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Biogenic amine content of boza: A traditional cereal-based, fermented Turkish beverage

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ABSTRACT

Boza is a fermented beverage made from millet, maize, wheat or rice. Biogenic amine contents of 10 boza samples from different manufacturers in Turkey were analysed for the first time, using HPLC after derivatisation with benzoyl chloride. Of the 11 biogenic amines under study, putrescine, spermidine and tyramine were detected in all boza samples. Tyramine was the prevailing biogenic amine. Tyramine concentrations of boza samples were between 13 and 65 mg/kg. Total biogenic amine contents of boza samples were between 25 and 69 mg/kg. Consequently, consumption of boza might represent a health risk for patients being treated with drugs containing monoamine oxidase inhibitor (MAOI). The pH values of boza samples were in the range from 3.16 to 4.02; total dry matters were from 15.3% to 31.1% (w/w); protein contents were from 0.50% to 0.99% (w/w). No significant correlations were detected between biogenic amine concentrations and pH, protein content and total dry matter content.

1. Introduction

Biogenic amines are basic nitrogenous organic compounds having a recognised activity and which may occur naturally in foods and beverages (Kirschbaum, Rebscher, & Brückner, 2000). They are generated either as a result of endogenous amino acid decarboxylase activity in raw food material or by the growth of decarboxylase-positive microorganisms under conditions favourable to enzyme activity (Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994; Hornero-Méndez & Garrido-Fernández, 1997). Biogenic amines are commonly found in many foodstuffs, particularly in products that involve a ripening or fermentation period, such as cheese, meat products, beer, wine and fish (Stratton, Hutkins, & Taylor, 1991; Slomkowska & Ambroziak, 2002).

Biogenic amines have important metabolic roles in living cells. Polyamines and putrescine are essential for growth, and other amines, like histamine, tyramine and serotonin, are involved in nervous system functions and the control of blood pressure (Lonvaud-Funel, 2001). Although they are needed for many critical functions in humans and animals, high concentrations of these amines can have toxicological effects (Ten Brink, Damink, Joosten & Huist In't Veld, 1990). Consumption of foods containing high amounts of biogenic amines may cause problems such as headaches, nausea, hypotension, hypertension, cardiac palpitation, etc. (Busto, Valero, Guasch, & Borrull, 1994; Romero, Gazquez, Bagur, & Sanchez-Vinas, 2000).

The most frequent foodborne intoxications caused by biogenic amines involve histamine (Bodmer, Imark, & Kneubühl, 1999). Several outbreaks of histamine poisoning have occurred after eating cheese and fish (Silla Santos, 1996). Furthermore, biogenic amines can react with nitrite and produce nitrosamines, many of which are known to be carcinogenic, mutagenic, teratogenic and embryopathic (Glória & Izquierdo-Pulido, 1999).

Under normal conditions biogenic amines from foods are converted to non-toxic products by enzymes of a fairly efficient detoxification system, which exists in the intestinal tract of mammals. Monoamine oxidase, diamine oxidase and histamine-*N*-methyltransferase are the enzymes which play an important role in the detoxification process (Ten Brink et al., 1990). Thus biogenic amines do not prove a health hazard to individuals, unless large amounts are ingested (Hornero-Méndez et al., 1997). However these enzymes are inhibited by ethanol or by the intake of monoamine oxidase inhibitors (MAOIs) in some drugs. In addition biogenic amines may represent a risk for individuals with a genetic deficiency of these enzymes (Lonvaud-Funel, 2001).

The threshold levels for intoxication in humans by biogenic amines are very difficult to establish, because they depend on individual responses and the presence of other amines (Halász et al., 1994; Ten Brink et al., 1990; Yongmei et al., 2007). It has been reported that 40 mg of biogenic amines per meal can be considered potentially toxic. However, not all biogenic amines are equally

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toxic; consequently, histamine, tyramine and β-phenylethylamine are of concern (Chiacchierini, Restuccia, & Vinci, 2006). The presence of 6 mg of tyramine in one or two usual servings is thought to be sufficient to cause a mild adverse event while 10–25 mg will produce a severe adverse event in those using MAOIs. For unmedicated adults, 200–800 mg of dietary tyramine is needed to induce a mild rise in blood pressure (McCabe-Sellers, Staggs, & Bogle, 2006). Ten Brink et al. (1990) reported that 100–800 mg/kg of tyramine in foods is toxic. Nout (1994) proposed acceptable levels for fermented foods of 50–100 mg/kg, 100–800 mg/kg and 30 mg/kg for histamine, tyramine and β-phenylethylamine, respectively, and a total of 100–200 mg/kg. Such levels could be regarded as acceptable also for non-fermented foods. Upper limits of 100 mg histamine/kg in foods and 20 mg histamine/l in alcoholic beverages were reported by Halász et al. (1994). Tailor, Shulman, Walker, Moss, and Gardner (1994) considered that a limit of 10 mg/l of tyramine is acceptable for alcoholic beverages.

Fermented foods and beverages are more susceptible to formation of biogenic amines, since several microorganisms are involved in the fermentation process and the raw materials used contain considerable amounts of proteins (Shalaby, 1996). Boza is a traditional fermented Turkish beverage made by yeast and lactic acid fermentation of millet, maize, wheat or rice semolina/flour, is a highly viscous suspension (Arıcı & Dağlıoğlu, 2002). For the preparation of boza, selected and cleaned cereal or combination of two or more cereals is milled to the size of semolina (300–800 μm) and cooked in an open or steam-jacketed boiler after water addition. The cooked material is transferred into suitable marble vessels for cooling. The cooled material is strained to remove bran, hull and other foreign materials. After sugar (sucrose) addition, the broth is fermented by adding previously fermented boza, sourdough or yoghurt as starter culture. Fermentation is carried out at 30 °C for 24 h. After fermentation, boza is cooled to refrigeration temperature, bottled in 1 kg plastic containers and should be consumed within 3–5 days (Evliya, 1990). In Turkey boza is usually consumed in winter and served with cinnamon and roasted chickpeas. It can be produced both on an artisanal and industrial scale. It is a nutritious beverage because of its lactic acid, protein, carbohydrate, fibre and vitamin content (Morcos, Hegazi, & El-Damhoughy, 1973). Alcohol content of Turkish boza samples was found to be in the range of 0.03–0.39% (w/v) (Köse & Yücel, 2003). According to related national regulations, beverages containing ethyl alcohol less than 5.0 g/l are accepted as non-alcoholic beverages (Anonymous, 1998). Boza-like products are Kenyan busaa, South African kaffir beer, Nigerian ogi, pito, skete, Egyptian bouza and Turkmenistan and Krim busa (Hayta, Alpaslan, & Köse, 2001).

Biogenic amine content of boza has not been studied before. Since it is a fermented beverage containing protein, which might be used by decarboxylase-positive microorganisms, the formation of various biogenic amines might be expected. Bacterial genera that are known to have decarboxylating ability include *Achromobacter*, *Aerobacter*, *Betabacterium*, *Clostridium*, *Escherichia*, *Lactobacillus*, *Proteus*, *Pseudomonas*, *Salmonella*, *Shigella*, *Streptecoccus* and *Pediococcus* (Tailor et al., 1994). Hancioğlu and Karapinar (1997) isolated several lactic acid bacteria, namely *Leuconostoc paramesenteroides, Lactobacillus sanfrancisco, Leuconostoc mesenteroides subsp. mesenteroides, Lactobacillus coryniformis, L. confusus, Leuconostoc mesenteroides subsp. dextranicum, Lactobacillus fermentum* and *Leuconostoc oenos* during fermentation of boza, and the yeasts isolated comprised *Saccharomyces uvarum* and *S. cerevisiae*. As is evident, the microflora of boza includes decarboxylase-positive microorganisms. *Lactobacillus sanfrancisco, Leuconostoc oenos* (Silla Santos, 1996) and *Leuconostoc mesenteroides* (Shalaby, 1996) were mentioned before as biogenic amine-producing bacteria. Consequently, the aim of the present study was to investigate the biogenic amine content of boza.

2. Materials and methods

2.1. Materials

2.1.1. Boza samples

Ten industrially produced and bottled boza samples from different manufacturers were purchased from local markets in different regions of Turkey.

2.1.2. Reagents and standards

Cadaverine dihydrochloride, tyramine, tryptamine, spermine, spermidine trihydrochloride, histamine dihydrochloride and agmatine sulphate were purchased from Sigma, (St. Louis, MO) while putrescine dihydrochloride was from Fluka (Bruchs, Switzerland); ethylamine hydrochloride was from Acros Organics (Geel, Belgium); methylamine hydrochloride, propylamine, isopentylamine, sodium hydroxide, benzoyl chloride, sodium chloride and chloroform were from Merck (Darmstadt, Germany); anhydrous sodium sulphate, methanol (HPLC grade) and diethyl ether (HPLC grade) were from Lab-Scan (Poch S.A.; Gliwice, Poland); sodium acetate.$3H_2O$ and hydrochloric acid were from J.T. Baker (Deventer, The Netherlands). Standard solutions of biogenic amines were prepared by dissolving known amounts in 25 ml of distilled water, and adding 15 μl of chloroform, in order to prevent microbial growth. These standard solutions were stored at 4 °C and contained 1 mg of free base form of biogenic amine in 1 ml. To prepare mixtures of standard biogenic amines, suitable volumes of standard solutions were mixed and diluted to 25 ml with distilled water.

2.2. Methods

2.2.1. Sample preparation

Fifteen grams of boza sample were suspended in 15 ml of 0.2 M hydrochloric acid. The resulting mixture was centrifuged at 2150*g* for 30 min. The aqueous layer was collected and derivatised prior to analysis by HPLC.

2.2.2. Derivatisation procedure

The procedures of Hornero-Méndez et al. (1997), and Özdestan and Üren (2004) were followed with modification. To a 5 ml aliquot of the extract in a glass tube were added 0.5 ml of distilled water, 3 ml of 5 M NaOH solution and 100 μl of benzoyl chloride. The mixture was shaken for 1 min using a vortex mixer and allowed to stand for 60 min at 25 °C. Following the addition of 5 ml of saturated sodium chloride solution the resulting derivatives were extracted three times with 5 ml aliquots of diethyl ether in a separating funnel. The upper organic phases were combined and dried with anhydrous sodium sulphate, decanted and evaporated under a stream of nitrogen. The residue was dissolved in 1 ml of methanol; 20 μl of the solution were injected into an HPLC. Chromatograms were obtained for two aliquots of the same boza extract that underwent the whole analytical procedure. Quantifications were performed by the standard addition method. For each boza sample two additional 5 ml aliquots of the extract were derivatised in the same way, except that 0.5 ml of mixture of standard biogenic amines was added into 5 ml of boza extract instead of 0.5 ml of distilled water. Quantifications were performed by comparing the peak areas obtained from the sample with those from the standard added sample.

2.2.3. Apparatus

Chromatographic experiments were performed using a Hewlett-Packard 1050 liquid chromatograph (Agilent, Santa Clara, CA) equipped with a Waters 486 variable wavelength UV–vis detector (Waters Corporation, Milford, MN) a gradient elution pump and a

Table 1
HPLC gradient profile for the separation of benzoyl derivatives of biogenic amines

Time (min)	Mobile phase composition (%, v/v)	
	% A	% B
0	90	10
4	70	30
10	70	30
12	60	40
16	40	60
19	30	70
22	0	100
24	0	100
30	90	10

Rheodyne 7125 injection loop of 20 μl (Rheodyne LLC., Rohnert Park, CA). The chromatographic column was Hichrom C_{18} (10 μm particle size, 300 × 3.9 mm ID, Hichrom Ltd., Theale, UK) thermostatted at 20 °C.

2.2.4. Chromatographic conditions

Chromatographic separations were achieved by using a binary gradient elution consisting of methanol and acetate buffer. Mobile phase was prepared as follows: solvent A – 0.05 M acetate buffer:methanol (60:40), solvent B – methanol. The pH of solvent A was adjusted to pH 8 and filtered through a Whatman (42) filter paper. The total separation time was 30 min. The flow rate was 1 ml/min and detection was performed at 254 nm. A new gradient elution programme was developed to obtain reliable peak resolution for boza samples. This HPLC gradient profile is summarised in Table 1.

2.2.5. Determinations of pH, total dry matter and protein content

The pH values of boza samples were measured with a digital pH meter. Total dry matter was determined at 70 °C and under 20–25 mm Hg pressure (Anonymous, 1992). Protein contents of boza samples were quantified according to Kjeldahl method (Anonymous, 1995).

2.2.6. Statistical analysis

Correlations between biogenic amine contents and other experimental results were realised by applying Microsoft Excel.

3. Results and discussion

To find the matrix effect of boza on determination of biogenic amines, peak areas for known amounts of biogenic amines added into boza sample were measured. These areas were compared with those for biogenic amines in standard solutions. Peak areas and standard deviations for 1 μg of biogenic amines injected, for boza sample and standard solution, respectively, were as follows: methylamine 176 ± 7, 190 ± 10; ethylamine 115 ± 2, 141 ± 8; propylamine 103 ± 4, 137 ± 6; putrescine 164 ± 30, 216 ± 22; cadaverine 55 ± 16, 122 ± 5; tryptamine 51 ± 6, 92 ± 7; isopentylamine 77 ± 5, 91 ± 6; spermidine 74 ± 8, 140 ± 7; spermine 46 ± 8, 155 ± 27; histamine 45 ± 8, 129 ± 16; tyramine 16 ± 3, 208 ± 33. These values were obtained as 6 replicates for boza samples and 3 replicates for standard solutions. As is seen, matrix effect was found to be considerably different for various biogenic amines. Recovery rate was the highest for methylamine and the lowest for tyramine, 92.6% and 7.7%, respectively. Consequently the standard addition method was used to determine biogenic amine contents of boza samples.

The types and the concentrations of biogenic amines detected in boza samples from different manufacturers are shown in Table 2. The chromatogram for standard biogenic amines and that for a boza sample are given in Fig. 1 and Fig. 2, respectively. pH, total dry matter content and protein content of each boza sample are shown in Table 3.

Table 2 shows that each kind of boza contained at least three biogenic amines. Putrescine, spermidine and tyramine were detected in all samples. Propylamine was not detected in any of the samples. Limits of detection for the individual biogenic amines were: methylamine 0.1 mg/kg, ethylamine 0.3 mg/kg, propylamine 0.2 mg/kg, isopentylamine 0.4 mg/kg, putrescine 0.2 mg/kg, cadaverine 0.3 mg/kg, tryptamine 0.8 mg/kg, spermidine 0.3 mg/kg, spermine 0.4 mg/kg, histamine 0.6 mg/kg and tyramine 1.8 mg/kg. Limits of detection values were calculated in mg biogenic amine/kg of boza, and to give a signal-to-noise ratio of 3. It was not possible to determine agmatine in boza samples due to interfering peaks. Among the 11 biogenic amines that were under study, tyramine was detected at the highest level in all samples. Tyramine contents of samples varied from 13 mg/kg to 65 mg/kg. In general, spermidine originates from raw materials with plant origin. Total biogenic amine contents of boza samples were between 25 mg/kg and 69 mg/kg.

The pH level is an important factor influencing amino acid decarboxylase activity (Silla Santos, 1996). It has been suggested that biogenic amine formation by bacteria is a physiological mechanism to counteract the acid environment (Teodorović, Bunčić, & Smiljanić, 1994). In particular, the amino acid decarboxylase activities were higher when pH was between 4 and 5.5. However, amine formation was found to depend on the growth activity of bacteria rather than the growth conditions (Gardini et al., 2001). pH values

Table 2
Biogenic amine contents (mg/kg) of boza samples

	Boza samples									
	1	2	3	4	5	6	7	8	9	10
Methylamine	ND	0.3 ± 0.1[a]	1.7 ± 0.1	ND	ND	0.2 ± 0.1	0.4 ± 0.1	ND	0.8 ± 0.1	0.2 ± 0.1
Ethylamine	ND	0.8 ± 0.3	5.0 ± 2.0	ND	ND	ND	ND	ND	1.7 ± 1.5	0.6 ± 0.2
Propylamine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Isopentylamine	ND	5.8 ± 1.6	6.2 ± 4.8	1.4 ± 1.0	2.1 ± 0.1	0.9 ± 0.4	1.8 ± 1.2	ND	ND	1.9 ± 0.5
Putrescine	3.4 ± 0.2	1.2 ± 0.2	5.6 ± 0.6	0.6 ± 0.1	0.8 ± 0.1	0.4 ± 0.1	2.8 ± 0.4	0.7 ± 0.2	1.0 ± 0.7	0.7 ± 0.2
Cadaverine	ND	1.1 ± 0.8	1.5 ± 0.8	ND	ND	ND	ND	ND	ND	ND
Tryptamine	ND	ND	3.3 ± 1.1	ND	ND	ND	ND	ND	ND	ND
Spermidine	1.4 ± 0.2	2.7 ± 0.1	3.5 ± 0.3	1.8 ± 0.1	1.2 ± 0.6	0.8 ± 0.2	1.5 ± 0.4	0.6 ± 0.1	2.0 ± 1.0	1.2 ± 0.2
Spermine	ND	3.0 ± 0.5	4.0 ± 0.1	ND	ND	1.0 ± 0.4	ND	ND	1.4 ± 0.9	0.5 ± 0.3
Histamine	N	2.2 ± 0.1	6.5 ± 0.4	ND	1.8 ± 0.8	1.6 ± 0.8	7.9 ± 0.3	ND	2.1 ± 1.8	1.5 ± 0.2
Tyramine	45 ± 26	18 ± 8	23 ± 2	65 ± 6	19 ± 7	55 ± 10	13 ± 4	52 ± 1	33 ± 3	41 ± 17
Total concentration	50	35	60	69	25	60	27	53	42	48

ND: not detected.
[a] Measurement uncertainties were given as standard deviations.

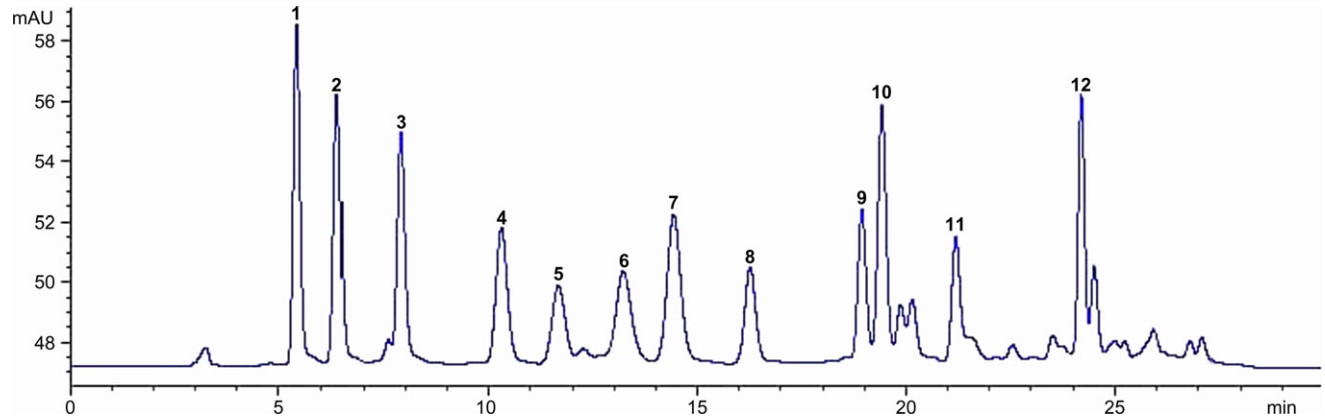

Fig. 1. HPLC chromatogram of standard biogenic amines. Peak identification: 1, methylamine; 2, ethylamine; 3, propylamine; 4, putrescine; 5, cadaverine; 6, tryptamine; 7, isopentylamine; 8, spermidine; 9, spermine; 10, histamine; 11, tyramine; 12, agmatine.

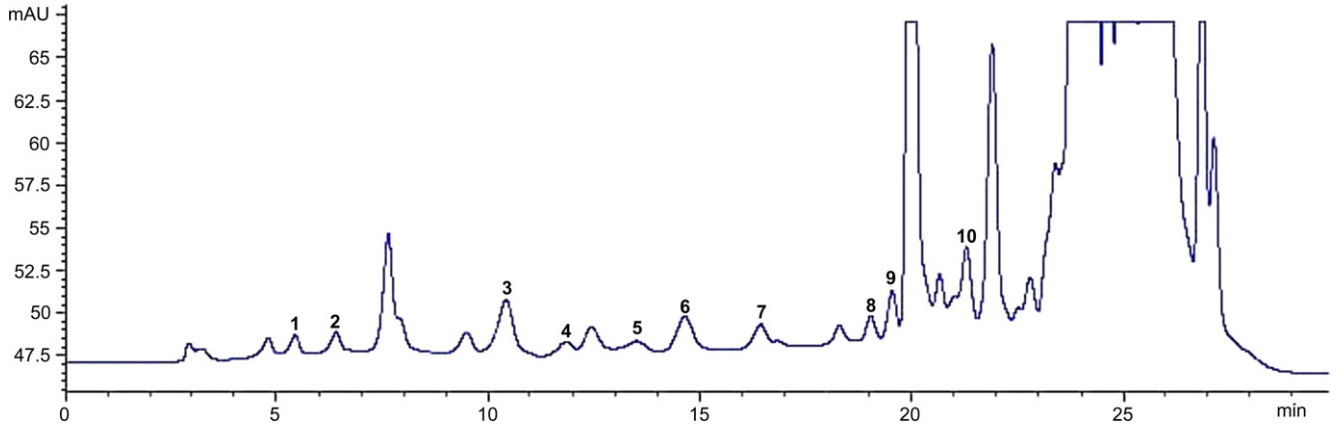

Fig. 2. HPLC chromatogram of a boza sample. Peak identification: 1, methylamine; 2, ethylamine; 3, putrescine; 4, cadaverine; 5, tryptamine; 6, isopentylamine; 7, spermidine; 8, spermine; 9, histamine; 10, tyramine.

Table 3
pH, total dry matter and protein contents of boza samples

	Boza samples									
	1	2	3	4	5	6	7	8	9	10
pH	3.96	4.02	3.38	3.49	3.51	3.16	3.29	3.81	3.54	3.49
% (w/w) total dry matter	16.1 ± 0.1[a]	23.8 ± 0.1	31.1 ± 0.1	20.0 ± 0.2	26.1 ± 0.2	24.4 ± 0.1	15.3 ± 0.1	24.1 ± 0.1	21.2 ± 0.1	23.0 ± 0.1
% (w/w) protein	0.69 ± 0.01	0.63 ± 0.01	0.51 ± 0.01	0.59 ± 0.02	0.50 ± 0.01	0.76 ± 0.03	0.84 ± 0.05	0.99 ± 0.02	0.57 ± 0.09	0.74 ± 0.03

[a] Measurement uncertainties were given as standard deviations.

of boza samples varied from 3.16 to 4.02. Compatible with these suggestions, no significant correlation was found between pH and amount of any of the biogenic amines detected in boza samples.

Since biogenic amine formation could occur by decarboxylation of amino acids, it was thought that the protein content and total dry matter content of boza could effect the biogenic amine formation. The protein contents of boza samples were between 0.50% and 0.99% (w/w); the total dry matter contents varied from 15.3% to 31.1% (w/w). But in this study no significant correlation was found between protein content and biogenic amine concentration, and between total dry matter content and biogenic amine concentration. The production of biogenic amines is an extremely complex phenomenon, depending on several variables such as raw materials, processing conditions, growth kinetics of microorganisms, and their proteolytic and decarboxylase activities, which interact with each others (Gardini et al., 2001). Protein content had a limited effect here, as proteolysis and peptidolysis to give free amino acids are necessary for biogenic amine production. Moreover, the amino acid composition of protein is different in the different cereals used for boza production. Because of all these interaction factors, affecting formation of biogenic amines, it was not possible to find correlations between biogenic amine concentrations and pH, protein content or total dry matter content.

4. Conclusion

The use of the standard addition technique for the quantification of biogenic amines provided reliable results. Using this method, we determined biogenic amine contents of 10 boza samples supplied from different manufacturers in different regions of Turkey. Tyramine was the prevailing biogenic amine in all boza sam-

ples. Considerable differences were observed among boza samples from different manufacturers. However, the concentrations of biogenic amines detected in Turkish boza were below the maximum permissible limits. Maximum tyramine concentration was 65 mg/kg and this value was below the 100–800 mg/kg that was proposed by Ten Brink et al. (1990), and Nout (1994), as an upper limit for tyramine. Maximum total biogenic amine concentration was determined as 69 mg/kg, which was less than the 100–200 mg/kg of legal limit given by Nout (1994). Biogenic amines contents of boza samples were compared with limits recommended for foods other than alcoholic beverages, as ethyl alcohol content of boza is less than 0.5% (w/v). Chiacchierini et al. (2006) reported that 40 mg biogenic amines per meal can be considered potentially toxic. As mentioned before, the presence of 10–25 mg of tyramine in one or two usual servings produces a severe adverse event in those using MAOI drugs (McCabe-Sellers et al., 2006). Consumption of boza is less than 0.5 kg per meal and this consumption level appears to be safe for unmedicated individuals, but patients being treated with MAOI drugs must limit their consumption of boza.

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