

## Free Amino Acid Composition of Tap Root in *Panax* species

Mee-Kyoung Lee and Hoon Park

Korea Ginseng and Tobacco Research Institute, Sinseongdong, Science Town, Daejeon 305-345

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**Abstract :** Free amino acid (FAA) compositions in the central part (pith-xylem : P-X) and the outer part (phloem-cortex : P-C) of root were investigated for *P. ginseng* (*P.g.*), *P. quinque-folium* (*P.q.*) and *P. notoginseng* (*P.n.*) by an amino acid analyzer. Total free amino acids content (TFAA) was highest in *P.g.* and lowest in *P.n.* The TFAA of P-Xs were higher than those of P-Cs in these *Panax* species except *P.n.* The higher the TFAA in P-X, the higher the ratio of TFAA in P-X to that in P-C. Seventeen free amino acids and ammonia were identified, and four unknown peaks appeared before the usual amino acids eluated. The total aspartic acid equivalent of these unknown peaks was corresponded to 77% of known TFAA in P-C of *P.n.*, 17% in *P.g.*, and 7% in *P.q.* The pattern of unknown peaks of *P.g.* was different from *P.q.* and similar to *P.n.* In all samples six major amino acids and ammonia accounted for 90~95% of TFAA. Arginine was comprised from 29% (*P.n.*) to 43% (*P.g.*) by  $\mu$ mole as amino acid and from 50 to 71% by  $\mu$ mole as nitrogen (N  $\mu$ mole) in TFAA. Ammonia was the second abundant one by  $\mu$ mole and the third by N $\mu$ mole. Histidine was the second by N $\mu$ mole. Proline was one of major FAA in *P.q.* Pattern similarity of FAA composition (excluding Arg and Am) by simple correlation was closer between P-C of *P.g.* and P-X of *P.q.* than between both P-Xs and quite different between the P-X of *P.g.* and that of *P.n.* The pattern similarities of major FAA percent abundance excluding Arg and Am were significant only between P-X and P-C of the same species. Arginine content ( $\mu$ mole) had positive correlation ( $r=0.859$ ,  $p=0.05$ ) with Arg/Am among species. Ammonia content was higher than arginine in *P.n.* Tryptophan content was greatest in *P.n.* among species and higher than lysine only in *P.n.* The ratios of TFAA to N(W/W) were in the range of 3.89~4.14 for TFAA and 3.61~3.92 for TFAA plus ammonia.

**Key words :** free amino acids, *panax* species, pattern similarity, pith-xylem, phloem-cortex, root

### Introduction

Free amino acids (FAA) of Korean white ginseng root were first identified by Gastner and Braun in 1963.<sup>1)</sup> FAA of fresh or white ginseng attracted attention as a quality factor of fresh ginseng extract,<sup>2)</sup> browning components for red ginseng process<sup>3-5)</sup> and comparison indices of *Panax* species.<sup>6,7)</sup> FAA study for nitrogen metabolism was hardly found except free amino acid change in various parts of *P. ginseng* during sprouting of new shoot.<sup>8)</sup> We reported the effect of early die back of stem on FAA composition of P-X and concluded that the FAA content could not be responsible for inside white of red ginseng

even though FAA are ingredients for browning.<sup>9)</sup> We also investigated FAA and protein amino acids to elucidate nitrogen metabolism of *Panax* species<sup>10)</sup> and report the results of FAA comparative analysis in this paper.

### Materials and Methods

#### 1. Ginseng Sample

*P.q.* and *P.n.* were purchased at Hong Kong. White ginseng (*P.g.*, 4-year-old) were chosen in small size-grade for possible matching with *P.q.* Roots eliminated rhizome were separated into central (pith-xylem) and outer (phloem-cortex) part after wet incubation at 40°C for 3 hours.

Samples were dried, ground to fine powder and kept in desiccator until used.

### 2. Free Amino Acid Extraction

Ginseng powder (0.2 g) was mixed with 50 ml of 75% ethanol, kept overnight at room temperature, refluxed at 70°C for 30 min. and filtered (Whatman No. 6), and the residue was extracted 2 times more. The combined extract was dried below 40°C in vacuo. The residue was dissolved in 5 ml of 0.2 M Na-citrate buffer (pH 2.2).

### 3. Free Amino Acid Analysis

Sample solution was filtered through 0.45 µm Millipore filter and applied to LKB 4150 Alpha Amino Acid Analyzer according to Mason *et al.*<sup>11)</sup> Eluents were 0.2 M Na-citrate buffers, pH 3.20, pH 4.25, pH 6.45, and 0.4 M NaOH.

### 4. Amino Acid Quantitation

Integrated peak area from amino acid analyzer was used with that of external standard amino acid. Unknown peaks eluted before aspartic acid were calculated as Asp equivalent. Tryptophan just before lysine peak was of lysine equivalent.

### 5. Pattern Similarity (PS)

Linear correlation of amino acid composition was used as pattern similarity. The trace amount of Phe in P-C of *P.g* was considered as 0.001 µmole for calculation. When threonine in one of the pair for PS included serine, the counterpart was treated in the same fashion. For PS of major amino acids, arginine and ammonia were excluded, so that remaining 5 amino acids were contained (Table 2). But numbers of pairs were 6 since there was one unequal pair. When numbers of major amino acids were 4 due to serine included threonine, the next abundant amino acid was used.

## Results and Discussion

### 1. Elution Pattern of Free Amino Acids

Liquid chromatograms of free amino acids of *Panax* species were shown in Figs. 1-2. The elu-

tion patterns were very similar between P-X and P-C in *P.g* (Fig. 1a and Fig. 2), only showing different peak height due to content. Such close similarities were shown between both parts of other species, *P.q* (Fig. 1b) and *P.n* (Fig. 1c). The elution patterns of P-Xs were shown in Figs. 1a, 1b and 1c. Whole visual elution patterns appeared to be very similar between *P.g* and *P.n* and quite different between *P.q* and other two. It was attributed to the unknown peaks (A, B, C, D, in Figs. 1-2) in the front part of chromatogram. The elution patterns of usual amino acids except unknown peaks seemed to be different among species.

### 2. Amino Acid Composition

Seventeen free amino acids were identified (Table 1). Tryptophan was not included in Table 1, because it was not added to standard solution and so calculated as lysine equivalent. In this case methionine was not detectable and cystine was detectable only for two samples (Table 1). Free methionine was reported in *P.g*,<sup>9)</sup> *P.n*,<sup>6)</sup> and trace in *P.q*.<sup>7)</sup> Proline content was specially high in *P.q* while phenylalanine was very low in *P.q* than *P.g* and *P.n*. Major amino acids were listed in the abundance order in Table 2. Ammonia was included because of its probable role of nitrogen metabolism. Most abundant amino acid was arginine and followed by ammonia in *P.g* and *P.q*, while ammonia was most abundant in *P.n*. Percent abundance of arginine was 43% for both P-X and P-C of *P.g*, 37 and 33% for *P.q*, and 29 and 27% for *P.n*. Ammonia was 17 and 19% for *P.g*, 17 and 24% for *P.q* and 30 and 29% for *P.n*.

Relationship between arginine and Arg/Am ratio from Table 1 was shown in Fig. 3. The higher the arginine content, the higher the Arg/Am ratio ( $r=0.859$ ,  $p=0.05$ ). Metabolic relation was not clear in *Panax* species. We found that ammonia was increasing especially in stem when new shoot started growth.<sup>8)</sup> According to liquid chromatogram of FAA of *P.g* ammonia peak appeared

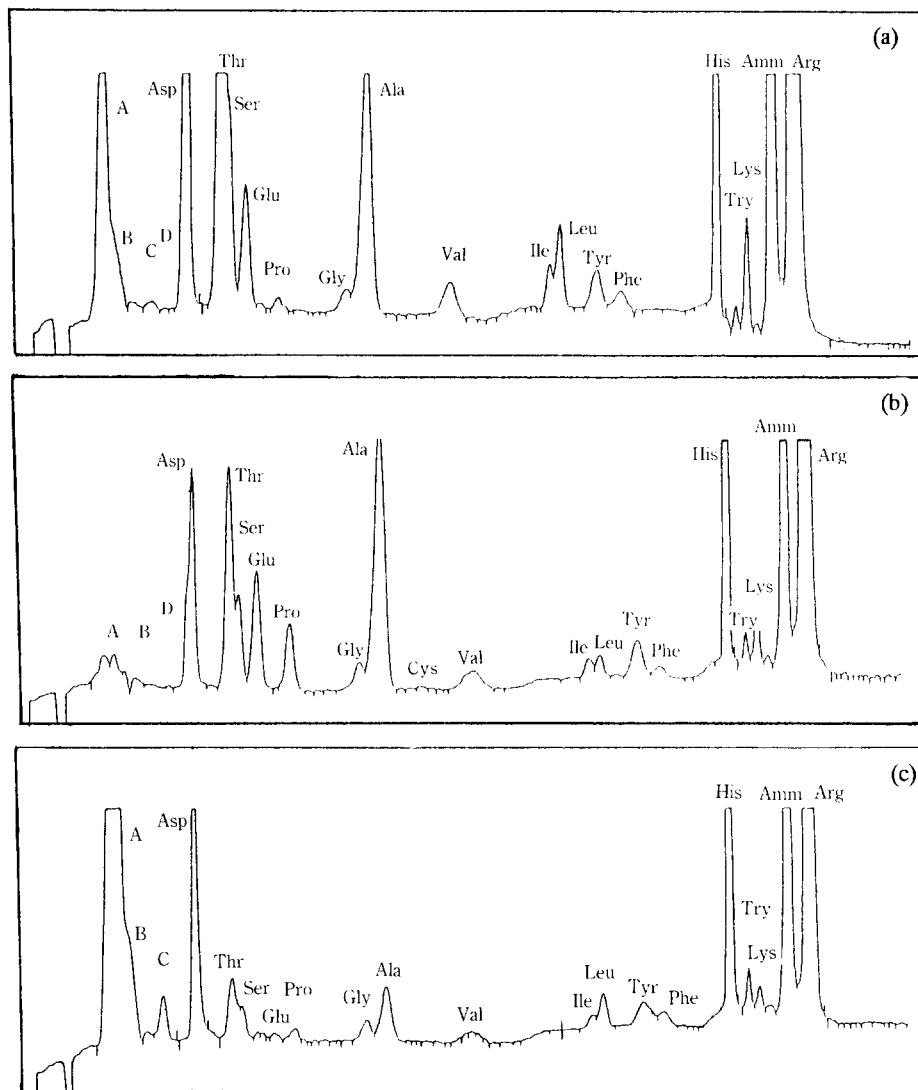


Fig. 1. Free amino acid chromatograms of pith-xylem of *Panax* sp root (a) *P. ginseng*, (b) *P. quinquefolium*, (c) *P. notoginseng* (A, B, C, D: unidentified peak).

to be the 2nd large next to arginine.<sup>6)</sup> It is well expected that Arg/Am ratio of root will be changed according to the growth stage of root. The fact that Arg/Am ratio was less than one in *P.n* might be related to warm weather of harvesting time. High content of arginine in *P.g* may indicate the preparation for long storage time, winter. Although ammonia content was so high it was excluded in most papers on free amino acid paper, because most papers on free amino acids

were for food or pharmacological studies. Since ammonia content was very high, the role of it should be investigated in comparison with nitrate.

The abundance of major FAA showed a slight difference between P-X and P-C in the same species and great difference between species. The abundance of nitrogen in major amino acids was shown in Table 3. The order of nitrogen abundance was arginine, histidine and ammonia regardless of part and species. Since arginine has

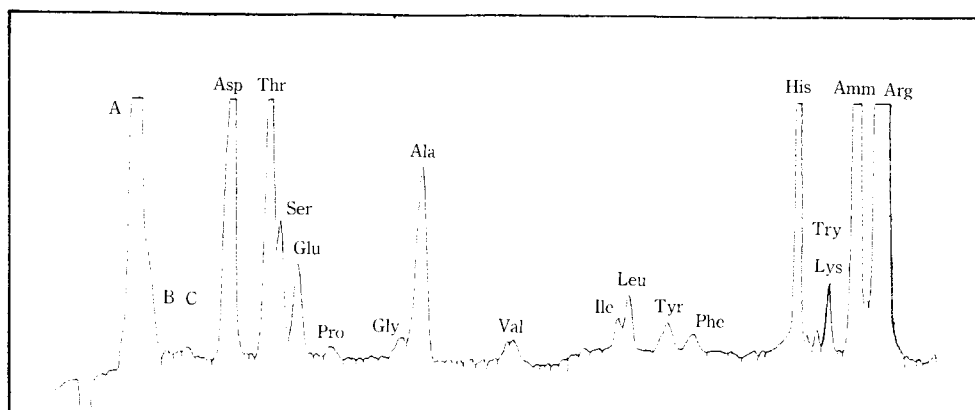


Fig. 2. Free amino acid chromatograms of phloem-cortex of *Panax ginseng* root (A, B, C, D: unidentified peak).

Table 1. Free amino acid composition of tap roots of *Panax* species (Unit:  $\mu$ mole/g, d.w.)

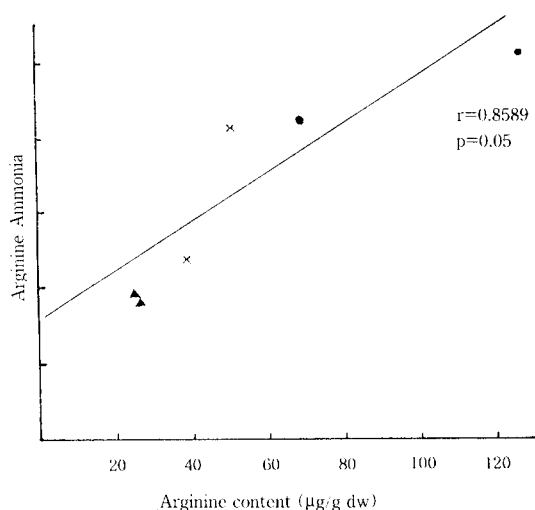
	<i>P. ginseng</i>		<i>P. quinquefolium</i>		<i>P. notoginseng</i>	
	P-X	P-C	P-X	P-C	P-X	P-C
Asp	17.75	11.82	7.05	5.00	7.41	9.00
Thr	37.08	10.04	7.89	4.87	2.35	2.39
Ser	*	3.81	2.80	2.56	*	1.96
Glu	5.70	3.09	4.45	4.93	0.162	0.477
Pro	1.47	1.65	6.50	8.08	1.33	1.57
Gly	1.50	1.05	1.28	1.39	0.984	0.945
Ala	21.31	9.52	15.57	9.87	3.47	3.70
Cys	T	0.790	T	T	0.249	T
Val	4.35	1.18	2.02	0.786	0.391	1.30
Ile	2.48	1.19	0.991	1.01	0.529	0.736
Leu	4.87	1.80	1.02	1.05	1.34	1.66
Tyr	3.05	1.09	1.81	1.78	1.81	0.723
Phe	2.28	0.814	0.174	T	0.717	1.85
His	16.65	11.18	14.45	10.0	14.78	16.14
Lys	2.08	0.941	0.900	0.536	0.352	0.363
Amm.	49.85	31.06	23.37	29.25	26.62	27.79
Arg	126.88	69.85	51.27	39.54	25.56	26.06

P-X : pith-xylem (central part), P-C : phloem-cortex (outer part), T : trace, \* : included into threonine.

Table 2. Percent abundance of major free amino acids of tap root of in *Panax* species (% in a.a  $\mu$ mole)

	<i>P. ginseng</i>		<i>P. quinquefolium</i>		<i>P. notoginseng</i>			
	P-X	P-C	P-X	P-C	P-X	P-C		
1	Arg	71.20	Arg	71.00	Arg	52.50	Arg	50.20
2	His	7.00	His	8.52	His	22.80	His	23.30
3	Am	6.99	Am	7.89	Am	13.70	Am	13.40
4	Thr	5.20*	Asp	3.00	Ala	3.81	Asp	4.34
5	Ala	2.99	Thr	2.55	Thr	1.78	Ala	1.78
6	Asp	2.49	Ala	2.42	Pro	1.21	Ala	1.15
7	Glu	0.80	Ser	0.97	Asp	0.93	Thr	1.00
Total		96.70		96.40		96.73		95.17

\* : including serine, P-X : pith-xylem (central part), P-C : phloem-cortex (outer part).



**Fig. 3.** Relationship between arginine content and arginine/ammonia ratio in root of *Panax* species. ● : *P. ginseng*, ▲ : *P. quinquefolium*, × : *P. notoginseng*.

4 nitrogen atoms, arginine amounted to 71% of total FAA nitrogen for both part of *P.g*, 64 and 61% for *P.q*, and 53 and 50% for *P.n*.

Ninhydrin positive peaks (Figs. 1 to 2, unknown A, B, C, D) were quantitated as aspartic acid equivalent in Table 4. The largest one was unknown A eluted first. The peak A was separated into two peaks in *P.q* and *P.n*. Peaks B and C were also separated into two peaks in P-C of *P.q*. The peak A was very high in *P.n* and *P.g*. Peak D was relatively high in *P.q*. The D peak in P-X of *P.q* was shown as a shoulder in aspartic acid peak (Fig. 1b). These unknown compounds seemed to be phosphoserine and urea according to the elution order,<sup>10</sup> though that method was a little different from this study. Total of unknowns was

**Table 3.** Percent abundance of free amino nitrogen (% in N µmole) of tap root in *Panax* species (% in a.a µmole)

	<i>P. ginseng</i>		<i>P. quinquefolium</i>		<i>P. notoginseng</i>							
	P-X	P-C	P-X	P-C	P-X	P-C						
1	Arg	71.20	Arg	71.00	Arg	63.50	Arg	60.90	Arg	52.50	Arg	50.20
2	His	7.00	His	8.52	His	13.40	His	11.50	His	22.80	His	23.30
3	Am	6.99	Am	7.89	Am	7.24	Am	11.30	Am	13.70	Am	13.40
4	Thr	5.20*	Asp	3.00	Ala	4.82	Ala	3.80	Asp	3.81	Asp	4.34
5	Ala	2.99	Thr	2.55	Thr	2.44	Pro	3.12	Ala	1.78	Ala	1.78
6	Asp	2.49	Ala	2.42	Asp	2.18	Asp	1.92	Thr	1.21	Thr	1.15
7	Glu	0.80	Ser	0.97	Pro	2.00	Glu	1.90	Tyr	0.93	Ser	1.00
Total		96.70		96.40		95.60		94.40		96.73		95.17

\* : including serine, P-X : pith-xylem (central part), P-C : phloem-cortex (outer part).

**Table 4.** Ninhydrin positive unknown peaks eluted before usual free amino acids of tap root in *Panax* species (Aspartic acid equivalent µmole/gdw)

Unknown peaks	<i>P. ginseng</i>		<i>P. quinquefolium</i>		<i>P. notoginseng</i>	
	P-X	P-C	P-X	P-C	P-X	P-C
A	17.20	21.50	(1) 1.13 (2) 1.02	(1) 1.70 (2) 1.39	34.41	(1) 16.53 (2) 32.89
B	0.442	0.205	0.499	(1) 0.509 (2) 1.12	0.315	0.892
C	0.473	0.210	0.00	(1) 0.783 (2) 0.246	1.52	2.39
D	0.046	0.368	1.76	0.564	0.00	0.00
Total	18.18	22.28	4.41	6.31	36.25	52.70
% to TFA	7.35	17.16	3.81	6.89	59.0	76.5

P-X : pith-xylem (central part), P-C : phloem-cortex (outer part), TFA : total free amino acid (1), (2) : separated first, and second peak, respectively.

greatest in *P.n* (59% and 77% of TFA in P-X and P-C), least in *P.q* (4 and 7%) and middle in *P.g* (7 and 17%). These unknowns may be very important not only in quantity but also for metabolic pathway.

### 3. Pattern Similarity (PS) of Free Amino Acid

Using simple correlation of FAA composition ( $\mu\text{mole/g}$ ) between parts of species the similarity of composition was analyzed. When arginine was included but ammonia was excluded, the PS was almost same among species.<sup>10)</sup> When arginine and ammonia were excluded because of the extremely high content, the PS between species appeared clearly (Table 5 lower half part). Similarity showed that P-X of *P.g* was quite different from *P.n* and *P.q*. It is interesting that PS ( $r=0.8335$ ,  $p=0.01$ ) between P-C of *P.g* and P-X of *P.q* tended to be higher than that ( $r=0.7671$ ,  $p=0.01$ ) between both P-Xs of *P.g* and *P.q*. According to the PS of FAA, *P.g* seemed to be closer to *P.q* than *P.n*. But by the unknown peaks *P.g* was a little closer to *P.n* than *P.q* (data

not shown). The PS of major FAA percent abundance (Table 5, upper part) was not significant between species but significant between parts of the same species. Similarities in tryptophan to lysine content were closer between *P.g* and *P.q* (Table 6). Tryptophan content was highest in *P.n*, but lysine was lowest. Consequently the lysine/tryptophan ratio was less than one in *P.n*.

### 4. Free Amino Acid Content

The total of free amino acids content was in decreasing order of *P.g*, *P.q* and *P.n* (Table 7). It was about 3 times in *P.g* than *P.n* and 2 to 3 times than *P.q*. Total FAA was higher in P-X than P-C for *P.g* and *P.q*, but it was reverse for *P.n*. Thus the higher the FAA content in P-X, the higher the ratio of FAA in P-X/P-C. The unknown amino acids content was also higher in P-X than P-C for *P.g* and *P.q*, but it was reverse for *P.n* (Table 4) as in the case of TFAA. TFAA plus unknown was still highest in *P.g*, but almost same in *P.q* and *P.n*.

**Table 5.** Pattern similarity of free amino acid of tap root among *Panax* species

		<i>P. ginseng</i>		<i>P. quinquefolium</i>		<i>P. notoginseng</i>	
		P-X	P-C	P-X	P-C	P-X	P-C
		(major amino acid, n=6)					
<i>P.g.</i>	P-X	-	0.8922**	0.5251	-0.1678	0.1138	0.2053
	P-C	0.9375****	-	0.6033	0.5509	0.4943	0.6858
<i>P.q.</i>	P-X	0.7671***	0.8335***	-	0.8437**	0.5119	0.5517
	P-C	0.6564**	0.7183***	0.9447****	-	0.4157	0.4349
<i>P.n.</i>	P-X	0.4312	0.6755**	0.6775**	0.6124**	-	0.9842****
	P-C	0.5113*	0.7590***	0.6900**	0.6193**	0.9867****	-
		(TFA except arginine,		n=13)			

\*\*\*\*, \*\*\*, \*\* and \*: significant at  $p=0.001$ , 0.01, 0.5 and 0.1, respectively. Ammonia was not included for pattern similarity.

**Table 6.** Free tryptophan content of tap root in *Panax* species

		<i>P. ginseng</i>		<i>P. quinquefolium</i>		<i>P. notoginseng</i>	
		P-X	P-C	P-X	P-C	P-X	P-C
Try (* $\mu\text{mole/g}$ )		0.375	0.306	0.351	0.259	0.515	0.615
Lys/Try		5.55	3.08	2.56	2.07	0.68	0.59

\*: Try., lysine equivalent, P-X: pith-xylem (central part), P-C: phloem-cortex (outer part).

**Table 7.** Total free amino acid content of tap root of *Panax* species

	<i>P. ginseng</i>				<i>P. quinquefolium</i>				<i>P. notoginseng</i>			
	P-X		P-C		P-X		P-C		P-X		P-C	
	μmol	μmol	μmol	μmol	μmol	μmol	μmol	μmol	μmol	μmol	μmol	μmol
TFAA (A)	247.4	37.19	129.8	19.74	115.8	17.31	91.4	13.37	61.4	9.39	68.9	10.34
TFAA <sub>(N)</sub> (B)	663.4	9.29	362.7	5.08	299.4	4.19	230.6	3.23	168.0	2.35	179.7	2.52
TFAA+Am (C)	297.2	38.04	160.9	20.27	139.2	17.71	120.7	13.87	88.1	9.84	96.7	10.81
TFAA <sub>(N)</sub> +Am <sub>(N)</sub> (D)	713.2	9.99	393.7	5.51	322.8	4.52	259.8	3.64	194.6	2.73	207.5	2.91
A/B	-	4.00	-	3.89	-	4.13	-	4.14	-	3.99	-	4.11
C/D	-	3.80	-	3.68	-	3.92	-	3.81	-	3.61	-	3.72

P-X : pith-xylem (central part), P-C : phloem-cortex (outer part), TFAA : total free amino acids, <sub>(N)</sub> : by nitrogen, Am : ammonia.

The conversion factors of nitrogen to TFAA ranged from 3.89 to 4.14 among parts and species for TFAA and from 3.61 to 3.81 for TFAA+ammonia (Table 7). Free amino acids content will greatly affect the crude protein content, when it is estimated from total nitrogen content.

## 요 약

高麗白蔘, 美國蔘과 田七根 胴體部の 中心部(수 및 도관)와 周皮部(물과부 및 유조직)에서의 遊離아미노산 조성을 액체 크로마토그래피로 분석하였다. 총 유리아미노산함량(TFAA)은 고려삼에서 가장 많고 전칠에서 가장 적었다. TFAA는 전칠을 제외하고는 주피부보다 중심부에서 높았다. 중심부의 TFAA가 많을수록 중심부/주피부의 TFAA 比가 높았다. 17개 유리아미노산과 암모니아를 동정하였다. 4개의 미지 피크가 일반 아미노산 앞에 용출되었다. Aspartic acid에 준하여 계산한 미지 아미노산 총함량은 주피부를 불태 전칠에서는 TFAA의 77%가 되었으며, 고려삼에서 17%, 미국삼에서 7% 였다. 미지 아미노산의 양상은 고려삼과 전칠이 유사하고 미국삼은 달랐다. 6개 주아미노산과 암모니아는 TFAA의 90~95%가 되었다. Arginine의 조성율은 전칠에서 TFAA의 29%로 최저였고, 고려삼에서는 43%, N μmole로는 50~71%로 최고였다. 암모니아는 아미노산 μmole로는 두번째, N μmole로 세번째였다. 히스티딘은 N μmole로 두번째의 다량 아미노산이었다. 프롤린은

미국삼에서 主要 아미노산에 포함되었다. 알지닌과 암모니아를 제외한 FAA의 양상 類似도는 고려삼의 주피부와 미국삼의 중심부간의 유사도가 이들의 중심부간 유사도보다 높았다. 고려삼과 전칠의 중심간에는 FAA의 양상 유사도에 유의성이 없었다. 주요 아미노산의 백분율로 본 양상 유사도는 알지닌과 암모니아를 제외한 경우 同種의 중심부와 주피부간에만 인정되었다. 種간에 알지닌이 많을수록 알지닌/암모니아 비가 높았다. 전칠에서만 알지닌이 암모니아보다 적었다(μmole의 경우). 트립토판은 전칠에서 가장 많고, 전칠에서만 라이신보다 많았다. 총 유리아미노산/질소(무계비) 값은 3.89~4.14 범위이고 암모니아를 합한 경우 3.61~3.92 범위였다.

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