy rice (*Oryza sativa*) of FARO 37 variety, obtained from National Cereal Research Institute Badeggi, Niger State, Nigeria was washed with tap water and soaked in 1000ml of tap water (1:2w/v) for 12h and then drained. The drained grains were couched by covering then with moist cloth for 4-5days at ambient temperature (30^{o}C) to germinate and then dried in the sun for 3days. The dried malted rice were steeped in 1% sodium metabisulphite solution for 5 minutes following which it was washed in tap water and ground to paste.

Pre-fermentation processing of cereals

1000g of cereal (millet (*Pennisetum typhoideum*) or combinations of millet+wheat (*Triticum aestivum*; mixed in ratio 4:1w/w) depending on the type of Kunun-zaki to be produced were washed using tap water and steeped in 2000ml of water in a plastic container for 24h at ambient temperature. After 24h of steeping, the grain was rewashed with clean water, drained and steeped in 2000ml of 1% sodium metabisulphite together with ginger (*Zingiber officinale;* 6g), black pepper (*Pipper spp*; 2g), clove (*Euginia spp.* 2g) for 5 minute after which it was rinsed with cooled boiled water and ground to paste (laboratory blender was sterilized with 5% sodium metabisulphite for 5min and rinsed with water).

Liquefaction and saccharification of gelatinized cereal starch

Two kilograms of cereal paste was divided into two (2) unequal portions (1:3v/v). The larger portion was gelatinized by the addition of boiling water (1:1v/v) in a plastic container and immediately 200g of ground malted rice paste was added and stirred vigorously (2-3min) at 76^{o}C for liquefaction and saccharification of the gelatinized cereal starch following which it was cooled to about 50^{o}C.

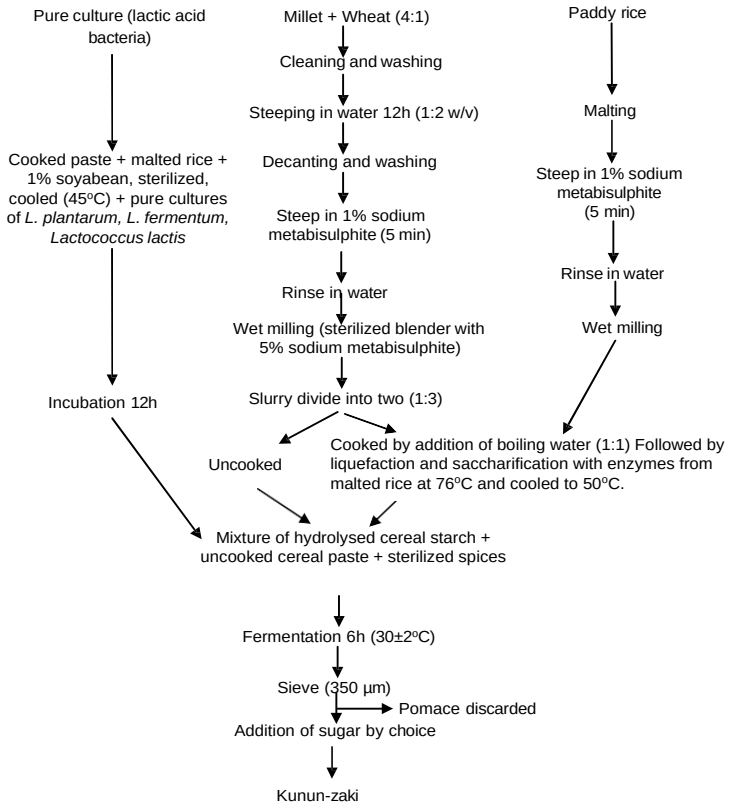

Figure 1. Flow diagram for the production of Kunun-zaki using malted rice and developed starter culture

Inoculation of hydrolyzed cereal starch with starter culture

100g of uncooked cereal paste (millet, or combinations of millet+wheat) was mixed with 150ml of 12h starter culture of LAB before addition to the 200g of hydrolyzed cereal starch (either millet or combinations of millet and wheat). This was mixed thoroughly for 1min before incubation for 6h at ambient temperature following which it was sieved (wire sieve was sterilized with 5% sodium metabisulphite for 5min and rinsed with water) to remove the pomace. The filtrate (ie Kunun-zaki) was transferred to 5l plastic jerry can (previously sterilized with 5% sodium metabisulphite) and stored in refrigerator (Figure 1).

Production of Kunun-zaki using natural fermentation process (uncontrolled fermentation)

Four types of Kunun-zaki were produced as described previously (Akoma *et. al.,* 2002) using the combinations of millet or millet+wheat with or without the addition of ground malted rice and fermented for 6h.

Control fermentation

For the control experiment, 100g of uncooked cereal starch (previously sterilized) was mixed thoroughly with 200g of hydrolyzed cereal starch, before addition to gelatinized cereal starch. This was incubated at ambient temperature (without addition of starter culture) for 6h to establish whether fermentation could take place. The six (6) types of Kunun-zaki produced in this study were replicated thrice.

Chemical analysis

PH and titratable acidity

The pH of Kunun-zaki during production (0, 2, 4, and 6h) was determined in triplicates using pH meter (TECPEL pH meter, model 705) after standardization with pH 4 and pH 7 buffers (BDH, England). The titratable acidity of Kunun-zaki during production (0, 2, 4, and 6h) was determined in triplicates by titrating 10ml of the sample with 0.1N sodium hydroxide to phenolphthalein end point (pink). The titratable acidity (expressed as % lactic acid) was calculated for each sample as described by Field (1977).

Proximate analysis

The moisture content, crude protein, crude fat and ash contents of the kunun-zaki samples were determined in triplicates as described by AOAC (1990).

Table 1. Characteristics of lactic acid bacteria[1] isolated from fermenting Kunun-zaki

Code	Growth in 0.02% sodium azide	Cultural characteristics	Cell morphology	Gram reaction	Catalase	Hot loop test[2]	Identification[3] by API 50 CHL kit
L01	+	Pin point colonies	Rod	+	-	+	*Lactobacillus fermentum*
L02	+	Pin point colonies	Rod	+	-	+	*Lactobacillus plantarum*
L03	+	Pin point colonies	Rod	+	-	+	*Lactobacillus plantarum*
L04	+	Pin point colonies	Cocci	+	-	-	*Leuconostoc meseteroides*
L05	+	Pin point colonies	Rod	+	-	+	*Lactobacillus fermentum*
L06	+	Pin point colonies	Cocci	+	-	-	*Leuconostoc meseteroides*
L07	+	Pin point colonies	Cocci	+	-	-	*Lactococcus lactis*
L08	+	Pin point colonies	Cocci	+	-	-	*Lactococcus lactis*
L09	+	Pin point colonies	Rod	+	-	+	*Lactobacillus pentoses*
L10	+	Pin point colonies	Cocci	+	-	-	Unidentified LAB

[1]The microbial isolates were obtained from three kunun-zaki preparation
[2]Gas (CO_2) evolution (Hot loop test): + heterofermentative, - homofermentative
[3]Degree of aroma production on 12h HCS-broth: +++L03, L05, L07; + L04, L01, L08;+ L02, L06, L09

Mineral analysis

The mineral contents (calcium, iron, magnesium, potassium and phosphorous) of the Kunun-zaki samples was determined in triplicates as described by AOAC (2006). Iron, magnesium and calcium were determined using Atomic Absorption Spectrophotometer (Buck Scientific, USA; Accusy 211); while potassium and phosphorus were determined using Jenwa flame photometer (UK, PF P7) and Jenwa colorimeter (UK, Spectronic, 20), respectively.

Organoleptic analysis

Quality attributes including appearance, aroma, taste and overall acceptability of six (6) types of Kunun-zaki produced in this study (millet, millet+wheat, millet+malted rice, millet+wheat+malted rice, millet+malted rice+starter culture, millet+wheat+malted rice+starter culture) were evaluated by 25 member panellist comprising of some trained students and lecturers who are familiar with the product using 7-point hedonic scale (where 1 = like extremely, 2 = like very much, 3 = like slightly, 4 = neither like nor dislike, 5 = dislike slightly, 6 = dislike very much and 7 = dislike extremely) as described by Larmond (1977). The sensory quality attribute of the control was not determined because it's objectionable odor.

Statistical analysis

Analysis of variance (ANOVA) was carried out for the sensory scores, proximate analysis, mineral analysis, pH and titratable acidity for the six (6) types of Kunun-zaki produced in this study and the control. The mean scores were computed and significant differences among the mean was determined (Duncan, P= 0.05) using 2006 Statistical Packages for Social Sciences (SPSS) for Windows version 15.0 (SPSS, 2006). Correlation coefficient (r) between pH and titratable acidity of the six types of Kunun-zaki was computed using Minitab statistical packages for windows version 15.1.0.0 (Minitab, 2005).

RESULTS

Characteristics of dominant fermenting micro-organism involved in Kunun-zaki production

Ten (10) species of lactic acid bacteria (LAB) comprising of five *Lactobacillus spp*, two *Leuconostoc spp*, two species of *Lactococcus* and one unidentified organisms were found to be dominant in Kunun-zaki production. The lactobacilli isolates were heterolactics since they were positive in the hot-loop test (Table 1). However, when these organisms were tested (singly and or in various combinations) for their ability to be developed as potential starter cultures for Kunun-zaki production, only L03, L05 and L07 showed a preferred aroma production when grown on HCS-broth (Data not shown but summarised as a footnote in Table 1).

Changes in chemical, nutritional and sensory quality attributes of Kunun-zaki during production pH and titratable acidity (% lactic acid)

The chemical quality attributes (pH and titratable acidity) of Kunun-zaki during production is shown in Table 2. Generally there was a steady drop in pH with corresponding increases in titratable acidity in all the products which showed a strong correlation (r) throughout the fermentation period. The drop in pH was more noticeable in the Kunun-zaki produced using combinations of millet+wheat+malted rice+starter culture which dropped from 3.92 (0h) to 3.18 (6h); this was

Table 2. pH and titratable acidity of Kunun-zaki produced using developed starter culture

	Fermentation period (h) [1,2,3]									
	pH				Titratable acidity (% lactic acid)				Correlation	
Kunun-zaki type	0	2	4	6	0	2	4	6	coefficient (r)	Significance
Millet+wheat	4.95±	4.63±	4.07±	3.78±	0.271±	0.415±	0.465±	0.545±	-0.955	P=0.045
	0.02^{b}	0.07^{b}	0.03^{b}	0.05^{b}	0.001^{bc}	0.005^{c}	0.005^{b}	0.005^{c}		
Millet	4.79±	3.79±	3.66±	3.54±	0.275±	0.445±	0.465±	0.500±	-0.998	P=0.002
	0.01^{c}	0.01^{c}	0.00^{d}	0.02^{d}	0.005^{bc}	0.005^{b}	0.005^{b}	0.010^{d}		
Millet+wheat+malted rice	4.94±	4.32±	3.90±	3.65±	0.280±	0.545±	0.565±	0.585±	-0.921	P=0.079
	0.02^{b}	0.01^{b}	0.07^{c}	0.01^{c}	0.010^{b}	0.005^{a}	0.005^{a}	0.005^{ab}		
Millet+malted rice	4.62±	4.30±	3.92±	3.81±	0.293±	0.535±	0.548±	0.560±	-0.862	P=0.138
	0.02^{d}	0.20^{b}	0.04^{c}	0.02^{b}	0.003^{b}	0.005^{a}	0.003^{a}	0.010^{bc}		
Millet+wheat+malted rice+starter culture	3.92±	3.88±	3.34±	3.18±	0.370±	0.385±	0.410±	0.600±	-0.793	P=0.207
	0.01^{f}	0.01^{c}	0.01^{e}	0.01^{e}	0.010^{a}	0.005^{d}	0.010^{c}	0.010^{a}		
Millet+malted rice+starter culture	4.42±	4.28±	3.63±	3.52±	0.385±	0.395±	0.415±	0.475±	-0.850	P=0.150
	0.02^{e}	0.07^{b}	0.01^{d}	0.01^{d}	0.005^{a}	0.005^{d}	0.005^{c}	0.005^{d}		
Control	5.44±	5.14±	4.98±	3.94±	0.255±	0.265±	0.295±	0.305±	-0.857	P=0.143
	0.04^{a}	0.15^{a}	0.01^{a}	0.01^{a}	0.005^{c}	0.005^{e}	0.005^{d}	0.005^{e}		

[1]Each value is the mean+standard error of triplicate determinations
[2]Different letters within the same column with each test are significantly different (p<0.05)
[3]Control: fermented sterilized ground millet paste+gelatinized millet starch without the addition of starter culture

Nutritional quality of Kunun-zaki

In Table 3 is shown the proximate and mineral contents of six types of Kunun-zaki produced in this study. Generally, the crude protein content (%) of all the products were low (1.02-1.14) The mineral content of the six types of Kunun-zaki as shown in Table 3 indicates that the Kunun-zaki produced using the combinations of millet+wheat+malted rice+starter culture was richer in iron, calcium, magnessiun and potassium content than the other products and was significantly different (p<0.05); the control fermentation was generally low in its mineral content (Table 3).

Sensory quality attributes of Kunun-zaki

The mean sensory scores of six types of Kunun-zaki as shown in Table 4 indicates that the Kunun-zaki produced with addition of starter culture to either millet+malted rice or millet+wheat+malted rice was generally preferred in all quality attributes and differed (p<0.05) from the other products.

DISCUSSION

Kunun-zaki is a fermented cereal beverage whose production is carried out using indigenous fermentation technology. Intensified efforts are ongoing in the developing world to isolate and characterise microorganisms responsible for the production of indigenous fermented foods (Abdelgadir et. al., 2001; Mathara et. al., 2004; Adnan and Tan, 2007). LABs associated with Kunun-zaki fermentation were isolated, characterised and three of these isolates two heterolactics (*Lactobacillus plantarum, L. fermentum*)

Table 3. Proximate and mineral content[1,2] of Kunun-zaki produced using developed starter culture

	Proximate content (%)					Mineral content (mg/100ml)				
Kunun-zaki type	**Carbohydrate**	**Ether extract**	**Ash**	**Crude protein**	**Moisture content**	**Iron**	**Calcium**	**Magnesium**	**Potassium**	**Phosphorus**
Millet+wheat	88.38± 0.07^{bc}	6.47± 0.15^{e}	3.93± 0.11^{c}	1.12± 0.01^{c}	80.5± 0.01^{g}	71.83± 0.09^{b}	8.67± 0.01^{b}	53.15± 0.40^{c}	169.37± 0.24^{c}	1.22± 0.01^{a}
Millet	87.37± 0.06^{c}	7.76± 0.03^{c}	3.92± 0.01^{c}	1.06± 0.01^{b}	87.3± 0.02^{b}	72.33± 0.27^{b}	4.69± 0.02^{f}	31.62± 0.06^{e}	149.37± 0.26^{e}	1.23± 0.01^{a}
Millet+wheat+malted rice	90.02± 0.13^{a}	3.95± 0.01^{g}	4.35± 0.01^{b}	1.09± 0.01^{b}	86.9± 0.03^{b}	52.73± 0.14^{d}	8.25± 0.03^{c}	54.47± 0.29^{b}	157.57± 0.23^{d}	1.21± 0.01^{a}
Millet+malted rice	88.61± 0.02^{b}	7.31± 0.02^{d}	2.90± 0.01^{d}	1.14± 0.01^{c}	85.7± 0.03^{d}	48.35± 0.23^{f}	5.21± 0.03^{e}	30.47± 0.27^{f}	145.90± 0.06^{f}	1.21± 0.01^{a}
Millet+wheat+malted rice+starter culture	87.21± 0.23^{c}	8.64± 0.01^{b}	2.89± 0.01^{d}	1.07± 0.01^{b}	84.6± 0.03^{f}	75.78± 0.13^{a}	9.72± 0.07^{a}	60.62± 0.20^{a}	217.03± 0.52^{a}	1.07± 0.01^{b}
Millet+malted rice+starter culture	87.21± 0.05^{c}	5.76± 0.01^{f}	2.92± 0.03^{d}	1.03± 0.03^{a}	86.0± 0.03^{a}	51.62± 0.22^{e}	6.78± 0.04^{d}	34.43± 0.12^{d}	186.83± 0.33^{b}	1.25± 0.01^{a}
Control[3]	85.88± 0.13^{d}	8.77± 0.02^{a}	4.48± 0.01^{a}	1.02± 0.02^{a}	84.9± 0.01^{c}	70.02± 0.07^{c}	5.18± 0.04e	30.03± 0.09^{f}	134.27± 0.48^{g}	0.88± 0.02^{c}

[1]Each value is the mean ± standard error of triplicate determinations
[2]Different letters within the same column with each test are significantly different ($p<0.05$).
[3]Control: fermented sterilized ground millet paste+gelatinized millet starch without the addition of starter culture

and the homolactics: (*Lactococcus lactis*; Table 1) were developed as starter culture by growing them in HCS-broth for 12h before inoculation into hydrolyzed cereal starch and fermented for 6h. It was observed that the colour of the substrate about 4h into the fermentation became lighter in colour (whiter); it is possible that the H_2O_2 produced by the heterolactics during fermentation in this study had a bleaching effect on the fermenting substrate. Hurst and Collins-Thompson (1979) cited by Steinkraus (1983) reported that heterolactics produces H_2O_2 during fermentation, when reduced nicotinamide adenine dinucleotide ($NADH_2$) is oxidized by flavin nucleotide in the presence of gaseous O_2 in acidic environment.

The marked decrease in pH with concomitant increases in titratable acidity as observed in this study (Table 2) especially in the Kunun-zaki produced using controlled fermentation process maybe due to the souring activity of the homolactics- *Lactococcus lactis* which are known to produce 100% lactic acid during fermentation of carbohydrates. The higher acidity of the Kunun-zaki produced using controlled fermentation process (Table 2) possibly enhanced its sensory quality attributes thereby making them more preferably to the rest product (Table 4). The implication of this study is that use of starter culture in the production of Kunun-zaki had affected the sensory quality of the product positively as compared with the Kunun-zaki produced using traditional method. Although the crude protein content of all the Kunun-zaki produced in this study was generally low (1.02-1.14%); the Kunun-zaki produced by the addition of starter culture to millet+wheat+malted rice showed a higher mineral content (Fe, Ca, Mg, K) and was significantly different ($p<0.05$) from the rest (Table 3). The mineral content of wheat as reported by Kent (1983) is generally higher than those of other cereals and this might have contributed positively to the mineral content of the product as observed in this study. LAB fermentation of food has been reported by other workers to improve the nutritional quality of foods resulting in increases in bioavailability of iron and release of phosphorus (Anderson, 1988; Lopez et al., 1983). Various reports have shown that Kunun-zaki produced using indigenous fermentation process has high counts of spoilage and

Table 4. Sensory quality attributes of Kunun-zaki produced using developed starter culture

Kunun-zaki type	Sensory scores[1,2]			
	Appearance	Taste	Aroma	Overall acceptability
Millet+wheat	1.80±0.08^{bc}	2.60±0.36^{ab}	2.00±0.18^{b}	2.60±0.36^{a}
Millet	2.60±0.28^{a}	3.40±0.44^{a}	2.80v0.15^{a}	2.80±0.30^{a}
Millet+wheat+malted rice	1.40±0.00^{cd}	2.60±0.36^{ab}	2.00±0.00^{b}	3.00±0.32^{a}
Millet+malted rice	2.20±0.20^{ab}	2.40±0.31^{ab}	2.80±0.40^{a}	3.00±0.34^{a}
Millet+wheat+malted rice+starter culture	1.40±0.10^{cd}	1.70±0.34^{b}	1.70±0.29^{b}	1.60±0.33^{b}
Millet+malted rice+starter culture	1.20±0.08^{d}	1.80±0.33^{b}	1.80±0.24^{b}	1.80±0.33^{b}

[1]Different letters within the same column with each test are significantly different ($p<0.05$).
[2]Each value is the mean ± standard error of 25-member panellist; using 7 point hedonic scale, where 1 = like extremely, 4 = neither like nor dislike and 7 = dislike extremely

pathogenic microorganisms which may be responsible for its short shelf-life (Onuorah et al., 1985; Umoh et al., 2004). It is possible that this problem could be reduced if starter cultures are employed in its fermentation process as done in the developed world. Use of starter cultures in the production of Kunun-zaki would encourage the industrialisation of the production process on sound scientific principle.

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