IMPLICATIONS OF STABILIZERS USED IN BRINE OF SOFT WHITE CHEESE ON THE AROMA PROFILE OF CHEESES

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Received / Geliş: 17.05.2022; Accepted / Kabul: 13.10.2023; Published online / Online baskı: 19.10.2023


ABSTRACT
This paper describes the compositional characterization of cheese brine and aroma profile of Soft White cheese which contains different stabilizers such as sodium caseinate, carrageenan and guar gum in the brine solutions. During the 60-day storage period, pH, titration acidity, dry matter, fat, salt and protein amounts of cheese brines were analyzed. The aroma profile of the cheese samples was examined by gas chromatography–mass spectrometry using a solid-phase microextraction technique and the proportional distribution of volatile compounds was revealed. The stabilizers in the brine increased the dry matter content in the cheese mass by binding the water and reduced salt penetration into the cheese mass. A total of 23 aromatic compounds were identified including 4 aldehydes, 5 alcohols, 4 esters, 1 ether, 5 hydrocarbons, 2 ketones and 2 organic acids in cheeses. Carrageenan and guar gum were found more effective than sodium caseinate in terms of flavor retention.

Keywords: Soft White cheese, brine, stabilizer, aroma compounds

ABSTRACT
Bu makalede, salamuradasında sodum kazeinat, karragenan ve guar gam gibi farklı stabilizörler içeren Yumuşak Beyaz peynirlerin aroma profilleri ve peynir salamuralarının bileşim özellikleri incelenmiştir. Peynir salamuralarının pH, titrasyon asitliği, kurumadde, yağ, tuz ve protein oranları 60 günlük depolama süresince analiz edilmiştir. Peynir örneklerinin aroma profili, gaz kromatografi-kütle spektrometri ile katı faz mikroekstraksiyon tekniği kullanılarak incelenmiş ve üçüncü bileşiklerin orantısal dağılımı ortaya çıkarılmıştır. Salamuradaki stabilizörler suyu bağlayarak peynir kitesindeki kurumadde oranını artırılmış ve kitleye tuz geçişini azaltmıştır. Peynirlerde 4 aldehit, 5 alkol, 4 ester, 1 eter, 5 hidrokarbon, 2 keton ve 2 organik asit olmak üzere toplam 23 aromatik bileşik tespit edilmiştir. Karragenen ve guar gam, aroma tutuculuğu açısından sodum kazeinattan daha etkili bulunmuştur.

Anahtar kelimeler: Yumuşak Beyaz peynir, salamura, stabilizör, aroma bileşikleri

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INTRODUCTION
Aroma is one of the most important criteria that determine cheese quality (Fox and Wallace, 1997). It was reported that more than 300 different volatile and non-volatile compounds are added to the cheese flavor. These compounds emerge as a result of cheese microflora, enzymes that are added to cheese or naturally found in cheese, and the effects of these enzymes on lactose, lipids and proteins (Fox et al., 1995). The aroma profile of cheeses is highly influenced by the water-soluble fraction, as it contains most of its aromatic compounds such as salts, amino acids and low molecular weight peptides in addition to some volatiles (Tavaria et al., 2003).

It is known that the flavors of foods are affected by the type and amount of stabilizer used (Malone et al., 2003). Many researchers have shown that in the presence of hydrocolloid, the viscosity of foods increases and the flavor perception decreases, and the use of hydrocolloid in increasing concentration gradually reduces the perceived aroma level (Pangborn and Szczesniak, 1974; Guichard, 2002). The main reason for this is that hydrocolloids increase the viscosity of foods and reduce the transfer of flavor compounds from the matrix to the vapor (Guichard et al., 2016). The use of stabilizers in milk and dairy products affects milk proteins and causes changes in the structure of the products (Lin et al., 2017). Modified starch (Benaoudaj et al., 2017), dietary fibers (Suvera et al., 2017), hydrocolloids (gum) (Murtaza et al., 2017) and some polysaccharides (Yousefi and Jafari, 2019) are used as stabilizers or thickeners in cheese making.

Sodium caseinate is defined as a milk protein and is often used as an emulsifier and stabilizer. It binds water and oil molecules together and positively affects the appearance of foods by reducing fat particles (Abdolmaleki et al., 2019). Sodium caseinate has many hydrophilic groups and hydrophobic groups that attract each other with water and oil analogs, respectively, thus having emulsifying and stabilizing capacities (Bai et al., 2019).

Carrageenan is a linear sulfated polysaccharide obtained from various species of edible red algae belonging to the Rhodophyceae family and is widely used as a thickener, stabilizer or gelling agent in food products, pharmaceutical applications and cosmetics. The molecular structure of carrageenan is based on a disaccharide repeat of alternating D-galactose and 3,6-anhydro-galactose (3,6-AG) units joined by α-1,4 and β-1,3 glycosidic linkages (Nogueira et al., 2019; Tang et al., 2019; Wurm et al., 2019).

Guar gum is a natural carbohydrate polymer obtained from the seed plant Cyamopsis tetragonoloba, widely grown in India and Pakistan. It consists mainly of galactomannan (80%). It has linear chains of (1-4)-β-D-mannopyranosyl units joined by (1-6)-α-D-galactopyranosyl distributed in side branches. In addition, guar gum functions as a highly compatible starting material forming with water-soluble vinyl functional monomers to obtain state-of-the-art bio-based materials due to its advantageous shelf life, biodegradability, superabsorbency and mechanical strength properties (Simiqueli et al., 2019; Yang et al., 2019).

In other studies, stabilizers were often added to cheese milk (Jana et al., 2010; Zomorodi et al., 2020) or used as coating material (Kampf and Nussinovitch, 2000; Goulart et al. 2019). These methods require a high amount of stabilizer use or an intensive labor force. Thus, economic losses, time and energy losses may occur. However, the amount of stabilizers used in brine is much lower and its application is a very simple process. For this reason, the above-mentioned disadvantages are reduced or prevented by the use of stabilizers in the cheese brine. However, the use of stabilizers in brine was found in only one study in the literature (Cankurt, 2019). In the research conducted by Cankurt (2019), guar gum, carrageenan, xanthan gum and gelatin were added to the White cheese brine and the physicochemical, textural, sensory and microbiological properties of the cheeses were examined. However, it was not investigated how the stabilizers in the brine affect the aroma compounds in the cheese.
Another innovative aspect of this research is that the aroma profile of soft white cheese, which is well known as a name of "white cheese with the consistency of Turkish delight" ("Lokum kıvamında Beyaz peynir" in Turkish) by the consumer but whose scientific properties are unknown, has been examined. There is no standard production method for this soft white cheese type in Turkey. Although this cheese variety is similar to the classic white cheese, it contains some differences in its process. In this context, the main difference is the application of pasteurization (82°C) at high temperatures (Aydemir and Kurt, 2020). In addition, thermophilic culture can be used together with mesophilic starter culture in these cheeses. White cheese with the consistency of Turkish delight is much softer than classic white cheese. Therefore, this cheese is structurally likened to Turkish delight in terms of softness.

In another part of this study, physicochemical, textural and sensory properties of soft white cheeses were determined. As a result of the research, by using stabilizer in brine, cheeses with higher dry matter and protein and less salt content were obtained and their textural properties were improved during storage. In addition, the stabilizers used did not affect the sensory properties of the cheeses (Özbek and Güzeler, 2022). In this study, it was aimed to determine the aromatic components of white cheese with unknown flavor profile, and the effects of using stabilizers in cheese brine on cheese flavor and brine composition were investigated. In this context, Soft White cheeses were stored in brine containing sodium caseinate, carrageenan and guar gum for 60 days, and brine compositions and aroma profile of cheeses were investigated on the 1st, 15th, 30th and 60th days of the storage.

MATERIAL AND METHOD

Raw materials
A mixture of milk from Simmental and Holstein cows obtained from the Güneydoğu Dairy Factory was used in cheese making. The milk used contained an average of 12.5% dry matter, 3.5% protein and 3.5% fat. Danisco Chozit TA 054 (France) (Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus helveticus) and MA 016 (Lactococcus lactis subsp. lactis and Lactococcus lactis subsp. cremoris) Feta cheese cultures (1:3 w/w) were used in the production of Soft White cheeses. Ajinomoto-ACTIVA YG microbial transglutaminase enzyme (100 g MTG/tonne of milk) was used. NATUREN® MANDRA 175 (175 IMCU value, 1/16,000 strength; contains minimum 80% chymosin enzyme, maximum 20% Pepsin enzyme) belonging to the company Chr-Hansen (Denmark) was used. Salt, calcium chloride, sodium caseinate, carrageenan and guar gum were obtained from the local producers.

Brine preparation
The amounts of stabilizers used in the research were determined by the trial productions and the best samples were selected as 0.05% carrageenan, 0.05% guar gum and 0.10% sodium caseinate, considering the sensory qualities of the samples. The compositions of the brines prepared at 38°C and 5.2 pH were shown in Table 1.

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Stabilizer Type</th>
<th>NaCl (%)</th>
<th>CaCl₂ (%)</th>
<th>Stabilizer (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>12.0</td>
<td>0.04</td>
<td>-</td>
</tr>
<tr>
<td>BRN14</td>
<td>Sodium caseinate</td>
<td>12.0</td>
<td>0.04</td>
<td>0.10</td>
</tr>
<tr>
<td>BRN76</td>
<td>Carrageenan</td>
<td>12.0</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>BRN58</td>
<td>Guar gum</td>
<td>12.0</td>
<td>0.04</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Cheese production
Cheese production was carried out in the Güneydoğu Dairy Factory (Kozan, Adana) in three replications. After the preliminary tests applied to cow’s milk, the milks were clarified and standardized to 3.5% fat content. Milk was pasteurized at 82°C for 6 minutes and cooled to incubation temperature (42°C). Mesophilic (0.075%) and thermophilic (0.025%) starter cultures, microbial transglutaminase enzyme (100
g/tonne, recommended by the manufacturer) and calcium chloride (0.16%) were added. Rennet was added to the milk at 42°C for curd formation in 70 minutes. The curd was broken (2-3 cm³), pressed for 1 hour (0.1 bar per cm²) and whey was removed. Then the cheeses were cut in 11x11 cm dimensions at pH 5.20-5.30. Cheese blocks were put in brine (pH 5.20 at 38°C) containing different stabilizers and rested for 6 hours. At the end of this period, the cheeses were placed in polypropylene packages, stabilizer-added brine was added to the cheeses and stored at 4-6°C for 60 days.

physicochemical analysis

Compositional analysis of cheese brines
pH measurements in brines were determined directly using the WTW 3110 brand pH meter (Hannon et al., 2003). Acidity determination was made according to alkali titration method and 0.1 N NaOH solution adjusted as alkali was used. Results were expressed as % lactic acid (TSE, 2002). Dry matter contents were determined using the gravimetric method and the results were expressed as percentage (%) (IDF, 2004a). Fat contents were determined according to Gerber method by using milk butyrometer (TSE, 2002). The fat in the dry matter amount was calculated by considering fat and dry matter contents of brine. Protein contents were calculated by multiplying the nitrogen amounts with a factor of 6.38 using the micro-Kjeldahl method and expressed as percentage (%) (IDF, 2004b). Salt contents were determined by the Mohr titration method according to Bradley et al. (1993) and the results are expressed as percentage (%).

Determination of aroma compounds of soft white cheeses
Aroma development in cheeses was determined by Solid Phase Microextraction (SPME) method. Aroma analyzes were carried out on the 15th day of storage. Cheese samples were frozen at -20°C. Then, 3.0 g of the frozen samples were weighed into a 20 mL vial. The samples were incubated at 40°C for 30 minutes. A solvent-free technique was used for the extraction of volatile substances (Pawliszyn et al., 1997). Extraction was performed with 1 cm 50/30 μm divinylbenzene-carboxen-polydimethylsiloxane (DVB/CAR/PDMS) (Supelco, Bellefonte, PA, USA) fiber-vial injection. The fiber was kept in the headspace for 30 minutes at 40°C, allowing the aroma substances to pass into the fiber structure. During desorption, the fiber was immersed in the injection block and kept at 250°C for 2 minutes. Helium was used as the carrier gas at a flow rate of 1 mL/min. HP-5MS (30 m × 0.25 mm × 0.25 μm; J&W Scientific, Folsom, CA, USA) column was used to separate the compounds. The applied furnace temperature program was: initially kept at 40°C for 2 minutes (desorption period), and the temperature was increased to 70°C at 5°C per minute and kept at this temperature for 1 minute. The temperature was then increased to 240°C with 10°C increments per minute and kept at this temperature for 30 minutes. The mass spectrometer was set at 33-450 amu (threshold value 1000) and the sampling rate was set at 1.11 scans per minute. Agilent 7000 Series Triple Quad GC-MS system was used to determine the volatile aroma compounds of cheeses. The 'Retention Index' (RI) values calculated by using the Wiley, National Institute of Standards and Technology (NIST) libraries and the retention times of each peak and the retention times of the hydrocarbon standard were taken as reference in the identification of aroma compounds that perform the separation.

Statistical analysis
The research was carried out as four different applications (SWC05, SWC64, SWC33, SWC17) and three replications. Statistical analyzes were performed using the ”SPSS 21.0 Package Program” and the results were compared by one-way analysis of variance (ANOVA) using the software SPSS 21.0, and the Duncan multiple comparison test was applied to significant differences whose level was assessed as p=0.05 (Efe et al., 2000).

RESULTS AND DISCUSSION
Composition of brines
During storage, pH, titration acidity, dry matter, fat and fat in dry matter, protein and protein in dry matter, and salt and salt in dry matter properties of brines were investigated. Data on the composition properties of brines were given in Table 2.
It was determined that the stabilizer type used did not cause significant statistical differences on the pH value of the brines (P > 0.05). During storage, the pH values of the brines increased, this increase was found to be significant only for the control sample (P < 0.05). It was concluded that the addition of stabilizer prevented changes in pH value during storage. Wagenaar and Dack (1958) reported that the addition of stabilizer did not cause a statistically significant change on the pH value and concentration of the cheese brine.

<table>
<thead>
<tr>
<th>Storage (days)</th>
<th>pH</th>
<th>Titratable acidity (L.a.%)</th>
<th>DM (%)</th>
<th>Fat (%)</th>
<th>Fat in DM (%)*</th>
<th>Protein (%)</th>
<th>Protein in DM (%)*</th>
<th>Salt (%)</th>
<th>Salt in DM (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>BRN14</td>
<td>BRN76</td>
<td>BRN58</td>
<td></td>
<td>BRN14</td>
<td>BRN76</td>
<td>BRN58</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5.34 ± 0.14K</td>
<td>5.47 ± 0.16K</td>
<td>5.38 ± 0.11K</td>
<td>5.41 ± 0.18K</td>
<td>0.58 ± 0.00L</td>
<td>0.40 ± 0.00L</td>
<td>9.68 ± 0.37K</td>
<td>9.83 ± 0.23K</td>
<td>10.04 ± 0.04K</td>
</tr>
<tr>
<td>15</td>
<td>5.41 ± 0.12K</td>
<td>5.40 ± 0.22K</td>
<td>5.37 ± 0.19K</td>
<td>5.31 ± 0.24K</td>
<td>0.79 ± 0.09K</td>
<td>0.56 ± 0.02L</td>
<td>11.03 ± 0.03L</td>
<td>11.92 ± 0.11L</td>
<td>11.36 ± 0.08K</td>
</tr>
<tr>
<td>30</td>
<td>5.44 ± 0.08K</td>
<td>5.53 ± 0.06K</td>
<td>5.45 ± 0.09K</td>
<td>5.46 ± 0.02K</td>
<td>0.65 ± 0.01L</td>
<td>0.65 ± 0.02K</td>
<td>11.08 ± 0.09L</td>
<td>10.72 ± 0.12L</td>
<td>10.49 ± 0.05K</td>
</tr>
<tr>
<td>60</td>
<td>5.60 ± 0.05K</td>
<td>5.52 ± 0.14K</td>
<td>5.48 ± 0.02K</td>
<td>5.41 ± 0.18K</td>
<td>0.54 ± 0.00M</td>
<td>0.40 ± 0.01M</td>
<td>13.88 ± 0.02K</td>
<td>12.98 ± 0.08K</td>
<td>13.36 ± 0.02K</td>
</tr>
<tr>
<td>1</td>
<td>0.58 ± 0.00LM</td>
<td>0.40 ± 0.00M</td>
<td>0.58 ± 0.06M</td>
<td>0.49 ± 0.04M</td>
<td>0.32 ± 0.03K</td>
<td>0.30 ± 0.00K</td>
<td>3.44 ± 0.54K</td>
<td>3.19 ± 0.10K</td>
<td>2.99 ± 0.01K</td>
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<td>15</td>
<td>0.79 ± 0.09K</td>
<td>0.56 ± 0.02L</td>
<td>0.70 ± 0.01L</td>
<td>0.72 ± 0.00K</td>
<td>0.32 ± 0.03K</td>
<td>0.32 ± 0.03K</td>
<td>2.87 ± 0.26K</td>
<td>2.68 ± 0.17L</td>
<td>2.93 ± 0.27K</td>
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<tr>
<td>30</td>
<td>0.65 ± 0.01L</td>
<td>0.65 ± 0.02K</td>
<td>0.88 ± 0.03K</td>
<td>0.73 ± 0.03K</td>
<td>2.86 ± 0.27L</td>
<td>2.89 ± 0.08L</td>
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<td>2.99 ± 0.01K</td>
<td>2.93 ± 0.27K</td>
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<td>0.99 ± 0.40N</td>
<td>0.88 ± 0.04N</td>
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<td>0.88 ± 0.19N</td>
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<td>19.97 ± 1.99LM</td>
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<td>27.82 ± 0.15K</td>
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<td>20.56 ± 1.08LM</td>
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<td>3.61 ± 0.02K</td>
<td>2.72 ± 0.04K</td>
<td>2.75 ± 0.14K</td>
<td>19.67 ± 0.04K</td>
<td>27.82 ± 0.15K</td>
<td>20.93 ± 0.23K</td>
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<td>1</td>
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<td>8.38 ± 0.31K</td>
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<td>7.24 ± 0.11K</td>
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<td>8.65 ± 3.06K</td>
<td>85.70 ± 3.92K</td>
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<td>8.32 ± 0.32K</td>
<td>7.03 ± 0.09K</td>
<td>8.14 ± 0.29K</td>
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<td>7.92 ± 0.36K</td>
<td>8.00 ± 0.46K</td>
<td>8.04 ± 0.40L</td>
<td>6.79 ± 0.15L</td>
<td>7.92 ± 0.36K</td>
<td>70.91 ± 3.75K</td>
<td>74.69 ± 5.02L</td>
<td>76.62 ± 4.24L</td>
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<tr>
<td>60</td>
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<td>7.85 ± 1.16K</td>
<td>7.92 ± 0.32K</td>
<td>7.97 ± 0.17L</td>
<td>68.57 ± 2.07LM</td>
<td>61.98 ± 1.43LM</td>
<td>61.06 ± 2.83LM</td>
<td>59.64 ± 1.18L</td>
<td>60.62 ± 2.07LM</td>
</tr>
</tbody>
</table>

BRN14: Sodium caseinate added brine, BRN76: Carrageenan added brine, BRN58: Guar gum added brine
a, b, c, d: Values that are shown in the same line with different exponential letters are different in terms of P < 0.05 level of significance.
K, I, M, N: Values that are shown in the same column with different exponential letters are different in terms of P < 0.05 level of significance.
*Fat in DM, Protein in DM and Salt in DM values were calculated by dividing these values by the total dry matter amount.
There was an increase in the acidity values of all brines during the initial stages of the storage, and these values decreased in the following days of storage ($P < 0.05$). The changes occurred depending on the stabilizer type used ($P < 0.05$). Yousefi and Jafari (2019) report that the acidity and gelation properties of the main product can vary significantly, depending on the type of hydrocolloids (amino acid or carbohydrate-based hydrocolloids) used in dairy products. While it was stated that polysaccharide-based hydrocolloids did not cause an effect on acidity, it was emphasized that amino acid-based hydrocolloids (such as gelatin or whey protein concentrate) could lower the acidity value more than the control sample without hydrocolloid addition. Sodium caseinate is in the amino acid-based hydrocolloid class (Abdolmaleki et al., 2019), and this can be explained by the fact that the titration acidity values of the sodium caseinate added brines were lower than the control sample in this study.

The addition of stabilizer caused statistically significant differences on the dry matter properties of the brines at each stage of storage ($P < 0.05$). In general, it was thought that the higher dry matter content of the control sample is related to the water retention of the stabilizers. Dry matter ratios of the brines generally increased during the storage period and the storage time was statistically effective on the dry matter contents of all brine samples ($P < 0.05$). In the study carried out by Messens et al. (1998), it was reported that the moisture content of cheese brine decreased during storage, and accordingly, the dry matter content increased. It was thought that the increase in dry matter during storage was due to the fact that the stabilizers in the brine transition to the cheese mass over time, and therefore, the water holding capacity of the brine decreases as the storage progresses. The increase in dry matter contents may also be caused by the mixing of the brine and cheese mass as a result of the disintegration in the cheese structure during storage and the inability to separate sufficiently during the analysis.

Fat contents of brines were very close to each other at each stage of storage, and they did not change depending on the use of stabilizers and the storage time ($P > 0.05$). It was observed that the fat in dry matter contents generally tended to decrease during storage significantly, especially on the 60th day of the storage ($P < 0.05$). This decrease was directly related to the increasing dry matter content during storage. The addition of stabilizer did not cause any change on the fat in dry matter contents of brines ($P > 0.05$).

The protein contents of brines which contain sodium caseinate, which is defined as a milk protein, were significantly higher than protein contents of other brines ($P < 0.05$). There was no significant difference between protein contents of other brines ($P > 0.05$). The protein contents of the brine samples increased during storage ($P < 0.05$). It was thought that an increase in the protein content of the brine occurs due to the increase in the ripening degrees of the cheeses during the storage period (Özbek, 2021). As stated by Gupta et al. (1973), the increase in the amount of soluble protein in the brine during storage might be occur due to the increase in the amount of casein and peptides in the brine due to the partial hydrolysis on the surface of the cheese curd. Similar results were obtained for protein in dry matter contents as well.

The salt content of the control brine during the storage period was significantly lower than the salt content of the other samples ($P < 0.05$). In this case, it was understood that the addition of stabilizer increases the salt content in the brine by decreasing the salt transfer to the cheese mass. As a result of the stabilizers binding the water in the brine, the moisture content in the brine increased and accordingly the salt penetration into the cheese mass decreased. When the stabilizer added brines were compared among themselves in terms of salt content, no statistical difference was found between the samples ($P > 0.05$). It was observed that the salt content of the brine decreased during storage. It is known that the salt content of the brine decreases during storage due to the passage of salt to the cheese mass as a result of diffusion, thus increasing the salt content of the cheese.
Many researchers reveal that there was an increase in salt content in brine cheeses during ripening and storage (Estrada et al., 2019; Kaminarides et al., 2019; Papademas et al., 2019). In the light of this information, it was thought that the decrease in salt content of brines during storage was normal. Similar results were obtained for the salt in dry matter contents as well.

Aroma profile
The aroma profiles of Soft White cheeses were shown in Table 3. Seven different groups of compounds were determined, namely aldehydes, alcohols, esters, ethers, hydrocarbons, ketones and organic acids in cheeses. It was determined that aldehydes were the most dominant group in Soft White cheeses, followed by esters and ketones. It was observed that the control sample was richer in terms of aldehydes and the cheese preserved in brine containing carrageenan was richer in terms of esters and ketones than the other samples. It was determined that alcohol, hydrocarbons, ether and organic acids observed in cheese samples were not dominant for Soft White cheeses.

<table>
<thead>
<tr>
<th>No</th>
<th>Compound</th>
<th>Molecular formula</th>
<th>RT</th>
<th>SWC05 (%)</th>
<th>SWC64 (%)</th>
<th>SWC33 (%)</th>
<th>SWC17 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3-Methyl-butanal</td>
<td>C₉H₁₈O</td>
<td>2.15</td>
<td>20.6a</td>
<td>29.66b</td>
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<tr>
<td>2</td>
<td>Acetaldehyde</td>
<td>C₆H₁₂O</td>
<td>2.73</td>
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<tr>
<td>3</td>
<td>Pentanal</td>
<td>C₅H₁₀O</td>
<td>2.24</td>
<td>2.33c</td>
<td>2.70b</td>
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<td>4</td>
<td>Butanal</td>
<td>C₄H₁₀O</td>
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<td>0.89a</td>
<td>3.93a</td>
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<tr>
<td>5</td>
<td>Isoamyl alcohol</td>
<td>C₃H₆O</td>
<td>3.11</td>
<td>3.08b</td>
<td>2.27c</td>
<td>3.44a</td>
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<tr>
<td>6</td>
<td>Cyclobutanol</td>
<td>C₇H₁₆O</td>
<td>3.63</td>
<td>-</td>
<td>0.20b</td>
<td>0.25a</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>(S)-2-amino,1-propanol</td>
<td>C₃H₆NO</td>
<td>9.30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.07a</td>
</tr>
<tr>
<td>8</td>
<td>1-Decanol</td>
<td>C₁₀H₂₂O</td>
<td>14.09</td>
<td>-</td>
<td>0.32c</td>
<td>-</td>
<td>-</td>
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<tr>
<td>9</td>
<td>2-Butyl-1-octanol</td>
<td>C₁₅H₃₂O</td>
<td>14.31</td>
<td>-</td>
<td>0.86a</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>Esters</td>
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<td></td>
</tr>
<tr>
<td>10</td>
<td>Formic acid, ethenyl ester</td>
<td>C₂H₂O₂</td>
<td>1.72</td>
<td>8.68a</td>
<td>6.45b</td>
<td>5.93b</td>
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<tr>
<td>11</td>
<td>Acetic acid, ethenyl ester</td>
<td>C₃H₄O₂</td>
<td>1.77</td>
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<td>4.69b</td>
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<tr>
<td>12</td>
<td>1,2,4-Benzenetricarboxylic acid, 1-2 dimethyl ester</td>
<td>C₁₁H₁₀O₆</td>
<td>4.83</td>
<td>-</td>
<td>0.43b</td>
<td>0.47a</td>
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<td></td>
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<tr>
<td>13</td>
<td>Phenylethyl acetate</td>
<td>C₁₀H₁₇O₂</td>
<td>15.20</td>
<td>-</td>
<td>0.62c</td>
<td>-</td>
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<tr>
<td>14</td>
<td>Styrene</td>
<td>C₆H₈</td>
<td>6.50</td>
<td>0.48b</td>
<td>0.72a</td>
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<td></td>
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<tr>
<td>15</td>
<td>1,3-dichloro-benzene</td>
<td>C₆H₄Cl₂</td>
<td>10.10</td>
<td>0.51b</td>
<td>0.58a</td>
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<td>16</td>
<td>Limonene</td>
<td>C₁₀H₁₆</td>
<td>10.61</td>
<td>1.91b</td>
<td>3.05a</td>
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<td></td>
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<tr>
<td>17</td>
<td>n-Undecane</td>
<td>C₁₃H₂₆</td>
<td>14.22</td>
<td>0.21c</td>
<td>0.35b</td>
<td></td>
<td></td>
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<tr>
<td>18</td>
<td>Tetradeacane</td>
<td>C₁₄H₃₀</td>
<td>17.19</td>
<td>0.80a</td>
<td>0.96b</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>19</td>
<td>Acetone</td>
<td>C₃H₆O</td>
<td>1.42</td>
<td>3.59d</td>
<td>10.31a</td>
<td>9.28b</td>
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<tr>
<td>20</td>
<td>Di-n-decylsulfone</td>
<td>C₂₀H₄₂O₅S</td>
<td>19.66</td>
<td>0.33c</td>
<td>0.10b</td>
<td>-</td>
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<tr>
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<td>Organic acids</td>
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<td></td>
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<tr>
<td>21</td>
<td>Butyric acid</td>
<td>C₄H₁₀O₂</td>
<td>4.10</td>
<td>-</td>
<td>0.46c</td>
<td>-</td>
<td>-</td>
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<tr>
<td>22</td>
<td>Hexanoic acid</td>
<td>C₆H₁₂O₂</td>
<td>9.39</td>
<td>0.26a</td>
<td>0.25a</td>
<td>0.07b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ether</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Triethylene glycol monododecyl ether</td>
<td>C₁₈H₃₆O₄</td>
<td>34.88</td>
<td>0.31c</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

RT: Retention time
SWC05 (control cheese), SWC64 (cheese with 0.10% of sodium caseinate in brine), SWC33 (cheese with 0.05% of carrageenan in brine) and SWC17 (cheese with 0.05% of guar gum in brine)

a, b, c, d: Values that are shown in the same line with different exponential letters are different in terms of P <0.05 level of significance.
A total of 23 aromatic compounds were detected in Soft White cheeses including 4 aldehydes (3-methyl butanal, acetaldehyde, pentanal, butanal), 5 alcohols (isoamyl alcohol, cyclobutanol, (S)-2-amino-1-propanol, 1-decanol, 2-buty1-1-octanol), 4 esters (formic acid ethenyl ester, acetic acid ethenyl ester, 1,2,4-benzentriarboxylic acid-1-2 dimethyl ester, phenyl ethyl acetate), 1 ether (triethylene glycol mono-dodecyl ether), 5 hydrocarbons (styrene, 1,3-dichloro-benzene, limonene, n-dodecane, tetradecane), 2 ketones (acetone, di-n-decylsulfone) and 2 organic acids (butyric acid, hexanoic acid).

**Aldehydes**

Among the aldehydes, the most observed compounds in the samples were 3-methylbutanal and acetaldehyde. In all Soft White cheese samples, 3-methylbutanal was detected. The differences observed between 3-methylbutanal levels of cheese samples were found to be statistically significant ($P < 0.05$). The characteristic aroma produced by *Streptococcus lactis* var. *maltigenes* in milk is mainly due to the production of 3-methylbutanal, which is derived from leucine (Morgan, 1970). It is known that 3-methylbutanal creates grass, malt and hazelnut odor in cheeses (Luo et al., 2018). Natrella et al., (2020), 3-methylbutanal was detected in Mozzarella cheese. Hydroxyacid dehydrogenases limit the conversion of α-keto acids to aroma compounds, and it is known that the panE gene in *Lactococcus lactis*, which encodes hydroxyacid dehydrogenase activity, inhibits the formation of 3-methylbutanal (Cadiñanos et al., 2013). In a study by Cankurt (2019), it was reported that the addition of carrageenan and guar gum to cheese brine increases the total lactic acid bacteria level in cheeses. In the light of this information, it was thought that the level of lactic acid bacteria may increase with the addition of stabilizers, and accordingly, it may limit the formation of 3-methylbutanal in cheese by increasing the hydroxyacid dehydrogenase activity.

Acetaldehyde, which gives green apples, walnuts and roasted odors to foods, is an important volatile compound for the aroma formation of dairy products such as yogurt, fermented milk and fresh cheeses (Evangelia et al., 2016). Acetaldehyde is commonly found in fermented milk products and has a distinctive sweet and pungent odor. It is formed by the lactose metabolism of lactic acid bacteria. It can also be produced in reversible ways through the action of the threonine aldolase enzyme on the amino acid threonine (Kaminarides et al., 2015). High acetaldehyde levels were detected in all cheeses and it might be related with use of *Lactococcus lactis* as a mesophilic culture. The differences between the acetaldehyde levels of the cheeses were statistically significant ($P < 0.05$). Evangelia et al., (2016) detected the presence of acetaldehyde in Galotyri cheese.

The differences between the butanal and pentanal levels of the cheeses were also statistically significant ($P < 0.05$). It has been reported that saturated aldehydes such as pentanal and butanal result from lipid oxidation and are associated with cardboard taste, oily and dirty flavors in dairy products. Saturated aldehydes are mainly produced by amino acid catabolism or decarboxylation of keto acids (Kaminarides et al., 2015). Pentanal (Liaw et al., 2010) and butanal (Attaie, 2009) were detected in different cheeses.

**Alcohols**

Alcohols were the groups in which the largest number of compounds were detected. Isoamyl alcohol was the compound with the highest level
in the alcohol group. The highest isoamyl alcohol level was detected in cheese in brine with guar gum and the lowest level was in control (P < 0.05). Branched-chain alcohols such as isoamyl alcohol are formed due to amino acid catabolism under the influence of lactococcal strains (Evert-Arriagada et al., 2013). Isoamyl alcohol is a compound formed as a result of the breakdown of lactose with the formation of acetyl-coA and leucine by the Erhlich pathway (Leclercq-Perlat et al., 2004). It was reported that isoamyl alcohol creates cream, fruit and alcohol odor in cheeses (Andrade et al., 2019). In the study conducted by Bas et al. (2019), it was determined that isoamyl alcohol is the dominant alcohol in White cheese. Other compounds in the alcohol group had low percentages and each compound was detected only in some cheeses. Cyclobutanol is formed as a result of the reduction of ketone compounds (Li et al., 2020), and it was detected in Divle Obruk cheese (Öztürkoğlu Budak et al., 2016) and Cheddar cheese (Liu et al., 2014). No study has been found in the literature on the presence of (S)-2-amino-1-propanol in any cheese variety. For this reason, it was determined that the only source of this compound was the addition of guar gum. It was reported that 1-decanol, which gives the cheese oil and straw odor, was detected in semi-hard goat cheeses (Poveda et al., 2008). 2-butyl-1-octanol was detected in Cheddar cheese (Göğüş et al., 2006) and Xinjiang Kazakh cheese (Xu et al., 2020).

Esters
Esters were one of the most dominant groups in Soft White cheeses after aldehydes and they had a wide variety. It was seen that the compounds with the highest ratios in the ester group were formic acid ethenyl ester and acetic acid ethenyl ester. It was determined that the highest ratios in terms of formic acid ethenyl ester (vinyl formate) were seen in cheese with sodium caseinate and the lowest level was in control (P < 0.05). In the literature, no research was found in which formic acid ethenyl ester was detected in any cheese variety. While the acetic acid ethenyl ester ratios of control and guar gum applied cheeses were statistically close to each other (P > 0.05), carrageenan applied cheese was found to be significantly higher than the other cheeses (P < 0.05). In the literature, no research was found that detected acetic acid ethenyl ester compound in cheese. However, Badings (1967) argues that because of the use of vinyl acetate emulsions in cheese packaging (Graiver et al., 2004), this component can be detected in cheeses and causes rib odor in cheeses.

Formic acid ethenyl ester and acetic acid ethenyl ester are in the vinyl ester group. According to EFSA (2010), vinyl esters are unstable compounds, they are unlikely to be found in foods, and vinyl alcohol parts of vinyl esters of fatty acids are generally converted to acetaldehyde as a result of hydrolysis by reaction with sucrose. Vinyl esters are mostly found in foods as aldehydes, but they can sometimes be detected in foods in very low amounts. In this research, it was seen that the compounds in the vinyl ester group were at high levels and it was thought that these compounds were likely to be converted to acetaldehyde in the later stages of storage. However, the high level detected was due to the fact that the analysis was carried out on the 15th day of storage. When the total values of the compounds in the vinyl ester group were examined, it was determined that the highest level was observed in carrageenan applied cheese, followed by guar gum applied, control and sodium caseinate applied cheeses, respectively. This ranking is the same for the acetaldehyde levels of cheeses. This supported that the compounds in the vinyl ester group were converted to acetaldehyde.

1,2,4-benzentricarboxylic acid, 1-2 dimethyl ester were detected only in carrageenan and guar gum applied cheeses, and phenyl ethyl acetate was detected in very low percentages only in sodium caseinate applied cheese. Cao et al. (2019) detected 1,2,4-benzentricarboxylic acid, 1-2 dimethyl esters in fermented milk products produced by the use of different oligosaccharides,
Shi et al. (2020) detected it in calcium sulfate added tofu samples. It was reported that phenyl ethyl acetate creates a rose odor in cheeses (Kirmaci et al., 2015) and was previously detected in Feta cheese (Kondyli et al., 2012).

**Hydrocarbons**

Although hydrocarbons do not contribute significantly to cheese flavor due to their high odor thresholds, they can act as precursors in the formation of other aromatic compounds that follow different degradation pathways (Bozoudi et al., 2018). Among the hydrocarbons, limonene and tetradecane were determined as the most dominant compounds in cheeses, while other hydrocarbons appeared in low percentages. The highest limonene content was observed in cheese preserved in brine with guar gum, followed by carrageenan applied cheese and control, respectively. Limonene was not detected in cheese preserved in brine with sodium caseinate. This difference observed between cheeses was found to be statistically significant (\(P<0.05\)). It was reported that the amount of limonene increases with the decrease in the amount of casein (Ni et al., 2020). The reason why limonene was not detected in sample sodium caseinate applied cheese might be due to its high caseinate content. Limonene is a monoterpen with a strong citrus odor and low detection threshold (Sable et al., 1997) and it is known to have antimicrobial properties (Khorsheidian et al., 2018). Fancello et al. (2020) reported that high amounts of limonene were detected in Ricotta cheese.

When the tetradecane and n-dodecane ratios were examined, it was determined that the highest ratio was observed in sodium caseinate applied cheese, and the lowest level was in control. Differences between cheese samples were found significant (\(P<0.05\)). Bozoudi et al. (2018) stated that tetradecane is formed by the degradation of compounds derived from plants. It is thought that the fact that carrageenan and guar gum are products of vegetable origin causes the tetradecane ratio in cheeses to be higher than the control sample. The highest rate was observed in sodium caseinate applied cheese, and it was associated with sodium caseinate addition causing an increase in the level of alkanes (Soong et al., 2019), which are hydrophobic compounds (Nordvi et al., 2007). It was reported that caseins exhibit a high hydrophobic character at pH 4.2, negatively affecting the retention of hydrophilic compounds, while promoting the retention of hydrophobic compounds in the protein matrix (Domingos et al., 2019). Pappa et al. (2020) stated that there was a significant amount of tetradecane in Kashkaval cheeses. Kavaz et al. (2013) detected n-dodecane in Ortu (herbaceous) cheeses.

While styrene was not detected in sodium caseinate applied cheese and 1,3-dichloro-benzene in control and sodium caseinate applied cheeses, these compounds were detected at the highest rate in guar gum applied cheeses (\(P<0.05\)). It was reported that styrene was also detected in Pecorino Siciliano Cheese samples (Gaglio et al., 2020). Styrene is an aromatic hydrocarbon used as a monomer in the production of many plastic materials widely used in food packaging. Various types of compounds released in the packaging material at storage temperature can affect food components by diffusion, so compounds such as methylbenzene, ethylbenzene, xylenes, styrene and 1,4-dichloro-benzene can be detected in food products stored in plastic package (Chiesa et al., 2010). In the literature, no study was found in which 1,3-dichloro-benzene was detected in any cheese variety. However, it has been known that 1,3-dichloro-benzene was a tri-isomer halogenated aromatic compound used as an intermediate in the production of dyestuffs (Aydın et al., 2005). Therefore, this compound was also thought to originate from the packaging.

**Ketones**

It was seen that the most dominant compound in the ketone group in Soft White cheeses was acetone. It was determined that the highest acetone ratio was observed in carrageenan applied cheese, followed by guar gum applied, control and sodium caseinate applied cheeses, respectively (\(P<0.05\)). In cheeses, acetone may originate from milk in relation to animal feed, or it may be formed as a result of thermal degradation of \(\beta\)-ketoacids (Pappa et al., 2020). Acetone was also
detected in Feta cheeses (Kondyli et al., 2012). Di-n-decylsulfone was only found in sodium caseinate and carrageenan applied cheeses in very low concentrations. This compound was not detected in control and guar gum applied cheeses. There was no evidence in the literature that di-n-decylsulfone detected in cheeses.

Ether and organic acids were found in very low percentages in Soft White cheeses and were not detected in all cheese varieties. One ether, triethylene glycol mono-dodecyl ether, was detected at a rate of 0.31% in cheese preserved in brine with the addition of sodium caseinate. No studies were found in which triethylene glycol mono dodecyl ether was found in a cheese variety.

Two organic acids, butyric acid and hexanoic acid, were detected in cheeses. While butyric acid was observed at the rate of 0.46% only in sodium caseinate applied cheese, hexanoic acid was detected in all cheese samples preserved in stabilizer added brine. While hexanoic acid levels were close to each other in sodium caseinate and carrageenan applied cheeses, it was determined that this value was significantly lower in guar gum applied cheese ($P < 0.05$). The presence of butyric acid (Nalepa et al., 2019) and hexanoic acid (Martino et al., 2019) in different cheese varieties was determined in many studies.

CONCLUSION
In this study, the diffusion of water in the brine to the cheese mass was slowed down by binding water by the stabilizers and the dry matter contents of the cheeses were kept high. The salt transfer from the brine was less in brine cheeses used stabilizers. As a result, thanks to the use of stabilizers, cheeses with higher dry matter content and lower salt content were obtained.

When the aroma properties of the cheeses were examined, it was determined that the cheese samples preserved in the sodium caseinate added brine generally had a different profile than the other cheeses. These cheeses had lower all aldehyde compounds than other cheeses. Some different compounds that were not detected in other cheeses such as 2-butyl-1-octanol, phenyl ethyl acetate, di-n-decylsulfone, butyric acid and triethylene glycol mono-dodecyl ether were detected in sodium caseinate applied cheese. On the contrary, compounds such as acetic acid ethenyl ester, limonene, styrene, 1,3-dichlorobenzene were detected in all other cheeses but not in sodium caseinate applied cheese. In this study, it could be said that carrageenan and guar gum were more effective than sodium caseinate in terms of flavor retention. It was also noteworthy that there were some volatile compounds in cheeses originating from packaging. It is important that there is no contamination from the materials to be used in food packaging to the food. For this reason, it is recommended that these materials be chosen more carefully by the manufacturers, considering their environmental and health effects.

CONFLICT OF INTEREST
Funding: This work was supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK) [Project 119O068]; the Çukurova University Research Fund [FDK-2018-10603].

ETHICAL STATEMENT
The authors state that no ethical approval was needed.

AUTHORS CONTRIBUTIONS
Çağla Özbek: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Visualization
Nuray Güzeler: Conceptualization, Methodology, Validation, Resources, Writing - Review & Editing, Supervision
All authors approved the final manuscript and accepted to be held responsible for the content.

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Brine stabilizers and soft cheese aroma

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