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Stability, nutritional composition, and antioxidant properties of surfactant-assisted enzymatically extracted tiger nut milk

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ABSTRACT

This study evaluated the effect of surfactant-assisted enzymatic extraction on the quality of tiger nut milk (TNM). TNM was extracted from tiger nuts using different concentrations of xylanase (0.010–0.100%) and Tween 20 (0.005–0.010%). The yield, stability, nutritional, antioxidant, and sensory properties of the samples were determined. The yield of TNM significantly increased, by 32.72–50.67%, following surfactant-assisted enzymatic extraction. Optimum yield and stability of TNM were obtained using 0.010% xylanase and Tween 20. Enzymatic extraction significantly increased total sugar and flavonoids, however, starch, dietary fibre, protein, carotenoids, lycopene, total phenolic content, and antioxidant properties reduced significantly. The incorporation of Tween 20 stabilised these parameters. There was no significant difference in panellists' preference for the control (sample extracted without enzyme and surfactant), enzymatically-extracted, and surfactant-assisted enzymatic extracted samples in mouthfeel and aroma, however, the surfactant-assisted enzymatic extracted sample was most preferred in colour, consistency, taste, and overall acceptability. Using surfactant-assisted enzymatic extraction could prove invaluable for the production of TNM.

KEYWORDS

extraction, functional drink, imitation milk, surfactant, xylanase

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1. INTRODUCTION

Tiger nut milk (TNM), popularly known as *horchata de chufa* in Spain and *kunun aya* in Nigeria, is a vegetable imitation of milk produced from tiger nut (*Cyperus esculentus* L.), a nut-like rhizome that is widely distributed in many parts of West Africa, Europe, and America (Clemente-Villalba et al., 2021). TNM is acceptable in many parts of the world due to its nutritional richness and bio-functionality (Rubert et al., 2017; Roselló-Soto et al., 2019), triggering research efforts in process modification (Kizzie-Hayford et al., 2018), shelf-life, and safety studies (Zhu et al., 2019). The main unit operations during conventional TNM production are soaking, wet-milling, and extraction (Muhammad et al., 2019). The extraction of TNM is facilitated by operations such as centrifugation, heating, high-speed mixing, and agitation (Kizzie-Hayford et al., 2014, 2018). Significant loss of nutrients is associated with these operations (Kizzie-Hayford et al., 2014). Besides, mechanical extraction is characterised by low yield, high downtime and production cost (Nguyen and Nguyen, 2018). Enzymatic extraction offers both technical and economic merits, and its use has increased drastically in the food industry (Garcia, 2018).

Hemicellulases, pectinases, and cellulases are the most common enzymes used in the food industry for juice extraction from fruits and vegetables (Marsol-Vall et al., 2021). Xylanase is the most important hemicellulase, which degrades xylan by randomly acting on the β-1,4-non-reducing terminal sections of the glycosidic bonds of glucose units (Silva et al., 2019). This disrupts the lignocellulosic cell wall by severing the linkages between hemicellulose, cellulose, and pectin (Toushik et al., 2017). Using xylanase for extraction and clarification of juice is well documented (Weber and Larsen, 2017; Ravindran et al., 2019), however, recent studies have shown that enzymatic extraction reduces bioactive compounds and alters physicochemical and sensory properties of the resulting juices (Weber and Larsen, 2017; Nguyen and Nguyen, 2018). Therefore, using surfactant-mediated enzymatic extraction could prove effective.

Surfactants are amphiphiles that are made up of discrete lipophilic and hydrophilic moieties, which form stable aggregates with monomers in an aqueous solution (Paleologos, 2017). Polyoxyethylene sorbitan monolaurate (Tween 20) is a non-ionic and non-toxic surfactant that is commonly used in the food industry, due to its availability, low cost, and ease of biodegradation (Eskandani et al., 2013). Recent studies have demonstrated an improvement in the yield and physicochemical properties of polysaccharides, phytochemicals, and bioactive compounds following the use of surfactants, in combination with other physical or biological techniques, for their extraction from plant materials (Amiri-Rigi and Abbasi, 2016; MohdMaidin et al., 2019). However, information on the use of surfactants for the enhancement of enzymatic extraction of juice from plants is sparse. The objective of this study was to evaluate the effect of surfactant-assisted enzymatic extraction on the yield, stability, nutritional composition, bioactivity, and sensory properties of TNM.

2. MATERIALS AND METHODS

2.1. Materials

Freshly-harvested yellow variety of tiger nuts was obtained from Agricultural Extension Workers at Wukari, Taraba State, Nigeria. Tween 20 (CAS No. 9005-64-5, Sigma-Aldrich, St.

Louis, MO631303, USA) was supplied by Ezek Roberts Ltd., Ibadan, Nigeria. Xylanase (produced from *Aspergillus oryzae*, Sigma-Aldrich, St. Louis, MO631303, USA) was procured from Bristol Plc., Lagos, Nigeria.

2.2. Surfactant-assisted enzymatic extraction of tiger nut milk

The tiger nuts were thoroughly washed with potable water to remove soil particles. Subsequently, 1 kg of cleaned tiger nuts was soaked in 4 L distilled water containing 10 mL 0.5% citric acid for 12 h. The nuts were separated and washed twice with potable water (20 °C) and milled with a Waring blender (Model 8010EX, Clarkson Inc., California, US) for 15 min to obtain a coarse mush. To facilitate the milling operation, 200 mL distilled water (20 °C) was added intermittently. Tiger nut milk was extracted from the mush with the aid of the xylanase enzyme with or without Tween 20. Accordingly, 50 g of tiger nut mush was weighed into seven different 250 mL beakers, and xylanase (0.010% or 0.100%) and Tween 20 (0.005% or 0.010%) were added. Samples obtained were as follows: X0T0 (TNM extracted without xylanase and Tween 20), X1T0 (TNM extracted with 0.010% xylanase), X1T1 (TNM extracted with 0.010% xylanase and 0.005% Tween 20), X1T2 (TNM extracted with 0.010% xylanase and 0.010% Tween 20), X2T0 (TNM extracted with 0.100% xylanase), X2T1 (TNM extracted with 0.100% xylanase and 0.005% Tween 20), and X2T2 (TNM extracted with 0.100% xylanase and 0.010% Tween 20). The preparations were mixed for 1 min and incubated at 40 °C for 4 h. After incubation, the mush was cooled to 20 °C, milled with a Waring blender for 15 min, and sieved with a 0.074-mm US standard sieve to obtain TNM. The samples were pasteurised at 80 °C for 2 min and cooled rapidly to room temperature (30 ± 2 °C). The freshly prepared samples were analysed immediately for nutritional and phytochemical composition and antioxidant and sensory properties. The TNM samples were refrigerated at 4 °C for 20 days and syneresis was determined every 2 days.

2.3. Determination of syneresis

Syneresis was determined using the centrifugal acceleration procedure described by Kizzie-Hayford et al. (2014). Briefly, 10 mL of TNM was poured into a centrifuge tube and centrifugation was done at 1200 × *g* and 4 °C for 10 min. Percentage syneresis was expressed as the ratio of the volume of serum in the tube after centrifugation to the volume of the sample taken.

2.4. Determination of macronutrients, phytochemicals, and antioxidant assay

Starch content was determined by treating TNM with 1 N KOH followed by neutralization with 1N HCl. The absorbance was read with the aid of a UV/VIS spectrophotometer (T60U, PG Instruments, Leicestershire, UK) at 720 nm and compared with a standard pure starch curve (Sadasivam and Manickam, 1996). Total sugar was determined using the phenol-sulphuric method and the reducing sugar by the dinitrosalicylic acid assay method (Sadasivam and Manickam, 1996). AOAC (2005) methods were used for the analysis of dietary fiber (number 991.43), protein (992.23), and fat (920.39). The aluminum chloride colorimetric method was used for the determination of total flavonoid content (Pontis et al., 2014). Total carotenoids and lycopene were determined spectrophotometrically (Branisa et al., 2014). Total phenolic content (TPC) was determined based on Folin–Ciocalteu's method using gallic acid as a standard (Álvarez et al., 2016). DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), and FRAP (ferric reducing antioxidant power) procedures

were employed for the antioxidant assay of TNM samples based on standard procedures (Pontis et al., 2014; Álvarez et al., 2016).

2.5. Sensory analysis

Evaluation of organoleptic properties of TNM was done based on a 9-point hedonic scale (Kizzie-Hayford et al., 2014). Samples were assessed for colour, mouthfeel, aroma, consistency, taste, and overall acceptability by 50 panellists comprising students and staff of the Faculty of Agriculture and Life Sciences, Federal University Wukari, Wukari, Nigeria. Coded samples at a temperature of 10 °C were presented to the panellists in individual booths illuminated with fluorescent light. Potable water was provided in between samples for palate cleansing.

2.6. Statistical analysis

All experiments and analyses, except sensory evaluation, were conducted in triplicates. The study design comprised two independent variables, xylanase and surfactant concentrations. The data generated were subjected to analysis of variance and Duncan multiple range test using the statistical package for the social scientists (version 23, IBM, Armonk, USA).

3. RESULTS AND DISCUSSION

3.1. Yield of tiger nut milk

Tiger nut milk yield as influenced by surfactant-assisted enzymatic extraction is presented in Fig. 1. Enzymatic treatment significantly increased the yield by 32.73–50.67%, likely to the activity of xylanase that degraded xylan by randomly acting on its β-1,4-non-reducing terminal sections of the glycosidic bonds of glucose units (Silva et al., 2019), reducing the water holding capacity of the macromolecules, such as xylan and starch, thereby increasing the release of TNM (Garcia, 2018). Ravindran et al. (2019) also reported an increase in yield of strawberry (*Fragaria ananassa*) and raspberry (*Rubus idaeus*) juices following extraction with xylanase. Results further showed an increased TNM yield following the incorporation of Tween 20. X1T1 and

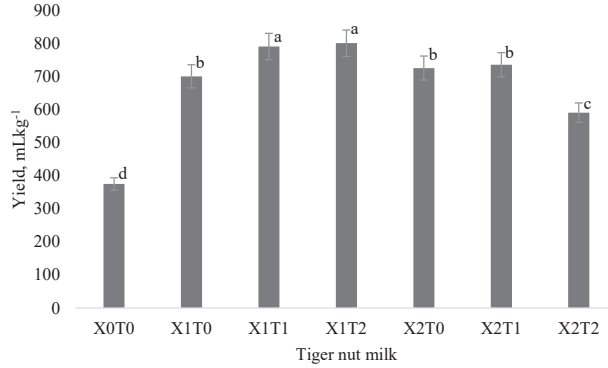

Fig. 1. Yield of surfactant-assisted enzymatically extracted tiger nut milk

X1T2 had the highest yield, likely to the enhancement of enzyme-substrate interfacial interaction following reduced surface tension that improved the degradation of complex macromolecules by xylanase (Paleologos, 2017).

3.2. Stability of tiger nut milk

The stability of TNM, as expressed in percentage syneresis, is presented in Fig. 2. Syneresis was observed in X0T0 (45%), X1T0 (30%), and X2T0 (40%) immediately after extraction, implying phase separation of these samples owing to the dissociation of solids following increased creaming and agglomeration of fat globules (Kizzie-Hayford et al., 2014). According to Kizzie-Hayford et al. (2014), the TNM matrix is a dispersion that is characterised by solutes with varying particle size, shape, molecular size, and charge, making it susceptible to phase separation. Results show that enzymatic extraction of TNM did not stabilise the product; however, this was achieved following surfactant inclusion. This was evident in the absence of phase separation (0% syneresis) in X1T1, X1T2, X2T1, and X2T2 after TNM production, likely to the formation of a stable dispersion of the lipophilic and hydrophilic components of the TNM by the surfactant (Sharma et al., 2015), in agreement with MohdMaidin et al. (2019) who reported an improvement in the stability of anthocyanins following the incorporation of Tween 20 during hydro-alcoholic extraction. During storage, the syneresis of X0T0, X1T0, and X2T0 increased significantly. X1T2 and X2T2 were 100% stable after the 4th and 6th day, respectively, however, syneresis was observed in X1T1 and X2T1 after the second day of storage. This showed that Tween 20 was solely responsible for phase stabilisation. At the advanced stage of storage (16th–20th days), lower syneresis was recorded for X1T2 relative to X2T2, hence, this sample was selected for further analysis.

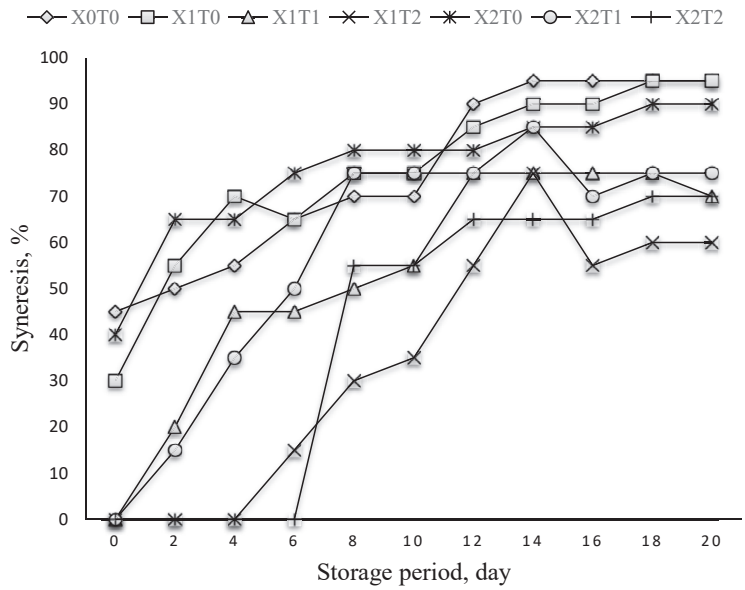

Fig. 2. Stability of surfactant-assisted enzymatically extracted tiger nut milk

Table 1. Selected macronutrients of tiger nut milk

Sample	Starch (g/100 mL)	Total sugar (mg/100 mL)	Reducing sugar (mg/100 mL)	Dietary fibre (g/100 mL)	Protein (g/100 mL)	Fat (g/100 mL)
Control	$1.50^{a} \pm 0.40$	$10.68^{c} \pm 0.15$	$5.72^{c} \pm 0.03$	$0.99^{a} \pm 0.03$	$4.19^{a} \pm 0.03$	$2.12^{a} \pm 0.02$
Xylanase-extracted	$1.13^{b} \pm 0.01$	$12.42^{b} \pm 0.23$	$6.26^{b} \pm 0.27$	$0.58^{c} \pm 0.02$	$3.03^{b} \pm 0.07$	$2.10^{a} \pm 0.01$
Xylanase+ surfactant-extracted	$1.55^{a} \pm 0.01$	$13.94^{z} \pm 0.49$	$7.30^{a} \pm 0.02$	$0.83^{b} \pm 0.04$	$4.21^{a} \pm 0.05$	$2.12^{a} \pm 0.04$

Presented data are means and standard deviation of three experimental data. Data having the same superscript within a column are not significantly ($P > 0.05$) different.

3.3. Selected macronutrients of tiger nut milk

The macronutrients of TNM were significantly influenced by extraction methods (Table 1). Starch content decreased by 24.67% and 16.67% following xylanase and xylanase-surfactant extractions, respectively, likely due to the degradation of tiger nut's lignocellulosic components (Toushik et al., 2017), suggesting improved stability and sensory attributes. This aligns with a reduction in starch content following the extraction of TNM by amylase (Djomdi et al., 2020). A higher starch content obtained for xylanase-surfactant extraction relative to the use of xylanase only could be due to the encapsulation of starch molecules by Tween 20, preventing further starch hydrolysis. The degradation of starch was evident in an increase in total and reducing sugars content, which might improve sweetness. Dietary fibre amounts decreased by 41.41% following enzymatic extraction, likely due to the degradation of lignin, hemicellulose, and cellulose contents following the random hydrolysis of β-1,4-non-reducing terminal regions of xylan by the action of xylanase (Ravindran et al., 2019). Meanwhile, the incorporation of Tween 20 increased the dietary fibre content in the xylanase-extracted TNM by 30.12%, probably because of the resistance of the micelle, formed following the encapsulation of xylan by Tween 20, to further degradation by xylanase. This implies increased functionality due to the prebiotic potential of dietary fibre in tiger nut (Mottawea et al., 2020). Enzymatic extraction reduced the protein content of TNM by 27.68%, likely due to the release of protein molecules after the degradation of cell-wall xylan by xylanase and consequent denaturation of the protein and peptide molecules during post-extraction operations such as pasteurisation (Silva et al., 2019). However, the addition of surfactant stabilised the protein, likely due to the encapsulating effect of the surfactant on protein molecules against oxidative reactions (Sharma et al., 2015). There was no significant difference among the samples in terms of fat content, suggesting resistance of the essential polyunsaturated fatty acids in TNM to enzymatic hydrolysis and surfactant action (Ezeh et al., 2016). The fat content (2.10–2.12 g/100 g) obtained for TNM in this study was within the standard value, 2.00%, recommended for the product (Sharma et al., 2015).

3.4. Phytochemicals and antioxidant properties of tiger nut milk

The phytochemical composition of TNM as influenced by extraction methods is presented in Table 2. Enzymatic extraction increased flavonoids by 116%, likely due to the secretion of

Table 2. Phytochemicals and antioxidant properties of tiger nut milk

Sample	Flavonoids (mg/100 mL)	Carotenoids (mg/100 mL)	Lycopene (mg/100 mL)	TPC (mg mL^{-1})	DPPH (μg/100 mL)	ABTS (mg/100 mL)	FRAP (mg/100 mL)
Control	$0.25^{b} \pm 0.00$	$1.65^{c} \pm 0.09$	$0.25^{b} \pm 0.01$	$0.14^{b} \pm 0.01$	$41.85^{bc} \pm 0.77$	$54.71^{a} \pm 0.73$	$121.78^{b} \pm 0.47$
Xylanase-extracted	$0.54^{a} \pm 0.02$	$3.20^{b} \pm 0.01$	$0.18^{c} \pm 0.03$	$0.12^{c} \pm 0.01$	$38.43^{c} \pm 1.04$	$51.54^{b} \pm 0.41$	$107.91^{c} \pm 1.12$
Xylanase+surfactant-extracted	$0.48^{a} \pm 0.01$	$3.73^{a} \pm 0.03$	$0.38^{a} \pm 0.00$	$0.18^{a} \pm 0.01$	$47.50^{a} \pm 0.57$	$55.32^{a} \pm 0.00$	$127.04^{a} \pm 0.46$

Presented data are means and standard deviation of three experimental data. Data having the same superscript within a row were not significantly ($P > 0.05$) different.

anthocyanin following the hydrolysis of glycosidic chains of xylan (Ravindran et al., 2019). However, total carotenoids and lycopene decreased by 48.44% and 28.00%, respectively, probably because of oxidative reactions triggered by co-enzymes, such as coumaroyl and malonyl (Álvarez, et al., 2016), corroborating Nguyen and Nguyen (2018) that a reduction in phytochemicals of mulberry (*Morus* spp.) juice occurred following its extraction with Pectinex and Viscozyme. Meanwhile, surfactant addition stabilised flavonoids, total carotenoids, and lycopene, likely because of the encapsulation of these compounds by Tween 20 leading to the formation of micelles that probably migrated into the product through molecular diffusion (Bi et al., 2011). TPC remained stable after enzymatic extraction, in agreement with a report that TPC of blackcurrant juice was not reduced after extraction with hemicellulase (Laaksonen et al., 2012). The incorporation of surfactant increased TPC by 28.57%, due to increased solubilisation and the consequent liberation of bound phenolic compounds in the cell wall following surface tension reduction by Tween 20 (Paleologos, 2017), indicating an improvement in bioactivity and, as a consequence, the production of important health biomarkers in the body. Enzymatic extraction of TNM significantly reduced DPPH, ABTS, and FRAP, probably because of the reduction in phytochemicals having antioxidant properties (Weber and Larsen, 2017), corroborating the report that the antioxidant capacity of asparagus juice reduced following extraction with laccase (Sun et al., 2005). The addition of surfactant increased DPPH and FRAP by 13.50% and 4.32%, respectively, likely due to the increase in TPC, improving the functionality and acceptability of the product by consumers that are interested in consuming functional foods.

3.5. Sensory attributes of tiger nut milk

The sensory attributes of freshly prepared TNM are presented in Table 3. There were no significant differences in mouthfeel and aroma, suggesting that the use of xylanase and Tween 20 for the extraction of TNM did not produce astringency-inducing compounds, contradicting a report that the acceptability of hemicellulase-extracted blackcurrant juice was reduced due to astringency (Laaksonen et al., 2012). The panellists showed a higher preference for the surfactant-assisted-xylanase-extracted TNM in colour, consistency, taste, and overall acceptability. The high preference for colour was likely to increase colloidal stabilisation owing to the reduction of phase separation (Paleologos, 2017). The preference for consistency could be linked with increased stability of the lipophilic and hydrophilic components of the samples (Sharma et al., 2015). The taste preference could be due to the higher sugar content of the sample (Section 3.3).

Table 3. Sensory attributes of tiger nut milk

Sample	Colour	Mouthfeel	Aroma	Consistency	Taste	Overall acceptability
Control	$8.20^{ab} \pm 1.78$	$7.00^{a} \pm 1.93$	$7.30^{a} \pm 2.00$	$7.38^{ab} \pm 2.14$	$6.70^{ab} \pm 2.40$	$7.14^{ab} \pm 2.25$
Xylanase-extracted	$7.38^{c} \pm 2.30$	$7.20^{a} \pm 2.31$	$7.62^{a} \pm 2.00$	$7.24^{ab} \pm 2.06$	$6.68^{ab} \pm 2.46$	$6.60^{b} \pm 2.45$
Xylanase+ surfactant-extracted	$8.68^{a} \pm 2.32$	$7.12^{a} \pm 2.36$	$7.48^{a} \pm 2.03$	$7.98^{a} \pm 2.00$	$7.70^{a} \pm 2.39$	$7.90^{a} \pm 2.37$

Presented data are means and standard deviations of fifty scores. Data having the same superscript within a column were not significantly ($P > 0.05$) different.

4. CONCLUSIONS

This study showed an improvement in the yield, stability, antioxidant and sensory properties of TNM following its extraction using the surfactant-assisted enzymatic procedure. The concentration of xylanase and Tween 20 influenced the yield and stability of TNM. Optimum yield and stability were obtained using 0.010% xylanase and Tween 20. The extraction of TNM with xylanase increased the yield of TNM, however, the stability, antioxidant and sensory properties were negatively affected. The inclusion of Tween 20 during extraction improved these properties.

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