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### RESEARCH ARTICLE

## PHYTOCHEMICAL, MICROBIOLOGICAL AND SENSORY CHARACTERISTICS OF TIGER NUT (CYPERUS ESCULENTUS L.) MILK PASTEURIZED AT DIFFERENT TIMES

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#### Abstract

Côte d'Ivoire is a country rich in agri-food resources, some of which are underutilized. This is the case with *Cyperus esculentus* L. (tiger nut), an unconventional and under-exploited tuber despite its good nutritional value. The overall objective is to determine its phytochemical and microbiological composition after pasteurization. The milk produced was divided into four portions. Three portions were pasteurized at 70°C for 10 min (LP10), 15 min (LP15), 20 min (LP20), and 25 min (LP25), and the remaining portion was unpasteurized (LNP). The phytochemical and microbiological characteristics were then determined. The results showed that pasteurization time did not influence the levels of polyphenols (5.90 to 7.33 mg GAE/100g) and flavonoids (0.15 to 0.21 mg QE/100g). However, the levels of vitamin C (26.67 to 66 mg/100g) and tannins (0.11 to 0.13 mg/100g) decreased with increasing pasteurization time. All microorganisms except *Staphylococcus* were counted at the LNP (GAM ( $2.8 \times 10^3$  CFU/mL); total coliforms ( $9.3 \times 10^2$  CFU/mL); yeasts and molds ( $5.6 \times 10^2$  CFU/mL)). Overall, the number of microorganisms decreased with increasing pasteurization time. Tiger nut milks were much appreciated by the panelists who indicated that all tiger nut-based drinks were good. This work paves the way for the valorization of tiger nuts.

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#### Introduction:-

A beverage is any liquid substance intended for human consumption for hydration, sustenance, and energy. According to Eke-Ejiofor and Beleya, (2018), beverages are liquids specifically prepared for human consumption. They can be homemade or industrially produced. The three main categories of beverages are stimulants, refreshers, and nutrients Eze and Njoku, (2018). Stimulant beverages such as tea and coffee are consumed to stimulate mental and physical activity. Refreshers such as water and juices are consumed to compensate for fluid loss in the body. Nutrients are consumed to provide nutrients to the body. This study focused on nutrient-rich beverages, specifically those prepared from tiger nuts. Tiger nuts, native to the Mediterranean basin, primarily Egypt, are herbaceous plants found in almost every part of the world (Bezerra et al., 2023). They belong to the Cyperaceae family and have the

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scientific name *Cyperus esculentus* (Ake-Assi, 1984). According to statistics from the Food and Agriculture Organization of the United Nations, global tiger nut production amounted to approximately 10 million tons, with a cultivated area of nearly 7 million hectares. In Côte d'Ivoire, national tiger nut production is estimated at nearly 16,000 tons per year (Tamboura, (2014). Yields per hectare range from 2.5 to 4 tons, with an average gross income per hectare of approximately 1,500,000 FCFA, primarily in the northern region (Abaejoh et al., 2006). In West Africa, this plant is known and cultivated for local consumption (Ong peamuru, 2013). In Africa in general, and in Côte d'Ivoire in particular, *Cyperus esculentus* is generally consumed raw or dried. Like peanuts or coconuts, its milk, oil, and flour are produced. Its derivative products are commonly found in markets. Plant-based milk is known for its antihypertensive, antidiabetic, antitumor, and antioxidant properties (Kampa, et al., 2021). The use of tiger nut milk is particularly recommended in cases of dyspepsia (indigestion), intestinal inflammation (colitis), or diarrhea (Kambire, (2015). Many consumers are turning to plant-based dairy products either for health reasons or as a lifestyle choice due to lactose intolerance in cow's milk. It should also be noted that most of the beverages consumed are carbonated and sweetened with artificial chemicals. Tiger nut milk production in Côte d'Ivoire is primarily artisanal and little known despite its nutritional and therapeutic potential. Once produced, the milk must be pasteurized to eliminate microorganisms. This study aims to investigate the effect of pasteurization time on the phytochemical, microbiological and sensory characteristics of tiger nut milk.

## Materials and Methods:-

### Materials:-

Tiger nut (*Cyperus esculentus*) (Figure 1) constitute the plant material used in this study. It purchased at the Korhogo main market in the Poro region. Korhogo is located in northern Côte d'Ivoire, 635 km from the city of Abidjan and between 9°27' north latitude and 5°38' west longitude. Once purchased, the tiger nut tubers are transported in coolers to the biochemistry laboratory at Peleforo GON COULIBALY University in Korhogo. All other chemicals and reagents used were of analytical quality.

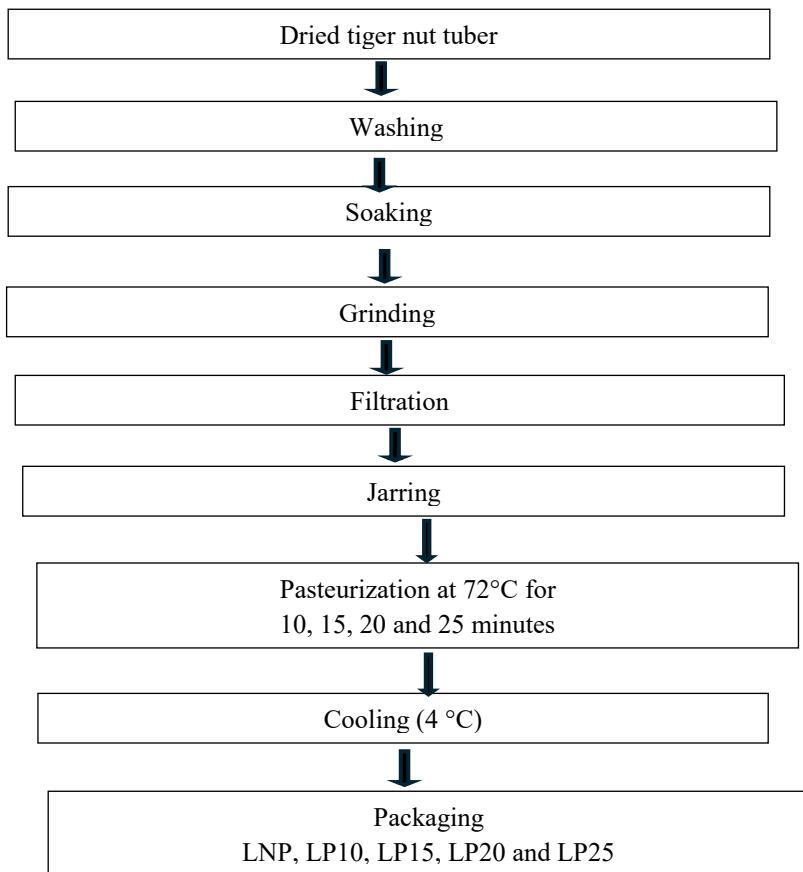
### Methods:-

#### Production of Tiger nut milk:-

The raw material (1 kg of dried tiger nuts) was cleaned with distilled water and then soaked in distilled water for 3 days. After soaking, the tubers were ground using a grinder containing 500 mL of water. The resulting powder was filtered through a white cloth. The milk was divided into five batches. The first four batches were pasteurized at 72 °C for 10, 15, 20, and 25 minutes, respectively. The fifth batch was not pasteurized. After pasteurization, the jars were cooled and stored in a refrigerator at 4 °C for subsequent analysis. Figure 2 shows the tiger nut milk production flowchart.



Figure 1 : Tiger nut tubers



**Figure 2 : Tiger nut milk production diagram.**

#### **Phytochemical properties of Tiger Nut Milk:-**

##### **Polyphenol determination:-**

The polyphenol content was determined according to the method of Singleton et al., (1999). The principle of the reaction is based on the reduction of Folin-Ciocalteu reagent during the oxidation of polyphenols. 1 mL of milk was mixed with 10 mL of 70% methanol. The resulting mixture was centrifuged at 1000 rpm for 10 min. The pellet was collected in 10 mL of 70% methanol and then centrifuged again. The supernatants were combined in a 50 mL volumetric flask and the volume was adjusted with distilled water. 1 mL of the methanolic extract was added to 1 mL of Folin-Ciocalteu reagent. After 3 min of stand, 1 mL of a 20% sodium bicarbonate ( $\text{Na}_2\text{CO}_3$ ) solution was added. The volume was adjusted to 10 mL with distilled water. The resulting mixture was incubated in the dark for 30 min. The optical density (OD) was read using a spectrophotometer at 725 nm against a blank. The amount of phenolic compounds was determined using a calibration curve established from a 1 mg/mL gallic acid solution.

##### **Flavonoid Determination:-**

The method of Marinova et al., (2005) was used for the determination of total flavonoids in the flours. To 0.5 mL of methanolic extract in a 25 mL volumetric flask, 0.5 mL of distilled water, 0.5 mL of aluminum chloride (10% w/v), and 0.5 mL of sodium acetate (1M) were successively added. The homogenized mixture was incubated for 30 minutes at room temperature. The absorbance reading was taken at 415 nm against the blank.

##### **Tannin determination:-**

The tannin content was determined according to the method of Bainbridge et al., (1996). To 1 mL of metabolic extract, 5 mL of vanillin reagent (0.1 mg/mL vanillin in 70% (v/v) sulfuric acid) was added. The resulting mixture

was incubated in the dark for 30 min. ODs were read at 500 nm against a blank. The amount of phenolic compounds was determined using a calibration curve established from a 1 mg/mL gallic acid solution.

#### Vitamin C determination:-

The vitamin C content was determined according to the method of Pongracz, (1971). 10 mL of milk was added to 10 mL of 20% methaphosphoric acid-acetic acid. The mixture is titrated with a 0.5 g/L solution of 2,6-DCPIP until a persistent pink color change is observed.

$$\text{Vitamin C (mg/100g)} = \frac{C (\text{DCPIP}) \times V_{\text{eq}} \times 5}{M_E} \times 100$$

C = DCPIP solution concentration

V<sub>eq</sub> = volume at the equivalence point

M<sub>E</sub> = sample mass

#### Microbiological Analyses:-

##### Inoculum Preparation:-

The stock solution was prepared by dissolving 1ml of the sample of tigernut milk in 9ml of sterile peptone water, serial dilution (10 fold) was carried out (1:10, 1: 100, 1:1000...10,0000) near the flame of a Bunsen burner. according to standard NF V 08-010 (AFNOR, 1996).

##### Isolation and Enumeration:-

Total bacterial count was determined using the method as described by Obasi et al., (2019). 0.1mL of the dilutions obtained were inoculated onto agar media specific to the target organisms and then incubated at the temperature and for the time required for each microorganism (Table 1). All enumeration was expressed as colony forming unit per milliliter (cfu/ml). The number of microorganisms was calculated using the following formula:

$$N = \sum \text{Colonies} / (n_1 + 0,1n_2) \times d \times v$$

**N:** number of microorganisms

**n<sub>1</sub>:** number of plates in the first dilution considered

**n<sub>2</sub>:** number of plates in the second dilution considered

**d:** the smallest dilution considered

**v:** volume of the inoculated sample

**Table 1: Culture medium and culture conditions of the microorganisms sought**

Germs	Medium	Type of sowing	Incubation	Standards
<b>Total coliforms</b>	Neutral crystal violet and red (VRLB) bile lactose agar	Depth	37 °C 24-48 h	ISO 4832
<b>Fecal coliforms</b>	Neutral crystal violet and red (VRLB) bile lactose agar	Depth	44 °C 24-48 h	ISO 4832
<b>Aerobic Mesophilic Germs (AMG)</b>	Plate count agar (PCA)	Depth	30 °C 48 h	NF/08-05
<b>Staphylococci</b>	Baird Parker (BP) with egg yolk and potassium tellurite	Spreading	37 °C 24-48 h	NF/V 08-057-1

Yeast/mold	Sabouraud +choramphenicol	Spreading	30 °C 72 h	NF/V 08-057-1
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### Sensory Analysis:-

Sensory evaluation is a unique source of information about products. It consists of measuring consumers' reactions to products in terms of appearance, aroma, taste, texture, and aftertaste, without taking into account the label, price, or other visual elements (Iwe, 2002). Acceptance and preference tests were conducted with 50 untrained panelists, comprised of employees and students from Peleforo Gon Coulibaly University in Korhogo, Côte d'Ivoire. The panelists' ages ranged from 22 to 67 years. The sensory analysis was based on a 9-point hedonic scale, according to the method of Curi et al. (2017). The hedonic scoring scale was arranged such that: 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely. The evaluation was based on quality parameters such as visual appearance, taste, color, odor, and overall acceptance. Panelists were randomly given approximately 30 mL of each type of tiger nut milk (pasteurized and unpasteurized) in clear plastic cups. They were asked to drink water before tasting the next sample.

### Statistical Analysis:-

Statistical analysis of the results was performed using STATISTICA 7 software. 1. The milk samples underwent analysis of variance (ANOVA) to determine the effect of pasteurization time on their properties. When a significant difference was observed, pairwise comparisons were made using Tukey's HSD test.

## Results and Discussion:-

### Results:-

#### Phytochemicals of Tiger Nut Milk:-

Table 2 shows the levels of phytochemical parameters in tiger nut milk. Analysis of the results in the table shows that the levels of polyphenols (5.90 to 7.33 mg GAE/100g) and flavonoids (0.15 to 0.21 mg QE/100g) in the different milks are all similar at the 0.05 threshold. However, the vitamin levels of LNP (62.67 mg/100g), LP10 (66 mg/100g), and LP15 (57 mg/100g) are significantly higher ( $p < 0.05$ ) than those of LP20 (36.70 mg/100g) and LP25 (26.67 mg/100g). It should also be noted that the vitamin C level drops significantly after 15 minutes of pasteurization. As for tannins, the levels of LNP (0.12 mg/100g) and LP10 (0.13 mg/100g) are significantly higher ( $p < 0.05$ ) than those of LP20 (0.11 mg/100g) and LP25 (0.11 mg/100g) but similar to that of LP15 (0.12 mg/100g).

**Table 2: Phytochemical parameters of tiger nut milk**

Parameters	LAIT				
	LNP	LP10	LP15	LP20	LP25
Flavonoids (mg QE/100g)	0.21 ± 0.01 <sup>a</sup>	0.15 ± 0,02 <sup>a</sup>	0.17 ± 0,04 <sup>a</sup>	0.19 ± 0.02 <sup>a</sup>	0.16 ± 0.01 <sup>a</sup>
Polyphenols (mg GAE/100g)	6.80 ± 0.76 <sup>a</sup>	7.33 ± 0,37 <sup>a</sup>	7 ± 0.65 <sup>a</sup>	5.90 ± 0.11 <sup>a</sup>	6.09 ± 0.63 <sup>a</sup>
Vitamin C (mg/100g)	62.67 ± 3.79 <sup>b</sup>	66 ± 1 <sup>b</sup>	57 ± 1 <sup>b</sup>	36.70 ± 0.75 <sup>a</sup>	26.67 ± 3.06 <sup>a</sup>

Tannin (mg/100g)	0.13 ± 0.01 <sup>b</sup>	0.13 ± 0.01 <sup>b</sup>	0.12 ± 0.01 <sup>ab</sup>	0.11 ± 0.01 <sup>a</sup>	0.11 ± 0.01 <sup>a</sup>
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Means assigned a different letter on the same line are significantly different at  $p < 0.05$ . LNP: Unpasteurized milk, LP10, LP15, LP20 and LP25: Milk pasteurized for 10, 15, 20 and 25 min respectively.

#### Microbiological Parameters of the Juices:-

Figure 3 shows the results of the count of these germs in tiger nut milk. All germs except *Staphylococcus* were counted in the LNP. Only mesophilic aerobic bacteria were counted in LP20 and LP25. Overall, the number of microorganisms decreased with increasing pasteurization time.

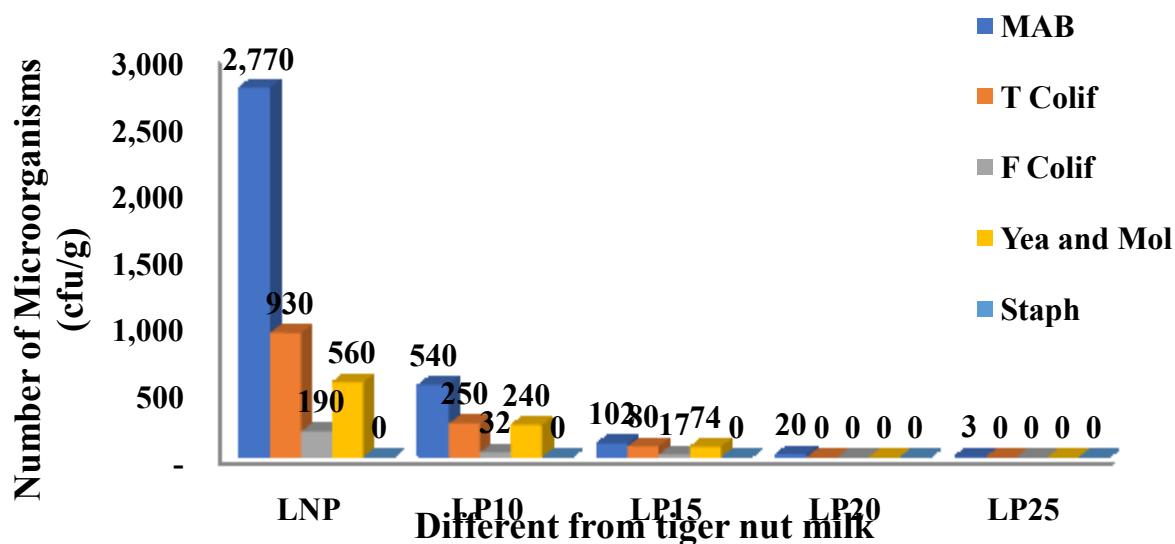


Figure 3: Germ count in tiger nut milk

#### Sensory Analysis:-

Sensory analyses were conducted to evaluate the visual appearance, taste, color, odor, and overall acceptability of pasteurized and unpasteurized tiger nut milks. The mean sensory evaluation scores for each sample are presented in Table 3. Analysis of the table shows that in terms of visual appearance (6.8 to 7.3), color (6.9 to 7.5), odor (7.1 to 7.6), and overall acceptability (7.6 to 7.8), there was no significant difference ( $p < 0.05$ ). Regarding taste, the values for LP15 (5.1), LP20 (5.3), and LP25 (5.4) were statistically higher than those for LNP (4.3) and LP10 (4.5). However, no significant difference ( $p < 0.05$ ) in taste was observed between LP15, LP20 and LP25 on the one hand and LNP and LP10 on the other.

Table 3: Mean scores for sensory evaluation of pasteurized and unpasteurized tiger nut milks

	LAIT				
	LNP	LP10	LP15	LP20	LP25
Visual appearance	7.2 <sup>a</sup>	7.3 <sup>a</sup>	7.2 <sup>a</sup>	7.1 <sup>a</sup>	6.8 <sup>a</sup>
Taste	4.3 <sup>a</sup>	4.5 <sup>a</sup>	5.1 <sup>b</sup>	5.3 <sup>b</sup>	5.4 <sup>b</sup>
Color	7.3 <sup>a</sup>	7.5 <sup>a</sup>	7.1 <sup>a</sup>	6.9 <sup>a</sup>	7.2 <sup>a</sup>
Odor	7.2 <sup>a</sup>	7.4 <sup>a</sup>	7.1 <sup>a</sup>	7.3 <sup>a</sup>	7.6 <sup>a</sup>

Overall acceptability	8.4 <sup>a</sup>	8.1 <sup>a</sup>	8.3 <sup>a</sup>	8.2 <sup>a</sup>	8.1 <sup>a</sup>
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### Discussion:-

The polyphenol content (5.90 to 7.33 mg GAE/100g) of tiger nut milk is higher than that of tiger nut milk powder ( $3.87 \pm 0.08$  mg GAE/100g) obtained by Kadjo et al., (2023) but lower than that found in the aqueous extract of tiger nut flour (211.5 mg GAE/100g) (Mai et al., 2022). These results show that tiger nuts could be rich in polyphenols. Polyphenols are molecules with antibacterial, anti-inflammatory, antithrombotic, anticancer, and neuroprotective properties (Amiot et al., 2009). Tiger nut milk could be used in milk production to replace cow's milk given its nutritional composition. The flavonoid content (0.15 to 0.21 mg QE/100g) in tiger nut milk is lower than that in tiger nut flour ( $289 \pm 1.53$  mg QE/100g) (Laziz and Ihadaden, 2021). It is true that tiger nut milk is less rich in flavonoids than tiger nut flour. However, consuming any food containing flavonoids is beneficial to the body. The vitamin C content of LNP is  $62.67 \pm 3.79$  mg/100 mL.

This content decreases as the pasteurization time increases. This could be explained by its heat sensitivity. Vitamin C is a reducing agent involved in antioxidant defenses (Frei, 2004). The vitamin C content (26.67 to 66 mg/100 g) in tiger nut milk would partially cover the average daily nutritional requirements of the population (15–100 mg) depending on the individual's age, with the exception of breastfeeding women (Mariotti et al., 2021). As for the tannin content (0.11 to 0.13 mg/100 g), it is lower than that found (53 mg/100 g) in studies conducted in Nigeria (Adedeji, 2016) on fermented tiger nuts. According to Kumari and Jain, (2012), the consumption of vegetables containing high levels of tannins and flavonoids has proven that these phytochemical compounds have numerous healing effects.

Regarding microbiological analysis, generally speaking, based on microbial loads, the different milk samples are classified as follows: LNP > LP10 > LP15 > LP20 > LP25. The number of each type of microorganism decreases with pasteurization time. This indicates that the pasteurization times and temperatures used in this study are adequate. The results show the presence of GAM (3 to  $27.7 \times 10^2$  CFU/mL), total coliforms (0 to  $9.3 \times 10^2$  CFU/mL), fecal coliforms (0 to  $1.9 \times 10^2$  CFU/mL), yeasts and molds (0 to  $5.6 \times 10^2$  CFU/mL), and the absence of *Staphylococcus*. The values for GAM, total coliforms, and yeasts and molds in this study are significantly lower than those found in tiger nut and date-based beverages ( $1.90 \times 10^3$  to  $1.26 \times 10^6$ ,  $3.20 \times 10^3$  to  $1.6 \times 10^6$ , and  $2.8 \times 10^3$  to  $7.25 \times 10^5$  CFU/ml, respectively) (Obasi and Mani, 2023)].

This difference is likely due to the unhygienic production conditions of this commercially available tiger nut and date-based beverage. The complete absence of *Staphylococcus* could be explained by the fact that the pasteurization temperature eliminated all vegetative forms. This confirms the findings of Jacob, (1990) which state that simple heat treatment of food (pasteurized juice, for example) is sufficient to destroy these *Staphylococcus* bacteria. It should be noted that the values obtained for GAM, total coliforms, and fecal coliforms following the enumeration are below the acceptable concentration described in the standard ( $5 \times 10^6$  and  $3 \times 10^3$  CFU/mL, respectively). Those for yeasts and molds and *Staphylococcus* are also below the acceptability standard ( $10^4$  and  $10^2$  CFU/mL, respectively). However, the fecal coliform value is within the acceptable range according to the standard ( $10^2 < \text{fecal coliforms} < 10^3$  CFU/mL).

The different pasteurization times showed no difference for most of the evaluated quality attributes, except for taste, which affected the organoleptic properties of tiger nut milk. Regarding taste preference, LNP and LP10 obtained the lowest average values, at 4.3 and 4.5 respectively, while LP5, LP20, and LP25 obtained the highest values, at 5.1, 5.3, and 5.2 respectively. All the milks were rated as "neither liked nor disliked to slightly disliked" in terms of taste. This is likely related to the fact that the milks were produced without additives. As for overall acceptance, the results indicated that the tiger nut milks were highly regarded by the panelists, who stated that all the tiger nut-based beverages were good.

**Conclusion:-**

This study determined the nutritional potential of unpasteurized and pasteurized tiger nut milk at different times. Generally, after 20 minutes, pasteurized tiger nut milk lost only vitamin C. The results of this study demonstrate that both pasteurized and unpasteurized tiger nut milk is a good source of polyphenols, flavonoids, and tannins. The number of each type of microorganism decreased with the pasteurization time. This indicates that the pasteurization times and temperatures used in this study were appropriate.

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