PROXIMATE COMPOSITION AND MICROBIAL LOAD OF VARIETIES OF MILLET-BASED *KUNUN ZAKI* DRINKS

AHMED EL-IMAM, A. M., & ISMAILA, Z.
Microbiology department, Faculty of Life Sciences,
University of Ilorin, P.M.B. 1515, Ilorin Nigeria.
E-mail: ahmedelimam.am@unilorin.edu.ng
Phone No: +234-806-937-6094

Abstract
Kunun zaki is a cereal-based non-alcoholic beverage widely consumed in Northern Nigeria. The microbial quality of different formulations of millet-based kunun zaki prepared using different ingredients and combinations was investigated. Sample A was prepared with millet, spices and sugar only; B sweet potatoes and less sucrose; C equal amounts of dates and potatoes while D contained potatoes, dates and tiger nuts and no sucrose. Proximate parameters were determined by standard methods. The samples were refrigerated for 14 days and bacterial, fungal and coliform counts were determined at intervals. The compositions were similar except for D which had the lowest calorific value, carbohydrates and lipids. Samples containing sucrose had higher bacterial counts with A having Log_{10} value of 7.9 by Day 9, while samples C and D had the least with 5.9 each. Sample B with the highest carbohydrate, lipid and ash contents also had the highest microbial burden. Sample C also had the least total coliform counts and microbial load. Bacterial counts surpassed fungal counts throughout the experiments, and coliforms, Staphylococcus sp, Lactobacillus, Bacillus and Listeria sp were recovered at different timepoints. Aspergillus sp and Saccharomyces were the fungi isolated. It is thus recommended that sucrose be replaced with other sweeteners and saccharifying agents in kunun zaki production as sucrose results in foods with high calorific values, and encourages bacterial proliferation which increase the risks of food borne diseases to consumers.

Keywords: *Kunun zaki*, sucrose, bacterial contamination, tigernuts, proximate analysis, composition

Introduction

Kunu is an important non-alcoholic beverage mostly found in Northern Nigeria but consumed all over the country. It is prepared from various grains (Nahemiah *et al.,* 2014) including sorghum (*Sorghum bicolor*), maize (*Zea mays*) and most commonly, the *gero* and *maiwa* varieties of millet (*Pennisetum glaucum*). It is usually prepared with various condiments such as potatoes (*Ipomoea* batatas), dates (*Phoenix dactylifera*) and tigernut (*Cyperus esculentus*); and spices including (*Zingiber officinale*), black pepper (*Piper nigrum*) grains of Selim (*Xylopia aethiopica*) and cloves (*Syzygium aromaticum*).

Kunu is consumed by people of all age groups and social classes and while it is generally made and consumed at home, it is increasingly being served as a refreshment in social gatherings. This drink's increasing popularity in Nigeria is due to declining purchasing power following an economic recession and inflation which has made soft drinks less affordable despite the continuous efforts of companies to decrease the retail sizes (and thus, prices).

It is also packaged in reused bottled water containers and hawked or sold in shops at prices ranging from N70 - N100 for a 75 cl bottle (in Ilorin Nigeria). In addition to its importance as a refreshing drink, Olasupo *et al.* (2002) reported that fermented cereals like *ogi* and *kunu*, are important weaning foods for infants.

The *kunu* preparation process includes washing and steeping the grains for between 12 - 24 hours, wet milling with the spices and gelatinization of about three-quarter of the mixture with boiling water, followed by pitching with the one-quarter ungelatinized part of the mixture. (Oranusi *et al.,* 2002*;* Umaru *et al.,* 2014). The slurry is then mixed and sieved before or after it is left to ferment for between 6-24 hours. The supernatant is then diluted (if required), sweetened to taste, then poured into containers and refrigerated immediately. As can be expected, the application of the hazard analysis and critical control point (HACCP) strategy to *kunu* production identified various critical control points (CCP) that could cause rapid deterioration. For instance, the gelatinization stage could cause spores to germinate, pitching could cause contamination, while an inadequately low pH could cause proliferation of organisms such as *B. cereus* (Oranusi *et al.,* 2003). These factors in addition to poor water quality and unhygienic handling practices can cause the final product to have a high microbial load.

Due to increasing health awareness, many consumers now prefer foods with low or no refined sugar and prefer foods containing different sweetening and saccharifying agents such as potatoes (Egbere *et al.,* 2008), honey and dates to sweeten their *kunu*. Thus, there are significant variations in the production of *kunun zaki* depending on taste, with some producers incorporating carbohydrate-rich saccharifying adjuncts such as sweet potatoes, dates and tigernuts with lower sugar contents, while others utilise only grains and sweeten appropriately with sugar. This expectedly results in variability in the nutritional profile of the *kunun zaki* (Table 1). Since its nutrient profile and high water activity make it a conducive environment for microbial growth it is subjected to rapid deterioration and can be a vehicle for food borne diseases and a large number of lactic acid bacteria, coliforms, moulds and yeasts have been implicated in the production of undesirable changes in *kunu* (Ndidi *et al*., 2012).

This study thus investigates the effects of various formulations and amounts of different ingredients in *kunun zaki* production on the proximate composition and microbial counts of the final *kunun zaki* product held at 4 ^{o}C over 14 days.

Materials and Methods

Millet, spices (ginger, cloves, black pepper, alligator pepper), sweet potatoes, tigernuts, dates, and sugar were procured from Oja-Oba market, Ilorin, Kwara State. All the materials were sorted and only those in good condition were used.

Kunun-zaki Preparation

The millet was washed wit tap water and steeped for 24 h decanted and rinsed, It was then mixed with washed spices namely ginger, cloves, black pepper and grains of Selim. Four different samples of *kunun-zaki* were produced in the laboratory and they contained various

amounts of potatoes, tiger nuts and dry dates in amounts similar to those commonly used in traditional production (See Table 1 for composition).

The slurry obtained was divided into two portions of a fifth which was kept aside, and four fifths which was gelatinized by the addition of boiling water. It was then covered and left to cool for about 90 minutes and pitched with the remaining slurry and stirred. The slurry was sieved, and the supernatant was diluted to the same final volume (results not shown), covered and left to spontaneously ferment for at least six hours at room temperature. This was then sweetened as needed with sugar and packed in bottles in the refrigerator.

The preparation steps for the various *kunun zaki* samples are demonstrated in the flowchart below (Figure 1).

Enumeration of microorganisms

Each sample was serially diluted appropriately with sterile distilled water before inoculation by the pour plate technique on Nutrient Agar (NA), Eosin Methylene Blue (EMB) and Potato Dextrose Agar (PDA) for the enumeration of total aerobic, coliform and fungal counts respectively. The NA and EMB plates were incubated for 24 h at 37 ^{o}C while PDA plates were incubated at room temperature (28 ± 3 ^{o}C) for seven days. Pure cultures of isolates were obtained and stored as slants in McCartney bottles at 4 ^{o}C pending identification.

Enumeration and Identification of Isolates

Microorganisms were counted in duplicates using the pour plating technique. Nutrient agar (NA) Potato dextrose agar (PDA), MacConkey agar (MCA), and de Mann, Rogosa and Sharpe agar (MRS) were used for the isolation of total viable bacteria, fungi, coliforms and lactic acid bacteria respectively. Counts were expressed as average colony forming units per milliliter (cfu/ml) along with the standard deviations. Representative colonies were purified and stored in agar slants at 4 ^{o}C pending identification. Bacterial isolates were identified phenotypically using the 9th edition of the Bergey's Manual of Determinative bacteriology while fungal isolates were identified based on colonial appearance and microscopic characteristics (Alexopoulos and Mims, 1979; Barnett and Hunter, 1987).

Proximate analyses

The crude protein, moisture, ash, crude fibre and lipid contents of the *kunun-zaki* samples were determined according to the AOAC (2000) methods. Carbohydrate content was measured using the estimation by difference method of Pearson (1976) and calorific values by the at water factor method of Hunt *et al.* (1987).

Determination of pH and titratable acidity

About 100 ml of each sample were dispensed into a beaker and thoroughly mixed. The pH was measured using a pH meter (Hanna's instrument, Padova, Italy). Titratable acidity (%) was determined by titrating 10 ml of samples against 0.2 N NaOH containing drops of 1% phenolphthalein indicator and calculated using the formula below:

$$\text{Acidity (\%)} = \frac{\text{volume of NaOH used} \times 0.2 \times 90 \times 100}{10 \text{ ml} \times 1000}$$

Results and Discussion

Proximate analyses revealed that the various *kunu* samples were of similar compositions (Table 1), and this was because while it was important to prepare the samples as is normally produced, care was taken to avoid considerable variation among samples which could affect the ability to compare them realistically. The moisture content of 60.9 – 69.3 % though high, as *kunu*n-zaki is a thin liquid especially compared to *ogi/akamu* which is a custard-like gel, is still lower than figures reported in literature (Table 2). This is due to the 200 g of potatoes added which increased the total solids content and lower quantities of water added during dilution, both of which produce a thicker and more nutritious *kunu* product, and this also explains the high ash contents. The lower ash contents in sample D is due to the lower amounts of adjuncts as only 100 g of potatoes was included. With a pH of 5.3, the millet-only *kunu*, sample A, was significantly less acidic than the other samples and this could be due to lower starch content. Sample B had the lowest pH at 3.2 and this is likely due to the starch from the high amount of added potatoes which may be hydrolysed into acidic compounds similar to what obtains in garri fermentation. There are previous reports that the formulation of *kunun-zaki* with nutritionally rich composites resulted in superior proximate compositions to the traditional beverage (Terna & Ayo, 2002; Oluwajoba *et al.,* 2013). The titratable acidity values were inversely related to the pH values and were similar to the findings of Efiuvwevwere and Akona (1995) who ascribed the observed acidity to the fermentation activities of *Lactobacillus leichmannii* and *L. fermentum*; and to values by Osuntogun and Aboaba (2004) but lower than those recorded by Terna *et al.* (2002a).

The drinks were found to be rich in carbohydrates and the composition similar to those reported by various other authors (Table 3). It is evident that the composition of *kunu* varies considerably widely and this could be due to the moisture contents (which influences most other parameters). The thickness of the final product is depends on the desired taste and skill of the producer. The carbohydrate and energy values were similar, except with sample D which was the lowest. *Kunu* D (with no sugar or dates) is also lowest in lipids and calorific value, this suggests that sample D is the most ideal for *kunu* consumers trying to reduce their calorie intake and weight. Conversely, *kunu* samples B and C is ideal for children and convalescents as they have high calorific value and lipids. The high acidity figures indicated that the fermentation was successful, and this intrinsic acidity helps decrease the risk of contamination by acid-sensitive pathogens.

The bacterial counts recorded in the samples are similar to the total aerobic counts of Log_{10} values of 4.18 – 8.79 recorded by Oranusi *et al.,* (2003). The low pH values of the drink indicate that only acidophiles such as bacteria may thrive in the drink and if held at refrigeration temperatures 4 ^{o}C – 8 ^{o}C) for short durations, then it is likely safe for consumption.

In all samples, bacteria were more numerous than fungi, except in sample D where the yeasts eventually became as numerous as the bacteria. Sample A had the highest bacterial and fungal counts overall, as the bacterial counts exceeded a Log_{10} count of 7.0 from Day 2 to 9 while fungal counts peaked at Log_{10} 6.0 on Day 2. This appears to be due to the high sucrose content which made it ideal for the proliferation of microbes, as it has been reported that osmotolerant and acid-tolerant yeasts and bacteria can grow in and spoil sugar-

sweetened drinks (Thompson, 2009). The mild pH of 5.3 is also favourable for fungal and some bacterial growth. Sample B had the next highest count at a Log_{10} figure of 8.29 by Day 9. This confirms that the sucrose content contributed to the high counts in *kunun-zaki.* The replacement of sugar completely with potatoes and dates resulted in a nearly 2-log reduction in bacterial counts (see graphs A and C in Figure 2). Contrarily, the replacement of dates with tigernuts in D did not affect bacterial counts, but increased yeast counts. It is thus clear that the presence of sucrose encourages bacterial proliferation; and the higher the sucrose content, the more prone *kunun zaki* is to bacterial spoilage.

It is observed that in all samples the fungal counts were more stable than the bacterial counts and after Day 2, all fungi recovered were yeasts putatively identified as *Saccharomyces sp. Aspergillus* species were only recovered on Days 1 and 2 and only from Sample A and all other isolates were uniformly isolated from all samples. Bacterial counts in all samples rose and then declined over time, presumably due to increased acidity which is unfavourable to bacteria.
There are several reports of coliforms in *kunun-zaki* (Ugwuanyi *et al.,* 2015; Olopade *et al.*, 2015; Asuquo *et al.,* 2017) and the source here could be from the grains and environment as tap water and strict hygiene practices were employed in the *kunu* preparation. Total coliform counts were around Log_{10} value 3.0 in all samples tested and were several orders lower than reported in literature such as up to 4.83 x 10^8cfu/ml a Log_{10} value of about 7.68 (Asuquo *et al.,* 2017) and 30 % occurrence of *Escherichia coli* in hawked *kunu* (Ugwuanyi *et al.,* 2015). Sample A had the highest count of 6.0 by Day 2 and this could be due to the low acidity of the traditional *kunu* product. The general trend in all samples is that the counts increased slightly, dipped between Days 2 and 4 and then persisted at low levels throughout the storage period.

The bacterial and fungal species isolated from the *kunu* samples are presented in Table 4. By Day 14 all samples had begun to produce off-odours. Similar to these findings, Ayo *et al*. (2004) have previously reported the recovery of *Enterobacter* sp.*,* and *Staphylococcus* species among others in *kunun zaki* sold in a polytechnic community in Nigeria. Similar to the findings of this study, Egbere *et al.* (2008) and Amusa and Odunbaku (2009) reported *Lactobacillus plantarum*, *Bacillus subtilis,* and other bacteria in freshly processed, hawked *kunun zaki* sold in Northern and southwestern Nigeria respectively, while Ugwuanyi *et al.* (2015) reported the presence of *Lactobacillus sp*, *Bacillus sp* and *E.coli*. The presence of *E. coli* and *Enterobacter aerogenes* indicates contamination with fecal origins and could have been introduced into the *kunu* through the water used to wet-mill the grains. Some strains of *E. coli* are acid-resistant (Gorden & Small, 1993) and this may explain their persistence through the initial fermentation. By Day 7, *E. coli* was no longer recovered from the *kunu* and this is similar to the findings of Oshoma *et al.* (2009) who reported that *E. coli* inoculated into *kunu*n-zaki survived for longer when the *kunu* was held at -4 ^oC than at a higher temperature, and this explains their eventual absence at refrigeration temperature. The continued fermentation at 4-8 ^oC made the *kunu* too acidic to continue to support *E. coli.*

However, while leaving *kunu* to ferment in the fridge might eliminate many bacteria over time, it encouraged the growth of an organism putatively identified as the human food

pathogen *Listeria sp* from initial non-recoverable levels which is a source of concern. The pathogens *Pseudomonas sp, Streptococcus sp, Salmonella sp* and *Shigella sp* and the mycotoxigenic mould *Fusarium sp* reported by other authors (Ugwuanyi *et al.,* 2015; Mbachu *et al*., 2014; Ogbonna *et al*., 2011; Edward & Ohaegbu, 2012) were not encountered in this study. This could be due to a thorough washing step which removed most of the mould spores; and an adequate fermentation process which made the *kunun zaki* non-conducive for the growth of most pathogens while sustaining *Lactobacillus sp* throughout the period of study.

Staphylococcus aureus produces toxins that could cause food poisoning and toxic shock syndrome. Some species of *Bacillus* can cause bacteremia/septicemia, endocarditis and respiratory tract infections. Some of the fungi isolated from *kunu* drink produce mycotoxins. For instance, *Penicillium, Fusarium* and *Aspergillus* species are known to produce mycotoxins in food products such as corn, rice, wheat etc (Ahmed El-Imam *et al.,* 2012; Dubey and Maheshwari, 2013). There is thus a need to maintain appropriate food handling practices during processing and the preparation of the *kunu* to eliminate the microbial contaminant and to improve the quality of the final product. There is also the need to employ adequate preservative measures to improve the shelf life of the *kunun zaki.*

Conclusion

The results of the study revealed that kunu prepared with less sugar, and had potatoes, dates and tigernuts as adjuncts were more acidic, had more minerals and lipids than the traditionally-prepared *kunu.* Also the addition of sugar resulted in higher bacterial counts in the *kunun zaki* samples whereas replacement of sucrose with other saccharifying/sweetening agents like dates and tigernuts resulted in a decrease in bacterial counts. It was observed that all the *kunun zaki* samples were contaminated with pathogenic organisms; and of particular note, coliform organisms which is however similar to findings in several studies involving *kunu,* where higher counts have even been reported. More research should be done to more accurately determine the status of *Listeria*, and investigate its source and fate in the various *kunu* samples.

It is thus recommended that *kunun zaki* production with non-sugar saccharifiers such as dates and tigernuts should be encouraged. There is a need to further improve sanitary conditions during the preparation and handling of *kunun zaki* to minimise microbial contamination. Finally, the Nigerian Agency for Food and Drug Administration and Control (NAFDAC) should sensitize citizens on the good manufacturing practices for this important beverage and ensure compliance with approved standards.

References

Ahmed-El-Imam, A. M., Ameh, J. B., & Abdullahi, I. O. (2012). Occurrence of fumonisins and deoxynivalenol in stored maize used in industrial productions in Zaria Nigeria. *African Journal of Food Science*, *6*(9), 249-252.

Alexopoulos, C. J., & Mims, C. W. (1979). *Introductory Mycology*. John Willey and Sons Inc. New York, U.S.A.

Amusa, N. A., & Odunbaku, O. A. (2009). Microbiological and nutritional quality of hawked *kunu* (a sorghum based non-alcoholic beverage) widely consumed in Nigeria. *Pakistan Journal of Nutrition, 8*(1), 20-25.

AOAC. Official Methods of Analysis (2000). 17th Ed. *Association of official analytical chemists.* Washington D.C., USA.

Ayo, J. A., Umianze, H., & Gaffa, T. (2004). Microbiological evaluation of "*Kunun Zaki"* and "*Zoborodo"* drink (beverages) locally produced and sold in a polytechnic community in Nigeria. *Nigeria Food Journal, 22*(1), 119-126.

Dubey, R. C., & Maheshwari, D. K. (2013). *A textbook of Microbiology.* Revised edition. S. Chad and Company Ltd. New Delhi, India.

Gorden, J., & Small, P. (1993). Acid resistance in enteric bacteria. *Infection and Immunity*, *61*(1), pp.364-367.

Edward, K. C., & Ohaegbu, C. G. (2012). The effect of ginger and garlic on the microbial load and shelf life of *Kunu*n-zaki. *Journal of Applied Pharmaceutical Science*, *2*(5), 150-153.

Efiuvwevwere, B. J. O. & Akona, O. (1995). The Microbiology of '*kunu*n-zaki', a cereal beverage from Northern Nigeria, during the fermentation (production) process. *World Journal of Microbiology and Biotechnology*, *11*(5), 491-493.

Egbere, O. J., Luka, J., Soo, J. N., Zumbes, H. I., Chollom, P., & Henry, U. I. (2008). A survey of local preparation methods and quality of Kunun-Zaki in two metropolitan towns of northern Nigeria. *Global Journal of Pure and Applied Sciences, 14*(4), 383-388.

Hunt, C., Burn, P., & Adamezul, Z. C. (1987). Proximate composition and fat type of three (3) popular takeaway meals. *International Journal of Food Science & Technology, 22,* 669- 759.

Mbachu, A. E., Etok, C. A., Agu, K. C., Okafor, O. I., Awah, N. S., Chidi-Onuorah, L. C., Ekwueme, V. C., Okpala, J., Ogbue M. O., & Ikele, M. O. (2014). Microbial quality of *kunu* drink sold in Calabar, Cross River state, Nigeria. *Journal of Global Biosciences*, *3*(2), 511-515.

Nahemiah, D., Bankole, O. S., Tswako, M., Nma-Usman, K. I., Hassan, H., & Fati, K. I. (2014). Hazard analysis critical control points (HACCP) in the production of Soy-kunun Zaki: A traditional cereal-based fermented beverage of Nigeria. *American. Journal of Food Science & Technology*, 2(6), 196-202.

Ndidi, U. S., Odoh, C. O., & Saleeman, A. O. (2012). Effect of storage conditions on the physicochemical properties and microbial load of *kunu*, soymilk, yoghurt and *zobo* drinks. *International Journal of Food Nutrition and Safety, 2*(1), 17.

Ogbonna, I. O., Opobiyi, M. Y., Katuka, B., & Waba, J. T. (2011). Microbial evaluation and proximate composition of *Kunun zaki*, an indigenous fermented food drink consumed predominantly in northern Nigeria. *Internet Journal of Food Safety, 13,* 93-97.

Olopade, B. K., Obiahu, N. V., Taiwo, O. S., & Oranusi, S. U. (2015). Microbiological and nutritional quality of *kunu-zaki* made from different grains. In: 5th International Biotechnology Conference, Exhibition and Workshop, July 8 – 10, 2015, Covenant University, Ota, Nigeria.

Olasupo, N. A., Smith, S. I., & Akinside, K. A. (2002). Examination of the microbial status of selected indigenous fermented foods in Nigeria. *Journal of Food Safety, 22*(2), 85.

Oluwajoba, O. S., Akinyosoye, F. A., & Oyetayo, O. V. (2013). Comparative sensory and proximate evaluation of spontaneously fermenting kunu-zaki made from germinated and ungerminated composite cereal grains. *Food Science and Nutrition 1*(4), 336-349.

Oranusi, S. U., Umoh, V. J., & Kwaga, J. K. P. (2003). Hazards and critical control points of *Kunun-zaki*, a non-alcoholic beverage in Northern Nigeria. *Food Microbiology, 20,* 127-132.

Oshoma, C. E., Aghimien, M. O., & Bello, Z. O. (2009). Growth and survival of *Escherichia coli* in *kunun zaki* during storage. *World Journal of Agricultural Sciences*, *5*(4), 494-497.

Osuntogun, B., & Aboaba, O. O. (2004). Microbiological and physico-chemical evaluation of some non-alcoholic beverages. *Pakistan Journal of Nutrition*, *3*(3), 188-192.

Pearson, D. (1976). *The chemical analysis of food.* Churchill Livingstone, Edinburgh.

Terna, G., Jideani, I. A., & Nkama, I. (2002a). Nutritional composition of different types of *Kunu* produced in Bauchi and Gombe States of Nigeria. *International Journal of Food Properties, 5*(2), 351-357.

Terna, G., Jideani, I. A., & Nkama, I. (2002b). Nutrient and sensory qualities of *kunu*n-*zaki* from different saccharifying agents. *International J. Food Science Nutrition, 53,* 109-115.

Thompson, S (2009). Microbiological spoilage of High-Sugar Products. In W.H. Sperber & M. P. Doyle (Eds.), *Compendium of the microbiological spoilage of foods and beverages* (pp. 252). New York, USA: Springer Science.

Ugwuanyi, R. C., Seghosime, R. A., & Gloria, T. O. (2015). Assessment of the microbiological quality of kunu zaki sold at Gariki, Enugu State, Nigeria. *International Journal of Microbiological Research, 6*(2), 138-144.

Umaru, G. A., Tukur, I. S., Akensire, U. A., Adamu, Z., Bello, O. A., Shawulu, A. H. B., & Audu, M. (2014). Microflora of *kunun zaki* and *Sobo* drinks in relation to public health in Jalingo Metropolis, North-Eastern Nigeria. *International Journal of Food Research, 1,* 16 – 21.

Table 1: Composition of the various *kunu* samples

Sample	Millet (kg)	Spices (g)	Sucrose (g)	Potato (g)	Dates (g)	Tiger nut (g)
A	1.0	129.0	100.0	--	--	---
B	1.0	129.0	50.0	200.0	--	---
C	1.0	129.0	--	100.0	100.0	---
D	1.0	129.0	--	100.0	--	60.0

Table 2: Proximate Analysis of various *Kunu* Samples

Sample	MC (%)	Carbo-hydrates (%)	Ash (%)	Total Protein (%)	Calorific Value (KJ/100g)	Crude lipids (%)	Crude Fibre (%)	% Acid	pH
A	69.25 ± 0.037^{a}	24.37 ± 0.10^{a}	4.88 ± 0.024^{a}	3.31 ± 0.30^{a}	1700.01 ± 208.45^{a}	1.22 ± 0.13^{a}	0.90 ± 0.13^{a}	2.35 ± 0.21^{a}	5.3
B	60.90 ± 5.60^{b}	25.34 ± 3.71^{a}	4.98 ± 0.002^{a}	5.20 ± 1.32^{b}	1781.05 ± 110.29^{a}	2.68 ± 0.70^{b}	0.99 ± 0.13^{b}	7.00 ± 0.13^{b}	3.2
C	61.39 ± 3.91^{b}	26.01 ± 3.10^{a}	4.82 ± 0.08^{a}	4.94 ± 0.91^{c}	1722 ± 3.91^{a}	1.79 ± 0.10^{c}	0.85 ± 0.09^{a}	2.68 ± 0.24^{c}	4.0
D	64.96 ± 0.20^{a}	28.6 ± 0.00^{a}	3.93 ± 0.013^{b}	1.54 ± 0.12^{d}	810.10 ± 4.47^{b}	0.18 ± 0.06^{d}	0.79 ± 0.001^{c}	2.83 ± 0.26^{c}	3.8

Values are means of triplicate measurements ± SD. Values with the same superscript are not significantly different at $p > 0.05$. See Table 1 for composition of samples A-D.

Table 3: Composition of *kunu*n-zaki as reported by various authors

Nutrient	Amount	Reference
Moisture	89.77-91.54 %; 87 – 91 %	Terna & Ayo, 2002; Terna *et al.,* 2002b
Carbohydrate	2.42 – 3.22; 2.66 – 7.92 %; 2.69 – 5.84 %	Terna & Ayo, 2002; Terna *et al.,* 2002a; Terna & Ayo, 2002; Terna *et al.,* 2002b;
Protein	4.48 - 5.39 %; 2.52 – 4.03 %; 3.19 – 7.86 %	Terna & Ayo, 2002; Terna *et al.,* 2002a; Terna & Ayo, 2002; Terna *et al,* 2002b
Fat	0.32 – 0.34; 2.66 – 7.92 %; 0.37 – 0.75 %	Terna & Ayo, 2002; Terna *et al.,* 2002a; Terna & Ayo, 2002; Terna *et al.,* 2002b;
Ash	1.22 – 1.30; 0.93 – 1.20 %	Terna & Ayo, 2002; Terna *et al.,* 2002b;
pH	4.92 – 5.43; 3.12 – 5.46; 5.79	Terna & Ayo, 2002; Terna *et al.,* 2002a; Osuntogun and Aboaba (2004)
Tit. Acidity	0.02 – 0.11 %; 2.30 %	Terna *et al.,* 2002a; Osuntogun and Aboaba (2004)

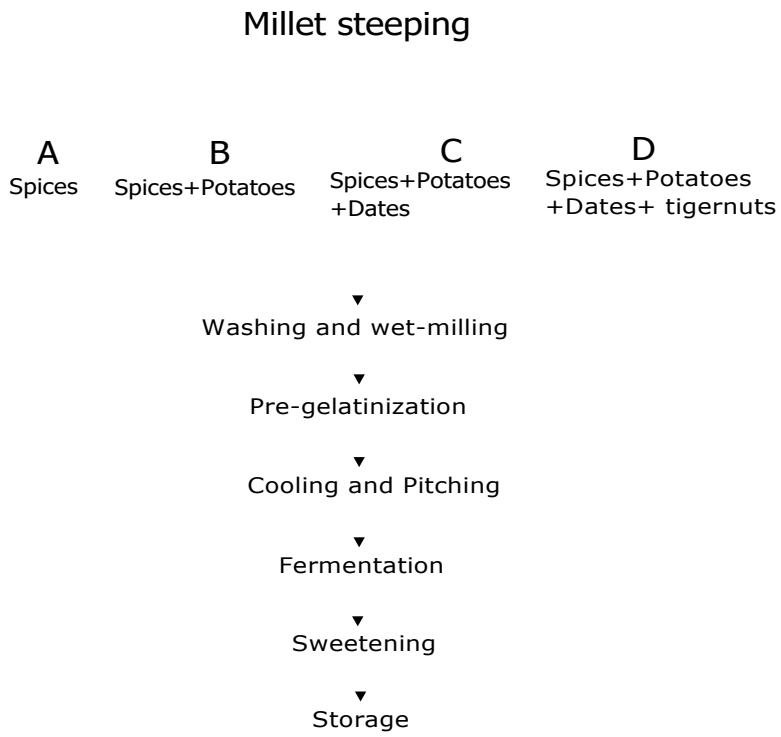
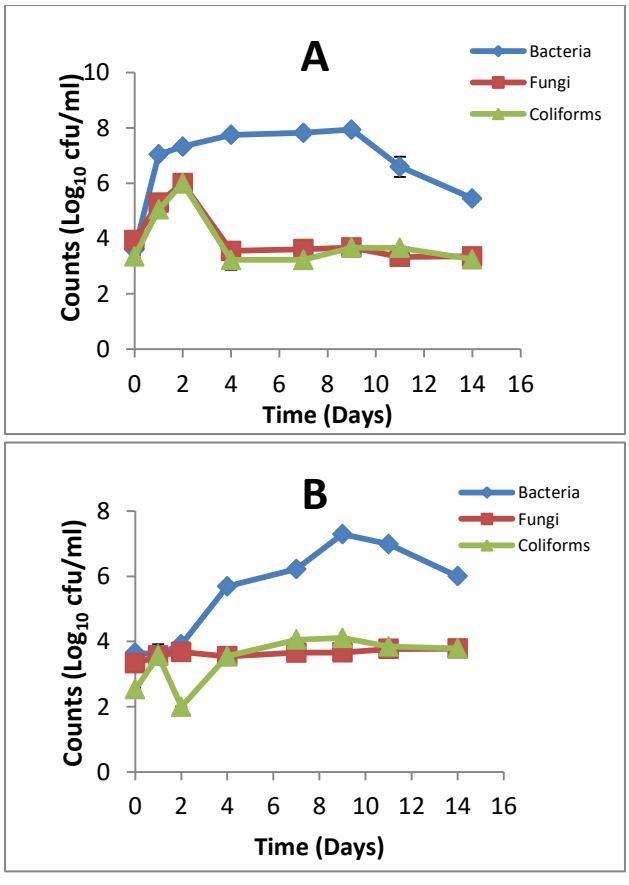


Figure 1: Preparation of the various *kunun zaki* samples. Proportions of various components as in Table 1.



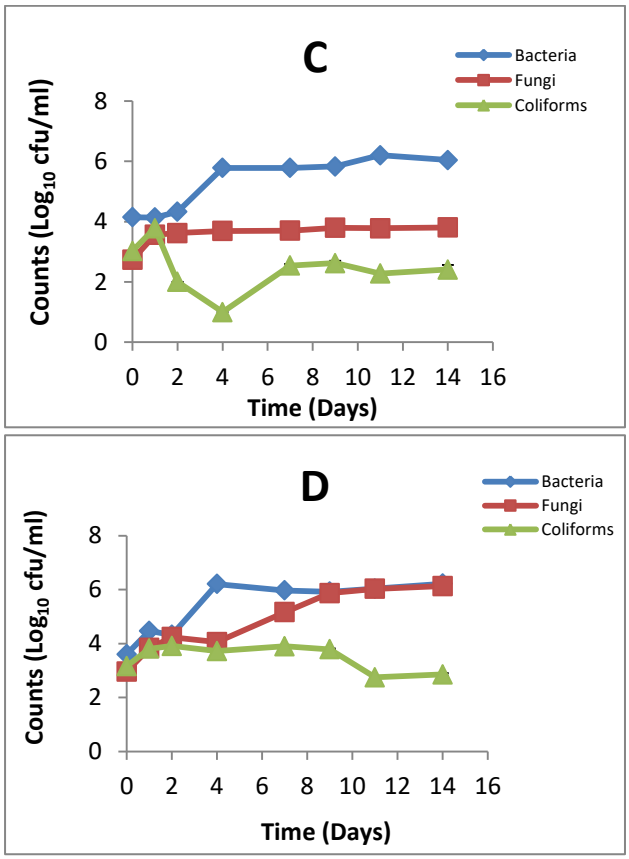


Figure 2: Log_{10} of bacterial and fungal counts of refrigerated samples A, B, C and D over 14 days. The values are averages of triplicates measurements ± SD (Some error bars are too small to be visible)

Table 4: Microorganisms recovered from *kunu* during refrigerated storage over 14 days

Microorganism	Day 0	Day 1	Day 2	Day 7	Day 9	Day 11	Day 14
Bacteria							
Lactobacillus sp	+	+	+	+	+	+	+
Bacillus sp	+	+	+	+	+	+	+
Staphylococcus aureus	+	+	+	-	-	-	-
E. coli	+	+	+	-	-	-	-
Staphylococcus epidermidis	+	+	-	-	-	-	-
Enterobacter	+	+	-	-	-	-	-
Listeria sp	-	-	-	+	+	+	+
Fungi							
Aspergillus flavus	-	+	+	-	-	-	-
Aspergillus terreus	-	-	+	-	-	-	-
Saccharomyces sp	+	+	+	+	+	+	+