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ORIGINAL ARTICLE

Antioxidant activity of labneh made from cashew milk and its combination with cow or camel milk using different starter cultures

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Abstract The aim of this study is to investigate the effects of three strains of probiotic *Lactobacillus* spp. i.e. *L. plantarum* (S1) *L. casei* (S2), and/or *L. rhamnosus* (S3) in co-cultures with *Streptococcus thermophiles* (St) and *L. delbrueckii* subsp. *lactis* (Ll) on the changes of total phenolic and flavonoid contents, and antioxidant activity of concentrated yogurt (labneh; L) made from individual cashew milk (EwM; 100:0 v/v) mixed with/without cow (Co) or camel (Ca) milk (75:25 v/v) during 0, 7, 14, and 21 days of storage. EwML100% S3 showed the highest total phenolic and flavonoid contents during 21 days of storage. Radical scavenging activity was improved in Ew-/Ca-ML (S2, S3, and S4) compared to control (containing St and Ll) on the 0th day. The maximum ferric reducing potential was observed in fresh Ew-/Ca-ML75% S1 (1.6 ± 0.05 mM Fe + 2 E/mL). All the starter cultures enhanced ($p < 0.05$) the chelating ability of EwML compared to control during the 7th and 14th day of storage. In conclusion, cashew milk labneh mixed with/without cow or camel milk containing probiotic *Lactobacillus* spp. can strengthen the health-promoting properties with antioxidant activity.

Keywords Labneh · *Lactobacillus planterum* · *Lactobacillus casi* · *Lactobacillus rhamnouses* · Antioxidant activity

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Introduction

Antioxidants are compounds that inhibit or delay the onset of oxidation and may be classified as natural or synthetic (Alenisan et al. 2017). There is an increasing demand for natural antioxidants due to the harmful and carcinogenic effects of synthetic antioxidants. Several studies have found that eating foods high in natural antioxidants can reduce the risk of malignancies, hypertension, diabetes, and cardiovascular diseases, particularly in underdeveloped countries where most people have limited finances and access to contemporary treatments (Mitterer-Daltoe et al. 2020; Macharia et al. 2022; Tain and Hsu 2022; Shori 2022).

Camel milk is a vital food supply for rural people in arid parts of Africa and the Middle East, where it may be consumed fresh or as fermented products (Shori 2017). There are around 28 million camels globally, and they produce about 3 million metric tonnes of milk each year (Polidori et al. 2021). The functional value of camel milk is greater than that of cow's milk. Dairy products derived from camel milk, including pasteurized milk, milk powder, fermented liquid milk, and cheese, are now widely available (Polidori et al. 2021). According to Alnohair (2021), camel milk has been used to treat a variety of metabolic and immunological disorders.

In recent years, the food industry has taken the lead in developing novel plant-based milk products. Alternatives to animal milk such as cashew milk are becoming increasingly popular among consumers (Olatidoye et al. 2020). Cashew milk is a good substitute for animal milk to produce certain dairy products. Cashew milk is used as a source of protein (5%), carbohydrates (5.95%), and fat (5.49%; Tamuno and Monday 2019). In addition, cashew milk contains calcium, potassium, and iron (4.75, 7.15, and 3.0 mg/100 g,

respectively). Magnesium and phosphorus contents in cashew milk are about 2.0 mg/100 g each (Tamuno and Monday 2019).

Dairy products have been considered healthy and natural and when consumed regularly, it can actually prevent disease (Shori et al. 2022d). The buffering capacity of milk ensures microbial viability during fermentation and refrigerated storage. Therefore, fermented dairy products could be a potential vehicle for delivering probiotic bacteria to customers (Gil et al. 2019). Concentrated yogurt, also known as labneh, is an excellent source of proteins and minerals (Al-Rimawi et al. 2020). Labneh can be made with a variety of milk, including cow, sheep, and goat milk (Serhan et al. 2016). Labneh is a semi-solid creamy textured product with an acidic taste, containing about 23–25% (w/w) of total solid content (Al-Rimawi et al. 2020). Labneh was found to have superior nutritional and medicinal properties compared to yogurt. It contains 2.5 times higher protein content, 50% more minerals, and much more lactic acid bacteria (LAB) than regular yogurt (Binda and Ouwehand 2019). Labneh is suitable for lactose-intolerant individuals because it has a low lactose content of ~ 6% (Al-Rimawi et al. 2020). Therefore, the aim of this study is to investigate the effects of three strains of probiotic *Lactobacillus* spp. (i.e. *L. rhamnosus*, *L. casei*, and/or *L. plantarum*) in co-cultures with *Streptococcus thermophilus* and *L. delbrueckii* subsp. *lactis* on the changes of total phenolic and flavonoid contents, and antioxidant activity of concentrated yogurt (labneh) made from individual cashew milk (100:0) mixed with/without cow or camel milk (75:25) during 0, 7, 14, and 21 days of storage.

Materials and methods

Materials

Pure strains of *S. thermophilus* St1342 and *L. delbrueckii* ssp. *lactis* ATCC 7830, *L. plantreum* ATCC 14917, *L. casei* (393) and *L. rhamnosus* ATCC 53103 were obtained from the Microbiological Resources Center (Cairo mircen) in Ain Shams University. All strains of bacteria were grown in selected media under anaerobic conditions at 37 ± 2 °C for 48 h. Cashew nuts, cow milk (Almarei®), and camel milk (Al-turath®) were purchased from a local food store. Anhydrous sodium carbonate, gallic acid (GA), (+)-catechin hydrate (CH), ethanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), folin-Ciocalteu reagent, potassium ferricyanide, ferrous chloride, ferric chloride, ferrozine, phosphate buffer, trichloroacetic acid (TCA), sodium nitrite, sodium hydroxide, and aluminum chloride were purchased from Sigma-Aldrich, St. Louis, MO, USA.

Methods

Preparation of starter culture

Streptococcus thermophilus, *L. casei*, *L. plantreum*, *L. rhamnosus*, and *L. delbrueckii* ssp. *lactis* were all stored at − 80 °C. Sterile 10 mL aliquots of MRS broth inoculated with 1% (v/v) of each strain individually were incubated at 37 °C except for *L. delbrueckii* ssp. *lactis* which was incubated at 42 °C (Shori et al. 2022b). The activated organisms after three successive transfers were used to prepare starter cultures for labneh making. The cultures were prepared by inoculating 1% (v/v) in 10 mL aliquots of sterile reconstituted skim milk (72 °C for 15 s) supplemented with 2% glucose and 1% yeast extract.

Preparation of cashew extract

Cashew nuts (10 g) were soaked in 100 mL of distilled water for 12 h (Shori et al. 2022b). The mixture was blended and filtered using a clean Muslin cloth, followed by centrifugation (4080 g, 4 °C) for 15 min. The supernatant was harvested and pasteurized at 90 °C for 5 min. The clear solution was refrigerated (4 °C) and used within 3 days as cashew milk in the making of labneh.

Preparation of labneh

Labneh was prepared according to Serhan et al. (2016) with some modifications. Labneh (L) was prepared from individual cashew milk (EwM) mixed with/without cow milk (CoM) or camel milk (CaM) in two different concentrations (100:0 and 75:25; v/v). In addition, 2% of starter culture (S) including S1 = *L. plantarum* (Lp), S2 = *L. casei* (Lc), S3 = *L. rhamnosus* (Lr), and S4 = (Lr, Lc, and Lp) in co-cultures with *S. thermophilus* (St) and *L. delbrueckii* subsp. *lactis* (Ll) was added to each formula. Then, all the mixtures were mixed thoroughly and incubated at 41 °C for 3 h. The prepared yogurt was centrifuged (4080 g; 4 °C) for 10 min and the whey was discarded. The solid product was collected namely (labneh) and stored at 4 ± 1 °C for 0, 7, 14, and 21 days. Labneh (control) containing co-cultures (St and Ll) was prepared in the same manner using EwM mixed with/without CoM or CaM in a ratio of 100:0 and 75:25 (v/v).

Preparation of labneh water extract

Ten grams of each labneh sample was homogenized with 2.5 mL dH_2O (Shori et al. 2018). The samples were acidified to pH 4.0 by adding HCl (0.1 M) and incubated in a water bath at 45 °C for 10 min. The samples were centrifuged (5000 g, 4 °C) for 10 min and the supernatant was then

adjusted to pH 7.0 using NaOH (0.1 M). This was followed by second centrifugation (5000 g, 4 °C) for 10 min and the clear supernatant was used for further analysis.

Total phenolic content (TPC)

2N Folin-Ciocalteu reagent (0.5 mL; 50% v/v) was added to 5 mL dH_2O, 1 mL of 95% ethanol, and 1 mL of labneh water extract or gallic acid standard solutions (10–60 µg/mL; Shori 2013). The mixture was kept at room temperature for 5 min before 1 mL of sodium carbonate solution (5% w/v) was added to the sample. The mixture was left in the dark at 25 °C for 1 h and the absorbance was measured at 725 nm using a spectrophotometer (Shimadzu UV Mini 1240, Duisburg, Germany). The total phenolic content was estimated against the gallic acid standard curve and expressed as µg gallic acid equivalent per gram fresh labneh (µg GAE/g fresh labneh).

Total flavonoid content (TFC)

The total flavonoid content was determined as described by Bouterfas et al. (2016). Labneh sample (250 µl) was mixed with 1.25 mL dH2O and 75 µl sodium nitrite for 5 min. Then, 150 µl of 10% aluminum chloride, 0.5 mL of 1 M sodium hydroxide, and 275 µl of dH2O were added to the mixture. The absorbance was read at 510 nm and a standard curve was prepared from a serial dilution (0–500 µg/mL) of catechin. The total flavonoid content was estimated against the catechin standard curve and expressed as µg catechin equivalent per gram fresh labneh (µg CE/g fresh labneh).

Determination of antioxidant activity

DPPH radical scavenging activity assay DPPH reagent (3 mL; 60 mM) dissolved in 95% ethanol was added to 250 µl of labneh water extract (Muniandy et al. 2016). The mixture was incubated in the dark at room temperature for 1 h. Absorbance (Abs) at 517 nm was measured against control (250 µl of ethanol instead of the extract) using a spectrophotometer (Genesys 10 UV–vis, Thermo Fisher Scientific, Inc., USA). The DPPH radical scavenging activity was calculated as below:

$$\text{Radical scavenging activity (\%)} = \left(\text{Abs}_{\text{control @ 517nm}} - \text{Abs}_{\text{sample @ 517nm}}/\text{Abs}_{\text{control@517 nm}}\right) \times 100$$

Ferric reducing antioxidant potential (FRAP) assay FRAP reagent (3.6 mL) containing 300 mM acetate buffer, 8 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) reagent, and 20 mM $FeCl_3$ solutions at a ratio of 10:1:1 was mixed with 400 µl of labneh water extract or iron(II) sulfate heptahydrate ($FeSO_4{\cdot}7H_2O$) standard solutions (0.3–1.0 µg/mL; Muniandy et al. 2016). The mixture was incubated in a water bath (37 °C) for 10 min before absorbance was read at 593 using a spectrophotometer (Genesys 10 UV–vis, Thermo Fisher Scientific, Inc., USA). The results were estimated against $FeSO_4{\cdot}7H_2O$ standard curve and expressed as mM Fe^{2+} equivalent/mL (mM Fe^{2+} E/mL).

Ferrous ion chelating (FIC) ability assay The 2 mM iron(II) sulfate hydrate ($FeSO_4{\cdot}xH_2O$) solution and 5 mM ferrozine solution were diluted 20 times prior to use (Shori 2020). Then, 1 mL of diluted $FeSO_4{\cdot}xH_2O$ solution was mixed with 1 mL of labneh water extract and 1 mL of diluted ferrozine solution. This was followed by incubation for 10 min at 25 °C. Absorbance (Abs) at 562 nm was measured against control (1 mL of dH_2O instead of the extract) using a spectrophotometer (Genesys 10 UV–vis, Thermo Fisher Scientific, Inc., USA). The FIC ability of samples was calculated as below:

$$\text{FIC ability (\%)} = \left(\text{Abs}_{\text{control @ 562nm}} - \text{Abs}_{\text{sample @ 562nm}} / \text{Abs}_{\text{control@562 nm}}\right) \times 100$$

Statistical analysis

All data were presented as means ± standard error (SE) and performed in duplicates for three batches (n = 3). IBM SPSS statistics software version 20.0 was used and one-way analysis of variance (ANOVA) as well as the significance of the mean differences were determined using Duncan's test ($p < 0.05$).

Results

Total phenolic and flavonoid contents of labneh

Table 1 shows the TPC and TFC in labneh made from individual cashew milk (100%) mixed with/without cow or camel milk (75:25) using three types of probiotics and their combination compared to control during 21 days of storage. Control labneh made from cashew milk showed significantly ($p < 0.05$) higher phenolic content (34.70 ± 1.90 µg GAE/g fresh labneh), followed by cashew and camel milk labneh (25.06 ± 4.70 µg GAE/g fresh labneh) at day 0 (Table 1). Comparatively, the presence of starter cultures S1, S2, S3, and S4 in all labneh samples displayed an increase ($p < 0.05$) in TPC compared to their respective controls during 21 days of storage. The higher phenolic content ($p < 0.05$) in all

Table 1 Total phenolic (µg GAE/g fresh labneh) and flavonoid (µg CE/g fresh labneh) contents of labneh (L) made from individual cashew milk (100%) and its mixture with cow or camel milk (75:25) in the presence of starter cultures *Lactobacillus plantarum* (S1), *L. casei* (S2), and *L. rhamnosus* (S3) and their combination (S4) compared to control during 21 days of storage at 4 °C

Samples	Total phenolic content (µg GAE/ g fresh labneh)				Total flavonoid content (µg CE/g fresh labneh)			
Day	0	7	14	21	0	7	14	21
EwML (control)*	34.7 ± 1.90^{a}	43.7 ± 9.00^{a}	62.5 ± 1.19^{a}	43.7 ± 0.10^{a}	30.5 ± 0.30^{a}	38.2 ± 0.80^{a}	41.5 ± 10.6^{a}	24.8 ± 1.80^{a}
EwML S1	42.2 ± 0.14^{b}	54.15 ± 0.10^{b}	92.7 ± 0.50^{b}	90.7 ± 0.10^{b}	33.5 ± 0.21^{a}	50.0 ± 0.90^{b}	75.9 ± 2.30^{b}	40.9 ± 0.31^{b}
EwML S2	41.4 ± 0.20^{b}	50.5 ± 3.50^{b}	88.8 ± 5.10^{b}	92.6 ± 4.88^{b}	31.6 ± 0.07^{a}	40.7 ± 0.90^{a}	42.4 ± 4.20^{a}	37.9 ± 0.49^{b}
EwML S3	49.2 ± 2.80^{c}	71.0 ± 0.39^{c}	107.7 ± 7.60^{c}	100.6 ± 2.81^{b}	40.4 ± 0.14^{b}	69.6 ± 0.87^{c}	106.4 ± 1.13^{c}	85.5 ± 1.13^{c}
EwML S4	42.3 ± 1.80^{b}	68.7 ± 0.39^{c}	97.0 ± 0.50^{b}	94.7 ± 0.77^{b}	33.9 ± 0.4^{a}	69.9 ± 1.06^{c}	87.6 ± 0.07^{d}	77.7 ± 4.45^{c}
Ew-/Co-ML (control)*	20.5 ± 2.60^{a}	31.6 ± 0.10^{a}	58.5 ± 1.10^{a}	32.4 ± 4.30^{a}	19.8 ± 0.14^{a}	24.4 ± 0.14^{a}	28.7 ± 3.40^{a}	21.1 ± 0.09^{a}
Ew-/Co-ML S1	22.15 ± 12.7^{a}	52.3 ± 1.50^{b}	86.7 ± 1.60^{b}	80.23 ± 0.82^{b}	25.1 ± 0.80^{b}	32.8 ± 0.49^{b}	55.5 ± 2.60^{b}	29.9 ± 0.3^{b}
Ew-/Co-ML S2	21.4 ± 4.03^{a}	50.8 ± 0.60^{b}	84.5 ± 1.60^{b}	88.5 ± 1.10^{b}	24.9 ± 0.18^{b}	30.3 ± 0.14^{b}	38.1 ± 0.87^{c}	24.7 ± 2.40^{c}
Ew-/Co-ML S3	24.5 ± 0.70^{b}	54.5 ± 0.70^{b}	102.7 ± 0.50^{c}	92.5 ± 1.19^{b}	33.5 ± 0.21^{c}	55.4 ± 0.25^{c}	99.5 ± 0.14^{d}	80.0 ± 1.13^{d}
Ew-/Co-ML S4	23.2 ± 1.55^{b}	53.32 ± 0.11^{b}	91.2 ± 46.8^{b}	84.5 ± 0.60^{b}	27.1 ± 0.60^{b}	58.2 ± 1.10^{c}	67.9 ± 1.06^{e}	44.4 ± 0.14^{e}
Ew-/Ca-ML (control)*	25.06 ± 4.70^{a}	35.7 ± 2.00^{a}	61.6 ± 1.10^{a}	38.7 ± 6.50^{a}	21.3 ± 1.16^{a}	27.5 ± 0.21^{a}	31.7 ± 0.20^{a}	23.9 ± 0.76^{a}
Ew-/Ca-ML S1	26.9 ± 1.90^{a}	52.3 ± 1.09^{b}	90.8 ± 6.40^{b}	86.2 ± 3.40^{b}	26.2 ± 1.60^{b}	34.0 ± 0.63^{b}	75.2 ± 5.20^{b}	30.4 ± 0.14^{b}
Ew-/Ca-ML S2	25.95 ± 2.33^{a}	45.2 ± 1.70^{c}	85.6 ± 4.93^{b}	91.13 ± 0.09^{b}	26.8 ± 5.16^{b}	33.4 ± 0.95^{b}	40.4 ± 0.98^{c}	28.9 ± 2.40^{b}
Ew-/Ca-ML S3	30.0 ± 6.08^{b}	68.6 ± 1.09^{d}	105.6 ± 6.12^{c}	94.5 ± 1.40^{b}	34.4 ± 0.14^{c}	67.9 ± 0.77^{c}	102.6 ± 1.50^{d}	81.5 ± 1.06^{c}
Ew-/Ca-ML S4	29.7 ± 4.90^{b}	65.7 ± 3.30^{d}	94.8 ± 1.80^{c}	87.9 ± 9.10^{b}	29.7 ± 2.60^{b}	67.7 ± 6.40^{c}	85.1 ± 0.42^{e}	65.6 ± 1.06^{d}

Data are presented as mean ± SEM. M

a,b,c,d means with different superscript letters in a column are significantly different ($p < 0.05$)

Cow- = Co, camel- = Ca, cashew- = Ew, milk = M, labneh = L, starter culture = S, *Lactobacillus plantarum* = 1, *L. casei* = 2, *L. rhamnosus* = 3, and 1 + 2 + 3 = 4

*Control containing *Streptococcus thermophilus* and *L. delbrueckii* subsp. *Lactis*

labneh samples was shown in the presence of S3 during the storage period (Table 1). All labneh samples showed a maximum increase ($p < 0.05$) in TPC on the 14th day, which was about twofolds, threefolds, and fourfolds higher than day 0 for all EwML, Ew-/Ca-ML, and Ew-/Co-ML; respectively. A non-significant reduction was noticed in all labneh samples on the 21st day (Table 1). However, control labneh made from individual cashew milk mixed with/without cow or camel milk showed a significant ($p < 0.05$) reduction in TPC at the end of storage.

The presence of starter cultures S1, S2, S3, and S4 increased ($p < 0.05$) TFC in fresh labneh made from 100% cashew milk (34–40 µg CE/g fresh labneh) compared to control (30.5 ± 0.30 µg CE/g fresh labneh; Table 1). However, only starter cultures S3 and S4 enhanced ($p < 0.05$) TFC in Ew-/Co-ML and Ew-/Ca-ML compared to their respective controls on the 0th day (Table 1). In addition, the increase in the TFC of labneh samples was influenced by the starter cultures used during the storage period (Table 1). In cashew milk labneh mixed with camel milk, S3 had the highest TFC ($p < 0.05$) followed by S4 as compared to other samples during the 7th and 14th day of storage. In addition, all labneh samples showed the highest TFC ($p < 0.05$) on the 14th day with maximum values shown in the presence of S3. However, all labneh samples showed no significant decline ($p > 0.05$) in TFC on the 21st day compared to their respective controls, which showed a sharp decrease ($p < 0.05$).

Antioxidant properties

DPPH radical scavenging activity of labneh

Figure 1 shows the DPPH radical scavenging activity in labneh made from individual cashew milk (100%) mixed with/without cow or camel milk (75:25) using three types of probiotics and their combination compared to control during 21 days of storage. EwML S1, S2, and S3 showed the highest radical scavenging activity (95–93%; $p < 0.05$) on the 0th day as compared to control (83.7 ± 0.40%; Fig. 1a). The presence of starter cultures (S1–S4) in cashew milk labneh did not significantly improve ($p > 0.05$) radical scavenging activity compared to control during 21 days of storage at 4 °C. Ew-/Co-ML (control) showed radical scavenging activity of 82.9 ± 0.11% on the 0th day (Fig. 1b). However, the presence of starter cultures increased ($p < 0.05$) the activity in Ew-/Co-ML between 90 and 93%. A slight reduction ($p < 0.05$) of radical scavenging activity was observed in Ew-/Co-ML S2 and S3 (82.1 ± 0.07 and 85.6 ± 0.05; respectively) compared to control (91.0 ± 0.02%) on the 7th day. Ew-/Ca-ML (S2, S3, and S4) showed significantly higher radical scavenging

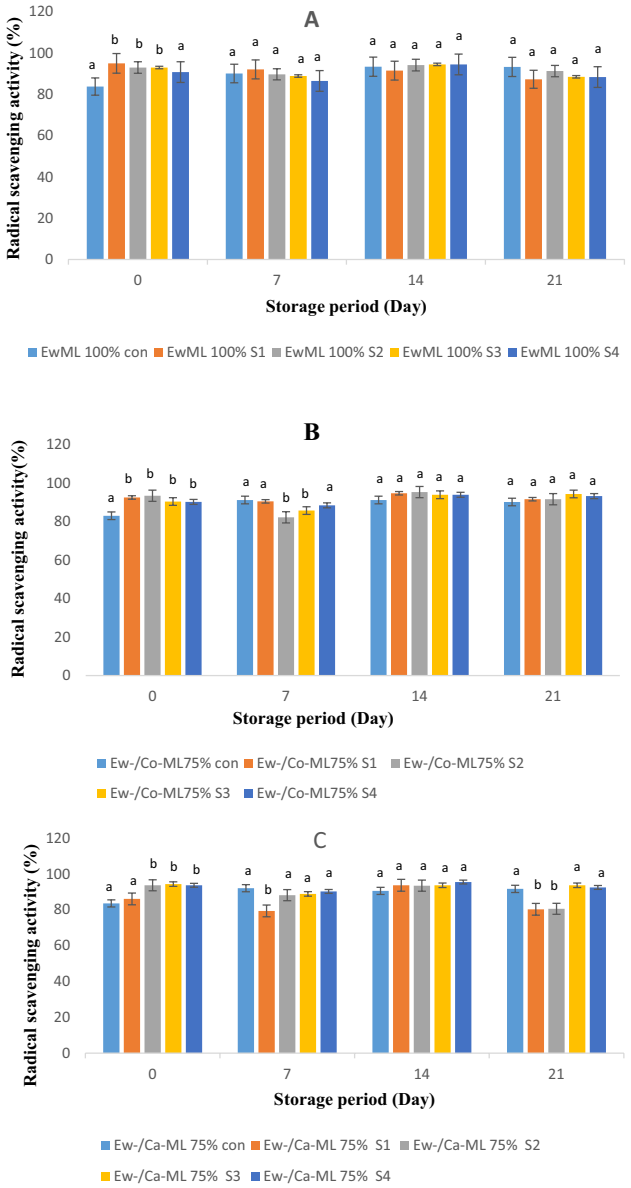


Fig. 1 Changes in radical scavenging activity of labneh (L) made from cashew milk (EwM; 100%; **A**) and its mixture with cow (Co; **B**) or camel (Ca; **C**) milk (M; 75:25) in the presence of starter cultures *Lactobacillus plantarum* (S1), *L. casei* (S2), and *L. rhamnosus* (S3) and their combination (S4) compared to control during 21 days of storage at 4 °C. Data are presented as mean ± SEM. The level of significance was preset at $p < 0.05$ compared to control at the same storage period

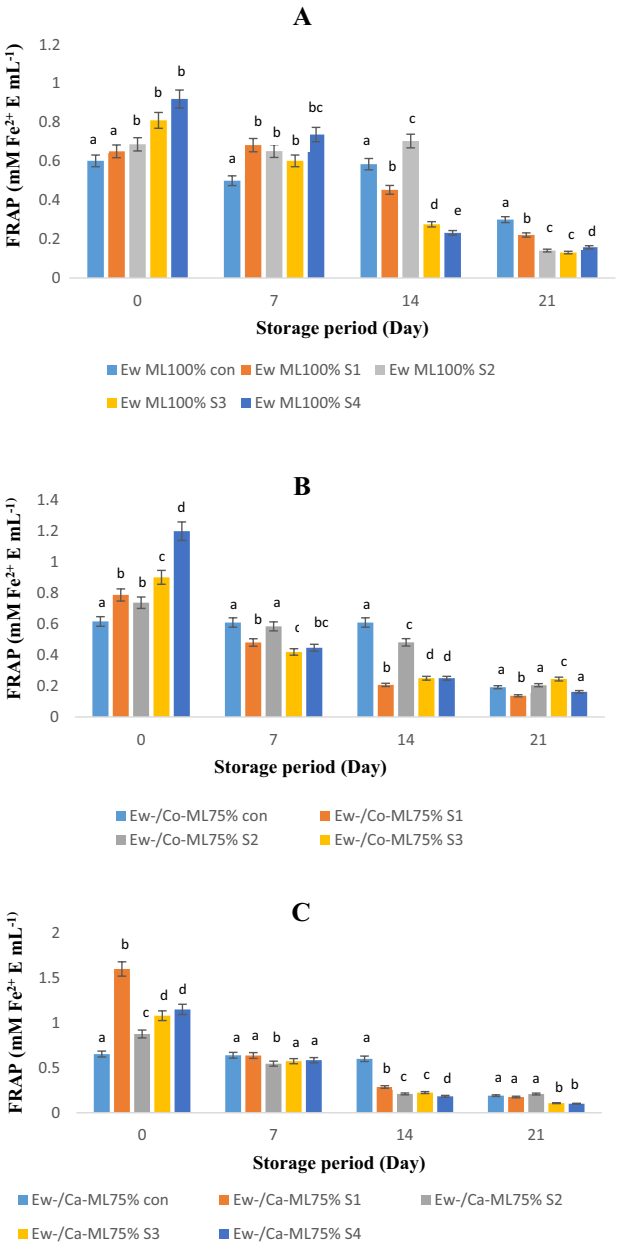


Fig. 2 Changes in ferric reducing antioxidant potential (FRAP) of labneh (L) made from cashew milk (EwM; 100%; **A**) and its mixture with cow (Co; **B**) or camel (Ca; **C**) milk (M; 75:25) in the presence of starter cultures *Lactobacillus plantarum* (S1), *L. casei* (S2), and *L. rhamnosus* (S3) and their combination (S4) compared to control during 21 days of storage at 4 °C. Data are presented as mean ± SEM. The level of significance was preset at $p < 0.05$ compared to control at the same storage period

activity (94%; $p < 0.05$) than control (86.1 ± 0.90%) on the 0th day (Fig. 1c). Ew-/Ca-ML S1 showed a significant reduction ($p < 0.05$) in radical scavenging activity compared to control on the 7th day but increased ($p < 0.05$) on the 14th day. Radical scavenging activity of Ew-/Ca-ML S1 and S2 was reduced by 11% ($p < 0.05$) compared to control at the end of storage (Fig. 1c).

Ferric reducing antioxidant potential (FRAP) of labneh

Figure 2 shows ferric reducing antioxidant potential of labneh made from individual cashew milk (100%) mixed with/without cow or camel milk (75:25) using three types of probiotics and their combination compared to control during

21 days of storage. EwML (control) showed ferric reducing potential of 0.602 ± 3.60 mM Fe^{+2} E/mL on the 0th day (Fig. 2a). Maximum ferric reducing potential was observed in EwML S4 (0.920 ± 0.00 mM Fe^{+2} E/mL), followed by EwMLS3 (0.810 ± 0.00 mM Fe^{+2} E/mL) on the 0th day. EwML S3 and S4 showed a significant decline ($p < 0.05$) in ferric reducing potential on the 7th day, whereas EwMLS2 maintained ferric reducing potential over 2 weeks (Fig. 2a). The presence of starter cultures (S1–S4) in Ew-/Co-ML increased ($p < 0.05$) ferric reducing potential compared to control on the 0th day with Ew-/Co-MLS4 showing the highest ferric reducing potential (1.200 ± 0.05 mM Fe^{+2} E/mL; Fig. 2b). Similarly, starter cultures (S1–S4) in Ew-/Ca-ML showed greater ferric reducing potential than controls on the 0th day with S1 showing maximum ferric reducing potential (1.600 ± 0.05 mM Fe^{+2} E/mL; Fig. 2c). Nevertheless, a significant ($p < 0.05$) loss of ferric reducing potential was observed in both Ew-/Co-ML and Ew-/Ca-ML using different starter cultures during 21 days of storage (Fig. 2b and c).

Ferrous ion chelation (FIC) of labneh

Figure 3 shows the ferrous ion-chelating (FIC) of labneh made from individual cashew milk (100%) mixed with/without cow or camel milk (75:25) using three types of probiotics and their combination compared to control during 21 days of storage. As compared to other samples, EwML (control) had the highest chelating ability (76.00 ± 0.30%) on the 0th day, which was followed by a reduction ($p < 0.05$) to 63% on the 14th day and an increase to 99.70 ± 0.05% on the 21st day (Fig. 3a). All EwML samples showed higher ($p < 0.05$) chelating ability than control during the 7th and 14th day of storage. EwMLS4 showed a significant reduction (23.90 ± 0.10%; $p < 0.05$) in chelating ability on the 21st day of storage. The addition of starter cultures did not affect ($p < 0.05$) the chelating ability of Ew-/Co-ML compared to control during 21 days of storage (Fig. 3b). However, the chelating ability of 7-day-stored Ew-/Co-MLS3 and 21-day-stored Ew-/Co-MLS1 (74.40 ± 1.50 and 92.50 ± 0.40%; respectively) was almost similar to that of control. Ew-/Ca-ML (control) showed significantly ($p < 0.05$) higher chelating ability than other samples on the 0th day (Fig. 3c). The chelating ability of Ew-/Ca-ML S1 and S3 increased ($p < 0.05$) to 95.00 ± 1.10% and 96.40 ± 0.10%; respectively on the 7th day and maintained these levels for 21 days of storage. In addition, Ew-/Ca-MLS2 showed higher chelating ability (99.33 ± 0.20%; $p < 0.05$) than the control (85.40 ± 0.10%) on the 21st day (Fig. 3c).

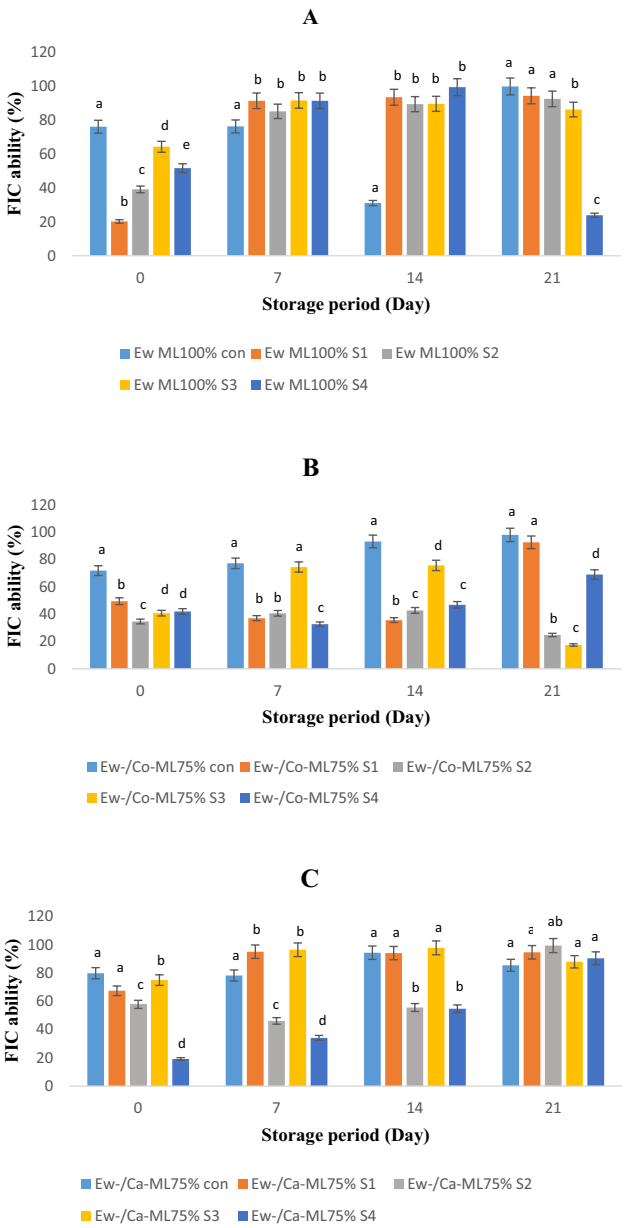


Fig. 3 Changes in ferrous ion-chelating (FIC) of labneh (L) made from cashew milk (EwM; 100%; **A**) and its mixture with cow (Co; **B**) or camel (Ca; **C**) milk (M; 75:25) in the presence of starter cultures *Lactobacillus plantarum* (S1), *L. casei* (S2), and *L. rhamnosus* (S3) and their combination (S4) compared to control during 21 days of storage at 4 °C. Data are presented as mean ± SEM. The level of significance was preset at $p < 0.05$ compared to control at the same storage period

Discussions

A number of scientific investigations have been conducted on the development and production of novel probiotic fermented foods with functional properties (Shori et al 2022c; Al-Sulbi and Shori 2022; Shori and Al zahrani 2022). Fermented foods are influenced by degradation, structural changes, and the development of new molecules that occur during fermentation which are related to several parameters such as microbial strains, substrate content, and fermentation conditions (Mikulajová et al. 2021). Lactic acid bacteria are

frequently used during fermentation to ensure the safety and stability of the final products and to promote health benefits to the host (Liptakova et al. 2017).

Plant-based milk (PBM) products have been developed to offer an alternative to animal-based dairy products and have become increasingly popular (Montemurro et al. 2021). Despite a lengthy history of non-dairy products, the development of new PBM products has seen a surge in interest because of their health benefits (Shori et al. 2022a). In our analysis, labneh made from 100% cashew milk had higher phenolic and flavonoid contents than labneh made from cashew milk mixed with cow or camel milk (Table 1). The inherent phenolic and flavonoid compounds in cashew milk could be responsible for high phenolic and flavonoid contents in the labneh. The composition of phenolic acids in cashew extract included caffeic acid, p-coumaric acid, ferulic acid, gallic acid, syringic acid, naringenin, catechin, and quercetin (Różańska et al. 2021). In addition, flavonoids in cashew extract such as 3-*O*-galactoside, 3-*O*-glucoside, 3-*O*-rhamnoside, 3-*O*-xylopyranoside, 3-*O*-arabinopyranoside and 3-*O*-arabinofuranoside of quercetin and myricetin, as well as kaempferol 3-*O*-glucoside were also identified (Salehi et al. 2019). Further study is required to determine the TPC and TFC as well as identify the phenolic and flavonoid compounds profiles in cashew milk before and after fermentation. The reduction of phenolic and flavonoid contents in labneh made from cashew milk mixed with cow or camel milk could possibly occur as a result of the interaction of milk proteins with reactive phenolic compounds from the cashew milk. However, the amalgamation of cashew and camel milk during labneh preparation with different strains of lactobacillus probiotics showed promising results for total phenols, suggesting the greater availability of nutrients and milk proteins.

Shori et al. (2022b) reported that the growth and metabolism of LAB have been enhanced in the presence of cashew milk. To ensure high-quality products, it is important to select the right starter cultures. The present study found that the addition of three strains of *Lactobacillus* spp and their combination increased the phenolic and flavonoid contents in labneh samples compared to control throughout storage periods. In addition, labneh samples containing *L. rhamnosus* exhibited the highest TFC and TPC among other samples during 21 days of storage. This indicated that the addition of *L. rhamnosus* in individual cashew milk and its mixture with cow or camel milk during fermentation contributed to an increase in phenolic and flavonoid contents in labneh which can lead to potential health benefits such as antioxidants. A similar observation was also reported for cashew milk yogurt fermented with *L. rhamnosus* (Shori et al. 2022b). A study conducted on soy-based milk fermented with *S. boulardii* and probiotic *Lactobacillus* spp. showed an increase in flavonoid content (especially isoflavone) by 95% (Rekha and Vijayalakshmi 2010). In addition, *Lactobacillus*-fermentation of soymilk increased phenolic acids, isoflavone, and antioxidant activity (Chavan et al. 2018). This phenomenon has been described as a general impact of LAB-induced acidification, which increases phenol solubilization and extractability. In addition, some LAB produces certain enzymes like feruroyl esterases that promote the release of antioxidant phenols, thereby improving phenolic content (Verni et al. 2019). Currently, there are several species of LAB that have been proved to produce feruloyl esterase including *L. johnsonii* (Lai et al. 2009), *L. plantarum* (Esteban-Torres et al. 2013), *Pediococcus acidilactici* (Kaur et al. 2014), *L. amylovorus* (Xu et al. 2017), *Bifidobacterium animalis* subsp. *lactis* (Fritsch et al. 2017) and *L. helveticus* (Song and Baik 2017). In addition, it is reported that *L. rhamnosus* can produce much lower but active feruroyl esterases during the fermentation of wheat bran, rye bran, corn pectin, or brewer's spent grain to release phenolic acids (Szwajgier and Jakubczyk 2011). Further study is needed to investigate the ability of the three strains of probiotic *Lactobacillus* spp. to produce feruroyl esterases during labneh fermentation from individual cashew milk and its combination with cow or camel milk. Higher protein content and dietary fiber in cashew milk fermented with different LAB strains may be associated with a higher phenolic content (Tlais et al. 2022). The decrease in TFC of labneh samples at the end of storage may be explained by the action of the starter culture to further break down the polymeric phenolics in the cashew milk. A similar observation was reported in a previous study (Shori et al. 2022b).

It was reported that fruits, vegetables, and nuts could exhibit antioxidant properties due to their phenolic and flavonoids compounds (Shori 2015). Rice-Evan et al. (1997) reported that phenolic and flavonoid compounds have redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. Thus, the redox potential of these compounds plays an important role in determining the antioxidant capacity of the plant. A variety of tests are used to determine antioxidant activity (Malomo et al. 2020). DPPH and FRAP are the most widely used methods to assess the antioxidant activity of food products. Antioxidants are chemical compounds that neutralize and scavenge free radicals, constantly generated in the body (Shori et al. 2022b). It was shown that the antioxidant content of goat's milk yogurt was 93% higher than camel milk yogurt when *Pediococcus pentosaceus* was used as a starter culture. This finding implies that probiotic bacteria boost yogurt's antioxidant properties (Balakrishnan and Agrawal 2014). In our current study, the labneh made from cashew and camel milk combination showed the highest chelating ability when fermented with different starter cultures. FIC assay was used to evaluate metal ion binding by antioxidant components. Ew-/Ca-ML was found to possess

the ability to prevent or inhibit reactions such as a Fenton's type reaction, which generates reactive hydroxyl radicals. In a previous study, tocopherols and tocopheryl acetate (vitamin E) were found to have antioxidant properties in cashew extract (Yang et al. 2009). Accordingly, the cashew milk labneh prepared in our study showed high antioxidant activity.

Conclusion

TPC and TFC were increased significantly in the presence of *L. rhamnouses* in all labneh samples during the two weeks of storage. The presence of starter cultures (S2, S3, and S4) in fresh Ew-/Ca-ML significantly increased radical scavenging activity. *L. planterum* enhanced ferric reducing potential in Ew-/Ca-ML. Cashew milk labneh mixed with/without cow or camel milk using three strains of *Lactobacillus* spp. and their combination has significantly boosted the total phenols and flavonoid content, as well as the antioxidant activity. The inoculation of *L. plantarum, L. casei*, and/or *L. rhamnosus* in labneh samples during storage could improve total phenolic and flavonoid content as well as act as a potential source of antioxidants. Therefore, labneh produced from cashew milk mixed with/without cow or camel milk using S1, S2, S3, or S4 can strengthen the health-promoting properties with anti-oxidant activity.

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References

Alenisan MA, Alqattan HH, Tolbah LS, Shori AB (2017) Antioxidant properties of dairy products fortified with natural additives: a review. J Assoc Arab Univ Basic Appl Sci 24:101–106

Alnohair SF (2021) Medical benefits of camel's milk: a comprehensive review. JPMA J Pak Med Assoc 71(3):933–937

Al-Rimawi F, Alayoubi M, Elama C, Jazzar M, Çakıcı A (2020) Use of cinnamon wheat germ and eucalyptus oils to improve quality and shelf life of concentrated yogurt (labneh). Cogent Food Agric 6(1):1807810

Al-Sulbi OS, Shori AB (2022) Viability of selected strains of probiotic *Lactobacillus* spp. and sensory evaluation of concentrated yogurt (labneh) made from cow, camel, and cashew milk. Food Sci Technol 42:e113321

Balakrishnan G, Agrawal R (2014) Antioxidant activity and fatty acid profile of fermented milk prepared by *Pediococcus pentosaceus*. J Food Sci Technol 51:4138–4142

Binda S, Ouwehand AC (2019) Lactic acid bacteria for fermented dairy products. Lactic acid bacteria. CRC Press, pp 175–198

Bouterfas K, Mehdadi Z, Elaoufi MM, Latreche A, Benchiha W (2016) Antioxidant activity and total phenolic and flavonoids content variations of leaves extracts of white Horehound (*Marrubium vulgare* Linné) from three geographical origins. Ann Pharm Fr 74(6):453–462

Chavan M, Gat Y, Harmalkar M, Waghmare R (2018) Development of non-dairy fermented probiotic drink based on germinated and ungerminated cereals and legume. LWT 91:339–344

Esteban-Torres M, Reverón I, Mancheño JM, de Las RB, Muñoz R (2013) Characterization of a feruloyl esterase from *Lactobacillus plantarum*. Appl Environ Microbiol 79(17):5130–5136

Fritsch C, Jänsch A, Ehrmann MA, Toelstede S, Vogel RF (2017) Characterization of cinnamoyl esterases from different Lactobacilli and Bifidobacteria. Curr Microbiol 74(2):247–256

Gil Á, Ortega RM (2019) Introduction and executive summary of the supplement role of milk and dairy products in health and prevention of noncommunicable chronic diseases: a series of systematic reviews. Adv Nutr 10(suppl_2):67–73

Kaur B, Chakraborty D, Kumar B (2014) Metabolic engineering of *Pediococcus acidilactici* BD16 for production of vanillin through ferulic acid catabolic pathway and process optimization using response surface methodology. Appl Microbiol Biotechnol 98(20):8539–8551

Lai KK, Lorca GL, Gonzalez CF (2009) Biochemical properties of two cinnamoyl esterases purified from a *Lactobacillus johnsonii* strain isolated from stool samples of diabetes-resistant rats. Appl Environ Microbiol 75(15):5018–5024

Liptáková D, Matejčeková Z, Valík L (2017) Lactic acid bacteria and fermentation of cereals and pseudocereals. Ferment Process 10:65459

Macharia JM, Mwangi RW, Rozmann N, Zsolt K, Varjas T, Uchechukwu PO, Wagara IN, Raposa BL (2022) Medicinal plants with anti-colorectal cancer bioactive compounds: potential game-changers in colorectal cancer management. Biomed Pharmacother 153:113383

Malomo SA, Niwachukwu ID, Girgih AT, Idowu AO, Aluka RE, Fagbemi TN (2020) Antioxidant and renin-angiotensin system inhibitory properties of cashew nut and fluted-pumpkin protein hydrolysates. Pol J Food Nutr Sci 70(3):275–289

Mikulajová A, Matejčeková Z, Mošovská S, Kohajdová Z, Valík Ľ, Hybenová E (2021) Fermented cranberry fortified buckwheat product—phenolic composition antioxidant and microbiological properties. Appl Sci 11(19):9241

Mitterer-Daltoé M, Bordim J, Lise C, Breda L, Casagrande M, Lima V (2020) Consumer awareness of food antioxidants synthetic vs natural. Food Sci Technol 41:208–212

Montemurro M, Pontonio E, Coda R, Rizzello CG (2021) Plant-based alternatives to yogurt: state-of-the-art and perspectives of new biotechnological challenges. Foods (Basel Switz) 10:316

Muniandy P, Shori AB, Baba AS (2016) Influence of green white and black tea addition on the antioxidant activity of probiotic yogurt during refrigerated storage. Food Packag Shelf Life 8:1–8

Olatidoye OP, Taofik AS, Samuel OA, Ain Ajisegiri ES (2020) Nutritional profile protein quality and biological value of raw and roasted cashew kernels (*Anacardium occidentale*) grown in southwest Nigeria. Croat J Food Sci Technol 12(1):11–19

Polidori P, Cammertoni N, Santini G, Klimanova Y, Zhang JJ, Vincenzetti S (2021) Nutritional properties of camelids and equids fresh and fermented milk. Dairy 2(2):288–302

Rekha CR, Vijayalakshmi G (2010) Bioconversion of isoflavone glycosides to aglycones mineral bioavailability and vitamin B complex in fermented soymilk by probiotic bacteria and yeast. J Appl Microbiol 109:1198–1208

Rice-Evans C, Miller N, Paganga G (1997) Antioxidant properties of phenolic compounds. Trends Plant Sci 2(4):152–159

Różańska MB, Siger A, Szwengiel A, Dziedzic K, Mildner-Szkudlarz S (2021) Maillard reaction products in gluten-free bread made from raw and roasted buckwheat flour. Molecules 26(5):1361

Salehi B, Gültekin-Özgüven M, Kırkın C, Özçelik B, Morais-Braga MFB, Carneiro JNP, Bezerra C, da Silva T, Coutinho H, Amina B, Armstrong L, Selamoglu Z, Sevindik M, Yousaf Z, Sharifi-Rad J, Muddathir A, Devkota H, Martorell V, Jugran A, Martins N, Cho WC (2019) Anacardium plants: chemical nutritional composition and biotechnological applications. Biomolecules 9(9):465

Serhan M, Mattar J, Debs L (2016) Concentrated yogurt (labneh) made of a mixture of goats' and cows' milk: physicochemical microbiological and sensory analysis. Small Rumin Res 138:46–52

Shori AB (2013) Antioxidant activity and viability of lactic acid bacteria in soybean-yogurt made from cow and camel milk. J Taibah Univ Sci 17(4):202–208

Shori AB (2015) Screening of antidiabetic and antioxidant activities of medicinal plants. J Integr Med 13(5):297–305

Shori AB (2017) Camel milk and its fermented products as a source of potential probiotic strains and novel food cultures: a mini review. PharmaNutrition 5(3):84–88

Shori AB (2020) Inclusion of phenolic compounds from different medicinal plants to increase α-amylase inhibition activity and antioxidants in yogurt. J Taibah Univ Sci 14(1):1000–1008

Shori AB (2022) Storage quality and antioxidant properties of yogurt fortified with polyphenol extract from nutmeg, black pepper, and white pepper. Electron J Biotechnol 57:24–30

Shori AB, Al zahrani AJ (2022) Non-dairy plant-based milk products as alternatives to conventional dairy products for delivering probiotics. Food Sci Technol 42:1–14

Shori AB, Rashid F, Baba AS (2018) Effect of the addition of phytomix-3+ mangosteen on antioxidant activity viability of lactic acid bacteria type 2 diabetes key-enzymes and sensory evaluation of yogurt. LWT 94:33–39

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

Shori AB, Aljohani GS, Zahrani AJ, Al-sulbi OS, Baba AS (2022b) Viability of probiotics and antioxidant activity of cashew milk-based yogurt fermented with selected strains of probiotic *Lactobacillus* spp. LWT 153:112482

Shori AB, Tin YP, Baba AS (2022c) Codonopsis pilosula and fish collagen yogurt: proteolytic, potential angiotensin-I converting enzyme (ACE) inhibitory activity and sensory properties. LWT 16:113729

Shori AB, Yong YS, Baba AS (2022d) Effects of medicinal plants extract enriched cheese with fish collagen on proteolysis and in vitro angiotensin-I converting enzyme inhibitory activity. LWT 159:113218

Song YR, Baik SH (2017) Molecular cloning, purification, and characterization of a novel thermostable cinnamoyl esterase from *Lactobacillus helveticus* KCCM 11223. Prep Biochem Biotechnol 47(5):496–504

Szwajgier D, Jakubczyk A (2011) Production of extracellular ferulic acid esterases by Lactobacillus strains using natural and synthetic carbon sources. Acta Sci Pol Technol Aliment 10(3):287–302

Tain YL, Hsu CN (2022) Oxidative stress-induced hypertension of developmental origins: preventive aspects of antioxidant therapy. Antioxidants 11(3):511

Tamuno ENJ, Monday AO (2019) Physicochemical mineral and sensory characteristics of cashew nut milk. Int J Food Sci Biotechnol 4(1):1

Tlais AZA, Kanwal S, Filannino P, Albiac MA, Gobbetti M, Di Cagno R (2022) Effect of sequential or ternary starters-assisted fermentation on the phenolic and glucosinolate profiles of sauerkraut in comparison with spontaneous fermentation. Food Res Int 156:111116

Verni M, Verardo V, Rizzello CG (2019) How fermentation affects the antioxidant properties of cereals and legumes. Foods 8(9):362

Xu Z, He H, Zhang S, Guo T, Kong J (2017) Characterization of feruloyl esterases produced by the four lactobacillus species: *L. amylovorus*, *L. acidophilus*, *L. farciminis* and *L. fermentum*, isolated from ensiled corn stover. Front Microbiol 8:941

Yang J, Liu RH, Halim L (2009) Antioxidant and antiproliferative activities of common edible nut seeds. LWT Food Sci Technol 42:1–8

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