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Biogenic Amine Content of Shalgam (Şalgam): A Traditional Lactic Acid Fermented Turkish Beverage

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Shalgam is a traditional Turkish fermented beverage. The biogenic amine contents of 20 shalgam samples from different manufacturers in Turkey were analyzed for the first time, using HPLC after derivatization with benzoyl chloride. Of the 10 biogenic amines under study, putrescine, cadaverine, histamine, and tyramine were detected in all shalgam samples. Putrescine was the prevailing biogenic amine. Putrescine concentrations were between 5.0 and 42.3 mg/L. Total biogenic amine contents were between 26.7 and 134.3 mg/L. Concentrations of biogenic amines were below the maximum permissible limits. pH values of shalgam samples were in the range from 3.15 to 4.25; acidities of shalgam samples were from 0.530% to 1.028% (w/v); total dry matter values were from 2.33% to 3.67% (w/w); total free amino acid contents were from 0.0074% to 0.0318% (w/v). Significant correlations were detected between biogenic amine concentrations and pH values, acidities, total dry matter contents, and total free amino acid contents.

KEYWORDS: Biogenic amines; Shalgam; Turkish beverage; HPLC

1. INTRODUCTION

Biogenic amines formed mainly by bacterial decarboxylation of amino acids are of particular interest, as at elevated concentrations they may cause a health hazard to individuals (*1*). The occurrence of biogenic amines is extremely widespread in a variety of food products, including fish and seafood, meat products, milk products, beer, wine, fermented vegetable products, and fermented soybean products (*2*−*4*). Many factors contribute to the presence and accumulation of biogenic amines in foods, such as availability of free amino acids, pH, water activity, salt content, temperature, bacterial density and synergistic effects between microorganisms (*5*, *6*), and, primarily, the presence of microorganisms possessing amino acid decarboxylase activity, such as *lactobacilli*, *enterococci*, *micrococci*, and many strains of *Enterobacteriaceae* (*7*, *8*). Histamine, tyramine, putrescine, cadaverine, tryptamine, 2-phenylethylamine, spermine, spermidine, and agmatine are the most important biogenic amines occurring in foods.

Low levels of biogenic amines in foods are not considered a serious risk. However, if the amount consumed is high enough or normal routes of amine catabolism are inhibited, various physiological effects may be the consequence, such as hypotension (in the case of histamine, putrescine, cadaverine) or hypertension (as in the case of tyramine), nausea, headache, rash, dizziness, cardiac palpitation, emesis, and even intracerebral hemorrhage, anaphylactic shock syndrome, and death, in very severe cases (*1*, *9*, *10*). Not all biogenic amines are equally toxic; histamine, tyramine, and 2-phenylethylamine are of major concern (*1*). Putrescine and cadaverine also play an important role in food poisoning, as they can potentiate the toxicity of histamine. Secondary amines react with nitrite to form nitrosamines, some of which are known as carcinogenic compounds. On the other hand, spermidine and spermine in foods originate generally from raw materials and are not produced by bacterial decarboxylation of amino acids. Monitoring the biogenic amine levels in foodstuffs and beverages is important not only from the toxicological point of view, but also they can be used as indicators of the degree of freshness or spoilage of foods (*4*).

The maximum allowable levels of biogenic amines in foods are very difficult to establish, because they depend on individual responses and the presence of other amines (*9*). Nout (*11*) proposed acceptable levels for fermented foods of 50−100 mg/kg, 100−800 mg/kg, and 30 mg/kg for histamine, tyramine, and 2-phenylethylamine, respectively, and a total of 100−200 mg/kg. Such levels could be regarded as acceptable also for nonfermented foods. Silla-Santos (*10*) proposed an acceptable level of 1000 mg/kg for total biogenic amine content. Upper limits of 100 mg histamine/kg of foods and 20 mg histamine/L of alcoholic beverages were reported by Halász et al. (*9*).

Alcoholic and nonalcoholic fermented beverages are produced in different parts of the world. Shalgam is a red colored, faintly cloudy and sour soft drink mainly consumed in southern Turkey. However, its consumption is currently increasing in other parts of Turkey, as well. Shalgam is produced on an industrial scale (*12*). Similar products to shalgam are kanji and lactofermented carrot juice (*13*, *14*). According to the Turkish Standards Institution (*15*), shalgam is defined as a beverage produced by lactic acid fermentation of black carrot (*Daucus carota* L.), turnip (*Brassica rapa* L.), salt, bulgur flour, and extract of sourdough. It is a nutritious beverage because of its lactic acid, mineral, anthocyanin, vitamin, and protein content (*12*).

For shalgam production, a standard manufacturing technique is not available, and processing differs from one plant to another.

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However, there are two main processing methods for shalgam production, the traditional method and the direct method. The traditional method consists of two stages: The first stage is called the first fermentation (sourdough fermentation), and the second stage is called the second fermentation (carrot or main fermentation) (*16*). The first fermentation is carried out for the enrichment of lactic acid bacteria and yeasts. Bulgur flour (3%), salt (0.2%), sourdough (0.2%), and an adequate amount of water are mixed. Then, the mixture is left for fermentation at room temperature for 3−5 days. The fermented mixture of bulgur flour and sourdough is extracted with adequate amounts of water three to five times (*16*−*18*). During the first fermentation, the acid content of the mixture considerably increases and consequently pH drops due to the activities of mainly lactic acid bacteria and yeasts being lowered. The extract from the first fermentation, which also includes sourdough flora, helps to give a good start to the main fermentation (*17*, *18*). The extract obtained from the first fermentation is combined with sorted and chopped black carrots (10−20%), salt (1−2%), if available sliced turnip (1−2%), and an adequate amount of water in a tank for the second fermentation. Traditionally, wooden vessels are used for the fermentation, although modern processes use fiberglass, plastic, or stainless steel tanks. Fermentation is naturally carried out for 3−10 days at ambient temperature, which can vary from 10 to 35 °C. During the fermentation, color compounds (anthocyanins) are extracted. Total acidity increases by the action of mainly lactic acid bacteria. At the end of the fermentation, a red colored, cloudy, and sour beverage is obtained (*16*−*18*). Fermented juice can also be seasoned by adding dried and powdered hot paprika. In some applications, pickled hot paprika is added to the mixture before fermentation. Clarification is not applied in shalgam production. Fermented product is marketed in sealed plastic bottles (*12*). It should be consumed within 3 months if stored at refrigeration temperature. The shelf life of shalgam is 1 year when pasteurization is applied.

For the direct method, there is no first fermentation (sourdough fermentation). Black carrots are sorted and cut into pieces. The chopped black carrots (10−20%), salt (1−2%), if available sliced turnip (1−2%), bakers' yeast (*Saccharomyces cerevisiae*) or sourdough (0.2%), and an adequate amount of water are transferred to a tank and allowed to ferment at ambient temperature (10−35 °C) for 3−10 days. Fermented juice is typically marketed in sealed plastic containers. Although there is no microbiological study on shalgam made by the direct method, lactic acid bacteria seem to be the dominant microorganisms in fermentation (*12*).

The microbiology of shalgam is complex and not known in detail. The fermentation is carried out naturally and involves mixed cultures of mainly lactic acid bacteria and yeasts. The fermentation of shalgam depends on microorganisms that are present on the surfaces of raw material and vessels used in the processes and in the sourdough extract (*16*, *17*, *19*). The total microbial load is dominated by lactic acid bacteria with yeast to a lesser extent during the fermentation. *L. plantarum*, *L. arabinosus*, *L. brevis*, and *L. paracasei* spp. were the dominant lactic acid bacteria during the fermentation of shalgam obtained by the traditional method (*19*, *20*). Shalgam producers do not inoculate the fermentation tanks with selected strains of lactic acid bacteria, because there are no commercial cultures available for shalgam fermentation. However, a method of inoculating up to 15% of shalgam from a previous production batch is sometimes used (*16*). The alcohol content of Turkish shalgam samples was found to be less than 0.3% (w/v) (*12*). According to related national regulations, beverages containing ethyl alcohol below the 0.3% are accepted as nonalcoholic beverages (*21*).

The biogenic amine content of shalgam has not been studied before. Since it is a fermented beverage containing free amino acids, which might be used by decarboxylase-positive microorganisms, the formation of various biogenic amines might be expected. Consequently, the aim of the present study was to determine the biogenic amine content of shalgam for the first time.

2. MATERIALS AND METHODS

2.1. Materials. *2.1.1. Samples*. Twenty industrially produced and bottled shalgam samples from different manufacturers were purchased from local markets in different regions of Turkey. All of the shalgam samples were pasteurized and analyzed within the first 2 months of shelf life. Nine of them were peppered.

2.1.2. Reagents. Cadaverine dihydrochloride, tryptamine, 2-phenylethylamine, spermidine trihydrochloride, spermine, histamine dihydrochloride, tyramine, and agmatine sulfate were obtained from Sigma (Steinheim, Germany). Methylamine hydrochloride and 1,7-diaminoheptane (internal standard, IS) were obtained from Merck (Schuchardt, Germany). Putrescine dihydrochloride was obtained from Fluka (Steinheim, Germany). The other reagents (sodium hydroxide, benzoyl chloride, sodium chloride, anhydrous sodium sulfate, cadmium acetate dihydrate, ninhydrin, ethanol, acetic acid, L-leucine, and distilled water) were supplied from Merck (Darmstadt, Germany), hydrochloric acid was from J. T. Baker (Deventer, Holland), methanol, acetonitrile, and diethyl ether (all of them HPLC grade) were from Lab-Scan (Dublin, Ireland), sodium acetate trihydrate was from Riedel (Germany), and phenolphthalein was from Panreac (Barcelona, Spain). The stock solution of biogenic amines contained, in 100 mL of 0.25 M HCl, 20 mg of agmatine, 30 mg of methylamine, putrescine, and cadaverine, 40 mg of tryptamine, 2-phenylethylamine, and spermidine, 50 mg of spermine, 90 mg of histamine, and 400 mg of tyramine. The stock solution was stored in a glass container at 4 °C. A standard solution of biogenic amines was prepared by diluting 4 mL of the stock solution to 25 mL with distilled water, and it was used daily. An internal standard solution was prepared by dissolving 25 mg of 1,7-diaminoheptane in 50 mL of 0.25 M HCl.

2.2. Methods. *2.2.1. Derivatization of Biogenic Amines*. Derivatization was achieved following the method of Özdestan and Üren (*4*). To 1.5 mL of shalgam sample in a glass tube was added 0.5 mL of distilled water, 0.1 mL of internal standard solution, 2.5 mL of 2 M NaOH solution, and 80 μL of benzoyl chloride. The mixture was shaken for 5 min using a vortex mixer, and after the addition of 1.5 mL of acetonitrile, the mixture was allowed to stand for 10 min at 25°C. Following the addition of 1.5 g of solid sodium chloride and vortexing for 1 min, resulting derivatives were extracted 3 times with 4 mL aliquots of diethyl ether. The upper organic phases were combined and dried with anhydrous sodium sulfate, decanted, and evaporated under a constant flow of nitrogen. The solid residue was dissolved in 1 mL of methanol and filtered through a 0.5 μm pore size filter, and 10 μL of the solution was injected into an HPLC column. Chromatograms were obtained for three aliquots of the same shalgam sample that underwent the whole analytical procedure. Quantifications were performed by the standard addition technique. A 1.5 mL portion of shalgam sample was derivatized with the addition of 0.5 mL of standard biogenic amines solution, 0.1 mL of internal standard solution, 2.5 mL of 2 M NaOH solution, and 80 μL of benzoyl chloride.

2.2.2. Chromatographic Conditions. Chromatographic separations of benzoyl derivatives were realized following the method of Yeğin and Üren (*22*) using a binary gradient elution consisting of methanol and acetate buffer. The 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 the solvent was 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.

2.2.3. Determinations of pH, Acidity, Total Dry Matter, and Total Free Amino Acid Content. pH values of shalgam samples were measured with a digital pH meter (*23*). Acidities of shalgam samples were determined with potentiometric titration using a 0.1 M NaOH solution (*24*). Total dry matter was determined with a refractometer (*25*). Total free amino acid contents of shalgam samples were quantified following the method of Folkertsma and Fox (*26*).

2.2.4. Apparatus. Chromatographic separations were performed by using an Agilent 1200 liquid chromatograph (Agilent, Santa Clara, CA) equipped with an Agilent 1200 diode array detector (DAD), a gradient elution pump and vacuum degasser, an autosampler system, and a thermostatted column compartment. The chromatographic column was Hichrom C_{18} (10 μm particle size, 300 mm × 3.9 mm i.d., Hichrom Ltd., Theale, U.K.) thermostatted at 20 °C. An HI 221 Microprocessor pH Meter (Hanna Instruments, Romania) was used for pH measurements. For the determination of total dry matter contents, a RFM 330 BS model refractometer (Bellingham+Stanley, U.K.) was used. Absorbance measurements for total free amino acid analyses were achieved with a Cary 50 UV−vis spectrophotometer (Varian, U.K.).

2.2.5. Statistical Analysis. Throughout the present study, all the experiments were performed in triplicate. Statistical analyses were realized with the SPSS 10.0 statistics package program. The statistical analysis of data was achieved by using one-way analysis of variance (ANOVA), Duncan post-test, paired *t* test, and Pearson correlation test. In all data analyses, a value of $P < 0.05$ was considered as statistically significant.

3. RESULTS AND DISCUSSION

The standard addition method and internal standard method were used together to calculate the biogenic amine contents of shalgam samples. The types and the concentrations of biogenic amines detected in 11 shalgam samples from different manufacturers are shown in **Table 1**. The biogenic amine contents of nine peppered shalgam samples are shown in **Table 2**. The chromatogram for standard biogenic amines and that for a peppered shalgam sample are given in **Figures 1** and **2**, respectively. To examine the method performance and the matrix effect of shalgam, recovery rates of biogenic amine determinations were estimated. Recovery rates varied from 80.3% to 99.8%. 99.8% recovery was found for methylamine, 97.0% for putrescine, 95.4% for cadaverine, 80.3% for tryptamine, 96.0% for 2-phenylethylamine, 93.7% for spermidine, 87.8% for spermine, 98.4% for histamine, 95.6% for tyramine, and 96.8% for agmatine. Detection limits of biogenic amines for the applied method were reported by Özdestan and Üren (*4*) as 0.5 mg/L or less, except for tyramine, which had a limit of detection value of 2.5 mg/L.

As is seen in **Table 1**, putrescine, cadaverine, histamine, and tyramine were detected in all samples. Spermidine was detected in all shalgam samples except one sample. Methylamine was not detected in any of the shalgam samples. The putrescine contents of samples varied from 5.0 mg/L to 33.3 mg/L. The average putrescine concentration of shalgam samples was 17.7 mg/L. The average histamine and tyramine concentrations of shalgam samples were 7.3 mg/L and 15.1 mg/L, respectively. Consequently, among the 10 biogenic amines under study, putrescine was detected at the highest level. The total biogenic amine contents of shalgam samples were between 26.7 mg/L and 134.3 mg/L. The average total biogenic amine concentration of shalgam samples was 70.2 mg/L. Significant differences were detected between individual and total biogenic amine concentrations of shalgam samples ($P < 0.05$). In **Table 1**, different matching letters in a column indicate significant differences. Correlations between biogenic amine contents of shalgam samples were tabulated in **Table 3**. As is seen in this table, statistically significant correlations were obtained between concentrations of various biogenic amines ($P < 0.05$).

Putrescine, cadaverine, histamine, and tyramine were detected in all peppered shalgam samples (**Table 2**). Spermidine was detected in all samples except one sample. Methylamine was not detected except in one sample. Putrescine was the prevailing biogenic amine. The putrescine contents of samples varied from 11.6 mg/L to 42.3 mg/L. The average putrescine concentration of peppered shalgam samples was 26.0 mg/L. The average histamine and tyramine concentrations of peppered shalgam samples were 8.6 mg/L and 11.6 mg/L, respectively. The total biogenic amine contents of peppered shalgam samples were between 42.8 mg/L and 130.3 mg/L. The average total biogenic amine concentration of peppered shalgam samples was 75.6 mg/L. Significant differences were detected between individual and total biogenic amine concentrations of peppered shalgam samples ($P < 0.05$). In **Table 2**, different matching letters in a column mean significant differences. As seen in **Table 3**, statistically significant correlations were obtained between amine contents for some biogenic amines ($P < 0.05$).

Biogenic amine contents of shalgam samples were compared with those of peppered ones by using the paired *t* test. Significant differences were detected between putrescine concentrations, tryptamine concentrations, and agmatine concentrations ($P < 0.05$). Peppered shalgam samples contained higher amounts of putrescine and tryptamine but a lesser amount of agmatine. The average total biogenic amine concentration of peppered shalgam samples was 75.6 mg/L, and this value was greater than 70.2 mg/L for shalgam samples.

The acidities, pH, total dry matter contents, and total free amino acid contents of shalgam samples were determined and tabulated in **Tables 4** and **5**. The pH values of shalgam samples varied from 3.15 to 4.25, and those of peppered ones varied from 3.18 to 3.85. According to the Pearson correlation test, a negative correlation was obtained between putrescine concentrations and the pHs of shalgam samples. Significant negative correlations were also obtained between putrescine concentrations and pHs and between spermidine concentrations and pHs of peppered shalgam samples ($P < 0.05$). Acidities of shalgam samples varied from 0.530% to 1.028% (w/v) as lactic acid. Acidities of peppered shalgam samples varied from 0.566% to 0.999%. Statistically significant correlations were obtained between putrescine concentrations and acidity; between 2-phenylethylamine and acidity; between spermidine and acidity; and between total biogenic amine concentration and acidity of shalgam samples. Significant correlations were also obtained between spermidine concentrations and acidity and between spermine and acidity for peppered shalgam samples ($P < 0.05$). It has been suggested that biogenic amine formation by bacteria is a physiological mechanism to counteract the acid environment. The pH level is an important factor influencing amino acid decarboxylase activity of bacteria. In particular, amino acid decarboxylase activities were higher when pH was between 4 and 5.5. However, biogenic amine formation was found to depend on the growth activity of bacteria rather than the growth conditions (*5*, *10*). In the present study, it was seen explicitly that, during the fermentation of shalgam and peppered shalgam, the pH of the medium decreased and the acidity and concentrations of biogenic amines increased as a result of activities of microorganisms.

Since biogenic amine formation could occur by decarboxylation of amino acids, it was thought that the total dry matter contents and total free amino acid contents of shalgam samples could affect the biogenic amine formation. Total dry matter contents of shalgam samples varied from 2.33% to 3.67% (w/w), and those of peppered shalgam samples varied from 2.50% to 3.50%. According to the results of Pearson correlation test, statistically significant correlations were obtained between putrescine concentrations and total dry matter contents, between cadaverine and total dry matter contents, between 2-phenylethylamine and total dry matter contents, and between total biogenic amine concentrations and total dry matter contents of shalgam samples ($P < 0.05$). Significant correlations were also obtained between spermidine concentrations and total dry matter contents, between spermine and total dry matter contents, and between total biogenic amine concentrations and total dry matter contents of peppered shalgam samples ($P < 0.05$). As is evident, activities of microorganisms and concentrations of biogenic amines were

Table 1. Biogenic Amine Contents (mg/L) and Standard Deviation Values of Shalgam Samples[a]

shalgam samples	methylamine	putrescine	cadaverine	tryptamine	2-phenylethylamine	spermidine	spermine	histamine	tyramine	agmatine	total conc
S1	ND	$33.3^{a} \pm 6.9$	$10.6^{a} \pm 2.9$	$3.1^{c} \pm 1.0$	$2.8^{b} \pm 2.5$	$2.8^{d} \pm 0.3$	$1.4^{b} \pm 1.2$	$9.0^{c} \pm 1.1$	$17.9^{abcd} \pm 7.6$	$3.3^{bc} \pm 3.4$	84.2^{b}
S2	ND	$11.6^{efg} \pm 4.3$	$5.0^{bc} \pm 1.2$	$3.2^{c} \pm 0.5$	$3.0^{b} \pm 0.5$	$17.1^{bc} \pm 10.2$	$19.1^{a} \pm 8.2$	$13.2^{b} \pm 1.5$	$13.6^{bcd} \pm 3.0$	ND	85.8^{b}
S3	ND	$20.9^{bc} \pm 1.1$	$8.5^{ab} \pm 5.6$	$4.5^{b} \pm 0.5$	$3.0^{b} \pm 2.5$	$6.1^{cd} \pm 3.2$	$0.6^{b} \pm 0.3$	$7.3^{cd} \pm 1.8$	$18.6^{abc} \pm 3.7$	$1.6^{bc} \pm 0.9$	71.1^{bcd}
S4	ND	$5.0^{g} \pm 0.8$	$2.3^{c} \pm 0.7$	$3.1^{c} \pm 0.4$	$0.9^{c} \pm 0.5$	$2.0^{d} \pm 0.3$	$1.8^{b} \pm 0.1$	$2.6^{f} \pm 0.9$	$21.8^{ab} \pm 8.3$	ND	39.5^{ef}
S5	ND	$26.5^{bc} \pm 2.0$	$8.7^{ab} \pm 1.0$	$7.0^{a} \pm 1.0$	$11.5^{a} \pm 1.0$	$52.7^{a} \pm 2.0$	ND	$5.8^{d} \pm 1.0$	$22.1^{ab} \pm 2.0$	ND	134.3^{a}
S6	ND	$13.5^{def} \pm 0.8$	$4.4^{bc} \pm 0.2$	$7.2^{a} \pm 0.1$	ND	ND	$3.2^{b} \pm 1.2$	$19.1^{a} \pm 1.1$	$9.2^{cde} \pm 0.9$	ND	56.6^{cde}
S7	ND	$18.0^{cde} \pm 3.5$	$6.0^{abc} \pm 6.4$	ND	ND	$8.2^{bcd} \pm 0.6$	$19.9^{a} \pm 16.5$	$5.3^{de} \pm 1.3$	$15.2^{bcd} \pm 9.7$	$3.7^{b} \pm 1.6$	76.3^{bc}
S8	ND	$20.4^{bcd} \pm 4.6$	$3.1^{c} \pm 0.3$	ND	ND	$9.0^{bcd} \pm 5.4$	$4.2^{b} \pm 1.4$	$7.1^{cd} \pm 1.4$	$8.5^{de} \pm 4.0$	$1.4^{bc} \pm 0.3$	53.7^{de}
S9	ND	$16.2^{cde} \pm 2.1$	$6.0^{abc} \pm 0.4$	ND	ND	$20.9^{b} \pm 18.3$	ND	$3.5^{ef} \pm 0.1$	$10.6^{cde} \pm 4.6$	$14.2^{a} \pm 0.8$	71.4^{bcd}
S10	ND	$6.8^{fg} \pm 1.0$	$1.5^{c} \pm 0.6$	ND	ND	$4.5^{cd} \pm 1.7$	$7.5^{b} \pm 0.6$	$2.7^{f} \pm 0.5$	$2.6^{e} \pm 0.3$	$1.1^{c} \pm 0.3$	26.7^{f}
S11	ND	$22.8^{bc} \pm 7.0$	$2.6^{c} \pm 1.6$	ND	ND	$9.4^{bcd} \pm 8.2$	$4.7^{b} \pm 2.6$	$5.3^{de} \pm 0.8$	$26.4^{a} \pm 4.2$	$2.1^{bc} \pm 1.8$	73.3^{bcd}

[a] ND = not detected. Different matching letters in a column mean significant differences according to Duncan test ($P < 0.05$).

Table 2. Biogenic Amine Contents (mg/L) and Standard Deviation Values of Peppered Shalgam Samples[a]

shalgam samples	methylamine	putrescine	cadaverine	tryptamine	2-phenylethylamine	spermidine	spermine	histamine	tyramine	agmatine	total conc
SP1	$0.8^{a} \pm 0.6$	$42.3^{a} \pm 3.1$	$9.6^{ab} \pm 2.6$	$4.1^{b} \pm 1.8$	$6.8^{a} \pm 6.8$	$9.6^{bc} \pm 8.4$	$5.4^{ab} \pm 4.6$	$10.7^{b} \pm 2.2$	$41.0^{a} \pm 6.6$	ND	130.3^{a}
SP2	ND	$11.6^{b} \pm 1.3$	$6.7^{ab} \pm 3.1$	$4.6^{b} \pm 1.5$	$2.7^{ab} \pm 0.4$	$4.0^{c} \pm 1.5$	$1.0^{b} \pm 1.8$	$5.0^{de} \pm 2.0$	$7.2^{bc} \pm 3.6$	ND	42.8^{b}
SP3	ND	$26.6^{ab} \pm 8.9$	$6.8^{ab} \pm 4.9$	$7.2^{b} \pm 1.3$	$1.9^{b} \pm 0.9$	$6.1^{c} \pm 4.3$	$2.1^{b} \pm 1.3$	$6.7^{cde} \pm 0.7$	$6.3^{bc} \pm 2.8$	$2.2^{b} \pm 0.7$	65.9^{b}
SP4	ND	$13.7^{b} \pm 0.2$	$12.3^{a} \pm 10.2$	$7.4^{b} \pm 0.4$	ND	$7.3^{bc} \pm 4.8$	$3.5^{b} \pm 0.6$	$18.0^{a} \pm 2.7$	$7.6^{bc} \pm 1.8$	$1.0^{c} \pm 0.1$	70.8^{b}
SP5	ND	$13.6^{b} \pm 0.2$	$10.0^{ab} \pm 8.6$	$17.2^{a} \pm 15.8$	$5.1^{ab} \pm 2.5$	$43.3^{a} \pm 5.0$	$9.0^{a} \pm 5.0$	$8.2^{bcd} \pm 3.2$	$3.8^{c} \pm 0.2$	ND	110.2^{a}
SP6	ND	$15.9^{b} \pm 1.8$	$6.8^{ab} \pm 2.4$	$8.6^{ab} \pm 2.1$	ND	ND	$2.8^{b} \pm 0.6$	$6.5^{cde} \pm 0.3$	$8.2^{bc} \pm 2.9$	ND	48.8^{b}
SP7	ND	$36.7^{a} \pm 11.3$	$1.8^{b} \pm 0.5$	ND	ND	$4.5^{c} \pm 1.3$	ND	$9.0^{bc} \pm 0.6$	$10.2^{bc} \pm 0.9$	ND	62.2^{b}
SP8	ND	$35.7^{a} \pm 14.2$	$3.6^{ab} \pm 0.2$	ND	ND	$15.6^{b} \pm 2.0$	$2.8^{b} \pm 0.4$	$9.3^{bc} \pm 2.8$	$8.8^{bc} \pm 0.6$	$0.7^{c} \pm 0.1$	76.5^{b}
SP9	ND	$37.8^{a} \pm 18.2$	$4.9^{ab} \pm 0.4$	ND	ND	$11.1^{bc} \pm 7.1$	ND	$4.4^{e} \pm 0.7$	$10.9^{bc} \pm 1.1$	$4.0^{a} \pm 0.8$	73.1^{b}

[a] ND = not detected. Different matching letters in a column mean significant differences according to Duncan test ($P < 0.05$).

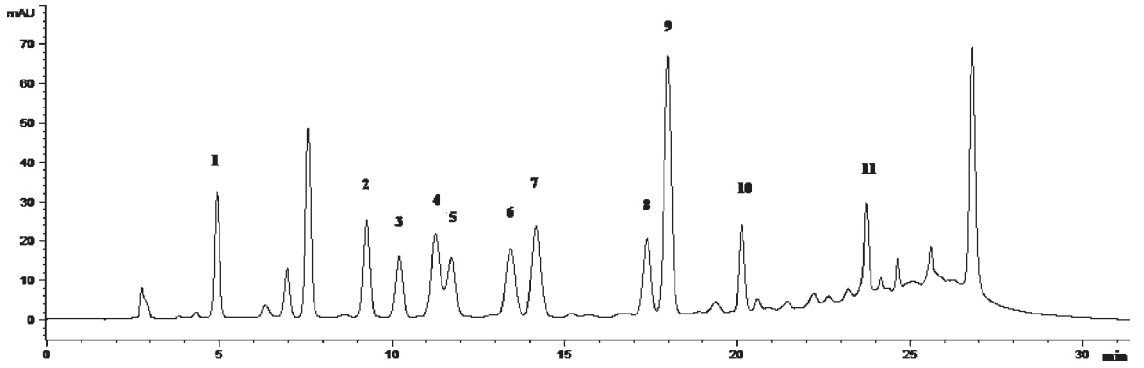

Figure 1. HPLC chromatogram of standard biogenic amines. Peak identification: 1, methylamine; 2, putrescine; 3, cadaverine; 4, tryptamine; 5, 2-phenylethylamine; 6, spermidine; 7, 1,7-diaminoheptane (IS); 8, spermine; 9, histamine; 10, tyramine; 11, agmatine.

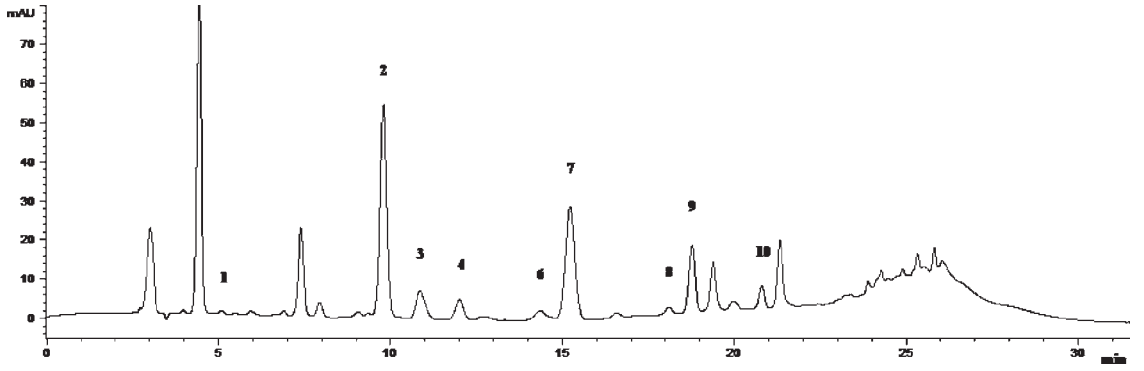

Figure 2. HPLC chromatogram of a peppered shalgam sample. Peak identification: 1, methylamine; 2, putrescine; 3, cadaverine; 4, tryptamine; 6, spermidine; 7, 1,7-diaminoheptane (IS); 8, spermine; 9, histamine; 10, tyramine.

Table 3. Correlations between Biogenic Amine Contents of Shalgam Samples and Biogenic Amine Contents for Peppered Shalgam Samples ($P < 0.05$)[a]

	putrescine	cadaverine	tryptamine	2-phenylethylamine	spermidine	spermine	histamine	tyramine	agmatine	total biogenic amine
putrescine		▲	□	▲	□	□	□	□	□	▲
cadaverine	□		□	▲	□	□	□	□	□	▲
tryptamine	□	■		▲	□	□	▲	□	□	▲
2-phenylethylamine	□	■	■		▲	□	□	▲	□	▲
spermidine	□	□	■	■		□	□	□	□	▲
spermine	□	■	■	■	■		□	□	□	□
histamine	□	□	□	□	□	□		□	□	□
tyramine	■	□	□	□	□	□	□		□	▲
agmatine	□	□	□	□	□	□	□	□		□
total biogenic amine	■	■	■	■	■	■	□	■	□	

[a] ▲ indicates a positive correlation between biogenic amines for shalgam samples. ■ indicates a positive correlation between biogenic amines for peppered shalgam samples. □ indicates no correlation.

higher when medium contained more substrate for lactic acid bacteria and yeasts.

Availability of free amino acids contributes to the presence and accumulation of biogenic amines in foods. Consequently, the total free amino acid contents of shalgam samples were determined and found to be between 0.0077% and 0.0311% (w/v). The total free amino acid contents of peppered shalgam samples varied from 0.0074% to 0.0318%. According to Pearson correlation test, significant correlations were obtained between tryptamine concentrations and total free amino acid contents and betweeen histamine concentrations and total free amino acid contents of shalgam samples. No statistically significant correlations were obtained between biogenic amines and total free amino acid contents of peppered shalgam samples. According to the results of Yongjin et al. (*27*), there was no significant correlation between free amino acids and formation of biogenic amines in fermented silver carp sausages. This result was in agreement with that of Hortos and Garcia-Regueiro (*28*), who reported no correlation between biogenic amines and amino acid contents. However, according to the results of Soufleros et al. (*29*), most of the biogenic amines were negatively correlated with their respective precursor but on the whole positively correlated with amino acids. Yeğin and Üren (*22*) determined the biogenic amine contents of 10 boza samples from different manufacturers in Turkey. Boza is a traditional fermented cereal product, and biogenic amine contents of boza samples are below the biogenic amine concentrations of shalgam samples. No significant correlations were detected between biogenic amine contents of boza and pH, protein contents, and total dry matter contents. 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

Table 4. pH, Acidity, Total Dry Matter, and Total Free Amino Acid Contents and Standard Deviation Values of Shalgam Samples[a]

shalgam samples	pH	acidity (%, w/v) (as lactic acid)	total dry matter (%, w/w)	total free amino acid (%, w/v as leucine)
S1	$3.21^{i} \pm 0$	$0.826^{c} \pm 0.020$	$3.67^{a} \pm 0.06$	$0.0083^{e} \pm 0.0004$
S2	$4.25^{a} \pm 0$	$0.530^{g} \pm 0.009$	$2.33^{i} \pm 0.06$	$0.0149^{c} \pm 0.0002$
S3	$3.31^{f} \pm 0.01$	$0.766^{d} \pm 0.005$	$3.33^{d} \pm 0.06$	$0.0145^{cd} \pm 0.0001$
S4	$3.70^{b} \pm 0.01$	$0.745^{d} \pm 0.027$	$2.70^{g} \pm 0$	$0.0085^{e} \pm 0.0007$
S5	$3.30^{f} \pm 0.01$	$1.028^{a} \pm 0.013$	$3.50^{b} \pm 0$	$0.0127^{cd} \pm 0.0014$
S6	$3.67^{c} \pm 0$	$0.557^{f} \pm 0.007$	$3.00^{f} \pm 0$	$0.0311^{a} \pm 0.0022$
S7	$3.15^{j} \pm 0.01$	$0.981^{b} \pm 0$	$3.40^{c} \pm 0$	$0.0210^{b} \pm 0.0019$
S8	$3.33^{e} \pm 0.01$	$0.825^{c} \pm 0$	$3.00^{f} \pm 0$	$0.0077^{e} \pm 0.0011$
S9	$3.53^{d} \pm 0$	$0.674^{e} \pm 0.003$	$3.10^{e} \pm 0$	$0.0146^{cd} \pm 0.0005$
S10	$3.26^{g} \pm 0$	$0.754^{d} \pm 0.014$	$2.50^{h} \pm 0$	$0.0126^{d} \pm 0.0008$
S11	$3.24^{h} \pm 0.01$	$0.767^{d} \pm 0.025$	$3.00^{f} \pm 0$	$0.0145^{cd} \pm 0.0013$

[a] Different matching letters in a column mean significant differences according to Duncan test ($P < 0.05$).

Table 5. pH, Acidity, Total Dry Matter and Total Free Amino Acid Contents and Standard Deviation Values of Peppered Shalgam Samples[a]

shalgam samples	pH	acidity (%, w/v) (as lactic acid)	total dry matter (%, w/w)	total free amino acid (%, w/v as leucine)
SP1	$3.31^{e} \pm 0.01$	$0.690^{d} \pm 0.023$	$3.30^{b} \pm 0.01$	$0.0229^{b} \pm 0.0016$
SP2	$3.85^{a} \pm 0$	$0.566^{e} \pm 0.003$	$2.50^{e} \pm 0.01$	$0.0106^{d} \pm 0.0005$
SP3	$3.23^{f} \pm 0.02$	$0.858^{b} \pm 0.020$	$3.50^{a} \pm 0.01$	$0.0126^{d} \pm 0.0034$
SP4	$3.71^{b} \pm 0$	$0.731^{c} \pm 0.013$	$3.10^{c} \pm 0.01$	$0.0074^{e} \pm 0.0007$
SP5	$3.21^{g} \pm 0$	$0.999^{a} \pm 0.022$	$3.50^{a} \pm 0.01$	$0.0184^{c} \pm 0.0004$
SP6	$3.64^{c} \pm 0.01$	$0.573^{e} \pm 0.009$	$3.10^{c} \pm 0.01$	$0.0318^{a} \pm 0.0001$
SP7	$3.18^{h} \pm 0$	$0.866^{b} \pm 0.005$	$3.00^{d} \pm 0.10$	$0.0127^{d} \pm 0.0013$
SP8	$3.21^{g} \pm 0.01$	$0.735^{c} \pm 0.013$	$3.10^{c} \pm 0.10$	$0.0118^{d} \pm 0.0018$
SP9	$3.52^{d} \pm 0.01$	$0.667^{d} \pm 0.003$	$3.30^{b} \pm 0.01$	$0.0169^{c} \pm 0.0017$

[a] Different matching letters in a column mean significant differences according to Duncan test ($P < 0.05$).

and decarboxylase activities, which interact with each other (*5*). Because of all these interaction factors, affecting formation of biogenic amines, significant correlations between total free amino acids and some of the biogenic amines were obtained for shalgam samples but not for peppered shalgam samples.

Shalgam is a nutritious beverage, and its consumption is currently increasing. The methylamine, putrescine, cadaverine, tryptamine, 2-phenylethylamine, spermidine, spermine, histamine, tyramine, and agmatine contents of 20 shalgam and peppered shalgam samples supplied from different manufacturers were determined. Putrescine, cadaverine, histamine, and tyramine were detected in all samples. Putrescine was the prevailing biogenic amine. The average putrescine concentrations of shalgam samples and peppered shalgam samples were 17.7 mg/L and 26.0 mg/L, respectively. The maximum putrescine and tyramine concentrations were 42.3 mg/L and 41.0 mg/L, respectively. The total biogenic amine contents of shalgam samples were between 26.7 mg/L and 134.3 mg/L. The average total biogenic amine concentration of shalgam samples was 70.2 mg/L. The total biogenic amine contents of peppered shalgam samples were between 42.8 mg/L and 130.3 mg/L with an average value of 75.6 mg/L. Considerable differences were observed between shalgam samples from different manufacturers. However, the concentrations of biogenic amines detected in shalgam and peppered shalgam were below the maximum permissible limits. Biogenic amine contents of shalgam samples were compared with limits recommended for nonalcoholic foods and beverages, as the ethyl alcohol content of shalgam is less than 0.3% (w/v). Consumption of shalgam is less than 300 mL per meal, and this consumption level appears to be safe for individuals. Putrescine and tyramine are the most abundant biogenic amines for shalgam and peppered shalgam samples. There are significant correlations between total biogenic amine contents and putrescine and tyramine concentrations (**Table 3**). Consequently, putrescine and tyramine together may be used as indicators of total biogenic amines in shalgam and peppered shalgam.

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