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Research Article/Araştırma Makalesi

Extending the shelf life of unsalted white cheese produced for special dietary preferences: role of essential oils and coating

Özel diyet tercihleri için üretilen tuzsuz beyaz peynirin raf ömrünün uzatılması: uçucu yağların ve kaplamanın rolü

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ABSTRACT

Unsalted white cheese is produced for individuals who have health problems and prefer it for special reasons. However, as salt is not used in its production and brining, its shelf life is limited. In this study, edible films prepared with different ratios of whey protein and essential oils were applied as coatings to unsalted white cheese. 5 different experimental groups were prepared and named as group C (control), group 1R (film containing 1% rosemary essential oil), group 3R (film containing 3% rosemary essential oil), group 1L (film containing 1% laurel essential oil), and group 3L (film containing 3% laurel essential oil). The microbiological, chemical, sensory and textural properties of the groups were analyzed after 0, 5, 10, 15, 20 and 25 days of storage at +4°C. On and after the 10th day of the storage period, the C, 1R, 3R, and 1L groups visually deteriorated and the analysis was stopped. However, the 3L group did not deteriorate until the end of the 25th day. In addition, it was determined that the 3L group gave the best results in terms of physical, chemical, microbiological, sensory, and textural analyses.

Key Words: Unsalted white cheese, shelf life, edible films, laurel essential oil, rosemary essential oil, whey protein

ÖZ

Tuzsuz beyaz peynir, sağlık sorunu yaşayan ve özel nedenlerle tercih eden bireyler için üretilmektedir. Ancak üretiminde ve salamurasında tuz kullanılmadığı için raf ömrü sınırlıdır. Bu çalışmada, farklı oranlarda peynir altı suyu proteini ve esansiyel yağlar ile hazırlanan yenilebilir filmler tuzsuz beyaz peynire kaplama olarak uygulanmıştır. 5 farklı deney grubu hazırlanmış ve grup C (kontrol), grup 1R (%1 biberiye uçucu yağı içeren film), grup 3R (%3 biberiye uçucu yağı içeren film), grup 1L (%1 defne uçucu yağı içeren film) ve grup 3L (%3 defne uçucu yağı içeren film) olarak adlandırılmıştır. Grupların mikrobiyolojik, kimyasal, duyusal ve tekstürel özellikleri +4C°'de 0, 5, 10, 15, 20 ve 25 günlük depolama sonrasında analiz edilmiştir. Depolama süresinin 10. günü ve sonrasında C, 1R, 3R ve 1L grupları görsel olarak bozulmuş ve analiz durdurulmuştur. Ancak 3L grubunda 25. günün sonuna kadar bozulma görülmemiştir. Ayrıca 3L grubunun fiziksel, kimyasal, mikrobiyolojik, duyusal ve tekstürel analizler açısından en iyi sonuçları verdiği belirlenmiştir.

Anahtar Kelimeler: Tuzsuz beyaz peynir, raf ömrü, yenilebilir filmler, defne uçucu yağı, biberiye uçucu yağı, peynir altı suyu proteini

Introduction

White cheese is a widely consumed product in Türkiye, Egypt, and Greece, albeit with different names (Feta, Batzos, Halloumi, Domiati, and Beyaz Peynir) and production methods (Albayrak and Duran, 2021; Geronikou et al., 2023). It is a type of cheese that is kept in brine, is usually white in color, and matures with a soft to semi-hard texture (Soltani et al., 2020). In Türkiye, it is among the most consumed cheese varieties (Erkaya-Kotan and Hayaloglu, 2024). Traditional taste habits and the fact that it is a semi-hard cheese type have increased the consumption of white cheese throughout the country. For this reason, it is known that individuals whose salt consumption is limited or prohibited for health reasons prefer to consume classical production white cheese despite all these negativities. On the other hand, white cheese consumption is high, especially in hospitals and elderly care centers, owing to its textural properties.

Salt is one of the most commonly added materials in food formulations, creating sensory-pleasing product forms. In cheese production, salt is used for various purposes, such as providing microbiological safety, flavor, and whey extraction. Although cheese varieties encounter salt at different production points, the most common use is brining (Glass et al., 2024). At this stage, high levels of salt (18-24%) are used especially during brine preparation to ensure that sufficient and safe amount of salt from the brine liquid can pass to the cheese (Lucey, 2021). However, dietary salt intake rates and their effects have been the subject of many studies. Heart diseases, hypertension, and kidney diseases can be prevented by reducing the sodium from salt components (He et al., 2020; Wang et al., 2020). In addition to its negative effects on health, the presence of high salt can negatively affect the presence of probiotic strains in white cheese (Rolim et al., 2020).

Rosemary (*Rosmarinus officinalis*) is a perennial herb that grows in the Mediterranean region and has been used in many areas for many years because of its strong antioxidant and antimicrobial effects. In contrast, it reduces inflammation, cancer, diabetes, and depression (Chen et al., 2024; Eid et al., 2022). Rosemary oil is an important commercial product of this plant that is used as a spice. Rosemary oil, which is used in many fields, can be used in the food sector for purposes such as antimicrobial and antioxidant effects and shelf life extension (Chen et al., 2024; Sirocchi et al., 2017). In contrast, α-pinene and β-pinene hydrocarbons provide rosemary oil with a pungent aroma (Formica et al., 2024). Its dominant aromatic structure may cause sensory limitations in its use as food. Laurel (*Laurus nobilis*) is a plant native to the Mediterranean region, usually used as a spice (Parthasarathy et al., 2008). The antimicrobial and antiseptic properties of laurel leaves and oils are well documented. In particular, their positive effects on foodborne pathogens have been identified (Özogul et al., 2015). Additionally, 1,8-cineole, linalool, α-terpinyl acetate, α-pinene, and β-pinene are the main components of laurel leaves (Stefanova et al., 2020).

Whey protein is a valuable byproduct of milk processing and is known for its ability to increase the stability of the products it is formulated into and its good carrier properties (Kong et al., 2022). In addition to valuable amino acid components, it is also valuable for emulsification and gelling (Wagner et al., 2020). Edible films generally aim to maintain food quality and extend shelf life; protein-based materials and carbohydrates are commonly used in the production of film coatings (Kang et al., 2021). Whey protein is preferred, especially in fat-based edible film coatings, owing to its high gelling and emulsification capacities.

The objective of this study was to investigate the possibility of extending the shelf life of unsalted white cheese, which has become compulsory for consumption for various reasons, especially health problems, and is used extensively, especially in hospital kitchens. In this context, films using rosemary and laurel essential oils and whey protein at acceptable sensory ratios were produced, and white cheeses were coated.

Total psychrophilic bacteria count, coliform group bacteria count, yeast and mold count, lactic acid bacteria count, pH, ash, moisture, titratable acidity, color, texture, and sensory analyses were performed on six different storage days (days 0, 5, 10, 15, 20, and 25).

Materials and Methods

The rosemary and laurel essential oils (Sigma-Aldrich) used in this study were 99% pure. Whey protein (70% protein) (Gemici Tic.), and glycerol (Tekkim Tic.) was obtained from local businesses.

White cheese production

For cheese production, 355 liters of milk was pasteurized using heat treatment. Then, 120 g of $CaCl_2$, 300 g of cheese starter culture, and coagulant rennet were added to the pasteurized milk. After the clot formation started, it was subjected to clot breaking, mixing and heating. After the curdling stage, the whey was separated, and the cheeses were molded and pressed.

Edible film production and coating of cheeses

Whey protein (5% w/v) was dissolved in distilled water and glycerol (5% w/v) was added. 1 N NaOH was added until the pH of the solution was 8 and pH was fixed at 8. The solution was then heated for 30 min until it reached 90±2^{o}C and sodium alginate (0.3% w/v) was added. In the first batch of edible films, 1% and 3% rosemary essential oil were added, and 1% and 3% laurel essential oil were added to the next batch. These ratios were determined based on the initial sensory evaluations and essential oil ratios at acceptable levels for consumers. The edible films were cooled to room temperature.

Unsalted white cheese samples were sliced into 10±0.5 g pieces after the edible film was formed. Approximately 10 g of unsalted white cheese sample was placed in sterile sealed containers. They were immersed in the film solution to cover the top and bottom and maintained for approximately 1.5 hours. After this stage five experimental groups were established and labeled as group C (control), group 1R (film with 1% rosemary essential oil), group 3R (film with 3% rosemary essential oil), group 1L (film with 1% laurel essential oil), and group 3L (film with 3% laurel essential oil). The samples were stored at +4^{o}C until the end of their shelf life for storage analysis. On day 0, analyses were started and samples were analyzed until day 25. This period was determined based on the deterioration time of the samples. 2 replicates were produced for each sample. All analyses were performed in parallel in triplicate.

Physicochemical analysis

The pH of the samples was determined by dissolving 1 g of the sample in 10 ml of distilled water and measuring with a pH meter.

The titration acidity value was calculated by titrating 25 ml of the filtrate obtained by crushing 10 g of cheese sample with distilled water with 0.1 N NaOH. The results determined using the formula expressed the acidity of the cheese in % in terms of lactic acid (AOAC, 2000).

Unsalted White cheeses coated with edible films and control samples were analyzed for moisture and ash contents during storage. For moisture determination, the samples were allowed to reach a constant weight in an oven at 105°C, whereas for ash determination, the samples were incinerated in a ash furnace until white ash residue remained (AOAC, 2000).

The color values of unsalted white cheeses were determined by determining the L^*, a^*, and b^* values using a color analyzer (Minolta Chroma Meter, CR-400).

Microbiological analysis

Unsalted white cheese samples were diluted 10^{-1} with sterilized physiological saline. For the rest of the analysis, the samples were diluted to 10^{-6} and inoculated on Plate Count Agar (PCA) medium for Total Psychrophilic Aerobic Bacteria Count using the pour plate method. The media were incubated at 10°C for 10 days, and the colonies were counted (log CFU/g). Lactic Acid Bacteria enumeration was performed using Man Rogosa Sharpe Agar (MRS) and M17 media. After inoculation, the medium was incubated at 37°C for 48 hours. Yeast and mold counts were inoculated on Potato Dextrose Agar (PDA) medium using the pour plate method. The medium was incubated at 10°C for 5-7 days. To determine the total coliform count, violet red bile agar (VRBA) medium was inoculated by double pouring. The medium was then incubated at 35°C for 24 h. Colonies were counted (log CFU/g) (Halkman, 2005).

Texture analysis

Texture analysis was performed to determine the effect of biofilm coating on cheese samples during storage. The analysis was performed using a texture meter (Taxt Plus 2-stable macrosystems). P/25 aluminum cylinder probe was used to determine textural properties. Device operating conditions were determined as test speed 1mm/s, pretest speed 5 mm/s, post test speed 1 mm/s and compression 25%. The values of parameters such as hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resilience were analyzed.

Sensory analysis

All of the unsalted white samples prepared within the scope of the study were sensory analyzed during the specified storage periods. The participants were selected as 25 males and 25 females who were educated in the food engineering department and trained on sensory panel. Sensory analyses were carried out with panelists who did not smoke and did not have any disease. The samples were evaluated in terms of external appearance, internal appearance, structure, odor and taste characteristics by 30 trained panelists (9=most liked, 1=least liked). On the common last day of storage, all samples were compared. Before the analysis, all panelists were informed about the raw materials used in the study and the scope of the study. They were asked whether they were allergic to any component subject to the study and the panel proceeded after approval was obtained.

Statistical analysis

The statistical difference between the sample results was determined by Anova and Tukey tests in Minitab17 package program.

Results and Discussion

Physicochemical analysis

Table 1. pH and titratable acidity of White cheese samples during the storage process

Sample	pH					
	Storage time (day)					
	0	5	10	15	20	25
C	6.64±0.05ᵃ	6.36±0.01ᶜ	*	*	*	*
1R	6.70±0.01ᵃ	6.34±0.02ᶜ	6.20±0.01ᵈ	5.88±0.04ᵈ	*	*
3R	6.74±0.01ᵇ	6.17±0.02ᵃ	5.90±0.02ᵇ	5.80±0.01ᶜ	*	*
1L	6.73±0.03ᵇ	6.61±0.02ᵈ	6.51±0.01ᵉ	*	*	*
3L	6.76±0.01ᵇ	6.24±0.01ᵇ	6.02±0.01ᶜ	5.71±0.05ᵇ	5.41±0.02ᵇ	5.14±0.06ᵇ
	Titratable Acidity (%)					
Sample	Storage time (day)					
	0	5	10	15	20	25
C	2.75±0.05ᵇ	1.78±0.08ᵇ	*	*	*	*
1R	1.80±0.05ᵃ	0.95±0.05ᵃ	2.48±0.03ᵇ	2.97±0.03ᶜ	*	*
3R	1.78±0.03ᵃ	0.97±0.06ᵃ	2.73±0.03ᶜ	2.92±0.03ᶜ	*	*
1L	2.63±0.19ᵇ	1.68±0.03ᵇ	2.58±0.10ᵇ	*	*	*
3L	2.48±0.42ᵇ	1.67±0.08ᵇ	2.55±0.05ᵇ	2.70±0.05ᵇ	2.88±0.03ᵇ	3.08±0.13ᵇ
C	2.75±0.05ᵇ	1.78±0.08ᵇ	*	*	*	*

C: Control; 1R: 1% rosemary essential oil; 3R: 3% rosemary essential oil; 1L: 1% laurel essential oil; 3L: 3% laurel essential oil. The difference between samples with different lowercase letters in the same row and samples with different uppercase letters in the same column is significant in itself for each analysis (p<0.05)

*samples were not analyzed due to sensory deterioration on the specified storage day

Table 1 shows the pH and titratable acidity values of unsalted white cheese samples. The pH values of the unsalted white cheese samples coated with edible films prepared by adding rosemary and laurel essential oil at different ratios were in the range of 5.14-6.76. When the experimental samples were evaluated between the groups according to the pH value chart, the statistical difference between group C and the other groups on day 0 was significant (P<0.05). When the changes between days were analyzed, a statistically significant difference was found between all days in the 3L sample (P<0.05). The free H+ ion in milk and other ions in equilibrium are defined as the actual acidity in milk and dairy products, and physical, chemical and microbiological changes in cheese production can be explained by changes in pH value (Yerlikaya and Karagözlü, 2014). When the effect of coating change on pH in unsalted white cheese samples coated with edible films prepared using whey powder protein, rosemary, and laurel essential oil was evaluated, a decrease was observed with storage. decrease in pH was observed from day 0 to day 25 of storage. It is thought that acid is released as a result of lactic acid bacteria breaking down lactose in milk; therefore, the pH decreases in the last stages of the storage process as a result of microbial activity. Yangılar (2015) reported that the initial pH values of cheddar cheese samples coated with control, chitosan, and chitosan/whey protein combination-based films was 5.31 and the pH values at 60 days were 5.49, 5.20, and 5.28, respectively. The results of this study were similar to our research results.

The titratable acidity values of the unsalted white cheese samples used in this study were in the range of 0.95-3.08% during the storage period. When the difference between groups was analyzed on day 0, the statistical difference between C, 1L and 3L groups and 1R and 3R groups was found to be significant (P<0.05). During the storage period, titratable acidity values decreased on the 5th day and increased. In the 3L group, the difference was statistically significant (P<0.05). When the titratable acidity values of unsalted white cheese samples coated with whey powder protein using rosemary and laurel essential oil were examined, it was found that the acidity increased towards the last days of storage, but the highest lactic acid value was found in group C. It is thought that the reason why the other groups have lower acid values than group C is due to the buffering properties of the amino acids in the whey. Kavas and Kavas (2014) applied edible film coating to curd cheese and reported that it improved acidity in cheeses and an increase in acidity was observed with the increase in mint essential oil ratio. Wagh et al., (2014) coated cheddar cheese with edible films using casein and whey concentrate and observed that the titration acidity increased in all samples at the end of 30 days of storage compared to the beginning. They reported that the difference between the control and coated groups was statistically significant. In another study, titratable acidity changes in Ricotta cheese coated with edible films containing chitosan and whey proteins were investigated. In the first week of the study, it was determined that titratable acidity increased in film-coated products compared to the control sample (Di Pierro et al., 2011).

Table 2. Moisture and ash content of white cheese samples during the storage process

Sample	Moisture Content (%)					
	Storage time (day)					
	0	5	10	15	20	25
C	59.72±0.45Ca	56.24±0.34Cb	*	*	*	*
1R	60.87±0.31^{Bc}	63.03±0.74^{Bb}	65.23±0.32^{Aa}	65.92±0.14^{Aa}	*	*
3R	62.39±0.55^{Ab}	63.20±0.30^{Bab}	63.95±0.16^{Ba}	64.07±0.21^{Ba}	*	*
1L	62.66±0.29^{Ab}	64.49±0.48^{Aa}	65.02±0.15^{Aa}	*	*	*
3L	62.27±0.30^{Ac}	63.50±0.42^{ABbc}	64.72±0.49^{ABab}	65.11±0.65^{Aa}	65.36±0.53^{a}	65.38±0.49^{a}
	Ash Content (%)					
Sample	Storage time (day)					
	0	5	10	15	20	25
C	1.42±0.01 C^{b}	1.45±0.01 C^{a}	*	*	*	*
1R	1.71±0.01^{ABc}	1.73±0.01^{ABbc}	1.74±0.01^{ABab}	1.75±0.01^{Aa}	*	*
3R	1.72±0.01^{Aa}	1.73±0.02^{ABa}	1.75±0.01^{Aa}	1.75±0.01^{Aa}	*	*
1L	1.68±0.02^{Ba}	1.69±0.01^{Ba}	1.71±0.02^{Ba}	*	*	*
3L	1.70±0.01^{ABa}	1.73±0.01^{Aa}	1.72±0.02^{ABa}	1.76±0.02^{Aa}	1.78±0.03^{a}	1.86±0.04^{a}
C	1.42±0.01 C^{b}	1.45±0.01 C^{a}	*	*	*	*

C: Control; 1R: 1% rosemary essential oil; 3R: 3% rosemary essential oil; 1L: 1% laurel essential oil; 3L: 3% laurel essential oil. The difference between samples with different lowercase letters in the same row and samples with different uppercase letters in the same column is significant in itself for each analysis (p<0.05)
*Samples were not analyzed due to sensory deterioration on the specified storage day

The moisture analysis values of unsalted white cheese samples varied between 56.24-65.92% (Table 2). Compared with group C, the moisture values in the other groups increased depending on the storage time. This increase was due to the coating material. An increase in moisture content was observed in the coated groups. According to the results of moisture analysis, when the experimental samples were evaluated between the groups, the statistical difference was found to be significant (P<0.05) on day 0 when C and 1R groups were compared with 3R, 1L and 3L groups. The moisture content of sample C was lower than that of the samples with the addition of essential oil to the coating. In the inter-day evaluations, the difference found in the increases on the 0th and 5th days and other storage periods of the 3L sample was found to be statistically significant (P<0.05). The moisture content of unsalted white cheeses coated with films prepared with rosemary and laurel essential oils increased during storage. On the other hand, the coated unsalted white cheeses had a higher moisture content than the control group. This may be attributed to the effect of the essential oils added to the coating and barrier properties of the whey protein powder. Whey proteins are known to enhance the water vapor barrier properties of coatings prepared with whey protein (Tang et al., 2003).

The ash analysis values of unsalted white cheese samples varied between 1.42-1.86%. When ash analyses were performed between the experimental sample groups, the statistical difference was found to be significant (P<0.05) when the C and 1L groups were compared with the 1R, 3R, and 3L groups on day 0. In all the unsalted white cheese samples prepared within the scope of the study, ash values increased towards the end of the storage period. It is thought that the amount of organic matter in the environment increases as a result of microbial activities, and accordingly, the amount of ash increases. In a similar study, the coating of soft cheese with thyme and green tea extracts and changes in the storage process were investigated. The amount of ash increased during the storage process, and the amount of ash in the samples was between 2.01 and 3.82% (Hazaa and Jassim, 2021).

Microbiological analysis

Table 3. Color properties of white cheese samples during the storage process

Sample	*L**					
	Storage time (day)					
	0	5	10	15	20	25
C	93.40±0.20^{Aa}	93.80±0.13^{Aa}	*	*	*	*
1R	91.81±0.18^{Bc}	92.17±0.05^{BCbc}	93.75±0.82^{Bb}	96.39±0.88^{Ba}	*	*
3R	91.04±0.27^{BCc}	90.29±0.26^{Dd}	93.04±0.23^{Bb}	99.60±0.33^{Aa}	*	*
1L	89.82±0.65^{Db}	91.81±0.28^{Ca}	92.79±0.21^{Ba}	*	*	*
3L	90.71±0.47^{CDe}	92.38±0.25^{Bd}	95.94±0.30^{Ac}	98.19±0.48^{Ab}	98.34±0.19^{b}	99.64±0.18^{a}
	*a**					
Sample	Storage time (day)					
	0	5	10	15	20	25
C	-0.01±0.13^{Ab}	0.13±0.01^{Aa}	*	*	*	*
1R	-0.29±0.10^{Bd}	0.14±0.03^{Ac}	0.33±0.02^{Ab}	1.73±0.07^{Ba}	*	*
3R	0.48±0.01^{BCb}	0.07±0.01^{BCd}	0.18±0.03^{BCc}	3.18±0.03^{Aa}	*	*
1L	-0.59±0.05^{Cc}	0.11±0.02^{ABb}	0.24±0.02^{Ba}	*	*	*
3L	-1.00±0.04^{Dd}	0.05±0.01 ^{Cc}	0.14±0.03^{Cb}	3.27±0.05^{Aa}	3.38±0.19^{a}	4.75±1.71^{a}
	*b**					
Sample	Storage time (day)					
	0	5	10	15	20	25
C	12.59±0.47^{Ca}	12.54±0.02^{Ca}	*	*	*	*
1R	14.66±0.02^{Ab}	14.77±0.08^{Aab}	15.12±0.09^{Ba}	9.80±0.30^{Ac}	*	*
3R	14.91±0.10^{Aa}	11.34±0.21^{Db}	11.84±0.11^{Cb}	9.18±0.85^{Ac}	*	*
1L	13.42±0.37^{Bb}	13.72±0.17^{Bb}	14.64±0.32^{Ba}	*	*	*
3L	13.72±0.25^{Bb}	13.90±0.02^{Bb}	15.65±0.12^{Aa}	6.62±0.58^{Bcd}	6.09±0.14^{d}	7.14±0.23^{c}

C: Control; 1R: 1% rosemary essential oil; 3R: 3% rosemary essential oil; 1L: 1% laurel essential oil; 3L: 3% laurel essential oil. The difference between samples with different lowercase letters in the same row and samples with different uppercase letters in the same column is significant in itself for each analysis ($p<0.05$)

*samples were not analyzed due to sensory deterioration on the specified storage day

Color values of the samples are shown in Table 3. *L** value represents whiteness and brightness. The *L** values of the samples were between 89.82-99.64, indicating that the samples were intensely white and bright. When evaluated between groups, the highest *L** value on day 0 was in group C and the lowest *L**value was in group 1L. When rosemary and laurel essential oil were added to unsalted white cheese samples coated with whey powder protein, the *L** (brightness and whiteness) value increased during storage. This was evaluated as an effect of the glossy structure of the coating material. In other studies, no change in the L value was observed in soft cheese samples during storage (Youssef et al., 2015), while the whiteness of Oaxaca cheese decreased during vacuum storage in the refrigerator (Fuentes et al., 2015). Changes in the preparation process of cheese varieties, ingredients added to the product formulation, and changes in coating materials affect the whiteness values.

*a** value represents redness and greenness indicators. The *a** values of the samples were in the range of -1.00-4.75. On day 0, *a** values were generally negative among the groups, with the lowest value in 3L and the highest value in 3R groups. Negative *a**values represent greenness and positive *a**values represent redness. The increase in *a** values towards the end of storage indicates an increase in redness. *b** value represents yellowness. The *b** values of the samples are in the range of 6.09-14.91. Whey protein powder had a yellowish color, and the yellowness value in cheese samples was high during the first analysis days. When the difference between 1R and 3R and the difference between 1L and 3L were compared between the groups, the statistical difference was found to be significant ($P<0.05$). While the *b** value of group C was the lowest, it increased as the amount of essential oil increased. The *a** (redness) values of all samples increased during storage. This is because an increase in yeast and mold values was observed during the last days of storage, which also increased the redness values. When the *b** (yellowness) values were examined, the yellowness values of the groups to which essential oils were added were higher than those of the control group. Rosemary and laurel essential oils were found to increase *b**(yellowness) values. In a study conducted by Civelek and Çağrı Mehmetoğlu (2019) on cheddar cheeses, Williopsis Saturnus var. Saturnus in the edible coating against yeasts and molds on the outside of the cheese reported *a** value between -0.74 and - 1.97 towards the end of 56 days of storage, and there was no change in the *b** value.

Table 4. Microbiological properties of White cheese samples during the storage process

Sample	Storage time (day)					
Total Psychrophilic Aerobic Bacteria (log kob/g)						
	0	5	10	15	20	25
C	<1	3.53±0.38^{Ba}	*	*	*	*
1R	<1	4.18±0.13^{ABa}	4.27±0.36^{Aa}	4.42±0.26^{Aa}	*	*
3R	<1	4.58±0.29^{Aa}	4.31±0.52^{Aa}	4.44±0.48^{Aa}	*	*
1L	<1	4.32±0.45^{ABa}	4.35±0.58^{Aa}	*	*	*
3L	<1	3.48±0.31^{Bab}	4.35±0.58^{Aa}	4.24±2.50^{Aab}	4.69±0.17^{a}	5.26±0.19^{a}
Total Yeast and Mold Count (log kob/g)						
	0	5	10	15	20	25
C	4.57±0.38^{Aa}	*	*	*	*	4.57±0.38^{Aa}
1R	4.67±0.19^{Aa}	4.94±0.12^{Aa}	4.97±0.06^{Aa}	*	*	4.67±0.19^{Aa}
3R	4.53±0.38^{Aa}	4.76±0.25^{Aa}	4.93±0.10^{Aa}	*	*	4.53±0.38^{Aa}
1L	4.18±0.05^{ABb}	4.55±0.24^{Aa}	*	*	*	4.18±0.05^{ABb}
3L	3.48±0.22^{Bb}	4.64±0.02^{Aa}	4.74±0.03^{Ba}	4.64±0.11^{a}	4.73±0.13^{a}	3.48±0.22^{Bb}
Lactic acid bacteria count *(Lactococcus spp.M17-medium)*						
	0	5	10	15	20	25
C	3.49±0.31^{Aa}	3.28±0.04^{Aa}	*	*	*	*
1R	2.79±0.19^{Ba}	3.01±0.03^{ABa}	2.85±0.04^{ABa}	2.82±0.10^{Aa}	*	*
3R	2.57±0.15^{Ba}	2.49±0.45^{Ba}	2.96±0.05^{Aa}	2.86±0.06^{Aa}	*	*
1L	2.35±0.04^{Ba}	2.58±0.25^{Ba}	2.46±0.10^{cb}	*	*	*
3L	2.46±0.13^{Bb}	2.60±0.19^{Bab}	2.74±0.03^{Ba}	2.81±0.07^{Aa}	2.84±0.03^{a}	2.85±0.04^{a}
Lactic acid bacteria count *(Lactobacillus spp.MRS medium)*						
	0	5	10	15	20	25
C	3.27±0.07^{Ab}	3.79±0.03^{Aa}	*	*	*	*
1R	2.25±0.02 ^{Cb}	2.34±0.03^{Cab}	2.38±0.04^{Ba}	2.33±0.08^{Bab}	*	*
3R	2.32±0.08 ^{Ca}	2.34±0.07^{Ca}	2.32±0.03^{Ba}	2.24±0.05^{Ba}	*	*
1L	2.79±0.13^{Ba}	2.45±0.09^{Cb}	2.32±0.04^{Bb}	*	*	*
3L	2.29±0.05^{Cc}	2.65±0.05^{Bb}	2.84±0.08^{Aa}	2.90±0.04^{Aa}	2.86±0.03^{a}	2.85±0.05^{a}

C: Control; 1R: 1% rosemary essential oil; 3R: 3% rosemary essential oil; 1L: 1% laurel essential oil; 3L: 3% laurel essential oil. The difference between samples with different lowercase letters in the same row and samples with different uppercase letters in the same column is significant in itself for each analysis ($p<0.05$)

*Samples were not analyzed due to sensory deterioration on the specified storage day

Table 4 shows the results of microbiological analysis of unsalted white cheese samples. The number of coliform bacteria in the unsalted white cheese samples during 25 days of storage at 4°C was below the detectable level and is therefore not shown in the table. According to the table, the psychrophilic aerobic bacteria values of the unsalted white cheese samples coated with films prepared with different ratios of rosemary and laurel essential oil were found below the detectable level on day 0 and ranged between 3.48 -5.26 (log_{10}cfu/g) during the storage period. On day 5, the 1R, 3R, and 1L groups were found to be statistically significant compared to the C and 3L groups (P<0.05). On day 5, the lowest value was observed in the 3L group, indicating that the antimicrobial activity of laurel essential oil against psychrophilic microorganisms was effective. In the inter-day evaluations, the difference in the 3L sample between days was not statistically significant (P>0.05). When the effects of rosemary and laurel oil on the number of psychrophilic bacteria in unsalted white cheese samples coated with whey powder protein were examined, the lowest number of psychrophilic bacteria was found in the 3L group on the 5th day of storage. The fact that the number of psychrophilic bacteria in the 3L sample was lower than that in the C sample can be interpreted as an inhibition of the microbial effect of the oil due to the high amount of laurel essential oil in the edible film coating. In addition, the fact that the shelf life of the cheeses produced with all coatings made with essential oils was longer than the control group unsalted white cheese was found valuable in terms of our study. The addition of essential oils to the coating formulation increased the shelf life of unsalted white cheeses coated with whey powder protein during cold storage. Similarly, in another study on essential oils, cheese samples coated with sodium alginate and essential oils showed poor microbial growth (total aerobic mesophilic flora, yeasts, and fecal coliforms) during storage (Mahcene et al., 2020). The addition of Mentha longifolia essential oil and pulegone to edible coatings significantly reduces bacterial growth in cheese (Shahdadi et al., 2023). This was attributed to the superior antimicrobial effects of the essential oils.

The yeast and mold counts of the samples varied between 3.48-4.97 (log_{10}cfu/g) during the storage period. When the experimental samples were evaluated between the groups, the statistical difference was found to be significant (P<0.05) on the 5th day when the C, 1R, 3R, and 1L groups were compared with the 3L group. In the inter-day evaluations, the difference in the 3L sample on the 5th day between the other days was statistically significant (P<0.05). When the effects of rosemary and laurel essential oil on yeast and mold counts in unsalted white cheese samples coated with whey powder protein were examined, an increase in yeast and mold counts was observed as the storage time increased. In the first days of storage, the lowest yeast and mold counts were observed in the 1L and 3L groups. Cheese samples coated with films prepared with essential oils were evaluated in terms of yeast and mold values, and they were determined to have a longer shelf life than uncoated cheese. This result indicates that coating unsalted white cheeses stored in cold storage with films produced with whey protein and laurel essential oil is more effective. The reason for this increase is thought to be that lactose in the environment is broken down into galactose and glucose by the starter culture, and the glucose formed supports the growth and nutrition of yeasts (Liu and Tsao, 2009). On the other hand, the coating applied to curd cheese retains moisture and preserves appearance and color by reducing yeast and mold growth during long-term storage (Mileriene et al., 2020).

The lactic acid bacteria count was analyzed for *Lactococcus spp*. in M17 medium and Lactobacillus spp. in MRS medium. When the experimental samples were evaluated between the groups according to the table of lactic acid bacteria values formed in M17 medium, the statistical difference was found to be significant (P<0.05) when the C, 1R, 3R, and 1L groups were compared with the 3L group on day 0. In the inter-day evaluations, the difference between the values of the 3L sample on day 0 and the other days was statistically significant (P<0.05). The values of lactic acid bacteria in MRS medium of unsalted white cheese samples varied between 2.24 - 3.79 (log_{10}cfu/g) during the storage period. When the experimental samples were evaluated between the groups, the statistical difference between the C and 1R groups and the other groups on day 0 was significant (P<0.05). When the effects of whey and rosemary-laurel essential oils on lactic acid bacteria were examined, it was found that 3L rosemary essential oil was more effective against lactic acid bacteria. Although the addition of essential oil and the coating process decreased the lactic acid numbers in cheese, this was not in the range to be expressed as inhibition of cheese. Even after the shelf life of the control sample was completed, owing to the prolonged shelf life, lactic acid bacteria ratios in the coated products were detected in valuable ranges. While examining the effects of Melissa officinalis essential oil on microorganisms in cheese, despite its positive effects, an inhibitory effect on lactic acid bacteria was detected, and it was concluded that this essential oil was not suitable for cheese production (Licon et al., 2020). In contrast, thyme essential oil does not negatively affect the growth or metabolic activity of lactic acid bacteria in cheeses coated with thyme (Marcial et al., 2016). The inhibitory effect is thought to be influenced by variables such as the antimicrobial effect of the essential oil and other raw materials used in the coating.

Texture analysis

Table 5 Textural properties of White cheese samples during the storage process

Sample			Hardness			
	Storage time (day)					
	0	5	10	15	20	25
C	1644.90±463.81^{ABa}	1701.92±463.73^{Aa}	*	*	*	*
1R	1139.71±217.10^{ABab}	1167.12±46.20^{Aa}	1020.32±126.19^{Aab}	789.21±85.26^{Ab}	*	*
3R	1743.10±574.92^{ABa}	1610.85±406.20^{ABa}	1159.45±81.00^{ABa}	876.01±77.45^{ABa}	*	*
1L	1936.59±239.83^{Aa}	1827.45±192.04^{Aa}	865.97±222.72^{Ab}	*	*	*
3L	809.49±358.19^{Bab}	1257.98±346.37^{Aa}	825.29±57.00^{Aab}	704.40±381.81^{Aab}	336.90±94.17^{b}	518.95±82.70^{b}
			Adhesiveness			
Sample	Storage time (day)					
	0	5	10	15	20	25
C	-13.73±8.31Cb	-54.92±38.28^{Aa}	*	*	*	*
1R	-5.44±3.97Db	-11.26±5.45C^{a}	-10.58±6.57^{Aa}	-9.78±6.32C^{a}	*	*
3R	-36.32±38.28^{A}b	-41.74±54.50B^{a}	-45.61±2.37^{A}a	-44.69±23.62^{Aa}	*	*
1L	-20.00±12.20Bb	-38.97±43.90B^{a}	-38.48±3.59^{A}a	*	*	*
3L	-13.26±1.34Cb	-13.00±1.70Cb	-24.19±29.30^{Aa}	-23.29±8.81B^{a}	-25.70±1.37^{a}	-26.27±4.68^{a}
			Gumminies			
Sample	Storage time (day)					
	0	5	10	15	20	25
C	1344.68±368.80^{Aa}	1386.31±370.05^{Aa}	*	*	*	*
1R	930.98±169.36^{Ba}	953.80±36.66B^{b}	818.62±89.75^{Bc}	634.01±61.07^{Bd}	*	*
3R	1454.11±467.42^{Aa}	1305.60±358.98^{Ab}	931.37±71.09^{Ac}	718.52±71.52^{Ad}	*	*
1L	1554.00±205.23^{Aa}	1442.61±145.97^{Aa}	703.49±165.94^{Cb}	*	*	*
3L	678.03±300.12^{Cd}	1011.60±260.55^{Ba}	672.74±49.28^{Db}	578.95±316.10^{Cc}	302.76±149.18^{e}	385.78±50.97^{d}
			Chewiness			
Sample	Storage time (day)					
	0	5	10	15	20	25
C	1217.82±361.90^{Aa}	1187.28±366.29^{Aa}	*	*	*	*
1R	846.39±171.73^{Ba}	857.53±55.12^{Ba}	738.22±70.13^{Ab}	570.99±61.40^{Ac}	*	*
3R	1321.90±400.55^{Aa}	1152.47±356.46^{Aa}	874.65±70.33^{Ab}	597.75±84.68^{Ac}	*	*
1L	1400.39±167.16^{Aa}	1296.50±170.72^{Aa}	616.24±125.76^{Bb}	*	*	*
3L	614.81±271.42^{Bb}	902.37±219.34^{Ba}	565.21±90.09^{Bc}	516.91±286.93^{Ac}	223.82±62.52^{d}	283.89±46.18^{d}

C: Control; 1R: 1% rosemary essential oil; 3R: 3% rosemary essential oil; 1L: 1% laurel essential oil; 3L: 3% laurel essential oil. The difference between samples with different lowercase letters in the same row and samples with different uppercase letters in the same column is significant in itself for each analysis ($p<0.05$)

*Samples were not analyzed due to sensory deterioration on the specified storage day

Table 5 presents the texture analysis results of the samples. No statistical difference was found between the springiness, cohesiveness, and resilience values ($P>0.05$); therefore, they are not presented in the table. The hardness value was determined to be in the range of 336.90-1936.59, although it decreased during storage. The adhesiveness values of unsalted white cheeses varied between -5.44 and -54.92. On day 0, when the difference between the groups was examined, the statistical difference between the C and 3L groups and the 1R, 3R, and 1L groups was found to be significant (P<0.05). The group with the highest value in the Gumminies parameter on day 0 was the 1L group, and the values ranged between 385.78 and 1554.00. In contrast, chewiness values of the cheeses ranged between 223.82 and 1400.39. Texture profile analysis of unsalted white cheese samples was performed using seven parameters: when the results were evaluated, the hardness of the samples decreased towards the end of storage. The texture profile analysis values of the samples (cohesiveness, springiness, and adhesiveness) did not vary depending on the concentration of essential oil contained in the sample and did not change the textural properties of the samples. The degree of hardness increased on the first day of storage. The reason for this is thought to be that the enzyme is still active during the ripening stage and the increase is thought to be due to the amount of dry matter. The degree of hardness decreased as the storage period increased. After the 5th day, a significant decrease in hardness was observed. It is believed that the hardness rate decreases as the moisture content increases. According to Akın et al., (2009), in their research on Kashar cheeses, the hardness data increased in the first days, but the hardness rates decreased on the 60th day. Biofilm coating on cheese reduces weight loss and hardness and protects cheese without seriously affecting its organoleptic properties (Mahcene et al., 2020). Edible coatings made from whey protein concentrate and cumin essential oil did not affect the color or texture of the samples during 28 days of storage (Nemati et al., 2023).

Sensory analysis

All unsalted white cheese samples experienced product deterioration at different times and their storage processes were completed. Therefore, a sensory evaluation graph was drawn for the last common storage day, Day 5 (Figure 1). In the sensory evaluation, 3L, 1R, and C received the highest scores for taste, whereas the lowest score was given to sample C for structure. On the other hand, samples coded 1L and 3 B received the lowest scores for odor, while 1L and 1R were the most preferred products in terms of external appearance. Sensory characteristics such as appearance, odor, flavor and texture influence consumer preferences for specialty cheeses (Lawlor and Delahunty, 2000). In the sensory evaluation of unsalted white cheese samples, group C scored the highest in odor and taste. Unsalted white cheese samples containing essential oils were less appreciated because they had a unique heavy and dominant aromatic odor and taste. The exterior appearance, interior appearance, and structure scored higher in the samples containing essential oil than in the C group.

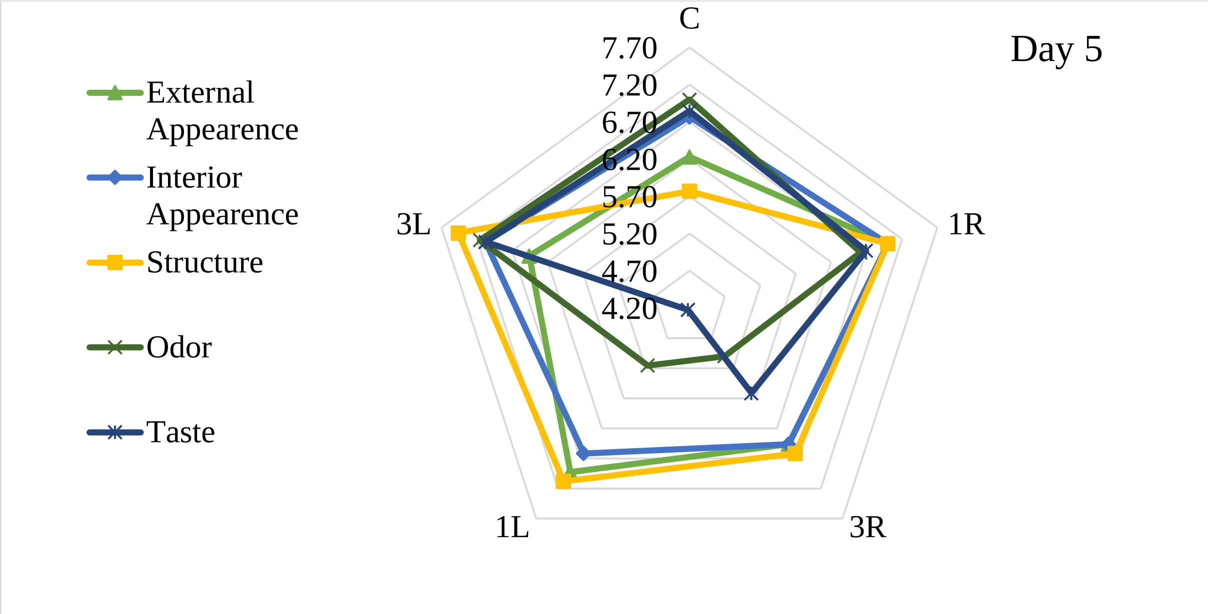

Figure 1. Sensory properties of White cheese samples at 5 day of storage

Conclusions

Based on the microbiological, physical, chemical, and sensory analyses, the 3L group was determined to be the most successful sample in the production of unsalted white cheese. The use of 3% laurel essential oil in whey protein-coated unsalted white cheese samples increases the shelf life and quality of the product. Because the product contains its own aromatic taste and odor, it is difficult to consume; however, the fact that whey protein and 3% laurel essential oil can be used in unsalted white cheese opens a new way to investigate the effects of these essential oils on different types of cheeses.

After this stage, studies can be carried out to obtain the most optimum product formulation by choosing different coating materials in unsalted white cheese. Different film materials can be used. In addition, synergistic effects can be studied with different essential oils. Every study is valuable for unsalted white cheese, which has a very short shelf life, and can support the solution of existing problems.

Availability of Data and Materials:

The data that findings of this study are available from the authors upon reasonable request.

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Author Contributions

ÖPC was responsible for the conception and design of the study. DU performed the experiments. DU and MGS wrote the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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