



Viability of selected strains of probiotic *Lactobacillus* spp. and sensory evaluation of concentrated yogurt (labneh) made from cow, camel, and cashew milk

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Abstract

The aim of this study is to investigate the effects of concentrated yogurt (labneh; L) made from cow (Co), camel (Ca), and cashew (Ew) milk (M) and their mixture (100% and 50%) using three strains of probiotic *Lactobacillus* spp. as starter culture (S) i.e. *Lactobacillus plantarum* (Lp; S1), *L. casei* (Lc; S2), *L. rhamnosus* (Lr; S3) or Lp, Lc, and Lr (S4) in co-cultures with *Streptococcus thermophilus* and *L. delbrueckii* subsp. *Lactis* on the changes of post-acidification and the viability of lactic acid bacteria (LAB) during 0, 7, 14, and 21 days of storage. In addition, sensory evaluation of all yogurt samples was run during the first day of storage. The lowest pH among all samples was seen in Ew-/Ca-ML50%S3 and Ew-/Co-ML50%S3 during the 21 days of storage. *S. thermophilus* increased to the highest numbers in Co-/Ca-ML50%S3 during 7th and 14th day as compared to other treated samples. All EwML100% were the most accepted by the panels compared to other samples. In conclusion, the combination of EwM with Co-/Ca-M during labneh preparation might enhance the viability of all different starter cultures especially S3 during the storage.

Keywords: concentrated yogurt (labneh); *Lactobacillus planterum*; *Lactobacillus casi*; *Lactobacillus rhamnouses*; viability.

Practical Application: Labneh made from different sources of milk has a great future potential to carry *Lactobacillus* spp., which can lead to the production of novel functional food.

1 Introduction

Nowadays, the maintenance of health and reducing diseases are becoming increasingly important. Many people awareness about consuming functional food for importance on health and diet (Mullin & Delzenne, 2017; Shori et al., 2022a). A functional food can be a natural product that contains useful biological components or a food obtained through a technological intervention that increases its level of biologically active compounds (Champagne et al., 2018). Biologically active compounds are components of foods that act positively on key body functions that are relevant to health. The benefits of function food included reduce the risk of developing diseases such as atherosclerosis, hypertension, myocardial infarction, diabetes, etc. (Aramesh & Ajoudanifar, 2017; Moghadam et al., 2018).

The microbes located in the intestines form a stable and diverse ecosystem that affects human health. However, certain bacteria found in the intestine may be useful in improving health through the consumption of live microorganisms such as probiotics. Fuller (1992) defined the probiotics as “live microbial food supplements, which beneficially affect the host by improving its intestinal microbial balance”. Probiotics are most commonly utilized for gastrointestinal effects, with the best-documented therapeutic benefits being the treatment and prevention of antibiotic-related diarrhea (Zendeboudi et al., 2020). However, their usage can be expanded to include oral, skin, and intestinal women's health, as well as treatment for liver problems, allergies, and metabolic diseases (Stavropoulou & Bezirtzoglou, 2020).

Many people around the world consume milk and its products. Cow's milk is usually consumed by the majority of the population and it is well believed that milk provides key nutrients such as fats, proteins, carbohydrates, calcium, selenium, riboflavin, vitamin B12, and pantothenic acid (vitaminB5; Garau et al., 2021). According to Shori et al. (2019) milk is considered an excellent medium to carry live and active cultures, so several studies in probiotic research show a lot of promise in the development of new products of functional foods based on milk (Ebrahimi et al., 2021; Shori et al., 2022a).

Camel milk is a good alternative to cow's milk for human nutrition (Shori & Baba, 2014; Singh et al., 2017). Middle Eastern, Asian, and African cultures have consumed camel milk for centuries as a medicinal drink. Camel milk contains low levels of fat and lactose contents, and high levels of volatile acids especially linoleic acids and polyunsaturated acids (Zouari et al., 2020; Kashaninejad & Razavi, 2021). It also contains protective proteins which have a potential role in enhancing the immune defense mechanism (Yagil, 2013; Khan et al., 2021).

Plant-based milk as well commonly known as non-dairy is milk obtained from seeds, grains, and nuts, which has a similar appearance to milk but does not contain fats and some compounds (Park, 2021). Plant milk can be used as a substitute for animal milk for its nutritional, functional, and sensory properties. Plant-based milk such as cashew milk contains proteins, healthy carbohydrates (low glycemic index), fatty

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acids, vitamins B and E, dietary fiber, antioxidants, and minerals such as calcium, potassium, iron, magnesium, and phosphorus (Chalupa-Krebszdzak et al., 2018; Shori et al., 2022b).

Dairy products have a character for being natural and healthy (Shori, 2021). Certain dairy products, when consumed on a regular basis, can actually prevent disease (Shori et al., 2021a). Concentrated yogurt, also known as labneh, is a fermented milk product that is consumed widely in the Middle East and Balkan regions (El-Sayed & El-Sayed, 2021). Labneh can be made from cow, sheep, or goat milk, but cow milk is the most common (Ismail et al., 2021). It is a semi-solid product with creamy consistency, acidic flavour, and has total solid content between 23 and 25 g/100g (Elkot et al., 2021). Labneh has nutritional and therapeutic properties considered comparable or even superior to yogurt. Therefore, the aim of this study is to investigate the effects of concentrated yogurt (labneh) made from cow, camel, and cashew milk and their mixture (100% and 50%) using three strains of probiotic *Lactobacillus* spp. i.e. *Lactobacillus rhamnosus*, *Lactobacillus casei*, or *Lactobacillus plantarum* in co-cultures with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *Lactis* on the changes of post-acidification and the viability of lactic acid bacteria (LAB) during 0, 7, 14 and 21 days of storage. In addition, sensory evaluation of all yogurt samples was run during the first day of storage.

2 Materials and methods

2.1 Materials and chemicals

Pure strains of *S. thermophilus* St1342, *L. delbrueckii* ssp. *Lactis* ATCC 7830, *L. plantarum* ATCC 14917, *L. casei* ATCC 393, and *L. rhamnosus* ATCC 53103 were obtained from the Microbiological Resources Center (Cairo Mircen) in Ain Shams University. Cashew nuts, pasteurized cow milk (Almarei®), and pasteurized camel milk (Al-turath®) were purchased from a local supermarket in Saudi Arabia, Jeddah. The de Man-Rogosa-Sharpe (MRS) agar and broth media for *Lactobacillus* growth, M17 agar for *S. thermophilus* growth, and all chemicals were obtained from Sigma Chemical Company (St Louis, MO, USA).

2.2 Preparation starter culture

S. thermophilus, *L. casei*, *L. plantarum*, *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* was incubated at 42 °C (Shori et al., 2022b). The activated organisms after three successive transfers were used for the production of cultures for labneh making. The cultures were prepared by inoculating 1% (v/v) in 10 mL aliquots of reconstituted skim milk (RSM) supplemented with 2% glucose and 1% yeast extract.

2.3 Preparation of cashew milk

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 (2000 rpm, 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.

2.4 Preparation of labneh

Labneh was prepared according to Serhan et al., (2016) with some modifications. Labneh (L) were prepared using milk (M) from cow (Co), camel (Ca), and cashew (Ew) and their mixture in two different concentrations (100:0 and 50:50; v/v). In addition, 2% of starter cultures (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) were 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 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.

2.5 Measurement of pH and titratable acidity (TA)

The pH was measured using a pH meter (Metler Toledo 320). For titratable acidity (TA), labneh was homogenized in water (1:9) and three drops of phenolphthalein were added to the mixture as an indicator (Shori et al., 2021b). The sample was titrated with 0.1N NaOH and this process was carried out under continuous stirring until the development of a consistent pink color. TA was calculated by converting the volume of NaOH using the below Equation 1.

$$TA \text{ (\%Lactic acid equivalent; LAE)} = 10 \times \frac{V_{NaOH} \times 0.009g \times 0.1 \times 100\%}{W} \quad (1)$$

10 = dilution factor, V_{NaOH} = volume of NaOH used to neutralize the lactic acid, 0.009 = conversion factor (1 mL NaOH (0.01N) neutralizes 0.009g of lactic acid), 0.1 = Normality of NaOH, W = weight of sample.

2.6 Viable cell counts (VCC) of LAB in labneh

One milliliter of each labneh sample was transferred into a tube containing 9 mL of sterile (0.15%) buffered peptone water. Then, serial dilutions were prepared up to 10^{-5} using peptone water (Afolabi et al., 2017). The spread counting method was used to determine the VCC of *lactobacillus* spp. and *S. thermophilus* using MRS and M17 agar; respectively. One milliliter of diluted labneh was spread on agar in a petri dish plate. All plates were parafilm and incubated inverted in an incubator at 37 ± 1 °C for 48 h. The VCC was calculated as follows (Equation 2).

$$CFU^* / mL = \frac{\text{Number of colonies formed} \times \text{dilution factor of sample}}{1mL \text{ of sample}} \quad (2)$$

*CFU: colony-forming unit.

2.7 Sensory evaluation

Sensory evaluation was done on the first day of storage as described by Shori et al. (2021c). The 13-member untrained panel of students and staff members of biological sciences, faculty of science, King Abdulaziz University with ages ranging between 20-40 years evaluated the labneh. Six attributes (color,

flavor, taste, texture, smell, and overall preference) were assessed based on a 10-point system (9-10 = very good, 7-8 = good, 5-6 = satisfactory, 3-4 = fairly satisfactory, 1-2 = unsatisfactory, and zero = defective).

2.8 Statistical analysis

All data were presented as means $\pm$ standard error (SE) and performed in duplicates for a total of 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 ($p < 0.05$).

3 Results and discussions

3.1 Post-acidification activity in labneh

The post-acidification characteristics (pH and TA) of labneh made from individual and mixtures of cow, camel, and cashew

milk (100% and 50%) using three types of probiotics and their combination were shown in (Table 1). All labneh samples showed a reduction in pH values compared to control. However, the pH in 7-day-old CoML100%S4 and CoML100%S1 at the 0th and 7th day were non-significantly different compared to their respective controls (Table 1). The lowest pH among all samples was seen in Ew-/Ca-ML50%S3 and Ew-/Co-ML50%S3 (3.79-3.46 and 3.8-3.51; respectively, $p < 0.05$) during the 21 days of storage. However, CoML100%S1 had the highest pH value among other samples (4.9-4.74, Table 1). In addition, the presence of S1, S2, and S4 in Ew-/Ca-ML50% and Ew-/Co-ML50% decreased significantly the pH values during the storage period. CoML100% (S2 and S3) and CaML100% (S3 and S4) were significantly ($p < 0.05$) lower in pH than respective controls during all days of storage whereas CoML100%S4 and EwML100% S3 showed lower pH on the 14th and 21st day.

In the present study, all labneh samples showed an increase in TA amount compared to control (Table 1).

Table 1. Changes in pH and titratable acidity (TA) of labneh made from cow-, camel-, and cashew- milk and their mixture (100% and 50%) in the presence of *Lactobacillus plantarum*, *L. casei*, *L. rhamnosus* compared to control during 21 days of refrigerated storage at 4 °C.

Samples	pH				TA			
	Day 0	Day 7	Day 14	Day 21	Day 0	Day 7	Day 14	Day 21
EwML 100% (control)*	4.35 $\pm$ 0.01	4.09 $\pm$ 0.01	4.08 $\pm$ 0.01	4.06 $\pm$.03	0.18 $\pm$.0	0.42 $\pm$ 0.05	0.45 $\pm$ 0.00	0.78 $\pm$ 0.05
EwML 100% S1	4.19 $\pm$ 0.1	4.08 $\pm$ 0.01	3.99 $\pm$ 0.01	3.93 $\pm$ 0.01	0.24 $\pm$ 0.05	0.27 $\pm$ 0.00	0.66 $\pm$ 0.05	0.78 $\pm$ 0.015
EwML 100% S2	4.23 $\pm$ 0.01	4.08 $\pm$ 0.03	4.08 $\pm$ 0.01	3.94 $\pm$ 0.01	0.21 $\pm$ 0.00	0.39 $\pm$ 0.05	0.69 $\pm$ 0.05	0.84 $\pm$ 0.10
EwML 100% S3	4.1 $\pm$ 0.00	4.04 $\pm$ 0.00	3.69 $\pm$ 0.03	3.59 $\pm$ 0.00	0.57 $\pm$ 0.05	0.96 $\pm$ 0.05	1.23 $\pm$ 0.05	1.26 $\pm$ 0.09
EwML 100% S4	4.13 $\pm$ 0.01	4.06 $\pm$ 0.01	3.93 $\pm$ 0.01	3.9 $\pm$ 0.01	0.36 $\pm$ 0.05	0.51 $\pm$ 0.10	0.96 $\pm$ 0.05	0.99 $\pm$ 0.00
CoML 100% (control)*	4.9 $\pm$ 0.00	4.85 $\pm$ 0.02	4.76 $\pm$ 0.02	4.76 $\pm$ 0.03	0.15 $\pm$ 0.04	0.27 $\pm$ 0.00	0.51 $\pm$ 0.05	0.54 $\pm$ 0.00
CoML 100% S1	4.9 $\pm$ 0.10	4.83 $\pm$ 0.01	4.75 $\pm$ 0.01	4.74 $\pm$ 0.01	0.42 $\pm$ 0.00	0.45 $\pm$ 0.05	0.7 $\pm$ 0.05	0.88 $\pm$ 0.00
CoML 100% S2	4.8 $\pm$ 0.20	4.6 $\pm$ 0.00	4.5 $\pm$ 0.05	4.4 $\pm$ 0.10	0.18 $\pm$ 0.00	0.51 $\pm$ 0.05	0.78 $\pm$ 0.13	0.81 $\pm$ 0.00
CoML 100% S3	4.6 $\pm$ 0.03	4.48 $\pm$ 0.00	4.45 $\pm$ 0.01	4.28 $\pm$ 0.00	0.42 $\pm$ 0.05	0.78 $\pm$ 0.00	0.85 $\pm$ 0.08	0.99 $\pm$ 0.00
CoML 100% S4	4.8 $\pm$ 0.05	4.8 $\pm$ 0.03	4.53 $\pm$ 0.02	4.40 $\pm$ 0.00	0.42 $\pm$ 0.05	0.61 $\pm$ 0.00	0.78 $\pm$ 0.05	0.9 $\pm$ 0.01
CaML 100% (control)*	4.95 $\pm$ 0.01	4.86 $\pm$ 0.01	4.75 $\pm$ 0.02	4.68 $\pm$ 0.08	0.18 $\pm$ 0.09	0.3 $\pm$ 0.05	0.51 $\pm$ 0.10	0.78 $\pm$ 0.05
CaML 100% S1	4.7 $\pm$ 0.01	4.7 $\pm$ 0.01	4.68 $\pm$ 0.05	4.53 $\pm$ 0.05	0.78 $\pm$ 0.05	0.78 $\pm$ 0.05	0.84 $\pm$ 0.05	0.93 $\pm$ 0.05
CaML 100% S2	4.87 $\pm$ 0.01	4.78 $\pm$ 0.00	4.73 $\pm$ 0.10	4.42 $\pm$ 0.03	0.21 $\pm$ 0.05	0.42 $\pm$ 0.05	0.72 $\pm$ 0.00	0.9 $\pm$ 0.00
CaML 100% S3	4.49 $\pm$ 0.01	4.48 $\pm$ 0.01	4.39 $\pm$ 0.05	4.3 $\pm$ 0.01	0.45 $\pm$ 0.15	0.51 $\pm$ 0.05	0.9 $\pm$ 0.00	0.99 $\pm$ 0.10
CaML 100% S4	4.6 $\pm$ 0.01	4.54 $\pm$ 0.05	4.37 $\pm$ 0.03	4.31 $\pm$ 0.02	0.45 $\pm$ 0.00	0.51 $\pm$ 0.10	0.87 $\pm$ 0.05	0.96 $\pm$ 0.05
Co-/Ca-ML 50% (control)*	4.53 $\pm$ 0.03	4.39 $\pm$ 0.00	4.28 $\pm$ 0.01	4.26 $\pm$ 0.01	0.18 $\pm$ 0.00	0.24 $\pm$ 0.05	0.69 $\pm$ 0.05	0.87 $\pm$ 0.05
Co-/Ca-ML 50% S1	4.5 $\pm$ 0.11	4.3 $\pm$ 0.20	4.3 $\pm$ 0.05	4.2 $\pm$ 0.01	0.18 $\pm$ 0.15	0.3 $\pm$ 0.10	0.81 $\pm$ 0.05	0.9 $\pm$ 0.00
Co-/Ca-ML 50% S2	4.52 $\pm$ 0.20	4.3 $\pm$ 0.02	4.18 $\pm$ 0.01	4.11 $\pm$ 0.01	0.43 $\pm$ 0.02	0.48 $\pm$ 0.00	0.63 $\pm$ 0.00	0.9 $\pm$ 0.00
Co-/Ca-ML 50% S3	4.49 $\pm$ 0.05	4.29 $\pm$ 0.05	4.13 $\pm$ 0.01	4.1 $\pm$ 0.02	0.63 $\pm$ 0.09	0.78 $\pm$ 0.05	0.87 $\pm$ 0.05	1.14 $\pm$ 0.05
Co-/Ca-ML 50% S4	4.5 $\pm$ 0.01	4.3 $\pm$ 0.01	4.14 $\pm$ 0.01	4.1 $\pm$ 0.01	0.43 $\pm$ 0.09	0.54 $\pm$ 0.05	0.87 $\pm$ 0.00	0.99 $\pm$ 0.00
Ew-/Co-ML 50% (control)*	4.53 $\pm$ 0.02	4.46 $\pm$ 0.05	4.21 $\pm$ 0.01	4.11 $\pm$ 0.01	0.09 $\pm$ 0.00	0.18 $\pm$ 0.00	0.66 $\pm$ 0.05	0.66 $\pm$ 0.05
Ew-/Co-ML 50% S1	3.84 $\pm$ 0.00	3.73 $\pm$ 0.01	3.62 $\pm$ 0.01	3.56 $\pm$ 0.01	0.09 $\pm$ 0.00	0.27 $\pm$ 0.00	0.75 $\pm$ 0.00	0.87 $\pm$ 0.05
Ew-/Co-ML 50% S2	3.87 $\pm$ 0.01	3.72 $\pm$ 0.01	3.6 $\pm$ 0.01	3.55 $\pm$ 0.00	0.15 $\pm$ 0.05	0.24 $\pm$ 0.05	0.54 $\pm$ 0.00	0.69 $\pm$ 0.05
Ew-/Co-ML 50% S3	3.8 $\pm$ 0.01	3.67 $\pm$ 0.00	3.56 $\pm$ 0.01	3.51 $\pm$ 0.01	0.84 $\pm$ 0.00	0.88 $\pm$ 0.05	1.2 $\pm$ 0.05	1.4 $\pm$ 0.20
Ew-/Co-ML 50% S4	3.82 $\pm$ 0.00	3.72 $\pm$ 0.01	3.59 $\pm$ 0.02	3.53 $\pm$ 0.01	0.57 $\pm$ 0.05	0.6 $\pm$ 0.05	0.75 $\pm$ 0.10	0.9 $\pm$ 0.00
Ew-/Ca-ML 50% (control)*	4.5 $\pm$ 0.03	4.15 $\pm$ 0.01	4.1 $\pm$ 0.00	4.01 $\pm$ 0.03	0.18 $\pm$ 0.09	0.27 $\pm$ 0.00	0.93 $\pm$ 0.05	1.14 $\pm$ 0.10
Ew-/Ca-ML 50% S1	3.8 $\pm$ 0.01	3.72 $\pm$ 0.04	3.58 $\pm$ 0.00	3.57 $\pm$ 0.03	0.27 $\pm$ 0.00	0.3 $\pm$ 0.05	0.93 $\pm$ 0.05	1.26 $\pm$ 0.15
Ew-/Ca-ML 50% S2	3.8 $\pm$ 0.01	3.76 $\pm$ 0.01	3.6 $\pm$ 0.01	3.59 $\pm$ 0.01	0.39 $\pm$ 0.05	0.39 $\pm$ 0.05	0.99 $\pm$ 0.00	1.2 $\pm$ 0.13
Ew-/Ca-ML 50% S3	3.79 $\pm$ 0.02	3.66 $\pm$ 0.00	3.47 $\pm$ 0.00	3.46 $\pm$ 0.01	0.88 $\pm$ 0.05	0.93 $\pm$ 0.05	1.2 $\pm$ 0.05	1.56 $\pm$ 0.05
Ew-/Ca-ML 50% S4	3.79 $\pm$ 0.017	3.7 $\pm$ 0.01	3.58 $\pm$ 0.00	3.54 $\pm$ 0.01	0.72 $\pm$ 0.05	0.84 $\pm$ 0.05	1.11 $\pm$ 0.05	1.4 $\pm$ 0.05

Data are presented as mean $\pm$ SEM. The level of significance was preset at $p < 0.05$ compared to control at the same storage period. Cow- = Co; camel- = Ca; cashew- = Ew; milk = M; labneh = L; starter culture = S; *Lactobacillus plantarum* = 1; *L. casei* = 2; *L. rhamnosus* = 3; and *L. plantarum* + *L. casei* + *L. rhamnosus* = 4. *Control containing *Streptococcus thermophilus* and *L. delbrueckii* subsp. *Lactis*.

Ew-/Ca-ML50%S3 showed the highest TA values (0.88 -1.56%LAE) followed by Ew-/Co-ML50%S3 (0.84-1.44%LAE) during storage days. Moreover, Ew-/Ca-ML50%S3 and S4 showed a significant increase ($p<0.05$) in TA compared to control on all days of storage (Table 1).

The acidification found in cow and/or camel milk labneh was related to lactose sugar that was converted to lactic acid by LAB (Shori, 2020; El-Shafei et al., 2020). The present results observed that the rate of acidification of LAB depends on the type of starter culture and milk. In addition, S3 showed more acidification in all treated labneh than other starter cultures. Many previous investigations have shown that *L. rhamnosus* can grow not only in dairy milk but also in non-dairy milk (plants water extracts and plant-based milk; Mauro & Garcia, 2019; Fatima & Hekmat, 2020). It is also able to lower pH because it has a high proteolytic capacity and may create exopolysaccharides in milk, making it a functional starter culture (Mishra et al., 2019). Therefore, the enhancement of the pH reduction in Ew-/Ca-ML50%S3 could occur due to the stimulation of *L. rhamnosus* growth (Sarkar, 2008). In comparison to cow and camel milk, cashew milk is believed to be a rich medium for *L. rhamnosus* growth. As noted by the results of TA and pH, S3 (*L. rhamnosus*) was more affected in all samples of labneh (Table 1). This could be confirmed by the highest lactic acid production by *L. rhamnosus* in comparison to other *Lactobacillus* strains in labneh samples. Beshkova et al. (2003) reported that *L. rhamnosus* possess increased access to sugar, which it can utilize as a carbon source for their growth. In addition, the mixing of cashew milk with both cow and camel milk during labneh preparation resulted in an increase in TA during 21 days of refrigerated storage (Table 1). Cashew milk is a rich source of protein (23%), carbohydrate (29.30%), essential fatty acids (44%), unsaturated fats (82%), and minerals (4.75 mg calcium, 7.15 mg potassium, 3.00 mg iron, and 2.00 mg magnesium and phosphorus (Olayinka et al., 2018; Bruno et al., 2020).

Post-acidification in labneh samples could occur during refrigeration because of the starter culture metabolic activity. Even at refrigerated storage temperature (0-5 °C), the activity of β -galactosidase produced by the LAB to cleave lactose is still active (Shori et al., 2012). This contributes to the accumulation of metabolic by-products such as lactic acid, acetic acid, citric acid, butyric acid, acetaldehyde, and formic acid produced by labneh starter culture (Shori & Baba, 2013; Muniandy et al., 2017).

3.2 Viability of LAB in labneh

Table 2 shows the viability of *Lactobacillus* spp. and *S. thermophilus* in labneh made from individual and mixtures of cow, camel, and cashew milk (100% and 50%) using three types of probiotics and their combination. The viability of *Lactobacillus* spp. and *S. thermophilus* in all samples showed an increase during the first three weeks of storage. The addition of *L. rhamnosus*, *L. casei*, or *L. plantarum* and their combination in labneh samples enhanced the viability of *Lactobacillus* spp. compared with controls throughout the storage periods (Table 2). Ew-/Ca-ML50%S1 showed the highest viability of

Lactobacillus spp. among other samples on 0 day of storage (7.66 ± 0.28 log cfu/mL; $p<0.05$). This followed by a significant increased to 8.20 ± 0.60 log cfu/mL on 14 day. Similarly, Ew-/Ca-ML50%S3 presented the highest VCC of *Lactobacillus* spp. on 7th and 14th day (8.11 ± 0.43 log cfu/mL and 8.38 ± 1.15 log cfu/mL; respectively) among all treated samples. In addition, *Lactobacillus* spp. in EwML100%S3 and CaML100%S3 were significantly increased in labneh during 0th and 14th day of storage compared with their respective controls. All samples showed significant reduction in *Lactobacillus* spp. counts on 21th day of storage with the lowest counts was shown in CoML100%S3 (6.33 ± 0.50 log cfu/mL).

The addition of S3 increased ($p<0.05$) the viability of *S. thermophilus* in all labneh samples compared with their respective controls during the 0th day of storage with the highest viability was shown in Ew-/Co-ML50%S3 and CoML100%S3 (Table 2). *S. thermophilus* increased to the highest numbers in Co-/Ca-ML50%S3 during 7th and 14th day as compared to other treated samples. The viability of *S. thermophilus* was significantly higher ($p>0.05$) in 7-old-day EwML100% (S1, S2, S3, and S4), 14-day-old Co-/Ca-ML50% (S1, S2, S3, and S4), and CoML100% (S3 and S4) on 0th and 7th day than their respective controls (Table 2). Likewise, the addition of S3 in CaML100% and Ew-/Co-ML50% improved ($p>0.05$) the viability of *S. thermophilus* (8.04 ± 0.03 and 8.14 ± 0.15 log cfu/mL; respectively) compared to their respective controls (7.41 ± 0.06 and 7.25 ± 0.06 log cfu/mL) for CaML100% and Ew-/Co-ML50%; respectively on the 7th day of storage. The VCC of *S. thermophilus* exhibited non-significant ($p<0.05$) reduction on the 21th days of storage for all treated samples (Table 2).

S. thermophilus grows faster and produces formate, pyruvate, and carbon dioxide which stimulate the growth of *Lactobacillus* spp. (Rul, 2017; Hadjimbei et al., 2020). In return, the proteolytic activity of *Lactobacillus* spp. tends to produce stimulatory peptides and amino acids which stimulate the growth of *S. thermophilus*. Although both bacteria can grow independently, the rate of acid production is much higher when these two bacteria are present at the same time. The present study found that the combination of cashew milk with cow/camel milk during labneh preparation increased the viability of *Lactobacillus* spp. and *S. thermophilus* during fermentation and refrigerated storage. Previous studies reported that *Lactobacillus* spp. and *S. thermophilus* can ferment plant-based milk such as soy milk, oat milk, cashew milk, and vegetables milk (Aboulfazli et al., 2016; Shori et al., 2022b; Yong et al., 2022). In addition, *Lactobacillus* spp. can hydrolyze oligosaccharides in plant-based milk by producing the -galactosidase enzyme (Singh & Vij, 2018). An earlier study found that the fermentation of cashew apple juice with *L. mesenteroides* increases oligosaccharide content (Rabelo et al., 2009; Vergara et al., 2010). Moreover, according to Fontes et al. (2009), cashew apple juice contains a high concentration of glucose and fructose, which could act as a carbon source for LAB growth. This indicated that mixing camel/cow milk with cashew milk could increase the viability of *Lactobacillus* spp. and *S. thermophilus* because of prebiotic

Table 2. Changes in viable cell counts (VCC) of *S. thermophilus* and *Lactobacillus* spp. of labneh (L) made from cow-, camel-, and cashew- milk and their mixture (100% and 50%) in the presence of *Lactobacillus plantarum*, *L. casei*, *L. rhamnosus* compared to control during 21 days of refrigerated storage at 4 °C.

Samples	Day 0		Day 7		Day 14		Day 21	
	<i>Lactobacillus</i> spp	<i>S. thermophilus</i>	<i>Lactobacillus</i> spp	<i>S. thermophilus</i>	<i>Lactobacillus</i> spp	<i>S. thermophilus</i>	<i>Lactobacillus</i> spp	<i>S. thermophilus</i>
EwML100% (control)*	6.63 ± 0.51	7.15 ± 0.23	7.11 ± 0.12	7.63 ± 0.54	7.54 ± 0.38	7.88 ± 0.21	7.54 ± 0.13	7.81 ± 0.20
EwML100% S1	7.04 ± 0.41	7.44 ± 0.38	7.25 ± 0.17	8.73 ± 0.10	8.11 ± 0.13	8.11 ± 0.15	7.74 ± 0.09	8.11 ± 0.16
EwML100% S2	7.20 ± 0.18	7.36 ± 0.20	7.23 ± 0.12	8.04 ± 0.06	7.86 ± 0.12	8.25 ± 0.04	7.74 ± 0.18	8.01 ± 0.08
EwML100% S3	7.20 ± 0.25	7.71 ± 0.14	7.57 ± 0.09	7.92 ± 0.09	8.20 ± 0.14	8.34 ± 0.26	7.90 ± 0.08	8.14 ± 0.17
EwML100% S4	7.20 ± 0.14	7.69 ± 0.28	7.32 ± 0.26	7.79 ± 0.02	7.69 ± 0.10	8.07 ± 0.13	7.50 ± 0.12	8.07 ± 0.48
CoML100% (control)*	6.43 ± 0.54	6.92 ± 0.21	6.76 ± 0.28	7.25 ± 0.21	6.83 ± 0.14	7.78 ± 0.11	6.49 ± 0.18	7.75 ± 0.25
CoML100% S1	6.97 ± 0.05	7.07 ± 0.16	6.99 ± 0.07	7.46 ± 0.59	7.14 ± 0.21	7.82 ± 0.03	7.11 ± 0.14	7.39 ± 0.1
CoML100% S2	7.00 ± 0.17	7.39 ± 0.12	7.14 ± 0.08	7.50 ± 0.47	7.69 ± 0.07	8.17 ± 0.10	7.63 ± 0.13	7.79 ± 0.15
CoML100% S3	6.49 ± 0.16	7.93 ± 0.10	6.59 ± 0.12	7.96 ± 0.06	6.64 ± 0.04	8.11 ± 0.05	6.33 ± 0.50	8.04 ± 0.05
CoML100% S4	7.19 ± 0.10	7.72 ± 0.26	7.46 ± 0.15	7.77 ± 0.03	7.68 ± 0.05	7.96 ± 0.02	7.53 ± 0.07	7.68 ± 0.04
CaML100% (control)*	6.32 ± 0.38	7.04 ± 0.04	6.85 ± 0.16	7.41 ± 0.06	6.95 ± 0.26	7.68 ± 0.60	6.65 ± 0.50	7.55 ± 0.10
CaML100% S1	6.65 ± 0.26	7.15 ± 0.15	7.04 ± 0.06	7.71 ± 0.13	7.49 ± 0.07	8.32 ± 0.10	7.30 ± 0.21	7.95 ± 0.02
CaML100% S2	6.60 ± 0.11	7.19 ± 0.10	6.85 ± 0.16	8.04 ± 0.12	7.51 ± 0.06	8.32 ± 0.11	6.95 ± 0.10	7.88 ± 0.11
CaML100% S3	7.20 ± 0.10	7.66 ± 0.56	7.39 ± 0.17	8.04 ± 0.03	7.86 ± 0.10	8.66 ± 0.05	7.59 ± 0.11	8.14 ± 0.14
CaML100% S4	6.39 ± 0.32	7.34 ± 0.23	6.86 ± 0.23	7.50 ± 0.09	7.47 ± 0.65	8.11 ± 0.08	7.34 ± 0.11	7.68 ± 0.11
Co-/Ca-ML50% (control)*	6.04 ± 0.02	7.00 ± 0.57	7.20 ± 0.10	7.50 ± 0.10	7.47 ± 0.06	7.67 ± 0.06	7.30 ± 0.02	7.50 ± 0.06
Co-/Ca-ML50% S1	7.14 ± 0.12	7.17 ± 0.12	7.23 ± 0.34	7.65 ± 0.16	7.63 ± 0.06	7.92 ± 0.13	7.43 ± 0.3	7.58 ± 0.08
Co-/Ca-ML50% S2	6.76 ± 0.13	7.34 ± 0.17	7.32 ± 0.18	8.14 ± 0.04	7.34 ± 0.08	8.25 ± 0.23	7.32 ± 0.22	7.86 ± 0.10
Co-/Ca-ML50% S3	7.14 ± 0.11	7.80 ± 0.04	7.50 ± 0.06	8.53 ± 0.19	7.85 ± 0.17	8.93 ± 0.13	7.78 ± 0.05	8.50 ± 0.19
Co-/Ca-ML50% S4	6.69 ± 0.33	7.69 ± 0.09	7.23 ± 0.08	8.04 ± 0.05	7.67 ± 0.06	8.17 ± 0.05	7.49 ± 0.19	7.90 ± 0.05
Ew-/Co-ML50% (control)*	6.65 ± 0.11	7.04 ± 0.15	6.90 ± 0.17	7.25 ± 0.06	7.55 ± 0.08	7.81 ± 0.05	7.32 ± 0.50	7.36 ± 0.11
Ew-/Co-ML50% S1	7.07 ± 0.85	7.44 ± 0.10	7.51 ± 0.06	7.74 ± 0.23	8.20 ± 0.40	8.38 ± 0.10	7.55 ± 0.14	8.11 ± 0.13
Ew-/Co-ML50% S2	6.99 ± 0.10	7.17 ± 0.07	7.20 ± 0.05	7.30 ± 0.17	7.69 ± 0.13	8.38 ± 0.13	7.68 ± 0.09	7.97 ± 0.05
Ew-/Co-ML50% S3	6.66 ± 0.11	8.00 ± 0.09	7.20 ± 0.30	8.14 ± 0.15	8.00 ± 0.10	8.17 ± 0.03	7.84 ± 0.06	8.02 ± 0.10
Ew-/Co-ML50% S4	6.93 ± 0.17	7.47 ± 0.09	7.11 ± 0.20	7.77 ± 0.06	8.03 ± 0.06	8.07 ± 0.07	7.74 ± 0.11	7.81 ± 26
Ew-/Ca-ML50% (control)*	6.65 ± 0.14	7.17 ± 0.10	7.30 ± 0.21	7.55 ± 0.80	7.70 ± 0.12	7.91 ± 0.13	7.53 ± 0.07	7.60 ± 0.03
Ew-/Ca-ML50% S1	7.66 ± 0.28	7.23 ± 0.17	7.75 ± 0.01	7.90 ± 0.12	8.20 ± 0.60	8.14 ± 0.14	7.97 ± 0.02	8.00 ± 0.05
Ew-/Ca-ML50% S2	7.04 ± 0.05	7.42 ± 0.10	7.50 ± 0.03	7.87 ± 0.07	8.20 ± 0.04	8.67 ± 0.03	7.74 ± 0.69	8.07 ± 0.07
Ew-/Ca-ML50% S3	7.23 ± 0.11	7.83 ± 0.08	8.11 ± 0.43	8.11 ± 0.04	8.38 ± 1.15	8.40 ± 0.24	7.87 ± 0.08	8.11 ± 8.0
Ew-/Ca-ML50% S4	7.20 ± 0.24	7.69 ± 0.08	7.53 ± 0.11	7.77 ± 0.06	8.00 ± 0.06	8.23 ± 0.10	7.78 ± 0.09	7.81 ± 0.08

Data are presented as mean ± SEM. The level of significance was preset at $p < 0.05$ compared to control at the same storage period. Cow- = Co; camel- = Ca; cashew- = Ew; milk = M; labneh = L; starter culture = S; *Lactobacillus plantarum* = 1; *L. casei* = 2; *L. rhamnosus* = 3; and *L. plantarum* + *L. casei* + *L. rhamnosus* = 4. *Control containing *Streptococcus thermophilus* and *L. delbrueckii* subsp. *Lactis*.

oligosaccharides present in cashew milk (Rabelo et al., 2009; Vergara et al., 2010).

L. rhamnosus (S3) showed a significant effect on labneh made from cow, camel, cashew milk, and their combination compared to other starter cultures. Lactose, galactose, and glucose were shown to be the most efficient carbon sources for *L. rhamnosus* (Watson et al., 2013; Lucatto et al., 2020). These bacteria showed high access to sugar, which they can utilize as a carbon source for their growth (Beshkova et al., 2003). Both cow and camel milk possess a high content of lactose (Mohamed et al., 2021). In addition, the glucose and fructose present in cashew milk could act as a carbon source for

L. rhamnosus growth (Fontes et al., 2009). Mishra et al. (2019) reported that *L. rhamnosus* is able to create exopolysaccharides in dairy milk. Similarly, Marazza et al. (2012) found that total isoflavone content rises as soymilk ferments with *L. rhamnosus* CRL981 which produced ~ 40 times more b-glucosidase than the *S. thermophilus* strain.

3.3 sensory evaluation

Table 3 shows the sensory evaluation of labneh made from individual and mixtures of cow, camel, and cashew milk using three types of probiotics and their combination. All treated samples showed no significant differences in color and aroma

Table 3. Sensory evaluation of labneh (L) made from cow-, camel-, and cashew- milk and their mixture (100% and 50%) in the presence of *Lactobacillus plantarum*, *L. casei*, *L. rhamnosus* compared to control on the first day of refrigerated storage at 4 °C.

Samples	Color	Flavor	Taste	Texture	Aroma	Overall acceptability
EwML 100% (control)*	6.33 ± 0.57	9.33 ± 0.58	9.30 ± 0.00	9.60 ± 0.57	8.60 ± 0.50	9.30 ± 0.56
EwML 100% S1	6.33 ± 0.57	9.00 ± 0.57	9.00 ± 0.50	9.60 ± 0.50	8.60 ± 0.50	9.20 ± 0.57
EwML 100% S2	6.33 ± 0.57	9.00 ± 0.00	9.00 ± 0.57	9.60 ± 0.57	8.60 ± 0.50	9.20 ± 0.50
EwML 100% S3	6.33 ± 0.57	9.00 ± 1.00	9.00 ± 0.57	9.60 ± 0.57	8.60 ± 0.50	9.20 ± 1.00
EwML 100% S4	6.33 ± 0.57	9.00 ± 0.57	9.00 ± 1.00	9.60 ± 1.00	8.60 ± 0.50	9.00 ± 0.00
CoML 100% (control)*	9.33 ± 0.58	8.66 ± 0.57	8.60 ± 1.00	9.60 ± 0.57	8.66 ± 0.56	8.30 ± 0.65
CoML 100% S1	9.33 ± 0.58	8.00 ± 1.00	8.00 ± 1.00	9.66 ± 0.50	8.66 ± 0.56	8.00 ± 0.50
CoML 100% S2	9.33 ± 0.58	8.00 ± 0.57	8.00 ± 1.00	9.60 ± 0.57	8.66 ± 0.56	8.00 ± 0.57
CoML 100% S3	9.33 ± 0.58	8.00 ± 1.15	8.00 ± 1.00	9.60 ± 1.00	8.66 ± 0.56	8.00 ± 1.50
CoML 100% S4	9.33 ± 0.58	8.00 ± 1.00	8.00 ± 1.00	9.66 ± 0.57	8.66 ± 0.56	8.00 ± 0.57
CaML 100% (control)*	9.33 ± 1.15	3.00 ± 0.00	3.00 ± 0.57	2.00 ± 1.15	5.33 ± 0.56	3.30 ± 0.57
CaML 100% S1	9.33 ± 1.15	3.00 ± 0.50	3.00 ± 0.57	2.00 ± 0.50	5.33 ± 0.56	3.30 ± 0.57
CaML 100% S2	9.33 ± 1.15	2.60 ± 0.57	2.00 ± 1.00	2.00 ± 1.00	5.33 ± 0.56	2.00 ± 0.57
CaML 100% S3	9.33 ± 1.15	2.60 ± 0.57	2.00 ± 1.50	2.00 ± 1.00	5.33 ± 0.56	2.00 ± 1.00
CaML 100% S4	9.33 ± 1.15	2.60 ± 0.57	2.00 ± 0.57	2.00 ± 1.00	5.33 ± 0.56	2.00 ± 1.00
Co-/Ca-ML 50% (control)*	8.66 ± 0.57	5.60 ± 1.53	5.00 ± 1.00	4.00 ± 1.00	5.66 ± 0.57	5.60 ± 1.00
Co-/Ca-ML 50% S1	8.66 ± 0.57	4.60 ± 0.57	4.60 ± 0.57	4.00 ± 1.00	5.66 ± 0.57	4.60 ± 0.57
Co-/Ca-ML 50% S2	8.66 ± 0.57	4.60 ± 0.57	4.60 ± 1.15	4.00 ± 0.57	5.66 ± 0.57	4.60 ± 0.57
Co-/Ca-ML 50% S3	8.66 ± 0.57	4.66 ± 1.50	4.60 ± 1.50	4.00 ± 0.57	5.66 ± 0.57	4.66 ± 1.50
Co-/Ca-ML 50% S4	8.66 ± 0.57	4.60 ± 0.57	4.60 ± 1.00	4.00 ± 1.50	5.66 ± 0.57	4.60 ± 0.57
Ew-/Co-ML 50% (control)*	7.00 ± 1.15	9.00 ± 0.57	9.00 ± 0.58	9.00 ± 1.52	9.00 ± 1.00	8.60 ± 1.00
Ew-/Co-ML 50% S1	7.00 ± 1.15	8.60 ± 1.00	8.00 ± 1.00	9.00 ± 0.58	9.00 ± 1.00	8.00 ± 1.00
Ew-/Co-ML 50% S2	7.00 ± 1.15	8.60 ± 1.00	8.00 ± 1.50	9.00 ± 1.15	9.00 ± 1.00	8.00 ± 0.57
Ew-/Co-ML 50% S3	7.00 ± 1.15	8.60 ± 0.57	8.00 ± 0.57	9.00 ± 0.00	9.00 ± 1.00	8.00 ± 0.57
Ew-/Co-ML 50% S4	7.00 ± 1.15	8.60 ± 0.57	8.00 ± 0.57	9.00 ± 0.00	9.00 ± 1.00	8.00 ± 0.57
Ew-/Ca-ML 50% (control)*	6.00 ± 1.70	6.00 ± 2.00	6.30 ± 0.57	6.600 ± 0.57	6.00 ± 0.00	6.700 ± 0.00
Ew-/Ca-ML 50% S1	6.00 ± 1.70	5.33 ± 0.57	6.00 ± 1.50	6.600 ± 0.57	6.00 ± 0.00	6.00 ± 0.00
Ew-/Ca-ML 50% S2	6.00 ± 1.70	5.33 ± 0.57	6.00 ± 1.00	6.600 ± 0.57	6.00 ± 0.00	6.00 ± 0.57
Ew-/Ca-ML 50% S3	6.00 ± 1.70	5.33 ± 1.00	6.00 ± 2.08	6.600 ± 1.00	6.00 ± 0.00	6.00 ± 0.57
Ew-/Ca-ML 50% S4	6.00 ± 1.70	5.33 ± 1.52	6.00 ± 0.57	6.600 ± 1.00	6.00 ± 0.00	6.00 ± 0.57

Data are presented as mean ± SEM. The level of significance was preset at $p < 0.05$ compared to control at the same storage period. Cow- = Co; camel- = Ca; cashew- = Ew; milk = M; labneh = L; starter culture = S; *Lactobacillus plantarum* = 1; *L. casei* = 2; *L. rhamnosus* = 3; and *L. plantarum* + *L. casei* + *L. rhamnosus* = 4. *Control containing *Streptococcus thermophilus* and *L. delbrueckii* subsp. *Lactis*.

as compared to their respective controls. All EwML100% and Ew-/Ca-ML50% samples showed the lowest score in color (satisfactory; Figure 1). CoML100% (S1, S2, S3, and S4) showed lower scores (8; good) for flavor and taste compared to control (9; very good). Taste and overall acceptability scores (3; fairly satisfactory) of CaML100% S1 were similar to control whereas CaML100% S2, S3, and S4 showed unsatisfactory scores for both taste and overall acceptability. The presence of different starter cultures decreased ($p < 0.05$) taste and overall acceptability scores (8; good) in Ew-/Co-ML50% compared to control (9; very good). The texture of labneh improved in all EwML100% samples. In addition, combining cashew milk with camel milk (Ew-/Ca-ML50%) enhanced ($p < 0.05$) the labneh texture compared to CaML100% alone using different starter cultures (Figure 1).

Flavor and texture are the most factors affecting the quality and acceptability of dairy products such as labneh (Delorme et al., 2021). Several parameters affect the Labneh flavor and texture, including the type of starting culture, the incubation temperature, the processing conditions (such as heat treatment and homogeneity), the structural properties of the milk, and the addition of probiotics (Costa et al., 2014; Clark & Mora García, 2017; Cais-Sokolińska et al., 2021). In the present study, CaML100% was failed to produce creamy labneh even after the addition of different types of probiotics. This could refer to the low content of κ -casein and β -lactoglobulin which plays an important role in the gelation process of concentrated yogurt (Al-Zoreky & Al-Otaibi, 2015). In addition, the texture of labneh could be greatly influenced by mixing cashew milk with camel milk.

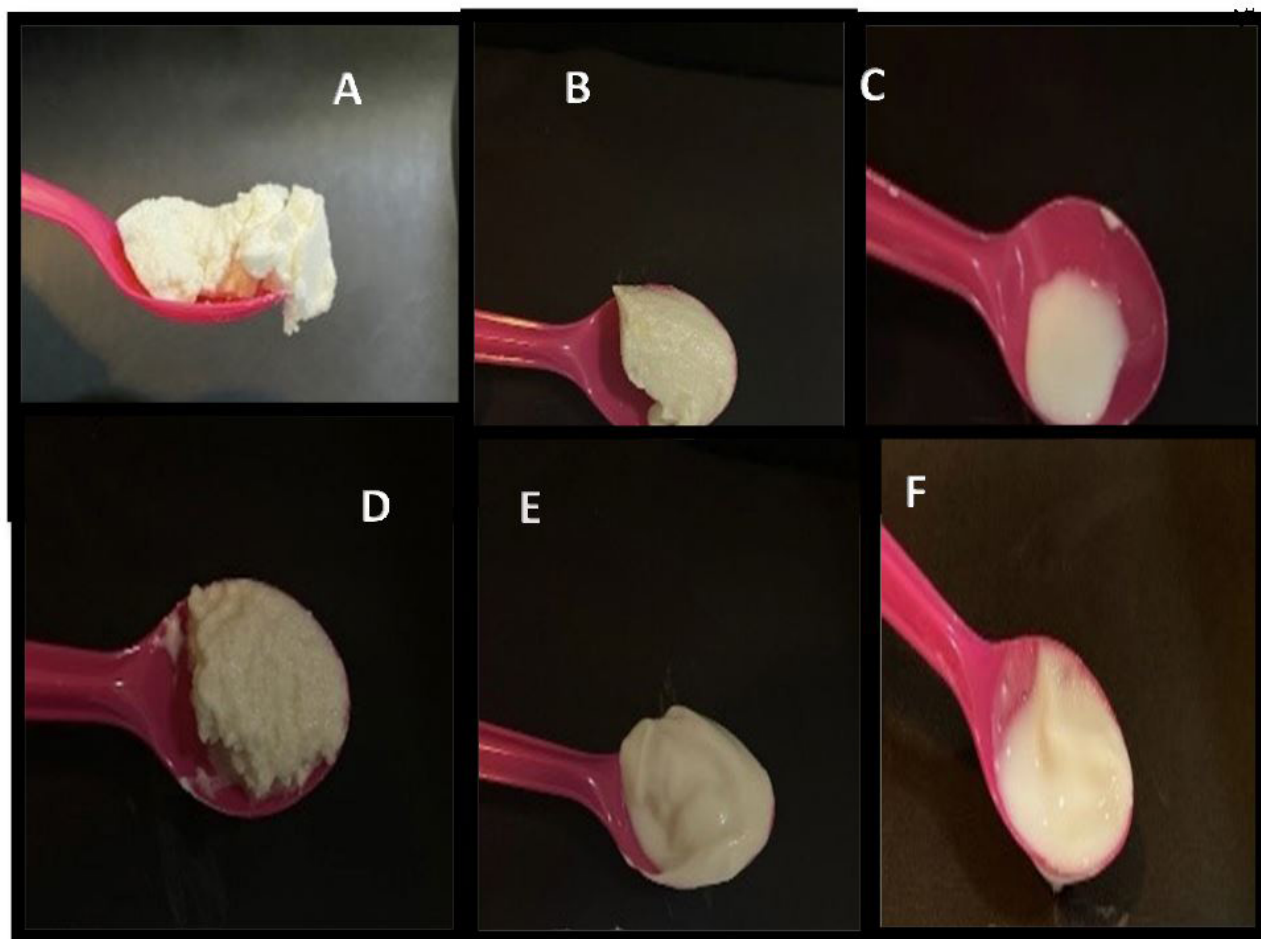


Figure 1. Visual appearance of labneh (control) made from cow-, camel-, and cashew- milk and their mixture (100% and 50%) in the presence of *Streptococcus thermophilus* and *L. delbrueckii* subsp. *Lactis*. (A) CoML100%; (B) EwML100%; (C) CaML100%; (D) Ew/Co-ML50%; (E) Ew/Ca-ML50%; and (F) Co/Ca-ML50%.

4 Conclusion

Influence of three strains of probiotic *Lactobacillus* spp. on post-acidification, the viability of LAB, and sensory properties of labneh made from cow, camel, and cashew milk and their mixture (100% and 50%) were studied. *L. rhamnosus* was the most affected starter culture in post-acidification of all labneh samples made from the three types of milk and their mixture during 21 days of storage. In addition, combining cashew milk with camel milk (50:50%) enhanced significantly TA of labneh during all storage days. All the starter cultures increased ($p < 0.05$) the VCC of *Lactobacillus* spp. and *S. thermophilus* in labneh samples during 21 days of storage compared to their respective controls. Labneh made from cashew milk (100%) using three types of probiotics and their combination was the most accepted by the panels compared to other samples. In addition, combining cashew milk with camel milk enhanced ($p < 0.05$) the labneh texture during storage periods. In conclusion, the combination of cashew milk with cow/camel milk during labneh preparation might enhance the viability of all different starter cultures especially S3 during 21 days of storage.

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