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ORIGINAL ARTICLE

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Effect of Lycopene Supplementation on the Physicochemical and Microbiological Properties of Fresh Goat Cheese

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ABSTRACT

Background: Lycopene is a bioactive carotenoid pigment of considerable nutritional and functional significance, predominantly derived from red-pigmented fruits and vegetables, most notably tomatoes and their processed derivatives. Its prospective application in food fortification and pharmaceutical formulations has attracted growing scientific interest, attributable to its well-documented antioxidant, anti-inflammatory, and health-promoting properties.

Aims: This study aimed to extract and purify lycopene from tomato concentrate and to systematically investigate the effects of its incorporation at varying concentrations on the physicochemical characteristics, and microbiological quality of fresh goat fresh cheese during refrigerated storage.

Material and Methods: Lycopene was extracted from tomato concentrate pulp via solvent extraction and subsequently purified through recrystallisation. The purity and identity of the recovered lycopene were confirmed by thin-layer chromatography (TLC). The purified lycopene was incorporated into fresh goat cheese at three mass ratios—0.01%, 0.05%, and 0.2%—with an unenriched sample (0%) serving as the negative control. All experimental specimens were stored at 4°C and subjected to systematic analysis over a four-week storage period. Physicochemical parameters—including pH, titratable acidity, dry matter content, moisture content, and syneresis— were evaluated through standard validated analytical methods. Microbiological analyses were conducted to enumerate total aerobic mesophilic flora, fecal coliforms, *Staphylococcus aureus*, *Salmonella-Shigella*, lactobacilli, lactococci, yeasts, and molds.

Results: The maximum lycopene extraction yield attained from tomato concentrate reached 0.0375 ± 0.60 g per 100 g of tomato concentrate, with a retardation factor (Rf) of 0.86, confirming acceptable chromatographic purity. A statistically significant increase ($p < 0.05$) in dry matter content and syneresis was observed across all lycopene enrichment levels relative to the control. Conversely, pH values, titratable acidity, and moisture content exhibited a statistically significant decrease ($p < 0.05$) in lycopene-enriched cheeses compared with the unenriched control. The incorporation of lycopene was associated with a measurable reduction in total mesophilic aerobic flora compared to control specimens. A concentration-dependent inhibitory effect was additionally observed against total and fecal coliforms, *S. aureus*, *Salmonella spp.*, yeast, and molds, suggesting an antimicrobial contribution of lycopene under the experimental conditions applied.

Conclusions: Lycopene incorporation exerted a significant and multifaceted influence on the physicochemical profile of fresh goat cheese whilst concurrently contributing to the maintenance of microbiological stability throughout refrigerated storage. Collectively, these findings underscore the considerable potential of lycopene as a natural bioactive additive for enhancing both the quality and the safety of fresh dairy products. Nevertheless, further investigation is warranted to comprehensively elucidate the effects of lycopene enrichment on the functional textural, and sensory attributes of fresh goat cheese prior to broader commercial application.

Keywords: Lycopene; Tomato Concentrate; Solvent Extraction; Purification; Goat Milk; Fresh Cheese.

Article Information

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1 INTRODUCTION

Numerous bioactive compounds present in plant matrices have attracted considerable attention in food production because of their nutritional and physiological benefits, including antioxidant, anti-inflammatory, and anti-cancer properties (Laranjeira *et al.,* 2022). Lycopene, a prominent nutraceutical, is recognized for its powerful antioxidant activity and plays a vital role in preventing diseases or alleviating their symptoms. It is also in high demand within the cosmetic industry. As a natural pigment, lycopene imparts a red color to fruits and vegetables, offering a safer alternative to synthetic food dyes in culinary and industrial applications. Beyond its coloring properties, lycopene protects living cells from oxidative damage and free radical attacks, contributing to the prevention of chronic diseases and age-related conditions (Rodriguez-Amaya, 2019). Additionally, lycopene acts as a preventive agent against certain carcinogens, largely attributable to its ability to regulate intestinal microbiota, cholesterol, and metabolism (Wiese *et al.,* 2019). As a result, lycopene is gaining recognition as an effective alternative to synthetic antioxidants (Li & Yu, 2023).

In recent years, the significance of goat's milk and its products in human nutrition gained growing importance in

human nutrition (Park, 2007). Goat milk is rich in proteins, amino acids, bioactive peptides, fatty acids, minerals, polyphenolic compounds, and vitamins, rendering it a valuable source of health-promoting nutrients (Lucas *et al.,* 2008). This milk stands out due to its unique properties, which distinguish it from other types of milk and offer various physiological benefits. It contains slightly less lactose than cow's milk and is often reported to be better tolerated by some consumers, although it is not suitable for individuals with lactose intolerance or cow's milk allergy unless otherwise tolerated. Additionally, goat milk possesses a high acidity level, which helps maintaining the body's natural acidity and serves as a potent antioxidant (Singh *et al.,* 2015). The milk is also recognized for its rich calcium content, along with phosphorus, both of which are crucial for bone strength and energy storage. Additional important nutrients found in goat milk include zinc, iodine, iron and copper (Stergiadis *et al.,* 2019), as well as vitamin A, thiamine, riboflavin, and niacin (Park, 2007).

Growth of the food market has driven the processing industry to innovate and develop novel products. Because dairy products are widely consumed, they are suitable vehicles for enrichment with natural antioxidants, which may help prevent or mitigate diseases associated with nutritional deficiencies. Plant materials are rich sources of natural antioxidants, including vitamins, micro- and macro-minerals, essential oils, phenols, saponins, and various phytochemicals such as tannins, flavonoids, and alkaloids (Sukhikh *et al.,* 2019). Although the enrichment of dairy products with natural bioactive compounds has been widely studied, the incorporation of lycopene specifically into fresh goat cheese remains relatively unexplored**.** This study aimed to formulate fresh goat cheese enriched with lycopene and to assess the effect of this addition on its physicochemical, nutritional, and microbiological properties during fermentation and storage.

2 Material and Methods

2.1 Extraction of Lycopene from Tomato Concentrate

2.1.1 Crystallization Method

Lycopene was extracted from a locally produced tomato concentrate (CAB brand, Amor Ben Amor) following the protocol of Hamed *et al.* (2022), with modifications. To remove water, 100 g of concentrate was mixed with 100 mL of acetone, stirred for 10 min, and filtered through Whatman No. 1 filter paper. The resulting filter cake was extracted with 100 mL of a petroleum ether-hexane-dichloromethane (2:1:1, v/v/v) under stirring for 15 min, and a few grams of magnesium sulphate ($MgSO_4$) were incorporated as a drying agent. After filtration of the thick suspension, a red-orange filtrate corresponding to a carotenoid oleoresin was recovered.

The oleoresin was concentrated by evaporation 50 °C, and 5 mL of dimethyl sulfoxide (DMSO) was added under stirring as an anti-solvent. Crude lycopene crystals formed immediately, and 10 mL of 96% ethanol was added to promote precipitation. The crystals were recovered by centrifugation (3000 rpm, 15 min), washed twice with 96% ethanol, and dried overnight at 40 °C. The extraction yield was calculated as the ratio of the mass of lycopene extracted (M_1) and the mass of raw material processed (M_0) (Hamed *et al.*, 2022):

$$Y\,(\%) = (M1/M0)100 \quad \ldots\ldots(1)$$

where Y is the yield (%), M_1 is the mass of lycopene extract (g), and M_0 is the mass of tomato concentrate (g).

This solvent-based extraction method, although effective at laboratory scale, may not be suitable for large-scale or food-grade applications and alternative food-safe extraction techniques should be explored for potential industrial application.

2.1.2 Thin-layer chromatography Characterization

Thin-layer chromatography (TLC), a qualitative technique, was employed to characterize the lycopene extract, following Baldermann *et al.* (2008) with modification. The stationary phase was silica gel, and the mobile phase was a mixture of petroleum ether, hexane, and acetone (5:4:1 v/v/v).

2.1.3 Collection of Goat Milk

Fresh goat milk was sourced on May 4, 2024 from a private breeder who raises goats employing traditional methods. The milk was collected manually after disinfection of the farmer's hands and the goats' udders. The samples were collected in bottles, stored in cooler boxes at 4 °C, and transported to the laboratory.

2.2 Quality Analysis of Raw Goat Milk

2.2.1 Physicochemical Analysis

The pH was measured with a pH meter on a 10 mL sample (AOAC, 2016). Titratable acidity was determined by titration with sodium hydroxide (NaOH) using phenolphthalein as the indicator (AOAC, 2016). Thermal stability was assessed with the boiling test, in which the milk was heated to boiling and examined for coagulation or precipitation, which would indicate acidification or other quality defects (AOAC, 2016). In the curdling test, the transition of the milk from a liquid to a semi-solid state, with

the formation of whey (liquid phase) and coagulum (solid phase), was observed (Wahyuningtyas *et al.,* 2023).

2.2.2 Microbiological Analyses

The microbiological quality of raw goat milk and cheeses was assessed according to the Algerian official standards (JORADP No. 35, 27 May, 1998). The following parameters were determined:

Total and thermotolerant (fecal) coliforms: Enumerated using standard plate count methods specified by JORADP, to evaluate hygienic quality.

Staphylococcus aureus: Detected using selective media and conditions recommended by JORADP, ensuring detection of pathogenic contamination.

Salmonella: Tested according to JORADP protocols to confirm its absence.

Total aerobic mesophilic flora (TAMF): The presence of Total Aerobic Mesophilic Flora (TAMF) was enumerated at 30 °C on plate count agar (PCA), inoculating 1 mL of each dilution by the pour-plate method (Guiraud, 2003).

Yeast and mold: Yeasts and molds were enumerated on oxytetracycline glucose agar (OGA) after incubation at 25 °C for 3–5 days. Results were expressed as CFU/mL for milk and CFU/g for cheese.

2.3 Manufacture of Fresh Goat Cheese

Following procedures similar to those described by Wang *et al.* (2023), raw goat milk was filtered through a sterile fine cloth to remove foreign particles, pasteurized at 63 °C for 30 min, and cooled to 32 °C. A mesophilic lactic starter culture (Chr. Hansen, Denmark) was added at 0.025 g /L, and the milk was incubated at 32 °C for 2 hours to activate the lactic acid bacteria. Calcium chloride was then incorporated at 1.4 mL/L to improve coagulation, together with bacterial rennet (Chr. Hansen, coagulant stick CHN 22) at 0.05g/L. The milk was maintained at an incubation temperature to coagulate et 32 °C for 10 hours. The curd was transferred to a sterile fine cloth and allowed to drain by hanging for 6 hours, until the desired moisture content and texture of fresh cheese were obtained.

After draining, lycopene, which is lipophilic, was first dissolved in melted butter. The lycopene-butter mixture was then incorporated into the fresh cheese under sterile conditions at varying concentrations, and the curd was manually stirred to ensure homogeneity. Four treatments were prepared to evaluate the effect of lycopene addition: Treatment 1, 25 g of butter (sample 1); Treatment 2, 25 g of butter plus 0.030 g of lycopene (sample 2), Treatment 3, 25 g of butter plus 0.061 g of lycopene (sample 3); and Treatment 4, 25 g of butter plus 0.122 g of lycopene (sample 4). The lycopene was incorporated into fresh goat cheese at specific concentrations based on preliminary trials to optimize its integration and maintain product quality. Only a limited number of lycopene concentrations were tested in this study. Future research should investigate a broader range of concentrations to evaluate their functional and sensory effects.

The finished fresh goat cheese was packed in opaque, food-grade polypropylene plastic jars to limit light and oxygen exposure, thereby preserving lycopene stability. The packed samples were stored under refrigerated conditions at 4 °C for 30 days (Chen *et al.,* 2025). Physicochemical and microbiological properties were monitored periodically during this storage period to evaluate product stability and quality.

The yield of the cheese was determined by calculating the percentage of the resulting sample weight relative to the weight of the milk used, as described by Cipolat-Gotet *et al.* (2013). The yield was calculated using the following formula:

$$Yield\ (\%) = \frac{Curd\ weight}{Milk\ weight}\ x\ 100 \ldots\ldots (2)$$

2.4 Physicochemical Analyses of Cheese

2.4.1 pH and Dornic Acidity

The pH was measured with a pH meter. Dornic acidity was determined by titrating 10 mL of the stock suspension with N/9 NaOH in the presence of phenolphthalein, the endpoint being a persistent light pink color (Guiraud, 2003).

2.4.2 Dry Matter and Moisture

Dry matter and moisture were determined by drying a 1-gram sample in a ventilated oven at 100 °C until a constant weight was reached (AOAC, 2005). Moisture was calculated from the weight loss, and dry matter by difference.

2.4.3 Fat Content

Fat content was determined by Soxhlet extraction according to AOAC Official Method 991.36. Approximately 2 g of finely ground cheese was placed in a cellulose thimble and extracted using 200 mL of hexane under reflux until extraction was complete. After removal of the solvent, the residue was dried at 100–105 °C to constant weight, and fat was expressed as a percentage of the initial sample weight.

2.4.4 Protein Content

Protein content was determined by the Lowry method (Lowry *et al.,* 1951), which quantifies soluble proteins colorimetrically. Cheese (1 g) was homogenized in 0.1 M phosphate buffer (pH 7.0) and centrifuged to remove

insoluble material. The supernatant was reacted with copper-based reagents, followed by Folin–Ciocalteu reagent, and absorbance was measured at 750 nm using bovine serum albumin (BSA) as the standard.

2.4.5 Syneresis

Syneresis, i.e., the expulsion of whey from the curd as a result of contraction of the protein network, was assessed by measuring the volume of whey released during storage, which reflects the physical stability and moisture retention of the curd, as described by Panthi *et al.* (2019).

2.5 Microbiological Analyses of Cheese

Total and thermotolerant coliforms, coagulase-positive *Staphylococcus aureus*, and yeasts and molds were enumerated, and the absence of *Salmonella* was verified according to JORADP (No. 35, 27 May, 1998). Total aerobic mesophilic flora at 30 °C was determined as described by Guiraud (2003). Lactic acid bacteria were enumerated on selective media: lactobacilli on MRS agar (de Man, Rogosa and Sharpe) incubated anaerobically at 37 °C for 24–48 h, and *Streptococcus* spp. on M17 agar by the double-layer pour-plate technique, incubated at 37 °C for 24–48 h (Guiraud, 2003). Viable counts were calculated as CFU/g = number of colonies × dilution factor / volume plated.

2.6 Statistical Analysis

The results of physicochemical and microbiological analyses of fresh goat cheese were initially analyzed using a factorial two-way ANOVA with complete randomization, followed by pairwise comparisons of means using the Newman-Keuls test (Stat Box 6.4). This approach was applied to identify significant differences between treatments at each storage time point.

3 Results

3.1 Lycopene Extraction Yield and TLC Characterization

Lycopene was extracted from Algerian tomato concentrate through the crystallization method (Figure 1). The yield, calculated from 100 g of raw material, was 0.0375 g per 100 g (0.0375%; Table 1). TLC analysis of the extract exhibited a retention factor (Rf) of 0.87, a value consistent with a nonpolar carotenoid such as lycopene, although TLC alone cannot establish the purity of the extract.

3.2 Preliminary Quality Assessment of Raw Goat Milk

3.2.1 Physicochemical Quality



Figure 1. Lycopene Extract

Table 1. Yield of Lycopene Extraction

Mass of plant matter (g)	Lycopene mass (g)	Yield (%)	Frontal ratio (Rf)
100	0.0375 ± 0.60	0.0375 ± 0.86	0.87

The mean pH of raw goat milk was 6.7 ± 0.12 and its titratable acidity was 16°D, within the range of 14–18°D as reported by the FAO (2014), indicating good milk quality.

3.2.2 Thermal Stability

The milk exhibited good thermal stability in the boiling test, remaining a single homogeneous phase without coagulation or precipitation.

3.2.3 Curdling Test

Coagulation of the milk resulted in a homogeneous curd of 230 g per liter of milk, which suggests that the milk is technologically suitable for the manufacture of fresh cheese.

3.2.4 Microbiological Quality

The microbiological quality of the milk was evaluated prior cheese manufacture to determine its hygienic status and suitability for processing. The total aerobic mesophilic flora (TAMF) count was 5×10^3 CFU/mL (3.70 log CFU/mL), which represents the natural microbial load of the raw milk. Total coliforms thermotolerant coliforms, *Staphylococcus aureus*, yeasts and molds were not detected, and *Salmonella* was absent from all samples, in compliance with the limits set by the JORADP standards (1998). These results indicate satisfactory hygienic conditions during milking, handling, and storage, and suggest that the milk was suitable for cheese

manufacture. They are in line with the absence of *Salmonella* in goat milk reported by Benkerroum and Tamine (2004).

3.3 Evolution of the Physicochemical Parameters of Lycopene-Enriched Fresh Goat Cheese during Storage

3.3.1 pH

The pH of all samples increased significantly during storage (Figure 2). Following the first week, the pH of samples S1, S2, S3, and S4 was 4.71, 4.64, 4.54, and 4.52, respectively, and after the fourth week it had increased to 5.33, 5.10, 5.01, and 4.95, respectively. The highest increase was observed in S1 (from 4.71 to 5.33) and the lowest final pH was recorded in S4 (4.95).

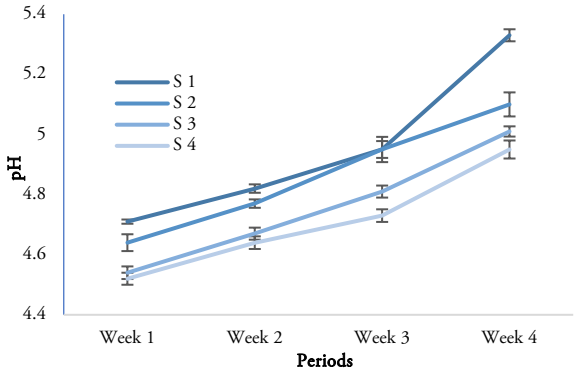


Figure 2. Changes in pH of Goat Cheese Samples Enriched with Different Lycopene Concentrations during 30 Days of Storage at 4°C

S1: 25 g of butter (Sample 1), S2: 25 g butter + 0.030 g lycopene (Sample 2), S3: 25 g butter + 0.061 g lycopene (sample 3), S4: 25 g butter + 0.122 g lycopene (sample 4)

3.3.2 Titratable Acidity

Dornic acidity increased significantly ($p < 0.01$) in all samples during storage (Figure 3). Values increased from approximately 53, 50, 47, and 40 °D in the first week to 80, 76, 78, and 79 °D at the end of storage, corresponding to the fourth week, for samples S1, S2, S3, and S4, respectively. This increase may be attributed to post-acidification, i.e., the conversion of residual lactose into lactic acid by lactic acid bacteria during refrigerated storage.

3.3.3 Dry Matter

Dry matter was stable at approximately 30% in all samples during the first week. It then increased significantly ($p < 0.05$), reaching 60, 50, 40, and 40% in S1, S2, S3, and S4, respectively, by the third week (Figure 4).

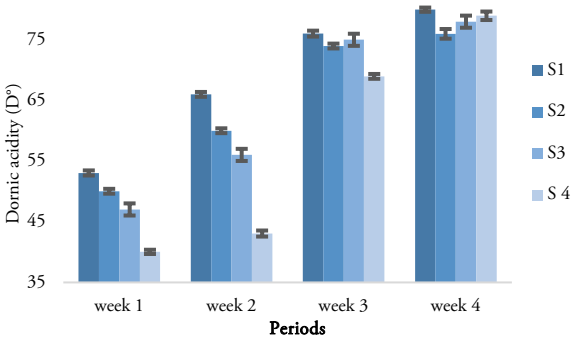


Figure 3. Evolution of Dornic Acidity of Fresh Goat Cheese Samples during Four Weeks of Refrigerated Storage

S1: 25 g of butter (Sample 1), S2: 25 g butter + 0.030 g lycopene (Sample 2), S3: 25 g butter + 0.061 g lycopene (sample 3), S4: 25 g butter + 0.122 g lycopene (sample 4).

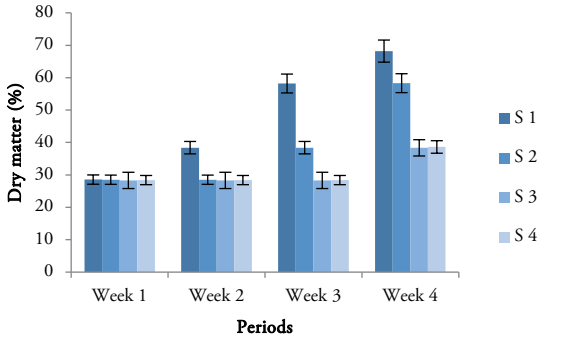


Figure 4. Evolution of Dry Matter Content of Fresh Goat Cheese Samples during Four Weeks of Refrigerated Storage

S1: 25 g of butter (Sample 1), S2: 25 g butter + 0.030 g lycopene (Sample 2), S3: 25 g butter + 0.061 g lycopene (sample 3), S4: 25 g butter + 0.122 g lycopene (sample 4).

3.3.4 Moisture

Moisture decreased significantly ($p < 0.05$) in all samples, to a degree that diminished with increasing lycopene concentration. The largest reduction was observed in S1, followed by S2, S3, and S4. (Figure 5).

3.3.5 Fat

Although the same amount of butter was added to all samples, fat content decreased significantly during storage ($p < 0.01$), and the decrease was more marked at higher lycopene concentrations (14.3, 13.6, and 9.15%; Figure 6).

3.3.6 Protein

Protein content did not change significantly during storage and remained at approximately 3% in all samples (S1–S4).

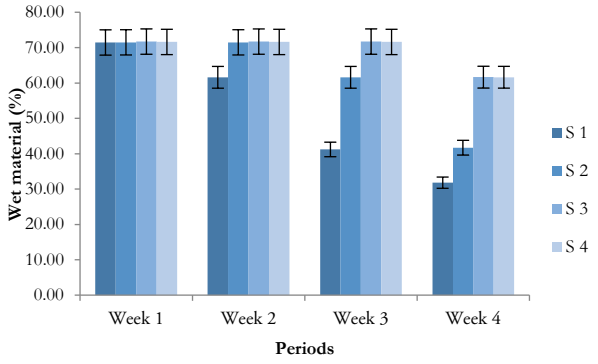


Figure 5. Evolution of Moisture Content of Fresh Goat Cheese Samples during Four Weeks of Refrigerated Storage

S1: 25 g of butter (Sample 1), S2: 25 g butter + 0.030 g lycopene (Sample 2), S3: 25 g butter + 0.061 g lycopene (sample 3), S4: 25 g butter + 0.122 g lycopene (sample 4).

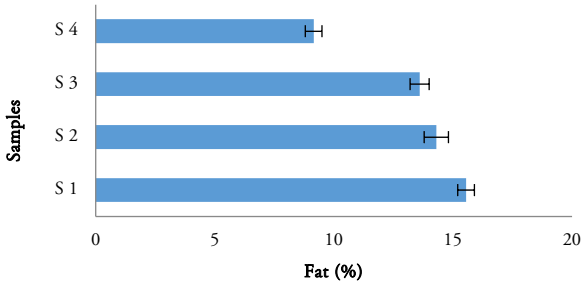


Figure 6. Fat Content of Cheese Samples

S1: 25 g of butter (Sample 1), S2: 25 g butter + 0.030 g lycopene (Sample 2), S3: 25 g butter + 0.061 g lycopene (sample 3), S4: 25 g butter + 0.122 g lycopene (sample 4).

3.3.7 Syneresis

The addition of lycopene significantly reduced whey exudation during the early storage period ($p < 0.01$), which may indicate a stabilizing effect on the curd structure. In all samples, however, syneresis increased gradually from day 7 to day 21 (Figure 7), reflecting the progressive separation of whey during storage.

3.4 Evolution of Microbial Counts During Storage

3.4.1 Total Aerobic Mesophilic Flora

TAMF counts were high in all samples throughout storage (Table 2) and exceeded the Algerian standard limit of 10^5 CFU/mL. It should be noted that, in fresh cheese, this count includes the lactic starter flora.

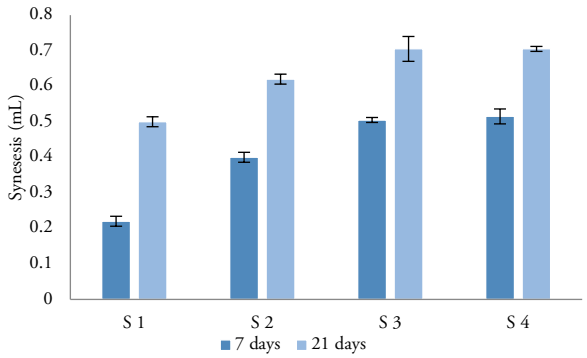


Figure 7. Syneresis of Fresh Goat Cheese Samples after 7 and 21 Days of Storage

S1: 25 g of butter (Sample 1), S2: 25 g butter + 0.030 g lycopene (Sample 2), S3: 25 g butter + 0.061 g lycopene (sample 3), S4: 25 g butter + 0.122 g lycopene (sample 4).

3.4.2 Coliforms

Neither total nor thermotolerant coliforms were detected in any sample throughout storage (Table 2).

3.4.3 *Staphylococcus aureus* and *Salmonella*

Staphylococcus aureus, sought on Chapman medium, was not detected at any time during storage. *Salmonella* was investigated on *Salmonella–Shigella* (SS) agar and was absent from all samples throughout the four weeks of storage. (Table 2).

3.4.4 Lactic Acid Bacteria

Lactobacilli were detected in the cheese samples throughout refrigerated storage, which indicates that they survived in the fresh cheese matrix; however, they were not detected in sample S4 at the end of storage. Streptococci/lactococci, enumerated on M17 agar, were detected in all samples from the first week; their counts increased during the second week and then decreased gradually.

3.4.5 Yeast and Molds

The fungal flora count in fresh cheese was performed on OGA medium. Microbiological analysis revealed an absence of yeasts and molds in all cheese samples (S1, S2, S3, and S4) during the first two weeks. However, contamination by yeasts and molds was observed on days 14, 21, and 30, with a significant increase in load across all samples.

4 Discussion

4.1 Lycopene Extraction Yield

The lycopene yield obtained in this study was 0.0375 g per 100 g of tomato concentrate (approximately 0.38 mg/g).

Table 2. Evolution of Microbial Counts of Lycopene-Enriched Fresh Goat Cheese During Storage (log CFU/g)

Samples	Microbiological Analysis	W1	W2	W3	W4
S 1	TAMF	7.12	7.14	7.17	7.26
	Total coliforms	<1	<1	<1	<1
	Fecal coliforms	<1	<1	<1	<1
	S. aureus	<1	<1	<1	<1
	S. Shigella	<1	<1	<1	<1
	Lactobacilli	7.98	8.23	10.18	10.38
	Streptococcus ssp	9.33	9.57	9.98	8.68
	Yeast and molds	<1	<1	Detected	Detected
S 2	TAMF	6.90	6.01	6.51	6.30
	Total coliforms	<1	<1	<1	<1
	Fecal coliforms	<1	<1	<1	<1
	S. aureus	<1	<1	<1	<1
	S. Shigella	<1	<1	<1	<1
	Lactobacilli	10.16	9.45	9.81	11.08
	Streptococcus ssp	8.26	9.04	10.17	7.38
	Yeast and molds	<1	<1	Detected	Detected
S 3	TAMF	6.45	6.33	6.08	5.90
	Total coliforms	<1	<1	<1	<1
	Fecal coliforms	<1	<1	<1	<1
	S. aureus	<1	<1	<1	<1
	S. Shigella	<1	<1	<1	<1
	Lactobacilli	11.00	11.63	11.86	10.60
	Streptococcus ssp	8.17	7.73	6.90	<1
	Yeast and molds	<1	<1	Detected	Detected
S 4	TAMF	6.56	6.60	6.60	6.54
	Total coliforms	<1	<1	<1	<1
	Fecal coliforms	<1	<1	<1	<1
	S. aureus	<1	<1	<1	<1
	S. Shigella	<1	<1	<1	<1
	Lactobacilli	12.20	11.86	10.11	<1
	Streptococcus ssp	8.08	8.29	9.08	<1
	Yeast and molds	Abs	Abs	Detected	Detected

W1: Week 1; W2: Week 2; W3: Week 3; W4: Week 4; TAMF: Total aerobic mesophilic flora; Values are expressed as log CFU/g. Results below the detection limit were reported as <1 log CFU/g. "Detected" indicates the presence of yeasts and molds. S1: 25 g of butter (Sample 1), S2: 25 g butter + 0.030 g lycopene (Sample 2), S3: 25 g butter + 0.061 g lycopene (sample 3), S4: 25 g butter + 0.122 g lycopene (sample 4).

Higher yields have been reported with hot-solvent extraction, for example up to 2.4 mg/g from tomato peel (Hatami & Ciftci, 2024). Direct comparison is limited, however, by differences in raw material, in lycopene content, and in the basis of calculation (fresh versus dry weight; crude crystals versus quantified lycopene). Solvent selection is critical: *n*-hexane provides the highest recovery but raises toxicity concerns (Deng *et al.*, 2021).

Alternative solvents have been explored to improve safety. Ethanol-based extraction has yielded 487.4 to 2773.53 µg/g (Mavridis *et al.*, 2025; Miranda *et al.*, 2023), offering a less toxic alternative at the cost of somewhat lower efficiency. Vegetable oils such as tomato seed oil (TSO), yielded 113.5 µg/g with reduced toxicity, although extraction is more complex and less cost-effective (Popescu *et al.*, 2022; Mutmainah *et al.*, 2026). The yield obtained here (approximately 375 µg/g) therefore lies below the ethanol range and above the TSO value.

These findings illustrate the trade-off between yield, safety, and scalability. Hot-solvent extraction is practical at laboratory scale, but its solvent toxicity and environmental impact limit industrial applications. Emerging food-safe techniques, including supercritical CO_2, ultrasound-assisted, and pulsed electric field extractions, offer promising alternatives to enhance both yield and sustainability (Deng *et al.*, 2021; Mutmainah *et al.*, 2026).

4.2 Primary Quality of Raw Goat Milk

4.2.1 Physicochemical Quality

The mean pH of the raw goat milk (6.7 ± 0.12) is within the range generally reported for fresh goat milk (Park, 2007) and slightly higher than the pH recently reported for raw goat milk from the M'Sila region of Algeria (6.4 ± 0.12), which was the lowest value among the cow, sheep, goat, and camel milks analyzed in that study (Salhi *et al.*, 2025). Milk pH is a key physicochemical parameter in cheese-making because it governs rennet coagulation, curd syneresis, and the acidifying activity of lactic acid bacteria (Fox *et al.*, 2017). Slight variations in milk pH have been linked to the production area and to breed, whereas diet and lactation stage further modulate milk composition (Salhi *et al.*, 2025). These results indicate that storage time affected the acidity balance of fresh goat cheese, while lycopene-enriched samples maintained slightly lower pH values than the control sample.

The titratable acidity observed in the present study (16 °D) is closely comparable to the value of 16.45 ± 0.79 °D recently reported for raw goat milk from Algeria (Salhi *et al.*, 2025). In that investigation, goat milk exhibited the lowest acidity among the ruminant milks examined, whereas sheep milk displayed the highest value (19.86 ± 0.92 °D).

4.2.2 Thermal Stability

The result of the boiling test is consistent with Guiraud (2003): milk is considered normal when it does not clot on boiling and flows along the wall of the tube without leaving a residue, whereas curdling, clot formation, or separation of a watery serum characterizes abnormal milk.

4.2.3 Microbiological Quality

The microbiological results indicate satisfactory hygienic quality and support the suitability of the milk for cheese manufacture. The presence of coliforms is often associated with enteropathogenic bacteria such as *Salmonella, Shigella, Yersinia*, and certain *E. coli* biotypes (Guiraud, 2003). In the

present study, total and thermotolerant coliforms, yeasts and molds, and *Salmonella* were not detected in the milk, in compliance with the standards of JORADP (1998).

4.3 Changes in Physicochemical Parameters of the Cheese during Storage

4.3.1 pH

The pH increased in all samples during storage, while remaining lower in the lycopene-enriched samples than in the control. The final values (4.95–5.33) are comparable to the pH values (5.25–5.31) reported for goat milk cheese during ripening (Moreira *et al.*, 2020), but higher than those reported for Ayib, an Ethiopian cottage cheese supplemented with garlic and ginger powders (Regu *et al.*, 2016). The decrease in pH of all goat cheese samples is primarily due to the action of lycopene on physicochemical parameters.

4.3.2 Titratable Acidity

Titratable acidity increased in all samples during refrigerated storage. This increase is consistent with post-acidification, a phenomenon frequently observed in fermented dairy products, in which lactic acid bacteria remain metabolically active during cold storage and continue to convert residual lactose into lactic acid (Guan *et al.*, 2025). Lactic acid bacteria play a central role in cheese acidification, both as starter cultures during coagulation and as part of the cheese microbiota during storage and ripening (Fox *et al.*, 2017). Moreira *et al.* (2020) likewise reported increased titratable acidity in goat milk cheese during ripening, which was attributed to carbohydrate degradation, organic acid production, and the activity of *Lactococcus* in lactose fermentation and lactic acid production. Therefore, the acidity evolution observed in the present study should therefore be interpreted primarily as a normal post-acidification process rather than as evidence of a strong antimicrobial effect of lycopene.

Lycopene and the butter carrier may have influenced the rate of acidification among formulations. Because lycopene is lipophilic and poorly soluble in water, its incorporation into dairy matrices usually requires a lipid carrier, which may affect its dispersion and interaction with the food matrix (Akhoond Zardini *et al.*, 2018). The butter may also have modified lactose diffusion and the distribution of bacteria. These effects remain hypothetical.

4.3.3 Dry Matter

Dry matter increased in all samples during storage, with lycopene-enriched samples exhibiting slightly higher values than the control. This suggests that lycopene contribute to the stabilization of the cheese matrix and to the reduction of moisture loss, likely through interactions with proteins and lipids that maintain structural integrity. Identical stabilization effects have been observed with other bioactive compounds in dairy matrices (Chen *et al.*, 2022). The dry matter levels in our study are consistent with reported ranges for goat cheese (Park, 2007; Say, 2022) confirming that lycopene incorporation does not compromise product quality. Overall, lycopene contributes to improved structural stability and moisture retention in fresh goat cheese.

4.3.4 Moisture

Moisture decreased in all samples, and the decrease was less pronounced as lycopene concentration increased. Lycopene may interact with proteins and lipids and alter the matrix in ways that improve water retention, as reported for other bioactive compounds incorporated into fresh goat milk cheese, in which jujube polysaccharide stabilized the secondary structure of the proteins and increased the water-binding capacity of the casein network (Wang *et al.*, 2023). Similarly, interactions between lycopene and cheese components may influence the cheese's microstructure. Chen *et al.* (2022) reported that syneresis in a lycopene-fortified yogurt emulsion was approximately twice that of plain yogurt after 7 days of storage, indicating that the incorporation of lycopene can restructure the matrix and increase the release of water in the form of serum. The presence of lycopene may also impact the stability of the cheese matrix. According to Alirezalu *et al.* (2019), the addition of bioactive compounds to dairy products can alter their rheological properties, affecting water retention depending on the concentration of these compounds.

4.3.5 Fat and Protein

Fat content decreased significantly ($p < 0.01$) during storage in all samples, even though the same amount of butter was incorporated to each formulation, and the decrease was more marked at higher lycopene concentrations. Such changes may be linked to lycopene's antioxidant properties, which can influence lipid stability through its interaction with unsaturated fatty acids and by quenching free radicals, thereby affecting lipid oxidation pathways in high-fat food matrices. Lycopene's antioxidant action has been shown to significantly reduce oxidative rancidity in milk fat systems, delaying lipid peroxidation during storage (Siwach *et al.*, 2016). Lycopene is a highly potent carotenoid antioxidant that can protect lipids against oxidative degradation, a primary factor in the deterioration of high-fat dairy products such as cheese. By stabilizing lipid fractions and reducing oxidative stress within the cheese matrix, lycopene may modulate the rate of fat loss during storage (Li & Yu, 2023). Although the direction and mechanisms in complex cheese systems require further investigation, recent evidence supports lycopene's role in improving oxidative stability in dairy matrices. Nevertheless, the total fat content of all cheese samples remained within the typical range reported for fresh

goat cheeses in the literature (Say, 2022), indicating that the addition of lycopene did not adversely affect overall lipid levels beyond expected variation.

Protein content remained stable (3%) across all cheese samples during storage, indicating that lycopene incorporation did not affect the protein matrix. Lycopene primarily interacts with the lipid fraction, enhancing antioxidant protection without altering protein structure. This aligns with reports showing that antioxidant addition generally affects oxidative stability rather than total protein content in dairy products (Siwach *et al.,* 2016). These findings indicate that lycopene can be incorporated into fresh goat cheese to provide antioxidant benefits while preserving protein content and only slightly affecting fat stability. At higher concentrations, minor effects on fat content during storage should be taken into account when optimizing functional cheese formulations.

4.3.6 Syneresis

Whey exudation increased in all samples during storage, particularly between days 7 and 21, which reflects the normal reorganization of the protein–fat network, as reported for other fresh cheeses such as Minas cheese (Sant'Ana *et al.,* 2013). Although lycopene is a lipophilic carotenoid and does not bind water directly like polysaccharides, its incorporation can indirectly stabilize the curd structure. This effect is achieved by protecting the lipid fraction from oxidation, which preserves interactions between fat globules and proteins and reduces early disruptions in the protein–lipid network that would otherwise facilitate whey separation. Lycopene's antioxidant activity has been shown to maintain lipid stability in dairy matrices (Chen *et al.,* 2022; Siwach *et al.,* 2016), supporting its role in moderating early syneresis without altering overall moisture dynamics. The increase in whey exudation over storage can also be attributed to acidification, as the gradual decrease in pH increases hydrogen ion concentration, reducing electrostatic repulsive forces between casein micelles. This promotes micelle aggregation and whey expulsion, consistent with mechanisms reported in dairy products (Fox *et al.,* 2000). Taken together, these observations indicate that while syneresis naturally progresses during storage due to acidification and matrix reorganization, lycopene incorporation can partially mitigate early whey loss, contributing to improved curd stability and structural integrity in fresh goat cheese. These findings highlight the potential of lycopene as a functional additive to enhance the physicochemical stability of dairy products without negatively impacting natural whey separation over time.

4.4 Microbial Counts during Storage

4.4.1 Total aerobic Mesophilic Flora

TAMF is an indicator of overall microbial quality and reflects bacteria originating from the raw material, processing, and storage (Guiraud, 2003). Recent studies support these observations; Kačániová *et al.* (2024) reported total viable counts and other microflora levels in fresh lump goat cheese under comparable storage conditions, revealing that aerobic-mesophilic bacteria generally remain within acceptable ranges compatible with good quality and shelf-life performance. As in our study, bioactive constituents or matrix-stabilizing factors can modulate microbial dynamics during storage, helping maintain cheese quality and safety.

This suggests that an increase in carotenoid compounds, particularly lycopene, leads to a reduction in the TAMF load, demonstrating lycopene's ability to lower the number of TAMF compared to the controls.

4.4.2 Total Coliforms

Our results are in agreement with the standards outlined in the Official Journal (JORADP. no.35 of May 27, 1998).

4.4.3 Fecal Coliforms

Our results are in compliance with the standards outlined in the Official Journal (JORADP No. 35 of May 27, 1998), which requires the absence of this microorganism. This reflects the aseptic conditions maintained during production. According to JORADP (1998), the tolerated level of fecal coliforms is 10^3 CFU/mL. The presence of coliforms in cheese is generally regarded as an indicator of poor hygiene during milking, processing, and storage (Metz *et al.*, 2020).

4.4.4 *Staphylococcus aureus*

The absence of *Staphylococcus aureus* can be attributed to effective sterilization during production, proper incorporation of lycopene, and overall good sanitary quality. The results are consistent with the recommendations for fresh cheeses as outlined in the Official Journal (JORADP, 1998).

4.4.5 *Salmonella-Shigella* Testing

Staphylococcus aureus and *Salmonella* are among the main pathogens associated with milk and dairy products (Fusco *et al.*, 2020); accordingly, only low counts of staphylococci are tolerated in cheeses, whereas *Salmonella* must be absent. Therefore, our results are consistent with the recommendations for fresh cheese outlined in the Official Journal (JORADP, 1998) and reflect the cleanliness of the environment during manufacturing as well as adherence to good manufacturing practices.

4.4.6 Lactic Acid Bacteria (LAB)

Our results are consistent with the high lactic acid bacteria counts (8.80 ± 0.92 log CFU/g) reported for Moroccan "Jben", a traditional fresh cheese in which *Lactococcus lactis* was the predominant species (Azzouz *et al.*, 2024), and with the dominance of *Lactococcus* in the microbiota of goat milk cheese during ripening (Moreira *et al.*, 2020). It can be concluded that the addition of lycopene helps maintain the Lactobacillus load in fresh cheese during 30 days of storage.

Our results indicate that lactococci decreased over the storage period, with no inhibition observed from the different concentrations of lycopene incorporated. The effect of plant-derived additives on lactic acid bacteria in fermented dairy products is variable: essential oils may adversely affect the development of lactic cultures, whereas, in other formulations, they have been associated with improved probiotic viability (Canbey *et al.*, 2025). These findings provide preliminary insight into the effects of lycopene; however, the study did not extensively evaluate its antimicrobial activity against lactic acid bacteria, which constitutes a limitation and warrants further investigation to fully elucidate its influence on these beneficial organisms.

4.4.7 Yeast and Molds

Yeasts and molds were detected in some samples from the third week of storage onward. This may be explained by the high moisture of fresh cheese, which renders it susceptible to microbial changes. The development of yeasts and molds is influenced by several factors, including water activity, pH, residual nutrients, oxygen availability, packaging, and storage temperature. Fungal contamination may alter flavor, texture, and appearance (Banjara *et al.*, 2015).

In the present study, the detection of yeasts and molds should not be interpreted as evidence of a direct antifungal effect or absence of antifungal activity of lycopene. Lycopene is primarily a lipophilic carotenoid antioxidant, and its main technological role in dairy matrices is more closely related to lipid oxidative stability than to antimicrobial inhibition. Lycopene has been reported to act as an efficient antioxidant because of its conjugated double-bond structure, which allows it to scavenge singlet oxygen and free radicals (Li & Yu, 2023). In dairy fat systems, lycopene enrichment has been associated with lower peroxide values and improved oxidative stability during storage (Salehin *et al.*, 2025).

Additionally, the peroxidation index of traditional butter was found to be high, resulting from intense fat hydrolysis and the release of free fatty acids. As fat degradation increases, so does the alteration of the cheese. Water content also plays a significant role in the preservation of fresh cheese. Cais-Sokołińska *et al.* (2024) reported that water loss, water activity, and the mobility of water molecules in butter change during storage, in parallel with a significant increase in cholesterol oxidation products in whey butter. Managing water content is therefore crucial in extending the shelf life of butter, as the degree of moisture dispersion and distribution serves as a key indicator for predicting storage stability. In other dairy matrices, water can also exert pro-oxidant effects; at elevated water activity levels, increased molecular mobility and the dissolution of catalytic metals can accelerate lipid oxidation. However, direct evidence for the specific mechanism of water facilitating pro-oxidant diffusion into the cream cheese matrix was not identified in the available literature. Moreover, lipid oxidation in emulsified fat systems is strongly governed by their microstructure, particularly the oil–water interface, so that a well-stabilized microstructure can help limit lipid oxidation in fatty products (Hennebelle *et al.*, 2024).

4.5 Limitations of the study

Despite the promising results, this study presents several limitations. The extraction and purification of lycopene were conducted using solvent-based methods, which may not be suitable for large-scale or food-grade applications without further optimization or validation for industrial safety standards. In addition, the study only examined three concentrations of lycopene in cheese, which may not encompass the full range of possible functional or sensory effects. Furthermore, the antimicrobial mechanisms of lycopene were not explored in detail, leaving gaps in understanding its specific modes of action against various microbial strains.

5 Conclusions

This study investigated the incorporation of lycopene extracted from tomato concentrate into fresh goat cheese and its effects on physicochemical and microbiological properties during 30 days of refrigerated storage. Lycopene-enriched samples exhibited improved oxidative stability and minor modulation of fat content, while protein levels remained stable throughout storage. Syneresis was slightly reduced in enriched samples during the early storage period, suggesting a stabilizing effect on the cheese matrix.

Microbiological analyses showed that lycopene-enriched cheeses maintained total viable counts and lactic acid bacteria populations within ranges typical for fresh goat cheese, indicating that lycopene does not adversely affect beneficial microbial populations. Spoilage microorganism counts were slightly lower in lycopene-enriched samples; however, direct antimicrobial tests were not performed, and the specific effects of lycopene on lactic acid bacteria require further investigation.

These findings suggest that lycopene may be incorporated into fresh goat cheese with limited effects on its basic composition, but the effects on oxidative stability and on microbial populations, including lactic acid bacteria, remain to be established. Future work should include oxidation indicators, targeted antimicrobial assays, sensory evaluation, and food-grade lycopene sources.

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