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Analyses of Free Amino Acids in Different Parts of Bean Sprouts by Different Cooking Methods and from Different Merchants

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Abstract

This study was performed to quantitatively analyze free amino acids in the bean sprouts of three different merchants by the parts and the cooking methods in order to evaluate the nutritional and sensory values of bean sprouts. Three merchant bean sprouts were analyzed from total, cotyledon and hypocotyls. Aspartic acid was the most common acid isolated from bean sprouts from all three merchants and was found more in the hypocotyls than the cotyledon. On the other hand, arginine, the second most common amino acid, was found more in the cotyledon than the hypocotyls while valine, the third or fourth most common amino acid in total bean sprout, occurred in a greater amount in hypocotyls than in cotyledons. After cooking, was the most concentrated amino acid in the liquid portion of both boiled bean sprouts and bean sprout soup was glutamic acid. Total bean sprouts from merchant C showed significantly higher contents of the most abundant amino acids, such as aspartic acid, arginine, alanine, serine, glutamic acid, isoleucine, leucine and tyrosine (p<0.05). After cooking, bean sprouts from merchant B showed less of a decrease in amino acid content in the solid parts than the products from merchants A and C. In conclusion, aspartic acid was the major amino acid in bean sprouts, regardless of the source, but after cooking, glutamic acid became the most abundant amino acid in the liquid part. Additionally, the pattern of release of the amino acids from the solid beans to the liquid portion during cooking was different with each merchant.

Key words: bean sprout, free amino acids, amino acid analyzer, aspartic acid, soybean

INTRODUCTION

Bean sprouts are one of the most common of the traditional Korean foods, and they provide many nutritionally important components with relatively low price. The consumption of bean sprouts has been increasing since annual gross production of 650,000 tons in 1997 with industrial market value of 4000 billion Korean won (1). Currently annual gross production reaches up to about 480,000 tons (2). Along with Kimchi, bean sprouts are one of the most popular vegetables in Korea, and, in fact, rank the 3rd highest consumed vegetable among Koreans. It is well accepted that diet can affect health by various mechanisms. Dietary factors, including the essential nutrients abundant in bean sprouts, such as protein (3,4), vitamin C (5), calcium (6), dietary fiber along with aspartic acid (7) and isoflavone (8) are attracting a lot of interest and increased consumption of bean sprouts is expected.

At this time, bean sprouts are sold for all purposes in Korea without any specification as to the type of sprout that is best suited for the different cooking purposes, such as making soup, cooked vegetables (namul), or making seasoned bean sprout dishes. In most cases, the quality of the bean sprout is often determined only by its outward appearance, without any objective and scientific quality control standards. The need to develop scientifically based, objective standards of determining the quality of a bean sprout and its best cooking use has been raised. Amino acid analyses are useful for establishing such standards since bean sprouts are a very good source of various amino acids. Many investigations (9-11) have been conducted to determine total amino acid content, although free amino acids in bean sprouts would be a greater contributor to flavor quality.

Therefore, this study analyzed the free amino acid content of bean sprouts in two different parts of the plant (cotyledon and hypocotyl) and from three different merchants. In addition to providing an evaluation of the nutritional values in different parts of bean sprouts, the results of this study provide baseline data for establishing standardized flavor components indices and a quality index. Koreans' most beloved bean sprout dishes were also analyzed to keep high standardized

quality for boiled bean sprouts and bean sprout soup.

MATERIALS AND METHODS

Materials

Three different commercial brands of bean sprout manufactured on the same day of analyses were purchased from a local market in Asan. All three different merchants used Poongsan bean and bean sprouts from all three merchants were cultivated for 5 to 6 days at 20°C. Purchased bean sprouts were washed with running tap water for 3 times and air-dried. The bean sprout was then separated into its components of cotyledon and hypocotyls with hair root. Three samples of bean sprouts from different merchants were washed, separated and freeze-dried, then analyzed as either total sprout, cotyledon or hypocotyl. The cooked bean sprouts were also analyzed after boiling for 3 min and the addition of ground green onion and garlic into the cooking water part. Boiled bean sprouts were prepared by putting 100 g bean sprout into 1 L of boiling water containing 0.4% salt and cooking for 3 min with a lid closed. After 3 min of cooking the solid parts were separated from the liquid part and the solid parts were washed promptly with cold water followed by air-drying. The solid parts were freeze-dried before analyses and the liquid parts were frozen and kept at -20°C until analyses. Bean sprout soup was prepared by putting 100 g of bean sprouts into 750 mL of boiling water, along with 3 g of table salt, 1/2 teaspoon of chopped garlic and 1/4 teaspoon of chopped green onion and boiling the soup with ingredients for 9 more min. After the additional boiling, the solid parts of the bean sprout soup were separated from the liquid part and the water was allowed to drip from the solid parts for 30 sec on top of the sieved mesh. The solid parts were then freeze-dried and the liquid part of the soup was frozen at kept at -20°C until the analyses.

Analyses of free amino acids

Half a gram of freeze-dried samples of each part were ground with mortar and pestle after 10 mL of 3% Trichloroacetic acid (TCA) solution was added. Ten mL of TCA solution and sea sand were added more as the sample was grounded and homogenized with vortex mixing. The homogenized sample was extracted with magnetic stirring bar at room temperature with lithium citrate buffer from freeze-dried bean sprouts after precipitate proteins with 3% trichloroacetic acid. After extraction, the sample was centrifuged at 15,000 rpm for 15 min and the supernatant was collected. The supernatant was diluted with lithium citrate dilution buffer (pH 2.3) and made up to 50 mL and injected into the amino acid analyzer after filtering through 0.45 µm syringe filter (2). Different dilution factors were applied depends on the sample parts.

The amino acid analyzer used was S7130, S5200, S2100 amino acid analyzer (Sykamm, Germany) and the columns used were cation separation column (LCA K07/Li 4.6×150 mm, Sykamm, Germany), ammonia filtration column (LCA K05/Li 4.6×100 mm, Sykamm,

Table 1. Operating conditions and data acquisition parameters of amino acid analyzer for the analyses of bean sprouts

Flow rate - Reactor 0.25 mL/min Pump 0.45 mL/min

Gas - Helium Quaternary Delivery Pump S 2100 Gradient Program B²⁾ (%) $C^{3)}$ (%) D⁴⁾ (%) $A^{1)}$ (%) Step Time (min) Flow (mL/min) 0 0 0.45 100 0 0 0 1 13.0 0.45 100 0 0 0 2 0.45 79.0 21.0 0 0 1.0 3 22.0 0.45 79.0 21.0 0 0 4 0 11.0 0.45 52.0 48.0 0 5 20.0 0.45 0 100 0 0 6 0 100 5.0 0.45 0 0 7 8.0 0.45 0 0 100 0 8 4.0 0.45 0 0 78 88 0.45 9 21.0 0 0 75 25 10 0.1 0.45 0 0 0 100 0 0 0 100 0.45 11 4.012 0.1 0.45 100 0 0 0 13 20.0 0.45 0 0 Total 129.2

¹⁾A: lithium citrate buffer A-1 0.12 N; pH 2.90. ²⁾B: lithium citrate buffer B-1 0.30 N; pH 4.20. ³⁾C: lithium citrate borate buffer C-4 0.30 N; pH 8.0. ⁴⁾D: regeneration solution 0.5 N.

Table 2. Free amino acid contents in the total bean sprouts by different merchants (dry basis, mg/100 g)

	A	В	С
Aspartic acid	241.8 ± 4.3^{b}	198.6 ± 1.8^{c}	412.9 ± 2.0^{a}
Arginine	$77.9 \pm 0.4^{\mathrm{b}}$	85.9 ± 1.5^{a}	90.9 ± 4.2^{a}
Alanine	70.1 ± 1.4^{c}	57.8 ± 0.2^{b}	52.1 ± 1.9^{a}
Valine	61.8 ± 1.4^{a}	43.8 ± 0.8^{c}	52.1 ± 0.1^{b}
Histidine	53.7 ± 1.6^{a}	40.0 ± 0.6^{b}	40.6 ± 1.4^{b}
Phenylalanine	52.6 ± 1.9	43.5 ± 0.8	47.0 ± 8.8
Serine	$44.5 \pm 1.4^{\text{b}}$	38.8 ± 0.4^{c}	48.5 ± 1.7^{a}
Glutamic acid	29.4 ± 9.9^{b}	32.2 ± 7.4^{b}	$58.0 \pm 3.3^{\text{a}}$
Threonine	39.1 ± 1.2^{a}	29.1 ± 0.9^{c}	33.2 ± 0.1^{b}
Isoleucine	37.5 ± 1.2^{a}	27.3 ± 1.1^{b}	36.6 ± 0.0^{a}
Leucine	$34.9 \pm 0.8^{\rm b}$	23.6 ± 0.6^{c}	60.8 ± 1.1^{a}
Tyrosine	16.7 ± 0.3^{b}	9.6 ± 0.2^{c}	24.7 ± 2.2^{a}
Glycine	15.4 ± 0.3^{a}	13.6 ± 0.2^{b}	9.6 ± 0.7^{c}
Tryptophan	13.5 ± 9.7	25.1 ± 9.6	26.9 ± 9.9
Methionine	5.1 ± 0.1^{b}	$4.3 \pm 0.2^{\rm b}$	9.6 ± 1.3^{a}
Cystine	1.9 ± 0.1^{b}	2.3 ± 0.4^{b}	6.7 ± 0.8^{a}

Means without a common superscript letter differs (p<0.05) with ANOVA and Tukey's post-hoc test.

Germany). Mobile phases were lithium citrate buffer (0.25 mL/min) with different pH's, and the flow rate of ninhydrin reagent was 0.5 mL/min with injection volume of 100 μ L. The temperature of the reactor was 130°C, and the temperature of the column was 54 \sim 85°C. All samples were analyzed in triplicate and the modified analysis condition (2) is described in Table 1.

The results of triplicates assays of the sprouts from the three different merchants were compared with ANOVA and a Tukey's post-hoc test.

RESULTS

The results of free amino acid analysis from total bean sprouts in different merchants were listed in Table 2. Free amino acid contents were also analyzed in the separate parts of bean sprouts (Tables 3 and 4) and compared by the different parts in Fig. 1. Although some amino acid levels differed from each merchant, aspartic acid was the most abundant amino acid in all three samples and arginine was the second most abundant amino acid. Aspartic acid was found most in both cotyledon and hypocotyls parts and arginine was found most in the cotyledon part while only small amounts were analyzed in the hypocotyls part of bean sprouts of three merchants. Except for the major two free amino acids, aspartic acid and arginine, valine, phenylalanine, serine and alanine were found in similar concentrations in the total sprout, although more of them were found in the hypocotyls regardless of the merchants; however, the order of concentration of each amino acid did differ by merchant. Merchant A showed significantly higher amount of as-

Table 3. Free amino acid contents in the cotyledon parts of bean sprouts by different merchants (dry basis, mg/100 g)

	A	В	C
Aspartic acid	115.0 ± 2.8^{c}	254.8 ± 2.2^{b}	354.1 ± 3.2^a
Arginine	94.9 ± 1.5^{c}	$109.4 \pm 6.3^{\mathrm{b}}$	153.0 ± 5.8^{a}
Glutamic acid	$37.2 \pm 6.3^{\text{b}}$	$35.9 \pm 6.4^{\rm b}$	64.9 ± 5.5^{a}
Phenylalanine	$26.1 \pm 0.7^{\rm b}$	$35.5 \pm 5.1^{\text{b}}$	46.9 ± 5.4^{a}
Serine	$26.5 \pm 0.1^{\mathrm{b}}$	28.5 ± 1.5^{b}	44.8 ± 8.5^{a}
Histidine	30.7 ± 1.0	32.0 ± 0.5	41.2 ± 8.6
Valine	20.5 ± 0.6^{b}	$26.0 \pm 4.4^{\text{b}}$	35.8 ± 8.7^{a}
Leucine	$22.3 \pm 0.6^{\circ}$	27.1 ± 2.4^{b}	34.2 ± 1.9^{a}
Isoleucine	15.5 ± 0.7	21.1 ± 4.8	29.3 ± 9.0
Alanine	20.9 ± 0.3	22.8 ± 2.3	26.4 ± 6.2
Threonine	15.2 ± 0.2	19.1 ± 3.1	24.3 ± 6.6
Tryptophan	9.1 ± 7.8^{b}	22.9 ± 2.9^{a}	18.4 ± 0.4^{b}
Tyrosine	11.2 ± 0.0	18.4 ± 3.7	21.8 ± 6.7
Glycine	7.2 ± 0.2	15.8 ± 5.5	10.8 ± 3.9
Methionine	4.1 ± 0.3	9.5 ± 3.9	8.4 ± 4.3
Cystine	3.1 ± 0.3	7.4 ± 4.4	7.0 ± 3.9

Means without a common superscript letter differs (p<0.05) with ANOVA and Tukey's post-hoc test.

Table 4. Free amino acid contents in the hypocotyls parts of bean sprouts by different merchants (dry basis, mg/100 g)

	A	В	С
Aspartic acid	$343.1 \pm 2.0^{\circ}$	346.9 ± 2.8^{b}	531.3 ± 2.6^{a}
Alanine	125.0 ± 1.1^{b}	167.7 ± 0.3^{a}	74.5 ± 1.1^{c}
Valine	101.8 ± 0.5^{c}	123.3 ± 0.6^{a}	$109.9 \pm 2.4^{\mathrm{b}}$
Serine	$68.8 \pm 1.5^{\text{b}}$	94.8 ± 0.2^{a}	91.5 ± 2.5^{a}
Phenylalanine	79.0 ± 2.2^{b}	90.9 ± 1.3^{a}	58.2 ± 2.7^{c}
Histidine	$69.6 \pm 1.7^{\text{b}}$	77.4 ± 0.9^{a}	43.6 ± 1.5^{c}
Threonine	$62.2 \pm 2.8^{\text{b}}$	71.6 ± 0.9^{a}	44.3 ± 1.9^{c}
Isoleucine	59.2 ± 2.6^{b}	$68.4 \pm 1.3^{\text{a}}$	45.0 ± 1.2^{c}
Glutamic acid	12.8 ± 1.0^{c}	27.7 ± 1.1^{b}	54.4 ± 8.7^{a}
Leucine	37.0 ± 0.7^{a}	28.2 ± 0.2^{b}	$21.9 \pm 7.1^{\text{b}}$
Arginine	22.9 ± 0.1^{a}	6.3 ± 0.1^{b}	$6.3 \pm 0.3^{\rm b}$
Tryptophan	21.6 ± 0.9^{a}	3.9 ± 0.0^{c}	13.1 ± 4.2^{b}
Tyrosine	14.2 ± 0.1	7.2 ± 0.0	16.9 ± 8.3
Glycine	10.0 ± 9.9	11.9 ± 0.1	6.1 ± 1.2
Methionine	2.0 ± 1.8^{b}	$2.9 \pm 0.1^{\rm b}$	8.0 ± 2.3^{a}
Cystine	0.8 ± 0.0^{a}	0.4 ± 0.0^{b}	nd ¹⁾

1)nd: not detected.

Means without a common superscript letter differs (p<0.05) with ANOVA and Tukey's post-hoc test.

partic acid, arginine, alanine, serine, glutamic acid, isoleucine, leucine, tyrosine, methionine and cystine in the total bean, while contents of valine, histidine, threonine and glycine were significantly lower than the bean sprouts of the other merchants (p<0.05). Overall, merchant C showed more variations and different tendencies of amino acids distributions while merchants A and B showed the similar tendency of distribution in the total bean sprout. Results from the cotyledons were quite different from the results of the total bean especially for the amino acid with lower concentrations, such as isoleucine, alanine, threonine, tyrosine, glycine, methionine and cysteine. These amino acid contents were not sig-

Fig. 1. Comparison of major free amino acid contents by different parts in bean sprouts of merchants A, B, and C.

nificantly different among the three merchants. Tryptophan content was significantly higher in the merchant B (p<0.05) while other major amino acids such as aspartic acid, arginine, valine histidine, phenylalanine, serine and leucine were significantly higher in the cotyledon parts of merchant C. Aspartic acid content in the hypocotyls of merchant C was significantly higher than merchants A and B as the total and cotyledon parts, but alanine, valine, serine, phenylalanine, histidine, threonine and isoleucine were significantly higher in merchant B than in merchants A and C (p<0.05). Other amino acids showed insignificant differences between the different merchants.

Table 5. Free amino acid contents in the liquid parts of the boiled bean sprouts and comparison by different merchants (mg/100 mL)

	Α	В	C
	A	D	
Glutamic acid	386.6 ± 1.1^{a}	387.9 ± 2.5^a	381.8 ± 0.5^{b}
Aspartic acid	$169.2 \pm 0.1^{\rm b}$	$155.2 \pm 0.3^{\circ}$	185.6 ± 0.9^{a}
Alanine	53.4 ± 0.6^{b}	53.9 ± 0.3^{b}	57.5 ± 0.6^{a}
Serine	$36.5 \pm 0.1^{\circ}$	$37.8 \pm 0.3^{\text{b}}$	44.1 ± 0.3^{a}
Valine	$40.4 \pm 1.1^{\mathrm{b}}$	41.6 ± 1.2^{b}	$43.5 \pm 1.0^{\text{a}}$
Arginine	29.8 ± 0.1^{c}	35.7 ± 0.1^{a}	32.1 ± 0.5^{b}
Phenylalanine	$27.7 \pm 0.5^{\mathrm{b}}$	$28.1 \pm 0.4^{\text{b}}$	31.8 ± 0.4^{a}
Isoleucine	24.1 ± 0.1^{c}	26.1 ± 0.5^{b}	27.9 ± 0.2^{a}
Threonine	24.9 ± 0.4^{b}	23.8 ± 0.1^{c}	27.2 ± 0.0^{a}
Histidine	25.2 ± 0.4^{a}	23.5 ± 0.2^{b}	25.5 ± 0.3^{a}
Leucine	17.4 ± 0.7^{c}	$20.0 \pm 0.1^{\rm b}$	21.9 ± 0.5^{a}
Tyrosine	10.1 ± 2.2	8.0 ± 0.4	11.3 ± 0.6
Glycine	7.4 ± 0.1^{a}	7.5 ± 0.1^{a}	7.1 ± 0.0^{b}
Methionine	1.6 ± 0.1^{b}	1.7 ± 0.0^{a}	1.5 ± 0.1^{b}
Tryptophan	0.7 ± 0.0^{a}	0.7 ± 0.1^{a}	0.4 ± 0.1^{b}
Cystine	0.7 ± 0.0^{a}	0.5 ± 0.0^{b}	0.5 ± 0.0^{b}

Means without a common superscript letter differs (p<0.05) with ANOVA and Tukey's post-hoc test.

The contents of free amino acids after boiling were also analyzed and the results are summarized in Tables 5 and 6. The amounts of free amino acids released into the liquid portion of the boiled bean sprouts were similar in all three different merchants. Therefore, if bean sprouts were boiled for 3 min, most amino acids stayed in the solid part and minor amounts were released into the boiling water. Kinds and contents of amino acids released into the boiling water varied dependent on the merchants, but amino acid released into the liquid part did not seem to be occur with any significance within 3 min of boiling. Sprouts from all three merchants released glutamic acid the most during boiling process.

Table 6. Free amino acid contents in the solid parts of the boiled bean sprouts and comparison by different merchants (dry basis, mg/100 g)

	A	В	С
Aspartic acid	112.5 ± 0.8^{c}	150.3 ± 0.1^{b}	199.6 ± 0.9^{a}
Glutamic acid	50.5 ± 1.2^{a}	$47.3 \pm 0.2^{\text{b}}$	$46.7 \pm 1.8^{\text{b}}$
Arginine	49.3 ± 7.6^{a}	33.0 ± 0.0^{b}	$37.6 \pm 0.8^{\mathrm{b}}$
Alanine	$30.7 \pm 0.2^{\circ}$	$35.6 \pm 0.0^{\rm b}$	37.6 ± 0.8^{a}
Valine	$24.8 \pm 2.6^{\rm b}$	26.1 ± 0.0^{b}	32.9 ± 2.8^{a}
Phenylalanine	$20.5 \pm 0.3^{\mathrm{b}}$	$20.7 \pm 0.0^{\mathrm{b}}$	29.8 ± 0.8^{a}
Histidine	$21.9 \pm 0.2^{\rm b}$	26.5 ± 0.0^{a}	26.9 ± 0.8^{a}
Serine	$19.8 \pm 0.2^{\mathrm{b}}$	23.3 ± 0.0^{a}	$20.4 \pm 0.6^{\mathrm{b}}$
Threonine	14.1 ± 0.1^{c}	17.4 ± 0.0^{a}	15.4 ± 0.5^{b}
Isoleucine	15.6 ± 0.2^{b}	16.5 ± 0.0^{a}	$15.2 \pm 0.4^{\rm b}$
Leucine	12.7 ± 0.2^{a}	10.1 ± 0.0^{b}	11.8 ± 0.8^{a}
Tryptophan	11.3 ± 0.1^{a}	11.4 ± 0.1^{a}	10.0 ± 0.5^{b}
Tyrosine	$5.8 \pm 0.0^{\rm b}$	$5.3 \pm 0.0^{\circ}$	6.7 ± 0.3^{a}
Glycine	5.2 ± 0.1^{a}	$3.9 \pm 0.0^{\rm b}$	4.1 ± 0.2^{b}
Methionine	2.3 ± 0.0^{a}	$1.6 \pm 0.0^{\rm b}$	1.9 ± 0.2^{b}
Cystine	0.3 ± 0.1^{c}	3.2 ± 0.0^{b}	4.3 ± 0.3^{a}

Means without a common superscript letter differs (p<0.05) with ANOVA and Tukey's post-hoc test.

Table 7. Free amino acid contents in the liquid parts of the bean sprout soups and comparison by different merchants (mg/100 mL)

			(mg/100 mL)
	A	В	С
Glutamic acid	494.9 ± 1.6^{a}	454.1 ± 4.7^{b}	454.5 ± 1.6^{b}
Aspartic acid	$327.1 \pm 7.3^{\mathrm{b}}$	331.9 ± 2.8^{b}	382.9 ± 1.5^{a}
Arginine	208.6 ± 2.4^{a}	$198.4 \pm 1.5^{\text{b}}$	208.7 ± 3.4^{a}
Alanine	106.5 ± 0.1^{b}	108.0 ± 0.4^{b}	114.4 ± 1.7^{a}
Serine	81.3 ± 1.0^{c}	$86.5 \pm 0.4^{\rm b}$	103.6 ± 0.1^{a}
Valine	$86.8 \pm 1.0^{\circ}$	90.7 ± 0.8^{b}	100.0 ± 0.0^{a}
Phenylalanine	$660 \pm 0.0^{\circ}$	70.4 ± 0.2^{b}	77.1 ± 0.2^{a}
Histidine	60.8 ± 2.2^{b}	62.7 ± 0.2^{b}	68.7 ± 1.5^{a}
Isoleucine	55.7 ± 0.4^{c}	62.6 ± 0.1^{b}	66.7 ± 1.0^{a}
Threonine	53.2 ± 0.3^{b}	49.6 ± 0.1^{c}	58.6 ± 0.8^{a}
Leucine	44.6 ± 0.2^{c}	55.4 ± 1.5^{a}	51.4 ± 0.3^{b}
Tyrosine	26.0 ± 0.1	26.6 ± 2.1	26.2 ± 1.4
Glycine	16.4 ± 0.1^{b}	18.3 ± 0.2^{a}	$15.7 \pm 0.0^{\circ}$
Methionine	5.0 ± 0.2^{b}	5.4 ± 0.1^{a}	4.7 ± 0.0^{b}
Tryptophan	2.6 ± 0.2	2.7 ± 0.1	2.7 ± 0.1
Cystine	2.0 ± 0.1^{a}	1.8 ± 0.0^{b}	1.5 ± 0.2^{b}

Means without a common superscript letter differs (p<0.05) with ANOVA and Tukey's post-hoc test.

Table 8. Free amino acid contents in the solid parts of the bean sprout soups and comparison by different merchants (dry basis, mg/100 g)

	A	В	С
Aspartic acid	186.8 ± 2.0^{c}	267.0 ± 1.2^{a}	195.8 ± 2.2^{b}
Glutamic acid	$59.4 \pm 0.2^{\circ}$	85.0 ± 0.4^{a}	$68.6 \pm 0.6^{\mathrm{b}}$
Arginine	46.4 ± 0.6^{b}	49.7 ± 0.2^{a}	49.1 ± 0.2^{a}
Alanine	17.7 ± 0.4^{b}	29.4 ± 0.7^{a}	30.0 ± 0.2^{a}
Serine	$20.8 \pm 0.2^{\rm c}$	29.5 ± 0.0^{a}	25.4 ± 0.4^{b}
Valine	18.2 ± 0.2^{c}	27.6 ± 0.1^{a}	21.8 ± 0.3^{b}
Threonine	12.9 ± 0.7^{c}	26.1 ± 0.5^{a}	23.3 ± 0.3^{b}
Histidine	19.5 ± 0.9^{b}	22.4 ± 0.2^{a}	19.4 ± 0.2^{b}
Phenylalanine	16.4 ± 0.5^{c}	22.4 ± 0.2^{a}	$19.5 \pm 0.2^{\mathrm{b}}$
Isoleucine	13.9 ± 0.2^{c}	18.7 ± 0.3^{a}	15.5 ± 0.1^{b}
Tyrosine	$8.8 \pm 0.2^{\rm c}$	13.3 ± 0.2^{a}	14.8 ± 0.2^{a}
Leucine	$9.9 \pm 0.3^{\rm c}$	14.0 ± 0.2^{a}	13.1 ± 0.1^{b}
Tryptophan	11.2 ± 0.6	11.6 ± 0.5	10.4 ± 0.7
Glycine	3.0 ± 0.2^{b}	7.5 ± 0.1^{a}	7.2 ± 0.2^{a}
Methionine	$nd^{1)}$	6.7 ± 0.2^{b}	7.3 ± 0.1^{a}
Cystine	nd	2.7 ± 0.4	2.4 ± 0.2

nd: not detected.

Means without a common superscript letter differs (p \leq 0.05) with ANOVA and Tukey's post-hoc test.

On the other hand, aspartic acid, the most abundant amino acid in bean sprouts, released the least from merchant B, although the original contents of aspartic acid in total bean sprout parts of merchant B was significantly lower than the other merchants (p < 0.05).

When the bean sprout was cooked as soup, free amino acid contents in both liquid and solid parts were analyzed and shown in Tables 7 and 8. Bean sprout soup was cooked longer than the boiled bean sprout and seasonings such as some green onions and garlic were added. Therefore the results of free amino acid contents in the

Fig. 2. Comparison of major free amino acid contents by different cooking methods in bean sprouts of merchants A, B, and C.

liquid and solid parts of bean sprout soup were different from the boiled bean sprouts. Moreover, more amino acids were analyzed in the liquid part of the soup for 9 min of cooking compared to the boiled water after three min of boiling (Fig. 2). However, similar amounts of free amino acids were analyzed in the solid parts when cooked both as soup and boiling was applied as a cooking method. More cooking time and added seasonings would contribute more free amino acids contents in the liquid parts of the soup compared with 3 min of boiling water.

DISCUSSION

Free amino acid content analyses were conducted on bean sprouts (total sprout and the cotyledon and hypocotyls components) from three different merchants to acquire baseline data for establishing standardized flavor component indices and a quality index. The order of the free amino acid concentrations in total sprouts were, from greatest to least, aspartic acid, arginine, alanine, valine, phenylalanine, histidine, serine, glutamic acid, isoleucine, threonine, leucine, tyrosine, tryptophane, glycine, methionine and cystine. However, amino acids other than the six mentioned in the results section as being the most common showed varying concentrations, depending on the different merchants. This discrepancy observed among the different merchants was more distinct when the concentrations of the amino acids were compared by the different parts of bean sprouts, and could be attributable to the different cultivating methods of each merchants, although all three merchants used the same kind of bean (Poongnam). Unfortunately, it was not possible to get specific information on the growing methods used by each merchant. The only information available was the cultivation dates. Many investigators (12-16) reported that different bean species used for bean sprout show different quality levels, sprouting conditions, growth characteristics and physiological changes. Therefore, the main contributor to the free amino acid content of bean sprout is the bean seed used for the sprouting, even with the same Poongnam bean; however, when and where to harvest would be other factors that contribute to the differences. A follow-up study will analyze and compare the free amino acids content according to the different bean seed species used for sprouting.

For the present study, we freeze-dried the samples before analyses and the contents of free amino acids were expressed as mg%. The results of the present study showed somewhat higher free amino acid contents compared to the other studies reported to date (2,10,11, 16-18). Since the purpose of the present study is to analyze free amino acids contents from the different bean sprout merchants and compare them after different cooking methods, we chose to freeze-dry the samples for the consistency and convenience of storage before the pretreatment of all samples. Thus, the higher contents analyzed in the present study than the previously reported studies were likely due to the different samples, analyses methods and pretreatment methods used. Usually, amino acids analyses were conducted after acid digestion, which releases amino acids. During the processes of acid elimination and concentration that take place before injection of samples into the analyzer, some amino acid

loss is expected. We chose to analyze free amino acid content rather than total amino acids, since our purpose was to identify the factors affecting flavor components indices and quality indexes, which are better explained by free amino acids (19).

When the amino acids contents were analyzed after boiling for 3 min, some amino acids contents observed in the solid parts were lower than the contents from the total part. Glutamic acid was the most possibly released amino acids from the solid parts out of free amino acids analyzed, although amounts of glutamic acid found in boiling water were different by the merchants. Overall, merchant B showed the least release from the solid parts after 3 min of boiling although the original amino acid contents before cooking were less in the merchant B than the other two merchants. When bean sprout soup was cooked, more amino acids were analyzed in the liquid parts of the soup. Free amino acid contents in the liquid portion of the soup are expected be higher than in plain boiling water because soup making requires a longer cooking time (9 min) and some seasonings were added. Both liquid and solid parts of soup are consumed, and the relatively higher concentration of free amino acids found in the liquid part of the soup is not considered to be nutrients losses.

Nutritional values of beans while in the seed stage and their beneficial health effects, as well as their nutritional values, are well documented (20-25). During the sprouting process of bean seeds, lipid contents diminish while protein, vitamins A and C and fiber contents increase, and carbohydrates transform into more easily digested monosaccharides (26,27). Therefore, more research is being proposed for developing the second generation bean products in the US and the western European countries, where the consumption of bean products is much lower than in the Asian countries (28-31). A more specific and systemized research focused on bean and bean sprout products are also essential and urgent in Korea, as well as other Asian countries. Asians have consumed bean products for a long time without fully investigating their many nutritional benefits. The present study focused on providing the basal data of bean sprouts to help develope standardized flavor indices and nutritional values for the expected increase of bean product consumption. In conclusion, we found that aspartic acid is the most abundant amino acid in all parts of bean sprout, regardless of the source. Aspartic acid concentration was higher in the hypocotyl than in the cotyledon. The next most common amino acid was arginine, which was found more in the cotyledon than in the hypocotyls while the third most abundant free amino acid was valine, which was found more in the hypocotyls than in the cotyledon part. Glutamic acid is the amino acid released most from the solid parts into the liquid part while cooking, either boiling plain or making soup, regardless of the merchant. Sprouts from merchant B showed the least amount of glutamic acid release while cooking, although the original aspartic acid contents were less than those of merchants A and C. Differences found from the different merchants might be attributable to the different methods used for sprouting, even if all three merchants used Poongnam bean. Therefore, follow-up studies are underway to compare free amino acid contents of different soybean seeds cultivated under identical methods.

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