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Determination of Biogenic Amines in Some Cheese Consumed in Hatay Region

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Abstract

Biogenic amines are biologically active, nitrogen based, low molecular mass substances and they consider as quality and toxicity indicators of foods. The amount of biogenic amines indicates also the freshness of the food products. These compounds form during production or storage of protein rich foods under unhygienic conditions. These amines are usually formed in protein rich foods by the act of microorganisms which has decarboxylase activity under inappropriate technological processing conditions especially while fermentation processes. Cheese, vogurt, kefir are among the most consumed fermented foods. Since these products are much consumed food products, the analysis of biogenic amines in them is very important in terms of consumer health and product quality. Biogenic amines taken in high amounts are known to cause headache, nausea and more serious food poisoning. There are studies both abroad and in our country on cheese varieties where biogenic amine formation is frequently observed. However, there is no study conducted in the Hatay region, which is extremely rich in terms of cheese varieties. Within the scope of this study, biogenic amine analyzes were performed on 20 cheese samples produced and consumed in the Hatay region after they were kept in the refrigerator at 4 °C for 30 days. Total biogenic amine concentrations were determined between 57.00–432.41 mg kg⁻¹. The samples with the highest total biogenic amine concentrations were blue-veined cheese (432.41 mg kg⁻¹) and moldy cheese (314.45 mg kg⁻¹). Total biogenic amine concentrations were the lowest in aged cheddar cheese (57.00 mg kg⁻¹) and knitted cheese (97.57 mg kg-1). All of the biogenic amines (spermidine, putrescine, cadaverine, histamine, tryptamine, tyramine, phenylethyl amine) were detected in all cheese samples examined.

Keywords:

Biogenic amine, cheese, spermidine, cadaverine, phenylethyl amine

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Introduction

Biogenic amines have many important functions in the body. They participate in the synthesis of proteins, hormones and nucleic acids. They support normal cell growth and proliferation. They ensure proper maintenance of blood pressure and body temperature (Naila et al., 2010). They are effective against aging and stress. They have an effect on membrane stability (Nuñez et al., 2016). However, they can cause toxic effects when taken in large quantities. Therefore, exogenous biogenic amines are considered to be anti-nutritional factors responsible for food poisoning, headaches, cold sweats or pseudo-allergic reactions (Ruiz-Capillas & Herrero, 2019). The high biogenic amine content is due to the presence of bacterial strains resulting from poor hygiene or lack of food safety. For this reason, it is extremely important to determine the amount of biogenic amines in foods. The body's natural defense mechanism tries to counteract the effect of excess biogenic amine through enzymes such as monoamine oxidase, diamine oxidase and polyamine oxidase. However, this natural mechanism is hindered by many factors such as taking antidepressants, analgesics and drugs used in the treatment of Alzheimer's and Parkinson's diseases, alcohol consumption, immune deficiencies, stomach dysfunction or excessive consumption. In addition, biogenic amines can accumulate in the tissues of the body and cause toxic effects (Özoğul & Hamed, 2018). It is very difficult to give precise limits on the toxicity of biogenic amines. Factors such as the type, amount and amine content of the food consumed and the presence of inhibitors make it difficult to determine the limits for the toxicity of biogenic amines. Biogenic amines over 40 mg taken at a meal are considered potentially toxic. Apart from these, putrescine and cadaverine, which are biogenic amines, can form carcinogenic nitrosamines with nitrous acid added to food products (Gardini et al., 2016).

Biogenic amines are found in various foods and beverages such as meat, fish, sausage, cheese, fermented vegetables, wine, beer, milk. The most common biogenic amines in foods are histamine, tyramine, putrescine, cadaverine, β-phenylethylamine, agmatine, tryptamine, serotonin, spermine and spermidine. Biogenic amine formation is affected by the type of food, microbial flora, storage conditions, and type of packaging (Akan & Demirağ, 2018). Cheese is the most suitable food for the formation of biogenic amines after fish. The most important factors that cause the formation of biogenic amines in cheese are the type and number of bacteria contained in the cheese, the interaction between microorganisms, and the proteolysis level of the cheese, its pH and salt concentration and storage temperature. Amino acids are formed as a result of proteolysis during the ripening of cheese. Sufficient amounts of these amino acids can lead to the formation of toxic biogenic amines. Therefore, as maturation progresses, biogenic amine formation may increase (Diler et al., 2018). Starter culture is widely used in the production of fermented foods. Studies on this subject have shown that the use of starter culture reduces the formation of biogenic amines (Kongkiattikajorn, 2015). Many microorganisms are also thought to be responsible for the formation of biogenic amines. It is known that lactic acid bacteria (Lactobacillus, Pediococcus, Streptococcus), some yeast species (Debaryomyces hansenii, Yarrowia lipolytica, Pichia jadinii, *Geotrichum candidum*), *Streptococcus lactis* and *Lactobacillus helveticus*, which are used as culture bacteria in the dairy industry and cheese, are effective in the formation of biogenic amines (Alvarez et al., 2014). It has been determined that the most suitable pH values in terms of biogenic amine formation are between 4.0 and 5.5, and when the pH drops below four, positive microorganisms cannot grow and therefore biogenic amine formation does not occur (Santos, 1996). Bacteria species are affected by salt concentration in different ways (Akan & Demirağ, 2018). Studies have shown that biogenic amines such as putrescine, cadaverine and tyramine are formed in foods that are stored for a long time at high temperatures (Şahin-Ercan et al., 2019).

In the literature, there are studies to determine the biogenic amine content of various cheeses consumed in different countries such as Greece, Italy, Egypt, Austria, Czech Republic, and Korea (Valsamaki et al., 2000; Schirone et al., 2013; Eleiwa et al., 2013; Bunkova et al., 2013; El-Zahar, 2014; Mayer & Fiecher, 2018; Kandasany et al., 2021). In our country, biogenic amine contents of some local cheeses such as Van herb cheese, Erzurum civil cheese, Urfa cheese, Kars old cheddar cheese, Mihaliç cheese, Erzincan tulum cheese, knitted cheese were investigated (Durlu-Özkaya, 2002; Yetişmeyen, 2005; Andıç et al., 2010; Diler et al., 2018; Şahin-Ercan, 2019). Although Hatay region is very rich in terms of cheese diversity and a large amount of cheese is consumed, there are no studies on this subject. The aim of this study was to determine the biogenic amine contents of 20 kinds of cheese consumed in Hatay region and to examine some chemical quality parameters.

Materials and Method

Reagents and Standards

Biogenic amine standards (spermidine, putrescine, cadaverine, histamine, tryptamine, tyramine, phenylethyl amine) were purchased from Sigma-Aldrich (Gillingham, UK). All chemicals used were of analytical reagent grade and were at least 99.5% pure. All of the cheese samples were obtained from local cheese and dairy shops and markets.

Preparation of Cheese Samples for Analysis

25 g cheese sample was taken and 25 ml of 0.1 M HCl was added to it. The homogenized mixture was centrifuged at 4000 rpm for 20 minutes at 4 °C. The supernatant was removed and 100 μ L of 2 M NaOH, 150 μ L of saturated NaHCO₃ and 1 ml of dansyl chloride were added. The mixture was incubated at 40 °C for 45 minutes. It was then incubated at room temperature for 10 minutes. Then, it was left at room temperature for ten minutes. 50 μ L of 25% NH3 was added and left at room temperature for another 30 minutes. 5 ml of 0.1 M ammonium acetate: acetonitrile (1:1) mixture was added. It was passed through a 0.45 μ m filter and injected into the HPLC system (Köse et al., 2011).

HPLC Analysis

Standard solutions of each biogenic amine (spermidine, putrescine, cadaverine, histamine, tryptamine, tyramine, phenylethyl amine) were prepared between 5-250 mg L^{-1} concentrations. Calibration lines were established using these solutions. In all cases, the correlation coefficients of linear functions were > 0.999.

HPLC analysis was performed on a Shimadzu Prominence CBM 20A (Kyoto, Japan) instrument. The C-18 reverse phase column (5 μ m, 250 x 4.6 mm) was used. A mixture of 0.1 M ammonium acetate (A): acetonitrile (B) was used as the mobile phase. The column temperature was set to 40 °C and the flow rate to 1 ml min⁻¹. A diode array detector (DAD) was used. All analyzes were repeated three times. Gradient elution has been applied and the program is given below (Table 1).

Table 1. Gradient elution program

Time (Min)	A (%)	B (%)
1	80	20
25	50	50
40	20	80

The limit of detection (LOD) measurement was performed on the standard solutions of biogenic amines between 5-250 mg L^{-1} concentrations. Using the ratio signal/noise recorded by the HPLC for the biogenic amine measurement in case of the standard solutions of biogenic amines, the detection limit was calculated. The limit of quantification (LOQ) was taken as three times the LOD. The values of retention time, correlation coefficient, LOD and LOQ of biogenic amines are listed in Table 2.

Table 2. The values of retention time, correlation coefficient, recovery, LOD and LOQ of biogenic amines

Biogenic amines	Retention time (min)	Correlation coefficient (R ²)	LOD (ng ml ⁻¹)	LOQ (ng ml ⁻¹)
Spermidine	12.5	0.9999	0.018	0.059
Putrescine	16.2	0.9999	0.014	0.046
Cadaverine	17.5	0.9998	0.010	0.033
Histamine	20.8	0.9994	0.005	0.016
Tryptamine	25.3	0.9999	0.005	0.016
Tyramine	26.0	0.9998	0.019	0.063
Phenylethylamine	27.2	0.9998	0.950	3.135

Physico-Chemical Analysis in Cheese Samples

Physicochemical analyzes such as dry matter, fat, salt, acidity, pH, protein determination were made in cheese samples. The dry matter content of the cheeses studied was determined by the oven drying method (Y1lmaz et al., 2015). Cheese samples were analyzed using reference chemistry methods for fat (Gerber), crude protein (Kjeldahl), dry matter (oven-drying), acidity (titrimetric) and salt (Mohr). pH was measured by digital pH meter in suspension diluted 1:1 (cheese:water) (AOAC International, 2016).

Results

The biogenic amines and their amounts detected in the analyzed cheese samples after they were kept in the refrigerator at 4 °C for thirty days are shown in Table 3. The average spermidine concentrations in the examined cheese samples ranged from 6.19 to 101.55 mg kg⁻¹, while the highest values were found in blue-veined cheese (101.55 mg kg⁻¹) and Edam cheese (47.21 mg kg⁻¹) ¹). It was observed that the average putrescine concentrations ranged from 0.84 to 25.96 mg kg⁻¹, the highest putrescine concentrations were found in blue-veined cheese (25.96 mg kg⁻¹) and vegan cheese (14.22 mg kg⁻¹), and the results obtained for the other samples were close to each other. Average cadaverine concentrations were found to be between 17.66–157.81 mg kg⁻¹, with the highest values again for blue-veined cheese (157.81 mg kg⁻¹) and Edam (112.95 mg kg⁻¹), parmesan (112.88 mg kg⁻¹) and vegan (112.76 mg kg⁻¹) cheese. Mean histamine concentrations were found between 2.57–13.46 mg kg⁻¹ and the highest histamine concentrations were found in blue-veined cheese (13.46 mg kg⁻¹) and vegan cheese (5.14 mg kg⁻¹). The values obtained for the other samples were found to be close to each other. Average tryptamine concentrations were determined between 1.36–49.55 mg kg⁻¹, while the highest amounts were found in creep cheese (49.55 mg kg⁻¹) and cubed cheese (31.64 mg kg⁻¹). Average tyramine concentrations were between 0.68–8.42 mg kg⁻¹, and the highest tyramine concentrations were found in Cara cheese (8.42 mg kg⁻¹) and stick cheese (8.34 mg kg⁻¹). Average phenylethyl amine concentrations were determined between 9.25 and 229.89 mg kg⁻¹, and the highest concentrations were found in moldy cheese (229.89 mg kg⁻¹) and stick cheese (170.82 mg kg⁻¹). Total biogenic amine concentrations were determined between 57.00 and 432.41 mg kg-1. The samples with the highest total biogenic amine concentrations were blueveined cheese (432.41 mg kg⁻¹) and moldy cheese (314.45 mg kg⁻¹). The samples with the highest total biogenic amine concentrations were blue-veined cheese (432.41 mg kg⁻¹) and moldy cheese (314.45 mg kg⁻¹). All of the biogenic amines were detected in all cheese samples examined. While the biogenic amines with the highest percentages in the cheese samples were determined as cadaverine and phenylethyl amine, the ones with the lowest percentages were determined as putrescine, histamine and tyramine.

Sample	Spermidine	Putrescine	Cadaverine	Histamine	Tryptamine	Tyramine	Phenylethyl amine	Total
Cottage cheese	8.57 ± 0.02	4.92 ± 0.01	90.54 ± 0.2	2.82 ± 0.004	5.85 ± 0.01	1.15 ± 0.002	9.25 ± 0.02	123.10 ± 0.2
Cubed cheese	7.18 ± 0.02	1.07 ± 0.002	87.35 ± 0.1	4.08 ± 0.01	31.64 ± 0.06	3.36 ± 0.005	85.24 ± 0.1	219.92 ± 0.1
Cara cheese	6.68 ± 0.01	0.89 ± 0.001	109.90 ± 0.2	4.74 ± 0.01	11.82 ± 0.02	8.42 ± 0.02	145.68 ± 0.2	288.13 ± 0.3
Creep cheese	8.17 ± 0.02	6.16 ± 0.01	92.37 ± 0.2	3.74 ± 0.006	49.55 ± 0.08	5.91 ± 0.01	46.63 ± 0.08	212.53 ± 0.2
Spiced skim- milk cheese	6.19 ± 0.01	0.91 ± 0.001	94.16 ± 0.2	2.57 ± 0.004	1.36 ± 0.002	2.80 ± 0.004	69.22 ± 0.1	177.21 ± 0.2
Moldy cheese	14.04 ± 0.03	4.09 ± 0.01	57.15 ± 0.08	2.73 ± 0.004	4.20 ± 0.01	2.35 ± 0.004	229.89 ± 0.4	314.45 ± 0.4
Knitting cheese	6.93 ± 0.01	1.11 ± 0.002	57.75 ± 0.08	2.70 ± 0.004	4.86 ± 0.01	1.40 ± 0.002	22.82 ± 0.04	97.57 ± 0.09
Stick cheese	14.42 ± 0.03	1.43 ± 0.002	75.03 ± 0.09	3.50 ± 0.006	4.26 ± 0.01	8.34 ± 0.02	170.82 ± 0.3	277.80 ± 0.3
String cheese	6.39 ± 0.01	0.84 ± 0.001	90.97 ± 0.2	3.12 ± 0.005	13.33 ± 0.02	1.37 ± 0.002	33.32 ± 0.06	149.34 ± 0.2
Old cheddar cheese	10.70 ± 0.02	1.12 ± 0.002	17.66 ± 0.03	3.53 ± 0.006	4.38 ± 0.01	0.68 ± 0.001	18.93 ± 0.03	57.00 ± 0.05
Fresh cheddar cheese	8.76 ± 0.02	0.92 ± 0.001	68.81 ± 0.1	3.23 ± 0.005	5.16 ± 0.01	1.23 ± 0.002	18.90 ± 0.03	107.01 ± 0.1
Full fat feta cheese	6.56 ± 0.01	0.95 ± 0.001	105.99 ± 0.2	2.67 ± 0.004	3.29 ± 0.005	3.08 ± 0.005	74.94 ± 0.1	197.48 ± 0.2
Semi-skimmed feta cheese	7.29 ± 0.01	1.01 ± 0.002	49.19 ± 0.08	3.51 ± 0.006	6.64 ± 0.01	1.52 ± 0.002	80.98 ± 0.2	150.14 ± 0.2
Parmesan cheese	7.72 ± 0.01	5.96 ± 0.01	112.88 ± 0.2	3.41 ± 0.005	5.05 ± 0.01	3.11 ± 0.005	72.81 ± 0.1	210.94 ± 0.2
Cheese with yoghurt	7.05 ± 0.01	1.14 ± 0.002	90.46 ± 0.2	2.94 ± 0.004	4.55 ± 0.01	5.89 ± 0.01	21.72 ± 0.04	133.75 ± 0.2
Gouda cheese with cumin	9.61 ± 0.02	1.62 ± 0.002	103.36 ± 0.2	3.60 ± 0.006	3.72 ± 0.006	5.85 ± 0.01	93.29 ± 0.2	221.05 ± 0.3
Gouda cheese	7.92 ± 0.01	1.70 ± 0.002	84.40 ± 0.1	3.97 ± 0.006	3.10 ± 0.004	2.71 ± 0.004	53.98 ± 0.09	157.78 ± 0.1
Edam cheese	47.21 ± 0.08	2.82 ± 0.004	112.95 ± 0.2	3.04 ± 0.005	2.76 ± 0.004	2.69 ± 0.004	58.04 ± 0.1	229.51 ± 0.2
Blue-veined cheese	101.55 ± 0.2	25.96 ± 0.05	157.81 ± 0.3	13.46 ± 0.03	4.42 ± 0.01	2.02 ± 0.004	127.19 ± 0.2	432.41 ± 0.4
Vegan cheese	16.11 ± 0.04	14.22 ± 0.03	112.76 ± 0.2	5.14 ± 0.01	8.62 ± 0.02	5.20 ± 0.01	103.30 ± 0.2	268.35 ± 0.3

Table 3. The biogenic amines and their amounts detected in the analyzed cheese samples (N=3) (mg kg-1)

The results obtained from the physico-chemical analyzes performed on cheese samples are given in Table 4. The percentages of total dry matter in the analyzed samples were found to be between 35.65% and 69.37%. While the fat content of the cheeses was determined between 8.4% and 30.3%, the salt ratios were determined between 1.4% and 6.78%. The lowest pH value was found as 3.94 and the highest pH value as 4.93 in the cheese samples examined. According to Turkish Food Codex standards, the acidity value in terms of lactic acid in cheese should not exceed 3%. The percentage lactic acid value did not exceed 3% in any of the cheese samples examined, and the values obtained were between 0.78% and 2.88%. Protein values were determined between 17.56% and 52.57%. The differences in the obtained values are due to the differences in the chemical compositions and fat amounts of the milk used in cheese making, the differences in the milk is obtained.

	Total Dry Matter (%)	Fat (%)	Salt (%)	рН	Lactic Acid (%)	Protein (%)
Cottage cheese	44.22 ± 0.06	21.0 ± 0.03	3.32 ± 0.01	4.70 ± 0.01	0.95 ± 0.01	29.94 ± 0.04
Cubed cheese	40.04 ± 0.06	19.2 ± 0.02	5.62 ± 0.01	4.05 ± 0.01	2.32 ± 0.01	30.12 ± 0.04
Cara cheese	54.57 ± 0.08	19.0 ± 0.02	5.55 ± 0.01	4.16 ± 0.01	2.24 ± 0.01	28.88 ± 0.04
Creep cheese	42.84 ± 0.06	22.0 ± 0.03	1.40 ± 0.01	4.93 ± 0.01	0.78 ± 0.01	26.36 ± 0.04
Spiced skim-milk cheese	36.24 ± 0.05	8.4 ± 0.01	4.60 ± 0.01	4.68 ± 0.01	2.32 ± 0.01	24.47 ± 0.03
Moldy cheese	54.32 ± 0.08	8.5 ± 0.01	4.22 ± 0.01	4.59 ± 0.01	2.36 ± 0.01	52.57 ± 0.07
Knitting cheese	60.92 ± 0.10	16.1 ± 0.02	3.27 ± 0.01	4.47 ± 0.01	1.73 ± 0.01	32.47 ± 0.05
Stick cheese	$35.88{\pm}0.05$	14.2 ± 0.02	6.78 ± 0.01	4.40 ± 0.01	2.48 ± 0.01	26.39 ± 0.04
String cheese	35.65 ± 0.05	16.0 ± 0.02	4.21 ± 0.01	4.31 ± 0.01	2.06 ± 0.01	28.07 ± 0.04
Old cheddar cheese	$46.41{\pm}0.07$	30.3 ± 0.04	5.38 ± 0.01	4.70 ± 0.01	2.60 ± 0.01	26.92 ± 0.04
Fresh cheddar cheese	42.58 ± 0.06	15.8 ± 0.02	$3.04{\pm}~0.01$	4.66 ± 0.01	2.42 ± 0.01	31.73 ± 0.05
Full fat feta cheese	45.60 ± 0.06	25.4 ± 0.03	4.45 ± 0.01	4.82 ± 0.01	2.66 ± 0.01	31.08 ± 0.04
Semi-skimmed feta cheese	37.94 ± 0.05	18.2 ± 0.02	4.02 ± 0.01	4.54 ± 0.01	2.48 ± 0.01	32.99 ± 0.05
Parmesan cheese	69.37 ± 0.10	29.0 ± 0.04	3.74 ± 0.01	4.46 ± 0.01	1.58 ± 0.01	45.78 ± 0.06
Cheese with yoghurt	44.87 ± 0.06	16.4 ± 0.02	3.42 ± 0.01	4.48 ± 0.01	1.53 ± 0.01	27.64 ± 0.04
Gouda cheese with cumin	58.30 ± 0.09	30.0 ± 0.04	2.85 ± 0.01	4.50 ± 0.01	1.87 ± 0.01	39.89 ± 0.06
Gouda cheese	55.07 ± 0.08	28.2 ± 0.04	2.10 ± 0.01	4.24 ± 0.01	1.44 ± 0.01	37.28 ± 0.05
Edam cheese	57.75 ± 0.08	18.1 ± 0.02	2.80 ± 0.01	3.94 ± 0.01	1.15 ± 0.01	37.22 ± 0.05
Blue-veined cheese	47.02 ± 0.07	19.0 ± 0.02	6.55 ± 0.01	4.14 ± 0.01	2.60 ± 0.01	17.56 ± 0.02
Vegan cheese	44.95 ± 0.06	20.0 ± 0.03	6.08 ± 0.01	4.61 ± 0.01	2.88 ± 0.01	28.36 ± 0.04

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Discussion

In the literature, there are many studies on the determination of biogenic amine contents of various cheeses consumed in different countries such as Greece, Italy, Egypt, Austria, Czech Republic, and Korea. In our country, biogenic amine contents of some local cheeses such as Van herb cheese, Erzurum civil cheese, Urfa cheese, Kars old cheddar cheese, Mihaliç cheese, Erzincan tulum cheese, knitted cheese were investigated. Although Hatay is very rich in terms of cheese variety and a large amount of cheese is consumed, there are no studies conducted on this subject.

In studies conducted abroad, 17.68-92.47 mg kg⁻¹ cadaverine, 11.89-22.57 mg kg⁻¹ phenylethyl amine, 9.66-111.18 mg kg⁻¹ histamine, 0-26.20 mg kg⁻¹ tryptamine, 59.34-70.77 mg

kg⁻¹ tyramine, 0-9.85 mg kg⁻¹ spermidine was found in Gouda cheeses (Kandasamy et al., 2021). In our study, the average values we determined in Gouda cheeses were 84.40 mg kg⁻¹ cadaverine, 53.98 mg kg⁻¹ phenylethyl amine, 3.97 mg kg⁻¹ histamine, 3.10 mg kg⁻¹ tryptamine, 2.71 mg kg⁻¹ tyramine, 7.92 mg kg⁻¹ spermidine. It is seen that the cadaverine, tryptamine and spermidine values we determined are similar, the phenylethyl amine values are high, and the histamine and tyramine values are low. Average 4.23 mg/100 g cadaverine, 4.63 mg/100 g histamine, 1.31 mg/100 g tryptamine, 8.30 mg/100 g tyramine, 5.13 mg/100 g putrescine, 23.60 mg/100 g total biogenic amine were determined in hard cheeses sold in Austria (Mayer and Fiechter, 2018). In semi-hard Italian cheeses, an average of 15.56 µg g-1 cadaverine, 9.51 µg g-1 phenylethyl amine, 28.55 µg g^{-1} histamine, 11.85 µg g^{-1} tryptamine, 29.89 µg g^{-1} tyramine, 7.71 µg g^{-1} spermidine, 75.87 µg g^{-1} 1 putrescine were determined (Innocente et al., 2007). The total biogenic amine content in Greek white cheeses was found to be 330 mg kg⁻¹ on average (Valsamaki et al., 2000). The values we determined in our white cheeses of similar structure are 7.29 mg kg-1 spermidine, 1.01 mg kg-1 putrescine, 49.19 mg kg⁻¹ cadaverine, 3.51 mg kg⁻¹ histamine, 6.64 mg kg⁻¹ tryptamine, 1.52 mg kg⁻¹ tyramine, 80.98 mg kg⁻¹ phenylethyl amine, 150.14 mg kg⁻¹ total biogenic amine. When the results are compared, it is seen that our spermidine values are similar, our phenylethyl amine values are high, and all other biogenic amine and total biogenic amine values are low. The total biogenic amine content was determined as 5.10 - 35.40 mg/100 g in blue-veined cheese samples (Mayer et al., 2010), and the result we found is $432.41 \text{ mg kg}^{-1}$.

In studies conducted in our country, 0.18 - 48.96 mg/100 g cadaverine, 0.22 - 0.37 mg/100 g phenylethyl amine, 0.39 - 94.76 mg/100 g histamine, 0.68 - 138.16 mg/100 g tyramine, 2.23 - 37.30 mg/100 g spermidine, 0.09 - 67.43 mg/100 g putrescine, 13.21 - 349.31 mg/100 g total biogenic amine were detected in old cheddar cheeses (Durlu-Özkaya, 2002; Yetişmeyen, 2005). The values determined in the old cheddar cheeses we examined were 17.66 mg kg⁻¹ cadaverine, 18.93 mg kg⁻¹ phenylethyl amine, 3.53 mg kg⁻¹ total biogenic amine. It is seen that all the values we found, except phenylethyl amine, are lower.

The amount of biogenic amines in cheeses varies depending on many factors such as the type of cheese, manufacturing technique, ripening time, time after production, the composition of microorganisms participating in the ripening process and their decarboxylase activity. For this reason, it is natural that the amounts of biogenic amines detected in cheese samples are different.

There is no information about the maximum amount of biogenic amines that can be found in cheese both in the world and in our country. EFSA conducted a qualitative risk assessment of biogenic amines in fermented foods in 2011 and stated that consumption of 25-50 mg of histamine per meal and 600 mg of tyramine per meal in adults did not cause any adverse health effects. According to these data, it is seen that the amounts of histamine and tyramine contained in the cheese samples examined are at levels that will not cause any problems. However, nowadays, when food quality and safety gain importance, it is necessary to determine the amount of biogenic amines that will not pose a health risk when at least foods suitable for the formation of biogenic amines are consumed.

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Author Contributions

S.Ş. and D.J. performed all the experiments and drafted the main manuscript text.

Conflict of Interest

The authors declare that they have no conflict of interest.

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