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Determination of biogenic amines in Korean traditional fermented soybean paste (Doenjang)

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ABSTRACT

Biogenic amines, produced by bacterial decarboxylation of amino acids, have been associated with toxicological symptoms in food products. Twenty-three samples of traditionally available Korean fermented soybean paste samples (Doenjang) were analyzed in order to determine the content of biogenic amines. Amines were extracted with 0.4 M perchloric acid and derivatized with dansyl chloride. Nine biogenic amines were separated from Doenjang samples by high performance liquid chromatography using gradient elution (acetonitrile and ammonium acetate), and detected with spectrophotometric UV–vis detection at 254 nm. The pH value of all the samples was ranged from 4.8 to 6.0, and the strong amino acid decarboxylase activity was found to be in an acidic environment. The mean values of biogenic amines (tryptamine, 2-phenyl–ethylamine, putrescine, cadaverine, agmatine, histamine, tyramine, spermidine and spermine) determined in 23 Doenjang samples were found to be 18.37, 82.03, 70.84, 34.24, 47.32, 26.79, 126.66, 74.41 and 244.36 mg%, respectively. The findings of this study enhance the safety of not only Doenjang but other salted and/or fermented food products.

1. Introduction

Biogenic amines are nitrogenous and low molecular weight organic bases of aliphatic, aromatic or heterocyclic structures that are synthesized and degraded during the cellular metabolism activities in microorganisms, plants and animals (Silla Santos, 1996; Tassoni et al., 2004). These amines are endogenous and indispensable components of living cells and are important in the cell proliferation and differentiation, regulation of nucleic acid function, protein synthesis, brain development, nerve growth and regeneration (Kalac and Krausová, 2005; Silla Santos, 1996; Tassoni et al., 2004). Biogenic amines are derived mainly from microbial decarboxylation of the corresponding amino acids (Awan et al., 2008; Silla Santos, 1996) through substrate – specific decarboxylase enzymes (Loukou and Zotou, 2003). Biogenic amines can also be found in a variety of foods, beverages and fermented food products especially in protein-rich foods e.g., fish and fish products, meat and meat products, eggs, cheeses, fermented vegetables, fruits, nuts, chocolate, soybean products and wine (Shalaby, 1996; Silla Santos, 1996).

Major biogenic amines found in food products are putrescine, cadavarine, histamine, tyramine, tryptamine, 2-phenyl–ethylamine, spermine, agmatine and spermidine. It has been found that the contents of biogenic amines are not significantly reduced by high temperature treatment (Shalaby, 1996; Silla Santos, 1996). The determination of biogenic amines in foods is of great interest not only due to their possible toxicity, but also due to acting as potential indicators to determine the quality of freshness or spoilage of food products (Awan et al., 2008; Silla Santos, 1996).

The amount and types of biogenic amines formed in fermented food products are strongly influenced by the food composition, microbial flora and by other parameters which allow bacterial growth during food processing and storage (Carelli et al., 2007). Low levels of biogenic amines in food are not considered a serious risk. However, when consumed in excessive amounts, they can cause severe toxicological effects in human beings.

Biogenic amines are also considered as precursors of carcinogens such as N-nitrosamine compounds. Several analytical methods for the determination of biogenic amines in foods have been described. These include thin layer chromatography (Shalaby, 1995), biosensors (Carelli et al., 2007; Keow et al., 2007), capillary electrophoresis (Paproski et al., 2002) and reversed phase high performance liquid chromatography (HPLC) (Lange et al., 2002; Moret et al., 2005; Oguri et al., 2007; Tassoni et al., 2004). HPLC is the most popular and frequently reported method for the separation and quantification of biogenic amines. Since many biogenic amines occurring in food show neither satisfactory absorption nor exhibit significant fluorescence properties, chemical derivatization is usually performed to increase the specificity and sensitivity. However, the reaction products have a short life and the methods require the preparation of amine samples before derivatization (Heideman et al., 1984).

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Fermented food products are widely consumed all over the world. Of particular interest to us are the indigenous fermented foods that are prepared in traditional Korean system from raw soybean. These items are normally consumed in Korea with fresh local herbs and eaten with rice. Various kinds of biogenic amines have been reported to find in Korean traditional fermented soybean paste samples (locally known as Doenjang) during fermentation. The level of biogenic amines present in Doenjang depends on ratio of raw material used, microbial composition, duration of fermentation, solvent used for amine extraction and many other factors. The biogenic amines analyzed in this study are shown in Table 1.

However, to the best of our knowledge, fewer reports are available on quantitative determination of biogenic amines in Doenjang samples. The objective of this research was, therefore, to extensively determine the level of different biogenic amines present in our 23 traditional fermented soybean paste samples of Doenjang.

2. Materials and methods

2.1. Standards and reagents

All chemicals and solvents used were of analytical and chromatographic grade. Spermidine trihydrochloride (SPD), putrescine dihydrochloride (PUT), histamine dihydrochloride (HIS), tryptamine hydrochloride (TRP), 2-phenyl–ethylamine (PHE), cadaverine dihydrochloride (CAD), spermine tetrahydrochloride (SPM), tyramine hydrochloride (TYR), agmatine sulfate (AGM) and acetone were purchased from Sigma–Aldrich. Dansyl chloride was obtained from Fluka. Sodium hydroxide, sodium hydrogen carbonate, ammonium hydroxide and perchloric acid were purchased from Junsei Chemicals. HPLC grade acetonitrile and ammonium acetate (0.1 M) were obtained from Merk.

2.2. Samples

A total of 23 traditional Korean fermented soybean paste samples of Doenjang were analyzed. A list of all soybean samples analyzed in this study is presented in Table. 2. All samples were collected from different area of Daegu-si and Gyeongsangbuk-do districts, Republic of Korea. Fermentation period of all Doenjang samples was near about one year. Collected samples were stored at 4 °C until analysis.

Table 2
List of the analyzed traditional fermented soybean paste samples of Doenjang.

Sample No.	Sample name	Collection period (month/year)	Color	pH[a]
1	Chungdo		Dark brown	5.0
2	Phalkong		Dark brown	4.8
3	Yeungju		Dark brown	4.9
4	Koreung-1		Dark brown	5.6
5	Wookog		Light brown	6.1
6	Ssanglim		Yellowish brown	6.5
7	Dukkog-1		Dark brown	5.7
8	Dukkog-2	04/2009	Dark brown	5.6
9	Koreung-2		Yellowish brown	6.5
10	Woonsu-1		Dark brown	5.5
11	Sanglim		Dark brown	5.3
12	Woonsu-2		Dark brown	5.0
13	Wookog-2		Dark brown	5.8
14	Munhwa		Dark brown	5.8
15	Andong-1		Light brown	5.4
16	Aphsan Halmai		Yellowish brown	6.2
17	Andong-2	10/2008–05/2009	Yellowish brown	6.0
18	Andong-3		Yellowish brown	5.2
19	So hi		Light brown	5.4
20	Koreung-3		Dark brown	5.6
21	Andong-4	04/2009	Light brown	6.4
22	Dukkog-3		Black dark brown	5.4
23	Yaungnyam		Dark brown	5.5

[a] pH expressed in mean values of triplicates.

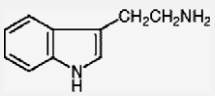
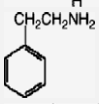
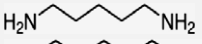
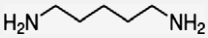
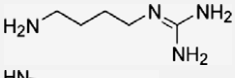
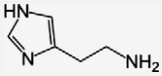
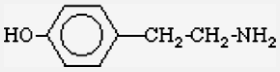
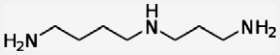
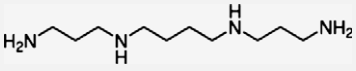
2.3. pH measurement

For the pH measurement of the samples, 25 ml of deionized distilled water was added to 5 g of Doenjang samples and then homogenized and filtered with Whatman paper (No. 2, Advantec, TOYO). pH was measured according to the procedure developed by Kim et al. (2005), using a pH meter (Orion 35 star pH Bechtop, Thermo electroncorporation).

2.4. Preparation of standard amine solution

Stock solutions of 1,7-diaminoheptane (internal standard) and all nine standard biogenic amines (TRP, PUT, HIS, TYR, SPM, PHE, AGM, CAD and SPD) were separately prepared at 50 mg/50 ml concentration in distilled water. Working solution was

Table 1
List of biogenic amines studied.

Compound name	Abbreviation	Molecular formula	Structure	Molecular weight	Precursor
Tryptamine	TRP	$C_{10}H_{12}N_2$		160.2	Tryptophan
2-Phenyl–ethylamine	PHE	$C_8H_{11}N$		121.2	Phenylalanine
Putrescine	PUT	$C_4H_{12}N_2$		88.2	Orthine, agmatine
Cadaverine	CAD	$C_5H_{14}N_2$		202.2	Lysine
Agmatine	AGM	$C_5H_{14}N_4$		130.2	Arginine
Histamine	HIS	$C_5H_{10}N_3$		111.1	Histidine
Tyramine	TYR	$C_8H_{11}NO$		137.3	Tyrosine
Spermidine	SPD	$C_{17}H_{19}N_3$		145.3	Arginine
Spermine	SPM	$C_{10}H_{26}N_4$		348.1	Arginine

prepared by diluting 1000 μl of each stock solution in distilled water to bring to a final volume of 10 ml. These solutions were stored at 4 °C until the use.

Dansyl chloride solution (10 mg/ml) was prepared by dissolving 100 mg in 10 ml of acetone and stored at 4 °C until the use. Separately a saturated solution of sodium hydrogen carbonate was prepared for the derivatization reaction.

2.5. Preparation of sample extracts

Extraction of fermented soybean paste samples and HPLC determination of biogenic amines were carried out according to the procedures developed by the modified method of Ben-Gigirey et al. (1998). To extract biogenic amines, 10 ml of 0.4 M perchloric acid containing a known amount of 1,7-diaminoheptane as an internal standard was added to 5 g of fermented soybean paste samples, and the mixture was homogenized for 3 min. The homogenate was centrifuged at 3000*g* at 4 °C for 10 min. The supernatant was collected and the residue was extracted twice with the same volume of 0.4 M perchloric acid solution. All supernatants were combined and the final volume was adjusted to 25 ml with 0.4 M perchloric acid. The extract was filtered through Whatman paper No. 1. One milliliter of each of the extract was used for the HPLC analysis followed by derivatization with dansyl chloride.

2.6. Derivitization of extracts and standards

Derivatization of biogenic amines was carried out according to developed method of Ben-Gigirey et al. (1998). One milliliter of each extracted sample or standard amine solution was mixed with 200 μl of 2 M sodium hydroxide and 300 μl of sodium hydrogen carbonate. Two milliliters of a dansyl chloride solution (10 mg/ml in acetone) was added to the mixture and then incubated in water bath at 40 °C for 45 min. One hundred micro-liters of 25% ammonium hydroxide was added to stop the reaction and to remove residual dansyl chloride. After 30 min incubation at room temperature, the final volume was adjusted to 5 ml by adding acetonitrile. Finally, the mixture was centrifuged at 2500*g* for 5 min and the supernatant was filtered through 0.2 μm-pore-size filter (Pall Co., Acrodisc Syringe Filters, 25 mm, 0.2 μm, Woongki Science Co., Seoul, Korea). The filtered supernatant was kept at −25 °C until assayed by HPLC. All samples were subjected to HPLC injection for two times.

2.7. Separation of biogenic amines by HPLC

Quantitative analyses of biogenic amines were carried out using a HPLC unit, consisted of two pumps and a UV–vis detector. Separation was achieved using a C_{18} Supelco column 5 μm (250 mm × 4.6 mm). The mobile phase was ammonium acetate (0.1 M; solvent A) and acetonitrile (solvent B) at the flow rate of 1 ml/min with gradient elution program for 35 min (Table 3). The sample volumes injected were 5, 10 and 20 μl (for getting data in replicates). The samples were monitored at 254 nm. Each HPLC run took about 35 min and afterwards the column was conditioned again with the mixture of 65% solvent A and 35% solvent B.

3. Results and discussion

3.1. pH measurement

The pH values of 23 traditional fermented soybean paste samples of Doenjang are shown in Table 2. The pH is an important factor for fermentation and formation of biogenic amines because amino acid decarboxylase activity remains stronger in an acidic environment (Silla Santos, 1996). We also confirmed that during organic acid examination, the contents of lactic acid were higher in all fermented soybean paste samples, thus growth of lactic acid bacteria may be higher causing decrease in pH value (unpublished data). In this study we found that the pH of all the samples was found in the range of 4.8–6.0, and these results are in strong agreement with previous findings of Sung et al. (1987). Silla Santos (1996) reported that the pH was an important factor influencing decarboxylase activity, and low pH about 3.0–6.0 was optimal for bacteria to produce decarboxylase. Silla Santos (1996) also found a higher tyramine level in mackerel when the pH was low. By these reports, we can conclude that amino acid decarboxylase activity was stronger in an acidic environment, being the optimum pH between 4.0 and 5.5 (Teodorovi et al., 1994). In addition to this, Kim et al. (2003) reported that the low pH of Doenjang samples, about 3.0–6.0, was effective for increasing the decarboxylase activity.

Table 3
Gradient elution program (flow rate 1.0 ml/min).

Time (min)	A (%)	B (%)
0.00	65	35
5.00	55	45
10.05	35	65
17.05	20	80
26.25	10	90
35.00	65	35

A, ammonium acetate; B, acetonitrile.

3.2. Content of biogenic amines in fermented soybean paste samples

The nine biogenic amines studied (TRP, PUT, HIS, TYR, SPM, PHE, AGM, CAD and SPD) are the most prevalent biogenic amines found in fermented traditional soybean paste samples in Korea. The biogenic amines were well resolved in C_{18} column with chromatographic conditions as described in Table 3. Amines were identified on the basis of their retention times by comparison with standard solutions. Retention times of amines were stable and consistently reproducible. Major side products of dansyl reaction were separated along with amines. The amines were separated with very good resolution and sharpness. Dansyl polyamines were completely eluted from the column after 25 min.

Quantitatively the most important biogenic amines in the present study determined were tryptamine, 2-phenyl–ethylamine, putrescine, cadaverine, agmatine, histamine, tyramine, spermidine and spermine. The contents of biogenic amine in 23 fermented soybean paste samples of Doenjang are listed in Table 4. The mean values of biogenic amines (tryptamine, 2-phenyl–ethylamine, putrescine, cadaverine, agmatine, histamine, tyramine, spermidine and spermine) determined in 23 Doenjang samples were found to be 18.37, 82.03, 70.84, 34.24, 47.32, 26.79, 126.66, 74.41 and 244.36 mg%, respectively. Histamine, a biogenic amine, was found to detect only in three of the Korean traditional Doenjang samples of Ssanglim, Koreung-2 and Koreung-3. From other studies carried out on another food products, some factors have been found, which affect the levels of histamine formation. For example, low temperature of storage (4 °C) does not favour histamine formation, which is determined by mesophilic bacteria that require temperature higher than 15 °C. Optimum temperature of histidine-decarboxylase enzyme activity has been reported as 30 °C (Joosten and Van Boekel, 1988). As described in Fig 1, the contents of spermine biogenic amine detected in our 23 traditional fermented soybean paste samples of Doenjang were comparatively higher than other biogenic amines (244.36 mg%) detected, while tryptamine was present in very least amount (Table 4). This may be due to the low tryptophan content in samples. Tyramine was found to be the main biogenic amine in fermented soybean paste samples representing 126.66 mg%. Tyramine originates from the decarboxylation of tyrosine and has been associated with Enterobacteriaceae (Halasz et al., 1994). However, relatively high levels of amines should be considered as indicators of microbial contamination during fermentation because their formation has been related to the presence of contaminating microorganisms, such as lactic acid bacteria (Zee et al., 1981; Halasz et al., 1994; Izquierdo-Pulido et al., 1996). Production of all the fermented soybean paste samples studied involved lactic acid fermentation, which could be implicated in amine formation. However, these samples were obtained from the traditional sources, their history was not known. Therefore, it can be assumed that the variation noticed among the samples in the content levels of biogenic amines might be due to the sources, processing conditions and degree of microbial contamination.

Biosynthesis of amino acids in fermented soybean paste is an enzymatic process which is catalyzed mainly by synthetases (e.g.

Table 4
Analysis of biogenic amines content in traditional Korean fermented soybean paste samples of Doenjang (mg/100 g).

Sample No.	TRY	PHE	PUT	CAD	AGA	HIS	TYR	SPD	SPM
1	280.81	ND	271.97	97.27	ND	ND	138.74	694.04	ND
2	ND	ND	ND	ND	66.06	ND	618.00	ND	ND
3	ND	ND	362.85	74.32	ND	ND	ND	ND	ND
4	ND	9.25	ND	ND	ND	ND	ND	ND	342.74
5	6.65	77.03	ND	ND	2.52	ND	ND	ND	286.60
6	ND	ND	53.00	56.39	ND	143.48	ND	ND	ND
7	ND	ND	ND	ND	550.84	ND	ND	ND	694.04
8	ND	ND	155.74	41.65	ND	ND	ND	ND	972.95
9	ND	ND	ND	ND	ND	193.10	ND	ND	543.69
10	ND	870.46	35.72	ND	ND	ND	423.11	62.93	ND
11	ND	618.89	ND	ND	ND	ND	ND	ND	ND
12	22.15	ND	ND	ND	ND	ND	ND	ND	ND
13	ND	311.14	29.53	ND	ND	ND	338.32	ND	418.88
14	ND	ND	57.46	ND	62.43	ND	ND	880.40	ND
15	ND	ND	429.23	323.55	ND	ND	ND	768.04	ND
16	113.00	ND	38.61	ND	ND	ND	ND	ND	ND
17	ND	ND	195.22	38.95	336.38	ND	434.63	ND	787.55
18	ND	ND	ND	ND	ND	ND	133.32	ND	ND
19	ND	ND	ND	ND	ND	ND	ND	ND	132.73
20	ND	ND	ND	ND	70.07	279.48	ND	ND	ND
21	ND	ND	ND	ND	ND	ND	165.53	ND	747.04
22	ND	ND	ND	56.53	ND	ND	661.61	ND	ND
23	ND	ND	ND	64.53	63	ND	ND	ND	ND
Mean	18.37	82.03	70.84	34.24	47.32	26.79	126.66	74.41	244.36

Results are given in mean values of duplicates.
ND: not detected.

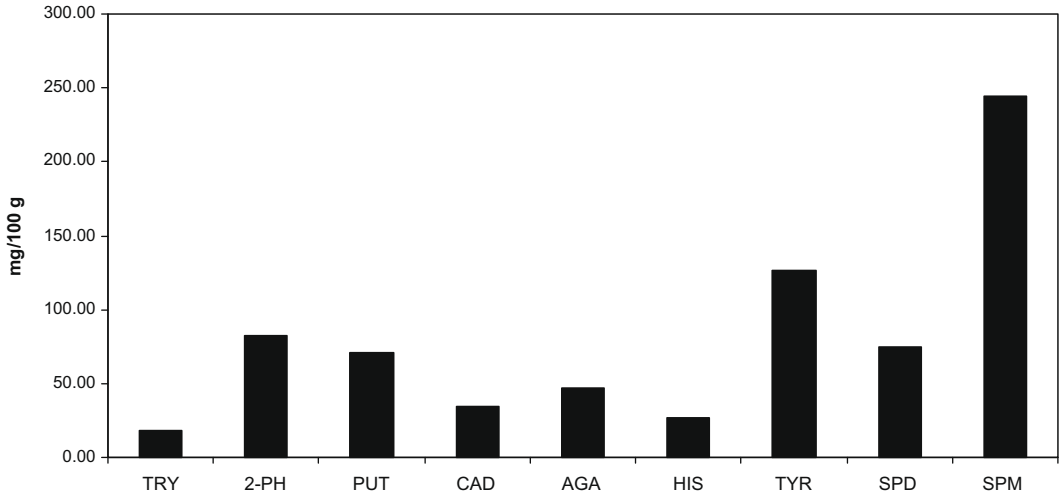


Fig. 1. Comparative values of various biogenic amines present in Korean traditional fermented soybean paste (Doenjang).

glutamine synthetase). Other amino acid metabolizing enzymes have been detected with higher levels, e.g., aspartate amino transferase and, especially, histidine-decarboxylase, glutamate decarboxylase and arginine-decarboxylase during the fermentation process in various fermented food products (Picton et al., 1993). The activity of decarboxylase enzyme depends on fermentation period and storing temperature. These decarboxylases may cause the production of hygienically significant contents of biogenic amines in the case of sufficiently high levels of relevant free amino acids as precursors (Picton et al., 1993). Herein this study, we focused on some specific biogenic amines and those production was enabled either by the presence of the relevant decarboxylases (histidine-, arginine- and glutamine-decarboxylase) or by the microflora present during fermentation process.

Studies have shown that biogenic amines in fermented soybean products are most likely formed by the lactic acid microflora that is active during fermentation (Kirschbaum et al., 2000; Stratton et al., 1991). The variability of biogenic amines levels in the fermented soybean paste samples has been attributed to the variations in manufacturing processes; variability in the ratio of soybean in the raw material, microbial composition, conditions and duration of fermentation (Shalaby, 1996). Nout et al. (1993) reported that the amines concentrations in fermented soybean paste samples were low or high depending on the applied manufacturing process: soaked soybeans, type of fermentative microorganisms, boiling or cooking and storage temperature.

Shalaby (1996) reported that fermented soybean products contained various and high levels of biogenic amines (HIS: 462 mg%; PUT: 1234 mg%; CAD: 634 mg%; TYR: 3568 mg%). An intake of over 40 mg biogenic amines per meal has been considered potentially toxic (Nout, 1994). In the present study, we found that Korean fermented soybean paste samples of Doenjang also contained various

biogenic amines, but showed relatively lower contents than those of previous reports for other kinds of soybean products (Chin and Koehler, 1983; Nout et al., 1993; Shalaby, 1996; Stratton et al., 1991).

Generally, biogenic amines in all kinds of food can be controlled by strict use of good hygiene in both raw material and manufacturing environments with corresponding inhibition of spoiling microorganisms. A better understanding of the mechanisms by which biogenic amines are being produced is necessary to prevent their formation. In fermented foods, the use of short fermentations with carefully selected active starter cultures instead of wild fermentations will help to prevent the formation of toxic amines.

4. Conclusions

It is well known that excess intake of biogenic amines causes several kinds of diseases like migraine, brain hemorrhage, heart failure, hypertension, abdominal cramps and flushing. In this study, we determined the overall amounts of biogenic amines in various Doenjang samples confirming the traditional value of such fermented food products. However, excessive intake of these food items on regular basis can not be recommended. Therefore, further study is warranted on traditional Korean fermented soybean paste samples of Doenjang to compare their biogenic amine contents in standardized manufacturing conditions so as to raise the safety concerns during fermentation process.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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