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ARTICLE

Various biogenic amines in *Doenjang* and changes in concentration depending on boiling and roasting

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Abstract Biogenic amines are formed by microorganisms during fermentation. Major biogenic amines found in food are histamine, tryptamine, 2-phenylethylamine, putrescine, cadaverine, tyramine, spermidine, and spermine. *Doenjang* is a traditional fermented food made of soybean and is widely used for cooking of various foods in Korea. During fermentation, harmful substances such as biogenic amines could be produced in *Doenjang*. In this study, we examined the types and quantities of biogenic amines in commercial *Doenjang* and analyzed the destructive effects of cooking on biogenic amines in *Doenjang*. Biogenic amines were identified by high-performance liquid chromatography with a fluorescence detector (HPLC-FLD). The concentrations of biogenic amines in commercial *Doenjang* depended on the manufacturer and ranged from none detected to 415.08 mg/kg. Putrescine and tryptamine were the most abundant biogenic amines in *Doenjang* samples, whereas cadaverine was not detected in any commercial samples. For all cooking conditions, tryptamine, 2-phenylethylamine, putrescine, and tyramine were detected in *Doenjang*, and their concentrations decreased significantly after 10 min of roasting. The total concentration of biogenic amines in *Doenjang* soup was not changed significantly by boiling. Therefore, roasting, unlike boiling, can be considered more effective at reducing the amount of biogenic amines in *Doenjang*.

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Introduction

Biogenic amines are reported to have possible toxicity because their high concentration can cause food poisoning [1, 2]. Although some biogenic amines show biological functions at a low concentration, a large amount of biogenic amines can lead to chronic diseases or food poisoning [3, 4]. Furthermore, consumption of certain biogenic amines may be risky because they can be converted to a carcinogen, such as N-nitrosamine [3]. They are also considered spoilage indicators because they can be used to determine the freshness or spoilage of foods [4, 5]. The formation of biogenic amines is primarily a consequence of the enzymatic decarboxylation of specific amino acids due to microbial enzymes or tissue activity. According to Vinci and Antonelli [6], the quantity of biogenic amines is also to be considered as a marker of the level of microbiological contamination in food. Some microorganisms have a specific decarboxylase. For example, most *Enterobacteriaceae* could produce putrescine and cadaverine and a few *Lactobacillus* are reported to have histidine decarboxylase [7]. Also, tyramine production in fermented sausages is related to some species of *Lactobacilli* and *Enterococci* [8].

Major biogenic amines reported in foods are histamine, tryptamine, 2-phenylethylamine, putrescine, cadaverine, tyramine, spermidine, and spermine [9, 10]. Putrescine, spermidine, and spermine are reported to promote tumor growth, whereas agmatine, spermidine, and spermine are known precursors of the carcinogenic nitrosamine [11, 12]. In addition, some biogenic amines, such as cadaverine and putrescine, enhance the toxicity of histamine, which can cause severe hematological diseases [13]. Therefore, it is important to analyze the types and amounts of biogenic amines in foods because of their possible toxicity [4].

Doenjang is a traditional fermented soybean product that has long been used in Korean foods in many ways. Commonly, it has been used as the main ingredient of Korean soup or stew soup with various vegetables and also serves as a sauce in various dishes [14, 15]. Biogenic amines are commonly found in fermented foods because their production is affected by the decarboxylase activity of microorganisms during fermentation [4, 16–19]. During fermentation, free amino acids that can act as precursors of biogenic amines are produced by proteolysis of proteins. Therefore, microorganisms with high proteolytic activity are believed to increase the risk of biogenic amine formation [7, 20]. Types and amounts of biogenic amines in fermented products are affected by the composition of food and the type and growth of microorganisms during processing and storage [21]. Due to their high protein content, soybeans pose a high risk of the presence of biogenic amines. Therefore, it is necessary to monitor the levels of biogenic amines in soybeans and their fermented products [3].

There are studies on analyzing biogenic amines in Doenjang [4, 20], but the change of their contents during cooking has received limited attention in the literature. Therefore, it is important to investigate the change of biogenic amines during cooking. In this study, we aimed to monitor the amount of biogenic amines (histamine, tryptamine, 2-phenylethylamine, putrescine, cadaverine, spermidine, and spermine) in commercial *Doenjang* on the market and to investigate the effects of cooking conditions on the change in biogenic amines in *Doenjang*.

Materials and methods

Chemicals and standards

Histamine dihydrochloride, tryptamine hydrochloride, 2-phenylethylamine, putrescine dihydrochloride, cadaverine dihydrochloride, tyramine hydrochloride, spermidine trihydrochloride, and spermine tetrahydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dansyl chloride was acquired from Sigma-Aldrich Co. (Buchs, Switzerland); and sodium hydroxide, sodium hydrogen carbonate, ammonium hydroxide, and perchloric acid were purchased from Daejung Chemical Co. (Siheung, Korea). HPLC grade solvents such as distilled water, acetone, and acetonitrile were obtained from Tedia Co. (Fairfield, OH, USA).

Sample collection and cooking methods

A total of 14 commercial *Doenjang* samples were purchased in retail markets in Korea. Collected samples were stored at −70 °C after homogenization until analysis. After screening analysis, the sample that contained the highest amounts of biogenic amines was used for further analysis to evaluate the effects of different cooking methods.

Boiling and roasting, the most common household cooking methods used for *Doenjang*, were tested in the study. For boiling, *Doenjang* (10 g) was mixed with water (40 mL), and the final volume was adjusted to 50 mL. Sample solutions were boiled at 96 °C for 10, 20, 30, 40, or 50 min. After heating, the sample solutions were cooled down to room temperature and used for extraction of biogenic amines. For roasting, 30 g of *Doenjang* was evenly spread on a tray 7 × 10 cm^2. The oven (Fujimak Combi Oven, Tokyo, Japan) was preheated at 180 °C, and the samples were cooked for 10, 20, or 30 min.

Extraction of biogenic amines

Extraction of biogenic amines from commercial (raw) *Doenjang* and cooked *Doenjang* samples was carried out by Shukla's method [20] with slight modifications. For extraction of biogenic amines, 0.4 M perchloric acid (20 mL) was mixed with 10 g of *Doenjang* samples (raw or baked) or 10 mL of boiled *Doenjang* solutions and homogenized for 3 min. The supernatant after centrifugation (3000× g, 4 °C for 10 min) was collected, and the precipitate was reextracted with 20 mL of 0.4 M perchloric acid and centrifuged again. All the supernatants were combined, and the final volume was adjusted to 50 mL with 0.4 M perchloric acid. After filtering using Whatman paper No. 1, 1 mL of the extract was used for derivatization with dansyl chloride.

Derivatization of extracts and standard amines

Derivatization of biogenic amines was conducted by the methods developed by Frias et al. [22] and Shukla et al. [4], with some modifications. An extract or standard solution (1 mL) was mixed with 2 M sodium hydroxide (200 µL) and saturated with sodium hydrogen carbonate solution (300 µL). The resulting solution was mixed with 1 mL of a dansyl chloride solution (10 mg/mL in acetone) and incubated at 40 °C for 45 min. Ammonium hydroxide (25%, 100 µL) was added to stop the reaction and to remove residual dansyl chloride. After incubation at room temperature for 30 min, 1 mL of diethyl ether was added two times to the mixture for extraction. The combined extracts were dried under a nitrogen stream, and the residues were resolved in acetonitrile (1.0 mL). The solution was injected into an HPLC system (Waters Co., Milford, MA, USA) for analysis after filtration through a 0.45-μm PVDF filter (Millipore Co., Bedford, Mass., USA).

HPLC analysis of biogenic amines

Quantitative analysis of biogenic amines was carried out by means of an HPLC system equipped with a Capcell Pak C18 column (4.6 × 250 mm, 5 μm i.d.; Shiseido, Kyoto, Japan) thermostated at 30 °C, a binary HPLC pump (Waters 1525), and a fluorescence detector (Waters 2475). The mobile phase consisted of 0.1 M ammonium acetate (solvent A) and acetonitrile (solvent B) at a flow rate of 0.8 mL/min with the following gradient elution program for 35 min: Solvent B was held at 35% for 5 min, ramped to 45% (5 min), 65% (10 min), 80% (17 min), 90% (26 min), and returned to 35% (28 min) and held there until the end of the run. The injection volume was 10 μL, and the excitation and emission wavelengths of the fluorescence detector were 325 and 525 nm, respectively. As shown in Fig. 1, identification of biogenic amines was conducted on the basis of their chromatographic retention time by comparison with retention time of standard compounds.

pH

For pH measurement in the samples, 10 g of a *Doenjang* sample was mixed with deionized water (20 mL) and homogenized for 3 min. After that, the sample solution was filtered using Whatman paper No. 2 (Advantec, Tokyo, Japan). Next, pH of the samples was measured using a pH meter (Beckman Coulter, FL, USA) by the method of Shukla et al. [20].

Statistical analysis

Data are expressed as mean ± standard deviation (SD) of triplicates. The significance of differences was evaluated by one-way analysis of variance (ANOVA) and Duncan's multiple-range test using the SAS software, version 8.0 for Windows (SAS Institute, Cary, NC, USA). The probability value of $p < 0.05$ was used to determine the significance of differences.

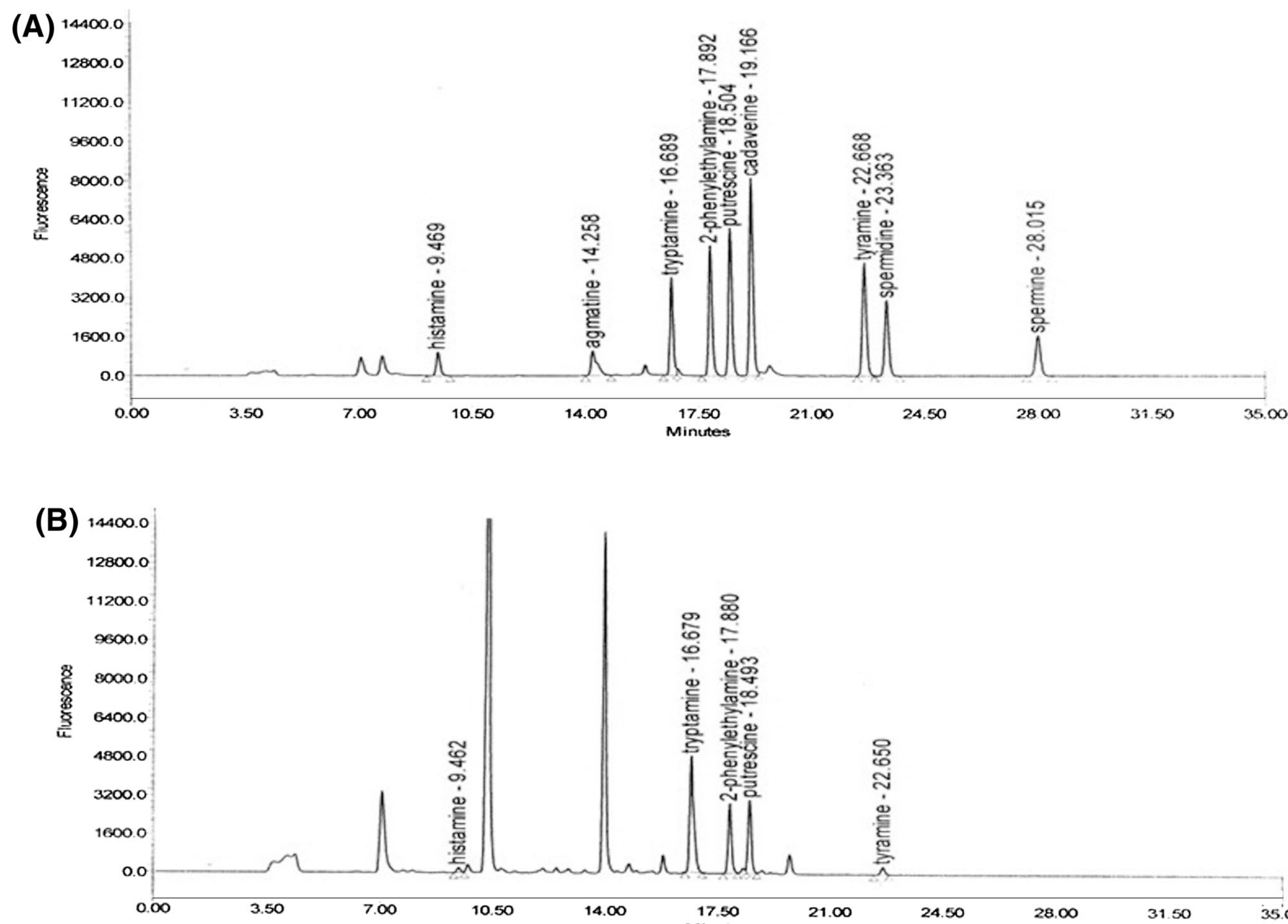

Fig. 1 HPLC chromatograms of biogenic amines: (**A**) a standard solution and (**B**) commercial *Doenjang* sample 1

Results and discussion

The pH measurement

The pH values of 14 commercial *Doenjang* samples ranged from 4.89 to 5.52, as shown in Table 1. It is known that pH is an important factor of biogenic amine formation, and optimal pH for this process is slightly acidic because bacteria produce more decarboxylase, and its activity is higher under acidic conditions [4, 23, 24]. Therefore, pH values of all the samples were assumed to be favorable for biogenic amine formation.

Contents of biogenic amines in commercial *Doenjang* samples

Commercial samples were analyzed in triplicate for the following eight biogenic amines: histamine, tryptamine, 2-phenylethylamine, putrescine, cadaverine, tyramine, spermidine, and spermine.

The concentrations of biogenic amines in commercial *Doenjang* samples ranged from ND to 415.08 mg/kg and varied depending on the manufacturer (Table 1). Kim et al. [25] reported total biogenic amine concentrations of 478.54–751.05 mg/kg in five commercial *Doenjang* samples, and Shukla et al. [4] reported that the total biogenic amine concentrations range from 2.22 to 179.27 mg/kg in traditional *Doenjang* samples.

Tryptamine and putrescine were detected in most of the samples analyzed, and their amounts were the highest among the biogenic amines detected. Histamine, spermidine, and spermine were not detected in some of the samples and, when detected, their concentrations were lower than those of tryptamine and putrescine. Cadaverine was not detected in any samples. Nuriez et al. [10] reported that fermented soybean foods, such as *Doenjang*, contain high levels of putrescine, tyramine, and histamine due to the metabolism and growth of microorganisms during fermentation. Lee et al. [3] also reported that putrescine has the highest concentration among the biogenic amines detected.

Sample 1 contained the highest total concentration of biogenic amines (415.08 mg/kg) compared with all other samples, which contained 146 mg/kg or less of total biogenic amines. In sample 14, no biogenic amines were detected. The differences in concentration and composition of biogenic amines were affected by variations in the *Doenjang* manufacturing process such as microorganisms employed for fermentation and the amount of soybeans used [4]. Therefore, it is important to suppress the formation of biogenic amines in fermented food during fermentation and storage. Control of putrescine, cadaverine, spermine, and spermidine amounts is especially important because they may contribute to the formation of carcinogenic nitrosamines and increase histamine toxicity [26, 27]. High concentrations of putrescine are rarely observed in fresh raw foods, but its concentration may increase considerably when such foods are stored improperly [28, 29]. The contamination by bacteria can cause formation of biogenic amines via decarboxylation of amino acids, which are the precursors of biogenic amines [30]. Byun and Mah [16] reported that the ratio of soybean to other grains in

Table 1 Biogenic amine contents (mg/kg) and pH of commercial *Doenjang* samples

Sample no.	HIS	TRP	PHE	PUT	CAD	TYR	SPD	SPM	Total biogenic amines	pH
1	ND	192.29	29.66	180.74	ND	12.38	ND	ND	415.08	5.46
2	ND	30.58	1.22	39.18	ND	<LOQ	8.40	ND	79.38	5.27
3	ND	4.79	1.01	64.03	ND	<LOQ	ND	ND	69.83	5.22
4	ND	4.33	ND	5.89	ND	<LOQ	ND	ND	10.22	5.23
5	12.81	23.00	6.10	82.72	ND	<LOQ	2.52	ND	127.15	5.04
6	11.35	24.01	71.67	14.10	ND	24.09	ND	ND	145.22	5.17
7	ND	12.16	ND	45.68	ND	0.17	11.30	7.21	76.52	4.89
8	ND	5.28	ND	3.15	ND	ND	ND	ND	8.43	4.92
9	ND	4.77	ND	0.53	ND	ND	ND	ND	5.30	5.52
10	ND	3.91	ND	0.49	ND	ND	ND	ND	4.40	5.33
11	ND	34.01	ND	ND	ND	25.11	ND	ND	59.12	5.08
12	ND	ND	ND	15.80	ND	ND	7.05	6.18	29.03	5.14
13	ND	ND	ND	7.03	ND	ND	ND	ND	7.03	5.13
14	ND	ND	ND	ND	ND	ND	ND	ND	ND	5.41

HIS histamine, *TRP* tryptamine, *PHE* 2-phenylethyl amine, *CAD* cadaverine, *TYR* tyramine, *SPD* spermidine, *SPM* spermine, *ND* not detected

Table 2 Biogenic amine concentrations in *Doenjang* soup (mg/L) after boiling at 100 °C for 10–50 min

	HIS	TRP	PHE	PUT	CAD	TYR	SPD	SPM	Total biogenic amines
0	ND	65.18 ± 4.31	20.47 ± 2.17[a]	670.05 ± 26.28	ND	53.40 ± 1.20	ND	ND	809.10 ± 49.10
10	ND	64.39 ± 6.97	17.92 ± 1.41[b]	627.49 ± 57.37	ND	54.28 ± 7.36	ND	ND	760.08 ± 52.35
20	ND	66.33 ± 1.38	17.87 ± 0.72[b]	623.15 ± 36.25	ND	54.47 ± 1.64	ND	ND	761.82 ± 34.41
30	ND	66.87 ± 0.91	19.34 ± 0.74[ab]	662.13 ± 27.52	ND	55.25 ± 3.47	ND	ND	803.60 ± 31.00
40	ND	64.11 ± 2.24	19.01 ± 0.94[ab]	591.96 ± 21.12	ND	53.83 ± 0.71	ND	ND	728.91 ± 22.47
50	ND	63.17 ± 0.31	17.24 ± 1.05[b]	620.32 ± 23.64	ND	53.39 ± 0.73	ND	ND	754.12 ± 25.81

HIS histamine, *TRP* tryptamine, *PHE* 2-phenylethyl amine, *CAD* cadaverine, *TYR* tyramine, *SPD* spermidine, *SPM* spermine, *ND* not detected

Means in the same column with different superscripts are significantly different ($p < 0.05$)

Miso affects the variation of amino acid precursors of biogenic amines, and consequently, leads to the differences in concentrations and types of the biogenic amines present. In addition, the formation of biogenic amines may be suppressed by decreasing storage temperature [8, 31].

Conventional *Doenjang* is exposed to contamination by microorganisms and is subject to formation of biogenic amines [25]. Therefore, we proceeded to the next step to examine the destructive effect of cooking conditions on the biogenic amines in *Doenjang* using the sample that contained the highest amounts of biogenic amines.

Influence of cooking methods on biogenic amine levels

The changes in biogenic amines in boiled *Doenjang* are shown in Table 2. According to Choe [32] and Oh and Kim [33], boiling is the cooking method most frequently used for *Doenjang*. Usually, it is boiled with various vegetables to make *Doenjang* soup. The total concentration of biogenic amines decreased slightly over time, but the change was not significant. In addition, amounts of heat-stable biogenic amines such as putrescine and tyramine [34] did not significantly decrease during the heating. Li et al. [35] reported that the concentrations of biogenic amines in sausages are decreased by boiling because these compounds get diluted in water. According to Shalaby [36], heat treatment enhances the extraction of biogenic amines into water. Nevertheless, because we analyzed biogenic amines in *Doenjang* soup, all dissolved biogenic amines were analyzed, and there were no significant changes in total amounts of biogenic amines under the influence of boiling. Therefore, it seems that the change in biogenic amines in various foods during boiling is mainly caused by the extraction of biogenic amines into water rather than by their destruction.

The changes in biogenic amines in *Doenjang* during roasting were determined after roasting at 180 °C for 10–30 min (Table 3). The total concentration of biogenic amines decreased significantly after 10 min of roasting, and no significant changes were detected during additional heating after the initial 10 min. Tryptamine and 2-phenylethylamine, which were reported to cause vascular diseases [8, 37], were affected by roasting the most. It was also found that roasting did not affect the amounts of putrescine and tyramine. Furthermore, because the contents of putrescine were the highest among biogenic amines detected, 20–30 min roasting did not cause significant change of total biogenic amines after 10 min roasting. Moret et al. [19] reported that tryptamine content decreased easily, as compared to other biogenic amines, during storage.

Kozova et al. [38] reported results similar to ours, i.e., they stated that cooking methods involving a high

Table 3 Concentrations (mg/L) of biogenic amines in *Doenjang* after roasting at 180 °C for 10–30 min

Time, min	HIS	TRP	PHE	PUT	CAD	TYR	SPD	SPM	Total biogenic amines
0	ND	126.61 ± 32.42[a]	35.32 ± 11.13[a]	258.63 ± 30.58	ND	7.38 ± 1.71[a]	ND	ND	427.94 ± 46.47[a]
10	ND	96.31 ± 20.60[ab]	26.60 ± 7.21[ab]	159.83 ± 16.58	ND	11.37 ± 3.87[a]	ND	ND	294.11 ± 34.70[b]
20	ND	85.41 ± 15.90[b]	20.84 ± 4.53[b]	225.70 ± 43.61	ND	9.24 ± 7.40[a]	ND	ND	341.19 ± 43.15[ab]
30	ND	88.34 ± 18.09[b]	23.77 ± 2.38[ab]	231.21 ± 32.19	ND	13.15 ± 5.11[a]	ND	ND	356.47 ± 47.58[ab]

HIS histamine, *TRP* tryptamine, *PHE* 2-phenylethyl amine, *CAD* cadaverine, *TYR* tyramine, *SPD* spermidine, *SPM* spermine, *ND* not detected

Means in the same column with different superscripts are significantly different ($p < 0.05$)

temperature such as roasting, grilling, and frying decrease biogenic amine concentrations more effectively than boiling does. In the present study, the concentrations of biogenic amines were decreased more strongly by roasting than by boiling. The concentrations of tryptamine and 2-phenylethylamine were reduced by roasting most significantly. Therefore, proper choice of a cooking method seems to be a more effective way to reduce biogenic amine amounts in food in comparison with increased duration of cooking.

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