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Research Article

Effect of sunflower oil and thyme essential oil on Kashkaval cheese quality during storage

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Abstract

The present study evaluated the effect of sunflower oil enriched with thyme essential oil (0.1% and 0.2%) as a surface treatment on the physicochemical, microbiological, metabolomic, and sensory characteristics of Kashkaval cheese during ripening and six months of refrigerated storage. The treated cheeses were analyzed for fatty acid composition, cholesterol content, organic acids, amino acids, microbial counts, volatile metabolites, and sensory properties. The application of sunflower oil and thyme essential oil maintained the stability of the fatty acid profile, reduced cholesterol content, and preserved the viability of lactic acid bacteria during storage. In addition, the treatments contributed to stable sensory quality and reduced the development of spoilage microorganisms. The results demonstrate the potential of sunflower oil combined with thyme essential oil, particularly at 0.1%, as a natural preservative system for improving the quality and shelf life of Kashkaval cheese. The study demonstrates the potential application of thyme essential oil as a natural preservative and functional ingredient for improving the nutritional profile, microbiological stability, and sensory quality of Kashkaval cheese during long-term ripening and storage.

Keywords

natural preservation; surface coating; functional dairy products; lipid stability; cholesterol reduction

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Introduction

Kashkaval is a traditional dairy product that is present in the diet of the Balkan Peninsula population. Today, to enrich the chemical composition, improve the quality and extend the shelf life, various functional additives of plant origin are included in the composition of dairy products, such as essential and glyceride oils, extracts and others (Adinepour et al. 2022; Dimitrova et al. 2019; Granato et al. 2018; Juric et al. 2022; Salama et al. 2021). It has been established that these functional food ingredients have a beneficial effect on human health, increase the quality of final products, and extend their shelf life (Balev et al. 2022; Granato et al. 2018; Keshavarzi et al. 2021). Among dairy products, Kashkaval has the potential to be effectively enriched with plant components, such as glycerides and essential oils, which have pronounced antimicrobial, antioxidant, and other biological properties (Ibrahim and Deen 2011; Mohanad et al. 2020). Various types of thyme are cultivated in Bulgaria, and common thyme (*Thymus vulgaris* L.). It is known that the composition of the essential oil obtained from plants grown under different climatic and geographical conditions varies, but its main components are the phenols thymol and carvacrol, which determine its highly pronounced antimicrobial and antioxidant effects (Aljabeili et al. 2018; Galgano et al. 2023; Mancini et al. 2015). Despite the increased interest in dairy products with functional plant-based additives, information on the use of a mixture of glyceride and essential oils in the preservation of Kashkaval is scarce.

Although essential oils have been extensively investigated as natural preservatives in dairy products, limited information is available regarding the combined application of sunflower oil and thyme essential oil as a surface treatment for Kashkaval cheese during long-term storage. The sunflower oil was used as a carrier medium for the essential oil and as a protective lipid coating capable of reducing moisture loss and oxidative changes. Furthermore, the effect of this treatment on metabolomic characteristics, microbiological stability, and sensory quality has not been sufficiently studied. Therefore, the aim of the present study was to evaluate the influence of sunflower oil enriched with thyme essential oil on the physicochemical composition, microbiological quality, metabolite profile, and sensory characteristics of Kashkaval cheese during ripening and refrigerated storage.

Materials and Methods

Materials. Sunflower oil, linoleic chemotype, supplied by Papas Olio AD, Bulgaria with the following indicators: acidity as oleic acid max 3%; peroxide value max 15 meq $O_2.kg^{-1}$; iodine value 110 - 143; saponification value 188 - 195; fatty acid composition: palmitic acid 5.5 - 8.5%, stearic acid 3 - 5.5%, oleic acid 20 - 40%, linoleic acid 40 - 70%, linolenic acid 0.5 - 1.5%.

Thyme essential oil obtained by hydrodistillation from the aerial parts of *Thymus common* L. variety "German Winter". The main components (over 2%) in the composition of which are: thymol (53.55%), γ- terpinene (11.13%), p-cymene (14.96%), borneol (2.75%), β-linalool (2.07%), and carvacrol (2.00%). It exhibits antioxidant activity against DPPH (165.91 mM TE.g^{-1}) and ABTS method (127.65 mM TE.g^{-1}) (Dobreva et al. 2024).

Raw cow's milk, supplied by the Academic Technological Complex of Thracian University – Stara Zagora, Bulgaria. The milk has the following parameters: dry non-fat residue 9.60±0.33%, proteins 3.33±0.08%, fats 3.60±0.10%, density 1.032±0.005 g.mL^{-1}, freezing point 0.580±0.030°C, pH=6.5. Raw milk meets the organoleptic, chemical and microbiological parameters (Regulation No 853 2004).

Starter culture for Kashkaval cheese – YO - Activ RSF -736® direct vat set (DVS) (CHR Hansen, Denmark), containing mesophilic and thermophilic microflora (*Lactobacillus helveticus*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis*, *Streptococcus thermophilus*) according to the manufacturer's requirements.

Rennet enzyme – chymosin CHY - MAX® Supreme (CHR Hansen, Denmark) (0.25 mL.10 L^{-1} milk), according to the manufacturer's requirements.

Calcium dichloride – with an initial concentration of 33%, purchased from Biokom Trendafilov Ltd., Sliven, Bulgaria.

Methods

Kashkaval preparation. After grading the raw cow's milk, it is subjected to thermization at a temperature of 63 - 65°C with a holding time of 15 - 20 s. After cooling to a temperature of 32 - 34°C, DVS starter is added to the milk in an amount of 1.5 g.10 L^{-1} of milk, calcium dichloride in an amount of 3 mL.10 L^{-1}, previously diluted with water in a ratio of 1:10, and subjected to coagulation by milk-coagulating enzyme (with a strength of 1:70000) in an amount of 0.25 mL.10 L^{-1}, previously diluted with water in a ratio of 1:10. Coagulation occurs at the same temperature for 20 - 30 min until a dense coagulum is formed. The processes of cutting, cooking the curd and draining the whey follow. The curd is cut into pieces for chedarization, which takes place at 35 - 40°C for 60 - 90 min until pH reaches 5.1. The chedarized curd is subjected to cooking, stretching and salting (in 10 - 12% salt solution with titratable acidity up to 15°T) at a temperature of 70 - 72°C. The processes of molding (0.200 kg) follow, then removing the cheese from the molds, blowing at a temperature of 10°C for 2 - 3 days, application of coating of linoleic sunflower oil with essential oil of thyme by spraying and vacuuming. After coating application, the cheese samples were vacuum packaged in polyethylene bags in order to minimize oxygen exposure during storage. Vacuum packaging was applied to reduce oxidative deterioration, moisture loss, and contamination by aerobic spoilage microorganisms, while ensuring stable storage conditions throughout the experimental period. The cheese samples ripen at a temperature of 10 - 12°C for 45 days. Samples were stored at 4±1°C throughout the experimental period of 6 months.

Determination of chemical and physical parameters. Acid value was determined titrimetrically by method described in BSS EN ISO 660 (2020). Peroxide value was determined iodometrically by method decribed in BSS EN ISO 3960 (2017). Oil content was determined by the Gerber volumetric method described in ISO 19662 (2018). Dry matter was analyzed by gravimetric method described in ISO 5534 (2004). pH was measured potentiometrically with a pH meter 7110 WTW (Germany). Titratable acidity was determined titrimetrically by method described in BSS 1111 (1980).

Determination of metabolites in Kashkaval cheese. The content of polar and nonpolar metabolites was determined by GC/MS analysis with Agilent 7890A instrument (Agilent Technologies Inc., Santa Clara, California, USA) under the following parameters: HP-5 ms column, 30 m × 250 mm × 0.25 μm; temperature 130°C/4 min, 15°C/min to 240°C for 3 min; helium as a carrier at a constant rate of 0.8 mL/min; split mode of 50:1. Retention times for the respective metabolites were compared to a reference library according to Golm Metabolome Database (Hummel et al. 2010) and the National Institute of Standards and Technology libraries (Manion et al. 2015).

Calculation of lipid quality indices. The iodine value (IV) and saponification value (SV) were calculated from the fatty acid composition data obtained by GC analysis. The iodine value was calculated according to the degree of unsaturation of fatty acids, while the saponification value was estimated based on the molecular weights of the identified fatty acids (Chira et al., 2009).

Microbiological analysis. Sample preparation for microbiological analysis was performed according to BSS EN ISO 6887-5 (2020). The number of *Lactobacillus* ssp. was according to BSS ISO 7889 (2005). *Lactococcus* ssp. and *Streptococcus thermophilus* were evaluated according to the procedure of BSS ISO 9232 (2005). The number of viable microorganisms was calculated using selective synthetic nutrient media MRS (Merck) and M 17 (Merck), respectively. Yeast and mold samples were inoculated into YCG (Merck) according to BSS EN ISO 6611 (2006).

Sensory analysis. It was conducted according to BSS 15612 (1983).

Statistical analysis. All experiments were performed in triplicate, with values in the tables averaged, and represented with their mean and standard deviation (SD). The experimental data were subjected to statistical evaluation using analysis of variance (ANOVA) with Statgraphics Centurion XVI Version 16.2.04 software (StatPoint Technologies, Inc., USA), and the significance was defined at $p < 0.05$. The analyses were performed after the standard ripening period (45 days), which was considered the initial evaluation point of the cheese samples.

Results and Discussion

To determine the influence of the mixture of sunflower oil and thyme essential oil on the storage of Kashkaval, two oil mixtures were used: sunflower oil linoleic chemotype and 0.1% thyme essential oil; sunflower oil linoleic chemotype and 0.2% thyme essential oil. The essential oil is chemotype thymol (thymol content 53.55%) (Dobreva et al. 2024).

After obtaining Kashkaval (Fig. 1), the samples were treated with the oil mixtures and vacuum-packed to prevent drying, humidification, contamination and stored under refrigerated conditions (4 ± 2°C) until analysis. Some physical and chemical parameters of the oil mixtures and Kashkaval samples were determined. It was also analyzed in terms of chemical composition, microbiological and sensory characteristics on the 45th day of production (after ripening), on the third and sixth month of storage.



Figure 1. Photographic image of the Kashkaval cheese samples (photo is copyrighted)

Physical and chemical characteristics.The chemical characteristics of the oil mixtures - acid and peroxide value , which are directly indicative of the quality and stability, have been determined (Table 1). The obtained low values indicate the high quality and oxidative stability of the vegetable oil blends (Farshoosh and Hoseini-Yazdi 2014; Wasowics et al. 2004).

The data on some physical and chemical indicators of Kashkaval (Table 2) indicate that the fat content shows a tendency to slightly decrease or stabilize depending on the amount of added thyme essential oil. In the control sample, the fat content decreased from 30.10% (at day 45) to 27.00% (at month 6).

The main reason for the change in fat content is the changes in lipolysis due to thyme essential oil and its main active ingredients thymol and carvacrol. In the samples with thyme, the fat content decreased less compared to the control, probably because the essential oil acts as a stabilizer of the fat phase. During ripening, Kashkaval loses some of its moisture, which leads to a change in the ratio between the components. A significant decrease in pH was observed in all samples by the 6th month. The control sample reached a value of 5.36, while the samples with thyme essential oil showed lower values – 5.10 (at 0.1%) and 4.86 (at 0.2%).

Table 1. Change in the acid and peroxide value of oil mixtures

Indicators	Control sample			Oil blend with 0.1% thyme essential oil			Oil blend with 0.2% thyme essential oil		
	45 days	3 months	6 months	45 days	3 months	6 months	45 days	3 months	6 months
Acid value, mgKOH.g^{-1}	0.20^{a}±0	0.28^{b}±0	0.30^{b}±0	0.22^{a}±0	0.28^{b}±0	0.30^{b}±0	0.19^{a}±0	0.23^{a}±0	0.25^{b}±0
Peroxide value, meqO_2.kg^{-1}	0.23^{a}±0	0.26^{b}±0	0.30^{b}±0	0.15^{c}±0	0.16^{c}±0	0.17^{c}±0	0.13^{c}±0	0.15^{c}±0	0.16^{c}±0
Saponification value*, mg KOH.g^{-1}	241	240	240	243	242	241	242	240	240
Iodine value, gI_2.100 g^{-1}**	35	36	35	36	37	36	36	38	36

$^{a, b, c}$ - indices showing significant differences ($p < 0.05$) between the mean values in the rows
*Computed from GC data;
**Computed from the GC data according to Chira et al. (2009)

This suggests that thyme essential oil can stimulate or support acid-forming processes during longer storage. In parallel with the decrease in pH, the titratable acidity increased progressively. At the end of the 6th month, the highest acidity was recorded in the sample with 0.2% thyme (240°T), followed by the sample with 0.1% (230°T) both exceeding the control (208°T). The observed preservative effect of thyme essential oil may be associated with the biological activity of thymol, which represents its major phenolic component. Thymol possesses strong antimicrobial activity due to its hydrophobic structure, which enables interaction with microbial cell membranes, leading to increased membrane permeability and leakage of intracellular constituents. In addition, thymol exhibits antioxidant activity through free radical scavenging and inhibition of lipid oxidation processes. These mechanisms contribute to the preservation of cheese quality and microbiological stability during storage. This is in full accordance with the data of other authors who performed surface treatment of Kasar cheese with thyme and clove essential oil fortified edible films (Kavas et al. 2015). The observed changes in the values of these indicators can be explained by the influence of the essential oil, especially its main component thymol (Burt 2004). The iodine values of the treated samples were slightly higher due to the increased proportion of unsaturated fatty acids contributed by sunflower oil, particularly linoleic acid. The saponification values showed only minor variation among treatments, indicating relatively small differences in the average molecular weight distribution of fatty acids. No major variations were observed regarding the saponification and iodine values in the control and thyme added Kashkaval samples across maturation; the obtained valued are consistent with typical saponification and iodine values for cheeses (Ivanova et al. 2022).

Chemical composition

Fatty acids. The Kashkaval samples were analyzed for fatty acid composition, organic and amino acid content during their storage. The change in fatty acids depending on the amount of essential oil and storage time is presented in Table 3. The obtained data show that the three types of samples are characterized by a similar fatty acid profile, but differ in some specific indicators. Saturated fatty acids such as palmitic, myristic and stearic acids dominate the profile, which is typical for hard types of cheese (Sadvari et al. 2025). Monounsaturated fatty acids, especially oleic and vaccenic, increase slightly during surface treatment, and this trend is valid for the entire storage period. The higher content of vaccenic acid in surface-treated cheeses is favorable, since it is a precursor of conjugated linoleic acid, which is characterized by a number of benefits for human health (Ivanova 2021). Higher values of oleic acid were found in samples of Tulum cheese packed in a natural small intestine bag (Klisović et al. 2020). In both cases, this indicates that the surface treatment or packaging has an effect and acts as a barrier to oxygen, which modifies the microbiological activity and, consequently, the metabolic profile of the product.

Table 2. Changes in physical and chemical parameters of Kashkaval

Indicators	Control sample			Kashkaval with 0.1% thyme essential oil			Kashkaval with 0.2% thyme essential oil		
	45 days of ripening	3 months of storage	6 months of storage	45 days of ripening	3 months of storage	6 months of storage	45 days of ripening	3 months of storage	6 months of storage
Fat content, %	$30.10^{a}\pm 0.2$	$28.60^{a}\pm 0.1$	$27.00^{b}\pm 0.3$	$30.40^{a}\pm 0.2^{b}$	$28.80^{a}\pm 0.1$	$28.60^{a}\pm 0.2$	$30.60^{a}\pm 0.1$	$28.90^{a}\pm 0.3$	$29.06^{a}\pm 0.2$
Dry matter, %	$62.52^{a}\pm 0.3$	$60.54^{b}\pm 0.3$	$60.82^{b}\pm 0.1$	59.35 ± 0.1	$59.08^{b}\pm 0.3$	$59.38^{b}\pm 0.3$	$60.1^{b}\pm 0.3$	$60.39^{b}\pm 0.2$	$61.23^{a}\pm 0.1$
pH	$5.68^{a}\pm 0.1$	$5.36^{a}\pm 0.1$	4.80^{b} 0.2	$5.36^{a}\pm 0.1$	$5.18^{b}\pm 0.2$	$5.10^{c}\pm 0.2$	$5.28^{b}\pm 0.2$	$5.04^{c}\pm 0.2$	$4.86^{c}\pm 0.2$
Titratable acidity, °T	$160^{a}\pm 1.2$	$180^{b}\pm 1.3$	$208^{c}\pm 2.0$	$162^{a}\pm 1.0$	$184^{b}\pm 1.3$	$230^{c}\pm 2.1$	$164^{a}\pm 1.1$	$182^{b}\pm 1.3$	$240^{c}\pm 2.2$

$^{a, b, c}$ - indices showing significant differences ($p < 0.05$) between the mean values in the rows

Polyunsaturated fatty acids such as linoleic and linolenic acids remain relatively stable. This is in disagreement with the data obtained by other authors, who found that the amount of these two fatty acids decreases during the storage of cheese in olive oil (Klisović et al. 2020). In other studies that followed the influence of natural small intestine packaging of Tulum samples, it even increased (Tomar et al. 2020). These differences are probably due to the application of different technological approaches (Najera et al. 2021). The ω-3:ω-6 ratio did not change significantly throughout the storage period for all the samples studied (around 1:6 - 1:7).

Although this ratio is below the optimal (up to 4:1), it is close to and significantly better than the average for the modern diet (about 15:1 to 20:1) (Simopoulos 2002). The ratio of saturated:unsaturated fatty acids was 4.82:1 in the control, 6.11:1 in the cheese with 0.1% thyme essential oil and 4.32:1 for the sample with 0.2% thyme essential oil. This indicates that the addition of thyme essential oil has a specific, nonlinear effect on this ratio.

The higher relative proportion of saturated fatty acids at the low concentration of thyme essential oil (0.1%) can be explained by partial lipolysis or insufficient antioxidant capacity to stabilize reactive unsaturatedbonds at this dose. In contrast, at a concentration of 0.2% thyme essential oil, the ratio improves significantly (4.32:1) even compared to the control. This effect is in line with the studies of Zantar et al. (2014) which indicate that the phenols thymol and carvacrol, main components of essential oils, act as powerful antioxidants that effectively "protect" polyunsaturated fatty acids from oxidative degradation.

The lower ratio of saturated fatty acids:unsaturated fatty acids in the sample with 0.2% thyme essential oil suggests that the higher dose of oil provides better lipid stability, preserving biologically valuable unsaturated fats. From a dietary point of view, this result is favorable, as it brings the profile of the cheese closer to the recommended intake of unsaturated fats, counteracting the high levels of saturated fatty acids typical of the modern diet (Simopoulos 2002).

Short-chain fatty acids were present in higher amounts in samples with thyme oil addition, especially in the sample with a higher amount of essential oil (0.2%). They have proven antimicrobial properties and significantly affect the flavor profile of the product (Bandic 2023; Kadry et al. 2023). Medium-chain fatty acids followed a similar trend to that of short-chain fatty acids. Long-chain fatty acids were slightly lower in the control and similar in samples with thyme essential oil addition. This suggests that the oil may inhibit certain biohydrogenation processes or help preserve these fatty acids. Similar effects of essential oils on the fermentation and biohydrogenation of fatty acids have been found in other studies, which show that the addition of essential oils (including thyme) alters microbial metabolism and lipid profile by inhibiting specific microbial populations (Günal et al. 2013; Vassiliou et al. 2023).

The change in the amount of cholesterol is presented in Fig. 2. The data indicate that the amount of cholesterol in the samples treated with an oil mixture of sunflower oil and thyme essential oil (0.1% and 0.2%) was lower than that in the control sample up to 3 months of storage. It is known that daily intake of large amounts of foods with high cholesterol content, such as some types of cheese and other dairy products, in which the content of polyunsaturated to saturated fatty acids is low (Kinik et al. 2005), can affect health (Dashti et al. 2003).

Therefore, more rational diets are sought or the inclusion of functional ingredients of plant origin in the composition of dairy products, which act as antioxidants. They reduce oxidative damage to blood vessels and reduce cholesterol content (Güney 2020). The values obtained for cholesterol are close to those traditionally found for similar cheeses on the Balkan Peninsula (Andrikopoulos et al. 2018; Asya Öz 2018). Usually its amount decreases in hard types of cheese that have undergone a long period of maturation and storage (Kisza and Juśkiewicz 1998).

The data in Fig. 2 show that its decrease is dynamic during storage, but a greater decrease was recorded in the presence of thyme essential oil in an amount of 0.2%. In the control samples, a slight increase (month 3) was observed, followed by a decrease in cholesterol (month 6). In the samples with 0.1%

Table 3. Change in the amount of fatty acids in Kashkaval**,** g.100 g^{-1}.

Fatty acids	Control sample			Kashkaval with 0.1% thyme essential oil			Kashkaval with 0.2% thyme essential oil		
	45 days of ripening	3 months of storage	6 months of storage	45 days of ripening	3 months of storage	6 months of storage	45 days of ripening	3 months of storage	6 months of storage
Butyric ($C_{4:0}$)	1.57^{a}±0.1	1.30^{b}±0.1	1.09^{c}±0.1	2.22^{d}±0.2	1.28^{b}±0.1	1.38^{b}±0.1	1.47^{a}±0.1	2.03^{d}±0.2	1.42^{b}±0.1
Caproic ($C_{6:0}$)	1.05^{a}±0.1	0.85^{b}±0	0.73^{b}±0	1.49^{c}±0.1	0.86^{b}±0	0.93^{a}±0	0.99^{a}±0	1.37^{c}±0.1	0.95^{a}±0
Capric ($C_{8:0}$)	0.62^{a}±0	0.50^{b}±0	0.43^{c}±0	0.88^{d}±0	0.51^{b}±0	0.55^{b}±0	0.58^{a}±05	0.80^{d}±0	0.56^{b}±0
Caprinic $C_{10:0}$)	1.10^{a}±0.1	0.84^{b}±0	0.76^{c}±0	1.55^{d}±0.1	0.89^{b}±0	0.97^{a}±0	1.03^{a}±0.1	1.42^{d}±0.1	0.99^{a}±0
Lauric ($C_{12:0}$)	1.26^{a}±0.1	1.00^{b}±0.1	0.88c±0	1.78^{d}±0.1	1.03^{b}±0.1	1.11^{b}±0.1	1.18^{b}±0.1	1.63^{d}±0.1	1.14^{b}±0.1
Myristic ($C_{14:0}$)	5.15^{a}±0.4	4.22^{b}±0.4	3.59^{c}±0.3	7.27^{d}±0.6	4.20^{b}±0.3	4.38^{b}±0.4	4.66^{b}±0.4	6.68^{d}±0.5	4.66^{b}±0.4
Palmitic ($C_{16:0}$)	10.72^{a}±0.9	8.50^{b}±0.8	7.48^{c}±0.6	15.14^{d}±1.2	8.75^{b}±0.7	9.13^{a}±0.7	9.70^{a}±0.8	12.85^{e}±1.0	9.71^{a}±0.8
Stearic ($C_{18:0}$)	4.29^{a}±0.3	3.66^{b}±0.3	2.99^{c}±0.2	6.06^{d}±0.5	3.50^{b}±0.3	3.65^{b}±0.3	3.88^{b}±0.3	4.51^{a}±0.4	3.88^{b}±0.3
Oleic ($C_{18:1}$)	3.45^{a}±0.3	3.50^{a}±0.3	3.77^{a}±0.3	4.24^{b}±0.3	5.41^{c}±0.4	5.00^{d}±0.4	5.06^{d}±0.4	5.25^{d}±0.4	4.90^{d}±0.4
Vaccenic ($C_{18:1\ trans`11}$)	0.80^{a}±0	0.75^{a}±0	0.52^{b}±0	0.70^{c}±0	0.43^{d}±0.	0.59^{b}±0	0.56^{b}±0	0.57^{b}±05	0.68c±0
Linoleic ($C_{18:2\ 2n6c}$)	0.99^{a}±0	0.89^{b}±0	0.67^{c}±0	0.93^{a}±0	0.76^{d}±0	0.84^{b}± 0	0.86^{b}±0	0.88^{b}±0	0.87^{b}±0
Linolenic ($C_{18:0\ 3n3}$)	0.15^{a}±0	0.12^{b}±0	0.10^{b}±0	0.14^{a}±0	0.12^{b}±0	0.13^{b}±0	0.13^{b}±0	0.14^{b}±0	0.13^{b}±0
Arachidonic ($C_{20:0}$)	0.13^{a}±0	0.10^{a}±0	0.09^{a}±0	0.18^{b}±0	0.10^{a}±0	0.11^{a}±0	0.12^{a}±0	0.17^{b}±0	0.12^{a}±0
Behenic ($C_{22:0}$)	0.11^{a}±0	0.09^{b}±0	0.07^{c}±0	0.15^{d}±0	0.09^{b}±0	0.09^{b}±0	0.10^{b}±0	0.14^{d}±0	0.10^{b}±0
SCFA*	2.62	2.15	1.82	3.71	2.14	2.31	2.46	3.40	2.37
MCFA	2.98	2.34	2.08	4.20	2.43	2.62	2.78	3.86	2.70
LCFA	20.39	16.57	14.22	28.80	16.64	17.36	18.45	24.35	18.47
SFA	26.00	21.06	18.11	36.72	21.21	22.30	23.71	31.60	21.16
MUFA	4.25	4.25	4.30	4.94	5.84	5.59	5.62	5.82	5.58
PUFA	1.14	1.01	0.77	1.07	0.88	0.97	0.99	1.02	1.01

*SCFA – short chain fatty acids; MCFA – medium chain fatty acids; LCFA – long chain fatty acids; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA - polyunsaturated fatty acids.

$^{a, b, c, d}$ - indices showing significant differences ($p < 0.05$) between the mean values in the rows

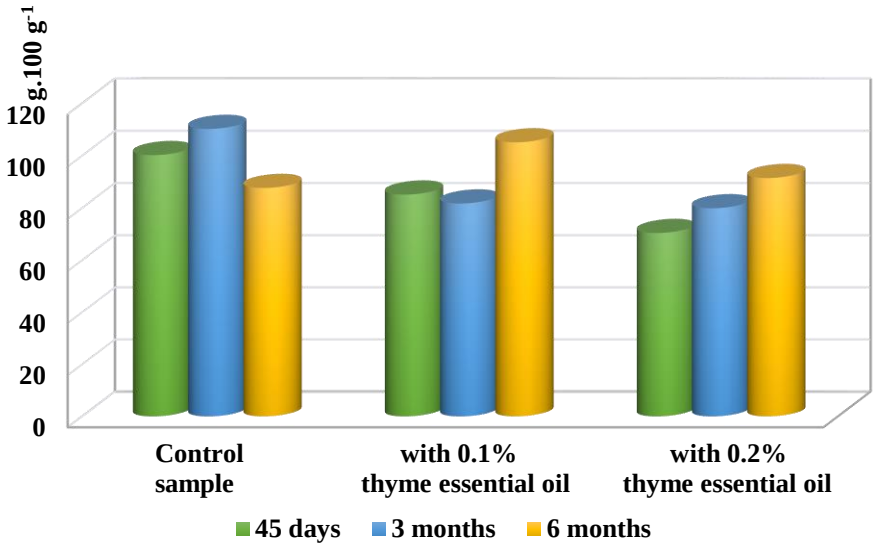


Figure 2. Change in cholesterol during ripening and storage and amount of thyme essential oil

essential oil, the opposite trend was observed – t he cholesterol content slightly increased at month 6, which may be due to the limitation of microbial activity and biochemical

transformations responsible for lipid changes. In the samples with 0.2% essential oil, cholesterol remained relatively stable throughout the period, showing less dynamics compared to the control.

This suggests that the surface treatment with thyme essential oil has a stabilizing effect on cholesterol, probably through its antimicrobial action, which limits microbial metabolic transformations of lipids during storage.

Organic acids. The change in the amount of organic acids is presented in Table 4. The highest content of lactic, oxalic, formic and citric acids was observed in both the control and the samples treated with oil mixtures. In all samples, a decrease in the amount of organic acids was observed during storage. Organic acids in fermented dairy products originate from normal biochemical metabolism (citric, orotic, uric and hippuric), bacterial growth (lactic, acetic, pyruvate, propionic and formic), hydrolysis of milk fats by lactic acid bacteria (e.g. free fatty acids such as acetic and butyric), as well as from their addition for technological purposes (e.g. citric and lactic) (Güler 2014; Silva et al. 2023). As a result of the vital activity of lactic acid microorganisms, various organic acids are produced, which have a direct impact on the taste and aroma of the finished product, but also affect the shelf life of the product (Andic et al. 2011). In the samples we studied, pyruvic, lactic, oxalic, uric, acetic, formic, hippuric and citric acid were found. This indicates that the surface treatment of the cheese affects the organic acid profile. A similar trend was found by other authors who produced Kasar cheese (Pasta-filata type), which was matured and stored under vacuum conditions and without packaging (Andic et al. 2011).

The results obtained show that lactic acid was found in the highest amounts, followed by oxalic, formic and citric acid. Data from other authors was found that confirm this trend, although there is no complete overlap in the organic acid profile, due to differences in the technological processes of the cheese types produced (Akarca et al. 2025). These authors found that the organic acid profile is affected both by the surface treatment of Kasar cheese with beeswax and by whether another type of oil is added – from black cumin and sesame.

In the present study, pyruvic acid decreased in all three samples studied. This is expected, since it is an intermediate metabolite in a number of biochemical transformations (Silva et al. 2023). Lactic acid is a major product of lactose fermentation, but during cheese ripening it is a substrate for subsequent chemical reactions, which is why its amount is significantly depleted already in the initial stages of storage (Atasever and

Mazlum 2024). This is in accordance with the study by Güler (2014), who found a similar profile of organic acids in Surk cheese (acid cheese) enriched with spices. Oxalic acid decreases during storage, reaching constant values. Citric acid is essential in the formation of the taste and aroma of cheese. In the present study, its amount decreases at the beginning of the storage period, then reaches constant values. This is probably due to the fact that the cheese is stored under vacuum with a specific surface treatment. The explanation for the difference with the data of other authors (Andic et al. 2011), who found a slight increase in this organic acid. This is probably due to differences in storage temperature as well as the fact that during the storage period a different amount and type of substrate for biochemical transformations was formed. Lactic, acetic and formic acids were found in higher amounts in cheeses with surface treatment with sunflower oil and slightly increased with increasing concentration of thyme essential oil.

This is in correlation with the studies of other authors who found similar trends for steamed cheeses stored under vacuum compared to others that were not packaged (Andic et al. 2011). This may be due to the fact that increasing the concentration of thyme essential oil enhances its antimicrobial effect, associated with the presence of phenolic compounds such as thymol and carvacrol (Dobreva et al. 2024). It has been established that they disrupt the integrity of cell membranes and suppress the enzymatic activity of microorganisms, limiting the intensity of fermentation processes by unwanted microflora (Burt 2004; Fox et al. 2017). Hippuric and uric acids are traditionally found in lower values in cheeses during their storage (Güler 2014; Fernandez-Garcia and McGregor (1994)). Their presence is probably due to the starter culture, which is traditionally used to make yogurt. Again, the trend is observed, which shows that in unprocessed Kashkaval the dynamics in the amount of these organic acids is more significant, while in cheeses with surface treatment this amount is smaller. The explanation for this is the change in the surface microenvironment in the processed cheeses, which further limits gas exchange and metabolic activity of the microflora and leads to a lower intensity of fermentation processes and the formation of organic acids compared to the control.

Amino acids. The change in the amount of amino acids is presented in Table 5. The data show that the amount of free amino acids in all samples decreases during storage, with the most significant decrease in the control. The results in Table 5 show a clear decrease in the amount of free amino acids in all samples with increasing storage period. This trend is most pronounced in the control. The decrease in their amount is probably due to ongoing microbiological and enzymatic processes during ripening, including decarboxylation, deamination and use of amino acids as a substrate for secondary metabolic reactions. In cheeses with added thyme essential oil (0.1% and 0.2%), a smaller decrease in most amino acids was observed, as well as higher values of essential amino acids compared to the control sample after 6 months of storage. This suggests that thyme essential oil exerts a specific effect on the proteolytic activity of the microflora and limits the degradation and subsequent metabolization of amino acids. Despite the limited number of studies in the field, data on similar types of studies are found, which show that the levels of amino acids are lower compared to the control (Erkaya-Kotan et al. 2023). These differences are likely due to the significantly higher levels of thyme oil applied in these samples - quince seed gel containing 0.5% to 2% thyme oil.

The higher degree of preservation of lysine, leucine, valine and phenylalanine in the treated samples indicates a high biological value of the product. Probably, the limited access of oxygen to the protein matrix also has an impact on the biochemical transformations of amino acids. The effect is similar for both applied concentrations of essential oil.

Microbiological analysis. The change in the values of microbiological indicators is presented in Fig. 3, 4 and 5. The changes in populations of lactic acid bacteria (LAB) in Kashkaval cheese in the presence of sunflower oil and thyme oil have not been previously studied. In this study, the determination of LAB counts in cheese samples was carried out to evaluate whether sunflower oil and thyme oil have a negative effect on these bacteria. The microbial changes during six months of storage of Kashkaval cheese surface-treated with sunflower oil and thyme essential oil are shown in Fig. 3 and 4. The count of *Lactobacillus* spp. in Kashkaval cheese samples was affected by the duration of storage and the concentration of thyme oil in the oil mixture used.

The results (Fig. 3) show that at the beginning of storage (45 days and 3rd months), samples with 0.1% thyme essential oil and with 0.2% thyme essential oil were characterized by an increased count of *Lactobacillus* spp. (from 7.7 to 8.3 log CFU.g^{-1}) compared to the control sample (around 6.8 log CFU.g^{-1}). This is probably due to the stimulating effect of sunflower oil and thyme essential oil, or to their provision of an additional source of fatty acids. At the 6th month of storage, all samples were characterized by a slight decrease in the count of *Lactobacillus* spp.

However, the treated samples maintained higher values (7.7 log CFU.g^{-1} for sample with 0.1% thyme essential oil and 7.5 log CFU.g^{-1} for sample with 0.2% thyme essential oil) compared to the control sample (6.2 log CFU.g^{-1}). These results are in agreement with findings from other studies, which show that the incorporation of some herbs, including thyme, into yogurts increased the count of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* during the storage period (El-nawawy et al. 1998). Gann (2013) reported that minimum inhibitory concentration (MIC) of thyme oil against some species of LAB was found to be 0.2%, which is consistent with the maximum concentration of thyme oil used in the present study (0.2%). In another study, it was reported that concentrations of 0.03% thyme oil were sufficient to inhibit *S. aureus* and *E. coli* (Hammer et al. 1999).

In the present study, the changes in the count of *Lactococcus* spp. and *Streptococcus* spp. (log CFU.g^{-1}) in Kashkaval cheese surface-treated with sunflower oil during storage (45 days, 3, and 6 months) are presented in Fig. 4. At the beginning of storage, the control sample showed the lowest count of *Lactococcus* spp. and *Streptococcus* spp. (6.8 log CFU.g^{-1}), while samples with 0.1% thyme essential oil and with 0.2% thyme essential oil exhibited higher values (8.1 and 7.7 log CFU.g^{-1}, respectively). These findings suggest that the applied treatment (surface oiling) does not exert an inhibitory effect on the starter culture but probably contributes to better preservation of bacterial viability during the early stage of storage. These results are comparable with data obtained from other studies (Akarca et al. 2025; Ivanov and Markova 2020). In the current study, the counts of *Lactococcus* spp. and *Streptococcus* spp. in the control sample decreased slightly, reaching 6.6 log CFU.g^{-1} during the storage period. On the other hand, sample with 0.1% thyme essential oil showed an initial increase until the third month, followed by a moderate decrease towards the end of the storage period. Sample with 0.2% thyme essential oil was characterized by a high degree of stability, with no significant changes in the microbial population throughout the storage period. These differences in microbial dynamics were probably due to the surface oil treatment, which may have reduced surface dehydration and produced more stable conditions in terms of water activity and oxygen access. This could help the survival of starter bacteria in the early stages of storage while stabilizing the population over the long storage period. By the sixth month, all samples reached similar levels of *Lactococcus* spp. and *Streptococcus* spp., indicating that surface treatment with sunflower oil and thyme oil mainly affects the rate of change in lactic acid bacteria counts rather than the final counts.

Changes in yeast and mold counts in the cheese samples throughout a six-month storage period are shown in Fig. 5. At the beginning of storage (45 days), the control sample and sample with 0.1% thyme essential oil were characterized by similar values (5.0 log CFU.g^{-1}), while sample with 0.2% thyme essential oil had a lower count (4.4 log CFU.g^{-1}). This decrease in the sample with 0.2% thyme essential oil may be associated with the higher concentration of oil and the antimicrobial effect against yeasts and molds. After 3 months of storage, an increase in the count of yeast and molds was observed in all samples. The lowest increase was recorded in sample with 0.2% thyme essential oil (4.5 log CFU.g^{-1}), while in control and in sample with 0.1% thyme essential oil the values reached 5.3 and 5.4 log CFU.g^{-1}, respectively. After 6 months of storage, an increase of yeast and mold counts in all samples was observed (Fig. 5). The control sample reached the highest value (6.5 log CFU.g^{-1}), followed by sample with 0.1% thyme essential oil (6.4 log CFU.g^{-1}) and sample with 0.2% thyme essential oil (6.3 log CFU.g^{-1}). The presence of thyme essential oil may explain the lower yeast and mold counts observed in treated samples during storage.

Table 4. Change in the amount of organic acids in Kashkaval

Organic acids, mg.100 g^{-1}	Control sample			Kashkaval with 0.1% thyme essential oil			Kashkaval with 0.2% thyme essential oil		
	45 days of ripening	3 months of storage	6 months of storage	45 days of ripening	3 months of storage	6 months of storage	45 days of ripening	3 months of storage	6 months of storage
Pyruvic ($C_3H_40_3$)	22.4^{a}±1.8	16.4^{b}±1.0	11.9^{c}±0.9	20.1^{a}±1.6	15.6^{b}±1.2	16.8^{b}±1.3	19.a±1.5	16.9^{b}±1.3	15.5^{b}±1.2
Lactic ($C_3H_60_3$)	669.3^{a}±52.8	440.2^{b}±40.0	135.9^{c}±12.4	614.6^{a}±48.5	339.6^{b}±26.8	193.1^{c}±15.3	558.2^{d}±44.0	414.3^{b}±32.7	176.5^{c}±13.9
Oxalic($C_2H_20_4$)	117.7^{a}±9.3	95.0^{b}±9.0	62.6^{c}±4.9	106.0^{a}±8.4	82.0^{b}±6.5	88.1^{b}±7.0	103.0^{a}±8.1	88.9^{b}±7.0	81.4^{b}±6.4
Uric ($C_5H_4N_40_3$)	5.2^{a}±0.4	4.2^{b}±0.4	2.8^{c}±0.2	4.7^{a}±0.4	3.7^{c}±0.3	3.9^{c}±0.3	4.6^{b}±0.4	3.9^{c}±0.3	3.6^{c}±0.3
Acetic ($C_2H_40_3$)	48.2^{a}±3.8	27.1^{b}±2.0	16.4^{c}±1.3	39.9^{d}±3.2	25.4^{b}±2.0	19.4^{c}±1.5	40.8^{e}±3.2	27.1^{b}±2.1	21.4^{c}±1.7
Formic (CH_20_2)	86.4^{a}±6.8	69.1^{b}±5.4	45.9^{c}±3.7	77.8^{d}±6.1	60.1^{e}±4.8	64.9^{e}±5.1	75.5±^{d}6.0	65.2^{e}5.1	59.7^{e}±4.7
Hippuric ($C_9H_9N0_3$)	16.2^{a}±1.3	13.1^{b}±1.2	8^{c}±0.7	14.4^{d}±1.2	11.3^{b}±0.9	12.2^{b}±1.0	14.2^{b}±1.1	12.3^{b}±1.0	11.2^{b}±0.9
Citric ($C_6H_80_7$)	64.6^{a}±6.0	44.5^{b} ±4.1	34.4^{c}±2.7	58.2^{d}±4.6	45.1^{b}±3.6	48.6^{b}±3.9	56.5^{d}±4.5	48.8^{b}±3.9	44.6^{c}±2.7

$^{a, b, c, d, e}$ - indices showing significant differences ($p < 0.05$) between the mean values in the rows

Table 5. Change in the amount of amino acids in Kashkaval

Amino acids, mg.g^{-1}	Control sample			Kashkaval with 0.1% thyme essential oil			Kashkaval with 0.2% thyme essential oil		
	45 days of ripening	3 months of storage	6 months of storage	45 days of ripening	3 months of storage	6 months of storage	45 days of ripening	3 months of storage	6 months of storage
Leucine*	4.60^{a}±0.4	4.10^{b}±0.4	2.45^{c}±0.2	4.14^{b}±0.3	3.20^{d}±0.3	3.18^{d}±0.3	4.02b±0.3	3.47^{e}±0.3	3.18^{d}±0.3
Isoleucine*	1.87^{a}±0.2	1.70^{b}±0.1	1.00^{c}±0.1	1.69^{b}±0.1	1.30^{d}±0.1	1.41^{e}±0.1	1.64^{b}±0.1	1.41^{e}±0.1	1.29^{d}±0.1
Methionine*	0.14^{a}±0	0.12^{a}±0	0.08^{b}±0	0.13^{a}±0	0.10^{b}±0	0.11^{b}±0	0.13^{a}±0	0.11^{b}±0	0.10^{b}±0
Phenylalanine*	5.29^{a}±0.4	4.11^{b}±0.4	2.81^{c}±0.2	4.76^{d}±0.4	3.68^{e}±0.3	3.97^{b}±0.3	4.62^{e}±0.4	3.99^{b}±0.3	3.65^{e}±0.3
Lysine*	8.93^{a}±0.7	6.41^{b}±0.6	4.75^{c}±0.4	8.04^{a}±0.6	6.22^{b}±0.5	6.71^{b}±0.5	7.81^{c}±0.6	6.74^{b}±0.5	6.17^{b}±0.5
Threonine*	2.19^{a}±0.2	1.80^{b}±0.1	1.17^{c}±0.1	1.97^{d}±0.1	1.53^{e}±0.1	1.65^{e}±0.1	1.92^{d}±0.1	1.66^{e}±0.1	1.52^{e}±0.1
Tyrosine*	0.23^{a}±0	0.17^{b}±0	0.13^{c}±0	0.21^{a}±0	0.16^{b}±0	0.18^{b}±0	0.21^{a}±0	0.18^{b}±0	0.16^{b}±0
Valine*	2.99±0.2	2.50±0.2	1.59±0.1	2.69±0.2	2.08±0.2	2.25±0.2	2.61±0.2	2.26±0.2	2.07±0.2
Alanine	5.50^{a}±0.4	4.00^{b}±0.3	2.92^{c}±0.2	4.95^{d}±0.4	4.81^{d}±0.4	3.83^{b}±0.3	4.81^{d}±0.4	4.15^{b}±0.3	3.80^{b}±0.3
Glycine	0.95^{a}±0	0.70^{b}±0	0.51^{c}±0	0.86^{d}±0	0.66^{e}±0	0.72^{b}±0	0.83^{d}±0	0.72b±0	0.66^{e}±0
Tryptophan	0.30^{a}±0	0.25^{b}±0	0.16^{c}±0	0.27^{b}±0	0.21^{d}±0	0.26^{b}±0	0.26^{b}±0	0.23^{d}±0	0.21^{d}±0
Serine	3.33^{a}±0.2	2.50^{b}±0.2	1.77^{c}±0.1	3.00^{d}±0.2	2.32^{e}±0.1	2.50^{e}±0.2	2.91^{d}±0.2	2.51^{e}±0.2	2.30^{e}±0.2
Aspartic acid	0.17^{a}±0	0.15^{a}±0	0.09^{b}±0	0.15^{a}±0	0.12^{c}±0	0.13^{c}±0	0.15^{a}±0	0.13^{c}±0	0.12^{c}±0
Arginine	2.09^{a}±0.2	1.55^{b}±0.1	1.11^{c}±0.1	1.88^{d}±0.1	1.45^{e}±0.1	1.57^{b}±0.1	1.83^{d}±0.1	1.58^{b}±0.1	1.44^{e}±0.1
Ornithine	0.20^{a}±0	0.18^{a}±0	0.10^{b}±0	0.18^{a}±0	0.14^{c}±0	0.15^{c}±0	0.17^{a}±0	0.15^{c}±0	0.14^{c}±0
Glutamine	0.33^{a}±0	0.29^{b}±0	0.17^{c}±0	0.29^{b}±0	0.23^{d}±0	0.25^{d}±0	0.29^{b}±0	0.25^{d}±0	0.23^{d}±0
Glutamic acid	4.65^{a}±0.4	4.13^{b}±0.4	2.47^{c}±0.2	4.18^{b}±0.3	3.24^{d}±0.3	3.49^{d}±0.3	4.06^{b}±0.3	3.24^{d}±0.3	3.21^{d}±0.3

* essential amino acid; $^{a, b, c, d}$ - indices showing significant differences ($p < 0.05$) between the mean values in the rows

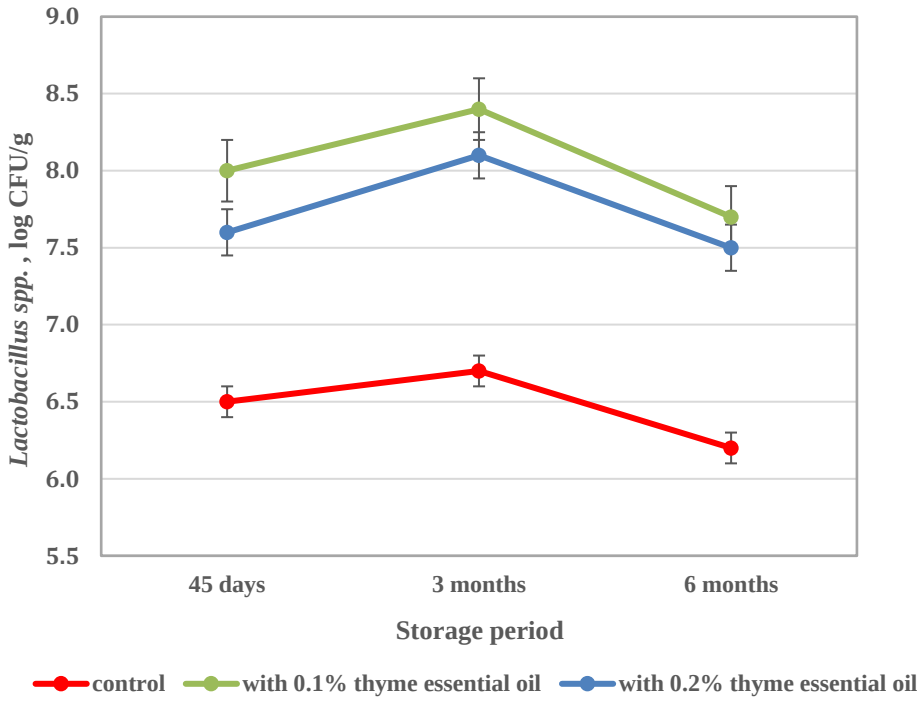


Figure 3. Changes in *Lactobacillus spp.* count of Kashkaval cheese surface-treated with sunflower oil and thyme oil during ripening and storage period

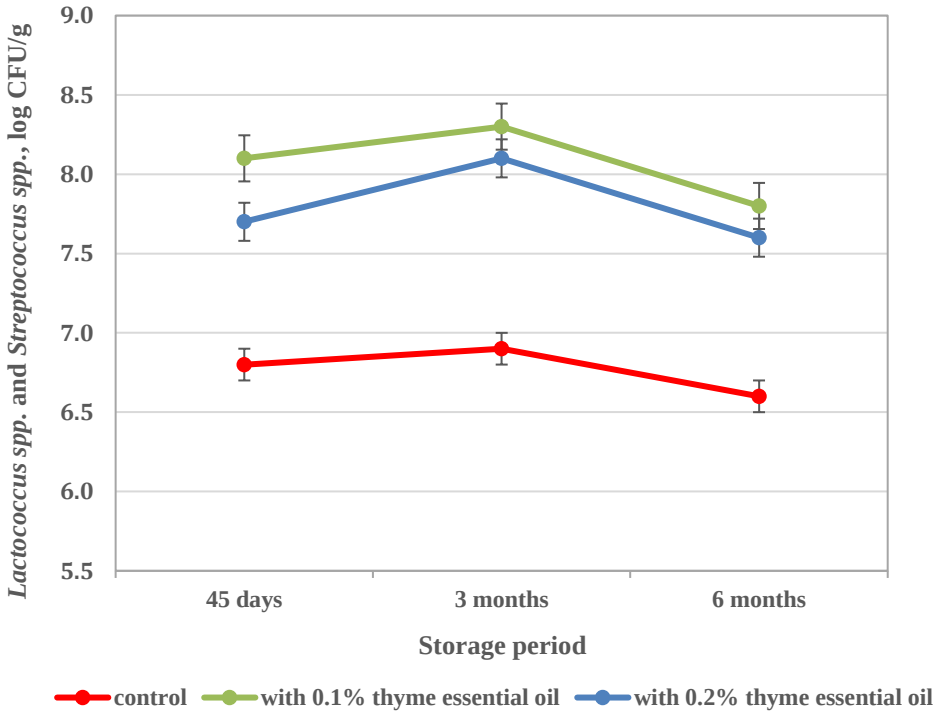


Figure 4. Changes in *Lactococcus spp. and Streptococcus spp.* count of Kashkaval cheese surface-treated with sunflower oil and thyme oil during ripening and storage period

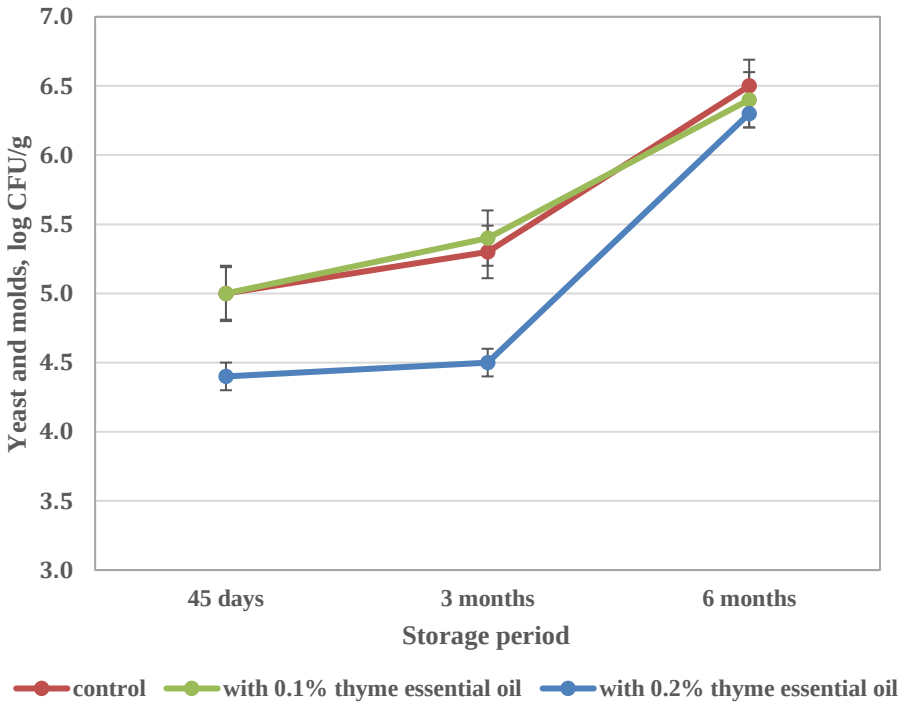


Figure 5. Changes in yeast and molds count of Kashkaval cheese surface-treated with sunflower oil and thyme oil during ripening and storage period

In the study reported by Erkaya-Kotan et al. (2023), it was shown that the coating of Kaşar cheese with quince seed mucilage and the increased levels of thyme oil significantly suppress mold growth throughout the ripening. The authors reported that until the 120th day of ripening, counts of yeasts and molds were lower than 2 log CFU.g^{-1} in the treated cheese sample with quince seed mucilage containing 2% TO. The findings are consistent with the results of the current study. The reported lower count of yeast and molds is probably due to the higher concentration of thyme oil (2%) compared to the 0.2% used in the present study.

Sensory evaluation. The sensory profiles of the controls and Kashkaval cheese samples depending on the amount of thyme essential oil are presented in Fig. 6, 7 and 8. Sensory characteristics data show that Kashkaval treated with an oil mixture with 0.1% thyme essential oil has similar values to those of the control. The appearance, taste and aroma are preserved, while being enriched by the added essential oil. The sample with 0.2% thyme essential oil was rated lower on all indicators, which is explained by its strong odor at a higher concentration. Similar results were also obtained by Martial et. al. (2016), who added oregano essential oil to enrich fresh cheese. Some researchers even recommend the limited use of essential oils in dairy products due to their influence on organoleptic properties, especially taste (Samah and Ahmed 2019). During storage of the samples, a uniform decrease in the scores for all sensory characteristics was observed. The slight increase in yellow coloration observed in treated cheeses may be associated with the natural pigments present in sunflower oil and thyme essential oil. In addition, the herbal aroma detected by panelists was likely related to volatile phenolic compounds, particularly thymol, which is the major aromatic constituent of thyme essential oil. At higher concentrations of thyme essential oil, the herbal aroma became more pronounced, which influenced the sensory perception of the samples. The color and surface of the sample changed, and deviations from the typical aroma-taste complex of ripe Kashkaval cheese occurred. The reason for this was the higher concentration of thyme oil in the oil mixture. The more intense aroma of the oil dominated the typical milky taste of Kashkaval At higher These results are in accordance with the data of Metwalli (2011).

According to Lopez-Cordova (2021), potato starch coatings and the flavoring agent carvacrol can be used as a natural preservation method for hard cheeses , improving the perception of taste and texture among consumers.

The data from the sensory characteristics conducted correspond to those for the content of organic acids. The values of lactic acid (Table 4) in the samples with essential oil, regardless of the amount, after 6 months of storage are significantly lower compared to those after the 45th day. This explains the lower scores for structure and consistency, as acidity is key to the texture of the cheese. The content of acetic acid also decreases after 6 months of storage, which also contributes to the less pronounced typical aroma of ripe cheese.

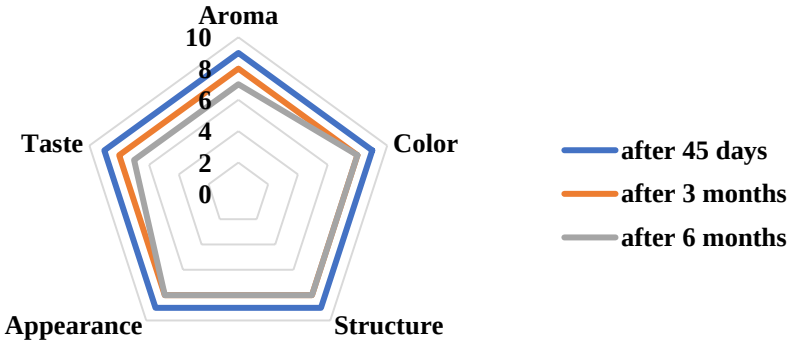


Figure 6. Sensory characteristics of Kashkaval controls during ripening and storage

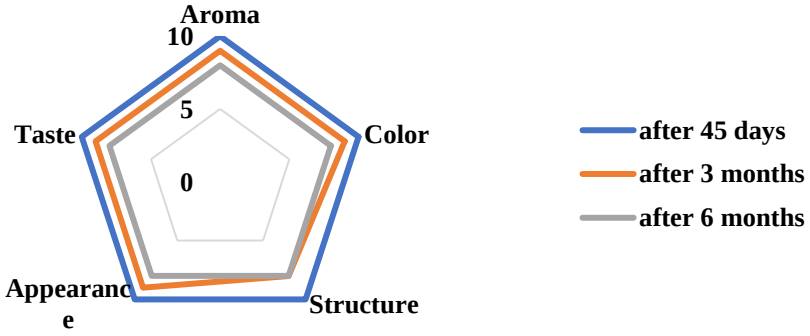


Figure 7. Sensory characteristics of Kashkaval treated with sunflower oil, linoleic type and 0.1% thyme essential oil during ripening and storage

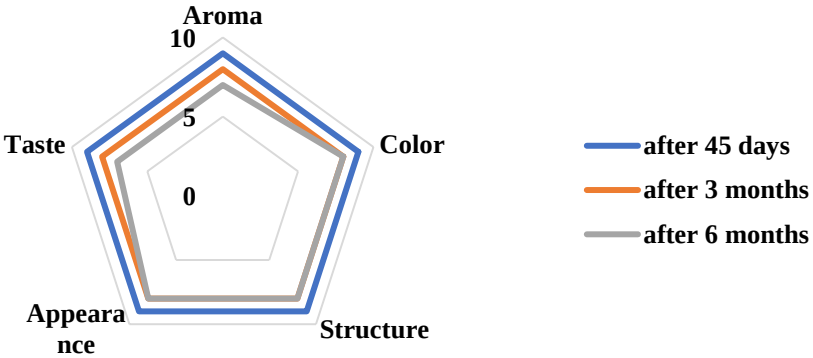


Figure 8. Sensory characteristics of Kashkaval treated with sunflower oil, linoleic type and 0.2% thyme essential oil during ripening and storage.

Conclusioncs

The present study demonstrates that surface application of an oil mixture of sunflower oil (linoleic type) and thyme essential oil is an effective approach for creating products with functional properties. The obtained results confirm the possibility of achieving a healthy fatty acid profile and a significant reduction in cholesterol, without disturbing the biochemical dynamics of storage within 6 months. The amount of monounsaturated fatty acids increases in the samples with surface treatment. The content of vaccenic acid, a trans-fatty acid with functional properties, increased during ripening, while the amount of polyunsaturated fatty acids does not decrease, and the ratio of ω-3:ω-6 fatty acids is not disturbed. The results show that surface treatment with thyme essential oil contributes to the stabilization of cholesterol content during storage and maintains low values.

Conditions are created for controlled microbiological activity, which leads to a more stable and healthy fatty acid profile during storage**.** Surface treatment with thyme essential oil contributes to slow amino acid transformations during storage and helps preserve the amino acid composition and nutritional value of the cheese. From a practical point of view, the obtained results demonstrate that surface treatment with sunflower oil and 0.1% thyme essential oil can be successfully applied in dairy industry practice as a natural preservation strategy for Kashkaval cheese. The treatment contributes to maintaining microbiological stability, preserving sensory quality, and improving the nutritional profile during six months of ripening and storage. Based on the obtained results, the application of 0.1% thyme essential oil is recommended as the most appropriate concentration due to its balanced effect on product quality and consumer acceptability.

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Author Contributions

Conceptualization, AS and KD; methodology, MI and IT; software, TV; validation, MI, IT and KD; formal analysis, MD, TV and II; investigation, MI, IT and MD-D; resources, TV; data curation, KD, AS and IT; writing - original draft preparation, KD, AS, MI and IT; writing - review and editing, AS and II; visualization, MD and TV; supervision, KD; project administration, KD; funding acquisition, KD. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest

The authors declare no conflicts of interest.

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