RJM Research Journal of Microbiology

OPEN ACCESS

Production and Assessment of Sensory Properties of Probioticated Tigernut and Soybean Milk Stored Under Ambient and Refrigerated Temperatures

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ABSTRACT

Background and Objective: The health benefits of probiotics have increased consumer's demand for probiotic foods. Several food products suggested as delivery vehicles for probiotics to consumer. The production and sensory properties of probioticated tigernut and soybean milk stored under ambient and refrigerated temperatures were investigated. **Materials and Methods:** Lactic Acid Bacteria (LAB) isolated from tigernut and soybean was assessed for probiotics properties. LAB 2 and 3 were identified as *Lactobacillus fermentum* and *L. plantarum*, respectively and found to possess 100% Cumulative Probiotic Potential (CPP) was used to prepare probioticated drinks. **Results:** Probiotication of the drinks with the two probiotics resulted in the reduction of pH of the drinks and an increase in Total Titratable Acidity (TTA). The probioticated drinks had a higher score for all sensory attributes assessed which made them compare favourably with dairy yoghurt as against un-probioticated drinks. The LAB count of the stored probioticated drink increased during the period of storage while coliforms were not detected before and during the period of storage. The probioticated tigernut and soybean drinks were also generally acceptable compared to the un-probioticated counterparts. **Conclusion:** Findings from this study revealed that probiotic milk from soybean and tigernut was accepted based on its sensory attributes and may have promising commercial potential for vegetarians and individuals suffering from lactose intolerance.

KEYWORDS

Production, non-dairy, milk, lactic acid bacteria, probioticated, sensory

INTRODUCTION

A growing public awareness of diet-related health issues and mounting evidence regarding the health benefits of probiotics have increased consumer's demand for probiotic foods[1-3]. Several food products including yoghurt, frozen fermented dairy desserts, spray-dried milk powder, cheeses, ice cream, freeze-dried yoghurt and fruit juices have been suggested as delivery vehicles for probiotics to consumer[1,4-6]. However, for the successful application of probiotics in foods, the probiotic used should be technologically compatible with the food and the food-manufacturing process[6,7]. The food itself should be able to maintain stability and viability of the probiotics during gastrointestinal transit[7,8], in addition to these, the

ISSN: 1816-4935 (Print)
https://doi.org/10.3923/rjm.2022.14.27

Received: 28 May 2022
Accepted: 17 Aug. 2022
Published: 01 Oct. 2022

foods containing the probiotic bacteria must maintain the characteristic sensory attributes of the traditional food, with this in mind, the most popular food delivery systems for probiotic have been fermented milk and yoghurt[1,6].

Although milk and milk products have been generally accepted as the best probiotic food carrier, some of the limitations associated with this product are, the inability to digest lactose in milk due to the absence of β-galactosidase, an enzyme needed for digestion of lactose in some individuals, the presence of high level of unsaturated fat and cholesterol, allergic reaction to the milk protein in some individual, unsuitability of the food for vegetarians and relatively high cost as compared to other products[1,9].

Plant-based products have been suggested as an alternative probiotic food carrier to dairy milk due to their low cost, availability, suitability for vegetarians and health benefits[10-12]. Moreover, 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 yoghurt (a milk product)[1,11]. Considering the limitations associated with dairy-based probiotics, this research was therefore designed to produce and assess the sensory properties of non-dairy probiotic milk using soybean and tigernut milk as carriers for the delivery of locally isolated probiotics and lactic acid bacteria.

MATERIALS AND METHODS

Study area: The study was carried out in Akure City, Ondo State from January, 2019 to June, 2019. Akure City is the capital of Ondo State which is one of the 36 states in Nigeria, it lies about 70°15' North of the equator and 50°15' East of the Meridian.

Sample collection: Soybean and tigernuts were purchased from Adedeji Market, Akure Ondo State Nigeria. They were sorted and graded to remove dirt and debris. Other samples which include, industrially produced soymilk, local/street vended soymilk, FUTA (Food Science and Technology Department)-produced tigernut milk, dairy milk and dairy yoghurt were also purchased in triplicates from street vendors, market places and beverage stores in different locations in Akure, Ondo State. After purchase, they were then refrigerated before the commencement of the analysis.

Preparation of soymilk: Soymilk was prepared according to the method of Afroz *et al.*[12], 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 (500 g in 1 L) for 12 hrs. It was then rinsed and blanched at 60°C in 1.25% $NaHCO_3$ for 30 min to remove the bitterness and anti-nutritional factors (trypsin inhibitor). The soybeans were washed, manually dehulled and rinsed. The soybean seeds were blended using a blender in a ratio of 3:1 (water to beans on a weight basis). The mixture was filtered using a cheesecloth, the filtrate is obtained as the milk while the residue was discarded. The milk obtained was then brought to a boil. Fifty grams of white granulated sugar was added to taste and the drinks were subsequently bottled and stored at ambient and refrigeration temperature.

Preparation of tigernut milk: Tigernut milk was prepared[13], 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 fiber, the water was changed 2-3 times to avoid bad smell. The soaked tigernuts with ginger were milled in a blender at the ratio of 3:1 (water to nuts on a weight basis). The mash obtained was then sieved twice with 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 min, sweetened, cooled to 4°C and refrigerated for further processes[14].

Isolation of microorganisms: Before the isolation, the raw materials which were tigernuts and soybean were each macerated using a sterile mortar and pestle. The different drinks samples purchased and the ones prepared were left to get accustomed to room temperature and shaken vigorously to suspend microbial content. Nine millilitres of distilled water was dispensed each into 10 clear test tubes and sterilized by autoclaving. After sterilization, each sample was serially diluted using sterile distilled water as diluents by weighing 1 g or 1 mL of the sample into the sterilized water, after which 1 mL of both 10^{-8} and 10^{-10} diluents were plated into the six different mediums (NA, PDA, EMB, MSA, SSA and MRS) in triplicate using the pour plate method. The plates were incubated at an appropriate temperature and time suitable for the organisms to be isolated[15,16].

After incubation, the colonies that developed on the nutrient agar plates were counted and used to determine the total viable count of the samples (CFU mL^{-1}). This was done by multiplying the counted numbers of colonies by the dilution used. The representative colonies or the distinct colonies on the different medium plates were then purified by sub-culturing on fresh nutrient agar using the streaking method to obtain pure cultures of the isolates. The pure cultures obtained were observed for their morphological characteristics and were then transferred into nutrient agar slants for further biochemical identification.

Isolation of lactic acid bacteria: Lactic Acid Bacteria (LAB) strains were isolated from the samples and prepared drinks were stored at ambient and refrigerated temperature. Here, 1 mL of each of the samples was diluted to appropriate 8-fold dilutions, isolation of LAB was carried out by plating 1 mL of the 5th and 8th diluents using the pour plate method on MRS agar and incubated at 30°C for 48 hrs. The number of the colony was counted for the total LAB count after incubation. The cultures were purified by repeated streaking. The process was repeated at the 1 week interval for 4 weeks. The isolates were maintained on an MRS agar slant and kept at 4°C.

Assessment of probiotic properties of *Lactobacilli* isolates: The probiotic potential of the isolated *Lactobacilli* was determined according to the method described by Prabhurajeshuwar and Chandrankanth[17] and Jung *et al.*[18]. 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.

Safety assessment of *Lactobacilli* isolates: The safety profile of the isolates of *Lactobacillus* was determined *in vitro* by their hemolytic activity, coagulase activity, DNase activity, gelatin liquefaction and antibiotic sensitivity[19,20]

Molecular identification of *Lactobacilli* with the highest probiotic potential: The *Lactobacilli* isolates with the highest probiotic potential were further characterized and identified using the DNA sequencing technique. The identification procedure is based on 16S rRNA gene sequence similarity[21].

Preparation of probioticated drinks: Probiotic soymilk and tigernut 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 4 groups, each group containing 4 bottles of the drinks. These four groups were for:

- **Group 1:** Control
- **Group 2:** *Lactobacillus plantarum*
- **Group 3:** *Lactobacillus fermentum*
- **Group 4:** Mix the culture of *L. plantarum* and *L. fermentum* in a ratio of 1:1

For the inoculation, 1% standardized culture each of *L. plantarum*, *L. fermentum* and *L. plantarum+L. fermentum* under aseptic conditions was introduced into their respective bottles as indicated above. However, the control samples were left un-inoculated and all the jars were then incubated at 37°C for 8 hrs for fermentation[1].

Physicochemical characteristics of the probioticated drinks: The pH and Titratable Acidity (TTA) of the probioticated milk were determined[22].

Determination of proximate composition of the probioticated non-dairy milk: The moisture content, ash content, crude protein, crude fat and crude fibre were determined using the methods of Wu and Wu[23]. Carbohydrate (CHO), a soluble carbohydrate was calculated by subtracting the sum of the percentage contents of moisture, crude protein, crude lipid, ash and crude fibre from 100.

Sensory evaluation probioticated non-dairy milk: The previous methods[13,24] were used to evaluate the organoleptic characteristics (colour, taste, flavour and texture) and overall acceptability of the probioticated non-dairy milk. Fifteen panellists comprised of microbiology undergraduate students, students from other Departments and some teaching and non-teaching staff members of Federal University of Technology Akure, Ondo State, Nigeria who are conversant with the products were used. A nine-point hedonic scale ranging from excellent or extremely like (score = 9) to very poor or extremely dislike (score = 0) was adopted[24,25]. The samples were presented in random order and the panellists were asked to pour a little of the product in their mouth for some seconds and grade it based on what they thought suitable to qualify it and water was used for rinsing the mouth in between samples.

Microbiological quality of the probioticated drinks: The microbiological quality of the product was determined by checking for the presence of coliforms in the drink and monitoring its microbial load during the storage period using the pour plate method according to the method of Adebayo-Oyetoro *et al.*[13], Yoon *et al.*[26] and Awaisheh *et al.*[27].

Statistical analysis: The data obtained were statistically analysed using SPSS version 20, the results 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 the mean of triplicate±standard deviation.

RESULTS

Total viable, total lactic acid bacteria, coliform and fungal counts of the samples: The total viable counts of both soybean and tigernut under different treatments, as well as those of t he drinks produced and purchased, are presented in Fig. 1. The unwashed tigernut had the highest bacterial load of 2.9×10^8 CFU g^{-1} and the least was seen in the blanched soybean 1.0×10^7 CFU g^{-1}. Unwashed tigernut (UT) had the highest count of 8.5×10^7 CFU mL^{-1} of LAB while the blanched soybean and tigernut (BB, BT) had no LAB growth. Moreover, the unwashed tigernut had the highest coliform count (1.2×10^7 CFU g^{-1}) and the blanched raw material had no coliform bacteria.

However, among the drinks analysed, the locally produced/street vended soymilk (LSM) had the highest bacterial count of 2.0×10^8 CFU mL^{-1} while the one produced by the well-established manufacturer had no growth in Fig. 2. However, as shown in Fig. 2, Dairy Yoghurt (DY) had the highest LAB count (4.0×10^7 CFU mL^{-1}). On the other hand, Dairy Milk (DM) and Industrially Produced Soymilk (ISM) had no LAB growth. The LSM has the highest coliform count (2.0×10^7) CFU mL^{-1}, while there were no coliforms in the other sample. The fungal counts of soybean and tigernut and the milk obtained from them are also presented in Fig. 1 and 2, respectively.

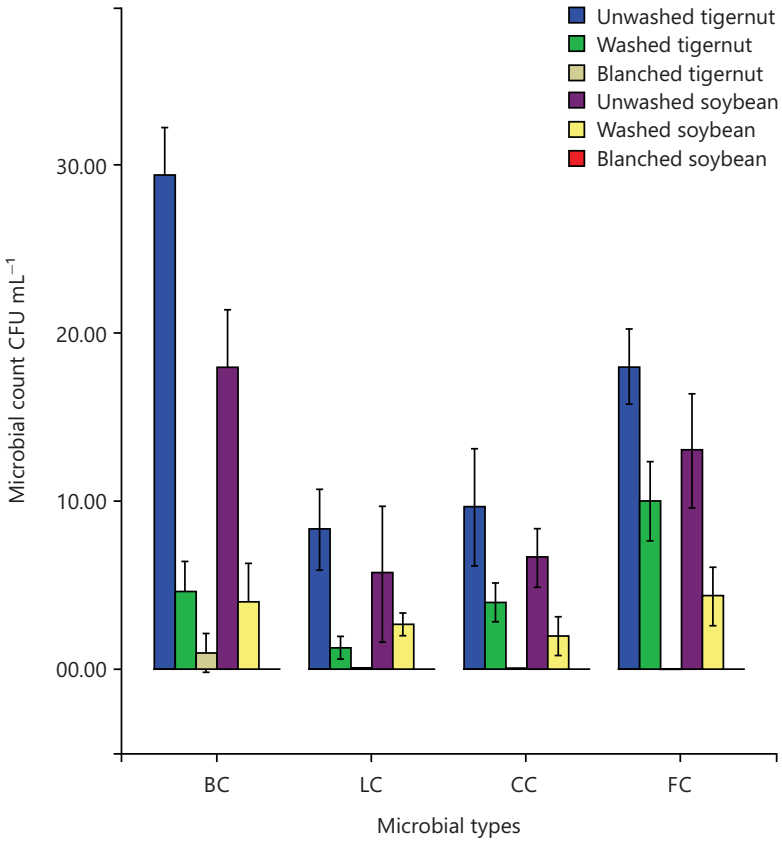

Fig. 1: Microbial load of the raw materials

BC: Bacterial count, LC: Lactic acid bacterial count, CC: Coliform count and FC: Fungal count

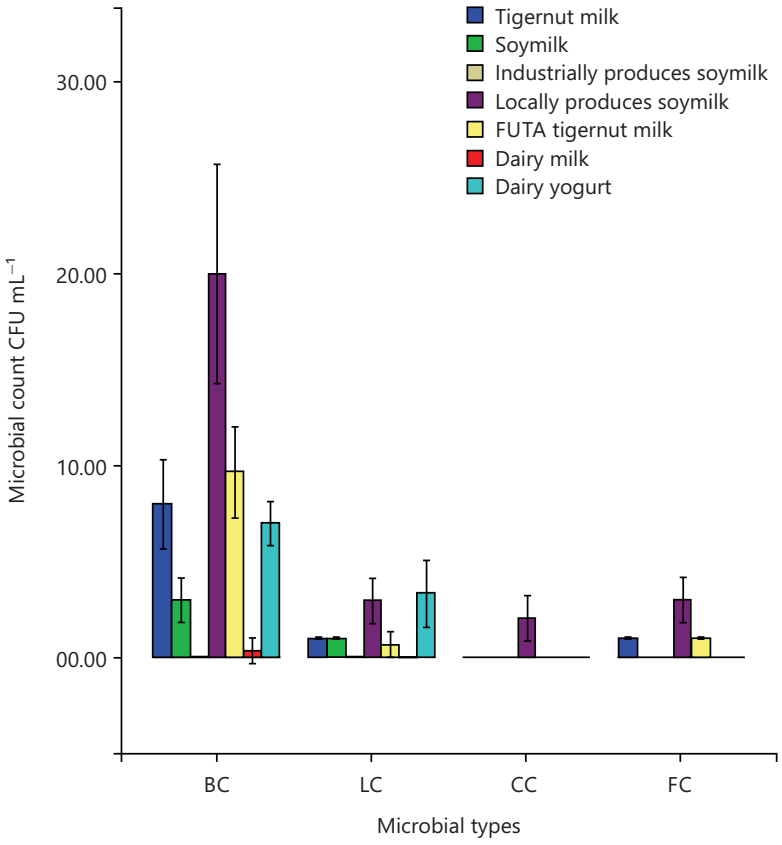

Fig. 2: Microbial load of the prepared and purchased milk and yoghurt samples

BC: Bacterial count, LC: Lactic acid bacterial count, CC: Coliform count and FC: Fungal count

Biochemical characteristics of Lactic Acid Bacteria (LAB) isolates: The biochemical of Lactic Acid Bacteria (LAB) isolates is shown in Table 1. The tentative identity of the isolates from their biochemical characteristics are *Lactobacillus casei*, *L. fermentum* and *L. plantarum*.

Probiotic property of isolated lactic acid bacteria: The probiotic properties of the isolated lactic acid bacteria were determined according to their tolerance to bile, acid, salt, growth at various temperatures and their antagonistic effect on pathogenic bacteria. The salt tolerance of isolates is presented in Table 2. It was observed that all the isolates tolerated 2, 3.5 and 6% salt concentration for 24 hrs of incubation. The acid tolerance assessment revealed that only LAB 3 survived for 2 hrs at pH 2, others did not grow at this pH but they all grew well at pH 3-8 in Table 3.

The influence of incubation temperature on the growth of LAB isolates also revealed that all grew at an incubation temperature of 15 and 37°C, however, only LAB 1 grew evidently at 45°C incubation temperature as shown in Table 4. Table 5 shows the bile salt tolerance of isolates. All the isolates tolerated bile salt concentration of 0.1-0.4% for 24 hrs while none of the isolates survived in 0.5% bile salt concentration.

Inhibitory property of the isolated LAB on selected pathogens: The inhibitory effect of LAB isolates on selected pathogens is shown in Fig. 3. The LAB isolate designated LAB 3 had a good inhibitory effect on all the isolates with the highest effect on *Escherichia coli* (18 mm) and the least effect was seen against *Pseudomonas aeruginosa* (12 mm).

Antibiotic sensitivity pattern of the isolated LAB: The sensitivity pattern of isolated LAB to different antibiotics is shown in Table 6. LAB isolate designated LAB 1 was found to be resistant to Streptomycin, Gentamycin and Chloramphenicol while LAB 2 and LAB 3 were found to be susceptible to all the tested antibiotics.

Safety of the isolated LAB: Table 7 shows results obtained for the *in vitro* assessment of the safety of the LAB isolates. All isolated LAB isolates tested negative for coagulase, haemolysis, DNase and gelatin liquefaction.

Table 1: Biochemical characteristics of Lactic Acid Bacteria (LAB) isolates

Isolates	Starch hydrolysis	NH_3 from arginine	Nitrate reduction	Rhamnose fermentation	Probable isolate
LAB 1	-	-	-	-	*Lactobacillus casei*
LAB 2	+	+	+	-	*Lactobacillus fermentum*
LAB 3	-	-	+	+	*Lactobacillus plantarum*

Table 2: Tolerance of the isolated LAB to different salt concentrations

	2% NaCl					3.5% NaCl					6% NaCl				
Isolates	1 hr	2 hrs	3 hrs	4 hrs	24 hrs	1 hr	2 hrs	3 hrs	4 hrs	24 hrs	1 hr	2 hrs	3 hrs	4 hrs	24 hrs
LAB 1	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
LAB 2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LAB 3	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-

Table 3: Growth of isolated LAB at different incubation temperatures

	15°C					30°C					45°C				
Isolates	1 hr	2 hrs	3 hrs	4 hrs	24 hrs	1 hr	2 hrs	3 hrs	4 hrs	24 hrs	1 hr	2 hrs	3 hrs	4 hrs	24 hrs
LAB 1	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
LAB 2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LAB 3	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-

Table 4: Tolerance of isolated LAB to different Acid concentration (pH)

	Acid concentration																								
	2					3					4					5					8				
Isolates	1 hr	2 hrs	3 hrs	4 hrs	24 hrs	1 hr	2 hrs	3 hrs	4 hrs	24 hrs	1 hr	2 hrs	3 hrs	4 hrs	24 hrs	1 hr	2 hrs	3 hrs	4 hrs	24 hrs	1 hr	2 hrs	3 hrs	4 hrs	24 hrs
LAB 1	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LAB 2	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LAB 3	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 5: Tolerance of isolated LAB to different bile salt concentrations

	Bile concentration																								
	0.1%					0.2%					0.3%					0.4%					0.5%				
Isolates	1 hr	2 hrs	3 hrs	4 hrs	24 hrs	1 hr	2 hrs	3 hrs	4 hrs	24 hrs	1 hr	2 hrs	3 hrs	4 hrs	24 hrs	1 hr	2 hrs	3 hrs	4 hrs	24 hrs	1 hr	2 hrs	3 hrs	4 hrs	24 hrs
LAB 1	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LAB 2	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LAB 3	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

https://doi.org/10.3923/rjm.2022.14.27

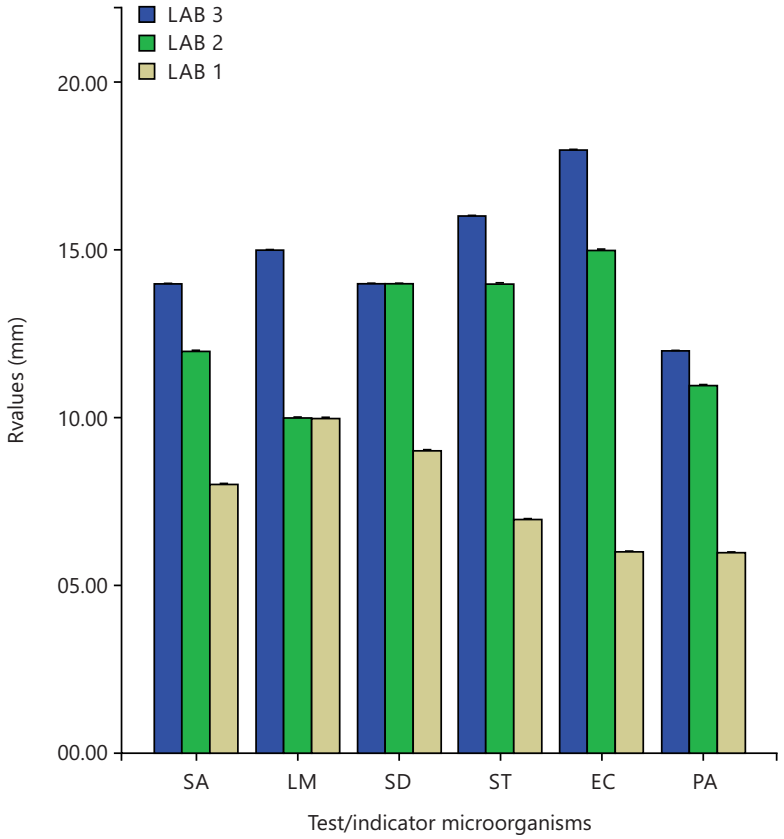

Fig. 3: Inhibitory effect of the isolated *Lactobacilli* against test bacteria

R: Width of clear zone, SA: *Staphylococcus aureus*, SP: *Streptococcus pyogens*, SD: *Shigella dysenteriae*, EC: *Escherichia coli* and PA: *Pseudomonas aeruginosa*

Table 6: Antibiotic sensitivity of the isolated LAB

	VN	E	S	CPX	GN	CH	TET	AMP
	S≥13 mm	S≥18 mm	S≥15 mm	S≥21 mm	S≥15 mm	S≥18 mm	S≥15 mm	S≥17 mm
Isolates	R≤12 mm	R≤13 mm	R≤12 mm	R≤15 mm	R≤12 mm	R≤12 mm	R≤11 mm	R≤16 mm
LAB 1	S	S	R	S	R	R	S	S
LAB 2	S	S	S	S	S	S	S	S
LAB 3	S	S	S	S	S	S	S	S

VN: Vancomycin, E: Erythromycin, S: Streptomycin, CPX: Ciprofloxacin GN: Gentamycin, CH: Chloramphenicol, TET: Tetracyclin and AMP: Ampicillin

Cumulative Probiotic Potential (CPP) of the isolated LAB: The results of the cumulative probiotic potential of the LAB isolates are presented in Table 8 isolate LAB 2 and 3 had the highest score which was 100% among the three isolated LAB. While LAB 1 had 75%.

Molecular identity of the isolated LAB: The molecular identity of LAB 2 and 3 are presented in Table 9. The lengths of amplified products or amplicons were 1561 bp and 1418 bp for LAB 1 and LAB 2, respectively. The BLAST analysis revealed *Lactobacillus fermentum* (LAB 1) *and Lactobacillus plantarum* (LAB 2) were closely related to *Lactobacillus fermentum* strain CIP 102980 (99.89%) and *Lactobacillus plantarum* strain CIP 103151 (99.80%), respectively. These two isolates were further characterized molecularly to their specie level and were used for the production of the probioticated drinks.

Sensory characteristics of the dairy and non-dairy milk products: Sensory characteristics of the entire samples, tigernut milk, soymilk, industrially produced soymilk, FUTA Tigernut Milk (FTM), Dairy Milk (DM), Dairy Yoghurt (DY), probioticated tigernut milk and soymilk (T1, T2, T12, S1, S2 and S12) is presented in

Table 7: Safety profile of the isolated Lactic Acid Bacteria (LAB)

	Safety parameters			
Isolates	Coagulase	Haemolysis	Dnase	Gelatin liquefaction
Lab 1	-	-	-	-
Lab 2	-	-	-	-
Lab 3	-	-	-	-

Key: Negative

Table 8: Cumulative Probiotic Potential (CPP) score (%) of the isolated *Lactobacilli*

	Grade		Isolated LAB and grade		
Probiotic properties			LAB 1	LAB 2	LAB 3
Tolerance to low pH	R=1	S=0	1	1	1
Tolerance of 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
Cumulative probiotic potential (%)	100		75	100	100

R: Resistant and S: Susceptible

Table 9: Molecular Identity of probiotic LAB isolates

Given name	Biochemical identities	Molecular identities of the closest relative	Accession number of the closest relative	Percentage similarity
LAB 1	*Lactobacillus fermentum*	*Lactobacillus fermentum* strain CIP 102980	NR_104927.1	99.89
LAB 2	*Lactobacillus palntarum*	*Lactobacillus plantarum* strain CIP 103151	NR_104573.1	99.89

Table 10: Coliform count of the non-dairy milk and their probioticated variants before and after storage at both refrigerated and ambient temperature (CFU mL^{-1})

		Coliform count at ambient temperature (CFU mL^{-1})		Coliform count at refrigeration temperature (CFU mL^{-1})	
Sample types	Varieties	Before storage	After storage	Before store	After storage
Tigernut milk	TM	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a
	T1	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a
	T2	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a
	T12	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a
Soymilk	SM	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a
	S1	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a
	S2	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a
	S12	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a	0.0±0.0^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 $p \leq 0.05$, T1: Tigernut milk probioticated with LAB 1, T2: Tigernut milk probioticated with LAB 2, T12: Tigernut milk probioticated with the mixture of LAB 1 and 2, S1: Soymilk probioticated with LAB 1, S2: Soymilk probioticated with LAB 2, S12: Soymilk probioticated with the mixture of LAB 1 and 2, TM: Non-probioticated tigernut milk/control and SM: Non-probioticated soymilk/control

Fig. 4. The DM and DY have the highest score of 8.00 and the least score for colour was observed in TM and FTM (6.33). Samples T1 and T2 have the highest score of 8.7 while sample ISM and FTM have the least score of 6.33 in terms of taste. In addition, sample DY had the highest score in flavour (8.7) while sample S2 has the least score (6.33). For texture, samples ISM, DM, DY, T1 and S1 have the highest score of 8.0, while S2 has the least score of 6.66. The overall acceptability revealed that samples T1 and T12 have the highest score of 8.7 while the least score was recorded in TM and FTM (7.0).

Microbiological quality of probioticated drink: Table 10 showed the microbial quality of probioticated non-dairy milk. Coliforms were absent in the probioticated milk stored at ambient and refrigerated

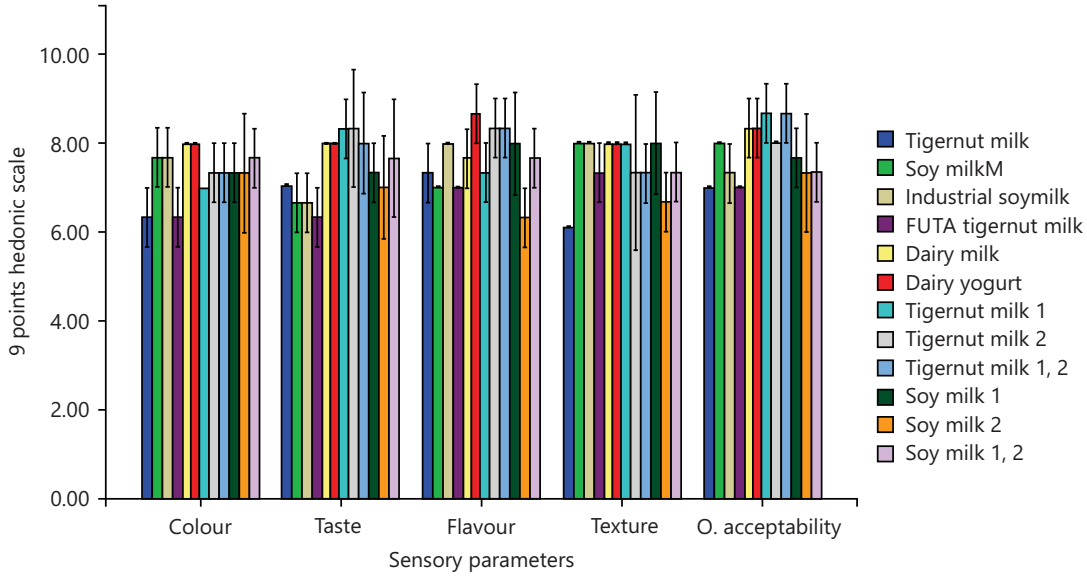

Fig. 4: Sensory evaluation of dairy milk and yoghurt, non-dairy drinks and their probioticated variants

Table 11: Total lactic acid bacteria count of the stored refrigerated drinks (10^8 CFU mL^{-1})

Type of samples	Varieties	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5
Tigernut	Control	1.00±0.58^{a}	1.00±0.00^{a}	1.33±0.33^{a}	1.00±0.00^{a}	0.33±0.33^{a}	0.67±0.33^{a}
	T1	5.33±0.33^{b}	6.33±0.33^{b}	7.00±0.00^{c}	7.33±0.33^{c}	7.00±0.00^{b}	6.67±0.58^{b}
	T2	5.67±0.33^{b}	6.00±0.00^{b}	6.33±0.33^{bc}	7.00±0.00^{bc}	7.00±0.00^{b}	6.33±0.58^{b}
	T12	7.33±0.33^{c}	6.33±0.33^{b}	6.00±0.00^{b}	6.33±0.58^{b}	6.33±0.33^{b}	7.00±0.00^{b}
Soymilk	Control	0.33±0.33^{a}	0.67±0.67^{a}	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}
	S1	5.67±0.33^{b}	6.00±0.00^{b}	6.33±0.33^{b}	6.00±0.00^{b}	6.67±0.33^{b}	7.00±0.00^{a}
	S2	6.00±0.00^{b}	7.00±0.00^{b}	6.00±0.00^{b}	6.00±0.00^{b}	6.67±0.33^{b}	7.00±0.00^{ab}
	S12	7.00±0.00^{c}	6.67±0.33^{b}	6.00±0.00^{b}	7.00±0.00^{b}	6.33±0.33^{b}	7.00±0.00^{b}

Data are presented as mean±standard error (where, n=3), values with the same superscripts are not significantly different at $p \leq 0.05$, T1: Tigernut milk probioticated with LAB 1, T2: Tigernut milk probioticated with LAB 2, T12: Tigernut milk probioticated with the mixture of LAB 1 and 2, S1: Soymilk probioticated with LAB 1, S2: Soymilk probioticated with LAB 2, S12: Soymilk probioticated with the mixture of LAB 1and 2, TM: Non-probioticated tigernut milk/control and SM: Non-probioticated soymilk/control

temperatures. However, the microbial load of the same drink stored at different temperature varies. There was no coliform in all the samples in Table 10 while, the lactic acid bacteria count ranged from 0.33×10^8 to 7.33×10^8 CFU mL^{-1} in Table 11.

DISCUSSION

This study evaluated the suitability of tigernut and soymilk in the production of non-dairy probiotic drinks and also monitored the effect of probiotication on microbial and sensory properties of the non-dairy milk. From the microbiological evaluation of the raw materials, it was observed that the total bacteria, lactic acid bacteria, coliform and fungi count of the unwashed tigernuts and soybean sample is higher than their washed and blanched counterpart. This high microbial load could be a result of pre-harvest contamination of both soybean and tigernut from the soil where they were harvested or due to postharvest contamination from the market or the handlers[28].

Locally vended soymilk had the highest microbial count and this high viable microbial load in these drinks could be attributed to inadequate hygienic measures in production or inadequate processing recontamination or non-aseptic handling and inadequate heat treatment[29]. Although the high viable bacterial counts obtained do not usually constitute a health risk they can serve as an indication of an overall lack of hygiene[28]. The presence of coliform in a particular sample can be used as an indicator to

determine its quality, safety and integrity[30]. However, the presence of coliforms in the unwashed raw materials and the street-vended soymilk constitute a public health concern as it could indicate the presence of other pathogenic organisms.

Three Lactic Acid Bacteria (LAB), *Lactobacillus casei*, *L. fermentum* and *L. plantarum*, were isolated from the raw soybean and tigernut and their milk. Sebastia *et al.*[28] had earlier isolated similar organisms from tigernut milk and other dairy products collected from Akure.

Microorganisms with potential probiotics application must survive in the bile salts environment of the intestine to play beneficial roles in the gut[31,32]. The three LAB isolates were able to tolerate and survived in growth medium supplemented with 0.1-0.4% concentration of bile salt, however, none survived in a higher concentration of bile salt. The result obtained is similar to the observation of Fijad[31]. This is expected and essential for probiotics since the physiological bile salts concentration of 0.3% is a level achieved normally in the human intestine.

The LAB isolates in this study were able to tolerate acidic pH of 3-5 and a basis pH of 8, however, only *Lactobacillus plantarum* was found to tolerate pH 2 for two hours of incubation. However, *Lactobacillus casei* and *Lactobacillus fermentum* present no visible growth at this pH. This is observation is contrary to the result of Halder *et al.*[33], who reported that *Lactobacillus plantarum* was unable to tolerate this low level of pH 2. The three LAB isolates also grew well at the lower temperature of 15 and 30°C, while *Lactobacillus casei* and *Lactobacillus plantarum* showed no growth at 45°C.

All the LAB isolates show a good inhibitory effect against spoilage and pathogenic microorganisms. *Lactobacillus plantarum* was found to antagonize all the test bacteria with the highest inhibition against *E. coli* (18 mm). This inhibition could be a result of the high amount of lactic acid produced which leads to a reduction in the pH of the growth environment, thus making it hostile to other microorganisms[34]. In addition to this, it has been well documented that the un-dissociated forms of these lactic acids also diffuse through the pathogenic bacteria cell membrane and dissociate inside the cell and releases H^+ which resultantly acidifies the cytoplasm to cause the collapse of the electrochemical proton gradient, leading to bacteriostasis and the eventual death of the susceptible bacteria[35].

All LAB isolates except *Lactobacillus casei* were susceptible to all the antibiotics used, this complies with the safety guidelines stated by Marchwińska *et al.*[36]. However, the resistance of *Lactobacillus casei* to some of the antibiotics agrees with the previous results[37]. The probiotic microorganisms must be safe and a safety test using haemolysis, DNA hydrolysis, coagulation of blood and gelatin liquefaction revealed that the *Lactobacillus* spp., with probiotic potential were safe. This is similar to the result obtained by Halder *et al.*[33] whose isolates were unable to liquefy gelatin and cause haemolysis.

The demonstration of Cumulative Probiotic Potential (CPP) of the *Lactobacilli* strains has been considered an improved criterion for probiotic validation[36]. The CPP obtained for the *Lactobacilli* strains used in this study was found to vary from one organism to the other. *Lactobacillus fermentum* and *Lactobacillus plantarum* had a CPP of 100% which made them fulfil the criteria of FAO/WHO[37]. Similarly, Halder *et al.*[33] also reported that *Lactobacillus plantarum* and some other isolates from curd had a CPP of 100%.

The overall acceptability of the probioticated drinks prepared was found to be more than average and this agrees with the reports of Adebayo-Tayo *et al.*[38] who indicated that the acceptability of milk blends from plant sources (tigernut, soy and groundnut milk) compared favourably with dairy milk. Also, El-Shenawy *et al.*[14] reported the acceptability of an equal proportion of blends of tigernut, soy and groundnut milk.

The microbial quality analysis revealed the absence of coliforms in non-dairy milk products. Yoghurt should contain a minimum of 4×10^6 CFU mL^{-1} LAB[36]. According to Terpou *et al*.[39], a high viable count ($>10^7$ CFU mL^{-1}) after 4 weeks of storage is important for maximum health benefits. The result from this study agrees with the guidelines of Terpou *et al*.[39] as the refrigerated probioticated drinks support and maintained the growth and survival of the probiotics microorganisms throughout the storage period with the minimal probiotic count of 3.39×10^7 CFU mL^{-1}. The Lactic Acid Bacteria (LAB) viability during cold storage is fundamental as it leads to the production of organic acids (mainly lactic acid) which consequentially lead to the reduction in pH value, it also ensures microbiological stability and prevents food-borne pathogens or spoilage microorganisms.

CONCLUSION

Conclusively, the production of soymilk and tigernut milk and the probiotication process were sufficient to produce probiotic drinks that met the standard, thus enabling it to compete with the commercial probiotic yoghurt in the market. The probioticated tigernut and soymilk drinks were also generally acceptable compared to the un-probioticated counterparts.

SIGNIFICANCE STATEMENT

This study discovered that probiotic drink produced from non-dairy sources, soybean and tigernut milk has promising marketability potential as they compete favourably with probiotic drink produced from dairy milk. The results from this study revealed that the non-dairy probiotic milk will be an ideal drink for vegetarians and individuals suffering from lactose intolerance.

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