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Proximate Composition and Microbiological Quality of "Kunu-Aya": A Locally Produced Non-Fermented Beverage in Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author FCA designed the study, wrote the protocol and packaged the final manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Kunu-aya" is non- fermented beverage locally produced from Cyperus esculentus (Tiger nut) and Phoenix dactylifera (Dates) based milk. This study determined the proximate composition and microbial quality of laboratory produced "Kunu-aya" following standard methods. Biochemical characterization method was carried out for identification of associated isolates. The results revealed that the "Kunu-aya" contains; moisture content was 59.44±0.05, Ash content of 5.02±0.07, crude fat 8.108±0.03, Crude fibre 9.16±0.04, crude protein 8.983±0.26, and carbohydrate 9.3±0.05 and the energy content was 182.69kcal. "Kunu-aya" also contains significant amounts of magnesium (Mg) 2.045±0.05, potassium (K) 40.506±0.05, phosphorus (P) 4.506±0.03, copper (Cu) 0.018±0.04, zinc (Zn) 48.001±0.01, iron (Fe) 0.408±0.04, sodium (Na) of 6.031±0.07 and calcium (Ca) 0.196±0.03. The total viable bacteria count of the sample was 4.0 x 104 CFU/ml and the total fungal count was 2.7x 102 CFU/ml. Species of Staphylococcus spp (50%), Bacilli spp. (16.7%)

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Streptococcus spp. (16.7%) and Micrococcus spp. (16.7%) are predominant bacteria contaminants of the beverage. Besides, fungi species of *Aspergillus spp.* (60%), *Penicillium spp.* (20%) and Rhizopus spp. (20%) were also isolated. The microbial load obtained exceeded the acceptable limit stipulated for dairy drinks. Based on the proximate and mineral contents of "Kunu-aya", its rich essential contents can provide nutrients for human nutrition. Therefore, the production of this beverage under strict hygiene conditions will improve the microbiological quality and reduce the health risk associated with its consumption.

Keywords: Tiger nut (Cyperus esculentus); dates (Phoenix dactylifera); kunu-aya; proximate composition; microbiological quality; hygienic environment.

1. INTRODUCTION

"Tiger nut (*Cyperus esculentus*) belongs to the division Magnoliophyta, class Liliopsida, order Cyperales, family Cyperaceae, and species *Cyperus esculentus* found to be a cosmopolitan, perennial crop of the same genus as the papyrus plant" (Hankus and Sarret, 2000). "The plant was introduced by the Arabs, first in the Valencia region. It is native to most of the Western Hemisphere, Southern Europe, Africa, Madagascar, the Middle East and the Indian subcontinent" [1]. "In Nigeria, Tiger nut is called "Aya" in Hausa, "Imumu" in Yoruba, and "Aki Awusa" in Igbo. Tiger nuts can be eaten raw, roasted, dried, or baked" [2,3].

"Date palm (*Phoenix dactylifera*) is a flowering plant species in the palm family, Arecaceae, cultivated for its edible sweet fruit. The origin of date palm is unknown, because of its long cultivation, it probably originated from lands around Iraq" [4]. Most species of date palm are cultivated in the tropical and subtropical regions worldwide [5]. "Date trees typically reach about 21-23 meters (69-75ft) in height, growing singly or forming a clump with several stems from a single root system" [6].

"Kunu-aya" is mostly consumed in the afternoon to cool the body from hot weather, it is cheap, popular, available, affordable, drink for both the poor and the rich [7]. "Tiger nut and date-based milk is a nutritive and energetic drink both for the old and young people" [1]. "It is rich in energy content (Starch, fat, sugar, and protein), minerals (phosphorus, potassium) and vitamins E and C" [8]. "Tiger nut milk is a traditional non-alcoholic beverage with a spicy nutty taste. Commonly, tiger milk is underutilized due to its short shelf life and lack of information on its nutritional potential" [9]. "Apart from being used as a beverage, tiger nut milk is thought to be beneficial to diabetic patients and those seeking to reduce cholesterol or lose weight" [10].

Locally produced Tiger nuts and dates are vulnerable to contamination arising from unhygienic handling of the nuts, processing, and packaging procedures. Consequently, the consumption of locally prepared tiger nuts and date-based milk could be a high source of infection and risk to human health. This study, therefore, determined the microbiological quality and proximate composition of tiger nuts and date-based milk.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Fresh tiger nuts, dates and all the other materials were purchased from Watt market, Calabar, Cross River State, Nigeria, and were of analytical standard.

2.2 Production of Tiger-nut and Date-based Milk

Tiger nut and date-based milk were prepared following the procedures of Djomdi and Ndjuenkeu [11]. Tiger nuts were sorted to remove broken, rotten nuts, dirts and foreign materials. The cleaned nuts were then washed in two changes with portable water, and soaked overnight. Other ingredients used in milk production such as date fruits and coconut were similarly processed. The entire ingredients were thoroughly washed in warm water. The cleaned tiger nuts, dates, and coconut were blended with 120ml of cooled boiled water into a slurry using a warring laboratory blender. The slurry was filtered using a muslin cloth to extract the milk. The milk extracted was transferred into sterile glass flak and stored for further analysis.

2.3 Proximate Composition Analysis of the Sample

2.3.1 Determination of the Moisture content

The moisture content of the sample (tiger nut and date-based milk, kunu-aya) was determined

following the guideline of the Association of Analytical Chemists [12]. Two grams (2.0g) of samples were weighed into three crucibles, labeled, and placed in a vacuum electrostatic oven at 79oC for 24 hours. The crucible and their content were cooled in a desiccator containing magnesium sulphate as a drying agent and the final weight was noted [12]. The calculation was done as shown below:

$$Moisture\ \% \ \frac{loss\ in\ weight \times 100}{initial\ weight\ of\ sample}$$

$$i.e \frac{W2-W3 \times 100}{W2-W1}$$

Where,

W1 = Initial weight of the crucible
W2 = Weight of crucible + sample before drying
W3 = Weight of crucible + sample after drying

2.3.2 Determination of crude fat content

Five grams (5.0g) of the sample was weighed into a small porcelain bowl and heated in an oven at 105^{o}C for 1 hour. After cooling, the dry tiger nut sample was transferred into a Soxhlet thimble. The sample was covered with glass wool and placed into a Soxhlet apparatus (fat extraction unity). A dry and clean fat extraction flask (pre-weighed) was placed into the extraction unit together with about 300ml of petroleum ether (boiling point 40-60^{o}C) and was allowed to reflux for 6 hours. Extraction was carried out on the tiger nut and date sample.

Finally, petroleum ether evaporated off and the flask dried in an oven at 105^{o}C for 1 hour and was then transferred into a desiccator to cool. The weight increase of the flask was estimated as corresponding to the fat content [12]. Fat was calculated using the formula:

$$\%\ fat = \frac{weight\ of\ fat \times 100}{weight\ of\ sample}\ i.e\ \frac{T-D \times 100}{w}$$

Where,

T = Final weight of flask used for extraction
D = Initial weight of flask used for extraction
W = Weight of sample used for extraction

2.3.3 Assessment of the protein content

Five grams (5.0g) of the sample was weighed into 250 ml standard Kjeldhal flask containing 1 table of Kjeldhal catalyst, some into bumping chip and 30ml of concentration H_2SO_4 was introduced into digestion rack and heated gently for one hour to prevent vigorous colouring and frothing. The flask was subjected to vigorously heating for 8 hours until a clear bluish colour was obtained. After the digestion the flask was cooled in tap water and qualitatively transferred into 100ml standard volumetric flask and make up to mark with distilled water. 10ml portion of the digested sample was measured into a semi micro Kjeldhal distillation apparatus and treated with 30ml of 40% NaOH solution. The ammonia evolved was steam distilled into 100ml beaker containing 10ml of 2% boric acid plus 2 drops of double indicator (0.1% methyl red and 0.1% methyl blue in 100ml of ethanol) [12].

The tip of the condensed receiver was immersed in the boric acid, double indicator and the dilution continued until about 3 times the original volume was obtained. The distillate was then titrated with 0.1ml Hel (Hydrochloric acid) solution until a purple-pink end point was reached. The % (percentage) nitrogen content in the sample was obtained with appropriate calculation.

A blank was also determined by carrying out the above procedure except that the sample was not used.

Calculation:

=% Crude protein =

$$\frac{\frac{N}{14}\ x\ Hcl\ sample - Hcl\ blank\ x\ DFG\ x\ NF}{weight\ of\ sample\ in\ milligrams}$$

Where,

DF = Dilution Factor
NF = Nitrogen Factor

2.3.4 Examination of fibre content

The procedure for the estimation of fibre was carried out in different stages: Acid digestion, filtration and rinsing, base digestion and ignition method [12].

Acid digestion: Five grams (5.0g) of samples was weighed out into 250ml beaker containing 2% H_2SO_4 and mixed well and heated for 30 minutes with constant steaming. After boiling, the sample was **filtered and washed** with distilled water to remove the acid content until the residue was acid-free.

Base digestion: The residue was further treated with 50ml of 2% NaOH solution and heated for 30 minutes with constant steaming. The residue was made base free by filtration and washing with distilled water. The residue left was treated with methanol and filtered. It was dried in a crucible of known weight, at 100^{o}C, this followed by ignition in furnace at 550^{o}C.

The weight of ash left after ignition was recorded and calculation done using the formula below:

% fibre =$\frac{weight\ of\ sample\ x\ 100}{initial\ weight\ of\ the}$

2.3.5 Determination of total carbohydrate content

Total Carbohydrate content in the sample was determined using differential method [12].

Total CHD = 100 – (% moisture + % Ash + % fat + % protein + % fibre)

2.3.6 Determination of ash content

Five grams (5.0g) of the samples were weighed into the crucible of known weight with the lid ignited in a muffle furnace for 2 hours at 550^{o}C. At the end of this process, the crucible and lid were cooled in the desiccator containing magnesium sulphate as a drying agent. The final weight was noted and calculated using the formula.

% Ash = $\frac{weight\ of\ ash\ in(g)x\ 100}{initial\ weight\ of\ the\ sample}$

i.e = $\frac{z-x\ \times 100}{initial\ weight\ of\ the\ sample}$

Z = Weight of crucible plus sample before drying
Y = Initial weight of crucible
X = Final weight of crucible after ashing

2.3.7 Determination of energy content of the sample

The energy value was calculated by the factor of 4, 9, and 4 with the following; the total CHO (Carbohydrate), % fat, and protein, i.e CHO by factor of 4, fat by factor of 9 and % protein by factor of 4 respectively [12].

2.3.8 Determination of Mineral elements of the sample using Atomic Absorption Spectrophotometer (AAS)

The sample was grounded with a manual blender, 1.0g of the ground sample was weighed into the Kjeldahl digestion flask. This was followed by the addition of 20ml of the strong acid mixture of combined 650ml of concentrated Nitric acid (HNO3), 200ml of Perchloric acid (PCA) and 30ml of concentrated Sulphuric acid (H2SO4). The mixture was then cooled under tap water. It was digested using Kjeldahl digesting rack by heated in the fume hood until the digestion solution gave a clear solution. The flask was allowed to cool under room temperature. The sample was then transferred into a 500ml volumetric flask with a stopper and made up to the mark with de-ionized water.

A 1.0ml of the digested sample was measured into different 100ml flask and made up to the mark with de-ionized water. The stock solutions were prepared as standards respectively. The digested sample was measured with their allowed cathode in AAS respectively. At the end of the Atomic Absorption Spectrophotometer analysis, the reading was obtained [12].

2.4 Microbiological Analysis

2.4.1 Enumeration of Total Heterotrophic Bacterial (THB) Counts

The Total Heterotrophic Bacterial count of the sample (Kunu-aya) was determined using the viable count method as described by Cheesbrough [13]. Serial dilutions (10^{-1} – 10^{-6}) were prepared from the liquid sample. Exactly one millimeter (1ml) was taken from each selected dilution (10^{2}, 10^{4}, 10^{6}) into sterile Petri dishes. The molten sterilized Nutrient and MacConkey agar was poured into the plates, swirled to spread the inoculums evenly within the agar medium and allowed to solidify. They were then incubated at 37^{o}C for 24 hours. Thereafter, plates with colony growth were counted and recorded in colony forming units (CFU/ml).

2.4.2 Determination of Total Fungi count

The spread plate method was used for determination of total fungi count. Fifteen grams (15 g) of sterilized molten Sabouraud Dextrose Agar (SDA) was poured onto sterilized Petri dishes and were allowed to solidify. Thereafter, 1ml of the selected diluents from the samples

was aseptically transferred onto the solidified SDA Petri dishes. The plates were swirled to ensure even distribution of the samples, and then incubated at 28-30^{o}C for 3 – 4 days. Colony growth were counted and expressed as colony forming unit per millimeter (CFU/ml).

2.4.3 Biochemical characterization and identification of isolates

All bacterial isolates were characterized and identified based on their cultural and biochemical characteristics following the method described by Cheesbrough [13]. Biochemical test conducted includes; Gram staining, Tripple sugar iron, indole, methyl red, citrate utilization, catalase, oxidase, Voges Proskauer and sugar fermentation.

Method for identification of fungi

3. RESULTS

The proximate composition of the produced milk revealed that the moisture content was 59.44±0.005%, Ash content 5.02±0.07%, Crude protein 8.783±0.26%, Crude fiber 8.108±0.03%, Crude fat 9.16±0.04%, and Carbohydrate content 9.3±0.05% as shown in Table 1. The energy content of the produced milk showed a value of 182.69kcal, arising from the percentage of fat, protein, and total carbohydrate.

Results are presented in mean ± standard deviation of triplicate value.

The analysis of the energy content of the produced milk showed a value of 182.69$k_{cal,}$ arising from the percentage values of fat, protein and total carbohydrate as shown in Table 2.

The mineral content of Kunu-aya is presented in Table 2. The result showed that iron, sodium, calcium, zinc, potassium, magnesium, phosphorus and copper are essential mineral element associated with the Tiger nut and Dates-based milk beverage.

Table 1. Proximate Composition of Kunu-aya produced from Tiger nut and Dates-based milk

Parameters	% Composition
Moisture	59.44±0.05
Ash	5.02±0.07
Crude protein	8.783±0.26
Crude fibre	8.108±0.03
Crude fat	9.16±0.04
Carbohydrate	9.3±0.05
Energy Value K_{cal}	**182.69K_{cal}**

Table 2. Energy contents of the produced milk sample

Parameters	Values
% Fat	72.854
% Protein	35.7
Total Carbohydrate	74.04
Energy Value K_{cal}	**182.69K_{cal}**

Table 3. Mineral elements of the produced milk sample

Mineral element	% Composition
Iron (Fe)	0.0405±0.04
Sodium (Na)	6.031±0.07
Calcium (Ca)	0.196±0.03
Zinc (Zn)	48.001±0.01
Potassium (K)	40.506±0.05
Magnesium (Mg)	2.045±0.05
Phosphorus (P)	4.506±0.03
Copper (Cu)	0.018±0.03

Results are presented in mean ± standard deviation of triplicate value

Table 4. Total Heterotrophic Bacterial Count

Samples	TVB Count (CFU/ml)	TF Count (CFU/ml)
Commercially made tiger nut milk	3.8×10^3	2.4×10^2
Self-made tiger nut milk	4.0×10^4	2.7×10^2
Locally hawked tiger nut milk	4.4×10^6	2.9×10^4

Key: TVBC – Total Viable Bacteria Count. TF- Total Fungi

Table 5. Biochemical characterization and identification of isolates

Samples	Cell morphology	Cell shape in Microscope	Gram reaction	Catalase	Indole	Methyl red	Voges proskaur	Citrate	Oxidase	Tripple sugar iron										Suspected Organisms
										Gas	Acid	Slant	Butt	H_2s	Slant	Butt	Glucose	Lactose	Sucrose	
1	Glisten	Cocci in chains	+	-	-	+	-	+	-	+	-	+	-	-	-	-	+	+	+	*Streptococcus spp*
2	Bluish	Bacilli with Central spore	+	+	-	-	+	+	-	+	+	+	-	-	-	-	+	+	+	*Bacilli spp*
3	Yellow	Cocci in clusters	+	+	-	+	+	+	-	+	-	+	-	-	-	-	+	+	+	*Staphylococcus spp.*
4	Grape like	Cocci in clusters	+	+	-	+	+	+	-	+	+	+	-	-	-	-	+	+	+	*Micrococcus spp.*
5	Big creamy	Cocci in clusters	+	+	-	-	-	+	-	+	-	+	-	-	-	-	+	+	+	*Staphylococcus spp.*
6	Small creamy	Cocci in clusters	+	+	-	+	-	+	-	+	-	+	+	-	-	-	+	+	+	*Staphylococcus spp,*

Table 6. Frequency and percentage occurrence of bacterial isolates

Isolates	No. of Occurrence	% Occurrence
Staphylococcus sp.	3	50
Bacillus sp.	1	16.7
Micrococcus sp.	1	16.7
Staphylococcus sp.	1	16.7
Total	**6**	**100**

Table 7. Characteristics and identification of fungal isolates

S/N	Macroscopic Characteristics	Microscopic characteristics	Presumptive Organisms
1	Rapidly growing on PDA starting with a white to yellowish mat of mycelia. Reverse side of plate is white.	Septate hyphae conidia are covered by double stigmata to form radiate head.	*Aspergillus* sp.
2	Typical blue-green mycelia with white edges. White on the reverse side of plate.	Septate hyphae conidia arranged like brown stick.	*Aspergillus* sp.
3	Colony irregular shape with purple edge. Mycelia became green-yellow as they aged. White on reverse side.	Septate hyphae conidia arranged like a mob-head.	*Aspergillus* sp.
4	Bluish-green mycelia with a white border. Reverse is white.	Septate hyphae conidia attached to sterigma.	*Penicillium* sp.
5	Dense white filamentous hyphae covering entire plate later turn grey or yellowish-brown. White on reverse plate.	Hyphae have few septa spores enclosed in a sporangia.	*Rhizopus* sp.

Table 8. Frequency and percentage of occurrence of fungal isolates

Isolates	No. of Occurrence	% Occurrence
Aspergillus spp	3	60
Penicillium spp	1	20
Rhizopus spp	1	20
Total	5	100

The enumeration of the total heterotrophic bacterial and the total fungal counts of the samples showed that the locally produced (hawked) tiger nut had higher bacterial load when compared to self-made tiger nut while the commercialized tiger nut sample yielded low bacterial count as when compared to the self-made tiger nut (Table 4).

Biochemical characterization and identification processes revealed that *Staphylococcus spp., Streptococcus* spp*., Micrococcus* spp. and *Bacillus* spp. were the suspected bacterial organisms found in the produced milk sample, and displayed in Table 5.

The percentage occurrence of the bacterial isolates obtained showed that *Staphylococcus* species had the highest prevalence (Table 6).

Aspergillus spp., *Rhizopus* spp. and *Penicillium* spp., were the organisms obtained from the total fungal screening (Table 7).

The percentage occurrence of fungal isolates observed in this study revealed that *Aspergillus* species had the highest of occurrence of 60% (Table 8).

4. DISCUSSION

The result of the proximate composition analysis and energy value of *Cyperus esculentus* and *Phoenix dactylifera* based milk drink (Kunu-aya) showed that it is a healthy and nutritious milk drink. Kunu-aya contained good quantity of carbohydrate, fat, fibre and protein content. The result obtained also showed that the drink is also a good source of magnesium, iron potassium, copper, zinc, calcium, sodium and phosphorus. These nutrients could significantly contribute to the body's metabolic processes and refreshing the body as well. The finding corroborates the reports of [14].

"Magnesium provides bone strength, aids enzyme, nerve and heart functions. Phosphorus enhances quick release of energy in the body and may combine with calcium for bone and teeth development. Recent studies on blood pressure showed that a diet rich in potassium and magnesium but low in sodium can lead to a decrease in blood pressure within days of beginning a specific diet" [15]. Tiger nut contains protective or preventive nutrients because it could supply adequate zinc, copper, iron. Zinc is an integral part of hormones and more than nearly 100 different enzymes. Zinc is important in many metabolic reactions and may play an important role in immunity, alcohol metabolism, sexual development and reproduction. Copper aids iron metabolism. It works with many antioxidants, enzymes especially those involved in protein metabolism and hormone synthesis.

Fibre plays an important role in the reduction of pressure and transit time of food through the body aiding in digestion. Fibre also aids in alleviation of flatulence problem. Thus, the fibre content present in the milk drink could serve as a way of treating indigestion, constipation and non-communicable diseases such as colon cancer, coronary heart disease and obesity [16].

The moisture content of Kunu-aya is high which indicates that it could perish easily due to microbial invasion. The result of the total microbial analysis showed that the samples; commercially prepared, self-prepared and locally prepared Kunu-aya drinks had high microbial count which exceeded the standard recommended by National Agency for Food and Drug Administration and Control (NAFDAC) who stipulates that mesophilic aerobic count of locally prepared beverages should not exceed 5.0 $\log_{10}$ CFU/ml [16]. The high microbial count observed in the drink could be attributed to inadequate hygienic conditions and methods used during the processing of the milk drink. Thus, this finding indicates that the tiger nut and date-based milk (Kunu Aya) is not safe for consumption. This is in agreement with the works of Francis et al. [17] who stated that all the ready-to-drink Tiger nut drinks Sold within Port Harcourt Metropolis are significantly contaminated thereby, making them not suitable for human consumption.

The bacterial isolates observed from the milk drink are *Bacilli spp. Streptococcus spp. Micrococcus spp.* and *Staphylococcus spp.* while the fungal isolates are *Rhizopus spp., Penicillium spp, and Aspergillus spp.* This observation, is in agreement with the work of other researchers who also isolated similar organisms from their works [18,17]. The high prevalence of *Staphylococcus spp.* could be explained by the fact that the bacteria exist as a normal flora of the skin. This corroborates the findings of Akusu et al. [19] who worked and reported that *Staphylococcus spp.* Is a normal flora of the skin. The presence of *Micrococcus spp.* found in the product may be as a result of fact that it is a normal flora of the skin and also present in places like soil, water, dust, even animal skin, which agrees with the work of Ganz et al. [20]. The presence of Bacillus species from tiger nut-based milk is also in line with the findings of Udeozor and Awonorin, [21] and Sherifah et al. [22]. Therefore, strict hygienic practices during production, handling and distribution of tiger nut drinks, use of sterilized plastic containers, natural antimicrobial preservatives, potable water and proper storage conditions should be enforced by relevant regulatory bodies to ensure adherence [23].

5. CONCLUSION

The research findings from the nutritive content of Kunu aya revealed that the milk drink is rich in energy giving nutrients (Carbohydrate and fats), phosphorus, potassium, magnesium and protective or preventive nutrients (Fibre, iron, copper and zinc). The milk drink also contains fair quantity of protein, calcium and sodium which makes the drink very essential and nutritional. The microbial analysis showed that the tiger nut and dates-based drink has total heterotrophic counts which exceeds the range stipulated by National Agency for Food and Drug Administration and Control (NAFDAC). The presence of *Staphylococcus spp. Micrococcus spp.* and *Bacillus spp* which are known normal flora of the skin indicates that the drink was not safe for humanconsumption, and could be traced to inadequate hygienic conditions during production processes.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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