Analysis of Amino Acids in Ginseng

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Abstract

Aiming to provide data and reference on illustrating the kinds and the contents of amino acids in ginseng, the analysis of amino acids was carried out. The dried ginseng was taken as the test sample. The method for analysis was then established based on high performance liquid chromatography combined with evaporative light scattering detection (HPLC-ELSD). The standard curves of 24 amino acids were established with the correlation coefficients being all greater than 0.99. Among all samples from various areas, 4-year-old ginseng obtained from Changbai had the highest content of total amino acids, while 6-year-old ginseng in North Korea had the lowest content. Among the detected amino acids, the content of cysteine was relatively high in all samples. The results also indicated that the contents of amino acids accumulated with the increasing cultivation ages.

Keywords

Dried ginseng · Amino acid · HPLC-ELSD

1.1 Introduction

Ginseng is rich in a variety of amino acids, most of which are essential amino acids [1–4]. The content of amino acid plays an important role in evaluating the quality of ginseng products. At present, several analytical methods have been developed to characterize and determine the amino acids in ginseng, including amino acid analyzer [5, 6], derivatization reversed-phase high performance liquid chromatography [7–9], etc. In this book, the method of high performance liquid chromatography-evaporative light scattering detection (HPLC-ELSD) was used to directly determine the amino acids in ginseng for the first time [10].

1.2 Materials and Instruments

1.2.1 Materials

The detailed information of the ginseng samples was attached in appendix.

Both methanol and acetonitrile were all of chromatographic grade (Fisher, Co. Ltd., America). Trifluoroacetic acid (TFA) (99.5%, Xiya Reagent Co. Ltd., China), heptafluorobutyric acid (98%, Aladdin Reagent Co. Ltd., China), and other reagents were of analytical grade. Ultrapure water was prepared by the water purification system (Changchun Laibopate Technology Development Co. Ltd., China).

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P. Li, J. Liu (eds.), *Ginseng Nutritional Components and Functional Factors*,
https://doi.org/10.1007/978-981-15-4688-4_1

Reference substances, including alanine (Ala), 2-aminobutyric acid, arginine (Arg), aspartic acid (Asp), cysteine (Cys), cystine (Cys-Cys), DL-3-(3, 4-dihydroxy phenyl) alanine (DL-3,4-DOPA), glutamate (Glu), glycine (Gly), histidine (His), L-hydroxyproline, leucine (Leu), isoleucine (Ile), norleucine (Nle), lysine (Lys), methionine (Met), ornithine (Orn), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Try), tyrosine (Tyr), and valine (Val) were all purchased from BDH Co. Ltd., United Kingdom. The purities of reference substances were all higher than 99.0%.

1.2.2 Instruments

LC-10AT-type high performance liquid chromatography (Shimadzu Corporation, Japan) equipped with SEDERE SEDEX 75-type evaporative light scattering detection evaporative light scattering detection (SEDERE Corporation, France), CBM-102 chromatography workstation (Shimadzu Corporation, Japan), AT-330 chromatographic column incubator (Tianjin Autoscience Instrument Co. Ltd., China), SpursilTM C18 chromatographic column (250 mm × 4.6 mm, 5 μm), R201D type electrothermal constant temperature water bath (Shanghai Yukang Science and Education Equipment Co. Ltd., China), FA1104N electronic balance (Shanghai Jinghua Technology Instrument Co. Ltd., China), FW177 high speed omnipotent pulverizer (Beijing Yongguangming Medical Instrument Co. Ltd., China), GZX-9076 MBE digital display air drying box (Shanghai Boxun Industrial Co. Ltd., China).

1.3 Experimental Methods

1.3.1 Preparation of Reference Solution

An accurately weighted quantity of 23 kinds of amino acid reference substances (except cystine) is dissolved in 0.01 mol/L HCl to produce a solution of 1.0 mg of references per mL, respectively, as mixed solution I.

An accurately weighted quantity of cystine is dissolved in 0.01 mol/L HCl and diluted with ultrapure water to 1.0 mg/mL, as solution II.

An accurately weighted quantity of aspartic acid, glycine, serine, hydroxyproline, threonine, alanine, glutamate, ornithine and cysteine is dissolved in 0.01 mol/L HCl to produce a solution of 1.0 mg of references per mL, respectively, as mixed solution III.

Same volume of solution I and II were mixed well to get mixed reference solution A (0.5 mg/mL).

Same volume of solution II and III were mixed well to get mixed reference solution B (0.5 mg/mL).

1.3.2 Preparation of Test Solution

Ginseng was, respectively, air-dried, grinded, and sieved (Chinese National Standard Sieve No. 6) to get the homogeneous powder. Accurately weigh about 0.1 g, transfer into a 50 mL stoppered conical flask, add 20 mL of 6.0 mol/L HCl, sealed with nitrogen, stir and warm to hydrolyze for 24 h at 110 °C. Allow it to cool, filter, evaporate the filtrate on a water bath at 95°Cto dryness, the residue was dissolved and diluted with ultrapure water to 5.0 mL, mix well and filter (0.45 μm), the successive filtrate as the test solution.

1.3.3 Chromatographic Conditions

The method for high performance liquid chromatography was applied, in which C18 column (4.6 mm × 250 mm, 5 μm) was used and its temperature was set at 25 °C. The mobile phase was composed of eluent A (acetonitrile: methanol =1:1) and eluent B (0.03% TFA solution containing 5 mmol/L heptafluorobutyric acid). The gradient elution is performed linearly (Table 1.1). Flow rate was 0.6 mL/min. The temperature of drift tube was set at 40 °C, the nitrogen

Table 1.1 Gradient elution program of HPLC

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0~15	0	100
15~30	0 → 15	100 → 85
30~50	15	85
50~55	15 → 35	85 → 65
55~65	35	65
65~66	35 → 0	65 → 100
65~76	0	100

Table 1.2 Regression equations with correlation coefficients (r) of 24 amino acids

Amino acid	Regression equation	Correlation coefficient
Gly and Ser	$y = 1.4901x + 12.352$	0.9933
Asp	$y = 1.2780x + 13.847$	0.9954
Hydroxyproline	$y = 1.2996x + 13.385$	0.9943
Ala	$y = 1.2778x + 13.883$	0.9917
Thr	$y = 1.3722x + 14.373$	0.9911
Orn	$y = 1.5757x + 13.407$	0.9964
Glu	$y = 1.2831x + 13.761$	0.9963
Cys	$y = 0.9031x + 11.767$	0.9952
Cys-Cys	$y = 2.0231x + 11.411$	0.9905
Pro	$y = 1.5068x + 12.197$	0.9912
2-aminobutyric acid	$y = 1.7195x + 12.116$	0.9971
Lys	$y = 1.4738x + 12.227$	0.9949
His	$y = 1.5225x + 12.420$	0.9951
Val	$y = 1.5053x + 13.333$	0.9975
DL-3,4-DOPA	$y = 1.7308x + 12.807$	0.9980
Arg	$y = 1.4284x + 14.098$	0.9982
Met	$y = 1.7324x + 11.256$	0.9995
Tyr	$y = 1.8810x + 10.928$	0.9991
Ile	$y = 1.8649x + 11.512$	0.9985
Leu	$y = 1.3980x + 12.557$	0.9998
Nle	$y = 1.3487x + 13.096$	0.9993
Phe	$y = 1.4552x + 13.807$	0.9991
Trp	$y = 1.5316x + 13.423$	0.9992

y natural logarithm of peak area, x natural logarithm of mass (μg)

flow rate was 2.9 L/min. Inject 15 μL solution into the column and record the chromatogram.

1.3.4 Drawing Standard Curve

Linearity determination was prepared by measuring accurately an amount of analyte separately. The treatment is a calculation of a regression line by the method of least squares of test results versus analyte concentrations.

Inject 4, 6, 8, 10, 12, 14, and 16 μL of solution A and 1, 2.5, 3.5, 5, 6, 7.5, and 9 μL of solution B, respectively, into the column and record the chromatogram.

The regression equations were obtained by taking the natural logarithm of the amount of the reference substance (μg) as the abscissa (x) and the natural logarithm of peak area of the reference product as the ordinate (y), which were shown in Table 1.2.

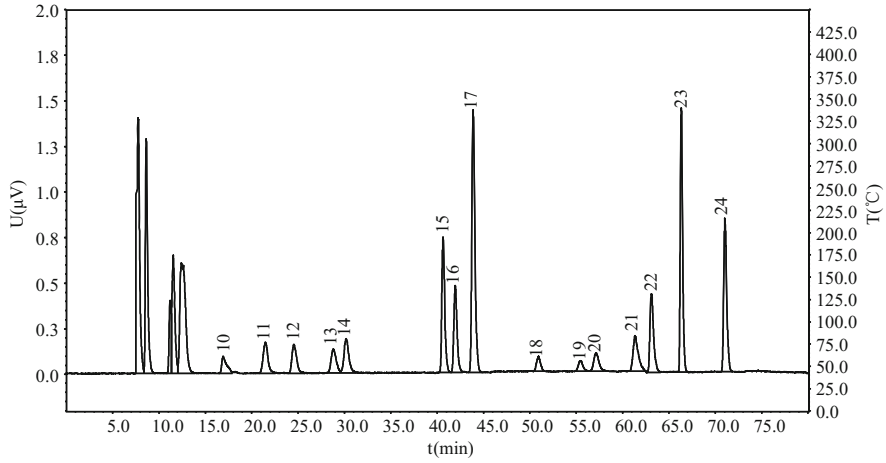

Fig. 1.1 HPLC-ELSD chromatogram of mixed standard solution A. *10* Cys. *11* Pro. *12* 2-aminobutyric acid. *13* Lys. *14* His. *15* Val. *16* DL-3,4-DOPA. *17* Arg. *18* Met. *19* Tyr. *20* Ile. *21* Leu. *22* Nle. *23* Phe. *24* Trp

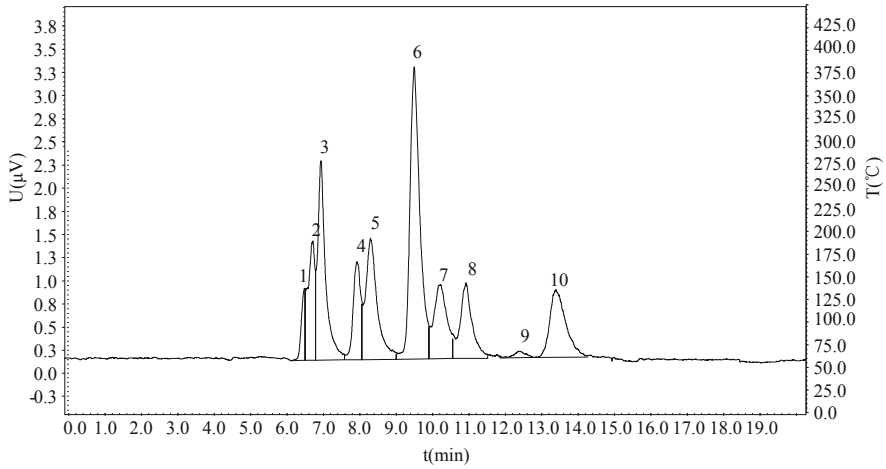

Fig. 1.2 HPLC-ELSD chromatogram of mixed standard solution B. *1* Gly. *2* Ser. *3* Asp. *4* hydroxyproline. *5* Ala. *6* Thr. *7* Orn. *8* Glu. *9* Cys. *10* Cys-Cys

The HPLC-ELSD chromatograms of mixed reference solution A and B were shown in Figs. 1.1 and 1.2, respectively.

1.3.5 Determination of Test Sample

The contents of amino acids in ginseng were determined based on the above method. The chromatograms of test samples were shown in Figs. 1.3 and 1.4.

1.4 Results and Discussion

1.4.1 The Contents of Amino Acids in Ginseng from Different Regions with Different Cultivation Ages

The results were shown in Tables 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, and 1.9.

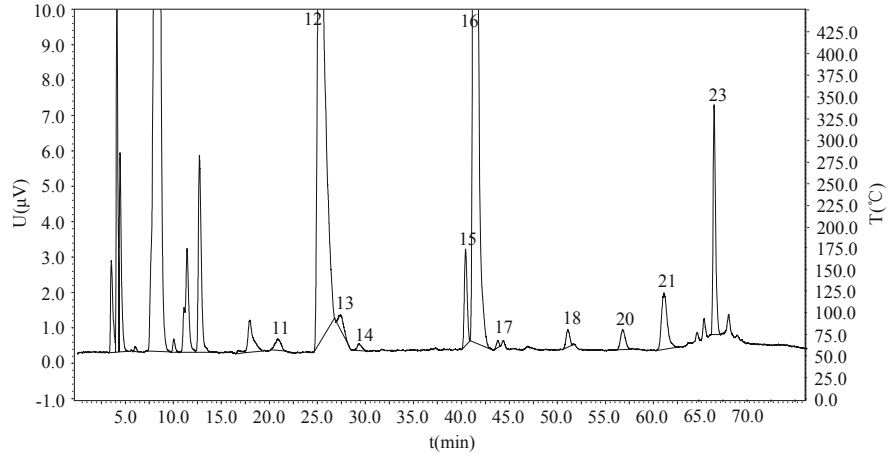

Fig. 1.3 HPLC-ELSD chromatogram of amino acids of 6-year-old ginseng from Wangqing. *11* Pro. *12* 2-aminobutyric acid. *13* Lys. *14* His. *15* Val. *16* DL-3,4-DOPA. *17* Arg. *18* Met. *20* Ile. *21* Leu. *22* Nle. *23* Phe

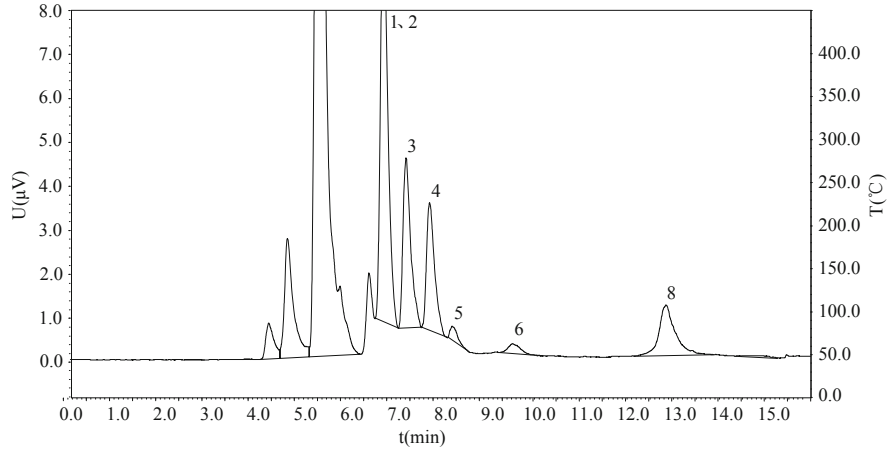

Fig. 1.4 HPLC-ELSD chromatogram of amino acids of 5-year-old ginseng from Kuandian. *1* Gly. *2* Ser. *3* Asp. *4* hydroxyproline. *5* Ala. *6* Thr. *8* Glu

1.4.2 Analysis of Amino Acid Contents in Ginseng from Same Regions with Different Cultivation Years

The results showed that the contents of amino acid accumulated with the increasing cultivation ages, as shown in Table 1.10, Figs. 1.5, 1.6, and 1.7.

1.4.3 Analysis of Essential Amino Acids in Ginseng from Same Regions with Different Cultivation Ages

The proportions of essential amino acids in the total amino acid of ginseng from Antu, Dunhua, Helong, Dadi Ji'an, and South Korea decreased with the cultivation age, while the proportions of

Table 1.3 Amino acids and total amino acids contents (%) of ginseng from Heihe, Hulin, Antu, and Changbai with different cultivation periods (year)

	Heihe	Hulin	Antu		Changbai		
Name of amino acid	4	4	4	5	4	5	6
Gly and Ser	1.00	4.61	2.73	0.95	1.57	1.57	0.89
Asp	0.36	1.20	0.88	3.06	0.95	0.56	0.33
L-hydroxyproline	3.09	1.53	0.10	1.99	1.87	0.44	1.68
Ala	0.51	0.10	0.29	0.13	0.34	2.98	0.02
Thr	0.11	0.11	–	–	0.02	0.20	0.17
Orn	–	–	0.08	–	–	–	–
Glu	–	0.80	0.27	0.29	0.02	0.46	0.24
Cys	0.28	–	2.56	2.42	21.67	0.92	3.63
Cys-Cys	–	–	–	–	–	–	–
Pro	0.16	0.23	0.28	0.18	0.15	0.18	0.23
2-aminobutyric acid	2.14	1.89	2.63	2.10	4.41	2.14	2.37
Lys	–	0.09	–	–	0.07	0.13	0.16
His	–	–	–	–	0.10	0.02	0.02
Val	0.02	0.07	–	–	0.19	0.16	0.20
DL-3,4-DOPA	3.15	2.79	2.10	2.44	2.41	2.30	2.37
Arg	0.03	0.03	0.13	0.03	0.02	0.05	0.04
Met	0.52	0.48	0.57	0.53	0.49	0.43	0.48
Tyr	–	–	–	–	–	–	–
Ile	0.44	0.45	0.39	0.45	0.47	0.39	0.45
Leu	0.55	0.57	0.47	0.46	0.57	0.50	0.60
Nle	–	–	–	–	–	–	–
Phe	0.36	0.42	0.44	0.36	0.38	0.35	0.39
Try	–	–	–	–	–	–	–
Total amino acids	12.75	15.35	13.93	15.39	35.70	13.78	14.25
Essential amino acids	2.02	2.18	1.87	1.81	2.19	2.15	2.44
EAA/TAA	15.81	14.22	13.45	11.74	6.14	15.61	17.10
Acidic amino acids	0.36	2.00	1.15	3.35	0.98	1.02	0.57
Neutral amino acids	12.36	13.23	12.65	12.01	34.53	12.56	13.46
Basic amino acids	0.03	0.13	0.13	0.03	0.19	0.20	0.22

Note: – means not detected

ginseng from Huadian and Jiaohe increased with the cultivation age, which showed no regularity (Tables 1.11 and 1.12).

1.4.4 Analysis of Total Amino Acids in Ginseng from Different Regions with Same Cultivation Ages

For 4-year-old ginseng, the regions with high-to-low contents of total amino acid were: Changbai, Jingyu, Shuangcha Ji'an, Dunhua, Xinbin, Fusong, Hulin, North Korea, Helong, Wangqing, Jiaohe, Antu, Huadian, Heihe, Hunchun, Hunchun, Kuandian (Fig. 1.8).

As for 5-year-old ginseng, the regions with high-to-low contents of total amino acid were: Helong, South Korea, Dadi Ji'an, Dunhua, Shuangcha Ji'an, Jiaohe, Antu, North Korea, Wangqing, Kuandian, Hunchun, Changbai, Fusong, Huadian (Fig. 1.9).

As for 6-year-old ginseng, the regions with high-to-low contents of total amino acid were:

Table 1.4 Amino acids and total amino acids contents (%) of ginseng from Dunhua and Fusong with different cultivation periods (year)

	Dunhua		Fusong		
Name of amino acid	4	5	4	5	6
Gly and Ser	4.39	4.17	5.94	3.17	1.51
Asp	0.72	3.79	1.32	0.13	0.54
L-hydroxyproline	1.31	2.20	0.25	1.05	0.41
Ala	0.06	0.31	0.03	0.07	4.79
Thr	0.14	–	–	0.07	0.10
Orn	–	0.08	–	–	–
Glu	–	0.42	0.73	–	0.54
Cys	6.40	2.74	1.65	1.77	–
Cys-Cys	–	–	–	–	–
Pro	0.17	0.22	0.15	0.19	0.14
2-aminobutyric acid	1.94	1.89	1.87	2.00	2.59
Lys	0.03	0.02	0.08	0.10	–
His	–	–	–	–	–
Val	0.17	0.10	0.11	0.18	0.15
DL-3,4-DOPA	1.84	2.01	2.20	2.13	1.62
Arg	0.02	0.03	0.02	0.09	0.05
Met	0.31	0.41	0.42	0.43	0.43
Tyr	–	–	–	–	–
Ile	0.38	0.42	0.39	0.43	0.45
Leu	0.49	0.59	0.47	0.46	0.44
Nle	–	–	–	–	–
Phe	0.28	0.33	0.32	0.33	0.32
Try	–	–	–	–	–
Total amino acids	18.65	19.73	15.95	12.59	14.06
Essential amino acids	1.80	1.86	1.79	2.00	1.89
EAA/TAA	9.67	9.45	11.20	15.88	13.41
Acidic amino acids	0.72	4.21	2.05	0.13	1.07
Neutral amino acids	17.89	15.47	13.81	12.25	12.94
Basic amino acids	0.05	0.05	0.09	0.20	0.05

Note: – means not detected

South Korea, Shuangcha Ji'an, Dadi Ji'an, Hunchun, Kuandian, Changbai, Fusong, Wangqing, Linjiang, North Korea (Fig. 1.10).

1.4.5 Analysis of Essential Amino Acids in Ginseng from Different Regions with Same Cultivation Ages

The results were shown in Figs. 1.11, 1.12, and 1.13.

1.4.6 Analysis of Acidic Amino Acid and Basic Amino Acid in Ginseng from Different Regions with Same Cultivation Ages

Acidic Amino Acid (1) For 4-year-old ginseng, the regions with high-to-low contents were: Jiaohe, Shuangcha Ji'an, Fusong, Hulin, Xinbin, Hunchun, Kuandian, Jingyu, Antu, Changbai, North Korea, Chongcha Hunchun, Dunhua, Wangqing, Huadian, Helong, Heihe. (2) For 5-year-old ginseng, the regions with high-to-low

Table 1.5 Amino acids and total amino acids contents (%) of ginseng from Hunchun and Huadian with different cultivation periods (year)

	Hunchun				Huadian	
Name of amino acid	4	5	6	4(CC)	4	5
Gly and Ser	0.75	2.46	5.24	2.29	1.02	0.41
Asp	1.18	0.88	0.47	0.71	0.25	0.06
L-hydroxyproline	0.23	0.05	1.92	0.08	0.42	2.76
Ala	0.04	0.26	0.12	0.19	3.08	0.23
Thr	0.18	–	–	–	0.09	0.05
Orn	–	–	0.05	0.06	–	–
Glu	0.37	–	–	0.03	0.34	0.10
Cys	1.01	3.01	2.18	2.31	2.56	0.27
Cys-Cys	–	–	–	–	–	–
Pro	0.25	0.25	0.38	0.18	0.11	0.12
2-aminobutyric acid	2.64	2.57	2.49	2.37	1.97	1.72
Lys	0.38	0.29	0.44	0.22	0.06	–
His	0.19	0.16	0.23	0.07	0.02	–
Val	0.32	0.26	0.32	0.23	0.17	0.16
DL-3,4-DOPA	2.73	2.09	2.64	1.78	1.70	1.58
Arg	0.03	0.02	0.03	0.02	0.07	0.01
Met	0.53	0.44	0.54	0.32	0.26	0.25
Tyr	–	–	–	–	–	–
Ile	0.51	0.42	0.51	0.36	0.36	0.37
Leu	0.64	0.52	0.64	0.41	0.39	0.38
Nle	–	–	–	–	–	–
Phe	0.44	0.38	0.45	0.33	0.28	0.29
Try	–	–	–	–	–	–
Total amino acids	12.42	14.06	18.65	11.95	13.16	8.76
Essential amino acids	2.99	2.30	2.91	1.85	1.62	1.50
EAA/TAA	24.11	16.33	15.60	15.52	12.30	17.18
Acidic amino acids	1.55	0.88	0.47	0.75	0.59	0.16
Neutral amino acids	10.28	12.70	17.49	10.91	12.42	8.59
Basic amino acids	0.60	0.47	0.69	0.30	0.15	0.01

Note: – means not detected

contents were: Dunhua, Shuangcha Ji'an, Antu, Dadi Ji'an, Korea, Kuandian, Jiaohe, Changbai, Hunchun, North Korea, Wangqing, Huadian, Helong, Fusong. (3) For 6-year-old ginseng, the regions with high-to-low contents were: Dadi Ji'an, Shuangcha Ji'an, Korea, Kuandian, Linjiang, Fusong, Wangqing, North Korea, Changbai, Hunchun.

Basic Amino Acid (1) For 4-year-old ginseng, the regions with high-to- low contents were: Hunchun, Xinbin, North Korea, Wangqing, Chongcha Hunchun, Kuandian, Changbai, Helong, Shuangcha Ji'an, Huadian, Antu, Hulin, Jingyu, Fusong, Jiaohe, Dunhua, Heihe. (2) For 5-year-old ginseng, the regions with high-to-low contents were: Hunchun, North Korea, Jiaohe, Wangqing, Dadi Ji'an, Changbai, Fusong, Shuangcha Ji'an, Korea, Kuandian, Dunhua, Helong, Antu, Huadian. (3) For 6-year-old ginseng, the regions with high-to-low contents were: Hunchun, North Korea, Kuandian, Wangqing, Shuangcha Ji'an, Linjiang, Korea, Fusong.

Table 1.6 Amino acids and total amino acids contents (%) of ginseng from Helong, Dadi Ji'an, and Shuangcha Ji'an with different cultivation periods (year)

	Helong		Dadi Ji'an		Shuangcha Ji'an		
Name of amino acid	4	5	5	6	4	5	6
Gly and Ser	2.60	5.00	0.65	0.94	1.06	1.50	2.62
Asp	0.42	0.15	2.16	1.69	3.66	3.36	1.32
L-hydroxyproline	0.12	0.52	1.43	1.82	1.28	1.80	0.70
Ala	0.23	0.09	0.00	0.12	0.10	0.13	0.35
Thr	0.04	0.13	0.13	0.15	0.18	0.17	0.00
Orn	–	–	–	–	–	–	–
Glu	0.16	–	–	–	–	–	0.23
Cys	4.31	11.23	6.99	7.78	8.37	5.91	7.80
Cys-Cys	–	–	–	–	–	–	–
Pro	0.23	0.19	0.24	0.23	0.17	0.22	0.23
2-aminobutyric acid	2.12	2.13	4.29	2.46	2.61	2.52	2.45
Lys	0.11	0.04	0.18	0.17	0.13	0.13	0.18
His	–	–	–	–	–	0.02	0.07
Val	0.21	0.20	0.26	0.23	0.22	0.24	0.24
DL-3,4-DOPA	2.18	1.74	2.38	2.22	1.69	1.82	2.49
Arg	0.06	0.01	0.03	0.02	0.02	0.04	0.04
Met	0.32	0.20	0.82	0.45	0.43	0.34	0.43
Tyr	–	–	–	–	–	–	–
Ile	0.42	0.39	0.50	0.44	0.37	0.44	0.41
Leu	0.49	0.41	0.64	0.58	0.47	0.57	0.55
Nle	–	–	–	–	–	–	–
Phe	0.33	0.32	0.44	0.37	0.31	0.37	0.33
Try	–	–	–	–	–	–	–
Total amino acids	14.32	22.74	21.15	19.69	21.07	19.58	20.43
Essential amino acids	1.90	1.68	2.97	2.40	2.11	2.26	2.15
EAA/TAA	13.29	7.39	14.04	12.18	10.01	11.54	10.50
Acidic amino acids	0.58	0.15	2.16	1.69	3.66	3.36	1.54
Neutral amino acids	13.58	22.55	18.78	17.81	17.25	16.03	18.60
Basic amino acids	0.16	0.05	0.21	0.19	0.15	0.19	0.29

Note: – means not detected

1.4.7 Analysis of Hydrolyzed Amino Acids in Ginseng from Different Regions with Same Cultivation Ages

There were more than 16 kinds of amino acids after hydrolysis. The contents of cysteine and aspartic acid were higher, while the contents of ornithine and histidine were the lowest. The ginseng from Jiaohe contained 20 kinds of amino acids, while those of Antu only contained 16 kinds. (Figs. 1.14, 1.15, 1.16, 1.17, 1.18, 1.19, and 1.20).

1.4.8 Cluster Analysis of Amino Acids in Ginseng from Different Regions with Different Cultivation Ages

Combining Ward Method, Chi-Square Metric and Squared Euclidean Distance in SPSS 22.0 software, the dendrogram (Fig. 1.21) of cluster analysis was established, in which the amino acid contents were the characteristic variable. The results showed that all ginseng samples could be classified into three categories when the distance used for clustering was 15.

The samples including 4-year-old ginseng from Hulin (2), 5-year-old ginseng from

Table 1.7 Amino acids and total amino acids contents (%) of ginseng from Jiaohe, Jingyu, Linjiang, and Tonghua with different cultivation periods (year)

	Jiaohe		Jingyu	Linjiang	Tonghua
Name of amino acid	4	5	4	6	4\5
Gly and Ser	0.63	0.52	2.21	0.60	2.94
Asp	3.71	0.62	1.01	0.56	0.37
L-hydroxyproline	1.55	5.06	–	0.14	2.06
Ala	0.14	0.56	0.27	0.45	0.16
Thr	0.03	0.12	0.01	0.15	–
Orn	–	0.09	–	–	–
Glu	0.18	0.53	0.20	0.61	0.31
Cys	1.66	3.93	12.94	–	12.52
Cys-Cys	–	–	–	–	–
Pro	0.19	0.23	0.16	0.20	0.27
2-aminobutyric acid	2.18	2.45	2.01	1.95	1.63
Lys	0.03	0.22	0.06	0.13	0.14
His	–	0.16	0.03	0.02	0.07
Val	0.12	0.28	0.08	0.05	0.17
DL-3,4-DOPA	2.24	2.64	2.55	2.49	2.16
Arg	0.05	0.08	0.03	0.03	0.04
Met	0.29	0.27	0.42	0.47	0.54
Tyr	–	–	–	–	–
Ile	0.42	0.43	0.40	0.45	0.48
Leu	0.43	0.66	0.53	0.54	0.62
Nle	–	–	–	–	–
Phe	0.34	0.34	0.32	0.37	0.41
Try	–	–	–	–	–
Total amino acids	14.18	19.18	23.22	9.20	24.90
Essential amino acids	1.65	2.32	1.81	2.16	2.35
EAA/TAA	11.65	12.12	7.80	23.45	9.45
Acidic amino acids	3.88	1.15	1.21	1.16	0.69
Neutral amino acids	10.22	17.57	21.90	7.85	23.96
Basic amino acids	0.08	0.46	0.12	0.18	0.25

Note: – means not detected

Changbai (6), 6-year-old ginseng from Fusong (12), 4-year-old continuous cropping ginseng from Hunchun (13), 4-year-old ginseng from Huadian (17), 6-year-old ginseng from Linjiang (29), 6-year-old ginseng from Wangqing (33), 4-, 5- and 6-year-old ginseng from Kuandian (34~36) and North Korea (38~40) were clustered into the first category. This category could be further divided into 2 groups when the clustering distance being 10. One group consisted of 4-year-old ginseng from Hulin (2), 4-year-old continuous cropping ginseng from Hunchun (13), 6-year-old ginseng from Linjiang (29), 6-year-old ginseng from Wangqing (33), 4- and 5-year-old ginseng from Kuandian (34~35), and 6-year-old ginseng from North Korea (40). The other group included the rest samples.

The samples including 4-year-old ginseng from Heihe (1), 5-year-old ginseng from Antu (4), 6-year-old ginseng from Changbai (7), 5-year-old ginseng from Dunhua (9), 5-year-old ginseng from Huadian (18), 5- and 6-year-old ginseng from Dadi Ji'an (21~22), 4- and 5-year-old ginseng from Shuangcha Ji'an (23~24) and

Table 1.8 Amino acids and total amino acids contents (%) of ginseng from Wangqing and Kuandian with different cultivation periods (year)

	Wangqing			Kuandian		
Name of amino acid	4	5	6	4	5	6
Gly and Ser	2.44	4.94	1.86	2.70	4.48	1.67
Asp	0.45	0.13	0.58	0.86	1.12	0.87
L-hydroxyproline	0.12	0.68	0.17	0.13	1.31	0.79
Ala	0.19	0.13	0.15	0.27	0.14	3.58
Thr	–	–	0.02	–	0.13	0.18
Orn	0.07	–	–	0.05	–	–
Glu	0.21	0.22	0.09	0.69	0.83	0.55
Cys	3.80	2.15	0.41	–	–	0.09
Cys-Cys	–	–	–	–	–	–
Pro	0.35	0.16	0.24	0.20	0.17	0.20
2-aminobutyric acid	2.27	2.42	2.25	1.93	1.61	1.96
Lys	0.25	0.23	0.25	0.16	0.12	0.17
His	0.09	0.09	0.12	0.07	–	0.19
Val	0.26	0.23	0.24	0.24	0.15	0.18
DL-3,4-DOPA	1.97	1.93	1.87	1.79	2.27	2.22
Arg	0.06	0.02	0.02	0.02	0.03	0.03
Met	0.40	0.15	0.36	0.34	0.50	0.51
Tyr	–	–	–	–	–	–
Ile	0.43	0.35	0.39	0.39	0.46	0.44
Leu	0.55	0.46	0.49	0.49	0.61	0.66
Nle	–	–	–	–	–	–
Phe	0.34	0.27	0.31	0.31	0.39	0.37
Try	–	–	–	–	–	–
Total amino acids	14.23	14.55	9.82	10.60	14.32	14.66
Essential amino acids	2.22	1.69	2.07	1.92	2.37	2.52
EAA/TAA	15.60	11.61	21.04	18.11	16.52	17.18
Acidic amino acids	0.66	0.34	0.67	1.55	1.95	1.41
Neutral amino acids	13.17	13.87	8.76	8.81	12.22	12.85
Basic amino acids	0.39	0.33	0.39	0.24	0.15	0.39

Note: – means not detected

Jiaohe (26~27) were clustered into the second category.

The rest samples were clustered into the third category. This category could be further divided into 2 groups when the clustering distance being 10. One group consisted of 4-year-old ginseng from Changbai (5), 4-year-old ginseng from Dunhua (8), 5-year-old ginseng from Helong (20), 4-year-old ginseng from Jingyu (28), 4- and 5-year-old ginseng from Tonghua (30), 5- and 6-year-old ginseng from South Korea (41~42). The other group included 4-year-old ginseng from Antu (3), 4- and 5-year-old ginseng from Fusong (10~11), 4- and 6-year-old ginseng from Hunchun (14~16), 4-year-old ginseng from Helong (19), 6-year-old ginseng from Shuangcha Ji'an (25), 4- and 5-year-old ginseng from Wangqing (31~32), and 4-year-old ginseng from Xinbin (37).

In short, when using 24 amino acid contents data as characteristic variable value, the results exhibited the similarity of the ginseng from different regions with different cultivation ages.

Table 1.9 Amino acids and total amino acids contents (%) of ginseng from Xinbin, North Korea, and South Korea with different cultivation periods (year)

	Xinbin	North Korea			South Korea	
Name of amino acid	4	4	5	6	5	6
Gly and Ser	3.29	1.99	1.62	0.71	5.05	4.68
Asp	1.41	0.77	0.69	0.37	1.95	1.43
L-hydroxyproline	0.22	0.65	0.64	–	1.16	1.05
Ala	0.32	3.41	3.89	0.19	0.26	0.17
Thr	–	0.22	0.15	0.01	–	–
Orn	0.03	0.92	0.63	0.12	–	–
Glu	0.15	0.06	0.07	0.20	0.13	–
Cys	5.78	–	–	–	7.30	8.97
Cys-Cys	–	–	–	–	–	–
Pro	0.27	0.25	0.27	0.28	0.20	0.18
2-aminobutyric acid	1.83	1.98	2.18	2.13	2.25	2.12
Lys	0.34	0.30	0.27	0.33	0.08	0.04
His	0.19	0.17	0.17	0.17	0.07	–
Val	0.30	0.31	0.28	0.27	0.20	0.19
DL-3,4-DOPA	2.25	2.06	2.06	1.96	1.92	1.58
Arg	0.03	0.02	0.02	0.03	0.03	0.03
Met	0.61	0.57	0.54	0.49	0.48	0.43
Tyr	–	–	–	–	–	–
Ile	0.51	0.47	0.45	0.47	0.45	0.40
Leu	0.68	0.61	0.57	0.59	0.51	0.47
Nle	–	–	–	–	–	–
Phe	0.42	0.41	0.39	0.37	0.34	0.30
Try	–	–	–	–	–	–
Total amino acids	18.61	15.19	14.89	8.71	22.39	22.04
Essential amino acids	2.87	2.90	2.65	2.54	2.07	1.83
EAA/TAA	15.40	19.10	17.81	29.13	9.23	8.32
Acidic amino acids	1.55	0.83	0.75	0.57	2.08	1.43
Neutral amino acids	16.50	13.87	13.67	7.61	20.13	20.55
Basic amino acids	0.56	0.50	0.46	0.52	0.17	0.06

Note: – means not detected

Table 1.10 Total amino acid contents (%) of ginseng from the same region with different cultivation periods (Year)

No.	Region	Contents in ginseng of different ages
1	Antu, Dunhua, Helong, Jiaohe	$CG_5 > CG_4$
2	Huadian	$CG_4 > CG_5$
3	Dadi Ji'an, South Korea	$CG_5 > CG_6$
4	Changbai, Fusong, Shuangcha Ji'an	$CG_4 > CG_6 > CG_5$
5	Hunchun	$CG_6 > CG_5 > CG_4 > CG_4$(CC)
6	Kuandian	$CG_6 > CG_5 > CG_4$
7	Wangqing	$CG_5 > CG_4 > CG_6$
8	North Korea	$CG_4 > CG_5 > CG_6$

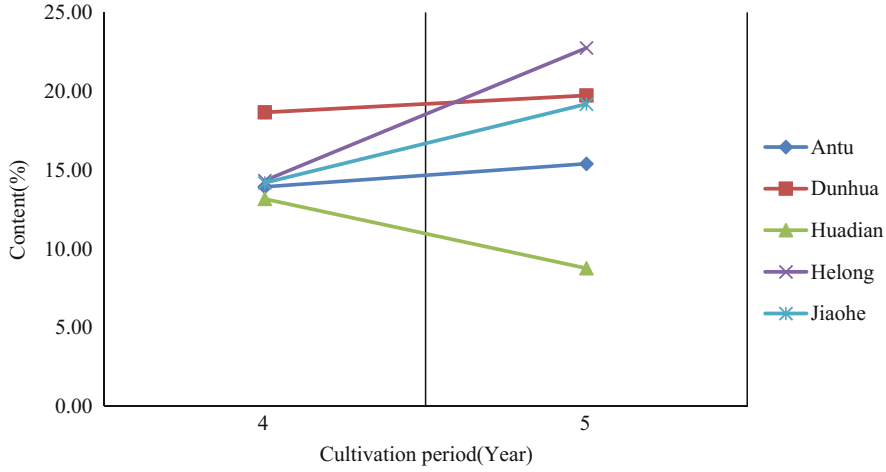

Fig. 1.5 The contents of total amino acids in ginseng of 4, 5 ages

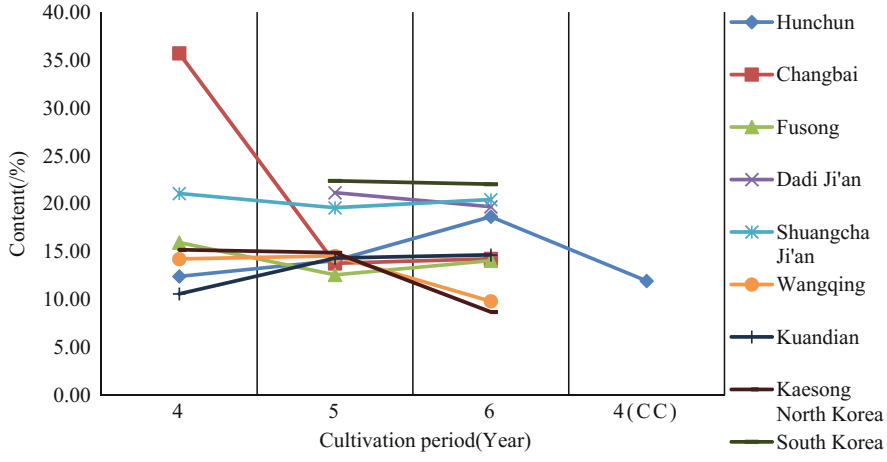

Fig. 1.6 The contents of total amino acids in ginseng of 4, 5, 6, 4(CC) ages

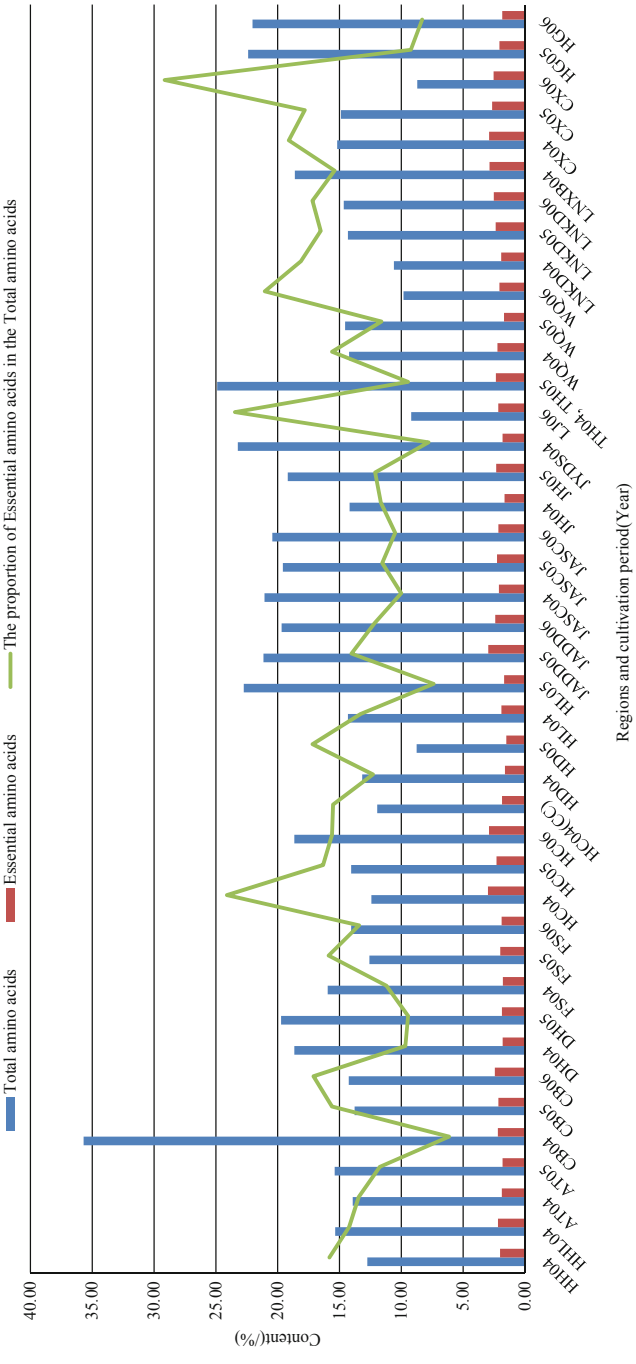

Fig. 1.7 Column diagram of total amino acids, essential amino acids of ginseng from different regions with different cultivation periods

Table 1.11 Essential amino acids contents (%) of ginseng from the same region with different cultivation periods (Year)

No.	Region	Contents in ginseng of different ages
1	Antu, Huadian, Helong	$CG_4 > CG_5$
2	Dunhua, Jiaohe	$CG_5 > CG_4$
3	Dadi Ji'an, South Korea	$CG_5 > CG_6$
4	Kuandian	$CG_6 > CG_5 > CG_4$
5	Changbai	$CG_6 > CG_4 > CG_5$
6	Fusong, Shuangcha Ji'an	$CG_5 > CG_6 > CG_4$
7	Hunchun	$CG_4 > CG_4(CC) > CG_6 > CG_5$
8	North Korea	$CG_4 > CG_5 > CG_6$

Table 1.12 The proportion of essential amino acids in the total amino acid of ginseng from the same region with different cultivation periods (Year)

No.	Region	The proportion of essential amino acids in the total amino acid
1	Changbai	$CG_6 > CG_5 > CG_4$
2	Fusong, Shuangcha Ji'an	$CG_5 > CG_6 > CG_4$
3	Hunchun	$CG_4 > CG_5 > CG_6 > CG_4(CC)$
4	Wangqing, North Korea	$CG_6 > CG_4 > CG_5$
5	Kuandian	$CG_4 > CG_6 > CG_5$

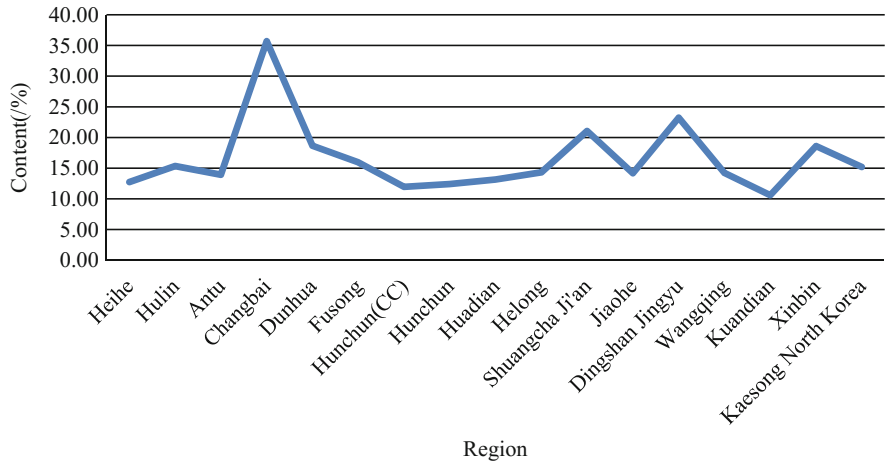

Fig. 1.8 Contents of total amino acids in 4-year-old ginseng

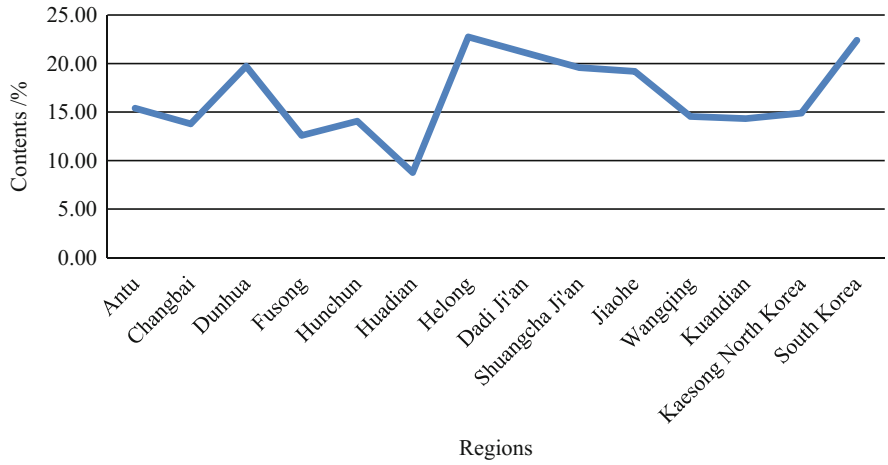

Fig. 1.9 Contents of total amino acids in 5-year-old ginseng

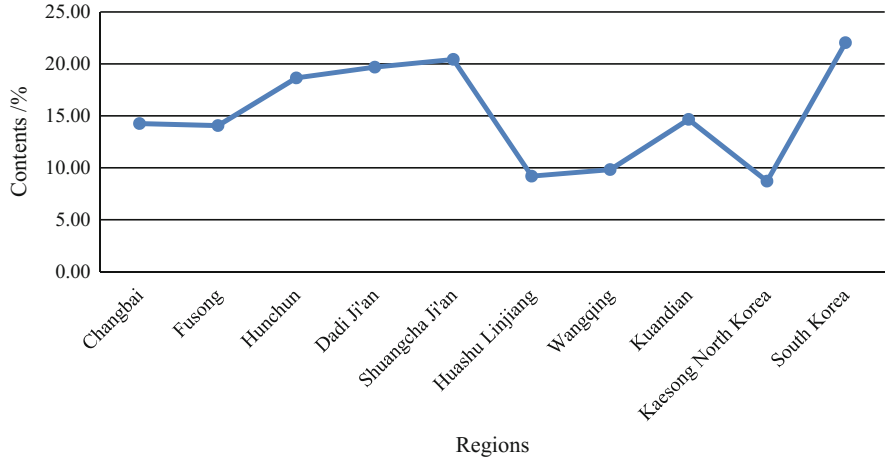

Fig. 1.10 Contents of total amino acids in 6-year-old ginseng

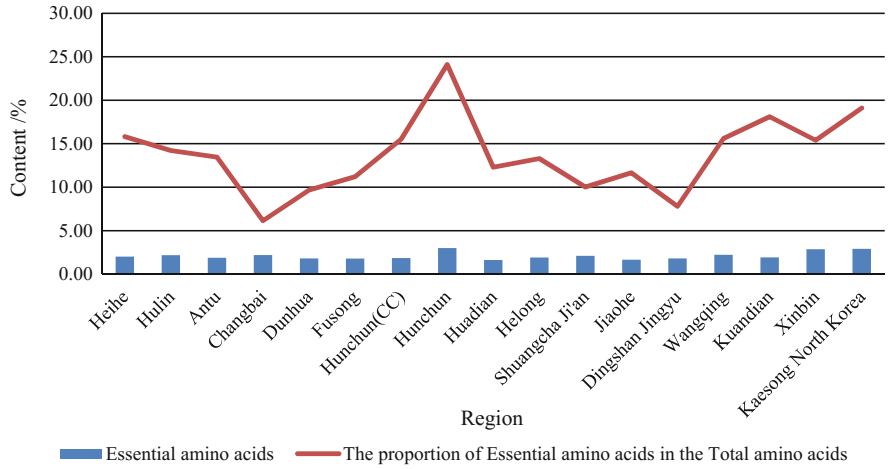

Fig. 1.11 The contents and proportions of essential amino acids in 4-year-old ginseng

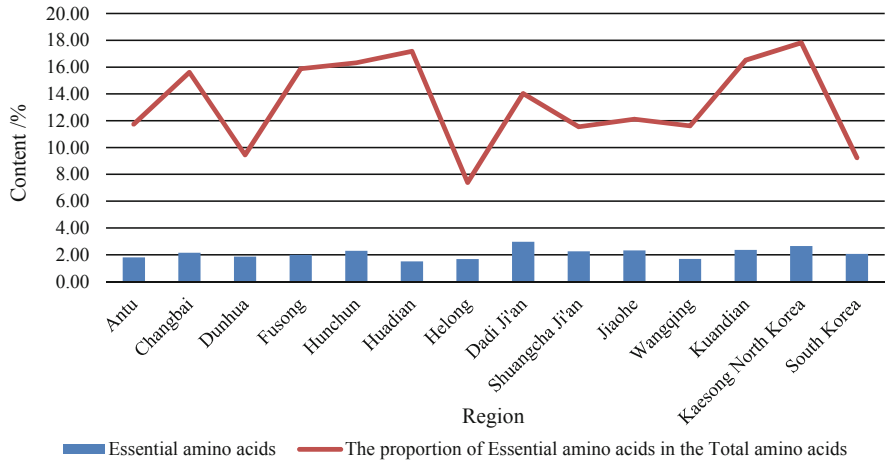

Fig. 1.12 The contents and proportions of essential amino acids in 5-year-old ginseng

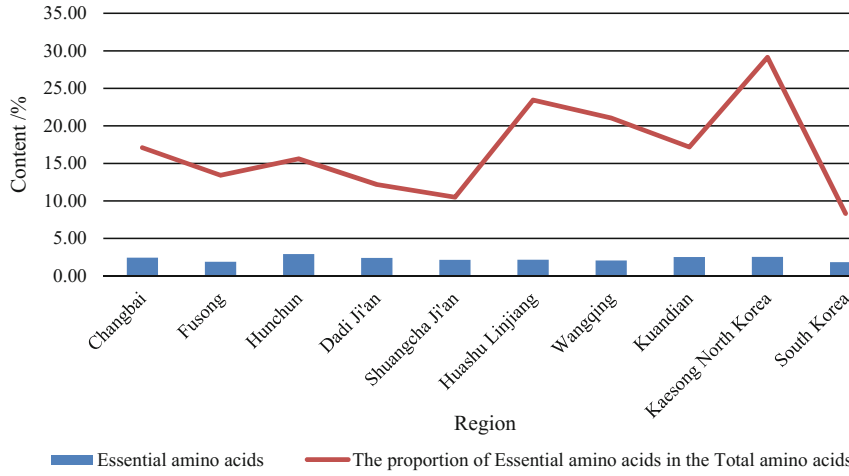

Fig. 1.13 The contents and proportions of essential amino acids in 6-year-old ginseng

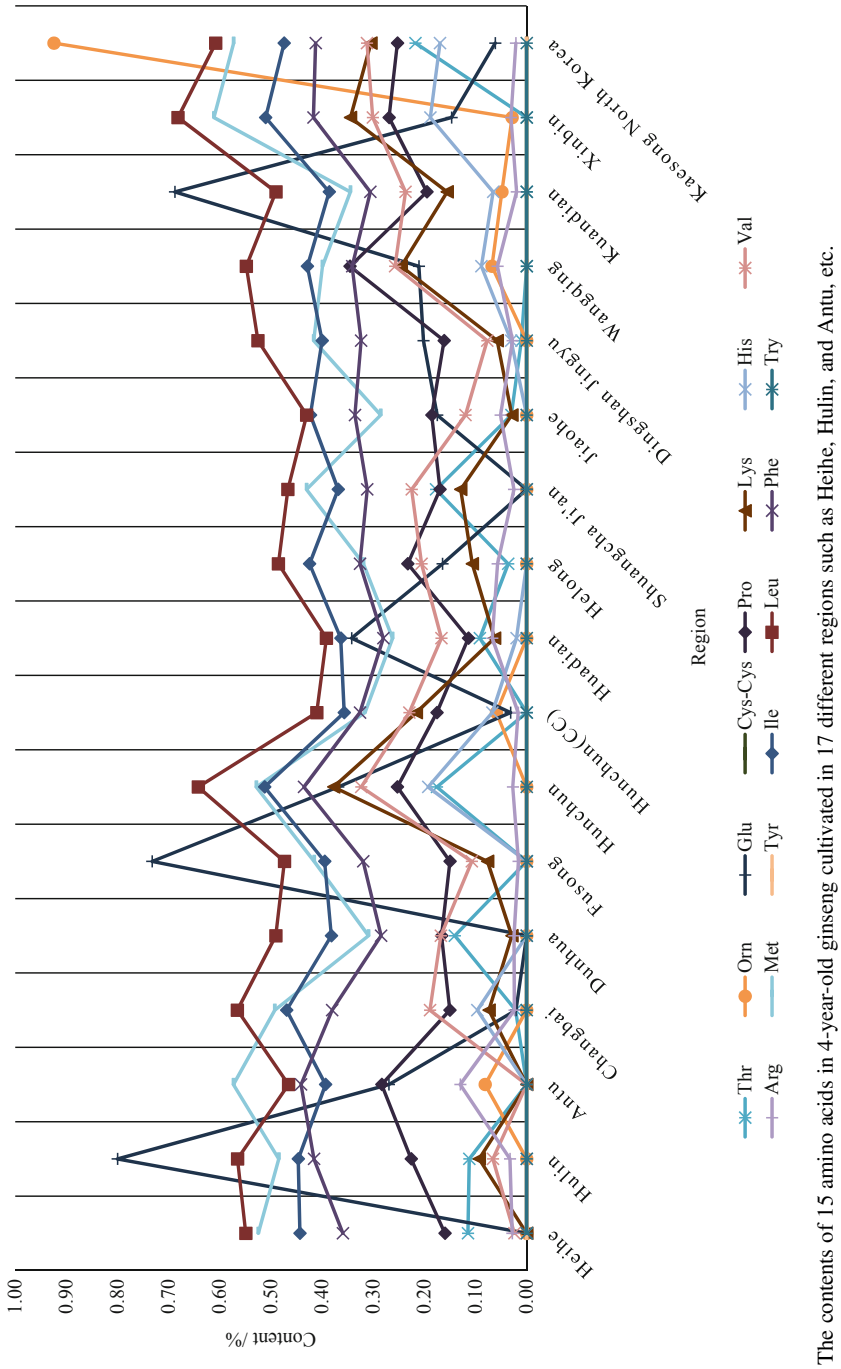

Fig. 1.14 The contents of 15 amino acids in 4-year-old ginseng cultivated in 17 different regions such as Heihe, Hulin, and Antu, etc.

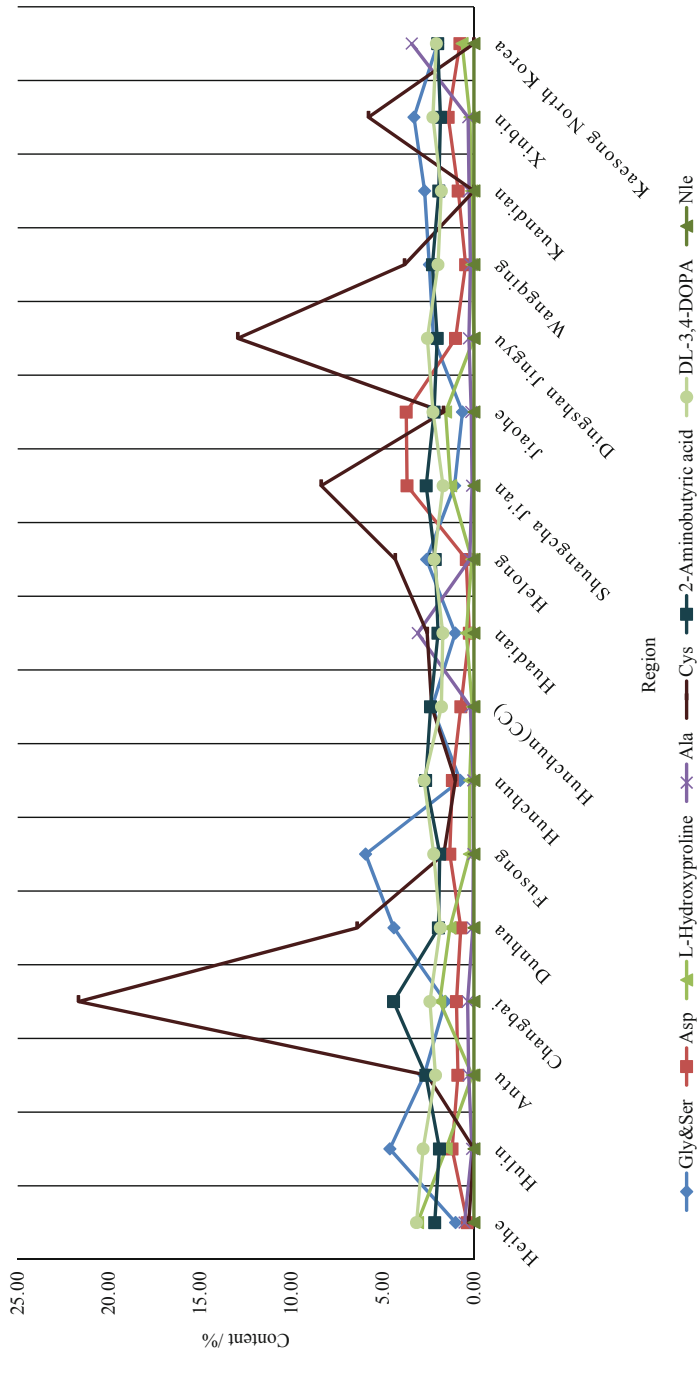

Fig. 1.15 The contents of 8 amino acids in 4-year-old ginseng cultivated in 17 different regions such as Heihe, Hulin, and Antu, etc.

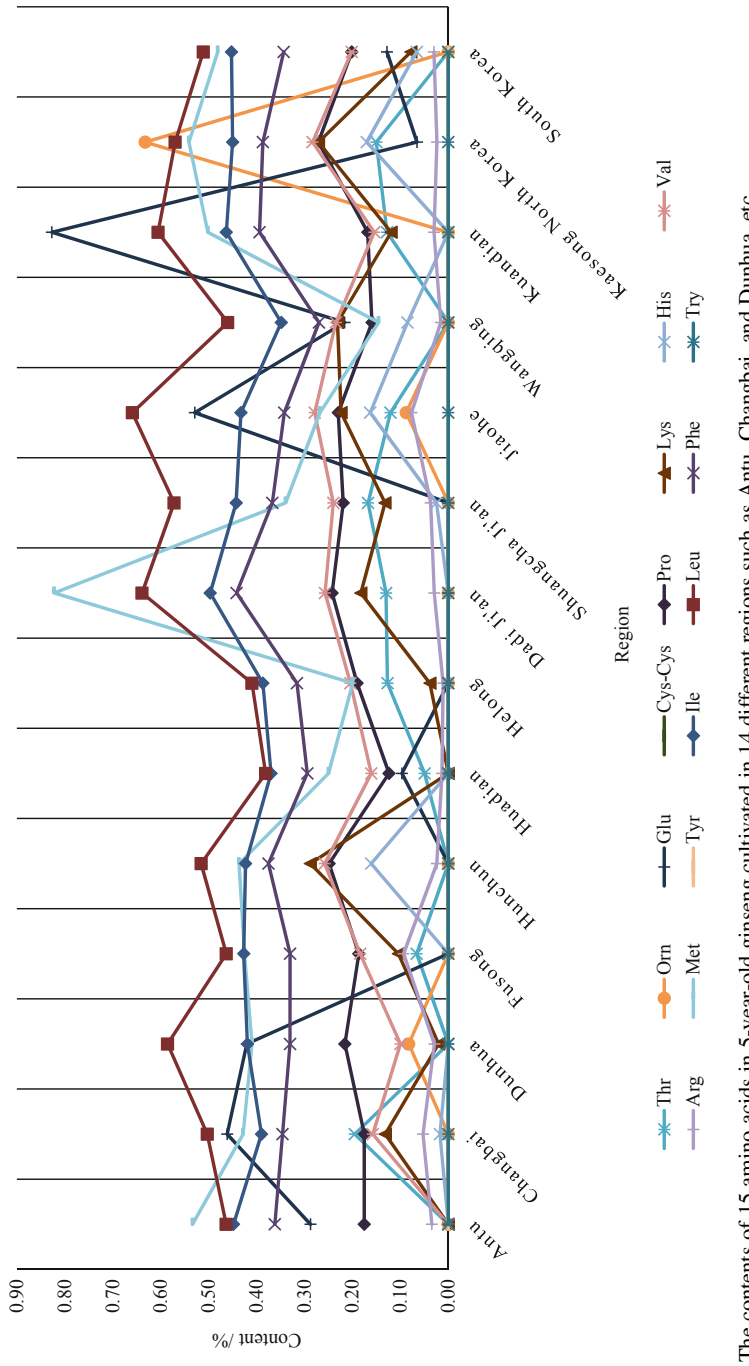

Fig. 1.16 The contents of 15 amino acids in 5-year-old ginseng cultivated in 14 different regions such as Antu, Changbai, and Dunhua, etc.

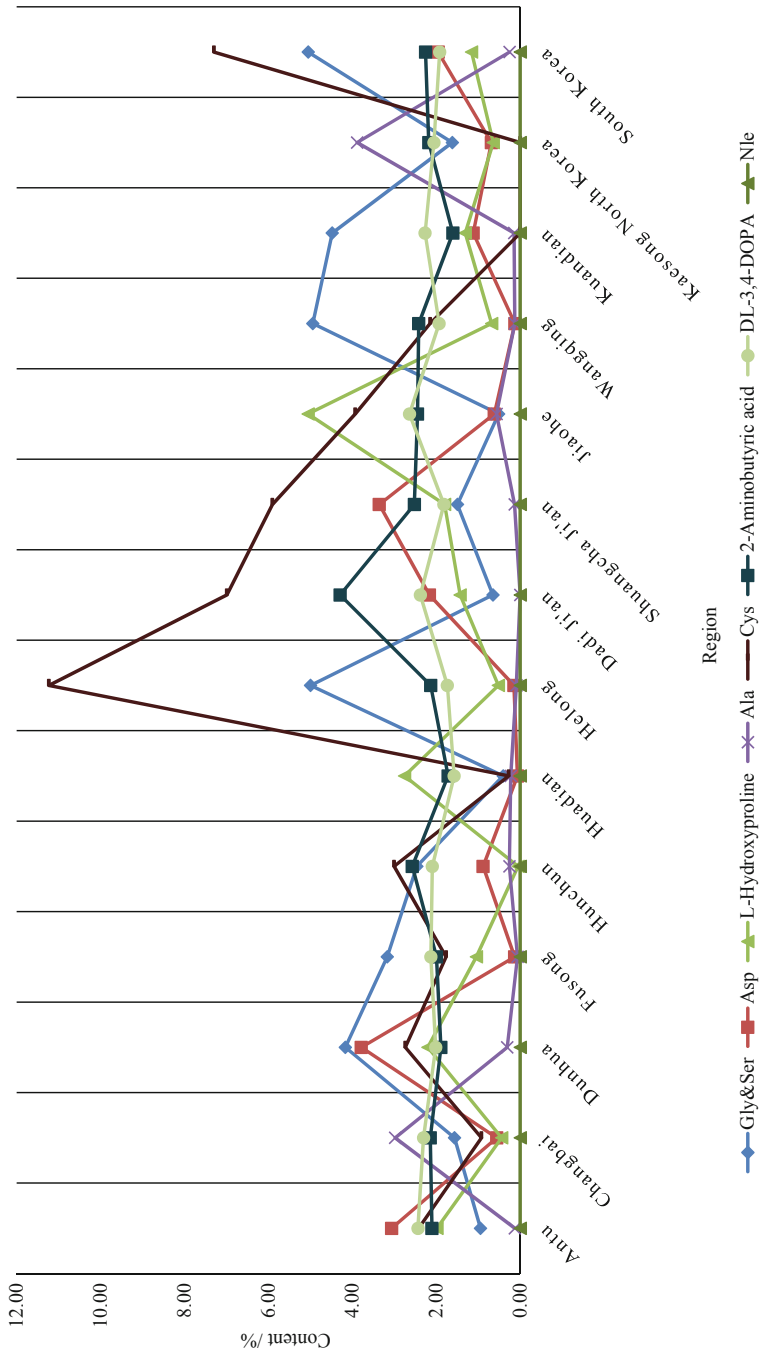

Fig. 1.17 The contents of 8 amino acids in 5-year-old ginseng cultivated in 14 different regions such as Antu, Changbai, and Dunhua, etc.

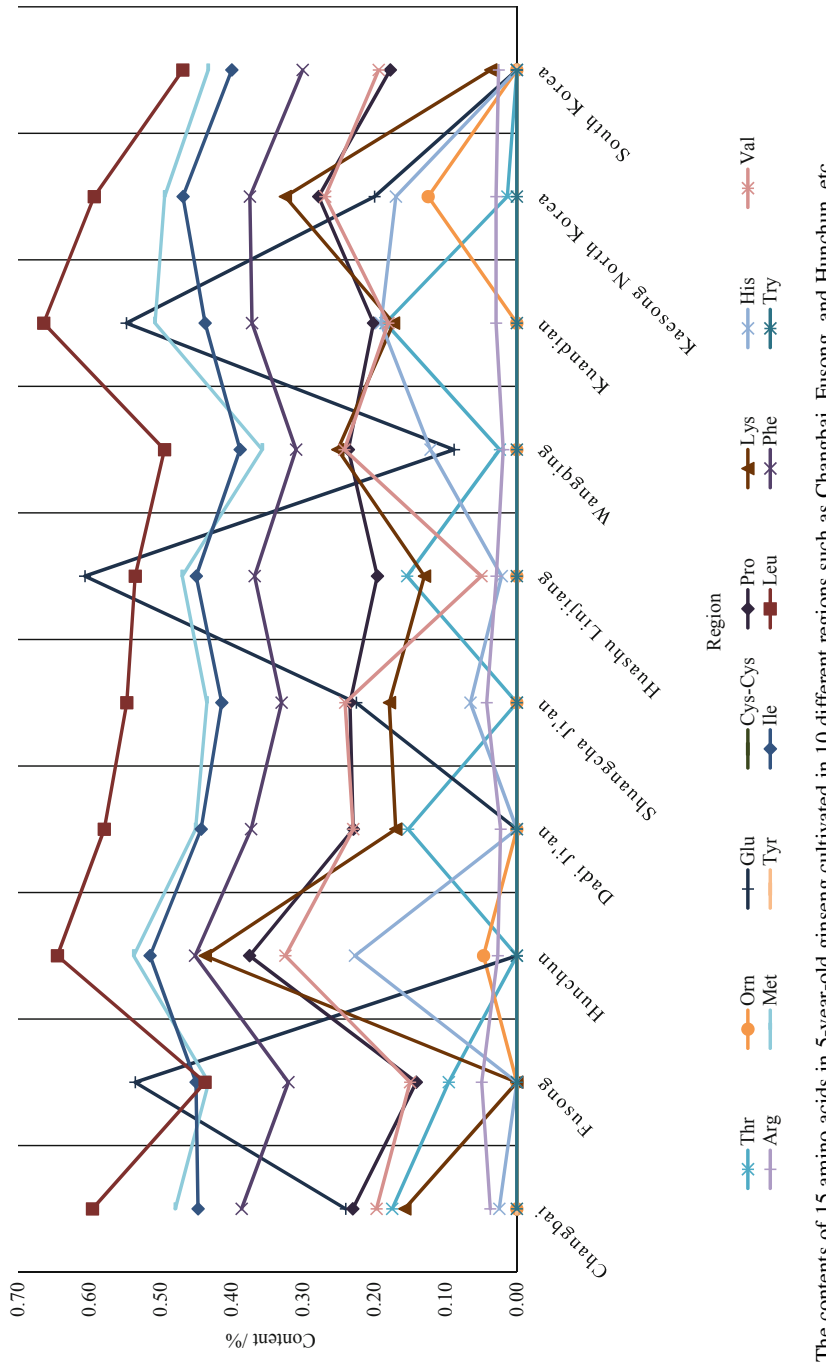

Fig. 1.18 The contents of 15 amino acids in 5-year-old ginseng cultivated in 10 different regions such as Changbai, Fusong, and Hunchun, etc.

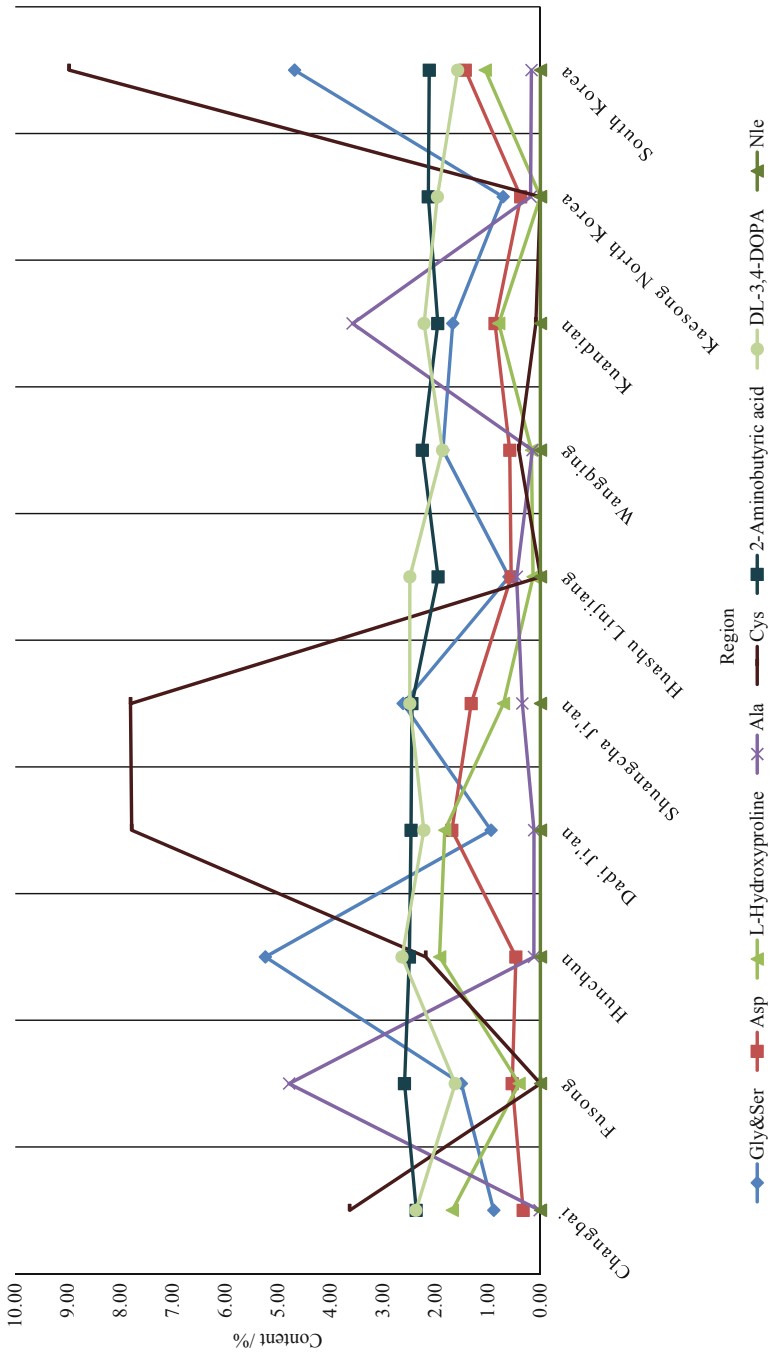

Fig. 1.19 The contents of 8 amino acids in 6-year-old ginseng cultivated in 10 different regions such as Changbai, Fusong, and Hunchun, etc.

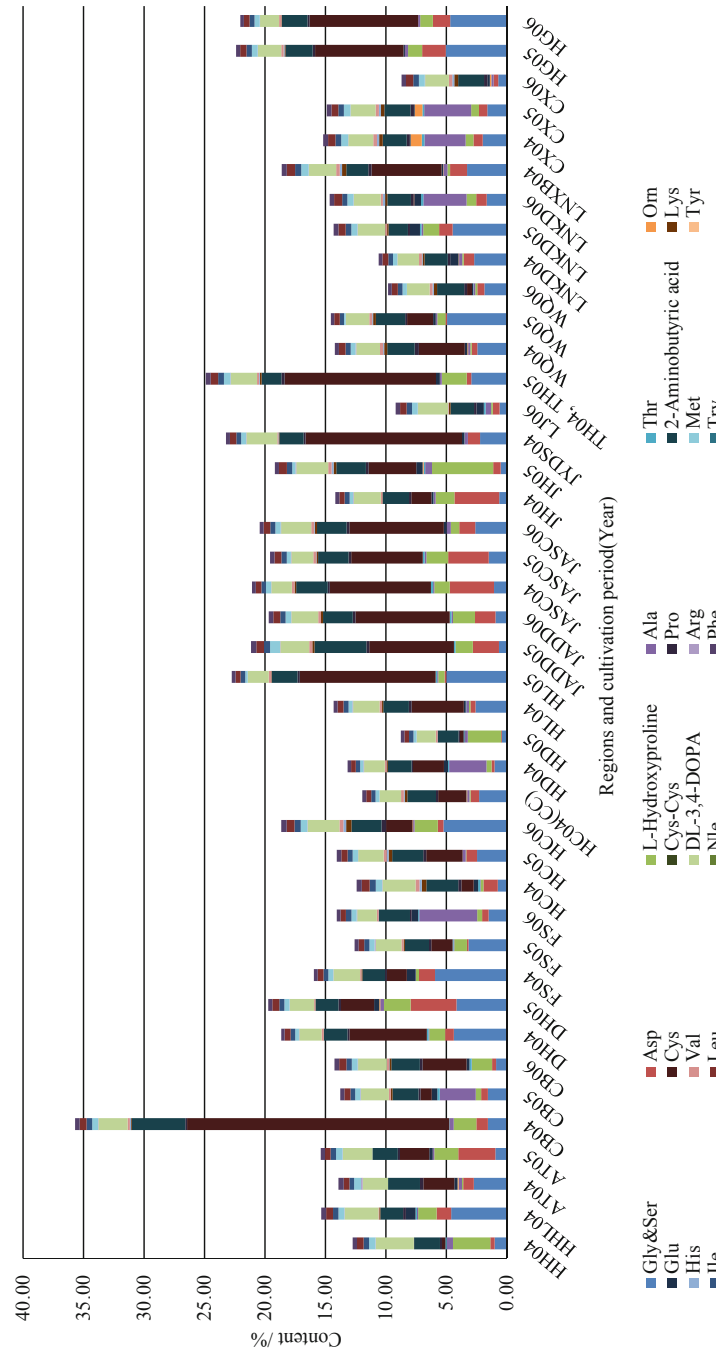

Fig. 1.20 The accumulation histogram of amino acid contents of different ages ginseng from different regions

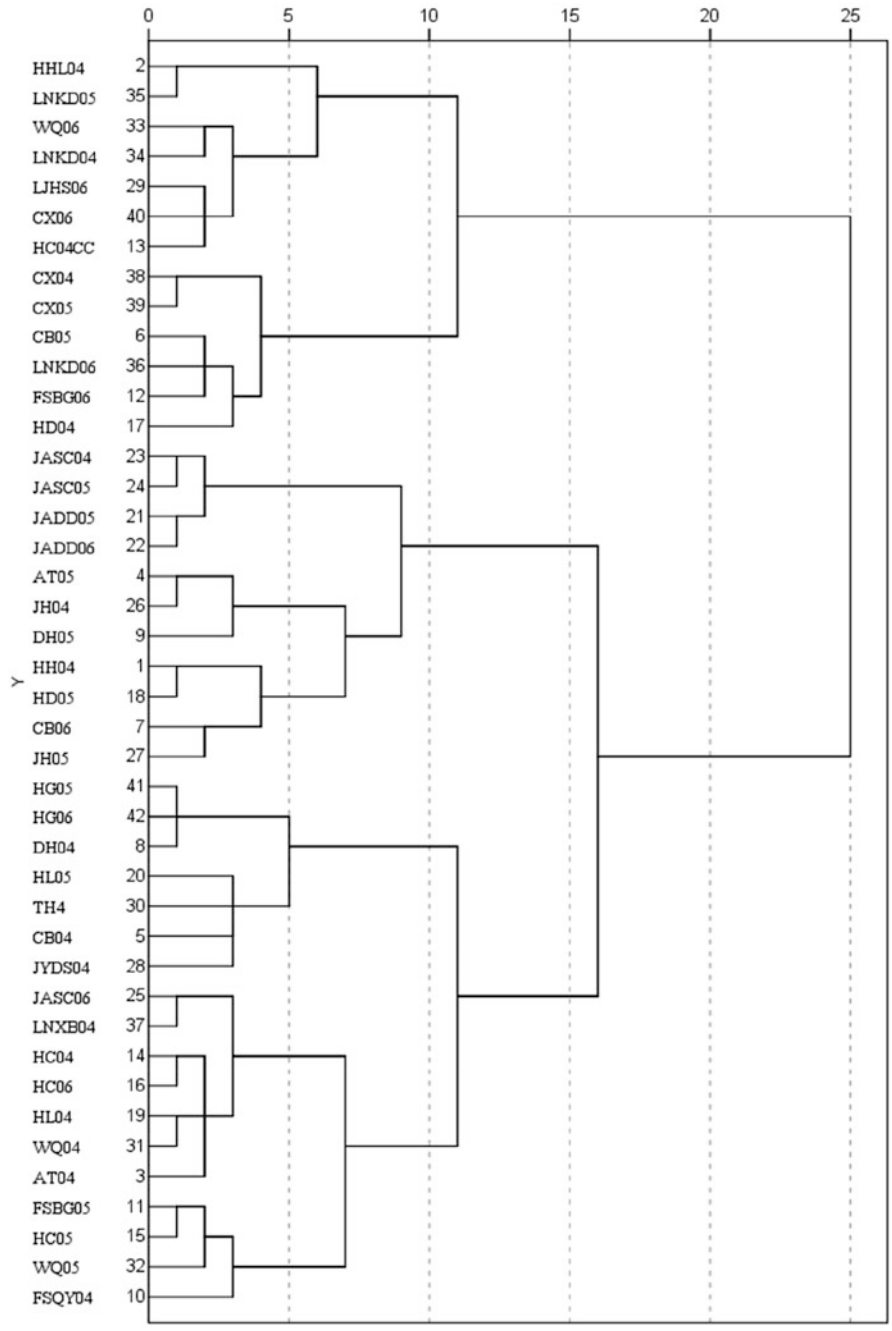

Fig. 1.21 Hierarchical graph of cluster analysis of amino acids in ginseng

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