

The Effects of Different Herb Species on Ripening and Sensory Properties of Kashar Cheese

Farklı Ot Türlerinin Kaşar Peynirinin Olgunlaştırma ve Duyusal Özellikleri Üzerine Etkileri

Engin AYDIN^{1*}, Zekai TARAKÇI²

Abstract

In this study investigated the effects of adding different herbs to Kashar cheese on the cheese's ripening process, physicochemical and sensory properties. A total of six types of cheese were produced using a control sample without herbs (CC) and nettle (*Urtica dioica*, G1), parsley (*Petroselinum crispum*, G2), lemon balm (*Melissa officinalis*, G3), mint (*Mentha piperita*, G4) and arugula (*Eruca vesicaria*, G5). The herbs were finely ground (1–3 mm) and added to the cheese at a concentration of 0.1% relative to the milk volume. After production, the cheeses were vacuum-packed and left to ripening for three months at 7±1 °C. Samples were taken at four different times (3, 30, 60 and 90 days) and analysed for dry matter, pH, fat, titratable acidity, salt and total protein content. In addition, ripening index such as water-soluble nitrogen (WSN, % of total nitrogen), degree of ripening and non-protein nitrogen (NPN, % of total nitrogen) ratio were also evaluated. Textural properties and electrophoretic casein profiles were also evaluated. Sensory analysis was conducted to determine consumer preference and overall acceptability. Statistical comparisons revealed that the addition of herbs affected the ripening characteristics and cheese composition over time and that there were differences between them. Among the herb-enriched cheeses, those containing parsley (G2) and mint (G4) were most favoured by the panelists. Overall, the study shows that adding herbs to Kashar cheese can improve its sensory properties and also accelerate the ripening process. This ripening process can contribute to shorter storage times and, consequently, reduced energy consumption. Furthermore, the addition of herbs could facilitate the development of Kashar cheeses with various flavours by offering an innovative approach to production in the dairy industry. These findings will support the development of alternative methods to traditional cheese-making processes and contribute to production practices that are both economically viable and environmentally sustainable.

Keywords: Kashar, Herb, Ripening, Sensory properties

¹*Sorumlu Yazar/Corresponding Author: Engin Aydın, Department of Cookery, Dereli Vocational School, Giresun University, Giresun, Turkey, E-mail: engin.aydin@giresun.edu.tr  OrcID: 0000-0003-4288-5911

²Zekai Tarakçı, Department of Food Engineering, Faculty of Agriculture, University of Ordu, Ordu, Turkey, E-mail: zarakci@odu.edu.tr  OrcID: 0000-0002-3828-3232

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Öz

Bu çalışmada, Kashar peynirine farklı otlar eklemenin peynirin olgunlaşma sürecini, fizikokimyasal ve duyuşal özellikleri üzerine etkileri araştırılmıştır. Ot içermeyen kontrol numunesi (CC) ve ısırgan otu (*Urtica dioica*, G1), maydanoz (*Petroselinum crispum*, G2), mendek (*Melissa officinalis*, G3), nane (*Mentha piperita*, G4) ve roka (*Eruca vesicaria*, G5) kullanılarak toplam altı çeşit peynir üretilmiştir. Otlar ince öğütölmüş (1–3 mm) ve süt hacmine göre %0,1 konsantrasyonda peynire eklenmiştir. Üretimden sonra peynirler vakumla paketlenmiş ve 7±1 °C'de üç ay olgunlaşmaya bırakılmıştır. Dört farklı zamanda (3, 30, 60 ve 90 gün) numuneler alındı ve kuru madde, pH, yağ, titre edilebilir asitlik, tuz ve toplam protein içeriği açısından analiz edildi. Ayrıca, suda çözünür azot (WSN, % toplam azot), olgunlaşma derecesi ve protein dışı azot (NPN, % toplam azot) oranı gibi olgunlaşma göstergeleri de değerlendirildi. Doku özellikleri ve elektroforetik kazein profilleri de değerlendirildi. Tüketici tercihi ve genel kabul edilebilirliği belirlemek için duyuşal analiz yapıldı. İstatistiksel karşılaştırmalar, otların eklenmesinin zaman içinde olgunlaşma özelliklerini ve peynir bileşimini etkilediği ve birbirlerinin arasında farkların olduğunu tespit etti. Otlarla zenginleştirilmiş peynirler arasında, maydanoz (G2) ve nane (G4) içerenler panelistler tarafından en çok beğenildi. Genel olarak, çalışma, Kashar peynirine otların eklenmesinin duyuşal özelliklerini iyileştirebileceğini ve ayrıca olgunlaşma sürecini hızlandırabileceğini göstermektedir. Bu olgunlaşma süreci, depolama sürelerinin kısalmasına ve dolayısıyla enerji tüketiminin azalmasına katkıda bulunabilir. Ayrıca, otların eklenmesi, süt ürünleri endüstrisinde üretime yenilikçi bir yaklaşım sunarak, çeşitli lezzete sahip Kashar peynirlerinin geliştirilmesini kolaylaştırabilir. Bu bulgular, geleneksel peynir yapım süreçlerine alternatif yöntemlerin geliştirilmesini destekleyerek ve hem ekonomik olarak uygulanabilir hem de çevresel olarak sürdürülebilir üretim uygulamalarına katkıda bulunacaktır.

Anahtar Kelime: Kaşar, Ot, Olgunlaşma, Duyusal özellikler

1. Introduction

Cheese is defined as a nutritious dairy product obtained by coagulating milk with rennet or organic acids, to which salt and sometimes harmless substances such as flavouring herbs and spices, and sometimes starter moulds are added, and which is consumed fresh or ripened depending on the variety (Atasoy and Akin, 1999). Cheese is the most widely consumed dairy product worldwide, providing biologically valuable proteins, fat-soluble vitamins (A, D, E, K) and essential minerals, particularly calcium and phosphorus. Furthermore, the breakdown of proteins during the cheese ripening process increases its digestibility and aids in the digestibility of other foods. Furthermore, some B-complex vitamins can also be synthesised during the ripening process (Demirci, 1990).

Approximately 50 different types of cheese are produced in Turkey, with the most economically significant in terms of production volume being White, Cheddar, Tulum, Otlu, Dil, Mihaliç, Circassian, Village Cheese, Civil and Lor cheeses (Delice et al., 2013). After white cheese, the most widely produced cheese variety in Turkey is Kashar cheese. (Keceli et al., 2006). Kashar cheese is a dense, non-porous cheese, typically moulded into various shapes such as cylinders or rectangles, and is usually pale yellow or yellow in colour. Kashar cheese is classified as “fresh cheddar” or “aged cheddar” depending on its ripening level, and as “full-fat” or “semi-fat” based on its fat content, defined as 45% and 20% fat in dry matter, respectively (TSE., 1999).

The ripening process of kashar cheese, which requires a long storage period, creates a significant working capital requirement. Storage costs and losses during the ripening process constitute a significant portion of the total production cost. Shortening the ripening period can help producers alleviate these financial burdens (Amos, 2007) (Uliescu et al., 2010). The ripening of cheese occurs through biochemical reactions, primarily glycolysis, lipolysis and proteolysis. Glycolysis is usually completed within the first few days to a week after production, while lipolysis and proteolysis continue throughout the ripening process (Fox and McSweeney, 1996). During proteolysis, chymosin breaks down α -1 casein, while plasmin breaks down β -casein, producing both water-soluble and insoluble peptides. Although water-insoluble peptides have a minimal effect on flavour and aroma, the water-soluble fraction, particularly short-chain peptides and amino acids, plays a critical role in flavour and aroma development (Atasoy and Akin, 1999).

The use of herbs to ripen Kashar cheese is relatively rare; however, it has been reported that herbs such as sirmo, thyme, mendo, heliz, siyabo and sov are frequently used in the production of other herb-flavoured cheese varieties to enhance flavour and aroma (Köse and Ocak, 2022). Research has shown that adding various herbs such as mint, thyme, cloves, black pepper and basil may contribute to increased storage stability in cheeses (Kačániová et al., 2024). Different fruit powders were added to Kashar cheese, and their positive effects on total phenolic content, antioxidant capacity, volatile compound composition and sensory properties were observed (Kilic and Koyuncu, 2024). In another study, it was found that adding different spices to a different type of cheese, Circassian cheese, affected its ripening (Gezmiş and Tarakçı, 2020). In addition, it has been reported that the addition of Ashwagandha plant powder positively affects the biological and physicochemical properties of Quarg cheese by increasing its phenolic and antioxidant capacity. (Singh et al., 2023). Previous studies have shown that the use of various herbs, spices and fruits in cheese formulations improves the physical and biochemical properties of the final product. Furthermore, the development of plant-enriched functional foods for dairy products will provide health benefits (Setyawardani et al., 2023).

Although it is known that various herbs and spices are used in many cheeses, studies investigating the effects of adding herbs to kashar cheese on its ripening process and sensory characteristics are limited. Therefore, this study aims to investigate the effects of different herbal additives on the ripening process and sensory characteristics of kashar cheese. Previous findings show that plant-based additives may play an important role in improving the ripening process and sensory characteristics of cheeses.

2. Materials and Methods

2.1. Properties of milk

The milk used in cheese production was sourced from the province of Ordu in the Central Black Sea Region. Kashar cheese was produced at the Ordu Kabataş Dairy Products Factory. Some characteristics of the milk used are as follows: pH 6.60, total dry matter 11.05%, fat 3.45% and protein 3%.

2.2. Herbs

The herbs added during Kashar cheese production (nettle (*Urtica dioica*), parsley (*Petroselinum crispum*), lemon balm (*Melissa officinalis*), mint (*Mentha piperita*) and arugula (*Eruca vesicaria*)) were sourced from the Giresun region. Before use, the herbs were dried in a drying oven at 40 °C for 24 hours to reduce their size and then added to whey at a concentration of 0.1% (w/v) by milk volume.

2.3. Kashar cheese production method

After initial quality checks were performed on raw cow's milk, it was heated to 37 ± 1 °C and 1% starter culture was added. Fermentation was then initiated using liquid rennet at a ratio of 1:15,000 (10-20 mL rennet per 100 kg of milk). The milk was left to rest for approximately 45 minutes until the pH value reached 6.30-6.35 to form curds. The curds were then separated from the whey and herbs such as nettle, parsley, lemon balm, mint and arugula were added at a rate of 0.1% based on the weight of the milk. After the herbs were added, the curd was pressed to remove excess whey and left to ferment at 15-20 °C for 15 minutes until the pH value reached 5.25-5.30. When the pH value of the curd reached 5.0-5.05 (60-65 °SH), the curd was prepared for the heating stage. Prior to heating, the curd was cut into 3-5 cm pieces using a special grating machine. The cut curd pieces were then transferred to an automatic heating tank containing hot brine (72-75 °C or 83-85 °C) with a salt concentration of 5-6%. The stretching process was carried out in hot water. After the heating process was completed, the curd mass was placed on a cheese processing table to cool and knead. The cheeses were then placed in moulds and left to rest and cool for 12 to 24 hours. Finally, they were vacuum-packed and left to ripening at 4-7 °C for 90 days.

2.4. Chemical and biochemical analyses

The chemical and biochemical properties of the cheese samples obtained were compared by performing numerous analyses. The dry matter content of the samples was kept in a laboratory oven at 105 °C until they reached constant weight and then weighed and calculated as a percentage. The total nitrogen concentration of the samples was determined using the Kjeldahl method, as described by (Case et al., 1985), employing a Micro-Kjeldahl analyzer (UDK-149, VELP Scientifica, Usmate, Italy), following the procedure of (Kurt et al., 2003). Total protein content was calculated by multiplying the total nitrogen value by a conversion factor of 6.38 (IDF, 1982). Fat content results were determined using the Gerber method (Kurt et al., 2003). The salt content was analyzed following the Mohr method, while pH values were measured with a digital pH meter (Starter 3100, USA), as described by (Case et al., 1985). Water-soluble nitrogen (WSN), ripening index (WSN/TN), and non-protein nitrogen (NPN) in 12% trichloroacetic acid were determined according to the methods outlined by (Kamaly et al., 1989; Bütikofer et al., 1993). Casein fractions were detected by electrophoresis, as revealed by (Creamer, 1991). The gels obtained by electrophoresis were stained with Coomassie Brilliant Blue and the gel images were transferred to a digital medium. The band intensity in the gels was analysed using TotalLab software (PHoretix, Newcastle upon Tyne, United Kingdom) to calculate the relative intensities of the casein fractions (α s1-casein and β -casein), and the percentage changes were calculated. (Ardö and Polychroniadou, 1999).

2.5. Sensory analysis

A group of 10 panelists from the Department of Food Engineering, Faculty of Agriculture, Ordu University, were selected for sensory analysis. The panelists were asked to evaluate the cheeses according to specific characteristics listed on the evaluation form, without being given any information about the herbs added to each cheese. Sensory quality was evaluated using a 10-point hedonic scale, where 1 meant 'unacceptable' and 10 meant 'very good'. The cheeses were coded as follows and presented to the panelists: 454 (Control cheese), 592 (Nettle cheese), 345 (Parsley cheese), 227 (Lemon balm cheese), 721 (Mint cheese), and 409 (Arugula cheese). Evaluation criteria included appearance (wet, dry, presence of cracks), texture (hard, soft, pasty, crumbly, granular), odor, taste (undesirable, salty, bitter, sour), and overall acceptability.

2.6. Statistical analysis

The statistical analysis of the findings obtained in this study was performed using Minitab 17.0 software. Analysis of variance (ANOVA) was performed to evaluate the significance of the procedures applied to the samples. According to the experimental design, Tukey's multiple comparison test was applied to determine the sources of significant variation and to separate effects with different impacts.

3. Results and Discussion

The chemical properties of the Kashar cheese samples showed significant changes during the ripening process. Vacuum packaging was found to increase the dry matter content during ripening, and this increase was statistically significant ($P < 0.05$). The highest dry matter content was observed in the G1 cheese sample. These results differ from previous studies reporting that the dry matter content of Kashar cheeses generally does not change significantly during ripening (Koca, 2002; Kececi et al., 2006). Differences in dry matter among samples may be related to variations in acidity, salt content, and the ability of the cheese to retain water (Üçüncü, 2008). Throughout the ripening process, the fat content of CC cheese increased similarly to other groups, but showed statistically significant differences compared

Table 1. Changes in the chemical content of cheese samples during ripening

	Cheese Types	Ripening Times (Days)			
		3	30	60	90
Dry Matter (%)	CC	46.01±0.48 ^{d, B}	49.21±0.11 ^{c, C}	51.72±0.32 ^{b, BC}	53.91±0.18 ^{a, A}
	G1	46.67±0.43 ^{d, AB}	49.88±0.16 ^{c, AB}	52.60±0.24 ^{b, A}	54.34±0.28 ^{a, A}
	G2	47.18±0.18 ^{d, A}	50.36±0.19 ^{c, A}	52.07±0.18 ^{b, AB}	54.17±0.06 ^{a, A}
	G3	46.55±0.12 ^{d, AB}	49.61±0.38 ^{c, BC}	51.81±0.16 ^{b, BC}	54.11±0.17 ^{a, A}
	G4	46.30±0.02 ^{d, B}	49.65±0.01 ^{c, BC}	51.12±0.32 ^{b, C}	53.02±0.42 ^{a, B}
	G5	46.78±0.14 ^{d, AB}	50.12±0.16 ^{c, AB}	51.61±0.32 ^{b, BC}	53.72±0.04 ^{a, A}
Fat (%)	CC	21.17±0.29 ^{c, B}	22.50±0.00 ^{b, A}	23.17±0.29 ^{a, A}	23.67±0.29 ^{a, A}
	G1	21.33±0.29 ^{c, B}	22.00±0.00 ^{b, A}	22.33±0.29 ^{b, BC}	23.67±0.29 ^{a, A}
	G2	21.17±0.29 ^{c, B}	22.17±0.29 ^{b, A}	22.67±0.29 ^{b, ABC}	23.50±0.00 ^{a, AB}
	G3	21.83±0.29 ^{b, AB}	22.00±0.50 ^{b, A}	22.33±0.29 ^{b, BC}	23.50±0.50 ^{a, AB}
	G4	21.67±0.29 ^{b, AB}	21.67±0.58 ^{b, A}	22.00±0.00 ^{ab, C}	22.83±0.29 ^{a, B}
	G5	22.17±0.29 ^{c, A}	22.33±0.29 ^{cb, A}	22.83±0.29 ^{b, AB}	23.50±0.00 ^{a, AB}
Titrateable acidity (La%)	CC	1.17±0.01 ^{d, A}	1.23±0.01 ^{c, A}	1.55±0.01 ^{b, A}	1.63±0.01 ^{a, B}
	G1	1.17±0.01 ^{d, A}	1.24±0.01 ^{c, A}	1.58±0.02 ^{b, A}	1.66±0.01 ^{a, A}
	G2	1.19±0.01 ^{d, A}	1.23±0.01 ^{c, A}	1.55±0.01 ^{b, A}	1.66±0.01 ^{a, A}
	G3	1.19±0.01 ^{d, A}	1.25±0.01 ^{c, A}	1.55±0.01 ^{b, A}	1.62±0.01 ^{a, B}
	G4	1.17±0.01 ^{d, A}	1.23±0.01 ^{c, A}	1.55±0.01 ^{b, A}	1.65±0.01 ^{a, AB}
	G5	1.17±0.01 ^{d, A}	1.24±0.01 ^{c, A}	1.56±0.01 ^{b, A}	1.62±0.01 ^{a, B}
pH	CC	6.16±0.02 ^{a, D}	6.01±0.01 ^{b, C}	5.71±0.01 ^{c, D}	5.70±0.00 ^{d, CD}
	G1	6.27±0.02 ^{a, A}	6.08±0.01 ^{b, B}	5.93±0.01 ^{c, B}	5.72±0.02 ^{d, CD}
	G2	6.23±0.01 ^{a, AB}	6.17±0.01 ^{b, A}	6.07±0.01 ^{c, A}	5.75±0.02 ^{d, BC}
	G3	6.19±0.01 ^{a, CD}	6.00±0.02 ^{b, C}	5.75±0.01 ^{c, C}	5.69±0.00 ^{d, D}
	G4	6.22±0.02 ^{a, BC}	6.13±0.02 ^{b, A}	6.05±0.02 ^{c, A}	5.79±0.02 ^{d, B}
	G5	6.21±0.01 ^{a, BC}	6.08±0.01 ^{b, B}	5.91±0.01 ^{c, B}	5.89±0.02 ^{c, A}
Salt (%)	CC	2.46±0.01 ^{d, A}	2.84±0.02 ^{c, B}	3.26±0.02 ^{b, B}	3.95±0.03 ^{a, B}
	G1	2.46±0.02 ^{d, A}	2.84±0.03 ^{c, B}	3.30±0.02 ^{b, AB}	3.98±0.03 ^{a, B}
	G2	2.45±0.05 ^{d, A}	2.85±0.03 ^{c, A}	3.31±0.02 ^{b, AB}	3.98±0.03 ^{a, B}
	G3	2.51±0.06 ^{d, A}	2.89±0.03 ^{c, B}	3.33±0.06 ^{b, AB}	4.09±0.03 ^{a, A}
	G4	2.48±0.01 ^{d, A}	2.86±0.01 ^{c, B}	3.37±0.02 ^{b, A}	3.99±0.02 ^{a, B}
	G5	2.61±0.03 ^{d, A}	2.87±0.08 ^{c, B}	3.34±0.02 ^{b, AB}	4.09±0.02 ^{a, A}
Protein (%)	CC	20.17±0.01 ^{d, E}	22.07±0.02 ^{c, A}	22.57±0.02 ^{b, E}	23.67±0.02 ^{a, E}
	G1	21.70±0.03 ^{d, A}	22.51±0.03 ^{c, A}	22.96±0.01 ^{b, C}	25.01±0.03 ^{a, A}
	G2	21.21±0.03 ^{d, C}	22.01±0.02 ^{c, A}	22.77±0.01 ^{b, D}	24.53±0.01 ^{a, D}
	G3	21.45±0.02 ^{c, B}	23.02±1.16 ^{b, A}	24.07±0.01 ^{ab, A}	24.92±0.01 ^{a, B}
	G4	21.25±0.01 ^{d, C}	22.46±0.01 ^{c, A}	23.63±0.02 ^{b, B}	24.87±0.03 ^{a, C}
	G5	20.56±0.02 ^{d, D}	21.77±0.01 ^{c, A}	22.97±0.01 ^{b, C}	24.52±0.02 ^{a, D}

CC: Control cheese, G1: Nettle added-, G2: Parsley added-, G3: Lemon balm added-, G4: Mint added-, G5: Arugula added cheeses. Values are means of two replicated trials ± standard deviation; different superscript lowercase letters indicate significant differences between cheese types and ripening time ($P < 0.05$).

to cheeses to which herbs were added at different stages. Therefore, while CC cheese showed similarities to some

groups in terms of fat content, it showed significant differences from other groups ($P < 0.05$) (Table 1). Fat levels showed a slight increase as ripening progressed, and this may be due to an increase in dry matter. In all cheeses, lactic acid (%) increases steadily from the 3rd day to the 90th day. The highest titration acidity was observed on the 90th day in G1 (stinging nettle cheese). In terms of pH, the control cheese generally had lower values than the cheeses with added herbs throughout the ripening process. At the end of the period, the cheese containing lemon balm reached the lowest pH value 5.69 (Table 1). In contrast, (Agboola and Radovanovic-Tesic, 2002), pH values ranging from 5.07 to 5.20 have been reported for local mint-flavoured Australian semi-hard cheeses, and variable pH levels have been observed during ripening. These values are lower than the pH levels indicated in this study. Previous studies have shown that pH values in kashar cheese decrease with ripening due to lactose breakdown and metabolite production (Kaminarides et al., 1999; Güven and Tatar Görmez, 2004). Salt content during ripening (Çakmakci and Çağlar, 1995) indicates that the increase in dry matter in kashar cheese causes a proportional increase in salt, which is higher at the beginning of ripening and lower in subsequent stages. Salt significantly affects the ripening of cheese by regulating water activity, microbial and enzymatic activities, and protein transformations (Holsinger et al., 1995). The salt content also increased over time and showed slight differences between different types of cheese. In this study, herb-added kashar cheeses showed the highest salt content at 4.09% (lemon balm and arugula) on day 90, while this rate was 3.95% in the control group (Table 1). The average salt content of the samples was $3.17 \pm 0.03\%$, in alignment with previous studies reporting salt values between 1.27–1.34% (Yaşar, 2007), 2.89–5.27% (Yılmaz, 2011), 1.03–1.91% (Çürük, 2006), and 2.10–4.10% (Bayram, 2018). Milk proteins, particularly casein, play an important role in the ripening of cheese, the development of its flavour, aroma and texture, and contribute to the cheese's water-binding capacity (Fox et al., 1993). An increase in protein content was observed during ripening, and a statistically significant difference ($P < 0.05$) was found between periods. At the end of the ripening period, cheeses containing nettle and lemon balm had significantly higher protein content than the control group (Table 1). The increase in protein content has been linked to bioactive compounds found in plants, particularly phenolics, which can enhance protein stability (Lončar et al., 2024). Previous studies report that the protein content of Kashar cheese varies widely, generally between 12.78% and 33.67% (Hayaloğlu, 2003; Güven and Tatar Görmez, 2004; Tarakçı and Bayram, 2020). The main chemical changes observed during ripening are shown in Table 1.

3.1. Biochemical changes in cheese

The water-soluble nitrogen content in cheese (WSN, % of total nitrogen) is an important indicator of proteolysis rate and ripening. It indicates the formation of low molecular weight nitrogen fractions resulting from casein hydrolysis. (Aydın, 1996). During the ripening process, the casein and para-casein molecules in the cheese structure undergo hydrolysis through rennet and microbial activity, breaking down into smaller compounds (such as amino acids, peptides, polypeptides, proteases, and peptones). This breakdown affects the natural structure of the cheese. That is, some of the nitrogenous compounds become water-soluble, and thus the water-based organic compound content increases during the ripening period (Çakmakci and Çağlar, 1995). In this study, WSN content increased significantly during ripening. ($P < 0.05$) (Table 2) showed that the effect of herb addition on WSN levels was significant on the 3rd and 60th days of ripening and insignificant in other periods. The WSN value was found to be in the range of 0.24 ± 0.01 to 0.75 ± 0.02 (Table 2). Consistent with these findings, other researchers have also observed an increase in WSN content during the ripening of kashar cheese (Çürük, 2006; Keceli et al., 2006). Among the cheeses with herb addition, G1 (nettle) exhibited significantly higher WSN, ripening index, and NPN values compared to the control cheese. (Table 2). This suggests a possible increase in early proteolysis. The ripening index (RI) increased steadily throughout the ripening period in both control and grass-fed cheeses, and was generally higher in the grass-fed groups. The NPN value was found to be highest in sample G1, at 4.34 ± 0.02 . There is a significant difference at the $P < 0.05$ level in terms of NPN (%) between cheese groups at each ripening stage (1, 30, 60, 90 days). The highest RI values were observed particularly in the G1 and G3 samples. The faster proteolysis in grass-fed cheeses is shown in the RI and NPN values. These findings suggest that herbs, particularly nettles, may contribute to the ripening of cheese. These findings indicate that NPN values in the control cheese were lower than those in herb-added cheeses, with statistically significant differences between cheese types and ripening stages ($P < 0.05$). In Circassian cheese with different added spices, a significant increase in the NPN ratio was observed during the ripening period, indicating the formation of peptides and free amino acids as a result of proteolytic activity (Gezmiş and Tarakçı, 2020). (Yılmaz, 2011) reported that WSN values ranged from 0.246% to 0.650% during ripening. (Gezmiş and Tarakçı, 2020) found that the addition of black cumin and black pepper accelerated ripening in Circassian cheese. While the lowest ripening index was in the control

cheese with a value of 6.95 ± 0.02 on the 0th day, the highest was in the nettle-added cheese with a value of 14.12 ± 0.01 on the 90th day. Significant differences in the ripening index were observed between periods during the ripening process among the cheese samples ($P < 0.05$) (Table 2). (Bayram, 2018) reported that WSN/TN values during storage ranged between $7.15 \pm 0.32\%$ and $9.93 \pm 0.08\%$, with an overall average of $8.71 \pm 0.18\%$ in fruit-added kashar cheeses. Another study found a WSN/TN ratio of $10.00 \pm 0.34\%$ in control cheese, while saffron-added cheeses showed ratios of $10.00 \pm 0.43\%$, $10.85 \pm 0.24\%$, and $11.42 \pm 0.32\%$ (Aktypis et al., 2018). The results of the present study suggest that the addition of herbs influenced the levels of nitrogen compounds during ripening, particularly at the early stages of ripening.

Table 2. Changes in the ripening index (WSN/TN), WSN, and NPN during ripening of cheeses

	Cheese Types	Ripening Times (Days)			
		3	30	60	90
WSN (%)	CC	$0.24 \pm 0.01^{d, B}$	$0.46 \pm 0.01^{c, A}$	$0.64 \pm 0.02^{b, B}$	$0.71 \pm 0.01^{a, A}$
	G1	$0.27 \pm 0.01^{d, A}$	$0.47 \pm 0.02^{c, A}$	$0.67 \pm 0.01^{b, AB}$	$0.75 \pm 0.02^{a, A}$
	G2	$0.25 \pm 0.01^{d, AB}$	$0.44 \pm 0.01^{c, A}$	$0.65 \pm 0.01^{b, A}$	$0.74 \pm 0.01^{a, A}$
	G3	$0.26 \pm 0.01^{d, AB}$	$0.47 \pm 0.02^{c, A}$	$0.67 \pm 0.01^{b, AB}$	$0.72 \pm 0.02^{a, A}$
	G4	$0.25 \pm 0.01^{d, AB}$	$0.46 \pm 0.01^{c, A}$	$0.66 \pm 0.01^{b, AB}$	$0.72 \pm 0.01^{a, A}$
	G5	$0.24 \pm 0.01^{d, B}$	$0.45 \pm 0.01^{c, A}$	$0.65 \pm 0.01^{b, AB}$	$0.72 \pm 0.01^{a, A}$
Ripening Index	CC	$6.95 \pm 0.02^{d, F}$	$9.92 \pm 0.02^{c, E}$	$12.49 \pm 0.01^{b, D}$	$13.24 \pm 0.04^{a, E}$
	G1	$7.95 \pm 0.01^{d, A}$	$10.86 \pm 0.02^{c, A}$	$12.85 \pm 0.02^{b, A}$	$14.12 \pm 0.01^{a, A}$
	G2	$7.17 \pm 0.02^{d, D}$	$10.24 \pm 0.01^{c, C}$	$12.66 \pm 0.02^{b, C}$	$13.74 \pm 0.01^{a, D}$
	G3	$7.32 \pm 0.01^{d, B}$	$10.65 \pm 0.01^{c, B}$	$12.74 \pm 0.01^{b, B}$	$13.79 \pm 0.02^{a, B}$
	G4	$7.12 \pm 0.01^{d, E}$	$10.08 \pm 0.01^{c, D}$	$12.31 \pm 0.02^{b, E}$	$13.97 \pm 0.02^{a, C}$
	G5	$7.24 \pm 0.01^{d, C}$	$10.27 \pm 0.01^{c, C}$	$12.53 \pm 0.02^{b, D}$	$13.41 \pm 0.03^{a, D}$
NPN (%)	CC	$1.51 \pm 0.02^{d, C}$	$2.62 \pm 0.01^{c, C}$	$3.71 \pm 0.02^{b, D}$	$4.25 \pm 0.02^{a, B}$
	G1	$1.58 \pm 0.02^{d, A}$	$2.67 \pm 0.02^{c, A}$	$3.78 \pm 0.01^{b, A}$	$4.34 \pm 0.02^{a, A}$
	G2	$1.55 \pm 0.01^{d, A}$	$2.67 \pm 0.01^{c, A}$	$3.76 \pm 0.01^{b, AB}$	$4.33 \pm 0.01^{a, A}$
	G3	$1.53 \pm 0.01^{d, B}$	$2.66 \pm 0.01^{c, AB}$	$3.75 \pm 0.01^{b, ABC}$	$4.30 \pm 0.01^{a, A}$
	G4	$1.52 \pm 0.01^{d, BC}$	$2.65 \pm 0.01^{c, B}$	$3.75 \pm 0.01^{b, BC}$	$4.28 \pm 0.01^{a, B}$
	G5	$1.52 \pm 0.01^{d, BC}$	$2.65 \pm 0.01^{c, AB}$	$3.73 \pm 0.01^{b, C}$	$4.28 \pm 0.01^{a, B}$

CC: Control cheese, G1: Nettle added-, G2: Parsley added-, G3: Lemon balm added-, G4: Mint added-, G5: Arugula added cheeses. Values are means of two replicated trials \pm standard deviation; different superscript lowercase letters indicate significant differences between cheese types and ripening time ($P < 0.05$).

3.2. Electrophoretic casein fractions in cheese

Thanks to the activities of proteolytic and other destructive enzymes in cheeses, proteins are broken down and converted into the large and small peptides, amino acids, and smaller organic molecules that compose them, and this hydrolysis can be monitored using various methods. Using one such method, gel electrophoresis, large gel peptides are detected (Ardö and Polychroniadou, 1999). The proteolysis of casein begins with the addition of rennet to milk; rennet plays an important role in the hydrolysis of α s1-casein and breaks this fraction down into α s1-I peptide. Another important casein is β -casein. Plasmin has a significant effect on the hydrolysis of β -casein (Caessens et al., 1999). Figure 1 presents images of gel electrophoresis analysis showing the ripening stages of cheese samples analysed using the urea-PAGE electrophoresis method.

Urea-PAGE electrophoretic analyses were conducted on cheese samples, and the results are presented in the form of electrophoretic bands. These bands illustrate the electrophoretic profiles of casein fractions in the cheeses at 3, 30, 60, and 90 days of ripening, respectively. The casein levels in the G1 sample on days 30 and 60 were lower than in other herb-added cheeses, suggesting that protease activity may have increased. It was observed that α s1-casein degradation was faster in the G1 and G4 cheese samples compared to the control group cheese (Figure 1). In contrast, G2 and G5 exhibited a more controlled and gradual proteolysis similar to the control cheese, showing a moderate effect on protein breakdown. G3 also showed a significant decrease in α s1-casein concentration, although not as pronounced as nettle and mint. As shown in Figure 1, the concentrations of β -casein and α s1-casein in the gels gradually decreased throughout the ripening period, with α s1-casein showing a more pronounced decrease compared to β -casein. In parallel, an increase was observed in the degradation products of

α s1-casein, supporting the findings of (Broyard and Gaucheron, 2015). Reviewing similar studies, (Atasoy and Türkoğlu, 2009) reported that, α s1-casein is hydrolysed more extensively than β -casein in all types of Urfa cheese produced from cow, sheep and goat milk. The band intensity of both β -casein and α s1-casein decreased during storage. Consistent with these findings, (Çürük, 2006) noted that there was a statistically significant and consistent decrease in β -casein levels during cheese storage. Similarly, (Yasar and Guzeler, 2011), in their study on Kashar cheese, they observed a statistically significant decrease in β -casein and α s1-casein concentrations, particularly after the 30th day of ripening. Along with this, there was an increase in the degradation products of α s1-casein. In all examined Kashar cheese samples, it was noted that α s1-casein and β -casein continuously decreased during the ripening process, while γ -casein and other degradation products, including the α s1-I peptide, increased (Irigoyen et al., 2000). Additionally, it has been observed that the amount of α s2-casein in cheese samples decreases during ripening. Studies have shown that α s2-casein hydrolysis is closely related to the activity of plasmin, a natural milk protease (Tarakci and Tuncturk, 2008).

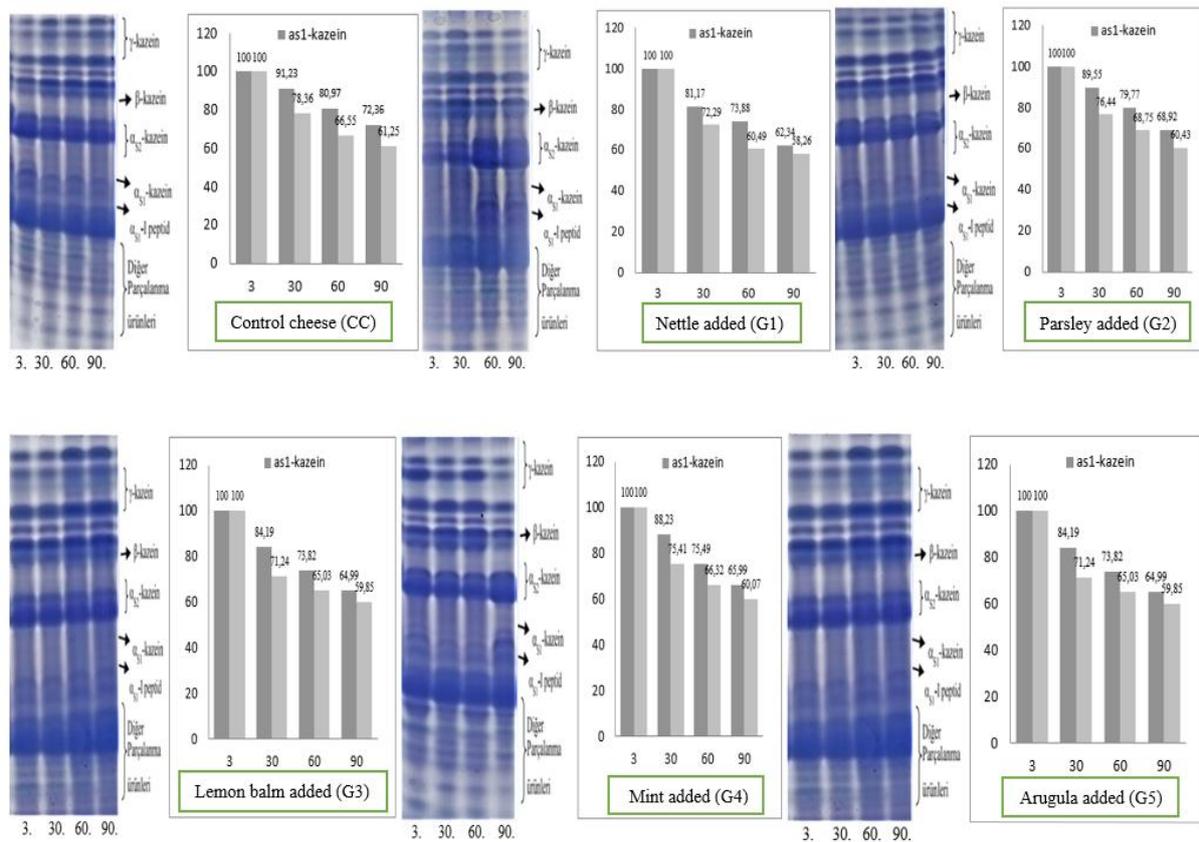


Figure 1. Urea-polyacrylamide gel electrophoresis profiles and graphics of experimental cheeses during ripening.

3.3. Sensory properties of kashar cheese

Sensory evaluation by the panelists indicated that scores for color and appearance, odor, structure and texture, taste and flavour, as well as overall acceptability generally increased throughout the ripening period (Table 3). The control cheese consistently scored high, particularly in color and appearance and structure and texture, reaching peak scores of 8.30 ± 0.10 and 8.73 ± 0.15 , respectively, at 90 days. However, G2 outperformed the control in several categories, achieving the highest scores in odor (9.07 ± 0.06 at 60 days), taste and flavour (9.10 ± 0.08 at 90 days), and general acceptability (8.40 ± 0.10 at 90 days), indicating that the herbs added to G2 significantly enhanced its sensory properties. G4 achieved better results, particularly in taste and aroma, reaching 8.63 ± 0.12 in 90 days and 8.30 ± 0.10 in overall acceptability. In contrast, the G1 sample received the lowest scores overall in sensory characteristics. Overall, the findings indicate that adding certain herbs, such as those in G2 and G4, can enhance the sensory properties of kashar cheese, while others may reduce its appeal. As shown in Table 3, sensory analysis revealed that the highest level of

appeal occurred on the 90th day of ripening. Statistical analysis confirmed that the addition of herbs caused a significant difference in the taste and flavour of the cheese samples ($P<0.05$) (Table 3). In general, across all groups (G1–G5), it is observed that the scores for colour-appearance, odour, structure-texture, taste-flavour and overall liking do not show significant fluctuations as the storage period increases (3–90 days), remaining at acceptable levels (Figure 2). In another study, (Fiol et al., 2016) that fresh cheese with nettles was liked and found acceptable by consumers. Additionally, it has been found that adding aromatic waters obtained from various plants such as thyme and garlic significantly improves the textural properties of cheeses (Sağdıç et al., 2017). The formation of characteristic cheese taste and aroma is attributed to the breakdown products of para- κ -casein (Fox et al., 1993). These aromas are formed by the combination of various volatile compounds produced by the breakdown of amino acids, peptides, fats and proteins. As the quantity and type of these compounds change during the ripening process, the taste and aroma of cheeses produced using different methods also differ significantly from one another (Çakmakci and Çağlar, 1995).

Table 3. Sensory evaluation in cheese samples

Cheese Types	Ripening Times (Days)				
	3	30	60	90	
Color and Appearance	CC	7.07±0.12 ^{c, AB}	6.97±0.25 ^{d, AB}	7.60±0.17 ^{b, C}	8.30±0.10 ^{a, C}
	G1	6.50±0.10 ^{b, C}	6.80±0.01 ^{c, AB}	7.37±0.15 ^{d, D}	7.60±0.10 ^{a, F}
	G2	7.00±0.20 ^{d, A}	7.30±0.01 ^{c, A}	7.80±0.01 ^{b, A}	8.43±0.06 ^{a, B}
	G3	6.70±0.10 ^{d, BC}	7.00±0.10 ^{c, A}	7.33±0.15 ^{b, D}	8.03±0.06 ^{a, D}
	G4	7.10±0.10 ^{c, AB}	6.77±0.25 ^{d, AB}	7.73±0.25 ^{b, B}	8.57±0.12 ^{a, A}
	G5	6.30±0.20 ^{d, C}	6.53±0.15 ^{c, B}	7.07±0.12 ^{b, E}	7.97±0.21 ^{a, E}
Odor	CC	7.63±0.12 ^{c, B}	8.50±0.10 ^{b, AB}	8.70±0.42 ^{a, B}	8.50±0.10 ^{b, C}
	G1	5.37±0.15 ^{d, E}	6.47±0.06 ^{b, AB}	6.67±0.21 ^{a, F}	6.17±0.06 ^{c, F}
	G2	8.67±0.15 ^{d, A}	8.83±0.12 ^{b, A}	9.07±0.06 ^{a, A}	8.70±0.10 ^{c, A}
	G3	6.67±0.15 ^{b, D}	6.63±0.06 ^{c, B}	6.77±0.15 ^{a, E}	6.43±0.12 ^{d, E}
	G4	8.67±0.06 ^{a, A}	8.40±0.10 ^{c, AB}	8.07±0.12 ^{d, C}	8.63±0.25 ^{b, B}
	G5	7.13±0.12 ^{c, C}	6.93±0.15 ^{d, AB}	7.27±0.15 ^{b, D}	7.20±0.26 ^{a, D}
Structure and Texture	CC	7.57±0.06 ^{c, A}	7.37±0.06 ^{d, B}	8.00±0.10 ^{b, A}	8.73±0.15 ^{a, A}
	G1	6.80±0.10 ^{d, D}	7.17±0.06 ^{c, C}	7.47±0.06 ^{b, C}	8.20±0.10 ^{a, C}
	G2	7.13±0.06 ^{d, C}	7.43±0.06 ^{c, A}	7.73±0.06 ^{b, B}	8.00±0.10 ^{a, D}
	G3	7.40±0.10 ^{c, B}	7.13±0.06 ^{d, C}	7.47±0.06 ^{b, C}	8.47±0.15 ^{a, B}
	G4	6.57±0.12 ^{d, E}	6.80±0.10 ^{c, D}	7.07±0.31 ^{b, D}	7.27±0.15 ^{a, F}
	G5	6.43±0.06 ^{c, F}	6.13±0.06 ^{d, E}	6.80±0.10 ^{b, E}	7.77±0.15 ^{a, E}
Taste and Flavour	CC	7.70±0.10 ^{c, B}	7.67±0.15 ^{d, C}	8.47±0.06 ^{b, A}	8.90±0.10 ^{a, B}
	G1	5.30±0.20 ^{d, E}	5.70±0.10 ^{b, F}	5.43±0.12 ^{c, F}	6.10±0.10 ^{a, E}
	G2	8.03±0.15 ^{d, AB}	8.67±0.21 ^{b, A}	8.37±0.25 ^{c, B}	9.10±0.10 ^{a, A}
	G3	6.66±0.10 ^{d, C}	6.90±0.10 ^{b, D}	6.80±0.10 ^{c, D}	7.23±0.15 ^{a, D}
	G4	8.80±0.10 ^{a, A}	8.20±0.10 ^{c, B}	8.13±0.15 ^{d, C}	8.63±0.12 ^{b, C}
	G5	6.07±0.06 ^{d, D}	6.30±0.10 ^{c, E}	6.60±0.10 ^{b, E}	7.20±0.10 ^{a, D}
General acceptability	CC	7.93±0.15 ^{d, A}	8.47±0.06 ^{b, A}	8.53±0.06 ^{a, A}	8.17±0.25 ^{c, A}
	G1	7.20±0.10 ^{b, D}	6.93±0.25 ^{c, D}	6.63±0.32 ^{d, E}	7.53±0.15 ^{a, B}
	G2	7.63±0.15 ^{c, B}	7.97±0.21 ^{b, B}	7.47±0.15 ^{d, C}	8.00±0.30 ^{a, A}
	G3	6.80±0.10 ^{d, F}	7.50±0.10 ^{b, C}	7.23±0.25 ^{c, D}	7.80±0.10 ^{a, AB}
	G4	7.53±0.15 ^{d, C}	8.00±0.10 ^{c, B}	8.30±0.10 ^{b, B}	8.40±0.10 ^{a, A}
	G5	7.10±0.10 ^{a, E}	6.70±0.10 ^{a, E}	7.47±0.15 ^{a, C}	7.90±0.10 ^{a, AB}

CC: Control cheese, G1: Nettle added-, G2: Parsley added-, G3: Lemon balm added-, G4: Mint added-, G5: Arugula added cheeses. Values are means of two replicated trials \pm standard deviation; different superscript lowercase letters indicate significant differences between cheese types and ripening time ($P<0.05$).

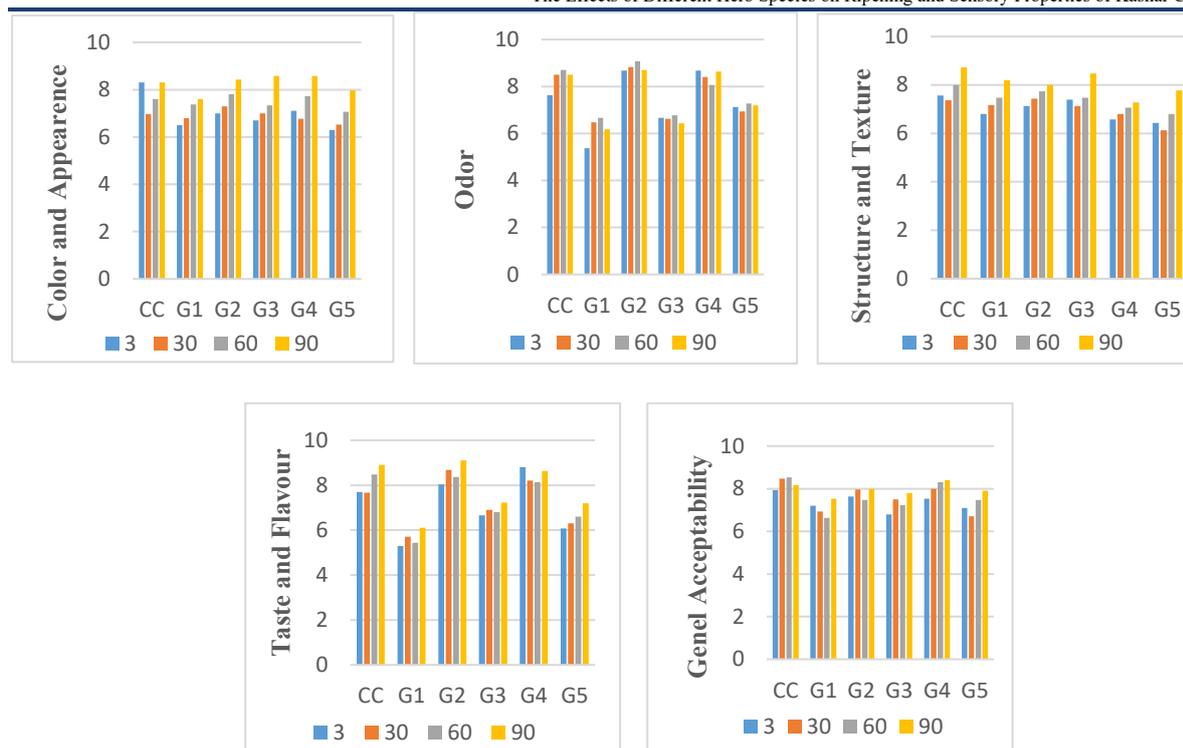


Figure 2. Sensory changes in cheese. CC: Control cheese, G1: Nettle added-, G2: Parsley added-, G3: Lemon balm added-, G4: Mint added-, G5: Arugula added cheese

4. Conclusions

Kashar cheese can be produced with the addition of various herbs. It has been demonstrated that the use of herbs in Kashar cheese production positively affects the cheese's chemical, biochemical, and sensory properties, depending on the type of herb used. In cheeses with herb additions, slight improvements in some sensory properties (particularly taste–aroma and odour) compared to the control group are noticeable; this indicates that herb additions can positively affect sensory quality. In particular, the addition of nettle and lemon balm has been associated with higher ripening indices and NPN values, and these herbs have been found to have an accelerating and supportive effect on cheese ripening. This finding indicates that the addition of herbs does not inhibit the ripening process but rather contributes positively to the ripening rate. The ripening process can be further investigated through methods such as increasing the herb ratio, using herbs in powder form, or incorporating herbs at different stages of cheese production. Adding herbs to Kashar cheese also encourages greater cheese consumption by increasing product diversity and offering consumers alternative options. Future studies may help reduce storage, labour, and energy costs by shortening the necessary ripening period in Kashar cheese production.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Planning: Aydın, E., Tarakçı, Z.; Materials and Methods: Tarakçı, Z., Aydın, E.; Data Collection and Processing: Aydın, E., Tarakçı, Z.; Statistical Analysis: Aydın, E.; Literature Review: Aydın, E., Tarakçı, Z.; Article Writing, Review and Editing: Aydın, E.

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