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Proximate, Mineral Contents and Microbial Analysis of Kunu-Zaki (A Non-Alcoholic Local Beverage) in Ogun State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author EAO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors UEO and OSO managed the analyses of the study. Author ODW managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Proximate, mineral and microbial analysis of kunu drinks obtained from three different cereal sources (maize, millet and guinea corn) were carried out. The proximate parameters determined include percentage moisture, ash, protein, total solid, pH and acidity, while Fe, Zn, Ca, K and Mn concentrations were also evaluated. Maximum proximate values in millet, guinea corn and maize were obtained to be 90.7, 87.7 and 90.04% for moisture contents, 2.0, 1.9 and 1.78% for ash contents, 6.4, 10.3 and 7.8% for total solid contents, 8.4, 5.8 and 3.32% for protein contents respectively. Maximum pH values were obtained to be 4.30, 5.00 and 4.20 for millet, guinea corn and maize respectively which indicate slight acidity of the various kunu drinks. Microbial analysis revealed that four species of bacteria and fungi were isolated and identified from the kunu drinks are *Escherichia coli, Staphylococcus aureus, Bacillus subtilis,* and *Streptococcus* sp, while the fungi isolated are *Mucor, Aspergillus flavus, Aspergillus niger* and *Aspergillus nidulans.* This article

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concludes that the enrichment of kunu-zaki with powder milk improved the nutritious values of the beverage.

Keywords: Cereals; Kunu; microbial; mineral contents; proximate.

1. INTRODUCTION

Kunu which is also known as kunu-zaki is the traditional Hausa name of a non-alcoholic beverage which is widely consumed in Nigeria. It's a major source of calories and supplied large proportion of energy in form of carbohydrates. Kunu is taken as an alternative to beverage and is mostly consumed throughout Nigeria, mostly in the North [1]. Kunu drink is relatively cheap due to the availability of its constituent materials. It is made from cereal grains such as maize (*Zea mays*), Millet (*Pennisetum typhoideum*) and Sorghum (*Sorghum vulgare*) [2-3]. Garlic, pepper and ginger are some other ingredients which are added to enhance its flavor, while honey or sugar is also added to serve as sweetener. Fura, Burukutu, Buwo, Burakosko, Akamu, Danwake, Bulum, Pito to mention but a few are other traditional foods which are often fabricated from cereals in the northern part of Nigeria [3,4]. Acha, guinea corn, maize and millet contain 70 to 77% of carbohydrate with little fat and protein and about 12% moisture contents in the whole grain [5]. The variety of drinks made from sorghum is a milky light-brown colour, whilst that is made from millet and maize is whitish in colour. Carbohydrate, proteins, minerals and fat can be obtained from cereals but carbohydrates are the prominent diet produced from cereals, while vitamins and proteins are less produced from cereals [6].

Minerals such as manganese, iron, zinc and copper can be obtained from the intake of kunu [6,7]. In Nigeria, particularly in the North, kunu is widely consumed by young and old as thirst quencher and also for refreshment at home, office or in the market place. Owning to the low protein contents, kunu is often supplemented [8]. One of the most commonly used supplements in Nigeria is milk. Kunu can be taken with bread or snacks. Some people prefer to take kunu drinks as an alternative to soft drinks which many believe to be associated with diabetes. Gaffa et al. [2] reported the microbiological quantity, consumption rate and nutrient contents of kunu. The high demand for kunu drinks is believe to be due to the presence of carbohydrates which serve as source of energy, protein (although of low content) and vitamins usually vitamins B [9-10]. When kunu beverage is exposed to air without refrigeration for two to three days, its nutritive value diminishes and this lead to the spoilage of the kunu product owning to enzyme actions, poor handling and frequent fermentation process of the carbohydrate contents [6,9]. According to healthdiaries.com, millet contains a polynutrients called lignin, which has cancer fighting properties and is beneficial in the treatment of hearth disease and also reduce the risks associated with diabetes. Ginger content in kunu helps to lowers cholesterol level and prevents the formation of blood cloths. Chronic inflammatory diseases such as *rheumatoid arthritis* can also be prevented through the intake of kunu [11].

The sale and consumption of this locally made non-alcoholic beverage is on the increase in most cities in Nigeria with little attention being pay to the quality of this food product. Thus, this present work aimed at the physicochemical and microbiological analysis of kunu beverage.

2. METERIALS AND METHODS

2.1 Sample Collection and Preparation

Millet, Guinea corn, Maize, Ginger, Sweet potatoes, Alligator pepper and Red pepper were all purchased from Ifo market in Ifo Local Government, Ogun State of Nigeria. About 1 kg of millet, guinea corn and maize were soaked separately in 10 L of cold water inside a plastic bow for 12 hours and was washed thoroughly with distilled water to remove dirt. Sliced sweet potatoes, ginger, red pepper, alligator pepper and cloves were added to each of maize, millet and guinea corn and was blended together with the aid of blender to form a thick paste. The mixture was transferred into 5 L bow and about 500 ml portion of each was set aside. 3 L of boiling water was then poured into each mixture in the bow and was stirred until it becomes thick and was then allowed to cool down. The 500 ml portion of each which was set aside was then added and it was thoroughly mixed together. The mixture was then sieved and sugar was added to improve the taste [12-13]. This was referred to as kunu-zaki and it was kept inside a refrigerator until further analysis.

2.2 Supplementation of Kunu- Zaki

In other to supplement the kunu product, about 100 ml of the prepared product was mixed with 5 g of powdered milk and the mixture was thoroughly stirred together and kept in refrigerator for further analysis. This was then referred to as modified kunu-zaki.

2.3 pH Determination

About 25 ml of the kunu drinks was mixed with 100 ml of distilled water in a 500 ml beaker and was thoroughly shaken and thereafter, left to stand for 20 minutes before it was filtered and the filtrate was used for the pH determination using pH meter (3305, Jenway model).

2.4 Acidity Determination

The water extracts method described by AOAC [14], was used for the acidity determination of the kunu-zaki products. Quickly, about 25 ml of the drinks was mixed with 100 ml of water in a conical flask with the flask loosely stopped and placed in a water bath for 60 minutes at a temperature of 50℃. The mixture was filtered and 25 ml portions were titrated with 0.1 M of NaOH solution using phenolphthalein as indicator.

2.5 Protein Content Determination

5 g of the kunu drinks was weighed into the digestion tube (kjeldahl tube). Two kjaldahl tablets and concentrated tetraoxosulphate (VI) acid were added and the tube was placed in the pre-heated digester at 400℃ for about 45 minutes until a clear solution was obtained. After digestion, the tube was removed from the digester, cooled and distilled with water. The tube was placed with the digested and diluted sample in the distillation units. The conical flask containing 25 ml of 2% boric acid was placed under the condenser outlet. 25 ml of 40% sodium hydroxide was added and distilled for 4 minutes. The ammonium borate solution formed was titrated with 0.01 M hydrochloric acid to purple grey end point [14].

2.6 Moisture Content Determination

Clean and dried crucible was placed in an oven for about 20 minutes, cooled in desiccators and weighed. 5 g of the kunu sample was weighed into the pre-weighed crucible and this was placed in an oven for 3 hour at 110℃ after which it was cooled in desiccators and re-weighed until the weight remained fixed. Loss of weight was equated to the moisture content [14].

2.7 Ash Content Determination

5 g of the kunu sample was weighed into previously ignited, cooled and weighed crucible. These were pre-ashed on a hot plate to eliminate fumes that may deposit in the furnace. This was then transferred into Muffle furnace at 580℃ for 4 hours. The ash was removed from the furnace, cooled and re-weighed [14].

2.8 Total Solid Determination

5 g of the kunu-zaki drinks was weighed into a flat-bottom flask and heated for 1 hour until the liquid evaporated leaving behind the solid. This was immediately transferred into an oven set at 1000℃ for 2 hours and re-weighed. The content was cooled and weighed. This process was done severally until a constant weight was obtained according to the method prescribed by AOAC, [14].

2.9 Microbial Analysis

2.9.1 Characterization and identification of the isolate

Cultured sample was used in the preparation of standard inocula and aseptically incubated onto sterile nutrient agar plates. These plates were incubated for 36 hours at 37℃ and thereafter characterized using gram staining reactions, motility indole ornithine, voges proskaour test, oxidase test, urease test, catalase test, triple sugar iron agar, methyl red, citrate test and coagulase test as described by Cheesbrough, [15].

2.9.2 Total Bacteria Count (TBC)

Standard microbiological methods were deployed in the TBC analysis. In each case, about 10 ml of the sample was aseptically introduced into 90 ml of sterile normal saline and thereafter mixed properly by further dilution to a concentration of 10^{-6}. 0.1 ml of the diluted sample was then used for the inoculation of freshly prepared media using spread-plate approach and thereafter incubated for 36 hours at a temperature of 37℃. Colonies were counted via digital colony counter (Labtronic, Indian) [1,14-16].

2.9.3 Fungi Count (FC)

The method described by Ikpoh et al. [1] was adopted. Briefly, pour plate method was used in plating on sabourand dextrose agar. Dilution was achieved by diluting 1 ml of kunu sample with 9 ml of water. 0.1 ml was thereafter plated out into molten sabourand dextrose agar plate in triplicates and was spined gently. The content was allowed to solidify and incubation was done at 28℃ for 72 hours.

2.10 Elemental Analysis

10 g of the kunu product was weighed into a conical flask containing 5 ml of concentrated tetraoxosulphate (VI) acid, 25 ml of nitric acid and 10 ml of hydrochloric acid and boiled on a hot plate until the solution was cleared. The mixture was allowed to cooled down and was then transferred into 1000 dm^3 volumetric flask and made up to the mark with distilled water. Metal contents of Ca, K, Fe, Mn and Zn were analysed using Atomic Absorption Spectrophotometer (ASS-Buck 210 VGP Model) [14].

2.11 Statistical Analysis

Data was expressed as mean (n=3). ANOVA was used to show significance difference at $p \leq 0.05$ among the various experimental groups.

3. RESULTS AND DISCUSSION

3.1 Proximate Analysis

Parameters obtained for the proximate analysis are presented in Table 1. The pH values obtained for the three samples of kunu-zaki analysed from this present work indicated that they are all acidic. The pH values range between 3.80 to 5.00 and the sample of maize which contained milk had the highest value. The pH ranges of the kunu obtained from millet range from 4.30 to 4.00 for kunu drinks with powdered milk and without milk. The pH values of the samples of guinea corn and maize were found to range from 4.82 to 5.00 and from 3.80 to 4.20 respectively. These values are within the range of 3.80 and 3.99 reported by Innocent et al. [16], 2.42 to 3.83 recorded by Otaru et al. [17], 5.25 to 5.65 reported by Amusa and Ashaye, [18]. The results for acidity analysis revealed that the values of the acidity from millet, guinea corn and maize were found between the range of 0.03 to 0.14, 0.06 to 0.08 and 0.02 to 0.18 respectively. The results also showed that the acidity properties of all the prepared kunu drinks increases with decrease in pH level. The results agree well with the observations of Adebayo et al. [6] and Essien et al. [19]. The acidity of the kunu drinks may be due to the added species and also to the presence of some bacteria such as *Lactobacillum, Acidophillus*, *Candida* species and *Saccharomyces cerevisiae* which help in acid fermentation of the kunu products and essential to human being [1,6,20]. The values of moisture contents in millet, guinea corn and maize without and with milk powder samples were found in the range of 90.70 to 82.90, 87.70 to 82.0 and 90.04 to 85.87% respectively. Sample from millet without milk recorded the highest value of moisture contents, while the least value was observed in the kunu sample of maize with milk. The percentage ash content of millet, guinea corn and maize were found to be within the range of 1.60 to 2.00, 1.30 to 1.90 and 1.48 to 1.78% with millet having the least value and maize having the highest ash content. These values were higher than 0.2% obtained by Otaru et al. [17] and 0.3 to 0.72% obtained by Essien et al. [19]. These results however agree with 1.00 to 2.00% obtained by Innocent et al. [16] and 1 to 2.0% recorded by Amusa and Ashaye, [18]. Analysis of the total solid showed that millet, guinea corn and maize without and with milk powder contain 4.30 to 6.40, 8.40 to 10.30 and 5.80 to 7.80% respectively. Samples with milk samples contain higher amount of total solid and this might be due to the added residue from the milk powder.

The results for the analysis of percentage protein showed kunu drinks from millet containing powdered milk had the highest protein value of 8.4%, followed by guinea corn sample with powdered milk of 5.8%, while sample from maize without milk powder had the lowest protein content of 2.18% as shown in Table 1. There was significant different ($p<0.05$) between the kunu product which contain the powdered milk and those in which milk were not added. The results indicated that kunu sample with powdered milk had higher proteins values. Essien et al. [19] reported that lost of protein during the processing of the drinks may be responsible for the low protein content observed. Hamad and Fields [21] opined that high value of protein content in cereals is often found in the germ and testa which are often sifted off during the preparation of kunu product. The results of the protein content analysis in this work were found to be

higher than the values recorded by Essien et al. [19] and Adebayo et al. [6].

3.2 Mineral Contents Analysis

The results obtained for the mineral content analysis of the kunu drinks gotten from millet, maize and guinea corns are shown in Fig. 1. The results showed that the concentration of K, Ca, Fe, Mn and Zn in the kunu drinks made from millet, guinea corn and maize were found to range from 0.114 to 0.442, 0.063 to 0.715, 0.148 to 0.691, 0.14 to 0.45 and 0.051 to 0.113 ppm respectively. In general, Ca from the sample of GCWM had the highest value of 0.71 ppm, while Zn has the least value of 0.042 ppm from the kunu sample of MMWM. Minerals are nutritionally important components in food and they could be classified as essential or non essential elements. Minerals are essential for health and as such are part of all aspect of cellular function and they are involved in structural components of human beings. Some mineral elements form an integral part of enzyme or protein structure. They are vital for normal growth, maintenance, effective immune system and prevention of cell damage [22]. Other functions of essential elements includes growth and production of bones, teeth, hair, blood, nerves, skin, vitamins, enzymes and hormones, blood circulation, cellular integrity, energy production and muscle contraction [22]. There exists a range of intake over which the supplies of mineral elements become toxic. However, above and below this rang (ceiling limit), toxic and deficiency effects are observed respectively [23]. As a result of this, it is imperative to establish mineral contents of food items and to estimate their daily dietary intake. Essential elements can be systemic toxins with specific neurotoxin, nephrotoxic, fetotoxic, and tetratogenic effects. Mineral elements can influence behaviour in human by impairing immune, mental and neurological function, influencing neurotransmitter production and utilization and altering other metabolic process in the body [24].

Table 1. Proximate analysis of Kunu Zaki products obtained from millet, maize and guinea corn

Proximate contents (%)	MMW	MMM	GCWM	GCM	MWM	MM
Moisture	90.70±0.15	82.90±0.17	87.70±0.12	82.0±0.15	90.04±0.18	85.87±0.11
Ash	1.60±0.01	2.00±0.10	1.30±0.03	1.90±0.02	1.48±0. 03	1.78±0.01
Total solid	4.30±0.07	6.40±0.13	8.40±0.41	10.30±0.06	5.80±0.81	7.80±0.21
Protein	3.40±0.03	8.40±0.23	2.60±0.14	5.80±0.05	2.18±0.02	3.32±0.01
pH	4.30±0.02	4.00±0.01	5.00±0.02	4.82±0.03	4.2±0.01	3.80±0.02
Acidity	0.034±0.01	0.082±0.04	0.220±0.01	0.180±0.02	0.148±0.01	0.093±0.03

Note: MMWM = Kunu drinks from millet without milk powder, MMM = Kunu drinks from millet without Milk powder, MWM = Kunu drinks from maize without milk powder, MM = Kunu drinks from maize with milk powder, GCWM = Kunu drinks from guinea corn without milk powder and GCM = Kunu drinks from guinea corn with milk powder

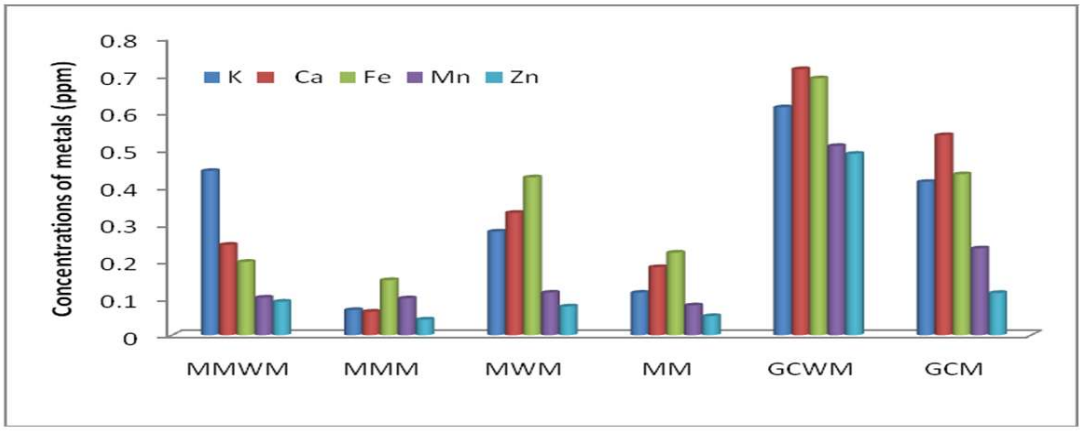

Fig. 1. Metal conentration of kunu drinks from different locations

3.3 Microbial Analysis

Four species of bacteria and fungi were isolated and identified from the kunu drinks and are presented in Figs. 2 and 3 respectively. The bacteria isolated were identified as *Escherichia coli, Staphylococcus aureus*, *Bacillus subtilis, and streptococcus* sp, while the fungi isolate are *Mucor, Aspergillus flavus, Aspergillus niger* and *Aspergillus, nidulans*. For the bacteria isolates, *E. coli* has the highest occurrence of 46% followed by *S. aureus* (37%), then by *streptococcus* sp (33%) and the least was observed to *B. subtilis* (6.4%). On the other hand, for the fungi isolate, the order of increase in percentage occurrence is given as *A. flavus* (33%) > *mucor* (30%) > *A. niger* (25%) > *A. nidulans* (5%) respectively. The presence of some of micro-organisms in the kunu drinks may be as a result of contaminations through human handling, the use of contaminated containers, washing with polluted water, unhygienic environment, acidic nature of the sample, nutritional composition, or substrate contamination [6,25,26]. All these could increase the presence of pathogens in the drink sample. According to Adebayo et al. [6], the presence of fungi in food may lead to poisoning and contaminated fungi result in the production of undesirable odour, changes in colour and taste of the sample. Many of fungi species isolated from the sample produce toxin. They stressed further that 'the fungi of the general *Aspergillu*s and yeast are predominant in the elaboration of toxins known as mycotoxins, a disease condition known as mycotoxincosis which develop when food containing microtoxins are eaten i.e. some strain of *Aspergillus* flavour and some species of yeast are potential carcinogens probable to disturb man when consumed'. The milk powder addition may have contributed to the increase in microbial load because the increase in pH of the kunu drinks which favours microbial growth and being a proteinuous liquid, it may serve as a medium for microbial growth. The presence of different microorganisms in kunu drinks has been widely reported in literature [1,6,16].

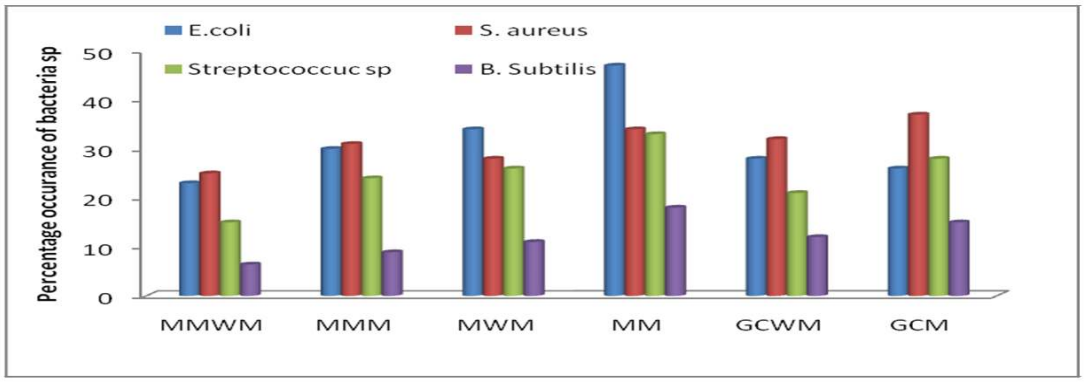

Fig. 2. Percentage occurance of bacteriaa sp at different locations

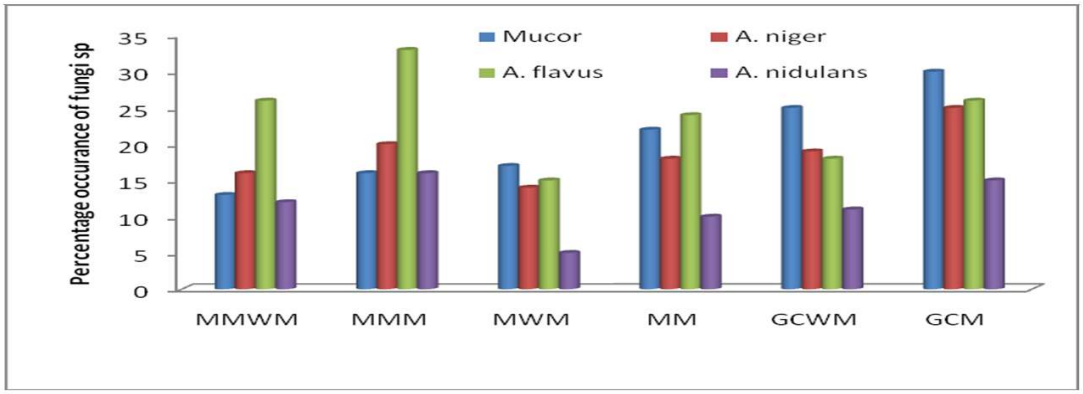

Fig. 3. Percentage occurance of fungi at different locations

4. CONCLUSION

In this study, guinea corn, maize and millet were used as different raw materials for the production of kunu-zaki drinks which were all supplemented with powder milk. Proximate analysis revealed moderate acidity and pH for all the sample tested which suggest that they are of good nutritive value and could serve as source of protein and energy to human body. Elemental analysis showed high level of Ca, K and Fe which are good for strong teeth and bone formation and also for blood supplement in the human body. Improved kunu-zaki enriched with powder milk gave a nutritious beverage as the level of milk powder addition increases protein contents and also the pH of the kunu-zaki drinks. Microbial activity in the kunu sample can be enhance by proper preservative method and by keeping them inside refrigerator.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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