

Physicochemical, Microbiological and Sensory Characteristics of Kunu Prepared from Millet, Maize and Guinea Corn and Stored at Selected Temperatures

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Abstract: Kunu beverages were prepared from three different cereals (millet, maize and Guinea corn). The process of cleaning, steeping, wet milling; wet sieving, settling, decantation and slurry recovery were applied in the preparation. The products obtained were analyzed for total solid, pH, protein, ash, acidity, moisture content and trace elements. The sensory properties (colour, Taste, Texture, flavour and general acceptability) were also carried out on a nine point hedonic scale where 1 -9 represents extremely poor to excellent respectively. The results obtained from physicochemical analysis shown that: millet, maize and Guinea corn products respectively have ; pH (5.00, 4.69, 4.66), total solid % (6.0, 6.0, 6.0), protein % (1.17, 1.07, 0.88), acidity (0.20, 0.62, 0.26), moisture content (94.0, 94.0, 94.0)% and trace element (lead ppm) (0.04, 0.05, 0.03), copper (ppm) (1.09, 0.55, 0.62) zinc (ppm) (3.30, 1.50, 2.19), calcium (ppm) (0.55, 4.92, 0.00), manganese (ppm) (1.05, 0.70, 0.51). The bacterial and fungi isolates were also determined which remained fairly constant in population within 96 hours of storage. All the samples became more acidic as the hours of storage increased, with the most significant changes occurring in samples kept at 25°C.

Key words: Beverage, cereals, Kunu, microbiological, physicochemical, sensory evaluation and trace elements

INTRODUCTION

Cereals are the most important source of the world's food and have significant impact in human diet throughout the world. In India and Africa, cereals products comprise 80% or more of the average diet. 50% in central and Western Europe and between 20-25% in the U.S. (Onwueeme and Sinha, 1991). The main cereals grown in Nigeria are maize, guinea corn, rice, millet and sorghum. These cereals can supply sufficient quantities of carbohydrates, fat, protein and many minerals, but diet consisting primarily of cereals is high in carbohydrate and deficient in vitamins and protein. Millet is the staple food of millions of people in drier parts of tropical Africa. It has been reported that air - dried grains contain approximately 12.4% water, 11.6% protein, 5% fat, 67.1% carbohydrate, 1.2% fibre and 2.7% ash. However, its protein is low in methionine (Onwueeme and Sinha, 1991). Millet are good sources of minerals e.g., calcium, iron, zinc, copper and manganese (Hulse *et al.*, 1980). Products from millet vary depending on people's taste and cultural preference. One of the common traditional products is kunu, a non alcoholic beverages made mainly from millet. It is of low viscosity and has a sweet-sour taste, milky cream appearance and is popular with people of northern Nigeria (Adeyemi and Umar, 1994). It is generally consumed on its own by adults as a thirst quencher or serves as refreshment in some communities.

It is sometimes used as a weaning drink for infants. However, since this drink is produced from cereals, its protein is incomplete and needs to be supplemented (Singh *et al.*, 1987).

The cereals are known under variety of name all over the world. In the Northern area of Nigeria Millet is known as JERO, OKA BABA in Yoruba land and in America, it is known as MILO. Also maize is known as MASARA in northern area and as AGBADO in Yoruba. Millet, maize and Guinea Corn require little rainfall. It does well in most soil except in pure sand and clay soil. The crop is successfully grown in well drained soil ranging in pH from 5.5 to 8.5. Rainfall amount of about 60-120 cm³ and a temperature about 27 °C will be favorable for the crop (Gama and Shemington, 1977). It can be stored for 6 months without any significant spoilage. In areas and cases where grains have been stored for more than 12 months without any treatments, losses of 15-20% have been reported (Gama and Shemington, 1977).

Millet, guinea corn and maize are used as food for man and animals. The seeds of the millet are used to make drink called pito in Ghana. In the southern part of Nigeria, the Yorubas used the sprouting seed of the red seeded varieties of millet, Guinea corn and maize for brewing local beer. In the northern area, the Hausa people used them to make kunu and burukutu. In Nigeria, the southern people used millet, guinea corn and maize in

preparing cold pap (ogi in Yoruba). Maize is also used to make popcorn, and it is processed in some industries to make corn-flake (Hobbs *et al.*, 1978).

Food beverages can be classified into two major classes namely, the alcoholic and the non-alcoholic beverages. Alcoholic beverages include wines of different sources, spirit e.g. Brandy, Whisky, Beer (Gama and Shemington, 1977). Non-alcoholic beverages includes carbonated and non carbonated beverages. The carbonated beverages are those made in the presence of carbon compound especially carbon dioxide. Examples include soda water, cocacola, ginger ale, tonic water, pepsi-cola etc. None carbonated beverages are those that are merely juice from fruits drink and nectars, vegetable juice, water chocolate drinks, coffee, tea, black currant etc. Kunu is a very nutritious, locally made beverages common among the northern Nigerian. Kunu is one of the complex mixtures which contain Macro- molecules such as protein, carbohydrates and lipids (Steven, 1964). The major important cereals which are used in the preparation of kunu are millet, maize, guinea corn and rice. During the preparation of kunu, the ingredients needed are ginger (*Zingiber officinalis*), Alligator pepper (*Aframomum melegueta*), red pepper (*Capsicum* species), black pepper (*Piper guineense*) and kakandoru or Eru. All these ingredients perform one function or the other in the course of the preparation. The most abundant constituent of kunu is water and it acts as the medium in which all other constituents are dissolved and contain only traces amount of in-organic substances. The nutritive value of kunu is highly due to the presence of protein, carbohydrates and some vitamin especially the vitamin B. (Chapman, 1982). Kunu is taken after meal as a supplement or to quench thirst. Kunu is widely accepted as food drink in some urban centre especially in the Hausa land (Ihekoronye and Ngoddy, 1985). It is greatly consumed by large number of people ranging from market women and men including young and old at home. The quality and quantity of the products depend largely on the quality of the ingredients and its proper handling in the course of production by the producer. The products could be obtained qualitatively after 2 days and it could be stored for another 3 days when refrigerated (Chapman, 1982). It has however been reported that, if kunu is kept overnight in hot season without being refrigerated, its quality begins to deteriorate and this may lead to the spoilage which when consume could constitute danger to health (Chapman, 1982). Spoilage of this product from observation occurs from improper handling, constant fermentation of the ingredients especially the carbohydrates and enzymatic action on the substrates (Chapman, 1982).

Currently in the country, soft drinks cost a lot. A bottle of 35cl costs an average price of N35.00. These so called soft drinks have little or no nutritive value because they are mostly of sugar and artificial concentrates. Kunu however seem to be highly nutritious with relatively low

cost of production and consumption. It is being prepared from our local cereals which are very common and are part of our staple food substances. The present study aimed at the production of this local beverage from three different cereals (millet, maize and Guinea corn). It is stored at room temperature to estimate its shelf life. The comparative analysis of the products was then carried out. This analysis involves the physicochemical, microbiological and sensory evaluation of the products. The result obtained was used to estimate the nutritive value.

MATERIALS AND METHODS

This study was carried out between November 2008 and February 2009 at Department of Chemistry, University of Ilorin, Nigeria and Department of Microbiology, Kwara State Polytechnic, Ilorin, Nigeria. The raw materials for the preparation of the Kunu were purchased from a major market in Ilorin, Nigeria. These materials include maize, millet and guinea corn, alligator pepper, black pepper, red pepper, kakandoro and Ginger.

Preparation of kunu: The sand and other solid impurities were removed from the Millet, Guinea corn and maize. It was then soaked separately for about 24 h after which the ingredients like ginger, alligator pepper, red pepper, black pepper and kakandoro were added. It was ground very well in a hygienic way and sieved with a very clean and white cloth. It was allowed to settle down and the filtrate was fermented for 24 h, during which the slurry was allowed to settle and sediment. The supernatant liquid was decanted and the residue was mixed with water and divided into two. Half of the residue was boiled and the second half was poured into it to produce kunu. After this, adequate water was added to meet the satisfactory level of the consumer i.e., not too watery and not too thick. The kunu was sieved again in order to remove the soluble material. Sugar was added into it and ready for consumption.

Physicochemical characteristics of kunu beverages:

Determination of pH kunu beverages: 10 ml of the kunu beverage was shaken with 100 ml of water and allowed to stand for a period of 30 min. The material was filtered and the pH of the filtrate was determined with a pH meter within the ranges of 6.0-6.8. Bleaching of the cereals with chlorine gas can cause a fall in the pH value (Ademoroti, 1966).

Determination of acidity in kunu beverages: Water extracts method (AOAC, 1990) was used in the determination. 18 ml of kunu was measured and shaken with 200 ml of CO₂ free water in a conical flask and placed in a water bath at 40°C for one hour with the flask loosely stoppered. It was filtered and 100ml of the clear

filtrate was titrated with 0.05M of NaOH solution with phenolphthalein indicator. The acidity of water extract increases during storage and is calculated as lactic acid or potassium dihydrogen phosphate (1 ml of 0.05M of NaOH = 0.0068 g of KH₂PO₄).

Determination of total solid in kunu: Five gram of kunu was weighed into a flat-bottomed metal dish and placed on boiling water for about 30 minutes until the liquid evaporated leaving the solid. It was then transferred into an oven maintained at 100 °C for 2½ h as W₂. It was then transferred to a desiccator, cooled and weighed. It was heated in the oven again for 1 hour, cooled and weighed. The process was continued until constant weight W₃, was obtained (AOAC, 1990). The total solid is calculated from Eq. (1):

$$\text{Total solid} = \frac{W_3 - W_1}{W_2 - W_1} \times 100 \quad (1)$$

Determination of protein in kunu beverage: The mole titration method (Pirie, 1975; Akoh, 1981) was used for the determination. 10ml of kunu was added to 0.05 ml of 0.5% phenolphthalein indicator. It was mixed and allowed to stand for a few minutes and neutralized with 0.1M NaOH to the standard pink colour. 2 ml of formalin was added, mixed and allowed to stand for few minutes. The new acidity produced was titrated with 0.1M NaOH to the same pink colour. Then 2ml of the formalin +10ml of H₂O were titrated separately with 0.17M NaOH as blank. Note: 1.95(a-b)% where: a- titre value, b-blank value

Determination of ash in kunu: The crucible dish was cleaned, dried ignited, cooled and weighed as W₁. 24.4 g of the kunu was weighed accurately and directly in the dish i.e. W₂. The substance was dried on a boiling water bath and the charred over a bursen flame or hot plate in fume cupboard until no more soot was given out. Then, it was then ashed with a muffle furnace at 500°C to obtain W₃ (AOAC, 1990). Percentage ash is calculated from Eq. (2):

$$\text{Ash (\%)} = \frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1} \quad (2)$$

Determination of trace elements in kunu: The determination of trace metal contaminants in kunu was carried out. These trace metals are lead, copper, zinc, manganese and calcium. The organic matter of the food was first destroyed dry ashing 24.4 g of kunu sample between 400-500°C for 5 h. This was followed by acid digestion by adding 20 ml of H₂SO₄ and 10ml of HNO₃ (2:1). The mixture was heated on bursen burner until the brown fumes subsided. Another 10 ml of H₂SO₄ and 5 ml of HNO₃ were added to the mixture. The addition of 10 ml HNO₃ was continued at 10 min interval and heating

continued until the solution becomes colourless. The digested sample was then analyzed for lead, zinc, calcium, manganese and copper using AAS.

NOTE: The weight of the kunu before ashing = 24.4g.

Determination of moisture content in kunu: This method is based on loss on dry at an oven temperature at 105 °C. Besides water the loss will include other matter volatile at 105 °C (AOAC, 1990; Akoh, 1981). Five gram of kunu was weighed into a pre-weighted flat dish (W₁) and dried at an oven temperature of 105 °C for 3 h as W₂. It was allowed to cool in an airtight desiccator and re-weighed. It was heated in the oven again for half an hour, cooled and weighed. The process was repeated until constant weight was obtained W₃ (AOAC, 1990). The percentage moisture was calculated from the equation 3

$$\text{Moisture (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1} \quad (3)$$

Microbial analysis: These were carried out by the pour plate method as described by Nigeria Distilleries Limited (NDL) using nutrient agar and potato dextrose agar for total aerobic bacteria and total yeast count respectively. Colony counts were done after the appropriate period of incubation. Distinct colonies from the poured plates were streaked unto fresh sterile nutrient agar plates to obtain pure colonies of bacterial and transferred to agar slants as stock culture for later use. The morphological characteristic of each pure bacterial colony was studied with emphasis on the pigmentation, colour, shape, edge, and elevation, optical characteristics that is opaque, translucent or transparent, the colony surface and consistency was later identified using Analytical Profile Index (API).

The fungal isolates were sub-cultured by transferring a bit of each typical colony from its edge on to a sterile fresh media and incubated at room temperature for 3-5 days. Morphological observations were based on colour, textures and the spreading rate of each colony on the potato dextrose agar plate and the microscope studies of each type were done for further identification.

Identification of fungi isolate was carried out by adding a drop of lacto phenol on cotton blue stain placed on a clean grease slide and a sterile inoculating needle used to tease out a fragment of the fungi and transferred into the stain on the slide and covered with a cover slip and examined with low power objective of the microscope x 40.

RESULTS AND DISCUSSION

Physicochemical characteristics: The physicochemical parameters determined are shown in Table 1. The three samples of kunu were found to be slightly acidic with pH (4.66, 4.69 and 5.00) for Guinea corn, maize and millet

Table 1: Physicochemical characteristics of kunu beverages

Kunu Source	pH	Total Soild %	Protein %	Acidity (KH ₂ PO ₄)g	ash%	Moisture Content %
1 Millet	5.00	6.0	1.17	0.0093	0.20	94.0
2 Maize	4.69	6.0	1.07	0.0165	0.62	94.0
3 Guinea corn	4.66	6.0	0.88	0.0129	0.26	94.0

Table 2: Essential and toxic trace elements in the kunu beverage

Kunu Source	Lead (ppm)	Copper (ppm)	Zinc (ppm)	Calcium (ppm)	Manganese (ppm)
Millet	0.04	1.09	3.30	0.55	1.05
Maize	0.05	0.55	1.50	4.92	0.70
Guinea corn	0.03	0.62	2.19	0.00	0.51

Table 3: Sensory properties of kunu

Sample	Colour	Taste	Texture	Flavour	General acceptability
Millet	8.0	7.0	7.5	7.6	7.9
Maize	6.4	5.5	7.0	7.5	6.1
Guinea corn	7.1	6.0	7.2	7.4	6.5

respectively. The acidity of the samples can be attributed to the added species. It can also be traced to the presence of some bacteria like lactobacillus, *Acidophilus*, *Candida* species and *Saccharomyces cerevisiae* which help in acid fermentation of kunu. These bacteria however are non pathogen as they are beneficial to human being. The percentages of protein in kunu were found to be 1.17% for millet, 1.07% for maize and 0.88% for guinea corn. The protein content of kunu made it to be more nutritious than any of the carbonated soft drinks (as coca cola, pepsi cola etc) which seem to have contained no protein content. Their major components are sugar, colouring and carbonated water. The total solid of kunu was found to be 6% for all the three samples (millet, maize and Guinea corn). The constant value for total solid may be due to the fact that the same procedures were followed for the three samples. It may also reflect the volatile composition of the samples. It has been revealed that the moisture and some other volatile organic compounds of the samples were as higher as 94%. The results also show the level of the viscosity of the samples as the slurry was diluted with water during preparation.

The acidity in kunu is calculated as potassium dihydrophosphate (KH₂PO₄). The results revealed that kunu from maize have the highest acidity (0.016524 g of KH₂PO₄), followed by Guinea corn (0.01292 g of KH₂PO₄) and then millet (0.009316 g of KH₂PO₄). It was observed that while the pH decreased, the total acidity increased with hours of storage. Moreover, it appears that the higher the storage temperature, the more pronounced the decrease in pH. This is expected, since the higher the storage temperature, the higher the rate of metabolism of sugar and hence the higher the rate of acid production by the relevant micro-organisms. These observations appear to be in agreement with those of earlier work (Fraizer, 1978) who reported the production of acid from sugar by various metabolism micro-organisms such as lactic acid bacteria, acetic and butyric acid bacteria.

The percentage ash in kunu varies from 0.20 to 0.62% but it was seen that maize has the greatest ash % with the value of 0.62% and millet has the lowest ash %

with the value of 0.20%. The importance of the ash content is that, it gives an idea of the amount of mineral elements present in the sample. It has also been reported that the value of ash is a useful and quality or grading assessment of certain edible materials (AOAC, 1990). These results were also supported by the level of trace elements in each of the samples.

Mineral contents: Table 2 show the results obtained for the major, essential trace and toxic elements. The major element is calcium while the trace elements are zinc, copper and manganese and toxic element is lead. The result revealed that calcium has the highest concentration (4.92 ppm) followed by zinc (3.30 ppm), then copper (1.09 ppm), then manganese (1.05 ppm) and the least is lead (0.04 ppm). The quantities of trace elements were relatively low for lead (0.04, 0.05 and 0.03 ppm), copper (1.09, 0.55 and 0.62 ppm), manganese (1.05, 0.70 and 0.51 ppm) compared with their tolerant limit. This is very good results from the biological point of view. However, calcium which is essential requirement for bone development and strong teeth and zinc which aid digestion and body functions were relatively high (zinc, 3.30, 1.50 and 2.19 ppm), (calcium, 0.55, 4.92 and 0.00). The concentration of manganese is relatively moderate because it becomes poisonous when taken in high concentration.

The changes that occurred in the sensory attributes of “kunu” samples stored at selected temperatures are as shown in Table 3, with respect to taste, odour, colour, texture, flavour and general acceptability (Lannoid, 1977; Duncan, 1955). All the samples remain acceptable only within the first 48hrs of storage at all temperatures and it can be stored in the refrigerator for five days without spoilage. This is also supported by the fact that the pH has fallen to acidic level within the same period. The colour and taste became unattractive after 48hrs of storage at room temperature.

Microbial result: Five species of bacteria and five different types of fungi were isolated and identified from fermented “kunu”. The micro-organisms isolated were identified as *Bacillus subtilis*, *Micrococcus species*, *Escherichia coli*, *Staphylococcus aureus*, *streptococcus* sp., *Mucor*, *Rhizopus stolonifer*, *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus, nidulans*.

Table 4: Colonial characteristics of bacterial isolates

Isolates	Shape	Elevation	Colour	Edge	Colony surface	Optical characteristics
A	Irregular	Flat	Creamy white	Entire	Rough	Opaque
B	Rhizoid	Umbonate	White	rhizoid	Wrinkled	Opaque
C	Round	Raised	Milky	Entire	Granular	Opaque
D	Regular	Raised	Yellow	Entire	Smooth	Opaque
E	Round	Flat	Cream	Entire	Rough	Opaque

Table 5: Colonial characteristics of fungal isolates

Isolate	Colour	Shape	Edge	Elevation	Surface
A	Cottony white	Irregular	Rough	Raised	Coarse
B	Fluffy white	Irregular	Rough	Raised	Fine
C	Black	Irregular	Rough	Raised	Coarse
D	Pale brown	Irregular	Rough	Upright	Coarse
E	Dark Green	Irregular	Rough	Raised	Coarse

Key: A = *Bacillus subtilis*, B = *Micrococcus* sp., C = *Escherichia coli*, D = *Staphylococcus* sp., E = *Streptococcus* sp.

The colonial characteristics, which aid in the identification of bacteria and fungi were observed. This includes the shape, elevation, colour, optical characteristics and edge. This is shown in Table 4 and 5.

The micro – organisms involved in the fermentation of millet, maize and guinea corn to produce kunu were isolated. Both bacteria and fungi were found to be involved in the process. The bacteria were *Bacillus subtilis*, *Micrococcus* sp., *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* sp., while the fungi were *Mucor*, *Rhizopus stolonifer*, *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus nidulans*. The presence of some of the bacteria may be due to contamination from the substrate, the air of the environment where the sample was produced, the hygienic state of the processing of the sample. The presence of fungi may be attributed to the acidic nature of the sample since it has been observed that yeast and mould are capable of utilizing organic acids. Also the presence of fungi in the food may lead to poisoning and contaminated fungi result in the production of undesirable odour, colour changes and even the taste of the sample will be changed. Many of fungi species isolated from the sample produce toxin. The fungi of the general *Aspergillus* and yeast are predominant in the elaboration of toxins known as mycotoxins, a disease condition known as mycotoxicosis 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 presence of these organisms in the sample may be due to the nutritional composition of the millet; these nutrients are present in different proportions.

As a method of preserving the “kunu” the steeped millet and spices can be air dried and ground into powder form and mix, when needed (Ijabadeniyi, 2006). The ginger and ‘Kanafuru’ serves as flavouring agents while the alligator pepper serves as antimicrobial agent. The result of this work was similar to the report of (Adeyemi and Umar, 1994) who worked on micro – organisms associated with the production of “kunu” using two different cereals (sorghum and millet) isolated eight

bacteria and six fungal species. These were *Bacillus subtilis*, *Bacillus pumilus*, *Escherichia coli*, *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, *Micrococcus* spp., *Staphylococcus aureus*, *Streptococcus* sp., *Mucor* sp., *Rhizopus* sp. and *Saccharomyces cerevisiae*. It was also in agreement with the observation of Akinrele *et al.* (1970), who worked on the fermentation studies of maize to produce a traditional African starch cake “Eko”. He isolated various species of bacteria, fungi and yeasts.

CONCLUSION

It is obvious that kunu is one of the locally made beverages that people rushed to purchase not only for its low price but also of what it does in the body. Kunu is a very nutritious beverage that can supply most of the nutrient requirement by the body. Also from the analysis, it was seen that kunu from millet gives the highest nourishment to the body; they are of more nutritive value and good source of energy because of their high amount of protein, normal total solid, moderate pH and acidity. They have high amount of calcium which help in healthy bone and strong teeth. The micro-organisms encountered in this study of indigenous fermented food drink (kunu) was as a result of contamination from one source or the other which include water, air, equipment or utensils used in processing, personal hygiene e.t.c. These bacteria are non pathogenic and human commensals, hence they cannot transfer disease.

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