INFLUENCE OF THERMAL ENZYMATIC HYDROLYSIS OF CEREAL STARCH ON THE PHYSICO-CHEMICAL QUALITY OF KUNUN-ZAKI (A fermented non-alcoholic cereal beverage).

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ABSTRACT: Millet grains were steeped in 1% sodium metabisulphite (1:2w/v) for 5min and subsequently washed and wet milled; the cereal paste was gelatinized with boiling water (1:1w/v, 76±2ºC) and immediately hydrolyzed separately either with α+β-amylases, α+amyloglucosidase or rice malt. The hydrolyzed cereal starch was inoculated with a 12h starter culture (2%v/w) of *Lactobacillus plantarum, L. fermentum,* and *Lactococcus lactis* and fermented for 6h. Chemical (pH, titratable acidity), physical (viscosity, S.G., total soluble solids) and sensory quality of the hydrolyzed cereal slurry and the fermented product were determined. The results obtained in this study show that the pH of the products decreased with concomitant increases in titratable acidity (% lactic acid) during production; however, the decrease in pH was more prominent in the 'kunun-zaki' produced from the cereal starch treated with α+β-amylases and this, differed from the other products ($p<0.05$). There was an increase in viscosity with a corresponding decrease in the total soluble solids (TSS) in all the samples throughout production; the decrease in TSS is an indication of an increase in the activity of the fermenting LABs. Furthermore, the sensory quality attributes of the three products were generally acceptable by the taste panelist in all the parameters evaluated (appearance, aroma, taste), however, the 'kunun-zaki' produced using the cereal starch treated with rice malt was preferred in taste and this was significantly different ($p<0.05$) from the other products. This study has shown that 'kunun-zaki' of acceptable quality could be produced within 7h, the marked reduction in the processing time of 'kunun-zaki' from 12-7h could encourage large-scale production of this popular drink.

Key word: Cereal starch, hydrolytic enzymes, starter culture, fermentation, 'kunun-zaki'

INTRODUCTION.

Enzymes are biological catalyst and have longed been employed in food processing (Poldermans, 1990) and the enzyme amylases has been employed in starch food processing industries to breakdown starch to simple sugars (Okafor, 1987). Amylases could be sourced from microorganisms (bacteria and fungi) and as well as from plants. However, amylases of plant origin are known to have higher productivity followed by fungal amylases. Enzymes that specifically catalyze the hydrolysis of α-1,6-glucosidic bonds or branched linkages of starch are known as debranching enzymes. Malted rice is excellent source of debranching enzymes and because of its high starch conversion rate has been used extensively to produce low calorie beer (Line *et. al.,* 1982). Yamada (1981) reported that the activity of debranching enzymes in rice seeds increased during the early stage of ripening and during germination. The role of starch debranching enzyme is to hydrolyze the α-1,6-glucan branches of amylopectin during germination of cereal seeds. Two distinct types of debranching enzymes occur in higher plants and these are R-enzymes, or pullulanases (Nakamura, 1996) and isoamylases (Manners, 1985).

'Kunun-zaki' is a refreshing non-alcoholic cereal beverage (Sopade and Kassum, 1992**)**. Traditionally, 'kunun-zaki' could be produced depending on the locality with millet (*Penniset*eum *typhoideum*), maize (*Zea mays*), sorghum (*Sorghum bicolor*), acha (*Digitalis exilis*) or wheat (*Triticum aestivum*) with or without the addition of saccharifying agent (sweet potato, malted cereal, extracts of *Cadaba farinosa* (Ayo and Okaka, 1998; Akoma *et. al.,* 2002; Gaffa *et. al.,* 2002). 'Kunun-zaki' production is basically a two-stage fermentation process. Steeping of cereal grain (primary fermentation) which usually last for 12-24h serves to soften the kernel of the grain; the nutrient that leaches out to the steep water encourages luxuriant growth of diverse kinds of microorganisms (Efiuvwevwere and Akoma, 1995; Odunfa, 1985). Secondary fermentation commences as soon as the hydrolyzed cereal starch is mixed with the uncooked cereal paste and this last between 8-12h. The dominance of the lactic acid bacteria is established with the lowering of pH with concomitant increases in titratable acidity (Efiuvwevwere and Akoma, 1995). Generally, 'kunun-zaki' production takes about 20-36h to complete.

Attempts have been made by several workers to shorten the processing time of 'kunun-zaki' production. Gaffa *et. al.* (2002) reported a reduction in the production time of 'kunun-zaki' from 24h to 12h by steeping the grains in 5% sodium metabisulphite solution (60ºC) for 3h. In their study, cereal grains were steeped in warm water (60ºC) and treated with 5% sodium metabisulphite for 3h and wet-milled. The gelatinized cereal starch was hydrolyzed with crude extracts of *Cadaba farinosa* fermented for 10h (Gaffa *et. al.,* 2002). Agarry *et. al*. (2010) reported shortening the secondary fermentation time of 'kunun-zaki' production from 8h to 6h using combinations of hydrolytic enzymes (malted rice) and locally developed starter culture comprising of *Lactobacillus plantarum, L. fermentum* and *Lactococcus lactis*. Obadina *et. al.* (2008) studied the effect of varying steeping time on the quality of 'kunun-zaki' produced using millet and reported that the crude protein and fat content of the product increased as the steeping time increased (12-48h) with corresponding decrease in the carbohydrate content. Furthermore, these workers reported that the sensory quality characteristics of the 'kunun-zaki' produced from the steeped grain at 12, 24, 36 and 48h did not differ but the organic acid content increased (Obadina *et. al*., 2008). The objective of this study is to determine the effect of shortening the processing time of 'kunun-zaki' production using hydrolytic enzymes on its chemical, physical and sensory quality attributes.

MATERIALS AND METHODS.

Preparation of ground malted rice paste

Ground malted rice was prepared as described by Nkama *et. al.* (2010). 500g paddy rice (*Oryza sativa*) was soaked in tap water (1:2w/v) for 12h and then drained. The drained grains were couched by covering them with moist cloth for 7days at ambient temperature (30±2ºC) to germinate and then dried in the sun for 3days. The dried malted rice were surface sterilized using 1% sodium metabisulphite solution for 5min following which it was washed in tap water and ground to paste.

Development of starter culture

Starter culture comprising of *Lactobacillus plantarum*, *L. fermentum* and *Lactococcus lactis* (isolated from 'kunun-zaki') was developed as described by Agarry *et. al.* (2010). Iml each of the pure culture of the organism in sterile saline suspension (equivalent to a no. 2 McFarland turbidity standard) was transferred to a 50ml Hydrolyzed Cereal Starch-broth (HCS broth: 500g gelatinized cereal starch was hydrolyzed at 76°C with 200g ground malted rice to which 2g of soy bean flour was added and sterilized at 121°C for 10min). This was incubated for 12h following which it was transferred to another 200ml HCS-broth and subsequently incubated at ambient temperature (30±2°C) for 12h (Agarry *et. al.*, 2010).

Determination of rice malt-cereal starch mixing ratio.

Five hundred gram (500g; wet weight) of millet paste was gelatinized with ca 500ml of boiling water and immediately the temperature was taken following which, it was hydrolyzed with varying amount of malted rice paste (150, 200, 250g; wet weight). The viscosity and specific gravity of the hydrolyzed starch determined. Sensory quality attributes (mouth-feel) of the 'kunun-zaki' produced following treatment with malted rice was evaluated by a 10-member taste panelist.

Enhanced production of 'kunun-zaki' using hydrolytic enzymes and starter culture.

Pre-fermentation processing of cereal.

One kilogram (1kg) of millet was washed with water and steeped in 2000ml of 1% sodium metabisulphite solutions (1:2w/v) together with ginger (*Zingiber officinale;* 6g), black pepper (*Pipper spp*; 2g), clove (*Euginia spp.* 2g) for 5min after which it was rinsed with cold boiled water and ground to paste (laboratory blender was sterilized with 5% sodium metabisulphite for 5min and rinsed with water); the paste was divided into two (2) portions (1:3).

Liquefaction and saccharification of gelatinized cereal starch.

Two third of the larger portion of the cereal paste was gelatinized with equal volume of boiling water (1:1v/v) and immediately 1000g of the gelatinized starch was liquefied (78±2°C) with 0.5ml of α-amylases (Novozymes, Switzerland) following vigorous stirring for 1min. The liquefied cereal starch was then divided into two portions of 500g each and then treated separately with either β-amylases (0.3ml at 65°C) or amyloglucosidases (0.3ml at 55°C); these were stirred vigorously for 1min for hydrolysis of the liquefied starch. The remaining portion of the cereal paste was gelatinized with boiling water and immediately, 500g of the gelatinized starch was hydrolyzed (76°C) with 200g of rice malt (2:5; rice malt-cereal starch).

Fermentation of hydrolyzed cereal starch.

The uncooked cereal paste was divided into three (3) portions and each set was added to a portion of the hydrolyzed cereal starch giving a total volume of 700ml of fermentable substrate. 100ml of 12h developed starter culture was added aseptically to each of the 3 portions and mixed thoroughly. This was allowed to ferment at 30±2°C for 6h (Figure 1) following which it was sieved and the sweet filtrate ('kunun-zaki').

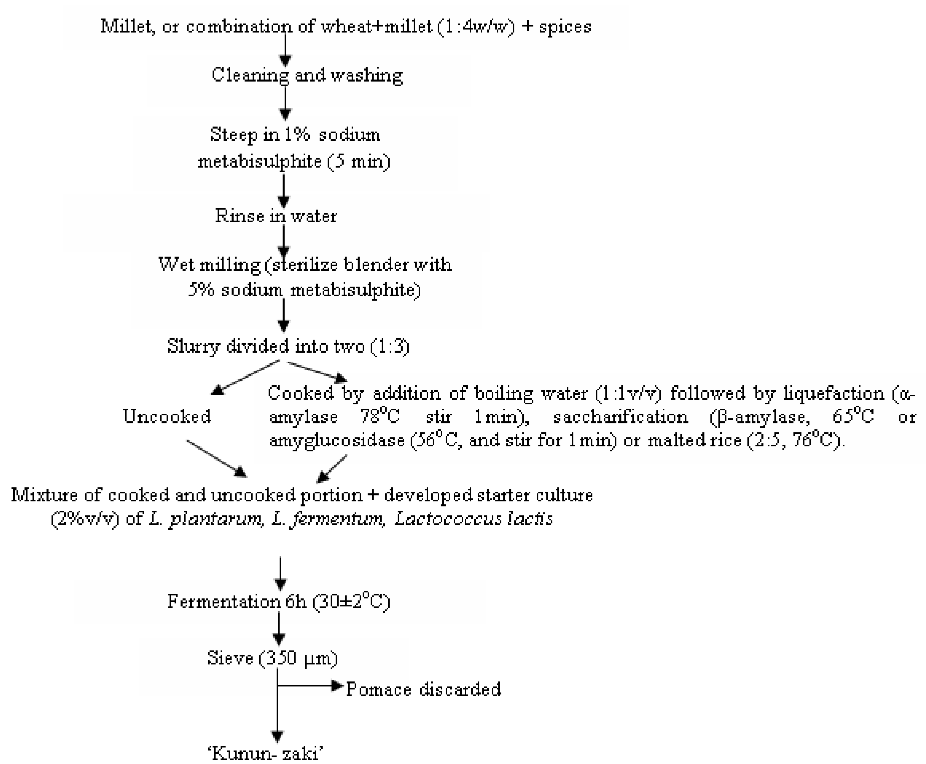

Figure 1: Flow diagram for the enhanced production of 'Kunun-zaki' using pure cultures of lactic acid bacteria and hydrolytic enzymes.

Chemical Analysis.

pH

The pH of 'kunun-zaki' during fermentation at 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).

Titratable acidity (% lactic acid).

The titratable acidity of 'kunun-zaki' during fermentation at 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).

Physical analysis.

Viscosity

The viscosity of the products during 'kunun-zaki' production following enzyme hydrolysis was determined using Rotational viscometer (NDJ-IA Rotational Viscometer, Shangai, China). The viscosity reading were taken in triplicates at the rotational speed of 60rpm using appropriate spindles (numbers 2, 3) at the temperatures of 60 and 30ºC. A 500ml beaker was used for all measurements with the viscometer guard leg on; 450ml of the sample was added to just cover the immersion grooves on the spindles' shaft. Viscosity values (cP; centipoises) were obtained by multiplying viscometer readings with appropriate factors as supplied by the viscometer manufacturer.

Total soluble solids (°Brix).

Total soluble solids of 'kunun-zaki' during production were determined in triplicates using Refractometer (CHASEbrand Refractometer) and the result expressed as degree brix (°Brix).

Specific gravity determination.

The specific gravity of 'kunun-zaki' during production was determined using a hydrometer (Universal Hydrometer for heavy and light liquids, TP-16°C, UK). 400ml of the 'kunun-zaki' was placed in a 500ml measuring cylinder and the hydrometer inserted and allowed to float. The readings were taken in triplicates and recorded.

Organoleptic analysis.

Quality attributes including appearance, aroma, and taste of the 'kunun-zaki' produced following hydrolysis with hydrolytic enzymes (α, β-amalyses, amyloglucosidase) and rice malt, were evaluated by 25 member taste-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).

Statistical analysis.

The sensory scores, total soluble solids, viscosity, specific gravity, pH and titratable acidity that were obtained in this study were subjected to statistical analyses (ANOVA). The mean scores were computed and significant differences among the mean determined using 2006 Statistical Packages for Social Sciences (SPSS) for Windows version 15.0 (SPSS, 2006).

RESULTS.

Rice malt-cereal starch mixing ratio.

The physical (viscosity, S.G) and sensory (mouth-feel) characteristics of the hydrolyzed starch using varying amounts of rice malt is shown in Table 1. The hydrolyzed substrates treated with rice malt-cereal starch ratio 2:5 and 1:2 did not differ ($p>0.05$) in S.G and viscosity but differed ($p<0.05$) in their sensory quality (Table 1).

Table 1: Viscosity, specific gravity and texture of hydrolyzed cereal starch[1,2,3] using varying amount of malted rice paste.

Mixing ratio w/w (Rice malt/cereal starch)	Gelatization temp °C	Liquefaction time (min)	Specific gravity	Viscosity (cPs) 60°C	Mouth-feel
3:10	76±2	3.5±0.25	1.173±0.270^{a}	401.67±7.26^{a}	5.0±0.5^{c}
2:5	76±1	3.0±0.05	1.112±0.001^{b}	183.33±6.01^{b}	1.5±0.3^{a}
1:2	76±2	2.0±0.05	1.101±0.001^{b}	163.33±8.82^{b}	2.8±0.5^{b}

[1]Volume of boiling water: 500ml
[2]Each value is the mean±SE of triplicate determination
[3]Different letters within the same column are significantly different ($p<0.05$)

Chemical and physical characteristics

The chemical (pH, titratable acidity) and physical (total soluble solids, viscosity, and specific gravity) quality characteristics of three types of 'kunun-zaki' produced in this study using hydrolytic enzymes are shown in Tables 2 and 3. There was sharp decrease in pH with corresponding increases in titratable acidity in all the products following mixture of the hydrolyzed starch with starter culture and uncooked paste (0h). Such decrease was more pronounced in the slurry treated with combinations of α+β-amylases which dropped from 5.63 (slurry) to 3.73 (0h) and subsequently dropped to 3.30 at the end of 6h fermentation (Table 2). Generally the pH of 'kunun-zaki' produced following hydrolysis with α+β-amylases and α-amylases+amyloglucosidase were not significantly different ($p>0.05$) both at the slurry stage and at the end of fermentation period (6h), but this differed ($p<0.05$) from the 'kunun-zaki' produced following hydrolysis with malted rice (Table 2). An increase in titratable acidity was noticed in the entire product during production and this differed significantly (Table 2).

Table 2: Chemical characteristics of enzyme treated gelatinized millet starch during 'kunun-zaki' production.

Treatment[3]/processing time	Analysis[1,2]	
	pH	Titratable acidity (% lactic acid)
Slurry (ground millet paste)		
α+β-amylases	5.63±0.03[a]	0.314±0.001[b]
α-amylases+amyloglucosidase (AMY)	5.60±0.06[a]	0.297±0.001[c]
Malted rice	5.13±0.09b	0.463±0.001a
0h		
α+β-amylases	3.55±0.01[b]	0.756±0.002[a]
α-amylases+AMY	3.50±0.01[c]	0.693±0.002[b]
Malted rice	3.61±0.01[a]	0.753±0.001[a]
2h		
α+β-amylases	3.55±0.01[b]	0.756±0.002[a]
α-amylases+AMY	3.50±0.01[c]	0.693±0.002[b]
Malted rice	3.61±0.01[a]	0.753±0.001[a]
4h		
α+β-amylase	3.48±0.01[a]	0.782±0.001[a]
α-amylases+AMY	3.49±0.01[a]	0.732±0.001[b]
Malted rice	3.45±0.03[b]	0.782±0.002[a]
6h		
α+β-amylases	3.30±0.06[a]	0.801±0.001[b]
α-amylases+AMY	3.20±0.00[a]	0.762±0.002[c]
Malted rice	3.03±0.03[b]	0.852±0.001[a]

[1]Each value is the mean ± standard error of triplicate determinations.
[2]Different letters within the same column are significantly different ($p<0.05$).
[3]Malted rice- cereal starch ratio; 2:5w/w

The total soluble solids of the three types of 'kunun-zaki' produced in this study as shown in Table 3 showed a decrease while the viscosity showed a concurrent increases during production and their mean values differed significantly (p<0.05). The specific gravity of the finished products (Table 3) shows that the 'kunun-zaki' produced following hydrolysis with α-amylase+ β-amylase was higher and differed significantly (p<0.05) from the other products.

Table 3: Physical characteristics of enzyme treated gelatinized millet starch during 'kunun-zaki' production.

Treatment[3]/processing time	Analysis[1,2,3]		
	Total soluble solids (°Brix)	Viscosity (cps)	Specific gravity
Slurry (ground millet paste)			
α+β-amylases	20.27±0.15[b]	230.7±5.81[a]	1.145±0.028[a]
α-amylases+amyloglucosidase (AMY)	20.83±0.44[b]	196.0±4.93[b]	1.118±0.001[a]
Malted rice	22.33±0.33[a]	183.3±6.01[b]	1.112±0.001[a]
0h			
α+β-amylases	15.33±0.33[a]	ND	ND
α-amylases+AMY	15.70±0.50[a]	ND	ND
Malted rice	15.73±0.50[a]	ND	ND
6h			
α+β-amylases	16.03±0.09[a]	241.7±4.01[a]	1.222±0.002[a]
α-amylases+AMY	14.23±0.12[b]	215.0±8.66[b]	1.203±0.003[b]
Malted rice	15.33±0.33[a]	208.0±1.15[b]	1.118±0.001[c]

[1]Each value is the mean ± standard error of triplicate determinations.

[2]Different letters within the same column are significantly different (p<0.05).

[3]ND- not determined

[4]Malted rice-cereal starch ratio; 2:5w/w

Sensory quality attributes

The mean sensory scores of the three types of 'kunun-zaki' produced using hydrolyzed enzymes are shown in Table 4. There were no significant difference (p>0.05) in all the products in appearance and aroma. However, the 'kunun-zaki' produced following hydrolysis with malted rice was generally preferred in taste and this differed significantly differed (p<0.05) from the other products.

DISCUSSION

'Kunun-zaki' is a popular non-alcoholic cereal beverage whose production is basically a home-based technology. However, intense research is ongoing by researchers in order to generate information that would be useful for its large-scale production. The results obtained in this study show that the pH of all the three (3) types of 'kunun-zaki' produced following hydrolysis of the millet starch with hydrolytic enzymes (α, β, amyloglucosidase or rice malt) showed a marked decrease with concomitant increase in titratable acidity (% lactic acid, Table 2).

Table 4: Sensory quality attributes of 'kunun-zaki' produced using enhanced processing techniques.

'Kunun-zaki' type	Sensory scores[1,2,3]		
	Appearance	Taste	Aroma
Slurry (ground millet paste)	2.0±0.00[a]	2.4±0.40[a]	1.8±0.20[a]
α+β-amylases	1.6±0.24[a]	2.0±0.32[a]	2.0±0.45[a]
α-amylases+amyloglucosidase (AMY)	1.8±0.20[a]	1.0±0.00[b]	1.8±0.37[a]
Malted rice			

[1]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.
[2]Different letters within the same column are significantly different ($p<0.05$).
[3]Malted rice-cereal starch ratio; 2:5w/w.

It has been established by other workers that increase in lactic acid bacteria growth in a fermenting food substrate results in increase in the organic acid content of the food (Efiuvwevwere and Akoma, 1995). The decrease in pH as observed in this study is as a result of increase in the activity of lactic acid bacteria on carbohydrates and other food nutrients to produce organic acid (lactic acid, acetion, acetaldehyde) which has been shown by other workers to improve the sensory and nutritional content of the food product (Steinkraus, 2002; Cooke *et. al*., 1987); and this must have accounted for the acceptable sensory quality of the 'kunun-zaki' produced in this study (Table 4).

The total soluble solids (TSS) of the slurry treated with rice malt as a source of the hydrolytic agent (Table 3) was significantly different ($p<0.05$) from the other treatments (α+ β-amylases, or α+amyloglucosidase). It has been reported that malted paddy rice is an excellent source of α-amylases, debranching enzymes (pullulanase-like enzymes), β-amylases and amyloglucosidase (Manners, 1985; Nakamura, 1996) and has the potential for achieving high starch conversion rate in sugar production (Ayernor and Ocloo, 2007). Therefore, the steady decrease in TSS during production in all the products indicates an increase in microbial activities (LABs) during fermentation which subsequently lowered the pH (Table 3). The diastatic activity of rice malt was optimal at a lower rice malt-cereal starch ratio (2:5) as reflected in their sensory quality (mouth-feel). The 'kunun-zaki' produced using a higher ratio of rice malt-cereal starch (1:2) was inferior in mouth-feel to the one produced using a lower ratio of the rice malt-cereal starch (2:5) as the product was observed to be watery (Table 1).

Furthermore, the viscosity of all the products as shown in Table 3 increased from the slurry stage up to the end of fermentation (6h). It is widely known that some LABs secrete exopolysaccharide (EPS) during fermentation of carbohydrate and these have been shown to contribute to the rheology and texture of the food and, also, impact a preservative effect (bacteriocin) on the fermented food product (Duru-Ozkaya *et. al*., 2007; Welman and Maddox, 2003). It is therefore possible that some of the LABs used as starter culture in this fermentation has the capacity to produce EPS and this could possible explain the increase in the viscosity as was observed at the end of the fermentation (Table 3). The rheological behavior of any food is an important factor when contemplating the design of equipment for large-scale production of the food. Sopade and Kassum (1992) reported that the rheological behavior of 'kunun-zaki' was more of a Newtonian fluid, implying that its viscosity would be fairly constant at each temperature. The results of this study has shown that 'kunun-zaki' of acceptable quality attributes (sensory) could be produced within 7h using combinations of hydrolytic enzymes (α, β, amyloglucosidase or rice malt) and starter cultures of LABs. The result of this study could encourage the large-scale production of 'kunun-zaki'.

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