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Utilization of Different Preservatives (Cloves and White Pepper) for Extending of Shelf Life and Nutritional Value of Tiger Nut Milk (Kunun Aya)

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Abstract

Tiger nut milk (Kunun aya) is a traditional beverage and its non-alcoholic widely consumed in Northern part of Nigeria. The beverage was prepared from tiger nut with addition of spices such as cloves, and white pepper at different concentration in order to find its natural preservatives, so as to be preserve for a longer period using natural material so as to find if its shelf life and nutritional content will be maintained. The samples were stored at 4° c for 2 days and the effects of those spices on their proximate, microbial and fungal counts were evaluated. Fresh tiger nut and the spices (cloves and white pepper) were purchased form Jalingo central market in Taraba state in the Northeastern part of Nigeria. The tiger nut milk seeds were sorted and all the unwanted materials which may affects the taste and keeping quality of the drink were removed, washed and rinsed with portable water and then soaked for eight hours(8hrs) to soften the fibre and to remove off-flavor. One kilogram of fresh tiger nut was blended four times in to slurry with water. The slurry was pressed using a muslin cloth to recover extract. Two samples of spiced drink were analyzed for total moisture, ash content, crude protein, crude

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fibre, crude fat, and carbohydrate. Results of the proximate analysis reveal a range of 80.0-89.5% and 79.8- 87.2% and for moisture of tiger nut milk with cloves and white pepper which is higher at tiger nut with cloves. Ash content ranged from 0.3 -0.6 and 0.6-0.8%, both shows no significant difference in both sample B(1g) of tiger nut milk with cloves and white pepper. For crude fat it ranges 2.5-4.8%, and 2.3-4.1%. The crude fibre shows 2.1-3.0 and 2.2-3.2%, then crude protein 3.3-3.7% and 3.0-3.9% and carbohydrate range 1.7-3.9% and 1.9-2.6% of tiger nut milk cloves and white pepper respectively with no significant difference in sample C(2g) and control in tiger nut milk with cloves under carbohydrate content. The bacterial count of tiger nut milk with cloves and white pepper range 1.2×10^4 -2.7×10^4 and 1.6×10^4-3.0×10^4 respectively. And fungal count 1.5×10^7 -2.1×10^7 and 1.8×10^7 -2.1×10^7 with no significant difference in sample B(1g) and C(2g) with both have 1.8×10^7. The study revealed that cloves and white pepper can be served as a natural preservative in tiger nut milk and at the same time maintain its nutritional qualities.

Keywords: Milk, Tiger Nut, White Pepper, Preservative and Clove

INTRODUCTION

Tiger nuts are sweet nut like root tubers of the perennial grass-like cyperaceous plant called *Cyperus esculentus L.* (Oke *et al.,* 2019). Tiger nuts are enrich in carbohydrate, minerals, fiber, proteins, lipids, ascorbic acids and tocopherols (Ekeanyanwu and Ononogbu, 2010Tiger nuts have been experimented to increase the fiber content in gluten-free bread and biscuits in an effort to take advantage of their nutritious potential (Zahra and Ahmed, 2014; Aguilar *et al*., 2015). Milk and milk products have benefits besides the standard "growing strong bones" benefit. Likewise, certain components in milk and dairy products are beneficial for the gastrointestinal and immunological systems. (Ha *et al*., 2003, Ajiduku *et al*., 2023). The process of producing milk like aqueous extracts from tiger nuts, called tiger nut milk, (TNM) or composites with other vegetable milk extracts for beverage was explored by few authors (Belewu, 2007; Oke *et al*., 2019). In Nigeria, Tiger nut is known as Aya in Hausa, Gangan in jukun, Ofio in Yoruba, and Akiausa in Igbo. There are three varieties of tiger nut namely; black, brown and yellow which are cultivated in the country and among these, only two varieties (yellow and brown) are readily available in the market The yellow variety is preferred to all other varieties because of its inherent properties like its bigger size and attractive colour. Three varieties of tiger nuts are grown throughout the nation: yellow,

brown, and black. Of these, only two are commonly found in outlets: yellow and brown. The yellow variety is valued above the other two due to its natural characteristics, which include its larger size and eye-catching color. The yellow variety also yields more milk upon extraction, contains lower fats and more protein and possess less anti nutritional factors especially polyphenols (Okafor and Nwachukwu, 2003).

The consumption and safety of non-carbonated drinks has become increasingly important and demand largely based on their value, flavour, aroma and colour. However, despite the increasing popularity of drinks made from plant origin, the storage stability and microbiological safety calls for concern (Ben-Nwadiba *et al.*, 2005). Tiger nut beverages are highly nutritious for human consumption, but their short shelf life may be caused by improper hygiene during production, packing, distribution, and storage, which could lead to microbial infection. It was also demonstrated that the beverage had enough nutrients to encourage microbial development and eventual spoiling. Numerous elements promote, inhibit, or restrict the growth of microbes in beverages.

Cloves exhibit antimicrobial properties due to their active constituents such as eugenol, incorporating cloves into tiger nut milk has been shown to inhibit the growth of spoilage microorganisms and delay the onset of oxidation, contributing to an extended shelf life. Clove has the attributes of the major vegetal sources of phenolic compounds such as flavonoids, hydroxibenzoic acid, hydroxicinamic acid and hydroxiphenyl propens. Eugenol is the main bioactive compound of clove, which is found in concentration ranging from 9381.70 to 14650 mg per 100 gram of fresh plant material (Neveu *et al.*, 2020). The antimicrobial activities of cloves have been proved against several bacterial and fungal strains. Sofia *et al.,* (2007) tested the antimicrobial activity of different Indian spices plant such as mint, cinnamon, mustard, ginger, garlic and clove.

White pepper (*Piper nigrum* L.) Was one of the first and most often used spices in human history, due to its distinctive pungency and depending on the time of harvest and processing strategy, it can be made as either black or white pepper (Omafuvbe *et al.*, 2004). Black pepper is the dried immature but fully developed fruit of pepper plants, whereas white pepper consists of the mature fruit devoid of the outer skin (pericarp) (Jeleń and Gracka, 2015). Black pepper has been used as a spice and in herbal medicines. It also applicable as a preservative and bio-control agent (Ahmad *et al.,* 2012). White pepper has many different applications too, such as a preservative, insecticide, anti-bacterial, anti-

fungal and anti-protozoa herb (Jeleń and Gracka, 2015). Especially, black and white peppers contain a bioactive constituents named piperine, also found in other members of the pepper family (Piperaceae), including long pepper (*Piper longum*). Piperine is the most present and proactive alkaloid in pepper. It is used as a therapeutic agent because of its many health benefits associated with its antioxidant, anti-inflammatory and drug activity-enhancing activities, respiratory effects and inhibitory action on lung metastasis (Gorgani, *et al.,* 2017). Antioxidants are widely used to prevent deterioration of oxidisable products such as food, cosmetics and pharmaceuticals. A tendency among modern consumers is the use of antioxidants obtained from natural sources, increased the interest in the research.

MATERIALS AND METHODS

Sample Collection

Fresh tiger nut (*Cyperus esculentus*), white pepper and Cloves were purchased from the Jalingo Central Market, Jalingo, Taraba State, Nigeria. Jalingo is a capital of Taraba state which was created in 1991 by federal Military Government of Nigeria. It is located between latitude 6^0 30' and 9^0 36' North and longitude 9^0 10' and 11^0 50' East.

Tiger nut drink preparation

One kilogram of Tiger nut was soaked in distilled water for eight hours (8 hrs). The soaked tiger nut was drained and blanched at 70^0C for five minutes mainly to inactivate enzymes that might cause clumping of the extract. The fresh tiger nut was blended several times into slurry with water six liters in a Q-link auto-clean blender. The slurry was than pressed using Muslin cloth to extract the milk. The extract was pasteurized at 72^0C for 7 minutes, homogenized and rapidly cooled. Two cups of sugar were equally added to the 6 Liters of the tiger nut milk (Maxwell *et al*., 2019).

Preparation of tiger nut milk with cloves

Cloves were added to 250 mL of tiger nut milk at different concentrations of 0.5 g, 1 g, and 2 g each. The experiment was repeated thrice. The spices and sugar were completely dissolved by vigorously stirring of the milk. The resulting tiger nut sample obtained was pasteurized at 70^0C for 30 minutes in a water bath with continuous stirring. The samples were allowed to cool and a representative sample was taken for analysis while the remaining

portion of the sample was stored in a refrigerator at 4^0C for further analysis (Maxwell *et al.*, 2019).

Preparation of tiger nut milk with white pepper (*piper nigrum*)

Two hundred and fifty milliliter (250 mL) of tiger nut milk was mixed with white pepper (*Piper nigrum)* at a varied concentration of 0.5g, 1g, and 2g each and was duplicated with *Piper nigrum*. The milk was stirred thoroughly to have the spices and sugar dissolve. The resulting tiger nut sample obtained was pasteurized at 70^0C for 30 minutes in a water bath with continuous stirring. The sample was allowed to cool and a representative sample was taken for analysis while the remaining portions of the sample were stored in a refrigerator at 4^0C for further analysis (Maxwell *et al.*, 2019).

Proximate analysis

Crude protein, moisture, total ash, crude fibre and carbohydrate content were determined using standard method as stated by AOAC (2005).

Determination of percentage moisture

Moisture content was analysed by oven drying procedur in accordance with Shumaila and Mahpara, (2009). Initial weight (W1) of the sample was precisely 2g, which was weighed in a dry crucible. After eight hours at 1000C in the oven, the crucible was taken out and its weight was measured. The crucible was taken out of the oven after eight hours and allowed to cool for thirty minutes in a desiccator. After cooling, the crucible was weight as final weight (W2). The percentage moisture was calculated using the formula below.

$$\% \text{ moisture} = \frac{W1-W2}{W_0} \times 100$$

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 600^0C 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 also taken in the crucible and labelled (W2). The sample was ignited over a burner using a blowpipe. After ignition, the crucible was placed in muffled furnace at 550^0C for 4 hours. After its shows ash, 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:

$$\% \text{ Ash} = \frac{W3 - W1}{W2} \times 100$$

Determination of Percentage Crude Fat

Dry extraction method was used for this analysis. Crude fat was determined by 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 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 switched on at 60^0C 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 was sequentially eight siphoning to ensure clean colourless fat-free solvent in the tube above the receiving flask. The content of the flask was subjected to evaporation, leaving only the fat extract in the flask and the flask was weighed as W2. The percentage crude fat was calculated as follows:

$$\text{Crude fat (\%)} = \frac{W1 - W0}{W2 - W0} \times 100$$

Determination of Percentage Crude Fiber

Exactly 2 g of the sample was weighed and labelled as W_0. It was then transferred to a porous crucible and placed into the fibre machine keeping the valve at 'off' position. Thereafter, 150ml of H_2SO_4 solution and few drops of acetone were added to the column. The cooler was then open to turn on the heating element (power at 90°C-100 °C). At boiling, the power was reduced to 30°C 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 is repeated using 150ml of KOH and later the samples was dried in the oven for an hour at 150^0C. After drying, the sample was allowed to cool 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 60^0C. Then it was washed completely, and then cooled and the percentage crude fiber was calculated as:

$$\text{Crude fibre (\%)} = \frac{W1 - W2}{W0} \times 100$$

Determination of Percentage Crude Protein

Two gram (2g) of the dried moisture-free sample was taken in digestion flask and 10ml of concentrated H_2SO_4 along with 8g of digestion mixture (K_2SO_4 and $CuSO_4$, 8:1) was added and mixed together by swirling in order to maintain homogeneity. The flask was heated to start digestion until the mixture turned blue-green in color. After 2 hours of digestion, the digest was allowed to cooled and transferred into 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 (NH4OH, yellow in color) 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 color 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(Musa and Hamza 2013).

% crude protein = 6.25 x %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 Percentage Carbohydrate Content

Carbohydrate was calculated by the difference [100 - (% water + % protein + % fat + % ash + % crude fibre)] (AOAC, 2005).

Microbial Screening

This was carried out according to methods described by Musa and Hamza, 2013

Determination of Total Count of Bacteria

The bacterial enumeration was carried out on nutrients agar (NA) and MacConkey agar (MA) using the pour plates method in accordance with Cheesbrough (2005). The samples were serially diluted in 10^{-4} folds after which 1ml of appropriate dilution was used to inoculate each of the plates in duplicate. The culture plates were incubated at 37^0C for 24 hrs initially, after which the samples was sub-cultured again for 48 hrs and 72 hrs respectively. After each culturing period of incubation, the colonies in each plate were counted using the GallenKamp colony counter.

Determination of Fungal Count

This was done on potato dextrose agar (PDA) using pour plate method. The samples were serially diluted, where 1ml of the diluents was used to inoculate the plates in duplicates. The plates were incubated at room temperature of 32^0c for 72 hours. After incubation, the colonies we recounted and screened by wet mount method on a microscope. The screened fungi were identified based on taxonomic schemes and descriptions by current atlas.

Data analysis

All evaluation tests were conducted in duplicates. Data that was obtained for the proximate composition, microbial plate counts were subjected to one way Analysis of Variance (ANOVA) and differences among the means were determined using Duncan multiple range test (DMRT). Statistical Package for Service Solution (SPSS) Version 23.0 was use to analyze the data and $p < 0.05$ was considered to be statistically significant.

RESULTS

Proximate analysis of tiger nut milk with cloves and white pepper as preservative

The efficacy of adding cloves and white pepper to tiger nut milk is presented in (Table 1 and 2) there is variation observed among the parameters evaluated and The Table 3 and 4 indicates the bacterial and fungal count of tiger nut milk with cloves and white pepper.

Table 1: Proximate analysis of tiger nut milk with clove as preservatives

Sample Concentration (g)	Moisture (%)	Ash (%)	Crude Fat (%)	Crude Fibre (%)	Crude Protein (%)	Carbohydrate (%)
A(0.5)	89.5±0.01[b]	0.6±0.01[a]	4.8±0.01[c]	2.1±0.01[a]	3.3±0.01[b]	1.7±0.01[d]
B (1)	80.0±0.01[a]	0.4±0.01[d]	3.8±0.02[a]	2.7±0.01[d]	3.5±0.01[c]	2.0±0.01[a]
C (2)	85.5±0.01[d]	0.3±0.01[b]	2.5±0.01[b]	3.0±0.01[c]	3.7±0.01[d]	3.9±0.01[b]
Control	84.5±0.01[c]	0.4±0.01[d]	4.5±0.01[d]	2.9±0.01[a]	3.6±0.01[a]	3.9±0.01[b]

KEY: A= 0.5 g Cloves + Tiger Nut Milk, B=1g of Cloves + Tiger Nut Milk, C= 2g 0f Cloves +Tiger Nut Milk. Values are mean ± standard error. Means on the same column with different superscript letter are significantly different ($p < 0.05$) while those with the same superscript letter are not significantly different ($p > 0.05$).

Table 2: Proximate analysis of tiger nut milks with white pepper as preservatives

Sample Concentration (g)	Moisture (%)	Ash (%)	Crude Fat (%)	Crude Fibre (%)	Crude Protein (%)	Carbohydrate (%)
A (0.5)	87.2±0.01[b]	0.7±0.01[a]	3.9±0.01[c]	2.2±0.01[a]	3.6±0.01[b]	2.0±0.01[d]
B (1)	81.6±0.01[a]	0.6±0.01[d]	4.1±0.02[a]	2.8±0.01[d]	3.9±0.01[c]	1.9±0.01[a]
C (2)	79.8±0.01[d]	0.8±0.01[b]	2.3±0.01[b]	3.2±0.01[c]	3.0±0.01[d]	2.2±0.01[b]
Control	83.6±0.01[c]	0.6±0.01[d]	3.9±0.01[d]	3.0±0.01[a]	3.8±0.01[a]	2.6±0.01[c]

KEY: A= 0.5 g Cloves + Tiger Nut Milk, B=1g of Cloves + Tiger Nut Milk, C= 2g 0f Cloves +Tiger Nut Milk. Values are mean ± standard error. Means on the same column with different superscript letter are significantly different ($p < 0.05$) while those with the same superscript letter are not significantly different ($p > 0.05$).

Table 3: Bacterial and fungal count of tiger nut milk with cloves

Samples concentration (g)	Mean bacterial count (cfu/ml)	Mean fungal count (cfu/ml)
A (0.5)	2.1×10^4	2.0×10^7
B (1)	1.8×10^4	1.7×10^7
C (2)	1.2×10^4	1.5×10^7
CONTROL	2.7×10^4	2.1×10^7

KEYS: CFU/ML= Colony Forming Unit per milliliter.

Table 4: Bacterial and fungal count of tiger nut with white pepper

Sample concentration in (g)	Mean bacterial count (cfu/ml)	Mean Fungal count (cfu/ml)
A (0.5)	2.3×10^4	2.1×10^7
B (1)	1.9×10^4	1.8×10^7
C (2)	1.6×10^4	1.8×10^7
Control	3.0×10^4	2.0×10^7

KEYS: CFU/ML= Colony forming unit per milliliter

DISCUSSION

There is a significant difference between the control and some of the test proximate parameters analyzed ($p<0.05$). Consequently, no significant difference was observed between B(1g) and A (0.5g) for ash content and crude fiber respectively. The outcome of the proximate composition of 100 g of tiger nuts in a study reported by Suleiman *et al.* (2018) showed the various components in a decreasing and slightly increasing order of moisture, carbohydrate, crude fat, crude fiber, crude protein, and ash contents. This implies that tiger nuts are high in moisture, crude fat and crude fiber and protein contents. The outcome of the proximate analysis in this study is contrary to the report of Madaki *et al.* (2018) who showed a high content of ash, crude fiber, crude fat, crude protein and carbohydrates in proximate compositions of raw and processed (dankuwa) tiger nuts (*Cyperus esculentus*).However, the protein content in this study is higher when compared to

studies on the nutritional value of tiger nut (*Cyperus esculentus*) and its products by Sabeh *et al.* (2019) and studies on the nutritional value of protein rich foods like soyabeans, cowspeas, pigeon peas, and some oil seeds, the protein values for un-gingered(2.83) and gingered(3.34) tiger nut drink are low(Obadesagbo*et al.*,2023).

The microbial count for bacterial count of samples A, B, C, and control decreased as the concentration of the preservatives increased with control having the highest microbial count because of the absent of the the target preservatives (cloves.) the (Table 4.5) shows how the bacterial count decreased as the concentration of the cloves increase. The sample A (0.5g) was recorded $2.1\times10^{4.}$ With sample B (1g), and C(2g) was recorded 1.8×10^{4} and 1.2×10^{4} respectively with the control showing the highest bacterial count which shows the absence of any preservatives and it was recorded 2.7×10^{4}. The presence of the bacterial count of tiger nut milk with white pepper as showed in Table 4.6, shows the decrease in bacterial count as concentration increased with sample A (0.5g) was recorded as 2.3×10^{4} while sample B(1g) was recorded as 1.9×10^{4}, sample C(2g) 1.6×10^{4} respectively with only Control that increase with 3.0×10^{4}. The results in this study is contrary to the work of Adejuyitan *et al.*, 2019), who isolated some diverse microbial species associated with tiger nuts as obtained from the study. However, the identified non-lactic acid bacteria (*E. coli*, *Bacillus* species and Proteus species) reported by Udeozor and Awonorium, 2014 which is contrary to this study which was carryout without been specific to the target bacteria while its only focus on bacterial count of cloves and white pepper as preservatives.

The fungal count of tiger nut milk with cloves as showed in the table 4.3 shows the decrease of fungal count as the concentration of the cloves increase. Sample A (0.5g) was 2.0×10^{7}. Sample B(1g) was $1.7\times10^{7}$and sample C(2g) was recorded 1.5×10^{7} respectively with control 2.1×10^{7}. The Fungal count of Tiger nut milk with white pepper as showed in the table 4.4 also shows the decrease as the concentration increase but sample B (1g) and C(1g) both shows the same result 1.8×10^{7}. Sample A (0.5g) was recorded 2.1×10^{7}while sample B(1g) and C(2g) both shows the same results 1.8×10^{7}respectively with control that shows 2.0×10^{7}. The relatively high microbial counts in this study is similar with the work of Obasi and Mani,2023 who showed a high microbial count was observed in the analyzed tiger nut milk drink. This was not surprising as the tiger nut milk production systems are sometimes done under unhygienic conditions, with no authorized agency to monitor their microbial quality and safety. The presence of high microbial load could also be as a result of contamination from the milling equipment used in the processing.

The highest bacterial (2.0×10^4 CFU/ml), (3.0×10^4CFU/ML) and fungal (2.1×10^7 CFU/ml), (3.0×10^7 CFU/ml) counts after the storage period were found in the cloves and white pepper spiced kunun aya. However, the microbial loads obtained were in compliance with International Standard Organization (ISO) statement that the count of milk at 21°C after storage at 5°C should be less than 10^5 (Kayode *et al*., 2017). It had also been stated that milk sample containing 5.00 x103 cfu/ml of bacteria is classified as good for consumption, 1.00 x104 to 4.00 x105 cfu/ml is fairly good and 2.00 x106 is passable and it's good for consumption.

CONCLUSION

The preservation of tiger nut milk with different preservatives such as cloves and white pepper hold a promise for extending its shelf life while maintaining its nutritional quality. Both cloves and white pepper have demonstrated the effectiveness in inhibiting the microbial growth and preserving food products. Additionally, those natural preservatives offer potential health benefits due to their antioxidant and microbial properties.

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