

SHORT COMMUNICATION

Determination of amino acid and free sugar contents in Korean traditional fermented soybean products of Doenjang and evaluation of their sensory attributes

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Abstract: This study quantitatively determined contents of amino acids and free sugars in soybean fermented samples (Doenjang) using amino acid autoanalyzer and HPLC, respectively. As a result, threonine, serine, glycine, alanine and lysine categorized as total sweet amino acids and free sugars (sucrose, maltose, glucose, galactose and fructose) were found in the range of 32.7 to 117.1 and 5.85 to 23.66mg/100 g, respectively. However, average of total sugar content was noted to be 71.63mg%. Three samples possessing the best sensory characteristics were selected for further study. The mean values of four sensory attributes such as sweet, sour, savory and bitter tastes of Doenjang samples were found to be 709.53, 210.25, 241.90 and 276.05. These findings confirm nutritive properties of fermented food products of Doenjang with improved quality and utilization.

Keywords: Doenjang; amino acids and free sugars; sensory evaluation; HPLC; anion-exchange chromatography

INTRODUCTION

Production of Doenjang is achieved by the fermentation of soybean using natural microorganisms (bacteria and fungi) which has been known as a protein-rich, health nutritional fermented supplement in Korea since decades. The initial ingredients, process of fermentation, and microorganisms significantly influence the quality of Doenjang products (Park *et al.*, 2002). The unique flavor and taste of Doenjang is resultant of decomposing product of soybean proteins used as a basic substrate for Doenjang fermentation by the action of microorganisms (Kang *et al.*, 2000).

Amino acids and free sugars play an important role in the flavor characteristics of soybean products and also in the consumer acceptability of its processed products. Free sugars present in various food products and vegetables are important components of taste, and they together with aroma play important role in maintaining the food quality and determining their nutritive value (Zhao *et al.*, 2015). The nature and concentration of these constituents in food products are of significant interest due to their direct influence on organoleptic properties. Therefore, food analysts are concerned on variations in nature and amount of these chemical components during fermentation, due to their impact on market quality of food products. Determination of amino acid and free sugar contents is one of the important parameters for assessment of commercial quality of fermented food products (Charro and Barreiro, 1957).

Separation of amino acids and free sugars is generally accomplished by chromatographic methods which include thin layer chromatography (Torrighiani *et al.*, 1988), overpressure layer chromatography (Simon-Sarkadi and Galiba, 1988), high performance liquid chromatography (HPLC) (Flores and Galston, 1982) and gas chromatography (Yamamoto *et al.*, 1982). However, in this study, quantitative determination of amino acids and free sugars was performed by automated-programmed amino acid autoanalyzer and HPLC, respectively. These techniques are most widely used due to their high speed, selectivity and reliability.

Though reports have confirmed presence of amino acids and free sugars in Doenjang samples, their relation with sensory attributes has not been discussed yet. Therefore, the objectives of the present study were to determine amino acid and free sugar contents of Doenjang samples and to correlate their sensory characteristics to identify the best sensory variables of Doenjang samples.

MATERIALS AND METHODS

Doenjang Samples

This study analyzed eighteen types of different Doenjang samples, which were procured from Daegu metropolitan city, Gyeongbuk province, Republic of Korea.

Standards and reagents

A highly purified and analytical grade chemicals and reagents for used. Standard free sugars (sucrose, glucose and fructose) were obtained from Sigma (St. Louis, MO).

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Maltose and galactose were obtained from Junsei Chemicals (Japan). For amino acid analysis in automatic amino acid analyzer, ion exchange column was pre-standardized using all essential amino acid standards.

Preparation of standard solution of free sugars

To prepare the standard solutions of free sugars, glucose, maltose, fructose, sucrose and galactose were dissolved in 100 mL distilled water at the concentration of 0.2%, and each solution was stored at 4°C until used.

Measurement of free sugar contents

Measurement of free sugar contents in 18 Doenjang samples was performed by our previously developed method (Shukla *et al.*, 2015). Ion-exchange column chromatographic technique was used to purify the extracted components of free sugars. For chromatographic separation, cation and anion resins exchangers were amberlite IR-120 and amberlite IRA-400, respectively. Completely dried sample of sugar (10 µL) dissolved in triple distilled water was manually injected in HPLC to obtain HPLC chromatograms.

Measurement of amino acid contents

To measure the contents of amino acids in 18 Doenjang samples, a previous method of Park *et al.* (2002) was adopted. The sample components absorbed after free sugar collection on amberlite IRA-400 were eluted with 150mL of eluting solvent (2N NH₄OH) at slow flow rate. The solution of eluted amino acids was completely dried under vacuum on a rotary evaporator (EYELA Rotary vacuum evaporator, N-N Series, Korea). Completely dried samples of amino acids (10µL) dissolved in lithium citrate buffer (0.20M) were manually injected in an amino acid autoanalyzer and chromatographs were analyzed.

Sensory evaluation

Sensory analysis was performed by an expert panel consisting of 4 female and 4 male, aged between 25-45 years. To perform sensory analysis, Doenjang samples were dissolved in water and kept at 40°C. During preliminary session, taste parameters were discussed by the panelists before descriptive analysis, before their use as sensory attributes. The overall sensory evaluation of Doenjang samples was ranked on the basis of 5-point scale from 1 (very bad) to 5 (very good).

Chromatographic conditions

Water's HPLC system (model 600E) was used for quantitative determination of content of free sugars fitted with refractive index detector (RI- Model 410) and a Sugar-Pak I column (6.5 × 300 mm). Calcium-EDTA buffer was used as a mobile phase and column temperature was 40°C with a flow rate of 0.5mL/min. Concentration of sugar in Doenjang samples was determined by the peak area of standard samples.

An amino acid analyzer (Hitachi), equipped with a cation exchange resin bed, was used for the separation and quantification of amino acids from *Doenjang* samples. The analysis conditions were as follows: buffer flow rate: 0.35 mL/min; ninhydrin flow rate: 0.3 mL/min; temperature: 30 to 70 °C; wavelength decreased to 440 nm from 570 nm; column length: 4.6 × 60 mm; injection volume: 10 µL.

STATISTICAL ANALYSIS

The IBM SPSS 19 program, from SPSS Inc., USA was used for statistical analysis. Data representing as mean standard deviation (S.D.) was analyzed using one-way ANOVA, and differences were considered significant at the level of $P < 0.05$.

RESULTS

Determination of free sugar contents

An HPLC chromatographic technique was employed to determine the content of free sugars in Doenjang samples. Standards solutions of individual sugar were chromatographed separately to determine the retention time of each sugar compound. Individual free sugar content was qualitatively determined by comparing the chromatograms of standard samples and by doping the samples with reference compound (fig. 1). Table 1 shows the amount of total free sugar contents in each type of Doenjang sample. It was observed that mean values of free sugars such as sucrose, maltose, glucose, galactose and fructose determined in 18 Doenjang samples were 11.57, 5.85, 14.68, 15.86 and 23.66 mg/100g, respectively. While the average of total sugar contents in Doenjang samples was found to be 71.63 mg%. The total amount of fructose was significantly higher than amount of other sugar compounds, while maltose was found to be in the least amount (5.85 mg/100 g) when compared with other sugar compounds.

Determination of amino acid contents

A total of 15 kinds of amino acids categorized in sweet, savory and bitter taste including threonine, serine, glycine, alanine, lysine, aspartine glutamic acid, cystein, methionine, isoleucine, tyrosine, phenylalanine, valanine, histidine and proline were analyzed. Total sweet amino acids determined in 18 Doenjang samples such as threonine, serine, glycine, alanine and lysine represented mean values of 32.7, 34.4, 42.2, 117.1 and 89.4 mg/100 g, respectively (table 2). The amino acid contents of savory taste (aspartine, glutamic acid and cystein) were noted to be 24.8, 136.4 and 0.80 mg/100g. In this study, contents of bitter taste compounds (methionine, isoleucine and leucine) in 18 Doenjang respectively, while contents of some other amino acids such as tyrosine, phenylalanine, valine, histidine and proline were found to be 21.8, 60.9,

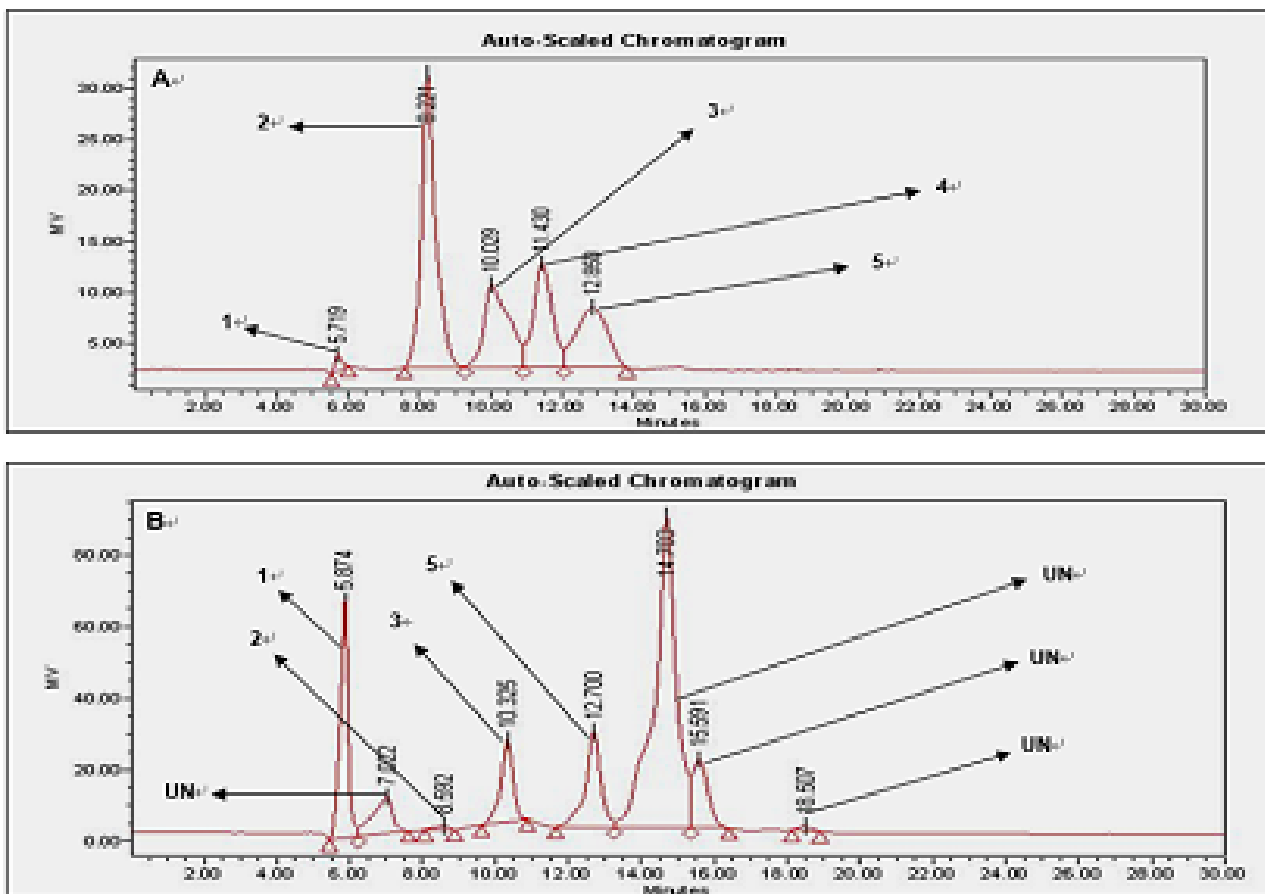


Fig. 1: HPLC chromatograms relative to: (A) standard solution of 5 free sugars; (B) Doenjang sample (1: sucrose; 2: maltose; 3: glucose; 4: galactose; 5: fructose).

Table 1: Free sugar contents of Korean fermented soybean paste samples of Doenjang (mg/100g).

Sample	Sucrose	Maltose	Glucose	Galactose	Fructose	Total
1	26.97±8.23*	0.81±0.01	12.17±0.01*	ND ^a	16.89±1.13	56.84
2	33.58±2.56*	1.18±0.02	46.82±5.10*	ND	90.89±8.13*	172.48
3	4.46±4.53	0.46±0.00	9.58±0.12	285.51±20.8*	53.99±5.12*	354.00
4	1.24±3.86	3.51±0.02*	17.20±2.13*	ND	12.59±1.20	34.54
5	2.78±4.21	0.00±0.00	0.47±0.03	ND	6.93±0.03	10.18
6	3.83±0.02	0.00±0.00	0.77±0.00	ND	0.31±0.00	5.00
7	85.46±1.53*	72.94±1.10*	36.59±4.63*	ND	47.08±0.13*	242.08
8	22.31±1.21*	4.82±0.07*	48.08±4.02*	ND	40.97±5.63*	116.18
9	1.00±0.01	0.73±0.02	3.03±1.12	ND	9.78±0.08	14.54
10	0.80±0.00	1.60±0.06	4.22±1.11	ND	2.66±0.01	9.28
11	8.09±0.03	0.26±0.00	28.22±0.70*	ND	31.32±5.41*	67.90
12	0.98±0.01	0.00±0.00	20.10±2.23*	ND	18.57±2.30	39.65
13	1.26±0.01	1.56±0.02	5.61±1.02	ND	0.24±0.01	8.66
14	2.83±0.30	0.54±0.01	8.79±1.01	ND	43.35±7.23*	55.51
15	4.67±0.06	13.82±0.23*	5.26±0.03	ND	1.67±0.05	25.42
16	2.17±0.02	0.36±0.01	6.04±0.01	ND	40.87±0.52*	49.45
17	3.49±0.01	2.51±0.01	7.05±0.05	ND	4.71±0.06	17.76
18	2.41±0.21	0.12±0.00	4.27±1.12	ND	3.06±0.01	9.85
Mean	11.57	5.85	14.68	15.86	23.66	71.63

^a Not detected; Values are mean ± S.D., n = 3, *P < 0.05 significant.

Table 2: Amino acid contents of Korean fermented soybean paste samples of Doenjang (mg/100 g).

Sample	Sweet				Savory				Total			Bitter			Total			Others				Total
	Thr.	Ser.	Gly.	Ala.	Lys.	Asp.	Glu.	Cys.	Total	Met.	Ileu.	Leu.	Total	Tyr.	Phe.	Val.	His.	Pro.				
1	15.6	18.5	12.5	29.8	24.9	70.4	ND	95.2	5.5	26.7	43.7	75.9	15.3	26.7	26.4	6.8	30.0	105.3				
2	111.9	133.2	73.7	105.1	143.2	42.6	357.1	ND	399.7	24.7	112.0	11.6	148.3	106.3	11.8	95.9	22.3	146.1	382.5			
3	98.3	84.8	53.5	139.8	144.8	26.2	354.9	0.4	381.5	25.6	108.6	159.0	293.3	8.4	123.5	102.9	43.3	113.7	391.8			
4	19.8	15.9	39.4	110.8	46.9	232.8	14.9	73.5	1.2	89.6	15.1	59.7	167.2	7.2	46.3	61.6	6.9	42.0	164.1			
5	ND ^a	1.6	57.7	90.0	121.3	270.5	2.3	187.8	1.1	191.3	31.5	107.3	171.1	309.9	6.6	83.4	93.0	1.4	24.3	208.8		
6	26.6	27.3	18.3	45.4	35.7	153.4	11.9	75.0	0.1	87.1	8.4	51.7	80.3	140.4	11.2	43.6	49.4	9.6	37.3	151.1		
7	63.7	55.8	54.9	124.5	124.7	423.7	38.2	126.6	ND	164.7	23.8	103.1	169.3	296.2	14.1	73.8	90.0	16.1	114.3	308.4		
8	86.8	85.7	53.6	85.0	79.0	390.0	35.6	227.6	ND	263.2	13.9	75.6	120.0	209.5	7.1	56.0	82.5	10.6	99.6	255.7		
9	4.4	12.6	24.1	82.6	72.5	196.1	33.3	141.6	ND	174.9	18.5	72.2	117.5	208.1	10.0	64.1	73.2	19.2	40.6	207.2		
10	18.7	19.1	15.1	51.6	39.2	143.8	15.1	75.2	ND	90.3	7.7	48.3	78.6	134.6	15.0	44.2	48.0	2.9	40.1	150.2		
11	36.0	44.2	30.6	120.2	86.2	317.2	13.7	152.0	ND	165.8	17.2	62.7	101.4	181.4	18.4	61.0	71.6	25.7	55.3	232.0		
12	19.8	19.8	17.4	103.7	67.4	228.3	6.5	121.6	ND	128.1	13.3	54.2	96.0	163.4	22.9	49.2	56.9	1.0	44.8	174.8		
13	ND	2.2	45.2	183.6	69.2	300.3	3.6	161.3	5.2	170.1	19.9	82.7	132.2	234.8	1.4	54.0	87.6	0.6	10.7	154.3		
14	70.6	87.0	84.7	285.0	164.5	691.8	40.5	38.1	2.5	81.0	26.8	117.5	181.0	325.4	15.6	105.3	136.6	0.7	102.0	360.1		
15	ND	1.3	26.1	100.5	66.5	194.3	3.0	6.5	0.2	9.7	16.5	51.7	112.6	180.7	0.3	60.7	59.4	0.3	34.2	155.0		
16	ND	0.9	105.1	245.0	198.8	549.9	125.9	16.4	1.5	143.8	27.6	107.4	162.7	297.7	22.7	68.3	138.7	10.2	106.2	346.1		
17	9.3	5.5	27.6	117.2	70.8	230.4	6.1	156.0	1.1	163.2	22.5	79.4	140.8	242.7	109.6	71.6	77.2	0.0	27.5	286.0		
18	6.7	3.9	19.9	87.7	53.8	172.0	2.2	113.7	0.8	116.6	16.8	59.4	104.7	180.9	0.8	52.1	57.7	0.0	20.7	131.3		
Mean	32.7	34.4	42.2	117.1	89.4	315.8	24.8	136.4	0.8	162.0	18.6	76.7	115.3	210.6	21.8	60.9	78.3	9.9	60.5	231.4		

^a Not detected. Values are mean ± S.D., n = 8, *P < 0.05 significant.

Table 3: Sensory description of taste and flavor compounds of Korean fermented soybean paste samples of Doenjang.

Sensory score	Group	Sample	Sweet			Sour			Savory			Bitter		
			Sample	Mean	S.D.	Sample	Mean	S.D.	Sample	Mean	S.D.	Sample	Mean	S.D.
3.9	I	8	506.22	170.22	170.22	263.21	209.48							
3.6		3	875.07	170.01	293.31									
3.6		14	747.29	290.52	325.36									
		Mean	709.53±187.30*	210.25±69.51	241.90±151.33	81.03	276.05±59.83							
3.4	II	2	739.66	343.72	148.28									
3.3		7	665.74	125.27	296.23									
3.0		16	599.36	200.02	297.73									
		Mean	668.25±70.1*	223.01±111.0	236.08±142.06	89.58	247.41±85.85							
2.9	III	4	267.34	183.48	167.18									
2.9		5	280.70	222.84	309.90									
2.9		9	210.64	63.32	208.12									
2.9		Mean	251.71±30.46*	225.85±111.6	154.72±44.9	163.16	231.98±60.4							
2.7	IV	12	267.94	93.09	163.40									
2.6		1	158.21	264.45	75.88									
2.6		15	219.69	275.79	180.74									
2.6		Mean	181.89	237.24	116.63	9.71	180.85							
2.4	V	10	206.94±47.9	217.85±84.5	150.22±50.2	87.42±53.5	134.63							
2.3		11	385.05	128.13	181.35									
2.3		6	158.36	87.06	140.45									
2.0		Mean	251.35±114.7*	227.84±86.3	172.80±46.2	128.30±45.8								

Values are mean ± S.D., n = 8, *P < 0.05 significant.

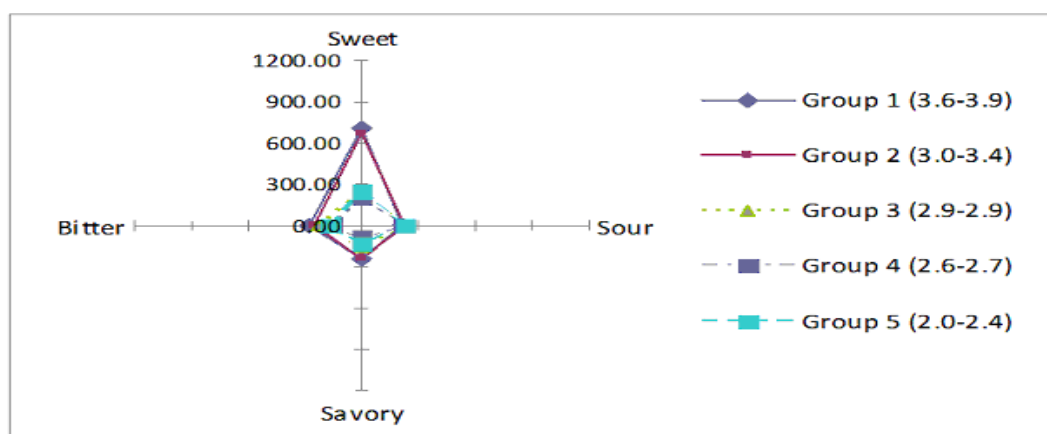


Fig. 2: Spider web diagram of sensory attributes of Korean fermented soybean paste samples of Doenjang.

78.3, 9.9 and 60.5 mg/100g, respectively (table 2). Glutamic acid was found to be the most abundant amino acid among all the tested samples of Doenjang, representing 136.4 mg/100g (table 2). However, mean values of total sweet, savory, bitter and other amino acids in our tested Doenjang samples were found to be 315.8, 162.0, 210.6 and 231.4 mg%, respectively (table 2).

Sensory evaluation

In the present study, we analyzed 18 Doenjang samples and determined their amino acid and free sugar contents responsible for sensory traits. On the basis of sensory score, we categorized 18 Doenjang samples into 5 groups (group I, II, III, IV and V) following the order from highest to lowest sensory score. As shown in table 3 and fig. 2, group I exhibited highest sensory score ranging from 3.6-3.9 followed by group II (3.0-3.4), group III (2.9), group IV (2.6-2.7) and group V (2.0-2.4). All the tested samples of Doenjang were analyzed for 4 tastes such as sweet, sour, savory and bitter. Sweet taste in Doenjang samples may contribute to the presence of amino acid and free sugar contents. Some amino acids are also responsible for savory and bitter tastes while volatile and non-volatile organic acid contents may contribute to sour taste in Doenjang samples.

Relationship of amino acid and free sugar compounds with sensory characteristics

A spider-web diagram confirms relationship between sensory attributes and taste components of Doenjang samples (fig. 2). Our results showed that isolation techniques used in this study to determine taste compounds are valid to detect quantitative differences among various Doenjang samples. Accurate determination of food components such as amino acids and free sugars in fermented foods and food products is dramatically needed for nutritional quality/safety consideration and for manufacturing requirements in terms of assessing flavor development.

DISCUSSION

Free sugars are key taste components and contribute to sweet factor of fermented foods (Kim *et al.* 1998). Park *et al.* (2002) reported free sugar contents in commercial fermented soybean paste samples using a similar extraction procedure, however their results differed from the results obtained in the present study. Further, Choi and Bajpai (2010) also reported free sugar components such as glucose, galactose, sucrose and lactose in non-germinated meju (MNG), germinated meju under light (MGD) and germinated meju under dark conditions (MGL). These findings were also differed from the findings of our study. These discrepancies could be attributed to the use of different raw material as well as variations in fermentation period, fermentation condition, microbial flora and their enzymatic activities.

Moreover, similar findings on the determination of total content of sweet amino acids (threonine, serine, glycine, alanine and lysine), savory taste amino acids (aspartic acid, glutamic acid and cysteine) and bitter taste amino acids (methionine, isoleucine and leucine) were reported by Park *et al.* (2002) in commercial fermented soybean samples. Choi and Bajpai (2010) compared amino acid contents in MNG, MGD and MGL and observed the presence of similar kinds of amino acids. The composition of total amino acids in MNG, MGD and MGL was found to be 2,611.1, 2,549.6 and 2,542 mg%, respectively. Decarboxylation of amino acids through substrate specific decarboxylase enzymes forms corresponding biogenic amine (Loukou and Zotou 2003). In the present study, it was confirmed that histidine was present in a very low amount (14.45 mg%) than other amino acids. The results from other studies have indicated that fermentation led to an overall increase in free amino acids and ammonia by 60 and 40-fold, respectively (Collar and Martinez, 1993). The net decrease in the level of some individual amino

acids after fermentation suggested that they were metabolized to a greater extent than replaced by proteolytic activity (Collar and Martinez, 1993). Similar findings were observed while sour dough fermentation indicating nutritional requirement of amino acids for bacteria and yeasts (Collar and Martinez, 1993).

Previously we determined organic acid profile of 18 Doenjang samples tested in this study (Shukla *et al.*, 2010). Finally, three samples from group I, numbered 3, 8 and 14, showing potent sensory scores than other four groups (group II, III, IV and V), were selected from 18 Doenjang samples. Their sensory profiles were obtained and are shown in fig. 2. The mean values of four sensory attributes such as sweet, sour, savory and bitter taste of group I were found to be 709.53, 210.25, 241.90 and 276.05, respectively (table 3). Veit-Kohler *et al.* (1999) found positive relationship among aroma volatiles and sensory attributes in tomatoes. In addition, Kato *et al.* (1989) confirmed increased contribution of amino acids in the taste and quality of variety of food products suggesting their eminent role in distinct flavor of Doenjang (Kato *et al.*, 1989).

As confirmed by Sousa *et al.* (2001), that glycine, alanine, serine, lysine, proline, and threonine amino acids may contribute to the sweet taste of Doenjang samples. Kim and Rhee (1993) reported occurrence of hydrolysis of soybean proteins resulting in the production of glutamic acid, aspartic acid, and nucleotides, which may also contribute to the monosodium glutamate taste of Doenjang samples. Hence, results presented in fig. 2 for the relationship of sensory attributes and taste compounds of Doenjang samples (sweet, umami, sour and bitter) could not be considered as threshold taste compound intensity. The spider-web defined discriminative preference in Doenjang samples using sensory attributes, suggesting that sensory data obtained during the process of sensory evaluation by expert panelists could be considered an important step for assessing further quality and nutritional value of Doenjang products. Quantitative comparison of desirable flavor compounds in addition to free sugars, amino acids and organic acids in Doenjang would be of great significance in selection of a better taste Doenjang.

In the present study, analysis of amino acids and free sugar contents was accomplished by the most sensitive methods employing amino acid autoanalyzer and HPLC technique. Such findings emerged with optimal and valuable outcomes on Doenjang samples that may lead to production of novel quality of Doenjang products with increased amount of amino acid and free sugar contents. Based on the above findings, it can be concluded that an accurate characterization of food components (amino acids and free sugars) and their relationship along with sensory data could be important factors to determine the

quality of fermented Doenjang products for further assessment.

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