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Microbiological and Chemical Quality Assessment of Tulum, Kashar, and Golot Cheeses and Determination of Biogenic Amines by HPLC

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Abstract

This study was conducted to determine the microbiological and chemical properties, as well as the biogenic amine contents, of tulum (Erzincan), kashar, and golot cheeses. A total of 90 cheese samples, consisting of 30 samples from each cheese type, were analyzed. The microbiological results indicated that golot cheese exhibited higher yeast and mold counts compared to the other cheese types. Chemical analyses showed that golot cheese had lower fat but higher moisture and protein levels. Biogenic amine concentrations were determined using high-performance liquid chromatography (HPLC), and the total biogenic amine content was found to be higher in tulum cheese than in the other cheese types. However, the levels of biogenic amines detected in all cheese samples remained within acceptable safety limits. Overall, this study provides the first comprehensive data on the biogenic amine content of golot cheese, highlighting that production and ripening conditions play a crucial role in microbial load and biogenic amine formation in traditional cheeses.

Keywords: Cheese, Kashar, Tulum, Golot, Microbiological and Chemical Properties, Biogenic Amines

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INTRODUCTION

Turkiye is a country known for a wide variety of cheeses. White cheese, kashar cheese, and tulum cheese are among the most commonly produced and consumed types. Other varieties, such as herb cheese, mihalic cheese, golot, and minci, are made locally with traditional methods in small-scale operations.[1]

Tulum cheese, produced throughout nearly all parts of Turkiye, comes in two varieties: dry and brined. It is packaged in materials such as goatskin, plastic containers, and tin cans. The cheese then matures in cellars, pits, and caves before being sold for consumption. Kashar cheese is made by coagulating raw milk with rennet, then boiling and kneading the curd. It is sold either fresh or aged with added bacteria. In terms of production technique and chemical properties, kashar cheese resembles some Italian and Balkan cheeses. Golot cheese is a regional cheese produced in Turkiye's Eastern Black Sea Region, specifically in the provinces of Rize, Trabzon, Artvin, and Bayburt, as well as in the Ispir district in Erzurum province. It is mainly made at home or in family-run businesses. Depending on the region, it is also known as kolot, kolote, or golot. Its production technique is similar to kashar cheese, but differs in that the milk's fat is removed and the curd is obtained through natural acidification. It is consumed fresh or after being salted and aged in wooden barrels.[1-3]

The process of making cheese involves a series of complex reactions. During cheese formation, enzymes produced by rennet and/or starter culture microorganisms naturally present in milk hydrolyze casein, resulting in the production of peptides and amino acids. Microbial decarboxylation of these amino acids produces biogenic amines, low-molecular-weight nitrogen-containing compounds.[4,5]

Biogenic amines, including histamine, tyramine, and putrescine, play crucial roles in various functions in humans and animals. However, when consumed in high concentrations or by susceptible individuals, they can cause side effects such as headaches, anaphylaxis, nervous system symptoms, organ failure, and death. Additionally, diamines (e.g., putrescine and cadaverine) can react with nitrites to form carcinogenic nitrosamines. Once formed, biogenic amines are heat-resistant and cannot be destroyed by cooking, smoking, freezing, or other preservation techniques.[6-8] Therefore, information on the levels of biogenic amines in cheese is necessary to assess potential health risks associated with their consumption. Furthermore, the presence of biogenic amines in food is a valuable indicator of the quality and hygiene of the raw materials used in food production.[7] The formation of biogenic amines can be affected by several factors, including the presence of decarboxylase-positive microorganisms in raw milk and the environment, the composition of free amino acids, proteolysis, pH, moisture, and salt concentration. Additionally, secondary factors such as post-ripening processes and packaging can affect the accumulation of biogenic amines.[9] The detection of biogenic amines in foods is essential because of their potential toxic effects and their role as indicators of food quality. These amines can be identified using various techniques such as thin-layer chromatography (TLC), gas chromatography, capillary electrophoresis (CE), and high-performance liquid chromatography (HPLC). HPLC is the most commonly used method because most biogenic amines have low volatility and lack chromophores.[10]

In this study, the microbiological and chemical properties of tulum (Erzincan), kashar, and golot cheeses obtained from different markets were examined, and their biogenic amine contents (tyramine, histamine, putrescine, and cadaverine) were determined using a newly developed High-Performance Liquid Chromatography (HPLC) method. Furthermore, the biogenic amine content of golot cheese, a unique Turkish cheese, was reported for the first time in this study, as it is not currently documented in the existing literature.

MATERIALS AND METHODS

This study examined 90 cheese samples: 30 tulum (Erzincan), 30 kashar, and 30 golot. The samples were collected from the market under aseptic conditions, then transported in a cold chain to the Food Hygiene and Technology Laboratory at Ataturk University's Faculty of Veterinary Medicine. The samples were then prepared for analysis.

Microbiological analyses

To analyze the microbiological properties of the cheese samples, 10 g of each sample was weighed into a sterile Stomacher bag, and 90 mL of Ringer's solution was added. The sample was then homogenized in a Stomacher device for 2 minutes to prepare a 10^{-1} dilution. One milliliter of the first dilution was transferred to tubes containing 9 mL of sterile Ringer's solution using a sterile pipette to create subsequent dilutions.[11]

The following microbiological counts were performed: *Pseudomonas* spp.[12]; total aerobic mesophilic bacteria[12,13]; coliform group bacteria[12]; *Lactobacillus* spp.,[14] *Lactococcus* spp. count[15]; psychrophilic bacteria count[16]; proteolytic and lipolytic microorganisms count[12]; and yeast-mold count.[13]

Chemical analysis

The water activity of the cheese samples was measured with an Aqua Lab 4TE device. The dry matter and ash contents were determined by the gravimetric method; the fat content by the Gerber method; and the salt content by the Mohr method.[17] The pH was measured using a pH meter (Inolab WTW). Acidity was expressed as a percentage of lactic acid, following the method reported by Tekinsen et al.[17] The protein content was determined using the ISO method.[18]

Determination of biogenic amines

The analysis of biogenic amines was conducted by high-performance liquid chromatography (HPLC), utilising an Ace C18 column (5 µm, 250 × 4.6 mm) with a 50 mM acetate buffer (pH 5). The mobile phase contains acetonitrile (25:75, v/v), the flow rate is 1 mL/min, the injection volume is 10 µL, and detection occurs at 254 nm. These parameters have been utilised to develop a methodology.[19]

Standard solutions were prepared using the newly developed method. Stock solutions of four biogenic amines (putrescine, cadaverine, histamine, and tyramine) were prepared at a concentration of 100 µg/mL in methanol (10 mg/100 mL methanol). Standard solutions of 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, 50.0, 75.0, and 100 µg/mL were obtained from the stock solutions. Then, 0.1 mL of each solution was transferred into a vial. Next, 10 mmol of Dansyl chloride (the standard reagent for UV detection at 254 nm for BA) and 0.65 mL of borate buffer solution (pH 9.5) were added. The mixture was vortexed for 5 seconds and left to stand at room temperature for 15 minutes to facilitate derivatization. Initially cloudy, the mixture cleared after 15 minutes. Validation tests for the method were performed.[19]

To prepare the samples for analysis, 1 g of each cheese was placed in a 15-mL test tube. One milliliter of methanol was added, and the tube was centrifuged at 6,000 rpm for five minutes. For derivatization, 0.1 mL of the clear supernatant was transferred to a vial. Then, 0.25 mL of dichloromethane and 0.65 mL of borate buffer solution (pH = 9.5) were added. The mixture, initially cloudy, became clear after 15 minutes. The samples were filtered through a 0.2 µm PTFE filter and introduced into the instrument for analysis.[19]

High-performance liquid chromatography (HPLC) analysis was conducted using an Agilent 1260 series HPLC system. This system included a gradient pump (G7111A), an autosampler (G7129A), and a UV detector (G71144A). An Ace C18 column (250 × 4.60 mm ID, 5 µm particle size) was employed.

Statistical analyses

The normality of the data distribution was assessed using the Shapiro-Wilk test, and the homogeneity of variances was evaluated using the Levene test. For data that met the parametric assumptions (i.e., shown as mean ± SD), a one-way analysis of variance (ANOVA) was applied, followed by Tukey's post hoc test to determine differences between groups. The independent-samples t-test was used to compare two groups of data that followed a parametric distribution. Data that did not meet the parametric assumptions [presented as Median (Minimum-Maximum)] were analyzed using the Kruskal-Wallis test and the Dunn multiple-comparison test. Pearson's correlation (r) or Spearman's correlation was used to assess the relationship between parameters. A significance level of $p < 0.05$ was considered for all statistical tests. Data analysis and interpretation were performed using SPSS (Statistical Package for the Social Sciences) version 26.0 software.[19]

Table 1. Microbiological analysis findings of cheese samples (log_{10} CFU/g)

	Sample	n	Min.	Max.	Mean	H
Pseudomonas spp.	Tulum	15	2.94	5.16	4.50	5.55
	Kashar	15	2.95	4.18	3.53	
	Golot	15	2.26	5.60	3.89	
Total Aerobic Mesophilic Bacteria	Tulum	30	4.84	7.56	6.75[a]	22.09**
	Kashar	22	3.57	6.90	4.77[c]	
	Golot	28	2.90	7.27	6.06[b]	
Coliform	Tulum	11	2.45	5.24	3.40[a]	9.28*
	Kashar	6	2.00	2.54	2.33[b]	
	Golot	13	2.48	4.80	3.15[a]	
Lactobacillus spp.	Tulum	28	5.29	7.19	6.33[a]	9.12*
	Kashar	13	3.53	6.73	5.22[b]	
	Golot	20	4.45	7.34	6.17[ab]	
Lactococcus spp.	Tulum	30	4.88	7.47	6.80[a]	14.23**
	Kashar	19	3.20	6.91	5.33[b]	
	Golot	24	3.45	7.43	6.90[a]	
Psychrophilic	Tulum	14	3.53	6.44	5.07	0.43
	Kashar	9	3.53	6.45	5.20	
	Golot	16	3.25	6.44	5.35	
Proteolytic	Tulum	15	2.48	3.90	3.04[a]	6.60*
	Kashar	11	2.48	3.53	3.30[a]	
	Golot	13	2.48	3.28	2.70[b]	
Lipolytic	Tulum	27	4.00	6.94	5.73[a]	9.96*
	Kashar	22	3.00	6.04	5.55[b]	
	Golot	20	3.78	7.20	5.88[a]	
Yeast-Mold	Tulum	22	2.90	4.98	3.85[b]	13.63**
	Kashar	17	2.95	5.19	3.92[b]	
	Golot	23	3.26	5.48	4.89[a]	

*: $P < 0.05$, **: $P < 0.001$, Different letters (a-c) indicate differences between groups. N: number of samples

RESULT AND DISCUSSION

Microbiological analysis of cheese samples

The numerical values pertaining to the enumeration of *Pseudomonas* spp., TAMB (Total Aerobic Mesophilic Bacteria), coliform group bacteria, *Lactobacillus* spp., *Lactococcus* spp., psychrophilic, proteolytic, lipolytic bacteria, and yeast-mould counts in the cheese samples examined are presented in Table 1. The analyses revealed that the number of *Pseudomonas* spp. (2.26-5.60 log_{10} CFU/g) was similar in all cheeses analysed. However, the number of *Pseudomonas* spp. tulum cheese samples were higher than that reported by Evren and Sivgin.[20] This discrepancy suggests that the contamination level in the tulum cheeses examined was high.

Moreover, the investigative findings indicated that the highest TAMB count was observed in tulum cheese (7.56 log_{10} CFU/g), followed by golot (7.27 log_{10} CFU/g) and kashar cheeses (6.90 log_{10} CFU/g). Despite the tulum cheese findings being lower than those documented in certain studies,[21] they were consistent with those reported in other studies.[22,23] The value found in the kashar cheese samples was lower than that reported in kashar cheeses by Oksuztepe et al.,[24] Erol,[25] and Celebi and Simsek.[26] The value for golot cheese was higher than the 2.99 log_{10} CFU/g reported by Tuncturk and Ozdemir.[3] The inconsistency observed in the total aerobic mesophilic bacteria count when compared with data obtained in other studies can be attributed to the microbiological properties of the milk, non-compliance with hygiene rules, production techniques, and the freshness of the cheese offered for consumption. An analysis of the coliform bacteria count in the cheese samples revealed a range of 2.00-5.24 $\log_{10}$ CFU/g. The yeast-mold counts in the examined tulum, kashar, and golot cheese samples ranged between 2.90 and 5.48 $\log_{10}$ CFU/g, and golot cheese was found to have the highest yeast-mold count. However, no significant difference was found in the yeast-mold counts between tulum and kashar cheeses (Table 1). The elevated yeast-mold count observed in golot cheese, compared with other cheeses, is hypothesised to be attributable to suboptimal hygiene conditions, particularly during product sale.

The present study found that tulum cheese contained a higher number of *Lactobacillus* spp. compared to kashar cheese. However, no significant differences were detected when comparing golot and tulum or golot and kashar. Despite the prevalence of *Lactococcus* spp. being similar in tulum and golot cheeses, the population levels in these cheeses were found to exceed those observed in kashar cheese. The data concerning the number of *Lactobacillus* spp. obtained from tulum cheese, the data were found to be similar to those reported by Kara and Akkaya[22] and Demir et al.[23] The number of *Lactococcus* spp. obtained in tulum cheeses were found to be higher than the data reported by some researchers.[22,23] Isik et al.[27] reported that mesophilic and thermophilic LAB populations ranged from 6.28 ± 0.14 to 8.08 ± 0.11 $\log_{10}$ CFU/g in kashar samples collected from local producers. In their 2009 study, Oksuztepe et al.[24] reported the number of *Lactobacillus* spp. in kashar cheese samples sold in the market at 7.02 $\log_{10}$ CFU/g, and the number of *Lactococcus* spp. at 6.81 $\log_{10}$ CFU/g. Tuncturk and Ozdemir[3] determined the average lactic acid bacteria count in golot cheese samples obtained from the market to be 3.07 $\log_{10}$ CFU/g. The presence of high numbers of lactic acid bacteria in cheese samples is advantageous, as they contribute to the characteristic taste and aroma of cheese whilst also preventing the growth of undesirable microorganisms. The disparities observed between the cheeses examined and the values reported in other studies can be attributed to the extent of heat treatment the milk undergoes during cheese production, as well as the degree of environmental contamination during production.

The number of psychrophilic bacteria was found to be 3.53-6.44 $\log_{10}$ CFU/g in tulum cheese, 3.53-6.45 $\log_{10}$ CFU/g in kashar cheese, and 3.25-6.44 $\log_{10}$ CFU/g in golot cheese. The average values were 5.07, 5.20, and 5.35 $\log_{10}$ CFU/g, respectively. In a study conducted by Kara and Akkaya[22] on Afyon tulum cheeses, the average psychrophilic bacteria count was determined to be 3.92 $\log_{10}$ CFU/g. In another study on kashar cheese, the average bacterial count was found to be 5.04-5.59 $\log_{10}$ CFU/g.[28] While these data are consistent with our findings, the study by Ozdemir and Demirci[29] found a higher count (3.41 $\log_{10}$ CFU/g). In a study conducted by Yazici and Dervisoglu[30] using golot cheese, the psychrophilic bacterial count (5.95 $\log_{10}$ CFU/g) was found to be higher than in our study. These results indicate that hygienic conditions are not adequately provided during the cheese production process.

The proteolytic bacterial counts in the examined tulum, kashar, and golot cheese samples were determined to be 2.48-3.90, 2.48-3.53, and 2.48-3.28 $\log_{10}$ CFU/g, respectively, with average values of 3.04, 3.30, and 2.70 $\log_{10}$ CFU/g. In a study by Evren and Sivgin,[20] the average proteolytic bacteria count in tulum cheese packaged in skin and plastic containers was reported to be 3.04 and 2.51 $\log_{10}$ CFU/g, respectively, which is consistent with the data presented in this study. However, Ozdemir and Demirci[29] reported, in their research to determine the proteolytic bacteria count in kashar cheese samples, an average bacteria count of 4.56 $\log_{10}$ CFU/g. This value is higher than the results obtained in this study. In another study by Yazici and Dervisoglu,[30] the microbiological characteristics of golot cheese were determined, and the proteolytic bacteria count was found to be 5.66 $\log_{10}$ CFU/g. This value is higher than the data obtained in this study. The observed variations in values may be attributed to factors related to the quality of the milk, the hygienic production process, and storage conditions.

The lipolytic bacterial counts in the tulum, kashar, and golot cheese samples were found to be 4.00-6.94, 3.00-6.04, and 3.78-7.20 $\log_{10}$ CFU/g, respectively, with average values of 5.73, 5.55, and 5.88 $\log_{10}$ CFU/g. Evren and Sivgin's[20] study found the average lipolytic bacteria count in tulum cheese packaged in leather and plastic containers to be 3.82 and 3.33 $\log_{10}$ CFU/g, respectively. A further survey of Ozdemir and Demirci[29] on kashar cheese samples determined this value to be 4.35

Table 2. Chemical properties of cheese samples

	Sample (n=30)	Min.	Max.	Mean ± SD	F
pH	Tulum	4.75	5.65	5.18 ± 0.25^b	23.64**
	Kashar	5.21	6.16	5.60 ± 0.26^a	
	Golot	5.00	6.36	5.68 ± 0.37^a	
Water activity-a_w	Tulum	0.87	0.97	0.94 ± 0.02^c	35.49**
	Kashar	0.93	0.98	0.96 ± 0.01^b	
	Golot	0.96	0.99	0.98 ± 0.01^a	
Dry matter (%)	Tulum	47.23	79.67	58.92 ± 6.04^a	25.74**
	Kashar	52.40	68.43	58.80 ± 4.27^a	
	Golot	38.29	59.35	50.17 ± 5.77^b	
Ash (%)	Tulum	2.53	5.70	4.10 ± 0.84	0.38
	Kashar	2.81	7.14	4.03 ± 0.84	
	Golot	2.55	5.56	4.21 ± 0.67	
Acidity (%)	Tulum	0.90	1.66	1.42 ± 0.22^a	134.06**
	Kashar	0.22	1.44	0.55 ± 0.31^b	
	Golot	0.18	0.86	0.48 ± 0.19^b	
Salt (%)	Tulum	1.64	4.21	2.74 ± 0.68^a	8.19
	Kashar	0.94	4.21	2.26 ± 0.81^b	
	Golot	0.47	4.21	1.96 ± 0.78^b	
Fat (%)	Tulum	12	45	29.97 ± 6.11^a	53.15**
	Kashar	17	30	25.27 ± 3.45^b	
	Golot	0	30	12.70 ± 9.26^c	
Protein (%)	Tulum	17.62	19.5	18.54 ± 0.56^c	64.01**
	Kashar	18.32	23.58	20.94 ± 1.41^b	
	Golot	18.82	23.12	21.69 ± 1.22^a	

**: P < 0.001, Different letters (a-c) indicate differences between groups

$\log_{10}$ CFU/g. The values obtained for the samples of cheese from tulum and kashar are lower than those obtained for the samples of golot cheese. Furthermore, the lipolytic bacteria count in golot cheese samples was higher than the 5.43 $\log_{10}$ CFU/g reported by Yazici and Dervisoglu.[30] The observed discrepancy between the data obtained and that reported in the extant literature may be attributed to variations in post-production cold chain conditions and packaging methodologies.

Chemical analysis of cheese samples

The results for pH, water activity, dry matter, ash, acidity, salt, fat, and protein, as analyzed in the cheese samples examined in the study, are shown in Table 2.

The results of the analyses revealed that the average pH values of the tulum, kashar, and golot cheese samples obtained from the market were 5.18 ± 0.25, 5.60 ± 0.26, and 5.68 ± 0.37, respectively (Table 2). These results are consistent with the literature data.[3,20,22,24-26,31-34]

The water activity value of Erzincan tulum cheese samples from the market was found to be 0.94 ± 0.02 on average. Erdem and Patir[34] reported that the average water activity value of Tulum cheese sold in Elazig province was 0.93 ± 0.03. In contrast, Erkan et al.[31] reported that the water activity values of shavak tulum cheese samples taken from different production sites in Elazig were on average 0.96 ± 0.03. It was observed that the values obtained in this study were consistent with those reported by previous researchers. Differences in water activity values may be attributed to variations in production techniques, maturation periods, and storage conditions of the examined cheese samples. The water activity value of the kashar cheese samples was found to be 0.96 ± 0.01 on average. Celebi and Simsek[26] reported that the water activity value of the 1st-day samples was 0.96 ± 0.01 on average, while the water activity value of the 90th day samples was 0.95 ± 0.01 on average in their study on kashar cheese samples. Oksuztepe et al.[24] determined that the average water activity in kashar cheese samples was 0.91 ± 0.03. Akgul et al.[32] reported that the average water activity in Kars kashar cheese samples was 0.94 ± 0.00. As a result of our analysis, the water activity value in golot cheese samples was determined to be 0.98 ± 0.01 on average (Table 2).

The dry matter content of tulum cheese samples was determined to be 58.92 ± 6.04%. This data is similar to the average values reported by Erkan et al.[31] (57.41%), Akgul et al.[32] (55.95%), and Akpinar et al.[35] (60.15%). The average dry matter content of kashar cheese was 58.80 ± 4.27%, and the data obtained were similar to those reported in previous studies.[25,36-38] The average dry matter content of the golot cheeses examined was 50.17 ± 5.77%, consistent with the literature.[3,39] The findings of this study show that the dry matter content of golot cheese is lower than that of tulum and kashar cheeses ($P < 0.001$) (Table 2). It is thought that removing fat from milk during the processing of golot cheese leads to a low dry matter content in the cheese.

The ash content in tulum cheese samples was determined to be an average of 4.1% ± 0.84. These data are similar to the results of the study conducted by Evren and Sivgin,[20] higher than the value reported by Erdem and Patir,[34] and lower than the values reported by Digrak et al.[40] and Kara and Akkaya.[22] The ash content of kashar cheese samples was determined to be between 2.81% and 7.14%, falling within the maximum and minimum values reported in the studies conducted by Oksuztepe et al.,[24] Yildiz and Kurdal,[37] Yuceer and Dogan,[41] and Sahiner and Yuceer.[42] The ash values of golot cheese samples ranged from 2.55% to 5.56%, with an average of 4.21% ± 0.67. One study recorded that the ash content of golot cheeses varied between 2.34% and 11%.[39] The variability in ash content is attributed to methodological differences during cheese production and the varying water and salt content of the cheeses.

The acidity levels in the tulum cheese samples ranged from 0.90% to 1.66%, with an average of 1.42% and a standard deviation of 0.22% (Table 2). The results are similar to those found by Digrak et al.[40] but higher than the 0.51% reported by Kara and Akkaya,[22] the 0.25% by Erkan et al.,[31] and the 0.96% and 0.97% observed by Evren and Sivgin.[20] They are lower than the 1.8% reported by Erdem and Patir[34] and the 1.95% found by Akgul et al.[32] The acidity value in the kashar cheese samples was found to be an average of 0.55 ± 0.31% in terms of lactic acid. Our findings were similar to those of some studies, while others found them to be higher or lower.[24,26,33,37,43,44] The acidity value of the golot cheese samples ranged from 0.18 to 0.86% in terms of lactic acid, with an average of 0.48 ± 0.19. This value was found to be lower than the 0.83% reported by Tuncturk and Ozdemir.[3] The differences observed when compared with the literature data can be attributed to various biochemical processes, including the starter culture used during production, yeast activity, dry matter, and the maturation period. In particular, while the acidity values of golot and kashar cheese samples were close to each other, the high acidity of tulum cheese can be attributed to the increase in acidity as a result of the activity of lactic acid bacteria during the long maturation process and the use of raw milk in the production of tulum cheese.

The average salt content in the examined tulum cheese samples was found to be 2.74 ± 0.68 (Table 2). When compared with previous studies, the data obtained were higher than those reported by Akgul et al.[32] and lower than those reported by some other researchers.[20,34,35] The salt content of the kashar cheese samples was determined to be between 2.26 ± 0.81%, which was lower than the values reported by Kocak et al.[45] (2.72%), Oksuztepe et al.[24] (2.74%), and Erol[25] (3.17%). The obtained data were higher than the 1.81% reported by Celebi and Simsek[26] but lower than the 3.73% reported by Cetinkaya.[43] The salt content of the golot cheeses examined ranged from 0.47% to 4.21%. This value is consistent with the literature data, as it was determined to be 0.41% to 9.08% by Dervisoglu and Yazici[39] and an average of 3.07% by Tuncturk and Ozdemir.[3] It was also found that the saltiness values of golot and kashar cheese were similar ($P > 0.05$). It is well known that the use of salt in cheese contributes to its flavor, has a preservative effect, reduces moisture content, affects texture and ripening, and thus impacts the overall quality of the cheese. Studies have also found that producers' knowledge and experience are essential factors in the variability of salt levels in cheeses.[19]

The fat content of tulum cheese samples averaged 29.97 ± 6.11%, while the fat content in dry matter averaged 50.56 ± 7.56% (Table 2). The fat content obtained in this study was found to be consistent with the data of previous researchers.[34,35] The fat content of the kashar cheese samples was calculated to be an average of 25.27 ± 3.45%, and the fat content in dry matter was calculated to be an average of 43.03 ± 65.69%. The data obtained in this study were found to be similar to those from previous studies.[25,32,35,38] In contrast, the fat and fat content in dry matter detected in kashar cheeses were found to be lower than those in some previous studies.[7,33,37,43] The fat content of golot cheese samples was calculated as an average of 12.7 ± 9.26%, and the fat content in dry matter was calculated as an average of 24.09 ± 17.29%. In contrast to the findings of Dervisoglu and Yazici,[39] who reported that the fat content in golot cheese ranged from 1% to 20%, Tuncturk and Ozdemir[3] found an average of 7.41%. The fat content data identified for golot cheese in this study were found to be higher than those reported by other researchers. The fact that the fat content data obtained in this study is higher than the findings of other researchers is thought to be due to some packaged golot cheeses purchased from the market being full-fat.

In this study, the protein content of tulum cheese samples was found to be an average of 18.54% ± 0.56. The data obtained were found to be lower than the data reported by other researchers in previous studies.[20,22,31,34,35] This difference can be attributed to the high fat content in the dry matter of the tulum cheese samples examined. The protein content of kashar cheese samples ranged from a minimum of 18.32% to a maximum of 23.58%, with an average of 20.94 ± 1.41% (Table 2). The protein content of kashar cheese was found to be similar to some literature data,[37,46] while lower than others.[33,36] The protein content of golot cheese samples was determined to be 21.69 ± 1.22 on average. Contrary to the findings of Dervisoglu and Yazici,[39] who reported that the protein content of golot cheeses ranged between 24% and 41%, Tuncturk and Ozdemir[3] determined the average value to be 35.68%. The study concluded that golot cheese had the highest average protein content among the cheese samples, followed by kashar and tulum cheese. This high value may be attributed to golot cheese having a lower fat content compared to other cheeses. The inconsistency observed between the findings of this study and those of other researchers is thought to stem from the fact that the golot cheese samples examined in this study had a higher fat content than those analyzed by other researchers.

Determination of biogenic amines in cheese samples

The biogenic amine levels in tulum, golot, and kashar cheese samples are shown in Table 3.

In tulum cheese samples, putrescine levels ranged from 0.13 mg/kg to 29.17 mg/kg, with a mean of 8.72 mg/kg. Cadaverine levels ranged from 0.07 to 32.5 mg/kg, averaging 7.01 mg/kg. Histamine was detected at concentrations ranging from 0 to 13.04 mg/kg, with an average concentration of 1.58 mg/kg. Tyramine ranged from 0.11 to 33.84 mg/kg, with a mean of 0.62 mg/kg. Erdem and Patir[34] reported histamine levels in tulum cheese samples from Elazig province, ranging from a minimum of 67.8 mg/kg to a maximum of 2510 mg/kg, with an average of 706.5 mg/kg. Putrescine was found in 21 kashar cheese samples, with a mean level of 0.15 mg/kg. Cadaverine was present in all kashar samples, with concentrations from 0.07 to 6.98 mg/kg and an average of 0.3 mg/kg. Histamine was detected in 15 of 30 kashar samples, with a mean level of 0.01 mg/kg. Tyramine was detected in 26 samples, with an average concentration of 0.1 mg/kg. Akgul et al.[32] reported in their study that in kashar cheeses, putrescine was 0.9 mg/kg, cadaverine was 1.8 mg/kg, histamine was 3.9 mg/kg, and tyramine was 6.8 mg/kg. In tulum cheese, the average values for tryptamine, phenylethylamine, putrescine, cadaverine, histamine, and tyramine were found to be 1.3, 1.6, 5.1, 4.5, 1.2, and 5.6 mg/kg, respectively. The literature shows a lack of studies on biogenic amine levels in golot cheese. Putrescine was detected in 27 golot cheese samples, with levels from 0 to 15.49 mg/kg and a mean of 0.21 mg/kg. Cadaverine was found in all golot samples, with concentrations ranging from 0.07 mg/kg to 3.55 mg/kg and an average

Table 3. Biogenic amine findings of cheese samples (mg/kg)

	Sample (n=30)	Min.	Max.	Mean	H
Putrescine	Tulum	0.13	29.17	8.72[a]	41.25**
	Kashar	<LOQ	6.49	0.15[c]	
	Golot	<LOQ	15.49	0.21[b]	
Cadaverine	Tulum	0.07	32.5	7.01[a]	21.48**
	Kashar	0.07	6.98	0.3[c]	
	Golot	0.07	3.55	0.58[b]	
Histamine	Tulum	<LOQ	13.04	1.58[a]	45.51**
	Kashar	<LOQ	0.58	0.01[b]	
	Golot	<LOQ	0.20	0[b]	
Tyramine	Tulum	0.11	33.84	0.62[a]	24.68**
	Kashar	<LOQ	1.37	0.1[c]	
	Golot	<LOQ	1.91	0.26[b]	

**: P < 0.001. Different letters (a-c) indicate differences between groups

of 0.58 mg/kg. Histamine was present in 14 golot cheese samples, with levels from 0 to 0.20 mg/kg. Tyramine was found in 29 samples, with an average concentration of 0.26 mg/kg (Table 3).

Compared to other samples, tulum cheese had the highest tyramine levels (P < 0.001) and the highest average putrescine value. This can be attributed to the use of raw milk in the production of tulum cheese and its longer maturation period compared to other cheeses. Although the histamine levels of golot and kashar cheeses were similar (P > 0.05), golot cheese showed higher cadaverine and tyramine levels than kashar cheese (P < 0.001). The increase in biogenic amine levels in cheese can be attributed to the effect of decarboxylase-positive microorganisms.[9] The presence of decarboxylase-positive bacteria in fermented products such as cheese depends on various factors. These include the raw material itself, the characteristics of the starter culture, or contamination during the production process. Despite the similarity of the techniques used in the production of kashar and golot cheeses, golot cheese is mostly produced in small family businesses where hygiene standards are generally inadequate. Furthermore, these products are usually sold unpackaged and are exposed to unhygienic conditions at the point of sale. The higher concentrations of cadaverine and tyramine in golot cheese compared to kashar cheese can be attributed to these hygiene conditions. In general, the concentrations of biogenic amines in the analyzed cheeses did not exceed the recommended threshold values.

CONCLUSION

In this study, a range of cheeses, including tulum (Erzincan), kashar, and golot, obtained from various markets under aseptic conditions, were subjected to a comprehensive array of microbiological and chemical analyses. Subsequently, their biogenic amine contents were determined. The findings demonstrated that tulum and kashar cheeses share similar physicochemical features, whereas golot cheese differs in having a lower fat and higher protein content. Golot also exhibited the highest microbial load, especially for yeast and molds, indicating possible hygiene deficiencies during production or sale. Among the analyzed cheeses, tulum showed the highest total biogenic amine concentrations, likely due to the use of raw milk and extended maturation, while kashar contained the lowest levels. Notably, the biogenic amine levels in all cheese types remained below safety thresholds for human consumption. This research provides the first documented data on the biogenic amine content of golot cheese, expanding current knowledge on Turkish traditional cheeses. To improve product quality and safety, it is recommended that producers adhere to strict hygiene protocols, standardize manufacturing processes, and routinely monitor biogenic amine levels as indicators of fermentation performance and overall quality.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

FB, and GA conceptualized the idea. FB performed experimentation. BY, and FB performed the HPLC analysis. FB wrote the manuscript. All authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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