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Effect of Steaming, Blanching, and High Temperature/High Pressure Processing on the Amino Acid Contents of Commonly Consumed Korean Vegetables and Pulses

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ABSTRACT: In the present report, the effects of blanching, steaming, and high temperature/high pressure processing (HTHP) on the amino acid contents of commonly consumed Korean root vegetables, leaf vegetables, and pulses were evaluated using an Automatic Amino Acid Analyzer. The total amino acid content of the samples tested was between 3.38 g/100 g dry weight (DW) and 21.32 g/100 g DW in raw vegetables and between 29.36 g/100 g DW and 30.55 g/100 g DW in raw pulses. With HTHP, we observed significant decreases in the lysine and arginine contents of vegetables and the lysine, arginine, and cysteine contents of pulses. Moreover, the amino acid contents of blanched vegetables and steamed pulses were more similar than the amino acid contents of the HTHP vegetables and HTHP pulses. Interestingly, lysine, arginine, and cysteine were more sensitive to HTHP than the other amino acids. Partial Least Squares-Discriminate Analyses were also performed to discriminate the clusters and patterns of amino acids.

Keywords: amino acids, vegetables, pulses, Automatic Amino Acid Analyzer, partial least squares-discriminate analysis (PLS-DA)

INTRODUCTION

Amino acids and their derivatives are significant biochemical compounds that form the building blocks of proteins, neurotransmitters, hormones, and nucleic acids and play a major role in nutrition, medicine, and plant protection. Among the amino acids, L-threonine (Thr), L-valine (Val), L-methionine (Met), L-isoleucine (Ile), L-leucine (Leu), L-phenylalanine (Phe), L-lysine (Lys), and L-tryptophan are designated as essential amino acids. The nutritive value of proteins is determined by the types and amounts of amino acids they contain. Awareness of amino acid content is important when assessing the protein quality of foods, determining protein structures, and identifying essential and limiting amino acids (1,2).

Vegetables and pulses have been consumed as side dishes in Korea and other East Asian countries since ancient times. Pulses are highly nutritional foods that have a protein content of 20∼35% on dry weight basis and contain lipids, sugars, crude fiber, calcium, vitamin B_1, and vitamin B_2 (3). Vegetables are also an important part of a healthy diet; it is estimated that sufficient vegetable consumption could save up to 2.7 million lives worldwide (4).

Vegetables and pulses are typically marketed and consumed in a variety of processed, rather than raw, forms. They are easily available in fully- or semi-processed forms such as easy-to-store instant foods; however, over-processing vegetables degrades their texture and destroys the nutrients that vegetables contain. Hence, it is important to process vegetables in a less destructive and more effective manner that maintains their nutrient content. During food processing, protein sources are affected by a variety of factors, including heat, oxidizing agents, organic solvents, alkalis, and acids (5,6). These factors can lead to the desulfuration, deamination, and/or isomerization of proteins, which reduces amino acid levels and affects the nutritive value of proteins (7). Thus, it is essential to evaluate the effects of processing conditions on the amino acid contents of vegetables and pulses.

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The field of amino acid analysis has grown rapidly since Moore (8) successfully used partition chromatography to separate amino acids. Recently, the development of an automatic analyzer greatly improved the speed and accuracy of amino acid analysis. The purpose of the present study was to evaluate the amino acid contents of six vegetable species (i.e., carrot, onion, garlic, crown daisy, cabbage, and perennial artemisia) and four pulse species (i.e., Daepung, Sunyu, Heukmi, and Cheongja 3) that are commonly consumed as side dishes in Korea. The vegetable species were processed by blanching or high temperature/high pressure processing (HTHP), and the pulses were processed by steaming or HTHP. The effects of these processing methods on the amino acid contents of vegetables and pulses were compared.

MATERIALS AND METHODS

Materials

Mature vegetable and pulses were purchased in three Korean cities: carrot, crown daisy, cabbage, Daepung, Sunyu, Heukmi, and Cheongja 3 were purchased in Suwon; onion and garlic were purchased in Mokpo; and perennial artemisia was purchased in Ganghwa, Korea.

Reagents and standards

All solvents were HPLC grade and purchased from Thermo Fisher Scientific, Inc. (Bremen, Germany). The amino acids mixture standard solution (0.1 mg/L), cysteic acid (98%), and 6 N hydrochloric acid (HCl) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Methionine sulfone (98%) was purchased from Alfa Aesar (Ward Hill, MA, USA). Hydrogen peroxide [30%, guaranteed reagent (GR)] and formic acid (98%, GR) were purchased from Junsei Chemical Co., Ltd. (Kyoto, Japan).

Sample processing conditions

The dried raw vegetables and pulses were blanched for 2∼15 min at 90°C (3,5) and steamed for 20 min at 125°C, respectively. HTHP was performed with an autoclave (A100, Sejung, Seoul, Korea) set to 125°C for two hours under hydraulic conditions (20 MPa). All the samples were lyophilized (LP-20, Ilshin Lab Co., Ltd., Seoul, Korea), and the freeze-dried samples were milled with a domestic grinder and stored in a freezer at −60°C until use.

Pretreatment of samples

Oxidation: For each sample, 10 mL of performic acid (1 mL of 30% H_2O_2 and 9 mL of formic acid) was added 30 mg of crude protein. The mixtures were incubated for 17 h in a refrigerator set to <5°C. After 17 h, each mixture was evaporated to dryness under N_2 to yield an oxidized sample (3,6-8).

Hydrolysis: 30 mg of crude protein from each sample was hydrolyzed by adding 40 mL of 6 N HCl. Each sample was covered with a N_2 blanket to prevent explosion due to oxygen liberation. Then, the samples were incubated for 22 h in an oven set to 110°C. After 22 h, the cold-released hydrolyzed samples were centrifuged at 3,000 rpm for 5 min, and 2 mL of the supernatant was collected and evaporated to dryness at 70°C. Then, 2 mL of sodium citrate dehydrate in water (pH 2.2) was mixed with the dried sample, and the hydrolyzed sample was filtered with 0.22 μm syringe filter with a hydrophilic PTFE membrane (Advantec, Hyundai Micro Co., Ltd. Seoul, Korea). The eluent was collected and stored until analysis (3,6-9).

Amino acid analysis

A Hitachi L-8900 Automatic Amino Acid Analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan) with a 4.6 mm (ID)×60 mm ion exchange column (Hitachi High-Technologies Corporation) was used to determine the amino acid profiles of the vegetable and pulse samples. The following analyzer settings were used for the analysis: buffer flow rate of 0.4 mL/min, reagent flow rate of 0.35 mL/min, reactor heater temperature of 135°C, column temperature of 75°C, auto-sampler temperature of 5∼8°C, run time of 35.3 min (sulfur-containing amino acids) or 56.3 min (all other amino acids), sample injection volume of 20 μL, and detection wavelength of 570 nm (proline) or 440 nm (all other amino acids). Protein hydrolysate buffer set (PH-SET KANTO, Kanto Chemical Co., Inc., Tokyo, Japan) and hydrochloric acid (Wako Pure Chemical Industries, Ltd.) were used as mobilephase solvents (10,11). A standard amino acid mixture of cysteic acid and methionine sulfone (20 μL/mL) was diluted to 100 μmol/L for amino acid quantification and calibration. An external standard was used to calculate the concentration of each amino acid. Amino acid concentrations are reported in g/100 g.

Statistical analysis

SIMCA P+ (version 11, MKS Umetrics AB, Umea, Sweden), a multivariate analysis software, was used to perform Partial Least Squares-Discriminant Analysis (PLS-DA) and acquire Variable Importance in the Projection (VIP) data.

RESULTS AND DISCUSSION

The effect of different processing methods on crude protein content was analyzed in this study (Table 1). The

Table 1. Average crude protein content and 30 mg equivalent of crude protein in root vegetables, leaf vegetables, and pulses prepared by different processing methods

Sample		Processing method	Average crude protein content (%)	30 mg equivalent of crude protein (mg)
Root vegetables	Carrot	Raw	4.08	7.36
		Blanching	4.43	6.78
		HTHP	4.24	7.07
	Onion	Raw	8.96	3.35
		Blanching	9.22	3.26
		HTHP	10.30	2.91
	Garlic	Raw	21.49	1.40
		Blanching	23.72	1.26
		HTHP	21.47	1.40
Leaf vegetables	Crown daisy	Raw	24.37	1.23
		Blanching	27.40	1.09
		HTHP	24.88	1.21
	Cabbage	Raw	12.26	2.45
		Blanching	13.50	2.22
		HTHP	11.90	2.52
	Artemisia capillaris	Raw	13.24	2.27
		Blanching	13.22	2.27
		HTHP	15.32	1.96
Pulses	Daepung	Raw	34.54	0.09
		Steaming	36.52	0.08
		HTHP	36.12	0.08
	Sunyu	Raw	35.11	0.09
		Steamed	36.73	0.08
		HTHP	36.98	0.08
	Heukmi	Raw	34.78	0.09
		Steaming	37.89	0.08
		HTHP	37.01	0.08
	Cheongja 3	Raw	35.94	0.08
		Steaming	39.05	0.08
		HTHP	38.32	0.08

results indicate that the crude protein content of the vegetable species tested varies widely (4.08~27.40% protein), while the protein content of pulses is very consistent (34.54~39.05% protein). Among the vegetables and processing methods tested, the blanched crown daisy had the highest crude protein content (27.40%), and the raw carrot had the lowest crude protein content (4.08%). Among the pulses and processing methods tested in this study, the steamed Cheongja 3 had the highest crude protein content (39.05%) and the raw daepung had the lowest crude protein content (34.54%). Young and Pellett (12) reported a mean protein content of 36.5% in beans, 1.2% in cabbage, 1.0% in carrot, and 1.2% in onion. Similarly, Woo et al. (13) reported that the mean amino acid content of beans was 40% (measured with ion-exchange chromatography). The crude protein and amino acid contents of the pulses (i.e., beans) analyzed in the present study are similar those reported by others; however, the total protein and amino acid contents of the vegetables analyzed in this study are slightly higher than previously reported. The crude protein content of blanched vegetables and steamed pulses was slightly higher than the crude protein content of raw vegetables and pulses, and there was a large difference in the amino acid content among each 30 mg of crude protein.

In the present evaluation, the total amino acid content of each of the raw vegetables and pulses was 3.38 g/100 g of carrot, 6.61 g/100 g of onion, 16.33 g/100 g of garlic, 21.32 g/100 g of crown daisy, 9.02 g/100 g of cabbage, 11.30 g/100 g of perennial artemisia, 29.36 g/100 g of Daepung, 29.84 g/100 g of Sunyu, 29.57 g/100 g of Heukmi, and 30.55 g/100 g of Cheongja 3. The total amino acid content of each blanched vegetable was 3.82 g/100 g of carrot, 6.41 g/100 g of onion, 18.53 g/100 g of garlic, 23.69 g/100 g of crown daisy, 10.27 g/100 g of cabbage, and 9.50 g/100 g of perennial artemisia. The total amino acid content of each of the steamed pulses was 31.04 g/100 g of Daepung, 31.22 g/100 g of Sunyu, 32.21 g/100 g of Heukmi, and 33.19 g/100 g of Cheongja 3. The total amino acid content of each of the HTHP vegetables and pulses was 2.78 g/100 g of carrot, 6.41 g/100 g of onion, 18.53 g/100 g of garlic, 23.69 g/100 g of crown daisy, 6.94 g/100 g of cabbage, 10.64 g/100 g of perennial artemisia, 30.70 g/100 g of Daepung, 31.43 g/100 g of Sunyu, 31.50 g/100 g of Heukmi, and 32.57 g/100 g of Cheongja 3.

With respect to individual amino acids, the aspartic acid (Asp) and glutamic acid (Glu) contents of the vegetables were 1.3 g/100 g (12.1%) and 2.0 g/100 g (21.3%), respectively, and the Asp and Glu contents of the pulses were 3.7 g/100 g (11.8%) and 5.8 g/100 g (18.5%),

Table 2. A comparison of the amino acid contents of root vegetables, leaf vegetables, pulses prepared by different processing methods

Sample		Processing method	Amino acid (%)																	Total amino acid content (g/100 g DW)
			Ile	Leu	Lys	Met	Cys	Phe	Tyr	Thr	Val	His	Arg	Ala	Asp	Glu	Gly	Pro	Ser	
Root vegetables	Carrot	Raw	4.32	6.34	6.49	0.67	1.34	4.04	2.16	4.05	5.26	1.75	4.04	12.63	14.64	20.40	3.94	3.15	4.78	3.38
		Blanching	4.42	6.84	7.15	0.94	1.10	4.19	2.31	4.28	5.45	1.85	4.36	11.51	14.35	18.71	4.24	3.37	4.93	3.82
		HTHP	5.22	7.91	0.87	0.89	1.04	4.97	2.61	4.19	6.55	1.72	0.71	14.34	12.79	22.76	5.00	3.85	4.59	2.78
	Onion	Raw	2.78	5.48	6.33	0.79	1.34	3.95	1.83	2.84	3.14	1.90	19.84	2.98	8.53	30.97	3.03	1.52	2.75	6.61
		Blanching	3.05	5.68	6.65	0.90	1.44	3.90	1.88	2.82	3.43	1.82	19.88	3.09	8.93	28.55	3.25	1.69	3.02	6.41
		HTHP	3.91	6.36	3.16	0.81	1.89	4.47	2.16	2.83	4.54	1.36	3.84	5.84	9.06	41.03	3.99	1.67	3.07	4.59
	Garlic	Raw	2.20	4.02	4.11	0.69	1.38	3.69	2.85	2.53	4.28	1.63	28.50	2.36	16.23	18.65	2.83	1.36	2.69	16.33
		Blanching	2.86	5.33	4.35	0.86	1.91	3.71	3.74	3.19	4.95	1.65	23.93	3.21	14.84	16.40	3.48	1.90	3.68	18.53
		HTHP	3.31	6.07	2.34	0.95	3.75	3.97	3.75	3.30	5.94	1.66	8.37	5.49	14.43	26.68	4.12	1.90	3.98	9.79
Leaf vegetables	Crown daisy	Raw	5.09	9.50	8.08	1.22	1.37	5.56	1.84	5.03	6.24	3.04	6.06	6.24	11.20	14.08	5.80	5.17	4.48	21.32
		Blanching	5.27	9.91	7.97	1.37	1.32	5.77	2.10	5.09	6.37	2.97	6.09	6.43	10.70	12.94	5.96	5.26	4.47	23.69
		HTHP	5.71	10.47	3.68	1.31	0.95	6.52	2.49	5.27	6.86	2.24	4.20	7.27	11.68	14.65	6.46	5.71	4.52	20.16
	Cabbage	Raw	3.82	6.44	7.15	1.49	1.98	3.56	2.32	4.23	5.15	2.70	5.90	5.78	10.77	26.17	4.03	3.55	4.95	9.02
		Blanching	3.93	6.72	7.44	1.43	1.85	3.73	2.47	4.31	5.30	2.44	5.88	5.48	10.81	25.51	4.14	3.64	4.91	10.27
		HTHP	4.98	8.45	2.55	1.71	2.16	4.92	3.22	5.03	6.66	2.31	1.66	8.40	10.61	22.00	5.08	5.10	5.16	6.94
	Perennial artemisia	Raw	4.83	8.89	8.02	0.68	0.67	5.27	3.32	5.21	6.14	2.36	5.80	5.86	12.96	14.48	5.09	5.80	4.61	11.30
		Blanching	5.07	9.37	8.51	0.93	0.60	5.33	3.27	5.44	6.36	2.20	5.61	6.25	11.29	14.33	5.32	5.33	4.78	9.50
		HTHP	5.15	9.57	2.68	1.11	0.80	5.83	3.32	5.45	6.95	1.48	4.46	6.90	13.82	15.59	5.73	6.31	4.84	10.64
Pluses	Daepung	Raw	4.83	8.29	6.73	1.44	2.13	4.92	3.51	4.13	4.82	2.57	7.56	4.43	11.63	18.41	4.28	5.18	5.14	29.36
		Steaming	4.82	8.30	6.67	1.36	2.11	4.94	3.51	4.14	4.81	2.59	7.54	4.44	11.51	18.53	4.29	5.24	5.19	31.04
		HTHP	5.00	8.61	5.55	1.40	1.34	5.25	3.59	4.28	4.94	2.77	5.87	4.64	11.95	19.50	4.48	5.49	5.36	30.70
	Sunyu	Raw	4.69	7.96	6.75	1.40	2.21	4.91	3.17	4.16	4.77	2.58	7.74	4.47	11.71	18.66	4.34	5.27	5.23	29.84
		Steaming	4.93	8.38	6.74	1.40	2.17	4.99	3.63	4.06	4.84	2.60	8.47	4.55	11.44	17.76	4.32	4.70	5.01	31.22
		HTHP	4.92	8.50	5.28	1.41	1.38	5.22	3.37	4.27	4.81	2.78	6.48	4.87	12.05	19.20	4.51	5.61	5.32	31.43
	Heukmi	Raw	4.74	8.16	6.70	1.37	2.30	4.85	3.44	4.10	4.58	2.49	8.26	4.40	11.87	18.01	4.30	5.31	5.11	29.57
		Steaming	4.71	8.17	6.64	1.34	2.24	4.85	3.48	4.10	4.55	2.50	8.32	4.43	11.80	18.10	4.28	5.34	5.15	32.21
		HTHP	4.97	8.53	5.38	1.41	1.44	5.16	3.56	4.28	4.76	2.78	6.49	4.65	12.13	19.05	4.50	5.62	5.30	31.50
	Cheongja 3	Raw	4.89	8.12	6.72	1.40	2.39	4.85	3.07	3.98	4.84	2.57	8.60	4.39	11.79	17.95	4.20	5.32	4.91	30.55
		Steaming	4.93	8.33	6.61	1.41	2.20	4.91	3.43	3.98	4.85	2.57	8.63	4.38	11.64	17.74	4.21	5.32	4.87	33.19
		HTHP	4.96	8.59	5.25	1.40	1.37	5.20	3.47	4.17	4.82	2.75	6.88	4.69	12.04	19.14	4.43	5.59	5.26	32.57

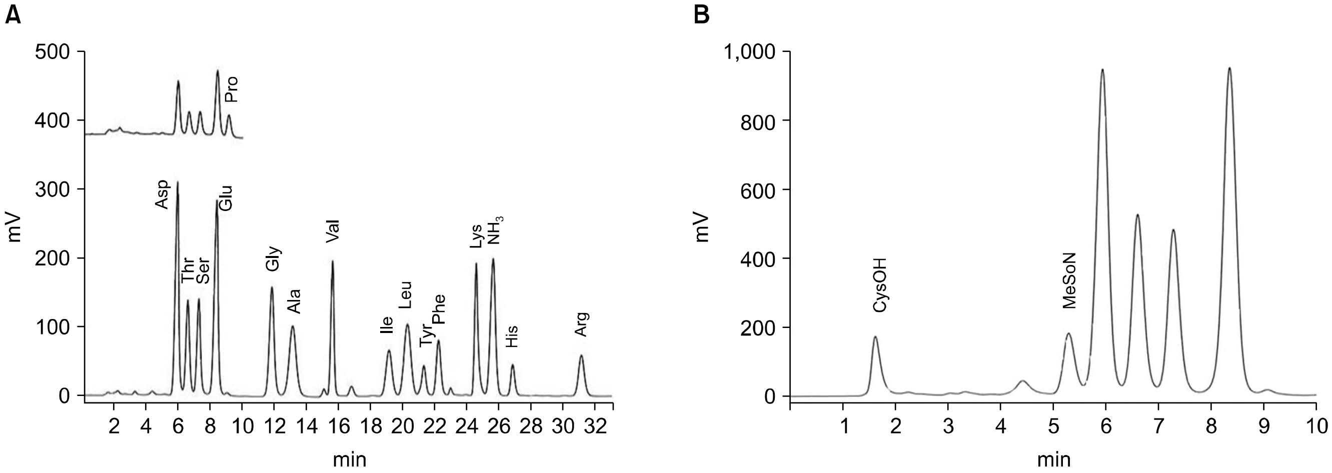

Fig. 1. Chromatogram of individual amino acids: (A) stable amino acids in perennial artemisia extract, (B) sulfur containing amino acids (CysOH: cysteic acid, MeSoN: methionine sulfone) in crown daisy extract.

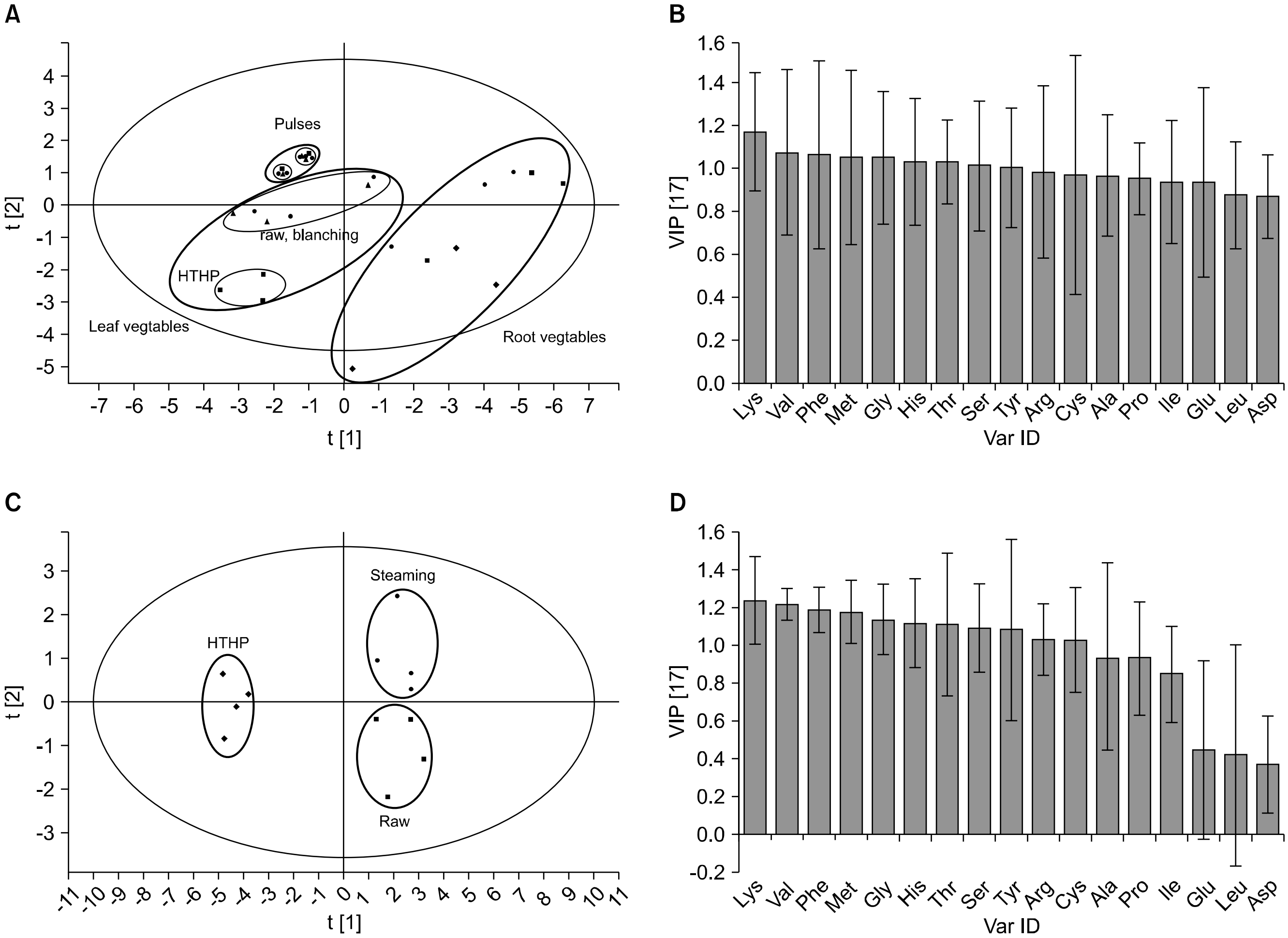

Fig. 2. The SIMCA PLS-DA score scatter plots of the amino acid concentrations of 6 vegetable species (root vegetables and leaf vegetables) and 4 pulse species: (A) total mapping of the vegetables and pulses; PC1=0.45, PC2=0.18, (B) VIP values of the vegetables and pulses, (C) specific mapping of the pulses; PC1=0.67, PC2=0.09, (D) VIP values of the pulses.

respectively. Lisiewska et al. (14) reported that Asp and Glu were the most prevalent amino acids in kale and leaf vegetable species. Similarly, we found that the Asp and Glu concentrations of vegetables and pulses were higher than the concentrations of any other amino acid. The cysteine (Cys) and Met contents were 0.1 g/100 g (1.0%) and 0.2 g/100 g (1.5%), respectively, in vegetables and 0.4 g/100 g (1.4%) and 0.6 g/100 g (1.9%), respectively, in pulses (Table 2, Fig. 1).

Adibi (2) reported that the plasma levels of branched-chain amino acids (BCAA, i.e., Leu, Ile, and Val) were more influenced by a day of starvation than other amino acids. In the present study, the Leu, Ile, and Val concentrations of HTHP vegetables and pulses were slightly higher than the BCAA concentrations of raw vegetables and pulses. In contrast, the Lys and arginine (Arg) concentrations of HTHP vegetables and pulses were markedly lower than the Lys and Arg concentrations of raw vegetables and pulses. This finding is consistent with a similar report (15) indicating that Lys is more sensitive to heat than other amino acids and that extrusion cooking, which is similar to HTHP is associated with Lys, Cys, and Arg losses.

A multivariate statistical analysis was used for more effective classification and for determination of the effect of processing method on the composition of each vegetable and pulse. Fig. 2 shows the PLS-DA scatter plots and graphical representations of the VIP values prepared by the SIMCA software. We explored PLS techniques for selecting variables (i.e., VIPs), that contributed to the separation observed on the PLS-DA scatter plots (16). Fig. 2A shows a PLS-DA plot for all of the vegetables and pulses tested in this study; distinct clusters of leaf vegetables, root vegetables, and pulses are apparent. The sum of principal component 1 (PC1) and principal component 2 (PC2) is 0.64, which indicates that PC1 and PC2 account for 64% of the total variance. As shown in Fig. 2B, Lys had the highest VIP value (1.17), followed by Val (1.07), Phe (1.06), Met (1.05), Gly (1.05), His (1.03), and Thr (1.04).

Fig. 2C shows a detailed PLS-DA plot for the pulses tested in this study. It should be noted that the raw and blanched processing methods form a single cluster, which implies that there is no significant difference in the amino acid compositions of raw and blanched pulses. The HTHP pulses form a separate cluster, which is indicative of a definite difference in the amino acid composition of pulses that have been prepared by HTHP. The sum of PC1 and PC2 is 0.75, indicating that PC1 and PC2 account for 75% of the total variance. As shown in Fig. 2D, Lys had the highest VIP value (1.24), followed by Cys (1.22), Phe (1.19), histidine (His) (1.17), glycine (Gly) (1.14), Arg (1.12), alanine (Ala) (1.11), Glu (1.09), Asp (1.08), Leu (1.03), Thr (1.03), proline (Pro) (0.93), serine (Ser) (0.93), and Ile (0.85). In HTHP pulses, the Lys, Cys, and Arg contents were decreased, but the Phe, His, Gly, Ala, Glu, Asp, Leu, Thr, Pro, Ser, and Ile contents were increased. Consequently, the most important variables for forming the HTHP cluster depicted in Fig. 2C are the individual amino acid increases and decreases that take place during HTHP.

In conclusion, the amino acid contents of commonly consumed Korean root vegetables, leaf vegetables, and pulses prepared by blanching, steaming, or HTHP were determined by automatic amino acid analysis. Seventeen amino acids were examined. The total amino acid content ranged from 3.38 g/100 g to 21.32 g/100 g in raw vegetables and from 29.36 g/100 g to 30.55 g/100 g in raw pulses. HTHP was associated with significant decreases in the Lys and Arg contents of vegetables and the Lys, Cys, and Arg contents of pulses. Interestingly, the amino acid patterns of blanched vegetables and steamed pulses are similar to that of raw vegetables and raw pulses, respectively. PLS-DA was also performed to discriminate the clusters and patterns of amino acids in raw and processed vegetables and pulses.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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