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Chemical and antioxidant properties of two non-dairy probiotic drinks produced from tigernut (*Cyperus esculentus*) and soybean (*Glycine max*)

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ABSTRACT

Probiotic drinks have become more popular and the desire for new flavours among consumers necessitates the development and production of improved probiotic drinks. In the present study, the reducing sugar, mineral content, antinutritional and antioxidant properties of non-dairy probiotic drinks produced from tigernut milk and soy milk were investigated. Probitication of the non-dairy milk results in the reduction of reducing sugars and antinutrient contents of the drinks. Probioticated tigernut milk sample (T2) has the least reducing sugar content (4.63%), while the highest was found in soy milk sample S2 (4.81%). However, there were significant increases in the mineral content and antioxidant properties of the probioticated non-dairy drinks compared to the non probioticated non-dairy drinks. The ferric reducing antioxidant power (FRAP), DPPH, and Fe^{2+} chelation activities of probioticated tigernut milk increased from 0.5% extract to 0.84% extract, 73.18% to 91.70% and 36.30% to 39.02% in samples T12, T2 and T1, respectively. The same increase was also observed in the probioticated soy milk compared with the unprobioticated. Results from this study revealed that probiotication enhances the mineral content and antioxidative capacity of milk from soybean and tigernut.

Introduction

The human gastrointestinal tract is a very important organ that helps in the digestion and absorption of food. It is a very important part of the body which is susceptible to many types of disorders caused mainly by infectious agents such as *Escherichia coli, Salmonella enteritidis* androtaviruses (Oyetayo, 2020). The Nobel laurate, Russian biologist Elie Metchnikoff (1845 – 1916) postulated that the toxic by-products of the bowel bacteria are responsible for many diseases. It has been reported that, when disease causing bacteria exceed good or friendly bacteria, health problems such as bloating, intestinal toxicity, constipation, and malabsorption of nutrients could be observed. Infectious microorganisms act in a certain way that allows them to cause diseases, hence the phrase "death begins in the colon" as postulated by Elie Metchnikoff (Nye, 2019).

Two mechanisms have been suggested for keeping the gastro intestinal tract (GIT) healthy and these are the consumption of probiotics and prebiotics. Probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (Patel et al., 2012), while prebiotics are nondigestible food ingredients, that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improving the host health (Gibson and Roberfroid, 1995).

Probiotics are live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance (O'Bryan et al., 2013; Prabhurajeshwar and Chandrakanth, 2017). In the last

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two to three decades, a growing public awareness of diet-related health issues and increasing evidence regarding health benefits of probiotics have encouraged consumers demand for probiotic foods (Vattem and Maitin, 2016; Amponsah et al., 2017).

Although dairy-based products have been suggested to be the main carriers for the delivery of probiotics (Afroz et al., 2016), other nondairy-based products can be exploited as a potential carrier of probiotic microorganisms (Kidist et al., 2019; Panghal et al., 2018; Sethi et al., 2016). There is an increasing demand for new flavours and tastes among consumers, since the majority of the probiotic products in the market do not meet the needs of all consumer groups, as they are mostly produced as yoghurts (a milk product) (Amponsah et al., 2017; Panghal et al., 2018). To satisfy the food needs of all groups of people, non-dairy probiotic productscan be an excellent choice (He and Hekmat, 2014; Sethi et al., 2016). Plant based products have been suggested as an alternative probiotic food carrier, due to their low cost, availability and health benefits (Min et al., 2019; Panghal et al., 2018; Afroz et al., 2017). Soybean and tiger nut, two plant based probiotic food carriers, have been suggested as an important replacement for dairy milk (Jung et al., 2016; Abd El-Gawad et al., 2015; Babashahi et al., 2015). The present study is aimed at evaluating the reducing sugars, mineral content, antinutritional and antioxidant properties of two non-dairy probiotic drinks produced from tigernut and soybean milk.

Materials and methods

Reagents used

The used microbiological media, reagents and equipment were of analytical grade, obtained from the Department of Microbiology, Federal University of Technology Akure, Ondo State, and reliable chemical supply stores in Akure, Nigeria.

Sample collection and source of lactic acid bacteria

Soybean and tigernuts were purchased from Adedeji Market, Akure Ondo State Nigeria. They were sorted and graded to remove dirt and debris. Lactic acid bacteria used as probiotic organisms were isolated from tigernut and soybean. Briefly, tigernuts and soybean were each macerated using a sterile mortar and pestle. Nine millilitres (9mL) of distilled water was dispensed into 10 clear test tubes, and sterilized by autoclaving. After the sterilization, each sample was diluted using the sterile distilled water as diluent (Oyeleke and Manga, 2008), by weighing 1g or 1mL of the sample into the sterilized water, after which 1mL from dilution factors 10^{-8} and 10^{-10} were placed on already prepared deMann Rogossa and Sharpe (MRS) agar in triplicates, using the pour plate method. The plates were incubated at appropriate temperature and time according to the methods described by Patel et al. (2012).

Assessment of probiotic properties of lactic acid bacteria

The probiotic potential of the isolated lactic acid bacteria was determined according to the method described by Prabhurajeshuwar and Chandrankanth (2017) and Jung et al. (2016). Some of the parameters checked for were: tolerance to sodium chloride, bile salt and low-pH, growth at different incubation temperatures and *in vitro* antagonistic activity against selected pathogens. Two lactobacilli designated LAB 2 and 3with the highest score (100%) were eventually used for the formulation of probiotic drink. The two isolates designated LAB 2 and LAB 3 were identified by molecular methods as *Lactobacillus plantarum* and *Lactobacillus fermentum,* respectively, following the method of Janda and Abbott (2007).

Preparation of soymilk

Soymilk was prepared according to the method of Afroz et al. (2016), with little modification. Soybean was sorted and cleaned to remove stones and damaged, deformed seeds. Then the dry soybean was washed and soaked in water (500g in 1 L) for 12 h. It was then rinsed and blanched at 60°C in 1.25% $NaHCO_3$ for 30 minutes in order to remove the bitterness and anti-nutritional factors (trypsin inhibitor). The soybeans were washed, manually dehulled and rinsed. Prepared soybean seeds and water were mixed in the ratio of 3:1 (water to beans on a weight basis) and blended using a blender. The mixture was then filtered using a cheese cloth. The obtained filtrate was milk, while the residue was discarded. The obtained milk was then boiled for a 1 to 2 minutes. Fifty grams (50g) of white granulated sugar was added to the boiled milk to enhance its taste and the milk was subsequently bottled and stored at the room and the refrigeration temperature.

Preparation of tigernut milk

Tigernut milk was prepared according to the method of Kayode et al. (2017), with little modification. Tigernut extract was prepared by sorting out all unwanted objects and other rotten nuts, washed and

blanched at 60°C in distilled water and soaked overnight in water containing 0.5% sodium bicarbonate to soften the fibers. The water was changed 2-3 times to avoid bad smell. The soaked tigernuts (900g) with ginger (100g) were milled in a blender in the ratio of 3:1 (water to nuts on a weight basis).Ginger was added to give a peppery taste. The mash obtained was then sieved twice through a neat cloth to separate the extract. It was further strained to obtain a fine consistency. The filtered extract was heated at 90 °C for 15 minutes, sweetened, cooled to 4°C and refrigerated for further processes (Udeozor, 2012). The recipes for producing the non dairy milk are presented in Table 1.

Table 1. Recipes for the production of soybean and tigernut milk (Kayode et al. (2017); Afoz et al. (2016))

Ingredients	Soymilk	Tigernut milk
Soybean (g)	1000	0
Tigernut/ginger(g)	0	1000
Sugar(g)	50	50
Water(mL)	3000	3000

Preparation of probiotic drinks

Probiotic soy and tigernut milk were prepared by filling 20 sterile bottles each with 500 mL soymilk, and another 20 sterile bottles were filled with tigernut milk. Each of the 20 bottles was divided into four groups, each group containing 4 bottles of the drinks. These four groups were for:
group 1: control, without probiotic bacteria; group 2: *L. plantarum;* group 3: *L. fermentum*; and group 4: mix culture of *L. plantarum,* and *L. fermentum* in the ratio 1:1. For the inoculation, 1% standardized culture each of L. plantarum, L. fermentum and L. plantarum + L. fermentum under aseptic condition was poured into their respective bottles as indicated above. However, the control samples were left uninoculated. All four groups of bottle were then incubated at 37 °C for 8 h for fermentation (Amponsah et al., 2017).

Determination of reducing sugars of the drinks

Non-dairy probiotic milk sample (0.2mL) was pipetted into a test tube and made up to 2 mL with distilled water. Standard glucose (100g) was dissolved in 100 mL of distilled water to serve as stock for the standard. Ten millilitres (10 mL) of the stock solution was diluted with distilled water to 100 mL to serve as the working standard solution. Then, 0.2, 0.4, 0.6, 0.8 and 1 mL of the working standard solution were pipetted into different test tubes and made up to 2 mL with distilled water. Distilled water (2mL) was pipetted into a separate test tube and used as a the blank. Alkaline copper tartrate reagent (1 mL) was added into each test tube and the test tubes placed in a boiling water bath for 10 minutes. The test tubes were removed from the water bath and cooled. Thereafter, 1 mL of arsenomolybdate reagent was added and made up to 10 mL with distilled water. After 10 minutes the test tubes were placed into a spectrophotometer and the absorbance reading was taken at a wavelength of 620 nm. The amount of reducing sugar present in the sample was calculated from the standard curve plotted (AOAC, 2016).

Determination of mineral composition of the drinks

The mineral content of the samples was determined by the procedure of AOAC (2016). Magnesium, calcium, sodium, potassium, phosphorous, iron, manganese, and zinc were determined using the atomic absorption spectrometer (Thermo Scientific S Series Model GE 712354) after digestion with a nitric- perchloric acid mixture (AOAC, 2016). Prior to digestion, 0.50 g of soymilk and tigernut milk samples were weighed into a 125 mL Erlenmeyer flask with the addition of perchloric acid (4 mL), concentrated HNO_3 (25 mL) and concentrated sulphuric acid (2 mL). The contents were mixed and heated gently in a digester (Buchi Digestion unit K-424, Germany) at low to medium heat, on a hot plate under perchloric acid fume hood and heating was continued until dense white fume appeared. The heating continued strongly for half a minute and then the contents were allowed to cool, followed by the addition of distilled water (50 mL). The solution was allowed to cool and filtered completely with a wash bottle into a Pyrex volumetric flask. The solution was read on the atomic absorption spectrometer.

Determination of the anti-nutritional composition of the drinks

Total cyanide

The method used for this assay is the one obtained from AOAC (2016). Four grams (4g) of the samples were soaked in a mixture containing 40mL of distilled water and 2mL of orthophosphoric acid. It was then mixed, closed with a stopper and left overnight at room temperature to set free the bounded hydrocyanic acid. The resulting samples were transferred into distillation flask and a drop of paraffin was added (antifoaming agent) together with broken chips (antibumps). The content was filtered to other

distillation apparatus and distilled. About 45mL of the distillate was collected in the receiving flask that contains 40mL of distilled water with 0.1g of NaOH pellet. The distillates were then transferred into 50mL volumetric flask and distilled water was added to make it up to the 50mL mark. 20mL of distillate was collected and then placed in the conical flask. 1.6mL of 5% potassium iodide solution was added and titrated against 0.01M of silver nitrate solution until faint turbidity that persisted is obtained.

Determination of total phenol

The total phenol content of the extract was determined by the method of Singleton et al. (1999). The extract (0.2mL) was mixed with 2.5mL of 10% Folin Ciocalteau's reagent and 2mL of 7.5% sodium carbonate. The reaction mixtures were subsequently incubated at 45°C for 40min, and the absorbance was measured at 700nm in the spectrophotometer, using gallic acid as standard phenol.

Determination of total flavonoid

The total flavonoid content of the extract was determined using a colourimeter assay. Here, 0.2mL of the extract was added to 0.3mL of 5% $NaNO_3$ at zero time. After 5min, 0.6mL of 10% $AlCl_3$ was added and after 6min, 2mL of 1M NaOH was added to the mixture followed by the addition of 2.1mL of distilled water. Absorbance was read at 510nm against the reagent blank and flavonoid content was expressed as mg rutin equivalent (Meda et al., 2005).

Determination of alkaloid

This was determined according to the method described by Harbone (1998). 5g of the sample was weighed into a 250mL beaker and 200mL of 10% acetic acid in ethanol was added and allowed to stand for 4min.This was filtered and extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was an alkaloid, which was dried and weighed.

Determination of trypsin inhibitor

Trypsin inhibitor was determined by following the standard method described by AOAC (2016). Trypsin was extracted by mixing 1g of the sample with 50mL of 0.01N NaOH at pH of 8.4-10 and allowing the mixture to stand for 3h, while stirring at intervals. 2mL of diluted extract was then dispensed into test tubes to which 2mL of cold trypsin solution (4mg in 200mL of 0.001M HCl) was added, and the tubes were placed in water bath at 37 °C, 5mL of benzoyl-DL-arginine-P-nitroanilide hydrochloride (BAPNA) (40mg was dissolved in 1mL of dimethyl sulfoxide and diluted to 100mL with tris buffer 0.05M, pH 8 and diluted to 100mL with tris buffer 0.05M, pH 8.2, pre-warmed to 37 °C) was added as substrate to each tube. After 10 min, the reaction was terminated by adding 30% acetic acid and the content of each tube was thoroughly mixed. Thereafter, the content of each tube was centrifuged 3000rpm and the absorbance of the filtrate was measured at 410nm against reagent blank. The reference was prepared in the same way as the sample, except that 2mL of distilled water was added in place of an extract.

Determination of tannins

Sample of one gram each was weighed in a beaker. Each was soaked with solvent mixture (80 mL of acetone and 20 mL of glacial acetic acid) for 5 h to extract tannin. The samples were filtered through a double layer filter paper to obtain the filtrates which were stored for further use. A standard solution of tannic acid was prepared ranging from 10 ppm to 30 ppm. The absorbances of the standard solution, as well as that of the filtrates, were read at 500 nm on a Spectronic 20, England spectrophotometer (Evans, 2009).

Determination of phytates

Two grams of each sample of tigernut and soybean milk were weighed into a 250 mL conical flask. A total of 100 mL of 2% hydrochloric acid was used to soak each sample in a conical flask for3 h. This was filtered through a double layer of hardened filter paper Whatman No. 3. 50 mL of each filtrate was placed in 250 mL beaker and 107 mL of distilled water was added in each case. 10 mL of 0.3% ammonium thiocyanate solution was added into each solution as an indicator (Wheeler and Ferrel, 1971). This was titrated with standard iron (III) chloride solution, which contained 0.000195g iron per mL. The endpoint is slightly brownish yellow, which persisted for 5 minutes. The percentage phytates were calculated using the formula:

$$\%phytate = \frac{X \cdot 1.19 \cdot 100}{0.000195}$$

Where X = titre value.

Determination of saponin

The spectrophotometric method was used for saponin determination as described by Evans (2009). About 2g of the finely ground sample was weighed into a 250 mL beaker and 100mL of 50% isobutyl alcohol was added. Shaker was used to shake the mixture for about 5h to ensure uniform mixture. The mixture was thereafter filtered with No. 1 Whatman filter paper into a 100 mL beaker containing 20mL of 40% saturated solution of magnesium carbonate ($MgCO_3$). The mixture obtained was again filtered through No.1 Whatman filter paper to obtain a clean colourless solution. 1 mL of the colourless solution was added into 50mL volumetric flask using pipette, and 2mL of 5% iron (III) chloride ($FeCl_3$) solution was added. It was allowed to stand for 30 min for the colour to develop. The absorbance was read against the blank at 380 nm.

Determination of oxalate

Oxalate was determined by soaking 1g of the sample in 75mL of 1.5N H_2SO_4 for 1h and then filtered through a No. 1 Whatman filter paper. Filtrate (25mL) was aliquoted and placed inside a conical flask and this was titrated hot (80-90 ºC) against 0.1 M $KMnO_4$, until a pink colour that persisted for 15 sec appeared (Kakade et al., 1974).

Determination of antioxidant property

Antioxidant assays were carried out on the non dairy milk samples as presented below.

Ferric-reducing property of non-dairy milk

The ferric reducing properties of the extract were determined using the method described by Oyaizu (1986). 0.25mL of the extract was mixed with 0.25mL of 200mM of sodium phosphate buffer pH 6.6 and 0.25mL of 1% KFC. The mixture was incubated at 50 °C for 20min.Thereafter, 0.25mL of 10% TCA was also added and centrifuged at 2000 rpm for 10min. 1mL of the supernatant was mixed with 1mL of distilled water and 0.1% of $FeCl_3$ and the absorbance was measured at 700nm.

Free radical scavenging ability of non-dairy milk against DPPH

The free radical scavenging ability of the extract against DPPH (1,1-diphenyl-2-picryhydrazyl) was determined using the method of Gyamfi et al. (1999). 1mL of the extract was mixed with 1mL of 0.4mM methanolic solution of the DPPH. The mixture was left in the dark for 30min before measuring the absorbance at 516nm. DPPH scavenging ability was calculated using the formula below.

$$DPPH\ scavenging\ activity\ (\%) = \frac{|Ab - (As - Abs)|}{Ab \cdot 100}$$

Where Ab is absorbance of blank, Abs is absorbance of sample and DPPH and As is absorbance of the sample without DPPH.

Fe^{2+}chelation activity of non dairy-milk

The ability of the extract to chelate Fe^{2+} was determined using a modified method of by Puntel et al. (2005). Briefly, 150mM $FeSO_4$ was added to a reaction mixture containing 168mL of 0.1M Tris-HCl pH 7.4, 218mL saline and extract and the volume was made up 1mL with distilled water. The reaction mixtures were incubated for 5min, before the additional of 13mL of 1,10-phenanthroline after which the absorbance were read at 510nm. Fe^{2+}chelation ability was calculated using the formula below.

$$Fe^{2+}\ chelating\ effect\ (\%) = \frac{(Ab - As)}{Ab} \cdot 100$$

Where Ab is the absorbance of the blank without the sample, while As is the absorbance in the presence of sample.

Statistical analysis

The data obtained were compared using analysis of Variance (ANOVA), and the tests of significance were evaluated using Duncan's multiple range test at $p \leq 0.05$. The results obtained were computed as mean of triplicate ± standard deviation.

Results

Cumulative probiotic potential (CPP) of the isolated LAB

The results of the cumulative probiotic potential of the LAB isolates used in the probiotication of the non-dairy milk are presented in Table 2. Isolates LAB 2 and 3 had the highest score, which was 100% among the three isolated LAB, while LAB 1 had 75%.

Table 2. Cummulative probiotic potential (CPP) score (%) of the isolated *Lactobacilli*

Probiotic properties	**Grade**		**Isolated LAB and grade**		
			LAB 1	LAB 2	LAB 3
Tolerance to low pH	R=1	S=0	1	1	1
Tolerance to Bile salt	R=1	S=0	1	1	1
Antagonistic effect	Yes =1	No =0	1	1	1
Antibiotic susceptibility	R=0	S=1* NS (number of S)	5	8	8
Safe	Yes =1	No = 0	1	1	1
Total	12		9	12	12
Cummulative probiotic potential(%)	100		75	100	100

Keys: R=Resistant, S=Susceptible

Table 3. Effect of probiotication on the reducing sugar content of the drinks (%)

Type of Samples	**Varieties**	**Reducing Sugar**
Tiger nut	TM	4.83 ± 0.00^{c}
	T1	4.69 ± 0.07^{ab}
	T2	4.63 ± 0.03^{a}
	T3	4.74 ± 0.00^{b}
Soymilk	SM	4.95 ± 0.00^{c}
	S1	4.79 ± 0.05^{b}
	S2	4.81 ± 0.05^{bc}
	S12	4.76 ± 0.00^{a}

Data are presented as mean± standard error (where n=3). Values in the same column with the same superscripts are not significantly different at $p\leq 0.05$.

Keys:T1= tigernut milk probioticated with LAB 2; T2 =tigernut milk probioticated with LAB 3; T12 = tigernut milk probioticated with the mixture of LAB 2 and 3; S1 = soymilk probioticated with LAB 2; S2 = soymilk probioticated with LAB 3; S12 = soymilk probioticated with the mixture of LAB 2and 3; TM = non probioticated tigernut milk/control, SM = non-probioticated soymilk/control.

Table 4. Mineral composition (mg/100 mL) of the non-dairy milk and their probioticated variants

Samples	Calcium	Magnesium	Iron	Zinc	Phosphorus	Sodium	Manganese	Potassium
TM	98.74 ± 0.26^{d}	94.69 ± 0.31^{d}	0.81 ± 0.01^{a}	3.88 ± 0.02^{d}	111.48 ± 0.52^{d}	233.25 ± 0.75^{d}	38.57 ± 0.29^{a}	200.94 ± 0.53^{c}
TI	102.25 ± 0.95^{e}	99.69 ± 0.31^{e}	0.95 ± 0.00^{b}	3.99 ±0.01^{de}	112.22 ± 0.40^{e}	235.01 ± 0.57^{e}	39.79 ± 0.20^{c}	209.55 ± 0.45^{d}
T2	102.77 ± 0.23^{e}	109.11± 0.49^{f}	1.08 ± 0.01^{c}	4.08 ± 0.02^{e}	113.01 ± 0.57^{f}	239.06 ± 0.52^{f}	40.33 ± 0.04^{cd}	214.69 ± 0.30^{e}
T12	109.81 ± 0.19^{f}	112.48 ±0.52^{g}	1.30 ± 0.00^{d}	4.92 ± 0.08^{f}	112.96 ± 0.04^{e}	239.68± 0.32^{f}	44.00 ± 0.57^{d}	214.99 ± 0.00^{e}
SM	45.78 ± 0.22^{a}	54.93 ± 0.07^{a}	1.88 ± 0.01^{e}	0.99 ± 0.00^{a}	88.85 ± 0.15^{a}	96.81 ± 0.19^{a}	38.98 ± 0.02^{ab}	105.47 ± 0.29^{a}
S1	48.43 ± 0.57^{b}	55.68 ± 0.52^{a}	1.27 ± 0.03^{d}	1.16 ± 0.02^{b}	91.81 ± 0.75^{b}	97.77 ± 0.23^{ab}	41.06 ± 0.53^{d}	109.61 ± 0.39^{b}
S2	50.74 ± 0.26^{c}	59.72 ± 0.28^{b}	1.81 ± 0.03^{e}	1.95 ± 0.03^{c}	91.68 ± 0.32^{b}	98.80 ± 0.20^{b}	43.76 ± 0.24^{d}	109.81 ± 0.19^{b}
S12	51.35 ± 0.33^{c}	62.97 ± 0.03^{c}	2.08 ± 0.06^{f}	2.07 ± 0.07^{c}	97.31 ± 2.69^{c}	101.14 ± 0.46^{c}	45.69 ± 0.31^{e}	110.68 ± 0.32^{b}

Data are presented as mean± standard error (where n=3). Values in the same column with the same superscripts are not significantly different at $p \leq 0.05$
Keys: T1= tigernut milk probioticated with LAB 2; T2 =tigernut milk probioticated with LAB 3; T12 = tigernut milk probioticated with the mixture of LAB 2 and 3; S1 = soymilk probioticated with LAB 2; S2 = soymilk probioticated with LAB 3; S12 = soymilk probioticated with the mixture of LAB 2and 3; TM = non- probioticated tigernut milk/ control, SM = non-probioticated soymilk/control.

Table 5. Antinutritional composition (mg/100 mL) of the non-dairy milk and their probioticated variants

Types of samples	Varieties	Phenol	Flavonoid	Tannin	Saponin	Trypsin	Alkaloid	Phytic acid	Cyanide	Oxalate
Tigernut milk	**TM**	0.60±0.00^{a}	0.04±0.00^{e}	0.36±0.00^{a}	1.49±0.01^{a}	14.86±0.00^{d}	27.01±0.11^{g}	6.60±0.00^{a}	167.18±2.14^{e}	2.70±0.00^{g}
	T1	0.64±0.00^{b}	0.016±0.00^{a}	0.25±0.01^{a}	1.34 ±0.06^{a}	13.1±0.06^{c}	23.6±0.25^{e}	6.30±0.42^{c}	103.50±2.36^{d}	2.25±0.00^{f}
	T2	0.69±0.00^{c}	0.02±0.00^{b}	0.21±0.00^{a}	1.22±0.02^{a}	11.15±0.60^{b}	18.5±0.05^{b}	5.76±0.00^{a}	84.40±0.38^{c}	0.86±0.04^{bc}
	T12	0.78±0.00^{d}	0.02±0.00^{c}	0.20±0.01^{a}	1.12±0.10^{c}	9.04±0.00^{a}	16.58±0.02^{a}	5.18±0.42^{a}	77.2±0.22^{b}	0.45±0.00^{a}
Soymilk	**SM**	0.92±0.00^{e}	0.04±0.00^{f}	0.41±0.00^{b}	1.64±0.12^{a}	32.04±0.06^{g}	30.79±0.06^{h}	11.95±0.42^{d}	0.00±0.00^{a}	2.03±0.05^{e}
	S1	0.96±0.00^{f}	0.04±0.00^{f}	0.28±0.01^{b}	1.02±0.10^{b}	21.84±0.21^{f}	26.14±0.08^{f}	8.64±0.40^{b}	0.00±0.00^{a}	0.77±0.04^{b}
	S2	0.95±0.00^{f}	0.05±0.00^{g}	0.26±0.02^{a}	1.22±0.22^{b}	16.74±0.31^{e}	21.45±0.03^{d}	7.44±0.39^{e}	0.00±0.00^{a}	0.95±0.04^{c}
	S12	1.01±0.00^{g}	0.05±0.00^{g}	0.20±0.00^{a}	1.56±0.00^{d}	15.01±0.07^{d}	19.61±0.09^{c}	5.71±0.00^{c}	0.00±0.00^{a}	1.13±0.04^{d}

Date are presented as mean± standard error (where n=3). Values in the same column with the same superscripts are not significantly different at p≤ 0.05.
Keys: T1= tigernut milk probioticated with LAB 2; T2 = tigernut milk probioticated with LAB 3; T12 = tigernut milk probioticated with the mixture of LAB 2 and 3; S1 = soymil probioticated with LAB 2; S2 = soymilk probioticated with LAB 3; S12 = soymilk probioticated with the mixture of LAB 2and 3; TM = non- probioticated tigernut milk/ control, SM non-probioticated soymilk/control.

Effects of probiotication on the reducing sugar content of the drinks

Table 3 shows the effects of probiotication on the reducing sugars concentration of the non-dairy drinks. There were reductions in the reducing sugar content of the drinks compared to their non-probioticated counterpart. Probioticated tigernut milk sample (T2) had the least reducing sugar content (4.63 ± 0.03^{a}), while the highest was found in soy milk sample S2 (4.81 ± 0.05^{bc}).

Effects of probiotication on the mineral composition of the drinks

Probiotication was found to increase the mineral content of the drinks when compared to their non probioticated variant as presented in Table 4. For tigernut milk, the highest increase was seen in Sample T12 for calcium, magnesium, iron, zinc and manganese, which were found to increase from 98.74 to 109.81, 94.69 to 112.48, 0.81 to 1.30, 3.88 to 4.92 and 38.57 to 44.00 (mg/100mL) respectively. For phosphorous, the highest increase was seen in T2, which increased from 111.48 to 113.01mg/100mL. However, for sodium, both samples T2 and T12 had the highest increase since there was no significant difference in their phosphorous content at $p\geq0.05$. The probioticated soymilk (S12) was found to contain the highest mineral composition which increased from 45.78 to 51.35, 54.93 to 62.97, 1.88 to 2.08,0.99 to 2.07, 88.55 to 97.31, 96.81 to 101.14 and 38.98 to 45.69 (mg/100mL) for calcium, magnesium, iron, zinc, phosphorous, sodium and manganese, respectively.

Effects of probiotication on the antinutritional composition of the drinks

Probiotication of the non dairy drinks led to the reduction of all the anti-nutrients quantified apart phenol which was found to increase from 0.60 to 0.78 and from 0.92 to 1.01 in sample T12 and S12 for soymilk and tigernut milk respectively, when compared to their non probioticated counterpart (Table 5). The least reduction in antinutritional composition as a result of probiotication was seen in sample T12 which had a reduction from 0.04 to 0.02, 0.36 to 0.20, 1.49 to 1.12, 14.86 to 9.04, 27.01 to 16.58, 6.6 to 5.18, 167.18 to 77.2 and 2.70 to 0.45 (mg/100mL) for flavonoid, tannin, saponin, trypsin, alkaloid, phytic acid, cyanide and oxalate respectively. Similarly, for soymilk, the least reduction was observed in sample S12 which reduced from 0.41 to 0.20, 1.64 to 1.56, 32.04 to 15.01, 30.79 to 19.67, 11.95 to 5.71 and 2.03 to 1.13 (mg/100mL) for tannin, saponin, trypsin, alkaloid, phytic acid and oxalate respectively. The flavonoid content of soymilk increased from 0.04 to 0.05 in sample S2 and S12, but there were no significant difference between the flavonoid content of S1, S2 and S12 at $p\geq0.05$.

Table 6. Antioxidant properties of the non-dairy milk and their probioticated variants

Types of samples	Samples	FRAP mgAAE/g extract	DPPH (%)	Fechelating (%)
Tigernut milk	**TM**	0.57 ± 0.00^{a}	73.18 ± 0.06^{f}	36.30 ± 0.24^{e}
	T1	0.84 ± 0.00^{fg}	91.13 ± 0.06^{g}	39.62 ± 0.24^{g}
	T2	0.85 ± 0.00^{g}	91.70 ± 0.06^{h}	38.70 ± 0.24^{f}
	T12	0.84 ± 0.00^{f}	62.28 ± 0.06^{e}	37.90 ± 0.24^{d}
Soymilk	**SM**	0.62 ± 0.00^{b}	56.63 ± 0.06^{d}	2.39 ± 0.24^{a}
	S1	0.64 ± 0.00^{c}	66.41 ± 0.06^{a}	6.92 ± 0.24^{c}
	S2	0.83 ± 0.00^{e}	67.09 ± 0.06^{b}	5.73 ± 0.24^{b}
	S12	0.75 ± 0.00^{d}	64.43 ± 0.06^{c}	5.25 ± 0.24^{b}

Keys: T1= tigernut milk probioticated with LAB 2; T2 =tigernut milk probioticated with LAB 3; T12 = tigernut milk probioticated with the mixture of LAB 2 and 3; S1 = soymilk probioticated with LAB 2; S2 = soymilk probioticated with LAB 3; S12 = soymilk probioticated with the mixture of LAB 2and 3; TM = non- probioticated tigernut milk/ control, SM = non-probioticated soymilk/control.

Effects of probiotication on the antioxidant properties of the drinks

Probiotication increased the antioxidant property of the samples as shown in Table 6. The FRAP. DPPH, a and Fe^{2+} chelation content of tigernut milk increased from 0.5% to 0.84%, 73.18% to 91.70% and 36.30% to 39.02% in sample T12, T2 and T1, respectively. This same trend was observed in the soymilk as the DPPH, and FRAP increased from 0.62 to 0.75, 56.63 to 64.43mg TAE/g extract, respectively in sample S12. The Fe^{2+} chelation also increased from 2.39 to 6.92 in S1.

Discussion

The desire for new flavours and tastes among consumers has necessitated the development and production of probioticated drinks that are not-dairy milk based. These non-dairy probioticated drinks may also be important in addressing lactose intolerance in individuals that cannot produce β-galactosidase for the digestion of lactose in milk. This present study, therefore, reports the use of tigernut milk and soy milk in the production of non-dairy probiotic drinks, and also monitored the effects of probiotication on the reducing sugar, mineral, antinutritional and antioxidant properties of the drinks.

The cumulative probiotic potential (CPP) has been suggested as improved criterion for probiotic validation (FAO/WHO, 2002). The CCP obtained for the two Lactobacilli, *Lactobacillus fermentum* and *Lactobacillus plantarum*, used in this study was 100%. A CPP of 100% (Table 2) was earlier observed for *Lactobacillus plantarum* and some other isolates from curd (Halder et al., 2017). A decrease in reducing sugar was also observed in this study. This might be due to the fact that probiotic microorganisms use monosaccharides like the available reducing sugars such as fructose, glucose, and galactose for energy production (Mohamed et al., 2019).

Plant seeds harboured some protective natural compounds known as anti-nutrients which serve as a defence mechanism against their seeds being eaten or to protect them against harsh environments or damage by microbes (Chikwendu et al., 2014). Series of researches have proved that anti-nutrients generally reduce nutrient utilization in food (Lopez et al., 2002; Okoye and Ene, 2018). Results obtained from this study revealed reductions in phytic acid, tannin, saponin, trypsin, oxalate, cyanide, and flavonoid of the probioticated drinks. This decrease in the level of phytic acid during fermentation may be attributed to the action of the enzyme phytase released during fermentation, which degrades phytate (Oyetayo and Oyetayo, 2013). Additionally, the decrease in the tannin levels in all probioticated drinks may also be attributed to the hydrolysis of tannin complexes during fermentation and their solubility in water. Oxalates affect calcium, magnesium and protein metabolism in men. They also may react with calcium to form calcium oxalates which are responsible for the formation of kidney stones in humans.

The nondairy milk from soybean and tigernut are rich in mineral elements (Table 4). These mineral elements are very important for cell functions at biological, chemical and molecular levels (Prashanth et al., 2015), but in some foods majority of these minerals are not utilizable. Probiotication by fermentation has been found to improve the bioavailability of minerals in food and the result from this research shows a significant increase in the mineral content of the probioticated drinks as against the control (Table 4). This increase might be the result of the release of minerals through the dephosphorylation of phytate in which the removal of phosphate groups from the inositol ring decreases the mineral binding strength of phytate and hence improves the availability of minerals (Gupta et al., 2015).

Previous researches have demonstrated that the antioxidative activity of fermented soy foods, such as miso, natto, and tempeh, was remarkably stronger than unfermented steamed soybean (Achoribo and Ong, 2019). Similarly, in this research the antioxidant properties of the probioticated drinks assessed through DPPH and iron chelation properties showed an increase in the antioxidant activity and a slight increase in FRAP, when compared to unfermented samples (SM and TM). Increase in total phenol may be responsible for this. Velioglu et al. (1998) had earlier reported that antioxidant activity of plant materials was well correlated with the content of their phenolic compounds. The observation is similar to the result of Sebastian et al. (2018), who reported a three-fold increase in the antioxidant property of the fermented soymilk as against the unfermented. This increase may be due to the increase of isoflavones in aglycone form during fermentation process (Roselló-Sotoet al., 2019). Isoflavones are known to protect cells from the damaging effects of free radicals (Vij et al., 2011). Main isoflavones in soybean are in glucosides form.However, in fermented soy food like miso, natto, soy sauce, or fermented soymilk, isoflavone glucosides are hydrolyzed using α-glucosidases into aglycone form. Isoflavones in aglycone form are absorbed faster and are higher in amount than their glucosides in humans (Marguerite et al., 2019).

Conclusions

The results obtained from this study revealed the reduction in the reducing sugars and antinutrient contents of the probioticated non-dairy drinks. However, a significant increase was observed in the mineral content and antioxidant properties of the probioticated non-dairy drinks. The production of soymilk and tigernut milk and the probiotication process resulted in the production of probiotic drinks that meet standard requirements. This, in essence, will makethese non-dairy probiotic drinksready to compete the traditional probiotic yoghurts in the market. Furthermore, probioticated tigernut and soymilk drinks will also be ideal for vegetarians and individuals suffering from lactose intolerance.

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