Science World Journal Vol 8 (No 2) 2013
www.scienceworldjournal.org
ISSN 1597-6343

COMPARATIVE ANALYSIS OF LOCALLY PREPARED 'KUNUN AYA' (TIGER-NUT MILK) CONSUMED BY STUDENTS OF KADUNA STATE UNIVERSITY, KADUNA-NIGERIA.

[1]Musa, A.A* and Hamza, A.

[1]Department of Biochemistry, Kaduna State University, Kaduna-Nigeria.

*E-mail: a.a.musa@kasu.edu.ng

ABSTRACT
Samples of locally prepared Tiger- nut Milk ('Kunun aya') were obtained from three different restaurants at Kaduna State University main campus which are often consumed by students. The samples were separately subjected to proximate analysis, pH determination and Aerobic Mesophilic Bacterial Count using standard techniques to ascertain and compare their nutrient content, pH and microbial load respectively. Results of the proximate analysis as determined using standard procedures of AOAC (2003) revealed sample A (obtained from Choice Restaurant) had nutrient content of 62.8% moisture; 21.5% fat 2.69% crude protein; 5.2% crude fibre; 4.0% ash and 3.81% nitrogen-free extract. Sample B obtained from Salb restaurant, had 79.3% moisture; 11.0% fat; 3.3% crude fibre; 3.25% crude protein; 1.5% ash and 1.65% nitrogen-free extract. The last sample from Toma restaurant, had nutrient content of 82.5% moisture; 8.5% fat extract; 3.5% crude fibre; 2.9% crude protein; 2.5% ash and 0.1% nitrogen-free extract (NFE). The pH range of the samples were between 3.5 – 4.5 while aerobic mesophilic bacterial count ranged between 1.04 x 102 – over 3.0 x 102 cfu/ml. Poor hygienic practice, Poor quality tiger-nuts and water used in the preparation might be the cause of high microbial loads recorded. The microorganisms recovered were Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Rhizopus nigircans and Penicilium sp.

Key words: Tiger-nut, Proximate Analysis, Nutrient content, Microbial Load.

INTRODUCTION
The search for lesser known and underutilized crops, many of which are potentially valuable as human and animal foods has been intensified to maintain a balance between population growth and agricultural productivity, particularly in the tropical and sub-tropical areas in the world (Odoemelan, 2003). Tiger-nut was found to be a cosmopolitan perennial crop of the same genus as the papyrus plant. Other names of the plant are Earth almond as well as Yellow-nut grass (Farhath et al., 2001; Belewu & Belewu, 2007). Tiger-nut has been cultivated since early times (chiefly in South Europe and West Africa) for its small tuberous rhizomes, which can be eaten raw, roasted, dried, baked or made into a refreshing beverage (tiger-nut milk) called "Horchata De Chufas". The plant is taxonomically classified under division Magnoliophyta; class Liliopsida; order Cyperales; family Cyperaceae; genus Cyperus and specie Cyperus esculentus. Non-drying oil (usually called chufa) is equally obtained from the rhizome. In West Africa, the plant is gathered from the wild while it is a troublesome weed in planted field in United States (Belewu & Belewu, 2007). The nut was found to be rich in myristic acid, oleic acid, and linoleic acid (Eteshola et al., 1996). In Egypt, it is used as a source of food, medicine and perfumes.

In Nigeria, tiger-nut is known as 'Aya' in Hausa, 'Ofio' in Yoruba and 'Akiausa' in Igbo. Three varieties (black, brown and yellow) are cultivated in the country and among these, only two varieties, (yellow and brown) are readily available in the markets. The yellow variety is preferred to all other varieties because of its inherent properties like its bigger size and attractive colour. The Yellow variety also yields more milk upon extraction, contains lower fat and more protein and possesses less anti-nutritional factors especially polyphenols (Okafor & Nwachukwu, 2003).

Tiger-nut milk popularly known in the Northern part of Nigeria as 'Kunun aya' is one of the indigenous, locally fermented, non-alcoholic beverage drinks that is widely consumed for its thirst-quenching and nutritive properties. Even though it is being consumed throughout the year, its extensive consumption is known to be during the dry season. (Okafor & Nwachukwu, 2003). It is locally prepared by washing the tiger-nut thoroughly in order to remove soil and dirt. Once they are washed, the nuts are then soaked for about 4 - 8 hours, after which they can be grinded along with coconut, date fruit or pineapple into a mash. During the process, some cold water is added in a ratio of 3 litres of water for unit kilogram of tiger-nuts; and the mixture is left to macerate. When the appropriate time is spent, it is pressed and sieved and then known quantity of sugar or honey can be added depending on volume obtained, the mixture is then filtered again to get the pure filtrate. Once this is done, it is often served cold (Ayo & Okaka 1998). Significant variations exist in the procedures depending on the desired taste and cultural habits that leads to differences in quality and stability. While some cultures prefer 'Kunun aya' with different fruit flavours, others prefer it with no sugar (Adeyemi &Umar, 1994). It is usually packaged and sold in a 1litre and 500ml plastic bottles.
Tiger-nut milk or 'Kunun aya' must be consumed within 2 - 24 hours at 40C – 100C due to its poor shelf life (Akoma et al., 2006). This drink is very cheap because the tiger-nuts and additives used in its production are easily and locally sourced. The packaging materials are also cheap and available. Furthermore, the methods of production are simple and cheap as no elaborate equipment and expertise are required (Agboola, 1987). The preparation of this beverage has become a technology in many homes particularly in rural communities and more recently in the urban areas where more women have developed the skill and commercial production which has helped to alleviate poverty amongst the people. However the high water content coupled with crude methods of production and packaging under improper sanitary conditions predisposes 'Kunun aya' to microbial contamination. A large number of lactic acid bacteria, coliforms, molds and yeast have been reportedly implicated in food spoilage as they use the carbohydrate content for undesirable fermentation processes (Gibbons & Pains, 1988).

Tiger-nuts have long been recognized for their health benefit as they have a high content of soluble glucose and oleic acid along with high energy content (starch, fats, sugar and proteins). This nut produces high quality oil of up to about 25.5% content and about 8% of protein. The nut is high in oil content and is valued for the nutritious starch content, dietary fibre and carbohydrates (Bibek, 2001). Tiger-nut is also an excellent source of some useful minerals such as iron and

calcium which are essential for body growth and development (Oladele & Aina, 2007). They also contain other mineral elements such as phosphorus, potassium sodium, magnesium, zinc and traces of copper and vitamins E and C (Oladele & Aina, 2007). It is believed that they help to prevent heart attacks, thrombosis and cancers, especially of the colon (Bibek, 2001). They are thought to be beneficial to diabetic patients (if sugar-free) and those seeking to reduce cholesterol or lose weight (Oladele & Aina, 2007). It was reported that tiger-nut is high in dietary fiber content, which could be effective in the treatment and prevention of many diseases including colon cancer, coronary heart diseases, obesity, diabetics and gastro intestinal disorders. Its tubers are also use as an aphrodisiac, carminative, diuretic and a stimulant (Aletor et al., 1995). Tiger-nuts have been reported to be used in the treatment of flatulence, indigestion, diarrhoea and dysentery. In addition, tiger-nut has been demonstrated to contain higher essential amino acids than those proposed in the protein standard by the FAO/WHO in 1985 for satisfying adult needs (Belewu & Adedunmi, 2008). It is against this background that this research was conducted with the aim of determining the qualities of a locally prepared 'Kunun aya' with the specific objectives of assessing its nutrient content, pH, and bacterial load and type and to possibly provide recommendation for its consumption.

MATERIALS AND METHODS

Collection of Samples

Samples of Kunun-aya were aseptically collected from three different most frequently patronised restaurants (labelled randomly as A, B and C) in the university main campus market. The samples were immediately taken to the Biochemistry and Microbiology laboratories of Kaduna State University for proximate analysis and microbial screening respectively.

Proximate Analysis

Determination of Percentage Moisture

Moisture content was determined by oven drying method in accordance with Shumaila & Mahpara (2009). Exactly 2 grams of the sample was weighed in a dry crucible as initial weight (W_1). The crucible was placed in the oven for 8 hours at 1000oC until a constant weight was obtained. After eight (8) hours, the crucible was removed from the oven and cooled in a desiccator for 30 minutes. After cooling, the crucible was weighed again as final weight (W_2). The Percentage Moisture was calculated using the formula below:

$$\% \text{ moisture} = \frac{W_1 - W_2}{W_0} \times 100$$

W_0 = weight of empty crucible
W_1 = weight of sample and crucible before heating
W_2 = weight of sample and crucible after heating

Determination of Percentage Ash

Ash content was determined by the use of muffle furnace. A clean empty crucible was placed in a muffle furnace at 6000C for 1 hour after which it was removed and allowed to cool in a desiccator. The initial weight was determined and recorded as W1. Two grams of the sample was taken in the crucible and labelled (W2). The sample was then ignited over a burner using a blowpipe. After ignition, the crucible was placed in muffled furnace at 5500C for 4 hours. After ashing, the crucible was removed (a grey white ash was observed, which indicates complete oxidation of all organic matter in the sample) and placed in a desiccator to cool. After cooling, the weight was determined as (W3). Percentage ash was calculated as:

$$\% \text{ Ash} = \frac{W_3 - W_1}{W_2} \times 100$$

W_1 = Initial weight of empty crucible
W_2 = weight of sample and crucible before heating
W_3 = weight of sample and crucible after heating

Determination of Percentage Crude Fat

Dry extraction method was used for this analysis. Crude fat was determined by ether extraction method using soxhlet apparatus. Two grams of moisture free sample was wrapped in filter paper, placed in fat-free thimble and then inserted into the extraction tube. The receiving beaker was initially washed and dried and then weighed as W1 and later transferred about 250ml of petroleum spirit into it, which was then fitted into the apparatus. The extractor was then switched on at 600C and water was allowed to run in from the tap by the use of a tube. This process was left for 6 hours after which there were sequentially eight siphoning to ensure clean colourless fat-free solvent in the tube above the receiving flask. The content of the flask was then subjected to evaporation, leaving only the fat extract in the flask and the flask was weighed as W_2. The percentage crude fat was then calculated as follows:

$$\text{Crude fat (\%)} = \frac{W_1 - W_0}{W_2 - W_0} \times 100$$

Determination of Percentage Crude fibre

Exactly 2 g of the sample was weighed and labelled as W0. It was then transferred to a porous crucible and placed into the fibre machine keeping the valve at 'off' position. Thereafter, 150ml of H2SO4 solution and few drops of acetone were added to the column. The cooler was then open to turn on the heating element (power at 90oC-100 oC). After boiling, the power was reduced to 30oC and left for 30 minutes. The valves were then opened to drain the acid and distilled water was used to rinse the column three times to ensure complete removal of acid from sample. The above procedure was repeated using 150ml of KOH and later the samples were dried in the oven for an hour at 1500C. After drying, the sample was cooled in the desiccator and weighed as (W1). This weighed sample is then placed in the furnace for oxidation of the organic matter for 3 hours at 600C. When this was ashed completely, it was then cooled and reweighed finally as (W2) and the percentage crude fibre was calculated as:

$$\text{Crude fibre (\%)} = \frac{W_1 - W_2}{W_0} \times 100$$

W_0 = Initial weight of sample
W_1= weight of mixture before heating
W_2 = weight of mixed sample after heating

Determination of Percentage Crude Protein

Measured 2g of the dried moisture-free sample was taken in digestion flask and 10ml of concentrated H2SO4 along with 8g of digestion mixture (K2SO4 and CuSO4, 8:1) were added and mixed together by swirling in order to maintain homogeneity. The flask was then heated to start digestion until the mixture turned blue-green in colour. After 2 hours of digestion, the digest was cooled and transferred to 100ml volumetric flask adding distilled water to make up the volume to the mark. After the digestion, distillation was then carried out using Markam still distillation apparatus and then10ml of the digest was introduced into the distillation tube before 10ml of 0.5N NaOH was gradually added through the same way leaving it for 10 minutes.

Ammonium produced in the process was collected as ammonium hydroxide (NH_4OH, yellow in colour) in a conical flask containing 20ml of 4% boric acid solution with few drops of modified methyl red indicator. The distillate was then titrated against standard 0.1N HCl solution until the appearance of pink colour was observed. Alongside the titration, a blank was also ran using the same 0.1N HCl. Percentage crude protein content of the sample was calculated and multiplied by a factor of 6.25.

$$\% \text{ crude protein} = 6.25 \times \%N$$

$$\%N = \frac{(S - B) \times N \times 0.014 \times D \times 100}{\text{Weight of the sample} \times V}$$

Where: S = sample of titration reading
B = blank titration reading
N = normality of HCl
D = dilution of sample after digestion
V = volume taken for distillation
0.014 = milli equivalent weight of nitrogen

Determination of Nitrogen-Free Extract
Nitrogen Free-Extract (NFE) was calculated by subtracting the sum total of all the other items in the proximate analysis from 100.
NFE = (100 - % moisture + % crude protein + % crude fat + % crude fibre + % ash)

Energy Calculation
The percent calories in selected samples was calculated by multiplying the percentage of crude protein and carbohydrate with 4 and crude fat with 9. The values were then converted to calories per 100gm of the sample.

MICROBIAL SCREENING

Determination of Total Count of Bacteria
The bacterial enumeration was carried out on nutrients agar (NA) and Mac Conkey agar (MA) all of oxoid grade using the pour plates method in accordance with Cheesbrough (2005). The samples were serially diluted after which 1ml of appropriate dilution was used to inoculate each of the plates in triplicates. The culture plates were then incubated at 370C for 24 hours. After 24 hours of incubation, the colonies in each plate were counted using the GallenKamp colony counter.

Isolation and Identification of Bacteria
Discrete colonies of the bacteria were selected and sub cultured from the mixed cultures before they were incubated at 370C for 24 hours. Colonies obtained were gram stained to identify the different organisms present in each culture plate

Biochemical tests
Biochemical tests were performed using standard methods (Cheesbrough, 2005).
\

Determination of Fungal Count
This was done on potato dextrose agar (PDA) using pour plate method. The samples were serially diluted, 1ml of the diluents were then used to inoculate the plates in triplicates. The plates were incubated at room temperature of 320c for 72 hours. After incubation, the colonies were counted and screened by wet mount method on a microscope. The screened fungi were then identified based on taxonomic schemes and descriptions by Ainsworth et al,. (1973) and Mislivec et al., (1992).

Isolation and identification of fungi
A sterile pipette was used to transfer few drops of serially diluted sample onto the surface of prepared potato dextrose agar. The innoculum was then spread out thinly and evenly on the surface using a sterile bent glass rod. The plates were then incubated at room temperature (27-320C) for 72 hours. Discrete colonies were identified by colonial and microscopic characteristics of wet mount preparation of lactophenol in cotton blue.

pH Determination
The pH of the fresh samples of Kunun aya was immediately determined using sterile probes of the pH meter (Jenway).

RESULTS
Table 1 shows proximate composition of 'Kunun aya' samples in which sample A was found to contain 62.8% moisture, 21.5% fat, 2.69% crude protein, 5.2% crude fibre, 4.0% ash and 3.81% nitrogen-free extract. Sample B had 79.3% moisture, 11.0% fat, 3.3% crude fibre, 3.25% crude protein, 1.5% ash and 1.65% nitrogen-free extract. The last sample had nutrient content of 82.5% moisture, 8.5% fat extract, 3.5% crude fibre, 2.9% crude protein, 2.5% ash and 0.1% nitrogen-free extract (NFE).

The mean pH values of the kunun aya samples and the extent of microbial contamination are shown in Table 2.

Table 1: proximate composition of 'Kunun aya'

Samples	% Moisture	%Crude fat	% Ash	% Crude fibre	% Crude protein	% Nitrogen Free Extract	CaloricValue(Kcal/100g) code
A	62.8	21.5	4.0	5.2	2.69	3.81	225
B	79.3	11.0	1.5	3.3	3.25	1.65	
C	82.5	8.5	2.5	5.2	2.9	0.1	

Results of pH determination showed that all the samples were acidic at the time of analysis. Sample C was found to have the lowest pH of 3.5 while Sample A had the highest pH (4.5). Total bacteria count ranged from 5.2x101 to 4.1x103 cfu/ml with sample A having the highest value, Fungal count on the other hand ranged from 1.8x102 to 7.2x102cfu/ml with sample C having the highest value.

The microbial isolates obtained (Table 3) from various kunun aya samples collected revealed that Staphylococcus aureus was isolated from all the three samples. None of the samples contain Streptococcus pyogenes, while Penicillium digitatum was found in both samples A and B. Sample A has some strains of Fusarium spp, while Monilia sitophila and Rhizopus nigricans were isolated from Sample C.

Table 2 : Mean Microbial Counts and pH value of 'Kunun aya'

Samples Code	TBC (cfu/ml) on NA	CC (cfu/ml) on MA	FC (cfu/ml) on PDA	pH
A	4.1×10^3	3.9×10^2	1.8×10^2	4.5
B	1.5×10^2	1.2×10^2	6.9×10^2	3.7
C	5.2×10^1	3.5×10^1	7.2×10^2	3.5

Key: TBC=Toatal Bacteria Count; CC=Coliform Count; FC=Fungal Count; NA=Nutreint Agar; MA=Mackonkey Agar; PDA=Potato Dextrose Agar

Table 3: Biochemical characterization of bacteria isolated from 'Kunun aya'

Isolate Reaction code	Gram's	Cat	Coag	Mot	Ind	MR	VP	Cit	Isolate identified
x	G-ve rod	+	-	+	+	+	-	-	*Escherichia coli*
y	G+ve cocci	+	+	-	-	-	+	-	*S. aureus*
z	G+ve cocci	-	-	-	-	-	+	+	Str. sp

Key: Cat= catalase; Coag= coagulase; Mot=motility; Ind=indole; MR=methyl red; VP=voges proskeur; Cit=citrate +=positive; -=negetive

DISCUSSION

All the samples were found to be acidic (pH 3.5 – 4.5). This level of acidity of kunun aya has been described by several researchers including Efiuvwevwere and Akoma (1995) and Akoma et al, (2006), who attributed this to the presence of certain species of lactic acid bacteria namely Lactobacillus leichmanni and Lactobacillus fermentum during the fermentation process. Similar high acidic pH values have been reported for zobo and for orange juice products (Bolarinwa et al, 2009) as well as burukutu and pito (Kolawole et al, 2007). Although, these classes of beverages are acidic in nature, the acidity tends to increase with increase in fermentation period resulting into spoilage. Consequently, the low pH values may have encouraged the growth of fungi and this could be responsible for the species of microorganisms isolated. The acidic nature of the samples may also be due to the fact that the kunun aya might have started undergoing spoilage even before the time of purchase, and such may lead to production of certain metabolites and could bring about reduction in pH of the product.

The gradual decrease in lipid content of samples observed in this work might be as a result of the spoilage microorganisms utilizing lipids for synthesis of cell membrane and other cellular organelles. The continuous utilization of the reducing sugars and extractable fat may have led to the increase in the crude fibre content proportion of the kunun aya samples. There is also an increase in the ash content of the three samples analyzed implying increase in mineral content of kunun aya. However, the highest crude protein content was found in Sample B compared to Samples A and C. Reasons for this might have been as a result of some of the additives added. Consequently, the protein content of Samples A and C were low probably because most of it might have been lost during processing. According to Farhath et al 2001, much of the proteins in cereals and other crops are usually located in the testa and germ, which are usually sieved off during processing. High fibre percentage in this work implies high energy constituent of the kunun aya samples which makes it a more energetic beverage drink. Kunun aya and other indigenous Nigerian non-alcoholic beverages, such as kunu zaki and simi have been reported to contain high nutritional values because of the raw materials from which they were made. Spices are usually added in small quantities to improve the taste and flavour. Also, these spices

have been reported to inhibit microbial growth (Frazier et al., 1978).

The pH of kunun aya is usually too low to allow the growth of pathogenic microorganisms, but the presence of Escherichia coli, Staphylococcus aureus and Streptococcus spp could be a matter of serious concern. S. aureus is a normal flora of the skin, nose, throat palms, hairs and mucus membrane and a common etiological agent of septic arthritis (Alice, 1976). E. coli is an important member of the coliform group, it is part of the normal flora of the intestine of human and vertebrates. Some strains of the E. coli can cause gastroenteritis, diarrhea in infants and urinary tract infections (Abegaz, 2007). The streptococci are normal flora of the throat and the buccal cavity. Adesiyan et al., (1983), reported in their studies on kunun aya that the presence of organisms like Bacillus cereus, S. aureus and E. coli could render a beverage unsuitable for human consumption. It is possible that contamination by these pathogens could have occurred during sieving and packaging, as well as most of the people involved in the production, packaging and sales do not take necessary precautions, and as such contamination could be very prominent. Contamination of food items by specific species of microorganisms is largely due to the presence of these organisms and their entrance into the food or beverage as a result of poor hygiene and sanitation (Bibek, 2001).

Fungi isolated were Penicillium digitatum, Fusarium sporotichoides, Rhizopus nigricaus and Monillia sitophila. The presence of these fungal species is associated with spoilage of the beverages (Kolawole et al, 2007).The microbial counts were high as a result of the non-aseptic handling procedures during the kunun aya preparation. The presence of some of these microorganisms is a pointer that prolong storage of the product under room temperature will result in food poisoning; both intoxication and infection. This high density of microorganisms causes spoilage and may be responsible for its short shelf life (Bolarinwa et al., 2009). Frazier et al., (1978), have noted that foods with pH of below 5.6 are more susceptible to fungal and acidophilic bacteria deterioration. The microbial activity utilizes the solid content of the samples to produce water that brings about increase in moisture content.

The presence of coliform bacteria in Kunun-aya 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 drinks.

Our results reveal that all the three different samples contained relatively high microbial load and is probably due to the improper handling of materials for preparation of kunun aya and unhygienic processing method used. Most of the bacteria strains isolated were pathogenic while the fungi were basically fermentative microorganism which contributed to the acidity of the tiger-nut milk. However, the samples contain high nutrient content making them nutritionally good for consumption. Effort should therefore be made to process kunun aya in a well sterile and hygienic condition to prevent microbial contamination.

This could be by employing improved quality and production technologies of these indigenous exotic beverages so that large scale production for consumption by different staffs and students would be enhanced. Producers and hawkers should embrace good sanitary practices during the preparation and sale of the products. Since spices have been reported to inhibit microbial growth (Zaika et al., 1983; Adegoke & Skura, 1994), the addition of spices to the processed kunun aya is highly advocated.

Preservation method must therefore be applied during sales and distribution to make the product wholesome for consumption. The information obtained from this work should form baseline data on which preservation procedure should be adopted in order to retain the quality of this product.

To safeguard students' health, government and school authorities should intervene by setting standards in the acquisition of raw materials, production procedures and techniques as well as health status of people. Producers and food vendors of kunun aya should be encouraged to utilize the technical assistance of National Agency for Food, Drugs Administration and Control (NAFDAC) towards attaining quality standards. Therefore, further studies are recommended to preserve the health of the consumers.

REFERENCES

Abegaz,K.(2007)."Isolation,Characterization and Identification ofLactic Acid Bacteria involved in Traditional Formulation of Borde, and Ethiopian Cereal Beverage". African Journal of Biotechnology, 6 (12): 1469 -1478.

Achi, O. K. (2005). "The Potential for Upgrading Traditional Fermented Foods Through Biotechnology". African Journal of Biotechnology 4 (5): 375 – 380.

Adesiyun, A. A., Adekeye, J., Umoh, O., and Nadaraga, J. U. (1983). "Bacteriology of Cereals". Journal of Hygiene 90: 195 – 205.

Adeyemi T. and Umar S. (1994). "Effect of Manufacture on the quality and characteristics of Kunun Zaki, a Millet Base Beverage". Niger Food Journal 12 34 – 40.

Agboola, S. D. (1987). "Storage and Preservation of Agricultural products in Nigeria". Niger Foods Journal 8:44 -52.

Ainsworth G. C., Sparrow, F. K. and Sussman A. S. (1973). "The Fungi Taxonomic Keys". Academic Press pp 13 – 67.

Akoma O., Jiya E. A., Akumka D. D., and Mshelia, E. (2006). "Influence of malting on the nutritional characteristics of Kunun Zaki". African Journal of Biotechnology 10 (5): 996 – 1000.

Aletor, M. Venus, A. and Adeogun, O. A. (1995). "Food Chemistry". Journal of food Science 53:475 – 477.

Alexopoules, C. J. (1979). " Introductory Mycology" (3rd Ed) John Wiley and Sons Inc., New York. p 632.

Alice, L. S. (1976). "Microbiology and Pathology" (11th Ed). C. V.Mosby Company. pp 202 – 203.

Ayo, J. A., and Okaka, J. C. (1998). "Interaction Effect of Cadaba Farinose extract and pH Levels on Some Physiological Properties of Kunun Zaki". Proceedings of the 22nd Annual NIFST Conference 23rd - 26th November, Abeokuta Nigeria. Pp 31 – 33.

A.O.A.C. (2003). "Official Methods of Analysis of the Association of Officials Analytical Chemists" (17th Ed.). Association of Official Analytical Chemists, Arlington Virginia.

Bamgbose, A. M., Eruvbetine, D., and Dada, W. (2003). "Utilization of Tiger-nut
(Cyperus rotundus L.) in the Diets for Cockerel Starters". Journal of Biological Resources Technology. 89: 245 – 248.

Barnett, H. L. and Hunter, B. B. (1972). " Illustrated Genera of Imperfect Fungi"
(3rd Ed.) Burgess Publishing Co. Minneapolis. pp 241.

Belewu, M. A., and Adedunni, A. O. (2008). "Preparation of Kunu from exploited rich food source tiger-nut (Cyperus esculentus)". Pakistan Journal of Nutrition, 7:109 – 111.

Belewu, M. A., and Belewu, K. Y. (2007). "Comparative Physiochemical Evaluation of tiger-nut, soybean and coconut milk sources". International Journal of Agricultural Science 9:785 – 787.

Bibek, R. (2001). "Fundamental Food Microbiology" (2nd Ed.) The C.R.C Press Ltd Washington D. C. pp 56 – 90.

Bokhari, M. H., and Ahmed, M. S. (1983). "Food Plants in Borno State, Nigeria". Ghulami Publishers. Pp 25 -26.

Bolarinwa, I. F., Adebiyi, A. O., Akinwande, B. A., and Ade-Omowaye, B.I.O. (2008). "Evaluation of Tiger-nut (Cyperus esculentus) – Wheat Composite Flour and Bread". African Journal of Food Science. 2:87 – 91.

Bolarinwa, I. F., Oladokun, F. M., Akande, E. A., Otunola, E. T and Adejuyitan, J. A. (2009). "Some Physiochemical Properties of flour obtained from fermentation of tiger-nut (Cyperus esculentus). sourced from a market in Ogbomoso, Nigeria". African Journal of Food Science. 3:51 – 55.

Buchanam, R. E., and Gibbons, N. E. (1974). "Bergey's Manual of Determinative Bacteriology". (8th Ed.) Baltimore Williams and Wilkins Co. pp 34 – 89.

Cheesbrough, M. (2002). "Biochemical Tests to Identify Bacteria".Laboratory Practices in Tropical Countries" Cambridge Ed. Pp 63 – 70.

Efiuvwevwere, B.J.O., and Akoma, O. (1995). "The Microbiology of Kunu Zaki. A Cereal Beverage from Northern Nigeria during the Fermentation (Production) Process". World Journal of Microbiology and Biotechnology. 11:491 – 493.

Eteshola, K.D. and Oraedu, V.T. (1996). "Nutrient content of Cyperus esculentus" . African Journal of pure sciences. 10:18-21.

Farhath, K., Sudarshanakrishna, K. R., Semwal, A. D., Vishwanathan, K. R., and Khanum, F. (2001). "Proximate Composition and Mineral Contents of Spices". Industrial Journal of Nutrition and Dietetics. 38:93 – 97.

Frazier, W. C., and Westhoff, D. C. (1978). "Food Microbiology" 17 d.). McGraw – Hill Book Co., New York. P 540.

Gibbons, .D and Pains, A. (1988). "Crops of the Drier Regions of the Tropics". Longman Scientific and Technical Co. England. p 157.

Giese, G. (1995). "Measuring Physical Properties of Foods". Journal of Food Technology. 2:49 – 50.

Hermandez, E. (1925). "Nutrient contents of foods". Spanish publishing press Ltd, Spain. pp 113.

Kolawole, A.O., and Afonja, I.K. (2007). "Microorganisms in foods" (2nd ed). Abel and Co Ltd, Los Angeles. pp 281-293.

Odoemelan, J.T. (2003). "Principles of Botany" (2nd ed). Mc Graw hill Company U.S.A. pp150-155.

Okafor, T.S., and Nwachukwu, E. (2003). "Phytochemical screening of Tiger-nut (Cyperus esculentus) of three different varieties". Journal of Biological sciences.
81:115-120.

Oladele,O. and Aina, T.O. (2007). "Analysis of edible crops". Journal of Agricultural science. 6:21-24.

Shumaila, G. and Mahpara, S. (2009). "Proximate composition and mineral analysis of cinnamon". Pakistan Journal of Nutrition. 8(9): 1456-1460.