# CO-FERMENTATION OF MILLET AND AFRICAN BREADFRUIT SEEDS FOR THE PRODUCTION OF KUNNU AND EFFECT ON MICROBIAL QUALITIES

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Abstract: This study conducted the co-fermentation of pearl millet (*Pennisetum glaucum L. R. Br.*) and African breadfruit (*Treculia africana* Decne) seeds for the production of nutritionally improved kunnu (tentatively called kunnu-ukwa). Appropriate technologies were used to process pearl millet and African breadfruit seeds in varying proportions to determine the blend that would give the required consistency. The final blend (70% millet and 30% African breadfruit seeds) were then used for the formulation of 'kunnu-ukwa' that requires little cooking. Samples were obtained at various stages of production and examined microbiologically for potential pathogens. Kunnu-ukwa recorded total viable counts of 3.09 -3.12 $\log_{10}$ cfu/mL and fungal counts of 1.52-1.62 $\log_{10}$ sfu/mL. *Bacillus cereus*, salmonellae and staphylococci were insignificant in the freshly produced products. Kunnu-ukwa and kunnu-zaki (control) were subjected to physicochemical and sensory evaluation. Kunnu-ukwa had a pH range of 4.50-4.56. Kunnu-ukwa had higher crude protein and ash contents than the control sample (5.92 vs 2.04%) and (4.91 vs 3.15%), respectively. Moisture, fibre and carbohydrate contents were comparable in both samples. The control sample recorded highest value for total solid (13.05%) and specific gravity (0.83%). Except for thiamine, kunnu-ukwa had higher values for vitamin C, riboflavin and niacin. No significant differences ($p>0.05$) were observed for mineral and sensory qualities of the samples. Overall, kunnu-ukwa recorded higher values than the control sample. Fortification of pearl millet with African breadfruit seeds for kunnu-ukwa production gave values of improved nutritional qualities and is recommended for use in areas where kunnu products are consumed and protein intake is inadequate.

**Keywords:** Co-fermentation, fortification, pearl millet, *Treculia africana*, food safety, biocompatibility

## Introduction

Kunun is a sweetened cereal-based, non-alcoholic beverage in Nigeria, prepared traditionally using millet, sorghum, maize or in a composite form as millet and maize grains usually in mixing ratio 1:2 (w/w) maize/millet (Odunfa and Adeyeye, 1985; Gaffa and Ayo, 2002). The manufacture and consumption of kunun is high, on a local scale, particularly in Northern Nigeria. The manufacturing process is very crude involving the use of household utensil and is still largely done in homes. Also, the method of production varies from different localities owing to preferences in consumer taste, texture or appearance (Gaffa *et al.*, 2002). Millet, dry or fresh sweet potato (*Ipomea batatas*) chips, ginger (*Zinigiber officinale*) and other locally sourced spices like clove, red or black pepper, are major ingredients required for the production of kunun. Brief fermentation usually occurs during kunun processing. This brief fermentation which usually occurs during steeping of the grains in water over an 8 – 48 h period is known to involve mainly lactic acid bacteria and yeast (Odunfa and Adeyeye, 1985). Microbial fermentation provides a way to food preservation, reduction in volume of materials to be transported, inhibition of microbial growth, improvement of appearance and taste of some foods and reduces the energy required for preparing food, ensures safer food products and enhancement of nutritive values (Onuorah *et al.*, 1987; Onilude *et al.*, 2004; Oyeyipo, 2011; Onasoga *et al.*, 2014). The unhygienic conditions of production are a great problem facing many traditionally fermented cereal foods in Africa (Sanni, 1999). Unhygienic processing conditions and environment, contaminated utensils and materials as well as poor personal hygiene of the food handlers are major sources of microbial contamination, leading to short shelf lives of these foods and, more significant, food safety hazards. Spontaneous cereal fermentations are usually carried out by lactic acid bacteria and yeast. Lactic acid bacteria are known to produce metabolites such as diacetyl, lactic and acetic acids, which are natural preservatives (Efiuvwevwere and Akoma, 1993; Ayo *et al.*, 2004; Akoma *et al.*, 2006). The final product can then be sweetened with sugar, bottled or sealed in small polythene bags for sale. This traditional process described is implemented in Kano and Niger states of Nigeria.

This non-alcoholic beverage is however becoming more widely accepted in several other parts of Nigeria including the south western parts of Nigeria owing to its refreshing qualities. Kunun is consumed anytime of the day by both adult and children as breakfast or food/drink complement. It is a refreshing drink usually used to entertain visitors, as an appetizer and is commonly served at social gathering (Gaffa *et al*, 2002; Akoma *et al.*, 2002). Onuorah *et al.* (1987) reported that kunnu is regarded as after meal drinks or refreshing drinks in rural and urban areas. Although there are various types of kunun processed and consumed in Nigeria, including, kunun-zaki, kunun-gyada, kunun-akamu, kunun-jiko, kunnu-amshau and kunun-gayamba, depending on ingredients and locality. However, kunun-zaki is the most commonly consumed (Abidoye *et al.*, 2016; Agarry *et al.*, 2010).

Cereals generally have low essential amino acid contents which may cause protein malnutrition, therefore their nutritional value are greatly limited. Direct amino acid supplementation has been shown to improve the nutritive value of corn-based products (Adeyemi and Umar, 1994; Ayo *et al.*, 2013). A major drawback of this approach is the fact that most corn-based products including "kunnu" are produced in homes, by traditional methods, and do not include direct amino acid supplementation. Steeping, milling and sieving are the processing steps during which considerable nutrient losses take place. Much of the protein in cereal grains is located in the testa and germ which are usually sifted off during processing. Grains such as millet are the main staple for millions of people in Nigeria and in Africa and are well known for poor quality and low concentration of their protein (Makinde and Oyeleke, 2012; Adelekan *et al.*, 2013; Ayo *et al.*, 2013).

Protein deficiency is still a major problem in Nigeria and in Africa, particularly, among the low-income groups (Oyeyipo, 2011; Onasoga *et al.*, 2014). Therefore, there is need to open up other sources of protein to alleviate this problem. *Treculia africana* Decne commonly called African breadfruit is a member of the Moracea family; widely grow in southern

states of Nigeria for it seeds and commonly called "ukwa" while the Polynesian breadfruit (*Artocarpus altilis*) is seedless. The African breadfruit seed contain 19-23% crude protein, 11% crude fat and are good source of potassium and phosphorus (Oyeyipo, 2011; Nwabueze, 2007). The fat or oil can be used for industrial purposes and also for human consumption due to its high food energy value. The high essential amino acid in African breadfruit seeds could make it suitable to fortification processes (Oyeyipo, 2011, Onasoga *et al.,* 2014). African breadfruit seeds have been used in a variety of ways traditionally and serve as low cost protein substitutes for poor families in some communities. Extensive work has been done to elucidate the nutritional qualities of the seeds of *Treculia africana* (Nwabueze, 2007; Onyekwelu and Fayose, 2007; Oyeyipo, 2011, Onasoga *et al.,* 2014). However there is need to diversify the use of the seeds by developing non-traditional foods or fortifying traditional foods which would enhance wider utilization of the seeds, otherwise, the use of the seeds may be limited to only traditional culinary preparations when the seeds have potentials for wider applications.

There is little or no information on the co-fermentation of pearl millet and African breadfruit seeds for production of kunnu-ukwa by traditional/laboratory processing methods of fermentation. Therefore, this study was aimed at formulating a cheap processing technique (co-fermentation) that can be practiced at household level to produce nutritionally enriched foods with low-cost, biocompatible and locally available raw materials.

## Materials and Methods

### *Source of pearl millet, African breadfruit seeds and other ingredients*

Pearl millet (*Pennisetum glaucum L. R. Br*), African breadfruit (*Treculia Africana* Decne) seeds, black pepper, red pepper (*Capsicumannum*), ginger (*Zingiber officinale*), cloves (*Syzygium aromaticum*) and granulated sugar were purchased from Mile 12 Market, Lagos State, Nigeria.

### *Production of kunnu-ukwa*

There are slight variations from locality in the methods adopted for kunnu production. Winnowing and hand sorting of the grains/seeds were carried out to remove damaged or bad grains/seeds. The method (Fig. 1) of Ayo *et al.* (2013) was adopted in the production of kunun-*ukwa*, with some modifications (Fig. 2). 70 g of cleaned pearl millet grains and 30 g of cleaned African breadfruit seeds were washed and steeped in clean water for 48 h to soften the grains/seeds. The grains/seeds were washed to remove stones and wet milled along with added spices (65 g ginger, 10 g red pepper and 15 g sweet potatoes) into slurry. Two-third of the slurry was mixed with 2500 mL of boiling water and stirred to form a gel; this was allowed to cool for 3 h. The remaining one-third of the slurry was added to the gel, mixed with cold boiled water (1000 mL) and left open to ferment for 12 hours. It was then sieved with a muslin cloth and the filtrate was sweetened with sucrose (250 g). Three (3) batches (A1, B2 and C3) of kunnu-ukwa was produced and evaluated in this study. Kunnu-zaki (control) was also produced and subjected to the same treatment as kunnu-ukwa, except that 100 g of pearl millet was used.

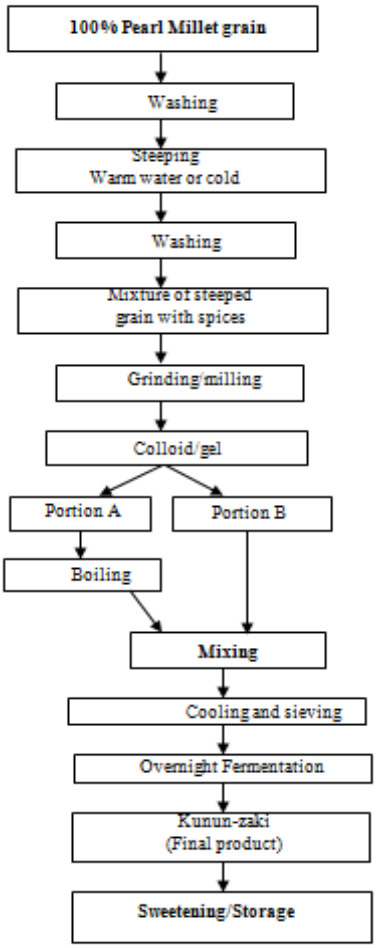


**Fig. 1: Flow diagram for the traditional kunun-zaki production process in Niger and Kano State of Nigeria**

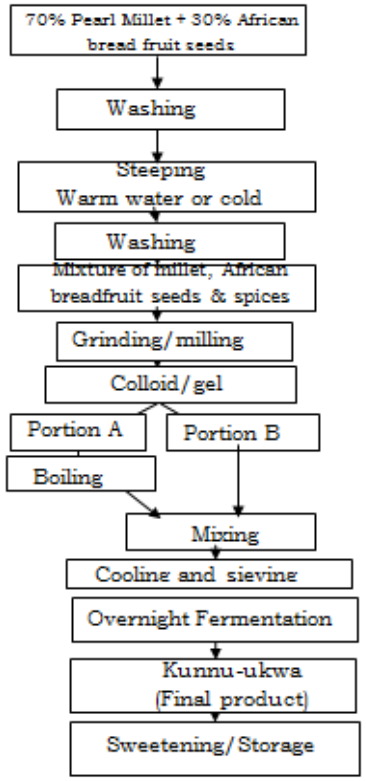


**Fig. 2: Flow diagram for the production kunnu-ukwa**

***Sample collection***

Samples were obtained at different stages of production for microbiological evaluation. Samples included grains/seeds, processing water, spices, swabs of utensils and ready-to-drink kunnu-ukwa. Triplicate samples of each material were collected. The samples were conveyed to the laboratory in sterile plastic packs (OK Plastic, Lagos, Nigeria) under ice. Approximately 10 g (or mL) of each material were collected per sample. Analyses were usually carried out within 6 hours of sampling. When delay occurs, the samples were refrigerated at 4ºC and analyzed within 24 h of collection.

***Microbiological evaluation***

Ten grams or milliliters (depending on sample type) of each sample for microbiological evaluation was aseptically transferred into 90 mL of 0.1% sterile peptone water, shaken thoroughly, and appropriate dilutions (up to $10^5$) were prepared for microbiological studies. Total viable counts (aerobic mesophiles) were made on Plate Count Agar (PCA, Oxoid, Hampshire, UK), while fungal counts (mold/yeast) were made on acidified Potato Dextrose Agar (PDA, Oxoid). PCA plates were incubated at 37ºC for 24 h, while the plates for fungal counts were incubated at 25ºC for 72 h. Coliforms were isolated by using MacConkey broth (Oxoid) and Eosin Methylene Blue agar (Oxoid), dilution of each sample was enriched in tetrathionate broth (Difco, Loveton Circle Sparks, MD) incubated at 37ºC for 6 h before it was inoculated on *Salmonella-Shigella* agar (Oxoid) for isolation of salmonellae. *B. cereus* wasisolated on mannitol/egg yolk/polymyxin agar. Mannitol/egg yolk/polymyxin agar was prepared by using peptone (Oxoid), meat extract (Oxoid), D-mannitol, sodium chloride, phenol red, agar-agar, egg yolk (Oxoid) and polymyxin B sulphate (Pfizer, New York, NY). The plates were incubated at 32ºC for 24 h. Gram-positive rods with halo zone of egg yolk precipitation were confirmed by using spore stain, catalase test, citrate test, Voges-Proskauer test and motility. All catalase-positive, motile organisms with ellipsoidal spores and positive Voges-Proskauer reaction were confirmed as *B. cereus*. *Staphylococcus* strains were isolated on mannitol salt agar (MSA) and Baird–Parker medium (Oxoid) for *S. aureus*. The coagulase and catalase tests were used to differentiate pathogenic *S. aureus* from nonpathogenic *staphylococci* (ICMSF, 1986; Oyeyipo, 2011).

***Proximate analysis***

Proximate analysis of moisture, ash, fat, carbohydrate, fiber and protein contents of kunnu-ukwa and kunun zaki (control) samples was carried out as previously described (AOAC, 2005) in which carbohydrate was determined by difference.

***Physical properties and vitamin contents***

Total solid was determined by evaporating 25 mL of kunnu-ukwa on a boiling water bath which was followed by drying to constant weight in an oven at 130°C for 2-3 h.

$$\%\, total\ solid = \frac{dry\ weight}{weight\ of\ sample}\ x\,100$$

Specific gravities, vitamin C, thiamine, riboflavin, and niacin were determined according to the methods of Association of Official Analytical Chemists (2005).

***Mineral analysis***

Analysis of potassium content of the samples was carried out using flame photometry, while phosphorus was determined by the phosphovanadomolybdate method (AOAC, 2005). The other elemental contents (Fe, Na, Ca, and Mg) were determined, after wet digestion of sample ash with an Atomic Absorption Spectrophotometer (AAS, Hitachi Z6100, Tokyo, Japan). All determinations were carried out in triplicates.

***Sensory evaluation***

The kunnu-ukwa and kunun zaki (control) samples were subjected to sensory evaluation to assess attributes such as appearance, viscosity, aroma, taste, and acceptability. A trained twenty member panel was used and scores were allocated to the attributes using a 9-point hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely). The data collected were subjected to statistical analysis to determine possible differences among samples.

***Statistical analyses***

All experimental determinations were made in triplicate. Data generated were subjected to statistical analyses by using SPSS 11.0 (SPSS Inc., Chicago, IL) for Windows. Means that were statistically different at 95% confidence level were separated by Duncan's multiple range tests.

**Results and Discussion**

The mean microbial values of samples collected during the various processing stages of kunnu-ukwa production are shown in Table 1. The grains/seeds recorded the highest counts of all groups of microorganisms taken. The swabs were next in number of organisms isolated for viable aerobic bacteria and staphylococci. Bacteria such as coliforms, *Salmonella, Bacillus* and *Staphylococcus* species are of public health importance. The isolation of these microorganisms is an indication of possible contamination resulting from the handling and processing environment (Umoh *et al.*, 2002).

The microbiological status of the freshly produced ready-to-drink kunnu-ukwa is shown in Table 2. No significant differences were observed in the microbiological profiles of the samples, kunnu-ukwa and kunnu-zaki (control) in this study. Total viable counts of aerobic mesophilic microorganisms ranged from 3.10 to 3.15 $\log_{10}$ cfu/mL. Counts on PDA showed a prevalence of yeast population ranging from 1.52 to 1.65 $\log_{10}$ cfu/mL and *Staphylococci* (1.06-1.23 $\log_{10}$ cfu/mL) whereas, coliforms (0.21-0.46 $\log_{10}$ cfu/mL), *Salmonellae* (0.24-0.43 $\log_{10}$ cfu/mL) and *Bacillus cereus* were also enumerated in the ready-to-drink kunun-ukwa and kunnu-zaki (control) samplesin this study. The non-isolation of coliforms, salmonella and *Bacillus cereus* could be attributed to the fermentation process, this is because when water is added to substrates, the microbial population in the substrates begins to grow and metabolize. As the microbial flora multiply, changes in pH and the general fermentation environment create conditions favourable for some organisms such as lactic acid bacteria and yeasts which can tolerate the acidic environment while inhibiting the growth of others such as the enteric organisms and *Bacillus* spp (Efiuvwevwere and Amadi, 1992; Obadina *et al.*, 2008; Onasoga *et al.*, 2014). The fermentation process of kunnu-ukwa production in this study had great impact on the final microbial load of the groups of microorganisms evaluated (Table 2). *B. cereus* was totally eliminated, while the other groups of pathogens, aerobicbacteria and fungi were reduced significantly.

**Table 1: Microbiological analyses of samples during *kunun-ukwa* processing ($log_{10}$ cfu/mL)**

| Sample | Total Counts | Fungal Counts | *Coliform* Counts | *Salmonellae* | *Bacillus cereus* | *Staphylococci* |
|---|---|---|---|---|---|---|
| Grains/seeds | $5.20^{c}$ | $4.32^{e}$ | $2.01^{e}$ | $1.11^{e}$ | $1.90^{b}$ | $2.15^{d}$ |
| Processing water | $2.02^{a}$ | $0.01^{a}$ | $1.40^{c}$ | $0.92^{d}$ | $0.00^{a}$ | $0.16^{a}$ |
| Mixed spice | $2.66^{a}$ | $1.35^{c}$ | $0.31^{a}$ | $0.45^{a}$ | $1.01^{a}$ | $1.31^{a}$ |
| Swabs | $5.38^{b}$ | $0.77^{b}$ | $0.90^{b}$ | $0.58^{b}$ | $0.21^{c}$ | $2.03^{c}$ |
| A1 | $3.10^{a}$ | $1.62^{b}$ | $0.21^{a}$ | $0.42^{c}$ | $0.00^{a}$ | $1.19^{d}$ |
| D | $3.15^{b}$ | $1.65^{d}$ | $0.46^{d}$ | $0.51^{c}$ | $0.00^{a}$ | $1.23^{b}$ |

Values are means of three replicates. Values followed by different superscripts within columns are significantly different by Duncan's multiple range tests ($P$> 0.05). A1= Kunnu-ukwa batch 1, D= Kunnu-zaki (Control)

**Table 2: Microbiological status of freshly produced *kunun-ukwa* ($log_{10}$ cfu/mL)**

| Sample | Total Counts | Fungal Counts | *Coliform* Counts | *Salmonellae* | *Bacillus cereus* | *Staphylococci* |
|---|---|---|---|---|---|---|
| A1 | $3.10^{ab}$ | $1.62^{a}$ | $0.21^{a}$ | $0.42^{a}$ | $0.00^{a}$ | $1.19^{a}$ |
| B2 | $3.12^{c}$ | $1.52^{a}$ | $0.21^{a}$ | $0.43^{b}$ | $0.00^{a}$ | $1.23^{abc}$ |
| C3 | $3.09^{a}$ | $1.61^{a}$ | $0.34^{a}$ | $0.24^{b}$ | $0.01^{a}$ | $1.06^{c}$ |
| D | $3.15^{bc}$ | $1.65^{b}$ | $0.46^{a}$ | $0.51^{b}$ | $0.00^{a}$ | $1.23^{ab}$ |

Values are means of three replicates. Values followed by different superscripts within columns are significantly different by Duncan's multiple range tests ($P$> 0.05), A1= Kunnu-ukwa batch 1, B2= Kunnu-ukwa batch 2, C3= Kunnu-ukwa batch 3, D= Kunnu-zaki (control)

**Table 3: Proximate analysis of the*kunun-ukwa* samples**

| *Kunun* | Proximate parameters | | | | | |
|---|---|---|---|---|---|---|
| | pH | MC (%) | Protein (%) | Ash (%) | Fiber (%) | Carbohydrate (%) |
| A1 | $4.52^{a} \pm 0.1$ | $85.47^{b} \pm 0.11$ | $5.86^{c} \pm 0.12$ | $4.85^{b} \pm 0.02$ | $0.72^{a} \pm 0.03$ | $3.10^{a} \pm 0.13$ |
| B2 | $4.50^{a} \pm 0.1$ | $85.25^{b} \pm 0.11$ | $5.81^{b} \pm 0.21$ | $4.84^{a} \pm 0.02$ | $0.73^{a} \pm 0.04$ | $3.17^{b} \pm 0.38$ |
| C3 | $4.56^{a} \pm 0.1$ | $85.17^{b} \pm 0.12$ | $5.92^{b} \pm 0.12$ | $4.91^{a} \pm 0.03$ | $0.78^{a} \pm 0.11$ | $3.12^{c} \pm 0.12$ |
| D | $4.42^{a} \pm 0.0$ | $88.53^{a} \pm 0.08$ | $2.04^{a} \pm 0.13$ | $3.15^{a} \pm 0.02$ | $1.85^{a} \pm 0.05$ | $3.35^{d} \pm 0.01$ |

MC, moisture content; A1= *kunun-ukwa batch 1*; B2= *kunun-ukwa batch 3*; C3= *kunun-ukwa batch 3*; D= *kunun-zaki (control)* .Values are mean ± standard deviation of three replicated samples. Values in columns with different superscript letters are significantly different ($P$< 0.05).

The results of the proximate analyses of kunun-ukwa and kunnu-zaki samples are shown in Table 3. pH values of the three kunnu-ukwa batches (A1, B2, C3) were 4.52, 4.50 and 4.56, respectively while kunnu-zaki recorded 4.42. No significant differences ($P$< 0.05) were recorded in the pH values of the samples. This slight difference in the pH of kunnu-zaki and kunnu-ukwa samples is probably due to the fortification process of kunnu-ukwa. Agarry *et al.* (2010) reported a pH value of 4.44 for kunun-zaki manufactured from millet and is similar to the pH (4.42) recorded for kunnu-zaki (control) in this study. The decrease in pH at the end of fermentation was due to the degradation of starch in the substrates by microorganisms with the production of various organic acids, consequently, lowering the pH of the substrates. Also, Adelekan *et al.* (2013) and Ayo *et al.* (2013), obtained values comparable to these during their studies on kunnu-zaki. The presence of lactic acid bacteria is very significant. The lactics degrade starch in the substrates with the production of various organic acids, and the lowering of pH. The yeast produces a variety of aldehyde and esters that are responsible for the characteristic desirable taste and aroma of the fermented products (Onasoga *et al.*, 2014; Oyeyipo, 2011).

Kunun-ukwa samples (A1, B2 and C3) recorded higher protein contents, 5.86, 5.20 and 5.92%, respectively, while thecontrol sample (D) recorded thelowest value (2.04%). The increase in protein content of kunnu-ukwa was due to the supplementation/fortification of the pearl millet with African breadfruit seeds used in the production of kunnu-ukwa, indicating that the African breadfruit seeds contributed to the protein contents of kunnu-ukwa samples. This is evident by the significant differences ($P$< 0.05) recorded in the protein contents of the kunnu-ukwa samples (A1, B2, C3) compared to the control (D). The improved protein contents of the kunun-ukwa samples obtained in this study may be beneficial to consumers from the economic point of view, as majority of the populace in the rural areas and some urban cities in Nigeria cannot afford high protein sources of foods such as meat, fish and egg.

Kunnu-ukwa samples recorded higher ash contents than kunnu-zaki sample in this study (Table 3). The increase in ash content of kunnu-ukwa samples in this study is probably due to the supplementation of the pearl millet with African breadfruit seeds. Makinde and Oyeleke (2012) reported similar increase in the ash contents of kunun-zaki following enrichment with extracts of sesame seeds. In contrast, Ogbonna *et al.* (2013) and Adelekan *et al.* (2013) reported higher values than those obtained in this study for ash contents of kunun-zaki. This is perhaps due to differences in types of cereal grains used in the manufacture of the beverage in the individual studies. Different cereal grains contain different compositions and contribute to the ash contents of the beverages, depending on their ash contents.

The fiber content of samples were statistically insignificant ($P$> 0.05), but lower values (0.72, 0.73 and 0.78%) were recorded for kunnu-ukwa samples (A1, B2 and C3), respectively, compared to the control sample (D) with the highest value of 1.85% (Table 2). This lower values could be attributed to the fortification/supplementation process that reduced the whole millet (100% w/w) by 30% with subsequent addition of 30% w/w of African breadfruit seeds to 70% w/w of pearl millet for the production of kunnu-ukwa coupled with the fact that the fiber content of pearl millet is higher than that of African breadfruit seed (Oyeyipo 2011; Belewu and Abodunrin 2006).

Also, there were no significant differences in the moisture and carbohydrate contents of samples in this study, however, lower values were observed for both parameters in kunnu-ukwa samples (A1, B2 and C3) than the control sample (D) (Table 3).

In Table 4 are the physical properties and vitamin contents of the kunun-ukwa samples. Total solids were 11.95%, 11.98% and 12.02%, for A1, B2 and C3, respectively while the control sample (D) recorded the highest value (13.05%). Higher values for total solids had been previously reported (Ayo *et al.*, 2013). The difference may be attributed to the type of grains, recipes and procedures employed for the production of kunnu in individual studies. A similar trend of decrease was observed in the specific gravity of kunnu-ukwa samples (A1= 0.73%, B2= 0.74% and C3= 0.76%) compared to the control sample (0.83%). In contrast, the supplementation/fortification process resulted in increased content of vitamin C, riboflavin and niacin in the kunnu-ukwa samples, whereas, the control sample (D) recorded highest for thiamine content (Table 4). This increases in vitamins will perhaps be of nutritional and health benefit to the consumers.

**Table 4: Physical properties and vitamin contents of thekunnu-ukwa samples**

| Sample | Physical properties and vitamins | | | | | |
|---|---|---|---|---|---|---|
| | Total Solid (%) | Specific Gravity ($g/cm^3$) | Vitamin C (mg/l00 g) | Thiamine (mg/kg) | Riboflavin (mg/kg) | Niacin (mg/kg) |
| A1 | $11.95^{a} \pm 0.12$ | $0.73^{a} \pm 0.03$ | $17.17^{a} \pm 1.02$ | $0.92^{bc} \pm 0.01$ | $0.65^{c} \pm 0.07$ | $1.01^{b} \pm 0.10$ |
| B2 | $11.98^{a} \pm 0.10$ | $0.74^{a} \pm 0.00$ | $16.99^{b} \pm 1.02$ | $0.96^{c} \pm 0.01$ | $0.65^{c} \pm 0.03$ | $1.05^{a} \pm 0.17$ |
| C3 | $12.02^{b} \pm 0.12$ | $0.76^{a} \pm 0.00$ | $17.21^{c} \pm 2.02$ | $0.95^{b} \pm 0.02$ | $0.63^{b} \pm 0.03$ | $1.03^{c} \pm 0.13$ |
| D | $13.05^{c} \pm 0.62$ | $0.83^{b} \pm 0.00$ | $13.76^{c} \pm 1.02$ | $1.02^{a} \pm 0.04$ | $0.52^{a} \pm 0.01$ | $0.99^{b} \pm 0.14$ |

A1=kunnu-ukwa batch 1; B2=kunnu-ukwa batch 2; .C3=kunnu-ukwa batch 3; D= kunnu-zaki (control). Values are mean ± standard deviation of three replicated samples. Values in columns with different superscript letters are significantly different ($P< 0.05$)

**Table 5: Mineral contents of thekunnu-ukwa samples**

| Sample | Minerals (mg/100 mL) | | | | | |
|---|---|---|---|---|---|---|
| | Na | Ca | K | Mg | P | Fe |
| A1 | $23..30^{a} \pm 0.2$ | $5.20^{d} \pm 1.2$ | $138.10^{a} \pm 0.9$ | $58.70^{c} \pm 1.0$ | $2.94^{d} \pm 0.0$ | $13.30c^{b} \pm 0.4$ |
| B2 | $23.20^{a} \pm 0.1$ | $5.20^{c} \pm 0.9$ | $139.70^{a} \pm 0.1$ | $59.50^{c} \pm 0.2$ | $2.91^{b} \pm 0.2$ | $13.54^{c} \pm 0.6$ |
| C3 | $23.17^{c} \pm 0.5$ | $5.40^{b} \pm 1.7$ | $137.30^{b} \pm 0.2$ | $59.10^{a} \pm 0.5$ | $2.99^{c} \pm 1.1$ | $13.61^{b} \pm 1.2$ |
| D | $23.10^{a} \pm 0.6$ | $5.80^{b} \pm 1.0$ | $142.60^{b} \pm 0.4$ | $53.70^{a} \pm 1.6$ | $2.20^{b} \pm 0.6$ | $14.69^{c} \pm 0.7$ |

A1=kunnu-ukwa batch 1; B2=kunnu-ukwa batch 2; .C3=kunnu-ukwa batch 3; D= kunnu zaki (control). Values are mean ± standard deviation of three replicated samples. Values in columns with different superscript letters are significantly different ($P< 0.05$).

**Table 6: Sensory attributes of the kunnu-ukwa samples**

| Sample | Attributes | | | | |
|---|---|---|---|---|---|
| | Appearance | Viscosity | Flavour | Taste | Acceptability |
| A1 | $7.2^{a} \pm 0.15$ | $6.9^{b} \pm 0.15$ | $6.9^{b} \pm 0.14$ | $7.2^{c} \pm 0.12$ | $7.4^{c} \pm 0.22$ |
| B2 | $7.1^{a} \pm 0.20$ | $6.6^{b} \pm 0.21$ | $6.9^{b} \pm 0.10$ | $7.4^{b} \pm 0.16$ | $7.2^{b} \pm 0.25$ |
| C3 | $6.9^{a} \pm 0.22$ | $6.9^{b} \pm 0.20$ | $6.8^{b} \pm 0.20$ | $7.1^{b} \pm 0.18$ | $7.3^{b} \pm 0.34$ |
| D | $7.4^{b} \pm 0.12$ | $7.1^{a} \pm 0.13$ | $6.6^{a} \pm 0.12$ | $6.8^{a} \pm 0.10$ | $6.7^{a} \pm 0.22$ |

A1=kunnu-ukwa batch 1; B2=kunnu-ukwa batch 2; .C3=kunnu-ukwa batch 3; D= kunnu-zaki (control). Values are mean ± standard deviation of three replicated samples. Values in columns with different superscript letters are significantly different ($P< 0.05$).

There were no significant differences in the mineral contents of the kunnu-ukwa and kunnu-zaki (control) samples in this study (Table 5). Except for sodium (Na), Magnesium (Mg) and phosphorus (P), kunnu-zaki recorded highest in other mineral contents (Ca, K, and Fe) evaluated. The values obtained for mineral content in this study were slightly different from that previously reported (Makinde and Oyeleke, 2012). As stated earlier, this could be attributed to the type of cereal grains, recipes and procedure(s) employed during the manufacture of the product (kunnu). Adelekan *et al.* (2013) reported closely similar values during their studies on kunnu-zaki. Similarly, the finding of Ogbonna *et al*. (2013) agrees with the values of Mg and Fe observed in this study. Minerals are of great values in diet as they play vital roles in body metabolism. For instance, Ca plays a role in the regulation of muscle contraction and impulse transmission in addition to bone and teeth development. Phosphorus is also important for bone growth, kidney function, cell growth, and maintenance of body's pH (Fallon and Enig, 2001). Also, potassium is vital in the synthesis of amino acids and proteins. Furthermore, Mg plays a major role in the relaxation of muscles and formation of strong bones and teeth, and also, plays crucial roles in almost all reactions involving transfer of phosphate which is very essential in the stability of nucleic acid structure and intestinal absorption (Appel 1999).

No significant differences ($P<0.05$) were observed in appearance, taste and overall sensory quality of samples by the panelists (Table 6), indicating that the fortification process did not adversely affect these parameters. Overall, kunnu-ukwa recorded highervalues in all attributes except for appearance (7.2±0.15 vs 7.4± 0.12) and viscosity (6.9±0.15 vs 7.1±0.13). Ratings on visual appearance and viscosity demonstrated that supplementation of pearl millet with African breadfruit seeds affected the colour and viscosity of the product.

Conclusively, the increases in the protein, vitamin and mineral contents recorded in this study for kunnu-ukwa samples (70% w/w pearl millet + 30% w/w African breadfruit seed) compared to kunnu-zaki sample (100% w/w pearl millet) is of great consideration and could therefore justify the use of the African breadfruit seeds in the fortification/supplementation process of kunnu-ukwa (a nutritionally improved traditional fermented beverage). The processes developed in this work will also help to overcome the constraints of promoting African breadfruit use and make it a common protein food in Nigeria. This can be used as a model for disseminating technologies developed in food fortification/supplementation in Africa, especially in Nigeria.

**Conflict of Interest**

Authors have declared that there is no conflict of interest reported in this work.

**References**


Abidoye OA, Taiwo K & Adeniran H 2016. Fortification of kunun zaki drink with cocoa powder. *Afr. J. Food Sci.,* 11(4): 112-123.

Adelekan AO, Alamu NU, Arisa Y O Adebayo YO & Dosa AS 2013. Nutritional, microbiological and sensory characteristics of malted soy--kunu zaki: an improved traditional beverage. *Adv. Microbiol.,* 3: 389–397.

Adeyemi IA & Umar S 1994. Effect of method of Manufacture in quality characterization of kunun-zaki: A millet based beverage. *Nig. Food J.,* 12: 33-40.

Agarry OO, Nkama I & Akoma O 2010. Production of kunun-zaki (A Nigerian fermented cereal beverage) using starter culture. *Intl. Res. J. Microbiol*., 1(2): 018-025.

Akoma O, Jiya EA, Akumka DD & Mshelia E 2006. Influence of malting on the nutritional characteristics of kunun-zaki. *Afr. J. Biotechnol*., 5(10): 996-1000.

Akoma O, Onuoha SA, Ajiboye MO, Akoma AO & Alawoki AM 2002. The nutritional and sensory quality characteristics of kunun-zaki produced with the addition of hydrolytic enzymes from malted rice (*Oryza sativa*). *J. Food Technol. Afr*., 7(1): 24-26.

AOAC 2005. Association of Official Analytical Chemists. Official Methods of Analysis of the Association of Analytical Chemists International, 18th ed. AOAC, Gaithersburg, MD.

Appel LJ 1999. Nonpharmacologic therapies that reduce blood pressure: a fresh perspective. *Clin. Cardiol*., 22: 1–5.

Ayo JA, Ayo VA., Yelmi B, Onuoha G & Ikani MO 2013. Effect of preservatives on microbiological qualities of kunnu zaki. *Intl. J. Agric. Sci. Res.,* 2: 124–130.

Ayo JA, Umianze H & Gaffa T 2004. Microbiological evaluation of "kunu-zaki" and "zoborodo" drink (beverage) locally produced and sold in Polytechnic community in Nigeria. *Nig. Food J*., 22: 119–126.

Belewu M A & Abodunrin OA (2006). Preparation of Kunnu from unexploited rich food source: tiger nut (*Cyperus esculentus*). *World J. Dairy Food Sci*., 1: 19–21.

Efiuvwevwere BJO & Akoma O 1995. The microbiology of 'kunun-zaki' a cereal beverage from northern Nigeria during the fermentation (production) process. *World J. Microbiol Biotechnol*., 11: 491-493.

Fallon S & Enig MG 2001. Nourishing traditions. The cookbook that challenges politically correct nutrition and the diet dictocrats. Revised 2nd Edn. New Trends Publishing, Inc., Washington DC, pp. 40–45.

Gaffa T, Jideani IA & Nkama L 2002. Traditional production, consumption and storage of kunu, a non-alcoholic cereal beverage. *Plant Foods Human Nutr.,* 57: 73–81.

Gaffia T & Ayo JA 2002. Innovations in the traditional kunun zaki production process. *Pakistan J. Nutr*., 1(5): 202-220.

ICMSF 1986. International Commission on Microbiological Specifications for Foods. Microorganisms in Foods 2. Sampling for Microbiological Analysis: Principles and Specific Applications, 2nd Ed., University of Toronto Press, Toronto, Canada.

Makinde F & Oyeleke O 2012. Effect of sesame seed addition on the chemical and sensory qualities of sorghum based kunun- zaki drink. *Afri. J. Food Sci. Technol*., 3: 204–212.

Nwabueze TU 2007. Nitrogen solubility index and amino acid profile of extruded African breadfruit (*Treculia africana)* blends. *Nig. Food J.,* 25: 23-35.

Obadina AO, Oyewole OB & Awojobi TM 2008. Effect of steeping time of milled grains on the quality of kununzaki (A Nigerian beverage). *Afri. J Food Sci*., 2: 33-36.

Odunfa S A & Adeyeye S 1985. Microbiological changes during the traditional production of ogibaba, a West African fermented sorghum gruel. *J. Cereal Sci*., 3: 173–180.

Ogbonna AC, Abuajah CI, Akpan MF & Udofia US 2013. A comparative study of the nutritional values of palmwine and kunnu-zaki. *Annals Food Sci. Techn.,* 14: 39–43.

Olaoye OA, Ubbor SC & Uduma EA 2016. Determination of vitamins, minerals, and microbial loads of fortified nonalcoholic beverage (kununzaki) produced from millet. *Food Sci. Nutr*., 4(1): 96–102.

Onilude AA, Sanni AI & Ighalo M I 2004. Process upgrade and the microbiological, nutritional and consumer acceptability of infant weaning food from fermented composite blends of cereal and soya beans. *J. Food, Agric. Env*., 2(3 & 4): 64-68.

Onuorah SI, Adesiyun AA & Adekeye JO 1987. Occurrence of Staphylococcus and coliforms in kunun-zaki in the utensils used in its preparation in Samaru, Zaria. *J. Food Agric.*, 1: 31-34.

Onyekwelu JC & Fayose OJ 2007. Effect of Storage Methods on the germination and proximate composition of *Treculia africana* seeds. University of Kassel-Witzenhausen and University of Gottingen conference on International Agricultural Research for Development Oct. 9-11.

Oyeyipo OO 2011. Studies on lafun fortified with African breadfruit-tempeh, MSc thesis, University of Port Harcourt, Port Harcourt, Nigeria.

Onasoga MF, Ayodele DO & Oyeyipo OO 2014. Chemical changes during the fortification of cassava meal (Gari) with African breadfruit (*Treculia africana*) Residue. *J. Appl. Sci. Env. Mgt.,* 18(3): 506-512.

Sanni AL, Onilude AA & Ibidapo T 1999. Biochemical composition of infant weaning food fabricated from fermented blends of cereal and soyabean. *Food Chem.,* 65: 35-39.

SPSS 2009, Statistical Package for Social Sciences. SPSS 1001 for windows. SPSS Inc., Chicago, Illinois.

Umoh VJ, Oranusi SU & Kwaga JKP 2004. The public health significance of pathogens isolated from Kunnu-zaki sold in retail outlets in Zaria, Nigeria. *Nig. Food J*. 22: 10–17.