JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

EFFECTS OF ADDITION OF DIFFERENT SPICES ON THE QUALITY ATTRIBUTES OF TIGER-NUT MILK (*KUNUN-AYA*) DURING STORAGE

Rowland Monday Kayode[*1], John Kolade Joseph[1], Mojisola Olanike Adegunwa[2], Adegbola Oladele Dauda[1], Sarafa Adeyemi Akeem[3], Bukola Idowu Kayode[2], Adeshola Ajoke Babayeju[1] and Stephen Orobola Olabanji[4]*

Address(es): Dr. Rowland Monday Kayode

[1]Division of Food Processing, Preservation, Microbial Biotechnology and Safety, Department of Home Economics and Food Science, University of Ilorin, Nigeria, +2348035850545.
[2]Department of Food Science and Technology, College of Food Science and Human Ecology, Federal University of Agriculture, Abeokuta, Nigeria.
[3]Department of Food Technology, University of Ibadan, Ibadan, Oyo State, Nigeria, +2347039314405.
[4]Department of Microbiology, University of Ilorin, Ilorin, Nigeria.

*Corresponding author: kayodermosnr@gmail.com

doi: 10.15414/jmbfs.2017.7.1.1-6

ARTICLE INFO

Received 13. 4. 2017
Revised 6. 5. 2017
Accepted 23. 5. 2017
Published 1. 8. 2017

Regular article

OPEN ACCESS

ABSTRACT

Kunun-aya is a traditional fermented non-alcoholic tiger-nut beverage widely consumed in the Northern parts of Nigeria especially during dry season. *Kunun-aya* was prepared from tiger-nut, coconut, date and spices such as cinnamon, cloves, coriander, ginger, rosemary and black pepper were added separately. The samples were stored at 4°C for 5 days and the effects of added spices on physicochemical, microbial and sensory properties of the samples were evaluated. The results of the pH (4.25–5.95) and titratable acidity (2.95–12.17) showed that all the samples were acidic throughout the storage period. Brix values ranged from 0.05–2.85% during the storage period. The moisture, protein, ash, fat, fibre and carbohydrate contents of *kunun-aya* ranged from 85.35–95.22%, 1.53–4.06%, 0.14–0.64%, 0.31–0.85%, 0.23–1.84% and 0.69–10.85%, respectively prior to storage and ranged from 79.50–98.24%, 0.27–2.56%, 0.04–2.25%, 0.24–0.42%, 0.09–3.74% and 1.05–17.34%, respectively after storage. The bacterial count of *kunun-aya* ranged from (1.1–5.4, 2.0–6.9, 3.6–9.8)×10^4 CFU/ml and fungal count from (1.0–6.4, 1.3–7.4, 5.2–9.5)×10^4 CFU/ml on days 1, 3 and 5, respectively. The phytochemical screening of the spices revealed that ginger contained alkaloid, glycoside, saponin, steroid, flavonoid and terpenoid but not tannin while other spices contained one or two of these phytochemicals. There was significant difference between the sensory attributes of the treated *kunun-aya* and the control samples with the control sample having the highest score ratings. Addition of spices had varying effects on the quality attributes of *kunun-aya* and could extend its shelf life for 5 days under refrigerated conditions.

Keywords: Physicochemical, Sensory property, Microbial load, Tiger-nut, Spice, Storage

INTRODUCTION

The ever increasing world's population coupled with high cost of animal protein has necessitated the search for underutilized cheap crops which can be easily processed and serve as a source of dietary protein and energy for human. Tiger-nut milk (*Kunun-aya*) is a refreshing high nutritive, energy drink produced mainly from tiger nut. Sometimes, fruits such as coconut, pineapple and date are added to impart flavour into the milk. Tiger-nut (*Cyperus esculentus*) belonging to the family, *Cyperaceae* is a good source of energy, fat, starch, fibre, glucose and protein (**Muhammed *et al.*, 2011**). Tiger-nut is also rich in vitamins, minerals and some digestive enzymes such as catalase, lipase and amylase (**Adejuyitan, 2011**). Raw tiger-nut had also been reported to contain certain phytochemicals and antinutritional factors such as alkaloids, cyanogenic glycosides, resins, tannins, sterols, oxalates, phytates and saponins (**Chukwuma *et al.*, 2010**). The absence of sodium, lactose sugar, casein protein, gluten and cholesterol in tiger-nut makes it suitable for consumption for people who are hypertensive, lactose intolerant or allergic to gluten (**Belewu and Abodunrin, 2008; Gambo and Da'u, 2014**). In addition, tiger-nut has been reported to contain higher essential amino acids than those proposed in the protein standard by the FAO/WHO in 1985 for satisfying adult needs (**Bosch *et al.*, 2005**). The use of tiger-nuts as stimulants, tonic and for the treatment of flatulence, indigestion, diarrhoea and dysentery had been documented (**David, 1986**).
Coconut (*Cocos nucifera)* of the family, *Arecaceae,* is a versatile fruit found throughout the tropics and subtropics and forms part of the daily diets of many people especially in Southeast Asia, the Caribbean and Northern South America. Coconut milk is the liquid that comes from the grated meat of a mature coconut (**Philippine Coconut Authority, 2014**). **Arumughan *et al.* (1993)** recommended the washing of the coconut meat with water containing 100 ppm hydrogen peroxide, followed by blanching at 80°C for 10 minutes to reduce the initial microbial load and to inactivate lipase enzyme. Emulsifier and stabilizer can be used to prevent the layer separation of the cream from the coconut milk during storage. Coconut milk are rich source of dietary protein, energy, calcium and fat such as myristic acid, oleic acid, lauric acid, linoleic acid, palmitic acid, and capric acid (**Belewu and Belewu, 2007**). Fresh coconut milk has a consistency and mildly sweet taste similar to that of cow's milk and can be stored for four weeks at 4°C.
Date (*Phoenix dactylifera L.*) belonging to the Arecaceae family represents an important economical and ecological culture for many countries in the North Africa and in the Middle East (**Ahmed *et al.*, 1995**). Date fruits contain about 80% sugar and less than 40% moisture content (**Borchani *et al.*, 2010; Hasnaoui *et al.*, 2011**). Its other nutritional components include ash, fibre, small amounts of protein and fat, potassium and trace elements such as boron, cobalt, copper, fluorine, magnesium, manganese, selenium, and zinc (**Al-Shahib and Marshall, 2003; Borchani *et al.*, 2010**). Spices have been used for thousands of centuries by many cultures to enhance the flavor, taste and aroma of foods. Early cultures also recognized the value of using spices in preserving foods and for their medicinal value (**Ene-Obong *et al.*, 2015**). The beneficial health effects of spices have been well documented (**Mensah *et al.*, 2009; Bhattacharjee and Sengupta, 2009**). Many spices have been reported to have antimicrobial properties, cholesterol lowering effects, anti-diabetic and anti inflammatory properties (**Suekawa *et al.*, 1984; Kwada and Tella, 2009**). 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**).

Recently, milk sources from plants such as tiger-nut are gaining strong interest from the researchers as well as increasing acceptability from consumers not only because they provide refreshment but also because of their nutritional and medicinal values. However, tiger-nut milk (*kunun-aya*) must be consumed within 2-4 hours at 40°C–100°C due to its poor shelf life (**Akoma *et al*., 2006**). Some researchers have reported the ability of pasteurization and addition of citric acid, ginger and garlic to extend the shelf-life of tiger-nut milk by minimum of 2-3 days (**Nwobosi *et al.*, 2013**). The present study was therefore designed to determine the effects of addition of spices such as cinnamon, cloves, coriander, ginger, rosemary and black pepper on the physicochemical, microbial and sensory properties of tiger-nut milk (*Kunun-aya*) produced from tiger-nut, coconut and date during storage.

MATERIALS AND METHODS

Collection of samples

Fresh tiger-nuts (*Cyperus esculentus*), date palm (*Phoenix dactylifera*) and coconut (*Cocos nucifera*) were purchased from Challenge, Ilorin, Kwara State, Nigeria. Ginger (*Zingiber officinale*), cloves (*Syzygium aromaticum*), coriander (*Coriandrum sativum*), cinnamon (*Cinnamomum verum*), black pepper *(Piper nigrum)* and rosemary (*Rosmarinus officinalis)* were bought from shoprite mall, Ilorin, Kwara State, Nigeria. The samples were properly packaged in polyethylene bags and brought to the Food Processing Laboratory, Department of Home-Economics and Food Science, University of Ilorin, Nigeria for processing.

Preparation of tiger-nut milk (*kunun-aya*)

The fresh tiger-nuts and dates were sorted and washed to remove soil, dirts and other unwanted substances. Approximately 3 kg of the tiger nut was steeped in distilled water for 8 hours. The tiger-nuts were drained and blanched at 70^{o}C for 5 minutes mainly to inactivate enzymes that might cause clumping of the extract. The tiger-nut was then ground with two medium sized coconut meats and 30 pieces of dates palms (after the seeds of the date have been removed) into a mash with the addition of 9L of water for efficient blending. The mixture was strained using a muslin cloth to separate the chaff from the milk. A 10g of gelatin was added as a stabilizer to the resulting tiger-nut milk in order to prevent layer separation **(NAFDAC, 2002)**.

Preparation and application of spices to *Kunun-aya*

The spices were washed, thinly sliced (about 4mm thickness), oven dried at 60°C for 48 hours and milled continuously in an hammer mill until a fine and smooth powders were obtained. The tiger nut extract at room temperature (28±2°C) was divided into seven (7) portions with volume of 300 ml each. One portion was set aside as the control while 1.6% (4.8 g) of ginger, cinnamon, coriander, cloves, black pepper and rosemary powder was added to each of the remaining 6 portions, respectively. The amount of spices used in this study were based on allowable standard as recommended by **NAFDAC (2002).** The resulting tiger-nut milk samples obtained were pasturized at 70^{o}C for 30 minutes in a water bath with continuous stirring. The samples were allowed to cool anda representative sample was taken from each sample for analysis to obtain day 1 results while the remaining portions of each sample were stored in a refrigerator at 4°C for further analysis.

Phytochemical screening of the spices

The quick reagent test methods of **Sofowora (1993)** and **Harbone (1973)** were used to screen the samples for flavonoids, terpenoids, tannins, saponins, steroids, alkaloids and glycosides.

Determination of pH and titratable acidity

pH meter was used to determine the pH of the samples (**Owoso and Ogunmoyela, 2001**). For the titratable acidity, 15ml of the sample was poured into a beaker and about three drops of phenolphthalein was added. The mixture was titrated against 0.1N NaOH until the solution turned pink. The amount of NaOH used was recorded (**AOAC, 2000**).

Titratable acidity (g/ml) = $\frac{titre\ value \times 0.09 \times 100}{Volume\ of\ sample}$

TSS (brix %) determination

The total soluble solids of the samples were determined using a refractometer according to the procedures described in **AOAC (2000)**. The refractometer was sterilized with distilled water and cotton wool. Three drops of well homogenized sample were taken on prism of refractometer and direct reading was taken.

Proximate composition

Moisture, crude protein, total ash, crude fibre and carbohydrate contents were determined using standard methods (**AOAC, 2005**). Briefly, oven drying method at 105^o C for 5 hours for moisture determination, micro-Kjeldahl method for crude protein, total ash was obtained by igniting 2 g sample at 550 ^{o}C for 4 hours using muffle furnace, crude fibre was determined using digestion method and carbohydrate was estimated by difference [100-(% water + % protein + % fat + % ash + % crude fibre)]. Crude fat was determined using standard soxhlet extraction method with diethyl ether as the solvent (**AOAC, 1995**).

Microbial plate counts

The total bacterial and fungal plate counts of the *kunun-aya* samples were carried out according to the method described by **Musa and Hamza (2013)**. The nutrient agar (NA) and the potato dextrose agar (PDA) used for the isolation of bacteria and fungi, respectively were prepared according to the manufacturer's instructions. The counts were expressed in cfu/ml.

Sensory evaluation

The sensory quality attributes including taste, appearance, texture, aroma and overall acceptability of the seven (7) *kunun-aya* samples were evaluated by 20 member panelists comprising of both students and staff members of the Department of Home Economics and Food Science, University of Ilorin. The panelists were instructed to score the coded samples based on 9-point hedonic scale with 1 as disliked extremely and 9 as liked extremely (**Ihekoronye and Ngoddy, 1985**).

Statistical analysis

All analyses were conducted in triplicates. Data obtained for the pH, TSS, titrable acidity, proximate composition, and sensory analyses of the samples were subjected to one way Analysis of Variance (ANOVA) and difference among the means was determined using Duncan multiple range test. Statistical Package for Social Sciences (SPSS) Version 16.0 (SPSS Inc., Chicago, IL USA) was used to analyze the data and *$p<0.05$* was considered to be statistically significant. Results were expressed as mean ±standard deviation.

RESULTS AND DISCUSSION

Phytochemical composition of the spices

The bioactive components of the spices used in the formulation of *kunun-aya* samples are shown in Table 1. The results revealed that while ginger contained all the investigated phytochemicals except tannins, cinnamon, cloves, coriander, rosemary and black pepper contained one or two of these bioactive constituents. This supported the assertion of **Akeem *et al*. (2016)** that spices are good sources of phytochemicals. The anti-dyspeptic, anti-flatulent, anti-bacterial, anti-fungal, anti-diabetic effects, as well as other health promoting effects such as anti-allergic, anti-cancer, anti-oxidant, anti-inflammatory, anti-thrombotic, vasoprotective, tumour inhibitory and anti-viral effects of these bioactive constituents had been documented and some of these properties promote their use as preservatives in foods (**Trease and Evans, 2002b; Srinivasan, 2005; Edeoga *et al*., 2006**).

pH, Titatable acidity and TSS of *kunun-aya* treated with different spices during storage

The effects of different spices and storage period on the pH values, titratable acidity and total soluble solids (TSS) of *kunun-aya* are shown in tables 2, 3 and 4, respectively. The pH values ranged from 4.25 in *kunun-aya* treated with cloves to 5.95 in the control, ginger and black pepper spiced *kunun-aya*. This is similar to 4.56–4.95 reported by **Awonorin and Udeozor (2014)** for tiger-nut soy-milk. Some other researchers had reported significantly higher pH values (6.12–6.71) for tiger-nut milk with or without spices (**Belewu and Belewu, 2007; Belewu and Abodunrin, 2008; Nwobosi *et al*., 2013; Bristone *et al*., 2015**). The low pH may be attributed to the inclusion of date and coconut in the formulation of the beverage. The *kunun-aya* treated with black pepper had the lowest titratable acidity (2.95 g/ml) while the highest (12.17 g/ml) was recorded for the control and the cloves spiced *kunun-aya*, and they were significantly different from each other. **Belewu and Abodunrin (2008)** had reported the titratable acidity of tiger-nut milk to be 8.10 g/ml. The pH values and the titratable acidity 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 the fermentation process (**Akoma *et al*., 2006**). An inverse relationship was observed between the pH values and the titratable acidity of *kunun-aya*; as the pH values generally decreased on day 2 of storage, the titratable acidity generally increased. Similar trend has been reported by earlier researchers (**Awonorin and Udeozor,**

2014). The general decrease in the pH on day two may be an indication of the increase in lactic acid bacteria as the fermentation progressed. Significant difference ($p<0.05$) existed among the pH values of the control, *kunun-aya* treated with rosemary and *kunun-aya* treated with cloves on the first day of storage. Although the *kunun-aya* treated with cinnamon had the least pH value (4.35), the pH values of all the samples including the control were comparable. The low pH is desirable because it inhibits the growth of pathogenic micro-organisms which may be hazardous to human health (**Ukwuru and Ogbodo, 2011**). The brix value which ranged from 0.05–2.85% is the measure of total soluble solids in the beverage samples. Although most of the values decreased on days 2 and 4, the effect of storage period on the total solid of the *kunun-aya* samples varied (Table 4). These decrease agreed with the assertion that as the total soluble solids (% Brix) and pH reduced, titratable acidity increased (**Awonorin and Udeozor, 2014**). The effect of the addition of spices during storage varied though the TSS of most of the samples increased (Table 4).

Table 1 Phytochemical composition of the spices used for *kunun-aya* treatment

Sample	Alkaloid	Glycosides	Saponin	Steroid	Flavonoid	Terpenoid	Tannin
Cinnamon	-	+	-	+	-	-	-
Cloves	-	-	-	-	+	-	+
Coriander	-	-	-	-	+	+	-
Rosemary	-	-	-	-	+	-	-
Black pepper	+	-	-	-	-	-	-
Ginger	+	+	+	+	+	+	-

+ indicates present while – indicates absent

Table 2 pH values of *Kunun-aya* treated with different spices during storage

Samples	Day 1	Day 2	Day 3	Day 4	Day 5
Control	5.95^{a} ±0.50	4.35^{c} ±0.50	4.25^{b} ±0.50	4.35^{b} ±0.50	4.45^{ab} ±0.50
KCM	5.75^{b} ±0.50	4.55^{b} ±0.50	4.35^{b} ±0.50	4.35^{b} ±0.50	4.35^{b} ±0.50
KRM	5.85^{ab} ±0.50	4.90^{a} ±0.00	4.65^{a} ±0.50	4.55^{a} ±0.50	4.55^{a} ±0.50
KGG	5.95^{a} ±0.50	4.55^{b} ±0.50	4.25^{b} ±0.50	4.45^{ab} ±0.50	4.45^{ab} ±0.50
KCV	5.55^{c} ±0.50	4.45^{c} ±0.50	4.35^{b} ±0.50	4.45^{ab} ±0.50	4.45^{ab} ±0.50
KCD	5.85^{ab} ±0.50	4.45^{bc} ±0.50	4.25^{b} ±0.50	4.55^{a} ±0.50	4.55^{a} ±0.50
KBP	5.95^{a} ±0.50	4.45^{bc} ±0.50	4.25^{b} ±0.50	4.45^{ab} ±0.50	4.45^{ab} ±0.50

Values in the same column with different superscript are significantly different ($p<0.05$). KRM= *Kunun-aya* spiced with rosemary, KCD= *Kunun-aya* spiced with coriander, KCV= *Kunun-aya* spiced with cloves, KCM= *Kunun-aya* spiced with cinnamon, KGG= *Kunun-aya* spiced with ginger, KBP= *Kunun-aya* spiced with black pepper.

Table 3 Titratable acidity (g/ml) of *Kunun-aya* treated with different spices during storage

Samples	Day 1	Day 2	Day 3	Day 4	Day 5
Control	5.05^{a} ±0.50	6.95^{e} ±0.01	5.65^{c} ±0.05	8.65^{g} ±0.05	8.27^{e} ±0.01
KCM	4.37^{e} ±0.01	6.55^{f} ±0.05	4.97^{e} ±0.01	10.43^{d} ±0.01	9.65^{c} ±0.01
KRM	4.85^{c} ±0.01	9.23^{a} ±0.01	5.35^{d} ±0.05	12.17^{a} ±0.01	11.95^{a} ±0.50
KGG	4.55^{d} ±0.01	8.05^{c} ±0.05	4.75^{f} ±0.05	9.65^{e} ±0.01	8.70^{d} ±0.00
KCV	5.95^{a} ±0.50	8.95^{b} ±0.05	12.410^{a} ±.01	10.67^{c} ±0.01	10.15^{b} ±0.50
KCD	4.45^{de} ±0.50	7.31^{d} ±0.01	3.25^{g} ±0.05	10.85^{b} ±0.01	11.95^{a} ±0.50
KBP	2.95^{f} ±0.50	5.05^{g} ±0.05	7.67^{b} ±0.01	9.17^{f} ±0.01	7.76^{f} ±0.01

Values in the same column with different superscript are significantly different ($p<0.05$). KRM= *Kunun-aya* spiced with rosemary, KCD= *Kunun-aya* spiced with coriander, KCV= *Kunun-aya* spiced with cloves, KCM= *Kunun-aya* spiced with cinnamon, KGG= *Kunun-aya* spiced with ginger, KBP= *Kunun-aya* spiced with black pepper.

Table 4 Total soluble solids (brix %) of *Kunun-aya* treated with different spices during storage

Samples	Day 1	Day 2	Day 3	Day 4	Day 5
Control	0.85^{d} ±0.05	0.05^{e} ±0.04	0.05^{e} ±0.04	0.05^{d} ±0.04	0.25^{a} ±0.05
KCM	1.85^{b} ±0.05	0.35^{d} ±0.05	1.65^{b} ±0.05	0.35^{c} ±0.05	1.65^{b} ±0.05
KRM	0.65^{e} ±0.05	1.15^{b} ±0.05	1.05^{c} ±0.05	0.85^{a} ±0.05	1.95^{a} ±0.05
KGG	0.85^{d} ±0.05	0.85^{c} ±0.05	0.55^{d} ±0.05	0.25^{c} ±0.05	1.85^{a} ±0.05
KCV	2.85^{a} ±0.05	1.35^{a} ±0.05	0.55^{d} ±0.05	0.95^{a} ±0.05	1.15^{c} ±0.05
KCD	1.15^{c} ±0.05	0.75^{a} ±0.05	2.25^{a} ±0.05	0.55^{b} ±0.05	1.05^{c} ±0.05
KBP	1.95^{b} ±0.05	0.85^{c} ±0.05	0.65^{d} ±0.05	0.55^{b} ±0.15	1.95^{a} ±0.05

Values in the same column with different superscript are significantly different ($p<0.05$). KRM= *Kunun-aya* spiced with rosemary, KCD= *Kunun-aya* spiced with coriander, KCV= *Kunun-aya* spiced with cloves, KCM= *Kunun-aya* spiced with cinnamon, KGG= *Kunun-aya* spiced with ginger, KBP= *Kunun-aya* spiced with black pepper.

Proximate composition of *kunun-aya* treated with different spices during storage

The effect of addition of different spices on *kunun-aya* prior to storage is presented in Table 5. The effect varied among the parameters. The results indicated that the moisture content which ranged from 85.35% in *kunun-aya* treated with black pepper to 95.44% in the control formed the major component of the *kunun-aya* samples and consequently, made it a good alternative to soft drinks in the supplying of water to human body. The values obtained were comparable to 81.71–86.45% and 92.44% reported by **Awonorin and Udeozor (2014)** and **Bristone *et al*. (2015)**, respectively but lower than 62.8–82.5% reported by **Musa and Hamza (2013)** for tiger-nut milk. There was significant difference ($p<0.05$) between moisture content of the control and the treated samples with the treated samples having significantly lower values. The addition of the spices in powdered form which might have increased the solid contents of the treated samples could be responsible for the reduction in moisture contents of the samples. High moisture content of *kunun-aya* could be responsible for its poor storage quality since high moisture content encourages microbial growth during storage (**Umar *et al*., 2006**). The *kunun-aya* treated with black pepper had the highest protein content (4.06%) while the lowest value (1.53%) was recorded for rosemary spiced *kunun-aya*. The crude protein obtained was comparable to value range of 2.69–3.25% reported by **Musa and Hamza (2013)** but higher than 0.83% and 1.03% reported by **Bristone *et al*. (2015)** and **Nwobosi *et al*. (2013)**, respectively. It was observed that the addition of cinnamon and black pepper significantly increased the crude protein content of the samples. This may be due to the high protein content of the spices (**Otunola *et al*., 2010**). However, the reduction in crude protein contents of the samples treated with rosemary and ginger was unexpected and this may be due to the binding and screening effects of the anti-nutritional factors such as tannins present in the spices (**Akeem *et al*., 2016**). The ash content which is the measure of mineral elements ranged from 0.14–0.64% in black pepper and cloves spiced *kunun-aya*, respectively. Earlier researchers had reported comparable values (0.20–0.50%) for the ash content of tiger-nut milk (**Belewu and Belewu, 2007; Belewu and Abodunrin, 2008; Bristone *et al*., 2015**). With the exception of *kunun-aya* treated with cloves, the ash contents of the treated samples were lower than the control. It was observed that addition of spices generally increased the fat content of the samples though that of coriander spiced sample (0.36%) was not significantly different from the control (0.31%). Spices such as pepper, ginger and garlic had been reported to contain considerable amount of fat (**Otunola *et al.*, 2010**). Fat contributes substantially to the energy value of food (**Umar *et al*., 2006**). The crude fibre of *kunun-aya* increased with the addition of rosemary and cloves but decreased with the addition of cinnamon, ginger, coriander and black pepper. The crude fibre of the samples with the exception cinnamon and black pepper spiced *kunun-aya*, was significantly different ($p<0.05$) from one another. However, the value range (0.23–1.84%) was higher than 0.20% reported by **Belewu and Abodunrin (2008)** and this may be attributed to the addition of coconut milk whose fat content had been reported to be high, with oleic and lauric acids being the dominant fatty acids (Belewu and Belewu, 2007). Fibre increases faecal output (digestion), reduces the faecal pH, incidence of colon cancer, diabetes, heart diseases, obesity and certain degenerative diseases (**Cummings *et al.*, 1996; Ingabire and Vasanthakaalam, 2011**). Interestingly, the carbohydrate content of *kunun-aya* generally increased with the addition of individual spices which made most of the values higher than 1.31–2.48% and 5.81% reported by **Awonorin and Udeozor (2014)** and **Bristone *et al.* (2015)**, respectively. Carbohydrate serves as a primary source of energy in the diets (**Grieshaber, 2013**).

After five (5) days of storage, most of the nutritional properties of the samples reduced (Table 6). The moisture content of the kunun-aya spiced with cinnamon, black pepper and rosemary increased while that of ginger, cloves and coriander decreased. With the exception of the ginger and black pepper spiced *kunun-aya* which had comparable moisture content, significant difference existed among the moisture content of the stored samples. The protein increased in the control, rosemary and ginger spiced *kunun-aya* but decreased in cinnamon, cloves, coriander and black pepper spiced samples. The protein content of 0.72–1.03% reported by **Nwobosi *et al*. (2013)** after four days of storage was within 0.27–2.56% obtained in this study.

Table 5 Proximate composition of treated *kunun-aya* samples before storage

Samples	Moisture content (%)	Crude protein (%)	Total ash (%)	Crude fat (%)	Crude fibre (%)	Carbohydrate (%)
Control	95.44 ^{a} ±0.01	2.16 ^{c} ±0.01	0.54 ^{ab} ±0.01	0.31 ^{d} ±0.01	0.86 ^{c} ±0.01	0.69 ^{f} ±0.10
KCM	85.75 ^{e} ±0.05	2.59 ^{b} ±0.01	0.14 ^{d} ±0.01	0.76 ±0.01	0.23 ^{f} ±0.01	10.52 ^{b} ±0.01
KRM	90.95 ^{b} ±0.05	1.53 ^{d} ±0.00	0.34 ^{c} ±0.01	0.85 ^{a} ±0.05	1.84 ^{a} ±0.01	4.49 ^{e} ±0.00
KGG	88.55 ^{c} ±0.05	1.96 ^{c} ±0.00	0.45 ^{bc} ±0.05			
KCV	86.04 ^{d} ±0.01	2.16 ^{c} ±0.01	0.64 ^{a} ±0.01			
KCD	85.44 ^{f} ±0.01	2.16 ^{c} ±0.01	0.45 ^{bc} ±.05			
KBP	85.35 ^{f} ±0.05	4.06 ^{a} ±0.05	0.15 ^{d} ±0.05			

Values in the same column with different superscript are significantly different ($p<0.05$). KRM= *Kunun-aya* spiced with rosemary, KCD= *Kunun-aya* spiced with coriander, KCV= *Kunun-aya* spiced with cloves, KCM= *Kunun-aya* spiced with cinnamon, KGG= *Kunun-aya* spiced with ginger, KBP= *Kunun-aya* spiced with black pepper.

Table 6 Proximate composition of treated *kunun-aya* samples after storage

Samples	Moisture content (%)	Crude Protein (%)	Total ash (%)
Control	90.15 ^{c} ±0.05	2.56 ^{a} ±0.01	0.24 ^{c} ±0.01
KCM	98.24 ^{a} ±0.01	0.27 ^{f} ±0.00	0.09 ^{c} ±0.01
KRM	92.05 ^{b} ±0.05	1.69 ^{c} ±0.01	0.09 ^{c} ±0.01
KGG	88.44 ^{d} ±0.01	2.13 ^{b} ±0.01	0.09 ^{c} ±0.01
KCV	80.75 ^{f} ±0.05	1.25 ^{e} ±0.01	0.04 ^{c} ±0.01
KCD	79.50 ^{e} ±0.50	1.50 ^{d} ±0.00	0.09 ^{c} ±0.01
KBP	88.05 ^{d} ±0.50	2.56 ^{a} ±0.01	2.25 ^{a} ±0.05

Values in the same column with different superscript are significantly different ($p<0.05$). KRM= *Kunun-aya* spiced with rosemary, KCD= *Kunun-aya* spiced with coriander, KCV= *Kunun-aya* spiced with cloves, KCM= *Kunun-aya* spiced with cinnamon, KGG= *Kunun-aya* spiced with ginger, KBP= *Kunun-aya* spiced with black pepper.

The control and the black pepper spiced samples had the highest protein contents which were significantly different from other samples. The ash content of black pepper spiced *kunun-aya* was observed to increase and significantly different from other samples while that of other samples generally decreased and were not significantly different ($p<0.05$) from one another. The fat content of all the samples decreased after storage, though the control sample had the significantly lowest value (0.29%). This is similar to the report of **Nwobosi *et al*. (2013)** on the influence of pasturization, preservatives and storage conditions on tiger-nut milk. The results showed that the fibre and carbohydrate contents of the samples varied significantly ($p<0.05$) from one another after storage. It was also observed that the fibre content of ginger, cloves and coriander spiced samples increased while that of the control, cinnamon, rosemary and black pepper spiced samples decreased. Similarly, there was an increase in the carbohydrate content of the control, cloves and coriander spiced samples and a reduction in cinnamon, ginger and black pepper spiced samples. The increase in the nutritional profile of some of the samples during storage may be attributed to the concentration of nutrients due to reduction in moisture content, inhibition of anti-nutritional factors due to reduction in pH as the fermentation progressed or breakdown of anti-nutrients as a result of microbial activities.

Microbial loads of *kunun-aya* treated with different spices during storage

The effects of storage time (day) on the bacterial and fungal counts of *kunun-aya* treated with different spices are presented in Figures 2 and 3 respectively. The bacterial count of *kunun-aya* samples ranged from (1.1–5.4, 2.0–6.9, 3.6–9.8) $\times 10^4$ CFU/ml and fungal count from (1.0–6.4, 1.3–7.4, 5.2–9.5) $\times 10^4$ CFU/ml on days 1, 3 and 5, respectively. It was observed that the bacterial and fungal counts of the samples generally increased with the storage period. The ginger and rosemary spiced *kunun-aya* had lower bacterial count than the control while the coriander, ginger, rosemary and black pepper had lower fungal count than the control after the five (5) days storage period.

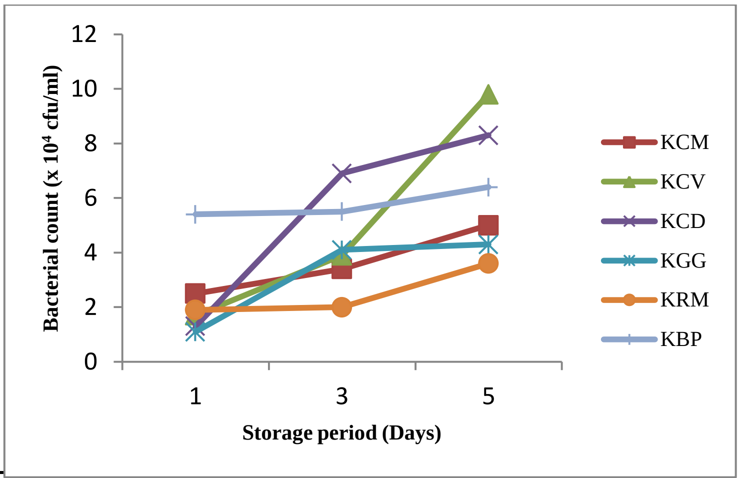

Figure 2 Bacterial count of *Kunun-aya* treated with spices during storage. KRM= *Kunun-aya* spiced with rosemary, KCD= *Kunun-aya* spiced with coriander, KCV= *Kunun-aya* spiced with cloves, KCM= *Kunun-aya* spiced with cinnamon, KGG= *Kunun-aya* spiced with ginger, KBP= *Kunun-aya* spiced with black pepper.

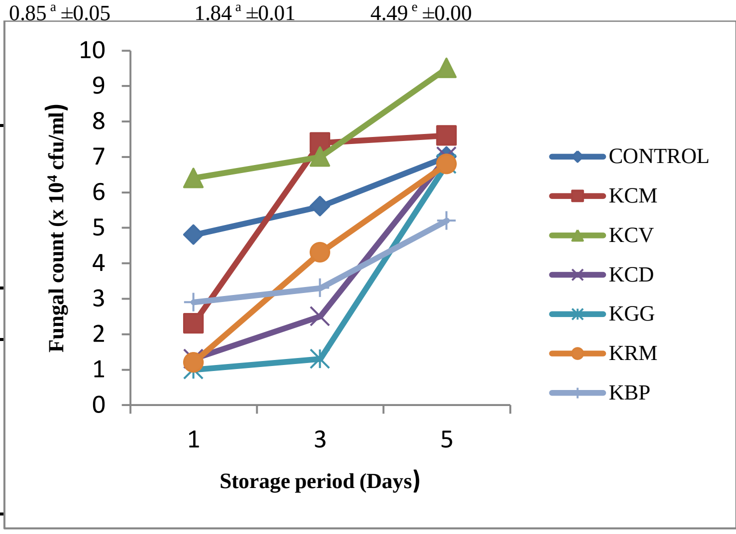

Figure 3 Fungal count of *Kunun-aya* treated with spices during storage. KRM= *Kunun-aya* spiced with rosemary, KCD= *Kunun-aya* spiced with coriander, KCV= *Kunun-aya* spiced with cloves, KCM= *Kunun-aya* spiced with cinnamon, KGG= *Kunun-aya* spiced with ginger, KBP= *Kunun-aya* spiced with black pepper.

The highest bacterial (9.8×10^4 CFU/ml) and fungal (9.5×10^4 CFU/ml) counts after the storage period were found in the cloves 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 (**Narang, 2004**). It had also been stated that milk sample containing 5.00 $x10^3$cfu/ml of bacteria is classified as good for consumption, 1.00 $x10^4$ to 4.00 $x10^5$ cfu/ml is fairly good and 2.00 $x10^6$ is passable, while over 2.00 x 10^7 cfu/ml is bad for consumption (**Ihekoronye and Ngoddy, 1985**). Hence, the *kunun-aya* samples were still fairly good for consumption and the storage could be extended beyond 5 days.

Sensory analysis of the *kunun-aya* samples

The effect of addition of spices on the sensory attributes of *kunun-aya* is shown in Table 7. There was significant difference *($p<0.05$)* between the organoleptic properties of the treated *kunun-aya* samples and the control with the control having the highest mean scores for taste, appearance, texture, aroma and overall acceptability. The low sensory mean scores of the treated samples may be due to the addition of the spice powders which might have caused a deviation in the sensory attributes that the panelists are used to. However, the results showed that the coriander and black pepper spiced samples were the most acceptable among the treated samples having no mean scores less than 6.0 (liked slightly). In terms of texture and aroma, no significant difference existed among the treated samples. Based on the results, the treated samples could be arranged in order of decreasing sensory acceptability as KBP>KCD>KGG>KRM>KCM>KCV.

Table 7 Mean sensory scores of *Kunun-aya* treated with spices

Samples	Taste	Appearance	Consistency	Aroma	Overall acceptability
Control	$8.27^{a} \pm 0.15$	$8.20^{a} \pm 0.18$	$8.00^{a} \pm 0.19$	$7.87^{a} \pm 0.17$	$8.53^{a} \pm 0.13$
KRM	$6.27^{b} \pm 0.25$	$6.00^{bc} \pm 0.22$	$5.93^{b} \pm 0.28$	$6.33^{b} \pm 0.23$	$6.13^{bc} \pm 0.35$
KCD	$6.5^{b}3 \pm 0.40$	$6.67^{b} \pm 0.23$	$6.47^{b} \pm 0.32$	$6.47^{b} \pm 0.32$	$6.67^{b} \pm 0.29$
KCV	$4.8^{c}0 \pm 0.50$	$5.67^{c} \pm 0.42$	$5.87^{b} \pm 0.41$	$5.67^{b} \pm 0.36$	$5.47^{c} \pm 0.36$
KCM	$5.67^{bc} \pm 0.44$	$6.07^{bc} \pm 0.21$	$6.27^{b} \pm 0.32$	$5.67^{b} \pm 0.32$	$6.13^{bc} \pm 0.26$
KGG	$5.9^{b}3 \pm 0.50$	$6.40^{bc} \pm 0.24$	$5.93^{b} \pm 0.21$	$6.27^{b} \pm 0.32$	$6.40^{b} \pm 0.21$
KBP	$6.67^{b} \pm 0.25$	$6.40^{bc} \pm 0.21$	$6.73^{b} \pm 0.32$	$6.00^{b} \pm 0.32$	$6.60^{b} \pm 0.31$

Values in the same column with different superscript are significantly different *($p<0.05$)*. KRM= *Kunun-aya* spiced with rosemary, KCD= *Kunun-aya* spiced with coriander, KCV= *Kunun-aya* spiced with cloves, KCM= *Kunun-aya* spiced with cinnamon, KGG= *Kunun-aya* spiced with ginger, KBP= *Kunun-aya* spiced with black pepper.

CONCLUSION

Tiger-nut milk (*kunun-aya*) with high nutritional value was produced from tiger-nut, coconut and date palm. The addition of spices as natural preservatives slowed down the multiplication of micro-organisms during the storage of *kunun-aya* at refrigerated condition and hence, made it consumable after the storage period (5 days). The amounts of spices added to the *kunun-aya* samples were not sufficient to completely inhibit microbial growth and retain the nutritional composition of *kunun-aya* during the storage period. For this reason, there is need for reformulation and standardization of the ingredients and natural preservatives used in the production of *kunun-aya* in order to retain or improve the quality during storage and also to encourage commercial scale production.

REFERENCES

Adejuyitan, J. A. (2011). Tiger-nut processing: its food uses and health benefits. *Am J Food Technol*, 6(3): 197–201. http://dx.doi.org/10.3923/ajft.2011.197.201.

Ahmed, I. S. A., Al-Gharibi, K. N., Daar, A. S. & Kabir, S. (1995). The composition and properties of date proteins. *Food Chemistry*, 53: 441-446. https://dx.doi.org/10.1016/0308-8146(95)99840-V.

Akeem, S., Joseph, J., Kayode, R. & Kolawole, F. (2016). Comparative phytochemical analysis and use of some Nigerian spices. *Croatian Journal of Food Technology, Biotechnology and Nutrition*, 11(3-4): 145-151.

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

Al-Shahib, W. & Marshall, R. J. (2003). The fruit of the date palm: its possible use as the best food for the future. *Int. J. Food. Sci. Nutr.*, 54: 247–259. https://dx.doi.org/10.1080/09637480120091982.

AOAC (1995). Official Methods of Analysis, (15th Edition) Washington DC, USA: Association of Official Analytical Chemists.

AOAC (2000). Official Methods of Analysis, (17th ed.) Washington, DC, USA: Association of Official Analytical Chemists International.

AOAC (2005). Official Methods of Analysis, (19th Edn.), Washington, DC, USA: Association of Official Analytical Chemists.

Arumughan, C., Balachanndran, C. & Sundaresan, A. (1993). Development of a process for coconut cream on a commercial scale. *Journal of Food Science and Technology*, 30(6): 408-412.

Awonorin, S. O & Udeozor, L. O (2014). Chemical Properties of Tiger nut-Soy Milk Extract. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, 8(3): 87-98.Belewu, M. A. & Belewu, K. Y. (2007). Comparative physico-chemical evaluation of tiger-nut, soybean and coconut milk sources. *Int. J. Agric. Biol.*, 9: 785- 787.

Belewu, M. A. & Abodunrin, O. A. (2008). Preparation of Kunun from unexploited rich food source: Tiger nut (*Cyperus esculentus*). *Pak. J. Nutr.*, 7(1), 109-111.

Bhattacharjee, S. & Sengupta, A. (2009). Spices in cancer Prevention: An overview. *Internet J. Nutr.Wellness*, 7(1).

Borchani, C. Besbes, S., Blecker, C., Masmoudi, M., Baati, R. & Attia, H. (2010). Chemical Properties of 11 Date Cultivars and Their Corresponding Fiber Extracts. *African Journal of Biotechnology*, 9(26): 4096- 4105.

Bosch, L., Alegría, A. & Farré, R. (2005). RP-HPLC determination of tiger nut and orgeat amino acid contents. *Food Sci. Tech. Int.*, 11(1): 33–40. http://dx.doi.org/10.1177/1082013205051266.

Bristone, C., Badau, M. H., Igwebuike, J. U. & Igwegb, A. O. (2015). Production and Evaluation of Yoghurt from Mixtures of Cow Milk, Milk Extract from Soybean and Tiger Nut. *World Journal of Dairy and Food Sciences*, 10(2): 159-169. http://dx.doi.org/10.5829/idosi.wjdfs.2015.10.2.94216.

Cummings, J. H., Beatty, E. R., Kingman, S. M., Bingham, S. A. & Englyst, H. N. (1996). Digestion and physiological properties of resistant starch in the human bowel. *Brit. J. Nutr.*, 75: 733.

David, F. (1986). Human nutritional dietary. Food Encyclopedia. 35-36.

Edeoga, H. O., Omosun, G. & Uche, L. C. (2006). Chemical Composition of *hyptis suaveolens* and *Ocimum gratissimum* hybrids from Nigeria. *Afr. J. Biotechnol.*, 5(10): 892-895.

Ene-Obong, H. N., Onuoha, N. O., Aburime, L. C. & Mbah, O. (2015). Nutrient composition, phytochemicals and antioxidant activities of some indigenous spices in Southern Nigeria. 11TH IFDC, Hyderabad, India. 1-31.

Gambo, A. & Da'u, A. (2014). Tiger nut (*Cyperus esculentus*): composition, products, uses and health benefits – a review. *Bayero Journal of Pure and Applied Sciences*, 7(1): 56 – 61. http://dx.doi.org/10.4314/bajopas.v7i1.11.

Grieshaber, M. (2013). Metabolic regulation of energy metabolism. *Exogenous and endogenous influences on metabolic and neural control*, 1: 225-242.

Harbone, J. B. (1973). Textbook of Phytochemical methods. London: Chapman and Hall, Ltd. 49-188.

Hasnaoui, A., Elhoumaizi, M. A., Hakkou, A., Wathelet, B. & Sindic, M. (2011). Physicochemical characterization, classification and quality evaluation of date palm fruits of some moroccan cultivars. *Journal of Scientific Research*, 3(10): 139-149. http://dx.doi.org/10.3329/jsr.v3i1.6062.

Chukwuma, E. R., Obiama, N. & Christopher, O. I. (2010). The phytochemical composition and some Biochemical effect of Nigerian Tiger nut (*Cyperus esculentus. L*) tuber. *Pakistan Journal of Nutrition*, 9(7): 709-715.

Ihekoronye, A. I. & Ngoddy, P. O. (1985). Integrated Food Science and Technology for the Tropics. London and Oxford: Macmillan Education Ltd. 105-112, 189-190, 343-351.

Ingabire, M. R. & Vasanthakaalam, H. (2011). Comparison of the nutrient composition of four sweet potato varieties cultivated in Rwanda. *Am. J. of Food and Nutr.*, 1(1): 34-38. http://dx.doi.10.5251/ajfn.2011.1.1.34.38.

Kwada, A. D. & Tella, I. O. (2009). Determination of infochemicals and the phytochemical screening of the foliage and stem bark of *Senna siamea (lam)* in Yola, Adamawa State. *J. Med. Plants Res.*, 3(9): 630- 634.

Mensah, J. K., Okoli, R. I., Turay A. A. & Ogie-Odia, E. A. (2009). Phytochemical analysis of medicinal plants used for the management of hypertension by Esan people of Edo State, Nigeria. *Ethnobot. Leaflets*, 13: 1273–1287.

Muhammad, N. O., Bamishaiye, E. I., Bamishaiye, O. M., Usman, L. A, Salawu, M. O., Nafiu, M. O. & Oloyede, O. B. (2011). Physicochemical Properties and Fatty Acid Composition of *Cyperus esculentus* (Tiger Nut) Tuber Oil. *Bioresearch Bulletin*, 5: 51- 54.

Musa, A. A. & Hamza, A. (2013). Comparative analysis of locally prepared "kunun aya" (Tiger nut milk) consumed by students of Kaduna state university, Kaduna, Nigeria. *Science World Journal*, 8: 13-18.

NAFDAC, (2002). Hand Book on Common Used Additive. African Publisher, Lagos, Nigeria.

Narang, S. P. (2004). Food Microbiology. New Delhi: A P H Publishing Corporation. pp.195.

Nwobosi, P. N. U., Isu, N. R. & Agarr, O. O. (2013). Influence of pasteurization and use of natural tropical preservatives on the quality attributes of tiger nut drink during storage. *International Journal of Food and Nutrition Science*, 2: 27-32.

Otunola, G. A., Oloyede, O. B., Oladiji, A. T. & Afolayan, A. J. (2010). Comparative analysis of the chemical composition of three spices – *Allium sativum L. Zingiber officinale Rosc*. and *Capsicum frutescens L*. Commonly Consumed in Nigeria. *Afri. J. Biotechnol.*, 41: 6927-6931.

Owoso, O. F. & Ogunmoyela, O. A. (2001). Chemical analysis of foods; An outline. Ibadan -Nigeria: Concept publications. pp. 24-25, 55.

Philippine Coconut Authority, (2014). Coconut milk. Philippine: http://www.pca.da.gov.ph/pdf/techno/coconut_milk.pdf.

Sofowora, E. A. (1993). Medicinal plants and traditional medicine in Africa. Ibadan: Spectrum Books Ltd. pp. 55–71.

Srinivasan, K. (2005). Spices as influencers of body metabolism: an overview of three decades of research. *Food Research International*, 38, 77-86. http://dx.doi.org/10.1016/j.foodres.2004.09.001.

Suekawa, M., Ishigie, A., Yusas, K., Sudo, K., Aburada, M. & Hosoya, E. (1984). Pharmacological studies on ginger. 1. Pharmacological actions of pungent constituents, (6)-gingerol and (6)-shogaol. *Pharmacol-Biol. Dyn*., 7(11): 836-848.

Trease, G. E. & Evans, W. C. (2002b). Phytochemicals. In: Pharmacognosy. 15th ed. Saunders Publishers, London. 42-44, 221- 229, 246- 249, 304-306,331-332, 391-393.

Ukwuru, M. U. & Ogbodo, A. C. (2011). Effect of processing treatment on the quality of tiger nut milk. *Pak. J. Nutr.,* 10: 95-100. http://dx.doi.org/10.3923/pjn.2011.95.100.

Umar, K. J., Hassan, L. G. & Garba, H. L. (2006). Nutritive value of some indigenous spices: Cloves (*Eugenia caryophyllus*) and African black pepper (*piper guineense*). *Chemclass Journal*, 3: 81-84.