



ISSN 2357-0725

<https://jsasj.journals.ekb.eg>

JSAS 2025; 10(1): 53-61

Received: 07-11-2024

Accepted: 11-03-2025

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Almond Milk as a Partial Replacement in the Manufacture of Yogurt

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Abstract

Almond milk is a recent invention used as an alternative to animal milk and milk products due to its nutritional value and health benefits. In this study, five formulations were prepared to manufacture yogurt of buffalo and almond milk at different rations (90:10), (80:20), (70:30), (60:40), and (50:50), respectively. Chemical composition, microbiological analysis, bioactive components (total phenols and antioxidant activity), texture profile analysis, microstructure, and sensory evaluation were determined. The obtained results indicated that the incorporation of almond milk into yogurt blends resulted in a significant increase of bioactive components in yogurt samples ($p < 0.05$). Results proved that almond milk has an anti-*Escherichia coli* effect. Sensory characteristics showed that all formulations used in fermented products were acceptable; the addition of 10% and 20% of almond milk to fermented products were most appreciated by the panelists. Results from the present study proved that it is possible to use almond milk as a vegetarian alternative to manufacture yogurt with a good acceptability and functional properties.

Key words:

Almond milk - Fermented Products - Bioactive Components.

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INTRODUCTION

Almond is a product of the well-known medicinal plant, which has been used for thousands of years to treat and prevent various illnesses. Asian nations are the original home of almond trees. Almond is a good food that improves health, as it has possessed high percentage of antioxidants, flavonoids, fatty acids, vitamins, proteins (25%), and necessary minerals are all present and have a remarkable content of tocopherol (vitamin E). Almonds' ability to decrease cholesterol is a result of their phytosterol content. Traditionally, almond has been used to treat a variety of conditions, including uterine pain, pityriasis, hysteria, infections of the brain, anemia, sleeplessness, headaches, sore throats, and renal problems. Almond milk is highly regarded for the nutrients it contains, including calcium, magnesium, copper, and vitamin E. People looking for a dairy-free or vegan choice may find it especially appealing. Health-conscious consumers are also drawn to unsweetened almond milk due to its lower calorie profile (Kim *et al.*, 2022). Over the past few decades, the food sector has grown in response to the rapidly changing requirements of customers. The health benefits of plant-based milk substitutes are valued by consumers of all ages, and as a result, consumption has recently increased by 60% to 70% (Aydar *et al.*, 2020). On the other hand, a lot of attention is being paid to plant-based milk products because of their possible health benefits. These useful plant-based milk substitutes may help ensure that the expanding population has easy access to a healthy, balanced diet in the future (Proserpio *et al.*, 2020). Because of changes in lifestyles and lactose intolerance and milk allergies, there has been an upsurge in demand for plant-based milk substitutes or plant basic milk alternatives in recent years (Reyes *et al.*, 2023). Plant basic milks are often positioned as an environmentally friendly alternative to dairy milk that can help lower consumers' environmental impacts. For instance, plant basic milks under the Silk brand claim that their drinks are less carbon-intensive than traditional dairy milk in the US (Berardy *et al.*, 2020). Life cycle assessment, a quantitative technique that calculates the embodied

environmental consequences of products based on the inputs and outputs throughout that product's life cycle, has been used to examine the sustainability of plant basic milks in comparison to dairy milk. Food life cycle assessments regularly demonstrate that animal-based diets have more environmental impact than plant-based ones (Kustar and Patino, 2021). Food fermentation is the process of employing bacteria in anaerobic environments to break down complex carbohydrates like starch and sugars into simpler molecules like alcohols, acids, and CO₂ (Paul *et al.*, 2020 and Shori, 2021). Most of the plant-based milk and extracts undergo spontaneous or controlled fermentation, which increases the production of bioactive compounds. Additionally, the increase in necessary amino acids during fermentation improves the digestion of the beneficial proteins in soybean by more than 40% (Ketnawa and Ogawa, 2019). Yogurt is a fermented dairy product that is widely popular in the dairy industry and offers medicinal and nutritional benefits. Yogurt is an essential source of high-quality proteins and minerals such as calcium. Yogurt has a high rate of digestion and nutrient bioavailability, which means it can help meet daily requirements for a variety of nutrients. Yogurt that contains probiotics is becoming more and more popular to consumers, widely utilized in the dairy business as a high-nutrient food. Consuming probiotic foods, such dairy products, may even be one of the promising therapeutic options for the prevention or treatment of novel pathogenic viruses (Fonseca *et al.*, 2020). Therefore, the current study aimed to evaluate the chemical composition, bioactive components, microbiological, microstructure, rheological and organoleptic properties of yogurt containing almond milk at different ratio.

MATERIALS AND METHODS

1. Materials

Fresh raw buffalo's milk was obtained from the herd of animal production, faculty of agriculture, Sohag University, Egypt. Almond seeds were purchased from the local market, Sohag governorate. Lg03 YF starter, which had *Streptococcus thermophiles* and *Lactobacillus*

bulgaricus, is used to manufacture buffalo yogurt and formulated yogurt, It was obtained from Hansen Corporation, Denmark, and stored at -20°C for future use.

2. Methods

2.1. Preparation of Almond milk and Formulations

Almond seeds were soaked in distilled water (5°C) overnight until they reached the

milky stage phase after removing the damaged grains, then left at room temperature for 15 min to get rid of excess water, mixed with distilled water in a ratio of 1:2 (w/w) using a blender, and filtered by using cheese cloth to get almond milk. Five formulations were made by replacing buffalo with almond milk in the following proportions (Table 1) (90:10), (80:20), (70:30), (60:40), and (50:50), respectively.

Table (1). Chemical composition and antioxidant activity of raw buffalo and almond milk.

	T.S%	Moisture%	Fat%	Protein%	Ash%	Acidity%	pH	Sp .Gr	T.ph.	Anti.act.
Buffalo's milk	19.79	80.21	8.5	4.7	0.85	0.17	6.69	1.038	39.31	222.86
Almond milk	13.89	86.11	7.5	0.44	0.46	0.12	6.90	1.013	233.4	708.40

Note: Values represent the mean, n=3 T.ph. = Total phenols (mg gallic acid/g) Anti.act.=antioxidant activity (ug trolox/g) Sp.Gr= Specific gravity

2.2. Yogurt manufacture

Formulations of yogurt were prepared in triplicated batches and heated at 75 °C for 15 min, swiftly cooled to 42 °C, inoculated with activated yogurt starter (Lg03) 1.5% (w/w), and incubated at 42 °C until full coagulation (nearly 3 h), then kept at 5 °C for 14 days (Yilmaz and Topcuoglu, 2022).

2.3. Chemical Analysis

Total solids, ash, protein, fat, titratable acidity and specific weight were determined according to AOAC, (2000). The pH value was measured using Orion mode 1410A, Boston, MA, and moisture was estimated mathematically.

2.4. Bioactive Components.

Total phenolic content was determined using the folin_ciocaltue method by El_Dein *et al.* (2022). The total phenolic content was expressed as mg of gallic acid equivalent per gram of extract. The antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay by Abu-El Khair *et al.* (2023) and Tavakoli *et al.* (2018).

2.5. Microbiological Analysis

Nutrient agar used for total counts, Macconkey agar base for counting coliform bacteria (*Escherichia coli*), PDA agar for molds and yeasts, M17 agar for counting *Streptococcus*

thermophiles, and MRS agar for *Lactobacillus bulgaricus*. *Streptococcus thermophiles* and coliform bacteria incubated aerobically at 37°C for 48 h by anaerobic incubation. *Lactobacillus bulgaricus* was enumerated by anaerobic incubation at 37°C for 48 hr. Molds and yeasts were incubated aerobically at 28 °C for 72 h (Topcuoglu *et al.*, 2020).

2.6. Texture profile analysis (TPA)

The textural properties of control (buffalo milk yogurt) and treatment (80% buffalo milk:20% almond milk) yogurt were evaluated using the textural analyzer (Mult-test *Id* Memesin, Food Technology Corporation, Slinfold, West Sussex, UK.) equipped with a 25 mm diameter perplex conical-shaped probe. The texture profile analysis (TPA) was done on yogurt samples as described by Abu-El Khair *et al.* (2023). The textural parameters were calculated from the force-time curve according to the definition given by IDF, (1991).

2.7. Microstructure

Scanning electron microscopy (SEM) was used to determine the microstructure of control (buffalo milk yogurt) and treatment (80% buffalo milk: 20% almond milk) yogurt, as described by Khalifa and Ibrahim, (2015) at the National Research Center, Giza, Egypt.

2.8. Sensory Evaluation

Sensory evaluation of all products was

run on the first day of storage. Samples were assessed by 15 panelists from the staff members of food and dairy science department, faculty of Agriculture, Sohag University. For the following parameters flavor (50), body and texture (30), appearance and color (20) and overall acceptability (100) as mentioned by Aktar, (2022).

2.9. Statistical analysis

Analysis of Variance (ANOVA) was performed using SAS 9.4 software. The means were compared using Duncan's multiple range test at $p < 0.05$ (Duncan, 1955).

RESULTS AND DISCUSSION

1. Chemical composition:

1.1. Chemical composition of buffalo and formulated yogurt:

Chemical composition buffalo and formulated yogurt were illustrated in table (2). The total solids content has decreased significantly ($p < 0.05$) by increasing the percentage of almond milk in formulated yogurt, as well as, total protein and ash decreased. Fresh control has the highest value of total solids (21.02%) compared with other formulated yogurt and during storage. PH values increased insignificantly by increasing the percentage of almond milk in different formulations, decreased during storage. On the other hand, acidity decreased by increasing the percentage of almond milk in different formulations, increased during storage. The moisture content of all samples gradually decreased in the storage, in the opposite, total protein and ash increased. This decrease may be due to the development of acidity as the storage period progresses. Above results had agreement with Yilmaz and Topcuoğlu, (2019).

Table (2). Chemical composition of buffalo and formulated yogurt during storage period.

Storage period	Measurements	T.S%	Moisture%	Fat%	Protein%	Ash%	Acidity%	pH
Fresh	Control	21.02 ^a	78.98 ^c	9.23 ^a	4.99 ^a	0.92 ^a	1.5 ^a	4.6 ^b
	T ₁	20.98 ^a	79.02 ^c	9.11 ^a	4.64 ^{ba}	0.89 ^{ba}	1.4 ^{ba}	4.7 ^b
	T ₂	20.40 ^a	79.60 ^c	9.02 ^a	4.19 ^{bc}	0.85 ^{ba}	1.3 ^{ba}	4.8 ^{ba}
	T ₃	19.79 ^{ba}	80.21 ^{bc}	8.94 ^a	3.74 ^{dc}	0.81 ^{bc}	1.2 ^{ba}	4.9 ^{ba}
	T ₄	19.08 ^{bc}	80.92 ^{ba}	8.82 ^a	3.27 ^{de}	0.76 ^c	1.1 ^b	5.0 ^{ba}
	T ₅	18.44 ^c	81.56 ^a	8.72 ^a	2.82 ^e	0.72 ^c	1.0 ^b	5.1 ^a
7 days	Control	21.32 ^a	78.68 ^d	9.36 ^a	5.06 ^a	0.93 ^a	1.9 ^a	4.3 ^a
	T ₁	21.28 ^a	78.72 ^d	9.24 ^a	4.71 ^a	0.90 ^b	1.8 ^{ba}	4.4 ^a
	T ₂	20.7 ^{ba}	79.3 ^{dc}	9.15 ^a	4.25 ^b	0.86 ^c	1.7 ^{ba}	4.5 ^a
	T ₃	20.09 ^{bc}	79.91 ^{bc}	9.08 ^a	3.8 ^c	0.82 ^d	1.6 ^{ba}	4.6 ^a
	T ₄	19.39 ^{dc}	80.61 ^{ba}	8.96 ^a	3.32 ^d	0.77 ^e	1.5 ^b	4.7 ^a
	T ₅	18.74 ^d	81.26 ^a	8.86 ^a	2.87 ^e	0.73 ^f	1.4 ^b	4.8 ^a
14days	Control	21.74 ^a	78.26 ^c	9.54 ^a	5.27 ^a	0.95 ^a	2.2 ^a	4.0 ^{bc}
	T ₁	21.69 ^a	78.31 ^c	9.41 ^a	4.80 ^{ba}	0.92 ^{ba}	2.1 ^{ba}	4.1 ^a
	T ₂	21.1 ^a	78.90 ^c	9.25 ^b	4.33 ^{bc}	0.88 ^{bac}	2.0 ^{ba}	4.2 ^{bac}
	T ₃	20.49 ^{ba}	79.51 ^{bc}	9.18 ^a	3.88 ^{dc}	0.84 ^{bdc}	1.9 ^{ba}	4.3 ^{bac}
	T ₄	19.79 ^{bc}	80.21 ^{ba}	9.14 ^a	3.39 ^{de}	0.79 ^{dc}	1.8 ^b	4.4 ^{ba}
	T ₅	19.14 ^c	80.86 ^a	9.05 ^a	2.9 ^c	0.75 ^d	1.7 ^b	4.5 ^a

B= buffalo's milk

A = almond milk

Control =fresh buffalo's milk

T1=90% B milk: 10% A

T2=80% B milk: 20% A

T3=70% B milk: 30% A

T4=60% B milk: 40% A

T5=50% B milk: 50% A

1.2. Bioactive Components

As shown in Table (3) there was a significant ($p < 0.05$) increase in phenolic content and antioxidant activity by increasing the added percentage of almond milk in formulations of

yogurt these, results in agreement with (Aydar *et al.*, 2020 and Vashisht *et al.*; 2024) . Control yogurt had the lowest value of phenolic content and antioxidant activity (37.76 mg Gallic acid/g and 209.6 ug trolox/g, respectively). T₅ had the

highest value of phenolic content and antioxidant activity (110.11 mg Gallic acid/g and 697.1 ug trolox/g, respectively). Topcuoglu and Yilmaz-Ersan, (2020) reviewed that raising the ratio of the almond milk from 0% to 100% resulted in a significant difference of total phenolic content and antioxidant activity, as the highest total phenolic content was found in (raw

milk50: almond milk50) sample, on the contrary, the lowest was in raw milk yogurt (control). Yilmaz-Ersan *et al.* (2018) revealed that antioxidant capacity may be attributed to the fortification by almond milk, due to its content particularly vitamin E, flavonols, flavanols, flavanones, anthocyanins, procyanidins, and phenolic acids

Table (3). Phenolic content and total antioxidant activity of fresh yogurt at different percentage of replacement.

Measurements	Control	T ₁	T ₂	T ₃	T ₄	T ₅
T.ph.	37.76 ^e	68.87 ^d	69.27 ^d	75.51 ^c	99.89 ^b	110.11 ^a
Anti.act.	209.6 ^f	427.6 ^e	515.6 ^d	573.98 ^c	682.9 ^b	697.1 ^a

Note: Values represent the mean, n=3

T.ph.= Total phenols (mg gallic acid/g)

B= buffalo's milk

Control =fresh buffalo's milk

T₂=80% B milk:20% A

T₄=60% B milk:40% A

Anti.act.=antioxidant activity (ug trolox/g)

A= almond milk

T₁=90% B milk:10% A

T₃=70% B milk:30% A

T₅=50% B milk:50% A

2. Microbiological properties of buffalo and formulated yogurt

To investigate the effect of almond milk on the microbiological properties, the formulations were storage for 14 days for total bacterial count, *Streptococcus thermophiles*, *Lactobacillus bulgaricus*, *Escherichia coli*, Molds & yeasts (table 4). The results observed that increases almond percentage in formulations lead up to decrease in total bacterial counts. Control yogurt had the highest counts of total bacteria (10.93) followed by T₁(10.90), the lowest counts of bacteria were in T₅(10.85). Total bacterial count in control yogurt significantly increased during storage, although there was a slight and insignificant increase during storage in all formulated yogurts after 14 days. Different rations of almond milk affected on *Streptococcus thermophiles* count where it varies according the percent of almond milk added. A significantly decreased (p>0.05) with almond milk increased in *Streptococcus thermophiles* count. Control yogurt had the highest counts (10.96) in comparison with yogurts incorporated almond milk. A significantly decreased (p>0.05) between control

and formulated yogurts after 7 and 14days of storage, despite there was insignificantly decreased (p>0.05) between control, T₁, T₂ and T₃ in fresh yogurt, which in agreement with Coşkun and Erol ,(2023) .During storage there was an increasing in *Streptococcus thermophiles* counts in control samples, on opposite, a slight decrease was noticed in formulated yogurts, but with almond milk increasing the decreasing increased, as in T₅ *Streptococcus thermophiles* in fresh was 10.86 in compared with 14 days stored were 10.72 a similar result indicated according to Topcuoglu and Yilmaz-Ersan, (2020). *Lactobacillus bulgaricus* counts decreased significantly(p>0.05) with almond increasing and during storage, despite for control yogurt as the number of *Lactobacillus bulgaricus* counts increased during storage. A similar result indicated by Mazzaglia *et al.* (2020). Molds and Yeast appeared only after 14 days in control yogurt (4.01), T₁ (3.63) correspondingly. The results demonstrated the anti-*Escherichia coli* effect of almond milk, since the microorganisms were exclusively detected in control (1.83cfu) after 14 days of storage.

Table (4) Microbiological properties of buffalo and formulated yogurt during storage period.

Storage Period	Measurements	T.C (log ml ⁻⁹)	<i>Streptococcus thermophiles</i> (log ml ⁻⁹)	<i>Lactobacillus bulgaricus</i> (log ml ⁻⁸)	Molds& Yeast (log ml ⁻¹)	E.C (log ml ⁻¹)
Fresh	Control	10.93 ^a	10.96 ^a	9.92 ^a	N.D	N.D
	T ₁	10.9 ^{ba}	10.94 ^{ba}	9.9 ^{ba}	N.D	N.D
	T ₂	10.89 ^{ba}	10.91 ^{bac}	9.88 ^{bac}	N.D	N.D
	T ₃	10.87 ^b	10.9 ^{bac}	9.87 ^{bdc}	N.D	N.D
	T ₄	10.86 ^b	10.88 ^{bc}	9.85 ^{dc}	N.D	N.D
	T ₅	10.85 ^b	10.86 ^c	9.83 ^d	N.D	N.D
7days	Control	11.03 ^a	11.04 ^a	9.96 ^a	N.D	N.D
	T ₁	10.92 ^b	10.93 ^b	9.88 ^b	N.D	N.D
	T ₂	10.9 ^b	10.9 ^{cb}	9.86 ^b	N.D	N.D
	T ₃	10.89 ^b	10.88 ^{cb}	9.85 ^{cb}	N.D	N.D
	T ₄	10.88 ^b	10.86 ^{cd}	9.82 ^{cb}	N.D	N.D
	T ₅	10.87 ^b	10.81 ^d	9.78 ^c	N.D	N.D
14days	Control	11.05 ^a	11.05 ^a	9.98 ^a	4.01	1.83
	T ₁	10.94 ^b	10.91 ^b	9.85 ^b	3.63	N.D
	T ₂	10.92 ^{cb}	10.86 ^{cb}	9.81 ^b	N.D	N.D
	T ₃	10.9 ^{cb}	10.84 ^c	9.79 ^{cb}	N.D	N.D
	T ₄	10.89 ^c	10.79 ^c	9.74 ^c	N.D	N.D
	T ₅	10.88 ^c	10.72 ^d	10.68 ^d	N.D	N.D

B= buffalo's milk

A= almond milk

Control =fresh buffalo's milk

T₁=90% B milk:10% AT₂=80% B milk:20% AT₃=70% B milk:30% AT₄=60% B milk:40% AT₅=50% B milk:50% A

3. Texture profile properties:

There were significant differences between control (buffalo's milk yogurt) and treatment (80 buffalo's milk: 20 almond milk) milk yogurt in all parameters ($p < 0.05$) as shown in Fig 1. In Generally, buffalo's milk yogurt was higher in all textural properties except for cohesiveness, (80 buffalo's milk:20 almond milk) milk yogurt was higher (0.22). Control's hardness (buffalo milk yogurt) was higher due to its high total solid's percentage, which was 19.79% Table (1). (Yilmaz and Topcuoglu, 2022).

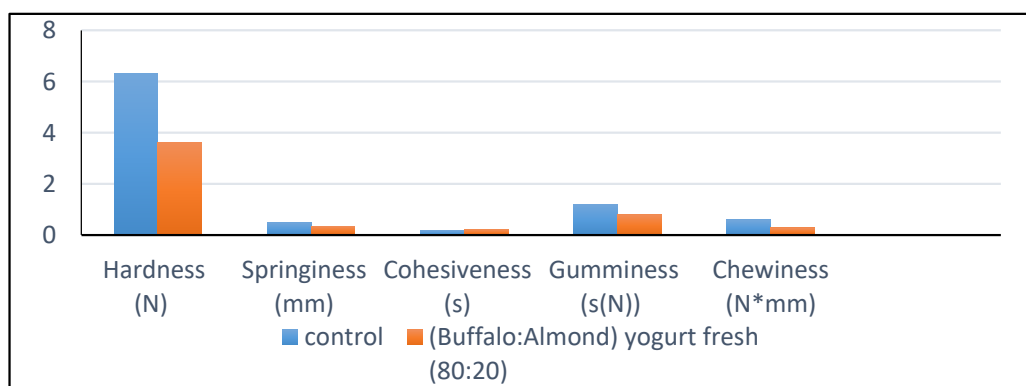


Figure 1. Texture profile characteristics of control (buffalo's milk yogurt) and formulated yogurt (80:20).

4. Microstructure properties:

Microstructure of control (fresh buffalo's milk) and T₂ are shown in Figure 2.

Microstructure analysis illustrated that the internal structure of T₂ was more cohesive than the control surface. Despite cohesive surface in

T₂, surface had more smoothly and systematically distributed casein, also less

porosity in casein network which has agreement with Xu *et al.* (2024).

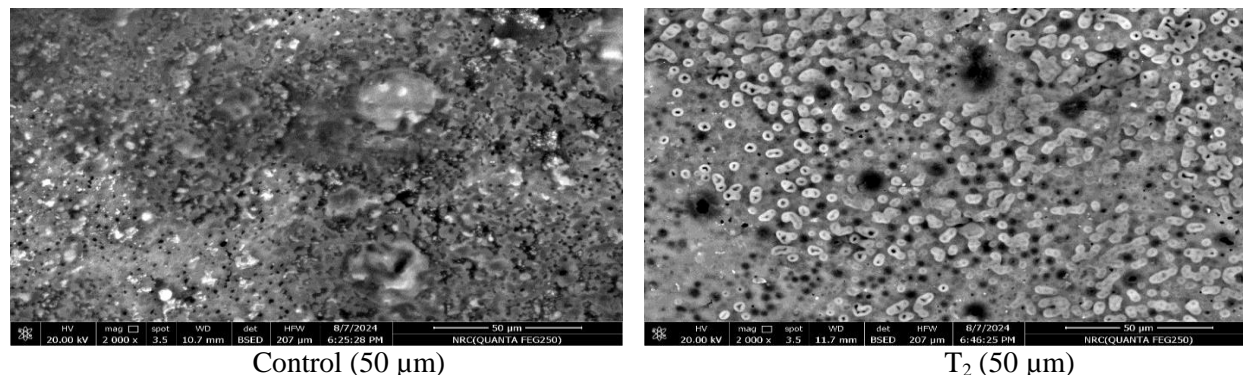


Figure 2. Microstructure of control (buffalo's milk yogurt) and formulated yogurt (80:20).

5. Sensory evaluation:

Sensory properties given by panelists are presented in table (5). Generally, there were statistically significant differences among samples ($p > 0.05$). The panelists' evaluations for all of the sensorial parameters were tended to be accepted. Control yogurt (91%) had the highest overall acceptability in comparison with formulated yogurts, all of the sensorial parameters decreased with almond milk

increased (Ilyasoglu and Yilmaz, 2019). During storage, flavor of formulated yogurt improved significantly ($p > 0.05$), on the contrary control yogurt. Overall acceptability optimized in T₃, T₄ and T₅ at the end of storage in comparison with fresh samples. As a conclusion, storage affected positively on flavor with increasing almond milk percentage in formulated yogurt, similar results observed by (Yilmaz and Topcuoglu, 2022).

Table (5). Sensory evaluation of buffalo and formulated yogurt during storage period

Storage Period	Measurements	Flavor (50)	Body and Texture (30)	Appearance & color (20)	Total (100)
Fresh	Control	45.7 ^a	26.7 ^a	19 ^a	91.4 ^a
	T ₁	42 ^b	26 ^a	18 ^a	86 ^b
	T ₂	42 ^b	26 ^a	18 ^a	86 ^c
	T ₃	40 ^b	22.7 ^b	17 ^{ba}	79.7 ^d
	T ₄	35 ^c	20 ^c	15 ^{bc}	70 ^e
	T ₅	33 ^c	18 ^d	13.7 ^c	64.7 ^f
7days	Control	44.2 ^a	27.8 ^a	19 ^a	91 ^a
	T ₁	42.3 ^a	24.4 ^{bc}	16.7 ^b	83.2 ^d
	T ₂	43 ^a	25.4 ^{bac}	16.5 ^b	84.9 ^c
	T ₃	43.9 ^a	26.3 ^{ba}	17 ^{ba}	87.2 ^b
	T ₄	41.4 ^a	23.8 ^{bc}	15 ^b	80.2 ^f
	T ₅	42.1 ^a	27.7 ^a	15.6 ^b	85.4 ^e
14days	Control	44 ^{bac}	26.7 ^a	18.4 ^a	89.1 ^a
	T ₁	42 ^{bc}	24.4 ^b	16.4 ^b	82.8 ^e
	T ₂	44.3 ^{ba}	25.1 ^{ba}	14.5 ^b	83.9 ^c
	T ₃	44.6 ^a	26.5 ^a	16.4 ^b	87.5 ^b
	T ₄	42.3 ^{bc}	24.5 ^b	16.7 ^{ba}	83.5 ^d
	T ₅	42.3 ^{bc}	23.3 ^b	15.7 ^b	81.3 ^f

B= buffalo's milk A= almond mil Control =fresh buffalo's milk T1=90% B milk: 10% A T2=80% B milk: 20% A T3=70% B milk: 30% A T4=60% B milk: 40% A T5=50% B milk: 50% A

CONCLUSION

The present study investigated the use of almond milk at different ratio in manufacture of yogurt was acceptable by panelists, as the content of almond milk increased; storage had a favorable overall impact. The result observed that significant increase in total phenolics and Total antioxidant activity by increasing the percentage of almond milk in formulations for yogurt. Not detecting of *Escherichia coli*, molds and yeast during storage has positive effect on shelf life and human health. It's highly recommended using almond milk in fortification of yogurt, and as a replacement for buffalo's milk.

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