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Free Amino Acid Composition of Korean Spinach (*Spinacia oleracea*) Cultivars as Influenced by Different Harvesting Time

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Abstract

BACKGROUND: There is lack of comprehensive compositional data of the amino acid profile of spinach with regard to different cultivars. A more detailed knowledge in this aspect will be of benefit in the future selection of spinach genotypes with improved nutritional quality.

METHODS AND RESULTS: The effects of cultivar type (Jeoncheonhu, Sagyejul, Namdongcho and Mustang) and harvest time (79th, 116th and 145th days after sowing or DAS) on the concentrations of free amino acids in field-grown spinach (*Spinacia oleracea*) were examined. About 35 different free amino acids were detected and quantified by the amino acid analyzer. Glutamic acid and proline were identified as the major amino acids, while α -amino adipic acid and α -aminobutyric acid were present in much lower concentrations. Spinach constituted 1468.4 mg/100 g total free amino acids (TAA), of which essential amino acids, neutral/acidic amino acids and sulphur containing amino acids constituted around 15, 45 and 2% of the TAA, respectively. The most limiting amino acids among the leafy vegetables – cysteine was recorded only in Mustang harvested at 116 DAS. Free amino acid contents did not differ significantly among the spinach cultivars and also at different harvest times.

CONCLUSION: The data show that, either of the spinach cultivars, preferably Mustang harvested on or after 116 DAS can serve as a significant source of nutritionally relevant amino acids to meet the demand of the growing populations.

Key words: Amino acid, Cultivar, Essential, Harvest time, Non-essential, Spinach

Introduction

Spinach (*Spinacia oleracea*) is one of the widely used nutrient rich leafy vegetables with a global production area and amount of nearly 890,000 ha and 14,000,000 ton, respectively (Citak and Sonmez, 2010). In Korea, spinach is the only leafy vegetable cultivated in open-field condition by farmers during winter season. A steady rise in consumer demand has been reflected by an expansion of Korean spinach production reaching 84170 ton in 2014 with a total production area of 5645 ha (KOSIS, 2014). Mostly, spinach has a high content of vitamins, organic acids, minerals, proteins and antioxidants of nutritive value. Of all the compounds, amino acids are the most important constituent of any plant products since some of the amino acids for e.g., histidine, lysine, leucine, isoleucine, methionine, phenylalanine, threonine and valine cannot be synthesized in human body and it is essential to obtain these from natural foodstuffs

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(Lisiewska *et al.*, 2011).

The chemical composition of horticultural crops are modified by several factors, among them, the type of cultivar and harvest times are the most important ones. Beis *et al.* (2002) stated that the level of amino acids in spinach levels was dependent on the age of leaves. Wu *et al.* (2007) reported that, depending on the type of cultivars, differences in the content of horticultural crops were significant. Much of the spinach related studies were focused mainly on its chemical composition depending on the dose of fertilizers applied and amount of light (Budwalda *et al.*, 2000). However, there is lack of comprehensive compositional data of its amino acid profile with regard to different cultivars. Nowadays four cultivars of spinach are commonly planted in Korea, including *Spinacia oleracea* cv. Jeoncheonhu, Sagyejul, Namdongcho and Mustang. So far, no data have been reported on the amino acid composition of these cultivars cultivated in Korea. Therefore, this research was focused on the analysis and comparison of the free amino acids of different spinach cultivars harvested at different times. A more detailed knowledge of the variability among the cultivars will be of benefit in the future selection of spinach genotypes with improved nutritional quality.

Materials and Methods

Materials

The investigated material consisted of leaves of spinach cultivars Jeoncheonhu, Sagyejul, Namdongcho and Mustang. The spinach was conventionally grown in an experimental field at Namhae-gun, Gyeongsangnam-do, South Korea (34° 46'N, 127° 57'E) between October 2014 and February 2015. The soil at this site had pH 7.1, EC 0.36 dS/m, total carbon 1.86%, total nitrogen (N) 0.16% and available phosphorous (P) 215.87 mg/kg. Exchangeable cations - potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}) and sodium (Na^+) were 0.86, 11.81, 2.83 and 0.16 cmol./kg soil, respectively. The land was prepared in a randomized manner consisting of three replicates and fertilized with inorganic fertilizers by adopting basal application and top-dressing. Urea, super phosphate and potassium chloride were the sources of N, P and K, respectively. Basal application of N, P and K fertilizers were at a rate of 10, 5.9 and 7.9 kg/ha. One dose of K fertilizer (4 kg/ha) was top dressed after one month of sowing.

The spinach received N fertilizer as top-dress at a rate of 5 kg/ha/month till the end of the harvest season. Harvest dates were chosen based on the leaf length (first harvest was made when the leaf length was over 7 cm). The 1st, 2nd and 3rd harvests were scheduled on the 79th, 116th and 145th days after sowing (DAS). On each harvest dates, yellow and damaged leaves were removed. The remaining leaves were hand-washed to render them free of soil, freeze dried and ground to a fine powder using liquid nitrogen. After powdering, the four cultivars of Korean spinach analyzed in this paper were vacuum packed in small bags and stored at -20°C until all assays were performed. All results represent averages of triplicate determinations and are expressed on dry weight basis.

Amino acids extraction and analysis

The content of amino acids were determined according to procedures described by Ishimoto *et al.* (2010) with some modifications. The L-8900 high-speed amino acid analyzer (AAA) (Hitachi High-Tech. Corp., Japan) was used for quantitative determination of the amino acid composition. Free amino acids from each spinach cultivar samples (0.10 g) were extracted by vigorous shaking for 1 h with 100 ml of 3% sulfosalicylic acid. The suspensions were then centrifuged at 3,500 rpm for 10 min at 4°C, and the resulting pellets were subjected to four additional rounds of extraction. The five extracts were combined, filtered with 0.45 mm syringe filter and analyzed for the soluble or free amino acids by the ninhydrin method using AAA. The calculations were carried out according to the external standard.

Statistical analyses

Statistical analyses allowing a mean comparison of the content of amino acids in the four spinach cultivars at the 0.05 probability level was carried out using Duncan's multiple range test (DMRT) using SAS software version 9.4 (SAS Institute Inc.).

Results and Discussion

Quality of horticultural crops is generally determined from the relative levels of several chemical constituents such as soluble sugars, vitamins, fatty acids and amino acids considered together. Selection of the right cultivar and harvest time are crucial for the production of high-quality crops (Wu *et al.*, 2007).

Table 1. Amino acid profile of four spinach cultivars in mg/100 g on dry weight basis

Amino acid	1 st harvest (79DAS)				2 nd harvest (116DAS)				3 rd harvest (145DAS)				Mean	SD	CV%
	JCH	SGJ	NDC	MST	JCH	SGJ	NDC	MST	JCH	SGJ	NDC	MST			
Major amino acids															
<i>Essential</i>															
Histidine	18.8 ± 0.6	16.1 ± 0.6	9.9 ± 0.3	18.0 ± 0.7	6.0 ± 0.1	9.1 ± 0.8	12.0 ± 0.4	10.3 ± 0.2	13.4 ± 0.1	18.4 ± 0.1	15.2 ± 0.2	10.3 ± 0.1	13.1	4.1	0.3
Isoleucine	20.6 ± 1.5	24.1 ± 0.5	20.2 ± 0.8	21.3 ± 0.8	17.0 ± 0.8	16.2 ± 1.8	22.1 ± 0.8	18.2 ± 1.2	22.4 ± 0.8	27.9 ± 0.2	25.3 ± 0.4	22.2 ± 0.1	21.5	3.3	0.1
Leucine	37.8 ± 2.5	28.4 ± 0.9	25.7 ± 0.9	32.6 ± 1.3	29.4 ± 0.5	25.5 ± 1.8	35.9 ± 1.1	31.9 ± 1.1	42.2 ± 0.7	46.7 ± 0.3	41.8 ± 0.4	40.1 ± 0.2	34.8	6.9	0.2
Lysine	23.4 ± 2.6	18.9 ± 0.8	15.5 ± 0.5	17.0 ± 0.6	22.8 ± 0.3	18.6 ± 1.4	26.9 ± 1.4	23.5 ± 0.6	29.2 ± 0.3	33.6 ± 0.3	29.5 ± 0.3	30.1 ± 0.1	24.1	5.8	0.2
Methionine	16.3 ± 1.5	24.8 ± 1.8	15.5 ± 0.7	18 ± 0.6	14.7 ± 1.3	16.0 ± 1.1	17.0 ± 0.4	14.3 ± 1.1	17.6 ± 1.3	27.9 ± 0.2	22.7 ± 0.4	20.7 ± 0.3	18.8	4.3	0.2
Phenylalanine	20.7 ± 2.1	17.4 ± 0.3	15.8 ± 1.3	17.4 ± 0.8	23.8 ± 1.2	18.1 ± 1.1	24.7 ± 0.8	22.2 ± 0.5	25.3 ± 0.1	27.4 ± 0.5	26.0 ± 0.5	24.2 ± 0.3	21.9	3.9	0.1
Threonine	19.8 ± 2.0	17.0 ± 0.6	15.3 ± 0.6	18.4 ± 0.7	16.4 ± 0.1	14.2 ± 1.2	20.7 ± 0.9	16.7 ± 0.4	20.0 ± 0.2	22.5 ± 0.2	21.4 ± 0.4	18.3 ± 0.1	18.4	2.5	0.1
Valine	56.5 ± 1.9	55.0 ± 1.7	49.2 ± 1.8	58.2 ± 2.1	34.4 ± 0.6	38.5 ± 1.2	53.1 ± 3.5	41.4 ± 3.5	51.7 ± 0.6	54.0 ± 0.3	57.0 ± 1.2	45.2 ± 0.5	49.5	7.8	0.1
<i>Non-essential</i>															
Alanine	62.9 ± 4.6	55.6 ± 6.1	62.5 ± 2.2	81.4 ± 4.0	40.1 ± 0.8	42.8 ± 3.5	57.5 ± 1.9	60.2 ± 1.6	54.3 ± 0.9	53.8 ± 0.5	64.2 ± 1.1	50.1 ± 0.2	57.1	10.7	0.1
Aspartic acid	148.3 ± 5.8	95.3 ± 8.2	157.0 ± 2.8	157.6 ± 5.8	91.8 ± 0.7	115.3 ± 11.1	108.2 ± 1.2	132.6 ± 3.8	67.0 ± 2.5	84.5 ± 1.9	122.5 ± 3.5	74.5 ± 0.4	112.9	31.4	0.2
<i>Conditionally essential</i>															
Arginine	135.1 ± 2.2	62.8 ± 2.9	57.1 ± 1.6	92.9 ± 3.0	21.2 ± 0.3	53.3 ± 3.9	64.3 ± 2.2	43.6 ± 1.9	88.5 ± 1.5	94.2 ± 0.8	75.7 ± 1.5	39.8 ± 0.3	69.0	30.5	0.4
Cysteine	nd	nd	nd	nd	nd	nd	nd	10.1 ± 2.9	nd	nd	nd	nd	0.8	2.9	3.4
Glutamic acid	474.8 ± 9.0	404.7 ± 5.0	431.6 ± 12.4	623.5 ± 21.0	231.5 ± 10	221.8 ± 22.1	302.0 ± 7.2	271.3 ± 6.2	236.8 ± 10.2	282.2 ± 9.4	292.1 ± 3.1	167.8 ± 3.4	328.4	130.5	0.4
Tyrosine	18.1 ± 1.5	20.7 ± 1.0	12.7 ± 0.9	16.2 ± 0.7	17.1 ± 1.1	14.1 ± 1.1	18.4 ± 0.7	16.9 ± 0.8	20.7 ± 0.4	23.3 ± 0.4	20.2 ± 0.4	18.9 ± 0.1	18.1	2.9	0.1
Glycine	15.2 ± 0.6	18.3 ± 0.9	11.3 ± 0.4	19.0 ± 0.7	8.8 ± 0.2	7.6 ± 0.7	8.9 ± 0.3	8.1 ± 0.2	9.9 ± 0.2	10.7 ± 0.1	8.5 ± 0.3	8.3 ± 0.1	11.2	4.0	0.3
Ornithine	5.3 ± 2.3	2.0 ± 0.1	1.1 ± 0.1	1.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.9 ± 0.2	0.2 ± 0.1	0.7 ± 0.1	0.7 ± 0.02	0.4 ± 0.01	0.4 ± 0.04	1.2	1.4	1.1
Proline	595.0 ± 7.4	752.7 ± 28.0	655.3 ± 17.1	955.2 ± 39.1	88.9 ± 1.6	153.7 ± 13.4	121.1 ± 2.5	169.6 ± 4.4	71.9 ± 1.4	115.5 ± 2	57.1 ± 0.8	104.2 ± 5.2	320.1	322.0	1.0
Serine	51.4 ± 2.5	44.0 ± 1.6	41.7 ± 1.1	55.2 ± 1.9	28.2 ± 0.5	34.0 ± 3.8	38.9 ± 0.9	41.7 ± 1.1	42.6 ± 0.7	50.4 ± 0.3	53.8 ± 0.6	41.4 ± 0.2	43.6	8.0	0.1
Minor amino acids															
<i>Essential</i>															
α-Aminoadipic acid	0.7 ± 0.4	nd	nd	nd	0.5 ± 0.2	nd	1.3 ± 0.1	nd	nd	nd	nd	nd	0.2	0.4	1.9
<i>Non-essential</i>															
γ-Aminobutyric acid	216.6 ± 8.4	267.0 ± 29.0	213.0 ± 5.8	250.5 ± 14	146.9 ± 9	132.2 ± 15.2	179.4 ± 4.1	247.4 ± 9.4	277.4 ± 4.2	260.7 ± 3.1	251.5 ± 5.1	242.5 ± 5.6	223.8	47.6	0.2
Phosphoserine	10.8 ± 3.2	6.0 ± 3.5	16.4 ± 0.8	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.7	5.4	1.9
Phosphoethanolamine	38.4 ± 3.7	39.9 ± 3.8	44.6 ± 3.4	35.0 ± 2.8	31.9 ± 0.4	34.9 ± 3.1	37.5 ± 5.3	35.4 ± 2.5	43.3 ± 1.2	44.0 ± 0.5	34.1 ± 4.5	30.4 ± 3.7	37.4	4.7	0.1
Cystathionine	1.2 ± 0.7	2.1 ± 0.6	3.6 ± 0.3	3.7 ± 0.1	nd	nd	1.3 ± 0.8	1.8 ± 0.5	nd	nd	nd	nd	1.1	1.4	1.2
β-Aminoisobutyric acid	2.2 ± 0.8	3.7 ± 1.5	nd	0.6 ± 0.2	7.4 ± 4.3	0.1 ± 0.1	2.2 ± 1.1	0.4 ± 0.3	nd	nd	2.6 ± 1.2	0.5 ± 0.3	1.6	2.2	1.3
β-Alanine	4.6 ± 0.8	5.7 ± 1.6	4.6 ± 0.2	3.9 ± 0.5	5.7 ± 1.3	1.9 ± 0.5	8.5 ± 2.3	4.9 ± 0.7	4.6 ± 0.01	5.0 ± 0.8	6.9 ± 0.4	5.7 ± 0.2	5.2	1.6	0.3
Sarcosine	4.9 ± 0.1	4.7 ± 0.7	2.4 ± 0.1	4.7 ± 0.2	2.6 ± 0.9	2.7 ± 0.1	2.3 ± 0.1	2.0 ± 0.6	2.8 ± 0.1	3.3 ± 0.1	3.2 ± 0.5	2.1 ± 0.05	3.1	1.0	0.3
1-Methylhistidine	0.6 ± 0.4	0.9 ± 0.3	nd	0.5 ± 0.1	nd	0.7 ± 0.2	3.6 ± 0.1	2.1 ± 0.1	1.8 ± 0.1	4.8 ± 0.8	4.6 ± 0.4	3.1 ± 0.4	1.9	1.7	0.9
3-Methylhistidine	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-
Carnosine	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-
Anserine	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-
α-Aminobutyric acid	1.1 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.8 ± 0.2	0.9 ± 0.1	1.1 ± 0.1	1.1 ± 0.02	1.3 ± 0.04	0.6 ± 0.2	1.1	0.2	0.2
Ethanolamine	18.9 ± 0.3	18.9 ± 0.7	16.4 ± 0.4	17.3 ± 0.7	17.0 ± 0.4	15.4 ± 1.6	16.8 ± 0.3	20.2 ± 0.6	19.6 ± 0.1	22.0 ± 0.2	20.4 ± 0.1	16.4 ± 0.1	18.3	2.0	0.1
Hydroxyproline	0.3 ± 0.1	0.1 ± 0.1	0.02 ± 0.1	0.1 ± 0.1	0.9 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.7 ± 0.1	0.6 ± 0.01	0.4 ± 0.04	0.6 ± 0.04	0.4	0.2	0.5
Conditionally essential															
Taurine	nd	nd	nd	1.3 ± 0.5	nd	nd	nd	0.9 ± 0.6	nd	nd	nd	0.7 ± 0.4	0.2	0.4	1.8
Citrulline	8.5 ± 1.8	5.8 ± 0.1	4.3 ± 0.2	7.4 ± 0.3	3.4 ± 0.1	4.5 ± 0.2	3.9 ± 0.1	5.2 ± 0.1	7.2 ± 0.01	6.6 ± 0.1	5.3 ± 0.5	4.7 ± 0.5	5.6	1.5	0.2

JCH - Jeoncheonhu; SGJ - Sagyejul; NDC - Namdongcho; MST - Mustang; nd - not detected; Data represent means ± standard error of three determinations

The free amino acid composition of the field-grown spinach cultivars Jeoncheonhu, Sagyejul, Namdongcho and Mustang harvested at designated time intervals (79th, 116th and 145th DAS) are presented in Table 1.

Nearly 35 different free amino acids were detected by AAA and quantified (Table 2; Fig. 1). The free amino acid levels were not variously distributed amongst both the spinach cultivars and at different harvest

Table 2. Free amino acid analyzer details of this study

Peak no.	Retention time (min)	Amino acid	
		Abbreviation	Trivial name
1	1.79	P-Ser	Phosphoserine
2	2.20	Tau	Taurine
3	3.14	PEA	Phosphoethanolamine
4	11.46	Asp	Aspartic acid
5	16.48	Thr	Threonine
6	18.11	Ser	Serine
7	22.88	Glu	Glutamic acid
8	28.19	Sar	Sarcosine
9	33.18	Pro	Proline
10	35.75	α -AAA	α -Amino adipic acid
11	37.75	Gly	Glycine
12	39.49	Ala	Alanine
13	41.08	Cit	Citrulline
14	42.42	α -ABA	α -Aminobutyric acid
15	45.16	Val	Valine
16	46.01	Cys	Cysteine
17	47.63	Met	Methionine
18	48.60	Cysthi	Cystathionine
19	50.80	Ile	Isoleucine
20	52.42	Leu	Leucine
21	54.56	Tyr	Tyrosine
22	58.42	Phe	Phenylalanine
23	62.57	β -Ala	β -Alanine
24	64.49	β -AiBA	β -Aminoisobutyric acid
25	69.47	γ -ABA	γ -Aminobutyric acid
26	77.30	AE	Ethanolamine
27	89.68	Hyp	Hydroxyproline
28	93.01	Orn	Ornithine
29	97.54	Lys	Lysine
30	101.04	1-Mehis	1-Methylhistidine
31	102.51	His	Histidine
32	108.16	3-Mehis	3-Methylhistidine
33	110.92	Ans	Anserine
34	114.31	Car	Carnosine
35	118.94	Arg	Arginine

times, and this could certainly be witnessed in the coefficient of variation percentage (CV%) which was quite low ranging from 0.1 to 3.4. Glutamic acid (22.4% of the total contents of free amino acids) and proline (21.8% of the total contents of free amino

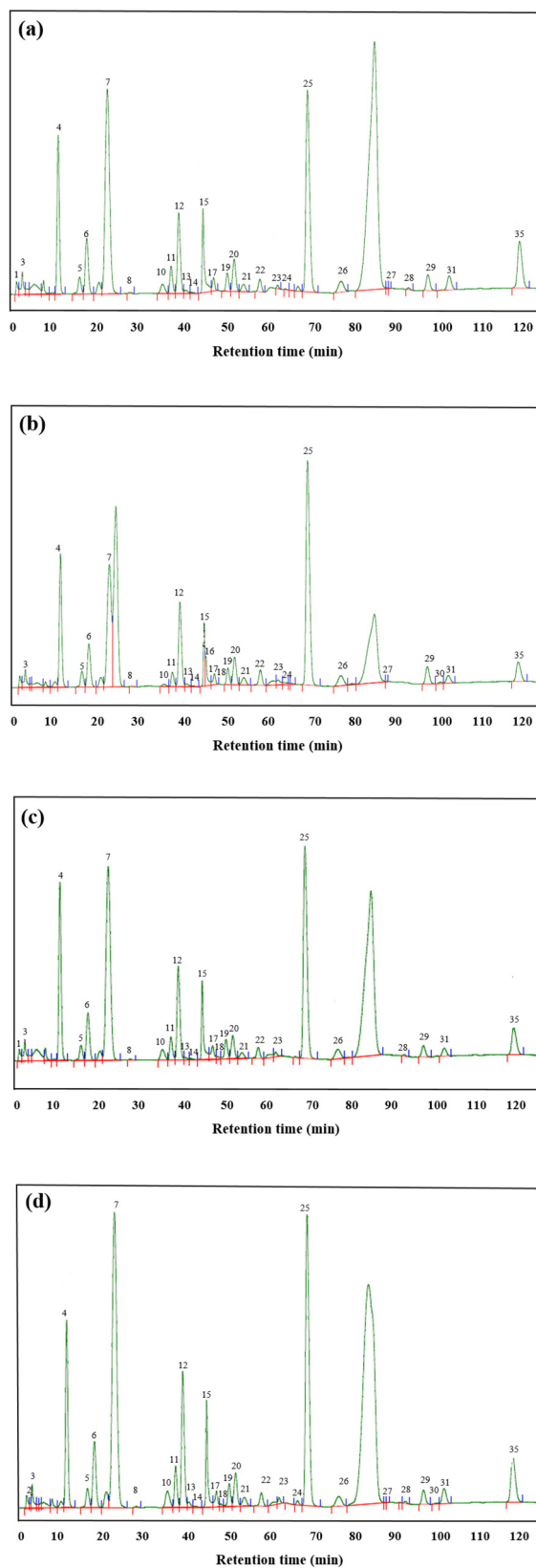


Fig. 1. Chromatograms of the free amino acids in spinach (Cultivars: a. Jeoncheonhu, b. Sagejul, c. Namdongcho and d. Mustang). In the figures, peak numbers refers to the free amino acids listed in Table 2

Table 3. Essential, conditionally essential, non-essential, neutral, acidic and basic amino acids (mg/100g) of the four spinach cultivars

Amino acid	1stharvest(79DAS)				2ndharvest(116DAS)				3rdharvest(145DAS)				Mean	SD	CV%
	JCH	SGJ	NDC	MST	JCH	SGJ	NDC	MST	JCH	SGJ	NDC	MST			
Total amino acid (TAA)	2030.1	2015.1	1905.2	2531.2	911.0	994.2	1192.0	1255.8	1173.6	1323.0	1265.0	1024.9	1468.4	516.8	0.3
Total conditionally essential amino acid (TCEAA)	1303.7	1311.4	1215.4	1772.7	400.1	489.8	558.6	568.1	478.4	583.9	513.6	386.4	798.5	467.6	0.5
Total non-essential amino acid (TNEAA)	511.2	501.6	522.2	557.2	345.5	347.8	419.1	508.8	472.9	480.3	512.1	427.0	467.1	68.2	0.1
Total essential amino acid (TEAA)															
With Histidine	215.1	202.0	167.5	201.3	165.2	156.4	214.2	178.9	222.2	258.7	239.1	211.4	202.6	30.9	0.1
No Histidine	196.2	185.8	157.5	183.2	159.2	147.3	202.2	168.5	208.7	240.3	223.9	201.0	189.5	28.0	0.1
Total neutral amino acid (TNAA)	976.0	1117.2	997.6	1341.0	361.0	421.0	470.5	486.4	430.5	513.6	443.3	432.8	665.9	339.9	0.5
Total acidic amino acid (TAAA)	843.2	772.1	803.0	1033.9	478.8	470.6	592.7	652.8	582.4	628.7	670.2	486.2	667.9	169.5	0.2
Total basic amino acid (TBAA)	210.9	125.7	104.5	156.2	71.1	102.4	128.6	116.5	160.6	180.6	151.3	105.8	134.5	38.8	0.2
Total sulphur amino acid (TSAA)	16.3	24.8	15.5	19.4	14.7	16.0	17.0	25.4	17.6	27.9	22.7	21.4	19.9	4.4	0.2
Total aromatic amino acid (TArAA)	57.8	54.3	38.5	51.7	47.0	41.3	55.2	49.8	59.6	69.1	61.5	53.5	53.3	8.5	0.1
% TCEAA	64.2	65.0	63.8	70.0	43.9	49.2	46.8	45.2	40.7	44.1	40.6	37.7	50.9	11.4	0.2
% TNEAA	25.1	24.8	27.4	22.0	37.9	34.9	35.1	40.5	40.3	36.3	40.4	41.6	33.9	7.0	0.2
% TEAA															
With Histidine	10.6	10.0	8.7	7.9	18.1	15.7	17.9	14.2	18.9	19.5	18.9	20.6	15.1	4.6	0.3
No Histidine	9.6	9.2	8.2	7.2	17.4	14.8	16.9	13.4	17.7	18.1	17.7	19.6	14.2	4.4	0.3
% TNAA	48.0	55.4	52.3	52.9	39.6	42.3	39.4	38.7	36.6	38.8	35.0	42.2	43.4	6.9	0.1
% TAAA	41.5	38.3	42.1	40.8	52.5	47.3	49.7	51.9	49.6	47.5	52.9	47.4	46.8	4.9	0.1
% TBAA	10.3	6.2	5.4	6.1	7.8	10.3	10.8	9.2	13.6	13.6	11.9	10.3	9.6	2.7	0.2
% TSAA	0.8	1.2	0.8	0.7	1.6	1.6	1.4	2.0	1.5	2.1	1.8	2.1	1.4	0.4	0.3
% TArAA	2.8	2.7	2.0	2.0	5.1	4.1	4.6	3.9	5.0	5.2	4.8	5.2	3.9	1.2	0.3
% Cysteine in TSAA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	40	0.0	0.0	0.0	0.0	3.3	11.5	3.4

JCH - Jeoncheonhu; SGJ - Sagyejul; NDC - Namdongcho; MST - Mustang

acids) were the most dominant amino acids in spinach. Acidic amino acids, γ -amino butyric acid (GABA) and aspartic acid occupied the third and fourth position in spinach (7.7 to 15.2% of the total contents of free amino acids). Hydroxyproline, α -amino adipic acid and α -aminobutyric acid were the least concentrated free amino acids in spinach cultivars with mean value of nearly 0.2 mg/100g. Valine and leucine (17.2 to 24.4% of the total contents of essential amino acids) were the dominant among the essential amino acids in spinach. There is considerable variation in the proportions of individual amino acids in this vegetable found by different authors (Budwala *et al.*, 2000; Beis *et al.*, 2002; Lisiewska *et al.*, 2011). But, in most cases glutamic acid had the highest proportion similar to that found in the present study. Lisiewska *et al.* (2008, 2009) however found the highest proportion of proline as much as 12% in kale leaves and brussels sprouts. Our trend in essential amino acids agreed with the results of Lisiewska *et al.* (2011), who found that valine and leucine were the most concentrated essential amino

acids in leafy vegetables. Still, few authors (Lisiewska *et al.*, 2008; Adeyeye *et al.*, 2010) had stressed lysine as one of the dominant essential amino acids in horticultural crops, for e.g., cocoa beans.

Various parameters are presented in Table 3. The total free amino acids (TAA) in spinach cultivars at different harvest time ranged between 911 to 2531.2 mg/100g with a CV% of 0.3. This range was 1.5-fold less than the TAA levels recorded by Lisiewska *et al.* (2011) in spinach. The reason for the different results between studies might be due to the type of soil, climate, sowing time, particular tissue under investigation or method used for analysis (Lee and Kader, 2000). The level of TAA in other freshly harvested consumables, for instance pear (Chen *et al.*, 2007) was less (9x) to the value of spinach highlighting that leafy vegetables could be one of the good sources of amino acids among the horticultural crops. The total conditionally essential amino acids (TCEAA, needed in times of illness and stress) range was higher to the total non-essential amino acids (TNEAA) as well as total essential amino acids

(TEAA) in the following pattern: TCEAA (50.9% of TAA) > TNEAA (33.9% of TAA) > TEAA with histidine (15.1% of TAA) > TEAA without histidine (14.2% of TAA). This result showed that TCEAA formed the bulk of the amino acids in spinach and this vegetable could be consumed as a high value food while sick. On a percentage basis, the observed trend of TEAA was not in agreement with the spinach studies of Lisiewska *et al.* (2011). The total neutral amino acids (TNAA) range (40 to 45% of TAA) was very close to the total acidic amino acids (TAAA), this pattern was also observed in cocoa beans by Adeyeye *et al.* (2010). Most of the neutral and acidic amino acids such as GABA are very useful and highly essential supplement for the wellbeing of humans. Generally these kinds of amino acids has various beneficial roles in humans: acts as a tranquilizer and is very effective in preventing stress/anxiety; regulates the nerve impulses and helps in maintaining muscle tone; increase the levels of growth hormones; breakdown body fats; capable of nourishing the infants; controls high blood pressure and effective in reducing chronic arthritis (Xbrain, 2016). Spinach is thus recommended as the important nutrient feedstock for vegetarians being the rich sources of TNAA/TAAA with health significance.

One of the most limiting amino acids in leafy vegetables are the sulphur containing amino acids particularly cysteine and methionine that are incorporated into proteins. Cysteine can spare part of the requirement for methionine and it is absent in several consumables, for e.g., melon, pumpkin seed and gourd seed (Adeyeye *et al.*, 2007). The experimentally determined total sulphur containing amino acids (TSAA) in spinach ranged from 0.7 to 2.1% of the total TAA. It is important to note that SAA deficiency can be met by consuming large quantities of spinach cv. Mustang harvested at 116 DAS, as it constituted cysteine to a maximum possible level around 40% of TSAA. The observed cysteine range was consistent with the results of Adeyeye *et al.* (2010) and Lisiewska *et al.* (2011). Another class of amino acids of dietary significance is aromatic amino acids that includes phenylalanine, tyrosine and histidine that are needed for the synthesis of thyroid hormones as well as proteins (Pencharz *et al.*, 2007). The estimated levels of total aromatic amino acids (TArAA) were better in spinach (3.9% of TAA) alike other horticultural crops (Lisiewska *et al.*, 2008; Adeyeye *et al.*, 2010).

It is important to note that no significant differences ($P \leq 0.05$) existed between the free amino acid contents (all of the associated parameters summarized in Table 3) of the four spinach cultivars as well at different harvest time. Hence, for the production of spinach with good quality either of the four cultivars Jeoncheonhu, Sagyejul, Namdongcho or Mustang and harvest times (79th, 116th or 145 DAS) are ideal. In consideration of the cysteine demand in leafy vegetables, using the cultivar Mustang may be appropriate.

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