Changes in the Chemical Composition of Turkish White Cheese According to Storage Temperature

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Abstract

The aim of this study is to highlight changes in the chemical composition, namely pH, total dry matter percentage (DM%), total nitrogen and protein, titratable acidity, salt content and biogenic amines, of Turkish white cheese according to storage temperature. The results confirmed that a decrease was observed in pH values of the samples during storage. Titratable acidity was observed to show a trend of increase ($P > 0.05$). As per these results, there was an increase in DM% in cheese samples stored at all three temperatures, namely 5, 15, and 25°C, on day 11, but a decrease was observed on day 31. An increased trend was seen in samples after day 31. In addition, an increased trend in the amount of salt in the samples, depending on time, was determined. The concentration of biogenic amines, tryptamine, 2-phenylethylamine, putrescine, cadaverine, tyramine, spermidine and spermine, with dominated biogenic amines relate to shelf life, were putrescine, cadaverine, and tyramine, and were increased based on storage temperatures.

Keywords: biogenic amines, proximate composition, Turkish white cheese

INTRODUCTION

Cheese is a dairy product produced with different form, texture and flavour by coagulating milk proteins, especially casein, and consumed fresh or after ripening (Üçüncü, 2008). It is one of the oldest fermented food products. Cheese, which has been produced and consumed for thousands of years, has known to be adapted to several different technical, social and economic conditions around the world. According to a recent study, in total there are more than 1400 traditional cheese all around the world, which shows an astonishing variety in terms of their production and ripening method, texture, taste and smell (Dugat-Bony et al., 2016).

A complex chain of microbiological and biochemical processes, namely glycolysis, proteolysis and lipolysis, involve during the production and ripening of cheese. Proteolysis is known to be important in the development of the texture and the microstructure of cheese, it also contributes to the flavour development by providing some reactants to the reactions in which products responsible in the taste and smell arise. Lipolysis, on the other hand, has a direct effect on taste by breaking down triglycerides and releasing fatty acids (Cinbaş, 2004).

If there is a balance in these steps, the cheese would possibly have the desired taste and smell, on the other hand undesirable taste and smell could arise as a reason of unfavourable conditions. Due to some problems during these complex microbiological and biochemical processes the consumption of the cheese won't be possible. The most serious and common cheese failure is bitter taste. Such problems negatively affect the

acceptability of the cheese (Çakmakçı and Şengül, 1995; Topçu, 2004).

Turkish white cheese is a variety of cheese, which is produced in brine and ripened generally for about 1 to 3 months, and packed in cubic or rectangular shape with a weigh of about 350 - 500 grams (Hayaloğlu et al., 2002). The type of cheese that ranks first in terms of both production and consumption in Turkey is white cheese (Üçüncü, 2008).

The main aim of this study was to show changes in the chemical composition of Turkish white cheese, where similar types are widely consumed, especially in Eastern European countries, such as Greece and Bulgaria.

MATERIALS AND METHODS

Cheese samples

The white cheese samples used in the study were obtained from a local factory, which were produced on the same day and having the same Lot Number. Cheese samples were divided into 100 grams of slices in aseptic conditions, vacuum packed, and stored in different temperatures (5, 15, and 25°C) in an incubator (Selecta, Spain). In order to determine total spoilage with microbiological observations, which are not given here, they were conducted. All tests in the study were done in triplicate, and data were expressed as the average.

pH determination

In addition, 10 g of cheese sample was blended with 10 mL of distilled water (dH_2O) with a pestle and mortar, and the pH of the slurry was measured by a pH meter (Fox et al., 2017).

Total dry matter analysis

Total dry matter (DM) was determined by drying 10 g of a cheese sample for 24 h at 102 ± 2°C until it reaches a stable weight (IDF, 1982).

The total dry matter percentage was calculated by using the formula defined in previous studies (AOAC, 2000).

Total nitrogen and protein determination

To determine total nitrogen (TN) and protein in the cheese sample, the protocol proposed by PanReac AppliChem, Chicago, IL, USA (2017) was used with modifications. As such, 0.2 g of cheese sample was placed into a digestion flask, while 20 mL of HCl (98%) and 10 g of Kjeldahl tablet were added to the sample. A digestion flask was placed in the unit and heated to 350 to 380°C to digest the sample. Then the sample was cooled and 25 mL of 10 N NaOH was added, and attached to the Kjeldahl apparatus. Sample ammonia was captured at 25 mL of 1% boric acid. A mixture of methyl red and bromocresol green was added to the boric acid and titrated with 0.1 N H_2SO_4. Nitrogen (N%) is expressed with the formula in the reference. The proteins in cheese samples were calculated, where 6.38 was taken as the protein factor.

Titratable acidity

Further, 5 g of cheese sample were macerated in 0.1 N NaOH. 6 drops of phenolphthalein were added and titrated, until a stable pink color was formed. Titratable acidity (TA) was calculated by the formula given in the reference (AOAC, 2000a).

Salt content

Also, 5 g of sample were added to 250 mL of a flask and 100 mL of boiling water was added. After stirring for 10 minutes, the temperature was decreased about 50 55° C, as potassium chromate was added and titrated with silver nitrate. The volume, in which the color change was observed, and the amount of salt was calculated as w/w% (AOAC, 2000b).

Biogenic amines

In order to extract biogenic amines from cheese samples, 2.0 g of cheese were weighed and 0.125 mL of 1000 ppm (1.7 diaminoheptane) was added. Next, 10 mL of 0.4 M $HClO_4$ was added and homogenized. The mixture was centrifuged at 3000 rpm at 4°C for 10 minutes, as the first extract was obtained by passing the aqueous phase through filter paper. The solid phase was homogenized again by adding 10 mL of 0.4 M $HClO_4$ and centrifuged at 3000 rpm at 4°C for 10 minutes. The second extract was again obtained by passing the aqueous phase through filter paper. The two extracts were collected in a 25 mL volumetric flask, while the final volume was completed at 25 mL with 0.4 M $HClO_4$ (Gürkan, 2013).

For determining the biogenic amine content of the cheese samples, the extracts had to be derivatized. Thus, 1 mL of the extract was taken, with 200 µL 2N NaOH and 300 µL saturated sodium bicarbonate ($NaHCO_3$) added. Then, 2 mL of dansyl chloride solution (10 mg DaCl / 1 mL acetoin) were added. The mixture was kept for 45 minutes at 45°C in water bath for derivatization (Mietz and Karmas, 1977). In order to remove excess DaCl, 100 µL of 25% NH_3 was added and kept at room temperature for 30 minutes (Vinci and Antonelli,

2002; Antolini et al., 1999). The volume was completed at 5 mL by adding acetonitrile. The whole mixture was centrifuged at 2500 rpm at 4°C for 5 minutes. After centrifugation, the aqueous phase was filtered through a 0.45 µm filter into vials and used in the high-performance liquid chromatography (HPLC) analysis (Gürkan, 2013).

HPLC analysis

Twenty µL samples were injected in a HPLC (Shimadzu) device with an Inertsil ODS-3 C18 5µm (4.6 x 250) column, set to 254 nm (Moret and Conte, 1996; Moret et al., 2005).

A buffer would serve as 2-nonanol (pH 8) in the HPLC mobile phase, and was prepared by 0.1 M Tris, 0.1 M acetic acid, and dH_2O at 40%, 20%, and 40%, respectively. Solution A was prepared by using 30 mL of 2-nonanol, 550 mL of acetonitrile, 420 mL of dH_2O, and B solution 2 mL of buffer, 900 mL of acetonitrile, and 100 mL of distilled water (Gürkan, 2013).

The gradient used was given in Table 1 and the flow rate was 0.75 mL/min.

Statistical analysis

R Studio, version 3.3.2 was used to conduct one-way analysis of variance (ANOVA) to analyze the results ($P = 0.05$). (Core R Team, 2019).

RESULTS AND DISCUSSION

To observe changes in samples stored at three different temperatures (5, 15, and 25°C), 4 different samples were taken for cheese stored at 15 and 25°C, and 5 samples for cheese stored at 5°C for overall shelf life quality tested at the start, middle, and final stages of storage.

pH variations

The pH changes in the samples during their shelf-life are given in Table 2. The pH analysis shows that the pH value was 5.10 on day 1, and remained rather constant for up to 53 days, with a range of 5.04 at S2 on day 11 and 5.08 at S4 on day 53 for samples stored at 5°C. The pH was dropped to 4.95 for the same cheese samples on day 100 (S6). A similar decline was detected in the samples at 15 and 25°C until S3 or day 31, and a

Table 1. HPLC solvent gradient flow.

Time (min)	Concentration Solvent A (%)	Concentration Solvent B (%)
0.01	95	5
15	70	30
20	37	67
21	0	100
35	0	100
36	95	5
45	95	5

Table 2. Changes in pH during storage at 5, 15, and 25°C.

Sample	5°C	15°C	25°C
S1 (Starting Day)	5.10 ± 0.02	5.10 ± 0.02	5.10 ± 0.02
S2 (Day 11)	5.04 ± 0.04	4.95 ± 0.05	5.02 ± 0.01
S3 (Day 31)	5.07 ± 0.02	4.96 ± 0.04	5.04 ± 0.00
S4 (Day 53)	5.08 ± 0.01	5.24 ± 0.03	5.27 ± 0.02
S5 (Day 68)	4.90 ± 0.04	4.90 ± 0.10	4.90 ± 0.05
S6 (Day 100)	4.95 ± 0.05	-	

"-": No result due to spoilage.

fluctuation scope was observed, which resulted in 4.90 for both cheese samples that were kept at 15 and 25°C in S5 on day 68. The same change level was observed previously by Memiši et al. (2014).

Akarca et al. (2016) conducted a study with mozzarella cheese, and our study show that the acidity was increased during the storage period. The results of the pH change show that it varies, and is approximately correlated to the change in lactic acid bacteria (LAB) in the samples.

According to Perveen et al. (2011), it is normal for pH levels to drop, as the product is in storage, attributed to the lactic/organic acid formation by the culture and probiotic LAB.

Kongo (2013) stated that cheese making is based on LAB application as a starter culture, with the increase in the amount of LAB causing an acidification in milk, in turn causing an increase in lactic acid, as well as an increase in LAB, creating a decrease in pH. For this reason, the pH drop in this study could probably be related to the increase in LAB.

Total dry matter percentage

The total dry matter (DM%) fluctuations throughout the shelf-life are given in Table 3.

Based on these data, it can be concluded that DM% spiked in the case of all cheese samples, kept at the previously-stated temperatures in S2 or day 11. This change is related to a steady drop in moisture, as reported by El Owni and Hamid (2008). Moreover, these values dropped in S3 or day 31, and thereafter a steady increase was observed in all cheese samples.

Akarca et al. (2016) showed that the total dry matter in the samples taken on days 5, 10, 15, 21, and 28 of mozzarella cheese tended to increase. Bojanić-Rašović et al. (2013) studied natural dried cheese, considered to be a semi-hard cheese, which found that the total dry matter showed a decrease first, then an increase in DM, as found in our study. These results reaffirm the conclusions highlighted by the previous studies.

In addition, these changes could be attributed to proteolysis, lipolysis, and fat content turned into whey, as reported by Hayaloglu et al. (2005).

Total nitrogen

Total nitrogen (TN) sample changes during their shelf-life are given in Table 4: this presents values between 1.504 and 2.315% with minor differences among cheese samples. According to

Table 3. Changes in DM% during storage at 5, 15, and 25°C.

Sample	5°C	15°C	25°C
S1 (Starting Day)	44.4 ± 0.6	44.4 ± 0.6	44.4 ± 0.6
S2 (Day 11)	45.3 ± 0.3	46.1 ± 0.6	47.0 ± 0.4
S3 (Day 31)	40.9 ± 0.9	42.6 ± 0.6	43.1 ± 0.1
S4 (Day 53)	44.8 ± 0.8	43.5 ± 0.5	43.7 ± 0.2
S5 (Day 68)	44.1 ± 0.1	46.5 ± 0.4	45.9 ± 0.9
S6 (Day 100)	44.4 ± 0.3	-	

"-": No result due to spoilage.

Table 4. Changes in TN (N%) during storage at 5, 15, and 25°C.

Sample	5°C	15°C	25°C
S1 (Starting Day)	1.976 ± 0.020	1.976 ± 0.020	1.976 ± 0.020
S2 (Day 11)	1.642 ± 0.100	2.160 ± 0.004	1.504 ± 0.021
S3 (Day 31)	1.986 ± 0.113	1.513 ± 0.048	1.772 ± 0.015
S4 (Day 53)	1.996 ± 0.132	1.922 ± 0.021	1.922 ± 0.044
S5 (Day 68)	1.862 ± 0.031	2.315 ± 0.037	2.039 ± 0.167
S6 (Day 100)	1.791 ± 0.028	-	-

"-": No result due to spoilage.

Tarakci and Kucukoner (2006), the likely reason for minor changes is the moisture.

In addition, Innocente (1997) showed that the amount of nitrogen in cheese is associated with cheese ripening. An increase in the amount of sample nitrogen indicates the change in time, especially in samples stored at 5°C; the total amount of nitrogen is approximately horizontal, showing that the change in temperature is slower. Since the change in total protein (%) was calculated by using TN (N%), the correlation between TN and total protein is logical.

Total protein

Table 5 presents values related to the total protein (TP) and changes in this study, which point to a decrease in certain cheese samples stored at different temperatures. That is, a decline in the TP content through storage might be attributed to the degradation of proteins, thereby causing formation of soluble compounds, reported by Abdalla et al. (1993). Such an observation is in line with the study done by Hayaloglu et al. (2005), which also pointed to a drop in TP level throughout the storage period for white cheese samples.

To the contrary, a rise in TP values was experienced in S5 on day 68 at 15 and 25°C, which agrees with the findings of El Owni and Hamed (2008) and Tarakci and Kucukoner (2006).

Titratable acidity

Based on the observations in Table 6, it can be proposed that the TA values of cheese samples from the beginning of the study to day 100 of storage were at 5°C, 15°C, and 25°C, which show a rising trend as time progresses.

Keesenkas et al. (2012) refers to a steady increase in TA values as a result of the generation of lactic acid and hydrogen, while Sadler and Murphy (2010) justify pH and TA as two vital and related factors when it comes to food analysis experiments.

On S3 or day 31, the TA values were not much different at 5°C. This value concerns those stored at 15°C and 25°C, and were specifically contrasted with those stored at 5°C, with the outcomes appearing identical.

On S5 or day 68, the top TA value was detected at 25°C. Some previous studies, such as that of Coşkun and Ondül (2004), point out that the TA is related to LAB activity increase.

Table 5. Changes in total protein (%) during storage at 5, 15, and 25°C.

Sample	5°C	15°C	25°C
S1 (Starting Day)	12.607 ± 0.031	12.607 ± 0.031	12.607 ± 0.031
S2 (Day 11)	10.479 ± 0.254	13.780 ± 0.119	9.597 ± 0.096
S3 (Day 31)	12.668 ± 0.055	9.652 ± 0.042	11.306 ± 0.180
S4 (Day 53)	12.735 ± 0.014	12.266 ± 0.102	12.260 ± 0.109
S5 (Day 68)	11.882 ± 0.060	14.771 ± 0.018	13.009 ± 0.114
S6 (Day 100)	11.425 ± 0.104	-	-

"-": No result due to spoilage.

Table 6. Changes in titratable acidity (%) during storage at 5, 15, and 25°C.

Sample	5°C	15°C	25°C
S1 (Starting Day)	1.26 ± 0.09	1.26 ± 0.09	1.26 ± 0.09
S2 (Day 11)	1.35 ± 0.07	1.53 ± 0.26	1.44 ± 0.07
S3 (Day 31)	1.31 ± 0.05	1.50 ± 0.16	1.43 ± 0.06
S4 (Day 53)	1.43 ± 0.08	1.43 ± 0.05	1.44 ± 0.03
S5 (Day 68)	1.53 ± 0.11	1.61 ± 0.08	1.62 ± 0.07
S6 (Day 100)	1.54 ± 0.11	-	-

"-": No result due to spoilage.

Table 7. Changes in salt content (w/w%) during storage at 5, 15, and 25°C.

Sample	5°C	15°C	25°C
S1 (Starting Day)	2.34 ± 0.13	2.34 ± 0.13	2.34 ± 0.13
S2 (Day 11)	2.57 ± 0.14	2.34 ± 0.07	2.34 ± 0.04
S3 (Day 31)	3.28 ± 0.14	3.51 ± 0.03	3.51 ± 0.06
S4 (Day 53)	3.51 ± 0.08	3.51 ± 0.13	3.74 ± 0.07
S5 (Day 68)	3.74 ± 0.05	3.74 ± 0.09	3.98 ± 0.17
S6 (Day 100)	2.57 ± 0.25	-	-

"-": No result due to spoilage.

Table 8. The results of the biogenic amine analysis (mg/L) on the starting day.

Number	Biogenic amine	Retention Time	Concentration
1	Tryptamine	0.000	0.000
2	2-phenylethylamine	23.006	2.756
3	Putrescine	24.253	1.007
4	Cadaverine	25.217	0.911
5	Tyramine	28.888	2.335
6	Spermidine	0.000	0.000
7	Spermine	36.652	0.129

Table 9. The results of the biogenic amine analysis (mg/L) at the end of shelf-life (day 100) for samples stored at 5°C.

Number	Biogenic amine	Retention Time	Concentration
1	Tryptamine	20.452	1.897
2	2-phenylethylamine	22.779	3.446
3	Putrescine	24.232	5.150
4	Cadaverine	25.209	2.255
5	Tyramine	28.879	5.822
6	Spermidine	0.000	0.000
7	Spermine	36.619	0.213

Table 10. The results of the biogenic amine analysis (mg/L) at the end of shelf-life (day 68) for samples stored at 15°C.

No.	Biogenic amine	Retention Time (min.)	Concentration (mg/L)
1	Tryptamine	20.066	3.791
2	2-phenylethylamine	22.742	5.073
3	Putrescine	24.214	31.038
4	Cadaverine	25.194	5.909
5	Tyramine	28.860	51.577
6	Spermidine	29.588	0.431
7	Spermine	36.592	0.251

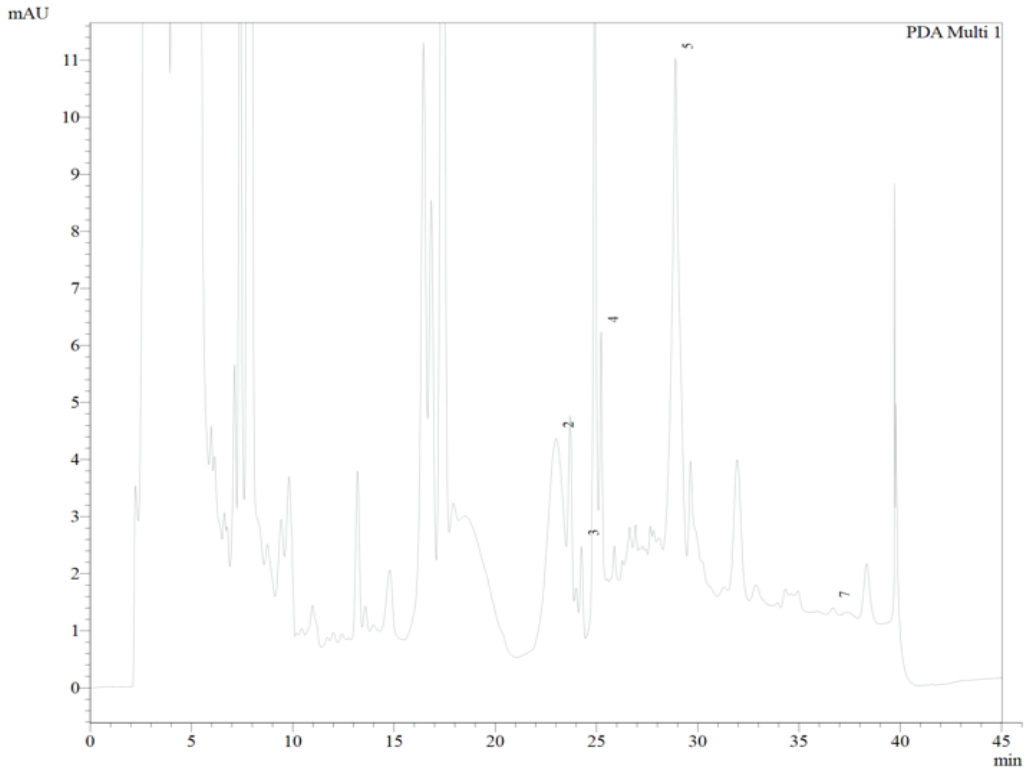

Figure 1. Chromatogram of the biogenic amine analysis on the starting day.

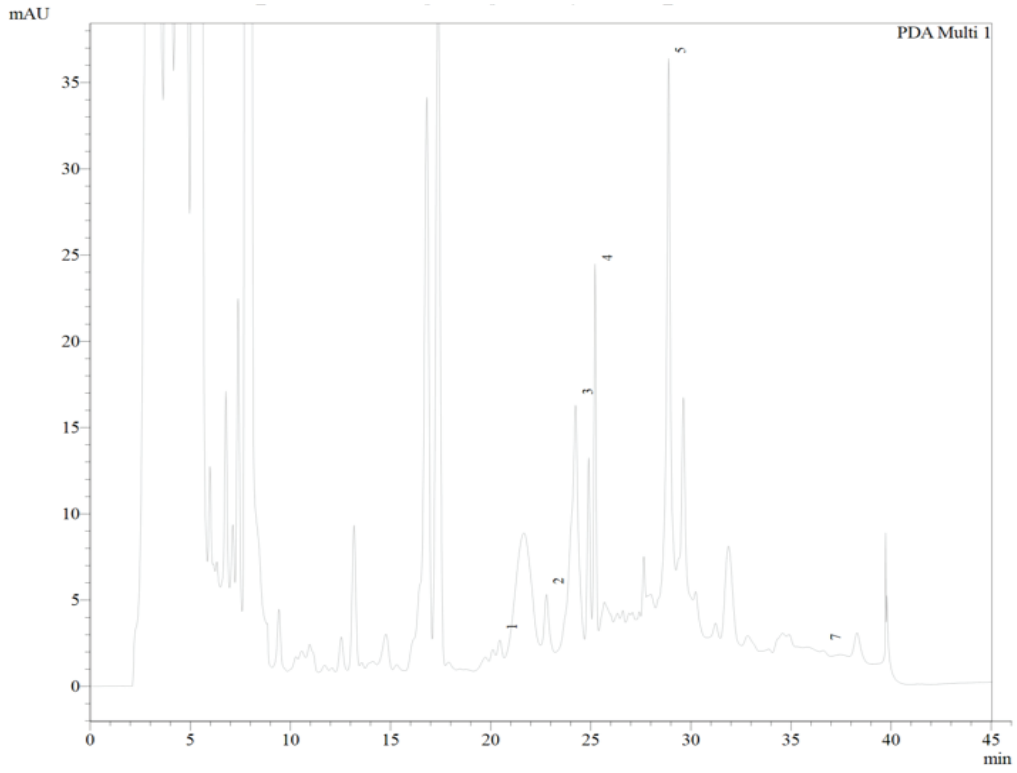

Figure 2 Chromatogram of the biogenic amine analysis at the end of shelf-life (day 100) for samples stored at 5°C.

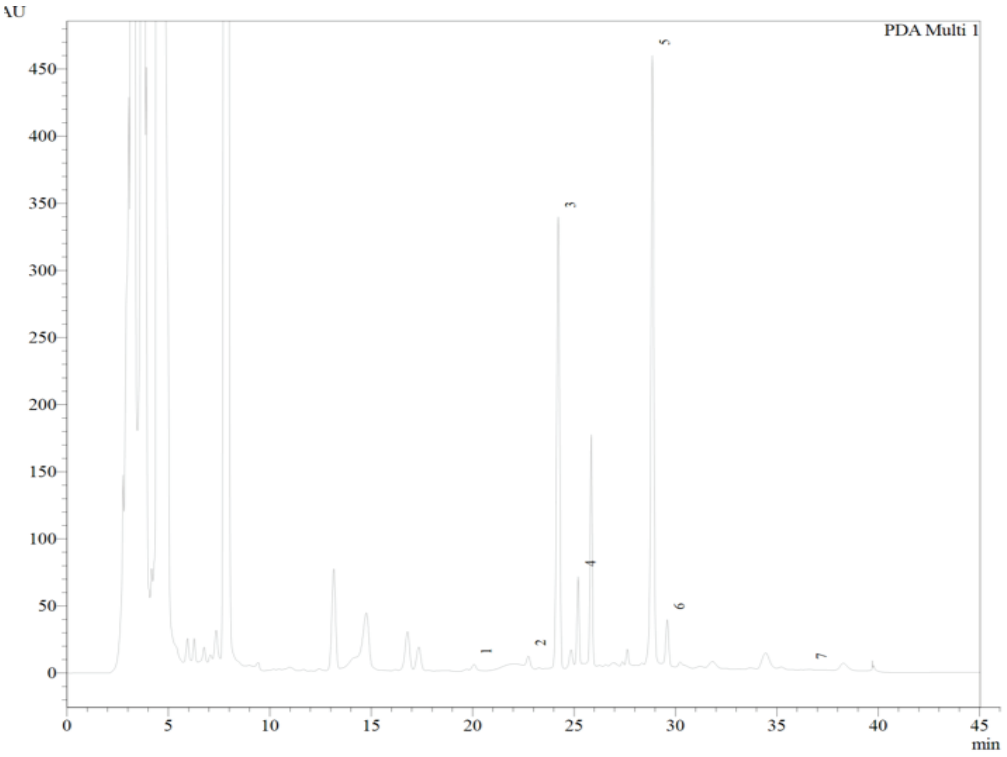

Figure 3. Chromatogram of the biogenic amine analysis at the end of shelf-life (day 68) for the samples stored at 15°C.

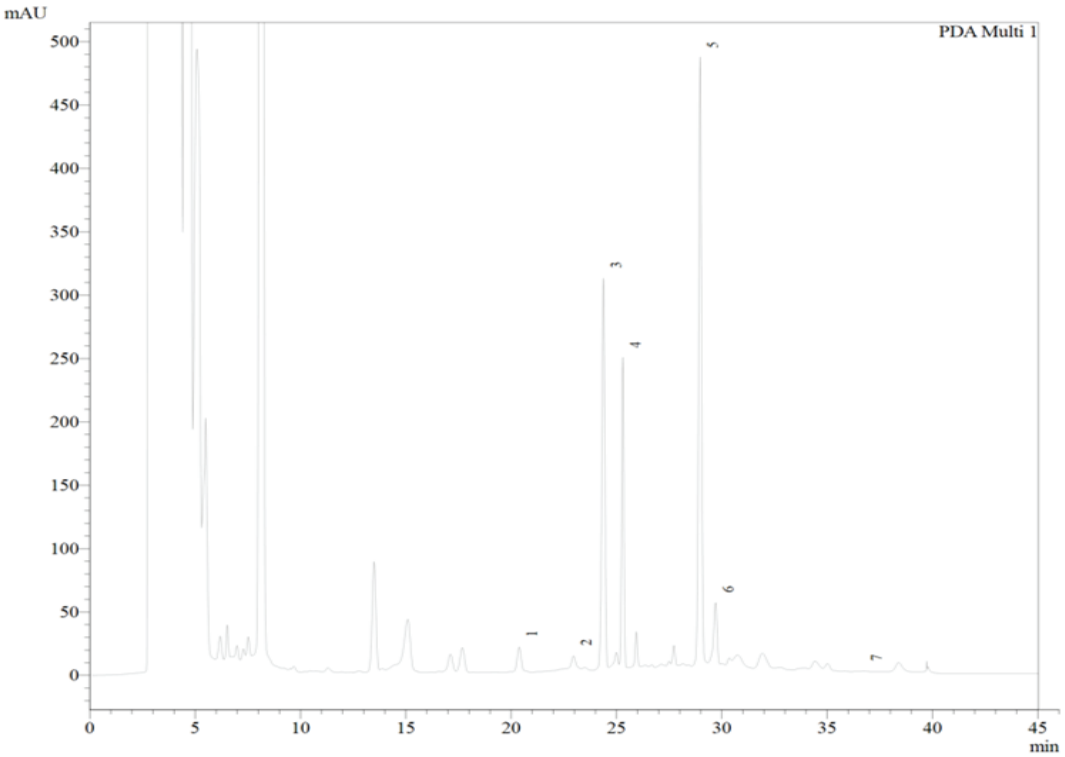

Figure 4. Chromatogram of the biogenic amine analysis at the end of shelf-life (day 68) for samples stored at 25°C.

Table 11. The results of the biogenic amine analysis (mg/L) at the end of shelf-life (day 68) for samples stored at 25°C.

No	Biogenic amine	Retention Time (min.)	Concentration (mg/L)
1	Tryptamine	20.376	7.954
2	2-phenylethylamine	22.951	7.277
3	Putrescine	24.372	27.489
4	Cadaverine	25.291	16.781
5	Tyramine	28.961	53.828
6	Spermidine	29.692	4.064
7	Spermine	36.730	0.517

Separately, El Owni and Hamed (2009) stated that TA values in the case of products stored at room temperature are higher versus cold-stored samples. The likely reason for this is proposed to be slowed growth and reduced bacterial activity.

Salt content

The cheese samples are tested for their salt content as well, as depicted in Table 7. Here, the salt content values on S5 or day 68 was 3.98% as the highest at 25°C and 2.34% as the lowest level of salt on S1 or day 1. In addition, the salt content spiked in all samples during storage. This trend has been confirmed by some other studies as well.

For example, Yetişmeyen et al. (1996) stored white cheese from pasteurized milk and examined some chemical parameters, including the amount of salt, where an increase in the amount of salt was also observed by 90 days in all samples used.

Similarly, Kurdal and Gürtunca (1996) observed salt content for cheese samples collected from the city of Bursa in Turkey to stand at 3.70% to 5.46%.

Kayagil and Candan (2009) examined the salinity of 4 various Turkish cheeses within the course of 30 days in storage, with salt levels reaching a peak of 9.82% in one case, and the lowest value of 7.72% in two others. An increase in salt content was observed in all three.

Biogenic amines

The biogenic amines found in samples on the starting day are given in Table 8 and the chromatogram of the analysis is given in Fig. 1. The end of shelf lives were determined according to the microbiological observations mentioned before, and the biogenic amines were tested at the end of the shelf lives of cheese samples.

The results of biogenic amines at the end of shelf-life (day 100) for the samples stored in 5°C is given in Table 9 and the chromatogram of the analysis is given in Fig. 2.

The results of biogenic amines at the end of shelf-life (day 68) for samples stored at 15°C is given in Table 10, while the chromatogram of the analysis is given in Fig. 3.

The results of biogenic amines at the end of shelf-life (day 68) for samples stored at 25°C is given in Table 11, while the chromatogram of the analysis is given in Fig. 4.

The results clearly demonstrate that the total values of biogenic amines are highest on day 68 for cheese samples kept at 25°C, followed by the same day for those kept at 15°C. The lowest value in this category was observed on day 1.

Innocente (1997) showed that the increase in biogenic amines is associated with cheese ripening. Therefore, these cheese samples indicate the amount of change, depending on time.

Öner et al. (2006) investigated the chemical changes in Turkish white cheese during ripening. They found that the biogenic amine values during this period were histamine 1.28; tyramine 10.55; they also reported that phenylethyleneamine was 0.74 mg/kg.

In another study, biogenic amine contents of different types of Turkish cheeses were assessed (Durlu-Özkaya, 2002). Biogenic amine content (tryptamine, phenylalanine, putrescin, cadaver, histamine, tyramine, spermine, and spermidine) of cheese samples was determined

by HPLC. According to research, cheese putrescin, cadaverine, histamine tyramine, and spermidine were predominant biogenic amines, while some 2-phenylethyleneamine, cadaverine, tyramine, and spermidine were also reported as predominant biogenic amines. In traditional cheeses, histamine and tyramine values were above the toxic level.

Statistical Analysis

Statistical analysis showed no significant difference between results of replicates ($p > 0.05$).

CONCLUSIONS

The results of the study confirmed that when the pH values on the last day of all cheese samples and the first day samples were compared, a decrease was observed, although an increase trend was present in the samples taken at mid-shelf lives. Also, titratable acidity was also observed to show an increase. The trend in pH and titratable acidity could be related to LAB. According to the results, there was an increase in DM% in the cheese samples stored at all three temperatures at day 11, but a decrease was observed at day 31. An increase was also observed in samples taken after day 31. In addition, an increased trend in the amount of salt in the samples, depending on time, was also determined. The concentration of biogenic amines, tryptamine, 2-phenylethylamine, putrescine, cadaverine, tyramine, spermidine, and spermine, were found to increase based on storage temperatures. It can be concluded that controlling the formation of biogenic amines in white cheese samples must be carefully observed.

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