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Taro leaves extract and probiotic lactic acid bacteria: A synergistic approach to improve antioxidant capacity and bioaccessibility in fermented milk beverages

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ABSTRACT

Taro leaves extract (TLE) was used to manufacture two fermented milk beverages in concentrations of 250 and 500 mg/L (FMTLE 250 and FMTLE 500). The polyphenolic profile via HPLC, volatile compounds via GC-MS, antioxidant, antimicrobial activities of TLE, physicochemical properties, probiotic survival, and bioaccessibility of the supplemented beverages were also evaluated. TLE presented phenolic and flavonoid levels of 130.56 ± 1.55 μg GAE/g and 53 ± 1.15 CE μg/g, respectively. Sulfated polysaccharides, triterpenoids, and tannins were recorded (26.5 ± 0.8 μg/g, 7.30 ± 0.37 μg/g, 0.26 ± 0.03 μg/g). IC_{50} of the TLE measured using DPPH· assay was 144.83 ± 2.19 μg/mL, while the ABTS*+ assay was 100.48 ± 1.45 for determination antioxidant activity. The highest antimicrobial activity of TLE was observed against *Salmonella enterica* and *Listeria monocytogenes* with an inhibition zone of 15.5 and 13.6 mm, respectively. The survival of *Lactobacillus paracasei* showed no statistical difference between the control and FMTLE 250 and FMTLE 500 ($P > 0.05$). Antioxidant potential increased, with probiotics were stable through the digestion processes of the supplemented beverages and the increasing polyphenol concentration in the beverages. Sensory evaluation showed the acceptance of FMTLE 250 and FMTLE 500 for consumer consumption. In sum, combining TLE and probiotics in fermented beverages provides an excellent food model with many health benefits.

1. Introduction

Taro, scientifically known as *Colocasia esculent* (L). Schott is a tropical root propagated vegetatively and belongs to the Araceae family (Sharma et al., 2020). Taro originates from Southeast Asia and is characterised by large green leaves, often referred to as elephant ears, which can grow up to 1–2 m in height during the growth period. Consuming taro has been shown to have various potential health benefits, such as reducing the risk of constipation and colon cancer, as noted in the study by Rojas-Sandoval and Acevedo-Rodríguez (2022). According to Ganesan et al. (2018) and Sharma et al. (2020) indicated that taro leaves have been found to contain various bioactive compounds, including flavonoids, steroids, β-sitosterol, saponin, arabinogalactan, and mono and digalactocyl diacylglycerols. These compounds have been linked to potential health benefits, such as hepatoprotective, hypolipidemic, antitumor, antimicrobial (antibacterial and antifungal), antidiabetic, and antimelanogenic effects. In addition, taro leaves have been found to exhibit antimicrobial properties attributed to the presence of cystatin (Abima Shazhni et al., 2018). It should be noted that both the leaves and tubers of the taro plant are toxic if consumed in their raw form due to the presence of acrid calcium oxalate, which must be eliminated by heating before consumption (Savage & Dubois, 2006). Plant-based vegetables contribute the highest amounts of natural phenolic compounds in the human diet, and their leaves, shells, and peels are especially rich in these compounds (Lin et al., 2016). Furthermore, by utilizing these agro-wastes in our study, we not only harness the health benefits of

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phytochemicals, such as scavenging free radicals, inhibiting lipid peroxidation, chelating metals, and play crucial roles in maintaining food products' stability and contributing to biological systems' defense mechanisms (Rathod et al., 2023), but also contribute to a decrease in agro-waste production. This reduction in waste is ecologically desirable as it minimizes environmental impact.

Combining bioactive compounds extracted from natural sources with functional lactic acid bacteria is a promising biotechnology strategy for expanding the market for functional beverages (Ruiz Rodríguez et al., 2021). Furthermore, adding exogenous functional compounds or microorganisms that produce biogenic compounds or possess probiotic characteristics is a common way to enhance the nutritional value of functional foods and beverages (Szydłowska & Sionek, 2022). To the best of our knowledge, this is the first study to specifically investigate the phytochemicals and antimicrobial properties of taro leaves and taro leaves extract. Additionally, it involved the development of a novel functional fermented milk beverage using the phenolic compounds from taro leaves and probiotic lactic acid bacteria (LAB). The study also assessed the physical and antioxidant characteristics of the developed products. Furthermore, the study examined the bioaccessibility of the bioactive compounds and the survival of probiotic lactic acid bacteria during an in vitro gastrointestinal digestion model.

2. Materials and methods

2.1. Materials

The pasteurized cow's milk was purchased from a local market in Asafra District, Alexandria Governorate, Egypt. CHR HANSEN's Lab, Inc. supplied a standard starter culture (YoFlex–YC-X11) containing *Streptococcus thermophiles* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Milwaukee, WI). *Lactobacillus paracasei* KC 39 isolate used in this research was previously isolated from Kareish cheese (Shehata et al., 2018). In 2018, Taro leaves were gathered from a Taro Farm in Kom Hamada, Beheira Governorate, Egypt. The leaves were then air-dried for 48 h at 40 °C in a ventilated oven and ground into a fine powder using the technique described by Shehata et al. (2021).

2.2. Chemical analysis of taro leaves

The moisture, total protein, and ash contents were analysed using the official methods of the Association of Official Agricultural Chemists (AOAC) (Association of Official Analytical Chemists AOAC, 2000). The automatic fibre analyzer (Fibre Analyzer, Model A200I, O'Neil Road, Macedon, USA) was used to determine the fibre content based on the Van-Soest and Wine (1967) method. The total lipids content was extracted and quantified using an automatic fat analyzer (Ankom Extractor, Model XT10I, O'Neil Road, Macedon, USA). All the analyses were executed in triplicate. The mineral contents of the prepared taro leaves, namely iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn), were determined using an Atomic Absorption Flame Emission Spectrophotometer (Analytik Jena Zeemit 700, Germany), following the method described by Association of Official Analytical Chemists AOAC (2000).

2.3. Extracts preparation

Ten grammes of taro leaves were extracted with 100 mL of deionized water (1:10 w/v) at room temperature by conventional extraction method for 6 h. The mixture was filtered through a Whatman No. 2 filter paper to remove leaves particles, followed by lyophilization (Dura-Dry MP freeze-drier FTS System, USA) at −50 °C for 15–20 h and kept extract in the refrigerator for further analysis.

2.4. Characterization of the taro leaves extract

Total soluble phenol and flavonoid content was determined according to Dewanto et al. (2002) and Sakanaka et al. (2005), respectively. Total phenolic content was expressed in μg of gallic acid equivalents (μg GAE) per g of sample. In contrast, total flavonoid content was expressed in μg catechol equivalents (μg CE) per g of dry sample. Sulfated polysaccharides were measured by the toluidine blue assay as described by Albano and Mourao (1986). The triterpenoid content was assessed colorimetrically via reaction of the triterpenoids with vanillin using ursolic acid as a standard (Bai et al., 2007). The antioxidant activity of the extract was assessed using the DPPH· assay, as described by Brand-Williams et al. (1995). Additionally, the ABTS*+ assay was adopted, using the method of ABTS (Re et al., 1999), to determine the extract's ABTS*+ radical cation scavenging activity was expressed as (μg ascorbic acid equivalents/mg extract).

2.5. Determination of the polyphenol profile by RP-HPLC

The phenolic content of the ethanol extracts of taro leaves was quantitatively analysed using an RP-HPLC system (Agilent1260; Santa Clara, CA, USA) by the method outlined by Tomaino et al. (2010) and main phenolic compounds expressed as mg/100 g of dry sample.

2.6. Antimicrobial activity the taro leaves extract

The antimicrobial activity was determined by agar-well diffusion method (Shehata et al., 2021) for taro leave extract against various pathogenic strains (*Bacillus cereus* ATCC 49064, *Staphylococcus aureus* NCTC 10788, *Listeria monocytogenes* ATCC 19116, *Salmonella* senftenberg ATCC 8400, *Yersinia enterocolitica* ATCC 23715, *Escherichia coli* BA 12296, *Fusarium culmorum KF846* and *Fusarium oxysporum ITEM 12591*) were obtained from Microbial Resource Center (MIRCEN), Ain Shams Univ. (ASU), Hadak Shobura, Cairo, Egypt. Results were expressed as the diameter (mm) of the inhibition zone. Ampicillin can be used as a positive control.

2.7. Manufacture of the fermented milk beverages

Pasteurized cow's milk beverages with and without taro leaf extract (FMTLE) had an enhanced viscosity by boiling the milk at 90 °C for 20 min. Taro leaves extract (TLE) was added to the pasteurized cow's milk at 250 mg/L (FMTLE 250) or 500 mg/L (FMTLE 500) (Dewanto et al., 2002). Inoculation with starter cultures (*Streptococcus thermophiles* and *Lactobacillus delbruecki* subsp. *Bulgaricus)* was carried out at a volume of approximately 1 mL/dL (v/v), corresponding to a log 9.0 cfu/mL, and functional strain *Lactobacillus paracasei* KC39. The mixture was incubated at 30 °C for 24 h. A control fermented milk beverage (FM) was produced using the same procedure but without the addition of TLE. The fermented beverages (FMTLE 250, FMTLE 500, and FM) were stored at 4 °C until further analysis; all were carried out in triplicate.

2.8. Physical analysis of the fermented beverages

The pH and titratable acidity of samples were determined using a pH meter and through titration with 0.1 mol L^{-1} NaOH, respectively (Kurt et al., 1996). A dynamic viscometer was utilized to determine the viscosity of each sample (Brookfield Model RVDI, USA). The color of fermented beverages was evaluated according to the method described by Francis (1983) using the colorimeter (Smart Color Pro, Advanced Automation Systems, Egypt).

2.9. GC-MS analysis of volatile compounds

The volatile compounds were determined according to the method described by Yufei et al. (2016) using steam distillation Extraction

(SDE). A 6890 gas chromatography-5973 mass selective detector (GC-MS, Agilent Technologies Inc., Santa Clara, CA, USA) was used to analyze the volatile compounds. The results were quantified based on the area counts obtained from Gas Chromatography-Flame Ionization Detector analysis (GC-FID).

2.10. Microbiological analyses

To estimate the LAB counts in both FMTLEs and FM samples, plating onto MRS agar media (for lactobacilli) or M17 agar media (for streptococci) (purchased from Oxoid, England) was performed, followed by cultivation of isolated colonies in MRS broth at 30 °C for 24 h in anaerobic condition (Shehata et al., 2019). Samples were collected on days 0, 7, 14, 21, and 30 of storage to determine the count of *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus paracasei*.

2.11. Simulated in vitro gastrointestinal (GI) digestion

The procedure of Minekus et al. (2014) was utilized to simulate the GI digestion conditions in vitro (Fig. 1). The components of salivary, gastric, and intestinal fluids are summarised in Table 1. Following these procedures, 2-mL aliquots were collected for further examination.

2.12. Sensory evaluation

The sensory evaluation, following the method of Hooda and Jood (2005), was conducted to compare the organoleptic properties of the samples with the control. The attributes evaluated included color, texture, taste, consistency, and overall acceptability of each sample.

2.13. Statistical analysis

Data were represented as mean ± standard error of the mean (SEM) using multiple comparisons one-way analysis of variance (ANOVA) Tukey's test with SPSS version16 software (IBM, SPSS Inc., Illinois, USA) and probability (p)-values 0.05 deemed statistically significant.

Table 1
Simulated digestion fluids used for in vitro gastrointestinal system simulation.

Constituents	Concentration (mol/L)	Simulated salivaryfluida (SSF) (pH:7)	Simulated gastric fluida (SGF) (pH:3)	Simulated intestinal fluida (SIF) (pH:7)
KCl	0.5	15.1 mL	6.9 mL	6.8 mL
KH_2PO_4	0.5	3.7 mL	0.9 mL	0.8 mL
$NaHCO_3$	1	6.8 mL	12.5 mL	42.5 mL
NaCl	2	–	11.8 mL	9.6 mL
$MgCl_2(H_2O)_6$	0.15	0.5 mL	0.4 mL	1.1 mL
$(NH_4)_2CO_3$	0.5	0.06 mL	0.5 mL	–
HCl	6	0.09 mL	1.3 mL	0.7 mL

All digestion fluids should be filled to 400 mL with distilled water.

3. Result and discussion

3.1. Proximate chemical composition

Results showed that the moisture, crude proteins, crude fibre, lipids, ash and carbohydrates were 6.06 ± 0.33, 7.13 ± 0.24, 14.4 ± 0.74, 0.633 ± 0.15, 4.2 ± 0.24 and 67.56 ± 1.52 g/100 g, respectively. The protein was higher than a previous study in taro leaves (6.54 ± 0.16 g/100 g) reported by Alcantara et al. (2013). The high concentration of complex carbohydrates and fibre is one of the nutritional benefits of taro leaves (Agu & Okolie, 2017). The increase in fibre content observed in taro powder in this study also resulted from moisture removal during drying, thus, raising the fibre concentration (Agoreyo et al., 2011). Various factors such as plant stage growth, climate conditions, and harvest date can be attributed to a diversity in gross chemical composition (El-Sohaimy et al., 2022). The higher ash value of the taro leaves (4.2 ± 0.24 g/100 g) shows that the leaves represent a good source of minerals and can therefore be used in diet supplementation to improve

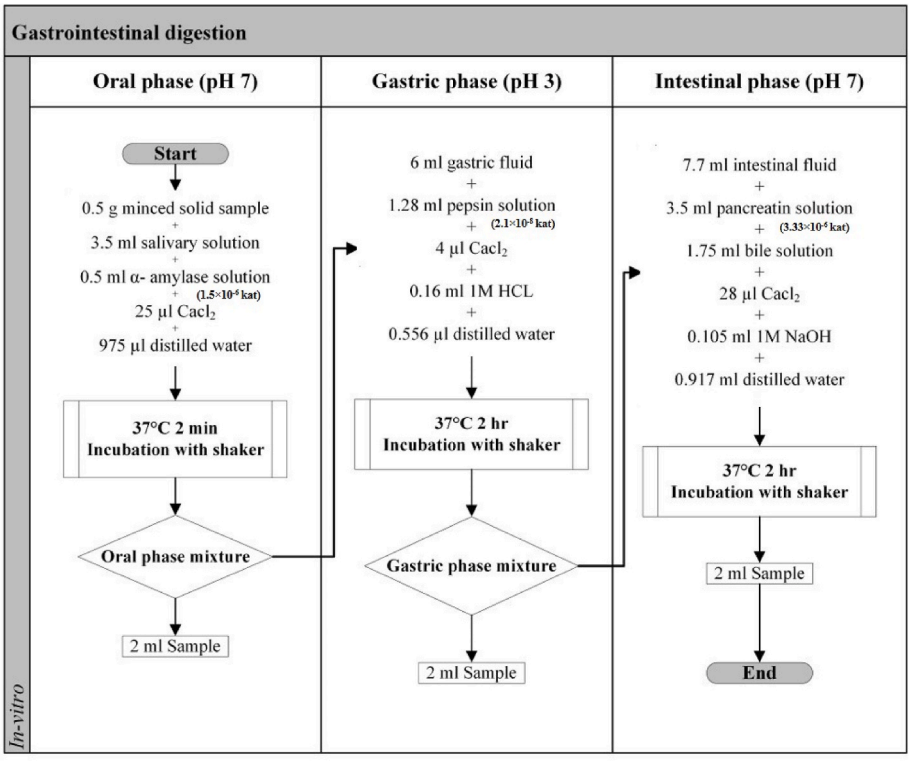

Fig. 1. Flow diagram of simulated in vitro gastrointestinal digestion.

dietary mineral quality. The potassium, calcium, zinc, copper, sodium, manganese, iron, and phosphorus concentrations in taro leaves were (106, 56, 40, 16, 15, 14, 12.5, and 12.5) mg/L dry matter, respectively. The level of potassium (K) is high compared to olive leaves 40 mg/L. Bananas 33 mg/L, Oranges 15 mg/L, Strawberries 17 mg/L, Tomatoes 20 mg/L (Labban et al., 2017). It is well known that increasing potassium intake reduces cardiovascular disease mortality (Aburto et al., 2013). Indeed, minerals play a vital role in strengthening bones, teeth, and the heart, and decreasing the risk of many diseases like hypertension or seizures.

The findings of the current investigation emphasized the adequate concentrations of essential minerals in taro leaves such as calcium that is important for healthy bone and teeth. Iron plays a crucial role by preventing anemia and potassium deficiency. Essential minerals are also responsible for the maintenance of an optimal pH balance and blood pressure regulation in the human body. The obtained results indicate that taro leaves are rich sources of micro and macronutrients that could be used as food supplements.

3.2. Bioactive compounds of taro leave water extract

The results of bioactive compounds revealed that TLE contain a total phenol content of 130.56 ± 1.55 μg GAE/g, a total flavonoid content of 53 ± 1.15 μg CE/g, sulfated polysaccharide content of 26.5 ± 0.8 μg/g, triterpenoids content of 7.30 ± 0.37 μg/mL and a tannin content of 0.26 ± 0.03 μg/g. Phenol content was relatively high (3.8 fold) compared to another study of taro leaf extract (Alcantara et al., 2013), possibly due to different methods of extraction of the leaves. This is the first study concerning the measurement of tannins, triterpenoids, and sulfated polysaccharides in taro leaf extract. Such bioactive compounds have been reported to have health benefits via their capability of scavenging harmful free radicals (Chaudhuri et al., 2014; Haile & Kang, 2019).

3.3. HPLC analysis

Phenolic compounds of the taro leaves extract via HPLC are represented in Table 2. Results revealed that TLE showed the highest content of *p*-Coumaric acid (46.49 mg/100 g) compared to the other compounds (Fig. S1). while the content of catechol, vanillin, rutin, and ellagic acid was recorded of 35.86, 31.90, 29.96 and 26.93 mg/100 g, respectively. Phenolic substances are commonly recognised as possible antioxidants, free radical scavengers, metal chelating agents, and regulators of lipid peroxidation (Kurutas, 2015; Pei et al., 2016). *p*-Coumaric acid, which occurs in high concentration in taro leave extract, has several biological effects such as antioxidant, anti-cancer, antimicrobial, antivirus, and anti-inflammatory (Pei et al., 2016). This finding may indicate the presence of different types of phenolic and flavonoid components in taro leaf extract, but unfortunately, no HPLC standards were injected, which may allow for additional studies regarding this subject. Bioactive compounds identified in this Egyptian species of taro leaves explain their biological activity against free radicals and microorganisms and give special support to take the first step in using this extract in food applications.

Table 2
HPLC analysis of phenolic compounds (mg/100 g) for taro leave extract (TLE).

Phenolic compound	TLE Conc. (mg/100 g)
Gallic acid	2.97
Catechol	35.86
p- Hydroxy benzoic acid	ND
Caffeine	13.28
Vanillic acid	ND
Caffeic acid	ND
Syringic acid	7.93
Vanillin	31.90
p- Coumaric acid	46.49
Ferulic acid	10.07
Rutin	29.96
Ellagic acid	26.93
Benzoic acid	12.31
o- Coumaric acid	0.38
Salicylic acid	ND
Cinnamic acid	ND

TLE: Taro Leaves Ethanol Extract; ND: not detected, LOQ = 0. 1 mg/kg.

3.4. Biological activity of taro leave extract

3.4.1. DPPH· assay

Table 3 demonstrates the capacity of taro leaf extract to scavenge DPPH· free radicals. The findings indicate that the extract's ability to scavenge is dependent on the dosage, with a low IC_{50} value of 144.83 ± 2.19 μg/mL indicating a good antioxidant capacity compared to the IC_{50} value of standard ascorbic acid (84.36 ± 2.31 μg ascorbic acid equivalents/ml extract). Abd El-Aziz et al. (2021) reported a positive correlation between the DPPH· assay and the phenolic compound content of plant extracts, with reference to the positive control, ascorbic acid.

3.4.2. ABTS*+ assay

The principal objective of this test is to evaluate the ability of various substances to scavenge the ABTS*+ radical. The IC_{50} values, which indicate the concentrations of extracts required to scavenge 50% of ABTS*+ radical, were used to express the antioxidant capacities of the samples. Table 3 displays the IC_{50} values for the ABTS*+ radical scavenging activity, which were found to be 100.48 ± 1.45 and 74.05 ± 0.47 μg ascorbic acid equivalents/ml extract for the taro leaf extract and the standard ascorbic acid, respectively. The results of this study showed fermented milk fortified with pomegranate peel extracts increased antioxidant activity, as has been previously noted in a study by Chan et al. (2018). Jyothi and Srinivasa (2018) pointed out that extracts of *Colocasia esculenta* had good antioxidant properties. Likewise, Simsek and El (2015) demonstrated that the scavenging activity of *C. esculenta* against ABTS*+ and DPPH· radicals resulted in an antioxidant capacity of 452 ± 72 mM TEAC/100 g and 244 ± 73 mM TEAC/100 g, respectively.

3.5. Antimicrobial activity

Table 4 displays the findings of the antimicrobial activity of the aqueous extract of taro leaves against eight pathogenic microorganisms.

Table 3
Radical scavenging activity of taro leaves extract (TLE).

Concentrations (μg/mL)	ABTS*+		DPPH·	
	TLE	AC	TLE	AC
7.81	47.36 ± 0.65	74.43 ± 0.94	21.37 ± 0.78	60.54 ± 0.32
15.62	50.86 ± 1.13	76.3 ± 0.98	41.11 ± 0.51	66.9 ± 0.37
31.25	55.63 ± 1.06	79.2 ± 0.88	43.33 ± 0.74	70.70 ± 1.22
62.5	58.30 ± 0.84	82.86 ± 1.16	52.12 ± 0.65	72.22 ± 1.73
125	62.21 ± 0.90	84.4 ± 0.53	54.44 ± 1.03	74.14 ± 2.06
250	65.61 ± 0.60	86.36 ± 0.74	55.95 ± 0.37	75.55 ± 1.88
500	68.31 ± 0.94	90.93 ± 0.61	59.49 ± 0.51	77.57 ± 0.74
1000	74.66 ± 0.87	91.25 ± 1.21	70 ± 0.24	79.69 ± 0.42
IC_{50} (μg/mL)	100.48 ± 1.45	74.05 ± 0.47	144.83 ± 2.19	84.36 ± 2.31

TLE: Taro Leaves Extract; AC: Ascorbic acid.

Table 4
Antimicrobial activity of taro leaves extract.

Pathogenic microorganisms	TLE (mm)
Gram positive bacteria	
Bacillus cereus ATCC 49064	16.21 ± 0.68^{b}
Staphylococcus aureus NCTC 10788	11.33 ± 0.84^{d}
Listeria monocytogenes ATCC 19116	9.30 ± 0.98^{e}
Gram negative bacteria	
Salmonella senftenberg ATCC 8400	18.53 ± 0.75^{a}
Yersinia enterocolitica ATCC 23715	14.36 ± 1.03^{bc}
Escherichia coli BA 12296	19.30 ± 1.02^{a}
Fungi	
Fusarium culmorum KF846	0^{f}
Fusarium oxysporum ITEM 12591	12.93 ± 1.17^{cd}

[a, b, c, d, e,] and [f]: data in the same column followed by different superscript letters differ significantly ($p < 0.05$).

The extract exhibited notable antimicrobial activity against all the microorganisms tested. The extract showed excellent activities against *E. coli* BA 12296 and *S. Senftenberg* ATCC 8400, with inhibition zones of 19.3 ± 1.02 mm and 18.53 ± 0.75 mm, respectively. The good antifungal activity of taro leave extract was observed against *Fusarium oxysporum* ITEM 12591 with an inhibition zone of 12.93 ± 1.17 mm. A smaller zone of inhibition (11.33 ± 0.84 mm) was observed by extract against *S. aureus* NCTC 10788. A previous study by Dutta and Aich (2017) showed that an ethanolic extract of *Colocasia esculenta* leaves has an inhibition zone of 8.24 ± 0.66 mm against *Staphylococcus aureus*, which is smaller than our extract value for the same species. The antibacterial and antifungal potential observed in this study could be attributed to the presence of bioactive components in the plant, as reported by Cowan (1999) and Mostafa et al. (2018). Overall, the extract showed better antibacterial (Fig. S3) and antifungal activities that may be exploited as natural preservatives in food products.

3.6. Physical properties of taro fermented milk

3.6.1. pH and acidity

The effects of TLE fortification of functional milk beverages on the pH and beverage acidity during storage are shown in Table 5. With increased storage time, pH levels of functional milk beverages with and without TLE significantly decreased ($p < 0.05$). This is consistent with other studies that produce fermented milk beverages complemented by phenolic compounds and functional lactic acid bacteria (El-Sohaimy et al., 2022; Servili et al., 2011). The pH values of FM ranged from 4.11 to 4.81. The average pH of TLE fortified functional milk beverages was 4.19–4.48. The higher count of lactic acid bacteria at the end of the storage period contributed to lower pH levels fortified with and without TLE, functional milk beverages. Furthermore, the acidity of beverages increased significantly ($p < 0.05$) independent of TLE concentration, with increasing storage time. For functional dairy beverages enhanced with and without TLE, the mean acidity values were between 0.010 and 0.091. This is due to the prolonged storage period, leading to an increase in lactic acid production, resulting in higher acidity and a reduction in pH levels.

Table 5
pH, TTA, and viscosity of FM, FM+TLE 250 and FM+TLE 500 during storage.

Fermented milk	FM		FMTL 250		FMTL 500	
	t_1	t_{30}	t_1	t_{30}	t_1	t_{30}
pH	4.81 ± 0.037^{a}	4.11 ± 0.09^{b}	4.79 ± 0.12^{a}	4.14 ± 0.12^{b}	4.80 ± 0.07^{a}	4.19 ± 0.12^{b}
TTA	0.10 ± 0.06^{b}	0.91 ± 0.07^{a}	0.10 ± 0.07^{b}	0.90 ± 0.13^{a}	0.13 ± 0.06^{b}	0.90 ± 0.01^{a}
Viscosity	289.27 ± 5.60^{a}	282.70 ± 2.85^{b}	287.67 ± 6.80^{a}	275.47 ± 4.50^{bc}	288.67 ± 5.50^{a}	270 ± 4.35^{c}

t1: storage for 1 day; *t*30: storage for 30 days; values are mean ± SD ($n = 3$). FM: the control fermented milk beverage; FMTLE 250: fermented milk beverages supplemented with taro leaves extract (250 mg/L); FMTLE 500: fermented milk beverages supplemented with taro leaves extract (500 mg/L).
[a, b,] and [c]: data in the same row followed by different superscript letters differ significantly ($p < 0.05$).

3.6.2. Viscosity

Viscosity is an important factor in deciding the appearance and density of a product in the food industry. The impact of TLE on the viscosity of functional milk beverages is shown in Table 5. After 30 days of fermentation, the viscosity of TLE 500 was significantly ($p < 0.05$) lower (270.00 ± 4.35 cP) than it was prior to storage (day one, 288.67 ± 5.50 cP). This may be associated with the TLE effect of electrostatic interaction on the aggregation of the casein network in yoghurt and on the flow resistance of the yoghurt matrix (Tamime & Robinson, 1999). Alwazeer et al. (2020) investigated that incorporation of plant extracts mainly decreases product viscosity by decreasing the milk protein's water-binding ability.

3.6.3. Color

The color parameters of fermented milk as a control and fermented milk supplemented with taro leaves extract are shown in Table 6 lightness (L*) significantly ($p < 0.05$) decreased when the TLE concentration increased in the fermented milk due to nature of taro leaves color. The (L*) value in fermented milk was 90.77 ± 0.045, while in FMTL 250, FMTL 500 were 87 ± 2.85, 84.94 ± 0.22, respectively. The (a*) value (redness) in fermented milk (FM) registered −8.57 ± 1.73 which was significantly ($p < 0.05$) increased in FMTL 250 and FMTL 500 with −2.13 ± 0.80, 0.38 ± 0.81, respectively. The (b*) value (yellowness) in fermented milk was 1.40 ± 4.98, which was significantly ($p < 0.05$) increased in FMTL 250 and FMTL 500 with 28.90 ± 1.49, 31.07 ± 0.32, respectively. Similarly, Mohamed et al. (2018) reported that utilisation of *Moringa oleifera* leaves extract in the cream cheese increased yellowness and redness of this product.

3.7. Volatile compounds of taro fermented milk

Volatile compounds are the main influencers of beverages flavours, hence its sensory characteristics (Arrizon et al., 2006). In this study, a total of fifteen flavour-active compounds of FMTLE 500, including various types of amino acids (1), alcohols (1), esters (4), hydrocarbons (3), aldehydes (2), and carboxylic acids (4), were identified by GC-MS coupled with flame ionization detection (GC-FID), and analysed during 24 h of manufacturing of fermented milk (Table 7). The major aroma volatile compounds were glycyl-D-asparagine (amino acid), followed by carboxylic acids; ester; trace concentrations of aldehyde and hydrocarbon compounds. Amino acid compound (glycyl-D-asparagine) showed high relative abundance (Fig. S2) in fermented milk. Carboxylic acid compounds of different types (n-hexadecanoic acid, linoleic acid, and erucic acid) were found in fermented milk. Mid-levels of alcohol compounds (eugenol) were found in fermented milk. It has been shown that

Table 6
Color analysis of functional fermented milk.

Fermented milk	L*	a*	b*
FM	90.77 ± 0.45^{a}	-8.57 ± 1.73^{c}	1.40 ± 4.98^{b}
FMTL 250	87 ± 2.85^{b}	-2.13 ± 0.80^{b}	28.90 ± 1.49^{a}
FMTL 500	84.94 ± 0.22^{b}	0.38 ± 0.81^{a}	31.07 ± 0.32^{a}

Values with different letter in the same row were significantly different at $p \leq 0.05$. L* is lightness; a* is redness; b* is yellowness. FM: the control fermented milk beverage; FMTLE 250: fermented milk beverages supplemented with taro leaves extract (250 mg/L); FMTLE 500: fermented milk beverages supplemented with taro leaves extract (500 mg/L).

Table 7
Volatile compounds of functional milk beverage fortified with 500 (FMTLE500) mg/L of taro leave extract (TLE).

ID#	R. Time (min)	m/z	Area	Height	Chemical Formula	Volatile Compound	Classification
1	4.188	18.00	196.4658	116.107	$C_6H_{11}N_3O_4$	Glycyl-d-asparagine	Amino acid
2	23.484	164.00	95.999	13.467	$C_{10}H_{12}O_2$	Phenol, 2-methoxy-3-(2-propenyl)-	Alcohols
3	27.114	163.00	94.589	8.534	$C_{15}H_{10}FNO_6$	Phthalic acid, 4-fluoro-2-nitrophenyl methyl ester	Ester
4	–	77.00	–	–	$C_8H_7NO_2$	1,3-Oxazetidin-2-one, 3-phenyl-	Ester
5	30.317	65.00	5355	1.159	$C_3H_6F_2$	Propane, 2,2-difluoro-	Hydrocarbon
6	31.374	57.00	5105	1.826	$C_6H_{12}O$	3-Pentanone, 2-methyl-	Aldehyde
7	31.591	149.00	826.306	151.496	$C_{12}H_{14}O_4$	Diethyl Phthalate	Ester
8	34.266	57.00	8717	2.638	C_6H_{14}	Butane, 2,2-dimethyl-	Hydrocarbon
9	36.986	57.00	11336	3.334	$C_{14}H_{29}I$	Tetradecane, 1-iodo-	Hydrocarbon
10	41.266	43.00	101.842	18.783	$C_{16}H_{32}O_2$	n-Hexadecanoic acid	Carboxylic acids
11	41.465	149.00	53.784	13.533	$C_{19}H_{26}O_4$	Phthalic acid, cyclobutyl heptyl ester	Ester
12	45.419	67.00	125.379	24.585	$C_{18}H_{32}O_2$	9,12-Octadecadienoic acid (Z,Z)-	Carboxylic acids
13	45.522	55.00	237.590	41.017	$C_{22}H_{42}O_2$	Erucic acid	Carboxylic acids
14	45.963	43.00	22.211	4.272	$C_{16}H_{32}O_2$	n-Hexadecanoic acid	Carboxylic acids
15	–	81.00	–	–	$C_8H_{10}O_2S$	Propanethioic acid, S-(2-furanylmethyl) ester	Ester
16	47.591	41.00	2.644	1.161	$C_7H_7NO_4$	1-[(1-Oxo-2-propenyl) oxy]-2,5-pyrrolidinedione	Ester
17	–	55.00	–	–	$C_5H_6O_2$	2-Propenoic acid, ethenyl ester	Ester
18	54.408	18.00	3.508	1.452	$C_4H_7NO_3$	anti-2-Acetoxyacetaldoxime	Aldehyde

glycyl-D-asparagine, diethyl ketone, and 2-methyl have antimicrobial activity (Kamal et al., 2015), and eugenol, is important as an anti-inflammatory modulator (Gherraf et al., 2017).

3.8. Probiotic viability

Fig. 2 illustrates changes in viable counts (log cfu/mL) of taro fermented milk during cold storage (4 °C) over 30 days. Based on a comparison between FMTLE 250 and FMTLE 500 to FM, the addition of TLE had a significant ($p < 0.05$) effect on the survival of the probiotic strain (Fig. 2). After 0, 7, 14, 21, and 30 days of storage, all the strains could be detected. The cell density of starter cultures and functional strains after inoculum was ca. 8.6 log cfu/mL. Cell densities of lactic acid bacteria at the end of fermentation decreased to ca. 1.6 log cfu/mL. During storage, the number of *Lactobacillus paracasei* KC39 decreased from 8.73 log cfu/mL to 7.02 log cfu/mL. Nualkaekul and Charalampopoulos (2011) stated that, at the end of their shelf-life, probiotics must have a concentration of at least 7.00 log cfu/mL in order to withstand adverse conditions in the gastrointestinal tract and to have sufficient amounts in the intestine to provide health benefits, which they achieved in our taro fermented milk. The combination of phenolic compounds and probiotics may create a new functionally enhanced food product with much more benefits than traditional fermented milk products.

3.9. Simulated in vitro gastrointestinal (GI) digestion

3.9.1. Survival of lactic acid bacteria in GI

Probiotics survival after GIT digestion is shown in Fig. 3. During the digestion process, *L. paracasei* in fermented milk FMTLE 500 presented higher survival rates (8.76 ± 0.55 log cfu/g (oral), 7.0 ± 0.20 log cfu/g (gastric) and 6.0 ± 0.50 log cfu/g (intestinal)) for all the digestion phases when compared to the other beverages ($p < 0.05$). The population of starter cultures (*L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*) in fermented milk as a control showed a non-significant ($p < 0.05$) reduction during the gastric phase (6.0 ± 0.50; 5.80 ± 0.30 log cfu/mL) and exhibited a significant ($p < 0.05$) reduction during the intestinal phase (4.96 ± 0.68; 5.23 ± 0.45 log cfu/mL), respectively. The same pattern was observed in probiotic bacteria. However, there was a non-significant reduction in viability in the gastric and intestinal phases. Like many other studies, the survival rate of probiotics at pH 1.5 was small enough to be negligible (García-Núñez et al., 2022; Shehata et al., 2016). From our results, supplementation of the fermented beverage with taro leave phenolic compounds may have the upper hand in probiotic viability and stability through aggressive digestion, which was previously studied by Lacey et al. (2014). On the other hand, the kefir-fermented beverage made with *Colocasia esculenta* extract showed a significant growth in lactic acid bacteria (LAB) and yeast populations, reaching 10^7 CFUml−1. Additionally, the beverage had a shelf life of 21 days (Pinto et al., 2020). In this context, Kandyliari et al. (2023) reported that herbal extracts have the potential to enhance various dairy products, offering potential benefits to human health. The investigation of fortified food products revealed a varying bioavailability range of antioxidants and phenolics following in vitro digestion, between 4% and 68%.

3.9.2. Bioaccessibility of antioxidant activity in GI

Fig. 3 shows the antioxidant activity of FM, FMTLE 250, and FMTLE 500 after oral, gastric, and intestinal digestion. The result showed that the antioxidant activity increased through digestion processes in all treatments and control beverages and in dose dependence with the initial phenolic concentration in case of FMTLE 250 and FMTLE 500 beverages. In case of FM the antioxidant potential using DPPH· assay

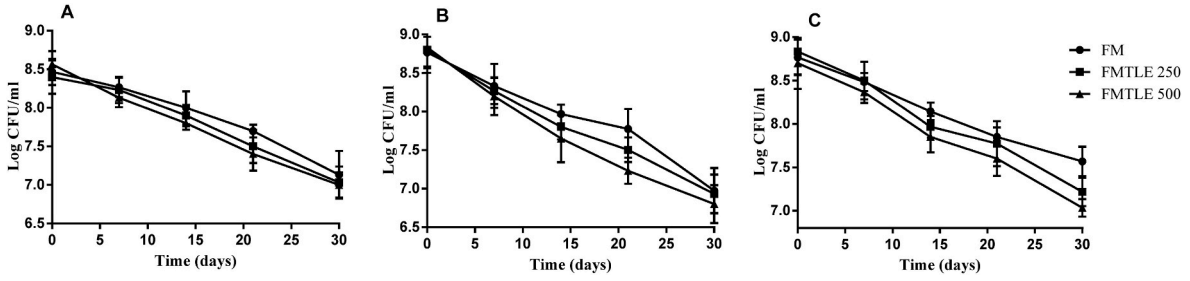

Fig. 2. Cell numbers of *Lactobacillus delbrueckii* subsp. *bulgaricus* (a), *Streptococcus thermophilus* (b) and *Lactobacillus paracasei* KC39 (c) during 30 days of storage at 4 °C of FMTL 250 (■), FMTL 500 (▲), and FM (●). FM: the control fermented milk beverage; FMTLE 250: fermented milk beverages supplemented with taro leaves extract (250 mg/L); FMTLE 500: fermented milk beverages supplemented with taro leaves extract (500 mg/L).

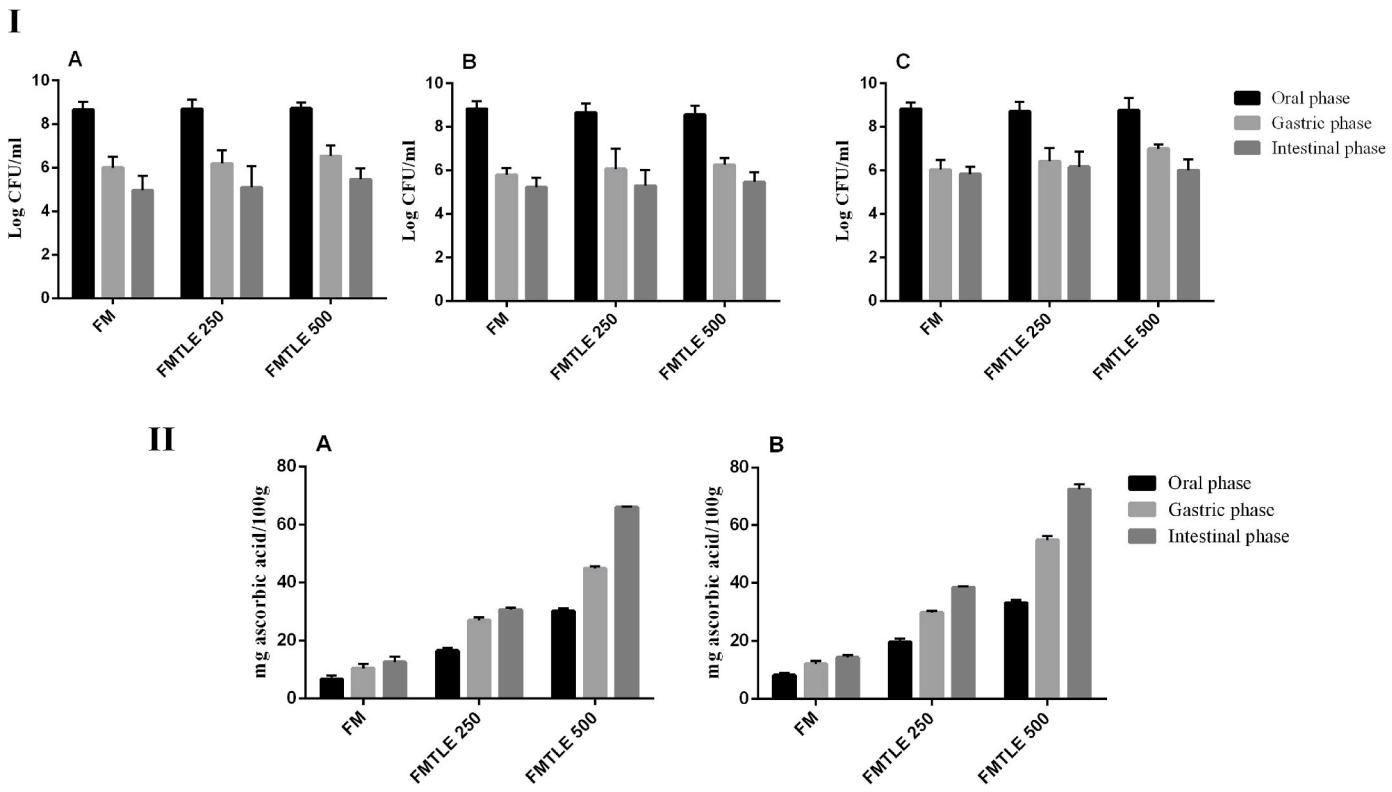

Fig. 3. Survival of LAB and antioxidant activity under oral, gastric and intestinal phases of functional milk beverages after in vitro GI digestion. **(I):** (A), *Lactobacillus delbrueckii* subsp. *bulgaricus*; (B), *Streptococcus thermophilus*; (C), *Lactobacillus paracasei* KC39; **(II):** (A), DPPH; (B), ABTS. Values are means of triplicates from two separate runs, n = 3. Values: mg ascorbic acid/100 g fermented milk. FM: the control fermented milk beverage; FMTLE 250: fermented milk beverages supplemented with taro leaves extract (250 mg/L); FMTLE 500: fermented milk beverages supplemented with taro leaves extract (500 mg/L).

significantly ($p < 0.05$) increased from 6.56 ± 1.42 to 12.5 ± 1.87 mg ascorbic acid/100 g. Moreover, FMTLE 250 the antioxidant, significantly ($p < 0.05$) elevated from 16.43 ± 1.10 to 30.65 ± 0.62 mg ascorbic acid equivalents/g extract. But FMTLE 500 the antioxidant potential increased from 30.16 ± 1.01 to 65.95 ± 0.36 mg ascorbic acid equivalents/g extract. This increase in antioxidant potential could be due to the effect of digestion enzymes during the intestinal phase, which may make it easier to extract phenolics and flavonoids (Bouayed et al., 2011; Salawu et al., 2020). This antioxidant behavior was confirmed by the results obtained by ABTS*+ assay.

3.10. Sensory properties

Fig. 4 shows that there was no substantial difference in sensory scores between TLE 250, TLE 500, and control fermented milk. The application of TLE (250 and 500 mg/L) does not affect sensory results such as odor, smell, and overall acceptability. However, all the beverages were overall acceptable (7–7.5) (Fig. 4). Based on these results, it is possible to add some fruit-derived ingredients to beverages in order to achieve greater sensory acceptability. Finally, the fortification of fermented milk by TLE 500 not show any undesirable odor, and this was confirmed by sensory evaluation and analysis of volatile compounds by GC-MS.

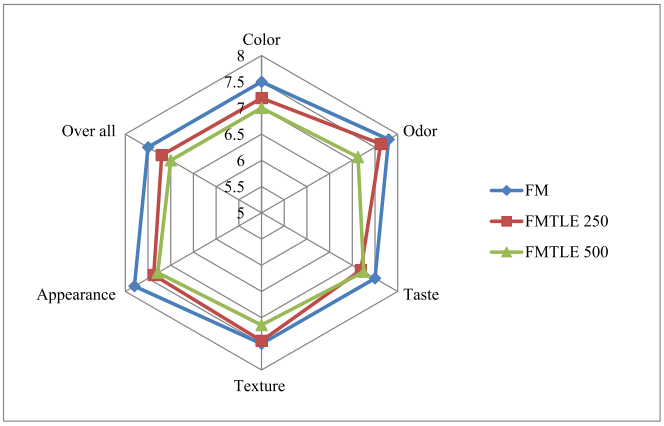

Fig. 4. Sensory properties of functional milk beverages; FM, FMTL 250 and FMTL 500. FM: the control fermented milk beverage; FMTLE 250: fermented milk beverages supplemented with taro leaves extract (250 mg/L); FMTLE 500: fermented milk beverages supplemented with taro leaves extract (500 mg/L).

4. Conclusions

This study marks the first investigation into the use of taro leaves to produce a novel functional fermented beverage with probiotic bacteria *Lactobacillus paracasei* supplementation. The fermented milk, fortified with taro leaves rich-phenolic compounds, demonstrated increased antioxidant potential and improved the survival of probiotics after simulated gastro-pancreatic digestion. As a result, these taro leaves polyphenol-fortified fermented milk may have the potential to protect the gastrointestinal tract from free radical injury. These promising findings suggest the need for further research on the use of taro leaves extract in food applications. Future studies should focus on the identification and purification of the bioactive compounds present in several taro species. Overall, this study provides an important contribution to the field of functional foods and highlights the potential health benefits of incorporating taro leaves into food products.

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CRediT authorship contribution statement

Mohamed G. Shehata: conceived and designed the experiments, carried out the experiments, the data, wrote the manuscript, and, Supervision, the work. **Nourhan M. Abd El-Aziz:** conceived and designed the experiments, carried out the experiments, the data, wrote the manuscript, and, Supervision, the work. **Taha Mehany:** wrote, revised and edited the manuscript. **Jesus Simal-Gandara:** conceived, Formal analysis, the data, revised and edited the manuscript, All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2023.115280.

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