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ORIGINAL ARTICLE

Potential antioxidant activities and bioactive compounds in probiotic tiger nut date palm yogurt fermented with lactic acid bacteria

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Abstract

The need for sustainable production of beverages has increased the interest in non-diary milk to produce yogurt. The functional profile of probiotic yogurt made from tiger-nut-date-palm milk (TNDPM) fermented with *Lactiplantibacillus plantarum* LB113 and *Pediococcus pentosaceus* L35 was investigated. Lactic Acid Bacteria (LAB) isolated from the natural fermentation of TNDPM were screened for their technological, probiotic, and safety potentials. Antioxidants and organoleptic attributes of the probiotic yogurt were investigated. The LAB from the fermentation process exhibited adequate production of metabolites, tolerance to simulated gastrointestinal conditions, and was considered safe. The yogurt fermented with a combination of *L. plantarum* LB113 and *P. pentosaceus* L35 had the highest DPPH scavenging activity (83.04%), ferric-reducing antioxidant function (0.66 mM $FeSO_4$), total phenolic content (56.08 mg/100 mL GAE) and total flavonoid content (4.10 mg/mL rutin). The sensory evaluation of the yogurt revealed that the overall sensory properties were influenced by the concentration of date palm milk (DPM) and the specific LAB strains used for fermentation. The beverage with a 20% concentration of DPM fermented with *L. plantarum* LB113 had the highest sensory attributes. The functional profile of this beverage makes it a promising option for individuals seeking a plant-based probiotic yogurt with potential health benefits.

Highlights

- The potential of lactic acid bacteria isolated from fermenting tiger nut and date palm milk as probiotic candidates.
- Combinations of tiger-nut-date palm milk fermented using probiotic microorganisms to make yogurt.
- The probiotic tiger-nut-date palm yogurt exhibited antioxidant activities, phenolics, and flavonoids.
- The potential of using probiotic tiger-nut-date-palm yogurt as a functional food with potential health benefits.

Keywords Probiotic yogurt · Tiger nut · Date palm · Lactic acid bacteria · Antioxidant activities · Bioactive compounds

Abbreviations

LAB	Lactic acid bacteria
TNDPM	Tiger-nut-date-palm milk
DPM	Date palm milk
TNM	Tiger nut milk
TTA	Total titratable acidity

Introduction

There has been a growing interest in developing functional beverages from plant-based sources in recent years because of their health advantages. The production and consumption of fermented beverages from these non-conventional sources align with the principles of sustainable development (Boukid et al. 2022). Tiger nuts (*Cyperus esculentus*) and date palm (*Phoenix dactylifera*) are sustainable and resilient crops that are gluten- and lactose-free with sufficient nutrients to meet individual dietary needs. Tiger nuts and date palm milk are functional foods with the potential to provide essential amino acids, maintain healthy cholesterol levels, and prevent various metabolic diseases, including cardiovascular diseases, gastrointestinal disorders, obesity, diabetes, and cancers (Vitali et al. 2023). However, despite the functional potential of these food crops, they need to be utilized and significantly valued more. This makes the development of a unique food product from these food

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crops imperative for consumers. Incorporating these crops into fermented beverages can promote responsible production and consumption, good health, and well-being.

The use of starter cultures and controlled fermentation processes enhances the sustainability of the production of functional beverages by ensuring consistent quality and longer shelf life, reducing food waste, minimizing the use of additives and preservatives, and prompting efficient resource use. The starter culture used in the controlled fermentation of beverages is lactic acid bacteria (LAB). The capacity of LAB to produce metabolites such as lactic acid, acetic acid, polyols, diacetyl, and γ-aminobutyric acid are characteristic values of LAB for food safety, shelf stability, enhancing food aroma, and other therapeutic purposes (Wang et al. 2021). The production of these metabolites is a valuable criterion for selecting starter cultures and probiotic strains for industrial and pharmaceutical purposes.

Probiotics are safe microorganisms that are important in the fermentation and preservation of food and are gaining widespread recognition for their beneficial effects on human health. These live microorganisms, such as bacteria and yeast, can positively influence the gut microbiota and improve the well-being of humans and animals (Byakika et al. 2020). Scientific research has shown that *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Leuconostoc*, *Pediococcus*, *Bacillus*, and *Streptococcus* are commonly sought-after probiotics (Banwo et al. 2021a; Assamoi et al. 2023). These genera of probiotics are well known for their role in the fermentation of food, inhibiting the growth of pathogenic microorganisms in food, enhancing the quality of food, and fortifying the therapeutic effect of food products (Yadav et al. 2016). In addition, the survival of LAB in gastric juice and bile salts is essential for viable probiotic microorganisms to exert their influence in the gastrointestinal tract (Shehata et al. 2016). Metabolic diseases are often associated with imbalances in the gut microbiota, leading to increased intestinal permeability, inflammation, and oxidative stress. The regular consumption of lactic acid bacteria through food beverages such as yogurts and kefir can help improve intestinal health and reduce the risk of metabolic diseases (Leeuwendaal et al. 2022). These selective actions of probiotics help maintain gut barrier function, competitive adherence to the mucosa and epithelium, and antimicrobial potential against harmful bacteria.

There are reports of the exploration of tiger nuts and other substrates to make fermented drinks (Amponsah et al. 2017; Assamoi et al. 2023). However, there is a dearth of information on the different combinations of both tiger nuts and date palms and the use of probiotic microorganisms to ferment these plant substrates into yogurt with antioxidant potential. This work is the first report involving the different combinations of tiger nuts milk and date palm drink fermented with probiotic microorganisms singly and in a consortium to make a functional yogurt. Therefore, this study explored the technological and probiotic capacities of LAB isolated from the spontaneous fermentation of tiger nut and date palm milk, the choice of strains for use as starter cultures, and the potential antioxidant activities, bioactive compounds, and sensory attributes of the probiotic yogurt made from tiger nut date palm milk.

Materials and methods

Spontaneous fermentation of tiger nut date palm milk

Tiger nut and date palm milk

Tiger nut and date palm milk were prepared according to the method described by Amponsah et al. (2017). Fresh tiger nut and date palm were bought from a retail market in Ibadan, Southwest Nigeria. The fruit crops were sorted to remove stones, pebbles, broken nuts, and foreign matter before soaking in water for 24 h. The steeped tiger nut and date palm were wetly milled, filtered, and allowed to ferment naturally for 24 h. The fermented milk samples were aseptically collected at 12 h intervals for physicochemical and microbiological exploration.

Microbiological analysis

Lactic acid bacteria (LAB) were isolated using deMann Rogosa Sharpe (MRS) agar (Lab M, Lancashire, UK) using the pour-plate technique and incubated anaerobically at 37 °C for 48 h. Isolates with distinct colonies were randomly selected and streaked out until pure cultures were obtained. The pure culture was phenotypically identified and stored in cryovial tubes containing sterile MRS broth (Lab M, Lancashire, UK) supplemented with 15% glycerol and kept at -20 °C (Banwo et al. 2021a).

Technological profile of the lactic acid bacteria isolates

Quantitative estimation of lactic acid produced was evaluated by titrating the supernatant of cell-free cultivation of selected isolates with 0.1 N NaOH and three drops of phenolphthalein as an indicator. Similarly, the supernatant of the isolates was titrated with 0.1 M HCl and bromophenol blue as an indicator to determine the quantity of diacetyl produced (Banwo et al. 2021a).

Probiotic potential and safety assessments of the selected lactic acid bacteria

Acid tolerance test

The survival of the selected LAB isolates in acidic conditions was examined using the method described by Shehata et al. (2016) with little modification. A 1 mL aliquot of overnight-grown LAB cultures was inoculated into MRS broth (Lab M, Lancashire, UK), adjusted to pH 3 with 1 M HCl, and incubated anaerobically at 37 °C for 6 h. The total viable bacteria count was performed at 0, 3, and 6 h using the pour plate method.

Survival in the simulated gastric juice

The survival of the selected LAB isolates in simulated gastrointestinal juice was determined, as reported by Assamoi et al. (2023). The isolates were grown in 5 mL of MRS broth (Lab M, Lancashire, UK) incubated at 37 °C for 16 h, followed by centrifugation at 5000 × *g* for 10 min. The obtained pellet was washed twice with phosphate-buffered saline (PBS) and resuspended in 0.5% saline. A 1% aliqot of the cel suspension was inoculated into the prepared simulated gastric juice (pH 2.0) and incubated at 37 °C for 3 h. The survival of the LAB isolates in simulated gastric juice was determined by viable plate counts at 0 and 3 h.

Survival in bile salt

The survival of the LAB isolates in bile salt was analyzed with a 1 mL aliquot of the overnight-grown LAB cultures inoculated into 9 mL of MRS broth (Lab M, Lancashire, UK) containing 0.3% (w/v) bile salt (Sigma–Aldrich, St. Louis, Missouri, USA). The cultures were incubated anaerobically at 37 °C for 3 h. The survival of the isolates in bile salt was determined by viable plate counts at 0 and 3 h (Shehata et al. 2016).

Bile salt hydrolase (BSH) activity

The LAB isolates were inoculated into MRS agar (Lab M, Lancashire, UK) plates containing 0.5% (w/v) sodium taurodeoxycholic acid salt (Sigma–Aldrich, St. Louis, Missouri, USA) and incubated under anaerobic conditions for 72 h. The BSH activity was indicated by the precipitation zone around the colonies (Shehata et al. 2016).

Fresh overnight isolates grown on MRS agar (Lab M, Lancashire, UK) plates were streaked onto blood agar plates supplemented with 5% defibrinated whole sheep blood (Thermo Fisher Scientific, UK). The plates were incubated anaerobically at 37 °C for 24 h. The plates were observed and characterized as hemolytic with a greenish zone around the colonies, partial hemolysis with a clear zone around the colonies, and non-hemolytic with no zone or reaction around the colonies (Banwo et al. 2021a).

Gelatinase assay

The LAB isolates were grown on MRS agar (Lab M, Lancashire, UK) plates for a period of 16 h and then streaked into nutrient agar (Oxoid, Basingstoke, UK) augmented with 1% gelatin. The plates were incubated anaerobically for 48 h at 37 °C, after which they were flooded with $HgCl_2$ solution and observed for clear zones around the colonies, which indicate positive gelatin hydrolysis (Banwo et al. 2021a).

DNase assay

The LAB isolates were examined for DNase activity using the plate assay method. Fresh overnight-grown cultures were inoculated on DNase agar (Oxoid, Basingstoke, UK) and incubated at 37 °C for 24 h. After that, the plates were flooded with HCl, and excess HCl was removed. After 5 min, the agar plates were observed for halo appearance around the colony, indicative of positive DNase activity (Banwo et al. 2021a).

Molecular characterization of the selected LAB

The genomic analysis of the LAB strains was performed through the DNA extraction of the selected isolates according to the procedure of the Big Dye Terminator v3.1 cycle sequencing kit, USA. The 16S rDNA gene sequence analysis of the selected LAB strains was amplified by polymerase chain reaction using the bacterial primers 16SF (5'-GTG CCA GCA GCC GCG TAA-3') and 16SR (3'-AGA CCC GGG AAC GTA TTC AC-5'). The PCR products were analyzed using 1.5% agarose gel electrophoresis and sequenced at the Department of Biosciences and Biotechnology, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The sequences obtained were analyzed using BioEdit software. The sequences were submitted to GenBank and were assigned accession numbers (Banwo et al. 2021a).

Fermentation of probiotic tiger nut date palm yogurt

Preparation of the probiotic culture

The pure cultures of the two selected LABs were inoculated into MRS broth (Lab M, Lancashire, UK) and incubated for

48 h. After 48 h, the broth was centrifuged at 6000 × *g* for 10 min. The cell pellet was washed twice with normal saline solution until colorless pellets were observed and finally suspended in equal volumes of the same solution. The inoculum size was enumerated using the viable count on MRS agar (Lab M, Lancashire, UK) (Chavan et al. 2018).

Production of probiotic yogurt from tiger nut and date palm milk

The functional beverage was developed from milk extracted from tiger nuts and date palms separately. The tiger-nut milk and date palm milk were made by soaking 500 g of tiger nut and 500 g of date palm in water for 24 h. The steeped fruit crops were wet milled with 1500 mL distilled water, and the resulting slurries were strained to obtain the milk. Tiger nut milk and date palm milk were then mixed at ratios of 80/20, 60/40, and 50/50 to create a composite milk. The respective composite milk was left in watertight bottles and pasteurized at 121 °C for 20 min (Fig. 1).

Controlled fermentation

The pasteurized beverages were allowed to cool and then inoculated with 1% (v/v) washed cells of the probiotic cultures. The fermentation process was allowed to occur at room temperature for 24 h. Three repeats of each setup were performed, and samples were obtained at 12 h intervals of fermentation for the physicochemical characterization, antioxidant activities, bioactive contents, and sensory properties of the beverage.

Physicochemical characterization of probiotic tiger-nut-date-palm yogurt

The total titratable acid (TTA) was determined by the titration of 25 mL of each beverage sample against 0.1 N sodium hydroxide (NaOH) solution using three drops of phenolphthalein as an indicator. The results were expressed as the quantity of lactic acid produced (Banwo et al. 2021b). The pH values of the beverages were measured using a pH meter (Mettler-Toledo, Massachusetts, USA). All procedures and measurements were performed in triplicate.

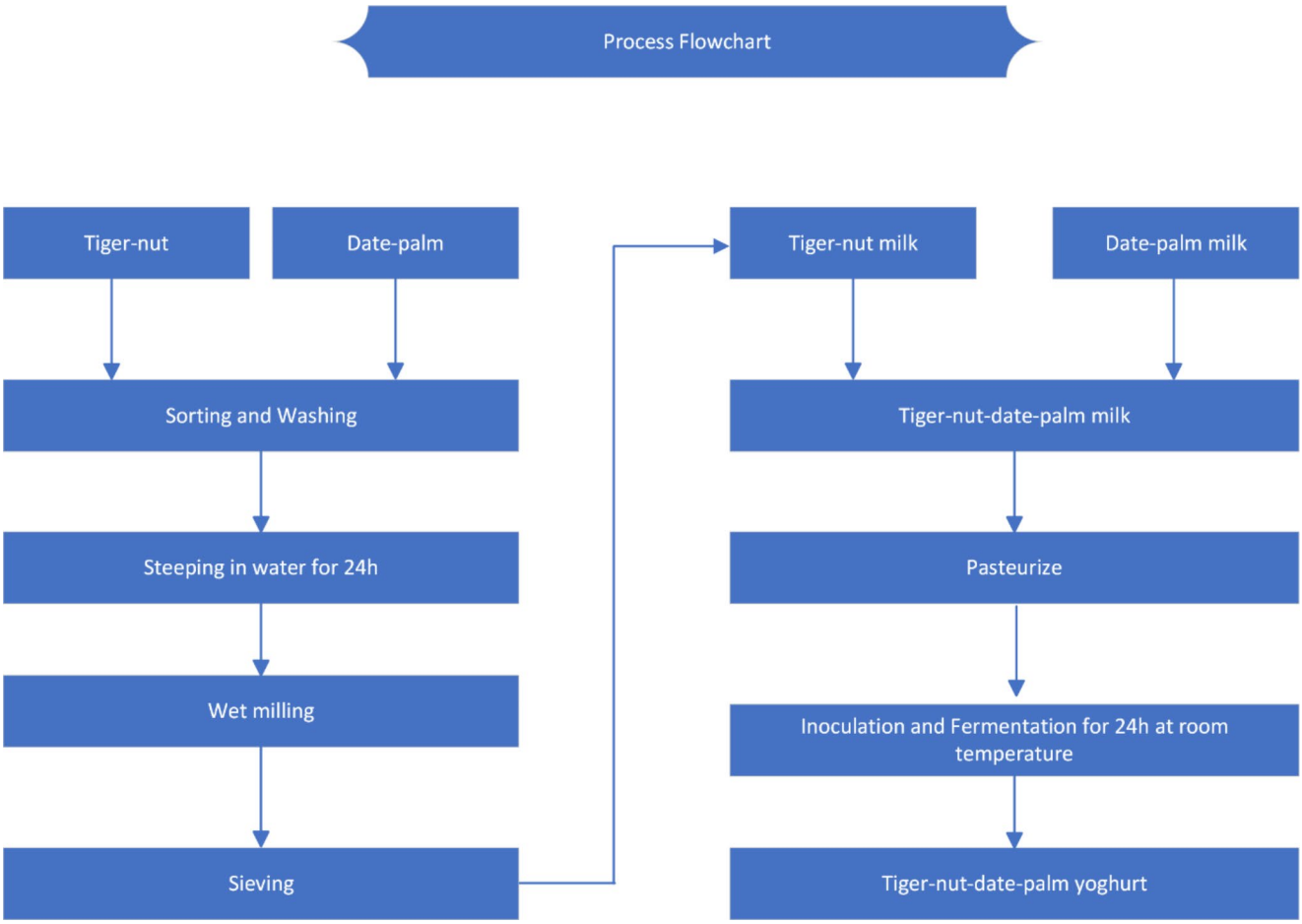

Fig. 1 Flowchart of the production of tiger nut date palm yogurt

Determination of total reduced sugar of the beverage

Total reducing sugar was determined by the method described by Miller (1959). A known volume of 3,4-dinitrosalicylic acid (DNSA) reagent (HiMedia, Mumbai, India) was added to 1 mL of each sample in a lightly capped test tube. The mixture was heated until it developed a red-brown color. A 1 mL potassium sodium tartrate solution was added to stabilize the color. The sample was cooled to room temperature, and the absorbance was measured at 575 nm using a visible spectrophotometer (Bibby, Essex, UK). The results are expressed as glucose equivalents.

Determination of the antioxidant activity of probiotic tiger nut date palm yogurt

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays

The scavenging rate of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was evaluated according to the method described by El-Said et al. (2014). One milliliter of the supernatant obtained from the beverage was mixed with 3 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent (0.2 mM) in a methanol solution. The mixtures were shaken and kept in the dark at ambient temperature for 30 min. Methanol was used as the blank, and DPPH methanol solution (0.1875 g/L) was used as the reference sample. After centrifugation (1400 × g, 10 min), the absorbance of the supernatant was measured at 517 nm (Bibby, Essex, UK). The free scavenging activity was calculated using the following formula with the assay conducted in triplicate:

Scavenging activity (%) = $[(Abs_{control} - Abs_{sample})/ Abs_{control}] \times 100$ (Banwo et al. 2021a).

Determination of ferric-reducing antioxidant activities

Samples (0.5 mL) were drawn under sterile conditions and mixed with an equal volume of 1.0% potassium ferricyanide containing phosphate-buffered saline (0.22 M, pH 7). The blend was incubated at 50 °C for 20 min; 10% trichloroacetic acid (0.5 mL) was added to the mixture. The mixture was centrifuged at 3000 × *g* for 5 min. A 1.5 mL aliquot of the supernatant was mixed with 0.2 mL distilled water containing 0.1% ferric chloride. The absorbance was measured at 700 nm using a spectrophotometer (Bibby, Essex, UK), and the ferric-reducing antioxidant activities were expressed as $FeSO_4$ equivalents (Banwo et al. 2021a).

Determination of the total phenolic and flavonoid contents

The total phenolic content was determined by mixing a 10 µL aliquot of each sample with 90 µL of distilled water and 100 µL of 10% Folin-Ciocalteu reagent solution. The blend was incubated for 3 min. Afterward, 2 mL of 2% sodium carbonate (Na_2CO_3) was added to the mixture and left to stand at room temperature for 30 min. The absorbance was measured at 750 nm (Bibby, Essex, UK). The results were expressed as gallic acid equivalent (mg GAE/g) deduced from a standard curve of different concentrations of gallic acid in methanol (Chavan et al. 2018; Djeridane et al. 2006).

The total flavonoid content of the samples was measured according to the method of Djeridane et al. (2006). A 1 mL aliquot of each sample was mixed with 1 mL of methanolic solution containing 2% Aluminum chloride ($AlCl_3$). The blend's absorbance was measured at 430 nm using a spectrophotometer (Bibby, Essex, UK) after incubation at room temperature for 15 min. The total flavonoid content was expressed as mg of rutin equivalents per g (mg RU/g).

Sensory analysis of the probiotic tiger nut-date palm yogurt

A 15-member panelist who has been trained on the standard procedure of sensory evaluation of a product evaluated the sensory characteristics (appearance, flavor, consistency, taste, and overall acceptability) of the beverage on a 9-point hedonic scale in the following sequence, with a nine as like extremely and one as dislike extremely (Sanni et al. 2013).

Statistical analysis

The results obtained in the study were evaluated using the descriptive mean and standard deviation. The test of significant differences established at $p \leq 0.05$ was conducted using analyses of variance (ANOVA). All data collected were analyzed using the statistical package for social science (SPSS, version 20.0). Each analysis was conducted independently three times to validate the results and reduce the error of bias.

Results and discussion

Microbial ecology of spontaneous fermentation of tiger nut and date palm milk

The health benefits attributed to fermented plant beverages are largely because of the beneficial microorganisms that conduct the process of fermentation. The microorganisms

that are primarily responsible for the fermentation of plant-based foods are lactic acid bacteria, acetic acid bacteria, fungi, and yeasts (Banwo et al. 2021a). The phenotypic characterization of the microbial community isolated from fermenting tiger nut and date-palm milk revealed that the LAB isolates belonged to the genera *Lactiplantibacillus* (formerly *Lactobacillus* 53.3%), *Lactococcus* (1.8%), *Pediococcus* (1.3%), *Leuconostoc* (93%), and *Streptococcus* (5.3%) (Results not shwn) (Fig. S1). The isolated LAB were gram-positive rods, catalase-negative, non-motile, and utilized vital sugars such as sucrose, glucose, maltose, fructose, and lactose. Previous studies have shown that the representative LAB microbial communities in the spontaneous fermentation of plant-based beverages are *Enterobacter*, *Lactobacillus*, *Streptococcus*, *Lactococcus*, *Leuconostoc*, and *Pediococcus* (Tangyu et al. 2023). The composition of these microbial communities is based on the substrate for fermentation, human practices, dynamics of the fermentation process, and other physiological conditions (Horlacher et al. 2023).

Technological capacities of LAB isolates

The highest concentration of lactic acid was produced by *Pediococcus* sp. L35 (2.12 g/L) and *Lactiplantibacillus* sp. DLB108 (2.12 g/L), while the highest concentration of diacetyl was produced by *Lactiplantibacillus* sp. DLB104 (5.81 g/L) and *Lactiplantibacillus* sp. LB113 (4.62 g/L) after 48 h of fermentation respectively (Table 1). Diacetyl is an important metabolite of LAB that enhances the flavor of fermented foods. All the isolates from fermented tiger nut date palm milk produced diacetyl. The highest concentration of diacetyl evident in *Lactiplantibacillus* sp. (formerly *Lactobacillus* sp.) agrees with the report of (Haryani et al. 2023), who stated that "*Lactococcus lactis*, *Lactobacillus* spp., *Streptococcus thermophilus*, and *Leuconostoc mesenteroides* are the most important diacetyl-producing LAB species". The production of lactic acid is a fermentative property of LAB that enhances its technological suitability. The production of lactic acid was in the range of 1.80–4.20 g/L, which was consistent with the lactic acid produced by the homofermentative species of LAB (Haryani et al. 2023).

Probiotic attributes of selected LAB

The survival of probiotic strains through the biological barriers in the gastrointestinal tract is essential for the ability of the strains to exert their therapeutic functions. These barriers are the acidic conditions in the stomach and gastric juice and bile salt in the ileum and duodenum. The in vitro tendency of the LAB isolates to survive in the acidic and bile conditions is shown in Table 2. There was a Log decline in the survival of the LAB isolates as the incubation period progressed. Nevertheless, the microbial count after the incubation period was above 7.0 log CFU/mL. This was typical of potential LAB isolates that could be regarded as probiotic strains (Banwo et al. 2021a).

Probiotic strains must survive in acidic stomach conditions with a pH of 3.0 or less for 2–4 h. Under this condition, *Pediococcus* spp. had the highest survival rate range (96.2–99.3%), followed by *Lactiplantibacillus* spp. (88.1–91.7%). The survival of the strains during their passage through the duodenum, which has a high concentration of bile salt, is critical for their selection as probiotic strains. The average bile concentration in the duodenum is 0.3%, which makes a bile concentration of 0.15–0.3% recommended as a reliable screening parameter for probiotic strains (Byakika et al. 2020). Unlike the survival of the isolates in acidic conditions, the highest survival rate under bile salt conditions was the *Lactiplantibacillus* group (98.6–99.0%), while *Lactococcus*sp. DLB103 had the lowest survival rate (95.5%).

The survival of probiotic LAB in the harsh condition of gastric juice present in the stomach and duodenum

Table 1 Metabolite production of LAB isolates

	Lactic acid produced (g/L)			Diacetyl produced (g/L)		
	Fermentation period (h)			Fermentation period (h)		
Isolate codes	24	48	72	24	48	72
Pediococcus sp. DLB52	4.20 ± 0.16	1.14 ± 0.03	0.54 ± 0.06	2.47 ± 0.15	3.33 ± 0.30	2.96 ± 0.08
Pediococcus sp. DLB114	1.80 ± 0.13	1.67 ± 0.06	0.90 ± 0.25	2.21 ± 0.23	3.66 ± 0.76	3.22 ± 0.15
Pediococcus sp. DLB117	1.89 ± 0.13	1.35 ± 0.25	1.35 ± 0.13	2.21 ± 0.08	2.80 ± 0.15	2.58 ± 0.15
Pediococcus sp. L35	2.70 ± 1.02	2.12 ± 0.06	0.81 ± 0.13	1.56 ± 0.53	2.37 ± 0.46	3.23 ± 0.15
Pediococcus sp. DLB120	1.94 ± 0.19	1.53 ± 0.89	0.59 ± 0.06	2.21 ± 0.23	3.01 ± 0.15	2.21 ± 0.98
Lactococcus sp. DLB103	2.25 ± 0.64	1.53 ± 0.25	3.29 ± 0.32	0.86 ± 0.30	2.37 ± 1.07	4.73 ± 0.15
Lactiplantibacillus sp. DLB104	2.12 ± 0.45	1.57 ± 0.19	1.76 ± 0.19	2.69 ± 0.46	5.81 ± 0.76	3.22 ± 0.15
Lactiplantibacillus sp. DLB108	2.16 ± 0.25	2.12 ± 0.06	1.40 ± 0.19	0.86 ± 0.30	1.61 ± 0.30	2.21 ± 0.08
Lactiplantibacillus sp. LB113	2.25 ± 0.76	0.85 ± 0.06	0.68 ± 0.06	3.39 ± 0.38	4.62 ± 0.30	4.30 ± 0.15
Streptococcus sp. DLB 105	1.80 ± 0.25	2.11 ± 0.06	1.71 ± 0.13	1.78 ± 0.38	2.58 ± 0.76	2.26 ± 0.15

Values are means of triplicate independent assessments

Table 2 The survival rate of the selected LAB isolates at pH 3.0 and 0.3% bile salt

	Acidic pH (3.0)			Bile salt (0.3%)		
	Microbial count (Log CFU/mL)			Microbial count (Log CFU/mL)		
	Incubation period (h)		Survival rate	Incubation period (h)		Survival rate
Isolate codes	0	6	(%)	0	3	(%)
Pediococcus sp. DLB52	8.29 ± 0.01	8.16 ± 0.02	98.4	7.92 ± 0.02	7.68 ± 0.04	96.9
Pediococcus sp. DLB114	7.54 ± 0.00	7.48 ± 0.01	99.3	7.66 ± 0.06	7.45 ± 0.01	97.3
Pediococcus sp. DLB117	7.43 ± 0.06	7.16 ± 0.01	96.4	7.81 ± 0.00	7.60 ± 0.01	97.3
Pediococcus sp. L35	7.55 ± 0.00	7.42 ± 0.02	98.2	7.79 ± 0.02	7.68 ± 0.03	98.6
Pediococcus sp. DLB120	7.44 ± 0.05	7.16 ± 0.01	96.2	7.83 ± 0.05	7.75 ± 0.04	98.9
Lactococcus sp. DLB103	8.49 ± 0.02	7.53 ± 0.07	88.6	7.66 ± 0.04	7.31 ± 0.07	95.5
Lactiplantibacillus sp. DLB104	8.50 ± 0.01	7.49 ± 0.18	88.1	7.12 ± 0.09	7.01 ± 0.10	98.6
Lactiplantibacillus sp. DLB108	7.22 ± 0.00	6.52 ± 0.06	90.3	7.78 ± 0.02	7.67 ± 0.02	98.6
Lactiplantibacillus sp. LB113	8.35 ± 0.01	7.66 ± 0.03	91.7	7.82 ± 0.03	7.74 ± 0.04	99.0
Streptococcus sp. DLB 105	8.46 ± 0.01	7.59 ± 0.02	89.7	7.63 ± 0.01	7.35 ± 0.09	96.3

Values are means of triplicate independent assessments

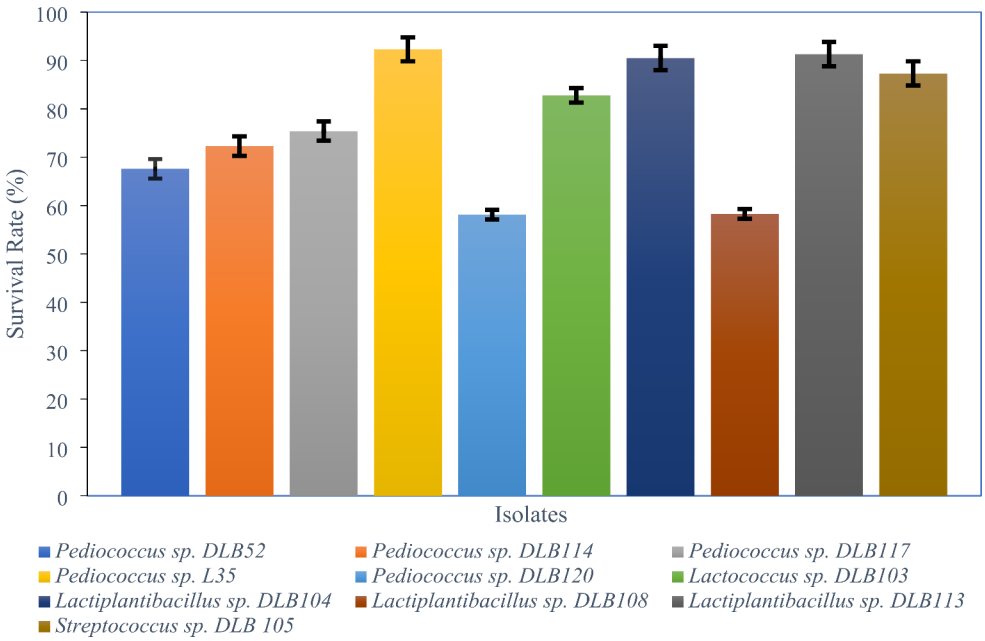

Fig. 2 The survival rate of the selected LAB isolates in simulated gastric juice

extends the ability of these strains to colonize the ileum, where their beneficial functions are imparted (Colombo et al. 2018). Four of the LAB isolates in this study had a survival rate above 80% (Fig. 2). However, *actiplantibacillus* sp. LB113 (91.3%) and *Pediococcus* s. L35 (92.3%) had the highest srvival percentages in gastric juice. LAB strains from the *Lactobacillus* and *Pediococcus* genera have been reported to have excellent tolerance to gastric juice conditions (Huligere et al. 2023). Therefore, *Lactiplantibacillus* sp. LB113 and *Pediococcus* sp. L35 were selected as the starter culture for the probiotic tiger-nut-date palm yogurt because of its consistency in the simulated gastrointestinal condition.

The production of bile salt hydrolase (BSH) by LAB isolates is a limiting factor that could hinder their choice as probiotic strains. The production of bile salt hydrolase increases the potency of probiotic LAB by conjugating bile salts in the duodenum (Byakika et al. 2020). However, there has been an argument regarding the BSH activity of LAB isolated from environments where bile salts are absent. While Sedláčková et al. (2015) argued that BSH activity is not present in LAB strains isolated from non-bile environments such as fruit and vegetable beverages, Shehata et al. (2016) opined that BSH activity could be detected in probiotic LAB-isolated environments where bile salts are absent. Nonetheless, the results of this study show that BSH activity was absent in the LAB isolated from fermenting tiger nuts and date palm milk. The BSH activity of LAB has been strongly linked to its ability to lower body cholesterol, which could be useful in the treatment of hypercholesterolemia (Byakika et al. 2020). This suggests that the BSH inactivity of the selected isolates in this study may not

possess the ability to lower body cholesterol. Otherwise, the survival in low pH, bile salt, and gastric juice makes the LAB isolates from tiger-nut and date-palm milk viable candidates for probiotic strains.

Safety assessment and molecular characterization of the selected LAB

Lactiplantibacillus sp. LB113 had the highest survival rate in acidic, bile, and gastric juice conditions, while *Pediococcus* sp. L35 had the highest survival rate in gastric juice conditions but maintained the second highest survival rate in acidic and bile conditions among the *Pediococcus* groups. The selected strains had good potential to survive bile salt and low pH conditions. In addition, the strains had excellent survival in gastric juice conditions, which shows their potential to colonize the ileum, where they can exert their actions. The strains were negative for gelatinase and DNase assays and were non-hemolytic. Thus, these strains are generally regarded as safe (GRAS) microorganisms and good candidates as probiotic strains (Banwo et al. 2021b). The identification of probiotic LAB strains by 16 S rDNA is an effective and low-cost method that is reliable for the identification of probiotic LAB. The molecular identification of the two selected LAB for controlled fermentation using 16 S rDNA gene sequencing revealed that the LAB strains were *Lactiplantibacillus plantarum* LB113 (Accession number: MW131681) and *Pediococcus pentosaceus* L35 (Accession number: MW131683).

Controlled fermentation of probiotic tiger nut date palm yogurt

The controlled fermentation of tiger-nut-date-palm yogurt was performed with an average inoculum size of 1.4×10^7 CFU/mL with the strains *Lactiplantibacillus plantarum* LB 113 and *P. pentosaceus* L35 singly and in a consortium. The controlled fermentation of beverages encourages the suppression of undesirable microorganisms and enhances the proliferation of desirable microorganisms. This is evident in the fermentation of cucumber beverages with *P. pentosaceus*, where the predominant microbes are of the genus *Pediococcus*, while the beverage inoculated by *L. casei* had a prevalence of the *Lactobacillus* genus. This proposition established the notion that the type of fermentation, strain used, and concentration of the starter culture determine the microbial community of the final product (Li et al. 2022; Świder et al. 2023).

Physicochemical characterization of probiotic tiger nut date palm yogurt

The physicochemical properties of the fermented tiger-nut-date-palm yogurt were analyzed to determine the pH and TTA of the milk. The results showed that the fermented milk had a pH within the desirable range and a TTA value that indicated successful fermentation. The LAB strains applied in the fermentation of the tiger-nut-date-palm yogurt beverage significantly reduced the pH of the beverage after 24 h. In contrast, the TTA of the beverage was significantly increased by the probiotic strains after 24 h of fermentation (Table 3). The decrease in the pH and increase in the TTA of the beverage depicts the production of lactic acid exerted by the utilization of carbon compounds in the metabolic pathway (Banwo et al. 2021a, b). The capacity of these probiotic strains to ferment a broad range of carbohydrates and produce organic acids is essential in the food industry to inhibit the growth of food-borne pathogens, improve the shelf life of the beverage, and enhance the stability and organoleptic acceptance of the beverage.

Total reducing sugar of the tiger nut date palm yogurt

The total reducing sugar content of the fermented tiger-nut-date-palm yogurt shows that the beverage that was allowed to undergo spontaneous fermentation had the highest reducing

Table 3 pH, TTA, reducing sugar, antioxidant activities and bioactive contents of tiger-nut-date-palm yogurt

	pH		TTA (g/L)						
Yogurt codes	0 h	24 h	0 h	24 h	Reducing Sugar (g/L)	DPPH (%)	FRAP (mM $FeSO_4$)	TPC (mg/100 ml GAE)	TFC (mg/ml Rutin)
TF1	5	4.55	0.32	0.36	4.49 ± 0.15	49.59	0.23	5.59	3.12
TC2	4.85	4.2	0.36	0.59	3.10 ± 0.09	64.31	0.4	30.23	3.53
TC4	4.95	4.7	0.27	0.29	2.65 ± 0.16	64.9	0.46	39.12	3.98
TE3	4.9	3.5	0.23	0.72	3.94 ± 0.13	80.1	0.51	42.34	3.83
TE4	5	3.9	0.27	1.13	3.65 ± 0.11	83.04	0.66	56.08	4.1

Values are means of triplicate independent assessments

TF1: spontaneously fermented yogurt containing v/v tiger-nut milk and date palm milk, TC2: yogurt fermented with *L. plantarum* LB113 containing 4/1 tiger-nut milk and date-palm milk, TC4: yogurt fermented with *L. plantarum* LB113 and *P. pentosaceus* L35 containing 4/1 tiger-nut milk and date-palm milk, TE3: yogurt fermented with *P. pentosaceus* L35 containing 3/2 tiger-nut milk and date-palm milk, TE4: yogurt fermented with *L. plantarum* LB113 and *P. pentosaceus* L35 containing 3/2 tiger-nut milk and date-palm milk. FRAP: ferric-reducing antioxidant power, DPPH: 2,2-diphenyl-1-picrylhydrazyl scavenging activity, TPC: total phenolic content, TFC: total flavonoid content

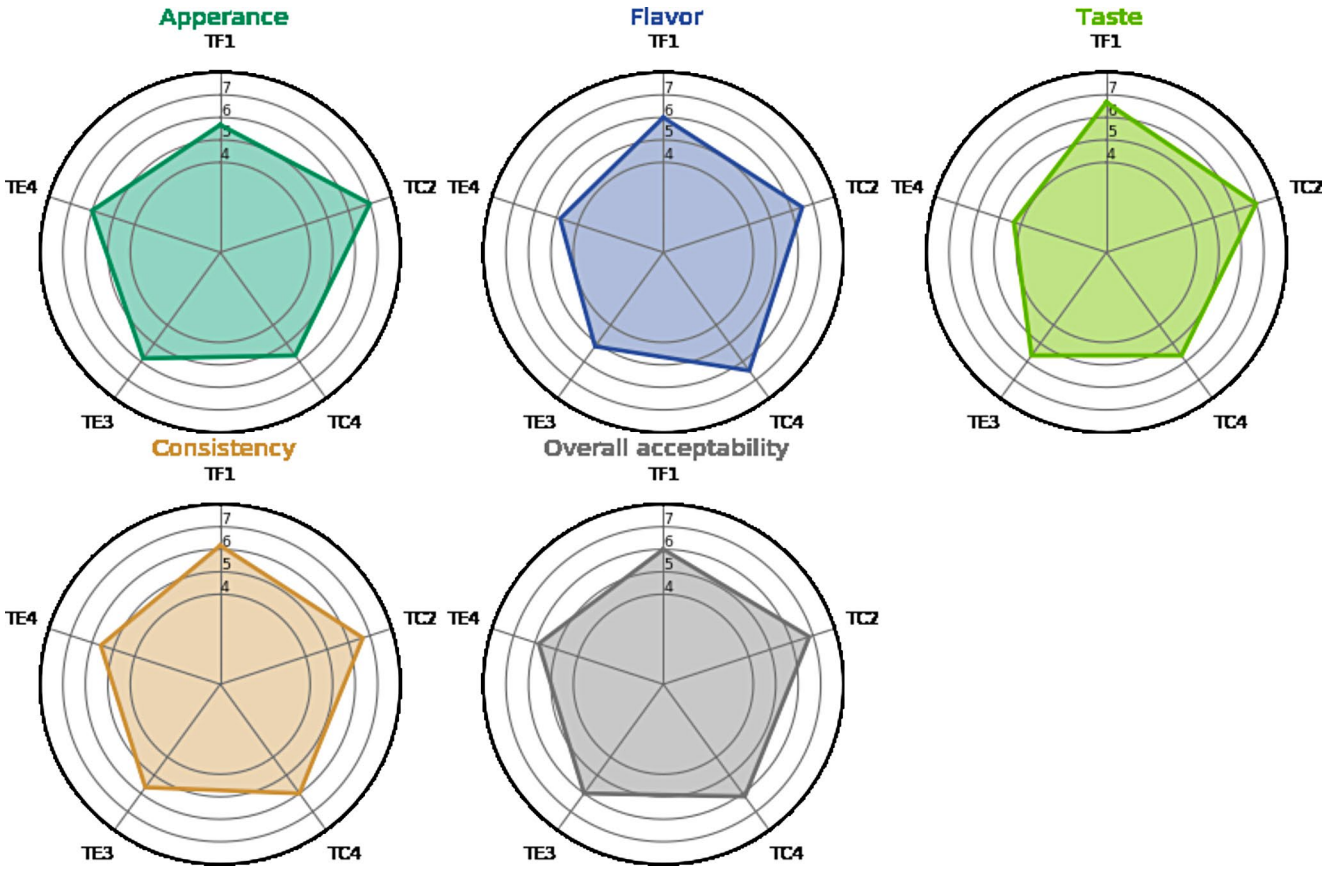

Fig. 3 Sensory evaluation of tiger nut date palm yogurt. TF1: spontaneously fermented yogurt containing v/v tiger-nut milk and date palm milk, TC2: yogurt fermented with *L. plantarum* LB113 containing 4/1 tiger-nut milk and date-palm milk, TC4: yogurt fermented with *L. plantarum* LB113 and *P. pentosaceus* L35 containing 4/1 tiger-nut milk and date-palm milk, TE3: yogurt fermented with *P. pentosaceus* L35 containing 3/2 tiger-nut milk and date-palm milk, TE4: yogurt fermented with *L. plantarum* LB113 and *P. pentosaceus* L35 containing 3/2 tiger-nut milk and date-palm milk

sugar. On the other hand, the beverage fermented with the constructive collaboration of the two probiotic strains had a lower sugar content than the beverage fermented with a probiotic strain. In another stride, the results show that an increase in the concentration of date palm in the beverage increased the reduced sugar content (Table 3). The addition of date extract to yogurt drinks has been studied to support the growth of LAB and increase the sugar content of the beverage. The high concentration of reducing sugars such as glucose and fructose in date palms, which could limit the growth of LAB, has led to the recommendation of date extract in yogurt drinks in the range of 30–35% (El-Said et al. 2014). However, the results of this study show that 40% of date extract coud be successfully added to yogurt drinks. In addition, the fermentation of yogurt drinks with *Lactiplantibacillus plantarum* and *P. pentosaceus* significantly reduced the sugar content of the beverage.

Bioactive compositions of tiger nut date palm yogurt

The antioxidant content of fruit crops employed in beverages is enhanced by metabolites of microorganisms that participate in the fermentation of the beverage. These antioxidant substances synthesized from metabolites of LAB can scavenge free radicals (Wang et al. 2021). The results of this study show that the combination of *P. pentosaceus* and *L. plantarum* in the fermentation of tiger-nut-date-palm yogurt increased the DPPH scavenging activities and ferric-reducing antioxidant power. Interestingly, the results showed that using probiotic LAB in the fermentation of yogurt significantly increased the antioxidant potency of the beverage compared with spontaneously fermented yogurt. These results confirmed that probiotic LAB can positively contribute to human health (Banwo et al. 2021a).

The presence of these natural sugars in fruit crops used for plant-based yogurt could help to improve the bioavailability and bioaccessibility of antioxidant compounds such

as polyphenols and flavonoids (Tavares et al. 2023). The LAB metabolizes phenolic acids through decarboxylation and reduction reactions. The phenol compounds synthesized from these reactions have strong antioxidant activities (Zhao et al. 2023). As shown in Table 3, the total phenolic content of the yogurt samples ranged from 5.59 to 56.08 mg/100 mL GAE, and the total flavonoid content ranged from 3.12 to 4.10 mg/mL rutin. The results showed that the yogurt sample fermented with *Lactiplantibacillus plantarum* LB113 had lower TPC and TFC than the yogurt sample fermented with *P. pentosaceus* L35. In a study, plant-based juice fermented with *Lactobacillus acidophilus* and *L. plantarum* had lower TFC than juice fermented with other LAB strains (Zhao et al. 2023) because of the presumed different responses and ability of LAB strains to produce hydrolytic enzymes (Banwo et al. 2021a). The results of the present study show that *P. pentosaceus* is a novel microorganism that could increase the bioavailability of antioxidant activities. Similarly, the results suggest that the combination of two or more probiotic strains in the fermentation of yogurt beverages will enhance its therapeutic benefits.

Sensory evaluation of the beverage

The development of food products from unconventional sources depends on consumer preference and acceptance. In the case of the probiotic yogurt developed from tiger-nut-date palm milk, the pilot sensory analysis indicated that the beverage was acceptable to consumers. The average hedonic scale was above 4 of 9 points for all sampled mixtures of the beverage (Fig. 3). The yogurt beverage with a lower concentration (20%) of date palm fermented with *Lactiplantibacillus plantarum* LB113 (TC2) had the highest sensory attributes. On the other hand, the probiotic with 40% date palm fermented with *L. plantarum* LB113 and *P. pentosaceus* L35 (TE4) had the lowest mean score for flavor, taste, consistency, and overall acceptability. The high acidic content of this yogurt could limit the acceptability of yogurt made from the nondairy substrate (Sanni et al. 2013).

Conclusion

This study highlighted the potential of tiger nut and date palm as a promising substrate for functional yogurt that offers promising health benefits. The probiotic potential of LAB isolated from fermented tiger nut and date palm milk showed that *L. plantarum* LB113 and *P. pentosaceus* L35 demonstrated significant probiotic ability for survival in gastrointestinal conditions. Additionally, the incorporation of probiotic *L. plantarum* LB113 and *P. pentosaceus* L35 in the tiger-nut-date palm milk was established to increase the bioactive compounds of the yogurt. In addition, the controlled fermentation process enhanced the sensory properties of the yogurt, making it a desirable choice for consumers. Thus, by harnessing the benefits of probiotic microorganisms from tiger nuts and date palm milk, we can create nutritious and accessible plant-based products that promote a healthy lifestyle, gut health, and overall well-being.

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Data availability the data can be made available upon request.

Code availability Not applicable.

Declarations

Ethics approval We declare that the research was conducted in an appropriate ethical and responsible manner. We declare that this manuscript is the original work of the authors, and it has not been published in any form or language.

Consent for publication Not applicable.

Consent to participate Not applicable.

Conflict of interest The authors declare that they have no competing interests.

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