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Profiles of the Biogenic Amines in Armola Cheese

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ABSTRACT

Armola is a traditional ewe's milk cheese of the Aegean Region of Turkey. It is a mild cheese that can be used as a sauce, as on tomato salad. The aim of this study was to describe the compositional characteristics and profiles of the biogenic amines (BAs) in Armola cheese.Twelve Armola cheese samples were randomly collected from small-scale producers in İzmir Province, Turkey. Armola cheeses were analyzed for BAs (histamine, tyramine, putrescine, cadaverine, tryptamine, phenylethylamine) pH, dry matter, fat, salt, and titratable acidity. After preparation of samples, BAs content was determined by the HPLC method.The results of our chemical analyses indicated that the cheese contained 40.0% mean dry matter, 17.38% fat, 3.23% salt, with an acidity of 36.93°SH and pH 4.50. From the 12 Armola cheese samples used in this study, levels of phenylethylamine were highest at 23.01 ± 3.19 mg/kg, followed by tryptamine (18.21 ± 2.62 mg/kg), putrescine (17.07 ± 1.37 mg/kg), tyramine (16.97 ± 2.9 mg/kg), histamine (6.41 ± 0.48 mg/kg), and cadaverine 0.67 ± 0.26 mg/kg), with tryptamine, tyramine, and putrescine being the most commonly found BA after phenylethylamine. When taken orally, the acute toxicity assessed for some of the BAs was >2000 mg/kg for tyramine and cadaverine, 2000 mg/kg for putrescine, and 600 mg/kg for spermine and spermidine. These results were well below the stated values. Phenylethylamine, tryptamine, putrescine, and tyramine were the predominant BAs in Armola cheese. The total BA content in the cheese samples was below the known toxic limits.

Key words: Cheese, traditional cheese, Armola cheese, chemical properties, biogenic amine

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1. INTRODUCTION

Seferihisar Armola cheese, which is purported to come from the Albanian culture, is a soft cheese unlike other traditional cheese varieties. It has a slightly salty and sour taste, spreadable consistency, and variable color from off white to slightly yellow. In Turkey, cheese is traditionally spread on bread and eaten at breakfast. As an option, olive oil, red pepper, and garlic can be added to a tomato salad and the cheese used as a sauce [1, 2].

Armola cheese is a local variety of traditional cheese that has been produced in villages and small-scale dairy farms for many years in the Seferihisar district in İzmir. During cheese production, separately kneaded Lor (60%), white cheese (20%), and yogurt (20%) are mixed, placed into a bag, and hung to strain [1].

Biogenic amines (BAs) are low molecular–weight organic compounds having the biological characteristics of aliphatic, aromatic, and heterocyclic structures [3, 4]. The control of BAs in foods is mainly to monitor food safety [5,6]; however, it is gaining importance in the ability to monitor food production or assess food quality and freshness.

BAs that are used as indicators of food deterioration are histamine, tyramine, tryptamine, phenylethylamine, cadaverine, and putrescine compounds that are formed as a result of the decarboxylation of the amino acids histidine, tyrosine, tryptophan, phenylalanine, lysine, and arginine, respectively [4, 7]. Fish and meat and their various products, eggs, some types of cheeses, fermented vegetables, soybean products, beer, and wine are among the foods that contain BAs [4].

BA concentrations in foods are affected by hygienic conditions and change during food processing and preservation. Because decarboxylation increases during the microbial decay of food, the presence of BAs is significant in terms of being an indicator of food deterioration. BAs that are significant in terms of food quality are putrescine, cadaverine,

spermine, spermidine, tyramine, tryptamine, histamine, and phenylethylamine [4]. Despite from being natural components of metabolism, a high intake of these amines from foods can cause adverse toxicological effects and jeopardize health [8]; therefore, the BA content in various foods is comprehensively investigated. The symptoms caused by the high intake of BAs, such as glottal fry, hives, nausea, fever, vomiting, sweating, high or low blood pressure, sore throat, thirst, lip swelling, or spots on the body, vary according to individual detoxification mechanisms [7, 9-12].

Histamine and tyramine are the most common BAs to cause adverse toxic effects [4]. It has been reported that >100 mg/kg histamine, 100–800 mg/kg tyramine, and >30 mg/kg phenylethylamine in foods or >2 mg/L of histamine in alcoholic beverages could cause toxic reactions [3]; however, according to Nout [13], Halasz et al. [3], and Karovıcová and Kohajdová [14], 10 mg histamine in a 100-g food sample can cause histamine poisoning; 10–80 mg tyramine can cause "cheese reaction" (6 mg if the patient is receiving monoamine oxidase inhibitor); and 3 mg phenylethylamine can cause a migraine. A ratio of 1000 mg amine/kg food is considered to be a health hazard [15].

Cheese is the most common food for BA contamination. In particular, proteolytic enzymes digest casein during cheese maturation, thereby increasing the amount of free amino acids. BAs are formed as a result of the decarboxylation of these amino acids by bacterial enzymes. The primary BAs in cheeses are histamine, tyramine, tryptamine, putrescine, cadaverine, and phenylethylamine [16].

Cheese is an ideal environment in which microorganisms can synthesize BAs, and the type and amount of BAs depend on various factors, such as the type of cheese, maturation time, and microorganism load. Different types of BAs have been determined in studies conducted on cheeses, and it has been observed that various BAs are present in the cheeses at different levels. The presence of histamine, tyramine, putrescine, and cadaverine in high amounts is presumed to be an indicator that the product has not been produced under the appropriate hygienic conditions but, rather, has been subjected to microbial contamination [4, 17].

Tyramine in food that is ingested in high amounts causes hypertension and heart failure, also known as "cheese reaction with a headache"; phenylethylamine causes hypertension, migraine-like headache, nausea, and vomiting. One study suggested that putrescine and cadaverine form carcinogenic compounds by reacting with nitrites [4].

Although there is no limit of BA content for milk and milk products in the Turkish Food Codex, the limit for water products is 100–200 mg/kg [18]. Studies conducted on milk, yogurt, curd cheese, and fresh cheese have reported that the amount of BAs should not exceed 10 mg/kg and should not exceed 15 mg/kg in other fermented products [19].

Studies have been conducted to determine the BA content in Turkish cheeses [16, 20-25] The technology for the production of Armola cheese [1,2] and its microbiological properties [26] have been studied, but there was no study on BA in Armola cheese.

The purpose of our study was to determine the quality of Armola cheese by considering the cheese's BAs by type and amount and whether their levels pose any risk of toxicity.

2. MATERIALS AND METHODS

Cheese production

In the production of the cheese, separately kneaded Lor (60%), white cheese (20%), and yogurt (20%) are mixed, placed into a bag, and hung to strain. In some villages, the raw milk for the production of the cheese is poured directly into an animal skin or cauldron and salted, and with time, as the acidity increases, the milk coagulates without intervention. As the milk coagulates inside the animal skin, a large proportion of the whey seeps out of the hide; however, if the milk coagulates inside a cauldron, the cheese is packed into an animal skin after the whey has been drained off.

Armola cheese samples

Twelve samples of Armola cheese were purchased from different small-scale producers in İzmir, Turkey, transported to the laboratory, and stored at 4°C for analyses.

Chemical analyses

The pH values of the cheeses were measured using a pH meter (Hanna Instruments Padova, İtaly). Titratable acidity was measured according to the methods of AOAC International [27]. Fat content was determined using the Gerber–Van Gulik method [28]. The total solids content was established using the gravimetric method [29]. Salt content in the form of NaCl was determined using the method of IDF-FIL [30]. All chemicals used for the analyses were of analytical grade and obtained from Merck (Darmstadt, Germany). All analyses were conducted in duplicate.

Biogenic amine determination

High-performance liquid chromatography (HPLC) and the methods of Anlı et al. [31] were used to determine BA content. A 25-g sample of the cheese was taken, and 25 mL 0.1 M hydrogen chloride was added. The homogenized mixture was centrifuged at 4000 rpm at 4ºC for 20 min. The supernatant was removed, and 100 weigh 5 g of the sample into the test tube and homogenize with 25 mL of 0.4 M perchloric acid. Centrifuge for 10 minutes at 3ºC 10000 rpm and filter with a 0.45 filtrem filter. Take 400 µL of the filtered sample and add 400 µL of Na_2CO_3 (2g / 100mL H_2O) and 400 µL of dansyl chloride (10mg / 1mL acetone) and incubate for 30 minutes in a 40ºC water bath. 200 NaL of Na-L-glutamate monohydrate (200 mg / 4 mL H_2O) is added and incubated for 1 hour at the same temperature. After incubation, 1 mL of acetonitrile is added to the mixture and centrifuged at 2500 rpm for 10 min. The supernatant is removed and injected into HPLC. 2 N sodium hydroxide, 150 µL saturated sodium bicarbonate, and 1 mL dansyl chloride were added. The mixture was incubated at 40ºC for 45 min and allowed to stand at room temperature for 10 min,

after which 50 µL 25% ammoniawas added. After allowing to stand at room temperature for another 30 min, 5 mL ammonium acetate: acetonitrile was added and then injected into the HPLC system. The mobile phase was acetonitrile and water. The flow rate was 1.0 mL/min and the column temperature was 40°C. The peaks were detected at 254 nm using the HPLC system with column prodigy ACE5 C-18 (250 x 4.6 mm) and a gradient pump, vacuum degasser, DAD detector, and computer, including the Agilent package program. The quantitative determinations were conducted using the standard internal method with peak heights. BA contents were expressed as mg/kg.

Table -1 Specifications of high-performance liquid chromatography.

Column	ACE5 C-18 (250 x 4.6 mm)
Column temperature	40°C
Flow rate	1 mL/min
Injection volume	50 µL
Recovery values	80%
Detector	DAD (SPD-M20A)
Column furnace	CTO-10ASVp
Pump	LC20 AT
Autosampler	SIL 20ACHT
Mobil phase	A: 0.1 M ammonium acetate: B: acetonitrile

Statistical Analyses

SPSS v. 19 (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. The obtained values are presented as the mean ± standard deviation (SD).

3. RESULTS AND DISCUSSION

The average values of the chemical components in the Armola cheese samples are listed in Table 2, and average values of the BA content are listed in Table 3. From the 12 Armola cheese samples used in this study, levels of 2-phenylethylamine were highest at 23.01 ± 3.19 mg/kg, followed by tryptamine (18.21 ± 2.62 mg/kg), putrescine (17.07 ± 1.37 mg/kg), tyramine (16.97 ± 2.9 mg/kg), histamine (6.41 ± 0.48 mg/kg), and cadaverine 0.67 ± 0.26 mg/kg), with tryptamine, tyramine, and putrescine being the most commonly found BA after phenylethylamine (Table 3).

Table -2 Characteristics of Armola cheese

Characteristic	**Mean Value**
Fat (%)	17.38 ± 0.41
Total solids (%)	40.00 ± 0.90
Salt (%)	3.23 ± 0.29
pH	4.50 ± 0.03
Acidity (°SH)	36.93 ± 2.45

Table -3 Biogenic amine contents in Armola cheese.

Biogenic amine	**Mean value (mg/kg)**
Tyramine	16.97 ± 2.9
Putrescine	17.07 ± 1.37
Cadaverine	0.67 ± 0.26
Histamine	6.41 ± 0.48
Tryptamine	18.21 ± 2.62
Phenylethylamine	23.01 ± 3.19
Total BA	82.34±5.42

When taken orally, the acute toxicity assessed for some of the BAs was >2000 mg/kg for tyramine and cadaverine, 2000 mg/kg for putrescine, and 600 mg/kg for spermine and spermidine. These results were well below the stated values.

Studies on tryptamine have not been found in the literature [20, 21, 32]. In a study conducted on tulum cheese, tryptamine was detected in all of the 20 samples analyzed at values of between 0.32 and 40.44 mg/kg [25]. The results of other studies are similar. High levels of histamine, tyramine, putrescine, and cadaverine in cheeses indicate that the product has been produced under unfavorable conditions and was subject to microbial contamination [17].

In their study on the presence of BAs in various hard, semihard, and soft cheeses, Aygün et al. [33] found 352 mg/kg histamine, 173 mg/kg tyramine, 74 mg/kg putrescine, and 123 mg/kg cadaverine in the hard cheeses. In the semihard cheeses, averages of 34 mg/kg histamine, 78 mg/kg tyramine, 73 mg/kg putrescine, and 15 mg/kg cadaverine were detected. In the soft cheeses, averages of 78 mg/kg histamine, 164 mg/kg tyramine, 179 mg/kg putrescine, and 234 mg/kg cadaverine were found. The results obtained in our study were significantly lower than these values.

According to the results of our study, the highest BA level detected in the cheese samples was 2-phenylethylamine at 23.01 ± 3.19 mg/kg, which was followed tryptamine, putrescine, tyramine, histamine, and cadaverine. Phenylethylamine

at 23.01 mg/kg, putrescine at 17.07 mg/kg, and tyramine at 18.21 mg/kg were below toxic levels in the Armola cheese samples. Contrary to expectations, the total BA content in the Armola cheese samples was below the determined limits for toxicity, which is believed to be a positive result.

4. CONCLUSIONS

BAs are small molecular–weight, aliphatic, aromatic, and heterocyclic substances. Histamine, putrescine, cadaverine, tyramine, tryptamine, phenylethylamine, spermine, and spermidine are the essential BAs. The formation of BAs in foods can cause several allergic reactions. It is essential that the BAs in fresh and processed foods be determined because of their potential toxic effects and as indicators of food freshness. High levels of histamine intake can cause food poisoning. For example, the intake of 8–40 mg histamine, 40–100 mg histamine, and >100 mg histamine intake can cause low, moderate, and severe food poisoning, respectively; therefore, the United States, Sweden, Austria, and the Netherlands have set legal limits for BAs (especially, histamine) in food. In Turkey, the only designated limit for histamine is that in fish.

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