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PRESERVATIVE EFFECTS OF GINGER (*Zingiber officinale*), TUMERIC (*Curcuma longa*) EXTRACT AND CITRIC ACID AND PASTEURIZATION ON THE NUTRITIONAL QUALITY AND SHELF LIFE OF TIGER-NUT NON-DAIRY MILK

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ABSTRACT

Tiger nut (*Cyperus esculentus*) non-dairy milk is a plant milk and a rich source of nutrients. However, milk from animal or plant is highly perishable. The focus of this study was to evaluate the influence of inclusion of preservatives and pasteurization on the quality of tiger nut milk. Five samples were produced: control (0 % preservative); natural preservatives (ginger or tumeric extract); combination of ginger and turmeric and manufactured citric acid. Samples were stored at room and refrigeration temperatures and evaluated for 30 days. Analyses performed include physico-chemical and nutritional composition, microbial load and general acceptability of tiger nut non-dairy milk samples using standard methods. Tiger nut non-dairy milk without preservative was generally accepted, while milk with ginger extract scored higher in appearance. Values ranged from (5.9 - 6.1), (5.2 - 6.8), (4.8 - 6.5); and (0.05 - 0.17 %); (0.04 – 0.15%); (0.05 – 0.17 %) for 0; 15 and 30 days respectively for pH and total titratable acidity. Total viable counts were (<1 to $5.9\pm1.8^a x10^6$ CFU/mL); enterobacteriaceae, (<1 to $5.4\pm0.7^a x10^6$ CFU/mL), Staphylococcal (3.3 ± 1.0^d $x10^5$ to $4.2\pm0.9^b x10^6$ CFU/mL) and fungal (<1 to $4.5\pm0.5^a x10^6$ CFU/mL). Moisture content (85.6 to 87.8 %), protein (3.7 - 4.3 %), ash (0.7– 1.8 %), crude fat (3.5 – 4.57 %). Sodium and potassium content ranged from (178.2 - 431.9 mg/kg) and (52.5– 416.2 mg/kg) respectively. The L* value ranged from (75.2–79.7). Conclusively, tiger nut non-dairy milk with natural preservative was as effective as manufactured citric acid in reducing the microbial content and maintaining the nutrients.

Contribution/Originality: This study contributes to the existing literature to the versatility of underutilized Tigernut. This crop can be converted to several food products including non-dairy milk. Natural preservative extracts were effective in preserving this nutritious plant milk. Preserving plant milk with turmeric extract is a novel idea to our knowledge.

1. INTRODUCTION

Milk is generally considered to be from an animal source. Although it is highly perishable, it contains many nutrients essential for the proper development and maintenance of the human body (Wattiaux, 1994). Its exceptional nutrient profile includes water, protein, fat, carbohydrates, cholesterol, minerals, vitamins and energy. Consumption of at least three servings of milk or milk products daily can have a positive impact on health and prevention of such diseases as osteoporosis, colon cancer, diabetes and help with weight management (Davoodi, Esmaeili, & Mortazavian, 2013). Recently however, animal milk has generated some controversies due to the presence of high milk fat and it's not so positive impact on health (De Oliveira Otto et al., 2018).

Plant milk has been proposed as substitute for animal milks in diets to combat coronary heart and cardiovascular diseases as well as malnutrition in poor regions of the world where animal milks are scarce and not affordable (Nsofor & Maduako, 1992; Nsofor. & Osuji, 1997). Soybean is commonly made into milk in developed countries. Recently, tiger nut is also being explored for plant milk in Africa and other developing economies. These plant milks have been documented to be highly nutritious, beneficial to health and cheap (Anderson, Johnstone, & Cook-Newell, 1995; Santo et al., 2010) considering the present dietary trend, which is geared towards low cholesterol and low saturated fatty diets (WHO/FAO, 2002).

Tiger nut (*Cyperus esculentus*) is an underutilized tuber of family Cyperaceae, which produces rhizomes from the base of the tuber that is somewhat spherical (De Vries, 1991). Tiger-nut has been reported as very healthy as it helps in preventing heart attacks, thrombosis, activates blood circulation and prevents cancer especially of the colon due to high content of soluble glucose (Osagie & Eka, 1998). Furthermore, tiger-nut was equally reported to have positive effect on cholesterol level due to high content of vitamin E. Consumption can benefit diabetic patients and those seeking to reduce cholesterol or lose weight (Chukwuma, Obioma, & Christopher, 2010; Yeboah, Mitei, Ngila, Wessjohann, & Schmidt, 2011). The high fibre content of the nut combined with a delicious taste, make them ideal for healthy eating (Osagie & Eka, 1998). The nut is also rich in minerals (phosphorus, potassium) and vitamins C and ideal for consumption for children, older persons and sportsmen (Martinez, 2003).

Tiger nut can be consumed raw, roasted, dried, baked or made into other food by products such as flour and yoghurt(Ajayi & Adisa, 2019; Oladele & Aina, 2007). It can also be used as flavouring agent for ice cream and biscuit (Cantalejo, 1997). There has not been a report of food allergies for tiger nut milk unlike soybean, peanut or nuts to our knowledge.

Preservatives are often used in foods generally to increase the shelf life and make the foods stable over a period of time in different quantity and concentrations. Preservatives can be natural or synthetic in nature. Among the currently used natural preservatives are sodium chloride (common salt) and spices such as ginger.

Traditionally, food preservation has three goals; the preservation of appearance, nutritional characteristics, and a prolongation of the time that the food can be stored. Hence, food preservatives can be defined as the "food additives used to inhibit the growth of microorganisms and prevent spoilage by different anti-oxidative reactions in order to maintain the quality, texture, consistency, taste, color, alkalinity or acidity" (Tuormaa, 1994; WHO, 1987).

Stabilizers, also called thickeners, gelling agents or hydrocolloids, can be obtained from different sources including animal connective tissues, sea and land plants and microorganisms (Imeson, 2010). Different stabilizers are used to overcome the problem of syneresis and to create desired texture and stability during processing and storage of milk and milk products.

Non- dairy milk made from tiger nut is prone to spoilage like cow milk after few hours of production unless preservatives are used. Since tiger nut milk is nutrient dense, healthy for individuals, particularly those that are lactose intolerant and has not been reported to elicit allergic reaction, improving the shelf life should be explored. Therefore, the objective of this study was to produce tiger nut non-dairy milk preserved with ginger or turmeric extracts and citric acid as natural and synthetic preservatives and compare the nutritional composition of the resulting non-dairy milk.

2. MATERIALS AND METHODS

2.1. Materials

Dried tiger nut (*Cyperus esculentus*), fresh ginger (*Zingiber officinale*) and tumeric (*Curcuma longa*) rhizomes, manufactured citric acid and gelatin were obtained from Kuto market in Abeokuta, Ogun State, Nigeria and immediately transported at ambient temperature to Food Science Laboratory at Bowen University.

2.2. Methods

2.2.1. Preparation of Aqueous Extracts of Ginger

About 1.2 Kg of fresh ginger rhizomes were sorted, washed, peeled and air-dried for 72 h. The dried ginger rhizomes were milled into a fine powder in an electric blender (MasterChef Crown star, Australia) and sieved. Then approximately 40 g of the powdered ginger was soaked in 200 mL of warm distilled water for 48 h at room temperature in a beaker and shaken vigorously at regular intervals according to Borode (2017). The extract obtained was filtered using muslin cloth to remove the residue and the filtrate was evaporated in a water-bath set at 40 ^{0}C. The resultant extract was used as natural preservatives in the tiger nut non-dairy milk samples.

2.3. Preparation of Aqueous Extracts of Turmeric

About 1 Kg of fresh turmeric rhizomes were sorted, washed, peeled and air-dried for 72 h and the dried turmeric was milled into a fine powder in an electric blender and sieved (MasterChef Crown star, Australia), following the protocol previously described for ginger.

2.4. Preparation of Tiger Nut Non-Dairy Milk

Tiger nuts (2.5 Kg) were sorted to remove dirt particles and spoilt nuts, washed with 40% alcohol to minimize contamination and prevent cell shrinking and then rinsed with sterile water (Nwobosi, Isu, & Agarry, 2013). The nuts were then soaked in 3,500 mL of water at ambient temperature for 12 h in order to soften the seeds. Soaked tiger nut was blanched at 70 ^{0}C for 5 min to inactivate enzymes that can cause clumping after extraction (Nwobosi et al., 2013). Then 2.5 Kg of the nuts was wet milled with 3.5 L of potable water and blended several times with a grinder (MasterChef Crown star, Australia), sieved with a muslin cloth and further sieved with about 4.0 L of water (1:3 tiger nut:water (w/v). Then, tiger nut non-dairy milk was stabilized with 15 g of gelatin to prevent separation (NAFDAC, 2002). Afterwards, the resulting tiger nut non-dairy milk was divided into the five (1.5 L) treatments.

Sample ABC: No (0 %) preservative added (Control). The non-dairy milk was divided into 6 PET bottles of 250 mL each.

Sample MAC: About 1.5 g of the ginger extracts was dissolved into 20 mL (w/v) of hot sterile water placed in a boiling water bath and stirred continuously until the extract is completely dissolved according to Borode (2017) with slight modification. The solution was then added to 250 mL of the tiger nut milk in a PET bottle, shaken vigorously before storage. There were 6 PET bottles as previously described.

Sample T34: Same process was followed for turmeric extract as with ginger extract.

Sample XYZ: Approximately 0.75 g each of ginger and tumeric extracts was used and similar process was followed as with other natural preservative.

PCB: About 18 mg of citric acid dissolved in 20 mL of warm water (w/v), and added to 250 mL of tiger nut milk per PET bottle as earlier described. There is a slight modification in the amount of manufactured citric acid used compared to the allowable recommended standard of 540mg/l (NAFDAC, 2002) based on recent toxicology findings of Sweis and Cressey (2018) that concluded that addition of manufactured citric acid in foods plays a potential role in eliciting inflammatory reactions in some consumers.

After filling the PET bottles with the various formulated tiger nut non-dairy milk, the samples were pasteurized immediately by immersing them into a water bath already set at 80 °C for 7 minutes according to Nwobosi et al. (2013). Then the samples were divided into two batches. One batch of sample was stored at room (32±2°C) and the other at refrigeration (4±2°C) temperature.

2.5. Sensory Evaluation Test

All samples were coded and served at room temperatures using clear plastic cups. The panelists were untrained prior to testing, but were instructed to score the tiger nut non-dairy milk samples based on a 5-point Hedonic scale, where 1 = dislike extremely, 2 = dislike moderately, 3 = neither like nor dislike, 4 = like moderately and 5 = like very much (Meilgaard, Carr, & Civille, 1991). The attributes assessed include appearance, taste, flavour, consistency and overall acceptability. Water and crackers were available for panel members to rinse their palates between samples, in order to prevent carry over effect and aid in removing flavour between tastes.

2.6. pH, Total Titratable Acidity (TTA) and Total Soluble Solids (TSS)

Previously calibrated digital pH meter (PHS-98108, Hanna) was used to determine the pH of the tiger nut non-dairy milk samples. The total titratable acidity (TTA) and total soluble solids (TSS) of the tiger nut non-dairy milk samples were determined using (AOAC, 2005) method. Readings for pH, TTA and TSS were taken in triplicates at weeks 0; 2; and 4 during product storage. TTA was calculated as lactic acid and expressed as percentage.

2.7. Microbiological Analyses

The microbiological analyses of tiger nut non-dairy milk samples were assessed for total viable, Staphylococcal, Enterobacteriaceae and fungal counts at weeks 0; 2; and 4 of storage as described by methods for the microbiological examination of foods. Briefly, about 1 mL of each milk sample was measured into 9 mL of peptone water and further serially diluted up to 10^{-5}. Using pour plate method, 0.5 mL of the last dilution was plated in duplicates using plate count agar (PCA), Mannitol salt agar (MSA), McConkey agar and Sabouraud dextrose agar (SDA) (Park scientific London, UK). Plates were incubated at 37 ^{0}C overnight for bacteria and 28 ^{0}C for 72 h for fungi and enumerated (American Public Health Association, 1992). The result was expressed as colony forming unit per ml (CFU/mL).

2.8. Proximate Composition of Tiger Nut non-dairy Milk Samples

Moisture, protein, ash and fibre contents were determined as described by (AOAC, 2005). Fat content was determined using Rose Gottlieb method as described by (AOAC, 2005). The carbohydrate content was determined by difference and energy was calculated using Atwater conversion factors in Kcal (4 Kcal, 4 Kcal, and 9 Kcal), for protein, carbohydrate and fat respectively.

2.9. Mineral Determination

The minerals analyzed were calcium, potassium, sodium and phosphorus. Mineral content was determined by (AOAC, 2005) method. The elements, calcium (Ca) and magnesium (Mg) were determined using Atomic Absorption Spectrometer (PG 990, United Kingdom) at wavelength of 422.7 nm and 285.2 nm respectively, and sodium (Na) and potassium (K) were determined by flame photometry method using a flame photometer (Jenway PFP7, United Kingdom) at wavelength of 589.0nm and 766.4nm respectively.

2.10. Physical Analysis (Colour Determination)

The colour of the tiger nut non-dairy milk samples was assessed by the method described by Hongbete, Mestres, Akissoe, and Nago (2009). The Commission Internationale de l' Eclairage (CIE) tristimulus L* a* b* parameters were determined using a colour meter CR-410 (Konica Minolta, Inc., Japan). The Hunter Lab colour coordinates system, L*(Lightness) a*(redness), b*(yellowness) values were recorded.

3. RESULTS AND DISCUSSION

3.1. Sensory Evaluation

The result for the sensory evaluation conducted is presented in Figure 1. For appearance scores ranged from (2.5 ±0.94 to 4.2 ±0.48); aroma (2.3 ± 0.85 to 3.4±0.82); taste (2.3 ±0.73 to 3.3 ±0.63); consistency (2.5 ±0.94 to 3.7±0.93); and general acceptability (2.6 ±0.75 to 3.4 ± 0.75). For appearance, MAC (tiger nut non-dairy milk with ginger extract) had the highest score (4.2 ±0.48). ABC (tiger nut non-dairy milk without preservative), MAC and PCB (tiger nut non-dairy milk with citric acid) were scored higher for consistency. ABC and MAC scored higher for aroma, while PCB scored (3.3±0.63) for taste. For general acceptability, ABC had (3.4± 0.75) and slightly higher than MAC. Tiger nut non-dairy milk with turmeric (T34) and combination of ginger and turmeric (XYZ) extracts consistently scored lower than other samples. There were significant differences between all samples.

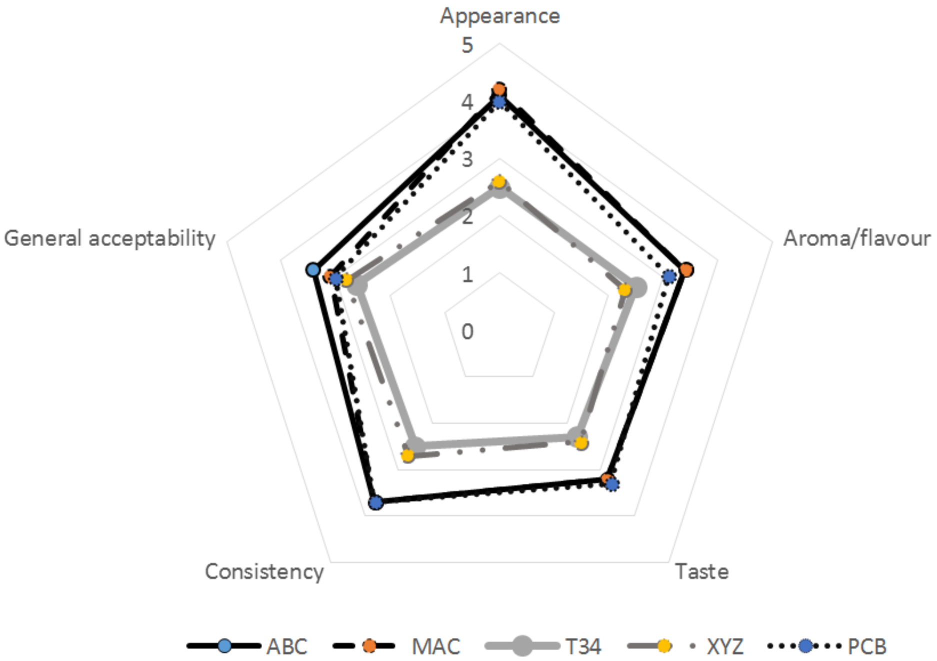

Figure-1. Sensory attributes of tiger nut non-dairy milk samples with preservatives. ABC= Tiger nut non-dairy milk without preservative (control); MAC= Tiger nut non-dairy milk with ginger extract; T34= Tiger nut non-dairy milk with turmeric extract; XYZ=Tiger nut non-dairy milk with combination of ginger and turmeric extracts; PCB = Tiger nut non-dairy milk with citric acid.

3.2. Physico-Chemical Properties and Microbial load (CFU/mL) of Tiger Nut Non-Dairy Milk

The pH of samples on day of production (day 0), ranged from (5.9 to 6.1), with tiger nut non-dairy milk without preservative (ABC) slightly higher than other samples Table 1. After two weeks of storage, the pH ranged from (5.4 to 6.1) and (5.2 to 6.8) for ambient and refrigeration temperatures of storage respectively. Samples with citric acid were observed to be lower in pH (5.4) and (5.2) for room and refrigeration temperature storage respectively. Similar trend as with 2 weeks of storage was also found after four weeks of storage. During storage, the pH of the samples further reduced particularly the samples stored at room (32±2 ^{0}C) compared to samples kept at refrigeration (4±2 ^{0}C) temperatures. As temperature increases, molecular vibrations also increase, improving the ability of water to dissociate thereby dropping the pH. The results in this study contradicts the pattern of pH observed by Nwobosi et al. (2013), in that the refrigerated samples consistently show higher pH value than the samples stored at room temperature. Also, in Table 1, initial total titratable acidity (TTA) ranged from (0.05 to 0.17 %) with tiger nut non-dairy milk with combination of turmeric and ginger extracts (XYZ) having the lowest value. After two weeks, TTA values for tiger nut non-dairy milk samples ranged from (0.10 to 0.15 %) for room temperature and (0.04 to 0.13 %) for refrigeration temperature storage. In addition, it was observed that after 2 weeks of storage, the refrigerated samples had slightly lower TTA compared to room temperature. After four weeks TTA ranged (0.09 to 0.17 %) and

(0.05 to 0.13 %) for room and refrigeration temperatures respectively. Similar trend was observed for TTA values in refrigerated samples as in 2 weeks storage. Degree Brix ranged from (12.7 to 14.7 ^{0}Brix) on the initial day, with non-dairy milk with turmeric extract (T34) having the highest value Table 1. It was observed that on the second week of storage, samples MAC and XYZ had the highest TSS values (8.3 ^{0}Brix) (14.7 ^{0}Brix) for room and refrigeration temperature respectively. Non-dairy milk stored at refrigeration was observed higher total soluble solid and the results obtained corroborated with that reported by Nwobosi et al. (2013).

3.3. Microbial Load

The result of the microbial load for the milk samples are presented in Table 1. On the day of production, for total viable ranged from (<1 to $4.5x10^{6}$ CFU/mL); Enterobacteriaceae, the count ranged from (<1 to 4.8×10^{6} CFU/mL); Staphylococcus ranged from ($3.3x10^{5}$ to $4.2x10^{6}$ CFU/mL); and fungal (<1 to $9.0x10^{5}$ CFU/mL). The source of microorganisms could be soil and dirt from the harvesting environment and partly inadequate washing before milling.

Table-1. Physico-chemical properties and microbial load (CFU/mL) of tiger nut non-dairy milk with preservatives during storage.

Samples	Physico- pH	Chemical[1] TTA	TSS	Microbial TVC	Load (CFU/mL) * Enterobacteriaceae	Staphylococcus	Fungal
Day 0							
ABC	6.1 ± 0.02^{a}	0.16 ± 0.00^{b}	13.0 ± 1.0	$4.5\pm5.7^{a}x10^{6}$	$4.8\pm2.1^{a}x10^{6}$	$4.2\pm1.1^{a}x10^{6}$	$9.0\pm0.1^{a}x10^{5}$
MAC	5.9 ± 0.01^{b}	0.17 ± 0.00^{a}	14.3 ± 1.5	<1	<1	$2.4\pm0.7^{b}x10^{6}$	<1
T34	6.0 ± 0.01^{a}	0.06 ± 0.00^{d}	14.7 ± 1.2	<1	$3.5\pm1.4^{b}x10^{6}$	$6.3\pm1.8^{d}x10^{5}$	$2.0\pm0.3^{b}x10^{5}$
XYZ	5.9 ± 0.02^{b}	0.05 ± 0.01^{d}	12.7 ± 1.2	$1.3\pm2.1^{b}x10^{6}$	<1	$2.5\pm0.7^{b}x10^{6}$	$5.5\pm0.6^{b}x10^{5}$
PCB	6.0 ± 0.02^{b}	0.14 ± 0.00^{c}	13.0 ± 1.0	<1	$3.5\pm2.1^{b}x10^{6}$	$3.3\pm1.0^{d}x10^{5}$	$1.0\pm1.4^{b}x10^{5}$
2 weeks @ 32 ± 2^{0}C							
ABC	5.9 ± 0.02^{b}	0.11 ± 0.00^{b}	7.3 ± 1.1	$5.6\pm1.3^{a}x10^{6}$	$5.4\pm0.7^{a}x10^{6}$	$3.3\pm2.1^{b}x10^{6}$	$4.3\pm1.1^{a}x10^{6}$
MAC	6.1 ± 0.02^{a}	0.15 ± 0.02^{a}	7.7 ± 0.6	$4.3\pm3.5^{b}x10^{6}$	$1.5\pm1.6^{b}x10^{6}$	$2.6\pm1.4x10^{6}$	$3.1\pm0.7^{b}x10^{6}$
T34	5.9 ± 0.04^{b}	0.10 ± 0.00^{b}	7.3 ± 1.5	$4.3\pm1.4^{b}x10^{6}$	$2.8\pm1.8^{b}x10^{6}$	$4.7\pm1.0^{a}x10^{6}$	$1.7\pm1.1^{d}x10^{6}$
XYZ	5.5 ± 0.03^{d}	0.13 ± 0.01^{a}	8.3 ± 1.5	$4.6\pm1.4^{b}x10^{6}$	$1.8\pm0.7^{b}x10^{6}$	$1.5\pm0.7^{c}x10^{6}$	$2.8\pm1.0^{c}x10^{6}$
PCB	5.4 ± 0.02^{e}	0.13 ± 0.01^{a}	7.6 ± 0.6	$4.8\pm1.4^{b}x10^{6}$	$2.8\pm0.6^{b}x10^{6}$	$4.2\pm0.9^{b}x10^{6}$	$2.7\pm0.7^{c}x10^{6}$
2 weeks @ 4 ± 2^{0}C							
ABC	6.3 ± 0.07^{b}	0.11 ± 0.01^{b}	11.0 ± 1.7^{b}	$5.3\pm0.2^{a}x10^{6}$	$2.8\pm1.4^{b}x10^{6}$	$1.4\pm0.6^{b}x10^{6}$	$3.2\pm1.8^{a}x10^{6}$
MAC	6.8 ± 0.02^{a}	0.13 ± 0.00^{a}	14.7 ± 2.3^{a}	$2.6\pm1.8^{c}x10^{6}$	$4.3\pm2.1^{a}x10^{6}$	$1.3\pm1.0^{b}x10^{6}$	$1.6\pm0.8^{c}x10^{6}$
T34	6.8 ± 0.09^{a}	0.06 ± 0.00^{c}	12.7 ± 1.2^{ab}	$2.7\pm0.4^{c}x10^{6}$	$2.3\pm1.8x10^{6}$	$2.5\pm0.7^{a}x10^{6}$	$1.1\pm0.8^{d}x10^{6}$
XYZ	5.6 ± 0.07^{c}	0.04 ± 0.00^{d}	10.7 ± 1.2^{b}	$4.2\pm0.7^{b}x10^{6}$	$1.1\pm1.4^{c}x10^{6}$	$1.2\pm0.7^{b}x10^{6}$	$2.1\pm0.9^{b}x10^{6}$
PCB	5.2 ± 0.03^{d}	0.12 ± 0.01^{b}	12.7 ± 1.5^{ab}	$4.3\pm0.7^{b}x10^{6}$	$2.6\pm1.4^{b}x10^{6}$	$3.4\pm3.6^{a}x10^{6}$	$1.1\pm1.7^{d}x10^{6}$
4 weeks @ 32 ± 2^{0}C							
ABC	4.8 ± 0.02^{b}	0.09 ± 0.00^{d}	5.7 ± 1.2^{bc}	$5.1\pm1.4^{b}x10^{6}$	$5.3\pm1.4^{a}x10^{6}$	$1.1\pm0.4^{d}x10^{6}$	$4.5\pm0.5^{a}x10^{6}$
MAC	5.9 ± 0.34^{a}	0.13 ± 0.01^{b}	7.8 ± 1.3^{a}	$5.2\pm2.1^{b}x10^{6}$	<1	$1.9\pm0.8^{b}x10^{6}$	$3.2\pm0.4^{b}x10^{6}$
T34	5.7 ± 0.02^{a}	0.12 ± 0.01^{c}	4.7 ± 0.6^{c}	$5.9\pm1.8^{a}x10^{6}$	$2.0\pm2.8^{b}x10^{5}$	$2.6\pm1.4^{a}x10^{6}$	$1.9\pm0.7^{d}x10^{6}$
XYZ	5.8 ± 0.02^{a}	0.17 ± 0.00^{a}	7.0 ± 1.0^{ab}	$4.1\pm1.8^{c}x10^{6}$	<1	$2.1\pm0.7^{b}x10^{6}$	$3.1\pm1.0^{b}x10^{6}$
PCB	4.9 ± 0.2^{b}	0.14 ± 0.02^{b}	6.0 ± 0.0^{b}	$5.0\pm0.7^{b}x10^{6}$	<1	$1.6\pm1.6^{c}x10^{6}$	$2.8\pm0.7^{c}x10^{6}$
4 weeks @ 4 ± 2^{0}C							
ABC	5.8 ± 0.02^{b}	0.10 ± 0.00^{c}	7.3 ± 1.2^{ab}	$4.1\pm1.4^{b}x10^{6}$	$3.6\pm1.7^{a}x10^{6}$	$9.3\pm2.5^{b}x10^{5}$	$3.6\pm0.7^{a}x10^{6}$
MAC	6.5 ± 0.04^{a}	0.11 ± 0.00^{b}	7.5 ± 2.1^{ab}	$4.8\pm0.7^{a}x10^{6}$	<1	$1.1\pm0.5^{b}x10^{6}$	$2.3\pm0.6^{c}x10^{6}$
T34	6.0 ± 0.05^{b}	0.10 ± 0.01^{c}	8.7 ± 0.6^{a}	$4.1\pm1.4^{b}x10^{6}$	<1	$1.6\pm0.6^{a}x10^{6}$	$1.4\pm1.0^{d}x10^{6}$
XYZ	6.5 ± 0.40^{a}	0.05 ± 0.00^{d}	8.3 ± 0.6^{a}	$3.1\pm1.4^{c}x10^{6}$	<1	$1.7\pm0.7^{a}x10^{6}$	$3.1\pm1.8^{b}x10^{6}$
PCB	4.8 ± 0.06^{c}	0.13 ± 0.01^{a}	5.7 ± 0.6^{b}	$4.6\pm0.7^{a}x10^{6}$	<1	$8.8\pm1.8^{b}x10^{5}$	$1.3\pm0.7^{d}x10^{6}$

Note: Physico-chemical[1] = Mean of triplicate; Microbial load * = Mean of duplicates; CFU=Colony Forming Unit; ABC= Tiger nut non-diary milk control; T34= Tiger nut non-dairy milk with tumeric; MAC=Tiger nut non-dairy milk with ginger; XYZ=Tiger nut non-dairy milk with combination of ginger and turmeric extract; PCB = Tiger nut non-dairy milk with citric acid.

After the non-dairy samples had been in storage for two weeks, in both ambient and refrigeration temperatures, all of the samples had growth. Total viable ranged from (2.6 to 5.6 $\times10^{6}$ CFU/mL), Enterobacteriaceae count ranged from (1.1 to 5.4 × 10^6 CFU/mL), Staphylococcus ranged from (1.2 to 4.7×10^6 CFU/mL); and fungal (1.1 to 4.3×10^6 CFU/mL). After four weeks of storage in ambient and refrigeration temperatures, tiger nut non-dairy milk with ginger extract, citric acid and combination of turmeric and ginger extracts preservatives were effective against Enterobacteriaceae.

After two and four weeks, it was observed that the microbial load for all samples were higher at room temperature than at refrigeration temperature because the activity of microbes were reduced during storage at refrigeration temperature.

3.4. Proximate Composition of Tiger Nut Non-Dairy Milk

The result of the proximate composition of the tiger nut non-dairy milk samples are presented in Table 2. There were no significant differences ($p < 0.05$) in moisture and protein contents, but significant differences were observed in ash, fat and carbohydrate contents of the samples. There was no detectable fiber content in all samples. Moisture content ranged from (85.6±1.9 to 87.8±1.9 %). Protein content ranged from (3.7±0.4 to 4.3±0.5 %). Ash content ranged from (0.7± 0.9 to 1.8± 0.4 %). Crude fat content ranged from (3.5±0.5 to 4.5± 0.5 %) and the carbohydrate content ranged from (2.2 ± 1.8 to 5.7± 1.3 %). The moisture content is within the range reported by Ezeonu, Tatah, Nwokwu, and Jackson (2016), for tiger nut milk. The crude protein values are also within the range of tiger nut milk (3.0 ± 0.1 to 5.5 ± 0.5 %) reported by Adgidzi and Abu (2010). The protein content of tiger nut seed is about (8 %) according to Adejuyitan (2011) hence reduction in the protein content of the milk could be attributable to processing. It was observed that the addition of the various preservatives did not have detrimental effect on the nutritional composition of the tiger nut non-dairy milk samples.

Table-2. Nutritional Composition of Tiger nut milk with preservatives.

Nutritional Composition (%)							
Sample	**Moisture**	**Protein**	**Crude fat**	**Ash**	**Crude Fibre**	**CHO**	**Energy (Kcal)**
ABC	86.7±0.0^a	3.7±0.4^a	4.2±0.8^a	0.7±0.9^c	ND	4.7±1.0^b	71.8
MAC	85.6±1.9^a	3.9±0.0^a	4.0±0.2^a	1.5±0.4^b	ND	5.1±2.3^a	72
T34	86.7±3.3^a	3.7±0.4^a	3.8±0.3^b	1.1±0.2^c	ND	5.7±1.3^a	72
XYZ	86.7±0.0^a	4.2±0.8^a	3.5±0.5^b	1.0±0.3^c	ND	4.6±0.7^b	66.7
PCB	87.8±1.9^a	4.3±0.5^a	4.5±0.5^a	1.8±0.4^a	ND	2.2±1.8^c	66.5

Note: Values are mean ± SD of triplicate; Duncan separation of means with same alphabets are not different ($p<0.05$) in each column; ABC= Tiger nut non-dairy milk (control); MAC= Tiger nut non-dairy milk with ginger extract; T34= Tiger nut non-dairy milk with turmeric extract; XYZ=Tiger nut non-dairy milk with combination of ginger and turmeric extracts; PCB = Tiger nut non-dairy milk with citric acid.

3.5. Mineral Composition

The result of the mineral composition of the samples are presented in Table 3. Calcium content ranged from (ND to 278.4 ± 0.00 mg/kg). Sodium content ranged from (178.2 ± 0.00 to 431.9 ± 0.00 mg/kg). Potassium content ranged from (52.5 ± 0.00 to 416.2 ±0.00 mg/kg) and magnesium content ranged from (108.5 ± 0.00 to 169.6 ± 0.00 mg/kg). Tiger nut non-dairy milk with turmeric extract (T34) was observed to have the lowest values in the four minerals analyzed. Although it has been documented that *Curcuma longa* and *Zingiber officinale* have similar levels for sodium, potassium, calcium and potassium (Taoheed et al., 2017) it is unclear why these minerals are significantly reduced in the milk with turmeric extract.

3.6. Colour Composition of the Samples

The mean values for the colour intensity of the tiger nut non-dairy milk samples are presented in Table 4. There were significant statistical differences ($p<0.05$) in the colour composition of all the samples except the a* value. The addition of turmeric extract increased the yellowness in T34 and XYZ samples. The L* values ranged from (75.2± 0.1 to 79.7 ± 0.1) for all samples. The a* value ranged from (-0.6± 0.09 to -4.1±0.02) and b* value ranged from (15.4±1.6 to 32.7±0.08). The lowest L* value was that of sample T34 that is because of the addition of turmeric in the milk sample. According to Surojanametakul, Satmalee, Saengprakai, Siliwan, and Wattanasiritham (2010), addition of turmeric extract increases the curcuminoid content as well as redness (a*) and yellowness (b*) values, while the lightness (L*) value tend to decrease. Although redness of tiger nut non-dairy milk was negative as indicated by the a* value, the colour should not negatively affect the food product it will be included (Sánchez-Zapata et al., 2012).

Table-3. Mineral content (mg/kg) of the tiger nut non-dairy milk samples.

Samples	Ca	Na	K	Mg
ABC	29.0± 0.00	379.9 ± 0.00	416.2±0.00	154.2± 0.00
MAC	278.4±0.00	431.9 ± 0.00	386.3±0.00	169.6±0.00
T34	ND	178.2 ± 0.00	52.5±0.00	108.5± 0.00
XYZ	106.9±0.00	315.9± 0.00	279.9±0.00	152.4± 0.00
PCB	23.3±0.00	326.5±0.00	276.1±0.00	138.7±0.00

Note: Values are mean ± SD of triplicate; Duncan separation of means with same alphabets are not different ($p<0.05$) in each column; ND = Not detected; ABC= Tiger nut non-dairy milk control; T34= Tiger nut non-dairy milk with turmeric; MAC= Tiger nut non-dairy milk with ginger; XYZ=Tiger nut non-dairy milk with combination of ginger and turmeric extract; PCB = Tiger nut non-dairy milk with citric acid.

Table-4. Colour composition of the milk samples.

Sample	L*	a*	b*
ABC	77.5 ± 0.14^{c}	-1.8 ± 0.6^{a}	15.4 ± 1.6^{d}
MAC	78.4 ± 0.20^{b}	-0.6 ± 0.09^{a}	17.8 ± 0.30^{c}
T34	75.2 ± 0.06^{e}	-1.7 ± 5.5^{a}	32.7 ± 0.08^{a}
XYZ	76.2 ± 0.18^{d}	-4.1 ± 0.2^{a}	29.5 ± 0.14^{b}
PCB	79.7 ± 0.09^{a}	-0.96 ± 0.2^{a}	17.3 ± 0.3^{c}

Note: Values are mean ± SD of triplicate; Duncan separation of means with same alphabets are not different ($p<0.05$) in each column; ABC= Tiger nut non-dairy milk control; MAC= Tiger nut non-dairy milk with ginger extract; T34= Tiger nut non-dairy milk with turmeric extract; XYZ=Tiger nut non-dairy milk with combination of ginger and turmeric extracts; PCB = Tiger nut non-dairy milk with citric acid.

3.7. Storability Study on Milk Samples

The milk samples stored for four weeks at room and refrigeration temperatures were monitored for changes in organoleptic attributes such as appearance, colour, texture and odour and reported in Table 5. Non-dairy milk stored at room temperature were observed to have signs of spoilage compared to those stored in the refrigeration temperature.

Table-5. Storability study of tiger nut non-dairy milk samples with preservatives.

Sample	Storage Temp.	Length of Storage	Sign of spoilage in tiger nut milk: Colour change	Odour Change	Texture	Appearance
ABC	32±2°C	4 weeks	NO	YES(Foul)	Slimy	Seperation
MAC			NO	YES(Foul)	Slimy	Seperation
XYZ			NO	YES(Foul)	Slimy	Seperation
T34			NO	YES(Foul)	Slimy	Seperation
PCB			NO	YES (Foul)	Slimy	Separation
ABC	4±2°C		NO	NO	Stringy	No Seperation
MAC			NO	NO	Stringy	No Seperation
XYZ			NO	NO	Stringy	No Seperation
T34			NO	NO	Stringy	No Seperation
PCB			NO	NO	Stringy	No Separation

Note: ABC= Tiger nut non-dairy milk control; T34= Tiger nut non-dairy milk with turmeric; MAC= Tiger nut non-dairy milk with ginger; XYZ=Tiger nut non-dairy milk with combination of ginger and turmeric; PCB = Tiger nut non-dairy milk with citric acid.

4. CONCLUSION

The use of the preservatives (ginger, turmeric and citric acid) helped to reduce the growth of the microorganism and hence, helped in maintenance of the product during storage. The nutritional composition of tiger nut non-dairy milk in this study is comparable to that of soymilk. Tiger nut non-dairy milk with citric acid maintained the highest protein, fat and ash contents compared to all others.

Appearance, Aroma, taste, colour of tiger nut non-dairy milk samples with preservatives rank side by side with tiger nut non-dairy milk without preservative. In general acceptability, tiger nut non-dairy milk without preservative was acceptable. This study also shows that pasteurization helped to extend the shelf life for a period of 4weeks at both ambient and refrigeration temperature. It also contributed to the overall quality and acceptability of the tiger nut non-dairy milk.

Therefore, tiger nut non-dairy milk can nutritionally replace milk produced from cow for people with lactose intolerance because tiger nut non-dairy milk doesn't contain lactose that cannot be digested by some humans.

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