PROXIMATE, MINERAL AND MICROBIAL ANALYSIS OF LOCALLY PRODUCED JUICE (KUNU, SOYMILK AND TIGERNUT)

ABSTRACT

Kunu, soymilk and tiger nut drinks are locally produced indigenous non-alcoholic beverages widely consumed in Nigeria. The beverages sold in Akwa was analysed for proximate, mineral and microbial analysis. The AOAC method of analysis was employed in the determination of proximate and mineral composition of the drinks. The following proximate results were obtained, soymilk contained 86% moisture, 2.22% ash, 0.07% fiber, 4.40% protein, 1.37% fat and 5.94% carbohydrate. For unu; 81% moisture, 1.85% ash, 0.53% fiber, 1.85% protein, 0.81% fat and 13.96% carbohydrate. For tiger nut; 84%moisture, 3.08% ash, 0.14% fiber, 2.70% protein, 1.95% fat and 11.03% carbohydrate. Mineral analysis of soy milk contained 127.89 Ca, 0.85 Fe, 17.60 P, 147.00 Mg and 11.37 P. Kunu contained 217.90 Ca, 2.37 Fe, 113.00 K, 106.05 Mg and 20.00 P. Tiger nut contained 221.00 Ca, 2.83 Fe, 135.00 K, 175.00 Mg and 75.10 P. Total bacteria count of soymilk, kunu-zaki and tigernut ranges from (0.70×10^6 to 1.97×10^6) (cfu/ml), (0.60×10^6 to 1.90×10^6) (cfu/ml), (0.40×10^6 to1.59×10^6) (cfu/ml) respectively. Faecal bacterial count of soymilk, kunu-zaki and tiger nut ranges from (3.00×10^4 to 5.90×10^4) (cfu/ml), (3.37×10^4 to 5.50×10^4) (cfu/ml), (1.15×10^4 to 5.13×10^4) (cfu/ml). Bacteria identify are *Klesiellaspp*, *Salmonella spp*, *Shigellaspp*, *E.coli*, *Vibrio spp*, *Staphylococcus aureus* and *Pseudomonas spp.*

Keywords: Bacteria, Drinks, Kunu, soymilk, tigernut

INTRODUCTION

1.1 Background of the Study

Kunun-zaki (Kunu) is a cereal based non-alcoholic fermented beverage mostly consumed in the Northern part of Nigeria. It can be produced either from millet (*Pennisetumtypoidum*), Sorghum (*Sorghum bicolor*), or maize (*Zea mays)*Akoma *et al.* (2006). Kunun-zaki is a Hausa word meaning sweet beverage. It is consumed

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Introduction
Material and methods
Results and discussion
Conclusion
Recommendations
References

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anytime of the day by both adults and children as a breakfast food drink. It is a refreshing drink usually used to entertain visitors; it also serves as an appetizer and is commonly served at social gathering (Amusa and Ashaye, 2009). Onuorah *et al*. (1987) reported kunun-zaki as being regarded as after meal drinks or refreshing drinks in rural and urban centres, it is sometimes used as a weaning drink for infants (Adebayo *et al.,* 2009). Preparation methods vary amongst people's taste and cultural preferences. Production of kunun-zaki is still on small scale and the beverage is widely found in the local market and at resorts (Innocent *et al.*, 2011). This non-alcoholic beverage is however becoming more widely accepted in several other parts of Nigeria, owing to its refreshing qualities (Amusa and Ashaye, 2009).

Generally, kunun-zaki production involves steeping of sorghum, millet or maize, wet milling, sieving and partial gelatinization of the slurry. Qualitatively it is obtained after 5 days and could only be stored for another 3 days when refrigerated. Reduction in processing time and an alternative technology to produce safer and shelf stable powdered kunu drink that would be reconstituted when needed will help to improve the availability of this indigenous food drink and more convenient, especially for people, who may not have time to go through the long process involved in kunun-zaki production. Hence the study of production of reconstitutable kunun-zaki, and its comparison with freshly prepared kunun-zaki.

Soymilk

Soymilk gotten from soybean (Glycine max) is a member of the family *legminosae* sub family *papilnonaceae* which have an exceptional nutritional and functional food profile. Soy-foods are considered to be nutritious and healthy based on their nutrient composition (UNUCED, 2016). It is an excellent source of protein and oil of good quality. It contains about (43%) protein, (21%) carbohydrates, (5%) minerals, (8%) moisture, (20%) fat, (4%) fiber (Ganshrao, 2016). Soybean is rich in calcium and vitamin B12. Tocopherols are an important constituent of soy oil, due both to the vitamin E supplied for human nutrition and their antioxidant properties.

Soy bean was introduced into Nigeria in 1908; it was first planted in Ibadan, Oyo State. Initially the crop was cultivated for export with the support and encouragement of Groundnut Board. Nigeria presently produces about 500,000 MT of Soybean annually making it the largest producer of the product on the African continent. Soybean is a legume which is produced in most the middle belt of the country with Benue state accounting for about 45% of the total production in country. Soymilk is an aqueous, white, creamy extract produced from soybeans which is similar to cow milk in appearance and consistency. It is a highly nutritious which contains protein, fat, carbohydrates vitamins and minerals. Soymilk beverage worldwide is credited to health benefits such as: low cholesterol and lactose, its ability to reduce bone loss and menopausal symptoms, prevention and

reduction of heart diseases and certain cancers. As this drink is cholesterol free and low in energy, it could enhance health benefits in terms of reducing body weight and blood lipids (kohli *et.al*. 2017).

Tigernut

Tiger nut "*Cyperusesculentuslativum"* is an underutilized tuber of family *Cyperaceae*, which produces rhizomes from the base of the tuber that is somewhat spherical. It is a tuber that grow freely and is consumed widely in Nigeria, other parts of west Africa, east Africa, parts of Europe particularly Spain as well as in the Arabian Peninsula (Abaejoh *et al.,* 2006). In many thousand years ago, tiger nut, in Spanish called chufa, was cultivated in region of chufa between Sudan and Egypt on the borders of the Nile River. There are documents that certify this product over 400 years ago. Proof of this is that on many occasion archeologists found earthen jars containing tiger nut in graves of pharaohs. (Obadina *et al*., 2008) previously, it was cultivated in the ancient Mesopotamia between the rivers Tigris and Euphrates. At the same time historical Persian and Arab documents mentioned the nutritive, digestive and dis-infective value of tiger nut. During the era the tiger nut milk was classified as medicinal drink due to it been highly energetic and diuretic, rich in mineral, predominantly phosphorus and potassium and also vitamins C and E (Abaejoh *et al.,* 2006). Tiger nuts tubers appear somewhat long or round in shape with a dimension of 8mm to 16mm, smaller size

however, are not used for human consumption. When hydrated, it is slightly harder (nut texture), but with a rather more intense and concentrated taste. Being cultivated through continuance irrigation, tiger nut has to be properly dried before storage. The drying process is completely natural,(i.e. sun drying) and the process can take up to one month. The dehydrating process ensures longer shelf life, preventing rot or any other bacterial infection securing their quality and nutritional level. Unfortunately, the dehydration process make the tiger nut skin wrinkled, a situation that limits its acceptability to some people (Belewu and Abodunrin, 2006). It is known in Nigeria as “Aya” in Hausa,”Ofio” in Yoruba and “Akiausa” in Igbo where these varieties (black, brown and yellow) are cultivated. Among these, the yellow variety is preferred over others because of its inherent properties such as large size, attractive color and fleshier nature. It also yield more milk upon extraction, contains lower fat and higher protein and less anti nutritional factors especially polyphenol (Okafor*et al*., 2003). Recently, there is awareness for increased utilization of tigernut (Belewu and Abodunrin, 2006; Belewu and Belewu, 2007).

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1.2 Statement of Problem

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Soymilk contains isoflavones (classified as phytoestrogen) which have a chemical structure similar to the hormone estrogen and binds to the estrogen receptor in the body. There are two estrogen receptors in the body. When isoflavones attach to one, they produce estrogen-like effects, but when they attach to the other, they have an anti-estrogen effect and because of this isoflavones in soy milk is link to breast cancer. The phytoestrogens may also have negative effects on thyroid function, especially in those with thyroid disease or subclinical thyroid disease or those who are deficient in iodine. Aside from isoflavone, soy milk also containsphylates which are anti-nutrients that can block the absorption of certain minerals, like iodine, zinc, iron, magnesium, copper and chromium. If consumed a lot together with eating processed foods that contain soy, this can increase the risk of developing nutritional deficiencies.

Due to the presence of anti-nutritional compounds such as phytates and oxalates in tiger nut; these anti-nutrients have specific effects on the body. Phytates may result in reduction of calcium and iron absorption, while oxalate could result in reduction of calcium formation and also, encouraging kidney formation.

1.3 Aim and Objectives of the Study

The aim of the study is to isolate *Staphylococcus aureus*found in locally produced Kunu, soy milk and tiger nut drink, also to carry out the nutritional and proximate compositions of the juices.

The objectives of the study include the following:

1. To determine the proximate composition such as: moisture content, ash content, crude fiber, crude fat, protein content, carbohydrate content.
2. To determine the mineral compositions which include: calcium, phosphorous, magnesium, iron and potassium.
3. To determine total bacteria count and faecal count.
4. To isolate and carry out microbial analysis on bacterial species

1.4 Justification of the study

In developing Nigeria, it has not been possible to have control over processing of hawked drinks because most vendors lack the adequate knowledge of food processing and adequate handling practices. As such, there is likely to be a high risk of chemical and microbial contamination. A large number of bacteria have been reportedly implicated in food spoilage as they used the carbohydrate content of food for undesirable fermentation processes (Ojoko *et al*., 2002; Amusa*etal*., 2005).Kunu, soymilk and tiger nut are rich beverages and food products rich in fiber, protein and Vitamin and a substitute for cow milk and other source of protein, cheaper than other source of protein. There is need to ensure that the milk

is hygiene prepared, free from bacteria or other spoilage organism.Therefore, it becomes very necessary to conduct this research to determine the bacterial load, proximate and mineral composition of these drinks in Awka, Anambra state.

1.6 Significances of the Study

Milk is an excellent source of most nutrients. In developing countries, the cost of diary milk is prohibitive. The high cost of milk in developing countries has led to the development of alternative source of milk from plant materials. Because of its underutilized less expensive and rural nature, it is hardly processed commercially and since it is processed locally, heat processing treatment like pasteurization, to combat pathogenic microorganisms in the juices.

The significant is to enable producers to improve hygienic condition handling of the drinks and a good knowledge of safe food, also to enlighten the public of various pathogenic organisms present hence increasing health awareness on the dangers of drinking the juices.

1.7 Scope of the Study

This study focuses on the bacterial strain of *Staphylococcus aureus* in locally produced juices (kunu, soy milk and tigernut) when poorly processed and stored, also to dictate the proximate and mineral composition of the drinks.

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MATERIALS AND METHODS

Materials

Oven, Kunu, Soymilk, Tigernut,Culture Media, Petri Dishes, Wire Loop, Bunsen Burner, Measuring Cylinder, Foil Paper, Incubator, Test Tube, Test Tube Rack, Cotton Wool, Masking Tape, Distilled Water, Refrigerator, Immersion Oil, Light Microscope, Slide, Dessicator, Muffle Furnace, Silica Dish, Kjeldahl Flask, Funnel, Soxhlet Apparatus, Filter Paper, Thimble, Retort Stand, Crucible, Weighing Balance,Tetraoxo-Sulphate (Vi) Acid, Boric Acid Indicator Solution, Sodium Hydroxide, Hydrochloric Acid, Petroleum Ether, Potassium Hydroxide, Phenolphthalein Indicator, Spectrophotometer, chronical flask.

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Sample Collection

Three samples each of kunu, soy milk and tiger nut drink was purchased from Awka market and taken to the laboratory for analyses.

Method

Moisture Content Determination

The AOAC (2002) method no. 945.38 will be used. 5g of the sample will be weigh .into clean, dry and pre weighed crucibles. The crucibles and their contents will be dry in the moisture extraction oven at 110^0C for 4 hours. The samples will be cool in desiccators and reweighed. The samples will be dried in the oven until a constant weight is obtained.

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$$\%\ Moisture\ content\ =\ \frac{Initial\ weight-\ weight\ of\ oven\ sample\ X\ 100}{\text{Initial weight of sample}}$$

Crude Fat Determination

Method no. 920.39A (AOAC, 2002) will be used. 5g of the air dried ground sample will be weighed into a filter paper, wrapped carefully and put in the sample holder of the soxhlet extraction apparatus. A clean dry and weighed soxhlet extraction flask will be half filled with N-hexane and the whole apparatus will be assembled together, and the flask placed on the heating mantle and heated at 60^0C.

The fat was extracted for three hours. Then, the sample holder will be disconnected and the extraction flask removed. The percentage fat contained will be determined thus:

$$\%\ Crude\ fat\ =\ \frac{weight\ of\ flask\ +\ oil-\ weight\ of\ empty\ flask\ X100}{\text{Initial weight of sample}}$$

Crude Fiber Determination

Method No. 942.05 (AOAC, 2002) will be used. 2g of defatted sample will be weighed into 250 ml beaker containing 200 ml of 0.125M tetraoxo-sulphate(iv) acid (Sulphuric acid). The mixture will be heated in a steam bath at 70^0C for hours, and then allowed to cool. The cooled mixture will be filtered using a muslin cloth over a Buckner funnel. The residue will be washed three times with hot water to

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remove the acid and then put in a beaker containing 200 ml of potassium hydroxide. The mixture will be heated as before over a steam bath for 2 hours. The solution will be filtered and the residue washed three times with hot water. The final residue obtained will be put in clean pre-weighed crucible and dried at 120^0C to a constant weight. The crucible with the dry sample will be put in a muffle furnace and ash at 550^0C for 30 minutes such that the sample became ash white. Percentage fiber will be calculated as followed:

$$\%\ Crude\ fiber\ = \frac{weight\ of\ oven\ dried\ sample - weight\ of\ ash\ X\ 100}{\text{Initial weight of sample}}$$

Method no. 955.04C called the Kjeldahl method will be used (AOAC, 2002). This method will be divided into three namely, digestion, distillation and titration.

Digestion: Approximately 0.1g of ground sample will be weighed into clean dried Kjeldahl flask for digestion, and 0.1g copper tetraoxo-sulphate(iv) crystals, 0.5g sodium tetraoxosulphate(iv) crystal and 25ml of concentrated H_2SO_4 acid will be added into the flask and some glass beads will be added into the flask content as anti-bumping agents. The Kjeldahl flask and its content will be transferred to the digesting chamber in a fume cupboard and digested. Digestion continued with constant rotation of the digestion flask until the sample changed colour (that is from black to light blue). The digestion flask will be removed from the digesting

chamber and allow cooling. The digest was made up to 100ml using distilled water and shaken vigorously to a homogenous solution.

Distillation: Out of the homogenous solution of the digest, 20ml will be transferred into a distillation flask using a pipette. Then 20ml of 40% sodium hydroxide solution will be added carefully down the side of the flask through a funnel.

Then 50ml of 2% boric acid solution will be pipetted into a receiving flask and two drops of methyl red indicator added. The distillation unit will be fitted such that the condenser is connected to the receiving flask with a glass tube, and the condenser cooled with constant supply of cold water from tap. Also, the tip of the glass tube will be immersed in the boric acid. The distillation unit is heated on a heating mantle for 35 minutes until the pink solution of the boric acid turned blue and the volume increased to about 100ml by the distillate.

Titration: Ten millilitres of the distillate will be titrated against 0.1N hydrochloric acid to a colour-less end point. A blank solution will also be titrated to get any trace of nitrogen in the blank. All the titre volumes were recorded. The percentage crude protein will be calculated as follows:

%Crude protein = % Nitrogen X 6.25

Ash Content Determination

The AOAC (2002) method No 942.05 will be used. Clean dried crucibles will be weighed on an electronic balance and 5g of sample weighed into the crucibles. The samples will be dry in the oven until constant weights are obtained.

Then, the samples will be transferred into the muffle furnace with a pair of tongs and ash at 550^0C 4 hours until ash was obtained. The sample will be removed from the furnace and cooled in desiccators, and reweighed. The percentage ash will be calculated as followed:

$$= \% \text{ Ash Content} = \frac{Weight\ of\ Ash\ X\ 100}{\text{Weight of sample (after oven drying)}}$$

Carbohydrate Content Determination

The carbohydrate content of the sample will be obtained by difference, that is, as the difference between the total summations of percentage moisture, fat, fiber, protein, ash and 100%.

Carbohydrate= 100 – (% moisture + % fat + % protein + % fiber + % ash).

Mineral Element Analysis

The mineral contents of the test samples will be determined by the dry ashextraction method following each specific mineral element as described by AOAC (2005). Twenty (20) grams of the samples will be burnt to ash (as in ash determination and the resulting ash will be dissolved in 100ml of dilute

hydrochloric acid (1MHCL) and then diluted to 100ml volumetric flask using distilled water. The solution will be used for the various analysis of mineral.

Determination of Calcium

Calcium contents of the test sample will be determined by the EDTA complex isometric titration. Twenty (20) ml of each extract will be dispersed into a conical flask and panels of the masking agents, hydroxytannin, hydrochlorate, and potassium cyanide will be added followed by 20ml of ammonia buffer (pH 10.0). A pinch of the indicator-Ferrochrome black will be added and the mixture will be shaken very well. It will be titrated against 0.02N EDTA solution. The calcium contents will be calculated using the formulae below.

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$$Calcium \quad (\boldsymbol{mg/100g}) = \frac{(Tv\ x\ 0.4008\ x\ 1000)}{\text{Vol of sample used}}$$

Determination of Magnesium

Exactly 10ml of the sample filtrate will be pipetted into 250ml conical flask after which 25ml of ammonia buffer solution will be added into the conical flask and will be properly mixed. Then a pinch of Erichrome black T indicator will be added and titrated with 0.02N of EDTA until the colour of the solution change.

$$Magnessium \quad (\boldsymbol{mg/100g}) = \frac{(Tv\ x\ 0.2432\ x\ 1000)}{\text{Vol of sample used}}$$

Determination of Potassium (K)

The concentrations of potassium (ppm) will be analyzed using UV-spectrophotometer at a wavelength of 766.5 nm, and the concentration in mg/100 g will be calculated using the following equation:

$$Potassium\ (\boldsymbol{mg/100g}) = \frac{Concentration\ (ppm)\ x\ Dilution\ factor\ X\ 1000}{\text{Wt of Sample}}$$

Determination of Iron (Fe)

The concentrations of chromium (ppm) wasanalysed using atomic absorption spectrophotometer at a wavelength of 243nm and the concentration in mg/100 g was calculated using the following equation:

$$Iron\ (\boldsymbol{mg/100g}) = \frac{Concentration\ (ppm)\ x\ Dilution\ factor\ x\ 1000}{\text{Wt of Sample}}$$

Determination of Phosphorus (P)

A 20 ml sample solution was put in a 100 ml volumetric flask. The solution was neutralized with ammonia and nitric acid solution (1:2). Twenty (20) ml of vanadate molybdate reagent was added and diluted to the mark. It was allowed to stand for ten minutes and absorbance read at 470nm in the ultra violet region and the mineral concentration in mg/100 g was calculated using the following equation:

$$Phosphorus\ (\boldsymbol{mg/100g}) = \frac{Concentration\ (ppm)\ x\ Dilution\ factor\ x\ 100}{\text{Wt. of Sample}}$$

Microbial Analysis

Preparations of Culture Media

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(a). Nutrient agar (NA): Nutrient Agar was prepared by dissolving 28 g of nutrient agar powder in 1000 ml of distilled water in a clean flask. The mouth of the flask was plugged with non-absorbent cotton wool wrapped with aluminum foil paper that was extended up to the neck of the flask. The flask was placed on a bunsen flame and allows to boil and mix completely. It was sterilized in an autoclave at 12IOC for 15 minutes and allowed to cool to 450C and aseptically dispensed into Petri dishes. Nutrient agar was used for the total bacterial aerobic plate count.

(b). Macron key Agar (MA): This agar was prepared by dissolving bile salt, Then 48.5 g of the powder was dissolved in 1000 ml of distilled water. The pH was adjusted to 7.8. It was autoclave at 121°C for 15 minutes and allowed to cool to a temperature of 45 - 50°C before pouring into plates. This was used to determine coliforms as described by Cheesbrough (2014). This is a selective and differential media designed to isolate and differentiate organism based on their ability to ferment lactose as described by Sebastia, *et al*., (2012).

(c) Corn meal agar (CMA): Corn meal agar was used to isolate yeast and it's prepared by dissolving 17 grams of corn meal powder in a 1000 ml of distilled water. The mixture was heated gently to dissolve the medium completely. 1 % of polysorbate was added and sterilized in autoclave at 121°C for 15 minutes. It was cool at room temperature before pouring into petri dish containing 1ml of the sample as described by Zumbes *et al.*, (2014).

(d). Potatoes dextrose agar (PDA): The medium PDA was prepared by using 39 grams of potatoes dextrose agar powder. It was dissolved in 1000 ml distilled water. It was heated to boiling, in order to get mixed completely. Then sterilized in an autoclave at 121°C for 15minutes, this particular media will be used to this particular media.

(e). Mannitol salt agar (MSA): The medium was prepared by dissolving 108 grams of mannitol salt agar in 1000 ml of distilled water, after which it was allowed to stand for 10 minutes, swirled to dissolve properly. The mixture was sterilized in an autoclave at 121°C for 15minutes and allowed to cool to a temperature of 45°C before pouring into the appropriate petri dish as described by Fowoyo (2012). Mannitol salt agar was used to determine and enumerate the bacteria *Staphylococcus auerus*.

How to Identify Bacteria Strain

(i). **Identification of microbial isolate**: Identification of the microbial isolate was performed using classical methods based on their morphological and biochemical characteristic with reference to systematic manual of bacteriology described by Cheesbrough (2014).

(ii). **Gram staining technique**: Gram staining reaction has the wide application that is capable of distinguishing virtually all bacteria into one of two large group — gram positive or gram negative. Smear of each isolate was made on the slide and heat fixed. Primary stain (crystal violet) was added in drops. Lugols iodine was added for 45 seconds decolorized with acetone and washed with water. It was then air dried examined at Xl00 under oil immersion as described by Bello *et al.*, (2014) Positive gram staining appears purple and negative grams staining appeared pink.

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(iii). **Motility**: The medium used for motility test (agar with concentration of 0.5%) was inoculated with test organism. A stab of each inoculate was made at the center of each tube. The tube at 37oC was incubated for 24 hours. A diffused growth at the place of inoculation was considered as positive and restricted growth was considered as negative.

(iv). **Citrate Test:** The citrate test was performed by inoculating into organic synthetic medium in which sodium citrate is the only sources of carbon and

energy. In sodium citrate broth (Koser's citrate medium), the presence of growth (turbidity) is a positive test result.

(v). **Indole Production:** Indole is produced in triptone broth by the enzyme of certain organisms. Triptone broth is rich in amino acid tryptophan which can be used by some bacteria as source of carbon, energy as well as nitrogen. Tryptophan is degraded to indole pyruvic acid and ammonia by some microorganisms. A loopful of test culture (Twenty four hour old) was inoculated into the triptone broth and incubated for two days. Into six milliliters of culture broth a three milliliters of Kovac's reagent was added from aqueous layer, colour change to red is a positive test.

(vi). **Urease Test:** Bacteria, particularly those growing naturally in an environment exposed to urine, may decompose urea by means of the enzymes urease. This ability was tested for using Christensen medium with heavy inoculation of the isolates was made and the agar slants in tube was observed after 24 hours and then incubated further for hours. 'This test was used to identify coli forms as reported by Musa and Hamm (2013).

(vii). **Coagulase test:** The use of blood plasma is being introduced in coagulase test. A loop full of human plasma was added to culture isolate on a slide. Positive isolate gave agglutination reagent with plasma. Test was also

carried out at 37°C for 24 hours' positive tubes showed coagulation of the plasma in the tube.

(viii). Catalase test: Catalase test was carried out using a drop of hydrogen peroxide. 2 ml of 3% hydrogen peroxide (H202) was placed in a clean test tube. A sterile wire loop was used to pick a colony of the test organism and mixed with 2 ml of 3% hydrogen peroxide (H2O2) in the test tube and observed for the production of gas bubbles which indicates a positive reaction. This test was used to identify *Staphylococcus aureus*.

(ix). **Oxidase test:** A few drops of kova's reagent were added to piece of filter paper on a petri dish. The bacteria isolates were then smeared on the filter paper with a glass rod. The paper was observed. Positive result gave a dark purple color while negative result showed no color change. This test was used to identify coliforms. As reported by James (2001)

RESULTS AND DISCUSSION

Table 1: Proximate Composition of the Drinks (Soy Milk, Kunu and Tiger Nut).

Sample	Moisture	Ash	Fiber	Protein	Fats	Carbohydrate
Soymilk	86.00	2.22	0.07	4.40	1.37	5.94

Kunu-zaki	81.00	1.85	0.53	1.85	0.81	13.96
Tigernut milk	84.00	3.08	0.14	2.70	1.05	11.03

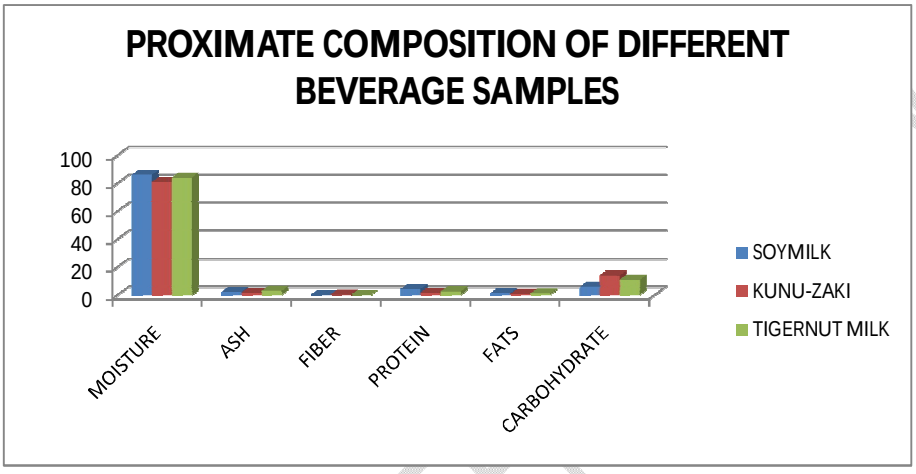

Figure 1: Proximate Composition of Different Beverage Sample

Moisture content of kunu 81.00%, % ash content is 1.85. This value were higher than 0.20% obtained by otaru *et al* (2013), but the results however agree with 2.00 to 3.00% obtained by innocent *et al* (2011). % content of crude fat, crude fiber, crude protein and carbohydrate were 0.81, 0.53, 1.85 and 13.96 respectively. Essien *et al* (2011) reported that loss of protein during processing of the drinks may be responsible for the low protein content observed. Different cereal types have abilities to contribute to the ash content of kunu- zaki as a result of the

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differences in their ash compositions. The high carbohydrate content of kunun-zaki indicates a good source of energy needed for human activity (Iwe, 2002).

Tigernut contain 84.00% moisture, 3.08% ash, 0.14% fiber, 2.70% protein, 1.05% fats and 11.03% carbohydrate. Soy milk contain 86.00 moisture, 2.22% ash, 0.07% fiber, 4.40% protein, 1.37% fat and 5.94% carbohydrate.

The ash content is an inorganic residue remaining after the removal of water and organic matter by heating in the presence of oxidizing agents. This gives a measure of the total amount of minerals in a food. Ash in dairy product is an important source of many minerals and vitamins and in low calorie density.

Table 2. Mineral Analysis Results of Kunu, Soy Milk and Tiger Nut

SAMPLE	Calcium	Iron	Potassium	Magnesium	Phosphorus
SOYMILK	127.89	0.85	17.60	147.00	11.37
KUNU-ZAKI	217.90	2.37	113.00	106.05	20.00
TIGERNUT MILK	221.00	2.83	135.00	175.00	75.10

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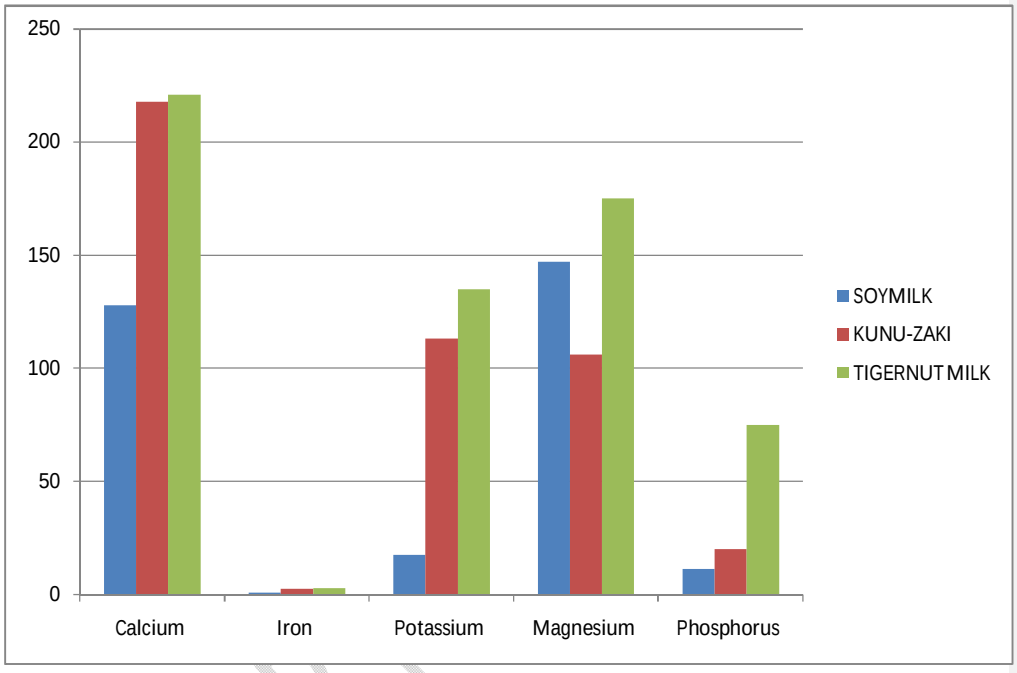

Figure 2: Graphical representation of mineral analysis of the drinks

The increase in the mineral content in the samples could therefore justify the need to enrich the beverage with source that are rich in other nutrients lacking in cereals

normally adopted in its production (Chowdhurys, and Punia, 2007). Minerals are of great importance in diet as they play important roles in body metabolism.

Potassium is an important mineral that conduct electricity in the body along with sodium chloride, calcium and magnesium. It is crucial to heart functions and plays a key role in skeletal and smooth muscle concentration. Magnesium works as an enzyme cofactor. It helps in the formation of DNA and RNA. It regulates the cholesterol production in the body. The body use 99 percent of its calcium to keep bones and teeth strong and healthy. It supports skeletal structure and function. The rest of the calcium in body plays a key role in cell signaling, blood clotting, muscle contraction and nerve functions. From the analysis, tiger nut is highly rich in mineral nutrients. Iron is required for growth and development. It also plays a central role in many biochemical processes in the body. These include oxygen transport and storage, assisting with immunity and contributing to enzyme systems. Phosphorus plays an important role in how the body uses carbohydrates and fats. It is also needed for the body to make protein for the growth, maintenance, and repair of cells and tissues.

Comment [a23]: Please provide references to some claims here.

From the graphical representation above, it shows that tigernut drunks is highly rich in minerals as it has the highest values for all. The drink with the least minerals is soy milk.

Table 3:Microbial Analysis

Sample	Total Bacterial count (cfu/ml)	Faecal Coliform counts (cfu/ml)
Soymilk 1	0.90 x 106	5.90 x 104
Kunu-Zaki1	0.60 x 106	5.50 x 104
Tigernut Milk 1	0.50 x 106	5.13 x 104
Soymilk 2	0.70 x 106	5.10 x 104
Kunu-Zaki2	1.35 x 106	3.37 x 104
Tigernut Milk 2	1.59 x 106	3.50 x 104
Soymilk 3	1.97 x 106	3.00 x 104
Kunu-Zaki3	1.90 x 106	3.50 x 104
Tigernut Milk 3	0.40 x 106	1.15 x 104

Comment [a24]: What's the difference between sample 1, 2 and 3??? And why different values for sample 1, 2 and 3 of the same juice?

The total bacteria counts (CFU/ml) of soy milk, kunu and tigernut ranged from (0.70×10^6 to 1.97×10^6), (0.60×10^6 to 1.90×10^6), (0.40×10^6 to1.59×10^6) respectively. The faecal bacteria count (CFU/ml) of soy milk, kunu and tigernut ranged from (3.00×10^4 to 5.90×10^4), (3.37×10^4 to 5.50×10^4), (1.15×10^4 to 5.13×10^4) respectively. The results indicate that fresh kunu presented a high bacteria count after 24hrs of incubation. The high colony count is an indication of spoilage as a consequence of either poor hygiene or poor quality of cereals and

water used. The presence of coliform bacteria in these drinks as determined in this research will be of public health concern because teaming populace, especially students, relies on these drinks as cheaper alternative to the bottled soft drink.

The bacterial isolate are seven and it include: *Staphylococcus spp, Vibrio spp,Pseudomonas spp, E. coli, Shigellaspp, Salmonella spp and Klebisella spp.*

Table 4: Morphological and Biochemical Characteristics Of Isolates

Parameters	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7
Colony Characterization	Milkish irregular shape with flat elevation	Pinkish circular with flat elevation	Yellowish circular with flat elevation	Whitish irregular shape with flat elevation	Yellowish irregular shape with flat elevation	Whitish irregular shape with flat elevation	Yellowish irregular shape with flat elevation
Cell characterization	Coci in clusters	Long rods in singles	Short rods in singles	Rods in clusters	Cocci in clusters	Rods in clusters	Cocci in clusters
Gram's Test	Positive	Negative	Negative	Negative	Positive	Negative	Positive
Motility Test	Negative	Negative	Negative	Positive	Positive	Positive	Positive
Catalase	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Coagulase	Negative	Negative	Negative	Negative	Positive	Negative	Positive
Citrate	Negative	Negative	Negative	Negative	Positive	Negative	Positive
Indole	Negative	Positive	Positive	Negative	Positive	Negative	Positive
Oxidase	Negative	Negative	Negative	Positive	Negative	Positive	Negative
Urease	Positive	Positive	Positive	Negative	Positive	Negative	Positive
Probable	*Klebsiellaspp*	*Salmonella*	*Shigellaspp*	*E- coli*	*Staphylococcus*	*Vibrio*	*Pseudomonas*

organism	*spp*	*spp*	*sp.*	*spp*	

The presence of *E. coli* in kunu indicates faecal contamination and may have serious health implications. *Pseudomonas* and *Klebsiella spp* have been implicated in the spoilage of food and beverages. Their presence in kunu, soymilk and tigernut is undesirable. There is then the need to maintain adequate hygienic conditions during processing and preparation of the beverages to eliminate these microbial contaminants and to improve on the quality of the final product. There is also the need to employ adequate preservative measures to improve the shelf-life of the beverages.

Comment [a25]: Reference

Table 5:Antibiotic Susceptibility Pattern of the Bacterial Isolate

		ANTIBIOTICS SENSITIVITY PROFILE											
SAMPLE	ISOLATES	CN	S	LC	CPX	RX	E	NOR	CH	OFX	PEF	AU	SXT
SOYMILK	*Klebsiellasp.*	S	S	R	S	R	I	R	R	R	R	R	I
	Vibrio sp.	S	S	I	S	I	R	S	S	S	R	R	R
	Staphylococcus sp.	R	R	R	R	R	R	R	R	S	R	R	R
KUNU-ZAKI	*Staphylococcus sp.*	R	R	S	I	R	R	R	R	I	S	R	R
	Escherichia coli	S	R	S	S	R	R	S	I	S	S	R	S
	Klebsiellasp.	S	R	R	R	R	R	R	R	S	S	R	R
	Salmonella sp.	R	S	R	S	R	R	I	R	S	S	R	S
	Vibrio sp.	I	R	R	S	R	R	S	R	S	I	R	R
TIGERNUT MILK	*Vibrio sp.*	R	S	R	I	R	R	S	R	S	S	R	R

Shigellasp.	R	S	R	R	R	R	R	R	S	R	R	R
Klebsiellasp.	S	S	I	S	R	S	S	S	S	S	S	S
Vibrio sp.	S	R	R	I	R	R	I	S	S	S	R	R

***N/B: R** = Resistant, **I** = Intermediate, **S** = Susceptible; **CN** - Gentamycin; **S** – Streptomycin; **LC** –Lincocin; **CPX**-Ciprofloxacin; **RX** – Rifampicin; **E** – Erythromycin; **NOR** - Norfloxacin; **CH**-Chloramphenicol; **OFX**-Ofloxacin; **PEF** - Pefloxacin; **AU**-Augumentin; **SXT**-Cotrimoxazole.*

Conclusion

The result of the study provides information on the nutritive values of all drinks. Soy milk has the highest moisture, fat and protein contents, kunu-zaki is highly rich in carbohydrate and fiber whereas tiger nut has a higher amount of ash content. Tiger nut drink is extremely rich in minerals from the graphical representation. The microbial content of these hawked beverages were high and were contaminated with microorganisms which are potentially pathogenic to man. This possess a threat to the general public, as these contaminants has ability to cause varying level of diseases, ranging from food borne illness and food poisoning due to *staphylococcus aureus.* The presence of these isolated organisms in the beverages analyzed could serve as an indicator for the need to promote awareness about possible health hazards that could arise due to handling and processing.

Comment [a26]: All drinks?

Recommendation

(i). It is recommended that local beverages should be adequately fortified so that the nutrients loss during processing would be replaced.

(ii). Regulatory agencies should intervene by setting standards in acquisition of raw material, production techniques as well as health status of personnel involved in the production process of non-alcoholic beverage widely consumed in Nigeria.

(iii). Education of the manufacturers and provision of basic facilities will greatly improve non alcoholic beverage drinks quality and safety.

(iv). More work should be carried out on the antibacterial resistance of all beverages.

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