Phytochemical,microbiologicaland sensorycharacteristics of tigernut (*Cyperus esculentus* L.) milkpasteurized at different times

Abstract

Côte d'Ivoire is a country rich in agri-foodresources, some of which are underutilized. This is the case with*Cyperus esculentus* L. (tigernut), an unconventional and under-exploited tuber despiteits good nutritional value. The overall objective is to determineitsphytochemical and microbiological composition afterpasteurization. The milkproducedwasdividedinto four portions. Three portions werepasteurized at 70°C for 10 min (LP10), 15 min (LP15), 20 min (LP20), and 25 min (LP25), and the remaining portion wasunpasteurized (LNP). The phytochemical and microbiologicalcharacteristicswerethendetermined. The resultsshowedthatpasteurization 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) decreasedwithincreasingpasteurization time. All microorganismsexcept Staphylococcus werecounted at the LNP (GAM (2.8 x 10^3 CFU/mL); total coliforms (9.3 x 10^2 CFU/mL); yeasts and molds (5.6 x 10^2 CFU/mL)). Overall, the number of microorganismsdecreasedwithincreasingpasteurization time.Tiger nutmilksweremuchappreciated by the panelistswhoindicatedthat all tigernut-based drinks were good. This work paves the way for the valorization of tigernuts.

Keywords: *Cyperus esculentus*, milk, pasteurization ; phytochemical and microbiologicalcharacteristics, sensoryanalysis

Introduction

A beverageisanyliquid substance intended for humanconsumption for hydration, sustenance, and energy. According toEke-Ejiofor andBeleya, (2018), beverages are liquidsspecificallyprepared for humanconsumption. They can behomemade or industriallyproduced. The three main categories of beverages are stimulants,refreshers, and nutrientsEze and Njoku, (2018). Stimulant beveragessuch as tea and coffee are consumed to stimulate mental and physicalactivity. Refresherssuch as water and juices are consumed to compensate for fluidloss in the body. Nutrients are consumed to providenutrients to the body. This studyfocused on nutrient-richbeverages, specificallythosepreparedfromtigernuts.

Tiger nuts, native to the Mediterranean basin, primarilyEgypt, are herbaceous plants found in almostevery part of the world (Bezerra et al., 2023). Theybelong to the Cyperaceaefamily and

have the scientificname Cyperus esculentus(Aké-Assi, 1984). According to statisticsfrom the Food and Agriculture Organization of the United Nations, global tigernut production amounted to approximately 10 million tons, with a cultivated area of nearly 7 million hectares.

In Côte d'Ivoire, national tigernut production isestimated at nearly 16,000 tons per year(Tamboura, (2014). Yields per hectare range from 2.5 to 4 tons, with an averagegrossincome per hectare of approximately 1,500,000 FCFA, primarily in the northernregion(Abaejoh et al., 2006). In West Africa, this plant isknown and cultivated for local consumption(Ongpeamuru, 2013).

In Africa in general, and in Côte d'Ivoire in particular, Cyperus esculentusisgenerallyconsumedraw or dried. Like peanuts or coconuts, itsmilk, oil, and flour are produced. Itsderivativeproducts are commonlyfound in markets. Plant-basedmilkisknown for its antihypertensive, antidiabetic, antitumor, and antioxidantproperties(Kampa, et al., 2021). The use of tigernutmilkisparticularlyrecommended in cases of dyspepsia (indigestion), intestinal inflammation (colitis), or diarrhea(Kambire, (2015).Manyconsumers are turning to plant-baseddairyproductseither for healthreasons or as a lifestyle choice due to lactose intolerance in cow'smilk. It shouldalsobenotedthatmost of the beveragesconsumed are carbonated and sweetenedwithartificialchemicals. Tiger nutmilk production in Côte d'Ivoire isprimarily artisanal and littleknowndespiteitsnutritional and therapeuticpotential. Once produced, the milk must bepasteurized to eliminatemicroorganisms. This studyaims to investigate the effect of pasteurization time on the phytochemical, microbiological and sensorycharacteristicsof tigernut milk.

Materials and Methods

Materials

Tiger nut (*Cyperus esculentus*) (**Figure 1**) constitute the plant materialused in thisstudy. It purchased at the Korhogo main marketin the Pororegion. Korhogoislocated 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 tigernuttubers are transported in coolers to the biochemistrylaboratory at Peleforo GON COULIBALY University in Korhogo. All otherchemicals and reagentsusedwere of analyticalquality.

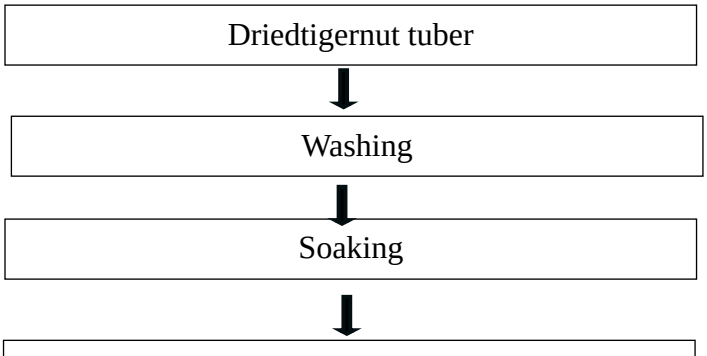
Methods

Production of Tiger nutmilk

The rawmaterial (1 kg of driedtigernuts) wascleanedwithdistilled water and thensoaked in distilled water for 3 days. Aftersoaking, the tubersweregroundusing a grinder containing 500 mL of water. The resultingpowderwasfilteredthrough a white cloth. The milkwasdividedinto five batches. The first four batcheswerepasteurized at 72 °C for 10, 15, 20, and 25 minutes, respectively. The fifth batch was not pasteurized. Afterpasteurization, the jars werecooled and

stored in a refrigerator at 4 °C for subsequentanalysis. **Figure 2** shows the tigernutmilk production flowchart.

Figure 1 : Tiger nuttubers



Phytochemicalproperties of Tiger Nut Milk

Polyphenoldetermination

The polyphenol content wasdeterminedaccording to the method of Singleton et al., (1999). The principle of the reactionisbased on the reduction of Folin-Ciocalteureagentduring the oxidation of polyphenols. 1 mL of milkwas mixed with 10 mL of 70% methanol. The resulting mixture wascentrifuged at 1000 rpm for 10 min. The pellet wascollectedin 10 mL of 70% methanol and thencentrifugedagain. The supernatantswerecombined in a 50 mLvolumetricflask and the volume wasadjustedwithdistilled water. 1 mL of the methanolicextractwasadded to 1 mL of Folin-Ciocalteureagent. After 3 min of stand, 1 mL of a 20% sodium bicarbonate (Na_2CO_3) solution wasadded. The volume wasadjusted to 10 mLwithdistilled water. The resulting mixture wasincubated in the dark for 30 min. The opticaldensity (OD) wasreadusing a spectrophotometer at 725 nm against a blank. The amount of phenolic compounds wasdeterminedusing a calibration curveestablishedfrom a 1 mg/mLgallicacid solution.

FlavonoidDetermination

The method of Marinova et al., (2005)wasused for the determination of total flavonoids in the flours. To 0.5 mL of methanolicextract in a 25 mLvolumetricflask, 0.5 mL of distilled water, 0.5 mL of aluminumchloride (10% w/v), and 0.5 mL of sodium acetate (1M) weresuccessivelyadded. The homogenized mixture wasincubated for 30 minutes at room temperature. The absorbance readingwastaken at 415 nm against the blank.

Tannin determination

The tannin content wasdeterminedaccording to the method of Bainbridge et al., (1996). To 1 mL of metabolicextract, 5 mL of vanillinreagent (0.1 mg/mLvanillinin 70% (v/v) sulfuricacid) wasadded. The resulting mixture wasincubated in the dark for 30 min. ODswereread at 500 nm against a blank. The amount of phenolic compounds wasdeterminedusing a calibration curveestablishedfrom a 1 mg/mLgallicacid solution.

Vitamin C determination

The vitamin C content wasdeterminedaccording to the method of Pongracz, (1971). 10 mL of milkwasadded to 10 mL of 20% methaphosphoricacid-aceticacid. The mixture istitratedwith a 0.5 g/L solution of 2,6-DCPIP until a persistent pinkcolor change isobserved.

$$\text{Vitamin C (mg/100g)} = \frac{C(\text{DCPIP}) \times Veq \times 5}{ME} \times 100$$

C = DCPIP solutionconcentration

Veq = volume at the equivalence point

ME = samplemass

Microbiological Analyses

Inoculum Preparation

The stock solution wasprepared by dissolving 1ml of the sample of tigernutmilk in 9ml of sterile peptone water, serial dilution (10 fold) wascarried 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 wasdeterminedusing the method as described by Obasi et al., (2019). 0.1mL of the dilutions obtainedwereinoculated onto agar media specific to the targetorganisms and thenincubated at the temperature and for the time required for eachmicroorganism(Table 1).All enumerationwasexpressed as colony forming unit per milliliter (cfu/ml).The number of microorganismswascalculatedusing the following formula:

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

N: number of microorganisms

n_1: number of plates in the first dilution considered

n_2: number of plates in the second dilution considered

d: the smallest dilution considered

v: volume of the inoculatedsample

Table 1: Culture medium and culture conditions of the microorganismssought

Germs	Medium	Type of sowing	Incubation	Standards
Total coliforms	Neutral crystal violet and red (VRLB) bile lactose agar	Depth	37° C 24-48 h	ISO 4832
Fecalcoliforms	Neutral crystal violet and red (VRLB) bile lactose agar	Depth	44 °C 24-48 h	ISO 4832
AerobicMesophilicGerms (AMG)	Plate count agar (PCA)	Depth	30 °C 48 h	NF/08-05
Staphylococci	Baird Parker (BP) witheggyolk 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

SensoryAnalysis

Sensoryevaluationis a unique source of information about products. It consists of measuringconsumers' reactions to products in terms of appearance, aroma, taste, texture, and aftertaste, withouttakingintoaccount the label, price, or othervisualelements (Iwe, 2002). Acceptance and preference tests wereconductedwith 50 untrainedpanelists, comprised of employees and studentsfromPeleforo Gon Coulibaly University in Korhogo, Côte d'Ivoire. The panelists' agesrangedfrom 22 to 67 years. The sensoryanalysiswasbased on a 9-point hedonicscale, according to the method of Curi et al. (2017). The hedonicscoringscalewasarrangedsuchthat: 9 = like extremely, 8 = like verymuch, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike verymuch, 1 = dislike extremely. The evaluationwasbased on

qualityparameterssuch as visualappearance, taste, color, odor, and overall acceptance. Panelistswererandomlygivenapproximately 30 mL of each type of tigernutmilk (pasteurized and unpasteurized) in clear plastic cups. Theywereasked to drink water beforetasting the nextsample.

StatisticalAnalysis

Statisticalanalysis of the resultswasperformedusing STATISTICA 7 software. 1. The milksamplesunderwentanalysis of variance (ANOVA) to determine the effect of pasteurization time on theirproperties. When a significantdifferencewasobserved, pairwisecomparisonswere made usingTukey's HSD test.

RESULTS AND DISCUSSION

Results

Phytochemicals of Tiger Nut Milk

Table 2 shows the levels of phytochemicalparameters in tigernutmilk. 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 differentmilks are all similar at the 0.05 threshold. However, the vitaminlevels of LNP (62.67 mg/100g), LP10 (66 mg/100g), and LP15 (57 mg/100g) are significantlyhigher ($p < 0.05$) thanthose of LP20 (36.70 mg/100g) and LP25 (26.67 mg/100g). It shouldalsobenotedthat the vitamin C level drops significantlyafter 15 minutes of pasteurization. As for tannins, the levels of LNP (0.12 mg/100g) and LP10 (0.13 mg/100g) are significantlyhigher ($p < 0.05$) thanthose of LP20 (0.11 mg/100g) and LP25 (0.11 mg/100g) but similar to that of LP15 (0.12 mg/100g).

Table 2: Phytochemicalparameters of tigernutmilk

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

Tannin (mg/100g)	0.13 ± 0.01 [b]	0.13 ± 0.01 [b]	0.12 ± 0.01 [ab]	0.11 ± 0.01 [a]	0.11 ± 0.01 [a]

Meansassigned a differentletter on the same line are significantlydifferent at $p < 0.05$.
LNP: Unpasteurizedmilk, LP10, LP15, LP20 and LP25: Milk pasteurized for 10, 15, 20 and 25 min respectively.

MicrobiologicalParameters of the Juices

Figure 3 shows the results of the count of thesegerms in tigernutmilk. All germsexcept Staphylococcus werecounted in the LNP. Onlymesophilicaerobicbacteriawerecounted in LP20 and LP25. Overall, the number of microorganismsdecreasedwithincreasingpasteurization time.

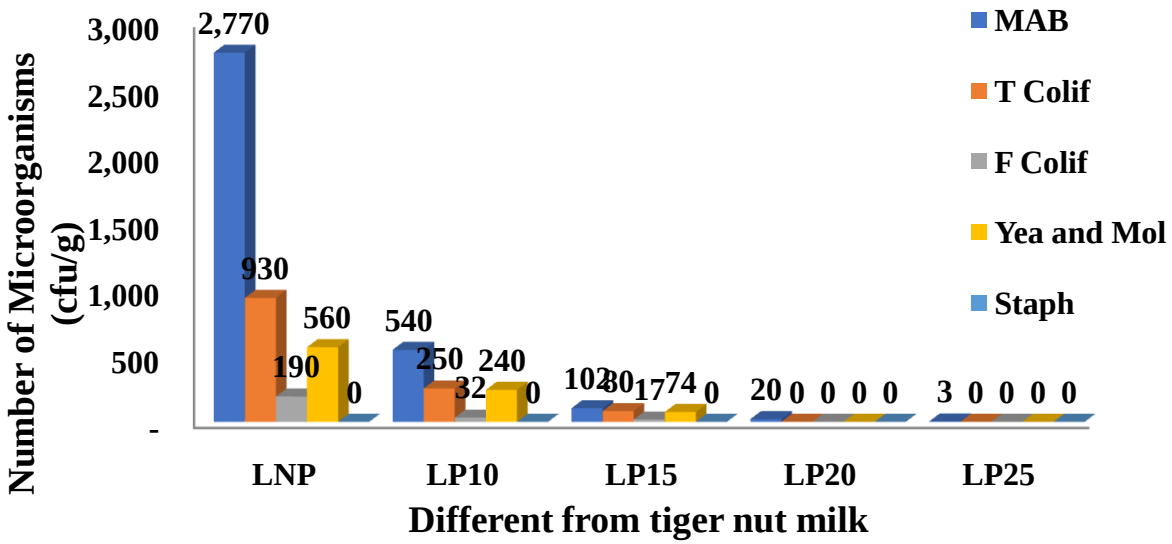


Figure 3: Germ count in tigernut milk

SensoryAnalysis

Sensory analyses wereconducted to evaluate the visualappearance, taste, color, odor, and overallacceptability of pasteurized and unpasteurizedtigernutmilks. The meansensoryevaluation scores for eachsample are presented in Table 3. Analysis of the table shows that in terms of visualappearance (6.8 to 7.3), color (6.9 to 7.5), odor (7.1 to 7.6), and overallacceptability (7.6 to 7.8), therewas no significantdifference ($p < 0.05$). Regarding taste, the values for LP15 (5.1), LP20 (5.3), and LP25 (5.4) werestatisticallyhigherthanthose for LPN (4.3) and LP10 (4.5). However, no significantdifference ($p < 0.05$) in taste wasobservedbetween LP15, LP20 and LP25 on the one hand and LPN and LP10 on the other.

Table 3: Mean scores for sensoryevaluation of pasteurized and unpasteurizedtigernutmilks

	LAIT				
	LNP	**LP10**	**LP15**	**LP20**	**LP25**
Visual appearance	7.2[a]	7.3[a]	7.2[a]	7.1[a]	6.8[a]
Taste	4.3[a]	4.5[a]	5.1[b]	5.3[b]	5.4[b]
Color	7.3[a]	7.5[a]	7.1[a]	6.9[a]	7.2[a]
Odor	7.2[a]	7.4[a]	7.1[a]	7.3[a]	7.6[a]
Overallacceptability	8.4[a]	8.1[a]	8.3[a]	8.2[a]	8.1[a]

Discussion

The polyphenol content (5.90 to 7.33 mg GAE/100g) of tigernutmilkishigherthanthat of tigernutmilkpowder (3.87 ± 0.08 mg GAE/100g) obtained by Kadjo et al., (2023) but lowerthanthatfound in the aqueousextract of tigernutflour (211.5 mg GAE/100g) (Mai et al., 2022). Theseresults show thattigernutscouldberich in polyphenols. Polyphenols are moleculeswithantibacterial, anti-inflammatory, antithrombotic, anticancer, and neuroprotectiveproperties(Amiot et al., 2009). Tiger nutmilkcouldbeused in milk production to replace cow'smilkgivenitsnutritional composition.

The flavonoid content (0.15 to 0.21 mg QE/100g) in tigernutmilkislowerthanthat in tigernutflour (289 ± 1.53 mg QE/100g)(Laziz and Ihadaden, 2021). It istruethattigernutmilkislessrich in flavonoidsthantigernutflour. However, consuminganyfoodcontainingflavonoidsisbeneficial to the body.

The vitamin C content of LNP is 62.67 ± 3.79 mg/100 mL. This content decreases as the pasteurization time increases. This couldbeexplained by itsheatsensitivity. Vitamin C is a reducing agent involved in antioxidantdefenses(Frei,2004).).The vitamin C content (26.67 to 66 mg/100 g) in tigernutmilkwouldpartially cover the averagedailynutritionalrequirements of the population (15–100 mg) depending on the individual'sage, with the exception of breastfeedingwomen(Mariotti et al., 2021). As for the tannin content (0.11 to 0.13 mg/100 g),

itislowerthanthatfound (53 mg/100 g) in studiesconducted in Nigeria (Adedeji, 2016). on fermentedtigernuts. According toKumari and Jain, (2012), the consumption of vegetablescontaining high levels of tannins and flavonoids has proventhatthesephytochemical compounds have numeroushealingeffects.

Regardingmicrobiologicalanalysis, generallyspeaking, based on microbialloads, the differentmilksamples are classified as follows: LNP > LP10 > LP15 > LP20 > LP25. The number of each type of microorganismdecreaseswithpasteurization time. This indicatesthat the pasteurization times and temperaturesused in thisstudy are adequate. The results show the presence of GAM (3 to 27.7 x 10^2 CFU/mL), total coliforms (0 to 9.3 x 10^2 CFU/mL), fecalcoliforms (0 to 1.9 x 10^2 CFU/mL), yeasts and molds (0 to 5.6 x 10^2 CFU/mL), and the absence of Staphylococcus. The values for GAM, total coliforms, and yeasts and molds in thisstudy are significantlylowerthanthosefound in tigernut and date-basedbeverages (1.90 x 10^3 to 1.26 x 10^6, 3.20 x 10^3 to 1.6 x 10^6, and 2.8 x 10^3 to 7.25 x 10^5 CFU/ml, respectively) (Obasiand Mani, 2023).]. This differenceislikely due to the unhygienic production conditions of thiscommerciallyavailabletigernut and date-basedbeverage. The complete absence of Staphylococcus couldbeexplained by the factthat the pasteurizationtemperatureeliminated all vegetativeforms. This confirms the findings of Jacob, (1990)which state that simple heattreatment of food (pasteurizedjuice, for example) issufficient to destroy these Staphylococcus bacteria. It shouldbenotedthat the values obtained for GAM, total coliforms, and fecalcoliformsfollowing the enumeration are below the acceptable concentration described in the standard (5×10^6 and 3×10^3 CFU/mL, respectively). Those for yeasts and molds and Staphylococcus are alsobelow the acceptability standard (10^4 and 10^2 CFU/mL, respectively). However, the fecalcoliform value iswithin the acceptable range according to the standard (10^2 <fecalcoliforms< 10^3 CFU/mL).

The differentpasteurization times showed no difference for most of the evaluatedqualityattributes, except for taste, whichaffected the organolepticproperties of tigernutmilk. Regarding taste preference, LNP and LP10 obtained the lowestaverage 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 milkswererated as "neitherlikednordisliked to slightlydisliked" in terms of taste. This islikelyrelated to the factthat the milkswereproducedwithout additives. As for overall acceptance, the resultsindicatedthat the tigernutmilkswerehighlyregarded by the panelists, whostatedthat all the tigernut-basedbeverageswere good.

Conclusion

This studydetermined the nutritionalpotential of unpasteurized and pasteurizedtigernutmilk at different times. Generally, after 20 minutes, pasteurizedtigernutmilklostonlyvitamin C. The results of thisstudydemonstratethatbothpasteurized and unpasteurizedtigernutmilkis a good source of polyphenols, flavonoids, and tannins. The number of each type of microorganismdecreasedwith the pasteurization time. This indicatesthat the pasteurization times and temperaturesused in thisstudywereappropriate.

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