El-Hadad *et al. Annals of Microbiology* (2024) 74:41
https://doi.org/10.1186/s13213-024-01785-4

Annals of Microbiology

UNIVERSITÀ DEGLI STUDI DI MILANO

ORIGINAL ARTICLE Open Access

Production and evaluation of probiotic brown yoghurt made from buffalo milk as an innovative functional dairy product

Sameh S. El-Hadad[1], Mahmoud Abd El-Aziz[1*], Mohamed T. Fouad[1], Ahmed F. Sayed[1] and Rehab S. Sayed[2]

Abstract

Purpose Brown fermented milk has become more popular with consumers due to its high nutritional value, creamy texture, delicious caramel flavor, and brownish color. Brown yoghurt (BY), made from buffalo milk fortified with probiotic bacteria was evaluated as an innovative functional dairy product.

Methods Standardized buffalo milk with a 1:1 protein/fat ratio was homogenized and browned at 97 ± 1 °C for 4 h. At 42 °C, it was inoculated with a 2.0% mixed starter culture and then divided into 4 portions. *Bifidobacterium bifidum* NRRL B-41410 and *Lacticaseibacillus rhamnosus* NRRL B-442, as probiotic bacteria, were added individually or in combination at a rate of 1.0% to create three treatments. The last portion without probiotics was served as a control BY.

Results *B. bifidum* showed the highest viable counts when added alone or in combination with *L. rhamnosus*, particularly on days 7 and 15. However, the addition of *B. bifidum* did not improve the physical and sensory properties of the BY, which were similar to those of the control. Adding *L. rhamnosus*, either alone (T3) or in combination with *B. bifidum* (T4), greatly improved the viscosity, hardness, flavor compounds, and sensory scores of the BY. The antioxidant activity against DPPH and ABTS radicals was also significantly enhanced. T3 and T4 also had a thicker body, a smoother and creamier texture, and a light caramel taste combined with a pleasant sour taste. Hydroxymethylfurfural (HMF) concentration in BY was affected slightly by bacteria strains and storage time.

Conclusions Standardized buffalo milk fortified with *L. rhamnosus* alone or in combination with *B. bifidum* can produce a higher-quality BY that is more acceptable as an innovative functional dairy product.

Keywords Brown yoghurt, Probiotic bacteria, Maillard reaction, Physiochemical properties, Sensory qualities

*Correspondence:
Mahmoud Abd El-Aziz
mabdelaziz69@yahoo.com
[1]Dairy Department, Food Industries & Nutrition Research Institute, National Research Centre, Giza, Egypt
[2]Regional Centre for Food and Feed, Agricultural Research Centre, Giza, Egypt

Introduction

Fermented milk, also known as cultured milk, has been consumed for centuries and is popular for its nutritional value and appealing flavor. Around 400 different types of fermented milk products are produced worldwide, such as yoghurt, Greek yoghurt, yammer, kefir, filmjölk, cultured buttermilk, cultured cream, and koumiss (Savaiano and Hutkins 2021; Turek and Wszołek 2021). Depending on the type of microorganism utilized [lactic acid bacteria (LAB), mold, or yeast]; lactic acid, acetic acid, carbon dioxide, diacetyl, acetaldehyde, alcohol, and many other compounds are produced throughout the fermentation process, giving the products their characteristic fresh taste and aroma (Zhenqiang 2022). In addition to enhancing the product's flavor, the fermentation process also increases the product's shelf life and improves its digestibility (Savaiano and Hutkins 2021).

Brown-fermented milk (BFM) or baked milk, which is produced by inoculating LAB after the Maillard reaction between milk and glucose at a suitable temperature, is fermented milk that is particularly well-liked in Russia, Belarus, and Ukraine (Ma et al. 2016). BFM is produced by holding milk at a high temperature for a long time (95–100 °C, 3–5 h), causing the Maillard reaction between lactose and milk proteins, which causes the milk to turn brown (Ni et al. 2017; Han et al. 2019; Li et al. 2023). The bacterial starter culture is then added to initiate the fermentation process after the Maillard reaction is complete. Apart from the color difference, this manufacturing process also leads to variations in taste, flavor, and nutritional value compared with other fermented products (Martins et al. 2000). These functional and sensorial differences occur due to chemical changes during the heating and fermentation process, which result in milk metabolomics profiles that vary depending on the type of milk and the phase of fermentation. Typically, the fat content of BFM made in industrial settings ranges from 3.5 to 4%; however, it is generally permitted to range from 0.5 to 8.9%. Additionally, it has at least 3% protein while usually has 4–5% carbohydrates (GOST 31455−2012). Recently, BFM became popular with consumers in China, Japan, Southeast Asia, Europe and other regions (Zhi-Yuan et al. 2010). Ma et al. (2016) reported that BFM has emerged as a new product in China since 2 year ago, with a variety of different forms; these include cooked fermented milk and Russian-style char-grilled fermented milk.

Yoghurt is one of the fermented dairy products that are most commonly consumed worldwide. Its strong consumer acceptability can be attributed, in particular, to its taste, nutritional value, and potential health benefits (Hadjimbei et al. 2022). It is available in several types, such as set or stirred yoghurt, full-fat, skimmed, and partially skimmed, as well as sweetened and flavored forms (Behare et al., 2016). Probiotics have also become more and more popular as beneficial ingredients around the world, particularly in dairy products. LAB has long been used as food starter cultures for food fermentations and as a provider of sensory properties. However, some of these bacteria, together with some members of the genus *Bifidobacterium*, have also been shown to have positive health effects on people when added to food or taken as supplements (Gao et al. 2021; González-González et sl., 2022). Probiotic bacteria have been clinically shown to have a wide range of health benefits, such as improved digestion, positive effects on the nervous system, enhanced immune responses, reduced blood cholesterol, vitamin synthesis, and protection against pathogenic microorganisms, among others (Markowiak and Śliżewska 2017; Sánchez et al. 2017). *Lacticaseibacillus rhamnosus* is one of the various species of *Lactobacillus* that are regarded as probiotics, and it has been the subject of numerous studies. It has been identified as a probiotic because of its ability to resist acid and bile, as well as its good growth characteristics that allow it to survive and persist in the gastrointestinal tract while preventing the growth and adherence of various pathogens (Mathipa-Mdakane and Thantsha 2022). *Bifidobacterium bifidum* is one of the species of *Bifidobacterium* that are essential to a healthy human gut microbiota. It produces acetic and lactic acid in the intestines (Hoffmann et al. 2021). *B. bifidum* is one of the bacteria that can help break down food (food digestion), absorb nutrients, and provide defense against harmful organisms. It also supports the immune system, preventing toxins and pathogens from going into the bloodstream (Chen et al. 2021; Mazziotta et al. 2023). However, BFM is not available in the Egyptian market, and buffalo milk represents approximately 44% of dairy production in Egypt (Sarhan and Al Damrawi 2022). Additionally, a lot of consumers may choose these products due to their distinct brown color and delicious cooked flavors. Probiotics can also be effectively transported by BFM, and their manufacture and consumption can help people's health. Variations in the starter cultures and probiotic combinations may also affect the probiotic viability in the final product due to antagonistic or symbiotic relationships, which may have an effect on the physiochemical and organoleptic properties of the product. Therefore, the aim of the study was to produce brown yoghurt (BY), as innovative dairy fermented milk made from buffalo milk fortified with probiotic bacteria, and evaluates its physiochemical, microbiological, and sensory qualities, as well as the effect of probiotic bacterial diversity on the product's technological and nutritional characteristics.

Materials and methods

Materials

Fresh buffalo milk was obtained from the farm of the Faculty of Agriculture, Cairo University, Egypt. Both starter cultures (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) and probiotic bacteria (*Bifidobacterium bifidum* NRRL B-41410 and *Lacticaseibacillus rhamnosus* NRRL B-442) were obtained from stock cultures at the Dairy Microbiology Lab, National Research Centre, Giza, Egypt. According to the procedure detailed by Tharmaraj and Shah (2003), each strain was individually activated using three transfers into MRS, then followed by further transfers into sterile 11% reconstituted skim milk powder. The 2,2-diphenyl-1-(2,4,6-trinitrophenyl)-hydrazinyl (DPPH, CAS: 1898-66-4), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS, CAS: 30931-67-0), semicarbazide hydrochloride (CAS: 563-41-7), 5-Hydroxymethyl-2-furaldehyde (HMF, CAS: 67-47-0) standard and analytical-grade zinc sulfate monohydrate (CAS: 7446-19-7) and potassium hexacyanoferrate (CAS: 14459-95-1) were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade methanol (CAS: 67-56-1) was procured from Merck in Darmstadt, Germany. All chemicals and reagents were analytical grade and obtained from different sources.

Methods

Brown yoghurt making

Standardized fresh buffalo milk (4.1% fat, 4.09% protein, 4.91% lactose, and 0.87% ash) was preheated to 65 °C, homogenized using a laboratory homogenizer (Polytron® PT 10–35 GT, Kinematica, Switzerland) at 21,000 rpm for 5 min, and then browned at 97±1 °C for 4 h in an electric oven (Li et al. 2023). The milk container was wrapped in foil to avoid evaporation. Browned buffalo milk was immediately cooled to 42 °C with cold water, then divided into 4 equal portions after being inoculated with 2.0% mixed starter culture (*L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*, at a ratio of 1:1). The first portion served as the control, while the second, third, and fourth portions were additionally inoculated with 1% *B. bifidum*, 1% *L. rhamnosus*, or 1% of their mixture as probiotic bacteria, respectively. All treatments were poured into 120 mL food-grade plastic cups and incubated at 42 °C until pH reached 4.6–4.7. The brown yoghurt (BY) samples were quickly cooled, stored at 5±1 °C and taken for testing on days 1, 7, 14, and 21 of the storage period.

Chemical analysis

Measurement of pH

The pH value of the BY samples was measured during the storage period using a laboratory pH meter with a glass electrode (HANNA, equipment, Portugal).

Determination of flavor compounds

The flavor compounds were determined by measuring the acetaldehyde and diacetyl content in the BY samples using the Conway microdiffusion-semicarbazide method of Less and Jago (1970; 1976). The carbonyl compounds react with the semicarbazide to form semicarbazone, which has an absorption peak at 224 nm for acetaldehyde and at 270 nm for diacetyl. A standard curve with a range of 5 to 50 µmol/100 g was used to calculate the acetaldehyde and diacetyl content in all BY samples.

Determination of hydroxymethylfurfural (HMF)

HMF was extracted from BY according the method of Shi et al. (2019) with some modifications. Briefly, 2 g of BY samples were weighed into 15 mL centrifuge tubes, added with 5 mL of methanol, and then vortex vigorously for 2 min. The extractives were centrifuged 6000 g for 20 min. The clear supernatant was poured into another centrifuge tube, and then 250 mL of Carrez I (15% potassium hexacyanoferrate solution) and 250 µL of Carrez II (30% zinc sulfate solution) were added. 2 mL of clear supernatant was put into a conical-bottom glass tube and dried at 40 °C using a nitrogen-pressure blowing concentrator after centrifugation also at 6000 g for 20 min. The remaining residue was re-dissolved with 2 mL of distilled water for further purification. UPLC H-Class Waters (Detector PDA, Column C18 phenomenex; 150 mm*4.6 mm*5 µm) were used to measure the HMF in purified samples. A 4:1 v/v acetonitrile-water combination was employed as the mobile phase for 20 min at a flow rate of 0.6 mL/min. The injection volume was 20 µL, and the UV detection wavelength was 284 nm. The concentration of HMF was determined by comparing the UV spectrogram and retention time value with those of the appropriate standards. The peak area values obtained from the various HMF standards were used to produce a standard curve with a concentration range of 2.5–25 µg/mL.

Antiradical activities

The antiradical activity of the BY samples was evaluated in filtrated whey using both stable DPPH and ABTS radical assays, according to Brand-Williams et al. (1995) and Re et al. (1999), respectively. 200 µL of BY filtered whey was added to 3.8 mL of DPPH (25 mg DPPH/L methanol) or ABTS (7 mM ABTS solution with 2.45 mM $K_2S_2O_8$) working solutions. A spectrophotometer (Shimadzu spectrophotometer, UV-Vis. 1201, Japan) was used to determine the degree of decolorization after an incubation period of 30 min in the dark at room temperature. The wavelengths used were 517 nm for the DPPH and 700 nm for the ABTS radical-scavenging assays. In the same way, control solutions—DPPH and ABTS without

whey were prepared. Both ABTS and DPPH scavenging activities were calculated using the formula shown below.

$$\text{BY antiradical activity } (\%) = \left[\frac{(A_0 - A_1)}{A_0}\right] \times 100$$

BY is brown yoghurt, A_0 is the absorbance of the control (DPPH or ABTS solution), and A_1 is the absorbance of the sample.

Viable counts of microorganisms

The BY sample was well mixed for 1 min to homogenize it before being diluted with buffered peptone water (BPW) for the microbiological examination. Ten-fold diluted homogenate samples were used as the inoculant for selective media plates. The pour-plate technique was used to count the bacteria after serial dilutions. The *L. delbrueckii* subsp. *bulgaricus* was counted on MRS agar after the pH was reduced to 4.58 with 1 M HCl, and the *S. thermophilus* was counted on M17 agar (Oxoid) after 48 h of aerobic incubation at 37 °C (Dave and Shah 1996). In anaerobic conditions, *B. bifidum* was counted using MRS-MRS-NNLP (MRS-nalidixic acid, neomycine sulphate, lithium chloride, and paromomycine sulphate) agar containing 0.05% of L-cysteine-HCl at 37 °C for 72 h, while *L. rhamnosus* was counted using MRS-V (MRS-vancomycine) agar containing 1 mg vancomycine/L and 1.1 mM bromocresol purple at 43 °C for 72 h, according to the method described by Tharmaraj and Shah (2003).

Physical properties

Apparent viscosity

The apparent viscosity of the BY samples was measured using a Brookfield digital viscometer (Model DV-II, Canada) with the measuring spindle 04 rotating at 12 rpm. The samples were completely stirred using a mixer with 2.6 cm blades (Heidolph No. 50 111, Type RZRI, Germany) at speed 5 for 20 s and then poured into a 100-mL glass cylinder. The viscosity was then measured at 7±1 °C. Each result was recorded in triplicate in mPa.s after a 30 s rotation.

Hardness

The BY samples were subjected to texture profile analysis (TPA) using a texture analyzer (TA-XT2 Texture Analyzer, Texture Technologies Crop, Scarsdale, NY) that was linked to a PC running texture analysis software. The moving crosshead was fitted with an artificial plastic cylinder (45 Perspex Cone, 432−081). The speed of penetration was set at 70 mm/min, and the plastic cylinder was inserted 20 mm below the surface of the BY sample, which was set on a flat holding plate at 5±2 °C. The hardness of BY samples was expressed in Newton (N).

Color attributes

The color attributes of BY samples were measured by Chroma Meter (Konica Minolta, model CR 410, Japan), calibrated with a white plate and light trap supplied by the manufacturer. The L*, a*, and b* color values were determined according to the International Commission on Illumination (CIE) Lab color space system (CIE 1976). L* represents a range of darkness from black (0) to white (100), a* for a range of redness (+) to greenness (-), and b* for a range of yellowness (+) to blueness (-). Each sample was measured three times, and the arithmetic means were calculated.

Sensory evaluation

The BY samples were evaluated on appearance, body & texture, and flavor by eleven male and female judges, all between the ages of 25 and 50 years, who were carefully selected from the staff of the dairy department at the National Research Centre (Egypt). The nine-point hedonic scale, as described by Shazly et al. (2022), ranges from extremely like (9 points) through like or dislike (5 points) to extremely dislike (1 point). BY samples were provided in three-digit-coded clear plastic containers, and they were tasted for the first time 15 min after being taken out of the refrigerator. In order to refresh their palates in between samples, water and unsalted crackers were provided.

Statistical analysis

Statistical analysis with Statistical Analysis System (SAS 2008) software was done using the General Linear Model (GLM) technique. Two-way analysis of variance (ANOVA) and Duncan's multiple comparison method were used to compare the means. A probability of $P \leq 0.05$ was chosen for establishing statistical significance. The results were presented as means±SE after three separate experiments were done.

Results and discussion

Viable counts of microorganisms

The viable counts of the starter cultures *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* as well as the probiotic bacteria *B. bifidum* NRRL B-41,410 (Fijan 2014) and *L. rhamnosus* NRRL B-442 (Steele 2022) for brown yoghurt (BY) made from buffalo milk during storage at 5±1 °C for 21 days are presented in Fig. 1. The viable counts of starter and probiotic bacteria were generally higher than the recommended limit of 10^6 cfu/g (Gueimonde et al. 2004), which is a crucial required in the final product that can be stored for up to 21 days. The highest viable counts of all bacteria in all BY samples were observed on day 7 of storage; following that, the counts began to decrease. *S. thermophilus* counts were more stable during the 21-day storage period, with either *B.*

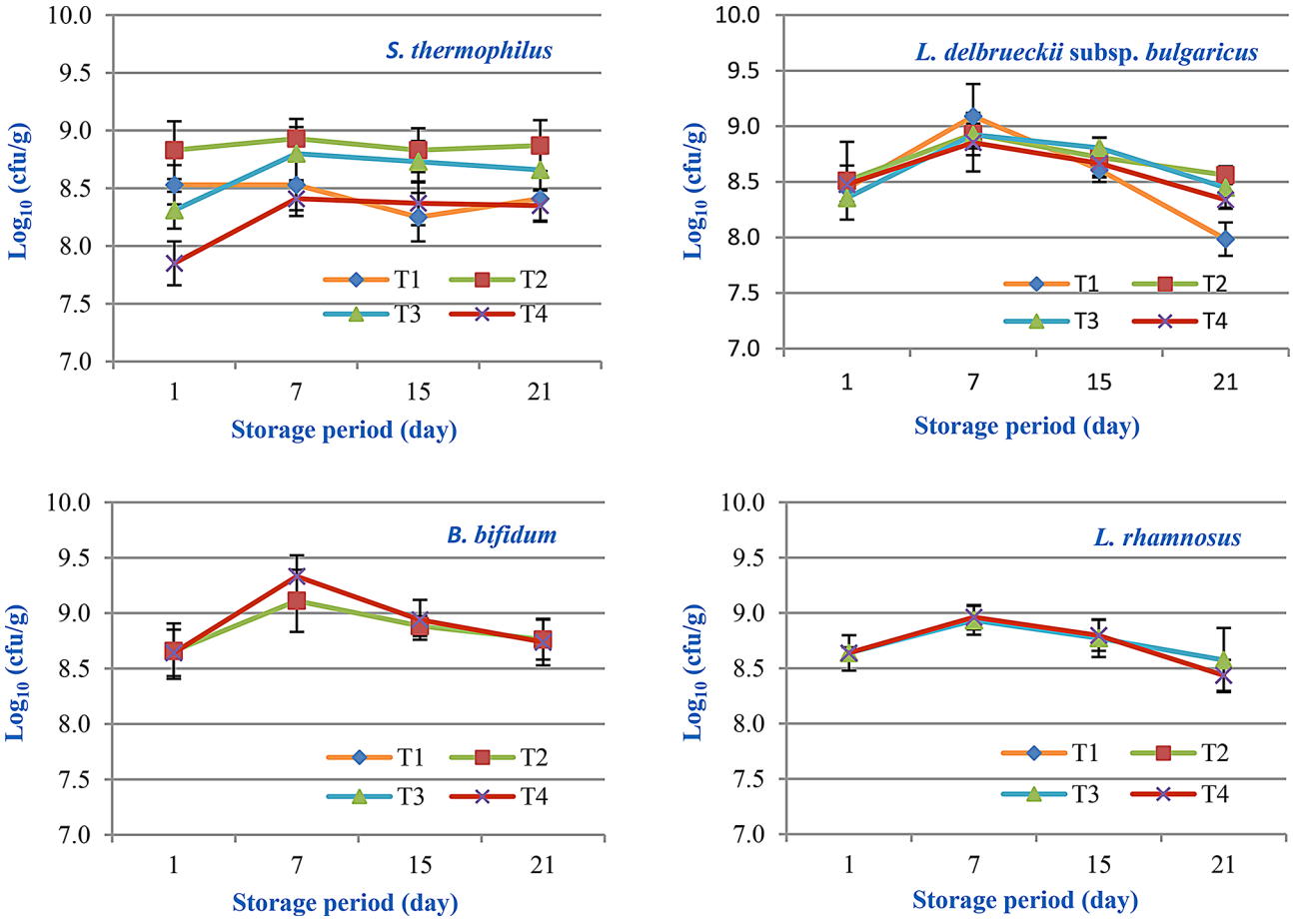

Fig. 1 Viable counts of bacterial strains in brown yoghurt made from buffalo milk fortified with probiotic bacteria during storage at 5 ± 1 °C for 21 days

bifidum (T2) or *L. rhamnosus* (T3) present. Similarly, Shori et al. (2022) showed that *L. rhamnosus* enhanced the viability of Lactobacillus spp. and *S. thermophilus* in yoghurt. With the exception of the *S. thermophilus* counts, the reduction reached statistical significance ($P \leq 0.05$) on day 21. Conversely, the highest counts of starters, *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, were found on the first day of storage in the probiotic yoghurt made with *aloe vera* gel (Ahmed et al. 2023) and on the third day of storage in the probiotic yoghurt made from ewe's milk (Shazly et al. 2022). Although most strains of *Bifidobacterium* grow slowly in milk due to their poor proteolytic activity, the viable counts of *B. bifidum* were the highest in the T2 and T4. A possible explanation for the increase in *B. bifidum* counts is the metabolic activity of *S. thermophilus* and *L. rhamnosus*, which supplies nutrients in the form of di-, tri-, and oligo-peptides (Liu et al. 2018; Li et al. 2020).

Chemical properties

Changes in pH and flavor compounds

The pH, flavor compounds measured as acetaldehyde and diacetyl (μmol/100 g sample), and antioxidant activity against DPPH radicals of BY fortified with probiotic bacteria during storage at 5±1 °C for 21 days are displayed in Table 1. Probiotic BY samples did not differ significantly from control BY samples in pH ($P > 0.05$), suggesting that neither *L. rhamnosus* nor *B. bifidum* contribute to increased acidity. Similarly, Jia et al. (2016) reported that *L. rhamnosus* produced modest acidification potential in goat milk. All BY samples showed a drop in pH during storage; the rate of drop was significant until day 15 ($P < 0.05$) and then non-significant. The decline in pH values is associated with the metabolic activity of bacteria, which have the capability of degrading lactose and producing more organic acids such as lactic and acetic acids. The pH values dropped considerably more during storage, reaching a range of 3.99 to 4.10, which was comparable to much research as well (Kang et al. 2019; Shazly et al. 2022; Zhu et al. 2023). As a result, neither the browning process nor the probiotic fortification had a significant effect ($P > 0.05$) on the fermentation process or the pH changes during storage.

The addition of *L. rhamnosus* alone (T3) or in combination with *B. bifidum* (T4) showed the highest concentrations of both acetaldehyde and diacetyl. The differences

Table 1 pH values and flavor compounds of probiotic yoghurt made from buffalo milk fortified with probiotic bacteria during storage at 5 ± 1 °C for 21 days

Brown yoghurt treatments	Storage periods (days)			
	1	7	15	21
pH				
T1	$4.56^{Aa} \pm 0.06$	$4.34^{Ab} \pm 0.07$	$4.09^{Ac} \pm 0.08$	$4.03^{Ac} \pm 0.04$
T2	$4.50^{Aa} \pm 0.06$	$4.33^{Ab} \pm 0.05$	$4.03^{Ac} \pm 0.07$	$4.01^{Ac} \pm 0.05$
T3	$4.55A^{a} \pm 0.04$	$4.29^{Ab} \pm 0.06$	$4.12^{Ab} \pm 0.08$	$4.10^{Ac} \pm 0.07$
T4	$4.52A^{a} \pm 0.06$	$4.28^{Ab} \pm 0.05$	$4.14^{Ac} \pm 0.06$	$4.07^{Ac} \pm 0.05$
Acetaldehyde (µmol/100 g)				
T1	$86.52^{Ba} \pm 5.71$	$78.93^{Aa} \pm 3.97$	$44.13^{Bb} \pm 3.01$	$40.40^{Ab} \pm 3.28$
T2	$84.82^{Ca} \pm 4.45$	$86.22^{Aa} \pm 4.37$	$43.66^{Bb} \pm 4.45$	$42.39^{Ab} \pm 5.19$
T3	$99.15^{Aa} \pm 6.31$	$90.59^{Ab} \pm 3.42$	$53.27^{Ac} \pm 2.82$	$45.17^{Ac} \pm 3.31$
T4	$97.78^{ABa} \pm 7.71$	$92.70^{Aa} \pm 4.56$	$48.38^{ABb} \pm 3.81$	$42.96^{Ac} \pm 4.02$
Diacetyl (µmol/100 g)				
T1	$15.11^{Bd} \pm 2.54$	$40.89^{Bc} \pm 2.31$	$62.55^{Ab} \pm 2.89$	$79.41^{Ba} \pm 6.74$
T2	$20.01^{ABd} \pm 2.51$	$37.78^{Bc} \pm 4.11$	$60.39^{Ab} \pm 5.05$	$81.70^{Ba} \pm 2.66$
T3	$22.78^{Ad} \pm 1.92$	$47.75^{A\,c} \pm 3.27$	$63.83^{Ab} \pm 2.71$	$89.68^{Aa} \pm 6.56$
T4	$23.11^{Ad} \pm 3.44$	$46.33^{Ac} \pm 3.51$	$64.56^{Ab} \pm 3.31$	$86.08^{ABa} \pm 3.41$

^{ABC}Means ($n = 3 \pm SE$) with different alphabets are significantly different between each type of yoghurt for a particular day of storage; ^{abcd}Means with different alphabets are significantly different within each type of yoghurt; T1, brown yoghurt fermented with starter culture; T2, brown yoghurt fermented with starter culture and *B. bifidum*; T3, brown yoghurt fermented with starter culture and *L. rhamnosus*; T4, brown yoghurt fermented with starter culture, *L. rhamnosus* and *B. bifidum*

between T3 and control BY (T1) were significant ($P \leq 0.05$) at days 1 and 15 for acetaldehyde content, whereas for daicetly, the differences were significant at days 1, 7, and 21 ($P \leq 0.05$). However, all of the BY samples had an acetaldehyde concentration that ranged from 84.82 ± 4.45–99.15 ± 6.31µmol/100 g on day 1 and decreased during storage to 40.40 ± 3.28–45.17 ± 3.31µmol/100 g on day 21. Conversely, the diacetyl concentration of BY samples ranged from 15.11 ± 2.54–23.11 ± 1.44 µmol/100 g on day 1 and significantly increased ($P < 0.05$) during storage to 79.41 ± 6.74–89.68 ± 6.56 µmol/100 g on day 21. These values were higher than the acetaldehyde concentrations that could be detected in traditional yoghurt made from various starter cultures, which were found to range from 12.09 to 43.60 µmol/100 g (Tamime and Robinson 2000). According to previous studies, the ranges of acetaldehyde concentrations were 9.30–40.70 µmol/100 g (Hernandez et al. 1995), 38.37–66.28 µmol/100 g in non-fat yoghurt from a high milk protein powder (Mistry and Hassan 1992), and 7.50–21.66 µmol/100 g in probiotic concentrated yoghurt fortified with CLA (Abd El-Salam et al. 2011). Han et al. (2019) reported that the distinctive flavor of a product is formed by flavor components such as carboxylic acids, aldehydes, alcohols, and ketones, which are produced during the browning stage and then increased during fermentation (Han et al. 2019). Similar, the concentrations of diacetyle found in BY samples were higher than those found in studies by Abd El-Salam et al. (2011) and Hassan et al. (2015), which were 2.54–7.35 and 0.92–3.22 µmol/100 g, respectively. Thus, BY fermented with starter culture alone or in combination with probiotics is characterized by a high concentration of flavor compounds.

Changes in HMF

After fermentation (1 day) and on day 15 of storage at 5 ± 1 °C, the HMF concentrations of BY as influenced by probiotic bacteria were determined. On day 1, T2, which fermented with *B. bifidum*, had the highest HMF concentration, followed by T4, which fermented with *L. rhamnosus* and *B. bifidum.* These findings might be explained by *B. bifidum* fermentation, which accelerates the breakdown of lactose into glucose and galactose and stimulates Maillard reactions. Similarly, Mottram et al. (2002) reported that methylglyoxal (MGO) and HMF levels increase as fermentation time increases. HMF levels increase due to the acidic conditions induced by starter culture fermentation, which accelerate the conversion of lactose and lead to the rapid formation of HMF through Maillard reactions. Conversely, On the other hand, T4, which contains *L. rhamnosus*, had the lowest HMF content; nevertheless, the differences were not statistically significant ($P > 0.05$), as illustrated in Fig. 2. In contrast to predictions, all BY treatments showed a small drop in HMF concentrations during cold storage. Han et al. (2019) found a similar finding after storage brown fermented milk at 4 °C for 21 days Albalá-Hurtado et al. (1998) reported that no variations in the HMF levels have been observed, which aligned with the values found in the baby milk kept at 20 °C but not in the milk kept at 37 °C, where an increase in HMF levels was detected.

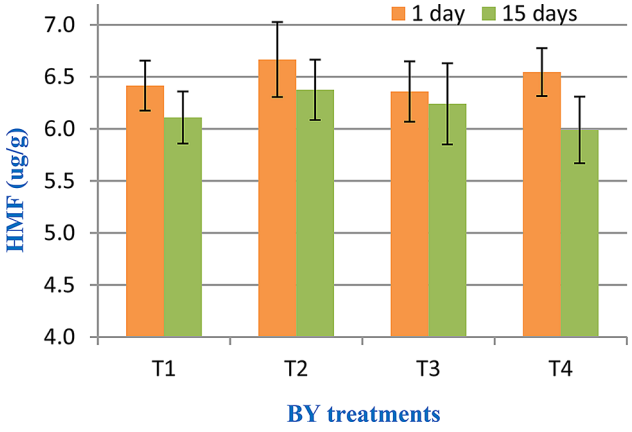

Fig. 2 Hydroxymethylfurfural of brown yoghurt made from buffalo milk fortified with probiotic bacteria during storage at 5 ± 1 °C for 15 days. T1, brown yoghurt fermented with starter culture; T2, brown yoghurt fermented with starter culture and *B. bifidum*; T3, brown yoghurt fermented with starter culture and *L. rhamnosus*; T4, brown yoghurt fermented with starter culture, *L. rhamnosus* and *B. bifidum*

Antiradical activities

As shown in Table 2, probiotic BY samples (T2, T3 and T4) exhibited higher antioxidant activity against DPPH and ABTS radicals compared to control BY. The proteolysis degree by probiotic strains and the type of peptides released are considered other factors that participated in the observed increment in the antioxidant activity (Taha et al. 2017). The antioxidant activity against DPPH radicals was more pronounced in T3, followed by T4 ($P \leq 0.05$). Similarly, Liu et al. (2018) found that *L. rhamnosus* significantly enhanced the DPPH radical scavenging behavior of cheddar cheese during the ripening period compared to the control group. *L. rhamnosus* may stimulate the production of smaller-molecule polypeptides through proteolysis. Moreover, *L. rhamnosus* has the ability to produce exopolysaccharides, which have antioxidant properties (Faraki and Rahmani 2020). In general, Hoffmann et al. (2021) reported that lactobacilli cell-free supernatants like *L. rhamnosus* exhibit strong antioxidant activities against DPPH radical scavenging, inhibition of linoleic acid peroxidation, hydroxyl radical scavenging and reducing power (RP) assays. As the time of storage increased, the antioxidant activity against DPPH radicals increased, the increase was significant ($P \leq 0.05$) at day 7 for T1 and T2, whereas for T3 and T4 the increase ($P \leq 0.05$) was significant at day 21. Increased antioxidant activity during storage could have been related to protein hydrolysis (Shazly et al. 2022). Similarly, Liu et al. (2018) found that *L. rhamnosus* significantly enhanced the DPPH radical scavenging behavior of cheddar cheese during the ripening period compared to the control group.

Physical properties

Apparent viscosity and firmness

According to a previous study (Akalın et al. 2012; Ichimura et al. 2023), the extended heating of the milk at high temperatures reduced the texture properties of the resultant fermented milk: viscosity and firmness. Viscosity plays a crucial role in consumer acceptance of yoghurt. Viscosity reflects the consistency and hardness of yoghurt samples; the higher, the better (Hasani et al. 2016). As shown Fig. 3, T3 and T4 exhibited higher viscosity and hardness ($P \leq 0.05$) in comparison to T1 and T2, indicating that *L. rhamnosus* fortification improved BY viscosity and hardness regardless of whether *B. bifidum* was present or not. For samples fortified with a combination of *L. rhamnosus* and *B. bifidum* (T4), the improvement in BY hardness was more noticeable. Yang et al. (2010) reported that capsular polysaccharide produced by *L. rhamnosus* (composed mainly of galactose and N-acetylglucosamine in a ratio of 5:1) was identified

Table 2 Antiradical activities of brown yoghurt made from buffalo milk fortified with probiotic bacteria during storage at 5 ± 1 °C for 21 days

Brown yoghurt treatments	Storage periods (days)			
	1	7	15	21
DPPH scavenging activity (%)				
T1	$8.84^{Bc} \pm 0.71$	$11.52^{Bbc} \pm 0.21$	$12.95^{Bab} \pm 1.11$	$15.71^{Ba} \pm 0.37$
T2	$10.75^{Bb} \pm 0.57$	$15.65^{ABa} \pm 1.31$	$14.73^{ABa} \pm 0.53$	$17.22^{ABa} \pm 0.73$
T3	$14.45^{Ab} \pm 0.93$	$17.72^{Aab} \pm 1.81$	$16.50^{Aab} \pm 0.66$	$19.99^{Aa} \pm 1.65$
T4	$13.19^{Ab} \pm 1.35$	$14.88^{Bab} \pm 1.45$	$17.79^{Aa} \pm 0.93$	$17.87^{ABa} \pm 1.21$
ABTS scavenging activity (%)				
T1	$33.30^{Bb} \pm 1.97$	$36.65^{Bab} \pm 2.36$	$38.55^{Ba} \pm 2.61$	$39.61^{Ba} \pm 2.28$
T2	$38.05^{Ab} \pm 1.24$	$45.34^{Aab} \pm 1.54$	$47.72^{Aa} \pm 1.47$	$47.56^{Aa} \pm 2.54$
T3	$40.92^{Ab} \pm 2.31$	$44.67^{Aab} \pm 1.25$	$45.56^{Aa} \pm 2.11$	$47.73^{Aa} \pm 2.24$
T4	$39.97^{Ab} \pm 0.97$	$44.48^{Aab} \pm 1.65$	$45.73^{Aa} \pm 2.15$	$49.02^{Aa} \pm 1.24$

^{ABC}Means ($n = 3 \pm SE$) with different alphabets are significantly different between each type of yoghurt for a particular day of storage; ^{abcd}Means with different alphabets are significantly different within each type of yoghurt; T1, brown yoghurt fermented with starter culture; T2, brown yoghurt fermented with starter culture and *B. bifidum*; T3, brown yoghurt fermented with starter culture and *L. rhamnosus*; T4, brown yoghurt fermented with starter culture, *L. rhamnosus* and *B. bifidum*

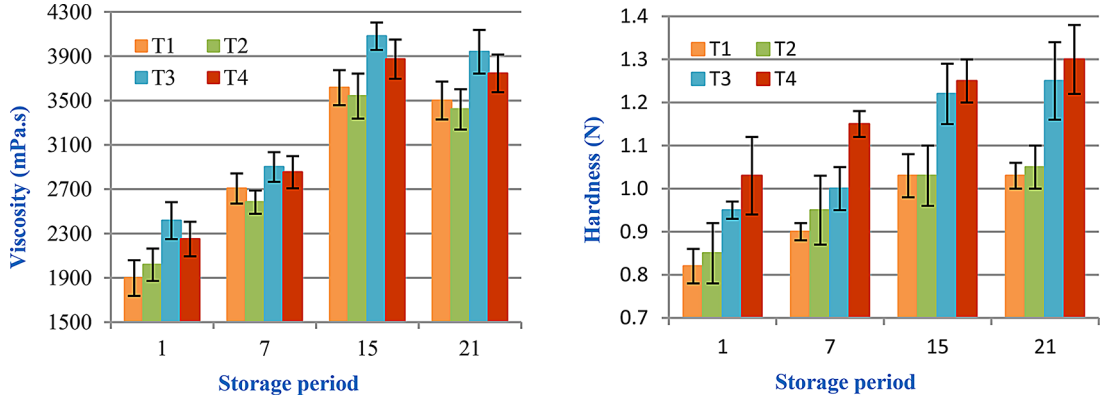

Fig. 3 Apparent viscosity and hardness of brown yoghurt made from buffalo milk fortified with probiotic bacteria during storage at 5 ± 1 °C for 21 days. T1, brown yoghurt fermented with starter culture; T2, brown yoghurt fermented with starter culture and *B. bifidum*; T3, brown yoghurt fermented with starter culture and *L. rhamnosus*; T4, brown yoghurt fermented with starter culture, *L. rhamnosus* and *B. bifidum*

Table 3 Color parameters of brown yoghurt made from buffalo milk fortified with probiotic bacteria during storage at 5 ± 1 °C for 15 days

Brown yoghurt treatments	Storage periods (days)	
	1	15
L*		
T1	82.77 ± 1.08^{ns}	82.02 ± 0.55
T2	82.72 ± 1.23	82.96 ± 1.08
T3	82.67 ± 1.06	83.61 ± 0.66
T4	82.56 ± 0.85	83.11 ± 0.98
a*		
T1	2.47 ± 0.16^{ns}	2.53 ± 0.13
T2	2.28 ± 0.29	2.21 ± 0.43
T3	2.38 ± 0.24	2.41 ± 0.17
T4	2.42 ± 0.22	2.33 ± 0.42
b*		
T1	18.63 ± 0.38^{ns}	18.79 ± 0.41
T2	18.64 ± 0.37	18.06 ± 1.08
T3	18.66 ± 0.41	17.76 ± 0.39
T4	18.86 ± 1.05	18.17 ± 0.54

Means (n = 3 ± SE) with the same letters are not significantly different at $P \leq 0.5$; ns, non-significant; T1, brown yoghurt fermented with starter culture; T2, brown yoghurt fermented with starter culture and *B. bifidum*; T3, brown yoghurt fermented with starter culture and *L. rhamnosus*; T4, brown yoghurt fermented with starter culture, *L. rhamnosus* and *B. bifidum*

during fermentation using both optical and transmission electron microscopy. *L. rhamnosus* can be used in the dairy industry to improve the rheological properties of fermented milk products by increasing their viscosity and water-holding capacity. Similarly, Jia et al. (2016) found that adding *L. rhamnosus* GG can improve the quality of goat milk yoghurt. *L. rhamnosus* grew and acidified milk, as well as being able to increase the viscosity of and confer a desirable texture to the fermented product (Salazar et al. 2009). However, the viscosity and hardness of BY samples were unaffected significantly ($P > 0.05$) by *B. bifidum* fortification alone. Over the storage period of 21 days, BY samples exhibited a continuous increase in viscosity until day 15 ($P \leq 0.05$) and then a slight decrease, whereas hardness continued to increase until day 21. Viscosity is increased because the particles are more swollen and attached over a greater area, and gel particles have stronger connections (Walstra et al. 1999). Also, Jia et al. (2016) reported that when the acidity value is too high, the protein gel becomes dehydrated. Thus, it reduced the yoghurt's capacity to hold water and caused the whey to dissolve, increasing the yoghurt's hardness. Such an effect has been confirmed by other researchers (Doleyres et al. 2005; Shazly et al. 2022).

Color attributes

On day 1, there are no differences in the L*, a*, and b* colors of BY, indicating that the fortification with probiotic bacteria had no effect on the color attributes during fermentation (Table 3). The lightness, redness, and yellowness degrees were within the range found by Han et al. (2019) and were between 82.56 ± 0.95–82.77 ± 1.08, 2.28 ± 0.29–2.47 ± 0.16, and 18.06 ± 1.08–18.86 ± 1.05, respectively. Additionally, both T1 and T2 did not exhibit any discernible changes in their color attributes during storage, which suggests that colored compounds were not formed. Han et al. (2019) found a similar finding with brown fermented milk stored at 4–7 °C. *L. rhamnosus* appears to have the capacity to bind or absorb some Maillard reaction products during storage, as indicated by the observation that T3 and T4 showed a slight rise in lightness and a decrease in yellowness. These results may be related to the ability of *L. rhamnosus* to produce capsular polysaccharides (Yang et al. 2010), which adsorb or reduce Millard reaction compounds. The color attributes—a slight increase in the degree of whiteness (L*) and a slight decrease in the degree of yellowness (b*)—are confirmed by a slight drop in the HMF concentration after storage at 5 ± 1 °C.

Table 4 Sensory evaluation of brown yoghurt made from buffalo milk fortified with probiotic bacteria during storage at 5 ± 1 °C for 21 days

Brown yoghurt treatments	Period of storage (day)			
	1	7	15	21
Appearance				
T1	8.18 ± 0.18^{ns}	8.09 ± 0.20	7.90 ± 0.19	7.81 ± 0.19
T2	8.00 ± 0.19	8.09 ± 0.21	7.81 ± 0.22	7.81 ± 0.23
T3	8.36 ± 0.20	8.36 ± 0.25	8.27 ± 0.19	8.27 ± 0.18
T4	8.27 ± 0.14	8.27 ± 0.14	8.27 ± 0.16	8.10 ± 0.12
Body & texture				
T1	7.81 ± 0.22^{Ba}	7.81 ± 0.21^{Ba}	7.63 ± 0.21^{Ba}	7.63 ± 0.23^{Ba}
T2	7.71 ± 0.26^{Ba}	7.81 ± 0.21^{Ba}	7.72 ± 0.20^{Ba}	7.63 ± 0.26^{Ba}
T3	8.45 ± 0.31^{Aa}	8.63 ± 0.28^{Aa}	8.63 ± 0.28^{Aa}	8.45 ± 0.15^{Aa}
T4	8.36 ± 0.18^{Aa}	8.63 ± 0.15^{Aa}	8.54 ± 0.17^{Aa}	8.45 ± 0.23^{Aa}
Flavor				
T1	7.17 ± 0.21^{Ba}	7.36 ± 0.20^{Ba}	7.36 ± 0.23^{Ba}	7.09 ± 0.24^{Ba}
T2	7.27 ± 0.22^{Ba}	7.36 ± 0.24^{Ba}	7.27 ± 0.19^{Ba}	7.18 ± 0.18^{Ba}
T3	8.36 ± 0.15^{Aa}	8.63 ± 0.24^{Aa}	8.63 ± 0.16^{Aa}	8.27 ± 0.22^{Aa}
T4	8.27 ± 0.19^{Aa}	8.45 ± 0.16^{Aa}	8.45 ± 0.14^{Aa}	8.27 ± 0.15^{Aa}

^{AB}Means ($n = 3 \pm SE$) with different alphabets are significantly different between each type of yoghurt for a particular day of storage; ^{ab}Means with different alphabets are significantly different within each type of yoghurt; T1, brown yoghurt fermented with starter culture; T2, brown yoghurt fermented with starter culture and *B. bifidum*; T3, brown yoghurt fermented with starter culture and *L. rhamnosus*; T4, brown yoghurt fermented with starter culture, *L. rhamnosus* and *B. bifidum*

Sensory evaluation

Table 4 shows the scores for probiotic BY made from buffalo milk during storage for 21 days at 5 ± 1 °C in terms of appearance, body & texture, and flavor. In general, all of the BY samples were characterized by a pleasant flavor (caramel flavor), a smooth texture, and a soft body. According to Li et al. (2020), the brown fermented milk has significantly more di- and tri-peptides, which contribute to a unique taste. With the exception of small whey droplets that appeared on the surface of T1 and T2 after two weeks of storage, there were no appreciable differences in the appearance among all of the BY samples. Additionally, no discernible change in flavor or texture attributes was found between T1 and T2 ($P > 0.05$), indicating that *B. bifidum* had no positive effect on sensory attributes of BY. Similarly, Tian et al. (2022) found that the flavor and taste of the yoghurt grown just with *B. longum* did not differ significantly from the yoghurt fermented with the starter culture. However, T3 and T4 were superior in terms of flavor—a light caramel taste combined with a desirable sour taste, a smoother and creamy texture, and a thicker body ($P < 0.05$). The quality of goat milk yoghurt was found to be improved by the inclusion of *L. rhamnosus* in the appropriate proportion (Jia et al. 2016). This suggests that adding *L. rhamnosus*, either with or without *B. bifidum*, can improve the sensory properties of BY. The sensory attributes of all BY samples changed little during storage, however on day 21, the taste appeared somewhat sour. During storage, similar and acceptable sensory qualities were observed, with a tendency for the quality to reduce with an extended storage period for day 21. Such an effect was found in fermented goat milk with *B. animalis* ssp. *lactis* or *B. longum* by Mituniewicz-Małek et al. (2017).

Conclusion

Standardized buffalo milk (~4.0% fat, ~4.0% protein) heated to 97 ± 1 °C for 4 h can be used to produce high-quality BY as an innovative dairy product with a more acceptable flavor and color. *B. bifidum* NRRL B-41,410 is not recommended to be added alone for making BY, but it can be added to mixed cultures as an adjunct to *L. rhamnosus* NRRL B-442 because of its health benefits. *L. rhamnosus* NRRL B-442 has probiotic properties, but it can also be technologically utilized to enhance the viscosity, hardness, flavor compounds, antioxidant activity and sensory properties of resultant BY. Brown fermented milks can be widely used in other dairy products, like frozen yoghurt, because they are well-liked by customers. Furthermore, the production of these products depends on Maillard reaction products, which have the potential to be harmful. As a result, the whole product must be biologically evaluated, not just individual components, to decide whether there will be positive or negative effects on health and thus limit or expand its production. Some natural substances, such as certain plant extracts, can also be utilized to reduce the production of HMF as long as they don't change the final product's taste or color.

Author contributions

Conceptualization: SSE, MA; Methodology: SSE, RSS, MA, MTF; Formal analysis and investigation: SSE, RSS, MA; Writing-original draft preparation: SSE, RSS, MA; Writing-review and editing: MA, SSE, RSS; Funding acquisition: AFS, SSE, MTF; resources: RSS, SSE, MTF; supervision: AFS, MA.

Funding

Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).

Data availability

All data generated or analyzed during this study are included in this published article.

Declarations

Consent for publication

All of the authors consent to the publication of this manuscript in Annals of Microbiology.

Competing interests

The authors declare no competing interests in publishing this manuscript.

Received: 2 August 2024 / Accepted: 19 November 2024
Published online: 24 December 2024

References

Abd El-Salam MH, Hippen AR, El-Shafie K, Assem FM, Abbas HM, Abd El-Aziz M, Sharaf O, El-Aassar M (2011) Preparation and properties of probiotic concentrated yoghurt (labneh) fortified with conjugated linoleic acid. Int J Food Sci Technol 46:1365–2621

Ahmed S, Noor A, Tariq M, Zaidi A (2023) Functional improvement of synbiotic yogurt enriched with *Lacticaseibacillus rhamnosus* and aloe vera gel using the response surface method. Food Production, Processing and Nutrition 5:38

Akalın AS, Unal G, Dinkci N, Hayaloglu AA (2012) Microstructural, textural, and sensory characteristics of probiotic yoghurts fortified with sodium calcium caseinate or whey protein concentrate. J Dairy Sci 95:3617–3628

Albalá-Hurtado S, Veciana-Nogues MT, Marine-Font A, Vidal-Carou MC (1998) Changes in furfural compounds during storage of infant milks. J Agric Food Chem 46:2998–3003

Behare P, Kumar H, Manda S (2016) Yogurt: Yogurt based products. Encyclopedia of Food and Health 2016: 625–631

Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. LWT – Food Sci Technol 28:25–30

Chen J, Chen X, Ho CL (2021) Recent development of probiotic *bifidobacteria* for treating human diseases. Front Bioeng Biotechnol 9:770248

CIE (1976) Official Recommendations on Uniform Colour Spaces, Colour Differences Equations, and Metric Colour Terms. Supplement No. 2 to CIE Publication, No.15, Paris

Dave RI, Shah NP (1996) Evaluation of media for selective enumeration of *Streptococcus thermophillus*, *Lactobacillus delbrueckii* ssp. *Bulgaricus*, *Lactobacillus acidophilus* and bifidobacteria. Journal of Dairy Science 79 (9):1529–1536

Doleyres Y, Schaub L, Lacroix C (2005) Comparison of the functionality of exopolysaccharides produced in situ or added as bioingredients on yoghurt properties. J Dairy Sci 88:4146–4156

Faraki A, Rahmani F (2020) The antioxidant activity of lactic acid bacteria and probiotics: a review. J Food Saf Hygiene 6:168–182

Fijan S (2014) Microorganisms with claimed probiotic properties: an overview of recent literature. Int J Environ Res Public Health 11(5):4745–4767

Gao J, Li X, Zhang G, Sadiq FA, Simal-Gandara J, Xiao J, Sang Y (2021) Probiotics in the dairy industry–advances and opportunities. Compr Rev Food Sci Food Saf 20:3937–3982

González-González F, Delgado S, Ruiz L, Margolles A, Ruas-Madiedo P (2022) Functional bacterial cultures for dairy applications: towards improving safety, quality, nutritional and health benefit aspects. J Appl Microbiol 133(1):212–229

GOST 31455 (2012) Ryazhenka. Technical conditions. International State Standard GOST 31455 – 2012, Ryazhenka. Specifications, Russian

Gueimonde M, Delgado S, Mayo B, Ruas-Madiedo P, Margolles A, Reyes-Gavilán CG (2004) Viability and diversity of probiotic *Lactobacillus* and *Bifidobacterium* populations included in commercial fermented milks. Food Res Int 37(9):839–850

Hadjimbei E, Botsaris G, Chrysostomou S (2022) Beneficial effects of yoghurts and probiotic fermented milks and their functional food potential. Food 11(17):2691

Han Z, Gao J, Wang X, Wang W, Dong J, Zhang Y, Wang S (2019) Formation and alterations of the potentially harmful Maillard reaction products during the production and storage of brown fermented milk. Molecules 24:272

Hasani S, Khodadadi I, Heshmati A (2016) Viability of *Lactobacillus acidophilus* in rice bran-enriched stirred yoghurt and the physicochemical and sensory characteristics of product during refrigerated storage. Int J Food Sci Technol 51:2485–2492

Hassan LK, Haggag HF, El-Kalyoubi MH, Abd EL-AM, El-Sayed MM, Sayed AF (2015) Physico-chemical properties of yoghurt containing cress seed mucilage or guar gum. Annals Agricultural Sci 60:21–28

Hernandez EJG, Estepa RG, Rivas IR (1995) Analysis of diacetyl in yoghurt by two new spectrophotometric and fluorimetric methods. Food Chem 53:315–319

Hoffmann A, Kleniewska P, Pawliczak R (2021) Antioxidative activity of probiotics. Archives Med Sci 17:792–804

Ichimura T, Kusaka M, Nakamura T (2023) The effect of high temperature heat treatment and homogenization on the microstructure of set yogurt curd networks. J Dairy Res 90:306–311

Jia R, Chen H, Chen H, Ding W (2016) Effects of fermentation with *Lactobacillus rhamnosus* GG on product quality and fatty acids of goat milk yoghurt. J Dairy Sci 99:221–227

Kang S-S, Kim MK, Kim Y-J (2019) Comprehensive evaluation of microbiological and physicochemical properties of commercial drinking yoghurts in Korea. Food Sci Anim Resour 39:820–830

Lees GJ, Jago GR (1976) Methods for estimation of acetaldehyde in cultured dairy products. Australian J Dairy Technol 24:181–185

Less GJ, Jago GR (1970) Formation of acetaldehyde from -deox-D.S.-phosphate in lactic acid bacteria. J Dairy Res 43:139–144

Li D, Zheng Y, Kwok LY, Zhang WY, Sun TS (2020) Metabolic footprinting revealed key biochemical changes in a brown fermented milk product using *Streptococcus thermophilus*. J Dairy Sci 103:2128–2138

Li H, Hang Y, Jiang Y, Li JX, Li C, Zhao Y, Li C, DuoJie R, Zulewska J, Li H, Yu J (2023) Application of tea polyphenols as additives in brown fermented milk: potential analysis of mitigating Maillard reaction products. J Dairy Sci 106:6731–6740

Liu L, Qu X, Xia Q, Wang H, Chen P, Li X, Wang L, Yang W (2018) Effect of *Lactobacillus rhamnosus* on the antioxidant activity of Cheddar cheese during ripening and under simulated gastrointestinal digestion. LWT 95:99–106

Ma ZM, Chu SX, Kang YF, Cao JQ (2016) Development of a brown yoghurt. Anhui Nongye Kexue 44:90–93

Markowiak P, Śliżewska K (2017) Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients 9:1021

Martins SIFS, Jongen WMF, van Boekel MAJS (2000) A review of Maillard reaction in food and implications to kinetic modelling. Trends Food Sci Technol 11:364–373

Mathipa-Mdakane MG, Thantsha MS (2022) *Lacticaseibacillus rhamnasus*: A suitable candidate for the construction of novel bioengineered probiotic strains for targeted pathogen control. Foods 11(6):785

Mazziotta C, Tognon M, Martini F, Torreggiani E, Rotondo JC (2023) Probiotics mechanism of action on immune cells and beneficial effects on human health. Cells 12:184

Mistry VV, Hassan HN (1992) Manufacture of nonfat yoghurt from a high milk protein powder. J Dairy Sci 75:947–957

Mituniewicz-Małek A, Ziarno M, Dmytrów I, Balejko J (2017) Short communication: Effect of the addition of *Bifidobacterium* monocultures on the physical, chemical, and sensory characteristics of fermented goat milk. J Dairy Sci 100:6972–6979

Mottram DS, Wedzicha BL, Dodson AT (2002) Food chemistry: Acrylamide is formed in the Maillard reaction. Nature 419:448–449

Ni D, Li L, Zhang HB, Zhang GW (2017) Development of brown drinking yoghurt. Zhongguo Rupin Gongye 45:56–58

Re R, Pellegrini N, Proteggente A (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26:1231–1237

Salazar N, Prieto A, Leal JA, Mayo B, Bada-Gancedo JC, de los Reyes-Gavilán CG, Ruas-Madiedo P (2009) Production of exopolysaccharides by *Lactobacillus* and *Bifidobacterium* strains of human origin, and metabolic activity of the producing bacteria in milk. J Dairy Sci 92:4158–4168

Sánchez B, Delgado S, Blanco-Míguez A, Lourenço A, Gueimonde M, Margolles A (2017) Probiotics, gut microbiota, and their influence on host health and disease, vol 61. Molecular Nutrition & Food Research, p 201600240

Sarhan H, Al Damrawi G (2022) An economic study of dairy production and consumption in Egypt. New Valley J Agricultural Sci 2(6):512–529

SAS (2008) Statistical Analysis System. The SAS system for windows, release 9.2 SAS Institute

Savaiano DA, Hutkins RW (2021) Yoghurt, cultured fermented milk, and health: a systematic review. Nutr Rev 79:599–614

Shazly AB, Khattab MSA, Fouad MT, Abd El Tawab AM, Saudi EM, Abd El-Aziz M (2022) Probiotic yoghurt made from milk of ewes fed a diet supplemented with *Spirulina platensis* or fish oil. Ann Microbiol 72:29

Shi J, Shao Z, Li H, Zhang L, Wang S (2019) Co-extraction and co-purification coupled with HPLC-DAD for simultaneous detection of acrylamide and 5-hydroxymethyl-2-furfural in thermally processed foods. Molecules 22:3734

Shori AB, Albalawi A, Al Zahrani AJ, Al-sulbi OS, Baba AS (2022) Microbial analysis, antioxidant activity, and sensory properties of yoghurt with different starter cultures during storage. Int Dairy J 126:105267

Steele C (2022) *Lactobacillus rhamnosus* GG: a review of clinical use and efficacy. Nutritional Med J 1(3):70–116

Taha S, El Abd M, De Gobba C, Abdel-Hamid M, Khalil E, Hassan D (2017) Antioxidant and antibacterial activities of bioactive peptides in buffalo's yoghurt fermented with different starter cultures. Food Sci Biotechnol 26:1325–1332

Tamime AY, Robinson RK (2000) Yoghurt Science and Technology. CRC, Washington, DC

Tharmaraj N, Shah NP (2003) Selective Enumeration of *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus, Bifidobacteria, Lactobacillus casei, Lactobacillus rhamnosus*, and *Propionibacteria*. Journal of Dairy Science 86:2288–2296

Tian R, Yu Z, Yu L, Tian F, Zhao J, Zhang H, Chen W, Zhai Q (2022) Effects of *bifidobacterium longum* ccfm5871 as an adjunct starter culture on the production of fermented milk. Food Bioscience 50 part B:102167

Turek K, Wszołek M (2021) Comparative study of walnut and *Camelina sativa* oil as a functional components for the unsaturated fatty acids and conjugated linoleic acid enrichment of kefir. LWT – Food Sci Technol 147:111681

Walstra P, Geurts TJ, Noomen A, Jellema A, Van Boekel MAJS (1999) Dairy technology: principles of milk properties and processes. Marcel Dekker, Inc, New York

Yang Z, Li S, Zhang X, Zeng X, Li D, Zhao Y, Zhang J (2010) Capsular and slime-polysaccharide production by *Lactobacillus rhamnosus* JAAS8 isolated from Chinese sauerkraut: potential application in fermented milk products. J Biosci Bioeng 110:53–57

Zhenqiang T (2022) The importance of the fermented milk products. Global J Dairy Farming Milk Prod 7:6–7

Zhi-Yuan XU, Yan WU, Guo BH, Zhou LH, Wang YY, Lian-Zhong AI (2010) Research and preparation of a brown milk drink with probiotic. Sci Technol Food Ind 31:242–244

Zhu H, Chen Z, Li G, Yao X, Hu Y, Zhao W (2023) Physicochemical, sensory, and antioxidant characteristics of stirred-type yogurt enriched with *Lentinula edodes* stipe powder. Food Sci Nutr 11:6231–6240

Publisher's note