



Assessment of the Preservative Effect of *Zingiber officinale* and *Syzygium aromaticum* on the Shelf Life of Kunun-Aya

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ABSTRACT

Spices and herbs have been reported to be potent source of natural antioxidants. Spices are known to impact flavor and improve overall organoleptic quality of foods. The use of naturally occurring materials like spices as preservatives has been proved to be a promising alternative to the use of chemicals. The effect of ginger and clove extract on the storability of kunun aya were investigated in this study. Fresh ginger and clove were collected, identified, air dried and extracted using distilled water. Kunun aya was prepared in the laboratory with the addition of the spices extracts at different concentrations. The samples were stored at room temperature and the effects of spices extracts on their proximate, physiochemical parameters, microbial and sensory properties were evaluated. The result of proximate analyses revealed the moisture content of the kunun aya to be between 83.98-80.54 in ginger spiced kunun aya and 81.67 to 78.15 in clove spiced kunun aya, the crude protein ranges from 3.96 to 4.52 in ginger spiced kunun aya and 3.07-3.93 in clove spiced kunun aya. The ash content ranges from 0.28-0.23 and 0.25-0.23 in both the ginger and clove respectively. The fat content ranges from 3.78-2.44 in clove and 3.54-2.31 in ginger spiced kunun aya and finally the carbohydrate content ranges from 8.24-12.35 in ginger and 11.24-14.87 in clove spiced kunun aya. Slight increase in temperature was recorded in all the samples but there was a marginal decrease in pH as storage time increases. The bacterial count of kunun aya treated with ginger extract ranged from 1.4×10^5 - 3.1×10^6 CFU/ml, the total fungal count ranged from 1.0×10^5 - 9.7×10^5 CFU/ml. Bacterial count of kunun aya treated with clove extract ranged from 1.8×10^5 - 3.5×10^6 CFU/ml while the total fungal count ranged from 8.0×10^4 - 8.6×10^5 CFU/ml. There was a significant difference between the sensory attributes of the treated kunun aya and the control samples with the control sample having the highest score rating.

Keywords: Spices, Antioxidants, Preservatives, Ginger, Cloves, Kunun aya.

INTRODUCTION

Tiger nut (*Cyperus esculentus*) are small wrinkled amber colored tiny tubers that grow just beneath the soil surface (Devries and Fueke, 1999). It is widely common in West Africa and Spain, Southern Europe, Madagascar, Middle East, Hawaii, Indonesia, China, Ukraine, sub- continent in India as traditionally cultivated foods and source of nourishment (Antonelli *et al.*, 2020). Tiger nut is among the cultivated crop used in

beverage preparation. In Spain, milk is extracted from it to form drink called *horchatade chufa* while in Nigeria particularly the northern part; the locally prepared milk extract drink is termed *Kunun Aya* (Ankomah, 2022). As such kununaya is a non-alcoholic beverage drink of milky appearance derived from the tubers of tiger nut plants mixed with water and sugar” according to Okafor and Nwachuku (2003). Tiger nut milk has a very short life of often less than few hours depending on the

condition of storage (Akoma *et al.*, 2006). High temperature and humidity significantly reduce the shelf life of the product (Nutso, 2014). As a result, tiger nut milk is often associated with significant microbial contamination, including bacteria and moulds (Onovo and Ogaraku, 2007; Nutso 2014). The short shelf life of raw tiger nut milk hinders widespread consumption of the beverage due to deteriorating effects of some microorganism on the milk (Abaejoh *et al.*, 2006).

Food spoilage refers to an irreversible modification in which food becomes not edible or its quality is compromised. Such changes can be driven by different factors, either physical (oxygen, temperature, light) and/or biological (enzymatic activity and microbial growth) (Liu *et al.*, 2019). Despite the current technologies available in the production chain (for instance freezing, pasteurization, drying, preservatives) it seems impossible to eliminate completely the risk of food spoilage (Gutierrez *et al.*, 2009). Lipid oxidation is one of the main issues of food spoilage. Hence, food industries have applied antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxy anisole (BHA) to prevent spoilage (stoilova *et al.*, 2007). However, their safety is doubtful and consumers are progressively demanding natural compounds. For this reason, spices represent a potent tool for the food industry, thanks to their natural properties (Hyldgaard *et al.*, 2012). Many spices have been reported to have antimicrobial properties, cholesterol lowering effects, anti-diabetic and anti-inflammatory properties (Suekawa *et al.*, 1986, Kwada and Tella, 2009). Spices and herbs have been reported to be potent source of natural antioxidants (Diniz do Nascimento *et al.*, 2020). The use of naturally occurring materials like spices as preservative has been proved to be a promising alternative to the use

of chemicals which have been shown to have adverse effect on consumers. Spices such as garlic, ginger, and pepper are good sources of nutrients, minerals and phytochemicals and could therefore serve as nutritional supplements (Otunola *et al.*, 2010).

Ginger (*Zingiber officinale*) belongs to the zingiberaceae family and native to South-East Asia. Ginger is used in many countries as a spice and condiment to give a pungent taste to food (Park and piz-zuto, 2002). Ginger rhizome has also been used in traditional medicine, because of its diversity in terms of phytochemicals (Adebayo *et al.*, 2021). Ginger and its constituents show antioxidant activity and prevent damage of macromolecules caused by free radicals/oxidative stress. Ginger also shows antimicrobial and other biological activities due to gingerol and paradol, shagoals and zingerone (Giriraju and Yunus, 2013). Cloves are aromatic flower buds of a tree in the family myrtaceae (*Syzygium aromaticum*) (Elisha *et al.*, 2022). Clove is native of Indonesia but nowadays is cultured in several parts of the world and is commonly used as a spice. Clove has been used for centuries as a primary preservative as well as flavoring agent for preserved foods. Cloves are high in antimicrobial, antifungal, antioxidant and antilarval properties. The primary substance in cloves that makes it so great for preserving food is called eugenol (Chaeibet *et al.*, 2007b).

MATERIALS AND METHODS

Plant Collection, Identification and Preparation

Fresh Ginger (*Zingiber officinale*) and Clove (*Syzygium aromaticum*) were purchased from Janguza Market, Kano State. All the plant materials were identified according to Demetrio *et al.*, (2015) at the Department of Plant Biology, Bayero University Kano. Herbarium accession number was given to

each of the plants used and *Cyperus esculentus* was identified as BUKHAN0367, *Syzygium aromaticum* as BUKHAN 0342 and *Zingiber officinale* as BUKHAN 0296.

The Clove and Ginger were washed with distilled water to remove all extraneous materials and adhering particles, the Ginger and Clove were later dried under shade environment for 3 weeks before grounding into fine powder using motor and pestle. The powder was then stored in a container until required for use.

Extraction of Bioactive Compounds from Ginger (*Zingiber officinale*) and Clove (*Syzygium aromaticum*)

Extraction was done according to Fatope *et al.* (2001) with modifications, in which 100g of each of the ginger and clove powder was weighed and soaked in 1 liter of distilled water for 7 days with regular shaking. The extract was thereafter passed through a fine cloth, cotton wool and then Whatman filter paper. The filtrate was later on evaporated at 50°C in oven and the final product was refrigerated until use.

Phytochemical Screening of Ginger (*Zingiber officinale*) and Clove (*Syzygium aromaticum*) extracts

Test for Tannins

A 0.5 g of the extract was mixed thoroughly with 10 ml distilled water and then filtered; 5 ml of the filtrate was added to 1 ml of 5% Ferric chloride solution. The appearance of blue black, greenish or blue green precipitate indicates the presence of tannins (Adwan and Mhanna, 2008).

Test for Flavonoids

A few drops of concentrated hydrochloric acid were added to a small amount of an alcoholic extract of the plant material. Immediate development of a red colour

indicates the presence of flavonoids (Dawoud *et al.*, 2013).

Test for Saponins

About 0.1 g of powdered plant material was boiled with 10 ml of water for 5 minutes then filtered. After cooling, 5 ml of filtrate was then diluted with water and shaken vigorously. The formation of persistent foam indicated presence of saponins (Adwan and Mhanna, 2008)

Test for Steroids

About 1 ml solution of the extract was added to 1ml sulphuric acid, the appearance of red colour indicates the presence of steroids (Adwan and Mhanna, 2008)

Test for Alkaloids

About 0.5 g of the extract was stirred with 5 ml of 1% hydrochloric acid on a steam bath and filtered. 1 ml of the filtrate was then treated with few drops of Mayer's reagent. A white or creamy white precipitate considered as an indication for the presence of alkaloids (Dawoud *et al.*, 2013).

Collection and Preparation of Tiger Nut for Laboratory Preparation of Kunun-Aya Drink

Four kilogram (4Kg) of fresh tiger nuts (*Cyperus esculentus*) was purchased from Janguza local market in Kano State for laboratory preparation of the drink. The tiger nuts tubers were sorted to remove dirt particles and spoilt nuts. This was followed by washing with 40% alcohol (ethanol) to reduce microbial contamination and prevent cell shrinking and then rinsed with sterile distilled water as described by Olosombo and Bakole (2020). The nuts were then soaked for 12 hours in sterile water at room temperature to soften the seed. The tiger nuts were thereafter collected and wet milled with sterile distilled water using functional and sterile laboratory

blender. The tiger nut mixture was sieved using a muslin cloth and kept aside in a covered container.

The extract from the tiger nut was kept at room temperature distributed in four different sterile bottle containers containing 1L volumes each by incorporating the preservative agents. This was achieved by measuring these varying concentrations of the extracts; 0.05g and 0.1g, of each of the ginger and clove extracts and 0.1g of sodium benzoate in the different sterile bottles.

In all, four types of tiger nuts drinks were produced:

- Tiger nut extract only (without treatment). Sample A
- Tiger nut + 0.05g/L of ginger and clove extracts. Sample B
- Tiger nut + 0.1g/L of ginger and clove extracts. Sample C
- Tiger nut + 0.1g/L sodium benzoate. Sample D

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

W_1 – Weight of empty crucible

W_2 – Weight of crucible + sample

W_3 – Weight of crucible after drying

Ash content

The crucible containing moisture-free samples was weighed as (W_2). It was then ashed for hours in a muffle furnace at 550°C until it

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

W_1 – Weight of empty crucible

W_2 – Weight of crucible + dried sample

W_3 – Weight of ash

Fat content

A known weight of dried tiger nut milk was mixed with petroleum ether in a conical flask.

All the samples were stored at room temperature, each of the sample was subjected to 2-hour analysis of proximate, pH, Temperature, Titrable acidity, Peroxide value, sensory evaluation and microbial enumeration consecutively 3-times adhering to the two-hour time frame

Proximate Analysis

The proximate composition of the tiger nut blends was determined using the methods of AOAC (1990).

Moisture content

A washed and dried crucible was weighed as W_1 . 10ml volume of tiger nut milk was added and weighed as and recorded as W_2 . The crucible and its content were then transferred unto a water bath, then to an oven and left for three hours at 105°C until the water completely dried. The crucible was cooled and weighed as W_3 . Percentage moisture was calculated as:

fully ashes (the colour changes to grey). It was then allowed to cool, and the weight recorded as (W_3). Percentage ash was calculated using the formula:

The mixture was extracted for hours on an orbital shaker. It then filtered on a pre-weighed conical flask recorded as W_2 . The percentage fat content was calculated as;

$$\text{Fat content (\%)} = \frac{(W_2 - W_1)}{W} \times 100$$

W_1 – Weight of empty flask

W_2 – Weight of flask + oil

W – Weight of sample used for extraction

Protein content

Ten millimeters (10ml) of the sample was pipetted into a conical flask, followed by addition of 3 drops of phenolphthalein indicator. The burette was filled with 0.1MNaOH to the mark without bubbles and titrated against experimental conical flask to a permanent faint pink colour. At this point, 2ml of formaldehyde was added to the experimental conical flask and the pink colour disappeared. The content was further titrated to a permanent faint pink colour. Blank was treated similar way. The volume of 0.1MNaOH used was recorded as the titre value.

$$\text{Protein content (\%)} = (T - B) \times 1.95$$

Where: T – Titre value for test samples

B – Titre value for blank

Carbohydrate

Percentage carbohydrate content was estimated by difference; i.e. having estimated all the other proximate compositions: % moisture, % ash, % fat and % protein, their sum was subtracted from 100.

Percentage carbohydrate = 100 - (%ash+ % fat+%moisture+%protein).

$$\text{TitrationAcidity (\%)} = \frac{(\text{Titre value} \times 0.09) \times 100}{(\text{Volume of sample})}$$

Determination of Peroxide value

Peroxide value is determined by the standard method of AOAC 1990). A known quantity of oil placed in a conical flask containing 10ml of solvent mixture (glacial acetic acid + chloroform in 2:1 ratio). A spatula full of powdered potassium iodide (KI) was added

Physicochemical Analyses of the Kunun-Aya

pH

The pH of each sample was determined using the method described by Nwachuckwu *et al.*(2010). 10 ml of each sample was diluted to 100 ml with distilled water and allowed to stand for few minutes. It was filtered and the pH of the filtrate measured with a pH meter (HI 209 pH meter by Hanna Instruments).

Temperature

The temperature of each sample was determined using well calibrated digital thermometer. The probe was inserted into the kunun aya and the reading was taken directly. Values were recorded in degree Celsius.

Determination of Titrable acidity

Titration acidity was determined using the method described by (AOAC 1990) with modification. Ten milliliter (10 ml) of each sample was measured into a conical flask, and then three drops of phenolphthalein indicator added and titrated against 0.1 M NaOH. Formation of pink colouration indicated the end point. Titration acidity was calculated using the formula:

and placed on the boiling water bath for 60seconds. The content was then transferred to another conical flask containing 20ml 5% KI and the flask was rinsed twice with 25ml portions of distilled water and the washing was added to the titration flask and titrated with 0.01 M $\text{Na}_2\text{S}_2\text{O}_3$ using starch as the

indicator. Blank was treated similar way. The peroxide value (PV) was calculated as

milliequivalent of peroxide per kilogram of fat.

$$\text{Peroxide value (mEqperoxide/Kg)} = \frac{1000 (V_2 - V_1)}{m} \times T$$

Where: m = mass of oil taken

V_2 = volume of 0.01M $\text{Na}_2\text{S}_2\text{O}_3$ used for test

V_1 = volume of 0.02M $\text{Na}_2\text{S}_2\text{O}_3$ used for blank

T = molarity of $\text{Na}_2\text{S}_2\text{O}_3$ (0.01 M)

Sensory Evaluation of the Kunun-Aya

Sensory quality in terms of appearance, taste/flavour, texture/consistency, aroma/smell and overall acceptability of tiger nut milk blended with various concentrations of the aqueous extracts of ginger (*Zingiber officinale*), clove (*Syzygium aromaticum*) and standard sodium benzoate were rated in comparison with the control at spaced hours using 10 semi-trained panelists. The samples were served in newly clean cups to each of the panelist with score sheet. The serving order for each of the panelist was randomized and clean water at room temperature was served for cleaning the palate between samples. A 9-point Hedonic scale was used in the assessment: 1 being 'like extremely' and 9 being 'dislike extremely' (1 = Like extremely, 2 = Like very much, 3 = Like moderately, 4 = Like slightly, 5 = Neither like nor dislike, 6 = Dislike slightly, 7 = Dislike moderately, 8 = Dislike very much, 9 = Dislike extremely).

Enumeration of Aerobic Mesophilic Bacteria and Fungi

To determine the counts of aerobic mesophilic bacteria and fungi, ten milliliters (10 ml) of each sample were serially diluted in ninety milliliters (90 ml) of sterile diluent until a 10^3 dilution was achieved (Cheesbrough, 2006). A 0.1 ml aliquot of the 10^3 dilution was then inoculated onto freshly prepared, surface-dried nutrient agar (NA) for bacterial counts, and onto Potato Dextrose Agar (PDA) for fungal counts using the pour plating method. Each type of agar was prepared in triplicate.

The nutrient agar plates were incubated at 37°C for 24 hours to facilitate bacterial growth. The Potato Dextrose Agar plates, used for fungal counts, were incubated at ambient temperature ($28 \pm 2^\circ\text{C}$) for 3-5 days to allow fungal colonies to develop. Fungal colonies were observed as discrete, often visible growths on the PDA plates. Colony counts for both bacteria and fungi were expressed in colony-forming units per milliliter (cfu/ml) of the sample (FAO, 2007).

Statistical Analysis

Data obtained were analyzed by Microsoft Excel 2016. The mean values were subjected to analysis of variance (ANOVA) to ascertain whether there's significant difference between the treated samples and the control using GraphPad Prism Software (GraphPad Inc., San Diego, CA, USA) (α) at $p < 0.05$.

RESULTS

Phytochemical Composition of Ginger and Clove Extracts

Table 1 shows the results of the phytochemical composition of ginger and clove extracts. Phytochemical screening carried out on the extract of clove showed the presence of flavonoids, tannins, saponins, steroids, glycosides, alkaloids and total phenols. While in ginger, alkaloids, flavonoids, phenols, saponins and steroids were present, but tannins and glycoside were found to be absent.

Table 1: Phytochemical composition of the extracts of ginger *Zingiber officinale* and clove *Syzygium aromaticum*

Phytochemicals	Ginger	Cloves
Flavanoids	+	+
Tannins	-	+
Saponins	+	+
Steroids	+	+
Glycosides	-	+
Alkaloids	+	+
Total phenols	+	+

Proximate Composition of Tiger-Nut Milk Blended with Extract of Ginger and Clove

The results of the proximate composition (moisture, ash, protein, fat and carbohydrate)

of tiger-nut milk blended with extract of ginger (*Zingiber officinale*) and clove (*Syzygium aromaticum*) at 0.05 g/L, 0.1 g/L and sodium benzoate (SB) as standard control at varied timings is presented in Tables 7 and 8 respectively. At 0 hour, the freshly prepared tiger nut milk blended with ginger was observed to contain proximate composition of 3.96 % protein, 83.98 % moisture, 0.28 % ash, 3.54 % fat and 8.24 % carbohydrate while the samples treated with clove contain 3.07 % protein, 81.67 % moisture, 0.25 % ash, 3.78 % fat and 11.24 % carbohydrate respectively (Table 2).

Table 2: Proximate composition of Tiger-nut milk blended with Ginger extract (*Zingiber officinale*)

Sample code	Proximate Composition (%)				
	Moisture	Ash	Protein	Fat	Carbohydrate
A0	83.98±0.00	0.68± 0.01	3.96±0.03	3.54±0.08	8.24±0.04
A2	81.83±0.17	0.28±0.01	4.05±0.03	3.27±0.05	10.62±0.10
A4	81.29±0.05	0.23±0.02	4.13±0.02	3.08±0.04	11.28±0.06
A6	80.54±0.36	0.23±0.03	4.27±0.08	2.63±0.14	12.35±0.05
B0	83.98±0.00	0.68± 0.01	3.96±0.03	3.54±0.08	8.24±0.04
B2	83.80±0.30	0.24±0.00	4.24±0.04	2.98±0.20	8.75±0.10
B4	82.81±0.20	0.23±0.02	4.37±0.02	2.52±0.02	10.06±0.03
B6	81.80±0.02	0.23±0.01	4.64±1.67	2.31±0.13	11.03±1.79
C0	83.98±0.00	0.68± 0.01	3.96±0.03	3.54±0.08	8.24±0.04
C2	85.22±0.04	0.23±0.01	4.28±0.12	2.82±0.10	7.47±0.07
C4	83.33±0.15	0.23±0.02	4.46±0.04	2.62±0.08	9.37±0.07
C6	81.78±0.08	0.22±0.02	4.57±0.05	2.27±0.02	11.17±0.01
D0	83.98±0.00	0.68± 0.01	3.96±0.03	3.54±0.08	8.24±0.04
D2	84.36±0.30	0.23±0.02	4.18±0.02	2.74±0.09	8.51±0.20
D4	82.26±0.06	0.23±0.01	4.44±0.06	2.51±0.04	10.58±0.19
D6	80.96±0.03	0.22±0.01	4.52±0.02	2.39±0.01	11.92±0.61

KEY;A-Kunun Aya without treatment, A0 = Treatment at 0hr, A2 = B-Kunun Aya + 0.05g/l of ginger extract,

C-Kunun Aya + 0.1g/l of ginger extract,

D – Kunun Aya + 0.01g/l of Sodium Benzoate.

The protein content obtained in samples treated with ginger ranged from 3.96 % to 4.52 %, while in clove, it ranged from 3.07% to 3.93 % Protein content slightly increased

from 3.96 % at 0 hours to 4.05 % in sample A, 4.24 % in sample D, 4.28 % and 4.18 % in sample B and C respectively in samples blended with ginger. Correspondingly, at 2

hours increase in protein content was observed in samples treated with clove from 3.07 % at 0 hours to 3.10 % in sample D and 3.22 % in sample A respectively. Subsequently at 4 hours increase in protein content in both sample A and D was observed, as well as in samples treated with clove from 3.41 % to 3.57 % at sample B and 3.53 % to 3.62 % in sample C respectively. Increased protein content was recorded in all samples

blended with ginger at 6 hours with sample A and D having 4.27 % and 4.64 %, and samples having 4.57 % and 4.52 % for sample B and C respectively. Similarly, relatively lower protein content was observed in samples treated with clove. Statistical result however, shows no significant difference between sample A and sample blended with ginger and clove B and C concentration and at varied time intervals (Table 3).

Table 3: Proximate composition of Tiger-nut milk blended with Aqueous extract of Clove (*Syzygium aromaticum*)

Sample code	Proximate Composition (%)				
	Moisture	Ash	Protein	Fat	Carbohydrate
A0	81.67±0.15	0.25±0.02	3.07±0.02	3.78±0.08	11.24±0.04
A2	79.31±0.11	0.25±0.01	3.10±0.01	3.23±0.07	14.12±0.04
A4	78.91±0.08	0.24±0.00	3.25±0.04	2.98±0.01	14.63±0.02
A6	78.57±0.10	0.23±0.02	3.57±0.07	2.60±0.05	15.03±0.02
B0	81.67±0.15	0.25±0.02	3.07±0.02	3.78±0.08	11.24±0.04
B2	80.15±0.05	0.24±0.03	3.22±0.03	3.35±0.04	13.04±0.02
B4	79.35±0.06	0.24±0.02	3.46±0.09	3.03±0.02	13.93±0.03
B6	79.33±0.06	0.23±0.00	3.91±0.07	2.84±0.03	13.69±0.09
C0	81.67±0.15	0.25±0.02	3.07±0.02	3.78±0.08	11.24±0.04
C2	79.64±0.02	0.24±0.02	2.41±0.01	3.46±0.04	13.26±0.03
C4	79.23±0.04	0.24±0.03	3.57±0.10	3.33±0.01	13.64±0.02
C6	78.15±0.01	0.23±0.02	3.82±0.04	2.93±0.05	14.87±0.07
D0	81.67±0.15	0.25±0.02	3.07±0.02	3.78±0.08	11.24±0.04
D2	79.25±0.10	0.24±0.01	3.53±0.37	3.12±0.05	13.87±0.33
D4	78.96±0.03	0.24±0.01	3.62±0.07	3.06±0.05	14.12±0.08
D6	78.53±0.13	0.23±0.01	3.93±0.06	2.44±0.08	14.87±0.03

KEY; A-Kunun Aya without treatment, B-Kunun Aya + 0.05g/l of ginger extract, C-Kunun Aya + 0.1g/l of ginger extract, D – Kunun Aya + 0.01g/l of Sodium Benzoate.

The moisture content at 2 hours was found to be 81.83 % in sample A, 83.80 % in sample D and 85.22 % and 84.36 % in sample B and C of ginger respectively. Similar trend was observed in all samples treated with clove at 2 hours. At 4 hours, slight decrease in the moisture content of all samples blended with ginger and clove was observed. Equally, at 6 hours, slight decrease in the moisture content

was observed in all samples with samples treated with ginger having higher values (80.54 – 81.80 %) than samples treated with clove (78.15 – 79.33 and). Statistical result however, shows a significant difference between the control and the various samples blended with ginger and clove at different time intervals. The ash content studied at 0 hour ranged from 0.25 % in clove to 0.28 %

in ginger. At 2, 4- and 6-hours' interval, ash content range of 0.24 % - 0.22 % was recorded in all samples under study.

The fat content of all samples under study decreases as storage time increases from 0 to 6 hours. The fat content obtained in samples treated with ginger ranged from 3.54 % at 0 hour which reduced to 2.27 % in sample B sample at 6 hours, while in clove, it ranged from 3.78% at 0 hour to 2.44 % in sample C sample at 6 hours. At 4 hours, fat content reduced slightly from 3.27 % in sample A to 3.08 %; 2.82 % to 2.62 in sample C and from 2.74 % to 2.51 in sample C respectively. Also at 4 and 6 hours, similar trend was observed in samples treated with extract of clove. At 6 hours, fat content decreased marginally from 2.98 % in sample A to 2.60 % and from 3.03 % in sample D to 2.84 %; 3.33 % to 2.93 in sample B and from 3.06 % to 2.44 in sample C respectively. Statistical result shows a significant difference between the study samples and the control at $p > 0.05$.

The carbohydrate content increased with increase in storage time. The results range between 14.12 % – 13.04 % in samples treated with clove at 2 hours with the sample A having the highest carbohydrate content and sample D the least, and in samples blended with ginger at 2 hours, sample A was found to contain the highest carbohydrate content of 10.62 % while sample B having the least of 7.47 %. Subsequently, at 4 and 6 hours, remarkable increase in carbohydrate content was recorded in all samples. Statistical result shows a significant difference between the study samples and the control at $p > 0.05$.

The Effects of Adding Aqueous Extracts of Ginger and Clove on Physicochemical Parameters

The effects of adding aqueous extracts of ginger (*Zingiber officinale*) and clove

(*Syzygium aromaticum*) on temperature, pH, titrable acidity and peroxide value on tiger-nut milk was studied at varied time intervals. Slight increase in temperature was recorded in all samples blends and control. Conversely, there was marginal decrease in pH as storage time increases from 0 to 6 hours. The pH obtained in samples treated with ginger ranged from 6.51 at 0 hour which decreased to 6.20 in sample A at 6 hours, while in clove, it ranged from 6.81 at 0 hour to 6.32 in sample A at 6 hours. At 4 hours, pH in samples blended with ginger reduced slightly from 6.42 in sample A to 6.36; 6.41 to 6.25 in sample B and from 6.24 to 6.13 in sample C respectively. Also, at 4 and 6 hours, similar trend was observed in samples treated with extract of clove. At 6 hours, pH decreased marginally in samples blended with clove from 6.52 in sample A to 6.32 and from 6.23 in sample D to 6.16; 6.32 to 6.14 in sample B and from 6.25 to 6.12 in sample C respectively (Table 4).

Statistical result of the study samples compared against control shows no significant difference at $p < 0.05$. increase in Total Titrable Acidity (TTA) was recorded in all the samples as storage time increases from 0 – 6 hours. The TTA obtained in samples treated with ginger ranged from 2.07 % at 0 hour which increased to 3.02 % in sample B at 6 hours, while in clove, it ranged from 2.43% at 0 hour to 3.39 % in sample C at 6 hours. At 4 hours, TTA of samples treated with ginger increased marginally from 2.39 in sample A to 2.57 %; 2.52 to 2.59 % in sample B and from 2.54 to 2.79 % in sample C respectively. Similar trend was observed in samples treated with extract of clove at 4 and 6 hours respectively. At 6 hours, TTA of samples treated with clove increased marginally from 2.61 % in sample A to 3.07 %; 2.72 % to 3.16 % in sample D; 2.66 to 3.24% in sample B and from 2.76 % to 3.39 % in C samples

respectively. Statistical result shows no significant difference between the study samples and the control at $p > 0.05$ (Table 5).

Table 4: Physicochemical Parameters of Tiger-nut milk blended with Aqueous extract of Ginger (*Zingiber officinale*)

	Temp ($^{\circ}$ C)	Ph	Titration acidity (%)	Peroxide value (mEq/Kg)
A0	18.00 \pm 0.00	6.51 \pm 0.00	2.07 \pm 0.09	10.00 \pm 1.00
A2	18.40 \pm 0.05	6.42 \pm 0.04	2.39 \pm 0.04	12.00 \pm 1.00
A4	19.00 \pm 0.00	6.36 \pm 0.02	2.57 \pm 0.13	15.00 \pm 1.00
A6	6.2 \pm 0.04	6.20 \pm 0.03	2.84 \pm 0.13	19.00 \pm 1.00
B0	18.00 \pm 0.00	6.51 \pm 0.00	2.07 \pm 0.09	10.00 \pm 1.00
B2	18.50 \pm 0.03	6.31 \pm 0.05	2.41 \pm 0.07	11.00 \pm 2.00
B4	18.80 \pm 0.08	6.21 \pm 0.03	2.88 \pm 0.18	14.00 \pm 2.00
B6	6.13 \pm 0.01	6.13 \pm 0.01	2.93 \pm 0.22	21.00 \pm 1.00
C0	18.00 \pm 0.00	6.51 \pm 0.00	2.07 \pm 0.09	10.00 \pm 1.00
C2	18.50 \pm 0.01	6.41 \pm 0.05	2.52 \pm 0.09	13.00 \pm 1.00
C4	19.00 \pm 0.00	6.25 \pm 0.01	2.59 \pm 0.07	20.00 \pm 1.00
C6	6.12 \pm 0.02	6.12 \pm 0.02	3.02 \pm 0.04	23.00 \pm 1.00
D0	18.00 \pm 0.00	6.51 \pm 0.00	2.07 \pm 0.09	10.00 \pm 1.00
D2	18.00 \pm 0.00	6.24 \pm 0.02	2.54 \pm 0.02	16.00 \pm 1.00
D4	18.60 \pm 0.14	6.13 \pm 0.03	2.79 \pm 0.09	21.00 \pm 0.00
D6	6.05 \pm 0.02	6.05 \pm 0.02	3.01 \pm 0.09	26.00 \pm 1.00

KEY; A-Kunun Aya without treatment, B-Kunun Aya + 0.05g/l of ginger extract, C-Kunun Aya + 0.1g/l of ginger extract, D – Kunun Aya + 0.01g/l of Sodium Benzoate.

Table 5: Physicochemical result of Tiger-nut milk blended with Aqueous extract of Clove (*Syzygium aromaticum*)

	Temp. ($^{\circ}$ C)	pH	Titration acidity (%)	Peroxide value (mEq/Kg)
A0	18.00 \pm 0.00	6.81 \pm 0.05	2.43 \pm 0.09	9.00 \pm 1.00
A2	19.00 \pm 0.00	6.61 \pm 0.03	2.52 \pm 0.00	11.00 \pm 1.00
A4	19.60 \pm 0.03	6.52 \pm 0.04	2.61 \pm 0.01	14.00 \pm 2.00
A6	20.00 \pm 0.00	6.32 \pm 0.03	3.07 \pm 0.13	22.00 \pm 2.00
B0	18.00 \pm 0.00	6.81 \pm 0.05	2.43 \pm 0.09	9.00 \pm 1.00
B2	19.20 \pm 0.01	6.56 \pm 0.02	2.50 \pm 0.03	14.00 \pm 2.00
B4	20.00 \pm 0.00	6.23 \pm 0.03	2.72 \pm 0.02	16.00 \pm 1.00
B6	21.00 \pm 0.00	6.16 \pm 0.02	3.16 \pm 0.02	20.00 \pm 2.00
C0	18.00 \pm 0.00	6.81 \pm 0.05	2.43 \pm 0.09	9.00 \pm 1.00
C2	18.50 \pm 0.03	6.74 \pm 0.04	2.58 \pm 0.02	13.00 \pm 1.00
C4	19.30 \pm 0.04	6.32 \pm 0.02	2.66 \pm 0.02	18.00 \pm 2.00
C6	22.00 \pm 0.00	6.14 \pm 0.02	3.24 \pm 0.11	21.00 \pm 2.00
D0	18.00 \pm 0.00	6.81 \pm 0.05	2.43 \pm 0.09	9.00 \pm 1.00
D2	18.80 \pm 0.03	6.62 \pm 0.02	2.52 \pm 0.02	16.00 \pm 1.00
D4	19.50 \pm 0.05	6.25 \pm 0.02	2.76 \pm 0.02	19.00 \pm 2.00
D6	20.50 \pm 0.03	6.12 \pm 0.02	3.39 \pm 0.02	24.00 \pm 1.00

KEY; A-Kunun Aya without treatment, B-Kunun Aya + 0.05g/l of ginger extract, C-Kunun Aya + 0.1g/l of ginger extract, D – Kunun Aya + 0.01g/l of Sodium Benzoate.

Peroxide value (PV) also recorded significant increase from 0 – 6 hours in all the samples as storage time increases. The PV obtained in samples treated with clove ranged from 9

mEq/Kg at 0 hour which increased to 20 mEq/Kg in sample D at 6 hours, while in ginger, it ranged from 10 mEq/Kg at 0 hour to 26mEq/Kg in sample C at 6 hours. At 4 hours,

PV of samples treated with ginger increased marginally from 12 in sample A to 15mEq/Kg; 13 to 20mEq/Kg in sample B and from 16 to 21 mEq/Kg in sample C respectively. Similar trend was observed in samples treated with clove at 4 and 6 hours respectively. At 6 hours, PV of samples treated with clove increased marginally from 14 in sample A to 22 mEq/Kg; 16 to 20 mEq/Kg in sample D; 18 to 21mEq/Kg in sample B and from 19 to 24 mEq/Kg in sample C respectively. Statistical result at $p>0.005$ shows no significant difference between the study samples and the control.

The effects of adding extracts of ginger (*Zingiber officinale*) and clove (*Syzygium aromaticum*) on the sensory attributes (appearance, flavour, texture, aroma and overall acceptability) of tiger nut at varied time interval is presented in Table 10 and 11 respectively. Significant decrease was recorded in all sensory attributes as storage time increases. There was an observed decline in the sensory attributes in samples blended with ginger as storage progressed at 2 hours' interval. Results obtained: for sample A: appearance 8.30 to 7.38, flavour 8.20 to 6.63, texture 7.90 to 6.63, aroma 7.90 to 5.63 and overall acceptability 8.00 to 5.88; for sample D: appearance 8.00 to 6.50, flavour 7.38 to 6.25, texture 7.63 to 6.13, aroma 7.75 to 5.13 and overall acceptability 7.88 to 5.88; for sample C extracts of ginger: appearance 7.00 to 5.33, flavour 6.38 to 4.11, texture 7.63 to 5.00, aroma 7.00 to 4.00 and overall acceptability 7.38 to 4.44; for sample C

extracts of ginger: appearance 6.50 to 4.00, flavour 6.50 to 4.13, texture 7.13 to 4.25, aroma 6.25 to 3.50 and overall acceptability 6.38 to 3.00 respectively.

In samples blended with clove, similar trend was recorded as storage time increased from 2 – 6 hours. Results obtained: for sample A: appearance 6.50 to 3.75, flavour 7.60 to 5.88, texture 7.30 to 5.25, aroma 7.50 to 5.38 and overall acceptability 7.70 to 5.63; for sample D: appearance 8.00 to 4.25, flavour 7.30 to 5.75, texture 7.50 to 5.50, aroma 6.80 to 5.25 and overall acceptability 7.60 to 5.38; for sample B extracts of clove appearance 7.90 to 3.75, flavour 7.50 to 5.13, texture 7.70 to 4.75, aroma 7.70 to 4.88 and overall acceptability 8.00 to 4.75; for sample C extracts of clove: appearance 8.00 to 3.63, flavour 7.10 to 4.38, texture 7.20 to 4.25, aroma 7.00 to 4.63 and overall acceptability 7.70 to 4.13 respectively. Statistical results of the organoleptic properties show significant difference at $p<0.05$ between the blended tiger nut milk samples and the control.

Figure 1, 2 and 3 shows the bacterial and fungal count of kununaya blended with ginger extracts. The total bacterial count ranged from 1.4×10^5 – 3.1×10^6 CFU/ml, the total fungal count ranged from 1.0×10^5 – 9.7×10^5 CFU/ml. Figure 4, 5 and 6 shows the bacterial and fungal count of kununaya blended with clove extract. The total bacterial count ranged from 1.8×10^5 – 3.5×10^6 CFU/ml while the total fungal count ranged from 8.0×10^4 – 8.6×10^5 CFU/ml.

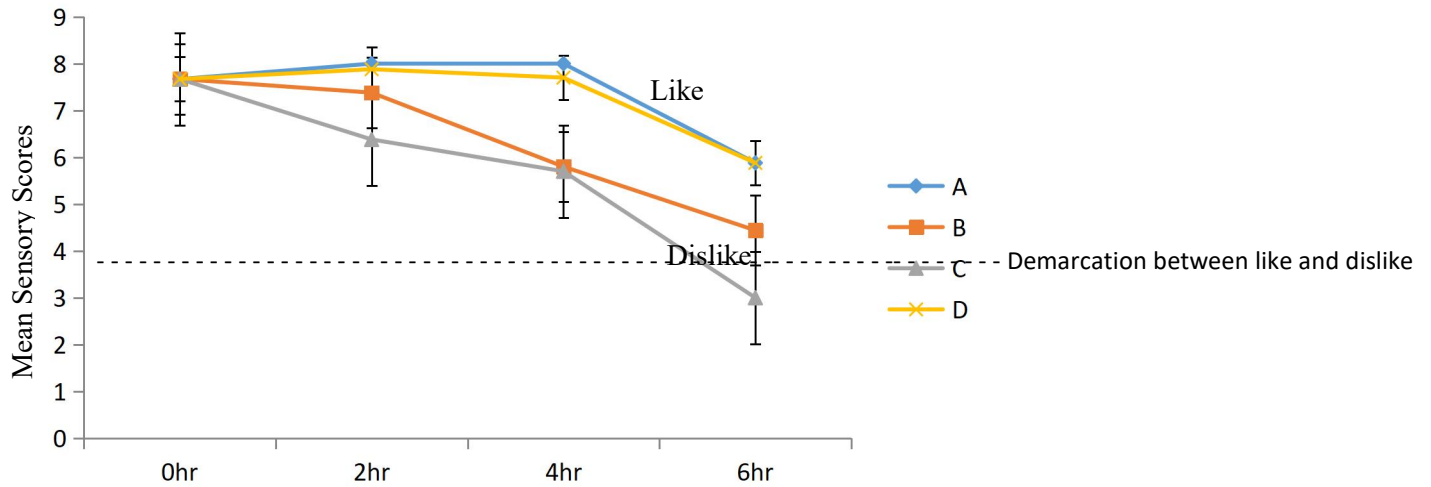


Figure 1: Overall Acceptability of Kunun Aya Treated with Ginger Extract.

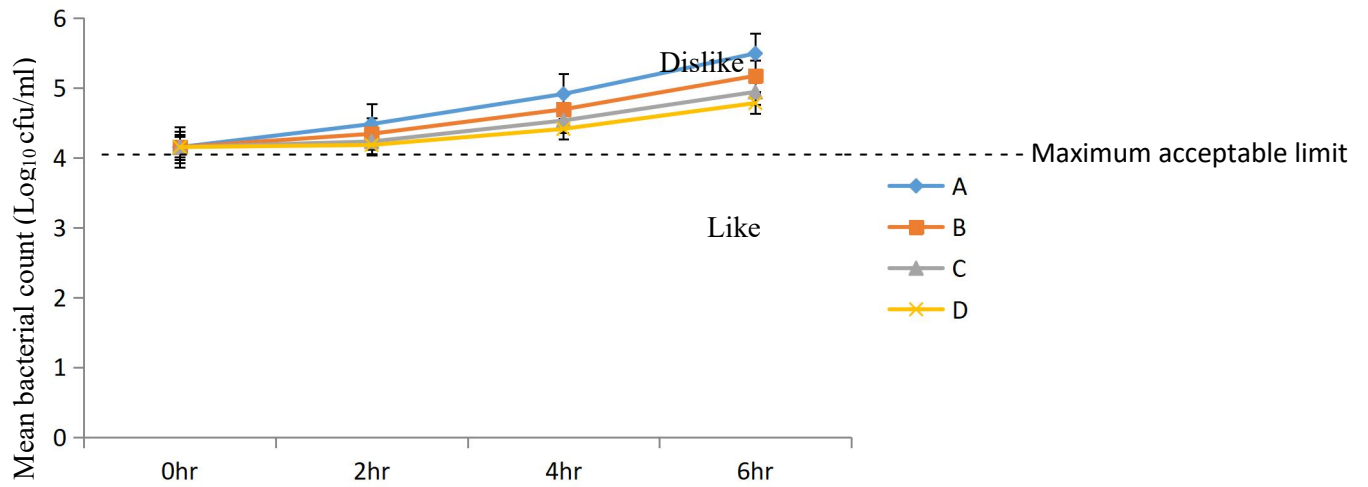


Figure 2: Bacterial Load of Kunun Aya treated with Ginger Extract.

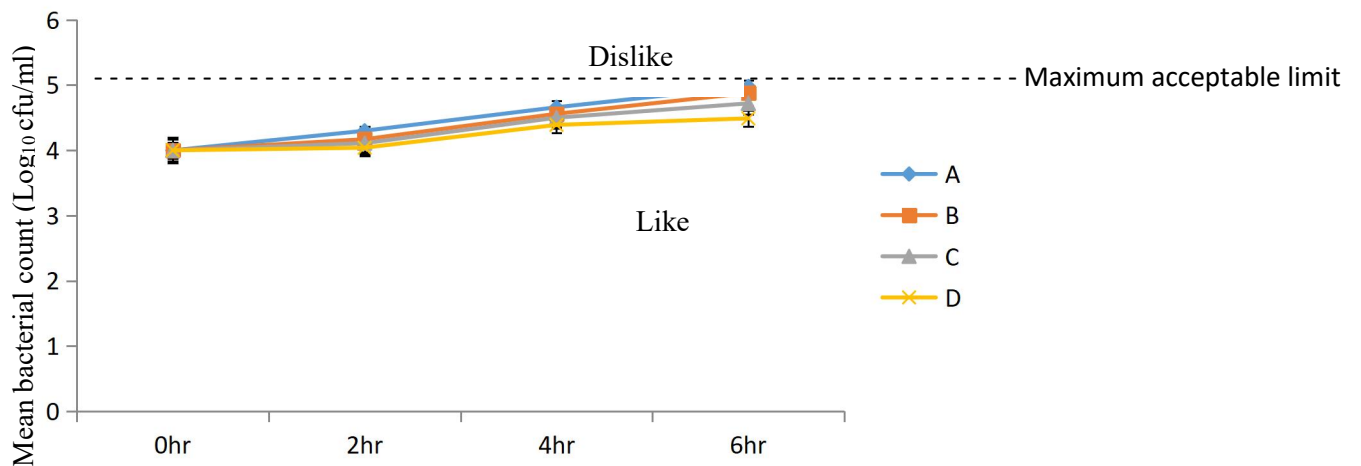


Figure 3: Fungal Load of Kunun Aya Treated with Ginger Extract.

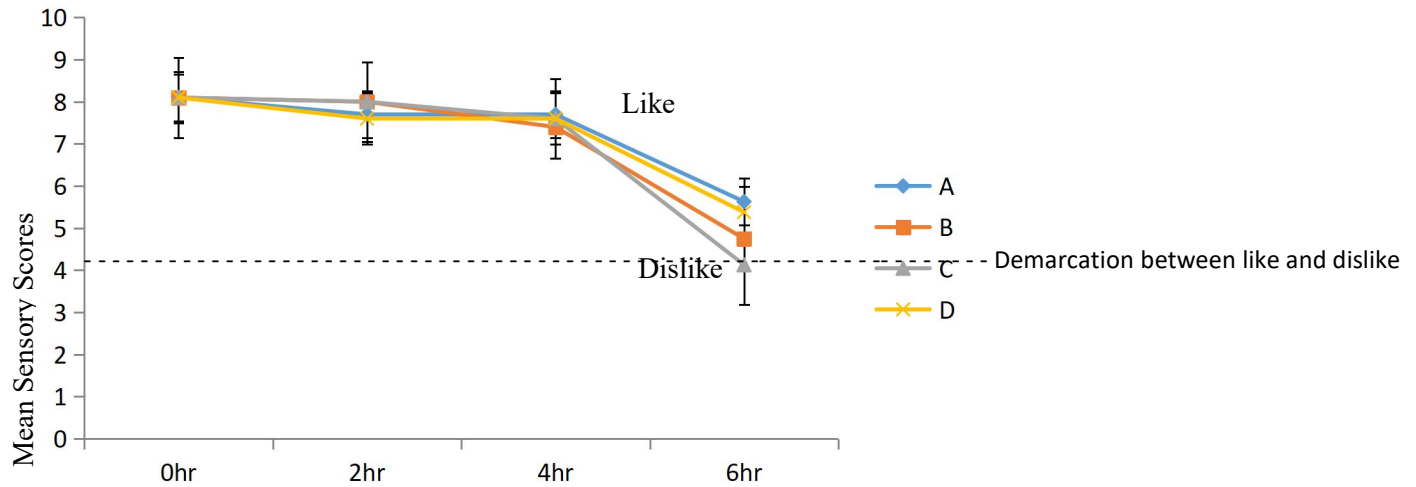


Figure 4: Overall Acceptability of Kunun Aya Treated with Clove Extract.

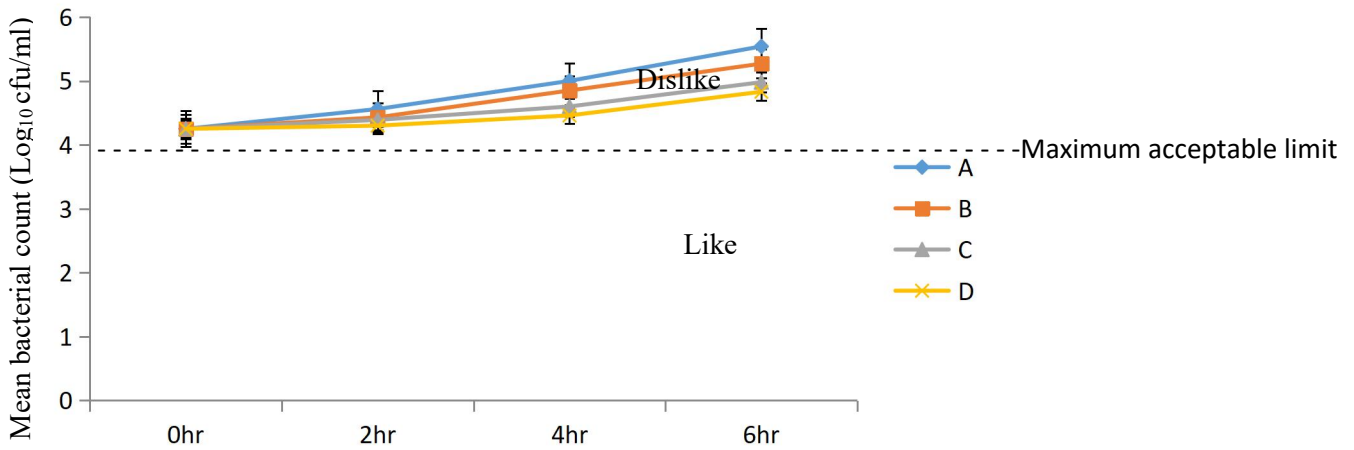


Figure 5: Bacteria Load of Kunun Aya Treated with Clove Extract

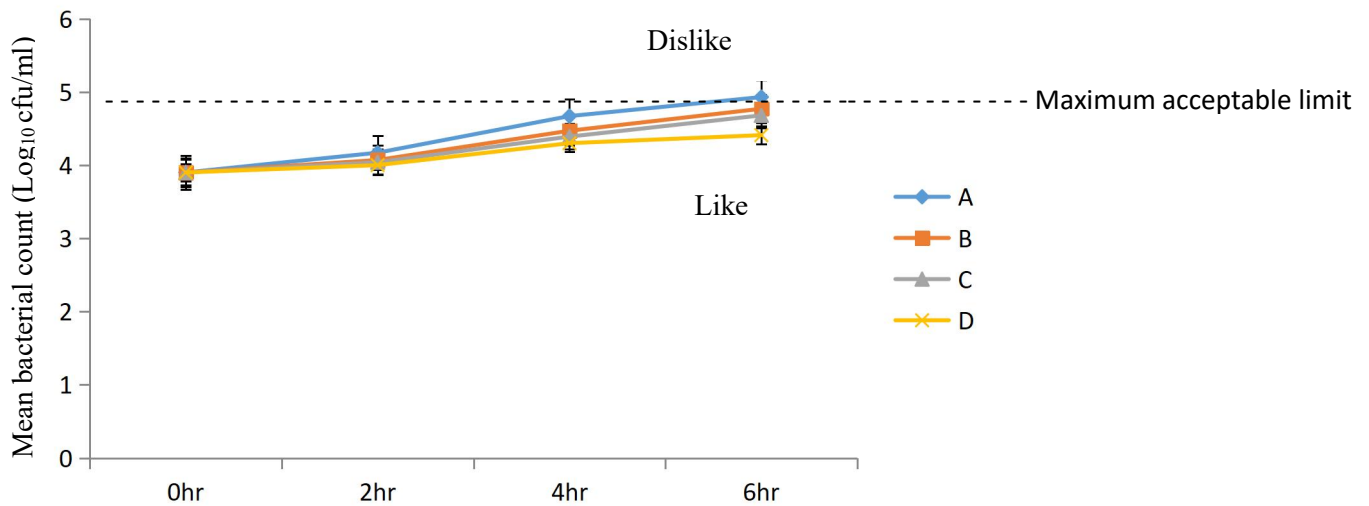


Figure 6: Fungal Load of Kunun Aya treated with Clove Extract.

DISCUSSION

Phytochemical screening carried out on the extract of clove (*Syzygium aromaticum*) showed the presence of all the phytochemicals in study, while in ginger (*Zingiber officinale*), alkaloids, flavonoids, phenols, saponins and steroids were present, while tannins and glycosides were found to be absent. These variations might be due to methods used for the analysis, geography and/or the solvent used for extraction. The presence of these constituents gives an indication of their medicinal value. The findings of ginger in this study agrees with the studies of (Osabor *et al.*, 2015) and conversely differs with the study of (Rowland *et al.*, 2017) whom found glycosides to be present in ginger, while alkaloids, saponins, flavonoids and steroids to be absent which were all present from the findings of this study.

The result of proximate analyses revealed the moisture content of the kunun aya to be between 83.98-80.54 in ginger spiced kunun aya and 81.67 to 78.15 in clove spiced kunun aya, this formed the major component of the kunun aya samples and consequently, made it a good alternative to soft drinks in supply of water to human body. The high moisture content associated with tiger-nut milk as observe in this study could be responsible for its poor storage quality and instability, as high moisture content encourages microbial growth and proliferation thereby predisposing kunun aya to bacterial and fungal attack (Onwuka 2005). The moisture contents obtained in this study are similar to the findings reported by (Awonorin and Udezor, 2014) and (Ukwuru and Ogbodo, 2011) but were higher than the values reported by (Musa and Hamza 2013).

The protein content obtained in samples treated with ginger ranged from 3.96 % to 4.52 %, while in clove, it ranged from 3.07% to 3.93 % which are relatively higher than the

range reported by (Kayode *et al.*, 2017). It was observed that addition of the spices significantly increased the crude protein content of the samples. This may be due to the high protein content of the spices (Ogunola *et al.*, 2010). Besides proteins functioning in the synthesis of new cells, repair of worn out tissues, enzymes, hormones, antibodies and other substances, proteins are also required for healthy functioning and development of the body and its protection (Cheesebrough 2006).

The ash content ranges from 0.28-0.23 and 0.25 to 0.23 in both the ginger and clove respectively

Ash content evaluation is an important marker in food analysis as it's generally taken to be a measure of the mineral content (Onwuka 2005). The average ash content of this study agrees with the findings of (Balewu and Abodunrin, 2008) but slightly lower than the finding of (Maxwell *et al.*, 2019).

The fat content of all samples under study decreases as storage time increases from 0 to 6 hours. The decrease in fat content agrees with the findings of (Isaac *et al.*, 2019) who reported decrease in fat content as fermentation period of tiger-nut milk progresses. Fat contributes substantially to the energy value of food. The decrease in fat content during storage could be due to increase in microorganisms utilizing the fat as fermentation period progresses (Obadina *et al.*, 2013). Statistical result shows no significant difference between the study samples and the control at $p > 0.05$. Lipids (fats and oil) in foods or drinks play important role to the body as they serve as energy storage of the body besides their role in maintaining healthy skin and hair. (Food Safety and Standards Authority of India, 2010).

Interestingly, carbohydrate content increased with increase in storage time. The result trend

obtained in this study corresponds with the research findings reported by (Maxwell *et al.*, 2019) though the carbohydrate content is marginally higher than what they obtained. Carbohydrates serve as the storage form of energy (glycogen) to meet the immediate energy demands of the body; provide necessary calories in the diet and promote the utilization of dietary fats (Balogun and Olatidoye 2012).

The effects of adding aqueous extracts of ginger (*Zingiber officinale*) and clove (*Syzygium aromaticum*) on temperature, pH, titrable acidity and peroxide value on tiger-nut milk was studied at varied time intervals. Slight increase in temperature was recorded in all samples blends and control. Conversely, there was marginal decrease in pH as storage time increases from 0 to 6 hours. The decrease in pH values obtained from this study might be as a result in increased lactic acid bacteria as fermentation progresses, same trend was reported by (Musa and Hamza, 2013) and (Rowland *et al.*, 2017) whom both reported relatively lower values than the pH obtained in this study.

Increase in Total Titrable Acidity (TTA) was recorded in all the samples as storage time increases from 0 – 6 hours. The titrable acidity and pH values showed that the kunun aya samples were generally acidic throughout the storage period. This acidity can be attributed to the production of lactic acid by some species of lactic acid bacteria (*Lactobacillus leichmanni* and *Lactobacillus fermentum*) during fermentation processes. The increase in TTA with respect to increased temperature, and inversely with pH, agreed with the studies of (Braide *et al.*, 2018) and (Awonorin and Udeozor, 2014) Statistical result at $p > 0.05$ shows no significant difference between the study samples and the control.

Peroxide value (PV) also recorded significant increase from 0 – 6 hours in all the samples as storage time increases. Peroxide value is an important index used to assess rancidity in lipids samples. Generally, fresh oil has PV < 10 mEq/Kg while oil with PV within 30 – 40 mEq/Kg range is vulnerable to rancidity (Onwuka 2005). The increase in PV agreed with the studies of (Adzidgi 2001)

The effects of adding extracts of ginger (*Zingiber officinale*) and clove (*Syzygium aromaticum*) on the sensory attributes (appearance, flavour, texture, aroma and overall acceptability) of tiger nut at varied time interval was studied. Significant decrease was recorded in all sensory attributes as storage time increases. The low sensory mean score recorded in the study samples might be that the concentrations of the extracts used might be higher for the products to be stored at ambient temperature unless refrigerated (Ali *et al.*, 2019), thereby predisposing the samples to rapider decline in the sensory attributes, hence the reasons for low scores by the panelists.

The sensory evaluation results thus inferred, that as storage time increase, the organoleptic properties of tiger nut milk are impaired thus affecting its acceptability by the consumers and predispose it to rapider spoilage. Flavor defects develop at higher rate than textural changes during storage and seem to be the earliest indicator of product failure (Al-kadamany *et al.*, 2002) The decline in sensory attributes of the tiger nut milk as storage time increases agrees with the work of (Kayode *et al.*, 2017) Deterioration in sensory quality as well as microbiological counts has been used as indices for the end of shelf life of dairy products (Muir and Banks, 2000) and (Al-kadamany *et al.*, 2002).

The rapid deterioration in shelf life of kunun aya and other African beverages is widely

acknowledged and is of great concern (Odufa, 1985; Dirar, 1993; Efiuvwev and Akoma 1995). The occurrence of diverse microbial genera and the remarkable high microbial load in the untreated control sample are a major cause of accelerated spoilage commonly experienced by brewers and consumers of these products. It was observed that microbial growth increased throughout the storage period. The increase in microbial load maybe due to storage time of product at ambient temperature which is a factor that may result in spoilage. This agrees with report by (Musa and Hamza, 2013). Microbial count of all the samples including control (sample A) at 0 hour and 2 hours were lower than that of 4 and 6 hours because of the effect of spices which tend to decrease the microbial count of the sample which correlates with work of (Kayode *et al.*, 2017) It is evident that treated samples have lower microbial load because the added spices have antimicrobial effect which are capable of destroying pathogenic bacteria (Ayo *et al.* 2003). Microbial count of all the samples increased during storage period with standard control (sample D) having the lowest microbial count during storage while microbial count increased at 4 hour and 6 hours but were too numerous to count in some plates. This can be attributed to storage environment of the samples at ambient temperature as reported by (Tembo *et al.*, 2008). The presence and increase in bacteria and fungi can be as a result of the natural spices which tends to deteriorates as storage period increased.

The microbial load obtained from the research suggests that the natural preservatives were less effective in prolonging the shelf life compared to the synthetic sodium benzoate preservative. The reason for this observation is not well understood but may be attributed to the insolubility of natural preservatives used in the study. This could synergize the

nutrient status of the kunun aya creating appropriate food base for the colonization of microorganisms. (Andargie *et al.*, 2008; Fasolin and Cunha, 2012). Sodium benzoate, a common chemical preservative showed a better bacteriostatic and fungistatic performance due to acidic condition of the kunun aya. It is also observed from this study that the spices used reduced the microbial load over storage duration when compared to the control sample A (Seetaramiah *et al.*, 2011). This may be due to phytochemical property of these plants (Tagoe *et al.*, 2010).

CONCLUSION

The addition of spices as natural preservatives sanitize the growth of microorganisms during storage period of the kunun aya at room temperature and hence, made it consumable after storage period (6hours).

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