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RESEARCH ARTICLE

Evaluation of the Biogenic Amine Content and Some Chemical and Microbiological Properties of Urfa and Van Herby Cheeses [1]

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Summary

In this study, the biogenic amine contents and some microbiological and chemical properties of Urfa and Van herby cheeses obtained from local supermarkets in Urfa and Van provinces were investigated. Tryptamine, β-phenylethylamine, putrecine, cadaverine, histamine and tyramine levels were detected in these cheeses. In addition some chemical properties (pH, total solids, fat-in-dry matter and salt-in-dry matter, protein, ripening coefficient, water soluble nitrogen and water activity) and some microbiological properties (total aerobic mesophilic bacteria, lactic acid bacteria and *Enterobacteriaceae*) were determined. Tyramine, cadaverine, histamine and putrecine were predominant biogenic amines in Urfa and Van herby cheese, respectively. β-phenylethylamine and tryptamine in Urfa cheese, were predominant in Van herby cheeses. The amounts of tyramine in Urfa and Van herby cheeses were found to be the highest with an average of 1.80 mg 100 g^{-1}, 0.56 mg 100 g^{-1}, respectively. None of the detected biogenic amines was higher than the toxic limit.

Keywords: *Biogenic amine, Enterobacteriaceae, Lactic acid bacteria, Total aerobic mesophilic bacteria, Urfa cheese, Van herby (otlu) cheese*

Urfa ve Van Otlu Peynirlerinin Bazı Kimyasal ve Mikrobiyolojik Özellikleri ve Biyojen Amin İçeriklerinin Değerlendirilmesi

Özet

Bu çalışmada, Urfa ve Van bölgelerindeki süpermarketlerden temin edilen Urfa ve Van otlu peynirlerinin biyojen amin içerikleri ve bazı mikrobiyolojik ve kimyasal özellikleri incelenmiştir. Bu peynirlerde triptamin, β-feniletilamin, putresin, kadaverin, histamin ve tiramin düzeyleri belirlenmiştir. İlaveten bazı kimyasal özellikleri (pH değeri, toplam kurumadde, kurumadde de yağ ve kurumadde de tuz içeriği, protein, olgunlaşma katsayısı, suda çözünür azot ve su aktivitesi) ve bazı mikrobiyolojik özellikleri (toplam aerobik mezofil bakteri, laktik asit bakterileri ve *Enterobacteriaceae* türleri) belirlenmiştir. Tiramin, kadaverin, histamin ve putresin her iki peynirde de dominant biyojen aminlerdir. β-feniletilamin Urfa peynirinde dominat iken triptamin Van otlu peynirinde dominanttır. Urfa ve Van otlu peynirlerinde en yüksek tiraminin miktarı sırasıyla 1.80 mg 100 g^{-1}, 0.56 mg 100 g^{-1} olarak saptanmıştır. Biyojen aminlerin hiçbiri toksik limitin üzerinde değildir.

Anahtar sözcükler: *Biyojen amin, Enterobacteriaceae, Laktik asit bakterisi, Toplam aerobik mezofil bakteri, Urfa peyniri, Van otlu peyniri*

INTRODUCTION

Biogenic amines are organic compounds of low molecular weight that exhibit biological activity and are usually produced by the decarboxylation of amino acids or by amination and transformation of aldehydes and ketones. The formation of biogenic amines in foods requires the availability of free amino acids, the presence of decarboxylase-positive microorganisms and conditions that enable bacterial growth [1]. Biogenic amines are organic

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substances present in food, which are responsible for toxicological risks and can be detrimental to health [2]. The presence of high levels of biogenic amines in foods may cause migraine, headaches, hypo or hypertension, heart palpitations, flushing, gastric and intestinal problems and pseudo allergic responses [3]. The most important biogenic amines occurring in foods and beverages are Histamine (HI), Phenylethylamine (PHE), Tyramine (TY), Tryptamine (TR), Putrescine (PU), Cadavarine (CA), Spermine (SM) and Spermidine (SD) [4,5]. These biogenic amines may be present in ripened cheeses and fermented products [6]. Cheese is one of the foods reported to have the highest content of amines, and it is the most prevalent product associated with amine poisoning [3]. Biogenic amines also can cause "cheese syndrome" and histamine intoxication related to tyramine and histamine respectively [7]. The factors which are related to the formation of biogenic amines in dairy products, are the microflora that can produce decarboxylases, microbiological quality of the milk, cheese production technology, cheese ripening conditions and hygienic conditions during production processes [1,8]. A high concentration of these amines could be used as an indicator of poor hygienic quality of cheese [7].

Urfa and Van herby cheeses are most important two traditional cheeses in Turkey. They are produced in the Eastern and South eastern Anatolia regions of Turkey. Urfa cheese is a semi hard brined Turkish type cheese manufactured from raw ovine milk or appropriate mixtures of ovine and caprine milk, without any starter culture [9]. The production of Urfa cheese is carried out from March to June in South Eastern Turkey [10]. The popularity of Urfa cheese has been increasing, and it is exported to Middle Eastern and Central Asian countries [11]. The annual production of Urfa cheese has been estimated to be approximately 35.000-40.000 tons [12]. The cheese making procedure of Urfa cheese is as following: raw milk is coagulated with rennet at 30-32ºC. When the coagulum is obtained, it is cut into small cubes and then drained in triangular moulds, called "*parzin*", which contain 250-400 g of curd. The parzins have finer pores than regular cheese cloth, and whey removal may not be completed lesser than 12 h at room temperature [13]. After removing the whey by gravity drainage, the curd is scalded with whey at around 90ºC/3 min. and stretched until it becomes well knit. Then it is shaped by hand in a cotton bag. Afterwards the cheese blocks are dry salted at room temperature, then they are stored in brine (15% NaCl) for 3-4 months [10,11,13].

Van herby (otlu) cheese is a very famous brined cheese variety in Turkey, and its popularity has been gradually increasing [13]. It is generally produced from raw sheep milk. In case that sheep milk is not available, a mixture of sheep and goat or cow milk can be used [14]. The local production has been being continued for more than 200 years [15]. Although its production is concentrated in eastern Turkey. Van herby cheese is marketed throughout Turkey and has a considerable export potential to the EU once its production is standardized [16]. Van herby cheese is traditionally produced as following: After the filtering of raw sheep milk, rennet is added at the milking temperature. After coagulation, it is cut into small pieces and the whey is removed. Van herby cheese is produced using herbs which give the cheese a characteristic appearance and aroma/flavour and also extend its shelf life [13,16]. Previously sliced herbs are added into the curd. As many as 25 kinds of herbs such as *Alliums* spp., *Thymus* spp., *Ferula* spp., *Anthriscus nemorasa*, etc can be used to make Van herby cheese. These herbs, can be added individually or as a mixture [16,17]. The amount of the added herb varies from 0.5 to 2 kg or from 0.5 to 3 kg per curd obtained from 100 L of milk [14,17]. Afterwards, the curd is mixed well to obtain a homogenous distribution of the herbs. Then the mixture of curd and herbs is pressed with a heavy object for approximately 3 hours to remove the whey sufficiently. The pressed cheese is cut into blocks and the blocks are salted with dry salt or ripened in brine. Then the cheese blocks are put into the bottom of a plastic container as the first layer. "Cacik" (a quark like product made from yoghurt), is placed over the cheese blocks, and is used to fill the spaces between blocks in the container in order to force out the air. When the container is entirely filled up with cheese, the top is covered with a clean cloth and the container is turned upside down and buried in the ground in a cool place. During ripening, the water drains from the cheese and absorb by the soil. This cheese is ripened for more than 3 months to obtain its characteristic taste and flavour [14]. However, this traditional production method has been replaced in dairies by brine-salting and the cheese is marketed in a vacuum-packed in containers [16].

In summary, Urfa and Van herby cheeses are economically and culturally important traditional products. These cheeses are usually manufactured using traditional methods. With the traditional manufacturing methods, high amounts of biogenic amines could be expected in these cheeses due to expected potentially high microbiological contents. To date, numerous studies concerning Urfa and Van herby cheeses have been published, describing their chemical, microbiological, and technological properties as well as antioxidant capacity and mineral contents [9-12,14,15,17-19]. However studies on the contents of the biogenic amines in Urfa and Herby cheese are not adequate. In one study, Durlu-Özkaya [3] obtained nine samples of Van herby cheese and nine samples of Urfa cheese from a supermarket in Ankara province and determined the biogenic amine content of these cheeses. On the other hand, the biogenic amine content of several other Turkish cheeses including white brined cheese, Tulum cheese, Civil cheese, Kaşar cheese etc, have been well characterized [3,20,21].

The objectives of this study are to determine some microbiological and chemical properties and the types and quantities of biogenic amines present in Urfa and Van

herby cheeses and, to correlate the biogenic amines with the other properties.

MATERIAL and METHODS

Materials

Twenty samples for each of two cheese types were purchased from different urban vendors of the Şanlıurfa and Van provinces of Turkey between May and June. Five hundred gram samples were obtained and kept on ice in an insulated container until being transferred to the laboratory. Then the samples were then analyzed in the laboratory of Dairy Technology Department of Ankara University.

Methods

Chemical Analyses

The total solid content of the cheese samples was determined by the gravimetric method [22], and fat content by the Gerber method using a Van-Gulik butyrometer [22]. The salt content of the cheeses was detected according to the Mohr method described by Hooi et al.[22]. The pH values were measured by using a pH-meter model Mettler Toledo (Analytical, Switzerland) fitted with a standard, combined with glass electrode. The total nitrogen (TN) and water soluble nitrogen (WSN) contents of samples were determined according to Gripon, Desmazeaud, Bars & Bergere [23]. Ripening coefficients were estimated as the percentage of WSN in TN. The water activity was measured using a Novasina thermoconstanter (Axair Ltd. Systems, Switzerland).

Microbiological Analyses

Samples were prepared according to AOAC methods [24]. The samples were aseptically opened, and homogenized with 0.1% peptone water for 90 s in sterile stomacher bags. Related dilutions were spread plated onto Plate Count Agar (Merck) for Total aerobic mesophilic bacteria (TAMB), MRS agar (Merck) for lactic acid bacteria (LAB) and Violet Red Bile Dextrose Agar (Merck) for *Enterobacteriaceae*. Results were calculated as CFU per gram [24,25].

Determination of Biogenic Amines Using HPLC

Samples were prepared according to the method used by Maijola and Eerola [26] and Durlu-Özkaya et al.[20]. Acetonitrile/ Percholoric acid (AP) solution (1:1) was used for precipitation of protein in the cheese and internal standard (IS) (1-7 diamino heptane, Sigma) was added into this mixture. After centrifugation of the mixture, the aqueous layer was removed and mixed with acetonitrile, bidistilled water, sodium carbonate solution and dansyl chloride, followed by agitation on a vortex mixer The samples were then maintained in a water-bath at 37°C for 30 min. Na-glutaminate solution (50 mg ml^{-1}) was added and the samples were agitated and then incubation in a water-bath at 37°C for 60 min. Acetonitrile (ACN) was added once again and the samples were centrifuged. The aqueous layer was used for determination of biogenic amine contents.

Standard solutions of each biogenic amine were prepared separately in AP solution. 5 ml from each of these standard solutions were dissolved in 20 ml of AP solution and 500 µl of IS was added into mixture. The chromatograms of biogenic amines were obtained according to Bütikofer, Fuchs, Hurni & Bosset [27]. High pressure liquid chromatographic (HPLC) system (Hewlett Packard, model 1100, CA, US) equipped with UV detector (HP-G 1314 A) was used for detection of biogenic amine. Measurements were performed at 254 nm, using a reversed phase Luna C_{18} column (250x 4.6 mm, 5 µm). A and B solutions as a mobile phase were used at a flow rate of 1 ml min^{-1} while the column temperature was set as 35°C. A and B mobile phases were prepared buffer/ethanol/acetonitrile/H_2O:7.5/ 50/75/117.5 mL and buffer/ethanol/acetonitrile/H_2O:0.5 /112.5/112.5/25 mL, respectively. Also buffer solution was prepared tris/acetic acid/ H_2O:2/1/2. The injection volume was 20 µl.

Statistical Analyses

Minitab version 13.2 (Minitab, Inc., State College, Pa.) was used for data analyses. A two-tailed *t* test at the 95% confidence interval was conducted to determine differences in microbial content and biogenic amines, and chemical properties and biogenic amines [28].

RESULTS

Mean, minimum and maximum values of water activity, pH, total solids, protein, water soluble nitrogen (WSN), ripening coefficient, fat-in-dry mater and salt-in-dry matter in Urfa and Van herby cheeses are given in *Table 1*.

The mean value of pH, total solids, fat in dry matter, salt in dry matter, protein, WSN and ripening coefficient in Urfa cheese were determined as 5.04±0.28, 47.25±1.08, 57.57±2.20, 12.99±1.69, 18.72±1.92, 0.46±0.11, 15.27±1.30, respectively.

The mean value of pH, total solids, fat in dry matter, salt in dry matter, protein, WSN and ripening coefficient in Van herby cheese were determined as 4.74±0.07, 52.14±0.74, 47.62±1.29, 12.10±0.72, 20.51±0.40, 0.49±0.02, 15.13±0.59, respectively.

The values of *Enterobacteriaceae*, LAB, TAMB, and individual biogenic amines in Urfa cheese are given *Table 2*.

The values of *Enterobacteriaceae*, LAB, TAMB and individual biogenic amines in Van herby cheese are given *Table 3*.

Table 1. *The results of chemical properties of Urfa and Van herby cheese*
Tablo 1. *Urfa ve Van otlu peynirlerin kimyasal analiz sonuçları*

Chemical	Urfa Cheese			Van Herby Cheese		
Properties	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**
Water Activity, (aw)	0.75	0.99	0.91±0.06	0.81	0.99	0.90±0.06
pH Value	4.68	5.76	5.04±0.28	4.22	5.19	4.74±0.07
Total Solids, (g 100 g^{-1})	41.04	59.74	47.25±1.08	45.24	57.38	52.14±0.74
Protein, (g 100 g^{-1})	14.73	22.19	18.72±1.92	17.26	24.21	20.51±0.40
WSN, (g 100 g^{-1})	0.33	0.65	0.46±0.11	0.39	0.64	0.49±0.02
Ripenning Coefficient, %	1.82	25.79	15.27±1.30	10.87	20.22	15.13±0.59
Fat in Dry Matter, (g 100 g^{-1})	44.33	85.45	57.57±2.20	35.33	59.57	47.62±1.29
Salt in Dry Matter, (g 100 g^{-1})	3.15	26.02	12.99±1.69	4.30	17.70	12.10±0.72

Table 2. *Microbiological properties and biogenic amines content of Urfa cheese samples*
Tablo 2. *Urfa peynirlerinin mikrobiyolojik özellikleri ve biyojen amin içeriği*

Samples	Bacteria (log cfu g^{-1})			Biogenic Amines (mg 100 g^{-1})						
	***Enterobacteriaceae* spp.**	**LAB**	**TAMB**	**TR**	**PHE**	**PU**	**CA**	**HI**	**TY**	**Total Amine**
1	5.48	7.90	7.95	nd	nd	0.27	0.13	nd	1.88	2.28
2	2.00	5.30	6.54	nd	nd	0.02	0.04	nd	nd	0.06
3	4.54	6.64	7.78	0.43	0.36	0.28	nd	nd	1.51	2.58
4	5.32	6.51	7.08	0.36	0.70	0.55	0.44	0.88	2.16	5.09
5	3.70	6.81	7.11	0.07	0.07	0.13	nd	0.23	0.34	0.84
6	3.48	5.46	7.49	nd	0.40	0.09	0.04	0.06	0.50	1.10
7	6.71	7.87	7.84	nd	0.06	0.08	0.12	0.09	0.21	0.56
8	4.48	6.84	7.20	nd	nd	nd	nd	nd	8.38	8.38
9	6.32	7.30	7.71	nd	0.22	0.23	0.16	0.37	0.40	1.38
10	4.04	6.45	7.83	nd	nd	nd	nd	nd	5.66	5.66
11	2.00	5.96	7.00	nd	3.75	nd	nd	nd	3.90	7.65
12	3.95	6.85	7.45	2.00	nd	0.09	0.10	0.13	1.45	3.77
13	2.00	5.15	6.08	nd	1.01	nd	nd	nd	nd	1.01
14	5.28	7.38	8.00	nd	0.07	0.08	0.50	nd	nd	0.64
15	5.80	7.71	7.87	0.31	0.13	0.14	2.28	0.57	3.33	6.76
16	5.95	6.93	7.34	nd	nd	nd	nd	0.37	4.21	4.58
17	3.84	7.65	7.60	0.09	0.17	0.14	0.07	0.11	0.74	1.32
18	2.00	5.76	5.86	nd	0.16	0.17	0.15	nd	0.34	0.82
19	2.00	4.85	6.56	nd	0.06	0.03	nd	0.14	0.46	0.69
20	3.48	7.46	8.65	nd	0.05	0.08	0.08	0.09	0.48	0.77
Min	**2.00**	**4.85**	**5.86**	**0.07**	**0.047**	**0.016**	**0.041**	**0.063**	**0.21**	**0.06**
Max	**6.71**	**7.90**	**8.65**	**2.00**	**3.75**	**0.55**	**2.28**	**0.88**	**8.38**	**8.38**
Mean	**4.12±0.35**	**6.64±0.21**	**7.35±0.15**	**0.16±0.10**	**0.36±0.18**	**0.12±0.03**	**0.21±0.11**	**0.15±0.05**	**1.80±0.50**	**2.80±0.58**

nd: *not determined,* ***LAB:*** *Lactic acid bacteria,* ***TAMB:*** *Total Aerobic Mezophylic Bacteria,* ***TR:*** *Tryptamine,* ***PHE:*** *Phenylethylamine,* ***PU:*** *Putrecine,* ***CA:*** *Cadaverine,* ***HI:*** *Histamine,* ***TY:*** *Tyramine*

Table 3. *Microbiological properties and biogenic amines content of Van herby cheese samples*
Tablo 3. *Van otlu peynirinin mikrobiyolojik özellikleri ve biyojen amin içeriği*

Samples	Bacteria (log cfu g^{-1})			Biogenic Amines (mg100 g^{-1})						
	Enterobacte riaceae spp.	LAB	TAMB	TR	PHE	PU	CA	HI	TY	Total Amine
1	<2.00	5.50	6.61	0.18	nd	1.01	1.22	0.49	0.53	3.43
2	<2.00	3.00	5.30	0.42	nd	0.52	0.92	0.77	0.81	3.44
3	<2.00	3.00	6.32	0.17	nd	0.17	2.17	0.36	0.51	3.38
4	<2.00	5.58	6.45	nd	nd	0.18	0.30	0.89	0.24	1.61
5	<2.00	5.34	5.97	0.11	nd	0.81	1.15	0.36	0.59	3.02
6	<2.00	3.00	4.00	nd	nd	0.26	0.29	0.96	0.12	1.63
7	4.20	5.34	6.18	0.32	nd	0.23	0.23	0.49	0.46	1.73
8	3.04	5.85	6.32	0.37	nd	0.56	0.92	0.64	0.68	3.17
9	3.11	5.90	7.81	nd	nd	0.12	0.07	0.14	0.25	0.58
10	4.49	7.08	8.20	nd	nd	0.04	0.08	nd	0.35	0.47
11	<2.00	5.48	5.63	nd	nd	0.05	0.03	0.19	0.14	0.41
12	<2.00	6.58	7.63	nd	nd	0.05	0.02	0.06	0.54	0.67
13	4.23	6.15	7.57	0.40	0.79	0.10	0.47	nd	nd	1.76
14	<2.00	5.59	7.18	nd	nd	nd	nd	nd	nd	nd
15	<2.00	5.26	5.85	0.60	0.20	0.18	0.11	0.20	1.15	2.44
16	2.30	4.20	7.18	0.58	0.27	0.09	0.44	0.11	1.04	2.53
17	<2.00	4.08	5.52	nd	nd	0.17	0.15	0.27	1.16	1.75
18	<2.00	4.43	4.70	0.65	0.36	0.21	0.12	0.18	1.38	2.90
19	2.30	3.30	6.59	0.31	nd	0.04	0.05	0.32	0.44	1.16
20	<2.00	4.36	5.04	nd	nd	0.11	0.13	0.08	0.76	1.08
Min	**<2.00**	**3.00**	**4.00**	**0.11**	**0.20**	**0.04**	**0.02**	**0.06**	**0.12**	**0.41**
Max	**4.49**	**7.08**	**8.20**	**0.65**	**0.79**	**1.01**	**2.17**	**0.96**	**1.38**	**3.44**
Mean	**2.48±0.19**	**4.95±0.27**	**6.30±0.25**	**0.21±0.05**	**0.08±0.04**	**0.25±0.06**	**0.44±0.13**	**0.33±0.07**	**0.56±0.09**	**1.86±0.25**

nd: *not determined,* ***LAB:*** *Lactic acid bacteria,* ***TAMB:*** *Total Aerobic Mezophylic Bacteria,* ***TR:*** *Tryptamine,* ***PHE:*** *Phenyl ethylamine,* ***PU:*** *Putrecine,* ***CA:*** *Cadaverine,* ***HI:*** *Histamine,* ***TY:*** *Tyramine*

DISCUSSION

The pH, total solids, fat- in-dry matter, salt- in- dry matter, protein, WSN and ripening coefficient in Urfa cheese are in accordance with the results reported by other researchers [9,11,29, 30]. These results indicated that Urfa cheese has similar properties as full fat, white-brined cheese [11]. However the salt-in dry matter is higher than in white-brined cheese. In traditional Urfa cheese production, producers prefer very high brine concentrations in order to provide microbial safety [11]. Therefore Urfa cheese has a very high salt concentration. Yalçın et al.[29], detected the mean of the salt content in dry matter in 30 Urfa cheese as 20.01%. The pH, total solids, fat- in- dry matter, salt-in- dry matter, protein, WSN and ripening coefficient in Urfa cheese results also show similarity with the results of previous studies [18,31,32]. Both Urfa and Van herby cheeses have a wide range of chemical properties. As a result of de-moisturing and/or biochemical changes during ripening, the compositional properties of cheeses were changed. In this regard, a significant difference between the chemical properties of fresh and ripened Van herby cheeses was found by Tarakçı et al.[18]. Another possible reason could be that a standard manufacturing procedure is not used by producers, since these cheeses are generally produced traditionally. İşleyici and Akyüz [31] reported that the microbial flora and chemical properties of Van herby cheese can vary due to the following reasons; production from different raw milk, post contamination after pasteurization, using different ripening techniques and conditions. In addition, Erkan et al.[33] reported that the microbiological properties of the herbs have not been investigated; therefore herbs could be one of the contamination points in herbed cheeses.

The mean value of *Enterobacteriaceae*, LAB, and TAMB determined from 20 Urfa cheese samples were found as 4.12±0.35 log cfu.g^{-1}, 6.64±0.21 log cfu.g^{-1}, 7.35±0.15 log cfu.g^{-1}, respectively. Özer et al.[34] and Yetişmeyen, Yıldız [30] found a higher TAMB count in Urfa cheese (6.1×10^9 cfu.g^{-1}and 1.0×10^9 cfu.g^{-1}, respetively). Atasoy et al.[35] reported that TAMB in Urfa cheese varied from 2.33×10^6 and 2.22×10^7 cfu.g^{-1}. Of twenty Urfa cheese samples, five cheeses were found not to contain *Enterobacterieceae* (<100 cfu.g^{-1}).

The mean value of *Enterobacteriaceae*, LAB and TAMB determined from 20 Van herby cheese samples were found as 2.48±0.19 log cfu.g^{-1}, 4.95±0.27 log cfu.g^{-1}, and 6.30±0.25 log cfu.g^{-1}, respectively. The TAMB counts for Van herby cheese were found to be lower than those obtained by Kurt and Akyüz [36], Coşkun and Öztürk [14] and İşleyici and Akyüz [31] (9.7×10^8 cfu.g^{-1}, 7.14 $\log_{10}$ cfu g^{-1}and 7.82 log cfu g^{-1}, respectively) but higher than that measured by Yetişmeyen et al.[32]. *Enterobacteriaceae* were not found in 13 of the samples of Van herby cheeses (<100 cfu. g^{-1}), and higher levels were detected in the rest of the samples.

SM and SD were not detected in the Urfa cheese samples. Similarly, Durlu-Özkaya [3] found that SM content was absent in some Turkish cheeses including Urfa and Van herby cheese. In Urfa cheese, TY was detected in 17 samples out of 20 (85%). Also Durlu-Özkaya [3] reported that TY was detected in 89% of cheeses. TY was found to be highest among the biogenic amines (max. 8.80 mg 100 g^{-1}) in Urfa cheeses. However, the TY amount did not exceed the tolerable limits for TY in cheese as reported (200 mg kg^{-1}) by Karovicova & Kohajdova [2]. It was found that TY (85%), PU (75%), PHE (70%), CA (60%) and HI (55%) were the predominant biogenic amines in Urfa cheese. Also TR was detected in 30% of Urfa cheeses. The mean values of TY, PHE and CA in Urfa cheeses were found to be high as 1.80±0.50 mg 100 g^{-1}, 0.36±0.18 mg 100 g^{-1}, and 0.21±0.11 mg 100 g^{-1}, respectively. Elsanhoty et al.[1] reported that the contents of TY, PHE and CA in cheese are associated with the number of *Enterobactericeae*. Also the accumulation of TY has been related to non starter lactic acid bacteria, mainly lactobacilli [5,37]. Durlu-Özkaya and Özkan [38] reported that the production of TY was also related to number of lactococci.

In this study, the data indicate that the number of both *Enterobacteriaceae* and LAB were very high in Urfa cheeses. The formation of these biogenic amines increased along with the high microbial counts of Urfa cheeses. The total amount of biogenic amines in detectable samples was 2.80±0.58 mg 100 g^{-1}. The biogenic amine content of Urfa cheeses is lower than that of the other studies reported in Turkey. In addition, the content of individual biogenic amines was in accordance with the levels obtained from Feta cheese [39]. But, on the contrary, it was lower than those reported elsewhere in various cheeses, like Domiati cheese [1], Manchego cheese [1,40]. Although the spices and herbs used in the manufacture of Van herby cheese have an antimicrobial effect, their own microflora must be taken into consideration [41]. It is reported that a positive correlation exists between biogenic amines, especially the content of CA, and the number of *Enterobacteriaceae* in cheese. *Enterobacteriaceae* may result in an increase in decarboxylase content [42]. In Van herby cheese, CA (95%), and PU (95%), TY (90%), HI (85%), TR (55%) were the predominant biogenic amines. However, PHE amount was detected fairly low (mean 0.08±0.04 mg 100 g^{-1}) in 20% of samples. Although the content of TY was found to be the highest with 0.56±0.04 mg 100 g^{-1}, none of biogenic amines exceed the tolerable limit. Toxic doses were given as 10 mg 100 g^{-1} for HI, 80 mg 100 g^{-1} for TY and 3 mg 100 g^{-1} for PHE, depending on the immune system of individuals [43]. However, in the study performed by Durlu-Özkaya [3], PHE was not detected in Van herby cheese and the amounts of other biogenic amines detected were found to be higher than those in this research. The higher content of CA, PU and HI in cheese batches from raw milk could be explained by the higher *Enterobacteriaceae* and lactobacilli counts found in those batches. These amines are commonly

associated with *Enterobacteriaceae* [37]. However, in this study, although *Enterobacteriaceae* and lactobacilli counts in Urfa cheese were approximately twice as high as those of Van herby cheese. CA, PU and HI contents in Van herby cheese were found to be higher.

Taking into account all of the chemical and microbiological results from Urfa and Van herby cheeses, there is a clear necessity for standardization in their production as seen by the large range of the cheese properties. In addition, the cheeses should be produced from pasteurized milk in order to reduce the bacteriological load. Since these types of cheeses are generally produced from raw milk, a high microbiological load results in formation of biogenic amines. Notably, the total biogenic amine content of Urfa cheese, which had high microbiological counts, was higher than the total biogenic amine content of Van herby cheese. However, generally the low levels of biogenic amine content in both type of cheeses could be attributed the low ripening index of the cheeses.

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