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THE EFFECT OF USING PUMPKIN SEED MILK AS A SUBSTITUTE FOR BUFFALO MILK ON THE PROPERTIES OF LABNEH

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ABSTRACT : This work aimed to study the effect of using pumpkin seed milk on the properties of different types of labneh. Labneh was produced from buffalo milk partially replaced by pumpkin seed milk at different ratios (0, 25, 50, and 75%). Labneh samples were examined for nutritional, physicochemical, microbiological, and antioxidant properties during 21 days of storage at 4°C. Labneh made from a mixture of buffalo milk and pumpkin seed milk showed higher acidity, total solids, fat, ash, protein, total volatile fatty acids, antioxidants, carotenoids, phenols, and total amino acid contents, than that made from buffalo milk only. The acidity values, total solids, protein, fat, ash, volatile fatty acids, and fiber contents increased for all treatments, while the antioxidants, carotenoids, and phenols contents decreased with the progress of storage. Labneh made from a mixture of buffalo milk and pumpkin seed milk also contained the highest number of *Str. Thermophilus* and *L. bulgaricus*. From the beginning of manufacturing until the end of the storage period, coliform bacteria, fungi, and yeasts were not detected in all treatments to which pumpkin seed milk was added. Labneh made from a mixture of buffalo milk and 50% pumpkin seed milk was more acceptable to all examiners. This suggests that pumpkin seeds can be used as an alternative source of milk in the labneh making, with improved nutritional value and shelf life.

Keywords: pumpkin seed, amino acids, Buffalo milk, Labneh.

INTRODUCTION

The possible health benefits of some foods have been acknowledged. Functional foods are food items or ingredients that provide benefits for the consumer's health and well-being beyond their fundamental nutritional worth. According to Jang *et al.* (2024), fermented milk containing probiotic bacteria is a well-known example of this type.

One of the traditional fermented dairy products, labneh, often referred to as strained or concentrated yoghurt is consumed globally, particularly in the Middle East, Turkey, and the

Balkans. It plays an important part in the family diet and has become more well-liked in recent years because of its preservation qualities and alleged nutritional advantages. However, labneh's organoleptic qualities which are greatly influenced by the processing method employed are significantly responsible for customer approval (Zahran *et al.*, 2024).

White or creamy, labneh has a smooth and soft texture, good spreadability, minimal syneresis, a clean flavor, and mild acidity. It is thought to have nutritional and therapeutic qualities that are comparable to or even better than those of ordinary yoghurt. Research

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indicates that compared to traditional yoghurt, labneh has a much higher amount of living microorganisms, 50% more nutrients, and 2.5 times more protein (Rocha *et al.*, 2014; Badawy, 2025).

Cereals and their constituents supply the vitamins, dietary fiber, protein, antioxidants, energy, and minerals necessary for human health, it is important to note that they have been recognized as functional foods (Atwaa *et al.*, 2022; Shahein *et al.*, 2022a). Additionally, probiotic bacteria can develop on fermentable materials found in grains (Atwaa *et al.*, 2020; Borrelli and Ficco, 2025). Part of the Cucurbitaceae family, pumpkins (*Cucurbita pepo* L.) are plump, nutritious vegetables that are grown in many parts of the world, including South-Central America, Mexico, Argentina, Chile, Europe, Asia (China and India), and Western America (Dotto and Chacha, 2020; and Singh and Kumar, 2024). Since the late 1800s, pumpkin seed oil has been utilized to treat urinary tract issues (Csikós *et al.*, 2021). Sterols, proteins, peptides, polysaccharides, para-aminobenzoic acid, carotenoids, and γ -aminobutyric acid are among the biologically active substances found in pumpkin (Grajzer *et al.*, 2024; and Faturoti and Ogidi, 2025).

Pumpkin seeds also include important fatty acids, primarily linoleic, stearic, oleic, and palmitic acids, as well as a high protein level. Additionally, it contains phytosterols including stigmasterol and sitosterol as well as non-essential amino acids. Additionally, tocopherol (vitamin E), fiber, microelements (Na, K, and Cr), and phenolic chemicals (triterpenoids, pyrazine, flavonoids, coumarins, and pigments) are all found in pumpkin seeds (Singh and Kumar, 2022, 2023, and 2024). Crucially, a pumpkin seed extract contains anti-arthritis, anti-tumor, anthelmintic, hepatoprotective, and antioxidant properties in addition to promoting wound healing and increasing hair development. Furthermore, pumpkin seed extracts have been shown to alleviate symptoms related to prostate and bladder issues (Altaf *et al.*, 2025; Bashir *et al.*, 2025).

According to a review of the literature, there is no information regarding the possible impact of including pumpkin seed milk in the production of fermented milk.

The goal of the current work was to assess the physicochemical features, microbiological

assays, phytochemical content, antioxidant activities, and sensory attributes of labneh manufactured with buffalo and pumpkin seed milk mixtures.

MATERIALS AND METHODS

The modified buffalo milk with 3 % fat was brought from the Dairy Processing Unit, Faculty of Agriculture, Zagazig University, Egypt. Lyophilized yogurt starter containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* obtained from Ch. Hansen's Laboratories, Denmark. Mature pumpkin fruits (*C. maxima*) weighing 4 ± 0.5 kg on average were harvested locally and submitted to the University of Zagazig's Department of Botany for identification. All chemicals used in this study were of reagent quality and were acquired from Sigma-Aldrich.

Preparation of pumpkin seed milk (PSM)

The pumpkin seeds were steeped for 20 hours at 4 °C in distilled water at a ratio of 1:3 (w/w). The seed coatings were carefully removed after soaking. After draining the soaking water, a kitchen paper towel was used to wipe away any remaining water from the exposed seeds. Then, the seeds were grounded with water at the ratio of 1:4 (w/v) in a blender (HR2101, Philips, Amsterdam, The Netherlands) for 3 min. Eight layers of cheesecloth were used to filter the slurry. A high-pressure homogeniser (JHG-54-P100, GEA Mechanical Equipment Italia S.P.A., Parma, Italy) was then used to homogenise the filtrate twice at 40 MPa (Yu *et al.*, 2023). Before being used within 24 hours of product formulation, pumpkin seed milk (PSM) was stored at a refrigerator temperature.

Preparation of pumpkin seed milk - Labneh (PSML)

The modified buffalo milk (3% fat) was divided into four equal portions; the first portion served as the control (C), the second portion was mixed with 25.0% PSM (T1), the third portion was mixed with 50.0% PSM (T2) and the fourth portion was mixed with 75.0% PSM (T3). The milk used for all treatments was homogenized at 55 - 60 °C for 2 min using a high-speed mixer (22,000 rpm), heat-treated in a thermostatically controlled water bath at 90 °C for 20 min directly, cooled to 45 °C and then inoculated with (0.1 g/L

of yoghurt mix) 2% of the yoghurt starter culture (*S. thermophiles* + *L. bulgaricus*) and incubated at 42 °C until it was completely coagulated. Thus, yoghurt was cooled to 5 °C and stored overnight, and then mixed with 2% of sodium chloride. The mixes were put into cheesecloth bags, which were hung in the refrigerator room at 5 °C for 18 h, to permit drainage of the whey. The fresh Labneh samples were packed into a small plastic jar and stored for 21 days at 5 ± 1 °C.

Chemical Composition and Physicochemical Analysis

The amounts of total solids, fat, protein, fiber, ash, and titratable acidity in the Labneh samples were measured by other methods (AOAC, 2010). Using a drying oven set to 100 °C for 24 hours, the total solids of the buffalo milk, pumpkin seed milk, and Labneh samples were measured. The Gerber method was utilized for milk and Labneh samples' fat content, whereas the Soxhlet apparatus method was used for pumpkin seed milk. The micro-Kjeldahl method was used to estimate the total nitrogen content (TN), and the percentage of TN was multiplied by 6.38 for milk components and 6.25 for pumpkin seed milk to get the protein level. A 5 g sample was heated to 550 °C for the entire night in a muffle furnace to measure the amount of ash present. The titratable acidity, represented as a percentage of lactic acid (%), was calculated by combining 10 g of Labneh with 10 mL of distilled water, titrating with 0.1 N NaOH, and using phenolphthalein as an indicator to reach a faintly pink end-point. A pH meter with a glass electrode (HANNA, Instrument, Portugal) was used to measure the pH values. The syneresis rate is calculated using the volumetric approach. To maintain the coagulum as intact as feasible, labneh samples were obtained using 40 mL ice cream scoops in one go and poured onto filter papers in a cone that was mounted over a cylindrical graduation. At the 90th minute, the amount of syneresis was determined in milliliters (mL) (Uzuner *et al.*, 2016). By Kosikowski (1977), the total volatile fatty acids (TVFA) were calculated. The total phenolic content (TPC; as mg GAE (gallic acid equivalents)/100 g), total carotenoid contents (TCC as mg/100g), and DPPH Inhibition (%) of the prepared Labneh treatments were assessed according to Maksimovic *et al.* (2005); de

Carvalho *et al.* (2012); and Apostolidis *et al.* (2007), respectively.

Determination of amino acids

According to Bhinder *et al.* (2019), the AAs of the samples were calculated using an HPLC (LC-30 AD Shimadzu) system outfitted with a C18 column (4.6 mm 250 mm, 5 µm) and fluorescence detector. The AAs standard H (Thermo Scientific, NCI0180) mixture was used to validate the procedure, and the concentration of AAs in mg/100 g DWB was determined.

Microbiological examinations

Lactobacillus delbrueckii ssp. *bulgaricus* and *Streptococcus thermophiles* numbers were determined in Labneh samples using the procedures outlined by Tharmaraj and Shah (2003). Standard methods were used to calculate the total number of bacteria, coliform bacteria, yeasts, and moulds (Marshall, 1992).

Sensory evaluation of labneh

The panel members were shown the labneh samples (coded with random numbers) on white plates during the day. They were instructed to rate each sample sequentially using a hedonic scale, where 1 represented the worst (1, very poor; 10 represents very good), covering a list of judged parameter attributes. The following attributes were evaluated: mouth, scent, and taste intensity; consistency by spoon (by gently mixing labneh with a spoon); and external appearance (by looking at labneh samples directly in the daylight). A spoonful of labneh was taken and spread out on the tongue to test for sweetness, mouth consistency, and the intensity of the flavor and smell. The sum of the evaluated parameters' scores was used to determine the overall acceptability. The eight panelists, who had been educated for 15 days, examined the labneh samples on the first, tenth, twentieth, and thirty days of storage in one-hour sessions, three times a week. In between sample evaluations, water was available for mouthwash (Tarakci *et al.*, 2011).

Statistical Analysis

Every experiment was conducted in triplicate, and SPSS (version 22) software (IBM, Armonk, NY, USA) was used to analyze the data. Duncan's tests ($p \leq 0.05$) were used to

compare mean value differences in order to establish the significant threshold.

RESULTS AND DISCUSSION

Chemical Composition of buffalo milk (BFM) and pumpkin seed milk (PSM)

Table 1 presents the approximate chemical composition of the PSM compared with the BFM. The PSM had a higher protein content (8.60% vs. 4.20 %), whereas it had a much higher fat content (7.30%) than BFM (3.0 %). The PSM was characterized by the unique presence of fibers and much higher levels of TPC (mg GAE/100 g), TCC, and DPPH

inhibition % activity than present in the BFM, i.e., 420.80, 5.50 and 78.60 vs. 3.40, 0.00 and 11.85, respectively. These results of approximate chemical composition are in line with those reported previously for PSM (Shahein *et al.*, 2022b; Wadeesirisak *et al.*, 2025) and BFM (Boro *et al.*, 2018; and Garau *et al.*, 2021). The low TPC, TCC and DPPH inhibition % detected in BFM were consistent with Khan *et al.*, (2017) who reported that BFM contains a lower antioxidant capacity. The high TPC, TCC, and DPPH inhibition % of PSM agreed with Shahein *et al.*, (2022b), who reported that PSM contains a higher total phenolic level.

Table 1. Chemical composition of buffalo milk and pumpkin seed milk

Components (%)	Milk	
	Buffalo milk	Pumpkin seed milk
Total Solids	14.80 ± 0.35	22.40 ± 0.26
Protein	4.20 ± 0.22	8.60 ± 0.12
Fat	3.00 ± 0.05	7.30 ± 0.09
Ash	0.76 ± 0.02	0.94 ± 0.06
Fiber	0.00 ± 0.00	5.60 ± 0.14
Total Phenol (mg GAE/100g)	3.40 ± 0.18	420.80 ± 3.60
Carotenoids (mg /100g)	0.00 ± 0.00	5.50 ± 0.04
% DPPH Inhibition	11.85 ± 0.55	78.60 ± 0.42

Chemical composition of labneh containing PSM during storage

Table 2 shows the chemical composition of labneh containing PSM during storage at 4 °C. Control labneh had a lower total solid (TS), protein, fat, ash, and fiber content. This difference was significant ($p \geq 0.05$) compared with the labneh containing PSM treatments. The TS, protein, fat, ash, and fiber content of labneh containing PSM increased gradually by increasing the mixing ratio, which was attributed to the high TS, protein, fat, ash, and fiber content of the prepared PSM compared with the BFM used in this study (Table 1). TS, protein, fat, ash, and fiber content of all treatments increased as the storage period progressed. These results are in agreement with those reported by Shahein *et al.*, (2022b), who found that partial replacement of camel milk with PSM by up to 50%, increased the TS, protein, ash,

and fiber contents of the resultant fermented camel milk compared with camel milk yogurt.

Titrateable acidity, pH, Syneresis, and Total volatile fatty acids of labneh containing PSM during storage

Furthermore, the titrateable acidity (TA) of the control labneh had the lowest value, according to the findings shown in Table 3. The idea that the fermentable PSM material enhances the viability of the starter culture may be the reason for the steady increase in acidity of labneh prepared from BFM combined with PSM when the mixing ratio was raised (Monica *et al.*, 2022; and Polyzos *et al.*, 2024). All of the treatments' pH levels showed the opposite trend from TA's. When compared to the control, labneh with PSM had the lowest pH and the greatest total TA% both at the beginning and after 21 days of storage. The TA% increased significantly in the labneh treatments by increasing the PSM level. The TA% increased and the pH value decreased in all

labneh treatments as the storage period progressed and until the end of the storage period. Similar results were obtained by Uzuner *et al.* (2016); Adebanye *et al.* (2017); Atwaa *et al.* (2020); and Shahein *et al.* (2022b), who found that partial replacement of animal milk with rice, Bambara, oat and pumpkin seed milk increased the TA and decreased the pH values of the resultant dairy products .

Table 3 provided the average syneresis readings for the labneh samples. On day 0, the T3 sample had the highest syneresis rate (33.60 mL), whereas on day 21, the C sample had the lowest syneresis rate (27.20 mL). The analysis of variance revealed that there was a significant difference ($p < 0.05$) between the storage days. The syneresis increased with an increasing percentage of added PSM. The syneresis increased in all labneh treatments as the storage period progressed and until the end of the storage period. Several investigations also documented a decrease in syneresis rates in labneh samples during the course of storage

(Guénard-Lampron *et al.*, 2020). This was linked to proteins' ability to retain water, and it was found that when pH levels dropped, proteins' ability to do so increased. When the pH fell to 4.00 during storage, Uzuner *et al.* (2016) likewise noted a decrease.

The total volatile fatty acid content of labneh containing PSM during storage is displayed in Table (3). Both initially and after 21 days of storage, The TVFA increased with an increasing percentage of added PSM, this may be attributed to the high-fat content of PSM. The addition of PSM to BFM increased the TVFA values of Labneh. The TVFA increased in all labneh treatments as the storage period progressed and until the end of the storage period. These findings are in line with those of Alshawhi *et al.* (2025), who reported that labneh treated with PSO had higher TVFA values than control. Given its high unsaturation, low peroxide value, and abundance of oleic acid and natural antioxidants (Fatureti and Ogidi, 2025).

Table 2. Chemical composition of labneh containing PSM during storage at 4 °C

Parameters	Storage period (Day)	Treatments			
		C	T1	T2	T3
Total solids %	0	25.30±0.54 ^{Db}	27.20±0.50 ^{Cc}	28.84±0.42 ^{Bb}	29.94±0.76 ^{Ab}
	7	25.70±0.42 ^{Db}	27.64±0.62 ^{Cb}	29.12±0.56A ^{Bb}	30.24±0.48 ^{Ab}
	14	26.20±0.65 ^{Da}	28.08±0.74 ^{Ca}	29.70±0.60 ^{Ba}	30.66±0.85 ^{Aa}
	21	26.32±0.48 ^{Da}	28.30±0.72 ^{Ca}	29.84±0.75 ^{Ba}	30.90±0.44 ^{Aa}
Protein %	0	10.52±0.22 ^{Db}	11.40±0.48 ^{Cb}	12.54±0.36 ^{Bb}	13.46±0.55 ^{Ab}
	7	10.60±0.30 ^{Db}	11.52±0.65 ^{Cb}	12.70±0.52 ^{Bb}	13.62±0.45 ^{Ab}
	14	10.94±0.28 ^{Da}	11.80±0.73 ^{Ca}	13.04±0.35 ^{Ba}	13.88±0.22 ^{Aa}
	21	11.06±0.24 ^{Da}	11.94±0.54 ^{Ca}	13.20±0.33 ^{Ba}	14.02±0.36 ^{Aa}
Fat %	0	9.20±0.12 ^{Da}	10.04±0.18 ^{Ca}	11.06±0.16 ^{Ba}	12.14±0.16 ^{Aa}
	7	9.25±0.14 ^{Da}	10.12±0.22 ^{Cb}	11.15±0.14 ^{Ba}	12.20±0.18 ^{Aa}
	14	9.30±0.11 ^{Da}	10.18±0.20 ^{Ca}	11.20±0.18 ^{Ba}	12.28±0.14 ^{Aa}
	21	9.40±0.16 ^{Da}	10.30±0.18 ^{Ca}	11.32±0.15 ^{Ba}	12.40±0.16 ^{Aa}
Ash %	0	1.18±0.03 ^{Dd}	1.22±0.02 ^{Cd}	1.28±0.01 ^{Bd}	1.32±0.03 ^{Ad}
	7	1.30±0.02 ^{Dc}	1.36±0.03 ^{Cc}	1.40±0.03 ^{Bc}	1.44±0.02 ^{Ac}
	17	1.33±0.04 ^{Db}	1.40±0.02 ^{Cb}	1.46±0.03 ^{Bb}	1.58±0.04 ^{Ab}
	21	1.38±0.03 ^{Da}	1.45±0.04 ^{Ca}	1.50±0.01 ^{Ba}	1.64±0.02 ^{Aa}
Fiber	0	0.00±0.0 ^{Da}	1.42±0.05 ^{Cd}	2.30±0.08 ^{Bd}	3.65±0.06 ^{Ad}
	7	0.00±0.0 ^{Da}	1.48±0.06 ^{Cc}	2.36±0.05 ^{Bc}	3.74±0.09 ^{Ac}
	17	0.00±0.0 ^{Da}	1.55±0.05 ^{Cb}	2.42±0.06 ^{Bb}	3.80±0.05 ^{Ab}
	21	0.00±0.0 ^{Da}	1.70±0.04 ^{Ca}	2.54±0.04 ^{Ba}	3.96±0.07 ^{Aa}

* The values are the mean ± SD of three calculations. Significant differences ($p < 0.05$) exist between means with different superscripts in the same row A–D and the same column a–c. Different superscript letters indicate statistically significant differences ($P \leq 0.05$) in the values (means ±SD). C: Control labneh, T1: labneh with 25% PSM., T2: labneh with 50 % PSM T3: labneh with 75 % PSM

Table 3. Titratable acidity, pH, Syneresis, and Total volatile fatty acids of labneh containing PSM during storage at 4 °C

Parameters	Storage period (Day)	Treatments			
		C	T1	T2	T3
Acidity%	0	1.04±0.03 ^{Dd}	1.06±0.05 ^{Cd}	1.08±0.03 ^{Bd}	1.06±0.02 ^{Ad}
	7	1.40±0.02 ^{Dc}	1.46±0.03 ^{Cc}	1.50±0.04 ^{Bc}	1.55±0.02 ^{Ac}
	14	1.82±0.03 ^{Db}	1.87±0.04 ^{Cb}	1.92±0.02 ^{Bb}	1.98±0.03 ^{Ab}
	21	2.15±0.02 ^{Da}	2.20±0.05 ^{Ca}	2.26±0.03 ^{Ba}	2.30±0.04 ^{Aa}
pH values	0	4.72±0.02 ^{Aa}	4.70±0.02 ^{Aa}	4.72±0.03 ^{Aa}	4.70±0.04 ^{Aa}
	7	4.50±0.03 ^{Ab}	4.44±0.03 ^{Ba}	4.40±0.05 ^{Ca}	4.33±0.02 ^{Da}
	14	4.24±0.04 ^{Ac}	4.20±0.04 ^{Bb}	4.14±0.03 ^{Cb}	4.08±0.04 ^{Db}
	21	4.12±0.03 ^{Ad}	4.06±0.02 ^{Bc}	4.03±0.01 ^{Cc}	4.00±0.05 ^{DBc}
Syneresis	0	28.70±0.94 ^{Da}	29.50±0.82 ^{Ca}	30.20±0.77 ^{Ba}	33.60±0.86 ^{Aa}
	7	28.10±0.66 ^{Da}	29.00±0.75 ^{Ca}	29.80±0.84 ^{Ba}	33.10±0.72 ^{Aa}
	14	27.90±0.76 ^{Db}	28.40±0.82 ^{Cb}	29.30±0.77 ^{Bb}	32.80±0.88 ^{Ab}
	21	27.20±0.58 ^{Db}	28.00±0.74 ^{Cb}	28.80±0.92 ^{Bb}	32.30±0.74 ^{Ab}
Total volatile fatty acids (mg 0.1 N NaoH/100g labneh)	0	20.0±0.62 ^{Dd}	21.70±0.58 ^{Cd}	23.50±0.46 ^{Bd}	25.70±0.35 ^{Ad}
	7	24.50±0.55 ^{Dc}	26.20±0.42 ^{Cc}	27.20±0.32 ^{Bc}	29.40±0.30 ^{Ac}
	17	28.00.74 ^{D^B}	29.30±0.50 ^{Cb}	30.80±0.44 ^{Bb}	32.20±0.54 ^{Ab}
	21	33.20±0.62 ^{Da}	34.70±0.48 ^{Ca}	35.50±0.40 ^{Ba}	36.80±0.35 ^{Aa}

* The values are the mean ± SD of three calculations. Significant differences ($p < 0.05$) exist between means with different superscripts in the same row A–D and the same column a–c. Different superscript letters indicate statistically significant differences ($P \leq 0.05$) in the values (means ±SD). C: Control labneh, T1: labneh with 25% PSM., T2: labneh with 50 % PSM T3: labneh with 75 % PSM.

Total phenolic, total flavonoids, total Carotenoids content, and % DPPH inhibition of labneh containing PSM during storage

The TPC, TCC, and % DPPH inhibition of labneh made from BFM mixed with PSM was increased by increasing the mixing ratio compared with the control labneh (Table 4). This might be attributed to the higher TPC. TCC, and % DPPH inhibition of PSM (Wadeesirisak *et al.*, 2025) vs. BFM (Khan *et al.*, 2017). The TPC, TCC, and % DPPH inhibition decreased in all labneh treatments as the storage period progressed and until the end of the storage period. These results are consistent with those reported by Shahein *et al.*, (2022b), who found that adding PSM to camel milk increased the TPC and % DPPH inhibition of fermented milk.

Microbiological examination of labneh containing PSM during storage

As the rate of supplementing labneh with PSM increased, the total count of bacteria,

Streptococci, and Lactobacilli of treatments rose significantly ($P \leq 0.05$) (Table 5). The stimulating impact of PSM, which contains a variety of components that may promote the growth of bacteria, including dietary fibers, fermentable carbohydrates, antioxidant compounds, vitamins, and minerals that may function as prebiotics, may be the cause of this rise (Polyzos *et al.*, 2024).

On the other hand, the total bacterial count and lactobacilli count gradually increased over the course of the storage period, reaching their maximum at 14 days of refrigerated storage. As the storage period went on, however, the counts decreased, possibly as a result of the effects of cold storage and developed acidity. That is consistent with the findings of Zahid *et al.* (2022); and Bakr *et al.* (2021). Streptococci count of labneh treatments rose until the first week of storage, after which they all decreased as the storage period progressed. In the meantime, the total bacterial count and Lactobacilli counts rose until the fourteenth day of the storage period.

Table 4. Total phenolic, total flavonoids, total Carotenoids content, and % DPPH inhibition of labneh containing PSM during storage at 4 °C

Parameters	Storage period (Day)	Treatments			
		C	T1	T2	T3
Total phenolic content (mg /100 g)	0	47.30±1.5 ^{Da}	52.40±1.4 ^{Ca}	53.80±1.6 ^{Ba}	57.20±1.4 ^{Aa}
	7	32.70±1.2 ^{Db}	45.60±1.5 ^{Cb}	48.50±1.4 ^{Bb}	51.40±1.8 ^{Ab}
	14	21.40±1.4 ^{Dc}	36.70±1.7 ^{Cc}	41.30±1.5 ^{Bc}	44.50±1.6 ^{Ac}
	21	18.60±1.3 ^{Dd}	28.40±1.8 ^{Cd}	33.90±1.6 ^{Bd}	39.20±1.4 ^{Ad}
Total Carotenoids (mg /100g)	0	0.00 ±0.0 ^{Da}	1.40±0.12 ^{Ca}	2.60±0.14 ^{Ba}	3.90±0.08 ^{Aa}
	7	0.00±0.0 ^{Da}	1.22±0.05 ^{Cb}	2.20±0.04 ^{Bb}	3.20±0.08 ^{Ab}
	14	0.00±0.0 ^{Da}	1.04±0.05 ^{Cc}	1.94±0.11 ^{Bc}	2.72±0.06 ^{Ac}
	21	0.00±0.0 ^{Da}	0.96±0.04 ^{Cd}	1.40±0.14 ^{Bd}	2.20±0.11 ^{Ad}
% DPPH Inhibition	0	22.40±1.2 ^{Da}	28.50±1.3 ^{Ca}	34.70±1.5 ^{Ba}	39.60±1.3 ^{Aa}
	7	17.80±1.5 ^{Db}	24.70±1.2 ^{Cb}	29.90±1.6 ^{Bb}	33.50±1.5 ^{Ab}
	14	13.50±1.3 ^{Dc}	19.40±1.4 ^{Cc}	22.70±1.5 ^{Bc}	28.30±1.4 ^{Ac}
	21	9.70±1.6 ^{Dd}	13.60±1.3 ^{Cd}	17.50±1.2 ^{Bd}	21.20±1.5 ^{Ad}

* The values are the mean ± SD of three calculations. Significant differences ($p < 0.05$) exist between means with different superscripts in the same row A–D and the same column a–c. Different superscript letters indicate statistically significant differences ($P \leq 0.05$) in the values (means ±SD). C: Control labneh, T1: labneh with 25% PSM., T2: labneh with 50 % PSM T3: labneh with 75 % PSM.

Table 5. Microbial examination (log10 cfu/g) of labneh fortified with PSO during storage at 4 °C

Property	Storage period (Day)	Treatments			
		C	T1	T2	T3
<i>Streptococcus thermophiles</i> (log cfu/g)	0	7.70±0.03 ^{Da}	7.80±0.05 ^{Ca}	7.88±0.07 ^{Ba}	7.95±0.06 ^{Aa}
	7	7.66±0.05 ^{Db}	7.72±0.04 ^{Cb}	7.84±0.07 ^{Bb}	7.88±0.03 ^{Ab}
	14	7.56±0.04 ^{Dc}	7.65±0.03 ^{Cc}	7.78±0.06 ^{Bc}	7.83±0.05 ^{Ac}
	21	7.48±0.04 ^{Dd}	7.58±0.06 ^{Cd}	7.69±0.04 ^{Bd}	7.76±0.04 ^{Ad}
<i>Lactobacillus bulgaricus</i> (log cfu/ g)	0	7.75±0.05 ^{Dc}	7.90±0.02 ^{Cc}	8.12±0.05 ^{Bc}	8.20±0.03 ^{Ac}
	7	8.02±0.04 ^{Db}	8.16±0.06 ^{Cb}	8.25±0.04 ^{Bb}	8.32±0.05 ^{Ab}
	14	8.09±0.03 ^{Da}	8.22±0.05 ^{Ca}	8.31±0.04 ^{Ba}	8.37±0.03 ^{Aa}
	21	7.80±0.06 ^{Dd}	7.90±0.03 ^{Cd}	8.00±0.05 ^{Bd}	8.11±0.07 ^{Ad}
Total bacterial (log cfu/ g)	0	7.85±0.08 ^{Dc}	8.00±0.05 ^{Cc}	8.12±0.03 ^{Bc}	8.25±0.06 ^{Ac}
	7	7.90±0.04 ^{Db}	8.07±0.03 ^{Cb}	8.18±0.05 ^{Bb}	8.32±0.04 ^{Ab}
	14	7.96±0.05 ^{Da}	8.15±0.04 ^{Ca}	8.30±0.03 ^{Ba}	8.36±0.05 ^{Aa}
	21	7.82±0.03 ^{Dd}	7.95±0.05 ^{Cd}	8.05±0.06 ^{Bd}	8.12±0.04 ^{Ad}
Mould &Yeast (log cfu/ g)	0	ND	ND	ND	ND
	7	ND	ND	ND	ND
	14	2.74±0.03 ^{Ab}	ND	ND	ND
	21	2.85±0.02 ^{Aa}	ND	ND	ND

* The values are the mean ± SD of three calculations. Significant differences ($p < 0.05$) exist between means with different superscripts in the same row A–D and the same column a–c. Different superscript letters indicate statistically significant differences ($P \leq 0.05$) in the values (means ±SD). C: Control labneh, T1: labneh with 25% PSM., T2: labneh with 50 % PSM T3: labneh with 75 % PSM.

Table (6) shows the impact of the storage duration and labneh containing PSM on the numbers of mould and yeast. It was observed that mould and yeasts were found in the control labneh after the storage period. This could be

because the production procedure for labneh was conducted under extremely hygienic conditions (Mpopo *et al.*, 2020). The fact that no coliforms were found in any of the laboratory treatments, whether they were fresh or in

storage, is evidence of the high standards of hygiene in laboratory production. In contrast, mould and yeasts were not found in any of the PSM-supplemented treatments; this could be because antifungal compounds are present and prevent the growth of mould and yeasts (Singh and Kumar, 2022).

Sensory properties of labneh containing PSO during storage

The impact of additional PSM on Labneh's sensory rating was explained by the findings in Table 6. Buffalo milk Labneh received the highest color and appearance scores. As more BFM was substituted with MSM, appearance and color values declined. These results shed light on how the type of milk affects Labneh's characteristics. Buffalo milk is the most popular dairy product among other milk varieties because of its bright white color, which the Egyptians find appealing. These findings are consistent with those of Ismail *et al.* (2017).

In terms of flavor, texture, and overall acceptability, the control sample yielded the highest flavor, texture, and overall acceptability values compared to the labneh containing PSM. Flavor, texture, and overall acceptability values were acceptable for the samples containing PSM up to 50% replacement, while the samples containing 75% PSM were not acceptable to the judges compared to the other treatments. This may be due to the appearance of a plant-based flavor and an undesirable texture with a replacement percentage above 50%.

As the storage period progressed, the sensory values for all labneh treatments increased until day 14 of storage, and then all sensory values decreased until the end of the storage period. These results are consistent with many studies that concluded that replacing animal milk with plant milk produces sensory-acceptable dairy products up to a 50% replacement rate (Uzuner *et al.*, 2016; Shahein *et al.*, 2022b; and Abd Elshafy *et al.*, 2025).

Table 6. Sensorial evaluation of experimental labneh samples during storage at 4 °C

Property	Storage period (Day)	Treatments			
		C	T1	T2	T3
Appearance and color	0	8.20±0.2 ^{Ac}	8.20±0.1 ^{Ac}	8.10±0.2 ^{Ac}	7.30±0.3 ^{Bb}
	7	8.50±0.1 ^{Ab}	8.40±0.2 ^{Bb}	8.20±0.1 ^{Cb}	7.50±0.2 ^{Db}
	14	8.70±0.3 ^{Aa}	8.50±0.1 ^{Ba}	8.30±0.4 ^{Ca}	7.80±0.1 ^{Da}
	21	8.20±0.2 ^{Ac}	8.10±0.3 ^{Bc}	8.00±0.2 ^{Cc}	7.20±0.2 ^{Dc}
Body and texture)	0	8.60±0.2 ^{Ac}	8.40±0.3 ^{Bc}	8.30±0.1 ^{Cc}	7.50±0.3 ^{Dc}
	7	8.70±0.1 ^{Ab}	8.50±0.2 ^{Bb}	8.40±0.3 ^{Cb}	7.60±0.2 ^{Db}
	14	8.80±0.3 ^{Aa}	8.70±0.3 ^{Ba}	8.50±0.1 ^{Ca}	7.80±0.3 ^{Da}
	21	8.50±0.3 ^{Ad}	8.20±0.3 ^{Bd}	8.10±0.4 ^{Cd}	7.40±0.1 ^{Dd}
Flavor	0	7.70±0.3 ^{Cc}	7.60±0.2 ^{Bc}	7.50±0.3 ^{Cc}	6.70±0.2 ^{Dc}
	7	7.90±0.2 ^{Ab}	7.80±0.4 ^{Bb}	7.60±0.1 ^{Cb}	6.80±0.3 ^{Db}
	14	8.20±0.1 ^{Aa}	8.00±0.1 ^{Ba}	7.80±0.4 ^{Ca}	7.00±0.2 ^{Da}
	21	7.60±0.4 ^{Ad}	7.40±0.3 ^{Bd}	7.30±0.2 ^{CD}	6.30±0.2 ^{Dd}
overall acceptability	0	8.30±0.1 ^{Ac}	8.10±0.2 ^{Bc}	8.00±0.4 ^{Cc}	7.20±0.3 ^{Dc}
	7	8.50±0.2 ^{Ab}	8.30±0.3 ^{Bb}	8.10±0.2 ^{Cb}	7.40±0.1 ^{Db}
	14	8.70±0.3 ^{Aa}	8.50±0.2 ^{Ba}	8.30±0.3 ^{Ca}	7.50±0.2 ^{Da}
	21	8.30±0.1 ^{Ac}	8.00±0.3 ^{Bd}	7.80±0.4 ^{Cd}	7.00±0.2 ^{Dd}

* The values are the mean ± SD of three calculations. Significant differences ($p < 0.05$) exist between means with different superscripts in the same row A–D and the same column a–c. Different superscript letters indicate statistically significant differences ($P \leq 0.05$) in the values (means ±SD). C: Control labneh, T1: labneh with 25% PSM, T2: labneh with 50% PSM, T3: labneh with 75% PSM.

Amino acids content (AA) of labneh

For protein synthesis, essential amino acids such as histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine, need to be present in the body in the right amounts at once, and can only be supplied from nutrition (Aristoy and Toldrá, 2024). There were 18 different types of amino acids found in the control labneh and the labneh + 50% PSM (Table 7).

In comparison to the control labneh, the labneh + 50% PSM substantially contained more of the essential amino acids histidine, isoleucine, leucine, phenylalanine, threonine, tryptophan, and valine ($p \leq 0.05$). The Lysine and methionine, content of labneh was higher than that of labneh + 50% PSM. Aspartate, serine, glutamate, glycine, alanine, and tyrosine, were among the non-essential amino acids with substantially greater concentrations in the labneh + 50% PSM than in the control labneh ($p \leq$

0.05). In contrast, labneh under control had more cysteine and proline than labneh plus 50% PSM. The labneh + 50% PSM ought to be regarded as highly nutritive labneh with a lot of high-quality protein.

Non-essential amino acid concentrations rose from 8.08 mg/g in control labneh to 11.15 mg/g in labneh + 50% PSM samples. The amount of essential amino acids was also higher in the labneh + 50% PSM samples than in the control labneh, rising from 10.44 mg/g in the control labneh to 15.40 mg/g. The control labneh and labneh + 50% PSM had amino acid compositions that were remarkably close to those of yoghurt and soy milk. This rise in amino acids appears to be connected to the rise in total protein found in yoghurt-like products. These findings support the findings of (Nadtochii *et al.*, 2020; Bakr *et al.*, 2024) who found that adding oat-chia seeds or chickpea flour to yoghurt improved the number of amino acids present in the final product.

Table 7. Effect of using PSM on free amino acids content (%) of fresh Labneh

Amino acids (%)		C	T2
Essential amino acids (AA)	Therionine (Thr)	0.82±0.3 ^b	0.94±0.3 ^a
	Valine (Val)	0.96±0.3 ^b	1.16±0.3 ^a
	Methionine (Met)	0.70±0.3 ^a	0.68±0.3 ^a
	Isoleucine (Ile)	0.88±0.3 ^b	1.02±0.3 ^a
	Leucine (Leu)	1.64±0.3 ^b	1.88±0.3 ^a
	Tryptophan	0.00	0.20±0.3 ^a
	Phenylalanine (Phe)	0.98±0.3 ^b	2.16±0.3 ^a
	Lysine (Lys)	1.52±0.3 ^a	1.34±0.3 ^b
	Histidine (His)	0.58±0.3 ^b	1.72±0.3 ^a
Total essential amino acids		8.08±0.2 ^b	11.15±0.1 ^a
Non-essential amino acids	Aspartic (Asp)	1.34±0.3 ^b	2.16±0.3 ^a
	Serine (Ser)	0.90±0.3 ^b	1.38±0.3 ^a
	Glutamic (Glu)	3.44±0.3 ^b	4.52±0.3 ^a
	Proline (Pro)	2.04±0.3 ^a	0.98±0.3 ^b
	Glycine (Gly)	0.40±0.3 ^b	1.22±0.3 ^a
	Alanine (Ala)	0.58±0.3 ^b	1.16±0.3 ^a
	Cystine (Cys)	0.32±0.3 ^{cd}	0.14±0.3 ^b
	Arginine	0.64±0.3 ^b	2.90±0.3 ^a
	Tyrosine (Tyr)	0.82±0.3 ^a	0.94±0.3 ^a
Total non-essential amino acids		10.44±0.3 ^b	15.40±0.5 ^a
Total amino acid		18.52±0.6 ^b	26.55±0.4 ^a

C: Control labneh, T2: labneh with 50 % PSM

Conclusion

It was found that Labneh was successfully made from 50 % buffalo milk and 50 % PSM mixture. Utilization of PSM increased values of total solids (TS), fat, ash, TN, TVFA, antioxidants, carotenoids, phenolic, and total amino acids contents. The results of sensory attributes evaluation showed that Labneh made from buffalo and PSM mixture was acceptable, especially at the beginning of the storage period. Nonetheless, additional research is necessary to address constraints like those pertaining to the interactions with dairy matrices, and widespread consumer acceptance in the context of dairy products. Future research ought to examine economic viability and long-term stability as well.

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تأثير استخدام لبن بذور اليقطين كبديل للبن الجاموسي على خصائص اللبنة

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يهدف هذا البحث الي دراسة تأثير استخدام لبن بذور اليقطين على خصائص اللبنة المختلفة. حيث تم إنتاج اللبنة من اللبن الجاموسي المستبدل جزئيًا بلبن بذور اليقطين بنسب مختلفة (0 و 25 و 50 و 75%). وتم فحص عينات اللبنة المصنعة من حيث الخصائص الفيزيوكيميائية والميكروبيولوجية والمضادة للأكسدة خلال التخزين لمدة 21 يوم علي درجة حرارة 4 درجات مئوية .

أظهرت اللبنة المصنعة من خليط اللبن الجاموسي و لبن بذور اليقطين أعلى نسبة حموضة وإجمالي المواد الصلبة والدهون والرماد والبروتين وإجمالي الأحماض الدهنية المتطايرة ومضادات الأكسدة والكاروتينات والفينولات ومحتوى الأحماض الأمينية الكلية بينما أظهرت اللبنة المصنعة من اللبن الجاموسي فقط أقل نسبة .

وزادت قيم الحموضة ومحتويات إجمالي المواد الصلبة والبروتين والدهون والرماد و الأحماض الدهنية المتطايرة و الألياف لجميع المعاملات، بينما انخفضت محتويات مضادات الأكسدة والكاروتينات والفينولات مع تقدم فترة التخزين.

كما احتوت اللبنة المصنعة من خليط اللبن الجاموسي ولبن بذور اليقطين على أكبر عدد من بكتيريا *Str. L. bulgaricus* و *thermophilus*.

في بداية التصنيع وحتى نهاية فترة التخزين، كذلك لم يكتشف وجود بكتيريا القولون والفطريات والخمائر في جميع المعاملات المضاف إليها لبن بذور اليقطين. كما أعطت اللبنة المصنعة من خليط اللبن الجاموسي مع لبن بذور اليقطين بنسبة 50% خصائص حسية مقبولة لدي جميع المحكمين . يستنتج من ذلك إمكانية استخدام بذور اليقطين كمصدر بديل اللبن في صناعة اللبنة، مع تحسين القيمة الغذائية ومدة الصلاحية.