Pingya Li
Jinping Liu *Editors*

Ginseng Nutritional Components and Functional Factors

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Preface

Panax ginseng C. A. Mey., belonging to the family Araliaceae, has some elegant names such as Huangjing, Dijing, God Grass, or the king of herbs in ancient times. As one of the three famous treasures of the Northeast of China, ginseng is considered a valuable therapeutic medicine and dietary supplement.

The earliest description about ginseng was recorded in "Shen Nong's Materia Medica" as follows: ginseng could be used to nourish internal organs, stabilize the psyche, prevent being horrified, remove evil influence, brighten eyes, benefit wisdom, and increase longevity. The nourishing and health-preserving efficacy of ginseng has been always highly praised and commended in traditional Chinese and Western medicine. But in some ways, there are still many vague concepts for the consumers. Aiming at providing the reference for making good use of ginseng, it is necessary to publish a book focused on the nutritional components. According to the place of origin, the ginseng grown in China is called "Chinese ginseng," grown in North Korea is called "Korean ginseng," grown in Japan is called "Japanese ginseng," and grown in Canada and the United States is called "American ginseng." China ginseng is grouped into three categories (wild ginseng, mountain-cultivated ginseng, and cultivated ginseng) according to the different growing environments and diverse cultivation methods. Wild ginseng grows in its native habitat, commonly in the primeval forest at the altitude of 1500–2000 m. The ginseng grown in Jilin province with the best quality is named as "Jilin Ginseng." It is the traditional belief that wild ginseng is more medicinally efficacious, and wild ginseng commanded much higher prices in the market. But the wild ginseng is nearing extinction now. Generally, wild ginseng grows for decades, even hundreds of years. The longer age, the thicker body, the better effect and the higher price. Since wild ginseng grows in the mountain with hard soil, it is greatly affected by the natural environment. All parts of wild ginseng show specific shapes with tight skin and deep grooves. Its body stretches in a shape similar to the Chinese character "八", with a proportionality of body, leg, and fibrous root. Cultivated ginseng is intensely cultivated under artificial shade structures. Due to the different processing methods, cultivated ginseng could be produced into different varieties such as dried raw ginseng, red ginseng, preserved fresh ginseng, etc. Mountain-cultivated ginseng, also called "Lin-Xia-Shan-Shen," refers to a method of growing ginseng in a hardwood forest environment under conditions without any other human intervention. It is

collected at least after 10–20 years or longer. As such, mountain-cultivated ginseng is indeed indistinguishable from the wild ginseng due to similar characteristics.

Since the 1950s, the research on ginseng has made great progress and rich achievements in cultivation, breeding new varieties, phytochemicals, structural modification, serum pharmacochemistry, pharmacology and toxicology, action mechanism, and innovation drug development. Among them, the books such as "Chinese Ginseng," "Compilation of Ginseng Research Progress," and "Standard Ginsenosides NMR Spectrum" are the representative achievements in different fields.

In 2012, ginseng was approved as a new resource food in China, which means ginseng became both medicine and food. As the new era of the ginseng industry is coming, the application scope is expanding and the industrial chain is extending.

As a participant in applying the new resource food, I deeply feel the responsibility for systematically studying the nutritional ingredients and functional factors in ginseng, and comparing the similarity or the difference between domestic ginseng and foreign ginseng. The aims are to provide scientific reference for the researchers and the consumers and to discover the mysteries of ginseng.

In this book, a total of 45 ginseng samples (from 3 to 5 years old) were collected from Jilin Province, Heilongjiang Province, Liaoning Province, and the cities in Korea. The contents of ginsenosides, polysaccharide, sterols, proteins, amino acids, flavonoids, nucleotides, organic acids, vitamins, and inorganic elements were analyzed and determined. As a result, the scientific data were obtained.

This book is divided into 11 chapters. The determination methods of ginseng nutrition and function factors including amino acids, proteins, saccharides, volatile oils, nucleosides, flavonoids, ginsenosides, organic acids, vitamins, inorganic elements, and sterols were established, and their contents in ginseng were determined respectively.

Thanks for the great support of Kangmei Xinkaihe (Jilin) Pharmaceutical Co., Ltd.

Due to our limited knowledge, there might be some mistakes and flaws in this book. Comments and suggestions are highly appreciated.

Changchun, People's Republic of China Pingya Li

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Jinping Liu is a professor at the School of Pharmaceutical Sciences, Jilin University, China. She received her Ph.D. degree from Shenyang Pharmaceutical University, China. Her career as a researcher began at the Institute of Frontier Medical science, Jilin University, China, where she worked as a Postdoctoral Fellow. She has also been a visiting scholar at Harvard Medical School, Harvard University, USA. She is currently the director of the Jilin Engineering Research Center of Ginseng/American Ginseng Innovative Drugs. Her work largely focuses on the research and development of active ingredients from traditional Chinese medicine.

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Analysis of Amino Acids in Ginseng

1

Hao Zhang, Yunhe Liu, and Pingya Li

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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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.

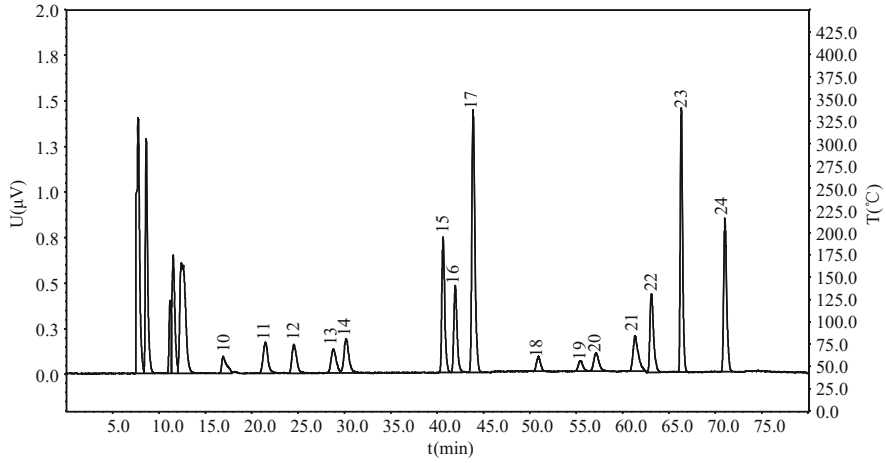

Fig. 1.1 HPLC-ELSD chromatogram of mixed standard solution A. *10* Cys. *11* Pro. *12* 2-aminobutyric acid. *13* Lys. *14* His. *15* Val. *16* DL-3,4-DOPA. *17* Arg. *18* Met. *19* Tyr. *20* Ile. *21* Leu. *22* Nle. *23* Phe. *24* Trp

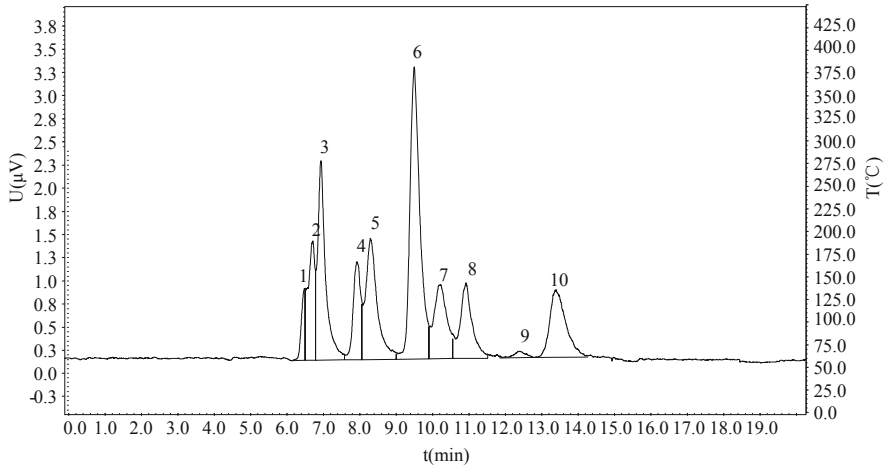

Fig. 1.2 HPLC-ELSD chromatogram of mixed standard solution B. *1* Gly. *2* Ser. *3* Asp. *4* hydroxyproline. *5* Ala. *6* Thr. *7* Orn. *8* Glu. *9* Cys. *10* Cys-Cys

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.

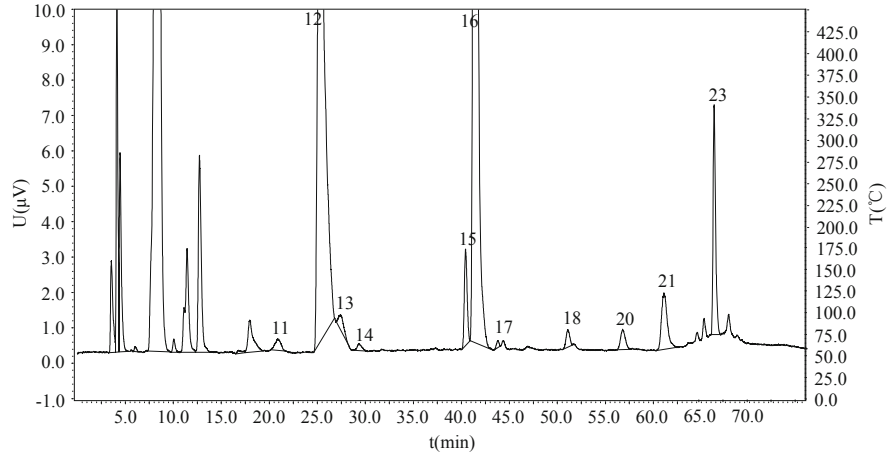

Fig. 1.3 HPLC-ELSD chromatogram of amino acids of 6-year-old ginseng from Wangqing. *11* Pro. *12* 2-aminobutyric acid. *13* Lys. *14* His. *15* Val. *16* DL-3,4-DOPA. *17* Arg. *18* Met. *20* Ile. *21* Leu. *22* Nle. *23* Phe

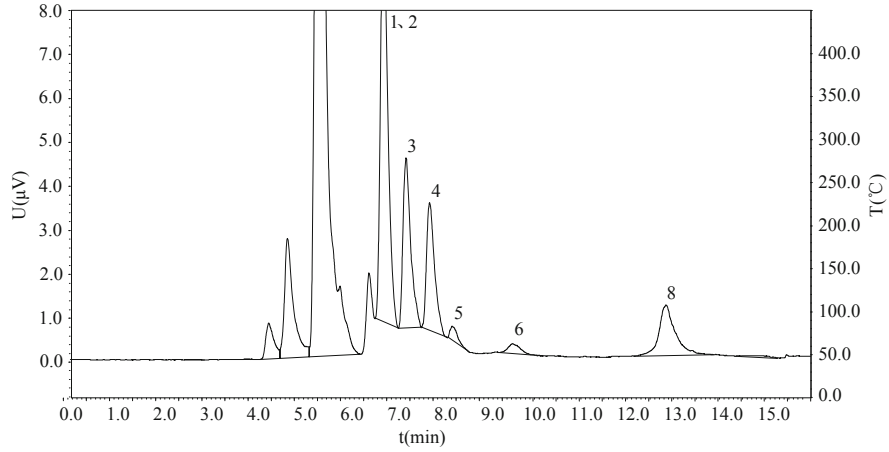

Fig. 1.4 HPLC-ELSD chromatogram of amino acids of 5-year-old ginseng from Kuandian. *1* Gly. *2* Ser. *3* Asp. *4* hydroxyproline. *5* Ala. *6* Thr. *8* Glu

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	$CG_4 > CG_5 > CG_6$

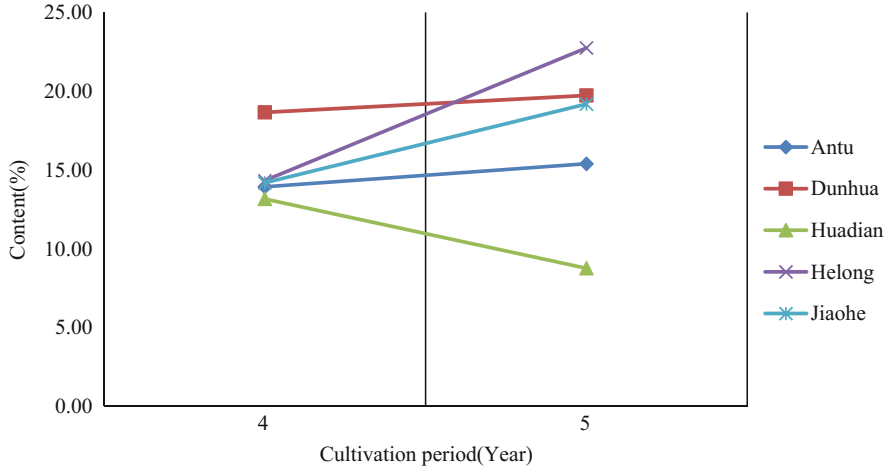

Fig. 1.5 The contents of total amino acids in ginseng of 4, 5 ages

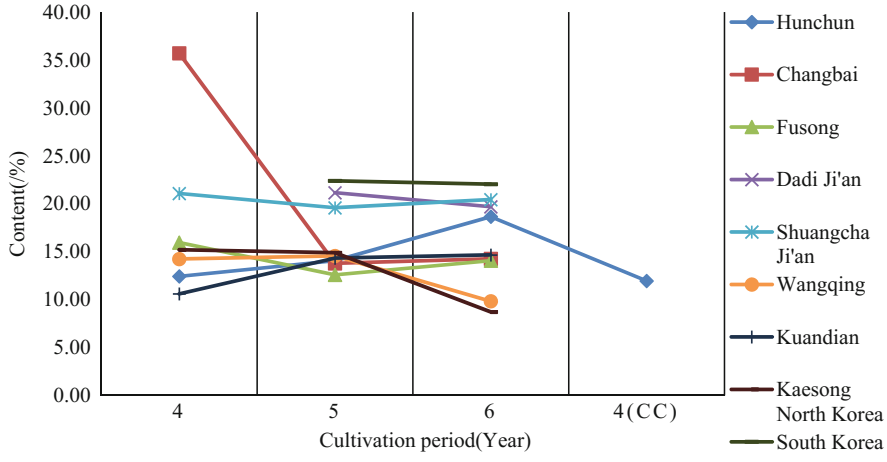

Fig. 1.6 The contents of total amino acids in ginseng of 4, 5, 6, 4(CC) ages

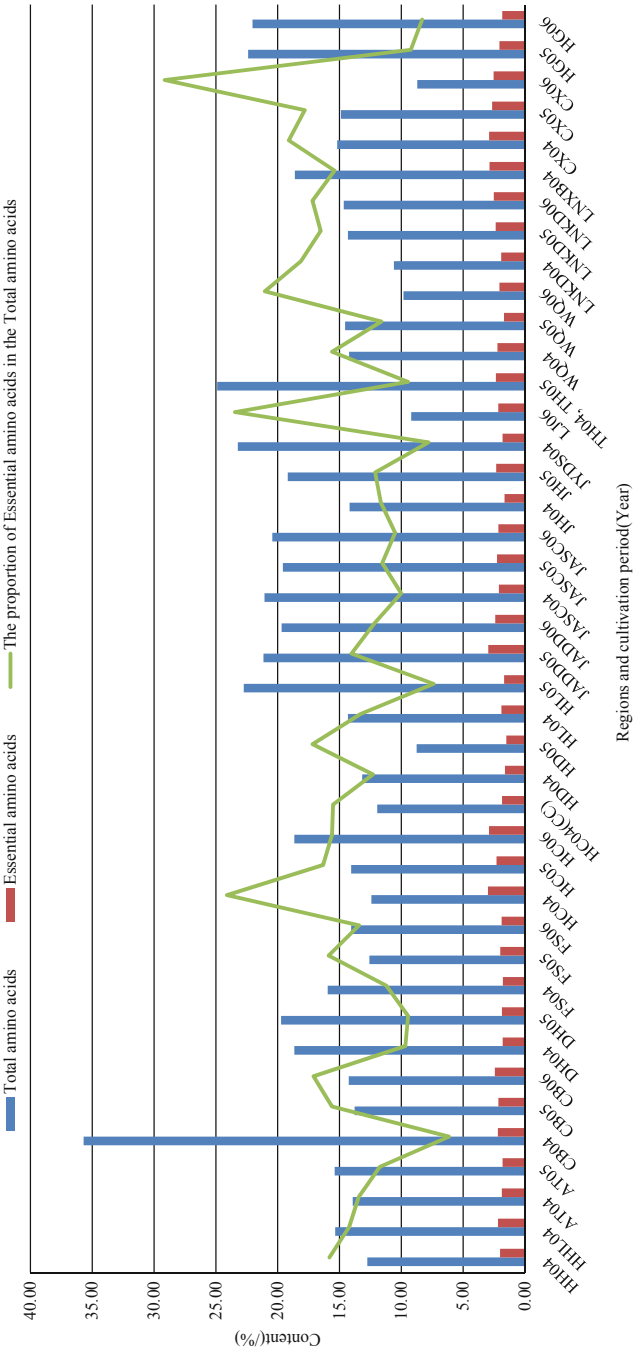

Fig. 1.7 Column diagram of total amino acids, essential amino acids of ginseng from different regions with different cultivation periods

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	$CG_4 > CG_6 > CG_5$

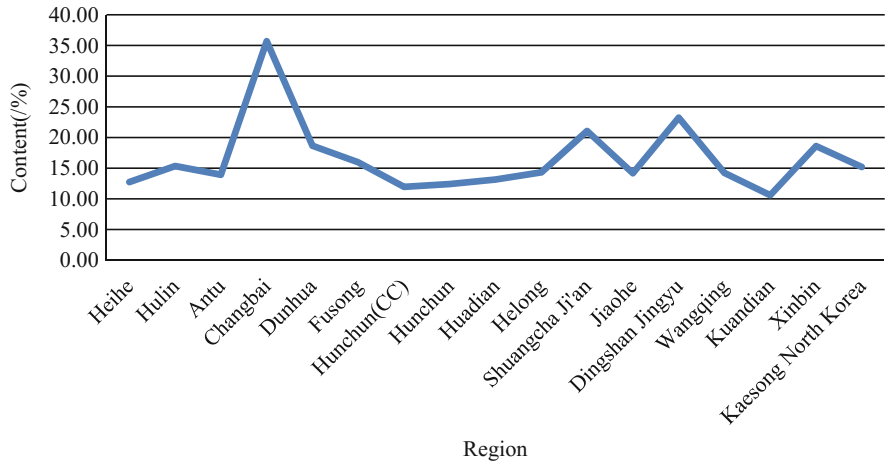

Fig. 1.8 Contents of total amino acids in 4-year-old ginseng

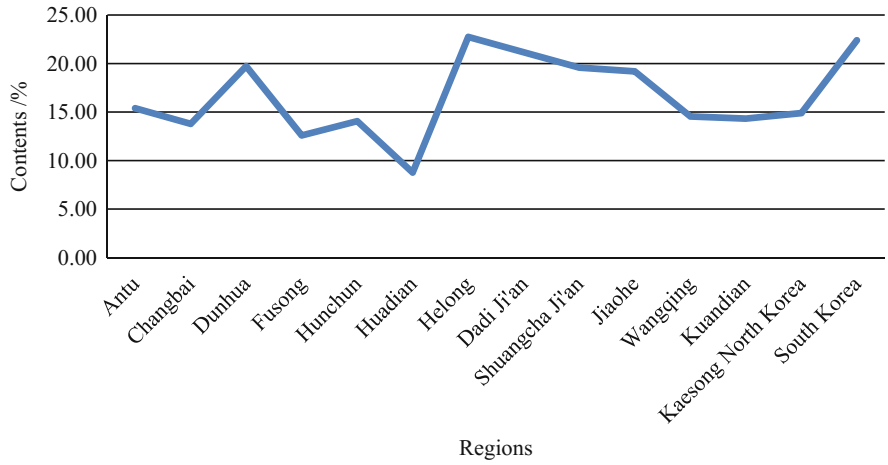

Fig. 1.9 Contents of total amino acids in 5-year-old ginseng

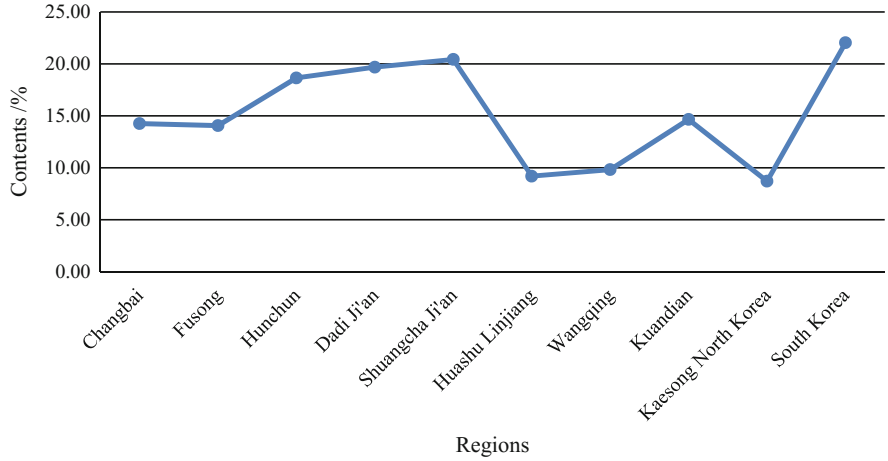

Fig. 1.10 Contents of total amino acids in 6-year-old ginseng

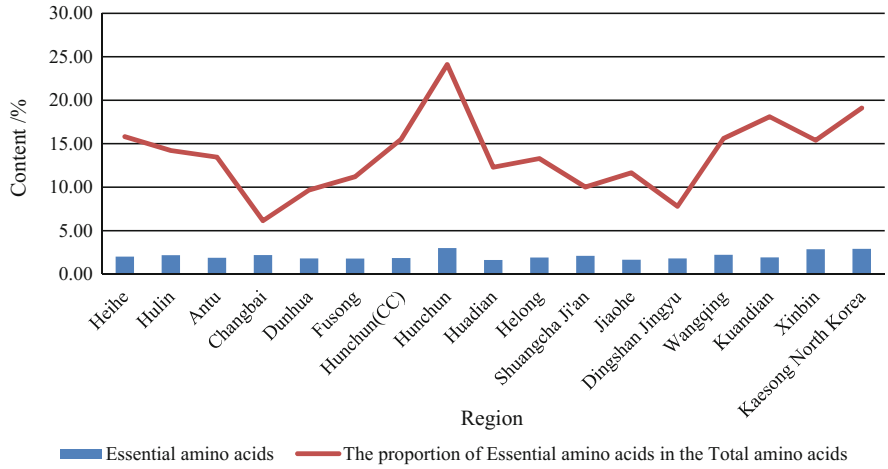

Fig. 1.11 The contents and proportions of essential amino acids in 4-year-old ginseng

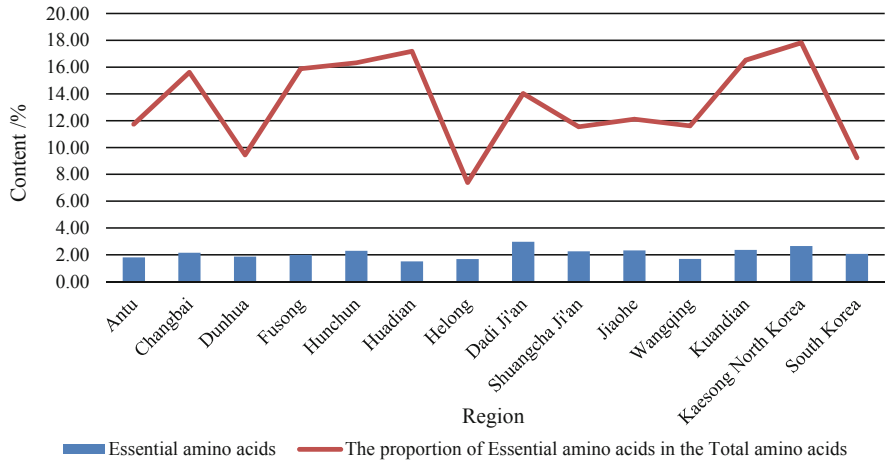

Fig. 1.12 The contents and proportions of essential amino acids in 5-year-old ginseng

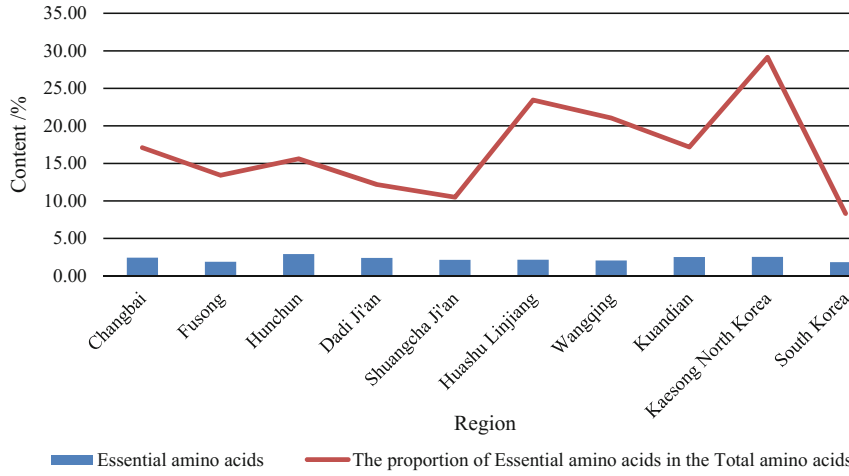

Fig. 1.13 The contents and proportions of essential amino acids in 6-year-old ginseng

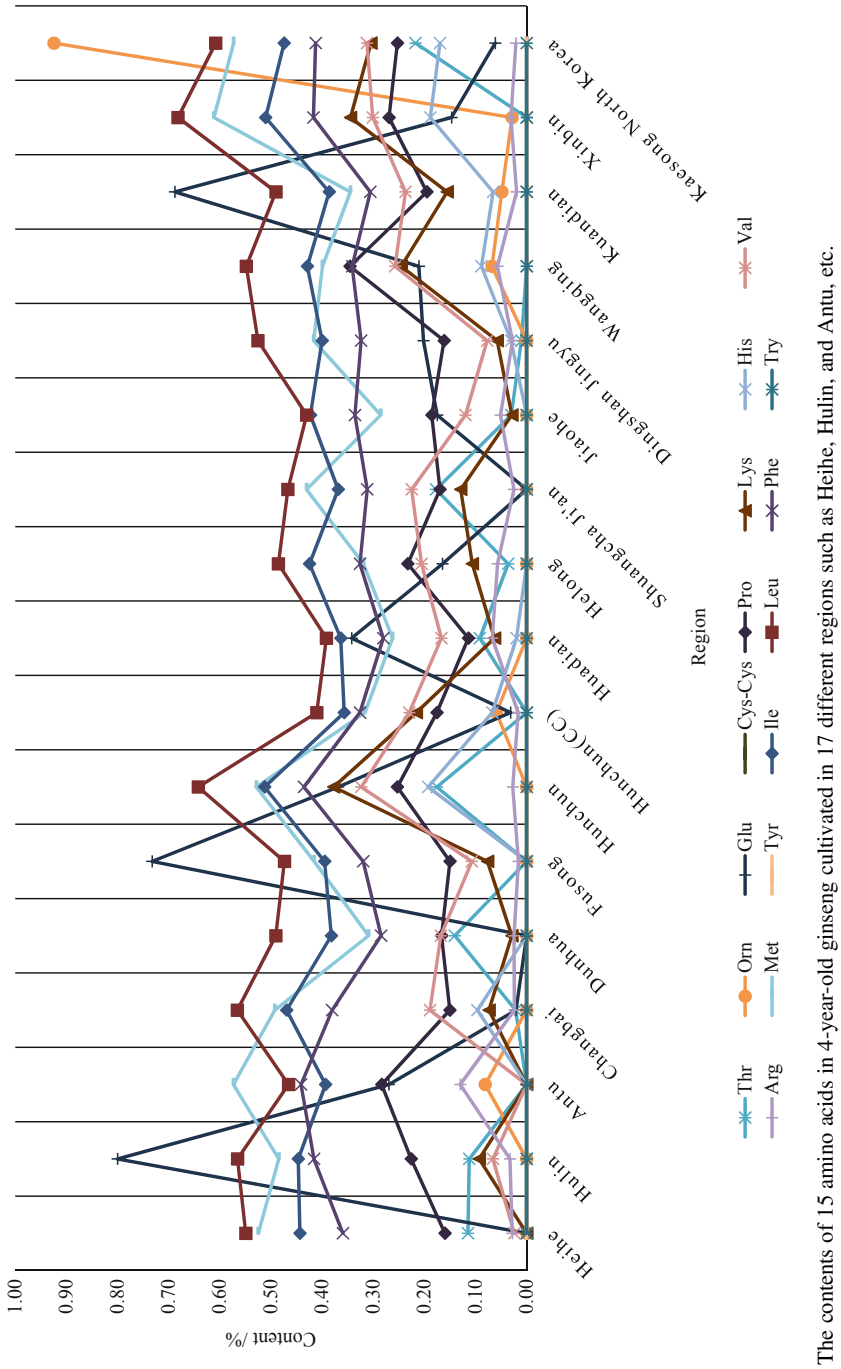

Fig. 1.14 The contents of 15 amino acids in 4-year-old ginseng cultivated in 17 different regions such as Heihe, Hulin, and Antu, etc.

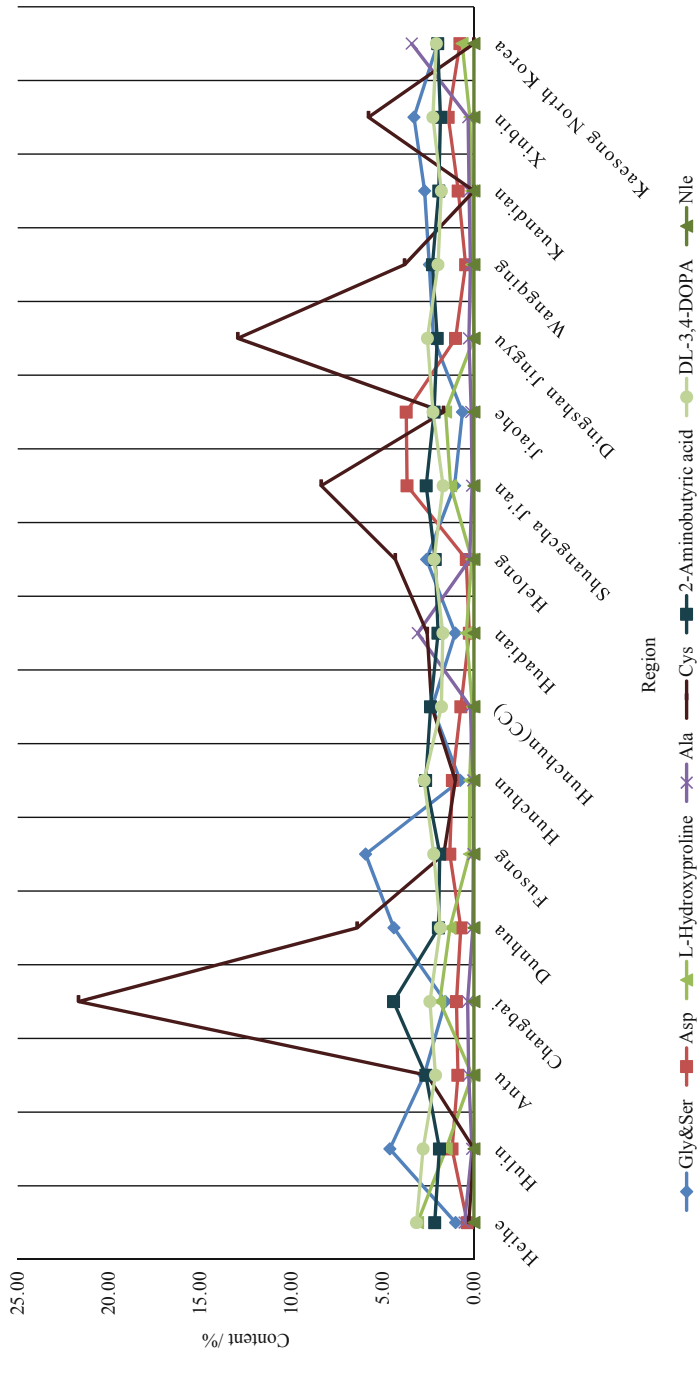

Fig. 1.15 The contents of 8 amino acids in 4-year-old ginseng cultivated in 17 different regions such as Heihe, Hulin, and Antu, etc.

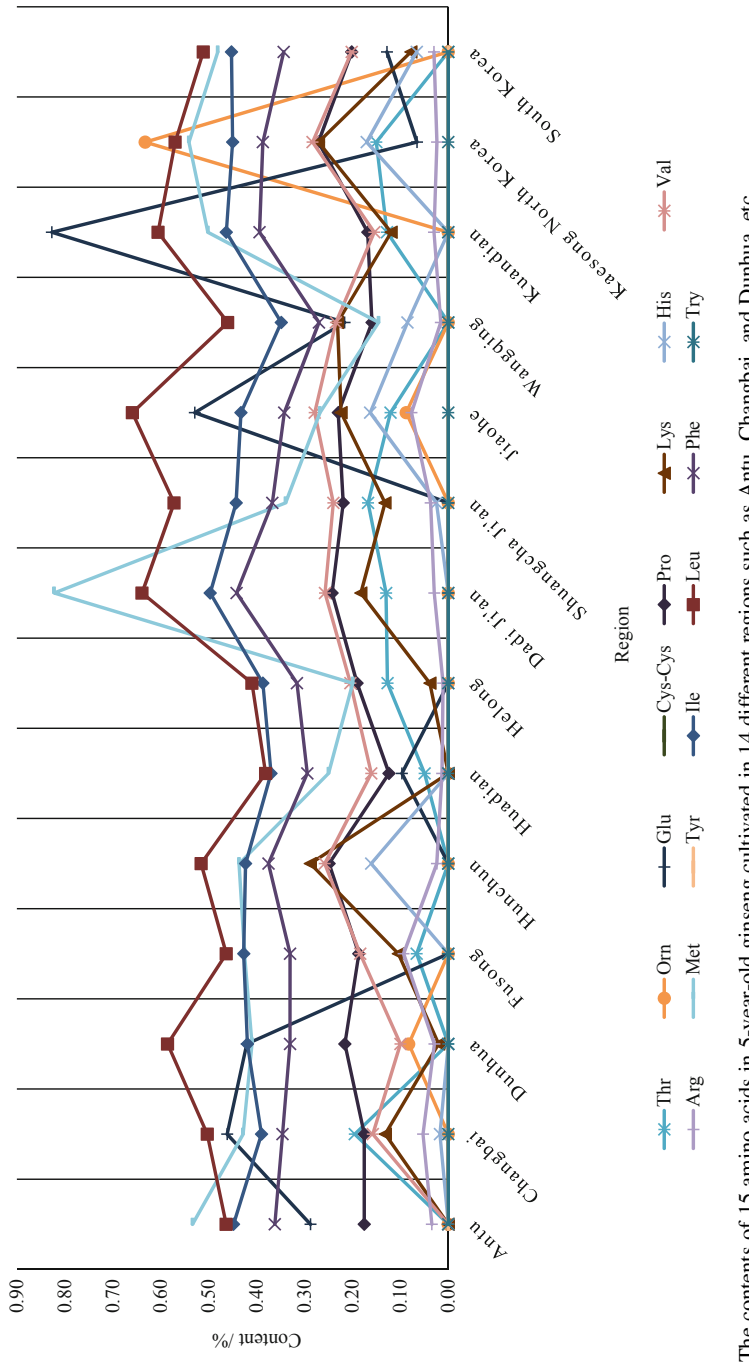

Fig. 1.16 The contents of 15 amino acids in 5-year-old ginseng cultivated in 14 different regions such as Antu, Changbai, and Dunhua, etc.

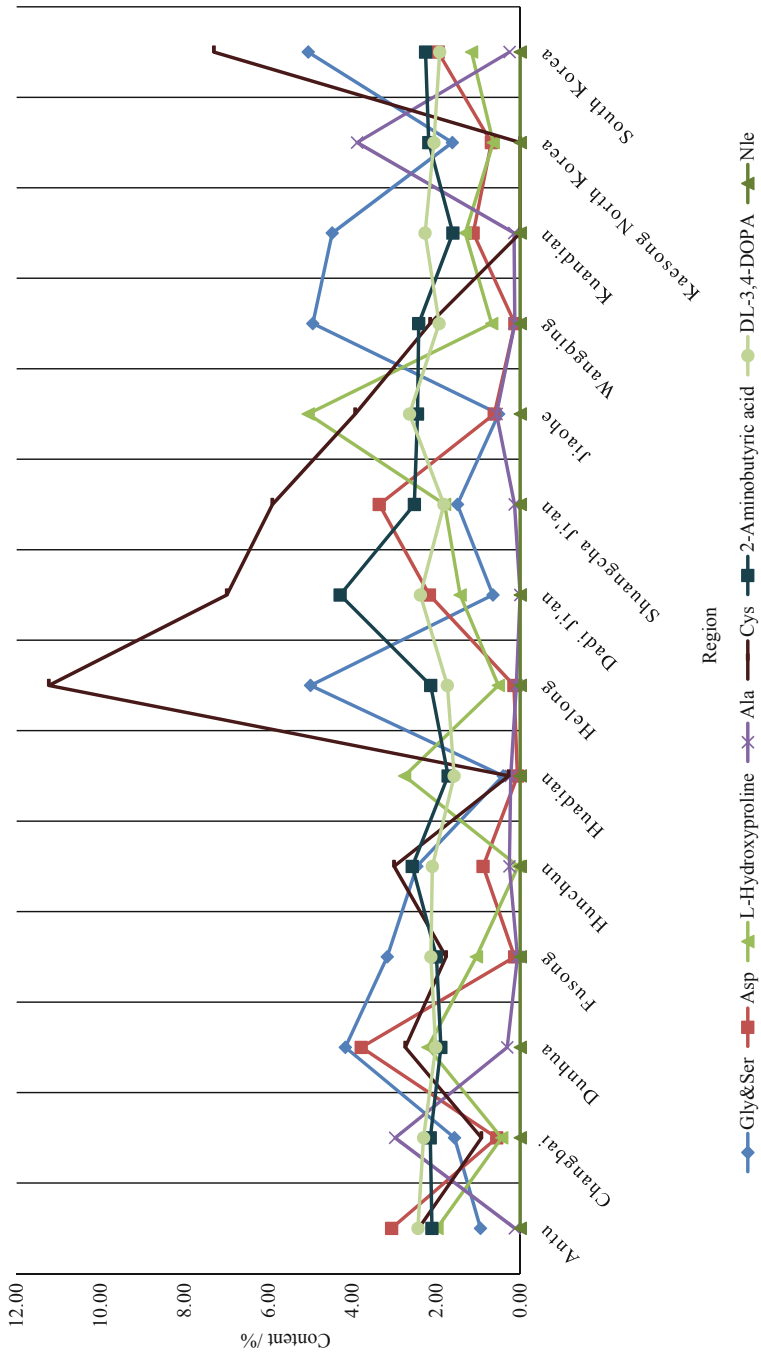

Fig. 1.17 The contents of 8 amino acids in 5-year-old ginseng cultivated in 14 different regions such as Antu, Changbai, and Dunhua, etc.

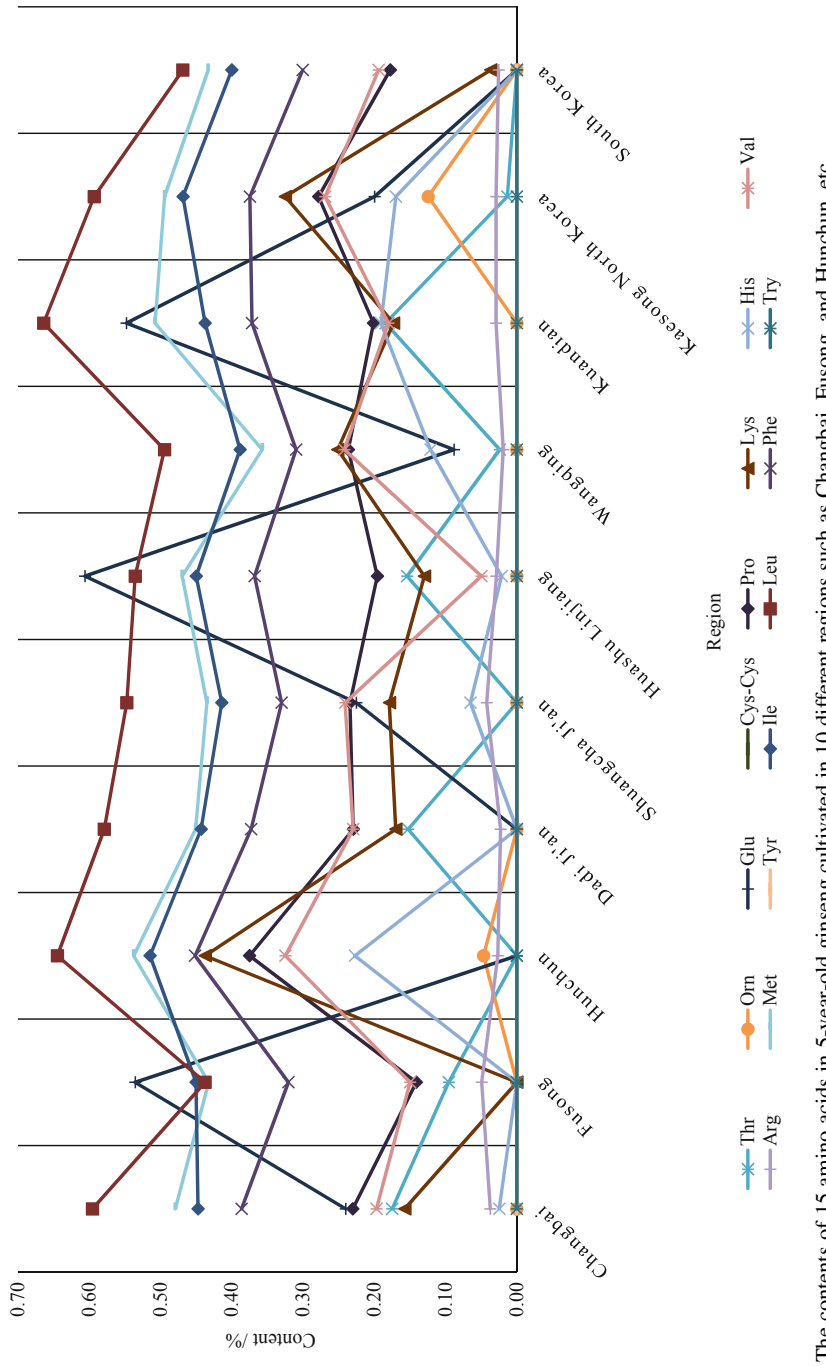

Fig. 1.18 The contents of 15 amino acids in 5-year-old ginseng cultivated in 10 different regions such as Changbai, Fusong, and Hunchun, etc.

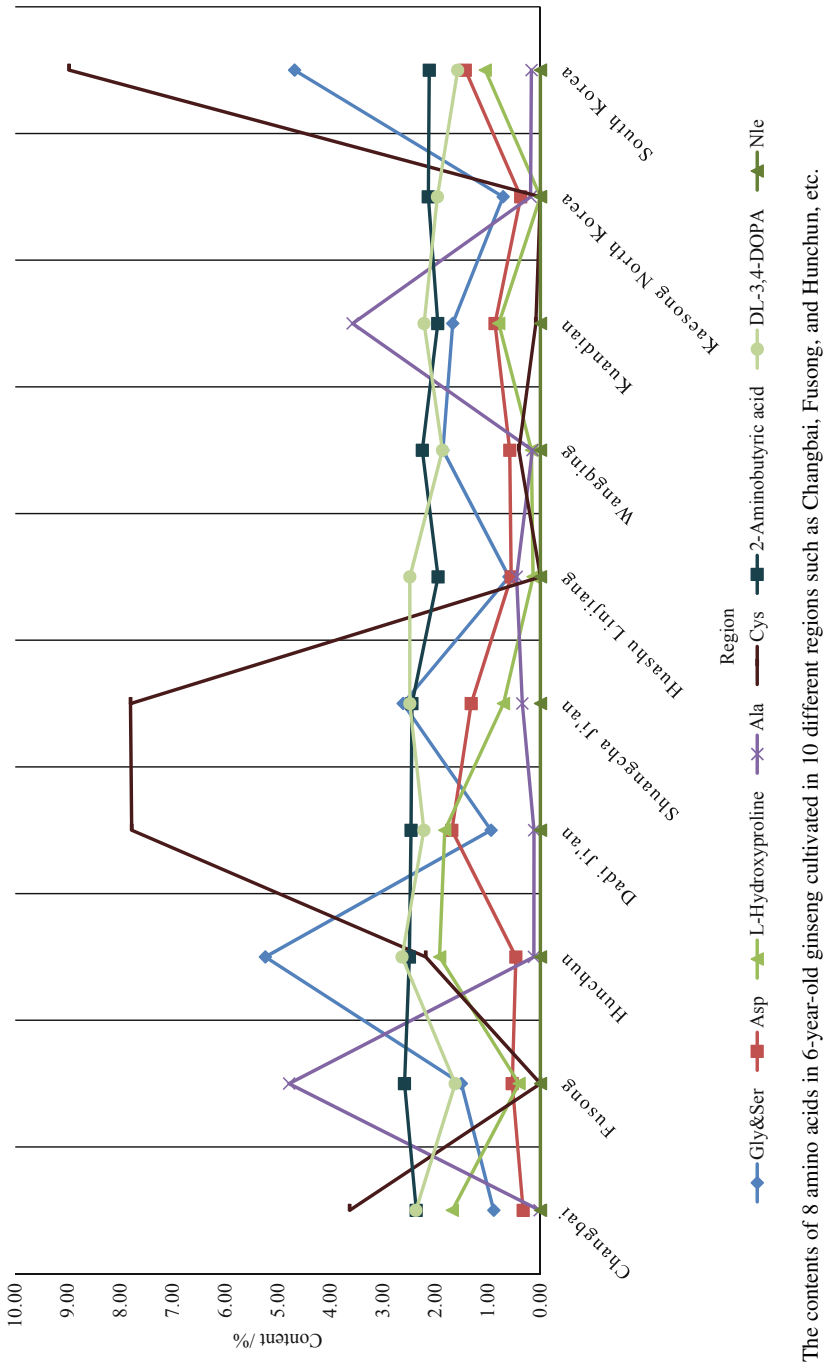

Fig. 1.19 The contents of 8 amino acids in 6-year-old ginseng cultivated in 10 different regions such as Changbai, Fusong, and Hunchun, etc.

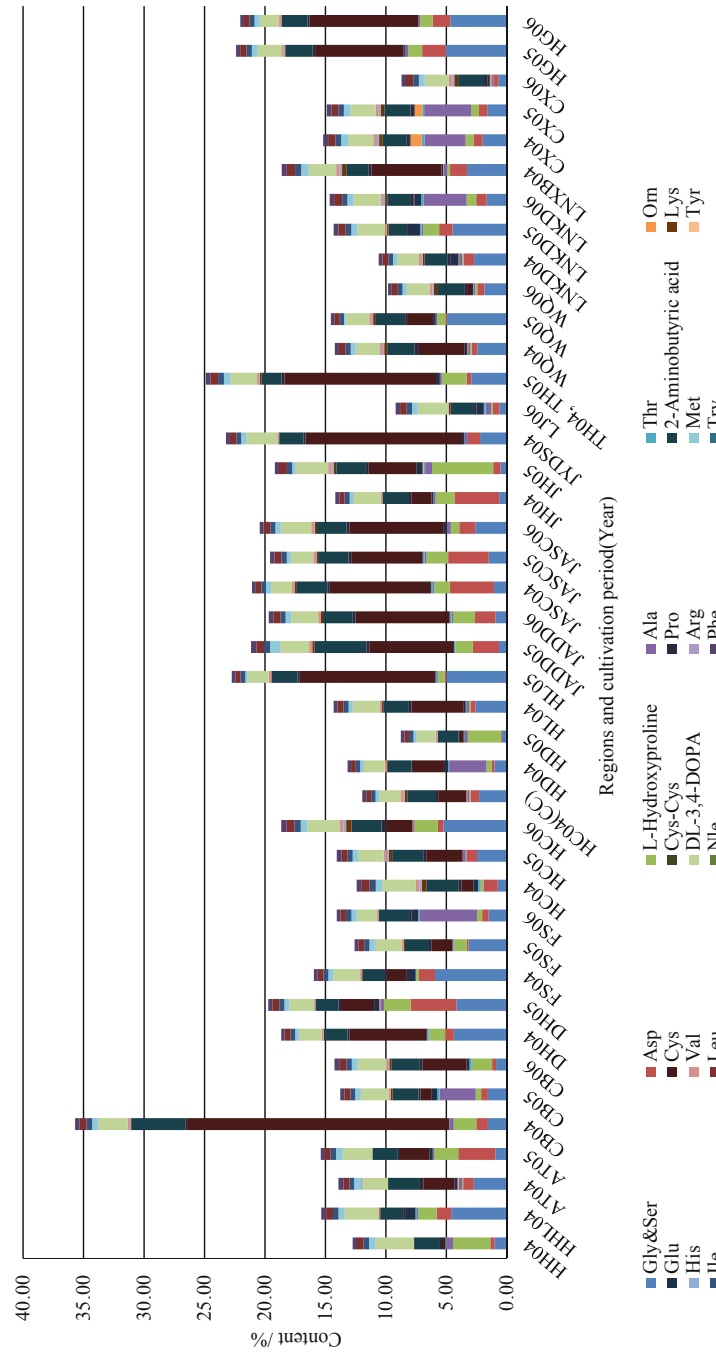

Fig. 1.20 The accumulation histogram of amino acid contents of different ages ginseng from different regions

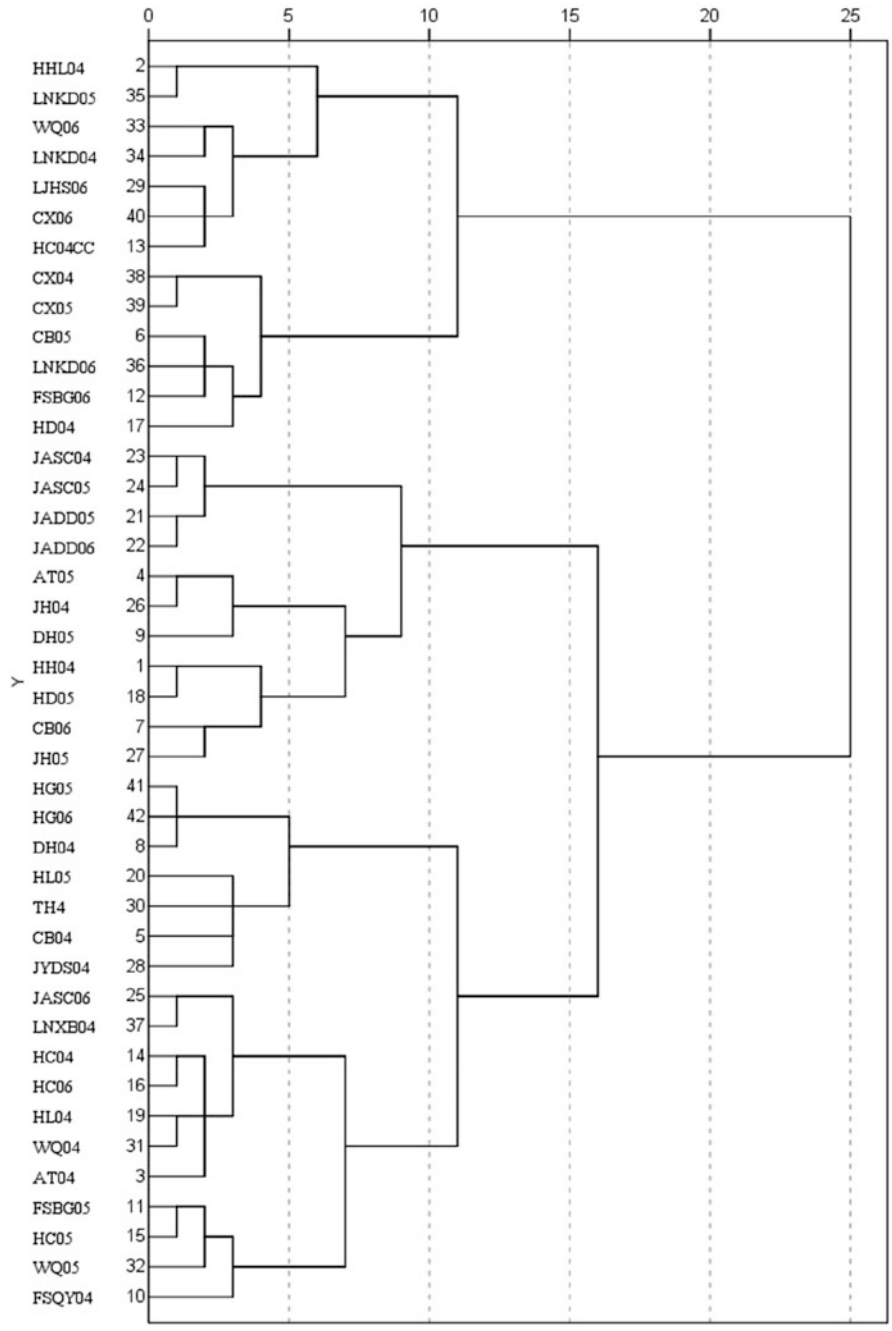

Fig. 1.21 Hierarchical graph of cluster analysis of amino acids in ginseng

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Analysis of Proteins in Ginseng

2

Zhidong Zhang, Zhongyao Wang, and Cuizhu Wang

Abstract

In order to provide data and scientific basis for clarifying the contents of protein in ginseng, the determination of protein was carried out. The dried ginseng was taken as the test sample. The Coomassie brilliant blue method was used to assay the total protein based on ultraviolet-visible spectrophotometry at 595 nm. The bovine serum albumin was used as the reference substance. The calibration curve of bovine serum albumin was produced by plotting the absorbance against concentration. The correlation coefficient was greater than 0.99 with the linear range between 0.021 and 0.147 mg. The results showed that the ginseng of different ages ranked in decreasing order based on the contents of protein was: 6-year-old ginseng, 5-year-old ginseng, and 4-year-old ginseng. It could be concluded that the contents of protein accumulated with the increasing cultivation ages.

Keywords

Dried ginseng · Protein · Ultraviolet spectrophotometry

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2.1 Introduction

As one of the major nutritional ingredients, the protein in ginseng has diverse pharmacological activities, especially in central nervous system and immune system [1, 2]. The common methods for protein analysis are Kjeldahl method [3], Biuret method [4], Lowry method [5], salicylic acid colorimetry, UV spectrophotometry [6, 7], etc. In this book, the ultraviolet spectrophotometry method was applied to determine the contents of protein.

2.2 Materials and Instruments

2.2.1 Materials

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

Bovine serum albumin and Coomassie brilliant blue G250 were purchased from Huishi Biochemical Reagent Co. Ltd., China. PEG 6000 (Tianjin Guangfu Fine Chemical Research Institute, China), Tris (201311120256), and HCl (Beijing Chemical Works, China) were all of analytical pure grade. Ultrapure water was prepared by the Water Purification System (Changchun Laibopate Technology Development Co. Ltd., China).

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2.2.2 Instruments

721 Ultraviolet-Visible Spectrophotometer (Shanghai Precision Scientific Instrument Co. Ltd., China), 98-1-B Electronic thermostat (Tianjin Test Instrument Co. Ltd., China), R201D type electrothermal constant temperature water-bath (Shanghai Yukang Science and Education Equipment Co. Ltd., China), Rotary evaporator (Shanghai Yukang Science and Education Equipment Co. Ltd., China), GF-254 chromatography silica gel plate (Zhejiang Taizhou Luqiao Sijia Biochemical Plastics Factory, China), FA1104N electronic balance (Shanghai Jinghua Technology Instrument Co. Ltd., China), FW177 High speed omnipotent pulverizer (Beijing Yongguangming Medical Instrument Co. Ltd., China).

2.3 Experimental Methods [8–10]

2.3.1 Preparation of Test Solution

Ginseng was, respectively, air-dried, grinded, and sieved (Chinese National Standard Sieve No. 4, R40/3 series) to get the homogeneous powder. Transfer about 5.0 g of powder, accurately weighed, into a 50 mL stoppered conical flask, add 75 mL of 10 mmol/L Tris-HCl buffer (pH 7.4, 0.15 mol/L NaCl), mixed well and allowed to stand for 20 h at 4 °C. Centrifuged at 5000 r/min for 30 min. The residue was filtered and extracted again by repeating the operation. Combined the filtrate, added PEG 6000 to produce a solution containing 20% of PEG 6000, mixed well and allowed it to stand overnight at 4 °C. Centrifuged at 5000 r/min for 30 min. The residue was filtered, dissolved and diluted to 50.0 mL with water, as the test solution.

2.3.2 Preparation of Reference Solution

An accurately weighted quantity of bovine serum albumin is dissolved in water to produce a solution of 1 mg per mL, as reference solution.

2.3.3 Drawing Standard Curve

Transfered 0.0, 0.02, 0.04, 0.06, 0.08, 0.10, 0.12, 0.14 mL of reference solution respectively into eight test tubes with stopper, diluted to 0.2 mL with water, then added 5.0 mL of coomassie brilliant blue solution, mixed immediately. Measure promptly the absorbance at 595 nm (Pharmacopoeia of the People's Republic of China (2015 Version), Appendix IV A), using No. 0 test tube as a blank. Calculate the linear regression equation from the absorbances (y) obtained versus the concentrations (x) of the reference solutions (Fig. 2.1, Table 2.1). The calibration curve was $y = 5.9218x - 0.0476$ ($R^2 = 0.9955$), indicating the method had good linearity in the range of 0.021~0.147 mg/mL.

2.3.4 Determination of Test Samples

Calculate the concentration of protein of the test solution according to the method in Sect. 2.3.3. The concentration of the protein in ginseng was calculated by the following formula:

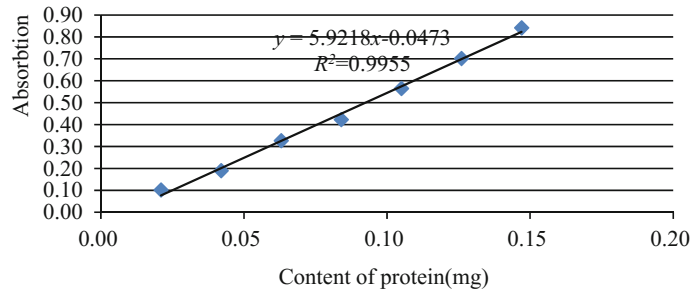

Fig. 2.1 The calibration curve of absorbance-protein levels

Table 2.1 The absorbance of standard substance

Standard solution volume/mL	0.02	0.04	0.06	0.08	0.10	0.12	0.14
Content of protein/mg	0.021	0.042	0.063	0.084	0.105	0.126	0.147
Absorbance 1	0.102	0.189	0.326	0.425	0.563	0.703	0.844
Absorbance 2	0.101	0.191	0.328	0.420	0.566	0.700	0.840
Average absorbance	0.102	0.190	0.327	0.423	0.565	0.702	0.842

$$\text{Formula : protein content } (\%) = \frac{m' \times V}{1000 \times V' \times m} \times 100\%$$

In formula: m—mass of the ginseng sample, g; V—constant volume, mL; m'—mass calculated by regressive equation of standard curve; V'—sample volume, mL. The final content of the protein in ginseng was the average value of test parallel samples.

2.4 Results and Discussion

2.4.1 Protein Contents in Ginseng from Different Regions with Different Cultivation Ages

The results were shown in Table 2.2.

2.4.2 Analysis of Protein Content in Ginsengs from Same Regions with Different Cultivation Years

The results showed that the contents of protein accumulated with the increasing cultivation ages (except Huadian), as shown in Table 2.3, Figs. 2.2 and 2.3.

2.4.3 Analysis of Protein Contents in Ginseng with Same Cultivation Years from Different Regions

The regions of 4-year-old ginseng ranked in decreasing order based on the contents of protein were Wangqing, Tonghua, Helong, Hulin, Antu, North Korea, Huadian, Quanyang, Xinbin, Shuangcha Ji'an, Heihe, Jingyu, Hunchun, Jiaohe, Dunhua, Changbai, Kuandian (Fig. 2.4).

The regions of 5-year-old ginseng ranked in decreasing order based on the contents of protein were South Korea, Tonghua, Helong, Linjiang, Dadi Ji'an, North Korea, Antu, Shuangcha Ji'an, Wangqing, Jiaohe, Jingyu, Fusong, Dunhua, Huadian, Kuandian, Hunchun, Changbai (Fig. 2.5).

The regions of 6-year-old ginseng ranked in decreasing order based on the contents of protein were South Korea, Linjiang, North Korea, Dadi Ji'an, Fusong, Hunchun, Wangqing, Shuangcha Ji'an, Changbai, Kuandian (Fig. 2.6).

In general, the protein contents of ginseng in South Korea, Tonghua, and Ji'an were higher than those in Jilin, Heilongjiang, and Liaoning.

Table 2.2 Protein contents of ginseng from different regions with different cultivation periods (%)

No.	Region	Cultivation period (year)	Sample 1(%)	Sample 2 (%)	Average content of protein (%)
1	Shuangcha Ji'an	4	1.04	0.95	0.99
2		5	1.03	1.00	1.01
3		6	0.77	0.68	0.73
4	Dadi Ji'an	5	0.93	1.58	1.26
5		6	1.31	1.41	1.36
6	Changbai	4	0.68	0.67	0.68
7		5	0.63	0.69	0.66
8		6	0.70	0.75	0.72
9	Fusong	4	1.02	1.13	1.08
10		5	1.09	0.84	0.97
11		6	1.11	1.09	1.10
12	Jingyu	4	1.08	0.81	0.94
13		5	1.10	0.90	1.00
14	Linjiang	5	1.32	1.33	1.33
15		6	1.43	1.42	1.43
16	Antu	4	1.21	1.18	1.19
17		5	1.15	1.33	1.24
18	Dunhua	4	0.64	0.91	0.77
19		5	0.85	0.95	0.90
20	Hunchun	4(CC)	1.34	1.25	1.30
21		4	0.93	0.96	0.94
22		5	0.74	0.76	0.75
23		6	1.08	1.02	1.05
24	Wangqing	4	1.49	1.60	1.54
25		5	1.04	0.97	1.00
26		6	1.00	1.00	1.00
27	Helong	4	1.18	1.29	1.24
28		5	1.34	1.35	1.34
29	Jiaohe	4	0.88	0.89	0.88
30		5	1.01	1.00	1.00
31	Huadian	4	1.17	1.02	1.10
32		5	0.91	0.88	0.89
33	Hulin	4	1.20	1.29	1.24
34	Heihe	4	0.98	1.01	0.99
35	Kuandian	4	0.46	0.47	0.46
36		5	0.84	0.78	0.81
37		6	0.62	0.57	0.60
38	Xinbin	4	1.00	1.12	1.06
39	North Korea	4	1.14	1.12	1.13
40		5	1.32	1.20	1.26
41		6	1.49	1.37	1.43
42	Tonghua	4	1.55	1.51	1.53
43		5	1.56	1.53	1.55
44	South Korea	5	1.95	2.12	2.04
45		6	1.81	1.94	1.87

Table 2.3 Analysis of protein contents of ginseng from the same region with different cultivation periods

No.	Region	Content
1	Shuangcha Ji'an	$CG_5 > CG_4 > CG_6$
2	Kuandian	$CG_5 > CG_6 > CG_4$
3	Changbai	$CG_6 > CG_4 > CG_5$
4	Hunchun	$CG_6 > CG_4 > CG_5$
5	North Korea	$CG_6 > CG_5 > CG_4$
6	Wangqing	$CG_4 > CG_5 > CG_6$
7	Fusong	$CG_6 > CG_4 > CG_5$
8	Dadi Ji'an	$CG_6 > CG_5$
9	Antu	$CG_5 > CG_4$
10	Dunhua	$CG_5 > CG_4$
11	Helong	$CG_5 > CG_4$
12	Jiaohe	$CG_5 > CG_4$
13	Huadian	$CG_4 > CG_5$
14	South Korea	$CG_5 > CG_6$
15	Linjiang	$CG_6 > CG_5$
16	Jingyu	$CG_5 > CG_4$
17	Tonghua	$CG_5 > CG_4$

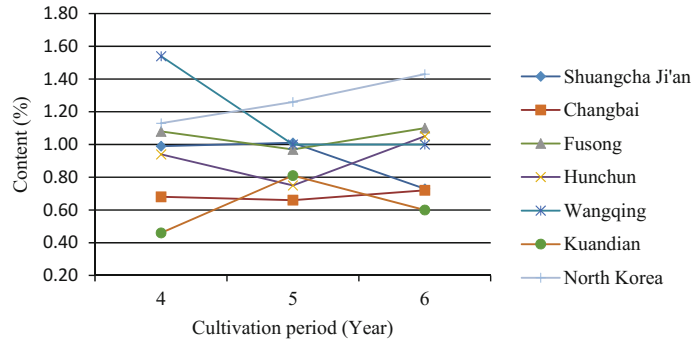

Fig. 2.2 Tendency chart of protein contents of ginseng from the same region with different cultivation periods

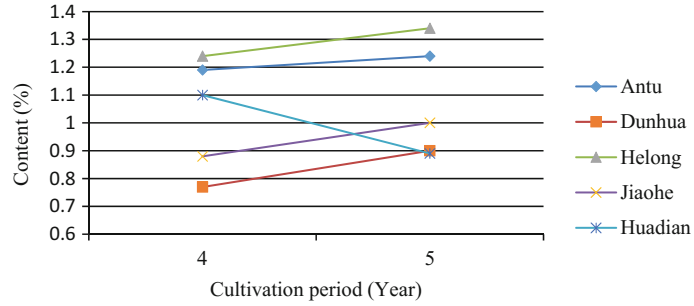

Fig. 2.3 Tendency chart of protein contents of ginseng from same region with different cultivation periods

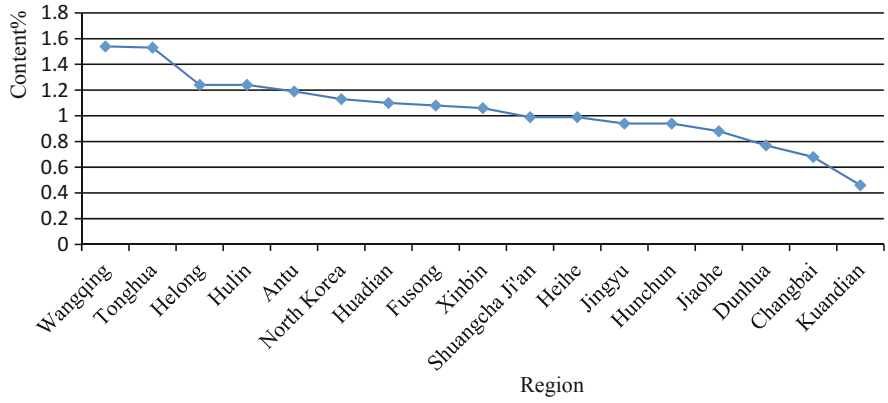

Fig. 2.4 Protein contents of 4-year-old ginseng

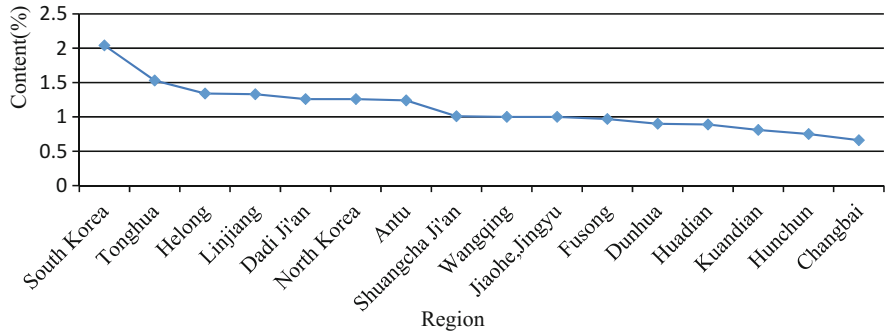

Fig. 2.5 Protein contents of 5-year-old ginseng

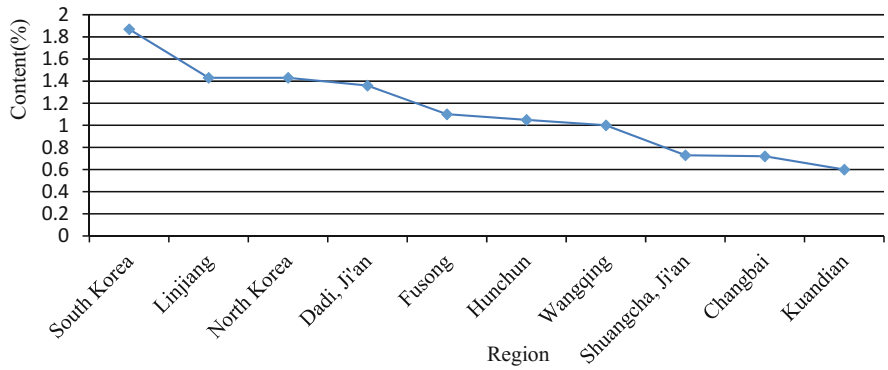

Fig. 2.6 Protein contents of 6-year-old ginseng

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Analysis of Saccharides in Ginseng

3

Cuizhu Wang, Jing Tan, Fulin Wu, and Ying Zhang

Abstract

In order to provide data clarifying the contents of saccharides in ginseng, the assay of total saccharides, polysaccharide, reducing saccharide, uronic acid, and monosaccharide was performed. Ginsengs from various regions and different cultivated periods were taken as the test samples. The methods were established based on DNS (reduction method), spectrophotometry, and HPLC-DAD. The established methods were used to determinate the contents of total saccharide, reducing saccharide, polysaccharide, uronic acid, and monosaccharide in each ginseng sample. For the ginsengs in same area, the contents of polysaccharide in 5-year-old ginseng were higher than 4-year-old ginseng, the contents of reducing saccharide increased with the increasing cultivation period. It could be concluded that contents of saccharides were different in different ages of ginseng from various areas.

Keywords

Ginseng · Saccharide · HPLC-DAD

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3.1 Introduction

The saccharides of ginseng are the main functional factor or active ingredient, and it plays an important role [1]. The methods for polysaccharides analysis include anthrone-sulfuric acid method [2], phenol-sulfuric acid method [3], colorimetric quantitative method, paper chromatography [4], ion exchange chromatography [5], enzymatic method [6], atomic absorption method [7], HPLC method [8, 9], DNS (reduction method) [10], Phosphorus Molybdenum Colorimetric Method, etc. This book uses DNS (reduction method) to analyze the total saccharide and reducing saccharide in ginseng. Ginseng polysaccharide and uronic acid were analyzed by spectrophotometry. Ginseng monosaccharide was analyzed by HPLC-DAD method.

3.2 Materials and Instruments

3.2.1 Materials

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

D-anhydrous glucose (China Food and Drug Control Institute, batch number: 110833–201,205), 3,5-dinitrosalicylic acid (analytical grade, China Pharmaceutical Group Chemical Reagent Co. Ltd., China), phenol and potassium sodium tartrate (analytical grade,

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Tianjin Guangfu Fine Chemical Research Institute), sodium sulfite (analytically grade, Tianjin Bodi Chemical Co. Ltd., China), iodine (analytical grade, Xilong Chemical Co. Ltd., China), potassium iodide (analytical grade, Tianjin Fuchen Chemical Reagent Factory), sodium hydroxide (analytical grade, Beijing Chemical Plant), distilled water.

3.2.2 Instruments

98-1-B Electronic Thermostat (Tianjin Test Instrument Co. Ltd., China), R201D Thermostat Water Bath (Shanghai Yukang Science and Education Equipment Co. Ltd., China), Rotary Evaporator (Shanghai Yukang Science and Education Equipment Co. Ltd., China), GF-254 Chromatography Silica Gel Plate (Zhejiang Taizhou Luqiao Sijia Biochemical Plastics Factory, China), FA1104N Electronic Balance (Shanghai Jinghua Tech Instrument Co. Ltd., China), 721 spectrophotometer (Shanghai Optical Instrument Factory), TGL-16aR High-speed Freezing Centrifuge (Shanghai Anting Scientific Instrument Factory, China), FW177 High Speed Universal Pulverizer (Beijing Yongguangming Medical Instrument Co. Ltd., China).

3.3 Experimental Methods

3.3.1 Assay of Total Saccharides in Ginseng

3.3.1.1 Preparation of the Reference Solution

Dissolve a quantity of D-anhydrous glucose, accurately weighed, in distilled water to produce a solution containing 1.04 mg of reference per mL, as reference solution.

3.3.1.2 Preparation of the Total Saccharides Test Solution

Ginseng was, respectively, air-dried, grinded, and sieved (Chinese National Standard Sieve No. 2, R40/3 series) to get the homogeneous powder. Transferred a quantity of powder (about 0.25 g), accurately weighted, to a 50 mL stoppered conical flask, added 7.5 mL of distilled water and 5 mL of 6 mol/L hydrochloric acid solution, mixed well, heated in a water bath at 100 °C to completely hydrolyze, as hydrolysis solution. Took a drop of the hydrolyzate to a white plate, the reaction ended if the blue color disappeared when added iodine-potassium iodide reagent. Cooled, neutralized with sodium hydroxide solution, filtered, centrifuged, the supernatant was diluted with distilled water to 50 mL, mixed well, as the test solution.

3.3.1.3 Preparation of DNS Chromogenic Agent

Add a quantity of 3,5-dinitrosalicylic acid about 3.15 g and 131 mL of 2 mol/L sodium hydroxide to 250 mL of hot water with 92.5 g of sodium potassium tartrate. Then, add 2.5 g of phenol and 2.5 g of sodium sulfite, stirred to dissolve, cooled, the solution was diluted with water to 500 mL, as the DNS chromogenic agent. It is stored in a brown volumetric flask, being used after 1 week.

3.3.1.4 Preparation of Iodine-Potassium Iodide Test Solution

Dissolve 0.5 g of iodine and 1.5 g of potassium iodide in 25 mL of water [11].

3.3.1.5 Drawing Standard Curve

Measured accurately 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 mL of the reference solution, respectively, to a 10 mL colorimetric tube, diluted with water to 3 mL, mixed well, added 0.75 mL of DNS solution, mixed well, heated on a boiling water bath for 5 min, allowed to cool to room temperature, diluted with water to 10 mL and mixed well. Measure the absorbance at 540 nm. Calculated the linear regression equation from the absorbances (y) obtained versus the concentrations (x) of the reference solution (Fig. 3.1, Table 3.1). The calibration curve was $y = 0.7734x - 0.0356$ ($R^2 = 0.9952$), indicating the method had good linearity in the range of 0.208 ~ 1.456 mg/mL.

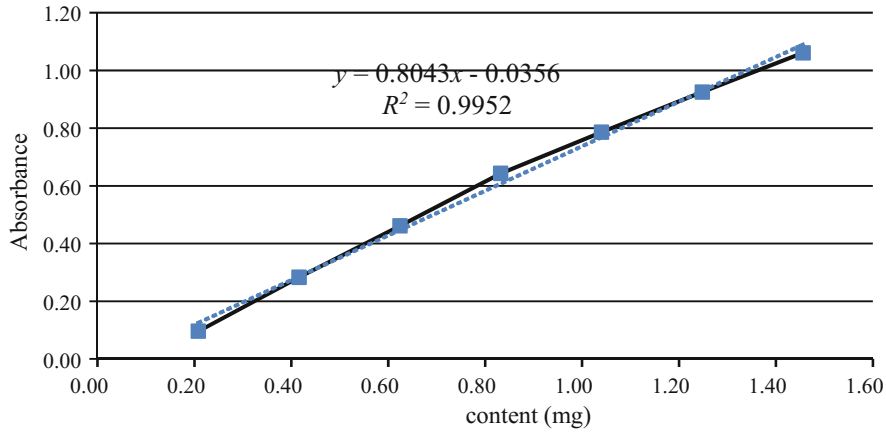

Fig. 3.1 The calibration curve of absorbance-glucose levels

Table 3.1 The absorbance of standard substance

Standard solution volume (mL)	0.2	0.4	0.6	0.8	1.0	1.2	1.4
Content of glucose (mg)	0.208	0.416	0.624	0.832	1.040	1.248	1.456
Absorbance 1	0.096	0.283	0.461	0.642	0.642	0.924	1.060
Absorbance 2	0.096	0.283	0.461	0.643	0.643	0.925	1.061
Average absorbance	0.096	0.283	0.461	0.643	0.786	0.925	1.061

3.3.1.6 Determination of Total Saccharide Content

Repeat the operation, using the substance being examined, 0.2 mL of the test solution was accurately measured, instead of the reference solutions, and calculated the concentration of total saccharide obtained from the equation. The concentration of the total saccharide in ginseng was calculated by the following formula:

$$\text{Total saccharide content } (\%) = \frac{C \times V}{1000 \times M} \times 100\%$$

M: mass of the ginseng sample (g); C: concentration of total saccharide (mg/mL); V: constant volume (mL).

The final content of the total saccharide in ginseng was the average value of test parallel samples.

3.3.2 Assay of Polysaccharides in Ginseng

3.3.2.1 Preparation of Polysaccharides in Ginseng

Ginseng was, respectively, air-dried, grinded, and sieved (Chinese National Standard Sieve No. 2, R40/3 series) to get the homogeneous powder. Transferred an accurately weighted 10 g of powder into a 500 mL round bottom flask, added 200 mL of water, mixed well, heated in a boiled water bath for 3 h, filtered, the residue was extracted twice with the repeated the operation. Combined the filtrate, vacuum evaporated and diluted with 95% ethanol to produce a solution containing 75% ethanol, allowed to stand overnight, filtered, the residue was washed with absolute ethanol, acetone, and diethyl ether successively, dried and weighted, as the polysaccharides.

3.3.2.2 Preparation of the Reference Solution

Dissolve a quantity of D-anhydrous glucose, previously dried to constant weight, accurately weighed, in water to produce a solution containing 100 μg of reference per mL, as reference solution.

3.3.2.3 Preparation of the Test Solution

Dissolve a quantity of polysaccharides, accurately weighted, in water to produce a test solution containing 100 μg per mL.

3.3.2.4 Drawing Standard Curve

Measured accurately 0.0, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, and 0.8 mL of the reference solution, respectively, to a 10 mL tube, diluted with 1 mL of water, 1 mL of 6% phenol and 5 mL of concentrated sulfuric acid, shaken well, heated in a boiling water bath for 15 min, cooled in ice water bath for 10 min, allowed to stand for 15 min in room temperature. Measure the absorbance at 490 nm. Calculate the linear regression equation from the absorbances (y) obtained versus the concentrations (x) of the reference solutions (Fig. 3.2, Table 3.2). the calibration curve was $y = 9.0571x - 0.0140$ ($R^2 = 0.9884$), indicating the method had good linearity in the range of 0.02 ~ 0.08 mg/mL.

3.3.2.5 Determination of Polysaccharide Contents

Repeat the operation, using the substance being examined, 0.5 mL of the test solution was accurately measured, instead of the reference solutions, and calculate the concentration of polysaccharide obtained from the equation. The concentration of the polysaccharide in ginseng was calculated by the following formula:

$$\text{Polysaccharide content } (\%) = \frac{C \times V \times m1}{1000 \times M \times m2} \times 100\%$$

m_1—crude polysaccharide sample amount (g); m_2—extracting quality of the crude polysaccharide (g); M—mass of the ginseng sample (g); C—concentration of polysaccharide (mg/mL); V—sample constant volume (mL).

The final content of the polysaccharide in ginseng was the average value of test parallel samples.

3.3.3 Assay of Reducing Saccharides in Ginseng

3.3.3.1 Preparation of the Reference Solution

Dissolve a quantity of D-anhydrous glucose, previously dried to constant weight, accurately weighed, in water to produce a solution containing 1.04 mg of reference per mL, as reference solution.

3.3.3.2 Preparation of the Reducing Saccharides Test Solution

Ginseng was, respectively, air-dried, grinded, and sieved (Chinese National Standard Sieve No. 2, R40/3 series) to get the homogeneous powder. Transferred a quantity of powder (about 0.25 g), accurately weighted, to a 50 mL stoppered conical flask, added 15 mL of distilled water, mixed well, heated in a water bath at 50 °C for 30 min, filtered. The residue was added 20 mL of distilled water and heated in a water bath at 50 °C for 30 min, filtered. Combined the filtrate, centrifuged, the supernatant was diluted with distilled water to 50 mL, mixed well, as the test solution.

3.3.3.3 Preparation of DNS Chromogenic Agent

Add a quantity of 3,5-dinitrosalicylic acid about 3.15 g and 131 mL of 2 mol/L sodium hydroxide to 250 mL of hot water with 92.5 g of sodium potassium tartrate. Then, add 2.5 g of phenol and 2.5 g of sodium sulfite, stirred to dissolve, cooled, the solution was diluted with water to 500 mL, as the DNS chromogenic agent. It is stored in a brown volumetric flask, being used after 1 week.

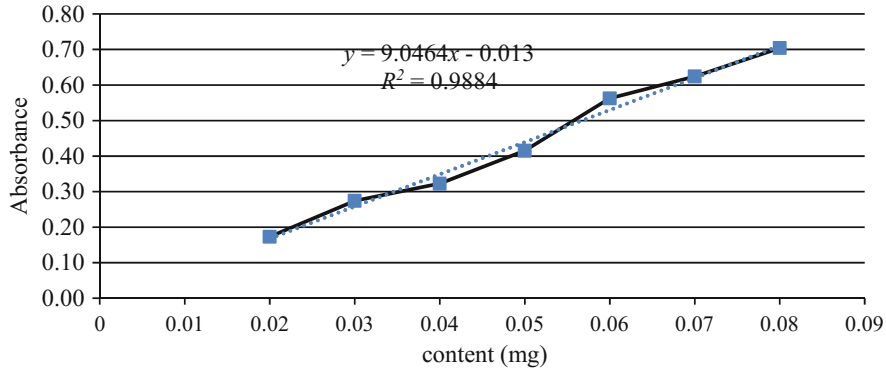

Fig. 3.2 The calibration curve of absorbance-glucose levels

Table 3.2 The absorbance of standard substance

Standard solution volume (mL)	0.2	0.3	0.4	0.5	0.6	0.7	0.8
Content of glucose (mg)	0.02	0.03	0.04	0.05	0.06	0.07	0.08
Absorbance 1	0.173	0.274	0.322	0.415	0.562	0.624	0.704
Absorbance 2	0.173	0.274	0.323	0.415	0.563	0.624	0.704
Average absorbance	0.173	0.274	0.3225	0.415	0.5625	0.624	0.704

3.3.3.4 Drawing Standard Curve

Measured accurately 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 of the reference solution, respectively, to a 10 mL colorimetric tube, diluted with water to 3 mL, mixed well, added 0.75 mL of DNS solution, mixed well, heated on a boiling water bath for 5 min, allowed to cool to room temperature, diluted with water to 10 mL and mixed well. Measure the absorbance at 540 nm. Calculated the linear regression equation from the absorbances (y) obtained versus the concentrations (x) of the reference solution (Fig. 3.3, Table 3.3). The calibration curve was $y = 0.7013x - 0.0952$ ($R^2 = 0.9952$), indicating the method had good linearity in the range of 0.208 ~ 1.456 mg/mL.

3.3.3.5 Determination of Reducing Saccharide Content

Repeat the operation, using the substance being examined, 0.8 mL of the test solution was accurately measured, instead of the reference solutions, and calculated the concentration of reducing saccharide obtained from the equation. The concentration of the reducing saccharide in ginseng was calculated by the following formula:

$$\text{Reducing saccharide content } (\%) = \frac{C \times V}{1000 \times M} \times 100\%$$

M: mass of the ginseng sample (g); C: concentration of reducing saccharide (mg/mL); V: constant volume (mL).

The final content of the reducing saccharide in ginseng was the average value of test parallel samples.

3.3.4 Assay of Uronic Acids in Ginseng

3.3.4.1 Preparation of Uronic Acids in Ginseng

Ginseng was, respectively, air-dried, grinded, and sieved (Chinese National Standard Sieve No. 2, R40/3 series) to get the homogeneous powder. Transferred an accurately weighted 10 g of powder into a 500 mL round bottom flask, added 200 mL of water, mixed well, heated in a boiled water bath for 3 h, filtered, the residue was extracted twice with the repeated the operation. Combined the filtrate, vacuum evaporated

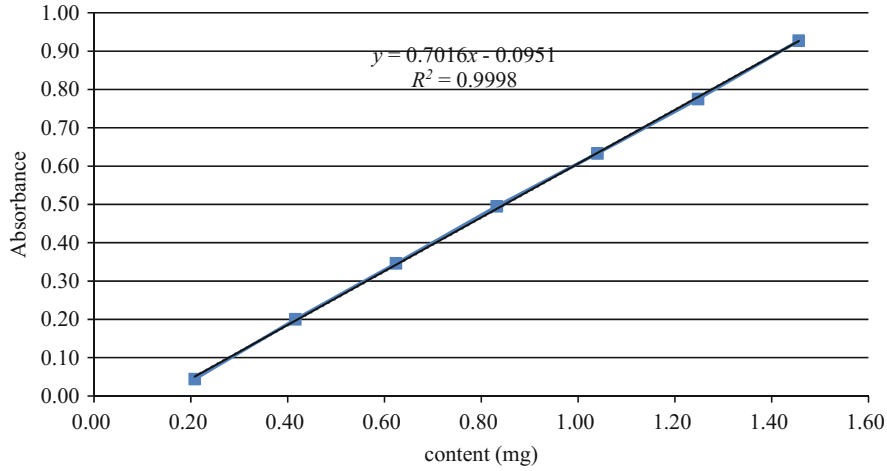

Fig. 3.3 The calibration curve of absorbance-glucose levels

Table 3.3 The absorbance of standard substance

Standard solution volume (mL)	0.2	0.4	0.6	0.8	1.0	1.2	1.4
Content of glucose (mg)	0.208	0.416	0.624	0.832	1.040	1.248	1.456
Absorbance1	0.044	0.200	0.346	0.494	0.633	0.774	0.926
Absorbance2	0.044	0.199	0.346	0.495	0.633	0.775	0.927
Average absorbance	0.044	0.200	0.346	0.495	0.633	0.775	0.927

and diluted with 95% ethanol to produce a solution containing 75% ethanol, allow to stand overnight, filtered, the residue was washed with absolute ethanol, acetone, and diethyl ether successively, dried and weighted, as the polysaccharides.

3.3.4.2 Preparation of the Reference Solution

Dissolve a quantity of D-galacturonic acid, previously dried to constant weight, accurately weighed, in water to produce a solution containing 100 μg of reference per mL, as reference solution.

3.3.4.3 Preparation of the Test Solution

Dissolve a quantity of polysaccharides, accurately weighted, in water to produce a test solution containing 100 μg per mL.

3.3.4.4 Drawing Standard Curve

Measured accurately 0.0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.35, and 0.45 mL of the reference solution, respectively, to a 10 mL tube, diluted with 1 mL of water and 6 mL of sodium tetraborate-concentrated sulfuric acid solution in an ice water bath, shaken well, heated in a boiling water bath for 6 min, allowed to cool to room temperature, added 80 μL of m-hydroxybiphenyl, shaken well, allowed to stand for 30 min in room temperature. Measure the absorbance at 525 nm. Calculate the linear regression equation from the absorbances (y) obtained versus the concentrations (x) of the reference solutions (Fig. 3.4, Table 3.4). the calibration curve was $y = 14.919x - 0.0429$ ($R^2 = 0.9909$), indicating the method had good linearity in the range of 0.006 ~ 0.056 mg/mL.

3.3.4.5 Determination of Uronic Acid Contents

Repeat the operation, using the substance being examined, 0.5 mL of the test solution was accurately measured, instead of the reference solutions, and calculate the concentration of uronic acid obtained from the equation. The

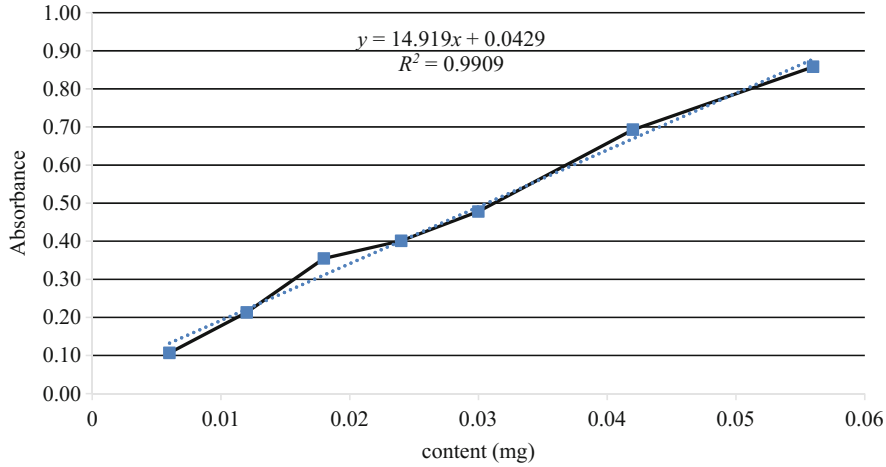

Fig. 3.4 The calibration curve of absorbance- galactose acid levels

Table 3.4 The absorbance of standard substance

Standard solution volume (mL)	0.05	0.1	0.15	0.2	0.25	0.35	0.45
Content of galactose (mg)	0.006	0.012	0.018	0.024	0.03	0.042	0.056
Absorbance1	0.106	0.212	0.355	0.401	0.478	0.693	0.858
Absorbance2	0.107	0.213	0.354	0.401	0.478	0.692	0.858
Average absorbance	0.107	0.213	0.355	0.401	0.478	0.693	0.858

concentration of the uronic acid in ginseng was calculated by the following formula:

$$\text{Uronic acid content}\ (\%) = \frac{C \times V \times m1}{1000 \times M \times m2} \times 100\%$$

$m1$—crude uronic acid sample amount (g); $m2$—extracting quality of the crude uronic acid (g); M—mass of the ginseng sample (g); C—concentration of uronic acid (mg/mL); V—sample constant volume (mL).

The final content of the uronic acid in ginseng was the average value of test parallel samples.

3.3.5 Assay of Monosaccharides in Ginseng

3.3.5.1 Preparation of the Mixed Reference Solution

Transferred a quantity of eight kinds of monosaccharide standard substances (2 mg), respectively, in the same tube, added 200 μL of 0.3 mol/L NaOH solution and 500 μL of 0.5 mol/L PMP methanol solution, mixed well, reacted in a water bath at 70 °C for 100 min, allowed to cool to room temperature, neutralized with 500 μL of 0.3 mol/L hydrochloric acid solution, diluted with water to 2 mL, and 2 mL of trifluoroacetic acid was added to remove unreacted PMP, repeat the operation three times. Filtered (0.45 μm) as the mixed reference solution, preserved in refrigerator.

3.3.5.2 Preparation of the Test Solution

Ginseng was, respectively, air-dried, grinded, and sieved (Chinese National Standard Sieve No. 2, R40/3 series) to get the homogeneous powder. Accurately weighed about 10 mg, add 2 mL of 2 mol/mL trifluoroacetic acid solution, sealed with nitrogen, stirred and warm to hydrolyze for 6 h at 100 °C. Measured a quantity of the hydrolysate (800 μL), dry it under nitrogen, and methanol was added to remove unreacted trifluoroacetic acid, the residue was treated with derivatization as the test solution.

Table 3.5 Eight kinds of monosaccharide reference substances and the retention time

Name of monosaccharide	Concentration (μg/μL)	Retention time (min)
Galacturonic acid	0.950	4.015
Gala	1.225	8.398
Glu	1.176	11.550
Ara	1.323	12.360
Xyl	1.250	13.605
Man	1.078	19.098
Fuc	1.337	26.556
Rha	1.029	37.553

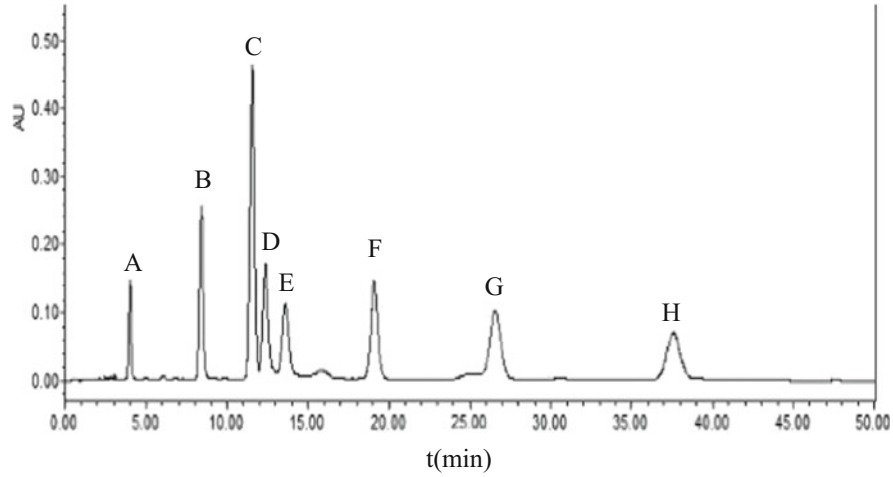

Fig. 3.5 HPLC chromatogram of eight monosaccharide standard substances. *A* Galacturonic acid; *B* Gala; *C* Glu; *D* Ara; *E* Xyl; *F* Man; *G* Fuc; *H* Rha

3.3.5.3 Chromatographic Conditions

Carry out the method for high performance liquid chromatography, using C18 (4.6 mm × 250 mm, 5 μm) column, and a mixture of 0.1 mol/L phosphate buffer (pH 6.7)-acetonitrile (95:5) as the mobile phase. The flow rate was 1 mL/min. Column temperature was 30 °C. Detection wavelength was 250 nm. Inject 20 μL of the reference solution into the column and record the chromatogram. As shown in Table 3.5 and Fig. 3.5.

3.3.5.4 Method Validation

The optimized method was validated by evaluating linearity, accuracy, precision, stability, and recovery.

Linearity was evaluated from the calibration curve obtained after analyzing reference solutions in increasing order of concentrations (4, 6, 8, 10, 12, 16, 20 μL, respectively). The abscissa (x) was the contents (μg) and the ordinate (y) was the peak areas (A) of reference substance. The standard curve was drawn to obtain the regression equation as shown in Table 3.6.

Precision was evaluated by analyzing the mixed reference solution six times ($n = 6$). the precision was expressed as the relative standard deviation (RSD) of each peak area of each monosaccharide. The results showed that the RSD was from 0.94% to 2.56%.

Accuracy was evaluated by analyzing the same test sample solution in replicated of six

Table 3.6 Regression equations with correlation coefficients (r) of eight monosaccharide standard substances

Name of monosaccharide	Regression equation	Correlation coefficient
Galacturonic acid	$y = 573{,}642x - 9991$	0.9991
Gala	$y = 2{,}561{,}780x - 194{,}599$	0.9999
Glu	$y = 10{,}050{,}845x + 521{,}424$	0.9991
Ara	$y = 894{,}548x - 197{,}626$	0.9994
Xyl	$y = 3{,}451{,}594x - 321{,}147$	0.9962
Man	$y = 6{,}225{,}977x - 581{,}491$	0.9997
Fuc	$y = 5{,}228{,}766x - 213{,}720$	0.9996
Rha	$y = 7{,}295{,}267x - 1{,}454{,}701$	0.9994

samples ($n = 6$). The accuracy was expressed as the relative standard deviation (RSD) of each peak area of each monosaccharide. The results showed that the RSD was from 1.07% to 2.82%.

Stability was investigated by analyzing the same test sample solution at 0, 2, 4, 8, 12, and 24 h, respectively. The stability was expressed as the relative standard deviation (RSD) of each peak area of each monosaccharide. The results showed that the RSD was from 1.65% to 4.87%.

Recovery was accessed by comparing the contents of eight monosaccharides before and after extraction at three levels in three replicates. The recovery was expressed as recovery rates and the RSD of recovery rates. The results showed that the recovery rates were in the range of 94.8~105.6%, with the RSD being 1.54~4.91%.

3.3.5.5 Determination of Monosaccharide Content

Inject 20 μL of the test solution into the column and record the chromatogram. The content of 8 kind of monosaccharides in samples was calculated by the area-content linear regression equation.

3.4 Results and Discussion

3.4.1 Proportion of Monosaccharides in Polysaccharides

The molar ratios of seven monosaccharides in polysaccharides are shown in Table.3.7.

3.4.2 The Total Saccharide Contents in Ginseng

3.4.2.1 The Total Saccharide Contents in Ginseng from Different Regions with Different Cultivation Periods

Total saccharide contents in ginseng from different regions with different cultivation periods were shown in Table 3.8.

3.4.2.2 The Total Saccharide Contents in Ginseng with Different Cultivation Periods from the Same Region

The total saccharide contents of ginseng with different periods from the same region were shown in Table 3.9. And the content trend graphs of the total saccharide were shown in Figs. 3.6 and 3.7.

3.4.2.3 The Contents of Total Saccharide in Ginseng from Different Regions with the Same Cultivation Period

The determination results were shown in Table 8.3.

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

Table 3.7 Molar concentration ratio of seven monosaccharide in ginseng polysaccharides

No.	Region	Cultivation period (year)	Molar concentration ratio of Xyl: Galacturonic acid: Glu: Ara: Gala: Man: Rha
1	Shuangcha Ji'an	4	1:7.94:8.82:79.31:1.07:10.46:9.17
2		5	1:7.11:7.22:29.18:0.24:1.62:1.35
3		6	1:4.57:9.62:153.84:4.90:21.68:11.06
4	Dadi Ji'an	5	1:3.19:0.80:12.74:0.04:0.50:0.74
5		6	1:6.14:3.43:76.04:0.09:3.79:3.65
6	Changbai	4	1:28.15:3.40:50.65:0.24:7.36:3.19
7		5	1:4.76:6.91:130.92:4.36:20.49:9.15
8		6	1:15.08:0.33:111.40:2.94:5.71:2.24
9	Fusong	4	1:2.07:1.49:28.84:0:3.01:0.98
10		5	1:2.73:2.47:64.00:1.11:5.89:3.62
11		6	1:7.50:3.37:83.44:0:2.73:4.06
12	Jingyu	4	1:3.83:5.97:107.85:0.62:10.13:2.41
13		5	1:13.24:6.28:92.85:0.98:12.11:5.24
14	Linjiang	5	1:15.23:8.24:98.24:0.56:18.23:20.24
15		6	1:14.42:7.09:107.54:1.20:19.41:24.79
16	Antu	4	1:6.65:9.30:107.54:1.20:19.41:3.93
17		5	1:3.50:2.89:79.63:0:5.31:2.32
18	Dunhua	4	1:4.09:6.60:62.56:4.79:1.28:4.31
19		5	1:6.67:4.34:56.35:0.21:0.91:11.55
20	Hunchun	4(CC)	1:4.03:3.85:48.56:1.52:8.10:1.50
21		4	1:5.91:4.75:74.10:0:2.46:2.89
22		5	1:32.46:10.50:40.58:1.20:20.61:6.33
23		6	1:15.51:19.30:173.76:3.65:3.41:6.92
24	Wangqing	4	1:2.91:2.29:42.57:0:4.14:5.33
25		5	1:8.12:13.12:128.39:1.03:5.05:3.83
26		6	1:12.62:19.47:167.06:2.77:5.34:1.26
27	Helong	4	1:38.13:9.56:67.19:0.40:4.97:7.22
28		5	1:1.01:1.31:36.55:0.14:5.22:1.86
29	Jiaohe	4	1:3.20:3.39:40.42:0.17:0.64:1.29
30		5	1:23.78:12.96:132.99:1.32:17.08:3.59
31	Huadian	4	1:29.24:20.97:139.00:4.15:20.75:28.76
32		5	1:4.71:3.93:62.42:1.34:2.55:1.38
33	Tonghua	4	1:7.16:7.33:70.96:0.39:1.41:2.24
34		5	1:23.78:12.23:120.58:0.52:10.28:3.49
35	Hulin	4	1:11.82:9.52:139.36:0:2.24:2.12
36	Heihe	4	1:1.49:1.33:20.34:0.12:3.66:0.79
37	Kuandian	4	1:4.69:6.93:72.16:9.80:5.11:135.82
38		5	1:3.62:6.63:137.46:15.99:12.29:3.59
39		6	1:1.52:3.88:67.68:0.49:3.66:2.98
40	Xinbin	4	1:0.62:1.18:35.40:0.93:1.86:2.49
41	North Korea	4	1:2.21:2.07:21.33:2.26:1.53:2.69
42		5	1:3.02:2.37:32.72:0.44:1.05:5.63
43		6	1:10.08:10.76:93.06:0.30:2.70:5.38
44	South Korea	5	1:3.31:1.80:6.96:0.16:0.42:1.24
45		6	1:1.01:1.38:7.05:0.12:1.55:2.93

Table 3.8 Total saccharide contents of ginseng from different regions with different cultivation periods (%)

No.	Region	Cultivation period (year)	Sample 1 (%)	Sample 2 (%)	Content of total sugar
1	Shuangcha Ji'an	4	59.12	56.33	57.72
2		5	33.48	44.51	30.00
3		6	65.22	62.34	63.78
4	Dadi Ji'an	5	70.98	69.15	70.06
5		6	57.80	57.04	57.42
6	Changbai	4	58.80	62.48	60.64
7		5	73.88	75.63	74.76
8		6	70.44	64.02	67.23
9	Fusong	4	69.26	70.27	69.77
10		5	57.77	57.77	57.77
11		6	39.79	46.32	43.06
12	Jingyu	4	62.44	61.97	62.21
13		5	63.22	62.11	62.67
14	Linjiang	5	67.21	60.21	63.71
15		6	68.51	48.29	58.40
16	Antu	4	57.11	56.72	56.91
17		5	56.38	56.94	56.66
18	Dunhua	4	49.20	59.00	54.10
19		5	55.13	47.17	51.15
20	Hunchun	4(CC)	59.56	63.95	61.75
21		4	61.03	46.80	53.92
22		5	62.34	63.37	62.85
23		6	52.14	59.51	55.82
24	Wangqing	4	47.47	51.76	49.61
25		5	64.10	60.73	62.41
26		6	64.16	61.72	62.94
27	Helong	4	60.21	60.21	60.21
28		5	57.15	70.33	63.74
29	Jiaohe	4	60.31	60.08	60.20
30		5	59.51	47.90	53.70
31	Huadian	4	75.21	67.73	71.47
32		5	66.50	55.54	61.02
33	Tonghua	4	57.73	54.80	56.27
34		5	59.00	57.21	58.11
35	Hulin	4	56.40	53.09	54.74
36	Heihe	4	50.26	59.91	55.09
37	Kuandian	4	55.64	39.09	47.37
38		5	58.13	61.26	59.69
39		6	49.00	64.77	56.88
40	Xinbin	4	41.11	44.32	42.72
41	North Korea	4	52.08	48.67	50.37
42		5	46.15	48.42	47.28
43		6	39.47	32.36	35.91
44	South Korea	5	66.06	42.50	54.28
45		6	49.54	43.98	46.76

Table 3.9 Analysis of total saccharide content of ginseng with different gown periods

No.	Region	Content
1	Shuangcha Ji'an	CG6 > CG4 > CG5
2	Kuandian, Changbai, Hunchun	CG5 > CG6 > CG4
3	North Korea, Fusong	CG4 > CG5 > CG6
4	Wangqing	CG6 > CG5 > CG4
5	Dadi Ji'an, South Korea, Linjiang	CG5 > CG6
6	Dunhua, Antu, Jiaohe, Huadian	CG4 > CG5
7	Helong, Tonghua, Jingyu	CG5 > CG4

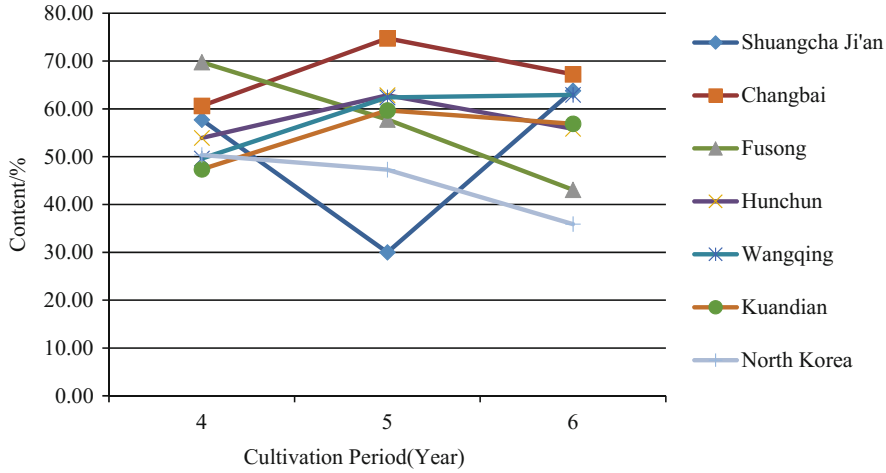

Fig. 3.6 The contents of total saccharide in ginseng of 4, 5, 6 ages

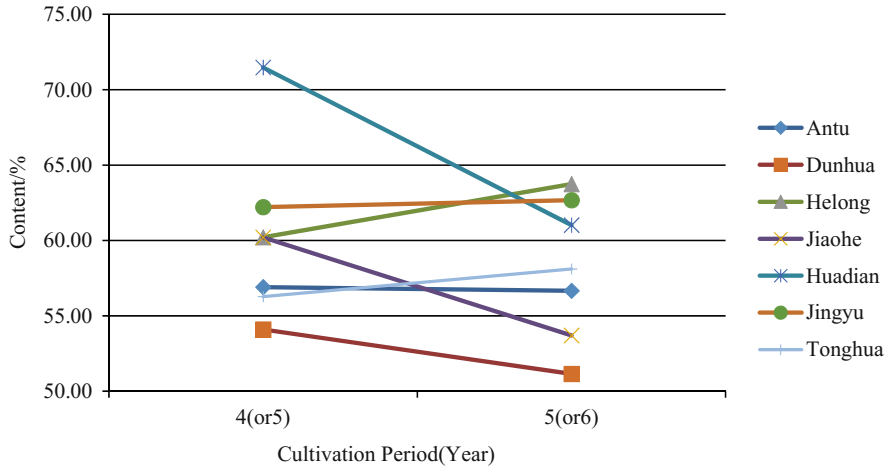

Fig. 3.7 The contents of total saccharide in ginseng of 4, 5 ages

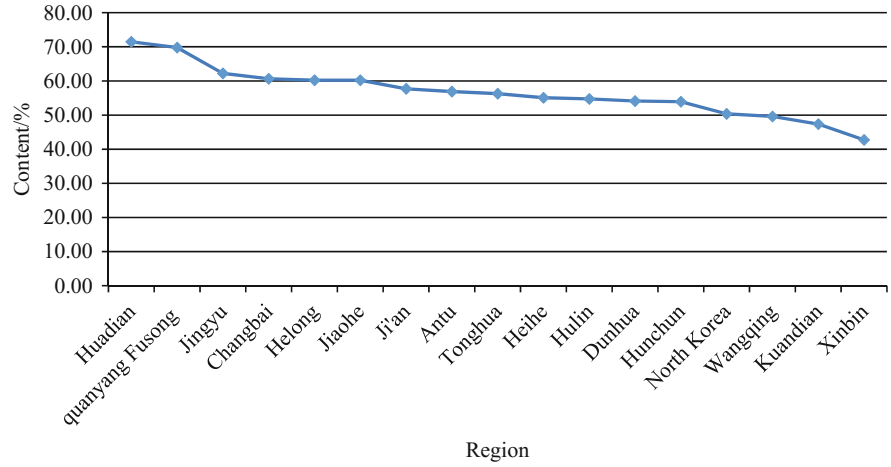

Fig. 3.8 Total saccharide contents of 4-year-old ginseng

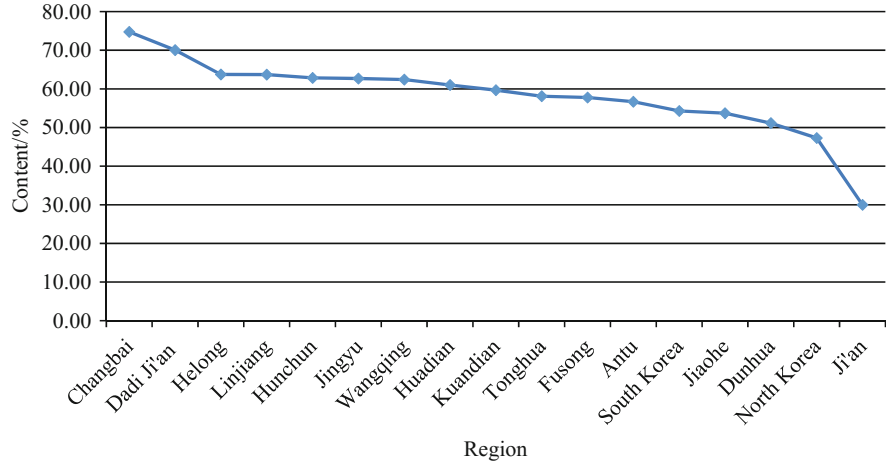

Fig. 3.9 Total saccharide contents of 5-year-old ginseng

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

The distributions of the total saccharides were shown in Figs. 3.8, 3.9, and 3.10, respectively.

3.4.3 The Polysaccharide Contents of Ginseng

3.4.3.1 The Polysaccharide Contents of Ginseng from Different Regions with Different Cultivation Periods

The polysaccharide contents of ginseng from different regions with different cultivation periods were shown in Table 3.10.

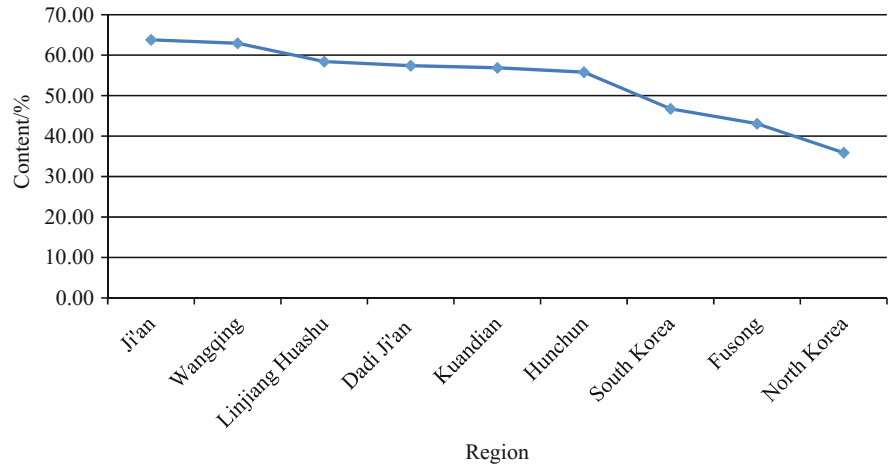

Fig. 3.10 Total saccharide contents of 6-year-old ginseng

3.4.3.2 The Polysaccharide Contents of Ginseng with Different Cultivation Periods from the Same Region

As shown in Table 3.10, the polysaccharide contents of ginseng with different cultivation periods from the same region were shown in Table 3.11. And the content trend graphs of the polysaccharide were shown in Figs. 3.11 and 3.12.

The overall trend was that the content in 5-year-old ginseng was higher than in 4-year-old ginseng. However, when compared the contents in 5-year-old ginseng with 6-year-old ginseng, the trend was not always same.

3.4.3.3 The Contents of Polysaccharide in Ginseng from Different Regions with the Same Cultivation Period

The determination result was shown in Table 3.10.

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

The distribution of the polysaccharide were shown in Figs. 3.13, 3.14, and 3.15, respectively.

3.4.4 The Reducing Saccharide Contents of Ginseng

3.4.4.1 The Reducing Saccharide Contents of Ginseng from Different Regions with Different Cultivation Periods

The reducing saccharide contents of ginsengs from different regions with different cultivation periods were shown in Table 3.12.

3.4.4.2 The Reducing Saccharide Contents of Ginseng with Different Cultivation Periods from the Same Region

As shown in Table 3.12, the reducing saccharide contents of ginseng with different periods from the same region were shown in Table 3.13. And

Table 3.10 Polysaccharide contents of ginseng from different regions with different cultivation periods (%)

No.	Region	Cultivation period (Year)	Sample 1/%	Sample 2/%	Content of polysaccharide (%)
1	Shuangcha Ji'an	4	26.56	21.01	23.78
2		5	25.06	20.49	22.78
3		6	25.28	15.16	20.22
4	Dadi Ji'an	5	27.63	22.60	25.12
5		6	25.93	19.42	22.68
6	Changbai	4	25.62	21.65	23.63
7		5	16.52	17.18	16.85
8		6	22.10	24.71	23.40
9	Fusong	4	20.56	23.96	22.26
10		5	16.19	15.53	15.86
11		6	25.90	29.38	27.64
12	Jingyu	4	23.20	35.95	29.58
13		5	24.22	27.88	26.05
14	Linjiang	5	18.22	17.56	17.89
15		6	16.29	17.31	16.80
16	Antu	4	28.16	17.67	22.91
17		5	22.66	23.98	23.32
18	Dunhua	4	13.93	19.16	16.55
19		5	22.92	26.08	24.50
20	Hunchun	4_{CC}	21.92	19.66	20.79
21		4	20.05	19.15	19.60
22		5	29.65	33.29	31.47
23		6	16.68	17.60	17.14
24	Wangqing	4	25.17	25.85	25.51
25		5	29.40	29.70	29.55
26		6	16.60	18.71	17.66
27	Helong	4	20.83	23.6	22.21
28		5	16.39	20.97	18.68
29	Jiaohe	4	13.68	17.67	15.68
30		5	21.70	25.10	23.40
31	Huadian	4	25.69	22.38	24.03
32		5	11.54	24.65	18.10
33	Tonghua	4	30.03	25.21	27.62
34		5	30.22	26.12	28.17
35	Hulin	4	27.59	31.43	29.51
36	Heihe	4	28.33	27.42	27.87
37	Kuandian	4	17.48	17.14	17.31
38		5	18.62	27.31	22.96
39		6	21.55	19.12	20.34
40	Xinbin	4	20.89	25.21	23.05
41	North Korea	4	25.52	27.22	26.37
42		5	19.67	16.30	17.99
43		6	36.86	20.66	28.76
44	South Korea	5	17.94	22.67	20.30
45		6	23.35	26.50	24.93

Table 3.11 Analysis of polysaccharide contents in ginseng with the different grown periods from the same region

No.	Region	Content
1	Shuangcha Ji'an	CG4 > CG5 > CG6
2	Kuandian	CG5 > CG6 > CG4
3	Changbai	CG4 > CG6 > CG5
4	North Korea, Fusong	CG6 > CG4 > CG5
5	Wangqing, Hunchun	CG5 > CG4 > CG6
6	Dadi Ji'an, Linjiang	CG5 > CG6
7	Dunhua, Antu, Jiaohe, Tonghua	CG5 > CG4
8	Helong, Jingyu, Huadian	CG4 > CG5
9	South Korea	CG6 > CG5

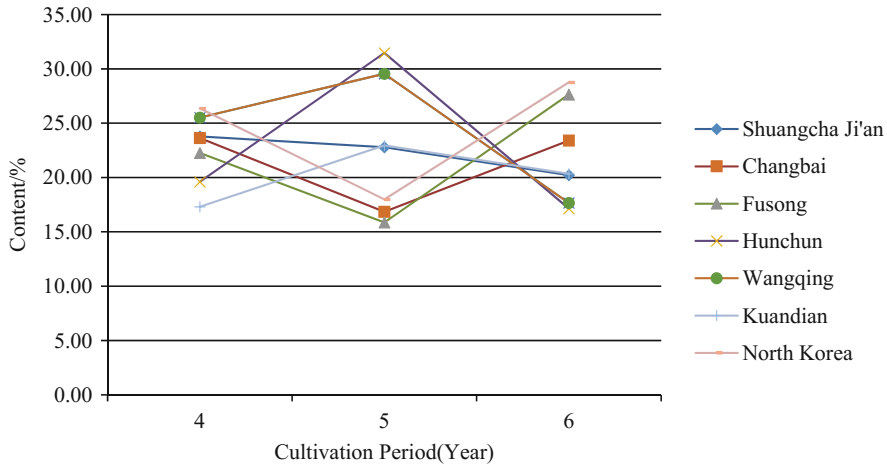

Fig. 3.11 The contents of polysaccharide in ginseng of 4, 5, 6 ages

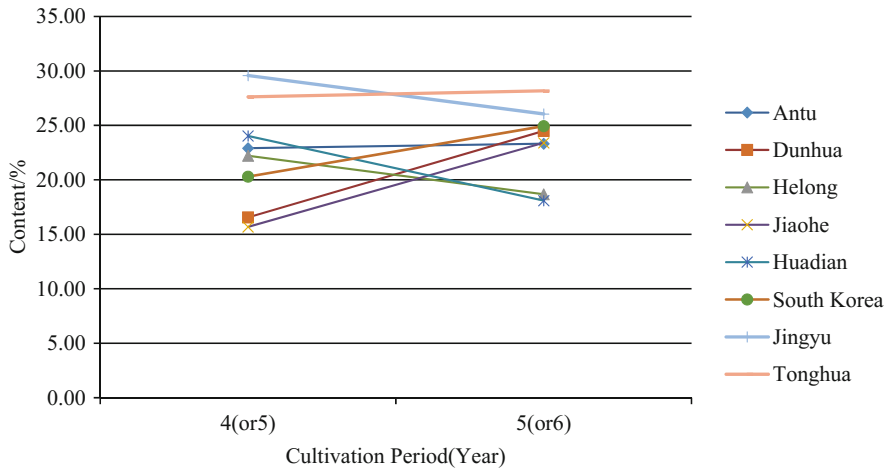

Fig. 3.12 The contents of polysaccharide in ginseng of 4, 5 ages

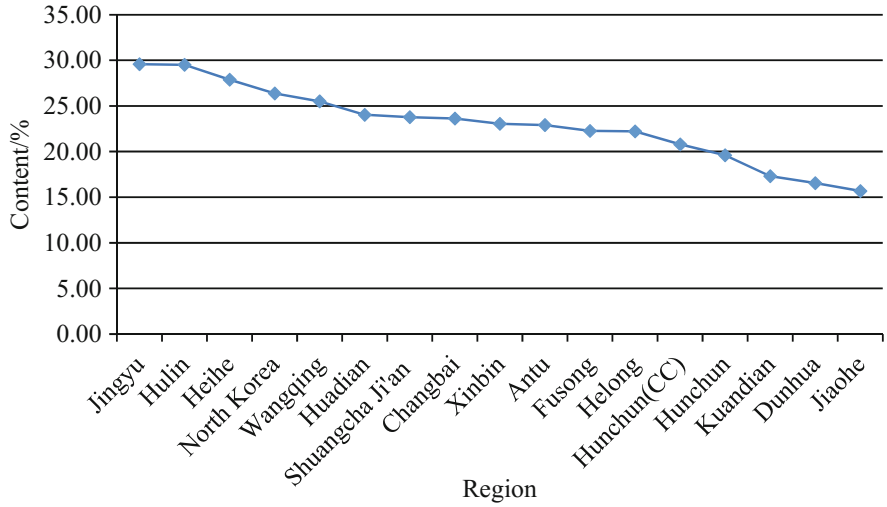

Fig. 3.13 Polysaccharide contents of 4-year-old ginseng

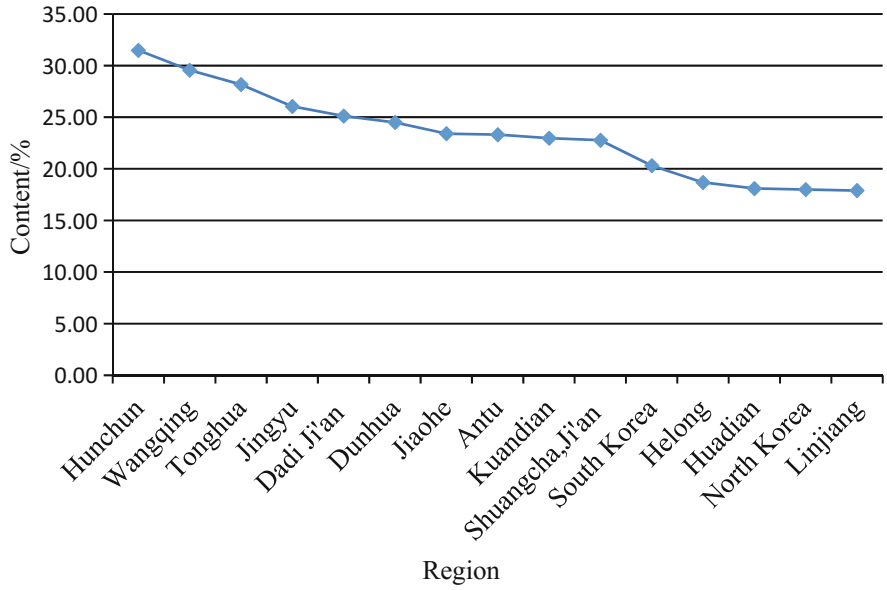

Fig. 3.14 Polysaccharide contents of 5-year-old ginseng

the content trend graph of the reducing saccharide was shown in Figs. 3.16 and 3.17.

The overall trend was that the content in 6-year-old ginseng was higher than in 5-year-old ginseng. And the lowest content was in 4-year-old ginseng.

3.4.4.3 The Contents of Reducing Saccharide in Ginseng from Different Regions with the Same Cultivation Period

The determination result was shown in Table 3.12.

(1) For 4-year-old ginseng, the regions with high-to-low contents were: Wangqing, Dunhua, Heihe, Quanyang Fusong, Helong, Hulin, Antu,

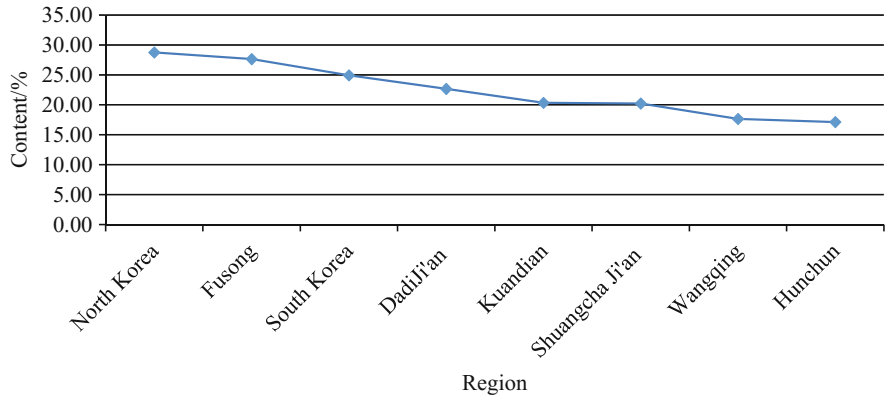

Fig. 3.15 Polysaccharide contents of 6-year-old ginseng

Changbai, North Korea, Xinbin, Jingyu, Shuangcha Ji'an, Kuandian, Huadian, Jiaohe, Hunchun. (2) For 5-year-old ginseng, the regions with high-to-low contents were: Dunhua, Jiaohe, Wangqing, Antu, Fusong, Kuandian, Linjiang, Helong, Tonghua, Jingyu, Dadi Ji'an, North Korea, Shuangcha Ji'an, Changbai, Huadian, Hunchun, South Korea. (3) For 6-year-old ginseng, the regions with high-to-low contents were: Wangqing, Shuangcha Ji'an, Huashu Linjiang, Kuandian, Dadi Ji'an, Hunchun, North Korea, Changbai, South Korea.

The distribution of the reducing saccharide were shown in Figs. 3.18, 3.19, and 3.20, respectively.

Fig. 3.19 Reducing saccharide contents of 5-year-old ginseng

3.4.5 The Uronic Acid Contents of Ginseng

3.4.5.1 The Uronic Acid Contents of Ginseng from Different Regions with Different Cultivation Periods

The uronic acid contents of ginsengs from different regions with different cultivation periods were shown in Table 3.14.

3.4.5.2 The Uronic Acid Contents of Ginseng in Different Cultivation Periods with the Same Region

The uronic acid contents of ginseng in different periods with the same region were shown in Table 3.15. And the content trend graph of the uronic acid was shown in Figs. 3.21 and 3.22.

3.4.5.3 The Contents of Uronic Acid in Ginseng from Different Regions with the Same Cultivation Period

The determination result was shown in Table 3.14.

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

The distribution of the uronic acid were shown in Figs. 3.23, 3.24, and 3.25, respectively.

Table 3.12 Reducing saccharide contents of ginsengs from different regions with different cultivation periods (%)

No.	Region	Cultivation period (year)	Sample 1 (%)	Sample 2(%)	Content of reducing saccharide
1	Shuangcha Ji'an	4	16.06	15.89	15.97
2		5	15.57	12.64	14.10
3		6	21.74	26.04	23.89
4	Dadi Ji'an	5	16.82	14.94	15.88
5		6	19.00	21.21	20.11
6	Changbai	4	15.65	24.56	20.11
7		5	14.48	13.26	13.87
8		6	10.32	10.08	10.20
9	Fusong	4	24.23	22.24	23.24
10		5	22.10	21.29	21.70
11		6	23.97	24.96	24.46
12	Jingyu	4	16.11	16.95	16.53
13		5	18.22	17.25	17.74
14	Linjiang	5	19.88	19.56	19.72
15		6	20.98	21.95	21.46
16	Antu	4	24.76	16.23	20.50
17		5	24.54	21.84	23.19
18	Dunhua	4	24.81	23.75	24.28
19		5	35.65	30.98	33.31
20	Hunchun	4(CC)	19.98	25.37	22.67
21		4	11.84	8.97	10.40
22		5	13.38	9.71	11.54
23		6	13.52	14.96	14.24
24	Wangqing	4	27.74	24.23	25.99
25		5	24.71	24.89	24.80
26		6	24.42	23.87	24.15
27	Helong	4	21.70	23.46	22.58
28		5	17.09	21.85	19.47
29	Jiaohe	4	12.63	11.52	12.08
30		5	22.40	27.77	25.09
31	Huadian	4	13.26	14.85	14.06
32		5	13.84	13.59	13.72
33	Tonghua	4	18.34	18.40	18.37
34		5	19.22	19.02	19.12
35	Hulin	4	22.39	21.73	22.06
36	Heihe	4	27.20	21.05	24.13
37	Kuandian	4	16.46	13.62	15.04
38		5	22.03	18.53	20.28
39		6	20.96	21.68	21.32
40	Xinbin	4	19.27	18.81	19.04
41	North Korea	4	18.98	19.26	19.12
42		5	11.71	16.73	14.22
43		6	13.57	13.29	13.43
44	South Korea	5	7.81	5.85	6.83
45		6	10.13	9.07	9.60

Table 3.13 Analysis of reducing saccharide content of ginseng of different ages

No.	Region	Content
1	Shuangcha Ji'an, Fusong	CG6 > CG4 > CG5
2	Kuandian, Hunchun	CG6 > CG5 > CG4
3	Changbai, North Korea, Wangqing	CG4 > CG5 > CG6
4	Jingyu, Antu, Dunhua, Jiaohe, Tonghua	CG5 > CG4
5	Helong, Huadian	CG4 > CG5
6	Dadi Ji'an, Linjiang, South Korea	CG6 > CG5

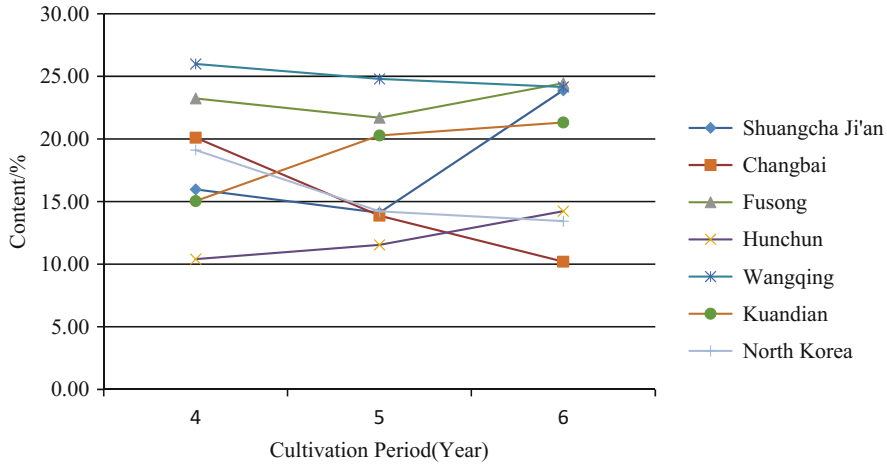

Fig. 3.16 The contents of reducing saccharide in ginseng of 4, 5, 6 ages

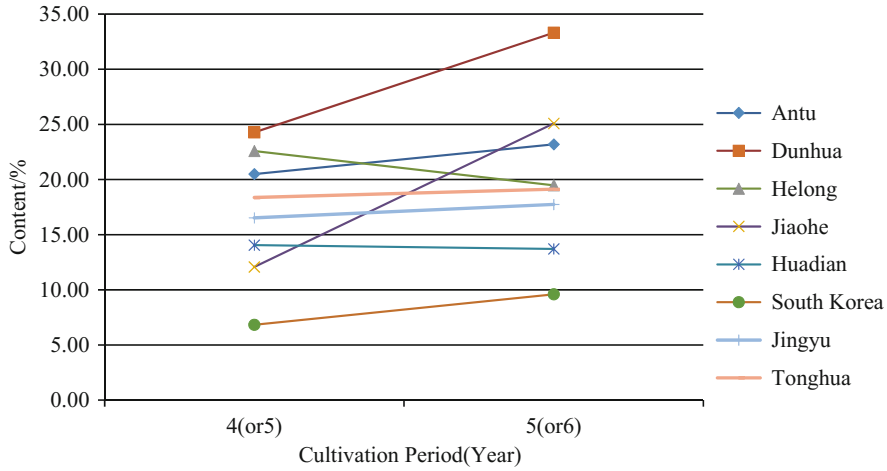

Fig. 3.17 The contents of reducing saccharide in ginseng of 4, 5 ages

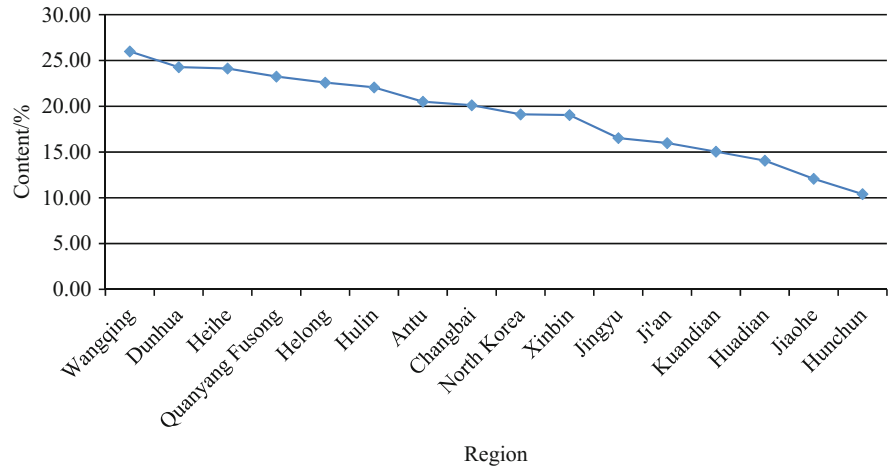

Fig. 3.18 Reducing saccharide contents of 4-year-old ginseng

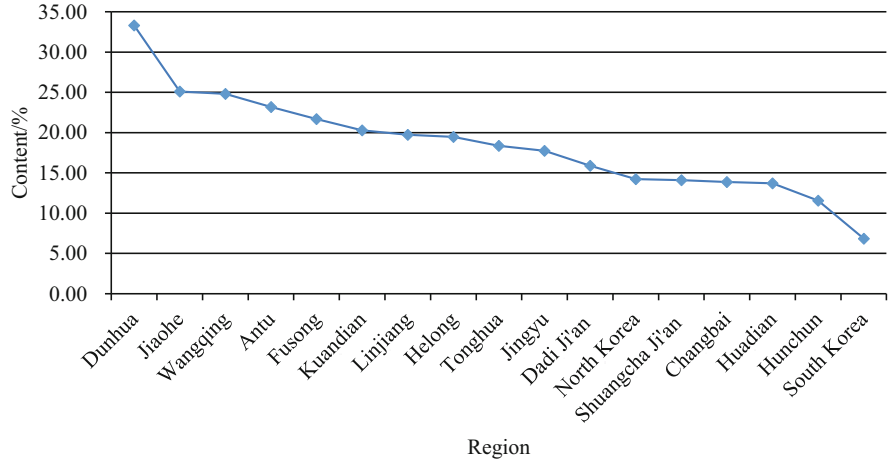

Fig. 3.19 Reducing saccharide contents of 5-year-old ginseng

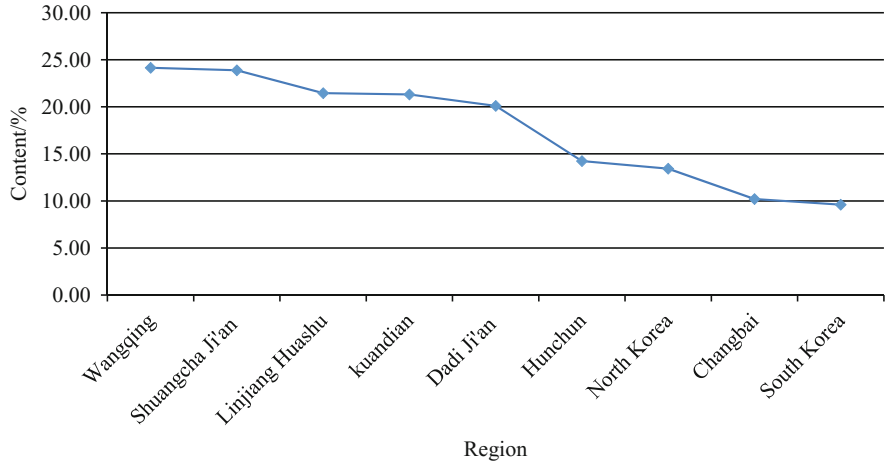

Fig. 3.20 Reducing saccharide contents of 6-year-old ginseng

Table 3.14 Uronic acid contents of ginsengs from different regions with different cultivation periods (%)

No.	Region	Cultivation period (Year)	Sample 1(%)	Sample 2 (%)	Content of uronic acid
1	Shuangcha Ji'an	4	1.54	1.45	1.49
2		5	0.52	0.93	0.73
3		6	1.15	1.01	1.08
4	Dadi Ji'an	5	0.73	1.07	0.90
5		6	1.97	1.65	1.81
6	Changbai	4	1.58	1.22	1.40
7		5	0.85	0.85	0.85
8		6	1.22	1.11	1.16
9	Fusong	4	1.36	1.17	1.27
10		5	0.96	0.91	0.94
11		6	0.93	1.07	1.00
12	Jingyu	4	1.18	1.61	1.40
13		5	1.10	1.60	1.35
14	Linjiang	5	0.95	0.92	0.93
15		6	0.92	0.96	0.94
16	Antu	4	1.23	1.26	1.25
17		5	1.05	1.84	1.45
18	Dunhua	4	0.85	1.24	1.05
19		5	1.46	1.95	1.71
20	Hunchun	4(CC)	1.40	1.58	1.49
21		4	1.24	1.65	1.45
22		5	1.86	1.95	1.91
23		6	1.56	1.44	1.50
24	Wangqing	4	1.28	1.65	1.47
25		5	1.07	1.27	1.17
26		6	1.16	1.57	1.37
27	Helong	4	0.83	0.80	0.81
28		5	0.47	1.22	0.84
29	Jiaohe	4	0.96	1.14	1.05
30		5	0.77	0.72	0.74
31	Huadian	4	1.30	1.14	1.22
32		5	1.15	1.59	1.37
33	Tonghua	4	0.90	1.17	1.04
34		5	0.89	1.09	0.99
35	Hulin	4	0.92	1.35	1.13
36	Heihe	4	1.67	1.52	1.60
37	Kuandian	4	1.14	1.03	1.09
38		5	1.04	0.99	1.02
39		6	0.67	0.76	0.72
40	Xinbin	4	0.71	0.77	0.74
41	North Korea	4	2.33	1.91	2.12
42		5	1.61	1.71	1.66
43		6	2.15	1.99	2.07
44	South Korea	5	1.80	1.91	1.85
45		6	1.84	2.11	1.97

Table 3.15 Analysis of uronic acid of ginseng content with different years in the same producing area

No.	Region	Content
1	Shuangcha Ji'an, Changbai, North Korea, Wangqing, Fusong	CG4 > CG6 > CG5
2	Kuandian	CG4 > CG5 > CG6
3	Hunchun	CG5 > CG6 > CG4
4	Dadi Ji'an, South Korea	CG6 > CG5
5	Jingyu, Tonghua, Jiaohe	CG4 > CG5
6	Dunhua, Antu, Helong, Huadian	CG5 > CG4

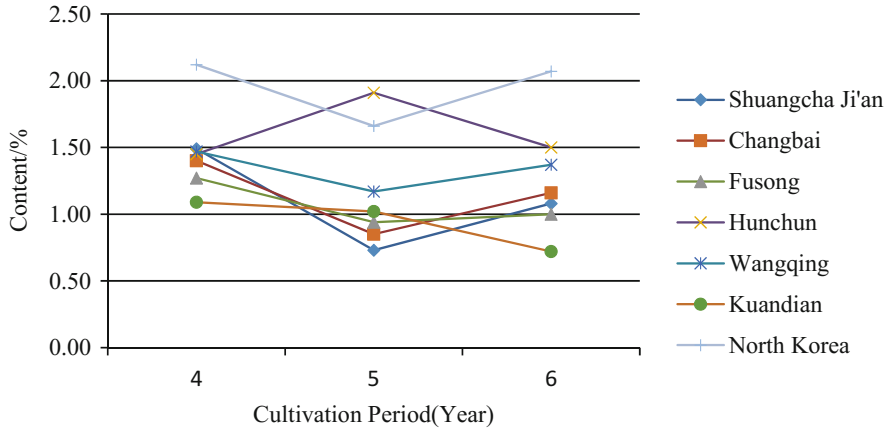

Fig. 3.21 The contents of uronic acid in ginseng of 4, 5, 6 ages

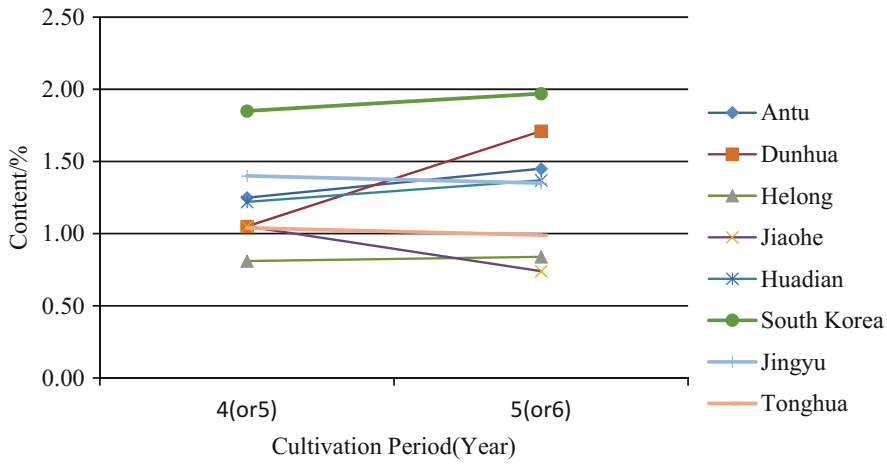

Fig. 3.22 The contents of uronic acid in ginseng of 4, 5 ages

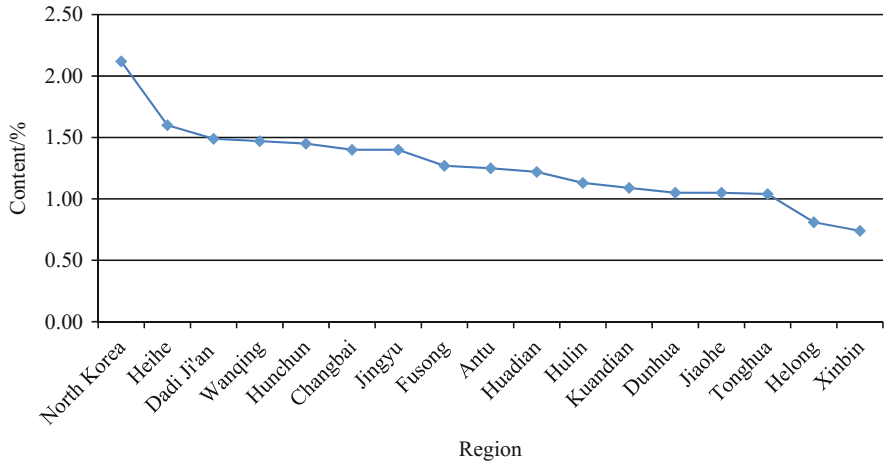

Fig. 3.23 Uronic acid contents of 4-year-old ginseng

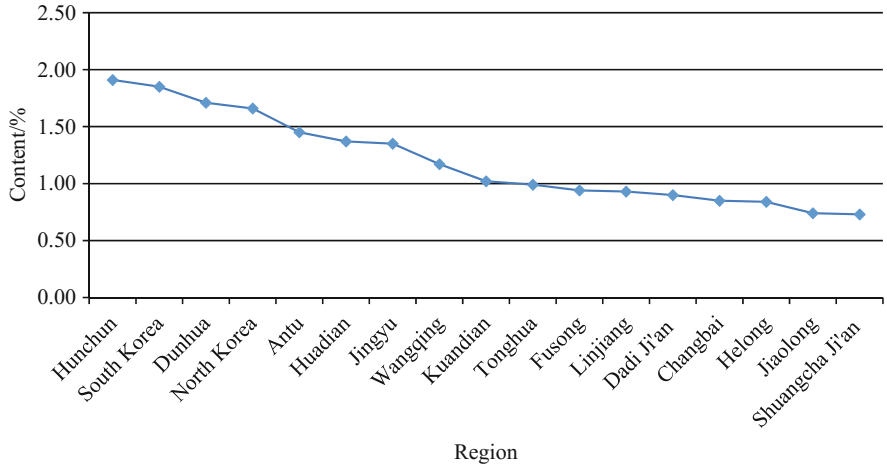

Fig. 3.24 Uronic acid contents of 5-year-old ginseng

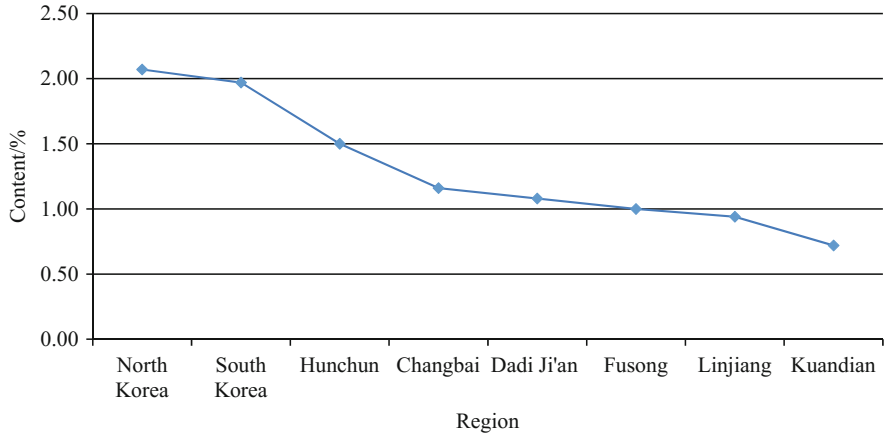

Fig. 3.25 Uronic acid contents of 6-year-old ginseng

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Analysis of Volatile Oils in Ginseng

4

Nanqi Zhang, Qinghai Dong, Qingxi Wang, and Jinping Liu

Abstract

In order to provide data for clarifying the contents of volatile oils in ginseng, the determination of volatile oils was performed, the fresh ginseng was taken as the test samples. The gas chromatography mass spectrometry (GC-MS) was used to determinate the contents of volatile oils in each ginseng sample. Among of all samples, the 4-year-old ginseng from Antu city was the richest in volatile oils. While the 6-year-old ginseng from Dadi Ji'an had the lowest contents of volatile oils. Among of the identified volatile oils, the shared components included β-farnesene, α-gurjunene, calarene, spathulenol, α-caryophyllene, and so on. In general, both the types and the contents of volatile oils in all ginseng samples were all different.

Keywords

Fresh ginseng · Volatile oils · GC-MS

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4.1 Introduction

The volatile oil of ginseng is one of the functional factors of ginseng. For ginseng from different regions with different grown periods, both the types and the contents of volatile oils are significantly different. Fresh ginseng, especially the ginseng with longer ages, emits a unique smell of pleasant aroma, which is a characteristic ingredient worthy of attention [1, 2]. The method most commonly used to analyze volatile components include gas chromatography-mass spectrometry (GC-MS) combined with headspace solidphase microextraction (HS-SPME) or steam distillation (SD) [3–10]. This book uses GC-MS technology to analyze the volatile components of ginseng.

4.2 Materials and Instruments

4.2.1 Materials

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

Anhydrous ether (Tianjin Tiantai Fine Chemicals Co. Ltd., China) and anhydrous sodium sulfate (Beijing Chemical Works) were all of analytical pure grade. Distilled water.

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4.2.2 Instruments

FW177 high speed universal pulverizer (Beijing Yongguangming Medical Instrument Co. Ltd., China); 5975/6890 gas chromatography-mass spectrometer (Agilent Technologies Co. Ltd., America); TC-15 sleeve thermostat (Zhejiang Xinhua Medical Equipment Factory, China); volatile oils extractor (Chengdu Changzheng Chemical Glass Co. Ltd., China).

4.3 Experimental Methods

4.3.1 Gas Chromatography-Mass Spectrometry Conditions

Agilent DB-WAX (60.0 m × 0.25 mm × 0.25 μm) quartz capillary column. High purity helium gas was used as the carrier gas, with the flow rate being 1 mL per minute. The split ratio was 30.9:1. The temperature of injection port was 250 °C. The column temperature was maintained at 80 °C for 3 min, then rised to 250 °C at a rate of 10 °C per minute. The temperature was kept at 250 °C for 10 min. The volume of injection was 1 μL. The temperature of vapanaer was 260 °C. The voltage of multiplier was 1200 eV.

Parameters of mass spectrometry: Electron impact ion source (EI), voltage of ion source was set at 70 eV, ion source temperature was 230 °C, scanning range was *m/z* 20–800. Mass Spectrometry Search Standard Library NIST 08.L.

4.3.2 Extraction of Volatile Components from Ginseng

According to Pharmacopoeia of the People's Republic of China (2015 Version, Appendix X D, method A) [11], fresh ginsengs were cut into pieces, accurately weighted 50 g in a 500 mL flask, added 100 mL of distilled water to extract volatile oils for 12 h. The yellowish-brown volatile oils were obtained and then were dissolved and extracted in anhydrous diethyl ether for three times. And the anhydrous sodium sulfate was added to remove water.

4.4 Results and Discussions

4.4.1 The Yields of Volatile Oil from Ginseng

The contents of volatile oil from ginseng cultivated in different regions with different ages are shown in Table 4.1.

4.4.2 Analysis of Yields of Volatile Oil from Ginseng of Different Ages Grown in Same Areas

The analysis results of the volatile oil in ginseng of different ages were shown in Table 4.2.

4.4.3 Analysis of Yields of Volatile Oil from Ginseng with Same Ages in Different Regions

For 4-year-old ginseng, the regions with high-to-low yields of volatile oil were: Antu, Shuangcha Ji'an, Changbai/Jiaohe/Dunhua/Jingyu/Xinbin/Hulin/Kuandian/Helong/Hunchun continuous cropping/North Korea, Wangqing/ Hunchun/Heihe/Fusong/Huadian.

For 5-year-old ginseng, the regions with high-to-low yields of volatile oil were South Korea, Tonghua/Dadi Ji'an, Hunchun/Antu/Helong/Kuandian/North Korea, Shuangcha Ji'an/Huadian/Fusong/Jiaohe/Dunhua/Wangqing.

For 6-year-old ginseng, the regions with high-to-low yields of volatile oil were South Korea, Kuandian, Shuangcha Ji'an/Fusong, Hunchun/North Korea, Dadi Ji'an/Changbai/Wangqing.

4.4.4 Analysis the Yields of Volatile Oil from Ginseng in Various Regions

Among of all samples, the 4-year-old ginseng sample collected from Antu had the highest

Table 4.1 Volatile oils contents of ginsengs from different regions with different cultivation periods (%)

No.	Region	Cultivation period (year)	Weight of volatile oils (g)	Content of volatile oils (%)
1	Shuangcha Ji'an	4	0.0304	0.06
2		5	0.016	0.03
3		6	0.0181	0.04
4	Dadi Ji'an	5	0.0232	0.05
5		6	0.0080	0.02
6	Changbai	4	0.0252	0.05
7		5	0.0203	0.04
8		6	0.0092	0.02
9	Fusong	4	0.0153	0.03
10		5	0.0148	0.03
11		6	0.0195	0.04
12	Jingyu	5	0.0227	0.05
13	Linjiang	5	0.0391	0.08
14	Antu	4	0.0043	0.09
15		5	0.0207	0.04
16	Dunhua	4	0.0251	0.05
17		5	0.0133	0.03
18	Hunchun	4(CC)	0.0179	0.04
19		4	0.0169	0.03
20		5	0.0208	0.04
21		6	0.0149	0.03
22	Wangqing	4	0.0169	0.03
23		5	0.0132	0.03
24		6	0.0082	0.02
25	Helong	4	0.0186	0.04
26		5	0.0194	0.04
27	Jiaohe	4	0.0252	0.05
28		5	0.0146	0.03
29	Huadian	4	0.0133	0.03
30		5	0.0152	0.03
31	Hulin	4	0.0190	0.04
32	Heihe	4	0.0164	0.03
33	Kuandian	4	0.0189	0.04
34		5	0.0188	0.04
35		6	0.0238	0.05
36	Xinbin	4	0.0220	0.04
37	North Korea	4	0.0178	0.04
38		5	0.0179	0.04
39		6	0.0149	0.03
40	Tonghua	5	0.0245	0.05
41	South Korea	5	0.0310	0.07
42		6	0.0252	0.06

yield of volatile oil, while the 6-year-old ginseng sample in Dadi Ji'an had the lowest yield. In addition, the average yield of volatile oils was 0.056% with the RSD being 26.0%. The experimental results indicated that there was a significant difference but no regular tendency of volatile oil contents, which means that there was no positive correlation between the contents of volatile oil and the ages of ginseng (Fig. 4.1).

Table 4.2 The analysis of volatile oils' yield from different ages of ginseng cultivated in the same regions

No.	Region	Yields
1	Shuangcha Ji'an	CG4 > CG6 > CG5
2	Dadi Ji'an, South Korea	CG5 > CG6
3	Changbai	CG4 > CG5 > CG6
4	Fusong, Kuandian	CG6 > CG5 = CG4
5	Antu, Dunhua, Jiaohe	CG4 > CG5
6	Hunchun	CG5 > CG4 = CG6
7	North Korea, Wangqing	CG4 = CG5 > CG6
8	Helong, Huadian	CG5 = CG4

4.4.4.1 Analysis of Volatile Oil from Changbai Ginseng

As shown in Table 4.3, 63 components were identified from 4-, 5-, and 6-year-old ginseng from Changbai.

In 4-year-old ginseng, 69 components were detected and 50 compounds were identified. The relative contents of 18 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene (12.88%), spathulenol (9.61%), calarene (8.57%), α-gurjunene (8.00%), palmitic acid (6.52%), and falcarinol (5.65%).

In 5-year-old ginseng, 17 components were detected and 11 compounds were identified with the relative contents being more than 1%. The volatile oil with high-to-low contents were palmic acid (32.17%), β-farnesene (4.89%), akardit I (3.94%), stibine, trimethyl- (3.59%), phenol, 2,4,6-tris (1-methylethyl) (3.47%), and terephthalic acid, di(2-ethylhexyl) ester (2.18%).

In 6-year-old ginseng, 52 components were detected and 33 compounds were identified. The volatile oil with high-to-low contents were β-farnesene (12.96%), Germacrene B (11.46%), α-gurjunene (10.23%), calarene (6.08%), falcarinol (5.91%), spathulenol (5.30%), α-caryophyllene (4.87%), and (−)-β-elemene (4.06%).

4.4.4.2 Analysis of Volatile Oils Results in Wangqing Ginseng

As shown in Table 4.4, 73 components were identified from 4-, 5-, and 6-year-old ginseng from Wangqing.

In 4-year-old ginseng, 115 compounds were detected and 61 components were identified. The relative contents of 20 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene (14.68%), calarene (9.21%), palmic acid (7.12%), α-gurjunene (6.89%), γ-elemene (5.95%), espatulenol (4.96%), α-caryophyllene (4.53%), β-panasinsene (4.04%), and falcarinol (3.68%).

In 5-year-old ginseng, 62 compounds were detected and 38 components were identified. The relative contents of 18 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene (15.31%), calarene (9.14%), α-gurjunene (8.94%), palmic acid (7.56%), γ-elemene (6.01%), espatulenol (5.15%), α-caryophyllene (4.50%), β-panasinsene (4.10%), and falcarinol (3.56%).

In 6-year-old ginseng, 55 compounds were detected and 43 components were identified. The relative contents of 17 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene (16.26%), α-gurjunene (9.85%), bicyclogermacrene (9.27%), calarene (9.02%), palmitic acid (6.69%), espatulenol (6.64%), α-caryophyllene (4.46%), β-panasinsene (4.19%), and falcarinol (3.48%).

4.4.4.3 Analysis of Volatile Oils Results in Kuandian Ginseng

As shown in Table 4.5, 52 components were identified from 4-, 5-, and 6-year-old ginseng from Kuandian.

In 4-year-old ginseng, 64 compounds were detected and 41 components were identified. The relative contents of 16 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene (17.69%), γ-elemene (12.27%), falcarinol (8.32%), α-gurjunene (7.56%), calarene (6.68%), α-caryophyllene

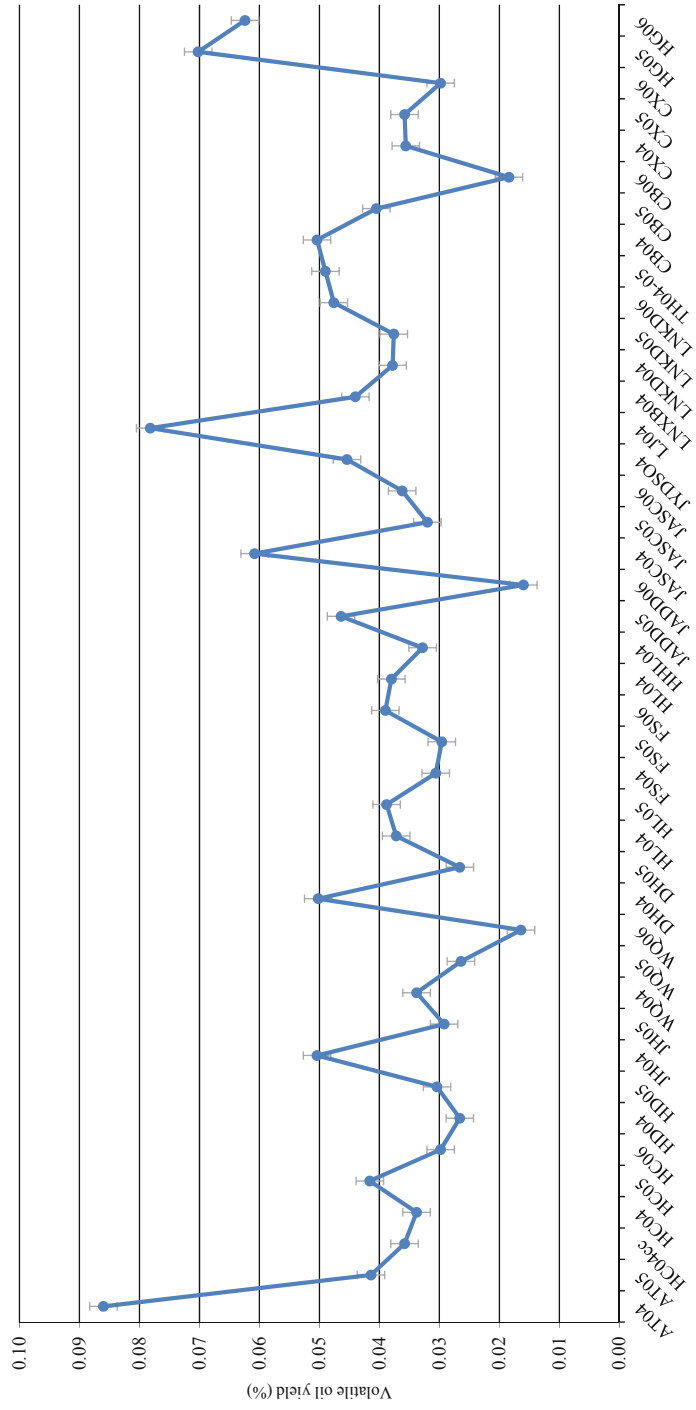

Fig. 4.1 Tendency chart of volatile oil yields from ginseng in different regions with different cultivation periods

Table 4.3 The information of volatile oil from Changbai ginseng

No.	Retention time (min)	Compound's name	Molecular formula	Relative content (%)		
				4-year-old	5-year-old	6-year-old
1	9.232	3,5-di-tert-butyltoluene	$C_{15}H_{24}$	0.19	–	0.22
2	9.564	1,5,5-trimethyl-6-methylenecyclohexene	$C_{10}H_{16}$	0.18	–	–
3	10.404	2,5,6-trimethyl-1,3,6-heptatriene	$C_{10}H_{16}$	–	–	0.66
4	10.410	3-methyl-6-(1-methylethylidene)-cyclohexene	$C_{10}H_{16}$	0.16	–	–
5	10.888	Cedrene-V6		0.18	–	–
6	11.359	1,2,3,6-tetramethylbicyclo[2.2.2]octa-2,5-diene	$C_{12}H_{18}$	–	–	1.76
7	11.365	2-(3-isopropyl-4-methyl-pent-3-en-1-ynyl)-2-methyl-cyclobutanone	$C_{14}H_{20}O$	1.43	–	–
8	11.510	*β*-panasinsene	$C_{15}H_{24}$	3.30	–	3.63
9	11.782	Naphthalene, 1,2,4*a*,5,8,8*a*-hexahydro-4,7- dimethyl-1-(1-methylethyl)-,(1α,4*aβ*,8*aα*)	$C_{15}H_{24}$	–	–	0.30
10	11.788	3,3,7,7-tetramethyl-5-(2-methyl-1-propenyl)tricy-cle [4.1.0.02,4]heptane	$C_{15}H_{24}$	0.49	–	–
11	12.108	11-methylene-2,4-dimethyl-3-azatricyclo[4.1.0.02,4] heptane	$C_{13}H_{19}N$	0.07	–	–
12	12.253	1,7-dimethyl-7-(4-methyl-3-pentenyl)-tricyclo[2.2.1.0 (2,6)]heptane	$C_{15}H_{24}$	0.07	–	–
13	12.356	(−)-aristolene	$C_{15}H_{24}$	0.25	–	0.14
14	12.447	Caryophyleine-(I3)	$C_{15}H_{24}$	–	–	0.17
15	12.706	(−)-*β*-elemene	$C_{15}H_{24}$	2.20	–	4.06
16	12.851	Calarene	$C_{15}H_{24}$	8.57	1.45	6.08
17	12.978	*β*-caryophyllene	$C_{15}H_{24}$	0.79	–	2.76
18	13.063	Eudesma-3,7(11)-diene	$C_{15}H_{24}$	0.53	–	–
19	13.310	*α*-gurjunene	$C_{15}H_{24}$	8.00	2.76	10.23
20	14.072	*β*-farnesene	$C_{15}H_{24}$	12.88	4.89	12.96
21	14.543	*α*-caryophyllene	$C_{15}H_{24}$	3.90	–	4.87
22	14.555	(1*S*)-(+)-3-carene	$C_{10}H_{16}$	–	1.11	–
23	14.682	l-caryophyllene	$C_{15}H_{24}$	2.10	–	0.88
24	15.026	Viridiflorene	$C_{15}H_{24}$	0.74	–	0.90
25	15.196	*β*-neoclovene	$C_{15}H_{24}$	0.91	–	1.01
26	15.383	(3*aS*,3*bR*,4*S*,7*R*,7*aR*)-7-methyl-3-methylidene-4-(propan-2-yl)octahydro-1H-cyclopenta[1,3]cyclopropa[1,2] benzene	$C_{15}H_{24}$	0.07	–	0.14
27	15.492	*cis*-*α*-bisabolene	$C_{15}H_{24}$	0.08	–	–
28	15.619	*β*-eudesmene	$C_{15}H_{24}$	0.56	–	1.42
29	15.721	(−)-*α*-selinene,	$C_{15}H_{24}$	0.43	–	0.54
30	15.884	Germacrene B	$C_{15}H_{24}$	–	–	11.46
31	15.890	Elixene	$C_{15}H_{24}$	3.98	–	–
32	15.897	2-methyl bicyclo[4.3.0]non-1(6)-ene	–	–	1.59	–
33	16.205	*δ*-cadinene	$C_{15}H_{24}$	0.38	–	0.42
34	17.733	*trans,trans*-2,4-*Nonadienal*	$C_9H_{14}O$	0.08	–	0.14
35	19.359	2,4,6-tris(1-methylethyl)phenol	$C_{15}H_{24}O$	–	3.47	2.69
36	21.497	*β*-selinene	$C_{15}H_{24}$	0.14	–	–
37	21.872	l-*β*-bisabolene	$C_{15}H_{24}$	0.21	–	0.29
38	22.422	Stibine, trimethyl-	C_3H_9Sb	–	3.59	–
39	22.579	1,2,3,4,5-pentachloro-benzene	C_6HCl_5	0.73	–	0.67
40	22.947	Globulol	$C_{15}H_{26}O$	0.63	–	–

(continued)

Table 4.3 (continued)

No.	Retention time (min)	Compound's name	Molecular formula	Relative content (%)		
				4-year-old	5-year-old	6-year-old
41	22.954	α-bulnesene	$C_{15}H_{24}$	–	–	1.07
42	23.074	α-longifolene	$C_{15}H_{24}$	0.63	–	–
43	23.461	[1α,3β,4β,5α,7β]-1,4-dimethyladamantane	C_9H_{16}	0.14	–	0.19
44	23.848	Spathulenol	$C_{15}H_{24}O$	9.61	–	5.30
45	24.059	α-patchoulene	$C_{15}H_{24}$	1.33	–	–
46	24.186	α-*cis*-himachalene	$C_{15}H_{24}$	0.21	–	–
47	24.361	Ginsenol	$C_{15}H_{26}O$	1.41	–	1.74
48	25.672	1,7,7-trimethyl-2-vinylbicy-clo[2.2.1]hept-2-ene	$C_{12}H_{18}$	1.75	–	–
49	26.990	Hexachlorobenzene	C_6Cl_6	1.00	–	0.48
50	27.110	1,2,4,5-tetrachloro-3-nitro-benzene	$C_6HCl_4NO_2$	0.22	–	–
51	30.035	*trans, trans*-methyl linoleate	$C_{19}H_{34}O_2$	–	1.70	–
52	30.101	Quintozine	$C_6Cl_5NO_2$	0.87	–	–
53	31.322	Terephthalic acid, di(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	–	2.18	–
54	31.026	Diisobutyl phthalate	$C_{16}H_{22}O_4$	0.13	–	–
55	32.095	1-nonadecene	$C_{19}H_{38}$	0.20	–	–
56	32.258	*N*′-methyl-*N, N*-diphenyl-urea	$C_{14}H_{14}N_2O$	1.54	–	–
57	32.264	1,1-diphenylurea	$C_{13}H_{12}N_2O$	–	3.94	–
58	33.750	1,2-benzenedicarboxylicacid, 1-butyl 2- (2-ethylhexyl) ester	$C_{20}H_{30}O_4$	0.11	–	–
60	37.841	Palmitic acid	$C_{16}H_{32}O_2$	6.52	32.17	3.27
61	39.218	Pentachloroaniline	$C_6H_2Cl_5N$	2.90	–	1.39
62	42.161	Falcarinol	$C_{17}H_{24}O$	5.65	–	5.91
63	48.735	Octaethylene glycol monododecyl ether	$C_{28}H_{58}O_9$	0.92	–	–

Note: "–" is not identified

Table 4.4 The information of volatile oil from Wangqing ginseng

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)		
				4-year-old	5-year-old	6-year-old
1	9.245	1-[4-(1,1-dimethylethyl)-2,6-dimethylphenyl]-ethanone	$C_{14}H_{20}O$	0.29	0.29	0.28
2	9.577	Bicyclo[3.1.0]hex-2-ene,4,4,6,6-tetramethyl-	$C_{10}H_{16}$	–	–	0.41
3	9.771	4-(2,7,7-trimethylbicyclo[3.2.0]hept-2-en-1-yl) but-3-en-2-one	$C_{14}H_{20}O$	0.85	0.82	0.83
4	10.302	Furfural	$C_5H_4O_2$	0.07	–	–
5	10.417	γ-pyronene	$C_{10}H_{16}$	0.34	–	0.56
6	10.798	9-aristolene	$C_{15}H_{24}$	0.23	0.25	–
7	10.895	Cedrene-V6	$C_{15}H_{24}$	0.30	0.32	–
8	10.901	2-isopropenyl-4*a*,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaph-thalene	$C_{15}H_{24}$	–	–	0.19
9	11.371	2,3,4,5-tetramethyltricyclo[3.2.1.02,7]oct-3-ene	$C_{12}H_{18}$	–	1.02	1.94
10	11.372	1,2,3,6-tetramethylbicyclo[2.2.2]octa-2,5-diene	$C_{12}H_{18}$	1.70	–	–
11	11.523	β-panasinsene	$C_{15}H_{24}$	4.04	4.10	4.19
12	12.115	Benzene,1-ethyl-2,4,5-trime	$C_{11}H_{18}$	0.16	0.10	0.11

(continued)

Table 4.4 (continued)

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)		
				4-year-old	5-year-old	6-year-old
13	12.363	(−)-aristolene	$C_{15}H_{24}$	0.37	0.36	0.29
14	12.707	1-methyl-2,4-di(prop-1-en-2-yl)-1-vinylcyclohexane	$C_{15}H_{24}$	–	1.32	2.85
15	12.725	*β*-elemene	$C_{15}H_{24}$	3.12	1.21	0.15
16	12.882	Calarene	$C_{15}H_{24}$	9.21	9.14	9.02
17	12.979	Bicyclo[5.2.0]nonane,4-ethe-nyl-4,8,8-trimethyl-2-methyl-ene	$C_{15}H_{24}$	–	–	0.98
18	12.991	l-caryophyllene	$C_{15}H_{24}$	1.04	–	–
19	13.070	Eudesma-3,7(11)-diene	$C_{15}H_{24}$	0.64	–	–
20	13.323	*α*-gurjunene	$C_{15}H_{24}$	6.89	8.94	9.85
21	14.085	*β*-farnesene	$C_{15}H_{24}$	14.68	15.31	16.26
22	14.308	Furfuryl alcohol	$C_5H_6O_2$	0.13	–	–
23	14.550	*α*-caryophyllene	$C_{15}H_{24}$	4.53	4.50	4.46
24	14.689	*β*-caryophyllene	$C_{15}H_{24}$	2.85	2.81	2.70
25	15.015	Viridiflorene	$C_{15}H_{24}$	0.85	0.87	0.88
26	15.190	*β*-neoclovene	$C_{15}H_{24}$	1.14	1.25	1.01
27	15.372	1*H*-cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methy	$C_{15}H_{24}$	–	–	0.18
28	15.474	*cis*-*α*-bisabolene	$C_{15}H_{24}$	0.17	–	0.10
29	15.601	*β*-eudesmene	$C_{15}H_{24}$	0.80	0.81	0.58
30	15.704	(−)-*α*-selinene	$C_{15}H_{24}$	0.53	0.32	0.43
31	15.879	Bicyclogermacrene	$C_{15}H_{24}$	–	–	9.27
32	15.885	*γ*-elemene	$C_{15}H_{24}$	5.95	6.01	–
33	16.079	*cis*-9-dodecenyl acetate	$C_{14}H_{26}O_2$	0.08	–	–
34	16.187	*δ*-cadinene	$C_{15}H_{24}$	0.51	0.30	0.44
35	16.520	*α*-bulnesene	$C_{15}H_{24}$	0.07	0.09	0.29
36	17.686	2,4-decadienal	$C_{10}H_{16}O$	0.05	–	–
37	19.317	2,6-di-*tert*-butyl-4-methylphenol	$C_{15}H_{24}O$	0.50	–	–
38	20.187	Benzylidene semicarbazide	$C_8H_9N_3O$	0.15	–	–
39	21.438	*γ*-selinene	$C_{15}H_{24}$	–	0.21	0.15
40	21.812	*cis*-nerolidol	$C_{15}H_{26}O$	0.33	0.10	0.19
41	22.356	3-cyclohexen-1-carboxalde-hyde,3,4-dimethyl-	$C_9H_{14}O$	1.64	–	–
42	22.888	(−)-globulol	$C_{15}H_{26}O$	1.16	1.25	1.21
43	23.009	Ledol	$C_{15}H_{26}O$	0.66	0.79	0.13
44	23.015	Eudesma-3,7(11)-diene	$C_{15}H_{24}$	–	–	0.66
45	23.395	4-butylanisole	$C_{11}H_{16}O$	0.19	0.21	0.17
46	23.782	Espatulenol	$C_{15}H_{24}O$	4.96	5.15	6.64
47	23.994	Bulnesol	$C_{15}H_{26}O$	1.31	1.01	–
48	24.108	(−)-*α*-selinene	$C_{15}H_{24}$	0.42	–	–
49	24.296	Ginsenol	$C_{15}H_{26}O$	1.73	1.89	1.64
50	24.543	(−)-*γ*-cadinene	$C_{15}H_{24}$	0.12	–	0.16
51	25.057	Torreyol	$C_{15}H_{26}O$	0.03	–	–
52	25.148	Methyl hexadecanoate	$C_{17}H_{34}O_2$	0.10	0.09	–
53	25.281	Caryophyllene oxide	$C_{15}H_{24}O$	0.12	–	–
54	25.595	8-cedren-13-ol	$C_{15}H_{24}O$	1.04	–	–
55	26.145	*α*-patchoulene	$C_{15}H_{24}$	0.13	–	1.22

(continued)

Table 4.4 (continued)

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)		
				4-year-old	5-year-old	6-year-old
56	27.383	4-methylene-6-(1-propenylidene)cyclooctene	$C_{12}H_{16}$	–	–	0.76
57	28.573	6-isopropenyl-4,8α-dimethyl-1,2,3,5,6,7,8,8α-octahydro-naphthalen-2-ol	$C_{15}H_{24}O$	0.18	–	–
58	28.809	Santalol	$C_{15}H_{24}O$	0.04	–	–
59	29.909	Methyl linoleate	$C_{19}H_{34}O_2$	0.18	0.25	–
60	30.900	Diisobutyl phthalate	$C_{16}H_{22}O_4$	0.10	–	–
61	31.927	5-octadecene	$C_{18}H_{36}$	0.23	0.30	–
62	31.933	Cyclohexadecane	$C_{16}H_{32}$	–	–	0.25
63	32.114	Diphenylamine	$C_{12}H_{11}N$	0.88	0.64	0.79
64	33.087	Tetradecanoic acid	$C_{14}H_{28}O_2$	0.11	0.12	0.19
65	33.177	Heptadecane	$C_{17}H_{36}$	0.05	–	–
66	35.129	Pentadecanoic acid	$C_{15}H_{30}O_2$	0.32	–	–
67	37.576	Palmitic acid	$C_{16}H_{32}O_2$	7.12	7.56	6.69
68	38.839	9-hexadecenoic acid ethyl ester	$C_{18}H_{34}O_2$	0.22	–	–
69	41.914	Falcarinol	$C_{17}H_{24}O$	3.68	3.56	3.48
70	45.600	Trans-13-octadecenoic acid	$C_{18}H_{34}O_2$	0.50	–	–
71	47.195	*n*-dioctyl phthalate	$C_{24}H_{38}O_4$	0.23	–	–
72	48.143	Linoleic acid	$C_{18}H_{32}O_2$	1.89	1.64	–
73	48.300	Dodecyl hexaethylene glycol monoether	$C_{24}H_{50}O_7$	–	–	1.05

Table 4.5 The information of volatile oil from Kuandian ginseng

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)		
				4-year-old	5-year-old	6-year-old
1	9.232	1-[4-(1,1-dimethylethyl)-2,6-dimethylphenyl] ethanone	$C_{14}H_{20}O$	0.30	0.31	–
2	9.233	3,5-di-tert-butyltoluene	$C_{15}H_{24}$	–	–	0.19
3	9.558	(1*E*,5*E*)-1,5-dimethyl-8-(1-methylethylidene)-1,5-cyclodecadiene	$C_{15}H_{24}$	0.60	–	–
4	10.404	Artemisia triene	$C_{10}H_{16}$	1.17	0.48	0.48
5	10.882	Cedrene-V6	$C_{15}H_{24}$	0.28	–	0.19
6	11.359	2,3,4,5-tetramethyltricyclo[3.2.1.02,7]oct-3-ene	$C_{12}H_{18}$	1.84	1.83	1.48
7	11.510	β-panasinsene	$C_{15}H_{24}$	4.34	4.47	3.55
8	11.788	β-patchoulene	$C_{15}H_{24}$	0.44	0.56	0.54
9	12.102	4-(2,7,7-trimethylbi-cyclo[3.2.0]hept-2-en-1-yl) but-3-en-2-one	$C_{14}H_{20}O$	0.12	–	–
10	12.108	11-methylene-2,4-dimethyl-3-azatricyclo [5.3.1.0 (4,9)]undec-2-ene	$C_{13}H_{19}N$	–	0.13	–
11	12.356	(−)-9-aristolene	$C_{15}H_{24}$	0.21	0.19	0.21
12	12.700	β-elemene	$C_{15}H_{24}$	4.87	3.83	3.24
13	12.845	Calarene	$C_{15}H_{24}$	6.68	6.56	8.19
14	12.972	β-chamigrene	$C_{15}H_{24}$	1.18	–	1.00
15	13.057	Eudesma-3,7(11)-diene	$C_{15}H_{24}$	0.51	–	–
16	13.305	α-gurjunene	$C_{15}H_{24}$	7.56	6.89	8.08

(continued)

Table 4.5 (continued)

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)		
				4-year-old	5-year-old	6-year-old
17	14.072	*β*-farnesene	$C_{15}H_{24}$	17.69	18.26	15.75
18	14.307	Furfuryl alcohol	$C_5H_6O_2$	0.08	–	–
19	14.543	*α*-caryophyllene	$C_{15}H_{24}$	5.36	5.82	5.30
20	14.676	l-caryophyllene	$C_{15}H_{24}$	3.53	5.06	3.47
21	15.020	Viridiflorene	$C_{15}H_{24}$	0.93	0.79	0.92
22	15.190	*β*-neoclovene	$C_{15}H_{24}$	1.17	1.34	1.07
23	15.377	1-isopropyl-7-methyl-4-methylene-1,2,3,4,4*a*,5,6,8*a*-octahydronaphthalene	$C_{15}H_{24}$	0.41	0.33	0.21
24	15.480	*cis*-*α*-bisabolene	$C_{15}H_{24}$	0.14	–	0.12
25	15.613	*β*-eudesmene	$C_{15}H_{24}$	1.08	1.44	0.68
26	15.709	(−)-*α*-selinene	$C_{15}H_{24}$	0.57	0.67	0.59
27	15.884	*γ*-elemene	$C_{15}H_{24}$	12.27	9.74	11.2
28	16.102	9-methyl-bicyclo[3.3.1]nonane	$C_{10}H_{18}$	–	0.11	–
29	16.199	*δ*-cadinene	$C_{15}H_{24}$	0.40	0.47	0.51
30	16.531	1,5,9-trimethyl-1,5,9-cyclododecatriene	$C_{15}H_{24}$	–	0.09	–
31	20.410	Spiro[4.5]decan-7-one,1,8-dimethyl-4-(1-met hylethyl)	$C_{15}H_{24}O_3$	–	–	0.41
32	21.872	l-*β*-bisabolene	$C_{15}H_{24}$	0.20	–	–
33	22.410	6-methy-5-hepten-3-yn-2-ol	$C_{11}H_{18}O$	–	1.67	–
34	22.585	1,2,3,4,5-pentachloro-benzene	C_6HCl_5	0.46	0.11	–
35	22.947	Globulol	$C_{15}H_{26}O$	0.81	–	1.22
36	23.068	*β*-selinene	$C_{15}H_{24}$	–	0.47	0.75
37	23.455	[1*α*,3*β*, 4*β*, 5*α*, 7*β*]-1,4-dimethyladamantane	$C_{12}H_{20}$	–	0.10	0.19
38	23.461	*N,N*-diethyl-1,4-phenylenediamine	$C_{10}H_{16}N_2$	0.14	–	–
39	23.842	Spathulenol	$C_{15}H_{24}O$	1.56	3.00	4.55
40	24.059	*α*-patchoulene	$C_{15}H_{24}$	–	1.60	0.15
41	24.174	2-methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane	$C_{15}H_{24}$	0.26	–	–
42	24.361	Ginsenol	$C_{15}H_{26}O$	1.50	1.91	2.11
43	25.231	2-methoxy-4-vinylphenol	$C_9H_{10}O_2$	–	0.09	–
44	27.008	Hexachlorobenzene	C_6Cl_6	0.43	0.10	–
45	27.842	1,4-dioxaspiro[4.5]decane	$C_8H_{14}O_2$	–	0.10	–
46	30.119	Quintozine	$C_6Cl_5NO_2$	0.10	–	–
47	31.026	Diamyl phthalate	$C_{18}H_{26}O_4$	–	0.11	–
48	32.035	2-tetradecene	$C_{14}H_{28}$	0.26	0.28	0.27
49	34.179	Pentachlorothioanisole	$C_7H_3Cl_5S$	0.30	0.16	–
50	37.883	Palmitic acid	$C_{16}H_{32}O_2$	–	2.80	2.87
51	39.231	Pentachloroaniline	$C_6H_2Cl_5N$	0.88	–	–
52	42.167	Falcarinol	$C_{17}H_{24}O$	8.32	6.65	8.29

(5.36%), *β*-elemene (4.87%), and *β*-panasinsene (4.34%).

In 5-year-old ginseng, 70 compounds were detected and 39 components were identified. The relative contents of 17 components were more than 1%. The volatile oil with high-to-low contents were *β*-farnesene (18.26%), *γ*-elemene (9.74%), *α*-gurjunene (6.89%), falcarinol (6.65%), calarene (6.56%), *α*-caryophyllene (5.82%), l-caryophyllene (5.06%),

β-panasinsene (4.47%), β-elemene (3.83%), espatulenol (3.00%), and palmitic acid (2.80%).

In 6-year-old ginseng, 53 compounds were detected and 32 components were identified. The relative contents of 16 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene (15.75%), γ-elemene (11.2%), falcarinol (8.29%), calarene (8.19%), α-gurjunene (8.08%), α-caryophyllene (5.30%), espatulenol (4.55%), β-panasinsene (3.55%), l-caryophyllene (3.47%), and β-elemene (3.24%).

4.4.4.4 Analysis of Volatile Oils Results in Jiaohe Ginseng

As shown in Table 4.6, 56 components were identified from 4- and 5-year-old ginseng from Jiaohe.

In 4-year-old ginseng, 47 compounds were detected and 25 components were identified. The relative contents of 16 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene (15.31%), α-gurjunene (7.92%), espatulenol (7.74%), α-caryophyllene (6.05%), falcarinol (5.67%), calarene (5.58%), and palmitic Acid (5.18). %), β-panasinsene (4.68%), γ-elemene (3.84%), β-elemene (3.74%), and β-caryophyllene (3.05%).

In 5-year-old ginseng, 80 compounds were detected and 52 components were identified. The relative contents of 19 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene (13.92%), γ-elemene (11.16%), α-gurjunene (10.27%), β-panasinsene (4.97%), α-caryophyllene (4.86%), calarene (4.82%), palmic acid (4.64%), espatulenol (4.38%), and falcarinol (3.29%).

4.4.4.5 Analysis of Volatile Oils Results in Shuangcha Ji'an Ginseng

As shown in Table 4.7, 54 components were identified from 4-, 5-, and 6-year-old ginseng from Shuangcha Ji'an.

In 4-year-old ginseng, 57 compounds were detected and 35 components were identified. The relative contents of 15 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene (16.30%), β-eudesmene (8.18%), calarene (6.78%), a-caryophyllene (6.20%), β-panasinsene (5.25%), falcarinol (5.15%), β- caryophyllen (3.75%), and palmitic acid (3.35%).

In 5-year-old ginseng, 51 compounds were detected and 28 components were identified. The relative contents of 16 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene (20.35%), falcarinol (12.47%), γ-elemene (6.29%), a-caryophyllene (6.24%), β-eudesmene (6.09%), l-caryophyllene (5.25%), β-elemene (5.09%), beta -panasinsene (3.94%), espatulenol (3.64%), and palmitic acid (3.56%).

In 6-year-old ginseng, 51 compounds were detected and 29 components were identified. The relative contents of 15 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene (15.27%), γ-elemene (11.66%), α-gurjunene (9.9 9%), falcarinol (6.11%), α-caryophyllene (5.82%), calarene (5.17%), espatulenol (4.66%), β-panasinsene (4.04%), valencen (3.39%), β-elemene (3.15%), and palmitic acid (3.02%).

4.4.4.6 Analysis of Volatile Oils Results in Dadi Ji'an Ginseng

As shown in Table 4.8, 76 components were identified from 5- and 6-year-old ginseng from Dadi Ji'an.

In 5-year-old ginseng, 115 compounds were detected and 61 components were identified. The relative contents of 18 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene (15.76%), γ-elemene (10.70%), α-gurjunene (7.88%), β-elemene (5.72%), α-caryophyllene (5.38%), and calarene (5.01%).), β-panasinsene (4.57%), falcarinol (3.43%), palmitic acid (3.31%), and espatulenol (3.03%).

In 6-year-old ginseng, 55 compounds were detected and 33 components were identified. The relative contents of 17 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene (14.84%), falcarinol (11.94%), palmitic acid (6.24%), α-caryophyllene (5.88%), β-eudesmene (5.31%), γ-elemene (4.79%), espatulenol (4.71%), β-elemene

Table 4.6 The information of volatile oil from Jiaohe ginseng

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)	
				4-year-old	5-year-old
1	9.245	e1-[4-(1,1-dimethylethyl)-2,6-dimethylphenyl]-thanone	$C_{14}H_{20}O$	0.29	0.36
2	10.309	3-furaldehyde	$C_5H_4O_2$	–	0.09
3	10.417	2,5,6-trimethyl-1,3,6-heptatriene	$C_{10}H_{16}$	0.22	0.48
4	10.798	9-aristolene	$C_{15}H_{24}$	–	0.25
5	10.895	Cedrene-V6	$C_{15}H_{24}$	0.23	0.36
6	11.372	1,2,3,6-tetramethylbicyclo[2.2.2]octa-2,5-diene	$C_{12}H_{18}$	1.97	2.38
7	11.523	*β*-panasinsene	$C_{15}H_{24}$	4.68	4.97
8	11.795	*δ*-selinene	$C_{15}H_{24}$	0.31	0.41
9	12.115	6-methyl-1,2,3,5,8,8*α*-hexahydronaphthalene	$C_{15}H_{24}$	–	0.15
10	12.363	(−)-aristolene	$C_{15}H_{24}$	0.16	0.16
11	12.447	Helminthogermacrene	$C_{15}H_{24}$	–	0.11
12	12.707	*β*-elemene	$C_{15}H_{24}$	3.74	2.68
13	12.852	Calarene	$C_{15}H_{24}$	5.58	4.82
14	12.979	Aromadendrene	$C_{15}H_{24}$	1.11	0.96
15	13.064	*α*-panasinene	$C_{15}H_{24}$	0.44	0.47
16	13.311	*α*-gurjunene	$C_{15}H_{24}$	7.92	10.27
17	14.061	*β-cis*-farnesene	$C_{15}H_{24}$	15.31	13.92
18	14.314	Furfuryl alcohol	$C_5H_6O_2$	–	0.15
19	14.538	*α*-caryophyllene	$C_{15}H_{24}$	6.05	4.86
20	14.671	*β*-caryophyllene	$C_{15}H_{24}$	3.05	2.34
21	14.846	*δ*-selinene	$C_{15}H_{24}$	–	0.13
22	15.015	Viridiflorene	$C_{15}H_{24}$	1.23	1.35
23	15.184	*β*-neoclovene	$C_{15}H_{24}$	1.33	1.34
24	15.317	Calarene	$C_{15}H_{24}$	–	0.09
25	15.493	*β*-vatirenene	$C_{15}H_{24}$	–	0.25
26	15.607	*β*-eudesmene	$C_{15}H_{24}$	0.70	0.67
27	15.704	(−)-*α*-selinene	$C_{15}H_{24}$	0.77	0.62
28	15.879	*γ*-elemene	$C_{15}H_{24}$	3.84	11.16
29	16.805	2-methyl-bicyclo[2.2.2]octane	C_9H_{16}	0.13	–
30	16.187	(−)-*β*-cadinene	$C_{15}H_{24}$	0.45	0.44
31	16.641	*E*, *E*-2,4-decadienal	$C_{10}H_{16}O$	–	0.06
32	17.233	*α*-methyl-4-isopropylstyrene	$C_{12}H_{16}$	–	0.22
33	17.680	2,4-decadienal	$C_{10}H_{16}O$	–	0.35
34	19.317	2,6-di-tert-butyl-4-methylphenol	$C_{15}H_{24}O$	–	2.68
35	20.193	2,3-dihydro-4-phenylazete	C_9H_9N	–	0.12
36	21.444	(−)-*α*-selinene	$C_{15}H_{24}$	–	0.16
37	21.819	*δ*-nerolidol	$C_{15}H_{26}O$	0.33	0.32
38	22.362	3,4-dimethyl-3-dyclohexen-1-carboxaldehyde	$C_9H_{14}O$	–	1.93
39	22.893	Longifolenaldehyde	$C_{15}H_{24}O$	0.86	–
40	22.894	(−)-globulol	$C_{15}H_{26}O$	–	1.00
41	23.015	*α*-longifolene	$C_{15}H_{24}$	0.60	0.79
42	23.402	4-butylanisole	$C_{11}H_{16}O$	–	0.18
43	23.782	Spathulenol	$C_{15}H_{24}O$	7.74	4.38
44	24.000	5,6,7,8,9-hexahydro-1,2,2,3-tetramethyl-2*H*-cyclopentacyclooc-tene	$C_{15}H_{24}$	–	1.58
45	24.133	*α*-bulnesene	$C_{15}H_{24}$	–	0.23

(continued)

Table 4.6 (continued)

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)	
				4-year-old	5-year-old
46	24.302	Ginsenol	$C_{15}H_{26}O$	–	1.54
47	25.154	Methyl hexadecanoate	$C_{17}H_{34}O_2$	–	0.28
48	26.151	α-patchoulene	$C_{15}H_{24}$	2.10	0.18
49	26.320	*cis*-α-copaene-8-ol	$C_{15}H_{24}O$	–	0.22
50	28.138	4-methyl benzoylacetone	$C_{11}H_{12}O_2$	–	0.21
51	28.586	6-isopropenyl-4,8α-dimethyl-1,2,3,5,6,7,8,8α-octahydro-naphtha-len-2-ol	$C_{15}H_{24}$	0.20	–
52	29.915	Methyl linoleate	$C_{19}H_{34}O_2$	–	0.18
53	31.939	Pentafluoropropionic acid tetradecyl ester	$C_{17}H_{29}F_5O_2$	–	0.10
54	32.126	*N*-methyl-*N*′, *N*′-diphenylurea	$C_{14}H_{14}N_2O$	0.94	–
55	37.612	*n*-hexadecanoic acid	$C_{16}H_{32}O_2$	5.18	4.64
56	41.914	Falcarinol	$C_{17}H_{24}O$	5.67	3.29

Table 4.7 The information of volatile oil from Shuangcha Ji'an ginseng

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)		
				4-year-old	5-year-old	6-year-old
1	9.238	1-[4-(1,1-dimethylethyl)-2,6-dimethylphenyl]-ethanone	$C_{14}H_{20}O$	0.39	0.25	–
2	10.314	Furfural	$C_5H_4O_2$	0.16	–	–
3	10.416	2,5,6-trimethyl-1,3,6-heptatriene	$C_{14}H_{20}O$	–	0.35	–
4	10.417	Artemisia triene	$C_{10}H_{16}$	–	–	0.68
5	10.797	3,4-dihydro-4,4,6,8-tetramethyl-coumarin	$C_{13}H_{16}O_2$	0.25	–	–
6	10.894	6*S*-2,3,8,8-Tetramethyltricyclo[5.2.2.0(1,6)]undec-2-ene	$C_{15}H_{24}$	0.34	0.24	0.25
7	11.365	2,3,4,5-tetramethyltricyclo[3.2.1.02,7]oct-3-ene	$C_{12}H_{18}$	2.08	1.54	1.86
8	11.516	β-panasinsene	$C_{15}H_{24}$	5.25	3.94	4.04
9	11.794	3*R*,4*aS*,5*R*,8*aS*-5,8*a*-dimethyl-3-(propan-2-yl)-1,2,3,4,4*a*,5,6,8a-octahydronaphthalene	$C_{15}H_{26}$	–	–	0.26
10	11.800	1*aR*-1*aβ*,2,3,3*a*,4,5,6,7bβ-Octahydro-1,1,3aβ,7-tetramethyl-1H-cyclopropa[a]naphthalene	$C_{15}H_{24}$	–	0.18	–
11	12.114	4-(2,7,7-trimethylbicyclo[3.2.0]hept-2-en-1-yl)but-3-en-2-one	$C_{14}H_{22}O$	0.13	–	–
12	12.362	9-aristolene	$C_{15}H_{24}$	0.20	–	0.26
13	12.453	β-elemene	$C_{15}H_{24}$	0.16	5.09	3.15
14	12.712	1-methyl-2,4-di(prop-1-en-2-yl)-1-vinylcyclohexane	$C_{15}H_{24}$	3.85	–	–
15	12.857	Calarene	$C_{15}H_{24}$	6.78	2.77	5.17
16	12.984	γ-neoclovene	$C_{15}H_{24}$	1.27	–	–
17	13.069	α-panasinsene	$C_{15}H_{24}$	0.48	–	–
18	13.316	α-gurjunene	$C_{15}H_{24}$	–	–	9.99
19	13.317	β-eudesmene	$C_{15}H_{24}$	8.18	6.09	–
20	14.072	β-*cis*-farnesene	$C_{15}H_{24}$	16.30	20.35	15.27
21	14.549	α-caryophyllene	$C_{15}H_{24}$	6.20	6.24	5.82
22	14.682	β-caryophyllene	$C_{15}H_{24}$	3.75	–	–
23	14.687	Valencen	$C_{15}H_{24}$	–	–	3.39
24	14.688	l-caryophyllene	$C_{15}H_{24}$	–	5.25	0.98

(continued)

Table 4.7 (continued)

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)		
				4-year-old	5-year-old	6-year-old
25	15.033	Viridiflorene	$C_{15}H_{24}$	0.83	0.56	0.92
26	15.196	*β*-neoclovene	$C_{15}H_{24}$	1.36	1.22	1.22
27	15.389	3*aS*,3*bR*,4*S*,7*R*,7*aR*-7-methyl-3-methylidene-4-(propan-2-yl)octahydro-1H-cyclopenta[1,3]cyclopropa[1,2]benzene	$C_{15}H_{24}$	0.19	0.15	–
28	15.492	*cis*-*α*-bisabolene	$C_{15}H_{24}$	–	0.17	0.10
29	15.624	*β*-eudesmene	$C_{15}H_{24}$	–	–	1.44
30	15.721	(−)-*α*-selinene	$C_{15}H_{24}$	0.61	0.67	0.57
31	15.896	*γ*-elemene	$C_{15}H_{24}$	–	6.29	11.66
32	16.120	*cis*-7-dodecenyl acetate	$C_{14}H_{26}O_2$	–	–	0.13
33	16.211	(−)-*β*-cadinene	$C_{15}H_{24}$	0.38	0.30	0.46
34	19.364	2,6-di-*tert*-butyl-4-methylphenol	$C_{15}H_{24}O$	–	2.79	1.53
35	21.884	*cis*-nerolidol	$C_{15}H_{26}O$	0.25	0.36	0.33
36	22.223	2-*tert*-butylthiophenol	$C_{10}H_{14}S$	0.14	–	–
37	22.422	*tert*-butylhydroquinone	$C_{10}H_{14}O_2$	1.62	–	–
38	22.965	1,2,3,6-tetramethyl-bicyclo[2.2.2]oct-2-ene	$C_{12}H_{20}$	–	0.59	0.92
39	22.966	*α*-bulnesene	$C_{15}H_{24}$	0.70	–	–
40	23.473	6-*tert*-butyl-*m*-cresol	$C_{11}H_{16}O$	–	–	0.18
41	23.860	5-ethyl-3-methyl-3,4-nonadien-6-yne	$C_{12}H_{18}$	1.40	–	–
42	23.861	Espatulenol	$C_{15}H_{24}O$	–	3.64	4.66
43	24.071	*α*-patchoulene	$C_{15}H_{24}$	–	2.12	–
44	24.373	Ginsenol	$C_{15}H_{26}O$	–	2.30	–
45	26.331	1-(3,3-dimethyl-but-1-ynyl)-1,2-dimethyl-3-methylene-cyclopropane	$C_{12}H_{18}$	0.38	–	–
46	27.642	*α*-Asarone	$C_{12}H_{16}O_3$	0.14	–	–
47	28.240	Bicyclo[2.1.1]hexane-5-carboxylic acid, 5-methoxy-3-methylene-, methyl ester	$C_{10}H_{14}O_3$	0.17	–	–
48	31.352	Terephthalic acid di(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	–	0.45	–
49	32.131	Cyclohexadecane	$C_{16}H_{32}$	–	–	0.34
50	33.376	9,9-ethylenedioxy-1-hydroxymethyltricyclo[3.3.1.0(3,7)] nonane	$C_{12}H_{18}O_3$	0.12	–	–
51	37.883	Palmitic acid	$C_{16}H_{32}O_2$	3.35	3.56	3.02
52	38.916	2,4-di-t-butyl-7,7-dimethyl-1,3,5-Cycloheptatriene	$C_{17}H_{28}$	1.16	–	–
53	42.185	Falcarinol	$C_{17}H_{24}O$	5.15	12.47	6.11
54	47.587	Phthalic acid mono-(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	0.99	–	–

(4.29%), *β*-panasinsene (3.18%), and calarene (2.94%).

4.4.4.7 Analysis of Volatile Oils Results in Hunchun Ginseng

As shown in Table 4.9, 102 components were identified from 4-, 5-, and 6-year-old ginseng and 4-year-old continuous cropping ginseng from Hunchun.

In 4-year-old continuous cropping ginseng, 90 compounds were detected and 73 components were identified. The relative contents of 17 components were more than 1%. The volatile oil with high-to-low contents were palmistic acid (22.66%), *α*-neoclovene (8.28%), linoleic acid (5.89%), *α*-gurjunene (3.36%), *β*-panasinsene (3.27%), 5-hepten-3-yn- 2-ol, 6-methy (3.13%), *α*-caryophyllene (2.90%), espatulenol (2.74%)

Table 4.8 The information of volatile oil from Dadi Ji'an ginseng

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)	
				5-year-old	6-year-old
1	9.232	3,5-di-*tert*-butyltoluene	$C_{15}H_{24}$	0.42	0.19
2	9.559	1,5,5-trimethyl-6-methylene cyclohexene	$C_{10}H_{16}$	0.55	–
3	9.571	4,4,6,6-tetramethyl-bicyclo[3.1.0]hex-2-ene	$C_{10}H_{16}$	–	0.17
4	10.145	2,3,3*a*,4-tetrahydro-3,3*a*,6-trimethyl-1-isopropyl-1H-indene	$C_{15}H_{24}$	0.23	–
5	10.308	Furfural	$C_5H_4O_2$	0.04	–
6	10.411	Artemisia triene	$C_{10}H_{16}$	0.86	–
7	10.417	α-terpinene	$C_{10}H_{16}$	–	0.20
8	10.646	Eudesma-3,7(11)-diene	$C_{15}H_{24}$	0.02	–
9	10.785	(−)-cyperene	$C_{15}H_{24}$	0.30	–
10	10.882	Cedrene-V6	$C_{15}H_{24}$	0.43	–
11	11.365	1,2,3,6-tetramethylbicyclo[2.2.2]octa-2,5-diene	$C_{12}H_{18}$	2.04	–
12	11.371	2,3,4,5-tetramethyltricyclo[3.2.1.02,7]oct-3-ene	$C_{12}H_{18}$	–	1.16
13	11.522	β-panasinsene	$C_{15}H_{24}$	4.57	3.18
14	11.800	1*aR*-1*a*β,2,3,3*a*,4,5,6,7*b*β-Octahydro-1,1,3*a*β,7-tetramethyl-1*H*-cyclopropa[*a*]naphthalene	$C_{15}H_{24}$	–	0.13
15	12.024	γ-selinene	$C_{15}H_{24}$	0.06	–
16	12.108	2-methyl-6-(2-propen-1-yl)-Phenol	$C_{10}H_{12}O$	0.19	–
17	12.253	1,7-dimethyl-7-(4-methyl-3-pentenyl)-tricyclo[2.2.1.0(2,6)] heptane	$C_{15}H_{24}$	0.09	–
18	12.356	9-aristolene	$C_{15}H_{24}$	0.19	–
19	12.725	β-elemene	$C_{15}H_{24}$	5.72	4.29
20	12.870	Calarene	$C_{15}H_{24}$	5.01	2.94
21	12.990	l-caryophyllene	$C_{15}H_{24}$	1.17	0.83
22	13.063	1,2,3,5,6,7,8,8*a*-octahydro-1-methyl-6-methylene-4-(1-methylethyl)-naphthalene,	$C_{15}H_{24}$	0.44	–
23	13.317	β-eudesmene	$C_{15}H_{24}$	–	5.31
24	13.329	α-gurjunene	$C_{15}H_{24}$	7.88	–
25	13.879	(−)-β-santalene	$C_{15}H_{24}$	0.03	–
26	14.084	β-*cis*-farnesene	$C_{15}H_{24}$	15.76	14.84
27	14.308	Furfuryl alcohol	$C_5H_6O_2$	0.06	–
28	14.561	α-caryophyllene	$C_{15}H_{24}$	5.38	5.88
29	14.688	*Bicyclo*[7.2.0]undec-4-ene,4,11,11-trimethyl-8-methylene-	$C_{15}H_{24}$	3.42	3.30
30	14.851	3,3,7,7-tetramethyl-5-(2-methyl-1-propenyl)tricyclo [4.1.0.02,4]heptane	$C_{15}H_{24}$	0.12	–
31	15.027	γ-gurjunene	$C_{15}H_{24}$	0.90	–
32	15.033	Viridiflorene	$C_{15}H_{24}$	–	0.46
33	15.196	β-neoclovene	$C_{15}H_{24}$	1.27	1.11
34	15.377	Germacrene D	$C_{15}H_{24}$	0.24	–
35	15.498	*cis*-α-bisabolene	$C_{15}H_{24}$	0.21	0.11
36	15.613	[1*S*-(1α,4α,7α)]-1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methyl ethenyl)-azulene	$C_{15}H_{24}$	0.68	–
37	15.715	(−)-α-selinene	$C_{15}H_{24}$	0.74	–
38	15.915	γ-elemene	$C_{15}H_{24}$	10.70	4.79
39	16.090	3-methyl-cyclooctene	C_9H_{16}	0.10	–
40	16.205	δ-cadinene	$C_{15}H_{24}$	0.52	0.30
41	17.715	2,4-decadienal	$C_{10}H_{16}O$	0.05	–

(continued)

Table 4.8 (continued)

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)	
				5-year-old	6-year-old
42	19.353	2,6-di-*tert*-butyl-4-methylphenol	$C_{15}H_{24}O$	1.72	2.31
43	20.229	2,3-dihydro-4-phenylazete	C_9H_9N	0.06	0.21
44	21.866	*cis*-nerolidol	$C_{15}H_{26}O$	0.35	0.43
45	22.948	(−)-globulol	$C_{15}H_{26}O$	0.77	–
46	22.966	α-bulnesene	$C_{15}H_{24}$	–	0.65
47	23.068	Ledol	$C_{15}H_{26}O$	0.59	–
48	23.087	γ-selinene	$C_{15}H_{24}$	–	1.30
49	23.455	4-butylanisole	$C_{11}H_{16}O$	0.15	–
50	23.848	Spathulenol	$C_{15}H_{24}O$	3.03	4.71
51	24.059	(+)-viridiflorol	$C_{15}H_{26}O$	1.52	–
52	24.071	α-patchoulene	$C_{15}H_{24}$	–	2.90
53	24.174	(1*S*,7*R*,8*aS*)-1,2,3,5,6,7,8,8*a*-octahydro-1,4-dimethyl-7-(1-methylethenyl)azulene	$C_{15}H_{24}$	0.32	–
54	24.361	Ginsenol	$C_{15}H_{26}O$	1.44	–
55	24.615	(−)-γ-cadinene	$C_{15}H_{24}$	0.12	–
56	24.960	l-α-cadinol	$C_{15}H_{26}O$	–	0.18
57	25.225	4-hydroxy-3-methoxy styrene	$C_9H_{10}O_2$	0.12	–
58	25.352	2,6-dimethyl-6-(4-methyl-3-pentenyl)-2-cyclohexene-1-carboxaldehyde	$C_{15}H_{24}O$	0.12	–
59	25.673	*cis*-α-copaene-8-ol	$C_{15}H_{26}O$	0.94	–
60	26.228	Selina-6-en-4-ol	$C_{15}H_{26}O$	0.26	–
61	26.325	1-(3,3-dimethyl-but-1-ynyl)-1,2-dimethyl-3-methylene-cyclopropane	$C_{12}H_{18}$	0.85	–
62	27.860	1,4-dioxaspiro[4.5]decane	$C_8H_{14}O_2$	–	0.19
63	28.228	α-copaene	$C_{15}H_{24}$	0.11	–
64	28.246	3-fluorobenzoic acid, 2-methyloct-5-yn-4-yl ester	$C_{16}H_{19}FO_2$	–	1.21
65	29.884	2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butenal	$C_{14}H_{22}O$	0.07	–
66	31.032	Diisobutyl phthalate	$C_{16}H_{22}O_4$	0.06	–
67	32.053	Cyclohexadecane	$C_{16}H_{32}$	0.15	–
68	32.119	1-pentadecanol	$C_{15}H_{32}O$	–	0.29
69	33.261	Myristic acid	$C_{14}H_{28}O_2$	0.09	–
70	33.763	Butyl octyl phthalate	$C_{20}H_{30}O_4$	0.04	–
71	33.769	1,2-benzenedicarboxylicacid,1-butyl2-(2-ethylhexyl) ester	$C_{20}H_{30}O_4$	–	0.39
72	35.340	Pentadecanoic acid	$C_{15}H_{30}O_2$	0.16	–
73	37.811	Palmitic acid	$C_{16}H_{32}O_2$	3.31	6.24
74	42.197	Falcarinol	$C_{17}H_{24}O$	3.43	11.94
75	48.602	Linoleic acid	$C_{18}H_{32}O_2$	1.18	–
76	48.765	Cyclohexano-15-crown-5	$C_{14}H_{26}O_5$	–	1.76

and 9, 12-octadecadienoic acid (Z,Z)-, ethyl ester (2.38%).

In 4-year-old ginseng, 39 compounds were detected and 27 components were identified. The relative contents of 20 components were more than 1%. The volatile oil with high-to-low contents were α-neoclovene (13.12%), palmic acid (10.06%), isolongifolene (6.57%), and espatulenol (5.78%), α-caryophyllene (5.57%), calarene (5.26%), 2,6-di-tert-butyl-4-methylphenol (4.65%), and β-panasinsene (4.02%).

Table 4.9 The information of volatile oil from Hunchun ginseng

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)			
				4-year-old (CC)	4-year-old	5-year-old	6-year-old
1	9.247	1-[4-(1,1-dimethylethyl)-2,6-dimethylphenyl]-ethanone	$C_{14}H_{20}O$	0.19	–	0.29	0.38
2	10.159	Cedrene	$C_{15}H_{24}$	0.09	–	0.92	–
3	10.309	Furfural	$C_5H_4O_2$	–	–	–	0.24
4	10.411	3-methyl-6-(1-methylethylidene)-cyclohexene	$C_{10}H_{16}$	–	–	–	0.38
5	10.425	Elixene	$C_{15}H_{24}$	–	–	0.84	–
6	10.799	(−)-aristolene	$C_{15}H_{26}$	0.16	–	–	0.25
7	10.896	3-(1,1-dimethylethyl)-*α*-methyl-benzenepropanal	$C_{14}H_{20}O$	0.21	–	0.27	0.33
8	11.373	2,3,4,5-*t*etramethyltricyclo [3.2.1.02,7]oct-3-ene	$C_{12}H_{18}$	1.17	1.60	2.04	2.37
9	11.525	*β*-panasinsene	$C_{15}H_{24}$	3.27	4.02	4.60	5.35
10	11.795	1*aR*-1*aβ*,2,3,3*a*,4,5,6,7*bβ*-octahydro-1,1,3*aβ*,7-tetramethyl-1*H*-cyclopropa[*a*]naphthalene	$C_{15}H_{24}$	–	–	–	0.31
11	11.802	3*R*,4*aS*,5*R*,8*aS*-5,8*a*-dimethyl-3-(propan-2-yl)-1,2,3,4,4*a*,5,6,8*a*-octah-ydronaphthalene	$C_{15}H_{26}$	0.18	–	–	–
12	12.117	4-isopropyl benzaldehyde	$C_{10}H_{12}O$	0.10	–	–	–
13	12.370	(−)-aristolene	$C_{15}H_{24}$	0.12	–	0.17	–
14	12.455	mayurone		0.04	–	–	–
15	12.709	(−)-*β*-elemene	$C_{15}H_{24}$	1.34	2.44	4.80	3.45
16	12.860	Calarene	$C_{15}H_{24}$	–	5.26	6.20	5.46
17	12.981	2-methylene-5-(1-methylvinyl)-8-methyl-bicyclo [5.3.0]decane	$C_{15}H_{24}$	0.51	–	–	–
18	12.987	1*R*,3*Z*,9*S*-4,11,11-trimethyl-8-methy-lenebicyclo [7.2.0]undec-3-ene	$C_{15}H_{24}$	–	–	1.19	–
19	13.058	*γ*-maaliene	$C_{15}H_{24}$	–	–	–	0.32
20	13.071	1*H*-cyclopropa[*a*] naphthalene,1*a*,2,3,5,6,7,7*a*,7*b*-octahydro-1,1,7,7*a*-tetramethyl-,(1*aR*,7*R*,7*aR*,7*bS*)-	$C_{15}H_{24}$	0.23	–	0.37	–
21	13.318	Isolongifolene	$C_{15}H_{24}$	–	6.57	–	–
22	13.319	*α*-gurjunene	$C_{15}H_{24}$	3.36	–	8.87	9.87
23	14.062	Neoclovene	$C_{15}H_{24}$	8.28	13.12	16.92	14.57
24	14.316	Furfuryl alcohol	$C_5H_6O_2$	0.03	–	–	–
25	14.545	*α*-caryophyllene	$C_{15}H_{24}$	2.90	5.57	6.20	6.21
26	14.671	l-caryophyllene	$C_{15}H_{24}$	–	–	–	3.15
27	14.678	*β*-caryophyllene	$C_{15}H_{24}$	0.85	–	–	1.10
28	14.685	β-farnesene	$C_{15}H_{24}$	–	2.00	3.91	–
29	15.017	(+)-ledene	$C_{15}H_{24}$	0.25	0.27	–	–
30	15.023	Viridiflorene	$C_{15}H_{24}$	–	–	0.78	0.71
31	15.192	*β*-neoclovene	$C_{15}H_{24}$	1.06	1.22	1.29	1.46
32	15.325	Seychellene	$C_{15}H_{24}$	0.06	–	–	–
33	15.379	(3*aS*,3*bR*,4*S*,7*R*,7*aR*)-7-methyl-3□-methylidene-4-(propan-2-yl)octahydro-1*H*-cyclopenta[1,3] cycl□opropa[1,2]benzene	$C_{15}H_{24}$	–	–	0.15	–
34	15.615	*β*-eudesmene	$C_{15}H_{24}$	–	–	0.63	0.67
35	15.621	*α*-bulnesene	$C_{15}H_{24}$	–	0.43	–	–
36	15.706	(−)-*α*-selinene	$C_{15}H_{24}$	0.36	0.41	–	0.56
37	15.712	Chamigrene	$C_{15}H_{24}$	–	–	0.59	–

(continued)

Table 4.9 (continued)

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)			
				4-year-old (CC)	4-year-old	5-year-old	6-year-old
38	15.873	γ-pyronene	$C_{10}H_{16}$	–	–	–	6.73
39	15.881	3,7,11,11-Tetrameth-ylbicyclo[8.1.0]2,6-undecadiene	$C_{15}H_{24}$	0.28	–	–	–
40	15.886	Bicyclogermacrene	$C_{15}H_{24}$	–	–	14.17	–
41	15.887	Germacrene B	$C_{15}H_{24}$	–	2.14	–	–
42	16.195	(−)-β-cadinene	$C_{15}H_{24}$	0.11	–	0.44	0.34
43	17.234	5,6-dimethyl tetralin	$C_{12}H_{16}$	0.11	–	–	–
44	17.687	2*E*,4*E*-2,4-octadienal	$C_8H_{12}O$	0.14	–	–	–
45	19.325	2,6-di-tert-butyl-4-methylphenol	$C_{15}H_{24}O$	2.04	4.65	1.47	–
46	20.195	2-cyanobenzaldehyde	C_8H_5NO	0.17	–	–	–
47	21.451	Tricyclazole	$C_9H_7N_3S$	0.11	–	–	–
48	21.826	*cis*-nerolidol	$C_{15}H_{26}O$	0.38	–	0.25	0.31
49	22.370	6-methy-5-hepten-3-yn-2-ol	$C_{11}H_{18}O$	3.13	–	–	–
50	22.515	1,2,3,4,5-pentachloro-benzene	C_6HCl_5	0.25	–	–	–
51	22.630	1*R*,3*Z*,9*S*-2,6,10,10-tetramethyl bicyclo[7.2.0] undeca-2,6-diene	$C_{15}H_{24}$	0.09	–	–	–
52	22.902	(−)-globulol	$C_{15}H_{26}O$	0.92	–	–	0.99
53	22.908	Longifolene	$C_{15}H_{24}$	–	0.94	0.87	0.72
54	23.016	Ledol	$C_{15}H_{26}O$	0.65	–	–	–
55	23.409	*cis*-1,4-dimethyladamantane	$C_{12}H_{20}$	0.11	–	–	–
56	23.790	Spathulenol	$C_{15}H_{24}O$	2.74	5.78	2.75	4.63
57	24.001	γ-gurjunene	$C_{15}H_{24}$	1.89	–	–	–
58	24.133	1,3*a*-ethano(1*H*)inden-4-ol,octahydro-2,2,4,7*a*-tetramethyl-	$C_{15}H_{26}O$	–	–	–	0.33
59	24.140	(−)-alloisolongifolene	$C_{15}H_{24}$	0.38	–	–	–
60	24.302	Ginsenol	$C_{15}H_{26}O$	–	–	–	2.21
61	25.161	Methyl hexadecanoate	$C_{17}H_{34}O_2$	–	1.17	–	0.39
62	25.826	Ethyl palmitate	$C_{18}H_{36}O_2$	–	0.67	–	–
63	26.901	Hexachlorobenzene	C_6Cl_6	0.24	–	–	–
64	27.383	2-methyl-5,7-dimethylene-1,8-Nonadiene	$C_{12}H_{18}$	–	–	–	0.54
65	27.639	2-methyl-2-bornene	$C_{11}H_{18}$	–	0.43	–	–
66	28.140	1-(4-methylphenyl)-1,3-butanedione	$C_{11}H_{12}O_2$	0.18	–	–	–
67	28.279	Tetracosane	$C_{24}H_{50}$	–	0.57	–	–
68	28.454	2′-fluoroacetophenone	C_8H_7FO	0.11	–	–	–
69	28.587	4-(1,5-dimethyl-1,4-diallyl)-1-methyl cyclohexene	$C_{15}H_{24}$	0.14	–	–	–
70	28.720	Octadecanoic acid methyl ester	$C_{19}H_{38}O_2$	0.05	–	–	–
71	29.161	6*E*-6-(*E*)-2-butenylidene-1,5,5-trimethyl-1-cyclohexene	$C_{13}H_{20}$	0.10	–	–	–
72	29.656	Elaidic acid ethyl ester	$C_{20}H_{38}O_2$	0.40	–	–	–
73	29.910	Methyl linoleate	$C_{19}H_{34}O_2$	1.54	2.70		0.70
74	30.460	Ethyl linoleate	$C_{20}H_{36}O_2$	2.38	1.91	–	–
75	30.913	Diisobutyl phthalate	$C_{16}H_{22}O_4$	0.14	–	0.36	–
76	31.499	Hexacosane	$C_{26}H_{54}$	–	1.02	–	–
77	31.940	5-octadecene	$C_{18}H_{36}$	0.35	–	–	–
78	31.951	Octadecyl monochloroacetate	$C_{20}H_{39}ClO_2$	–	–	–	0.29

(continued)

Table 4.9 (continued)

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)			
				4-year-old (CC)	4-year-old	5-year-old	6-year-old
79	32.128	Diphenylamine	$C_{12}H_{11}N$	0.51	0.46	0.36	1.70
80	32.653	1,6-dicyclohexyl hexane	$C_{18}H_{34}$	0.14	–	–	–
81	33.088	Myristic acid	$C_{14}H_{28}O_2$	0.30	–	–	–
82	33.203	Heptacosane	$C_{27}H_{56}$	0.78	1.41	–	–
83	33.209	Hentriacontane	$C_{31}H_{64}$	–	–	0.23	–
84	33.600	Butyl 2-ethylhexyl phthalate	$C_{20}H_{30}O_4$	–	–	–	0.29
85	34.019	1,2,3,4,5-pentachloro-6-(methylthio)-benzene	$C_7H_3Cl_5S$	0.31	–	–	–
86	34.206	2,4-dimethyl hexane	C_8H_{18}	0.06	–	–	–
87	34.563	9-hexacosene	$C_{26}H_{52}$	0.08	–	–	–
88	34.629	Heptadecyl cyclohexane	$C_{23}H_{46}$	0.07	–	–	–
89	35.167	Eicosane	$C_{20}H_{42}$	1.53	–	–	–
90	35.783	7-hexyldocosane	$C_{28}H_{58}$	–	2.69	–	–
91	36.997	2,6-di-tert-butyl-hydroquinone	$C_{14}H_{22}O_2$	0.40	–	–	–
92	37.626	Palmitic acid	$C_{16}H_{32}O_2$	22.66	10.06	3.63	10.81
93	38.605	*cis*-9-hexadecenoic acid	$C_{16}H_{32}O_2$	0.55	–	–	–
94	38.840	9-hexadecenoic acid	$C_{16}H_{30}O_2$	0.79	–	–	–
95	38.997	Pentachloroaniline	$C_6H_2Cl_5N$	0.62	–	–	–
96	40.314	Docosane	$C_{22}H_{46}$	0.61	–	–	–
97	41.928	(−)-falcarinol	$C_{17}H_{24}O$		–	3.82	–
98	41.940	Falcarinol	$C_{17}H_{24}O$	2.03	–	–	–
99	44.308	15-crown-5	$C_{10}H_{20}O_5$	0.29	2.80	–	–
100	45.625	Oleic acid	$C_{18}H_{34}O_2$	0.67	–	–	–
101	45.879	Octaethylene glycol monododecy l ether	$C_{28}H_{58}O_9$	0.36	–	–	–
102	48.169	Linoleic acid	$C_{18}H_{32}O_2$	5.89	–	–	–

In 5-year-old ginseng, 47 compounds were detected and 31 components were identified. The relative contents of 15 components were more than 1%. The volatile oil with high-to-low contents were α-neoclovene (16.92%), bicyclogermacrene (14.17%), α-gurjunene (8.87%), α-caryophyllene (6.20%), calarene (6.20%), β-elemene (4.80%), β-panasinsene (4.60%), β-farnesene (3.91%), falcarinol (3.82%), palmitic acid (3.63%), and espatulenol (2.75%).

In 6-year-old ginseng, 46 compounds were detected and 35 components were identified. The relative contents of 15 components were more than 1%. The volatile oil with high-to-low contents were neoclovene (14.57%), palmitic acid (10.81%), α-gurjunene (9.87%), γ-pyronene (6.73%), α-caryophyllene (6.21%), calarene (5.46%), β-panasinsene (5.35%), espatulenol (4.63%), β-elemene (3.45%), and l-caryophyllene (3.15%).

4.4.4.8 Analysis of Volatile Oils Results in Huadian Ginseng

As shown in Table 4.10, 59 components were identified from 5- and 6-year-old ginseng from Huadian.

In 5-year-old ginseng, 33 compounds were detected and 26 components were identified. The relative contents of 15 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene (24.12%), palmic acid (19.91%), β-bisabolene (8.35%), *cis*–αbisabolene (5.79%), ar-curcumene (4.27%), butylated hydroxytoluene (3.25%), isoledene (3.07%), and calarene (2.92%).

Table 4.10 The information of volatile oil from Huadian ginseng

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)	
				4-year-old	5-year-old
1	10.309	Furfural	$C_5H_4O_2$	–	0.28
2	10.762	α-Copaene	$C_{15}H_{24}$	–	0.29
3	11.034	1,3,5-triisopropylbenzene	$C_{15}H_{24}$	0.44	–
4	11.100	Clovene	$C_{15}H_{24}$	0.36	–
5	11.372	2,3-dihydro-7-methoxy-4-methyl-1*H*-1,5-benzodiazepin-2-one	$C_{11}H_{12}N_2O_2$	–	0.24
6	11.523	*β*-panasinsene	$C_{15}H_{24}$	–	0.92
7	12.701	*β*-elemene	$C_{15}H_{24}$	–	0.28
8	12.852	Calarene	$C_{15}H_{24}$	2.92	0.39
9	12.985	4-methyl-4-(2-methyl-2-propenyl)tricyclo[3.3.0.0(2,8)]octan-3-one	$C_{13}H_{18}O$	–	0.20
10	13.094	*γ*-limonene	$C_{15}H_{24}$	0.88	–
11	13.330	Viridiflorene	$C_{15}H_{24}$	0.43	–
12	14.091	*β*-farnesene	$C_{15}H_{24}$	24.12	43.30
13	14.200	γ-elemene	$C_{15}H_{24}$	1.57	–
14	14.399	Isoledene	$C_{15}H_{24}$	3.07	–
15	14.544	*α*-caryophyllene	$C_{15}H_{24}$	–	1.34
16	14.562	Chamigrene	$C_{15}H_{24}$	0.77	–
17	14.756	Acoradiene	$C_{15}H_{24}$	0.91	–
18	14.870	*δ*-selinene	$C_{15}H_{24}$	2.31	–
19	15.088	Fenchene	$C_{10}H_{16}$	0.91	–
20	15.191	*β*-neoclovene	$C_{15}H_{24}$	–	0.28
21	15.360	[*S*-(*R**,***S****)]-5-(1,5-dimethyl-4-hexenyl)-2-methyl-1,3-cyclohexadiene	$C_{15}H_{24}$	–	0.44
22	15.366	a-chamigrene	$C_{15}H_{24}$	1.36	–
23	15.487	l-*β*-bisabolene	$C_{15}H_{24}$	8.35	12.06
24	15.704	(−)-*α*-selinene	$C_{15}H_{24}$	–	0.17
25	15.752	*α*-cedrene	$C_{15}H_{24}$	0.98	0.16
26	15.910	Elixene	$C_{15}H_{24}$	1.03	–
27	16.085	1-bromo-5-heptadecene	$C_{17}H_{33}Br$	–	0.08
28	16.188	*δ*-cadinene	$C_{15}H_{24}$	2.58	2.81
29	16.459	*cis*-*α*-bisabolene	$C_{15}H_{24}$	5.79	–
30	16.465	[*S*-(*R**,*S**)]-3-(1,5-dimethyl-4-hexenyl)-6-methylenecyclohexene	$C_{15}H_{24}$	–	2.80
31	16.526	ar-curcumene	$C_{15}H_{22}$	4.27	–
32	16.870	Longipinene	$C_{15}H_{24}$	0.51	–
33	17.692	*trans,trans*-2,4-Decadien-1-al	$C_{10}H_{16}O$	–	0.12
34	19.335	Butylated hydroxytoluene	$C_{15}H_{24}O$	3.25	–
35	19.680	Dehydro-ar-ionene	$C_{13}H_{16}$	–	0.21
36	19.782	3-(4-methyl-3-pentenyl)-3-cyclohexene-1-carboxaldehyde	$C_{13}H_{20}O$	–	0.33
37	20.193	Benzylidenesemicarbazide	$C_8H_9N_3O$	–	0.75
38	21.819	*cis*-nerolidol	$C_{15}H_{26}O$	–	1.04
39	22.374	2,6-dimethyl-3-cyclohexene-1-carboxaldehyde	$C_9H_{14}O$	–	0.39
40	22.471	*β*-patchoulene	$C_{15}H_{24}$	–	0.15
41	24.326	Ginsenol	$C_{15}H_{26}O$	0.98	–
42	24.501	Cedrene-V6	$C_{15}H_{24}$	–	0.36

(continued)

Table 4.10 (continued)

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)	
				4-year-old	5-year-old
43	24.622	Farnesyl alcohol	$C_{15}H_{26}O$	–	0.15
44	24.803	γ-selinene	$C_{15}H_{24}$	0.47	0.42
45	25.075	(−)-hinesol	$C_{15}H_{26}O$	–	0.34
46	25.154	Methyl hexadecanoate	$C_{17}H_{34}O_2$	–	0.16
47	25.824	Ethyl palmitate	$C_{18}H_{36}O_2$	2.32	–
48	26.580	4-(1,5-dimethyl hexene-4-yl)cyclohex-2-enone	$C_{14}H_{22}O$	–	0.29
49	29.915	Methyl linoleate	$C_{19}H_{34}O_2$	–	0.20
50	30.459	Ethyl linoleate	$C_{20}H_{36}O_2$	1.14	–
51	31.939	Hexadecanol	$C_{16}H_{34}O$	–	0.45
52	32.120	Diphenylamine	$C_{12}H_{11}N$	–	2.14
53	32.688	Farnesyl cyanide	$C_{16}H_{25}N$	–	0.21
54	33.123	Myristic acid	$C_{14}H_{28}O_2$	–	0.19
55	33.600	Phthalic acid, butyl hexyl ester	$C_{16}H_{22}O_4$	–	0.14
56	34.549	Dodecylheptaglycol	$C_{26}H_{54}O_8$	–	0.13
57	37.606	Palmitic acid	$C_{16}H_{32}O_2$	19.91	7.32
58	41.920	(+)-falcarinol	$C_{17}H_{24}O$	–	7.01
59	48.2880	18-crown-6	$C_{12}H_{24}O_6$	–	1.00

In 6-year-old ginseng, 60 compounds were detected and 40 components were identified. The relative contents of 10 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene (43.30%), l-β-bisabolene (12.06%), palmic acid (7.32%), falcarinol (7.01%), δ-cadinene (2.81%) and cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [*S*-(*R**, *S**)]- (2.80%).

4.4.4.9 Analysis of Volatile Oils Results in Heihe and Hulin Ginsengs of Heilongjiang Province

As shown in Table 4.11, 56 components were identified from 4-year-old ginseng from Heihe and Hulin.

In 4-year-old ginseng from Heihe, 73 compounds were detected and 43 components were identified. The relative contents of 17 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene (17.32%), γ-elemene (8.61%), α-gurjunene (7.74%), calarene (6.41%), falcarinol (5.79%), α-caryophyllene (5.19%), espatulenol (4.52%), palmitic acid (4.33%), β-panasinsene (3.89%), β-elemene (3.52%), and β-caryophyllene (3.09%).

In 4-year-old ginseng from Hulin, 47 compounds were detected and 32 components were identified. The relative contents of 17 components were more than 1%. The volatile oil with high-to-low contents were espatulenol (14.51%), β-farnesene (14.46%), palmitic acid (8.19%), α-gurjunene (7.87%), calarene (6.44%), α-caryophyllene (5.13%), falcarinol (4.66%), and β-panasinsene (3.76%).

4.4.4.10 Analysis of Volatile Oils Results in South Korea Ginseng

As shown in Table 4.12, 46 components were identified from 5- and 6-year-old ginseng from South Korea.

In 5-year-old ginseng, 92 compounds were detected and 41 components were identified. The relative contents of 15 components were more than 1%. The volatile oil with high-to-low contents were α-neoclovene (12.28%), α-gurjunene (6.59%), β-panasinsene (5.36%), α-caryophyllene (4.48%), β-elemene (4.09%),

Table 4.11 The information of volatile oil from Heihe and Hulin ginseng

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)	
				4-year-old	5-year-old
1	9.238	p-hexylacetophenone	$C_{14}H_{20}O$	0.25	0.20
2	9.570	1,5,5-trimethyl-6-methylenecyclohexene	$C_{10}H_{16}$	0.23	–
3	10.416	Artemisia triene	$C_{10}H_{16}$	0.33	–
4	10.797	9-aristolene	$C_{15}H_{24}$	0.18	–
5	10.894	3-(1,1-dimethylethyl)-α-methyl benzenepropanal	$C_{14}H_{20}O$	0.23	–
6	10.900	1,3,5-triacetylbenzene	$C_{12}H_{12}O_3$	–	0.17
7	11.371	2,3,4,5-tetramethyltricyclo[3.2.1.02,7]oct-3-ene	$C_{12}H_{18}$	2.12	1.44
8	11.522	*β*-panasinsene	$C_{15}H_{24}$	3.89	3.76
9	11.800	6-isopropyl-4,8*α*-dimethyl-1,2,3,7,8,8*α*-hexahydronaphthalene	$C_{15}H_{24}$	–	0.21
10	12.259	1,7-dimethyl-7-(4-methyl-3-pentenyl)-tricyclo[2.2.1.0(2,6)]heptane	$C_{15}H_{24}$	0.06	–
11	12.368	(−)-aristolene	$C_{15}H_{24}$	0.17	–
12	12.712	*β*-elemene	$C_{15}H_{24}$	3.52	2.82
13	12.857	Calarene	$C_{15}H_{24}$	6.41	6.44
14	12.984	*γ*-neoclovene	$C_{15}H_{24}$	0.97	–
15	13.316	*α*-gurjunene	$C_{15}H_{24}$	7.74	7.87
16	14.084	*β*-farnesene	$C_{15}H_{24}$	17.32	14.46
17	14.555	*α*-caryophyllene	$C_{15}H_{24}$	5.19	5.13
18	14.688	*β*-caryophyllene	$C_{15}H_{24}$	3.09	–
19	14.689	*α*-aromadendrene	$C_{15}H_{24}$	–	1.94
20	15.032	Viridiflorene	$C_{15}H_{24}$	0.77	0.28
21	15.202	*β*-neoclovene	$C_{15}H_{24}$	1.20	1.19
22	15.498	*α-trans*-bergamotene	$C_{15}H_{24}$	0.20	–
23	15.624	*β*-eudesmene	$C_{15}H_{24}$	0.65	–
24	15.721	(−)-*α*-selinene	$C_{15}H_{24}$	–	0.66
25	15.727	*α*-bulnesene	$C_{15}H_{24}$	0.68	0.68
26	15.896	Terpinolene	$C_{10}H_{16}$	–	0.22
27	15.902	*γ*-elemene	$C_{15}H_{24}$	8.61	–
28	16.211	*δ*-cadinene	$C_{15}H_{24}$	–	0.32
29	16.217	(−)-*β*-cadinene	$C_{15}H_{24}$	0.45	–
30	16.549	*β*-elemene	$C_{15}H_{24}$	0.09	–
31	17.739	2*E*,4*E*-2,4-octadienal	$C_8H_{12}O$	0.09	–
32	19.364	2,6-*di-tert*-butyl-4-methylphenol	$C_{15}H_{24}O$	1.57	–
33	21.884	*cis*-nerolidol	$C_{15}H_{26}O$	0.35	0.40
34	22.126	Decahydro-1,1,4,7-tetramethyl-4*α*H-cycloprop[*e*]azulen-4*α*-ol	$C_{15}H_{26}O$	0.12	–
35	22.965	Globulol	$C_{15}H_{26}O$	1.03	–
36	23.086	*α*-longifolene	$C_{15}H_{24}$	0.71	1.01
37	23.473	*cis*-1,4-dimethyladamantane	$C_{12}H_{20}$	0.18	0.21
38	23.860	Espatulenol	$C_{15}H_{24}O$	4.52	14.51
39	24.071	*α*-patchoulene	$C_{15}H_{24}$	1.95	–
40	24.072	Bulnesol	$C_{15}H_{26}O$	–	2.45
41	24.186	(−)-isolongifolol	$C_{15}H_{26}O$	0.49	–
42	24.192	2,4,4-trimethyl-3-(3-methylbuta-1,3-dienyl) cyclohexanone	$C_{14}H_{22}O$	–	0.41
43	26.240	Selina-6-en-4-ol	$C_{15}H_{26}O$	–	0.24

(continued)

Table 4.11 (continued)

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)	
				4-year-old	5-year-old
44	26.337	1-(3,3-dimethyl-but-1-ynyl)-1,2-dimethyl-3-methylene-cyclopropane	$C_{12}H_{18}$	1.03	–
45	28.681	6-isopropenyl-4,8α-dimethyl-1,2,3,5,6,7,8,8α-octahydro-naphthalen-2-ol	$C_{15}H_{24}$	0.15	0.35
46	30.041	Methyl linoleate	$C_{19}H_{34}O_2$	0.12	–
47	32.125	7-hexadecene	$C_{16}H_{32}$	0.17	–
48	32.137	Cyclohexadecane	$C_{16}H_{32}$	–	0.21
49	32.276	4-benzylpyridine	$C_{12}H_{11}N$	–	1.85
50	33.768	Phthalic acid isobutyl 3-methylphenyl ester	$C_{16}H_{22}O_4$	–	0.39
51	33.769	Phthalic acid-2-methylphenyl octyl ester	$C_{16}H_{22}O_4$	0.17	–
52	37.865	*n*-hexadecanoic acid	$C_{16}H_{32}O_2$	4.33	8.19
53	42.197	Falcarinol	$C_{17}H_{24}O$	5.79	4.66
54	47.605	Phthalic acid mono-(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	–	1.52
55	48.692	Methyl-9,12-heptadecadienoate	$C_{18}H_{32}O_2$	–	2.33
56	48.753	3,6,9,12,15,18,21-heptaoxatricosane-21,23-diol	$C_{16}H_{34}O_9$	0.96	–

Table 4.12 The information of volatile oil from South Korea ginseng

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)	
				5-year-old	6-year-old
1	9.094	9-methyltetracyclo[7.3.1.0(2.7).1(7.11)]tetradecane	$C_{15}H_{24}$	0.81	–
2	9.691	β-clovene	$C_{15}H_{24}$	–	0.45
3	10.784	Cedrene-V6	$C_{15}H_{24}$	0.79	–
4	11.209	2,3,4,5-tetramethyltricyclo[3.2.1.02,7]oct-3-ene	$C_{12}H_{18}$	2.39	–
5	11.423	β-panasinsene	$C_{15}H_{24}$	5.36	–
6	11.437	Eudesma-3,7(11)-diene	$C_{15}H_{24}$	–	2.71
7	11.623	(−)-β-cadinene	$C_{15}H_{24}$	0.63	–
8	11.865	(−)-cyperene	$C_{15}H_{24}$	0.09	–
9	11.942	4-isopropyl-benzaldehyde	$C_{10}H_{12}O$	0.31	–
10	12.284	2-methylene-4,8,8-trimethyl-4-vinyl-bicyclo[5.2.0]nonane	$C_{15}H_{24}$	0.20	–
11	12.596	(−)-β-elemene	$C_{15}H_{24}$	4.09	–
12	12.738	Calarene	$C_{15}H_{24}$	3.98	1.91
13	12.890	β-patchoulene	$C_{15}H_{24}$	0.36	–
14	13.187	α-gurjunene	$C_{15}H_{24}$	6.59	8.89
15	13.449	Viridiflorene	$C_{15}H_{24}$	0.56	–
16	13.946	α-neoclovene	$C_{15}H_{24}$	12.28	–
17	13.951	Clovene	$C_{15}H_{24}$	–	3.83
18	14.422	α-caryophyllene	$C_{15}H_{24}$	4.48	–
19	14.555	l-caryophyllene	$C_{15}H_{24}$	1.88	–
20	15.016	β-neoclovene	$C_{15}H_{24}$	1.89	–

(continued)

Table 4.12 (continued)

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)	
				5-year-old	6-year-old
21	15.148	Naphthalene,1,2,3,5,6,7,8,8a-octahydro-1,8α-dimethyl-7-(1-methyle-thenyl)-, (1*S*,7*R*,8α*R*)-	$C_{15}H_{24}$	0.21	–
22	15.440	β-eudesmene	$C_{15}H_{24}$	0.76	–
23	15.710	1,5-dimethyl-8-(1-methylethylidene)- 1,5-cyclodecadiene	$C_{15}H_{24}$	0.64	–
24	16.056	δ-cadinene	$C_{15}H_{24}$	0.49	–
25	16.360	Longifolene	$C_{15}H_{24}$	0.22	–
26	17.087	Arsonous dichloride, As-methyl-	CH_3AsCl_2	0.14	–
27	17.558	*trans*-2,4-decadien-1-al	$C_{10}H_{16}O$	0.10	–
28	17.814	2-fluoro-4-hydroxybenzaldehyde	$C_7H_5FO_2$	0.10	–
29	19.158	9-cedranone	$C_{16}H_{23}O$	0.25	–
30	21.667	*cis*-nerolidol	$C_{15}H_{26}O$	0.68	–
31	22.235	3-methoxybenzyl alcohol	$C_8H_{10}O_2$	–	12.87
32	22.728	(+)-α-elemene	$C_{15}H_{24}$	1.29	–
33	22.764	α-patchoulene	$C_{15}H_{24}$	–	4.50
34	23.240	Uncineol	$C_{15}H_{26}O$	0.39	–
35	23.835	[1,2,4]triazolo[1,5-α]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino, ethyl ester	$C_9H_{11}N_5O_2S$	3.07	–
36	24.327	γ-selinene	$C_{15}H_{24}$	1.37	3.84
37	25.016	Methyl hexadecanoate	$C_{17}H_{34}O_2$	0.53	–
38	25.119	β-guaiene	$C_{15}H_{24}$	0.44	–
39	25.433	1,7,7-trimethyl-2-vinylbicyclo[2.2.1]hept-2-ene	$C_{12}H_{16}$	1.82	–
40	25.691	Ethyl palmitate	$C_{18}H_{36}O_2$	1.05	4.78
41	25.989	Guaia-3,9-diene	$C_{15}H_{24}$	0.68	–
42	27.978	Isolongifolenone	$C_{15}H_{22}O$	0.44	–
43	28.370	7*R*,8*R*-8-hydroxy-4-isopropylidene-7-methylbicyclo[5.3.1]undec-1-ene	$C_{15}H_{24}$	0.67	–
44	28.380	6-isopropenyl-4,8α-dimethyl-1,2,3,5,6,7,8,8α-octahydronaphthalen-2-ol	$C_{15}H_{24}O$	0.65	–
45	30.319	Ethyl-9 *cis*, 11 *trans*-octadecadienoate	$C_{20}H_{36}O_2$	1.23	–
46	33.420	1-(methylpropyl)-4-(1′,1′,2′-trichloro-3′-ethlally)benzene	$C_{15}H_{19}Cl_3$	0.21	–

calarene (3.98%) and [1,2,4]triazolo[1,5-a] pyrimidine-6- carboxylic acid (3.07%).

In 6-year-old ginseng, 21 compounds were detected and 9 components were identified. The relative contents of 8 components were more than 1%. The volatile oil with high-to-low contents were 3-methoxybenzyl alcohol (12.87%), α-gurjunene (8.89%), ethyl palmitate (4.78%), α-patchoulene (4.50%), β-selinene (3.84%), and clovene (3.83%).

4.4.4.11 Analysis of Volatile Oils Results in North Korea Ginsengs

As shown in Table 4.13, 88 components were identified from 4-, 5-, and 6-year-old ginseng from North Korea.

In 4-year-old ginseng, 92 compounds were detected and 43 components were identified. The relative contents of 17 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene, 16.89%, γ-elemene (7.92%), calarene (6.34%), α-gurjunene (6.29%), α-caryophyllene (5.68%),

Table 4.13 The information of volatile oil from North Korea ginseng

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)		
				4-year-old	5-year-old	6-year-old
1	9.214	1-[4-(1,1-dimethylethyl)-2,6-dimethylphenyl]ethanone	$C_{14}H_{20}O$	0.49	0.35	0.32
2	9.565	3- ethenyl-2,5-dimethyl-1,4-hexadiene	$C_{10}H_{16}$	–	0.31	–
3	9.927	Elixene	$C_{15}H_{24}$	–	–	0.03
4	10.151	3,4-dihydro-4,4,6,8-tetramethyl coumarin	$C_{13}H_{16}O_2$	–	0.16	–
5	10.296	Furfural	$C_5H_4O_2$	0.11	–	–
6	10.392	Artemisia triene	$C_{10}H_{16}$	–	–	0.60
7	10.398	α-terpinene	$C_{10}H_{16}$	0.45	–	–
8	10.410	1,5,5-trimethyl-6-methylene cyclohexene	$C_{10}H_{16}$	–	0.44	–
9	10.773	2-(3-isopropyl-4-methyl-pent-3-en-1-ynyl)-2-methyl cyclobutanone	$C_{14}H_{20}O$	–	–	0.24
10	10.791	1*aR*-1*aβ*,2,3,3*a*,4,5,6,7*bβ*-octahydro-1,1,3*aβ*,7-tetramethyl-1*H*-cyclopropa[*a*]naphthalene	$C_{15}H_{24}$	–	0.24	–
11	10.869	β-chamigrene	$C_{15}H_{24}$	–	–	0.30
12	10.876	Cedrene-V6	$C_{15}H_{24}$	0.45	2.02	–
13	10.960	2,10,10-trimethyltricyclo[7.1.1.0(2,7)]undec-6-en-8-one	$C_{14}H_{20}O$	–	–	0.04
14	11.347	2,3,4,5-tetramethyltricyclo[3.2.1.02,7]oct-3-ene	$C_{12}H_{18}$	–	–	1.86
15	11.353	1,2,3,6-tetramethylbicyclo[2.2.2]octa-2,5-diene	$C_{12}H_{18}$	2.12	–	–
16	11.498	β-panasinsene	$C_{15}H_{24}$	5.46	4.74	3.98
17	11.770	1*aR*-1*a*,2,3,3*a*,4,5,6,7*bβ*-Octahydro-1,1,3*aβ*,7-tetramethyl-1*H*-cyclopropa[*a*]naphthalene	$C_{15}H_{24}$	–	–	0.38
18	11.776	α-neoclovene	$C_{15}H_{24}$	0.57	0.72	–
19	12.090	4-(2,7,7-trimethylbicyclo[3.2.0]hept-2-en-1-yl)but-3-en-2-one	$C_{14}H_{22}O$	–	–	0.15
20	12.096	2-methyl-6-allylphenol	$C_{10}H_{12}O$	0.20	–	–
21	12.108	2-cyclopropylidene-1,7,7-trimethyl bicyclo[2.2.1] heptane	$C_{13}H_{20}$	–	0.12	–
22	12.235	1,7-dimethyl-7-(4-methyl-3-pentenyl)-tricyclo[2.2.1.0 (2,6)]heptane	$C_{15}H_{24}$	0.04	–	0.05
23	12.338	(−)-aristolene	$C_{15}H_{24}$	0.56	0.26	0.15
24	12.694	(−)-β-elemene	$C_{15}H_{24}$	4.20	3.03	4.75
25	12.845	Calarene	$C_{15}H_{24}$	6.34	8.13	4.63
26	12.966	l-caryophyllene	$C_{15}H_{24}$	1.28	3.26	1.03
27	12.972	Aromadendrene	$C_{15}H_{24}$	–	1.14	–
28	13.051	Eudesma-3,7(11)-diene	$C_{15}H_{24}$	0.46	–	–
29	13.057	α-panasinsene	$C_{15}H_{24}$	–	0.56	–
30	13.304	α-gurjunene	$C_{15}H_{24}$	6.29	8.32	7.68
31	14.072	β-farnesene	$C_{15}H_{24}$	16.89	14.05	14.66
32	14.295	Furfuryl alcohol	$C_5H_6O_2$	0.10	–	–
33	14.537	α-caryophyllene	$C_{15}H_{24}$	5.68	5.18	4.83
34	14.676	4,11,11-trimethyl-8-methylene bicyclo[7.2.0]undec-4-ene	$C_{15}H_{24}$	3.49	–	3.06
35	14.833	6-isopropyl-4,8α-dimethyl-1,2,3,7,8,8α-hexahydro naphthalene	$C_{15}H_{24}$	–	–	0.09
36	15.008	Viridiflorene	$C_{15}H_{24}$	0.71	0.85	0.77
37	15.177	β-neoclovene	$C_{15}H_{24}$	1.42	1.22	1.15

(continued)

Table 4.13 (continued)

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)		
				4-year-old	5-year-old	6-year-old
38	15.365	(3*aS*,3*βR*,4*S*,7*R*,7*aR*)-7-methyl-3-methylidene-4-(propan-2-yl)octahydro-1*H*-cyclopenta[1,3]cyclopropa[1,2]benzene	$C_{15}H_{24}$	0.32	–	0.29
39	15.383	Germacrene D	$C_{15}H_{24}$	–	0.28	–
40	15.480	*cis*-*α*-bisabolene	$C_{15}H_{24}$	–	0.13	0.19
41	15.600	*β*-eudesmene	$C_{15}H_{24}$	0.62	–	0.60
42	15.613	10*S*,11*S*-himachala-3(12),4-diene	$C_{15}H_{24}$	–	0.57	–
43	15.703	(−)-*α*-selinene	$C_{15}H_{24}$	0.65	–	0.63
44	15.715	2-isopropenyl-4*α*,8-dimethyl-1,2,3,4,4*α*,5,6,8*a*-octahydro-naphthalene	$C_{15}H_{24}$	–	0.54	–
45	15.890	*γ*-elemene	$C_{15}H_{24}$	7.92	12.81	11.29
46	16.084	6,9-heptadecadiene	$C_{17}H_{32}$	–	–	0.09
47	16.096	Cyclodecene	$C_{10}H_{18}$	0.18	–	–
48	16.192	*δ*-cadinene	$C_{15}H_{24}$	0.40	0.46	0.47
49	16.331	2-isopropyl-5-methyl-9-methylene[4.4.0]dec-1-ene	$C_{15}H_{24}$	–	–	0.06
50	16.519	Trimethyl cyclododecatriene	$C_{15}H_{24}$	–	–	0.13
51	17.715	(2*E*,4*E*)-2,4-octadienal	$C_8H_{12}O$	0.06	–	–
52	19.340	2,6-di-tert-butyl-4-methylphenol	$C_{15}H_{24}O$	2.61	1.91	2.32
53	21.860	Peruviol	$C_{15}H_{26}O$	0.28	0.20	0.29
54	22.398	3,4-dimethyl-3-cyclohexen-1-fomaldehyde	$C_9H_{14}O$	–	–	1.43
55	22.416	1-(2-hydroxy-6-methoxyphenyl)-ethanone,	$C_9H_{10}O_3$	–	1.15	–
56	22.579	Pentachlorobenzene	C_6HCl_5	–	1.01	–
57	22.941	(−)-globulol	$C_{15}H_{26}O$	0.60	–	0.79
58	22.954	*β*-eudesmene	$C_{15}H_{24}$	–	1.03	–
59	23.062	Ledol	$C_{15}H_{26}O$	–	–	0.59
60	23.068	Longifolene	$C_{15}H_{24}$	0.40	–	–
61	23.448	*cis*-1,4-dimethyladamantane	$C_{12}H_{20}$	0.10	–	–
62	23.449	*o*-Isopropylphenetole	$C_{11}H_{16}O$	–	–	0.14
63	23.836	Spathulenol	$C_{15}H_{24}O$	1.85	1.66	3.14
64	24.047	*α*-patchoulene	$C_{15}H_{24}$	1.44	–	1.38
65	24.162	*α*-bulnesene	$C_{15}H_{24}$	–	–	0.31
66	24.174	1,7-dimethyl-4-(1-methylethyl) spiro[4.5]dec-6-en-8-one	$C_{15}H_{24}O$	0.25	–	–
67	24.186	*N*-(1-cyanoethyl)(7,7-dimethyl-2-oxobicyclo [2.2.1] hept-1-ylmethanesul	$C_{13}H_{20}N_2O_3S$	–	0.17	–
68	24.349	5-(1,3,5-trimethyl-4-pyrazolyl)amino-1,2,4-triazol-3-amine	$C_8H_{13}N_7$	–	–	1.37
69	24.355	Ginsenol	$C_{15}H_{26}O$	1.21	1.30	–
70	24.615	(−)-*γ*-cadinene	$C_{15}H_{24}$	0.04	–	–
71	25.213	2-methoxy-4-vinylphenol	$C_9H_{10}O_2$	0.12	–	0.12
72	25.660	1,7,7-trimethyl-2-vinylbicyclo[2.2.1]hept-2-ene	$C_{12}H_{18}$	–	–	0.87
73	26.313	1-(3,3-dimethyl-but-1-ynyl)-1,2-dimethyl-3-methylene-cyclopropane	$C_{12}H_{18}$	–	–	1.17
74	26.996	Hexachlorobenzene	C_6Cl_6	–	0.78	–
75	28.645	7*R*,8*R*-8-hydroxy-4-isopropylidene-7-methylbicyclo [5.3.1]undec-1-ene	$C_{15}H_{24}O$	–	–	0.11
76	30.010	Methyl linoleate	$C_{19}H_{34}O_2$	0.09	–	0.07

(continued)

Table 4.13 (continued)

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)		
				4-year-old	5-year-old	6-year-old
77	30.101	Quintozine	$C_6Cl_5NO_2$	–	0.59	–
78	32.028	15-heptadecenal	$C_{17}H_{32}O$	–	–	0.18
79	32.029	Cyclotetradecane	$C_{14}H_{28}$	0.16	–	–
80	33.255	Myristic acid	$C_{14}H_{28}O_2$	–	–	0.08
81	34.167	1,2,3,4,5-pentachloro-6-(methylthio)-benzene	$C_7H_3Cl_5S$	–	1.25	–
82	35.327	Pentadecylic acid	$C_{15}H_{30}O_2$	–	–	0.32
83	37.792	Palmitic acid	$C_{16}H_{32}O_2$	3.12	2.67	4.22
84	39.116	15-crown-5	$C_{10}H_{20}O_5$	–	–	0.49
85	39.212	Pentachloroaniline	$C_6H_2Cl_5N$	–	1.95	–
86	42.167	Falcarinol	$C_{17}H_{24}O$	4.49	4.29	5.69
87	45.961	Tetraethylene glycol monoethyl ether	$C_{10}H_{22}O_5$	–	–	0.36
88	48.547	Linoleic acid	$C_{18}H_{32}O_2$	–	–	1.72

β-panasinsene (5.46%), falcarinol (4.49%), β-elemene (4.20%), and palmitic acid (3.12%).

In 5-year-old ginseng, 62 compounds were detected and 40 components were identified. The relative contents of 21 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene (14.05%), γ-elemene (12.81%), α-gurjunene (8.32%), calarene (8.13%), α-caryophyllene (5.18%), β-panasinsene (4.74%), falcarinol (4.29%), l-caryophyllene (3.26%), β-elemene (3.03%), and palmitic acid (2.67%).

In 6-year-old ginseng, 102 compounds were detected and 54 components were identified. The relative contents of 20 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene (14.66%), γ-elemene (11.29%) α-gurjunene (7.68%), falcarinol (5.69%), α-caryophyllene (4.83%), β-elemene (4.75%), calarene (4.63%), palmitic acid (4.22%), β-panasinsene (3.98%), and espatulenol (3.14%).

4.4.4.12 Analysis of Volatile Oils Results in Helong Ginseng

As shown in Table 4.14, 60 components were identified from 4- and 5-year-old ginseng from Helong.

In 4-year-old ginseng, 49 compounds were detected and 32 components were identified. The relative contents of 18 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene (13.72%), α-gurjunene (10.59%), γ-elemene (9.96%), calarene (7.47%), palmitic acid (6.42%), α-caryophyllene (5.15%), β-panasinsene (4.76) %), falcarinol (4.32%), and β-elemene (3.33%).

In 5-year-old ginseng, 84 compounds were detected and 47 components were identified. The relative contents of 17 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene (15.24%), calarene (7.94%), palmic acid (6.86%), espatulenol (6.45%), falcarinol (6.39%), α-gurjunene (5.92%), α-caryophyllene (5.60%), β-panasinsene (4.73%), and β-caryophyllene (3.06%).

4.4.4.13 Analysis of Volatile Oils Results in Dunhua Ginseng

As shown in Table 4.15, 61 components were identified from 4- and 5-year-old ginseng from Dunhua.

In 4-year-old ginseng, 60 compounds were detected and 41 components were identified. The relative contents of 23 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene (11.45%), espatulenol (6.14%), falcarinol (6.13%), palmitic acid (5.47%), calarene (5.21%), β-eudesmene

Table 4.14 The information of volatile oil from Helong ginsengs

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)	
				4-year-old	5-year-old
1	9.231	1-[4-(1,1-dimethylethyl)-2,6-dimethylphenyl]-ethanone	$C_{14}H_{20}O$	–	0.31
2	9.243	3,5-di-*tert*-butyltoluene	$C_{15}H_{24}$	0.33	–
3	9.564	4,4,6,6-tetramethyl-bicyclo[3.1.0]hex-2-ene	$C_{10}H_{16}$	–	0.25
4	10.144	9-aristolene	$C_{15}H_{24}$	–	0.14
5	10.313	3-furaldehyde	$C_5H_4O_2$	0.15	–
6	10.403	1*R*-2,2-dimethyl-3-methylenebicyclo[2.2.1]heptane	$C_{10}H_{16}$	–	0.05
7	10.422	2,5,6-trimethyl-1,3,6-heptatriene	$C_{10}H_{16}$	0.59	–
8	10.802	α-gurjunene	$C_{15}H_{24}$	0.40	–
9	10.881	3-(1,1-dimethylethyl)-α-methyl-benzenepropanal	$C_{14}H_{20}O$	–	0.31
10	10.899	3-cyano-4-(2,2-dimethyl-ethenyl)-6,6-dimethyl-3,4-dehydro-2-piperidon	$C_8H_8N_2O$	0.28	–
11	11.352	1,3-bis(1-methylethyl)-benzene	$C_{12}H_{18}$	2.21	1.77
12	11.503	β-panasinsene	$C_{15}H_{24}$	4.76	4.73
13	11.781	[*S*,(−)]-2,3,4,4α,5,6-hexahydro-1,4α-dimethyl-7-(1-methylethyl)-naphthalene	$C_{15}H_{24}$	–	0.54
14	11.799	α-neoclovene	$C_{15}H_{24}$	0.43	–
15	12.095	2-allyl-4-methylphenol	$C_{10}H_{12}O$	–	0.14
16	12.119	*n*-methyl-2-benzoxazolamine	$C_8H_8N_2O$	0.11	–
17	12.240	1,7-dimethyl-7-(4-methyl-3-pentenyl)-tricyclo[2.2.1.0(2,6)]heptane	$C_{15}H_{24}$	–	0.07
18	12.343	(−)-aristolene	$C_{15}H_{24}$	0.22	0.25
19	12.693	β-elemene	$C_{15}H_{24}$	3.33	2.71
20	12.844	Calarene	$C_{15}H_{24}$	7.47	7.94
21	12.965	Aromadendrene	$C_{15}H_{24}$	1.04	1.16
22	13.062	Eudesma-3,7(11)-diene	$C_{15}H_{24}$	0.46	–
23	13.298	α-gurjunene	$C_{15}H_{24}$	10.59	5.92
24	14.053	β-farnesene	$C_{15}H_{24}$	13.72	15.24
25	14.530	α-caryophyllene	$C_{15}H_{24}$	5.15	5.60
26	14.663	β-caryophyllene	$C_{15}H_{24}$	2.81	3.06
27	15.001	Viridiflorene	$C_{15}H_{24}$	0.78	0.44
28	15.171	β-neoclovene	$C_{15}H_{24}$	1.13	1.41
29	15.467	*cis*-α-bisabolene	$C_{15}H_{24}$	–	0.13
30	15.593	β-eudesmene	$C_{15}H_{24}$	1.47	0.64
31	15.696	Selina-3,11-diene	$C_{15}H_{24}$	–	0.64
32	15.708	γ-selinene	$C_{15}H_{24}$	0.43	–
33	15.865	γ-elemene	$C_{15}H_{24}$	9.96	1.78
34	16.083	*Z*, *Z*-10,12-hexadecadienal	$C_{16}H_{28}O_2$	–	0.10
35	16.180	δ-cadinene	$C_{15}H_{24}$	0.39	0.36
36	19.315	2,6-di-tert-butyl-4-methylphenol	$C_{15}H_{24}O$	–	0.15
37	21.817	*cis*-nerolidol	$C_{15}H_{26}O$	–	0.30
38	22.898	(−)-globulol	$C_{15}H_{26}O$	–	0.76
39	23.013	Longifolene	$C_{15}H_{24}$	–	0.55
40	23.019	(−)-isoaromadendrene-(V)	$C_{15}H_{24}$	0.65	–
41	23.786	Spathulenol	$C_{15}H_{24}O$	–	6.45
42	23.998	α-patchoulene	$C_{15}H_{26}O$	1.38	1.78
43	24.300		$C_{15}H_{26}O$	1.32	1.96

(continued)

Table 4.14 (continued)

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)	
				4-year-old	5-year-old
		1,4-ethano-3*aH*-inden-3*a*-ol,octahydro-2,2,4,7*a*-tetramethyl-, (1*S*,3*αR*,4*R*,7*αS*)-			
44	25.291	*β*-humulene (6CI,7CI)	$C_{15}H_{24}$	–	0.07
45	26.868	6-methylenespiro[4.5]decane	$C_{11}H_{18}$	–	0.12
46	27.762	Phosphoric acid trioctyl ester	$C_{24}H_{51}O_4P$	–	0.13
47	29.587	*E*-3-methylpent-2-en-4-yn-1-ol	C_6H_8O	–	0.18
48	30.578	1,6,10-dodecatrien-3-ol,3,7,11-trimethyl-,[*S*-(*Z*)]-	$C_{15}H_{26}O$	–	0.06
49	30.928	Diisobutyl phthalate	$C_{16}H_{22}O_4$	–	0.15
50	31.931	Cyclotetradecane	$C_{14}H_{28}$	0.19	–
51	31.937	Cyclohexadecane	$C_{16}H_{32}$	–	0.28
52	32.118	3-methyl-1,1-diphenyl-urea(6CI,7CI,8CI)	$C_{14}H_{14}N_2O$	1.14	–
53	32.148	Diphenylamine	$C_{12}H_{11}N$	–	0.93
54	33.127	Tetradecanoic acid	$C_{14}H_{28}O_2$	–	0.11
55	37.623	Palmitic acid	$C_{16}H_{32}O_2$	6.42	6.86
56	41.955	Falcarinol	$C_{17}H_{24}O$	4.32	6.39
57	45.676	3,6,9,12,15,18-Hexaoxatriacontan-1-ol	$C_{24}H_{50}O_7$	–	0.48
58	47.253	Dicyclohexyl phthalate	$C_{20}H_{26}O_4$	–	0.85
59	48.214	Linoleic acid	$C_{18}H_{32}O_2$	–	2.59
60	48.274	3,6,9,12-tetraoxatetradecan-1-ol	$C_{10}H_{22}O_5$	2.51	–

Table 4.15 The information of volatile oil from Dunhua ginseng

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)	
				4-year-old	5-year-old
1	9.245	1-[4-(1,1-dimethylethyl)-2,6-dimethylphenyl]ethanone	$C_{14}H_{20}O$	0.17	–
2	9.255	3,5-di-*tert*-butyltoluene	$C_{15}H_{24}$	–	0.21
3	10.428	2,5,6-trimethyl-1,3,6-heptatriene	$C_{10}H_{16}$	–	0.24
4	10.901	1,3,5-triisopropylbenzene	$C_{15}H_{24}$	0.16	0.20
5	11.366	2,3,4,5-tetramethyltricyclo[3.2.1.02,7]oct-3-ene	$C_{12}H_{18}$	1.23	–
6	11.376	1,2,3,6-tetramethylbicyclo[2.2.2]octa-2,5-diene	$C_{12}H_{18}$	–	1.56
7	11.517	*β*-panasinsene	$C_{15}H_{24}$	3.18	3.74
8	11.795	6-isopropyl-4,8*α*-dimethyl-1,2,3,7,8,8*α*-hexahydro naphthalene	$C_{15}H_{24}$	0.27	–
9	11.805	*α*-neoclovene	$C_{15}H_{24}$	–	0.37
10	12.363	9-aristolene	$C_{15}H_{24}$	0.12	0.16
11	12.373	(−)-aristolene	$C_{15}H_{24}$	–	0.18
12	12.701	*β*-elemene	$C_{15}H_{24}$	1.88	0.14
13	12.711	1-methyl-2,4-di(prop-1-en-2-yl)-1-vinylcyclohexane	$C_{15}H_{24}$	–	3.23
14	12.852	Calarene	$C_{15}H_{24}$	5.21	6.66
15	12.979	l-caryophyllene	$C_{15}H_{24}$	0.76	–
16	12.983	Aromadendrene	$C_{15}H_{24}$	–	0.97
17	13.068	Eudesma-3,7(11)-diene	$C_{15}H_{24}$	–	0.41
18	13.305	*β*-eudesmene	$C_{15}H_{24}$	4.85	1.39
19	13.316	*α*-gurjunene	$C_{15}H_{24}$	0.13	7.47

(continued)

Table 4.15 (continued)

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)	
				4-year-old	5-year-old
20	14.061	*β*-farnesene	$C_{15}H_{24}$	11.45	14.42
21	14.538	*α*-caryophyllene	$C_{15}H_{24}$	4.17	5.52
22	14.671	l-caryophyllene	$C_{15}H_{24}$	2.15	3.20
23	15.015	Viridiflorene	$C_{15}H_{24}$	0.48	0.68
24	15.185	*β*-neoclovene	$C_{15}H_{24}$	0.91	1.14
25	15.704	(−)-*α*-selinene	$C_{15}H_{24}$	0.41	–
26	15.708	2-isopropenyl-4*α*,8-dimethyl-1,2,3,4,4*α*,5,6,8*α*-octahydronaphthalene	$C_{15}H_{24}$	–	0.59
27	15.873	Germacrene B	$C_{15}H_{24}$	1.91	–
28	15.878	*γ*-elemene	$C_{15}H_{24}$	–	5.58
29	16.187	(−)-*β*-cadinene	$C_{15}H_{24}$	0.27	–
30	16.192	*δ*-cadinene	$C_{15}H_{24}$	–	0.43
31	19.317	2,6-di-*tert*-butyl-4-methylphenol	$C_{15}H_{24}O$	0.22	–
32	20.193	2,3-dihydro-4-phenylazete	C_9H_9N	0.17	–
33	21.817	l-*β*-bisabolene	$C_{15}H_{24}$	–	0.29
34	22.892	(−)-globulol	$C_{15}H_{26}O$	–	1.17
35	22.894	*δ*-longifolene	$C_{15}H_{24}$	1.24	–
36	23.782	Espatulenol	$C_{15}H_{24}O$	6.14	8.63
37	24.302	Ginsenol	$C_{15}H_{26}O$	1.20	2.12
38	24.815	2,6,10,14-tetramethyl octadecane	$C_{22}H_{46}$	0.26	–
39	26.574	Tricosane	$C_{23}H_{48}$	0.82	–
40	27.267	Di(cyclopentanonyl-2)methane	$C_{11}H_{16}O_2$	–	0.13
41	27.744	Cyclopentanone ethylene ketal	$C_7H_{12}O_2$	–	0.12
42	28.271	Tetracosane	$C_{24}H_{50}$	1.85	–
43	28.584	6-isopropenyl-4,8*α*-dimethyl-1,2,3,5,6,7,8,8*α*-octahydro-naphthalen-2-ol	$C_{15}H_{24}O$	–	0.19
44	29.903	Heneicosane	$C_{18}H_{38}$	3.14	–
45	30.898	Diisobutyl phthalate	$C_{16}H_{22}O_4$	–	0.20
46	31.492	Hexacosane	$C_{26}H_{54}$	3.70	–
47	31.611	7*β*-ethyl-8*β*-hydroxy-2,6-dimethylbic-yclo[4.4.0]dec-1-ene	$C_{14}H_{24}O$	–	0.21
48	31.919	5-octadecene	$C_{18}H_{36}$	–	0.27
49	31.927	Octadecyl trifluoroacetate	$C_{20}H_{37}F_3O_2$	0.27	–
50	32.120	Diphenylamine	$C_{12}H_{11}N$	0.92	1.45
51	32.652	Hexadecane	$C_{16}H_{34}$	0.15	–
52	33.208	Heptacosane	$C_{27}H_{56}$	3.94	–
53	34.199	7-hexylicosane	$C_{26}H_{54}$	0.40	–
54	35.183	Octadecane	$C_{18}H_{38}$	3.59	–
55	37.498	Docosane	$C_{22}H_{46}$	2.47	–
56	37.618	Palmitic acid	$C_{16}H_{32}O_2$	5.47	5.77
57	40.295	Triacontane	$C_{30}H_{62}$	2.02	–
58	41.896	Falcarinol	$C_{17}H_{24}O$	6.13	–
59	43.739	Heptadecane	$C_{17}H_{36}$	1.81	–
60	48.208	Linoleic acid	$C_{18}H_{32}O_2$	–	1.63
61	53.472	Octaethylene glycol monododecyl ether	$C_{28}H_{58}O_9$	1.33	–

(4.85%), α-caryophyllene (4.17%), heptacosane (3.94%), hexacosane (3.70%), octadecane (3.59%), β-panasinsene (3.18%), and heneicosane (3.14%).

In 5-year-old ginseng, 63 compounds were detected and 36 components were identified. The relative contents of 17 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene (14.42%), β-panasinsene (3.74%), 1-methyl-2,4-di (prop-1-en-2-yl)-1-vinylcyclohexane (3.23%), calarene (6.66%), α-gurjunene (7.47%), α-caryophyllene (5.52%), l-caryophyllene (3.20%), γ-elemene (5.58%), espatulenol (8.63%), and palmitic acid (5.77%).

4.4.4.14 Analysis of Volatile Oils Results in Antu Ginsengs

As shown in Table 4.16, 74 components were identified from 4- and 5-year-old ginseng from Antu.

In 4-year-old ginseng, 41 compounds were detected and 33 components were identified. The relative contents of 18 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene (14.82%), α-gurjunene (9.44%), calarene (6.93%), germacrene B (5.91%), espatulenol (5.89%), palmitic acid (4.86%), α-caryophyllene (4.77%), 2,6-di-tert-butyl-4-methylphenol (4.29%), β-panasinsene (3.93%), and β-elemene (3.03%).

In 5-year-old ginseng, 113 compounds were detected and 36 components were identified. The relative contents of 20 components were more than 1%. The volatile oil with high-to-low contents were palmitic acid (22.66%), followed by β-farnesene (8.28%), linoleic acid (5.89%), calarene (3.79%), α-gurjunene (3.36%), β-panasinsene (3.27%), α-caryophyllene (2.90%), espatulenol (2.74%), ginsenol (2.73%), 9,12-octadecadienoic acid (Z,Z)-ethyl ester (2.38%), and falcarinol (2.03%).

Table 4.16 The information of volatile oil from Antu ginseng

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)	
				4-year-old	5-year-old
1	9.247	1-[4-(1,1-dimethylethyl)-2,6-dimethylphenyl]ethanone	$C_{14}H_{20}O$	–	0.19
2	10.425	3-methyl-6-(1-methylethylidene)cyclohexene	$C_{10}H_{16}$	0.29	–
3	10.896	3-(1,1-dimethylethyl)-α-methylbenzenepropanal	$C_{14}H_{20}O$	–	0.21
4	11.373	2,3,4,5-tetramethyl tricyclo[3.2.1.02,7]oct-3- ene	$C_{12}H_{18}$	–	1.17
5	11.374	5-hydroxy-3-methyl-1-indanone	$C_{10}H_{10}O_2$	1.73	–
6	11.525	β-panasinsene	$C_{15}H_{24}$	3.93	3.27
7	11.802	3*R*,4α*S*,5*R*,8α*S*-5,8α-dimethyl-3-(propan-2-yl)-1,2,3,4,4α,5,6,8α-octahydro naphthalene	$C_{15}H_{26}$	0.26	0.18
8	12.117	4-isopropylbenzaldehyde	$C_{10}H_{12}O$	–	0.10
9	12.370	(−)-aristolene	$C_{15}H_{24}$	–	0.12
10	12.709	(−)-β-elemene	$C_{15}H_{24}$	3.03	1.34
11	12.860	Calarene	$C_{15}H_{24}$	6.93	3.79
12	12.987	4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinylcycloheptane	$C_{15}H_{24}$	0.76	–
13	13.071	Eudesma-3,7(11)-diene	$C_{15}H_{24}$	0.41	–
14	13.319	α-gurjunene	$C_{15}H_{24}$	9.44	3.36
15	14.062	β-farnesene	$C_{15}H_{24}$	14.82	8.28
16	14.545	α-caryophyllene	$C_{15}H_{24}$	4.77	2.90
17	14.678	z-caryophyllene	$C_{15}H_{24}$	2.00	0.85
18	15.017	(+)-ledene	$C_{15}H_{24}$	0.69	0.25
19	15.192	β-neoclovene	$C_{15}H_{24}$	1.05	1.06

(continued)

Table 4.16 (continued)

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)	
				4-year-old	5-year-old
20	15.325	Seychellene	$C_{15}H_{24}$	–	0.06
21	15.615	(+)-aromadendrene	$C_{15}H_{24}$	0.48	0.43
22	15.706	1,2,3,4,4α,5,6,8α-octahydro-4α,8-dimethyl-2-(1-methylethenyl) naphthalene	$C_{15}H_{24}$	0.39	0.36
23	15.881	3,7,11,11-tetramethyl bicyclo[8.1.0]2,6-undecadiene	$C_{15}H_{24}$	–	0.28
24	15.882	Germacrene B	$C_{15}H_{24}$	5.91	–
25	16.195	(−)-β-cadinene	$C_{15}H_{24}$	0.32	0.11
26	17.234	5,6-dimethyltetralin	$C_{12}H_{16}$	–	0.11
27	17.687	2*E*, 4*E*-2,4-octadienal	$C_8H_{12}O$	–	0.14
28	17.700	2, 4-decadienal	$C_{10}H_{16}O$	0.40	–
29	19.325	2,6-di-*tert*-butyl-4-methylphenol	$C_{15}H_{24}O$	4.29	2.04
30	20.195	Hydrazinecarboxamide,2-(phenylmethylene)-	$C_8H_9N_3O$	–	0.17
31	20.201	2,3-dihydro-4-phenylazete	C_9H_9N	2.54	–
32	21.270	1,5-dimethyl-6-methylenespiro[2.4]heptane	$C_{10}H_{16}$	–	0.12
33	21.826	*cis*-nerolidol	$C_{15}H_{26}O$	–	0.38
34	22.515	1,2,3,4,5-pentachloro benzene	C_6HCl_5	–	0.25
35	22.902	(−)-globulol	$C_{15}H_{26}O$	–	0.92
36	22.908	α-bulnesene	$C_{15}H_{24}$	1.72	–
37	23.016	Ledol	$C_{15}H_{26}O$	–	0.65
38	23.029	β-eudesmene	$C_{15}H_{24}$	0.98	–
39	23.409	1-(1,1-di-methylethyl)-4-methoxy benzene	$C_{11}H_{16}O$	–	0.11
40	23.790	Spathulenol	$C_{15}H_{24}O$	5.89	2.74
41	24.001	α-patchoulene	$C_{15}H_{24}$	1.86	1.89
42	24.309	Ginsenol	$C_{15}H_{26}O$	–	2.73
43	24.799	Docosane	$C_{22}H_{46}$	–	0.17
44	25.149	Methyl hexadecanoate	$C_{17}H_{34}O_2$	0.65	0.80
45	25.705	α-cadinol	$C_{15}H_{26}O$	0.46	–
46	25.814	Ethyl palmitate	$C_{18}H_{36}O_2$	0.54	1.64
47	26.152	α-patchoulene	$C_{15}H_{24}$	–	0.18
48	26.563	Hexadecane	$C_{16}H_{34}$	–	0.37
49	26.901	Hexachlorobenzene	C_6Cl_6	–	0.24
50	27.379	Patchoulane(7CI)	$C_{15}H_{26}$	0.91	–
51	28.267	Octadecane	$C_{18}H_{38}$	–	0.42
52	29.656	9*E*-9-octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	–	0.40
53	29.789	β-cedren-9-α-ol	$C_{15}H_{24}O$	–	0.17
54	29.910	Methyl linoleate	$C_{19}H_{34}O_2$	1.02	1.54
55	30.460	Ethyl linoleate	$C_{20}H_{36}O_2$	1.79	2.38
56	30.913	Diisobutyl phthalate	$C_{16}H_{22}O_4$	–	0.14
57	31.493	Hexacosane	$C_{26}H_{54}$	–	0.74
58	31.940	5-octadecene	$C_{18}H_{36}$	–	0.35
59	32.128	Diphenylamine	$C_{12}H_{11}N$	0.55	0.51
60	32.653	Heptadecyl cyclohexane	$C_{23}H_{46}$	–	0.14
61	33.088	Myristic acid	$C_{14}H_{28}O_2$	–	0.30
62	33.203	Heptacosane	$C_{27}H_{56}$	–	0.78
63	34.019	1,2,3,4,5-penta-chloro-6-(methylthio)benzene	$C_7H_3Cl_5S$	–	0.31

(continued)

Table 4.16 (continued)

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)	
				4-year-old	5-year-old
64	35.167	Eicosane	$C_{20}H_{42}$	–	1.53
65	36.997	2,6-di-*tert*-butyl-hydroquinone	$C_{14}H_{22}O_2$	–	0.40
66	37.626	Palmitic acid	$C_{16}H_{32}O_2$	4.86	22.66
67	38.605	*cis*-9-hexadecenoic acid	$C_{16}H_{30}O_2$	–	0.55
68	38.840	9-hexadecenoic acid	$C_{16}H_{30}O_2$	–	0.79
69	38.997	Pentachloroaniline	$C_6H_2Cl_5N$	–	0.62
70	40.314	Docosane	$C_{22}H_{46}$	–	0.61
71	41.940	(+)-falcarinol	$C_{17}H_{24}O$	–	2.03
72	43.770	Eicosane	$C_{20}H_{42}$	–	0.65
73	45.625	Oleic acid	$C_{18}H_{34}O_2$	–	0.67
74	48.169	Linoleic acid	$C_{18}H_{32}O_2$	–	5.89

4.4.4.15 Analysis of Volatile Oils Results from 6-Year-Old Ginseng in Linjiang, 4-Year-Old Ginseng in Jingyu, 4- and 5-Year-Old Ginseng in Tonghua

As shown in Table 4.17, 59 components were identified from 6-year-old ginseng from Linjiang, 4-year-old ginseng from Jingyu, 4- and 5-year-old ginseng from Tonghua.

In 6-year-old ginseng from Linjiang, 69 compounds were detected and 42 components were identified. The relative contents of 18 components were more than 1%. The volatile oil with high-to-low contents were γ-elemene (15.74%), β-farnesene (12.65%), α-gurjunene (10.90%), calarene (8.68%), β-panasinsene (5.34%), α-caryophyllene (4.98%), β-elemene (3.31%), palmic acid (3.06%), (−)-isocaryophyllene (3.04%), and falcarinol (2.70%).

In 4-year-old ginseng from Jingyu, 59 compounds were detected and 38 components were identified. The relative contents of 18 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene (14.60%), β-Eudesmene (10.43%), γ-elemene (9.00%), calarene (6.97%), α-caryophyllene (5.69%), β-panasinsene (4.55%), espatulenol (4.44%), palmitic acid (4.05%), β-elemene (3.94%), falcarinol (3.89%), and l-caryophyllene (3.19%).

In 4- and 5-year-old ginseng from Tonghua, 44 compounds were detected and 22 components were identified. The relative contents of 15 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene (20.09%), a-caryophyllene (7.52%), γ-elemene (6.82%), falcarinol (6.22%), α-gurjunene (6.25%), β-elemene (4.69%), β-panasinsene (4.52%), l-caryophyllene (4.36%), palmitic acid (3.97%), calarene (3.57%), espatulenol (3.56%), 2,6-di-tert-butyl-4-methylphenol (3.37%), and ginsenol (2.82%).

4.4.4.16 Analysis of Volatile Oils Results from 4-Year-Old Ginseng in Xinbin and 6-Year-Old Ginseng in Fusong

As shown in Table 4.18, 72 components were identified from 4-year-old ginseng from Xinbin and 6-year-old ginseng from Fusong.

In 4-year-old ginseng from Xinbin, 92 compounds were detected and 54 components were identified. The relative contents of 16 components were more than 1%. The volatile oil with high-to-low contents were β-farnesene (15.70%), γ-elemene (11.00%), α-gurjunene (8.41%), calarene (6.71%), α-caryophyllene (4.60%), β-panasinsene (4.17%), β-elemene (3.89%), palmic acid (3.03%), and espatulenol (2.77%).

Table 4.17 The information of volatile oil from Linjiang, Jingyu, and Tonghua ginseng

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)		
				Linjiang 6-year-old	Jingyu 4-year-old	Tonghua 4-and5-year-old
1	9.226	1-[4-(1,1-dimethylethyl)-2,6-dimethylphenyl] ethanone	$C_{14}H_{20}O$	0.34	0.30	0.28
2	9.558	4,4,6,6-tetramethyl bicyclo[3.1.0] hex-2-ene	$C_{10}H_{16}$	0.45	–	–
3	10.302	Furfural	$C_5H_4O_2$	0.21	–	–
4	10.404	Artemisia triene	$C_{10}H_{16}$	0.91	–	–
5	10.415	1,5,5-trimethyl-6-methylenecyclohexene	$C_{12}H_{12}O_3$	–	–	0.26
6	10.416	6,6-dimethyl-3-methyl-enebicyclo[3.1.1]heptane	$C_{10}H_{16}$	–	0.54	–
7	10.785	γ-maaliene	$C_{15}H_{24}$	0.22	–	–
8	10.882	3-(1,1-dimethylethyl)-α-methyl-Benzenepropanal	$C_{14}H_{20}O$	0.28	–	–
9	10.894	Cedrene-V6	$C_{15}H_{24}$	–	0.22	–
10	11.359	1,2,3,6-tetramethylbicy-clo[2.2.2]octa-2,5-diene	$C_{12}H_{18}$	2.15	–	–
11	11.371	2,3,4,5-tetramethyltricy-clo[3.2.1.02,7]oct-3-ene	$C_{12}H_{18}$	–	1.97	1.77
12	11.504	β-panasinsene	$C_{15}H_{24}$	5.34	4.55	4.52
13	11.782	δ-selinene	$C_{15}H_{24}$	0.64	–	–
14	11.794	α-neoclovene	$C_{15}H_{24}$	–	0.42	–
15	12.114	1,2-dihydro-4-methoxy-methyl-6-methyl-2-oxo-3-pyridinecarbonitrile	$C_9H_{12}N_2O_3$	–	0.12	–
16	12.247	1,7-dimethyl-7-(4-meth-yl-3-pentenyl)-tricyclo [2.2.1.0(2,6)]heptane	$C_{15}H_{24}$	0.08	–	–
17	12.350	(−)-9-aristolene	$C_{15}H_{24}$	0.30	0.39	–
18	12.452	1,5,9-trimethyl-1,5,9-cyclododecatriene	$C_{15}H_{24}$	–	0.18	–
19	12.700	(−)-β-elemene	$C_{15}H_{24}$	3.31	3.94	4.69
20	12.851	Calarene	$C_{15}H_{24}$	8.68	6.97	3.57
21	12.972	γ-neoclovene	$C_{15}H_{24}$	1.10	–	–
22	12.978	(−)-alloaromadendrene	$C_{15}H_{24}$	–	–	2.02
23	13.069	Eudesma-3,7(11)-diene	$C_{15}H_{24}$	–	0.50	–
24	13.310	α-gurjunene	$C_{15}H_{24}$	10.90	0.13	6.25
25	14.060	β-farnesene	$C_{15}H_{24}$	12.65	14.60	20.09
26	14.313	Furfuryl alcohol	$C_5H_6O_2$	0.05	–	–
27	14.543	α-caryophyllene	$C_{15}H_{24}$	4.98	5.69	7.52
28	14.676	(−)-isocaryophyllene	$C_{15}H_{24}$	3.04	1.09	–
29	14.688	l-caryophyllene	$C_{15}H_{24}$	–	3.19	4.36
30	15.020	Viridiflorene	$C_{15}H_{24}$	1.07	0.88	–
31	15.183	β-neoclovene	$C_{15}H_{24}$	1.14	1.32	0.18
32	15.377	(3*aS*,3*bR*,4*S*,7*R*,7*aR*)-7-methyl-3-methylidene-4-(propan-2-yl)octahydro-1*H*-cyclopenta[1,3] cyclopropa[1,2]benzene	$C_{15}H_{24}$	0.24	–	–
33	15.389	(−)-β-cadinene	$C_{15}H_{24}$	–	0.19	–
34	15.498	*cis*-α-bisabolene	$C_{15}H_{24}$	–	0.11	–
35	15.612	β-eudesmene	$C_{15}H_{24}$	0.63	10.43	0.60
36	15.709	(−)-α-selinene	$C_{15}H_{24}$	0.52	–	0.78
37	15.721	α-bulnesene	$C_{15}H_{24}$	–	0.64	–
38	15.890	γ-elemene	$C_{15}H_{24}$	15.74	9.00	6.82
39	16.102	9-methyl bicyclo[3.3.1]nonane	$C_{10}H_{18}$	0.11	–	–
40	16.199	δ-cadinene	$C_{15}H_{24}$	0.49	0.44	0.33
41	17.727	*trans,trans*-2,4-Decadien-1-al	$C_{10}H_{16}O$	0.05	–	–

(continued)

Table 4.17 (continued)

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)		
				Linjiang 6-year-old	Jingyu 4-year-old	Tonghua 4-and5-year-old
42	19.352	2,6-di-*tert*-butyl-4-methylphenol	$C_{15}H_{24}O$	2.08	2.03	3.37
43	21.872	*cis*-nerolidol	$C_{15}H_{26}O$	0.24	0.32	0.48
44	22.120	*δ*-viridiflorol	$C_{15}H_{26}O$	0.11	–	–
45	22.953	(−)-globulol	$C_{15}H_{26}O$	0.80	–	–
46	23.080	Longifolene	$C_{15}H_{24}$	0.63	0.65	–
47	23.461	*cis*-1,4-dimethyladamantane	$C_{12}H_{20}$	0.14	0.14	–
48	23.854	Spathulenol	$C_{15}H_{24}O$	1.37	4.44	3.56
49	24.065	α-patchoulene	$C_{15}H_{24}$	–	1.83	–
50	24.367	Ginsenol	$C_{15}H_{26}O$	1.12	1.87	2.82
51	24.953	l-*α*-cadinol	$C_{15}H_{26}O$	0.10	0.11	–
52	26.325	1,3-dichloropropane	$C_3H_6Cl_2$	1.75	–	–
53	27.636	*cis*-*β*-asarone	$C_{12}H_{16}O_3$	–	0.35	–
54	32.113	1-nonadecene	$C_{19}H_{38}$	0.15	–	–
55	32.137	1-docosanol	$C_{22}H_{46}O$	–	0.20	–
56	37.847	Palmitic acid	$C_{16}H_{32}O_2$	3.06	4.05	3.97
57	42.185	Falcarinol	$C_{17}H_{24}O$	2.70	3.89	6.22
58	47.574	Phthalic acid, mono-(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	0.96	–	–
59	48.741	15-Crown-5	$C_{10}H_{20}O_5$	–	1.37	–

Table 4.18 The information of volatile oil from Xinbin and Fusong ginseng

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)	
				Xinbin 4-year-old	Fusong 6-year-old
1	9.232	4′-*tert*-butyl-2′,6′-dimethylacetophenone	$C_{14}H_{20}O$	0.30	–
2	9.241	2,2′-isopropylidenebis(5-methylfuran)	$C_{13}H_{16}O_2$	–	0.12
3	9.939	Artemisia triene	$C_{10}H_{16}$	0.04	–
4	10.302	Furfural	$C_5H_4O_2$	0.12	–
5	10.404	1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-cyclohexane	$C_{15}H_{24}$	0.81	–
6	10.425	2,5,6-trimethyl-1,3,6-heptatriene	$C_{10}H_{16}$	–	0.20
7	10.785	2-(3-isopropyl-4-methyl-pent-3-en-1-ynyl)-2-methyl-cyclobutanone	$C_{14}H_{20}O$	0.22	–
8	10.882	Cedrene-V6	$C_{15}H_{24}$	0.30	–
9	11.129	[1*S*-(1*α*,4*α*,7*α*)]-1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl) azule	$C_{15}H_{24}$	0.04	–
10	11.359	1,2,3,6-tetramethyl bicyclo[2.2.2]octa-2,5-diene	$C_{12}H_{18}$	1.86	–
11	11.373	1-(2,4,6-trimethylphenyl)-ethanon	$C_{11}H_{14}O$	–	1.41
12	11.510	*β*-panasinsene	$C_{15}H_{24}$	4.17	2.51
13	11.788	*α*-neoclovene	$C_{15}H_{24}$	0.53	–
14	11.802	(3*R*,4*aS*,5*R*,8*aS*)-5,8*a*-dimethyl-3-(propan-2-yl)-1,2,3,4,4*a*,5,6,8*a*-octahydronaphthalene	$C_{15}H_{26}$	–	0.18

(continued)

Table 4.18 (continued)

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)	
				Xinbin 4-year-old	Fusong 6-year-old
15	12.102	4-(2,7,7-trimethylbicyclo[3.2.0]hept-2-en-1-yl)but-3-en-2-one	$C_{15}H_{24}O$	0.14	–
16	12.247	1,7-dimethyl-7-(4-methyl-3-pentenyl)-tricyclo[2.2.1.0(2,6)] heptane	$C_{15}H_{24}$	0.04	0.13
17	12.350	9-aristolene	$C_{15}H_{24}$	0.24	–
18	12.440	4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-cycloheptane	$C_{15}H_{24}$	0.22	–
19	12.706	*β*-elemene	$C_{15}H_{24}$	3.89	2.20
20	12.851	Calarene	$C_{15}H_{24}$	6.71	4.28
21	12.978	l-caryophyllene	$C_{15}H_{24}$	1.08	–
22	12.987	2-methylene-5-(1-methylvinyl)-8-methyl-bicyclo[5.3.0] decane	$C_{15}H_{24}$	–	0.55
23	13.057	*β*-panasinsene	$C_{15}H_{24}$	0.54	–
24	13.310	*α*-gurjunene	$C_{15}H_{24}$	8.41	12.40
25	14.072	*β*-farnesene	$C_{15}H_{24}$	15.70	10.84
26	14.307	2-methylene-cyclopropanecarboxylic acid	$C_5H_6O_2$	0.08	–
27	14.543	*α*-caryophyllene	$C_{15}H_{24}$	4.60	3.88
28	14.682	4,11,11-trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene	$C_{15}H_{24}$	2.98	–
29	15.020	Viridiflorene	$C_{15}H_{24}$	0.90	0.52
30	15.190	*β*-neoclovene	$C_{15}H_{24}$	1.09	0.81
31	15.377	Germacrene D	$C_{15}H_{24}$	0.25	–
32	15.486	*cis*-*α*-bisabolene	$C_{15}H_{24}$	0.15	–
33	15.627	*β*-humulene (6CI,7CI)	$C_{15}H_{24}$	–	0.54
34	15.709	2-isopropenyl-4*a*,8-dimethyl-1,2,3,4,4*a*,5,6,7-octahydronaphthalene	$C_{15}H_{24}$	0.56	0.43
35	15.890	*γ*-elemene	$C_{15}H_{24}$	11.00	6.15
36	16.096	Cyclodecene	$C_{10}H_{18}$	0.19	–
37	16.116	3-methyl-cyclooctene	C_9H_{16}	–	0.11
38	16.199	*δ*-cadinene	$C_{15}H_{24}$	0.50	0.50
39	19.352	2,6-di-tert-butyl-4-methylphenol	$C_{15}H_{24}O$	1.84	–
40	21.500	(−)-*α*-selinene	$C_{15}H_{24}$	–	0.18
41	21.866	*cis*-nerolidol	$C_{15}H_{26}O$	0.27	–
42	21.886	*trans*-geranylgeraniol	$C_{20}H_{34}O$	–	0.26
43	22.114	Palustrol	$C_{15}H_{26}O$	0.12	–
44	22.947	Globulol	$C_{15}H_{26}O$	0.81	–
45	22.962	Longifolene	$C_{15}H_{24}$	–	1.24
46	23.068	*β*-eudesmene	$C_{15}H_{24}$	1.19	–
47	23.089	*γ*-selinene	$C_{15}H_{24}$	–	0.90
48	23.455	4-ethenyl-3-hydroxy-2,4,7,14-tetramethyl tricyclo[5.4.3.0 (1,8)]tetradecan-6-one	$C_{20}H_{32}O_2$	0.17	–
49	23.469	*cis*-1,4-dimethyladamantane	$C_{12}H_{20}$	–	0.20
50	23.842	Spathulenol	$C_{15}H_{24}O$	2.77	15.86
51	24.053	*α*-patchoulene	$C_{15}H_{24}$	1.21	–
52	24.180	Longi-*β*-camphenyl alcohol	$C_{15}H_{26}O$	0.26	–
53	24.361	2,5-di-tert-butylhydroquinone	$C_{14}H_{22}O_2$	1.22	–

(continued)

Table 4.18 (continued)

No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)	
				Xinbin 4-year-old	Fusong 6-year-old
54	24.376	Ginsenol	$C_{15}H_{26}O$	–	2.08
55	24.615	Cadina-4(14),5-diene	$C_{15}H_{24}$	0.06	–
56	25.896	Ethyl palmitate	$C_{18}H_{36}O_2$	0.10	–
57	26.333	4,4*a*,5,7*a*-tetrahydro-8,8-dimethyl-1,4-methano-1*H*-cyclopenta[*d*]pyridazine	$C_{10}H_{14}N_2$	–	1.55
58	27.134	(1*a*,4*a*,4*aa*,7*aa*)-5-undecyne	$C_{11}H_{20}$	0.09	–
59	27.841	1,4-dioxaspiro[4.5]decane	$C_8H_{14}O_2$	0.06	–
60	30.023	Methyl linoleate	$C_{19}H_{34}O_2$	0.08	–
61	30.566	*Z, Z*-9,12-octadecadienoic acid ethyl ester	$C_{20}H_{36}O_2$	0.07	–
62	31.026	Di-*n*-hexyl phthalate	$C_{20}H_{30}O_4$	0.07	–
63	31.346	Terephthalic acid-2-ethyl-hexyl octyl ester	$C_{24}H_{38}O$	0.13	0.45
64	32.053	Cyclohexadecane	$C_{16}H_{32}$	0.19	–
65	32.279	3-methyl-1,1-diphenylurea	$C_{13}H_{12}N_2O$	–	1.43
66	33.336	Tetradecanoic acid	$C_{14}H_{28}O_2$	–	0.30
68	35.352	Pentadecanoic acid	$C_{15}H_{30}O_2$	0.34	–
69	37.829	Palmitic acid	$C_{16}H_{32}O_2$	3.03	5.71
70	42.206	Falcarinol	$C_{17}H_{24}O$	–	4.11
71	47.613	Phthalic acid mono-(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	–	2.25
72	48.674	Linoleic acid	$C_{18}H_{32}O_2$	0.92	–

In 6-year-old ginseng from Fusong, 50 compounds were detected and 33 components were identified. The relative contents of 16 components were more than 1%. The volatile oil with high-to-low contents were espatulenol (15.86%), α-gurjunene (12.40%), β-farnesene (10.84%), γ-elemene (6.15%), palmic acid (5.71%), calarene (4.28%), falcarinol (4.11%), α-caryophyllene (3.88%), β-panasinsene (2.51%), β-elemene (2.20%), and ginsenol (2.08%).

In summary, most of the volatile components of ginseng from different regions with different cultivation periods have β-farnesene, α-gurjunene, calarene, spathulenol, α-caryophyllene, α-panasinsene, β-panasinsene, α-neoclovene, β-neoclovene, α-elemene, β-elemene, γ-elemene, α-selinene, β-selinene, γ-selinene, α-cadinene, β-cadinene, palmitic acid, falcarinol, ginsenol.

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Analysis of Nucleosides in Ginseng

5

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Abstract

In order to provide data for clarifying the contents of nucleosides in ginseng, the assay of nucleosides was performed. The dried ginseng was taken as test sample. The method for nucleoside analysis was established based on high performance liquid chromatography combined with photodiode array detection (HPLC-PDA). The standard curves of nucleoside were established with the correlation coefficients being all greater than 0.999. The established method was used to determinate the contents of nucleosides in each ginseng sample. The contents of five nucleosides in 45 ginseng samples from different regions with different cultivation years were calculated, and the results were analyzed and clustered. It was found that the contents of five nucleosides in ginseng were related to the region and the cultivation years.

Keywords

Fresh ginseng · nucleoside · HPLC-PDA

5.1 Introduction

Nucleosides are essential components with a variety of biological activities such as anti-tumor, anti-viral, anti-convulsion, anti-arrhythmia, anti-platelet aggregation, and immune regulation [1–4]. There are few studies on nucleosides due to the low contents in ginseng [5, 6]. However, in order to interpret the peculiar effects of ginseng based on comprehensive functional factors or active ingredients, the analysis of nucleoside should be performed. The method of high performance liquid chromatography combined with photodiode array detector (HPLC-PDA) was used to assay the nucleoside contents of ginseng [7–10].

5.2 Materials and Instruments

5.2.1 Materials and Reagents

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

Uracil (98%, Beijing Yingzena New Chemical Technology Research Institute, China; Beijing New Chemical Technology Research Institute, China), Uridine (9.0%, Changchun Food and Drug Inspection Institute, China), Adenosine (For determination of content, China Food and Drug Testing Institute; Changchun Food and Drug Inspection Institute, China), Adenine (99.4%, China Food and Drug Control Institute,

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Changchun Food and Drug Inspection Institute, China), guanosine (99.0%, Changchun Food and Drug Inspection Institute, China). Chromatographic grade acetonitrile (Fisher, Co. Ltd., America), ultrapure water, and other reagents are all of analytical grade purity.

5.2.2 Instruments

1525 High Performance Liquid Chromatograph (Waters Co. Ltd., America), 2998 Photo-Diode Array Detector (Waters Co. Ltd., America), AT-330 Chromatographic Column Incubator (Tianjin Autoscience Instrument Co. Ltd., China), R201D Thermostat Water Bath (Shanghai Yukang Science and Education Equipment Co. Ltd., China), SHZ-D(III) Circulating Water Vacuum Pump (Gongyi Yuhua Instrument Co. Ltd., China), FA1104N Electronic Balance (Shanghai Jinghua Tech Instrument Co. Ltd., China), KQ3200V Ultrasonic Cleaner (Kunshan Ultrasonic Instrument Co. Ltd., China), PHS-3C Digital PH-Meter (Shanghai Yoke Instrument Co. Ltd., China), TGL-16aR High-speed Freezing Centrifuge (Shanghai Anting Scientific Instrument Factory, China), FW177 High Speed Universal Pulverizer (Beijing Yongguangming Medical Instrument Co. Ltd., China), RCT-3200 Water Purification System (Changchun Laibopate Technology Development Co. Ltd., China).

5.3 Experimental Methods

5.3.1 Preparation of Mixed Reference Solution

Dissolve a quantity of uracil, accurately weighted, in 50% methanol to produce a solution containing 0.25 mg of reference per mL, as solution I.

Dissolve a quantity of adenine, accurately weighted, in 50% methanol to produce a solution containing 0.25 mg of reference per mL, as solution II.

Dissolve a quantity of uridine, accurately weighted, in 50% methanol to produce a solution containing 0.51 mg of reference per mL, as solution III.

Dissolve a quantity of guanosine, accurately weighted, in 50% methanol to produce a solution containing 0.30 mg of reference per mL, as solution IV.

Dissolve a quantity of adenosine, accurately weighted, in 50% methanol to produce a solution containing 0.26 mg of reference per mL, as solution V.

Measured accurately 150 μL of solution I, 50 μL of solution II, 230 μL of solution III, 220 μL of solution IV, and 280 μL of solution V, diluted with water to produce a solution containing 0.0375 mg of uracil, 0.0125 mg of adenine, 0.1173 mg of uridine, 0.066 mg of guanosine, and 0.0728 mg of adenosine per mL as the mixed reference solution.

5.3.2 Preparation of Test Solutions

Ginseng was air-dried, grinded, and sieved (Chinese National Standard Sieve No. 6, R40/3 series) to get the homogeneous powder. Transferred 1.0 g powder to a stoppered conical flask, added 10 mL of water, weighed the total quantity. Ultrasonicated for 1 h, allowed to cool, added water to make up the loss of weight, mixed well, centrifuged (5000 r/min) for 10 min, filtered (0.45 μm), and used the filtrate as the test solution.

5.3.3 Chromatographic Conditions

Carried out the method for high performance liquid chromatography, using SpursilTM C18 (4.6 mm × 250 mm, 5 μm) column. Acetonitrile as mobile phase A and 0.01% aqueous formic acid solution as mobile phase B. Gradient elution procedure: 0% A from 0 to 10 min, 0–1% A from 10 to 15 min, 1–5% A from 15 to 25 min, 5% A from 25 to 35 min. The flow rate was 0.8 mL/min. Detection wavelength was 254 nm. Column temperature was at 25 °C. Injected 10 μL onto the column and recorded the chromatogram. The

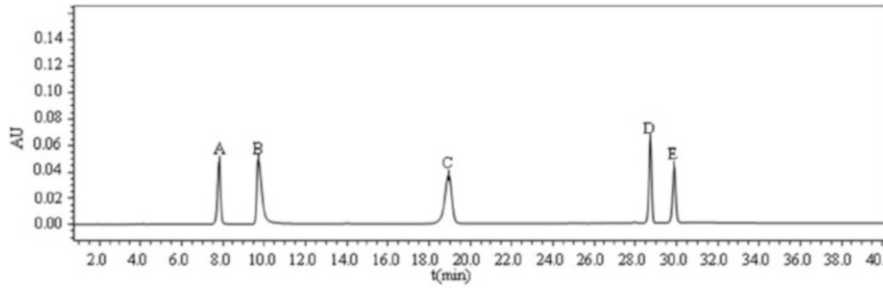

Fig. 5.1 HPLC chromatogram of a mixed reference solution of five nucleosides. *A* Uracil; *B* Adenine; *C* Uridine; *D* Guanosine; *E* Adenosine

Table 5.1 Linear equations and correlation coefficients of five nucleosides

Name of nucleosides	Regression equation	Correlation coefficient
Uracil	$y = 5 \times 10^6 x - 13{,}905$	0.9998
Adenine	$y = 6 \times 10^6 x - 26{,}545$	0.9999
Uridine	$y = 2 \times 10^6 x - 13{,}023$	0.9992
Guanosine	$y = 3 \times 10^6 x - 21{,}509$	0.9999
Adenosine	$y = 3 \times 10^6 x - 42{,}102$	0.9999

HPLC chromatogram of the mixed reference solution containing five nucleosides was shown in Fig. 5.1.

5.3.4 Drawing of Standard Curve

Injected 1, 2, 4, 6, 8, 10, and 12 μL of the mixed reference solutions, respectively, into the column and recorded the chromatogram under the chromatographic condition. The linear regression equations were calculated from the concentration (x) of the reference solutions versus the peak area (y) (Table 5.1).

5.3.5 Determination of Nucleosides in Ginseng

The content of each nucleoside in ginseng sample was calculated by the linear regression equation. The HPLC chromatogram of five nucleosides in 5-year-old ginseng from Kuandian was shown in Fig. 5.2.

5.4 Results and Discussion

5.4.1 Contents of Five Nucleosides in Ginseng from Different Regions with Different Cultivation Years

Contents of five nucleosides in ginseng from different regions with different cultivation years are shown in Table 5.2. The content accumulation histogram of nucleoside in ginseng is shown in Fig. 5.3.

5.4.2 Analysis of Five Nucleosides in Ginseng from Same Region with Different Cultivation Years

The tendencies of various contents (Figs. 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 5.10, 5.11, 5.12, and 5.13) of five nucleosides were drawn from the data. The analysis of the nucleoside contents in ginseng with different grown years and different areas is shown in Table 5.3. The difference on adenine contents in ginseng from different regions was

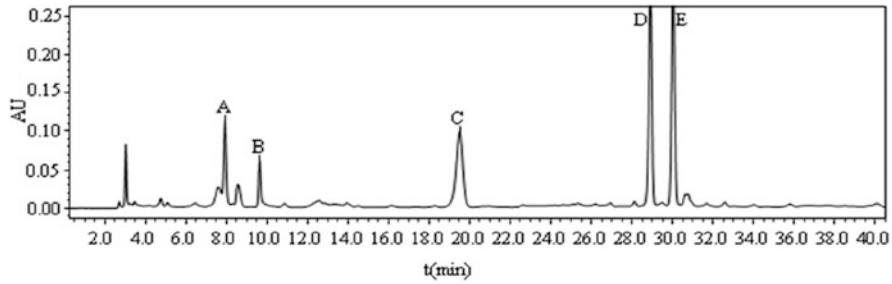

Fig. 5.2 HPLC chromatogram of five nucleosides in 5-year-old ginseng from Kuandian. *A* Uracil; *B* Adenine; *C* Uridine; *D* Guanosine; *E* Adenosine

not obvious. The contents of uracil and uridine in 3-year-old ginseng from different regions are similar. The contents of guanosine and adenosine in ginseng from different regions increased with the increasing of the cultivation period.

5.4.3 Analysis of Five Nucleosides in Ginseng from Different Regions with Same Cultivation Years

The determination result was shown in Table 5.2.

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

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

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

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

Table 5.2 Contents of five nucleosides in ginseng from different regions with different cultivation years (‰)

No.	Region	Cultivation period (year)	Uracil	Adenine	Uridine	Guanosine	Adenosine
1	Heihe	4	0.10	0.05	0.60	0.54	0.58
2	Hulin	4	0.12	0.05	0.54	0.47	0.52
3	Antu	4	0.08	0.07	0.57	0.46	0.41
4		5	0.07	0.06	0.57	0.48	0.48
5	Changbai	4	0.10	0.07	0.58	0.55	0.62
6		5	0.12	0.06	0.37	0.44	0.51
7		6	0.05	0.06	0.75	0.46	0.60
8	Dunhua	4	0.07	0.07	0.57	0.51	0.48
9		5	0.10	0.06	0.52	0.43	0.46
10	Fusong	4	0.05	0.04	0.39	0.45	0.48
11		5	0.04	0.04	0.35	0.46	0.50
12		6	0.03	0.02	0.23	0.55	0.59
13	Hunchun	4	0.05	0.03	0.59	0.46	0.46
14		5	0.04	0.03	0.53	0.44	0.44
15		6	0.07	0.03	0.60	0.46	0.49
16		4(CC)	0.06	0.03	0.48	0.44	0.48
17	Huadian	4	0.01	0.02	0.49	0.22	0.24
18		5	0.01	0.02	0.55	0.24	0.32
19	Helong	4	0.08	0.05	0.51	0.41	0.44
20		5	0.05	0.05	0.66	0.39	0.43
21	Dadi Ji'an	5	0.06	0.05	0.57	0.41	0.48
22		6	0.07	0.05	0.65	0.50	0.62
23	Shuangcha Ji'an	4	0.06	0.04	0.62	0.56	0.63
24		5	0.05	0.05	0.75	0.54	0.65
25		6	0.14	0.06	0.49	0.47	0.54
26	Jiaohe	4	0.03	0.04	0.57	0.38	0.46
27		5	0.07	0.04	0.48	0.36	0.36
28	Jingyu	4	0.05	0.04	0.47	0.43	0.44
29		5	0.06	0.03	0.50	0.44	0.45
30	Linjiang	5	0.08	0.04	0.56	0.45	0.51
31		6	0.09	0.05	0.57	0.46	0.52
32	Tonghua	4	0.12	0.04	0.53	0.50	0.56
33		5	0.03	0.05	0.61	0.50	0.50
34	Wangqing	4	0.04	0.03	0.60	0.47	0.47
35		5	0.04	0.03	0.51	0.45	0.42
36		6	0.04	0.03	0.52	0.40	0.35
37	Kuandian	4	0.04	0.03	0.61	0.46	0.53
38		5	0.07	0.05	0.55	0.49	0.53
39		6	0.05	0.03	0.57	0.42	0.51
40	Xinbin	4	0.11	0.07	0.83	0.75	0.78
41	North Korea	4	0.05	0.03	0.28	0.21	0.24
42		5	0.01	0.02	0.73	0.65	0.71
43		6	0.02	0.01	0.78	0.66	0.74
44	South Korea	5	0.01	0.04	0.51	0.47	0.68
45		6	0.01	0.05	0.52	0.45	0.70

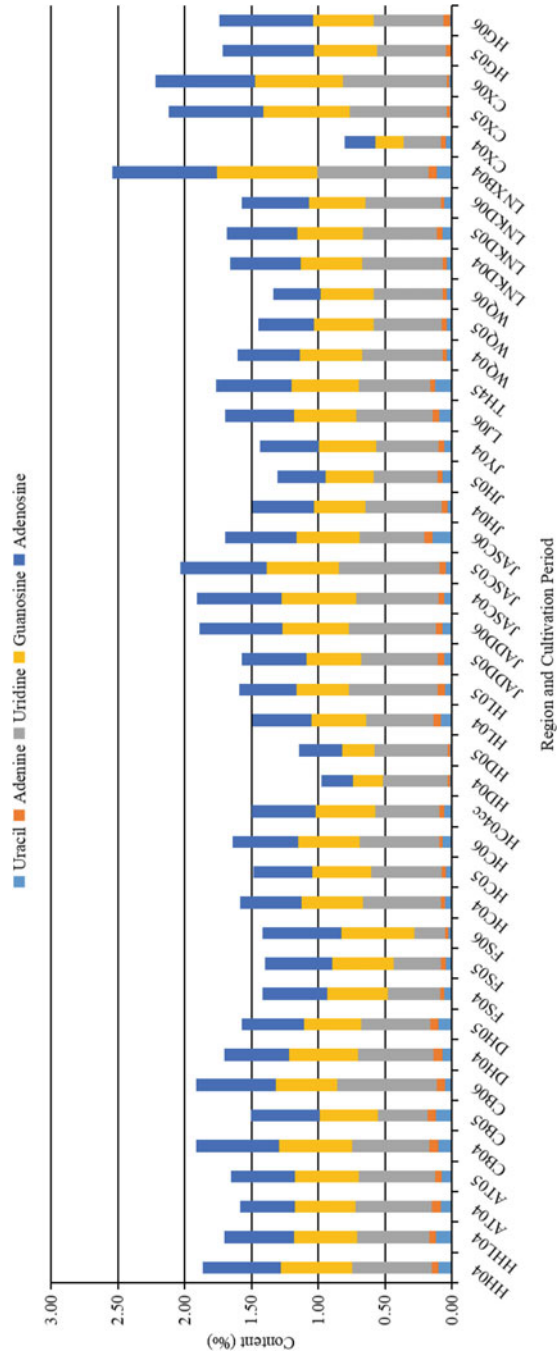

Fig. 5.3 The content accumulation histogram of nucleoside in ginseng from different regions with different cultivation years

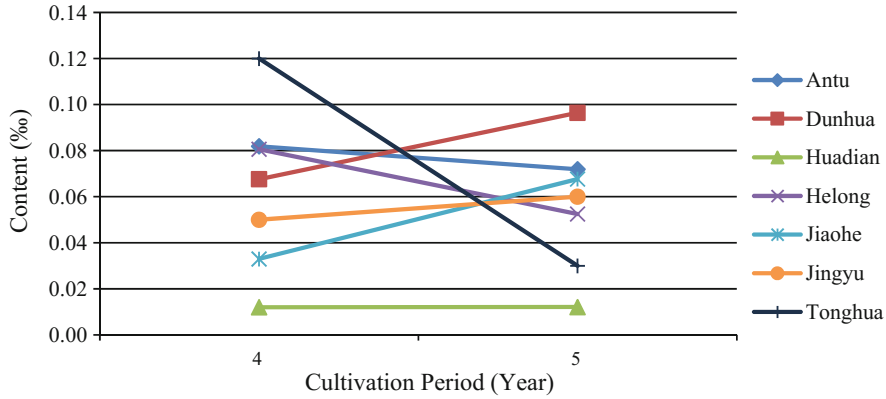

Fig. 5.4 The contents of uracil in ginseng of 4, 5 years

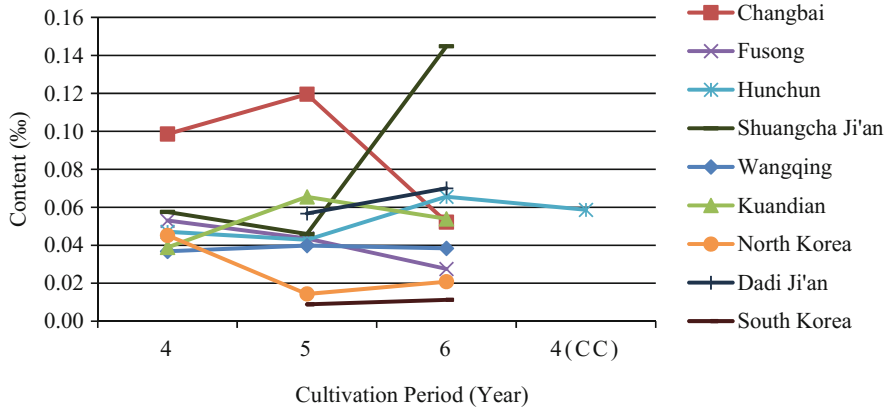

Fig. 5.5 The contents of uracil in ginseng of 4, 5, 6, 4 (CC) years

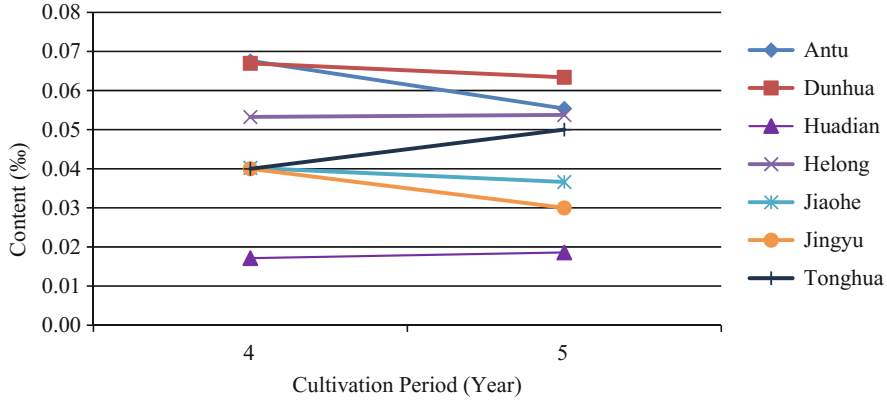

Fig. 5.6 The contents of adenine in ginseng of 4, 5 years

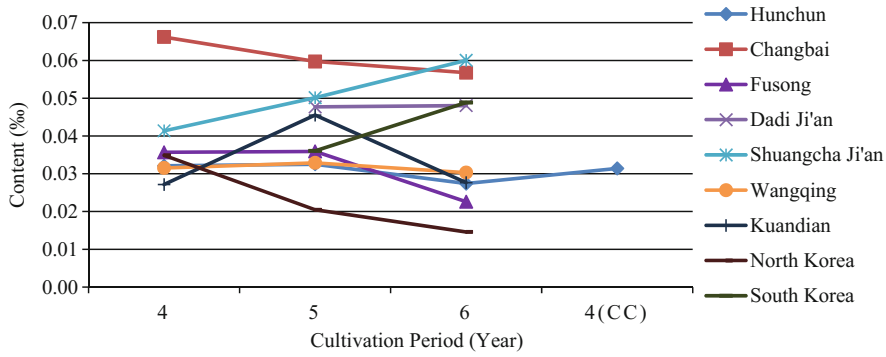

Fig. 5.7 The contents of adenine in ginseng of 4, 5, 6, 4 (CC) years

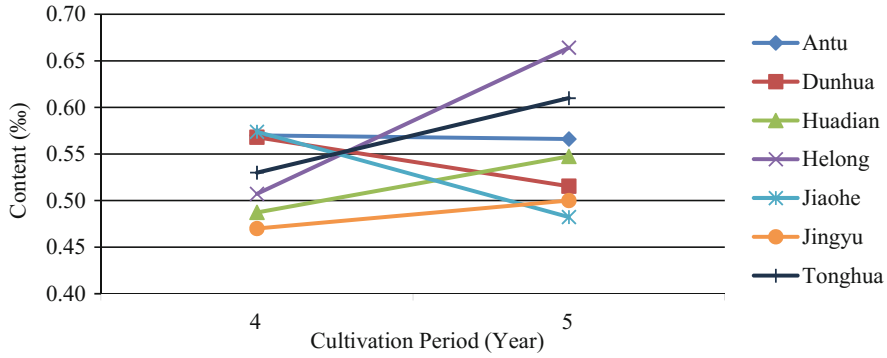

Fig. 5.8 The contents of uridine in ginseng of 4, 5 years

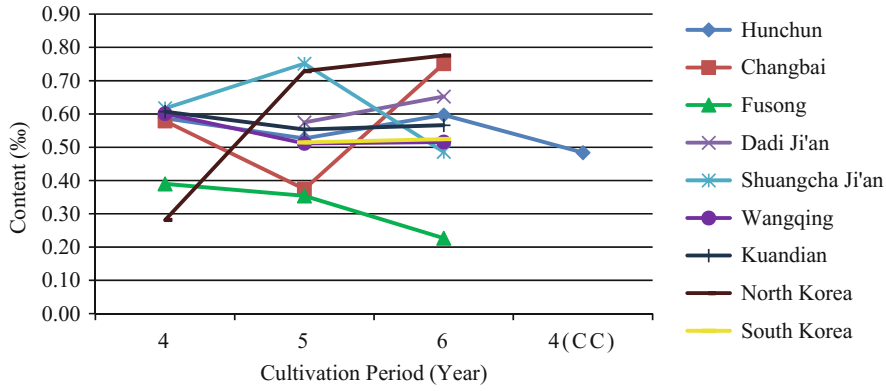

Fig. 5.9 The contents of uridine in ginseng of 4, 5, 6, 4 (CC) years

Shuangcha Ji'an, Linjiang/Hunchun/Changbai, South Korea, Kuandian, Wangqing.

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

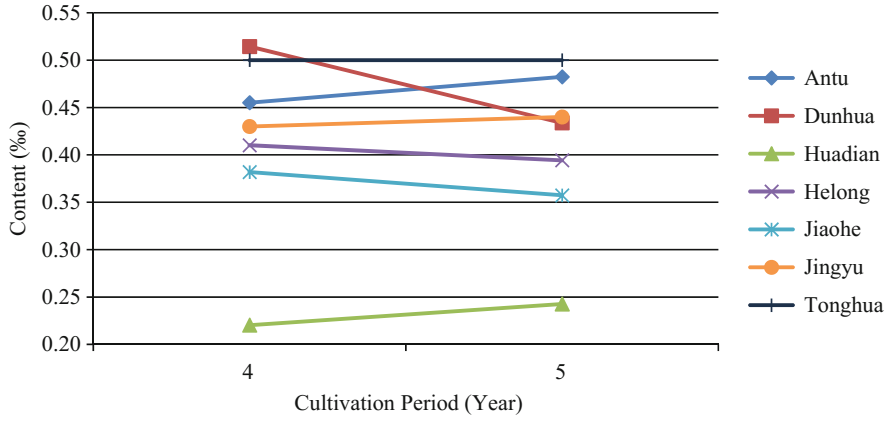

Fig. 5.10 The contents of guanosine in ginseng of 4, 5 years

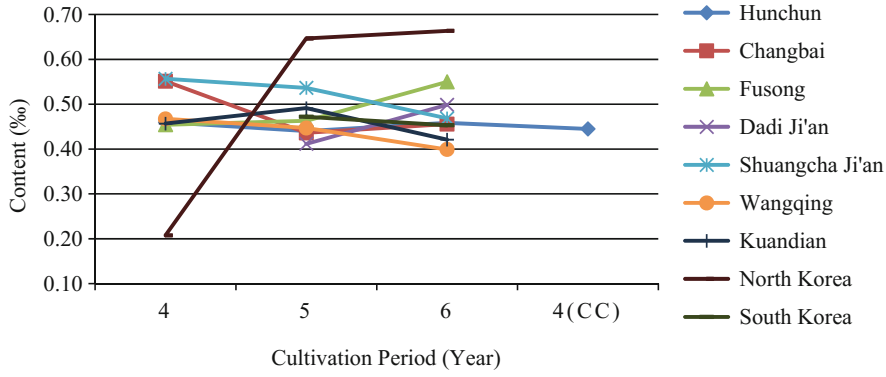

Fig. 5.11 The contents of guanosine in ginseng of 4, 5, 6, 4 (CC) years

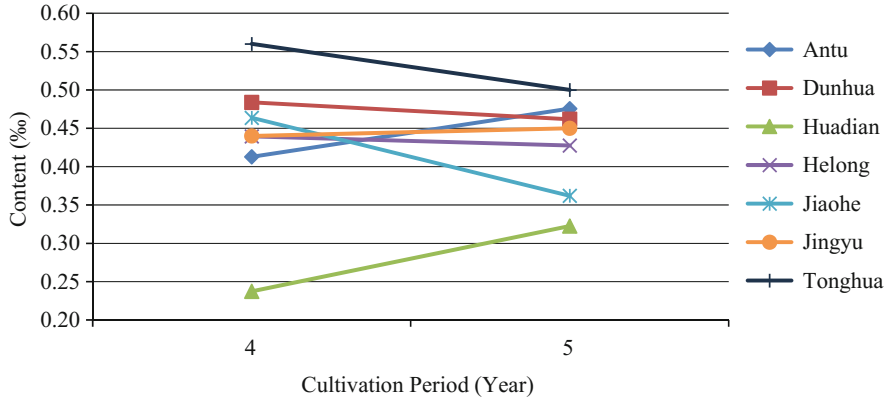

Fig. 5.12 The contents of adenosine in ginseng of 4, 5 years

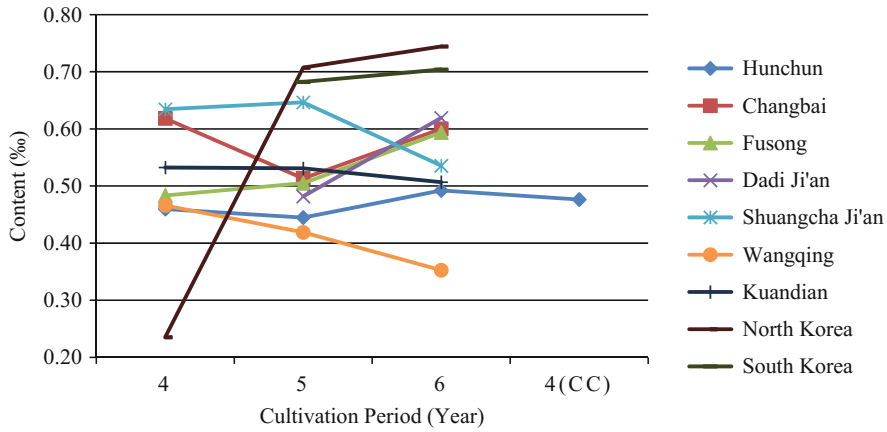

Fig. 5.13 The contents of adenosine in ginseng of 4, 5, 6, 4 (CC) years

Table 5.3 Analysis of five nucleosides in ginseng with different cultivation years

No.	Nucleoside	Region	Content
1	Uracil	Dunhua, Jiaohe, Jingyu	CG5 > CG4
		Antu, Helong, Tonghua	CG5 < CG4
		Dadi Ji'an, Linjiang	CG6 > CG5
2	Uridine	Huadian, Helong, Jingyu, Tonghua	CG5 > CG4
		Dunhua, Jiaohe	CG5 < CG4
		Linjiang, Dadi Ji'an, South Korea	CG6 > CG5
3	Guanosine	Antu, Huadian, Jingyu	CG5 > CG4
		Dunhua, Helong, Jiaohe	CG5 < CG4
		South Korea	CG5 > CG6
		Dadi Ji'an, Linjiang	CG6 > CG5
4	Adenosine	Antu, Huadian	CG5 > CG4
		Dunhua, Helong, Jiaohe, Tonghua	CG5 < CG4
		Dadi Ji'an, Linjiang, South Korea	CG6 > CG5

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

The distribution of five nucleosides was shown in Figs. 5.14, 5.15, and 5.16, respectively.

5.4.4 Hierarchical Cluster Analysis of Five Nucleosides in Ginseng from Different Regions with Different Cultivation Periods

Combined Ward method, chi-square metric and squared Euclidean distance in SPSS 22.0 software, the dendrogram (Fig. 5.17) of cluster analysis was established with nucleoside contents as 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 HH04 (1), HHL04 (2), AT04 (3), AT05 (4), CB04 (5), CB05 (6), DH04 (8), DH05 (9), FS04 (10), FS05 (11), HC04cc (13), HL04 (19), JASC04 (23), JASC06 (25), JH05 (27), JY04 (28), LJ06 (29), TH45 (30), LNKD05 (35), LNXB04 (37), CX04 (38) were clustered into the first category. The samples including FS06 (12), CX05 (39), CX06 (40), HG05 (41), HG06 (42) were clustered into the second category. The rest samples were clustered into the third category, which could be

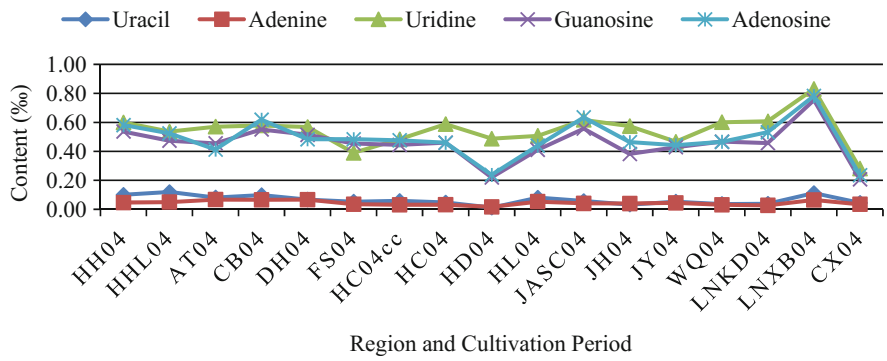

Fig. 5.14 Nucleoside contents of 4-year-old ginseng

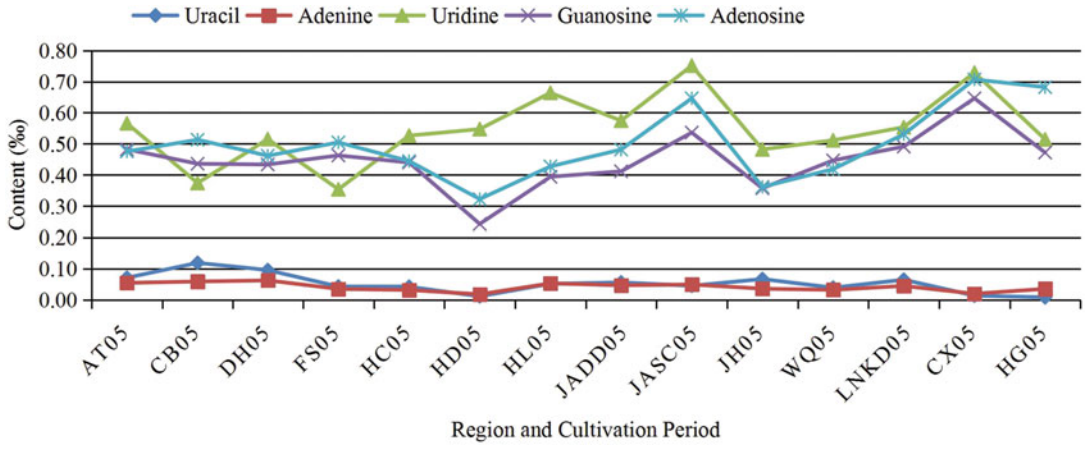

Fig. 5.15 Nucleoside contents of 5-year-old ginseng

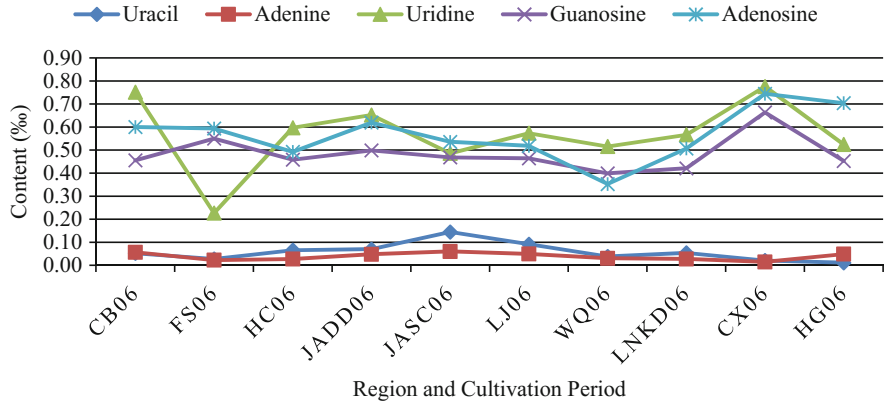

Fig. 5.16 Nucleoside contents of 6-year-old ginseng

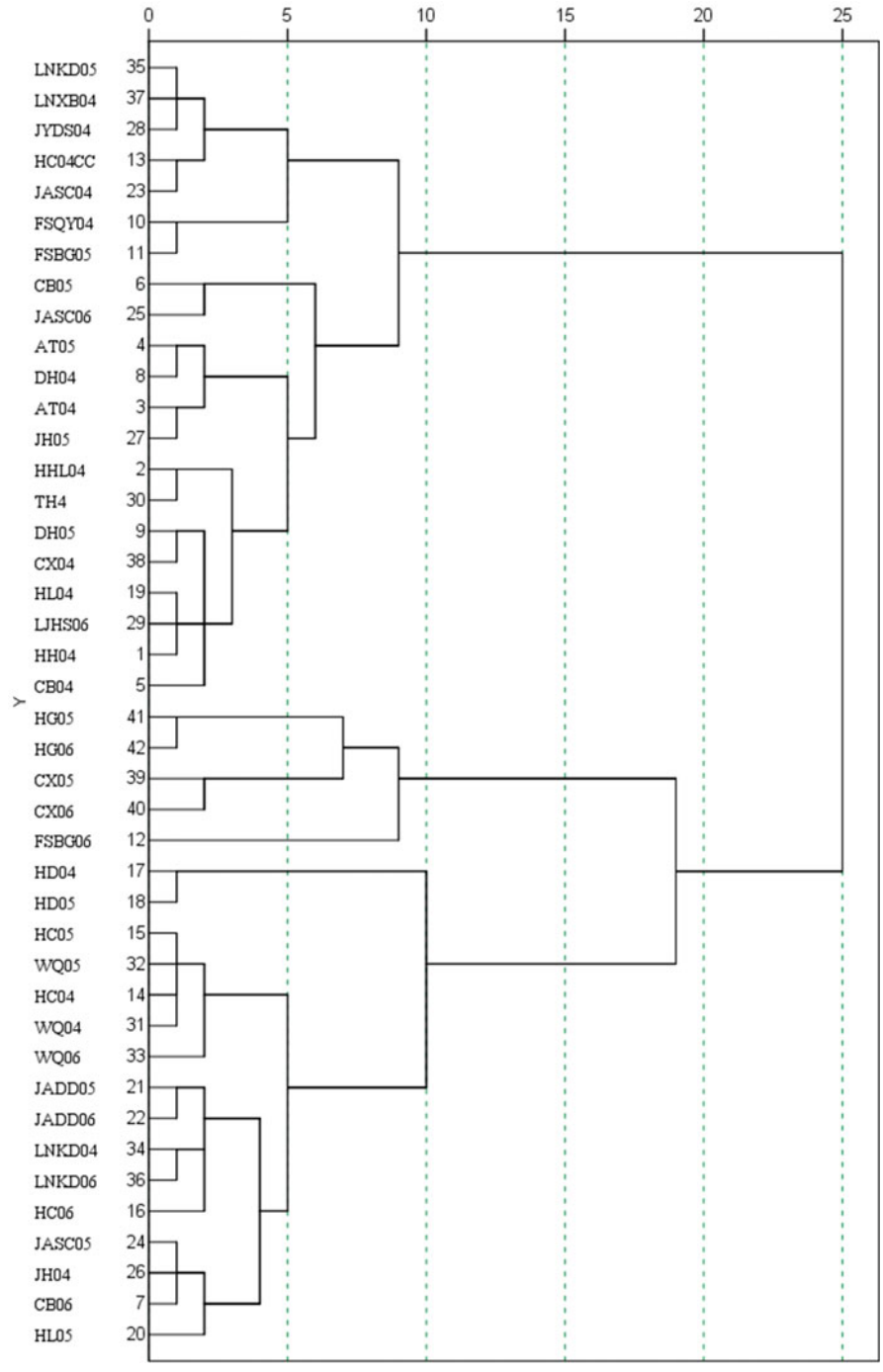

Fig. 5.17 Hierarchical graph of cluster analysis of nucleosides in ginseng

further divided into two groups when the clustering distance being 10. One group was consisted of HD04 (17) and HD05 (18). The other group included the rest samples.

According to the hierarchical cluster analysis, which was established with nucleoside contents as the characteristic variable, the similarity between ginseng from different regions with different cultivation years can be found.

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Analysis of Flavonoids in Ginseng

6

Pingya Li, Nanqi Zhang, Huanhao Ma, and Na Yang

Abstract

In order to provide data for clarifying the contents of flavonoids in ginseng, the assay of flavonoids was performed. The dried ginseng was taken as the test sample. The content of flavonoids was determined by ultraviolet-visible spectrophotometry method. Measured the absorbance at 500 nm. Rutin was used as the reference substance. The calibration curve of rutin was produced by plotting the absorbance against concentration. The correlation coefficient was greater than 0.999 with the linear range 38.6~270.23 μg. The results showed that the 4-year-old ginseng obtained from North Korea had the highest content of total flavonoids, while the 4-year-old ginseng in Changbai with the lowest content. The experimental results indicated that there was no regular tendency of total flavonoids contents in all samples, which means that there was no positive correlation between the content of total flavonoids and the ages of ginseng.

Keywords

Died ginseng · Flavonoid · Ultraviolet spectrophotometry

6.1 Introduction

Flavonoid aglycones and kaempferol are the main flavonoids in ginseng. Flavonoids were reported to exert anti-inflammatory [1, 2], anti-oxidant, anti-cancer [3, 4], and liver protective [5] effects and played important roles in cardiovascular and immune systems [6, 7]. In recent years, there were few studies on the flavonoids in ginseng. The common methods for flavonoid assay included spectrophotometry [8], high performance liquid chromatography- diode array-detector and mass spectrometry detector (HPLC-DAD-MS), [9] and high performance liquid chromatography- ultraviolet detector (HPLC-UV) [10]. In this book, the ultraviolet spectrophotometry method was applied to determine the contents of total flavonoids.

6.2 Materials and Instruments

6.2.1 Materials

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

Reference substance of rutin was purchased from Food and Drug Verification Research

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Institute of China. Sodium nitrite (Tianjin Guangfu Technology Development Co. Ltd., China), aluminum nitrate (Tianjin Guangfu Technology Development Co. Ltd., China), and sodium hydroxide (Beijing Chemical Works, China) were all of analytical pure grade. Ultrapure water was prepared by the Water Purification System (Changchun Laibopate Technology Development Co. Ltd., China).

6.2.2 Instruments

FW177 High speed omnipotent pulverizer (Beijing Yongguangming Medical Instrument Co. Ltd., China), KQ3200V Ultrasonic cleaner (Kunshan Ultrasonic Instrument Co. Ltd., China), Rotary evaporator (Shanghai Yukang Science and Education Equipment Co. Ltd., China), FA1102N electronic balance (Shanghai Jinghua Tech Instrument Co. Ltd., China), 722 ultraviolet visible spectrophotometer (Shanghai Precision Scientific Instrument Co. Ltd., China).

6.3 Experimental Methods

6.3.1 Preparation of Reference Solution

Dissolve an accurately weighted quantity of rutin with 70% ethanol to produce a solution of about 0.2 mg per mL, as reference solution.

6.3.2 Preparation of Test Solution

Ginseng was air-dried, grinded, and sieved (Chinese National Standard Sieve No. 2, R40/3 series) to get the homogeneous powder. To 2 g of powder, accurately weighed, add 40 mL of 67% ethanol and shake thoroughly. Allow to stand for 20 min. The mixture was ultrasonicated for 50 min, allowed to cool, filtered. The filtrate was transferred into flask, and the residue was extracted twice again. The extractions were combined, concentrated and evaporated to dryness. Added 70% ethanol, shaked to dissolve the residue, and diluted to volume 10.0 mL. All of the above solutions were prepared in duplicate. The solutions were filtered with a syringe filter (0.45 μm), and used the filtrate as the test solution.

6.3.3 Drawing Standard Curve

Transferred, respectively, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 mL of rutin standard solution into seven 10 mL stoppered conical flask, diluted each to 2.0 mL with 70% ethanol, then added 0.3 mL of 5% $NaNO_2$ solution to each flask, mix immediately and stand for 6 min. Next added 0.3 mL of 10% $Al(NO_3)_3$ solution and stand for 6 min. Cooled to room temperature, added 2 mL of 4% NaOH solution, shake thoroughly. Allow it to stand for 10 min. Cooled to room temperature. Measured the absorbance at 500 nm (Pharmacopoeia of the People's Republic of China (2015 Version), Appendix VA). Calculate the linear regression equation from the absorbances (y) obtained versus the concentrations (x) of the reference solutions (Fig. 6.1, Table 6.1). The method had good linearity in the range of 38.6~270.23 μg ($r = 0.9996$).

6.3.4 Determination of Test Samples

Repeated the operation, using the substance being examined, 1.0 mL of the test solution was accurately measured, instead of the reference solutions. Then the concentration of the total flavonoids in ginseng was calculated by the regression equation of calibration curve.

6.4 Results and Discussion

6.4.1 The Content of Total Flavonoids in Ginseng from Different Regions with Different Cultivation Ages

The results were shown in Table 6.2.

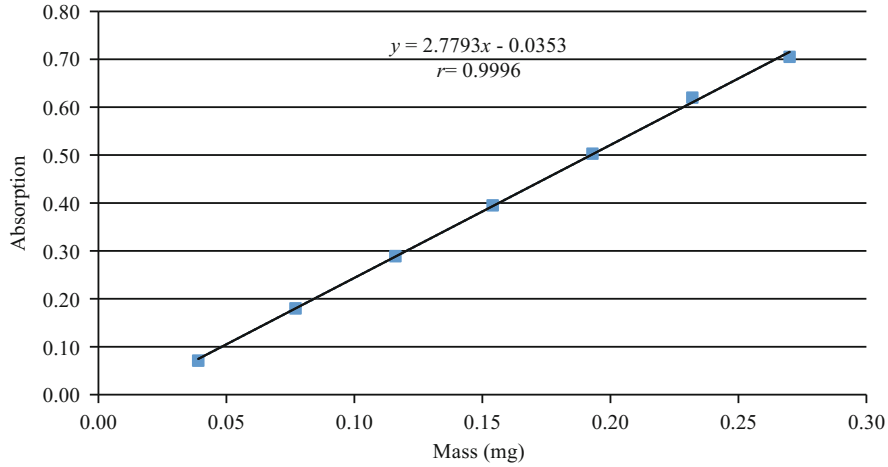

Fig. 6.1 Calibration curve of absorbances (y) versus the concentrations (x)

Table 6.1 The absorbance of standard substance

Standard solution volume (mL)	0.2	0.4	0.6	0.8	1.0	1.2	1.4
Content of rutin (mg)	0.039	0.077	0.116	0.154	0.193	0.232	0.270
Absorption 1	0.070	0.179	0.288	0.394	0.500	0.619	0.705
Absorption 2	0.072	0.180	0.289	0.395	0.505	0.621	0.705
Average absorption	0.071	0.180	0.289	0.395	0.503	0.620	0.705
Regression equation	$y = 2.7742x - 0.0337$ ($r = 0.9996$)						

6.4.2 Analysis of Total Flavonoid Contents in Ginseng from Same Regions with Different Cultivation Years

The results were shown in Table 6.3.

6.4.3 Analysis of Total Flavonoids Content in Ginseng with Same Cultivation Years from Different Regions

The regions of 4-year-old ginseng ranked in decreasing order based on the contents of total flavonoids were: North Korea, Huadian, Dunhua, Wangqing, Kuandian/Heihe, Shuangcha Jian Jingyu/Xinbin/Antu, Fusong, Jiaohe, Hulin, Hunchun, Helong, Changbai.

The regions of 5-year-old ginseng ranked in decreasing order based on the contents of total flavonoids were: Huadian, Hunchun, North Korea, South Korea, Shuangcha Ji'an, Changbai, Tonghua, Jingyu/Linjiang, Fusong/Helong, Jiaohe/Wangqing/Kuandian, Antu, Dunhua, Dadi Ji'an.

The regions of 6-year-old ginseng ranked in decreasing order based on the contents of total flavonoids were: Changbai, Fusong, Linjiang, Dadi Ji'an, South Korea, North Korea, Shuangcha Ji'an, Kuandian, Hunchun, Wangqing.

6.4.4 Analysis of Total Flavonoids Content in Ginseng from Different Regions with Different Cultivation Ages

Among of all samples from various areas, the 4-year-old ginseng obtained from North Korea had the highest content of total flavonoids (0.116%) while the 4-year-old ginseng in Changbai with the lowest content (0.065%). In addition, the average content of total flavonoids was 0.090% with the RSD being 15.0%. The

Table 6.2 Total flavonoid contents of ginseng

No.	Region	Cultivation period (year)	Sample 1 (%)	Sample 2 (%)	Content of total flavonoids (%)
1	Shuangcha Ji'an	4	0.088	0.097	0.093
2		5	0.104	0.104	0.104
3		6	0.086	0.072	0.079
4	Dadi Ji'an	5	0.069	0.081	0.075
5		6	0.087	0.095	0.091
6	Changbai	4	0.069	0.061	0.065
7		5	0.108	0.087	0.098
8		6	0.100	0.128	0.114
9	Fusong	4	0.089	0.092	0.091
10		5	0.088	0.086	0.087
11		6	0.095	0.095	0.095
12	Jingyu	4	0.080	0.104	0.092
13		5	0.081	0.105	0.093
14	Linjiang	5	0.092	0.093	0.093
15		6	0.094	0.094	0.094
16	Antu	4	0.103	0.080	0.092
17		5	0.075	0.088	0.081
18	Dunhua	4	0.101	0.101	0.101
19		5	0.077	0.076	0.077
20	Hunchun	4 (CC)	0.077	0.080	0.079
21		4	0.067	0.073	0.070
22		5	0.130	0.097	0.114
23		6	0.075	0.066	0.070
24	Wangqing	4	0.099	0.092	0.096
25		5	0.090	0.081	0.086
26		6	0.071	0.063	0.067
27	Helong	4	0.075	0.068	0.072
28		5	0.086	0.087	0.087
29	Jiaohe	4	0.099	0.078	0.089
30		5	0.087	0.085	0.086
31	Huadian	4	0.124	0.106	0.115
32		5	0.111	0.121	0.116
33	Hulin	4	0.093	0.076	0.085
34	Heihe	4	0.088	0.098	0.093
35	Kuandian	4	0.085	0.101	0.093
36		5	0.088	0.083	0.086
37		6	0.077	0.070	0.074
38	Xinbin	4	0.092	0.092	0.092
39	North Korea	4	0.117	0.115	0.116
40		5	0.110	0.110	0.110
41		6	0.078	0.082	0.080
42	Tonghua	4	0.089	0.096	0.093
43		5	0.093	0.095	0.094
44	South Korea	5	0.105	0.111	0.108
45		6	0.088	0.088	0.088

Table 6.3 The comparison of total flavonoid contents of ginseng from same regions with different cultivation years

No.	Region	Content
1	Shuangcha Ji'an	$CG_5 > CG_4 > CG_6$
2	Dadi Ji'an	$CG_6 > CG_5$
3	Changbai	$CG_6 > CG_5 > CG_4$
4	Fusong	$CG_6 > CG_4 > CG_5$
5	Antu	$CG_4 > CG_5$
6	Dunhua	$CG_4 > CG_5$
7	Hunchun	$CG_5 > CG_6 = CG_4$
8	Wangqing	$CG_4 > CG_5 > CG_6$
9	Helong, Jingyu, Jiaohe	$CG_5 > CG_4$
10	Linjiang	$CG_6 > CG_5$
11	Huadian	$CG_5 > CG_4$
12	Kuandian	$CG_4 > CG_5 > CG_6$
13	Tonghua	$CG_5 > CG_4$
14	North Korea	$CG_4 > CG_5 > CG_6$
15	South Korea	$CG_5 > CG_6$

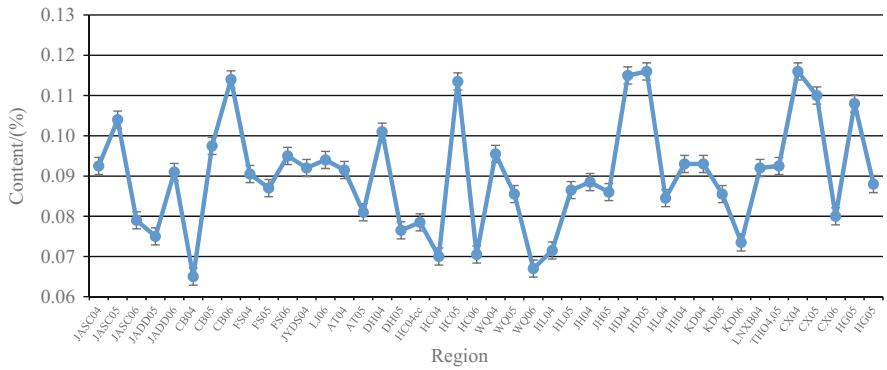

Fig. 6.2 Tendency chart of total flavonoids contents

experimental results indicated that there was significant difference but no regular tendency of total flavonoids content, which means there was no positive correlation between the content of total flavonoids and the ages of ginseng (Table 6.2, Fig. 6.2).

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Analysis of Ginsenosides in Ginseng

7

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Abstract

Aiming to provide reference on illustrating the kinds and the contents of ginsenosides in ginseng, the analysis of ginsenosides was carried out. The dried ginseng was taken as test sample. The method of total ginsenosides of ginseng from different regions with different cultivation periods was determined by ultraviolet-spectrophotometry, and 19 ginsenosides were determined by high performance liquid chromatography combined with ultraviolet detectors (HPLC-UV). The standard curves of ginsenosides were established with the correlation coefficients being greater than 0.99, which were used to determine the contents of total ginsenosides and 19 monomeric ginsenosides in each sample, respectively. For the ginseng in same area, the ginsenoside content in 5-year-old ginseng was the highest, and the ginsenoside content in 4-year-old ginseng was obviously higher than the 4-year-old continuous cropping ginseng. For the ginseng from various areas, the contents of total and monomeric ginsenosides were different. This chapter further explored the relationships between the ginseng's quality and the cultivation periods or producing regions. It is significant to ginseng's quality control.

Keywords

Dried ginseng · Total ginsenosides · Monomeric ginsenosides · Ultraviolet-spectrophotometry · HPLC-UV

7.1 Introduction

Ginseng, the king of herbal in the Orient, has always been receiving a lot of attention not only as a therapeutic medicinal herb but also as a health supplement [1–3]. Ginsenosides, as the most important functional factor and active component of ginseng, have been studied mostly on diverse bioactive effects [4–6]. More than 50 ginsenosides including 6 major ginsenosides (Rb1, Rb2, Rc, Rd, Re, and Rg1) have been found from *Panax ginseng* [7, 8]. As part of the phytochemical research on ginseng, the determination of ginsenoside contents in different ginseng samples was carried out in this chapter. The methods of analysis included ultraviolet-spectrophotometry method for determining the contents of total ginsenosides [9, 10], HPLC-UV method for determining the contents of monomeric ginsenosides.

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7.2 Materials and Instruments

7.2.1 Materials

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

Both methanol and acetonitrile were all of chromatographic grade (Fisher, Co. Ltd, USA), and 95% medical ethanol was analytical pure grade (Beijing Chemical Works, China); phosphoric acid was of chromatographic grade (Tianjin Guangfu Fine Chemical Research Institute, China); ultrapure water was prepared by the Water Purification System (Changchun Laibopate Technology Development Co. Ltd, China); Ginsenoside Re (99.0%, 20140202, National Local Joint Engineering Research Center of Natural Drug, China); Ginsenoside Rg_1, Rf, Rh_1(S), Rb_1, Rc, F_1, Rb_2, Rb_3, F_2 were purchased from Jilin University College of Chemistry and passed the test. Ginsenoside Rg_2, Rd, 20(S)-Rg_3, 20(R)-Rg_3, Rh_2, PPT, PPD were isolated in our laboratory and identified by spectroscopic data; oleanolic acid and ginsenoside Ro were purchased from National Institutes for Food and Drug Control.

7.2.2 Instruments

98-1-B Electronic Thermostat (Tianjin Test Instrument Co. Ltd., China); GF-254 Chromatography Silica Gel Plate (Zhejiang Taizhou Luqiao Sijia Biochemical Plastics Factory, China); FA1102N Electronic Balance (Shanghai Jinghua Tech Instrument Co. Ltd., China); 721 Spectrophotometer (Shanghai Optical Instrument Factory, China); D101 Macroporous Adsorption Resin (Bengbu Tianxing Resin Co. Ltd., China); R201D Thermostat Water Bath and Rotary Evaporator (Shanghai Yukang Scientific and Educational Equipment Co. Ltd., China), SHZ-D (III) Circulating Water Vacuum Pump (Gongyi Yuhua Instrument Co. Ltd., China), FA1102N Electronic Balance (Jinghua Tech Instrument Co. Ltd., Shanghai, China), KQ-250B Ultrasonic Cleaner (Kunshan Ultrasonic Instrument Co. Ltd., China); RCT-3200 Water Purification System (Changchun Laibopate Technology Development Co. Ltd., China); High Performance Liquid Chromatograph (Waters Model 1525 Binary HPLC Pump, Waters 2998 Diode Array Detector, Waters, USA); Empower 3 Chromatography Workstation (Waters, USA); Unitary C_{18} analytical column (250 mm × 4.6 mm, 5 μm).

7.3 Experimental Methods

7.3.1 Determination of Total Ginsenoside Content

7.3.1.1 Preparation of Reference Solution

Dissolve an accurately weighed quantity of Re reference substances in methanol to produce a solution of 1.0 mg of references per mL.

7.3.1.2 Preparation of Test Solutions

Ginseng was, respectively, air-dried, grinded, and sieved (Chinese National Standard Sieve No. 3, R40/3 series) to get the homogeneous powder. The fine powder was accurately weighed about 1 g. The weighted powder was then extracted with 100 mL of water at 100 °C, 2 h for the first time and 1.5 h for the second time. After filtered, the filtrates were combined and were added to D101 macroporous resin column (2.9 cm × 20 cm). The adding rate was 2 mL/min. After adsorbed on resin for 4 h, water was used to wash the column till colorless. Then 80% ethanol, till to no saponin reaction. The 80% ethanol eluent was concentrated, dried to dryness. The residue was dissolved and diluted with methanol to 10.0 mL, mix well and filter (0.45 μm), the successive filtrate as the test sample 1 and 2 in parallel.

7.3.1.3 Drawing of 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.

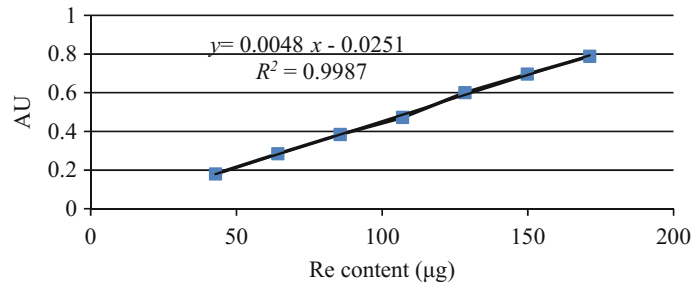

Fig. 7.1 The calibration curve of absorbance-Re levels

Table 7.1 The absorbance of Re standard substance

Standard solution volume (μL)	40	60	80	100	120	140	160
Content of Re (μg)	42.8	64.2	85.6	107.0	128.4	149.8	171.2
Absorption 1	0.180	0.284	0.382	0.472	0.600	0.695	0.787
Absorption 2	0.180	0.284	0.385	0.472	0.600	0.696	0.788
Average absorption	0.180	0.284	0.384	0.472	0.600	0.696	0.788
Regression equation	$y = 0.0048x - 0.0250$ ($r = 0.9993$)						

Measured accurately 40 μL, 60 μL, 80 μL, 100 μL, 120 μL, 140 μL, 160 μL of the reference solution, respectively, to a test tube with stopper, diluted with water to 200 μL, mixed well, added accurately 0.5 mL of 1% vanillin perchloric acid test solution, heated in a water bath at 60 °C for 15 min, allowed to cool immediately in an ice water bath for 2 min, added 5.0 mL of 77% sulfuric acid solution, shaked well. Measured the absorbance at 540 nm (Chinese Pharmacopoeia, 2015 edition, general rule 0401) with the corresponding reagent as a blank. Calculated the linear regression equation from the absorbances (y) obtained versus the concentrations (x) of the reference solution (Fig. 7.1). The calibration curve is shown in Table 7.1, indicating the method had good linearity in the range of 42.8–171.2 μg.

7.3.1.4 Determination of Total Ginsenosides Content

Repeated the operation, using the substance being examined, 50 μL of the test solution was accurately measured, instead of the reference solutions, and calculated the concentration of total ginsenoside obtained from the equation. The concentration of the total ginsenoside in ginseng was calculated by multiplying with 0.84. The content of the total ginsenosides was the average value of test parallel samples.

7.3.2 Determination of Monomer Ginsenoside Contents

7.3.2.1 Preparation of Reference Solution

Reference solution was prepared by dissolving a quantity of nineteen ginsenosides, previously dried to constant weight, accurately weighed, in methanol to produce a solution, as reference solution. The concentration and retention time in HPLC chromatogram of each ginsenoside are shown in Table 7.2.

7.3.2.2 Preparation of Test Solutions

As same as the preparation of test solution in Sect. 7.3.1.2.

7.3.2.3 Selection of Chromatographic Conditions

The reversed-phase high performance liquid chromatography (RP-HPLC), the most commonly used method, was selected to separate and detect the ginsenosides, the moderately polar compounds. Nineteen ginsenoside reference substances selected in this study all have the

Table 7.2 Concentration and retention time of 19 monomer ginsenosides (sapogenin)

Name of ginsenoside	Concentration (μg/μL)	Retention time (min)
Rg_1	0.24	25.44
Re	0.24	26.76
Rf	0.30	64.50
Rh_1	0.30	75.43
Rg_2	0.22	76.14
Rb_1	0.50	85.29
Rc	0.20	88.95
Ro	0.20	90.00
F_1	0.26	91.10
Rb_2	0.28	92.61
Rb_3	0.16	94.14
Rd	0.28	99.74
20(*S*)-Rg_3	0.11	110.20
20(*R*)-Rg_3	0.14	110.68
PPT	0.21	113.11
F_2	0.28	117.41
Rh_2	0.46	118.53
Oleanolic acid	0.21	130.16
PPD	0.21	131.79

characteristic absorption at 203 nm. Acetonitrile, instead of methanol, was selected as the organic solvent in mobile phase due to the little interference at 203 nm. When acetonitrile-ultrapure water was used as mobile phase, the peaks of Rc, F_1, Rb_2, and Rb_3 were tailed and asymmetrical. Meanwhile, ginsenoside Ro, the characteristic component of ginseng root, was not detected well under the condition. According to the literature, it was found that the tailed peak of ginsenoside Ro, the oleanolic acid type ginsenoside, might be caused by the interaction of the carbonyl group with the stationary phase in chromatographic column. Finally, the 0.05% phosphoric acid was added to the mobile phase of the water phase to weaken the interaction between the compounds and the stationary phase and to improve the separation effect and the peak shape. The results showed that the sharp peak of Ro appeared, and the tailing peaks of Rc, F_1, Rb_2, and Rb_3 were improved greatly. So, the chromatographic condition of 19 monomer ginsenosides is as follows:

Waters 1525 High Performance Liquid Chromatograph (Waters, USA); Waters 2998 Diode Array Detector (Waters, USA); Column: Unitary C_{18} (4.6 mm × 250 mm, 5 μm); Column temperature: 30 °C; Detection wavelength: 203 nm; Flow rate: 1.3 mL/min; Mobile phase: gradient elution with acetonitrile (A)—0.05% aqueous phosphoric acid (B). The gradient elution procedure is shown in Table 7.3, with all components peaked within 135 min.

7.3.2.4 Method Validation

The optimized method was validated by evaluating linearity, accuracy, precision, stability, and recovery.

Linearity was evaluated from the calibration curve obtained after analyzing standard solutions in increasing order of concentrations (4 μL, 6 μL, 10 μL, 15 μL, 20 μL, respectively). The abscissa (x) was the contents (μg) and the ordinate (y) was the peak areas of reference substance. The standard curve was drawn to obtain the regression equation as shown in Table 7.4. The chromatograms of reference substance and 5-year-old samples from Antu are shown in Figs. 7.2 and 7.3, respectively.

Precision was evaluated by analyzing the reference solution five times ($n = 5$). The precision was expressed as the relative standard deviation

Table 7.3 Gradient elution program of HPLC

Time (min)	Phase A (%)	Phase B (%)
0–14	20 → 21	80 → 79
14–24	21 → 22	79 → 78
24–36	22 → 23	78 → 77
36–52	23 → 25	77 → 75
52–55	25 → 28	75 → 72
55–78	28 → 30	72 → 70
78–96	30 → 35	70 → 65
96–112	35 → 60	65 → 40
112–127	60 → 90	40 → 10
127–134	90 → 100	10 → 0
134–138	100	0
138–139	100 → 20	0 → 80

Table 7.4 Regression equations with correlation coefficients of 19 monomer ginsenosides

Name of ginsenoside	Regression equation	Correlation coefficient
Rg_1	$y = 184298x + 32631$	0.9949
Re	$y = 482551x - 32854$	0.9994
Rf	$y = 244310x - 21295$	0.9996
Rh_1	$y = 332110x - 186009$	0.9764
Rg_2	$y = 488636x - 47502$	0.9998
Rb_1	$y = 209461x - 59556$	0.9991
Rc	$y = 336372x + 1684.4$	0.9993
Ro	$y = 314065x + 7629.2$	0.9980
F_1	$y = 243529x + 26805$	0.9998
Rb_2	$y = 357689x + 7374$	0.9990
Rb_3	$y = 450705x + 11464$	0.9988
Rd	$y = 189484x - 13213$	0.9996
20(*S*)-Rg_3	$y = 561904x - 21244$	0.9972
20(*R*)-Rg_3	$y = 330046x + 13333$	0.9996
PPT	$y = 151996x + 7562.1$	0.9951
F_2	$y = 870097x - 55070$	0.9999
Rh_2	$y = 282995x - 2520.4$	0.9997
Oleanolic acid	$y = 690881x - 35931$	0.9997
PPD	$y = 445875x + 32102$	0.9967

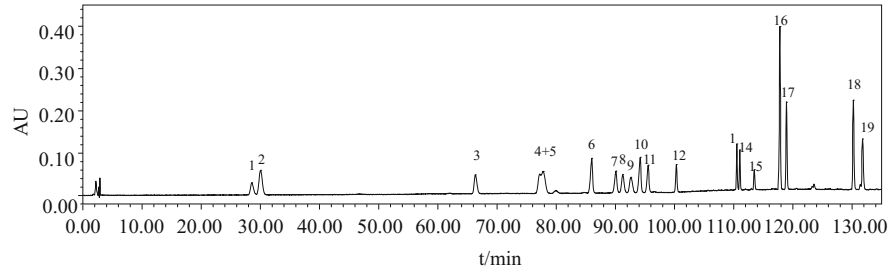

Fig. 7.2 HPLC chromatogram of nineteen ginsenosides standard substances. (1) Rg_1; (2) Re; (3) Rf; (4) S-Rh_1; (5) Rg_2; (6) Rb_1; (7) Rc; (8) Ro; (9) F_1; (10) Rb_2; (11) Rb_3; (12) Rd; (13) 20S-Rg_3; (14) 20R-Rg_3; (15) PPT; (16) F_2; (17) Rh_2; (18) oleanolic acid; (19) PPD

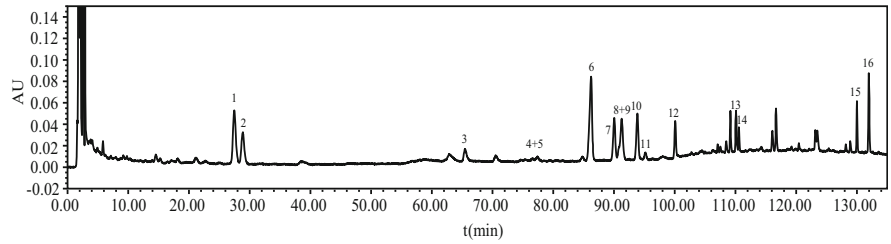

Fig. 7.3 HPLC chromatogram of ginsenosides from 5-year-old ginseng in Antu. (1) Rg_1; (2) Re; (3) Rf; (4) S-Rh_1; (5) Rg_2; (6) Rb_1; (7) Rc; (8) Ro; (9) F_1; (10) Rb_2; (11) Rb_3; (12) Rd; (13) 20S-Rg_3; (14) 20R-Rg_3; (15) oleanolic acid; (16) PPD

(RSD) of each peak area of each ginsenoside. The results showed that the RSD was from 0.86% to 1.87%.

Accuracy was evaluated by analyzing the same test sample solution in replicates of five samples ($n = 5$). The accuracy was expressed as RSD of the total amount of 19 ginsenosides. The results showed that the RSD was 2.97%.

Stability was investigated by analyzing the same test sample solution at 0 h, 4 h, 8 h, 12 h, and 24 h, respectively. The stability was expressed as RSD of the total amount of 19 ginsenosides. The results showed that the RSD was 1.03%.

Recovery was accessed by comparing the contents of 19 ginsenosides before and after extraction at three levels in three replicates. The recovery was expressed as recovery rates and the RSD of recovery rates. The results showed that the recovery rates were in the range of 94.1–103.4%, with the RSD being 0.97–5.32%.

7.3.2.5 Determination of Monomeric Ginsenoside Content

Injected 15 μL of the test solutions, respectively, into the column and recorded the chromatogram. The peak areas of ginsenosides in the sample were all within the linear range of the standard curve. Compared with the linear regression equation of ginsenoside peak area-content, the contents of 19 monomeric ginsenosides were calculated. The results and analysis are shown in Part 2.

7.4 Results and Discussions

7.4.1 The Analysis of Total Ginsenoside Contents of Ginseng

7.4.1.1 Total Ginsenoside Contents of Ginseng from Different Regions with Different Cultivation Periods

Total ginsenoside contents of ginseng from different regions with different cultivation periods are shown in Table 7.5.

7.4.1.2 Result Analysis

The total ginsenoside contents of ginseng varied greatly, which are shown in Table 7.5 and Fig. 7.4.

7.4.1.2.1 The Analysis of the Mean Value of Total Ginsenosides of Ginseng with Different Cultivation Periods

The average content of total saponins in all samples was 2.86%. The average contents of total saponins in 4-, 5-, and 6-year-old ginseng were 2.83%, 2.90%, and 2.84%, respectively, with RSD (%) of 19.92%, 19.92%, and 11.04%. The results showed that the ginseng with the high-to-low total saponin contents was 5-, 6-, and 4-year-old ginseng, as shown in Table 7.5.

Table 7.5 Total ginsenoside contents of ginseng from different regions with different cultivation periods (%)

Region	Content of total ginsenosides (%)		
	4-year-old	5-year-old	6-year-old
Heihe	3.08	–	–
Hulin	2.21	–	–
Antu	3.35	3.55	–
Changbai	3.47	2.03	3.19
Dunhua	2.02	2.60	-
Fusong	2.26	2.79	3.12
Hunchun (CC)	2.74	–	–
Hunchun	3.54	2.78	2.55
Huadian	4.24	4.56	–
Helong	2.38	3.40	–
Dadi Ji'an	–	2.63	2.59
Ji'an (CC)	2.33	2.50	2.59
Jiaohe	2.81	3.03	–
Jingyu	2.80	3.03	–
Linjiang	–	3.00	2.75
Tonghua	2.91	3.01	–
Wangqing	2.79	3.04	3.45
Kuandian	3.12	2.28	2.67
Xinbin	2.52	–	–
North Korea	2.43	2.40	2.94
South Korea	-	2.59	2.58
Average $\bar{x}$	2.83	2.90	2.84
Standard deviation SD	0.56	0.58	0.31
$\bar{x} \pm$ SD	2.83 ± 0.56	2.90 ± 0.58	2.84 ± 0.31
Relative standard deviation RSD (%)	19.92	19.92	11.04

Note: "–" not detected

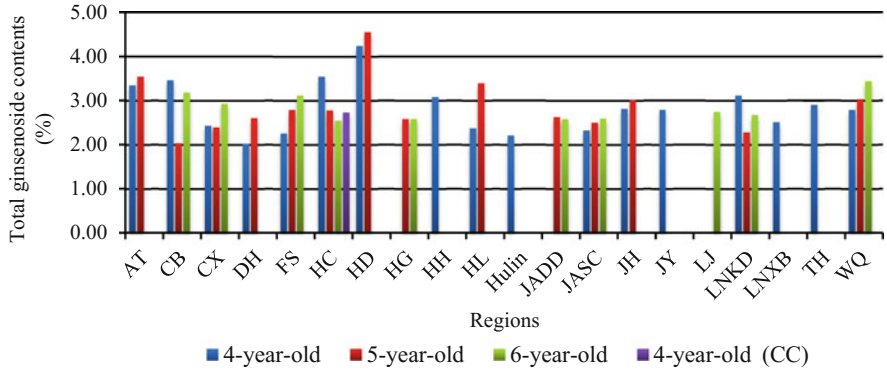

Fig. 7.4 The content accumulation histogram of total ginsenosides of ginseng from different regions with different cultivation periods

Table 7.6 The trend of total ginsenoside contents of ginseng from different regions with two or three cultivation periods (%)

Cultivation periods	No.	Regions	Content (%)
2	1	Antu, Dunhua, Huadian, Helong, Jiaohe, Jingyu, Tonghua	$CG_5>CG_4$
	2	Dadi Ji'an, Linjiang, South Korea	$CG_5>CG_6$
3	3	Changbai, Kuandian	$CG_4>CG_6>CG_5$
	4	Fusong	$CG_6>CG_5>CG_4$
	5	Shuangcha Ji'an, Wang qing	$CG_6>CG_5>CG_4$
	6	North Korea	$CG_6>CG_4>CG_5$
	7	Hunchun	$CG_4>CG_5>CG_{4cc}>CG_6$

7.4.1.2.2 The Analysis of Total Ginsenosides of Ginseng from Same Regions with Different Cultivation Periods

It was shown that the trend of total ginsenoside contents of ginseng from the same region was irregular with different cultivation periods. The trend of total ginsenoside contents of ginseng from different regions with two or three cultivation periods (%) is shown in Table 7.6. It could be concluded that in the sampling regions with only two kinds of cultivation periods, 5-year-old ginseng samples have obvious advantage in the contents of total ginsenosides.

7.4.1.2.3 The Analysis of Total Ginsenosides from Continuous Cropping or Common Planting Ginseng

Continuous cropping refers to continuously cultivating the same crop in the same field. For the total ginsenosides content of ginseng collected from Hunchun, the content (3.54%) of 4-year-old ginseng was significantly higher than the content (2.74%) of 4-year-old ginseng continuous cropping.

7.4.1.2.4 The Analysis of Total Ginsenosides of Ginseng from Different Regions with Same Cultivation Periods

As shown in Table 7.5, three samples with the higher contents of total ginsenosides were the ginseng of Huadian 5-year-old (4.56%), Huadian 4-year-old (4.24%), and Antu 5-year-old (3.55%).

The areas of 4-year-old ginseng ranked in decreasing order based on the contents of total ginsenoside were: Huadian, Hunchun, Changbai, Antu, Kuandian, Heihe, Tonghua, Jiaohe, Jingyu, Wangqing, Hunchun (CC), Xinbin, North Korea, Helong, Shuangcha Ji'an, Fu Song, Hulin, Dunhua.

The areas of 5-year-old ginseng ranked in decreasing order based on the contents of total ginsenoside were: Huadian, Antu, Helong, Wangqing, Jiaohe/Jingyu, Tonghua, Linjiang, Fusong, Hunchun, Dadi Ji'an, Dunhua, South Korea, Shuangcha Ji'an, North Korea, Kuandian, Changbai.

The areas of 6-year-old ginseng ranked in decreasing order based on the contents of total ginsenoside were: Wang Qing, Changbai, Fusong, North Korea, Linjiang, Kuandian, Shuangcha Ji'an, Dadi Ji'an, South Korea, Hunchun.

The relationships between the total ginsenoside contents of ginseng from different regions with different cultivation periods are shown in Figs. 7.5, 7.6, and 7.7, respectively. The contents of total ginsenosides in ginseng from different regions with different cultivation periods are ranked in Figs. 7.8, 7.9, and 7.10.

7.4.2 The Analysis of 19 Monomer Ginsenoside Contents of Ginseng

7.4.2.1 Contents of 19 Monomer Ginsenosides (Including Sapogenins) of Ginsengs

The contents of 19 monomer ginsenosides (including sapogenins) of ginseng from different regions with different cultivation periods are shown in Table 7.7, 7.8, 7.9, 7.10, 7.11, 7.12, and 7.13.

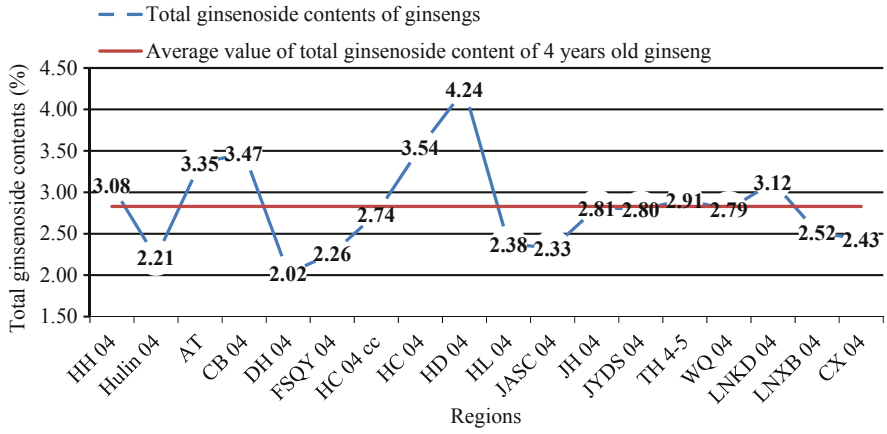

Fig. 7.5 Total ginsenoside contents of 4-year-old ginseng

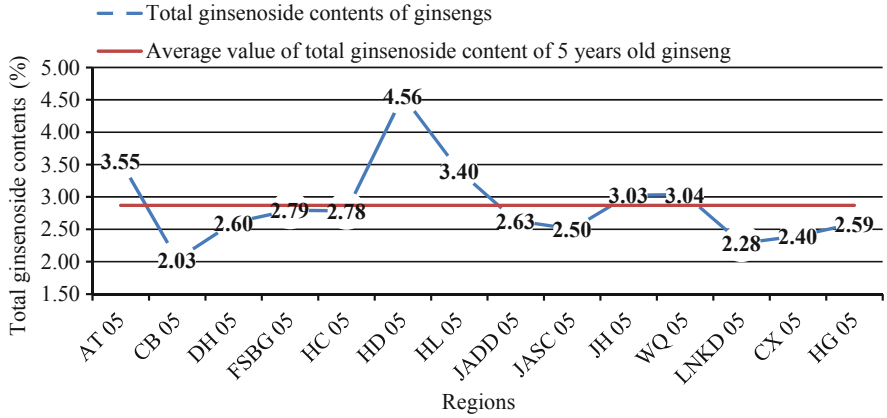

Fig. 7.6 Total ginsenoside contents of 5-year-old ginseng

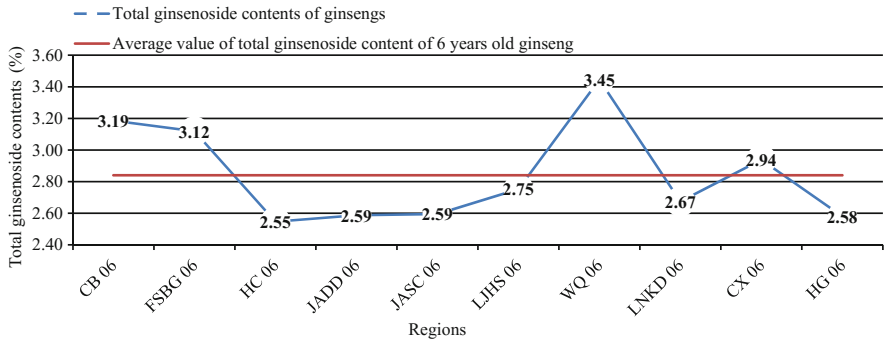

Fig. 7.7 Total ginsenoside contents of 6-year-old ginseng

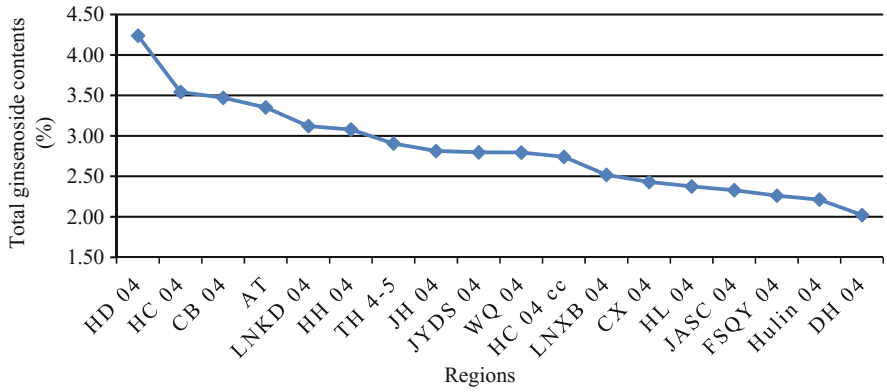

Fig. 7.8 Sequenced total ginsenoside contents of 4-year-old ginseng

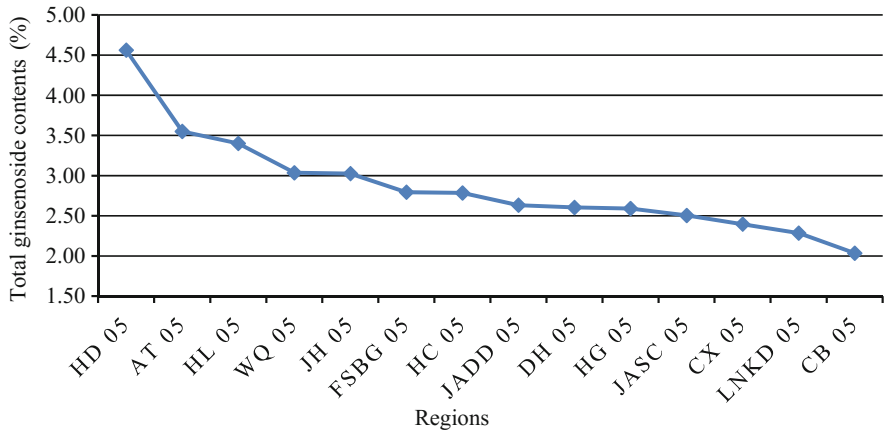

Fig. 7.9 Sequenced total ginsenoside contents of 5-year-old ginseng

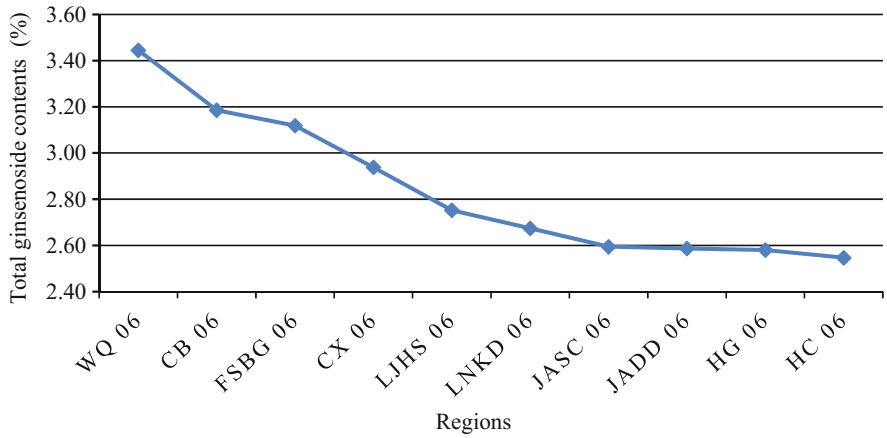

Fig. 7.10 Sequenced total ginsenoside contents of 6-year-old ginseng

Table 7.7 Contents of 19 monomer ginsenosides (including sapogenin) of ginseng from Heihe, Hulin, Antu, and Changbai (%)

	Heihe	Hulin	Antu		Changbai		
Ginsenoside	4-year-old	4-year-old	4-year-old	5-year-old	4-year-old	5-year-old	6-year-old
Rg_1	0.2775	0.3235	0.4260	0.4659	0.3615	0.3232	0.3442
Re	0.1165	0.1251	0.1309	0.1061	0.1199	0.0958	0.1267
Rf	0.0781	0.0773	0.1101	0.0908	0.0928	0.0806	0.0931
Rh_1+Rg_2	0.0272	0.1165	0.0343	0.0230	0.1092	0.0419	0.0729
Rb_1	0.3741	0.5152	0.5928	0.6145	0.5041	0.3671	0.5863
Rc	0.1516	0.1914	0.1684	0.1412	0.1649	0.1598	0.1602
Ro	0.0195	0.0170	0.1888	0.0017	0.1137	0.0102	0.0267
F_1	0.0166	0.0797	0.0005	0.1581	0.0017	0.0369	0.1656
Rb_2	0.1481	0.1971	0.1879	0.1497	0.1678	0.1553	0.1560
Rb_3	0.0135	0.0214	0.0197	0.0152	0.0159	0.0105	0.0179
Rd	0.1824	0.1940	0.2025	0.1546	0.2443	0.2186	0.1702
20(*S*)-Rg_3	0.0020	0.0021	0.0020	0.0021	0.0022	0.0021	0.0020
20(*R*)-Rg_3	0.0552	0.0151	0.0252	0.0342	0.0119	0.0388	0.0326
PPT	–	–	–	–	–	–	–
F_2	–	–	–	–	–	–	–
Rh_2	0.0061	–	–	–	–	–	–
Oleanolic acid	0.0123	0.0079	0.0117	0.0377	0.0169	0.0259	0.0125
PPD	0.0411	0.0444	0.0776	0.0958	0.1102	0.0890	0.0852

Note: "–" not detected

Table 7.8 Contents of 19 monomer ginsenosides (including sapogenin) of ginseng from Dunhua and Fusong (%)

	Dunhua		Fusong		
Ginsenoside	4-year-old	5-year-old	4-year-old	5-year-old	6-year-old
Rg_1	0.3056	0.3591	0.4216	0.5091	0.5413
Re	0.0913	0.1191	0.1343	0.1511	0.1708
Rf	0.0747	0.0940	0.0860	0.1147	0.1075
Rh_1+Rg_2	0.0345	0.0403	0.0383	0.0486	0.0836
Rb_1	0.3886	0.5104	0.6493	0.8329	1.2168
Rc	0.1049	0.1648	0.1674	0.2086	0.2551
Ro	–	0.1428	0.0210	0.0202	0.0321
F_1	–	–	0.0858	0.1049	0.2697
Rb_2	0.1060	0.1779	0.1789	0.2199	0.2742
Rb_3	0.0096	0.0186	0.0190	0.0226	0.0313
Rd	0.1425	0.2520	0.1680	0.2340	0.3780
20(*S*)-Rg_3	0.0020	0.0020	0.0027	0.0028	0.0027
20(*R*)-Rg_3	0.0171	0.0212	0.0261	0.0317	0.0575
PPT	–	–	–	–	–
F_2	–	–	–	–	–
Rh_2	–	–	–	–	–
Oleanolic acid	0.0060	0.0084	0.0079	0.0088	0.0056
PPD	0.0622	0.1069	0.1027	0.0786	0.0765

Note: "–" not detected

Table 7.9 Contents of 19 monomer ginsenosides (including sapogenins) of ginseng from Hunchun and Huadian (%)

	Hunchun				Huadian	
Ginsenoside	4-year-old	5-year-old	6-year-old	4-year-old (CC)	4-year-old	5-year-old
Rg_1	0.4129	0.3952	0.3842	0.4188	0.1957	0.1873
Re	0.1361	0.1211	0.1233	0.0742	0.5210	0.4261
Rf	0.1009	0.0878	0.0969	0.0729	0.0709	0.0419
Rh_1+Rg_2	0.0799	0.0303	0.0607	0.0204	0.0409	0.0448
Rb_1	0.5234	0.5801	0.6064	0.4254	2.8742	2.5193
Rc	0.2059	0.1885	0.1906	0.1130	0.2110	0.1139
Ro	0.0251	0.0170	0.0147	0.0086	–	0.0999
F_1	0.0139	0.1227	0.1232	0.0985	0.1048	–
Rb_2	0.2103	0.1947	0.1996	0.1085	0.0257	0.0100
Rb_3	0.0251	0.0206	0.0211	0.0119	0.0312	0.0186
Rd	0.2455	0.2454	0.2022	0.1636	0.4422	0.4006
20(*S*)-Rg_3	0.0021	0.0021	0.0571	0.0021	0.0027	0.0021
20(*R*)-Rg_3	0.0504	0.0485	0.0247	0.0185	0.0291	–
PPT	0.0012	–	–	–	–	–
F_2	–	–	–	–	–	–
Rh_2	–	–	–	–	–	–
Oleanolic acid	0.0082	0.0074	–	0.0092	0.0224	0.0190
PPD	0.1108	0.0811	–	0.0986	0.1477	0.0972

Note: "–" not detected

Table 7.10 Contents of 19 monomer ginsenosides (including sapogenins) of ginseng from Helong, Dadi Ji'an, and Shuangcha Ji'an (%)

	Helong		Dadi Ji'an		Shuangcha Ji'an		
Ginsenoside	4-year-old	5-year-old	5-year-old	6-year-old	4-year-old	5-year-old	6-year-old
Rg_1	0.4217	0.6080	0.5158	0.4136	0.3080	0.2761	0.4361
Re	0.1220	0.1773	0.1240	0.1058	0.1053	0.1058	0.1201
Rf	0.0991	0.1451	0.0962	0.0959	0.0645	0.0978	0.1188
Rh_1+Rg_2	0.0297	0.1418	0.0801	0.4536	0.0573	0.0526	0.0705
Rb_1	0.4813	0.9852	0.6475	0.5495	0.4852	0.4192	0.7452
Rc	0.1755	0.2973	0.1533	0.1544	0.1525	0.1258	0.1773
Ro	0.0476	0.2213	0.0459	0.0384	0.0310	0.0347	0.0402
F_1	0.1601	–	0.1662	0.1116	0.0187	0.1719	0.3663
Rb_2	0.1689	0.3233	0.1350	0.1416	0.1518	0.1092	0.1882
Rb_3	0.0174	0.0328	0.0171	0.0119	0.0131	0.0094	0.0219
Rd	0.2394	0.3879	0.1065	0.1321	0.1914	0.1047	0.1799
20(*S*)-Rg_3	0.0301	0.0020	0.0020	0.0021	0.0020	0.0020	0.0027
20(*R*)-Rg_3	0.0384	0.0412	0.0324	0.0602	0.0172	0.0440	0.0370
PPT	–	–	–	–	–	–	–
F_2	–	–	–	0.0068	–	–	–
Rh_2	–	–	–	–	–	–	–
Oleanolic acid	0.0207	0.0230	0.0217	0.0161	0.0234	0.0124	0.0223
PPD	0.0513	0.0534	0.0669	0.1026	0.0375	0.0555	–

Note: "–" not detected

Table 7.11 Contents of 19 monomer ginsenosides (including sapogenins) of ginseng from Jiaohe, Jingyu, Linjiang, and Tonghua (%)

	Jiaohe		Jingyu	Linjiang	Tonghua
Ginsenoside	4-year-old	5-year-old	4-year-old	6-year-old	5-year-old
Rg_1	0.4509	0.6103	0.3775	0.3897	0.3794
Re	0.1397	0.1779	0.1373	0.0964	0.1564
Rf	0.0970	0.1457	0.1192	0.0824	0.1015
Rh_1+Rg_2	0.0286	0.1423	0.2128	0.1545	0.0478
Rb_1	0.7157	0.9888	0.5741	0.6167	0.6362
Rc	0.1708	0.2984	0.1698	0.1166	0.1999
Ro	0.0544	0.2221	0.1655	0.0054	0.0275
F_1	0.1210	–	–	0.2444	0.1466
Rb_2	0.1629	0.3245	0.1765	0.1346	0.2109
Rb_3	0.0195	0.0330	0.0213	0.0180	0.0218
Rd	0.1720	0.3893	0.1760	0.1314	0.2284
20(*S*)-Rg_3	0.0020	0.0020	0.0020	0.0027	0.0020
20(*R*)-Rg_3	0.0374	0.0413	0.0322	0.0285	0.0223
PPT	0.0203	–	–	–	0.0138
F_2	–	–	–	–	–
Rh_2	–	–	–	–	–
Oleanolic acid	0.0190	0.0231	0.0077	0.0079	0.0176
PPD	0.0704	0.0536	0.0428	0.0659	0.0671

Note: "–" not detected

Table 7.12 Contents of 19 monomer ginsenosides (including sapogenins) of ginseng from Wangqing and Kuandian (%)

	Wangqing			Kuandian		
Ginsenoside	4-year-old	5-year-old	6-year-old	4-year-old	5-year-old	6-year-old
Rg_1	0.3760	0.5048	0.4617	0.5060	0.3108	0.4410
Re	0.1105	0.1110	0.1384	0.2130	0.1491	0.1729
Rf	0.1477	0.1270	0.1270	0.1167	0.0850	0.1269
Rh_1+Rg_2	0.0703	0.1302	0.1053	0.2004	0.1191	0.1621
Rb_1	0.4720	0.6030	0.5853	1.0047	0.4793	0.6736
Rc	0.1439	0.1898	0.1952	0.2795	0.1750	0.2317
Ro	0.0421	0.0400	0.0317	0.0735	0.0701	0.0989
F_1	0.1242	0.1797	0.0960	0.1989	0.0760	0.2541
Rb_2	0.1445	0.1904	0.1986	0.2866	0.1618	0.2121
Rb_3	0.0135	0.0198	0.0187	0.0292	0.0193	0.0233
Rd	0.2799	0.2912	0.3693	0.3475	0.2409	0.2508
20(*S*)-Rg_3	0.0020	0.0021	0.0021	0.0027	0.0020	0.0027
20(*R*)-Rg_3	0.0240	0.0356	0.0739	0.0689	0.0331	0.0369
PPT	–	–	–	0.0213	0.0087	–
F_2	–	–	–	–	0.0050	–
Rh_2	–	–	–	–	0.0071	–
Oleanolic acid	0.0159	0.0184	0.0160	0.0076	0.0068	0.0078
PPD	0.0632	0.1023	0.0640	0.0647	0.0733	0.1004

Note: "–" not detected

Table 7.13 Contents of 19 monomer ginsenosides (including sapogenins) of ginseng from Xinbin, North Korea, and South Korea (%)

	Xinbin	North Korea			South Korea	
Ginsenoside	4-year-old	4-year-old	5-year-old	6-year-old	5-year-old	6-year-old
Rg_1	0.4146	0.3491	0.4726	0.4279	0.5612	0.3691
Re	0.1777	0.2239	0.1583	0.1617	0.1440	0.1160
Rf	0.1123	0.1294	0.1400	0.1072	0.1230	0.0933
Rh_1+Rg_2	0.1922	0.1113	0.0575	0.0311	0.0505	0.0205
Rb_1	0.6476	0.8640	0.7075	0.6527	0.7547	0.5705
Rc	0.2131	0.2261	0.1961	0.1442	0.1665	0.1674
Ro	0.0683	0.1626	0.1785	0.0538	0.0360	0.0510
F_1	0.0791	0.0070	–	0.1038	–	–
Rb_2	0.2095	0.2320	0.2152	0.1536	0.1758	0.1593
Rb_3	0.0196	0.0272	0.0221	0.0172	0.0203	0.0180
Rd	0.2564	0.2553	0.2651	0.1728	0.1178	0.0996
20(*S*)-Rg_3	0.0027	0.0020	0.0020	0.0020	0.0022	0.0022
20(*R*)-Rg_3	0.0380	0.0327	0.0437	0.0299	0.0526	0.0790
PPT	0.0170	–	–	–	0.0298	0.0153
F_2	–	–	–	–	0.0066	0.0078
Rh_2	–	–	–	–	0.0050	0.0050
Oleanolic acid	0.0094	0.0120	0.0120	0.0154	0.0057	0.0058
PPD	0.0650	0.0033	0.0443	0.0405	0.3810	0.1540

Note: "–" not detected

7.4.2.2 Result Analysis

7.4.2.2.1 The Type of Measured Monomer Ginsenosides

According to the structural skeleton, ginsenosides could be divided into three categories: dammarane type, ocotillol type, and oleanolic acid type. And the dammarane type ginsenosides could be further divided into protopanaxadiol subtype and protopanaxatriol subtype, the difference between the two subtypes is that whether there is –OH at C-6. Protopanaxatriol subtype has the –OH at C-6, while protopanaxadiol subtype has no –OH at C-6. Different types of ginsenosides have different properties, which are of great significance to the pharmacological activities of ginseng. Ocotillol type ginsenoside is the characteristic component of *Panax quinquefolium,* it has not appeared in *Panax ginseng*. The skeleton structures of various types of ginsenosides are shown in Fig. 7.11.

The ginsenosides (or sapogenins) analyzed in this book are as follows: the protopanaxadiol subtype (ginsenoside Rb_1, Rb_2, Rb_3, Rc, Rd, Rg_3, Rh_2, F_2, and PPD), the protopanaxatriol subtype (ginsenoside Re, Rf, Rg_1, Rg_2, Rh_1, F_1, and PPT), and oleanolic acid type (ginsenoside Ro and oleanolic acid).

7.4.2.2.2 The Ratios of Monomer Ginsenoside Content of Ginseng

The contents of 19 monomer ginsenosides (or sapogenins) in ginseng were determined. The results showed that ginsenoside Rg_1, Re, Rf, Rg_2, Rh_1, Rb_1, Rc, Rb_2, Rb_3, Rd, 20(S)-Rg_3 were all existed in ginseng. Most of the samples contained ginsenoside Ro, F1, 20(R)-Rg3, oleanolic acid, and sapogenin PPD. The detection probability of ginsenoside F_2, Rh_2, and PPT was slightly lower, only appearing in ginseng samples from Kuandian and South Korea with 5 years cultivation period, South Korea with 6 years cultivation period.

As shown in Table 7.7 and Fig. 7.12, there were abundant monomer ginsenosides in ginseng. Among the 17 ginsenosides and 2 sapogenins, the contents of ginsenosides Rb_1, Rg_1, Rd, Rc, and Rb_2 were all very high, reaching a maximum of 28.74 mg/g (Rb_1 in Huadian 4-year-old ginseng). The contents of ginsenosides varied greatly with

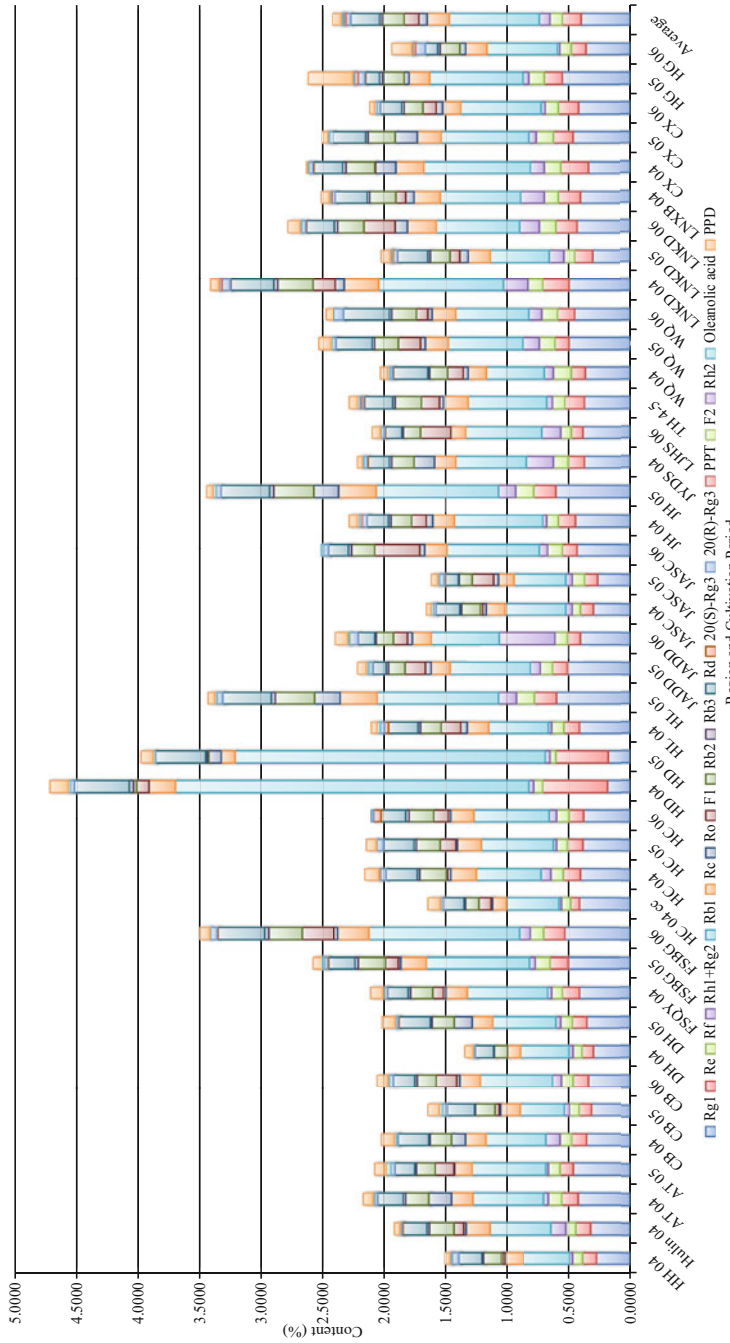

Fig. 7.11 Structural skeletons of ginsenosides

the regions and periods of ginseng. The contents of sapogenin PPT, ginsenosides F_2 and Rh_2 were lower than 0.373 mg/g and were not detected in some samples. The average proportions of monomer saponins are shown in Fig. 7.13.

7.4.2.2.3 The Analysis of Monomer Ginsenosides of Ginseng from Different Regions

1. The contents of monomer ginsenosides in 4-year-old ginseng samples from different

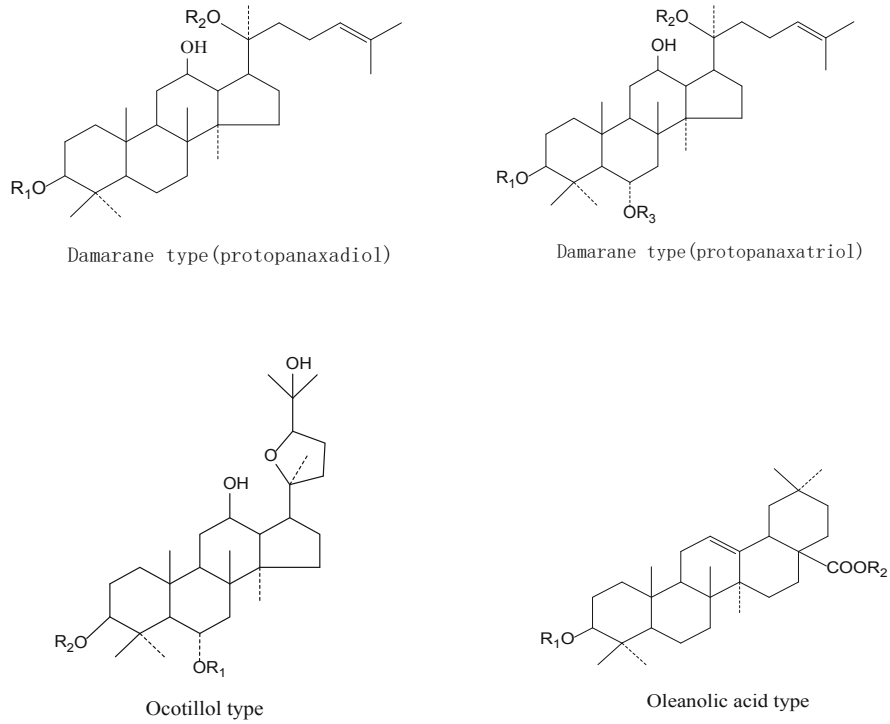

Fig. 7.12 The content accumulation histogram of 19 monomer ginsenosides (or sapogenins) of ginseng from different regions with different cultivation periods

regions were different, just as shown in Figs. 7.14, 7.15, 7.16, and 7.17. The contents of Re, Rd, PPD, and Rb_1 were significantly higher in ginseng from Huadian 4-year-old ginseng, while the contents of Rg_1 and Rb_2 were significantly lower. The contents of Rd, Rb2, Rc, 20(R)-Rg_3, Rb_1, Rg_1, Re, Rh_1 + Rg_2, F_1, and PPT in Kuandian 4-year-old ginseng were higher. The contents of Rb_2, Rd, Rg_1, and Ro fluctuated greatly with different regions, while the contents of other ginsenosides fluctuated slightly.

2. The contents of monomer saponins in 5-year-old ginseng samples from different areas are different, just as shown in Figs. 7.18, 7.19, 7.20, and 7.21. Among them, the contents of Rd, Rb_1, and Re in 5-year-old ginseng of Fusong were significantly higher, while the contents of Rc, Rb_2, Rg_1, and Rf were significantly lower and more special; the contents of Rg_1 and PPD in 4, 5-year-old ginseng samples of Tonghua were significantly higher, the contents of Rb_2, Rd, Rg_1, and Ro fluctuated greatly with different origins, while the contents of other saponins fluctuated slightly.

3. The contents of monomer ginsenosides in 6-year-old ginseng samples from different regions were different, just as shown in Figs. 7.22, 7.23, 7.24, and 7.25. Ginsenoside Rd, one of the main metabolites of protopanoxadiol type ginsenoside, has the unique effects on cardio-cerebrovascular, nervous, and immune system and plays a strong role in analgesic and neuroprotective effects. So the high content of Rd has practical significance. The results showed that 6-year-old ginseng samples from Hulin had the highest content of Rd. The contents of Rb_1, Rb_2, Rc,

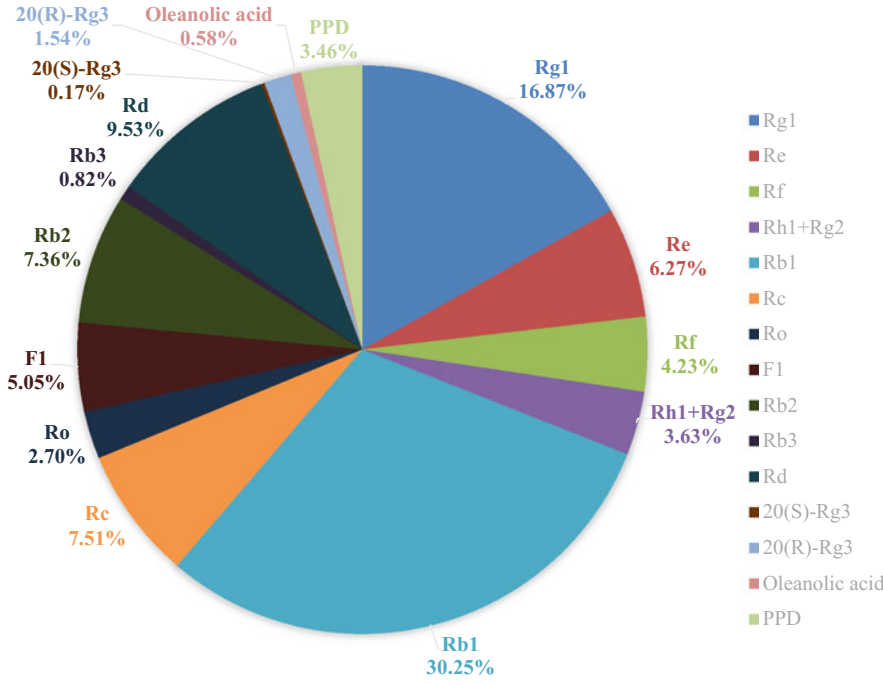

Fig. 7.13 Average proportions of main ginsenosides(or sapogenins)

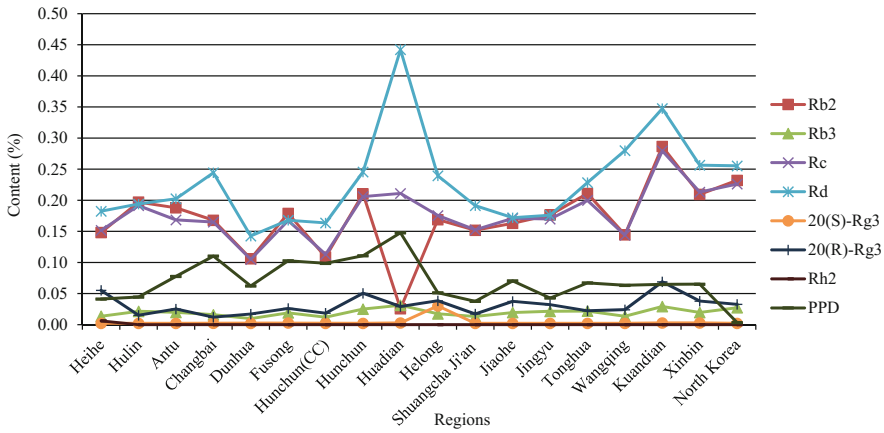

Fig. 7.14 Content variation of PPD subtype ginsenosides in 4-year-old ginseng from different regions (Rb_1 excepted)

Rg_1, and F_1 were also higher. The contents of Rd and F_1 were significantly higher in Hunchun continuous-cropping ginseng samples, and the contents of Rh_1+Rg_2 in Changbai 6-year-old ginseng samples were significantly higher. The contents of Ro in Hunchun 6-year-old ginseng samples were significantly higher.

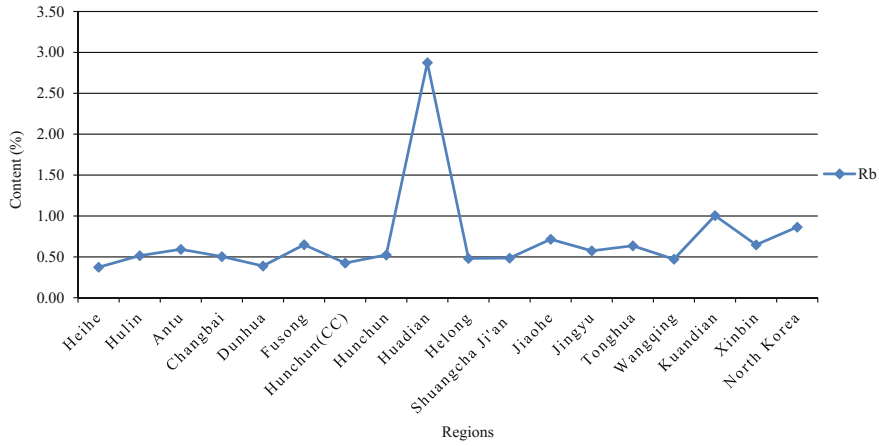

Fig. 7.15 Content variation of Rb_1 in 4-year-old ginseng from different regions

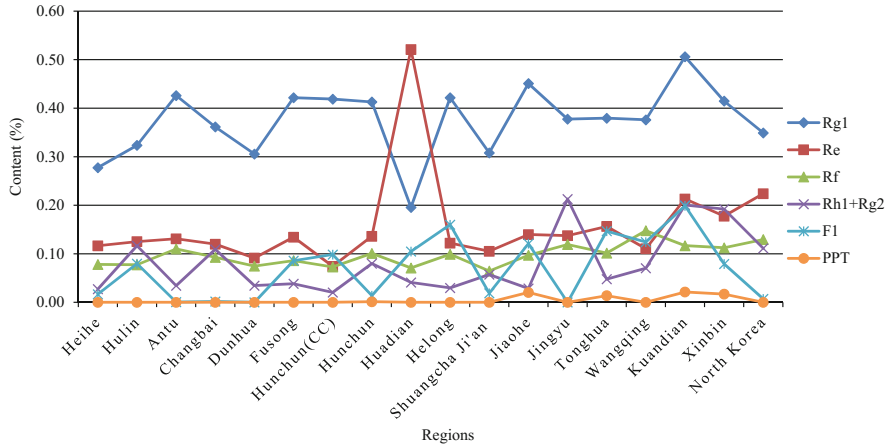

Fig. 7.16 Content variation of PPT subtype ginsenosides of 4-year-old ginseng from different regions

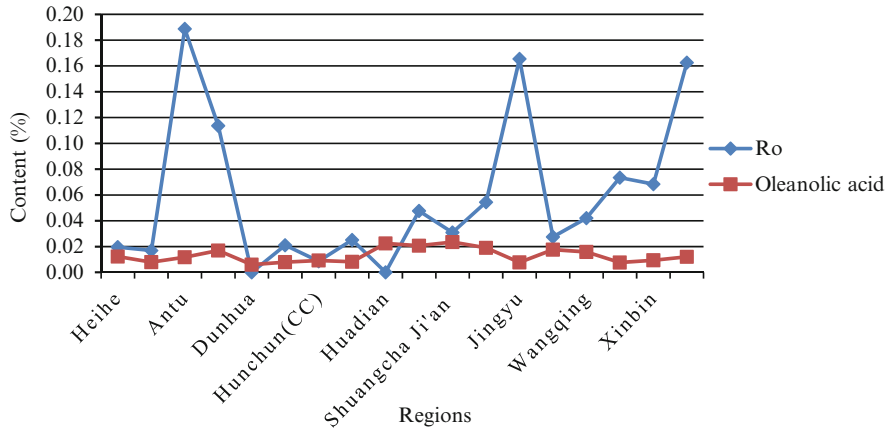

Fig. 7.17 Content variation of oleanolic acid type ginsenosides of 4-year-old ginseng from different regions

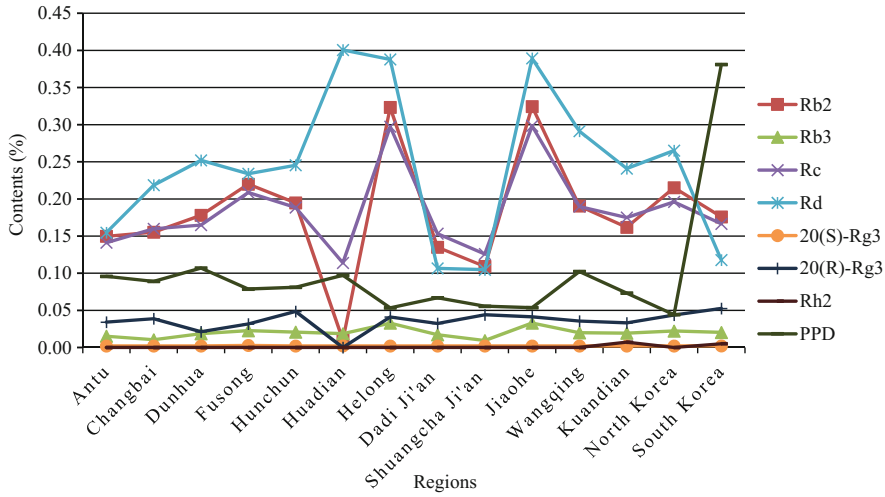

Fig. 7.18 Content variation of PPD type ginsenosides (or sapogenin) of 5-year-old ginseng from different regions (Rb_1 excepted)

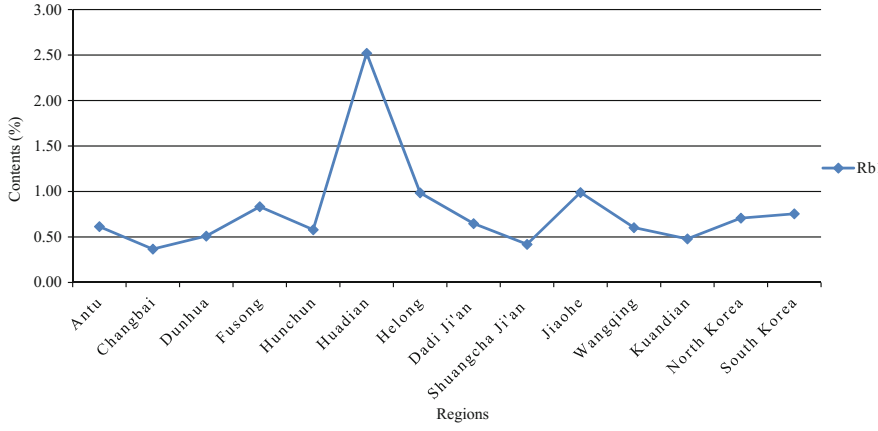

Fig. 7.19 Content variation of Rb_1 of 5-year-old ginseng from different regions

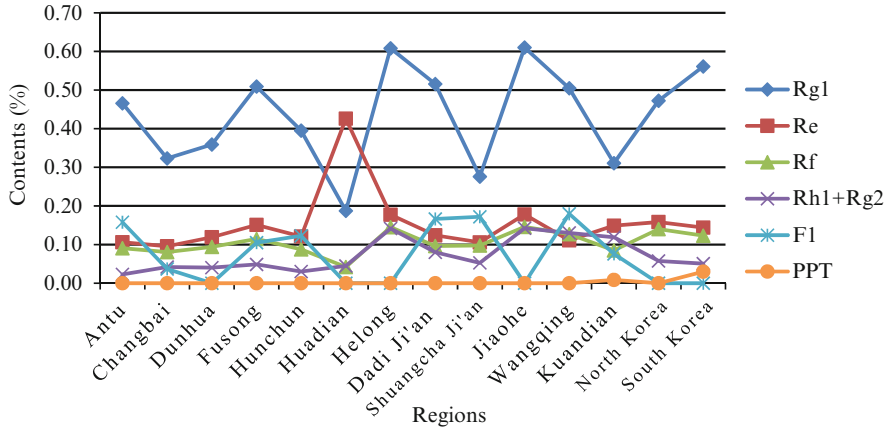

Fig. 7.20 Content variation of PPT subtype ginsenoside of 5-year-old ginseng from different regions

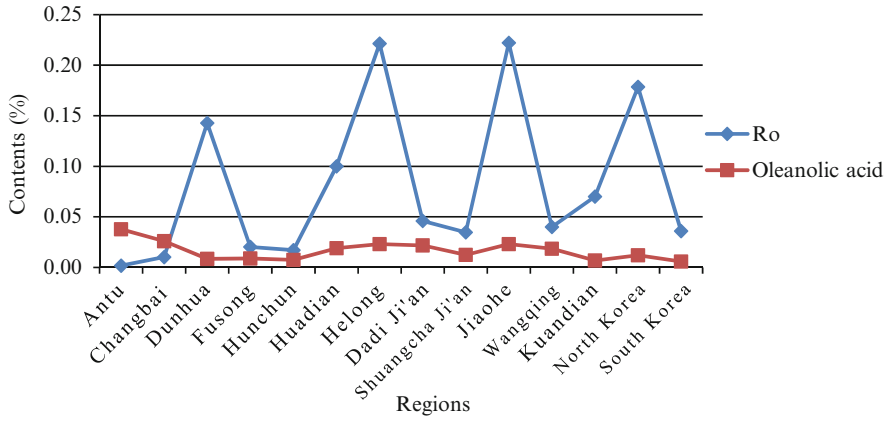

Fig. 7.21 Content variation of oleanolic acid type ginsenosides of 5-year-old ginseng from different regions

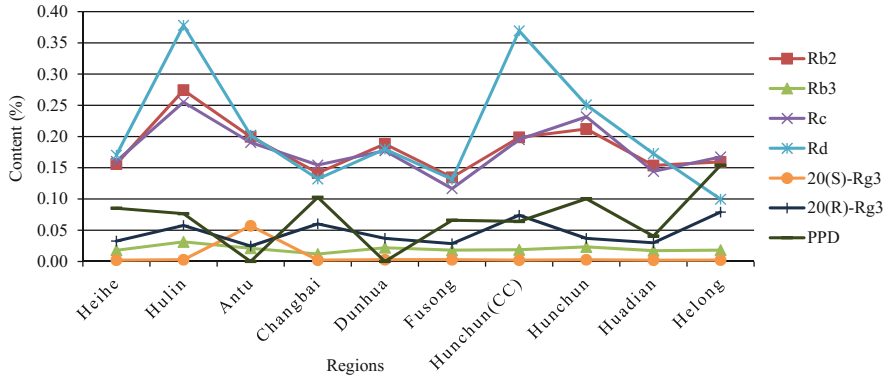

Fig. 7.22 Content variation of PPD type ginsenosides (or sapogenins) of 6-year-old ginseng from different regions (Rb_1 excepted)

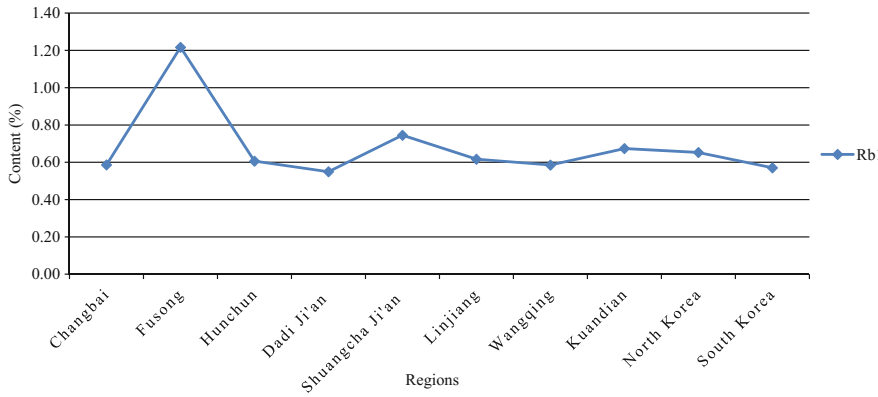

Fig. 7.23 Content variation of Rb_1 of 6-year-old ginseng from different regions

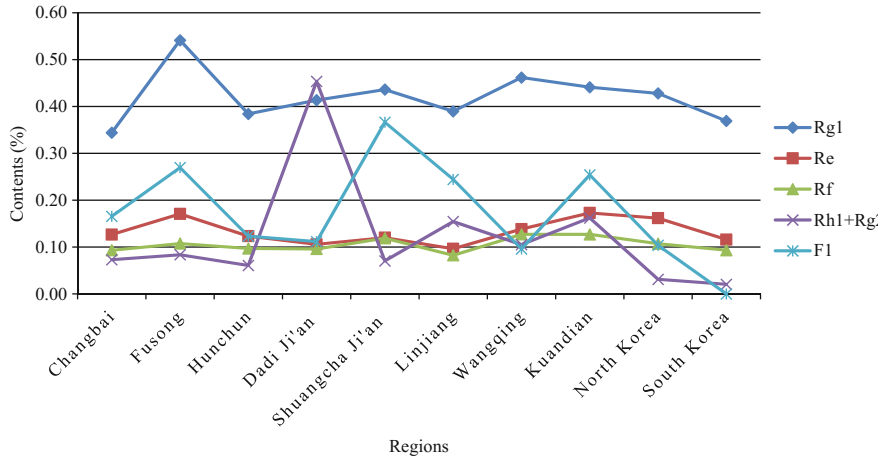

Fig. 7.24 Content variation of PPT type ginsenosides of 6-year-old ginseng from different regions

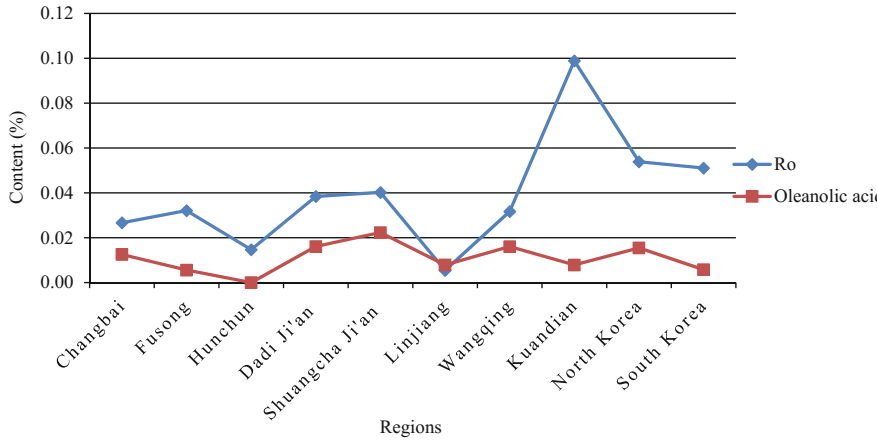

Fig. 7.25 Content variation of oleanolic acid type ginsenosides of 6-year-old ginsengs from different regions

7.4.2.2.4 System Cluster Analysis of Monomer Ginsenosides (or Sapogenins) from Different Ginseng Samples

1. Aims
   In order to further explore the relationships between the quality of ginseng and different growing periods and regions, system cluster analysis was carried out in this chapter according to the contents of monomer ginsenosides. The reasons are as follows: (1) Monomer ginsenosides are the characteristic and the main bioactive components of ginseng, which is of great significance to its quality. (2) Understanding the correlation of ginseng quality with different samples is helpful to increase the selectivity of ginseng purchasing, not limited to ginseng products from specific producing regions.
2. The steps of cluster analysis method
   The system clustering method in SPSS 22.0 statistical software was used to cluster 19 ginsenosides of different ginseng samples

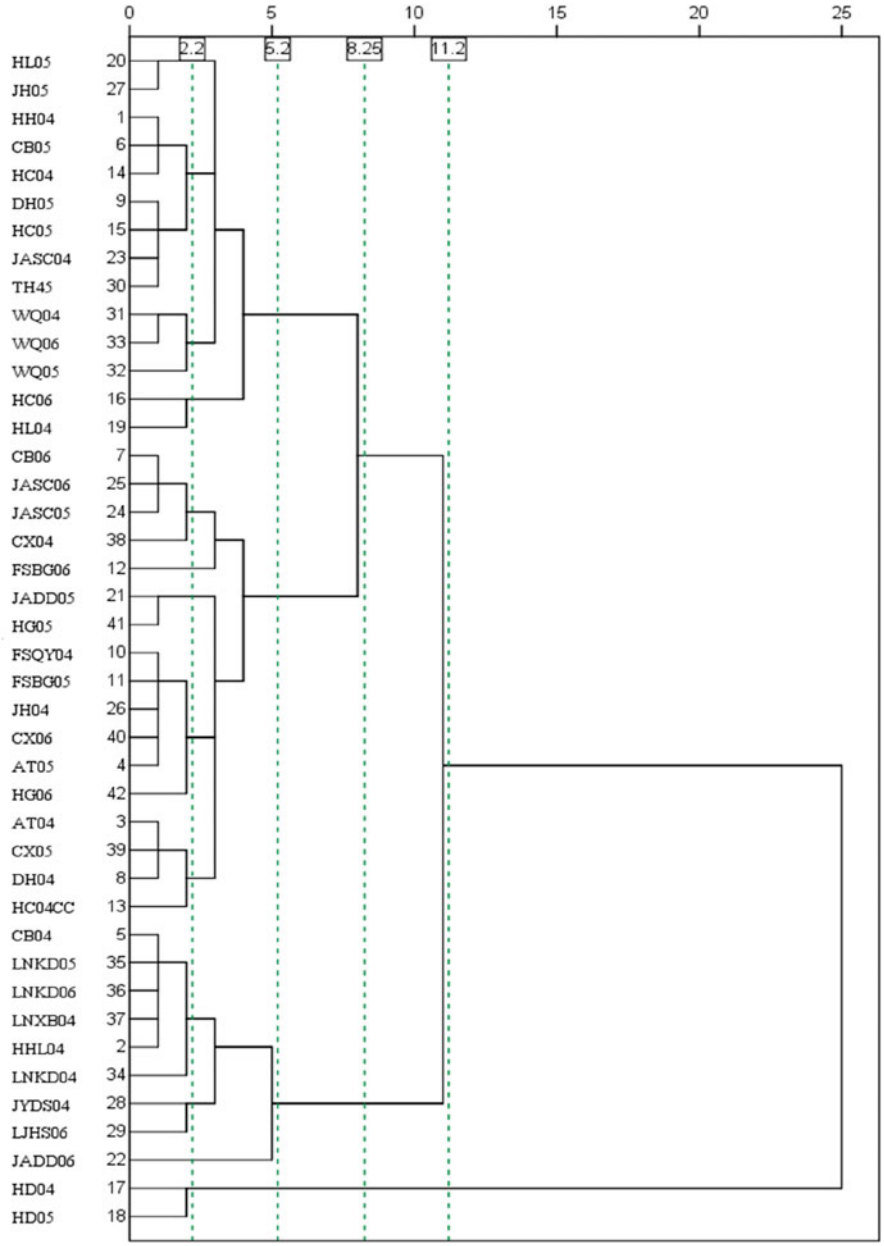

Fig. 7.26 Hierarchical graph of system cluster analysis of monomer ginsenosides (or sapogenins) from different ginseng samples

based on the contents. The minimum clustering number was 2 and the maximum clustering number was 42. Drawing the vertical pedigree map and adopting Ward's clustering method, the measurement was carried out by counting method. After Chi-square measurement, the pedigree chart was established (Fig. 7.26).

3. The results analysis of systematic clustering
   The results showed that when the clustering distance was $L_1 = 11.2$, all ginseng samples

4-year-old 5-year-old 6-year-old

Fig. 7.27 Maps of system cluster analysis by cultivation ages of ginseng

could be grouped into two categories: one category was 4-year-old and 5-year-old ginseng from Huadian and the other category was the other samples. The results showed that the quality and the contents of monomer ginsenosides of 4-year-old and 5-year-old ginseng from Huadian were similar, but they were different from those from other regions.

When the clustering distance was $L_2 = 8.25$, all ginseng samples can be grouped into three categories. That is, except samples from Huadian, ginseng samples from other regions could be subdivided into two categories. With the shrinking of clustering distance, the number of clustering categories is also increasing. These different categories show the subtle differences in ginsenosides of ginseng monomer samples from different origins. When the clustering distance $L_4 = 2.2$, the clustering results are as follows: (1) HL05, JH05; (2) HH04, CB05, HC04, DH05, HC05, JASC04, TH04, TH05; (3) WQ04, WQ05, WQ06; (4) HC06, HL04; (5) CB06, JASC06, JASC05, CX04; (6) FSBG06; (7) JADD05, HG05; (8) FS04, FS05, JH04, CX06, AT05, HG06; (9) repeated cropping of AT04, CX05, DH04, and HC04; (10) CB04, XB04, KD04, LNKD05, LNKD06, HHL04; (11) JY04, JYDS05, LJ05, LJHS06; (12) JADD06; (13) HD04, HD05.

In the above categories of ginseng samples, the monomer ginsenosides were similar in composition and content changes. The results of cluster analysis showed that there was no essential difference in the contents and compositions of ginsenosides between the 5-year-old and 6-year-old ginseng samples collected from Jinshan County, Zhongqing Nandao, South Korea, and those from China.

When the clustering distance is $L_3 = 5.2$, all ginseng samples could be grouped into four categories. The clustering results of 4-, 5-, and 6-year-old ginseng samples can be displayed on maps, respectively. Different categories could be distinguished by different colors, and ginsenosides could be distinguished by the type and content of ginseng ginsenosides in ginseng regions. The results are shown in Fig. 7.27.

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Analysis of Organic Acids in Ginseng

8

Fangli Zhong, Dongjin Xu, Hao Zhang, and Junli Liu

Abstract

In order to provide data of the contents of organic acids in ginseng, the analysis of organic acids was performed. The dried ginseng was taken as the test sample. The method for organic acid analysis was established based on high performance liquid chromatography combined with the ultraviolet detector (HPLC-UV). The standard curves of 7 organic acids had been established with the correlation coefficients being all greater than 0.99. The established standard curves were used to calculate the contents of organic acids in each ginseng sample. For the ginseng grown in the same area, there was an overall trend that the content of organic acid in 5-year-old ginseng was higher than that in 4-year-old ginseng. For the ginseng collected from various areas, the contents of organic acids were different. In short, the distribution of 7 organic acids in 45 samples was different but lacking regularity.

Keywords

Ginseng · Organic acid · HPLC-UV · Determination

8.1 Introduction

Organic acid is one of the functional and active ingredients of ginseng [1, 2]. In previous references, the organic acids were reported to have some effects such as anti-inflammatory [3], anti-oxidant [4], anti-fatigue [5], anti-viral [6], and cardioprotective activities [7, 8]. Although the reports on the activities of organic acids were not as many as saponins, flavonoids, or polysaccharides, organic acids still are the important components of ginseng. In this book, the content of organic acids in ginseng was determined by HPLC-UV according to "Determination of Organic Acids in Food" (GB/T 5009. 157-2003).

8.2 Materials and Instruments

8.2.1 Materials

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

Acetonitrile was of chromatographic grade (Fisher, Co. Ltd., America). Both ammonium phosphate dibasic and phosphoric acid (85.0%) were of chromatographic grade (Tianjin Guangfu Fine Chemical Research Institute, China). Other

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reagents were all of analytical grade. Ultrapure water was prepared by the Water Purification System (Changchun Laibopate Technology Development Co. Ltd., China).

Reference substances including succinic acid (99%) and maleic acid (98%) were all purchased from Beijing Yingzena New Chemical Technology Research Institute; both citric acid and vanillic acid were purchased from National Institute for the Control of Pharmaceutical and Biological Products of China; cinnamic acid and fumaric acid (99.4%) were purchased from National Institutes for Food and Drug Control of China; tartaric acid (99.0%) was purchased from Changchun Food and Drug Administration.

8.2.2 Instruments

1525 High Performance Liquid Chromatograph (Waters Corporation, America), 2998 Diode Array Detector (Waters Corporation, America), AT-330 Chromatographic Column Incubator (Tianjin Autoscience Instrument Co. Ltd., China), RCT-3200 Water Purification System (Changchun Laibopate Technology Development Co. Ltd., China), R201D Thermostat Water Bath (Shanghai Yukang Science and Education Equipment Co. Ltd., China), SHZ-D (III) Circulating Water Vacuum Pump (Gongyi Yuhua Instrument Co. Ltd., China), FA1104N Electronic Balance (Shanghai Jinghua Tech Instrument Co. Ltd., China), KQ3200V Ultrasonic Cleaner (Kunshan Ultrasonic Instrument Co. Ltd., China), PHS-3C Digital PH-Meter (Shanghai Yoke Instrument Co. Ltd., China), TGL-16aR High-speed Freezing Centrifuge (Shanghai Anting Scientific Instrument Factory, China), FW177 High Speed Universal Pulverizer (Beijing Yongguangming Medical Instrument Co. Ltd., China), YHG-400-IIFar Infrared Fast Drying Chamber (Shanghai Yuejin Medical Devices Co. Ltd., China).

8.3 Experimental Methods

8.3.1 Preparation of Mixed Reference Solution

Dissolve a quantity of tartaric acid, maleic acid, citric acid, fumaric acid, and succinic acid, accurately weighted respectively, in ultrapure water to produce a solution containing 0.5 mg of tartaric acid, 0.01 mg of maleic acid, 1.0 mg of citric acid, 0.01 mg of fumaric acid, and 1.5 mg of succinic acid per mL as mixed reference solution A.

Dissolve a quantity of vanillic acid and cinnamic acid, accurately weighted respectively, in mobile phase to produce a standard stock solution containing each of 1.0 mg per mL. Then the stock solution was diluted with ultrapure water to produce a solution of 0.005 mg of vanillic acid and 0.005 mg of cinnamic acid per mL respectively, as mixed reference solution B.

8.3.2 Preparation of Test Solution

Ginseng was air-dried, grinded, and sieved (Chinese National Standard Sieve No. 2, R40/3 series) to get the homogeneous powder. Weigh accurately in a 50 mL stoppered conical flask, add 20 mL of 80% ethanol, and sonicate for 30 min. After being centrifuged (5000 r/min) for 10 min, the supernatant was transferred into a 50 mL volumetric flask, and the residue was repeatedly extracted with 10 mL of 80% ethanol for two times. The supernatants were also transferred to the same volumetric flask, diluted with 80% ethanol to volume, mixed well as the extract solution.

10 mL of extract solution was accurately measured to an evaporating dish and evaporated on a water bath at 70 °C to dryness. Added 0.2 mL of phosphoric acid (1 mol/L) to the residue and transfered to a 10 mL volumetric flask, diluted with ultrapure water to 10 mL, mixed well and filtered (0.45 μm), the filtrate as the test solution.

8.3.3 Chromatographic Conditions

Condition A Carry out the method for high performance liquid chromatography, using XAqua C_{18} (4.6 mm × 250 mm, 5 μm) column. The mobile phase was the 0.01 mol/L diammonium hydrogen phosphate solution adjusted to pH 2.7 with 1 mol/L phosphoric acid. The flow rate was 0.8 mL/min. Detection wavelength was 210 nm. Column temperature was 30 °C. The volume injected for samples was all 10 μL for each run

and the chromatogram was recorded. The chromatographic peaks of each component of the mixed reference solution A reached baseline separation [9], as shown in Fig. 8.1.

Condition B Carry out the method for high performance liquid chromatography, using Unitary C_{18} (4.6 mm × 250 mm, 5 μm) column. Methanol as mobile phase A and 0.5% glacial acetic acid solution as mobile phase B. The gradient elution is performed linearly (Table 8.1). The flow rate was 1.0 mL/min. Detection wavelength was 260 nm. Column temperature was at 30 °C. Inject 10 μL onto the column and record the chromatogram. The chromatographic peaks of each component of the mixed reference solution B reached baseline separation, as shown in Fig. 8.2.

8.3.4 Draw of 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 2, 5, 8, 10, 12, 15, and 18 μL of the mixed reference solutions A respectively into the column and record the chromatogram under the chromatographic condition A.

Inject 1, 2, 4, 6, 8, 10, and 12 μL of the mixed reference solutions B respectively into the column and record the chromatogram under the chromatographic condition B.

The regression equations were obtained by taking the amount of the reference substance (μg) as the abscissa (x) and the peak area of the reference product as the ordinate (y), which were shown in Table 8.2.

8.4 Results and Discussions

8.4.1 Organic Acid Contents of Ginseng from Different Regions with Different Cultivation Periods

Organic acid contents of ginseng from different regions with different cultivation periods were shown in Table 8.3.

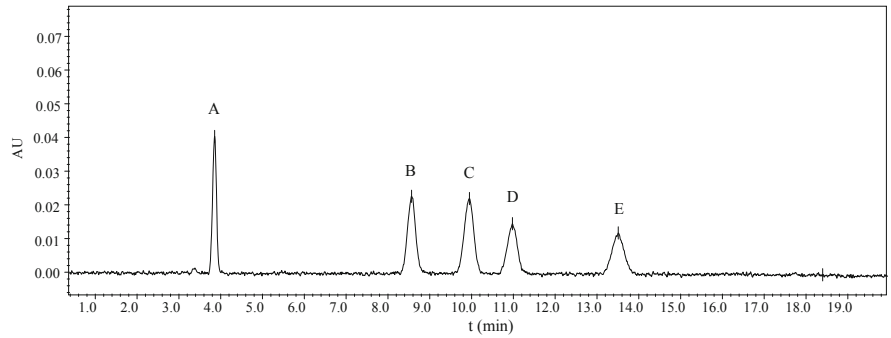

Fig. 8.1 HPLC chromatogram of mixed standard solution A. *A* tartaric acid; *B* maleic acid; *C* citric acid; *D* fumaric acid; *E* succinic acid

Table 8.1 Gradient elution program of HPLC

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0~18	25	75
18~20	25 → 75	75 → 25
20~46	75	25

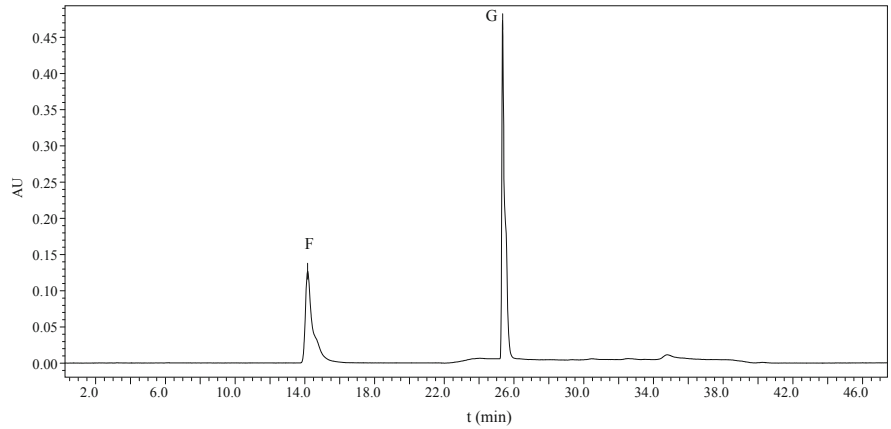

Fig. 8.2 HPLC chromatogram of mixed standard solution B. *F* vanillic acid; *G* cinnamic acid

Table 8.2 Regression equations with correlation coefficients of seven organic acids

Organic acid	Regression equation	Correlation coefficient
Tartaric acid	$y = 131{,}343x + 18{,}832$	0.9991
Maleic acid	$y = 8{,}179{,}440x + 25{,}039$	0.9994
Citric acid	$y = 83{,}332x + 34{,}973$	0.9978
Fumaric acid	$y = 5{,}830{,}140x + 26{,}287$	0.9984
Succinic acid	$y = 40{,}651x + 27{,}705$	0.9984
Vanillic acid	$y = 3{,}758{,}961x - 982$	0.9982
Cinnamic acid	$y = 4{,}768{,}380x + 827$	0.9995

8.4.2 The Total Contents of Organic Acids of Ginseng from Different Regions with Different Cultivation Periods

The total contents of seven organic acids in different sample could be calculated based on the data in Table 8.3. The results were shown in Fig. 8.3.

8.4.3 Organic Acid Contents of Ginseng from the Same Region with Different Cultivation Periods

The contents of seven kinds of organic acid in ginseng from the same regions with different cultivation periods were shown in Table 8.4. And the content trend graph of each organic acid was shown in Figs. 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 8.10, 8.11, 8.12, and 8.13.

8.4.3.1 The Contents of Organic Acids in Ginseng from Different Regions with the Same Cultivation Periods

The determination result was shown in Table 8.3.

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

Table 8.3 Organic acid contents of ginseng from different regions with different cultivation periods

No.	Region	Cultivation period (Year)	Tartaric acid	Maleic acid	Citric acid	Fumaric acid	Succinic acid	Vanillic acid	Cinnamic acid
1	Heihe	4	–	39.26	1924.74	76.95	–	2.95	2.40
2	Hulin	4	–	52.06	2481.76	59.97	–	4.81	8.71
3	Antu	4	–	34.66	1749.65	9.24	–	2.26	7.87
4		5	–	45.32	1956.09	65.34	–	2.89	19.52
5	Changbai	4	–	47.83	1421.42	538.95	–	2.01	8.66
6		5	–	59.02	1001.86	364.81	432.63	5.08	7.59
7		6	–	37.15	2746.40	118.93	–	4.80	17.25
8	Dunhua	4	–	28.67	1067.73	24.18	–	3.80	14.37
9		5	–	71.38	2756.32	69.82	–	2.63	9.59
10	Fusong	4	–	45.32	3517.63	50.07	–	4.34	2.83
11		5	–	36.60	2125.35	87.18	–	3.64	10.93
12		6	–	34.32	2502.01	61.82	–	3.87	15.49
13	Hunchun	4	–	40.55	231.44	470.90	9.32	5.69	16.86
14		5	–	30.87	806.93	635.71	–	1.83	8.52
15		6	–	37.91	730.58	611.49	–	2.62	26.39
16		4(CC)	–	50.84	759.68	916.11	–	3.82	16.08
17	Huadian	4	–	22.27	2804.74	22.09	502.47	4.56	5.58
18		5	–	22.25	2966.79	12.63	–	7.19	32.46
19	Helong	4	–	57.16	2430.05	6.47	–	2.91	16.52
20		5	–	46.08	2861.02	15.78	485.00	4.35	7.48
21	Dadi Ji'an	5	–	28.33	20.85	368.31	–	2.29	13.79
22		6	17.68	52.53	472.58	616.01	–	3.24	48.62
23	Shuangcha Ji'an	4	81.44	27.35	–	247.92	–	3.00	2.25
24		5	–	31.53	–	487.87	–	4.56	13.40
25		6	–	55.65	–	403.47	–	3.05	20.29
26	Jiaohe	4	–	17.19	1118.91	578.21	–	3.90	12.35
27		5	2.95	32.68	–	662.19	–	2.02	15.91
28	Jingyu	4	–	25.02	2945.47	56.38	–	1.60	5.44
29		5	–	28.06	3034.45	57.65	–	1.62	5.82
30	Linjiang	5	–	45.23	2315.23	165.23	–	3.45	36.80
31		6	–	44.15	2271.62	157.55	–	3.72	36.49
32	Tonghua	4	–	52.24	189.63	846.47	–	3.22	58.80
33		5	–	60.23	199.01	854.0.43	–	3.25	58.99
34	Wangqing	4	–	37.39	36.58	465.81	–	3.05	15.82
35		5	–	48.17	710.42	593.15	166.63	2.62	5.35
36		6	–	47.43	954.18	542.87	167.97	3.33	5.37
37	Kuandian	4	53.81	24.66	589.71	563.23	–	3.34	8.96
38		5	141.30	62.20	543.44	737.17	–	4.36	13.20
39		6	97.10	29.00	419.19	634.81	–	6.60	6.36
40	Xinbin	4	–	58.18	6915.84	121.47	–	3.71	34.01
41	North Korea	4	–	41.01	1858.11	554.05	1964.04	3.54	11.89
42		5	–	22.88	2125.09	596.27	–	1.95	30.56
43		6	–	41.44	1322.62	631.51	–	3.06	14.73
44	South Korea	5	–	11.67	2811.14	111.03	–	7.99	11.91
45		6	–	7.85	3435.45	100.03	–	4.52	9.80

"–" Undetected

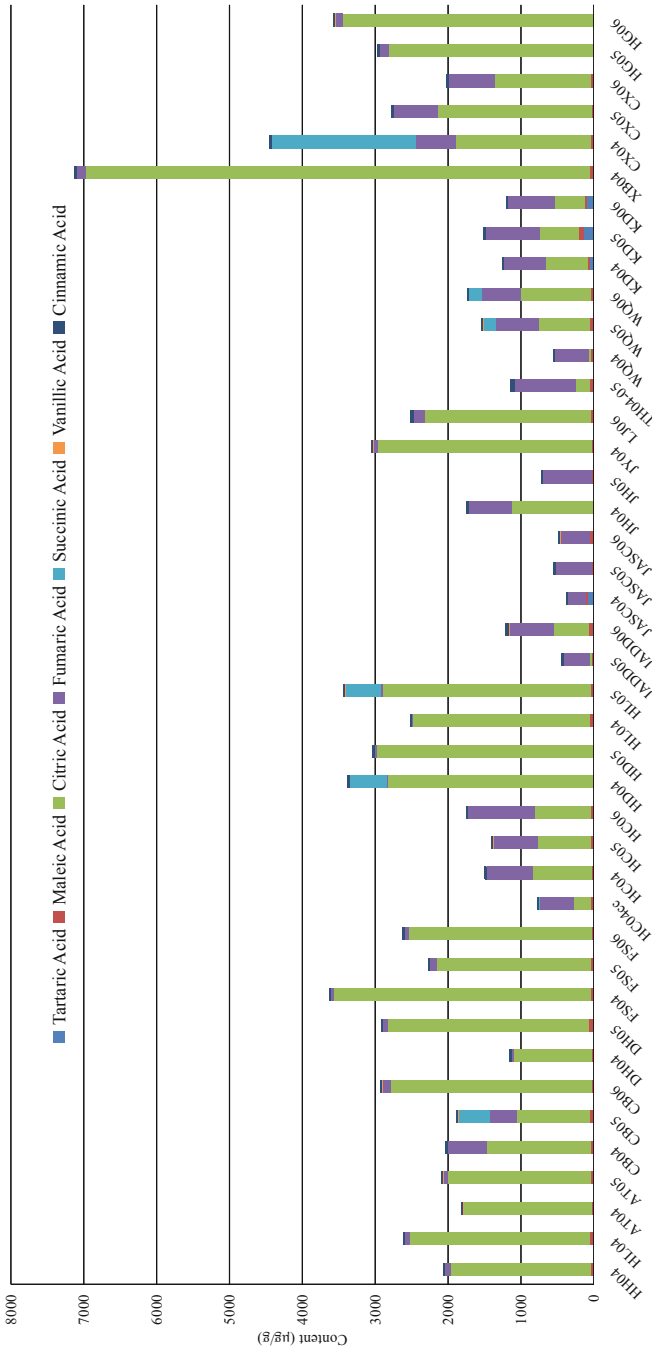

Fig. 8.3 The content accumulation histogram of organic acids of ginseng from different regions with different cultivation periods

Table 8.4 The analysis of organic acid contents in ginseng from the same regions with different cultivation periods

Organic acid	Region	Contents in ginseng of different ages
Maleic acid	Antu, Dunhua, Jiaohe, Tonghua, Jingyu	$CG_5 > CG_4$
	Huadian, Helong	$CG_4 > CG_5$
	Dadi village in Ji'an city	$CG_6 > CG_5$
	South Korea	$CG_5 > CG_6$
	Shuangcha village in Ji'an city	$CG_6 > CG_5 > CG_4$
	Hunchun	$CG_4 > CG_6 > CG_5$
	Changbai	$CG_5 > CG_4 > CG_6$
	Kuandian, Wangqing	$CG_5 > CG_6 > CG_4$
	Fusong	$CG_4 > CG_5 > CG_6$
	North Korea	$CG_6 > CG_4 > CG_5$
Citric acid	Antu, Dunhua, Huadian, Helong, Tonghua, Jingyu	$CG_5 > CG_4$
	Dadi village in Ji'an city, South Korea	$CG_6 > CG_5$
	Linjiang	$CG_5 > CG_6$
Fumaric acid	Antu, Dunhua, Jiaohe, Tonghua, Jingyu, Huadian, Helong, Jiaohe	$CG_5 > CG_4$
	Dadi village in Ji'an city	$CG_6 > CG_5$
	South Korea	$CG_5 > CG_6$
Vanillic acid	Antu, Tonghua, Jingyu, Huadian, Helong	$CG_5 > CG_4$
	Dunhua, Jiaohe	$CG_4 > CG_5$
	Dadi village in Ji'an city	$CG_6 > CG_5$
	South Korea	$CG_5 > CG_6$
Cinnamic acid	Antu, Tonghua, Jingyu, Huadian, Jiaohe	$CG_5 > CG_4$
	Dunhua, Helong	$CG_4 > CG_5$
	Dadi village in Ji'an city	$CG_6 > CG_5$

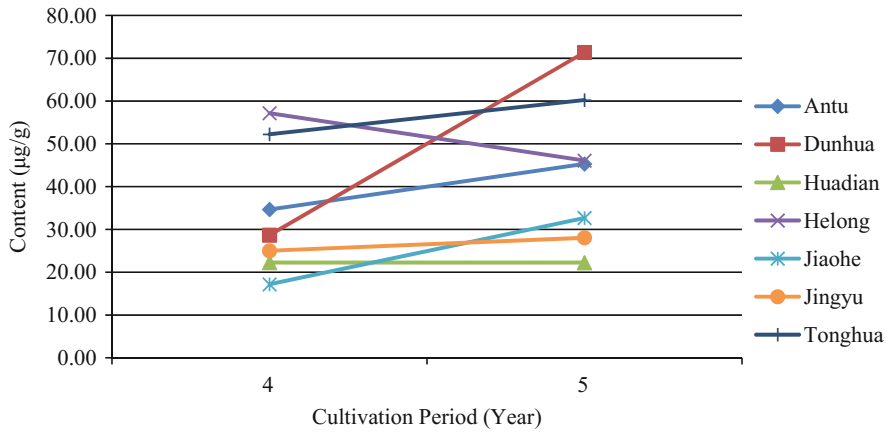

Fig. 8.4 The contents of maleic acid in some ginseng of 4, 5 ages

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

Citric acid (1) For 4-year-old ginseng, the regions with high-to-low contents were: Shuangcha Ji'an, Xinbin, Fusong, Jingyu, Huadian, Hulin, Helong, Heihe, North Korea, Antu, Changbai, Jiaohe, Dunhua, Hunchun,

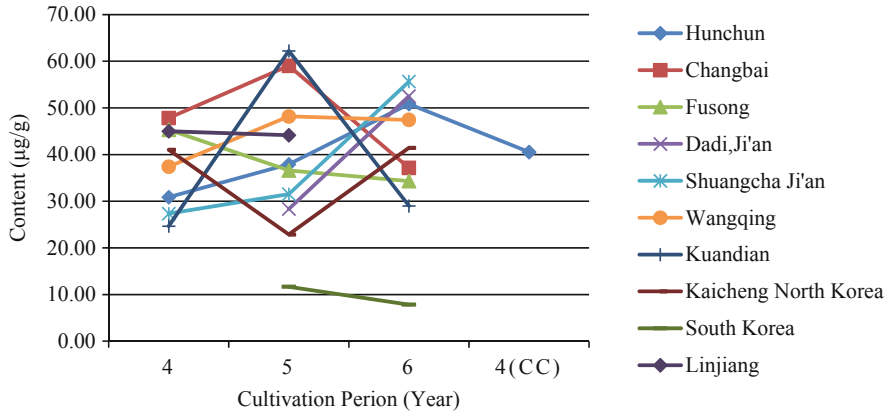

Fig. 8.5 The contents of maleic acid in some ginseng of 4, 5, 6, 4 (CC) ages

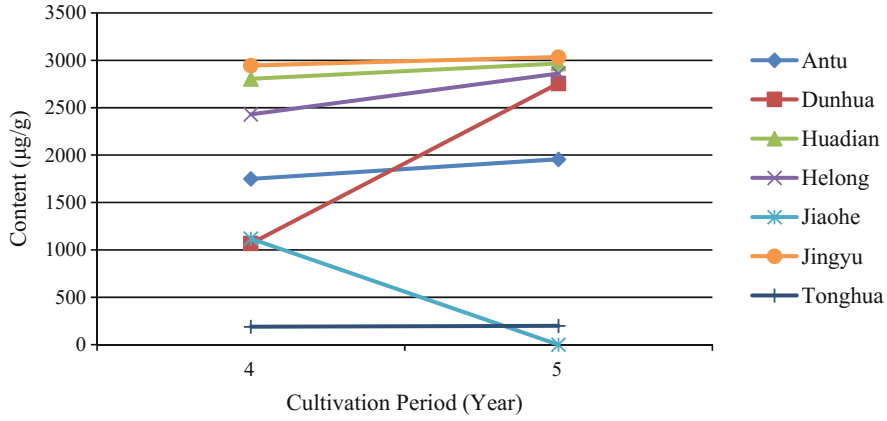

Fig. 8.6 The contents of citric acid in some ginseng of 4, 5 ages

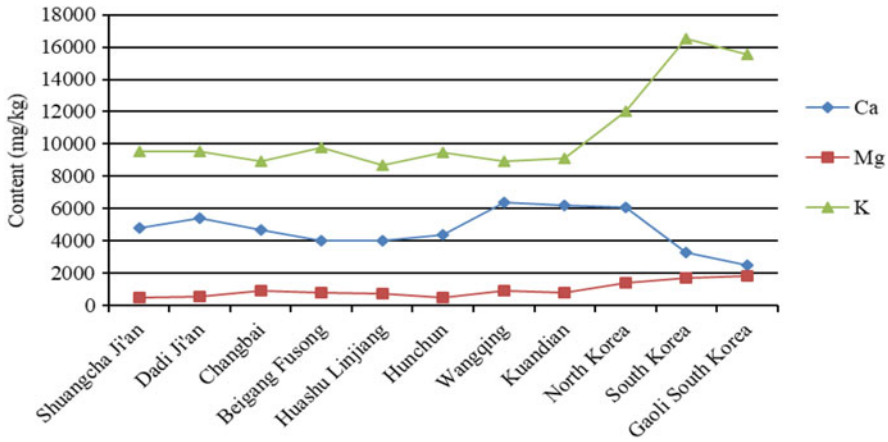

Fig. 8.7 The contents of citric acid in some ginseng of 4, 5, 6, 4 (CC) ages

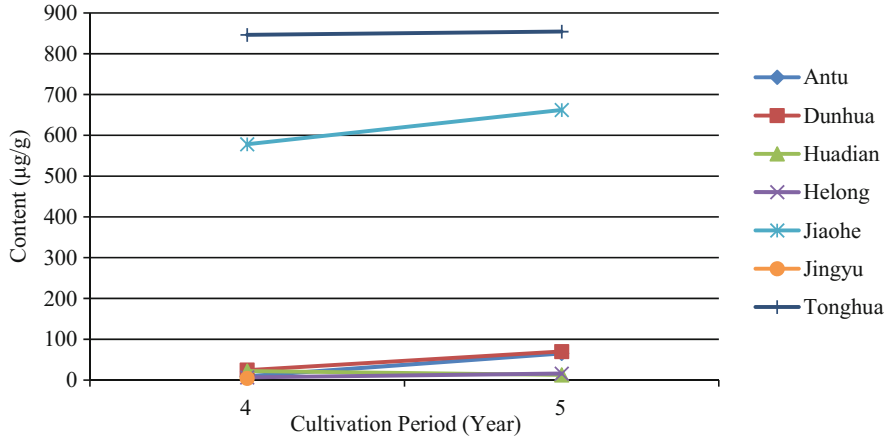

Fig. 8.8 The contents of fumaric acid in some ginseng of 4, 5 ages

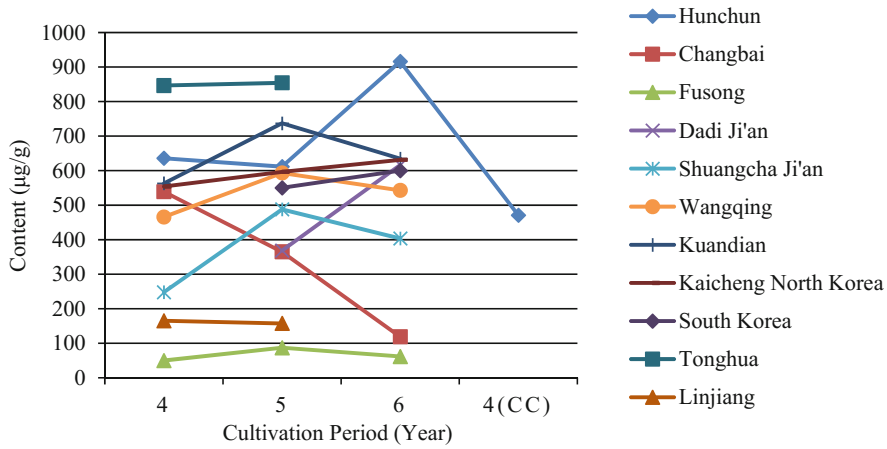

Fig. 8.9 The contents of fumaric acid in some ginseng of 4, 5, 6, 4 (CC) ages

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

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

Vanillic acid (1) For 4-year-old ginseng, the regions with high-to-low contents were: Hunchun (CC), Hulin, Huadian, Fusong, Jiaohe, Dunhua,

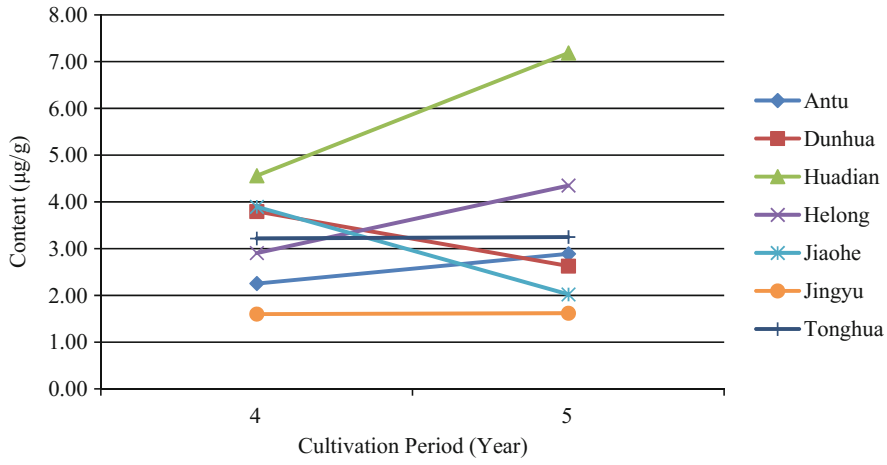

Fig. 8.10 The contents of vanilloid in some ginseng of 4, 5 ages

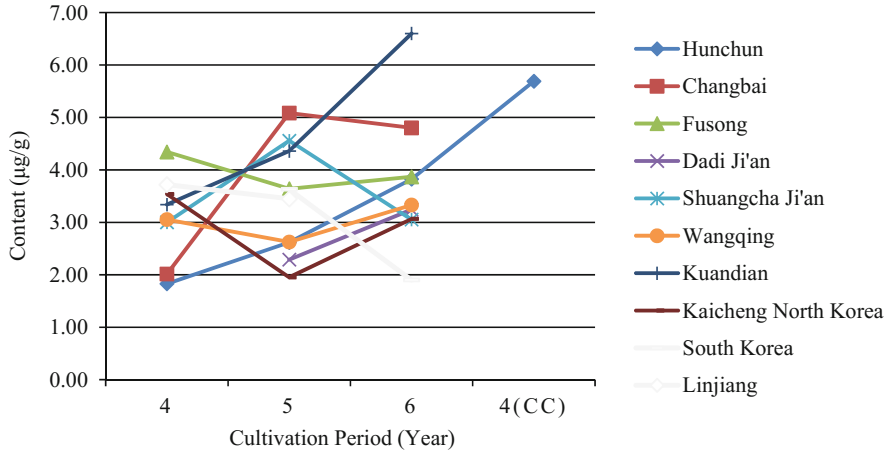

Fig. 8.11 The contents of vanilloid in some ginseng of 4, 5, 6, 4 (CC) ages

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

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

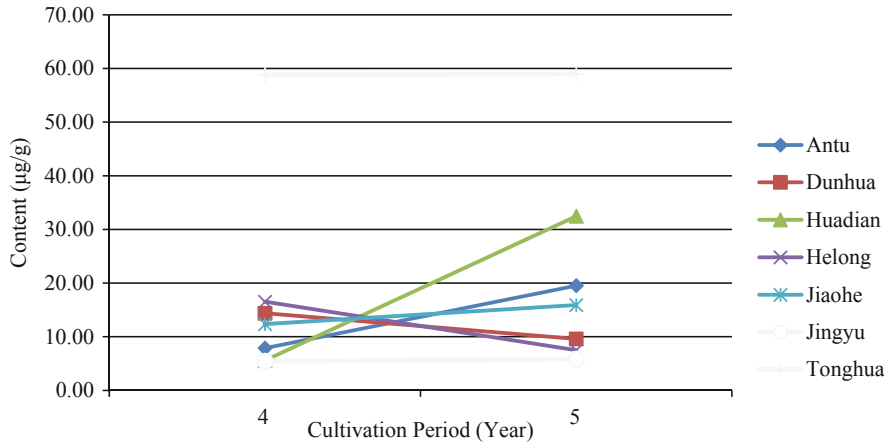

Fig. 8.12 The contents of cinnamic acids in some ginseng of 4, 5 ages

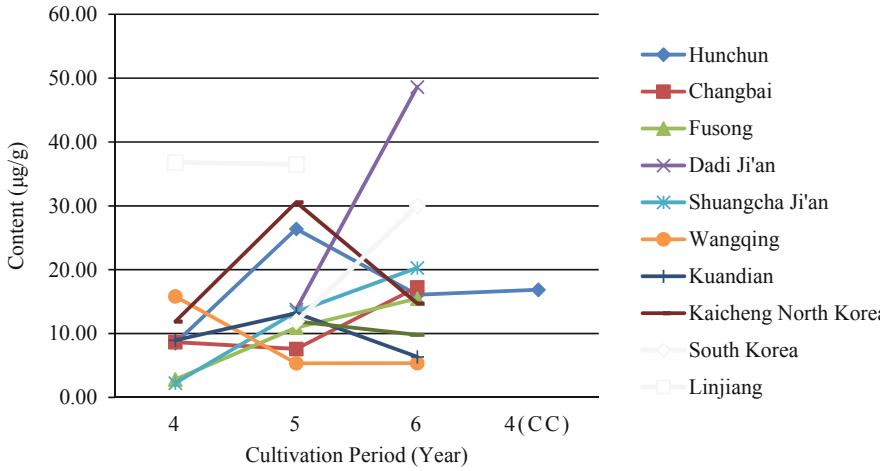

Fig. 8.13 The contents of cinnamic acids in some ginseng of 4, 5, 6, 4 (CC) ages

The distribution of some organic acids was shown in Figs. 8.14, 8.15, 8.16, 8.17, 8.18, and 8.19, respectively.

8.5 Hierarchical Cluster Analysis of Organic Acids in Ginseng from Different Regions with Different Cultivation Periods

Combined Ward method, chi-square metric, and squared Euclidean distance in SPSS 22.0 software, the dendrogram (Fig. 8.20) of cluster analysis was established with organic acid contents as the characteristic variable. The results showed that all ginseng samples could be classified into two categories when the distance used for clustering was 10. The samples including HH04 (1), HHL04 (2), AT04/05 (3~4), CB06 (7), DH04/05 (8~9), FS04, FS05/06 (10~12), HD04/05 (17~18), HL04/05 (19~20), JYDS04/05 (28), LJ05/06 (29), LYNX04 (37), and HG04/05 (41~42) were clustered into one category. The rest samples were clustered into another category, which could be further divided into two groups when the clustering distance being 5. One group was consisted of JADD05 (21), JASC04/05/06 (23~25), JH05 (27), TH04/05 (30) and WQ04 (31). The other group included the rest samples.

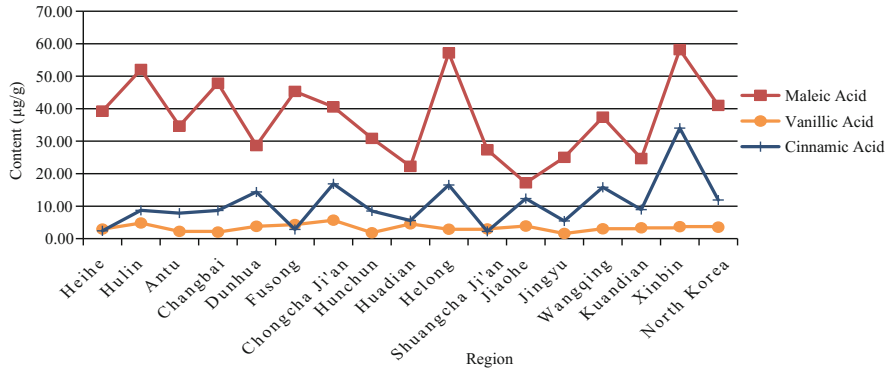

Fig. 8.14 The contents of maleic acid, vanillic acid, and cinnamic acid in 4-year-old ginseng from various areas

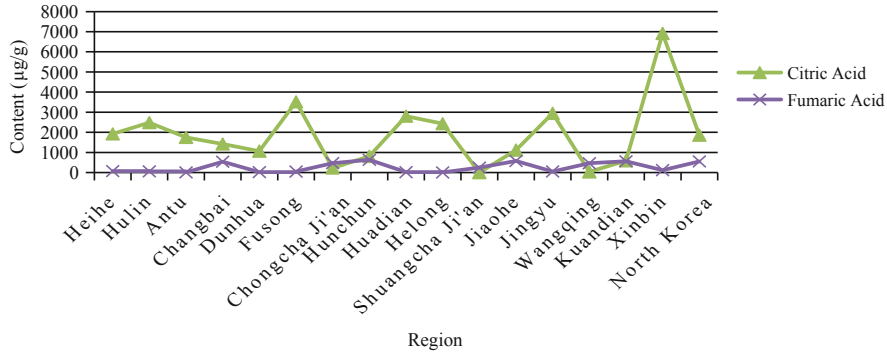

Fig. 8.15 The contents of citric acid and fumaric acid in 4-year-old ginseng from various areas

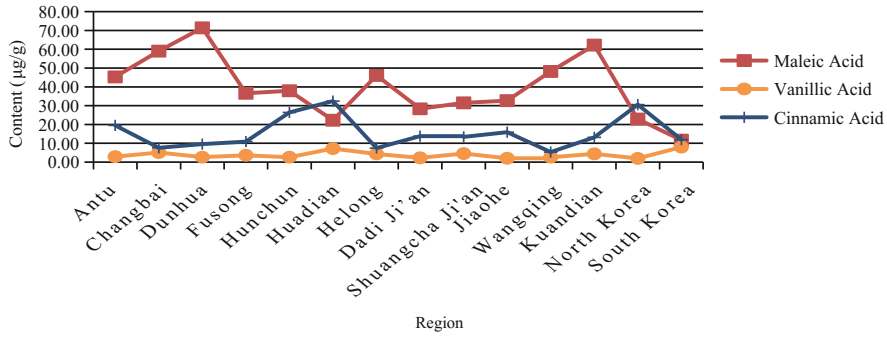

Fig. 8.16 The contents of maleic acid, vanillic acid, and cinnamic acid in 5-year-old ginseng from various areas

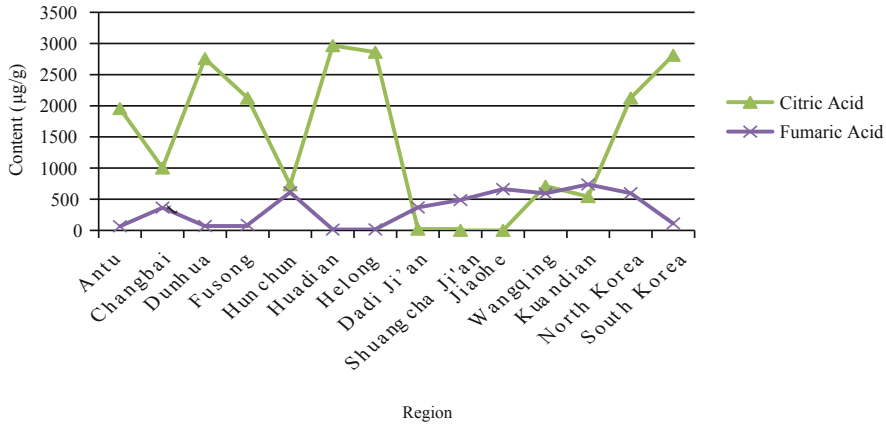

Fig. 8.17 The contents of citric acid and fumaric acid in 5-year-old ginseng from various areas

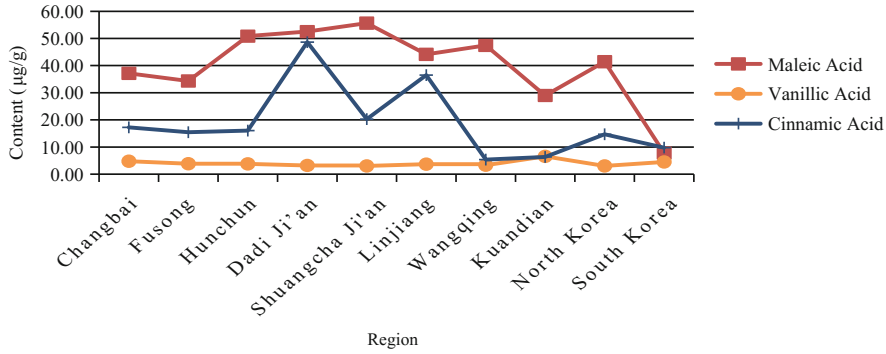

Fig. 8.18 The contents of maleic acid, vanillic acid, and cinnamic acid in 6-year-old ginseng from various areas

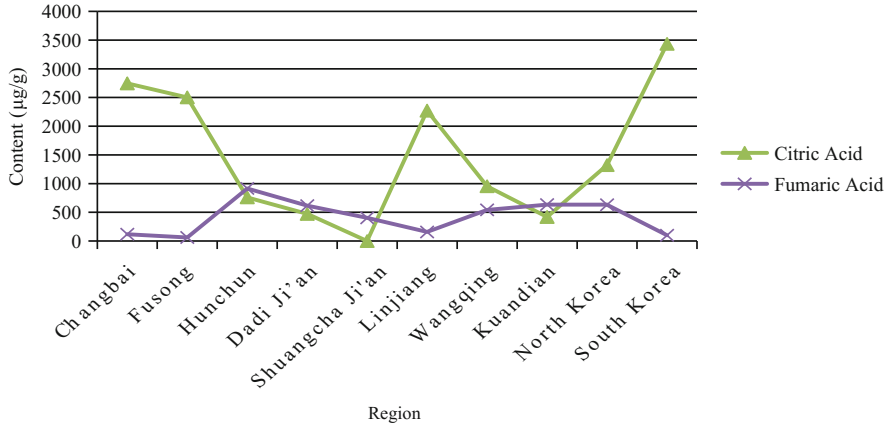

Fig. 8.19 The contents of citric acid and fumaric acid in 6-year-old ginseng from various areas

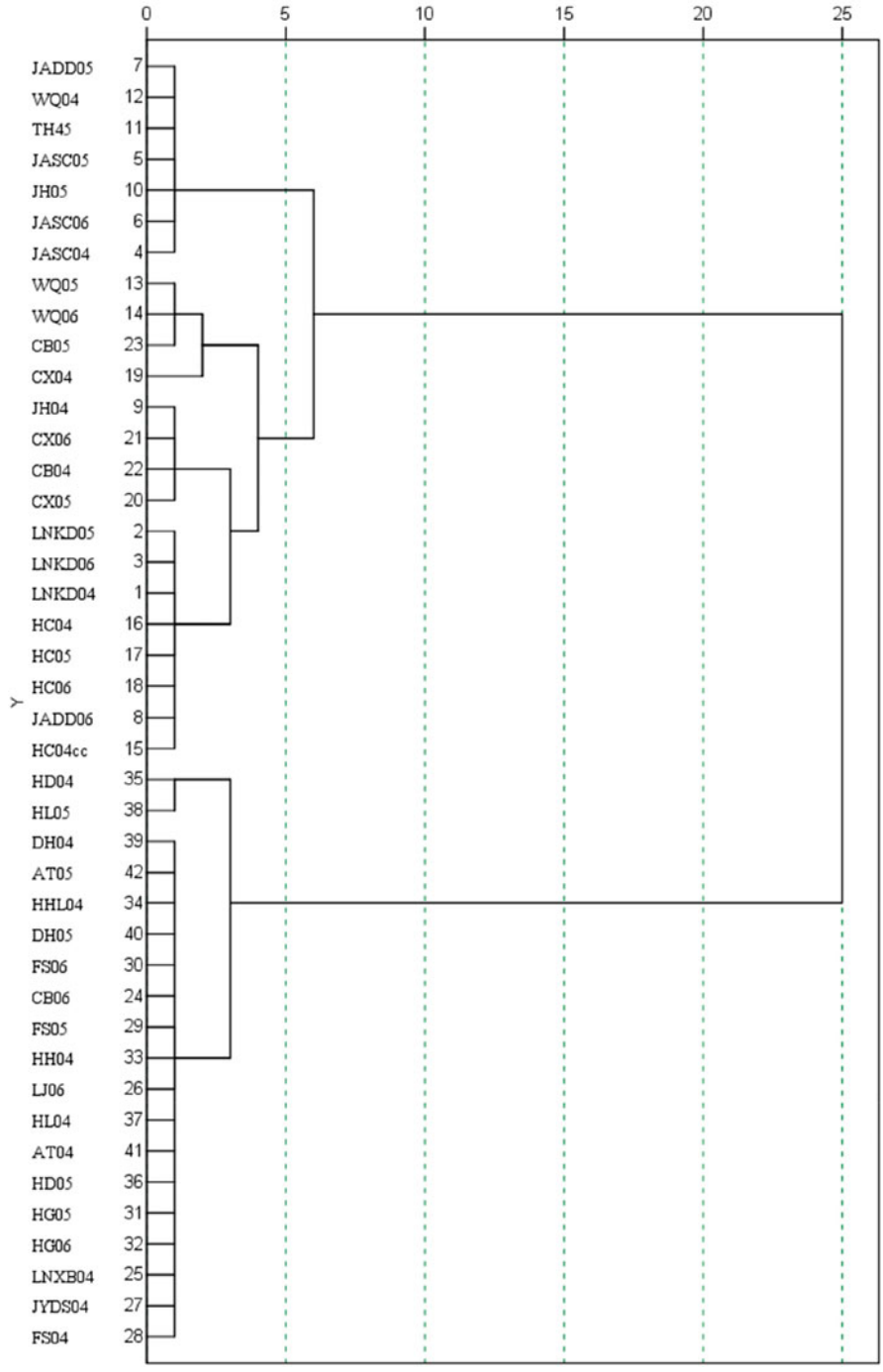

Fig. 8.20 Hierarchical graph of cluster analysis of organic acids in ginseng

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Analysis of Vitamins in Ginseng

9

Zhuo Li, Jinluan Chen, Han Wang, and Pingya Li

Abstract

Aiming to provide reference on illustrating the kinds and the contents of vitamins in ginseng, the analysis of vitamins was carried out. The dried ginseng was taken as test sample. The method for analysis was then established based on high-performance liquid chromatography combined with diode array detector (HPLC-PDA). The standard curves of ten vitamins were established with the correlation coefficients being all greater than 0.99. The established method was finally used to determine the contents of vitamins in each sample. Among all samples from various areas, both the 4-year-old ginseng from Heihe city and the 5-year-old ginseng from Korean were rich in vitamins. While the 4-year-old ginseng obtained from Fusong County had the lowest content of vitamins. Among the detected vitamins, the contents of vitamin B_1, calcium pantothenate, biotin, and vitamin C were relatively high in all samples. In general, the distribution of ten vitamins in 45 samples was different but lacks regularity.

Keywords

Dried ginseng · Vitamin · HPLC-PDA · Determination

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9.1 Introduction

Vitamin, one of the functional components, plays an important role in maintaining the survival of organisms [1, 2]. As the essential nutrient, it is widely involved in the metabolism of organisms [3, 4]. Ginseng is rich in vitamins [5], the common methods for vitamin analysis include titration [6], spectrophotometry [7], and HPLC [8]. In this book, the method of high-performance liquid combined with diode array detector (HPLC-PDA) was used to detect ten kinds of water-soluble vitamins in dried ginseng. RP-HPLC with the gradient elution was used to separate and determine the contents of vitamins.

9.2 Materials and Instruments

9.2.1 Materials

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

Both methanol and acetonitrile were all of chromatographic grade (Fisher, Co. Ltd., USA). Triethylamine, potassium dihydrogen phosphate, hydro-chloric acid, and sodium hydroxide (Xilong Chemical Co. Ltd., Guangdong, China) were all of analytical pure grade. Ultrapure water was prepared by the Water Purification System (Changchun Laibopate Technology Development Co. Ltd., China). Precise pH (pH 2.5–4.0,

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pH 1.4–3.0, and pH 2.7–4.7) papers were also used (Beijing Chemical Factory, China).

Reference substances, including vitamin C (99.0% or higher), vitamin B_1 (97.0%), vitamin B_2 (99.1%), vitamin B_6 (100.0%), folic acid (91.1%), calcium pantothenate (99.0%), niacinamide (99.9%), nicotinic acid (99.9%), biotin (99.6% or higher), and vitamin B_{12} (90.2%), were purchased from the Food and Drug Verification Research Institute of China.

9.2.2 Instruments

FA1104N electronic balance (Shanghai Jinghua Technology Instrument Co. Ltd., China), DK-2000-III L type electrothermal constant temperature water-bath (Tianjin Test Instrument Co. Ltd., China), KQ-250B ultrasonic cleaner (Kunshan Ultrasonic Instrument Co. Ltd., China), SHZ-D-III circulating water vacuum pump (Gongyi Yuhua Instrument Co. Ltd., China), TG.L-16aR centrifuge (Shanghai Anting Scientific Instrument Factory, China), BCD-237KC refrigerator (Hangzhou Huari Refrigerator Co. Ltd., China), RCT-3200 water purification system (Changchun Laibopate Technology Development Co. Ltd., China), High-performance liquid chromatography consisted of Waters 1525 binary HPLC pump and Waters 2998 Diode Array Detector (Waters, USA), Empower 3 chromatography workstation (Waters, USA), Unitary C_{18} Analytical Column (250 mm × 4.6 mm, 5 μm) (Acchrom Technology Co. Ltd., China).

9.3 Experimental Methods

9.3.1 Preparation of Reference Solution

An appropriate amount of vitamin C, B_1, B_2, B_6, B_{12}, folic acid, calcium pantothenate, niacinamide, niacin, and biotin was accurately weighed, respectively. All the weighed reference substances were then dissolved in 0.05 M potassium phosphate buffer at 25 °C to obtain a mixed reference solution containing ten vitamins. The final concentration of each reference substance and the corresponding retention time in HPLC chromatogram are listed (Table 9.1).

9.3.2 Preparation of Test Solutions

Ginseng was, respectively, air-dried, grinded, and sieved (Chinese National Standard Sieve No. 3, R40/3 series) to get the homogeneous powder. Then the fine powder was accurately weighed (2 g) and was put into 50 mL stoppered conical flask. Alpha-amylase (0.5 g) and water (30 mL) were then added in. After filled with high purity N_2, the stoppered conical flask was incubated for 30 min at 50 °C being kept in dark place. The incubated solution was then adjusted to pH 2.0 with 6 mol/L or 1 mol/L HCl solution and was placed for 1 min. Then the solution was adjusted to pH 4.7 with 6 mol/L or 1 mol/L NaOH solution and extracted in an ultrasonic bath (power of 250 W, frequency of 40 kHz) 10 min. After being filtered, the solution was diluted with ultrapure water to 50 mL. After filtered through a syringe filter (0.45 μm), the extraction solutions were injected directly into the HPLC system. All of the above solutions were prepared in duplicate and were stored at 4 °C prior to LC analysis.

9.3.3 Chromatographic Conditions

Column temperature was set at 25 °C. Detection wavelength was set at 210 and 264 nm. The mobile phases were composed of eluent A {0.05 mol/L potassium dihydrogen phosphate buffer containing 0.2% triethylamine–acetonitrile (97:3, v/v)} and eluent B (methanol) with flow rate of 1.3 mL/min. The gradient elution conditions are listed in Table 9.2. The run time was 30 min. The volume injected for samples were all 20 μL for each run.

Table 9.1 Final concentration and retention time of each standard substance

Name	Concentration (μg/μL)	Retention time (min)
Vitamin C	0.204	2.835
Vitamin B_1	0.098	4.234
Niacin	0.076	4.536
Calcium pantothenate	0.100	4.806
Vitamin B_6	0.098	5.220
Niacinamide	0.072	6.348
Folic acid	0.140	7.722
Biotin	0.064	9.036
Vitamin B_{12}	0.094	16.463
Vitamin B_2	0.078	21.573

Table 9.2 Gradient elution program of HPLC

Time (min)	Phase A (%)	Phase B (%)
0~20	90 → 60	10 → 40
20~30	60 → 90	40 → 10

9.3.4 Method Validation

The optimized method was validated by evaluating linearity, accuracy, precision, stability, and recovery.

Linearity was evaluated from the calibration curve obtained after analyzing standard solutions in increasing order of concentrations (4, 6, 8, 10, 12, 16, 20 μL, respectively). The abscissa (x) was the contents (μg) and the ordinate (y) was the peak areas (A) of reference substance. The standard curve was drawn to obtain the regression equation as shown in Table 9.3.

Precision was evaluated by analyzing the reference solution five times ($n = 5$). the precision was expressed as the relative standard deviation (RSD) of each peak area of each vitamin. The results showed that the RSD was from 1.99% to 4.78%.

Accuracy was evaluated by analyzing the same test sample solution in replicates of five samples ($n = 5$). The accuracy was expressed as RSD of the total amount of ten vitamins. The results showed that the RSD was 4.77%.

Stability was investigated by analyzing the same test sample solution at 0, 4, 8, 12, and 24 h, respectively. The stability was expressed as RSD of the total amount of ten vitamins. The results showed that the RSD was 4.83%.

Recovery was accessed by comparing the contents of ten vitamins before and after extraction at three levels in three replicates. The recovery was expressed as recovery rates and the RSD of recovery rates. The results showed that the recovery rates were in the range of 92.6~104.7%, with the RSD being 1.97~5.22%.

9.4 Results and Discussion

The chromatograms of the reference substance under 210 and 264 nm are shown in Figs. 9.1 and 9.2, respectively. The wavelength with the higher response was selected as detection wavelength according to the response of each vitamin. That is to say, vitamins C, B_1, and B_2 were detected and calculated at the wavelength of 264 nm, and other vitamins were detected and calculated at the wavelength of 210 nm. The contents of each vitamin in ginseng samples were calculated based on the linear regression equation. The contents of ten vitamins in various ages of ginseng from different producing areas are listed in Table 9.4. It could be concluded that the contents of ten vitamins detected in ginseng of different ages from various regions were different and irregular.

Table 9.3 Regression equations and correlation coefficients of ten vitamins

Name of vitamin	Regression equation	Correlation coefficient
Vitamin C	$y = 1{,}670{,}161x - 362{,}454$	0.9962
Vitamin B_1	$y = 570{,}295x - 7140$	0.9986
Niacin	$y = 1{,}337{,}982x - 33{,}745$	0.9996
Calcium pantothenate	$y = 93{,}498x - 7416$	0.9742
Vitamin B_6	$y = 1{,}165{,}737x - 63{,}820$	0.9976
Niacinamide	$y = 1{,}618{,}501x - 4305$	0.9991
Folic acid	$y = 1{,}180{,}119x - 78{,}368$	0.9989
Biotin	$y = 223{,}359x - 26{,}217$	0.9482
Vitamin B_{12}	$y = 1{,}340{,}543x - 29{,}599$	0.9963
Vitamin B_2	$y = 1{,}758{,}956x - 2438$	0.9983

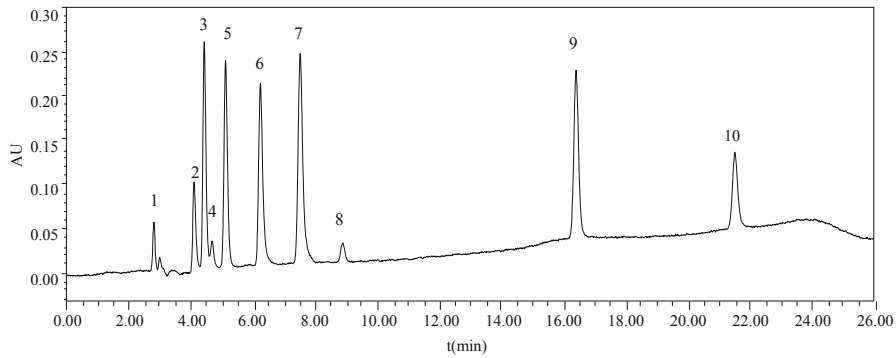

Fig. 9.1 HPLC chromatogram of ten vitamin reference substances under 210 nm. *1* Vitamin C. *2* Vitamin B_1. *3* Niacin. *4* Calcium pantothenate. *5* Vitamin B_6. *6* Niacinamide. *7* Folic acid. *8* Biotin. *9* Vitamin B_{12}. *10* Vitamin B_2

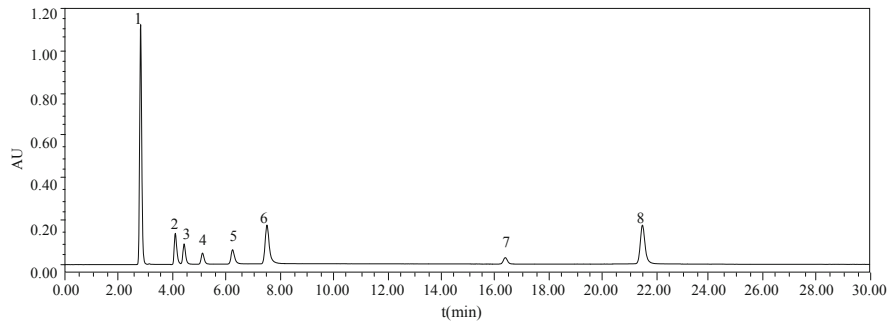

Fig. 9.2 HPLC chromatogram of ten vitamin reference substances under 264 nm. *1* Vitamin C. *2* Vitamin B_1. *3* Niacin. *4* Calcium pantothenate. *5* Vitamin B_6. *6* Niacinamide. *7* Folic acid. *8* Biotin. *9* Vitamin B_{12}. *10* Vitamin B_2

Among all samples from various areas, both the 4-year-old ginseng from Heihe city and the 5-year-old ginseng from Korean were rich in vitamins. While the 4-year-old ginseng obtained from Fusong County had the lowest content of vitamins. Among the detected vitamins, the contents of vitamin B_1, calcium pantothenate, biotin, and vitamin C were relatively high in all samples. In general, the distribution of ten vitamins in 45 samples was different but lacks regularity.

Table 9.4 Vitamins in ginseng of different ages in various regions

Region	Cultivation age (year)	Content of each vitamin (%)										Total content (%)
		Vitamin C	Vitamin B_1	Niacin	Calcium pantothenate	Vitamin B_6	Nicotinamide	Folate	Biotin	Vitamin B_{12}	Vitamin B_2	
Heihe	4	0.0942	0.0148	0.0067	1.1857	0.0206	0.0058	0.0143	0.0721	–	0.0006	1.4149
Hulin	4	0.0731	0.1385	0.0241	0.0165	0.0152	0.0015	0.0231	0.6734	0.0133	0.0004	0.9792
Antu	4	0.0725	0.0957	0.0039	–	0.0557	0.0024	0.0129	0.0526	–	0.0015	0.2971
	5	0.0397	0.1471	0.0060	0.3892	0.0182	0.0028	0.0114	–	–	0.0006	0.6150
Changbai	4	0.0399	0.1475	0.0081	–	0.0305	0.0027	0.0173	0.0468	–	0.0009	0.2937
	5	0.0334	0.0538	0.0053	0.0428	0.0118	0.0103	0.0222	0.0475	–	0.0008	0.2279
	6	0.0589	0.1174	0.0313	–	0.0114	0.0020	0.0124	0.0554	–	0.0006	0.2893
Dunhua	4	0.0714	0.1002	0.0056	0.0189	0.0664	0.0020	0.0131	0.0418	–	0.0006	0.3199
	5	0.0343	0.0607	0.0058	0.2506	0.0116	–	0.0232	0.0418	–	0.0004	0.4284
Fusong	4	0.0384	0.0043	0.0084	0.0235	0.0155	0.0055	0.0185	0.0369	–	0.0009	0.1519
	5	0.0698	0.0920	0.0047	0.0416	0.0556	0.0028	0.0132	0.5923	0.0079	0.0009	0.8807
	6	0.0538	0.0597	0.0107	0.0268	0.0424	0.0073	0.0122	–	–	0.0038	0.2167
Hunchun	4_{CC}	0.0389	0.0405	0.0058	0.0396	0.0188	0.0034	0.0323	0.0306	–	0.0004	0.2103
	4	0.0518	0.0168	0.0059	0.1326	0.0094	0.0043	0.0265	0.0935	–	0.0004	0.3411
	5	0.0555	0.0373	0.0059	0.2662	0.0241	0.0010	0.0163	0.0629	–	0.0011	0.4704
	6	0.0680	0.0031	0.0074	0.4965	0.0128	0.0013	0.0303	0.0575	–	0.0005	0.6774
Huadian	4	0.0413	0.0984	0.0148	–	0.0125	0.0023	0.0124	0.1585	–	0.0004	0.3406
	5	0.0788	0.2171	0.0242	0.0165	0.0153	0.0015	0.0232	0.6766	0.0134	0.0008	1.0676
Helong	4	0.0438	0.0615	0.0082	0.0419	0.0173	0.0030	0.0185	0.0630	–	0.0007	0.2578
	5	0.0340	0.0157	0.0080	0.1829	0.0130	0.0068	0.0138	0.0397	–	0.0006	0.3146
Dadi, Ji'an	5	0.0731	0.0392	0.0116	0.0216	0.0274	0.0039	0.0112	0.1279	0.0092	0.0014	0.3265
	6	0.0367	0.0863	0.0147	0.8423	0.0162	0.0036	0.0178	0.0366	–	0.0004	1.0547
Shuangcha, Ji'an	4	0.0471	0.1990	0.0082	0.0590	0.0139	0.0027	0.0141	0.0592	–	0.0009	0.4040
	5	0.0601	0.1479	0.0060	0.0354	0.1070	0.0038	0.0137	0.6002	–	0.0010	0.9753
	6	0.0358	0.0046	0.0057	0.1252	0.0236	0.0031	0.0189	0.0365	–	0.0012	0.2547
Jiaohe	4	0.0522	0.1202	0.0051	–	0.0386	0.0019	0.0106	0.1046	–	0.0007	0.3340
	5	0.0479	0.1387	0.0148	0.0303	0.0214	0.0020	0.0148	0.1848	–	0.0007	0.4553
Jingyu	4	0.0403	0.1249	0.0084	0.0534	0.0134	0.0023	0.0200	0.0992	–	0.0005	0.3625
Linjiang	6	0.0601	0.1579	0.0062	0.0244	0.0224	0.0021	0.0130	0.8747	0.0100	0.0010	1.1718

(continued)

Table 9.4 (continued)

Region	Cultivation age (year)	Content of each vitamin (%)										Total content (%)
		Vitamin C	Vitamin B_1	Niacin	Calcium pantothenate	Vitamin B_6	Nicotinamide	Folate	Biotin	Vitamin B_{12}	Vitamin B_2	
Tonghua	4	0.0705	0.0219	–	–	0.0116	–	0.0123	0.0576	–	0.0007	0.1745
	5	0.0710	0.0200	–	–	0.0132	–	0.0132	0.0585	–	0.0005	0.1513
Wangqing	4	0.0729	0.1824	0.0050	0.1050	0.1202	0.0090	0.0147	0.1413	–	0.0008	0.6512
	5	0.0818	0.1338	0.0081	0.0241	0.0134	0.0011	–	0.0326	–	0.0006	0.2957
	6	0.0493	0.0804	0.0125	0.0608	0.0211	0.0073	0.0153	0.0570	–	0.0007	0.3044
Kuandian	4	0.0479	0.1883	0.0118	0.0278	0.0173	0.0037	0.0141	0.0813	–	0.0011	0.3933
	5	0.0913	0.2003	0.0138	0.0308	0.0303	0.0047	0.0171	0.0913	–	0.0021	0.4816
	6	0.0818	0.1711	0.0054	0.0558	0.0629	0.0048	0.0134	0.0524	–	0.0040	0.4516
Xinbin	4	0.0540	0.0535	0.0114	0.4919	0.0543	0.0042	0.0194	0.0497	0.0062	0.0005	0.7451
North Korea	4	0.0424	0.1718	0.0089	0.7765	0.0463	0.0022	0.0138	0.0419	–	0.0007	1.1044
	5	0.0445	0.1073	0.0082	0.0322	0.0836	0.0011	0.0253	0.5834	–	0.0009	0.8864
	6	0.0461	0.1357	–	–	0.0922	–	0.0125	0.1876	–	0.0012	0.4755
South Korea	5	0.0497	0.1130	0.0062	0.0793	0.0197	0.0026	0.0284	0.9107	0.0117	0.0011	1.2225
	6	0.0319	0.1318	0.0093	0.9757	0.0178	0.0094	–	0.0378	–	0.0007	1.2145
Average		0.0550	0.1008	0.0096	0.2007	0.0322	0.0037	0.0172	0.1823	0.0102	0.0009	0.5603

Note: "–" means not detected

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Analysis of Inorganic Element in Ginseng 10

Liu Han, Cuizhu Wang, Xuejun Li, and Hailin Zhu

Abstract

In order to provide data for clarifying the contents of inorganic element in ginseng, the assay of inorganic elements was performed. The dried ginseng was taken as the test sample. The method of determination of 24 inorganic elements in ginseng was established based on Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The results showed that ginseng was rich in K, Mg, Ca, Fe, Na, Mn, Ba, Sr, Zn, Cu, Cr, Ni, Pb, Li, Cd, Co, and As, while it was poor in Sn, Mo, Ge, Se, Sb, and Hg. Furthermore, with 24 inorganic element contents as the characteristic variable, the hierarchical cluster analysis showed that the ginseng with various ages from different areas was classified into two categories when the distance used for clustering was 10.

Keywords

Ginseng · Inorganic element · ICP-MS · Analysis

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10.1 Introduction

There are various inorganic elements in ginseng, including macro- and micro-elements [1–3]. Many elements are key components of enzyme or protein, closely related to the growth, development, metabolism, and immune function of organism [4–7]. Common methods for the analysis of inorganic elements were Atomic Absorption Spectrometry (AAS) [8], Polarography [9], Fluorescence Method [10], and Inductively Coupled Plasma-Mass spectrometry (ICP-MS) [11]. In this book, the ICP-MS was used to determine the contents of inorganic elements.

10.2 Materials and Instruments

10.2.1 Materials

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

Spectrographic grade reagents were used to prepare reference stock solution with the concentration being 1 mg/mL. The stock solution would be diluted with deionized water to produce the solution with required concentration. Both HNO_3 and $HClO_4$ were of guaranteed grade, and the water for experiment was deionized water.

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10.2.2 Instruments

AA-7000 Graphite Furnace Atomic Absorption Spectrometer (Shimadzu Corporation, Japan), MARS Microwave Digestion Apparatus (CEM, USA), FA1104N Electronic Balance (Shanghai Jinghua Technology Instrument Co. Ltd., China), FW177 High Speed Universal Pulverizer (Beijing Yongguangming Medical Instrument Co. LTD, China), RCT-3200 Water Purification System (Changchun Laibopate Technology Development Co. LTD, China), GZX-9076 MBE Digital Display Air Drying Box (Shanghai Boxun Industrial Co. LTD, China).

10.3 Experimental Methods

10.3.1 Preparation of Test Solution

Dissolve a quantity of ginseng powder (1.0 g), accurately weighed, in 15 mL concentrated nitric acid and 5 mL concentrated perchloric acid to produce a solution. Then the solution was heated on the electric hot plate until white smoke appeared and complete nitrification. Allow it to cool, the residue was dissolved in 5% HNO_3, and diluted with deionized water to 100 mL.

10.3.2 Determination

Inject 20 μL of the test solution into the column and record the chromatogram under the condition in Table 10.1.

10.4 Results and Discussion

10.4.1 Inorganic Element Contents of Ginseng from Different Regions with Different Cultivation Periods

Inorganic element contents of ginseng from different regions with different cultivation periods are shown in Tables 10.2, 10.3, 10.4, 10.5, 10.6, and 10.7.

10.4.2 The Total Contents of Inorganic Elements of Ginseng from Different Regions with Different Cultivation Periods

The elements with content higher than 1000 mg/kg were: K (5923~20,958 mg/kg), Mg (1250~12,715 mg/kg), and Ca (1275~7259 mg/kg).

The elements with content in range of 10–1000 mg/kg were: Fe (45.9~260 mg/kg), Na (18~1031 mg/kg), Mn (13.96~119.73 mg/kg), Ba (10.5~95.9 mg/kg), Sr (4.8~68.2 mg/kg), Zn (9.14~32.27 mg/kg), and Cu (3.2~20.04 mg/kg).

The elements with content in range of 0.01–10 mg/kg were: Cr (0.09~2.39 mg/kg), Ni (0.25~10.09 mg/kg), Pb (0.01~1.8 mg/kg), Li (0.02~0.81 mg/kg), Cd (0.01~0.65 mg/kg), Co (0.03~0.72 mg/kg), and As (0.01~0.24 mg/kg).

The elements with content lower than 0.01 mg/kg were: Sn (<0.05 mg/kg), Mo (<0.02 mg/kg), Ge (<0.02 mg/kg), Se (<0.01 mg/kg), Sb (<0.01 mg/kg), and Hg (<0.005 mg/kg).

Table 10.1 Graphite furnace operating procedures

Procedure	Temperature (°C)	Heating time (s)	Retention time (s)	Flow of argon gas (L/min)
Drying	110	5	25	300
Ashing	600	3	15	300
Atomization	1300	1	5	0
Burning-out	2300	1	3	300

Table 10.2 Inorganic element contents of ginseng from Ji'an and Changbai with different cultivation periods (mg/kg)

Region	Shuangcha Ji'an			Dadi Ji'an			Changbai	
Cultivation period (year)	4	5	6	5	6	4	5	6
Ca	4696	4601	4828	6144	5395	5097	2773	4712
Mg	7841	11,723	9519	7468	9546	11,425	8063	8933
Na	19	128	69	114	130	79	20	52
K	7841	11,723	9519	7468	9546	11,425	8063	8933
Fe	157.3	81.5	63.5	45.9	53.8	99.8	59.8	76.0
Mn	30.04	68.24	42.65	27.27	18.29	97.89	119.7	39.19
Zn	11.23	12.71	14.34	9.31	12.79	15.69	20.44	19.92
Cu	7.17	7.72	6.06	7.28	6.04	3.20	5.10	7.63
Pb	0.16	0.14	0.14	0.09	0.25	0.13	0.05	0.10
Cd	0.07	0.10	0.07	0.07	0.08	0.16	0.18	0.08
Hg	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
As	0.05	0.04	0.02	0.03	0.02	0.03	0.02	0.02
Cr	1.85	0.40	0.51	0.24	0.40	0.26	0.28	0.63
Li	0.10	0.05	0.02	0.02	0.02	0.09	0.10	0.05
Co	0.06	0.14	0.06	0.07	0.08	0.14	0.15	0.06
Se	<0.01	0.02	0.03	0.03	0.03	<0.01	<0.01	0.03
Mo	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Ni	1.10	1.00	1.14	0.81	1.19	1.48	2.39	2.39
Ge	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Sr	28.0	23.0	20.6	33.2	21.1	23.0	14.4	38.5
Sn	0.08	0.06	0.50	0.23	0.19	0.09	0.12	0.12
Sb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Ba	51.1	19.6	27.6	39.5	31.9	34.3	23.2	45.1
Al	498.3	206	103.5	114.4	82.2	153.8	131.2	90.0

Table 10.3 Inorganic element contents of ginseng from Fusong, Jingyu, Linjiang, and Antu with different cultivation periods (mg/kg)

Region	Fusong			Jingyu	Linjiang	Antu	
Cultivation period (year)	4	5	6	4	6	4	5
Ca	4751	4500	4030	6011	4021	5486	4197
Mg	8272	9189	9790	9198	8697	8781	5923
Na	90	36	41	66	22	68	119
K	8272	9189	9790	9198	8697	8781	5923
Fe	93.4	76.4	104.1	80.9	65.8	198.5	71.4
Mn	33.78	69.45	43.28	31.02	56.88	39.44	45.64
Zn	21.64	18.59	32.13	14.84	20.65	14.00	9.40
Cu	9.18	7.40	7.20	5.98	9.79	9.37	7.46
Pb	0.17	0.15	1.80	0.26	0.23	0.26	0.01
Cd	0.08	0.13	0.11	0.08	0.12	0.06	0.06
Hg	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
As	0.02	0.03	0.04	0.04	0.03	0.05	0.02
Cr	0.50	0.48	1.05	0.42	0.39	0.28	0.49
Li	0.07	0.06	0.1	0.04	0.08	0.13	0.08

(continued)

Table 10.3 (continued)

Region	Fusong			Jingyu	Linjiang	Antu	
Cultivation period (year)	4	5	6	4	6	4	5
Co	0.17	0.21	0.14	0.11	0.13	0.10	0.15
Se	0.03	<0.01	0.02	0.02	0.05	<0.01	<0.01
Mo	<0.02	<0.02	<0.02	<0.02	0.08	<0.02	<0.02
Ni	4.78	3.07	4.09	1.29	4.2	10.09	4.26
Ge	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Sr	36.4	30.8	33.5	56.3	39.1	50.6	52.9
Sn	0.12	0.12	0.13	0.12	0.12	0.13	0.23
Sb	<0.01	<0.01	0.03	<0.01	<0.01	<0.01	<0.01
Ba	53.5	34.3	62.5	36.3	71.0	49.1	45.1
Al	147.2	174.2	200.8	287.6	126.2	495.9	139.2

Table 10.4 Inorganic element contents of ginseng from Dunhua and Hunchun with different cultivation periods (mg/kg)

Region	Dunhua			Hunchun		
Cultivation period (year)	4	5	4 (CC)	4	5	6
Ca	4710	4330	4119	5139	6088	4365
Mg	7606	8492	7839	8824	8244	9501
Na	24	347	18	36	29	61
K	7606	8492	7839	8824	8244	9501
Fe	94.0	57.7	87.8	65.2	65.1	108.8
Mn	42.99	57.02	33.15	34.79	15.89	26.31
Zn	14.50	19.85	12.61	17.69	14.41	15.92
Cu	7.96	8.03	6.18	7.77	6.68	9.86
Pb	0.14	0.05	0.09	0.05	0.21	0.22
Cd	0.10	0.06	0.1	0.15	0.16	0.13
Hg	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
As	0.03	0.01	0.03	0.01	0.02	0.04
Cr	0.47	0.23	0.57	0.66	0.40	0.23
Li	0.07	0.05	0.06	0.03	0.03	0.08
Co	0.11	0.06	0.04	0.06	0.03	0.04
Se	0.04	0.03	<0.01	0.02	<0.01	0.03
Mo	<0.02	<0.02	0.14	0.23	0.26	0.13
Ni	3.48	4.76	5.23	1.56	1.23	1.21
Ge	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Sr	68.2	49.8	34.5	42.8	55.9	41.8
Sn	0.23	0.34	0.15	0.22	0.07	0.04
Sb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Ba	63.6	59.4	37.0	43.2	19.0	22.2
Al	184.8	85.9	140.9	40.6	70.6	42.8

Table 10.5 Inorganic element contents of ginseng from Wangqing, Helong, and Jiaohe with different cultivation periods (mg/kg)

Region	Wangqing			Helong		Jiaohe	
Cultivation period (year)	4	5	6	4	5	4	5
Ca	5867	7295	6370	5752	6615	6806	6806
Mg	9588	7426	8923	10,968	11,892	7236	6841
Na	83	34	66	63	87	182	189
K	9588	7426	8923	10,968	11,892	7236	6841
Fe	70.0	66.2	61.9	61.5	60.2	83.7	62.8
Mn	16.25	13.96	16.82	23.53	18.79	36.13	25.50
Zn	11.18	14.77	14.49	9.14	12.22	19.00	21.82
Cu	5.63	5.59	4.99	5.29	5.47	10.87	9.52
Pb	0.09	0.16	0.09	0.07	0.09	0.16	0.10
Cd	0.06	0.08	0.07	0.06	0.04	0.11	0.09
Hg	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
As	0.02	0.03	0.03	0.02	0.03	0.13	0.03
Cr	0.26	0.28	0.17	0.11	0.21	0.20	0.26
Li	0.04	0.02	0.03	0.03	0.03	0.06	0.05
Co	0.07	0.05	0.04	0.05	0.04	0.06	0.05
Se	<0.01	<0.01	<0.01	<0.01	0.02	<0.01	0.03
Mo	0.04	<0.02	0.04	0.16	0.53	<0.02	<0.02
Ni	0.52	0.56	1.06	0.39	0.33	2.80	1.90
Ge	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Sr	49.1	55.0	42.4	50.9	43.7	40.1	38.9
Sn	0.04	0.04	0.02	0.02	0.02	0.03	0.03
Sb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Ba	30.7	33.5	27.2	28.4	26.9	73.7	72.9
Al	53.6	44.3	28.5	25.8	41.1	49.3	34.5

Table 10.6 Inorganic element contents of ginseng from Huadian, Hulin, Heihe, Kuandian, and Xinbin with different cultivation periods (mg/kg)

Region	Huadian		Hulin	Heihe	Kuandian			Xinbin
Cultivation period (year)	4	5	4	4	4	5	6	4
Ca	2654	1275	4672	5566	5793	4142	6232	5182
Mg	11,281	9457	9404	9289	8819	9032	9137	11,247
Na	159	195	160	351	30	37	24	64
K	11,281	9457	9404	9289	8819	9032	9137	11,247
Fe	160.7	86.3	56.8	170.4	95.5	82.0	75.8	83.0
Mn	108.00	94.72	52.87	51.07	28.15	29.38	34.25	49.78
Zn	32.27	25.14	13.64	15.80	19.68	25.82	11.62	13.22
Cu	7.04	7.74	7.69	20.04	7.75	10.18	8.20	6.91
Pb	0.27	0.17	0.08	0.11	0.23	0.10	0.39	0.56
Cd	0.65	0.31	0.07	0.03	0.11	0.05	0.09	0.16
Hg	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
As	0.10	0.05	0.02	0.07	0.04	0.04	0.03	0.13
Cr	0.28	0.60	0.22	0.63	0.26	0.17	0.22	0.23
Li	0.32	0.23	<0.01	0.17	0.04	0.03	0.03	0.02
Co	0.72	0.64	0.07	0.12	0.35	0.06	0.06	0.11

(continued)

Table 10.6 (continued)

Region	Huadian		Hulin	Heihe	Kuandian			Xinbin
Cultivation period (year)	4	5	4	4	4	5	6	4
Se	0.12	0.08	0.02	0.04	<0.01	<0.01	<0.01	0.03
Mo	<0.02	<0.02	0.07	<0.02	<0.02	<0.02	<0.02	<0.02
Ni	6.99	5.82	1.16	3.83	2.63	0.61	0.77	1.14
Ge	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Sr	24.9	18.1	35.8	30.3	30.5	11.1	24.6	16.8
Sn	0.21	0.14	0.07	0.02	<0.01	<0.01	<0.01	0.04
Sb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Ba	85.8	53.9	48.0	40.5	63.5	22.8	44.7	26.0
Al	107.2	77.0	27.1	162.6	59.2	16.1	49.5	59.0

Table 10.7 Inorganic element contents of ginseng from North Korea, Tonghua, and South Korea with different cultivation periods (mg/kg)

Region	North Korea		Tonghua		South Korea	
Cultivation period (year)	4	5	6	4, 5	5	6
Ca	6196	5084	6082	4122	2609	3288
Mg	12,715	1322	1397	1250	1629	1701
Na	298	856	217	655	329	150
K	12,715	9225	12,035	12,791	14,569	16,543
Fe	118.6	59.3	60.1	49.9	62.1	103.2
Mn	53.00	31.38	48.88	62.78	50.65	29.21
Zn	17.66	18.21	15.61	14.4	28.08	27.97
Cu	8.33	7.62	9.51	9.26	11.47	9.52
Pb	0.46	0.08	0.33	0.07	0.50	0.27
Cd	0.14	0.23	0.11	0.18	0.04	0.02
Hg	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
As	0.06	0.03	0.04	0.02	0.09	0.07
Cr	2.39	0.18	0.14	0.11	0.14	0.09
Li	0.06	0.06	0.02	0.03	0.09	0.12
Co	0.17	0.08	0.12	0.26	0.07	0.18
Se	0.06	0.02	0.03	<0.01	0.12	0.11
Mo	<0.02	<0.02	<0.02	<0.02	0.43	<0.02
Ni	0.96	1.29	0.73	0.61	0.45	0.34
Ge	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Sr	27.8	24.4	27.4	22.7	24.0	9.4
Sn	<0.01	<0.01	<0.01	0.05	0.02	<0.01
Sb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Ba	32.5	41.9	28.7	10.5	95.9	66.3
Al	53.8	33.1	22.2	13.9	103.9	198.5

10.4.3 Differences in the Content of Inorganic Elements of Ginseng from Different Regions with Different Cultivation Periods

For the 4-year-old ginseng, the content of some inorganic elements is shown as follows (Figs. 10.1, 10.2, and 10.3). The contents of Ca, Mg, and K in ginseng are shown in Fig. 10.1; the contents of Mn, Fe, Zn, Cu, Al, Ba, and Sr are shown in Fig. 10.2; the contents of Pb, Cd, As, Cr, Li, and Co are shown in Fig. 10.3. It could be concluded that the ginseng from Ji'an, Antu, Huadian, and Heihe had the higher content of inorganic elements than from other areas.

For the 5-year-old ginseng, the contents of some inorganic elements are shown in Figs. 10.4, 10.5, and 10.6. The contents of Ca, Mg, and K in ginseng are shown in Fig. 10.4; the contents of Mn, Fe, Zn, Cu, Al, Ba, and Sr are shown in Fig. 10.5; the contents of Pb, Cd, As, Cr, Li, and Co are shown in Fig. 10.6. It can be concluded that the ginseng from Ji'an, Changbai, and Huadian had the higher content of inorganic elements than from other areas (Figs. 10.7, 10.8, and 10.9).

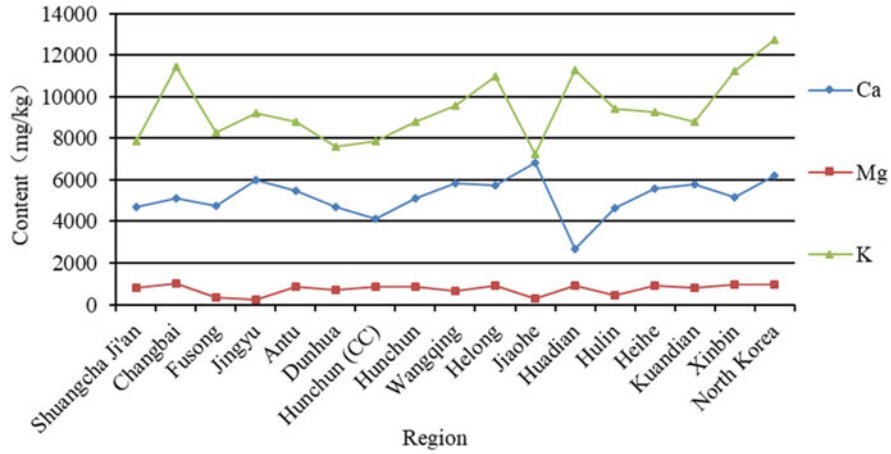

Fig. 10.1 The contents of Ca, Mg, and K of 4-year-old ginseng

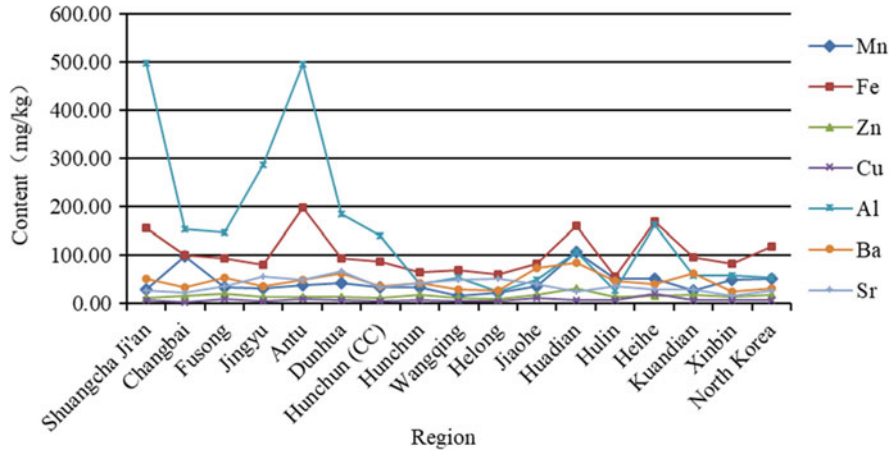

Fig. 10.2 The contents of Mn, Fe, Zn, Cu, Al, Ba, and Sr of 4-year-old ginseng

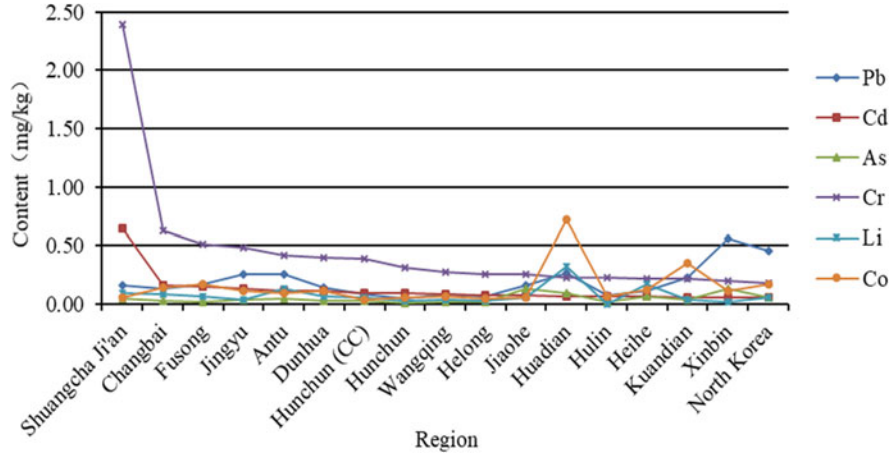

Fig. 10.3 The contents of Pb, Cd, As, Cr, Li, and Co of 4-year-old ginseng

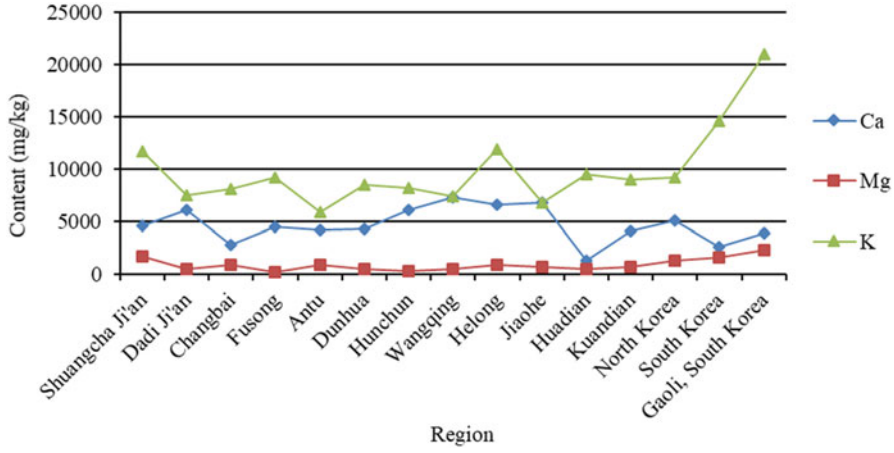

Fig. 10.4 The contents of Ca, Mg, and K of 5-year-old ginseng

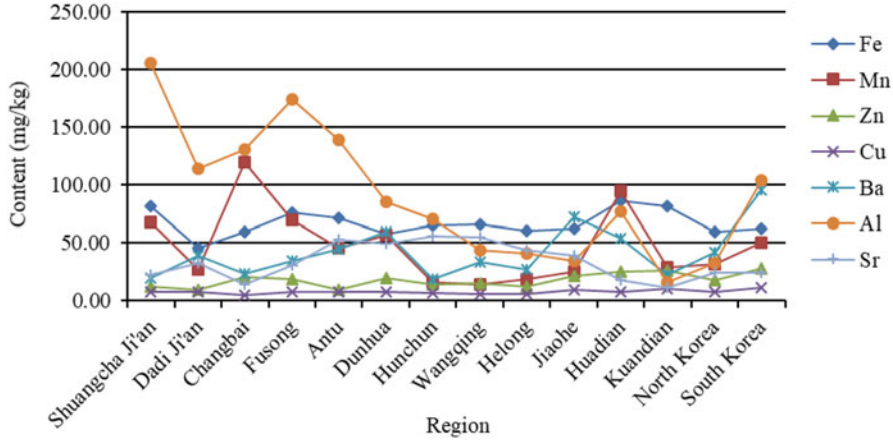

Fig. 10.5 The contents of Mn, Fe, Zn, Cu, Al, Ba, and Sr of 5-year-old ginseng

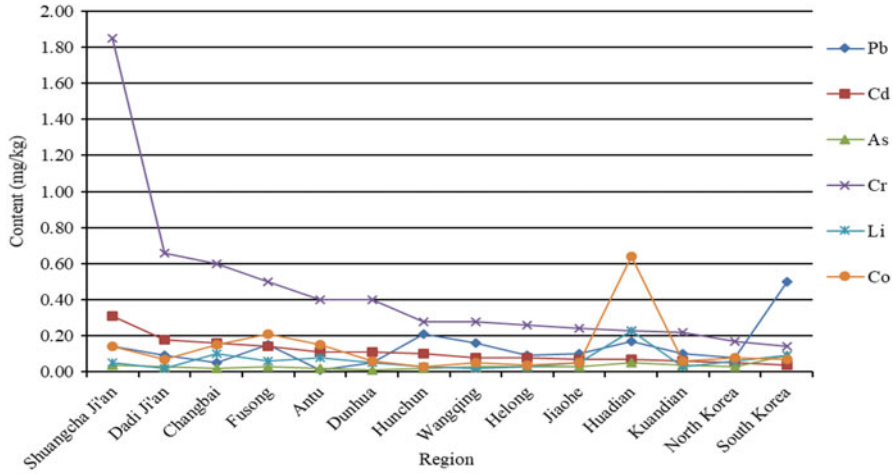

Fig. 10.6 The contents of Pb, Cd, As, Cr, Li, and Co of 5-year-old ginseng

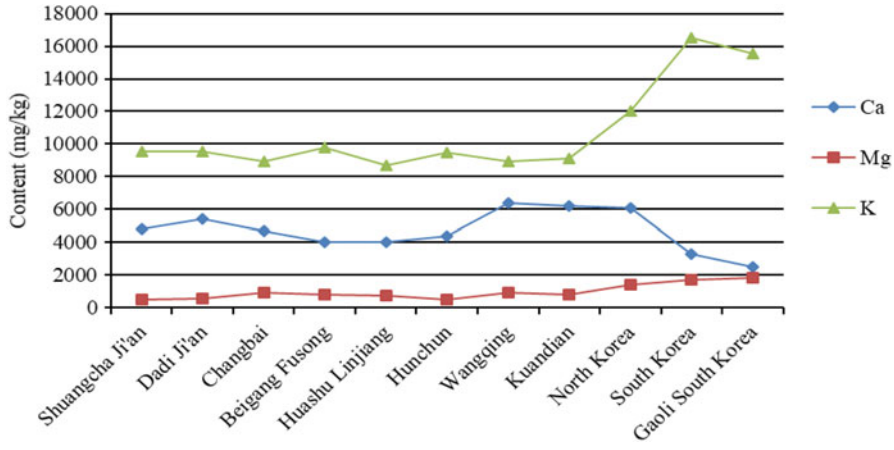

Fig. 10.7 The contents of Ca, Mg, and K of 6-year-old ginseng

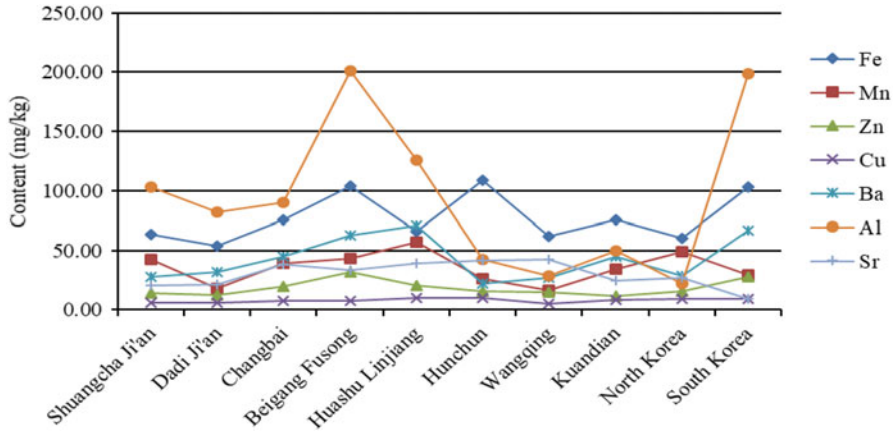

Fig. 10.8 The contents of Mn, Fe, Zn, Cu, Al, Ba, and Sr of 6-year-old ginseng

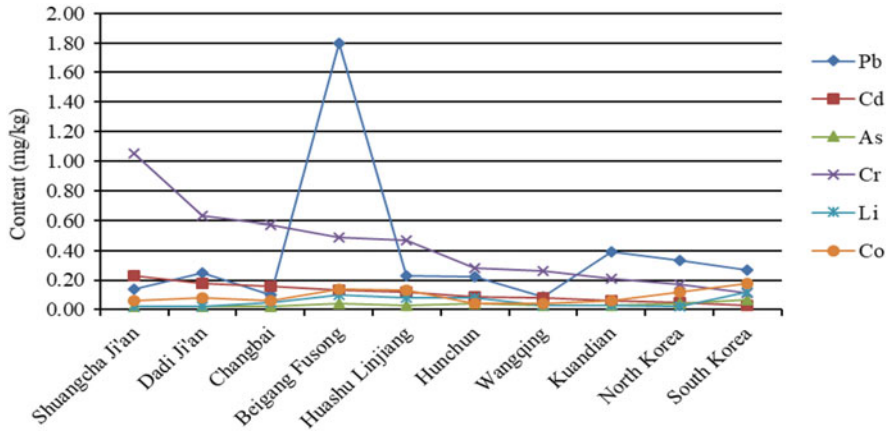

Fig. 10.9 The contents of Pb, Cd, As, Cr, Li, and Co of 6-year-old ginseng

10.5 Hierarchical Cluster Analysis of Inorganic Elements of Ginseng from Different Regions with Different Cultivation Periods

Combining Ward Method, Chi-Square Metric and Squared Euclidean Distance in SPSS 22.0 software, the dendrogram (Fig. 10.10) of cluster analysis was established, in which the contents of 24 inorganic elements were the characteristic variable. The results showed that all ginseng samples could be classified into two categories when the distance used for clustering was 10. The samples include TH4-5 (30), CX05 (39), CX06 (40), HG05 (41), and HG06 (42). The rest samples were clustered into the other category, which could be further divided into two groups when the clustering distance is 5. One group consisted of HH04 (1), AT04/05 (3~4), DH04/05 (8~9), HC05 (15), JADD05 (21), JASC04 (23), JH04/05 (26~27), JY04 (28), WQ05/06 (32~33), and KD04/KD06 (34, 36). The other group included the rest samples. In conclusion, the similarity of samples with different cultivation periods from different regions can be analyzed when the variances of 24 inorganic elements were used as the characteristic variables by hierarchical cluster analysis.

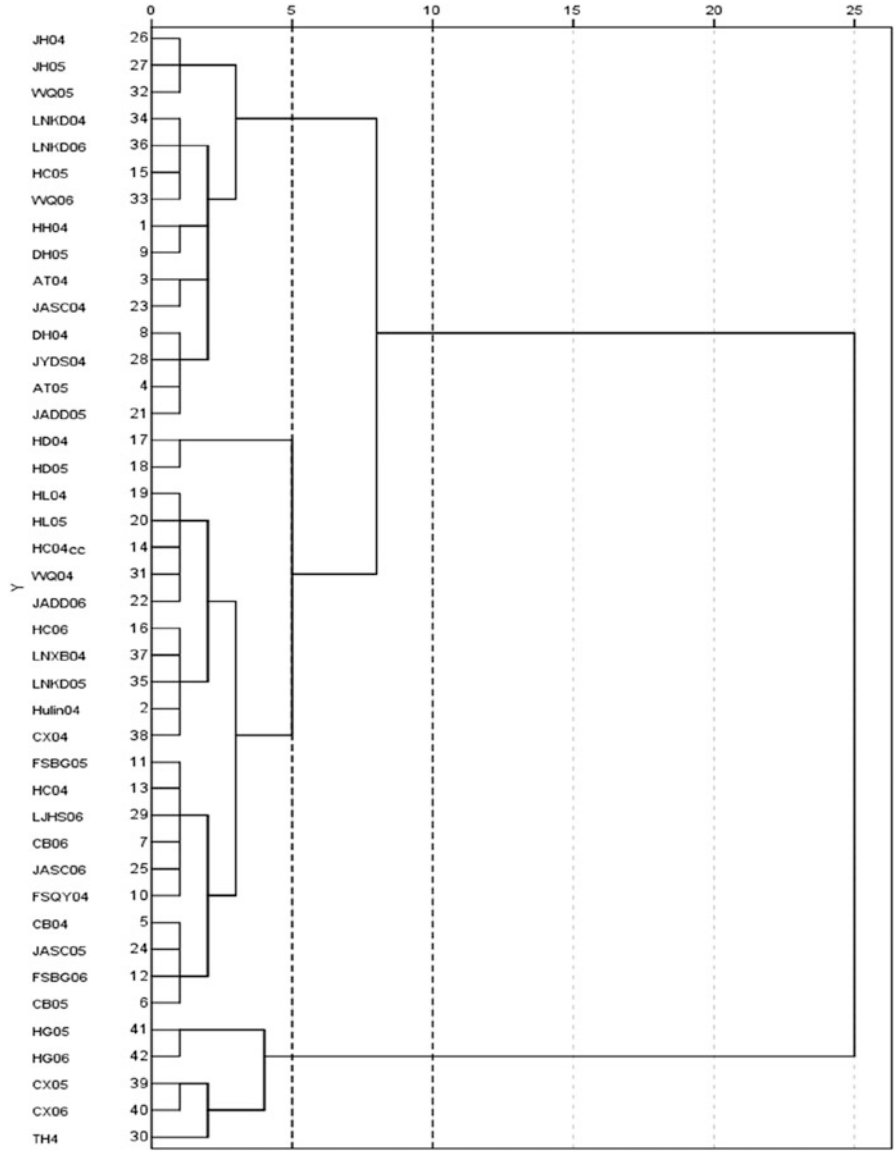

Fig. 10.10 Hierarchical graph of cluster analysis of inorganic elements in ginseng

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Analysis of Sterols in Ginseng

11

Chuangui Liu, Jinluan Chen, Yu Si, and Jinping Liu

Abstract

In order to provide data for clarifying the contents of sterols in ginseng, the determination of sterols was performed. The fresh ginseng was taken as the test samples. The methods for total sterols and monomeric sterol were established based on spectrophotometry-H_3PO_4-H_2SO_4-$FeCl_3$ coloration and high performance liquid chromatography-evaporative light scattering detection (HPLC-ELSD), respectively. In the chapter, the content of total sterols is determined by spectrophotometry-H_3PO_4-H_2SO_4-$FeCl_3$, and the monomeric sterol was detected by HPLC-ELSD. For the ginseng in same area, the sterol content of 5-year-old ginseng was the highest, and the sterol content of 6-year-old ginseng was the lowest. For the ginseng from various areas, the contents of sterol were different. In general, the distribution of sterol in 45 samples was different but lacks regularity.

Keywords

Ginseng · Sterol · HPLC-ELSD

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11.1 Introduction

Sterols are a kind of important natural active substance, which is classified into animal sterols, plant sterols [1, 2], and fungal sterols according to their raw materials. The plant sterols mainly included sitosterol, stigmasterol, and campesterol [3]. Plant sterols have a wide range of physiological activities and biological activities [4] in regulating blood lipids and lowering blood cholesterol. The determination methods of sterol content are spectrophotometry, HPLC method, and so on [5]. The content of total sterols in ginseng samples was determined by spectrophotometry [6]. The content of monomer sterol was analyzed by HPLC [7].

11.2 Materials and Instruments

The content of total sterols in ginseng samples is determined by spectrophotometry at the wavelength of 440 nm. And H_3PO_4-H_2SO_4-$FeCl_3$ solution [8] is used as a chromogenic agent.

The monomeric sterol in the sample was detected by a high performance liquid phase-evaporation photodetector (HPLC-ELSD).

11.2.1 Materials

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

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Anhydrous ethanol, ether, sodium hydroxide, acetic anhydride, $FeCl_3$, phosphoric acid, and concentrated sulfuric acid were all of analytical grade, methanol was of chromatographical grade. β-sitosterol (HPLC $\geq$98%) and stigmasterol (HPLC $\geq$95%) were purchased from Beijing Yingzena New Chemical Technology Research Institute, China.

11.2.2 Instruments

R201D Thermostat Water Bath (Shanghai Yukang Science and Education Equipment Co. Ltd., China), Rotary Evaporator (Shanghai Yukang Science and Education Equipment Co. Ltd., China), SHZ-D (III) Circulating Water Vacuum Pump (Gongyi Yuhua Instrument Co. Ltd., China), FA1102N Electronic Balance (Shanghai Jinghua Tech Instrument Co. Ltd., China), 721 Spectrophotometer (Shanghai Optical Instrument Factory, China).

LC-10AT Liquid Chromatography (Shimadzu Corporation, Japan), CBM-102 Chromatography Workstation (Shimadzu Corporation, Japan), SEDERE SEDEX 75 Evaporative Light Scattering Detector (SEDERE Corporation, France).

11.3 Experimental Methods

11.3.1 Determination of Total Sterol Contents

11.3.1.1 Screening of Methods for Total Sterol Extraction and Purification

11.3.1.1.1 Screening of Methods to Extract Total Sterols

Generally, there are two forms of sterols in plants, including free form and combined form. The free form can be directly extracted by a low-polar organic solvent such as diethyl ether or petroleum ether; the combined sterol is generally esterified with other molecules, and its solubility is different from that of free sterol. In order to ensure sufficient extraction of the sterols, the impurities are removed as much as possible. The saponification extraction step is generally carried out to convert them into free unsaponifiables as much as possible. The extraction methods included alcohol solution saponification extraction method, extraction saponification method, saponification extraction method, and extraction non-saponification method. In this study, thin layer chromatography (TLC) and high performance liquid chromatography-evaporative light scattering detector (HPLC-ELSD) are used to analyze the difference of sterol content. The results showed that the contents of sterol extracted by alcohol solution saponification extraction are the highest.

11.3.1.1.2 Screening of Solvents for Extraction

The structure of sterol is similar to triterpenoid saponin, which is also rich in ginseng. But the solubilities of sterol and triterpenoid saponin are different. Triterpenoid saponins are generally insoluble in ether and petroleum ether, while sterols are soluble in the above solvents. So sterols could be extracted from the mixture by using a low-polar organic solvent, such as diethyl ether and petroleum ether, as the extraction solvent. This chapter compared the ultrasonic extraction efficiency by using ether, petroleum ether, and cyclohexane as the solvents. The results showed that the total sterol content in the ether extract is the highest, but the color of the ether extract is the deepest one due to the highest contents of impurity. Considering the specificity of chromogenic reaction of H_3PO_4-H_2SO_4-$FeCl_3$ system and the small volume of the added sample, the influence of impurities is neglected. Finally, diethyl ether is selected as the extract solvent.

11.3.1.1.3 Screening of Times for Extraction

After each extraction, the extract was detected by thin layer chromatography (TLC). At the same time, the β-sitosterol reference solution and the extracted sample are spotted. The petroleum ether-diethyl ether (7:3) is used as the developing solvent, and the 10% H_3PO_4-H_2SO_4-$FeCl_3$ solution is used as the color reagent. Both the

β-sitosterol reference solution and the test solution would appear purple-red dot. The sample is extracted with 100 mL diethyl ether each time, and the sixth extract is confirmed to have no color reaction by TLC. Therefore, the ginseng was extracted five times, and 100 mL diethyl ether was used every time.

11.3.1.2 Screening of Methods for Total Sterol Determination

The common methods for determining total sterols are acetic anhydride-concentrated sulfuric acid spectrophotometry, H_3PO_4-H_2SO_4-$FeCl_3$ color spectrophotometry, digitonin precipitation method, and so on. The colorimetric principle of acetic anhydride-concentrated sulfuric acid method is based on the sterol reacting with strong acid, and the color change (Liebermann–Burchard reaction) occurs under the condition of anhydrous. Finally, it is green. Triterpenoid saponins can also undergo such color reaction, but in the end they appear red. The coloring principle of H_3PO_4-H_2SO_4-$FeCl_3$ is that sterol can form a stable purple-red compound under the action of concentrated sulfuric acid and Fe^{3+}, which has characteristic absorption peaks in a specific wavelength range. But different types of sterols will display a little different color. The principle of the digitonin precipitation method is that the sterol compound can be precipitated by insoluble molecular complex with steroidal saponin, and the reaction can occur in any sterol (such as β-sitosterol, stigmasterol, ergosterol, etc.) containing a C_3-position β-OH.

Spectrophotometry-H_3PO_4-H_2SO_4-$FeCl_3$ coloration method was chosen to determine the contents of total sterols in this chapter. The operation steps are as follows:

Accurately measured the sterol ethanol solution, added the H_3PO_4-H_2SO_4-$FeCl_3$ reagent, determined the absorbance of the samples and the sterol reference substance with series of concentrations at maximum absorption wavelength (λ_{max}) under the same conditions. Then draw the standard curve of the absorbance-sterol amount. The total sterol content was calculated from the calibration curve.

The screening of the maximum absorption wavelength is as follows: taking the β-sitosterol as the reference substance of the reaction, the color reaction is carried out according to the above procedure. The absorbance of the reaction system was rapidly determined by every 10 nm or 20 nm in the wavelength range of 400–800 nm. The results of wavelength scanning are shown in Fig. 11.1. Since the color reagent is light yellow, the absorbance of the blank solution is also determined to exclude the background interference after adding the chromogenic reagent. The maximum absorption wavelength λ_{max} of the reference solution and the sample solution is 440 nm, and the absorbance of the blank solution is close to 0. Because the influence is small, wavelength of 440 nm is selected as detected wavelength.

11.3.1.3 Determination of Total Sterol

11.3.1.3.1 Preparation of H_3PO_4-H_2SO_4-$FeCl_3$ Color Reagent

Dissolved an accurately weighed $FeCl_3$ about 2.5 g, in 85% of concentrated phosphoric acid and diluted to 100 mL, stored at 4 °C in the dark, as the stock solution.

Measured accurately 7.5 mL of iron storage solution and dilute with concentrated sulfuric acid to 50 mL. Allowed to store at room temperature in the dark for 6–8 weeks.

11.3.1.3.2 Preparation of Reference Solution

Dissolved a quantity of β-sitosterol, accurately weighted, in anhydrous ethanol to produce a solution of 1.0 mg/mL, as the reference solution.

11.3.1.3.3 Preparation of Test Solution

Ginseng was, respectively, air-dried, grinded, and sieved (Chinese National Standard Sieve No. 6, R40/3 series) to get the homogeneous powder. Weighed accurately 5 g of the fine powder, added 12 g of NaOH and 150 mL 95% ethanol, respectively, mixed well and refluxed in

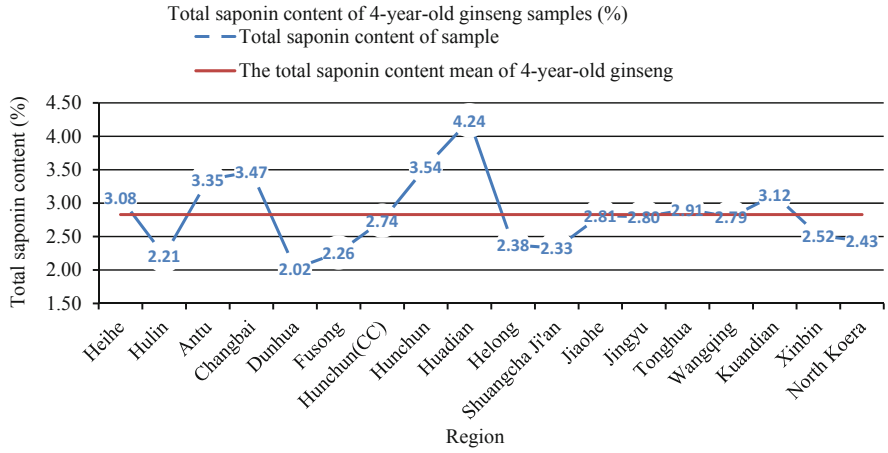


Fig. 11.1 H_3PO_4-H_2SO_4-$FeCl_3$ colorimetry wavelength scanning

a water bath at 85 °C for 4 h, then allowed to cool at room temperature, filtered. The residue was extracted again, filtered. The filtrate was combined, dried under vacuum, transfered to separatory funnel with 250 mL water and extracted with 100 mL ethylether for five times. The ethylether layer was combined, washed with water to pH 7.0 and dried. The residue was dissolved and transferred to 10 mL volumetric flask, diluted with anhydrous ethanol to 10 mL, mixed well and filtered (0.45 μm), the filtrate as the test solution.

11.3.1.3.4 Drawing the Standard Curve

Measured accurately 0.4 mL, 0.6 mL, 0.8 mL, 1.0 mL, 1.2 mL, 1.4 mL, and 1.6 mL of the reference solution, respectively, to a colorimetric tube with stopper, diluted with absolute ethanol to 7 mL, allowed to stand in an ice water bath, added 3 mL H_3PO_4-H_2SO_4-$FeCl_3$ colorimetry, mixed well, heated in a water bath at 50 °C for 20 min, allowed to cool to room temperature, diluted with absolute ethanol to 10 mL, mixed well, the absorbance was measured at 440 nm by UV-visible spectrophotometry [Chinese Pharmacopoeia 2010 edition, Appendix VA] with the corresponding chemical as a blank. The absorbance results are shown in Table 11.1. Measured the absorbance at 440 nm. Calculated the linear regression equation from the absorbances (y) obtained versus the concentrations (x) of the reference solution (Fig. 11.2). The regression equation was obtained: $y = 0.5579x - 0.0742$ ($R = 0.9990$), indicating the method had good linearity in the range of 0.4152–1.6608 mg.

11.3.1.3.5 Determination of the Test Solution

Repeated the operation, using the substance being examined, 0.7 mL of the test solution was accurately measured, instead of the reference solutions, and calculated the concentration of total sterols obtained from the equation. The total sterol contents were calculated according to the following formula. The final content of the total sterol in ginseng sample was the average value of the test parallel samples.

$$\text{Total sterol content}\ (\%) = \frac{m' \times V}{1000 \times V' \times m} \times 100\%$$

m sample quality (g); V constant volume (mL); m' the mass (mg) of β-sitosterol in the sample corresponding to the absorbance A; V' sampling volume (mL) on measuring the absorbance.

Table 11.1 The calibration curve of absorbance- β-sitosterol levels

Standard solution volume (mL)	0.4	0.6	0.8	1.0	1.2	1.4	1.6
Content of β-sitosterol (mg)	0.415	0.623	0.830	1.038	1.246	1.453	1.661
Absorption 1	0.147	0.282	0.403	0.498	0.620	0.716	0.868
Absorption 2	0.148	0.282	0.403	0.498	0.620	0.714	0.868
Average absorption	0.148	0.282	0.403	0.498	0.620	0.715	0.868
Regression equation	$y = 0.5579x - 0.0742$ ($R = 0.9990$)						

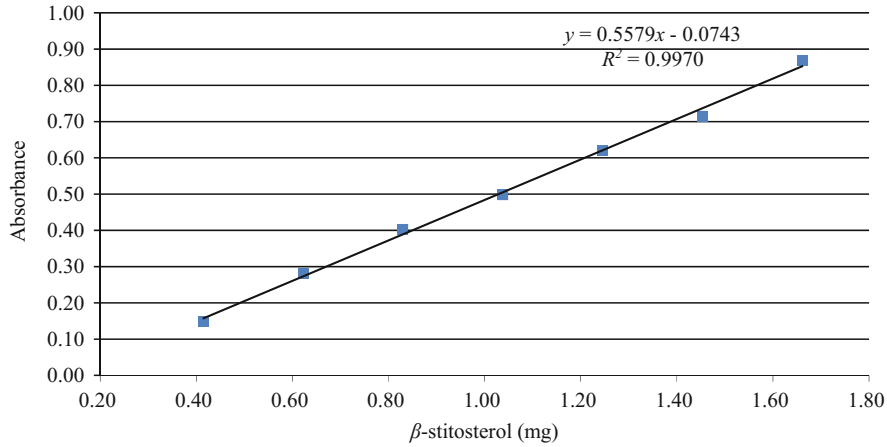


Fig. 11.2 Calibration curve of absorbance—the mass of β-sitosterol levels

11.3.2 Determination of Monomeric Sterol Contents in Ginseng Samples

11.3.2.1 Preparation of Reference Solutions

Dissolved a quantity of β-sitosterol, accurately weighted, in anhydrous ethanol to produce a solution of 0.98 mg/mL, as reference solution A.

Dissolved a quantity of stigmasterol, accurately weighted, in anhydrous ethanol to produce a solution of 0.116 mg/mL, as reference solution B.

The retention times of β-sitosterol and stigmasterol in HPLC chromatogram are shown in Table 11.2.

11.3.2.2 Preparation of Test Solutions

The same as in Sect. 11.3.1.3.3.

11.3.2.3 Chromatographic Conditions

Shimadzu LC-10AT high performance liquid chromatography, using Diamonsil C_{18} (4.6 mm × 250 mm, 5 μm). Column temperature was set at 30 °C. Drift tube temperature was set at 40 °C. Carrier gas flow rate was set at 2.50 L/min. The mobile phases were composed of eluent A (methanol) and eluent B (water) with flow rate of 1.0 mL/min. The gradient elution conditions are listed in Table 11.3. The run time was 50 min. The volume injected for samples were all 15 μL for each run. The chromatographic peaks of each component of sterols reached baseline separation.

11.3.2.4 Method Validation

11.3.2.4.1 Precision, Repeatability, and Stability

Precision was evaluated by analyzing the reference solution five times ($n = 5$). The precision was expressed as the relative standard deviation (RSD) of each peak area of each sterol. The results showed that the RSD was from 1.30% to 2.90%

Repeatability was evaluated by analyzing the same test sample solution in replicates of five

Table 11.2 Concentration and retention time of monomeric sterol standard solution

Name of sterol	Concentration (mg/mL)	Retention time (min)
Stigmasterol	0.116	40.031
β-Sitosterol	0.980	42.216

Table 11.3 Gradient elution program of HPLC

Time (min)	Phase A (%)	Phase B (%)
0–20	50 → 100	50 → 0
20–45	100	0
45–46	100 → 50	0 → 50

samples ($n = 5$). The repeatability was expressed as RSD of the stigmasterol and β-sitosterol. The results showed that the RSD was 2.93% and 3.06%, respectively.

Stability was investigated by analyzing the same test sample solution at 0, 4, 8, 12, 24 h and 2 weeks, respectively. The stability was expressed as RSD of the stigmasterol and β-sitosterol. The results showed that the RSD was 2.03% and 3.16%, respectively, which indicated that the test solution had good stability within 2 weeks.

11.3.2.4.2 Investigation of Sample Recovery

Recovery was accessed by comparing the contents of stigmasterol and β-sitosterol before and after extraction at three levels in three replicates. The recovery was expressed as recovery rates and the RSD of recovery rates. The results showed that the recovery rates were in the range of 91.2–106.5%, with the RSD being 1.39–4.78%.

11.3.2.4.3 Draw of Standard Curve

Injected accurately 2 μL, 4 μL, 6 μL, 8 μL, 10 μL, 12 μL, and 14 μL Stigmasterol reference solution, respectively, into the column and recorded the chromatogram.

Injected accurately 1 μL, 2 μL, 10 μL, 15 μL, and 20 μL of the β-sitosterol reference solution, respectively, into the column and recorded the chromatogram.

The regression equations were obtained by taking natural logarithm of the amount of the reference substance (μg) as the abscissa (x) and natural logarithm of the peak area of the reference product as the ordinate (y), which were shown in Table 11.4. The HPLC chromatogram of β-sitosterol and stigmasterol reference is shown in Fig. 11.3.

11.3.2.4.4 Determination of Monomeric Sterol

Injected accurately 15 μL test solution into the column and recorded the chromatogram. The peak area of the monomeric sterol peak in the test sample was within the linear range of the standard curve, and the content of every monomer sterol in the ginseng sample was calculated by the linear regression equation of the monomer sterol peak area-content.

11.4 Results and Discussion

11.4.1 Total Sterol of Ginseng

11.4.1.1 Total Sterol Contents of Ginseng from Different Regions with Different Cultivation Periods

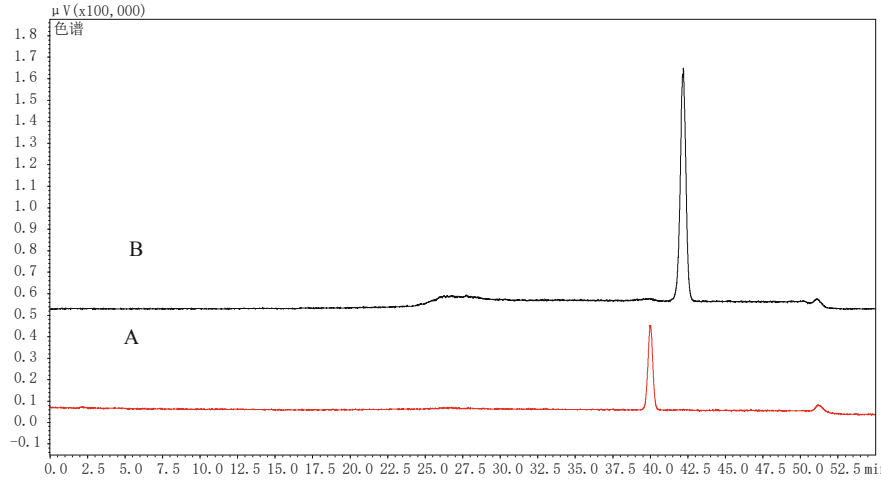
Total sterol contents of ginseng from different regions with different cultivation periods are shown in Table 11.5.

11.4.1.2 Analysis of the Total Sterols in Ginseng with Different Periods

The ginseng with the highest total sterol content was North Korean 5-year-old ginseng

Table 11.4 Regression equations with correlation coefficients of sterols

Name of sterol	Retention time (min)	Regression equation	Correlation coefficient
Stigmasterol	39.872 ± 0.139	ln(S) = 1.7935 ln(m) + 12.944	0.996
β-sitosterol	42.135 ± 0.065	ln(S) = 1.5198 ln(m) + 13.981	0.997



A Stigmasterol; *B* *β*-Sitosterol

Fig. 11.3 HPLC chromatogram of sterol standard substances. (**a**) Stigmasterol; (**b**) *β*-Sitosterol

(0.66%) and Huadian 4-year-old ginseng (0.62%). The ginseng with the lowest total sterol content was Tonghua Jilin 4-year-old ginseng (0.16%). The average value of the total sterol content of all samples was 0.35% ± 0.10%. The average sterol contents of 4-, 5-, and 6-year-old ginseng were 0.34%, 0.35%, and 0.33%, respectively. The grown ages of ginseng with high-to-low sequence of total sterol contents are 5-year-old, 4-year-old, 6-year-old, as shown in Table 11.6.

The change tendency of total sterols with ages in ginseng samples from the same origin is not stable (Fig. 11.4).

11.4.1.3 The Analysis of Total Sterols in Ginseng from Different Regions

Sequenced total sterol contents of 4-,5-, and 6-year-old ginseng are shown in Figs. 11.5, 11.6, and 11.7.

11.4.2 The Analysis of Stigmasterol

11.4.2.1 The Contents of Stigmasterol in Ginseng from Different Regions with the Different Cultivation Periods

Contents of stigmasterol in ginseng from different regions with different cultivation periods are shown in Table 11.7.

11.4.2.2 Analysis of Stigmasterol in Ginseng

The mean of stigmasterol content in all samples is 0.0166% ± 0.0082%. The stigmasterol content differs greatly between different samples and RSD is 48.27%. The highest sample is Hunchun 5-year-old ginseng (0.0335%) and the lowest sample is Changbai 5-year-old ginseng (0.0062%). The content of 4-,5-, and 6-year-old ginseng is 0.0142%, 0.0191%, and 0.0165%, respectively. The trend of the total content is CG5 > CG6 > CG4.

For 4-year-old ginseng, the regions with high-to-low contents were: Antu, Changbai, Hunchun,

Table 11.5 Total sterol contents of ginseng from different regions with different cultivation periods (year)

No	Region	Cultivation period (year)	Content of sterol (%)		
			Parallel sample 1	Parallel sample 2	Average value
1	Heihe	4	0.3588	0.3066	0.3327
2	Hulin	4	0.3944	0.2306	0.3125
3	Antu	4	0.2119	0.3175	0.2647
4		5	0.2866	0.3009	0.2938
5	Changbai	4	0.3347	0.3347	0.3347
6		5	0.2115	0.2661	0.2388
7		6	0.3671	0.2869	0.3270
8	Dunhua	4	0.4004	0.3120	0.3562
9		5	0.2516	0.2363	0.2439
10	Fusong	4	0.2658	0.4597	0.3627
11		5	0.2301	0.3493	0.2897
12		6	0.2895	0.2961	0.2928
13	Hunchun	4(CC)	0.4016	0.4979	0.4497
14		4	0.4021	0.3098	0.3559
15		5	0.3749	0.3198	0.3474
16		6	0.4675	0.3549	0.4112
17	Huadian	4	0.5662	0.6661	0.6161
18		5	0.5780	0.3663	0.4721
19	Helong	4	0.2347	0.1780	0.2064
20		5	0.2542	0.3168	0.2855
21	Dadi, Ji'an	5	0.4175	0.4061	0.4118
22		6	0.2407	0.2407	0.2407
23	Shuangcha, Ji'an	4	0.2544	0.2837	0.2691
24		5	0.3312	0.2788	0.3050
25		6	0.3835	0.3406	0.3620
26	Jiaohe	4	0.4812	0.4071	0.4441
27		5	0.4821	0.3906	0.4363
28	Jingyu	4	0.3703	0.4659	0.4181
29		5	0.3515	0.3525	0.3520
30	Linjiang	5	03544	0.3498	0.3521
31		6	0.3371	0.3371	0.3371
32	Tonghua	4	0.1690	0.1462	0.1576
33		5	0.3522	0.3510	0.3416
34	Wangqing	4	0.2907	0.3148	0.3028
35		5	0.3148	0.2775	0.2961
36		6	0.2065	0.2761	0.2413
37	Kuandian	4	0.3785	0.3230	0.3508
38		5	0.2550	0.1726	0.2138
39		6	0.2383	0.2961	0.2672
40	Xinbin	4	0.3783	0.4334	0.4059
41	North Korea	4	0.3375	0.3951	0.3663
42		5	0.3769	0.5143	0.4456
43		6	0.4168	0.5276	0.4722
44	South Korea	5	0.6892	0.6349	0.6621
45		6	0.3658	0.4045	0.3852

Table 11.6 The analysis of total sterol contents in ginseng from the same regions with different cultivation periods

No	Region	Content
1	Changbai	CG4 > CG6 > CG5
2	Fusong	CG4 > CG6 > CG5
3	Hunchun	CG4(CC) > CG6 > CG4 > CG5
4	Shuangcha Ji'an	CG6 > CG5 > CG4
5	Wangqing	CG4 > CG5 > CG6
6	Kuandian	CG4 > CG6 > CG5
7	North Korea	CG6 > CG5 > CG4
8	Antu	CG5 > CG4
9	Dunhua	CG4 > CG5
10	Huadian	CG4 > CG5
11	Helong	CG5 > CG4
12	Dadi Ji'an	CG5 > CG6
13	Jiaohe	CG4 > CG5
15	South Korea	CG5 > CG6
16	Jingyu	CG4 > CG5
17	Tonghua	CG5 > CG4
18	Linjiang	CG5 > CG6

Wangqing, Helong, Fusong, Jingyu, Huadian, Hulin, Dunhua, Tonghua, North Korea, Xinbin, Huangcha Ji'an, Heihe, Kuandian, Jiaohe.

For 5-year-old ginseng, the regions with high-to-low contents were: Hunchun, Fusong, Antu, Jiaohe, South Korea, Shuangcha Ji'an, Helong, Huadian, Wangqing, Jingyu, Tonghua, Linjiang, North Korea, Dadi Ji'an, Dunhua, Kuandian, Changbai.

For 6-year-old ginseng, the regions with high-to-low contents were: Dadi Ji'an, Changbai, South Korea, Shuangcha Ji'an, Linjiang, Wangqing, Hunchun, Fusong, North Korea, Kuandian.

11.4.3 The Analysis of β-sitosterol

11.4.3.1 The Contents of β-sitosterol in Ginseng from Different Regions with the Different Cultivation Periods

Contents of β-sitosterol in ginseng from different regions with different cultivation periods are shown in Table 11.8.

11.4.3.2 Analysis of β-sitosterol in Ginseng

The mean of β-sitosterol content in all samples is 0.0358% ± 0.0192% and higher than stigmasterol. The β-sitosterol content differs greatly between different samples and RSD is 53.74%. The highest sample is Hunchun 5-year-old ginseng (0.0828%) and the lowest sample is Kuandian 5-year-old ginseng (0.0110%). The content of 4-, 5-, and 6-year-old ginseng is 0.0290%, 0.0440%, and 0.0340%, respectively. The trend of the total content is CG_5, CG_6, CG_4.

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

For 5-year-old ginseng, the regions with high-to-low contents were: Hunchun, Antu, Jiaohe, South Korea, Fusong, Helong, Jingyu, Shuangcha Ji'an, Linjiang, Tonghua, Wangqing, North Korea, Huadian, Dadi Ji'an, Dunhua, Changbai, Kuandian.

For 6-year-old ginseng, the regions with high-to-low contents were: Dadi Ji'an, South Korea, Changbai, Shuangcha Ji'an, Linjiang, Wangqing, Hunchun, Fusong, North Korea, Kuandian.

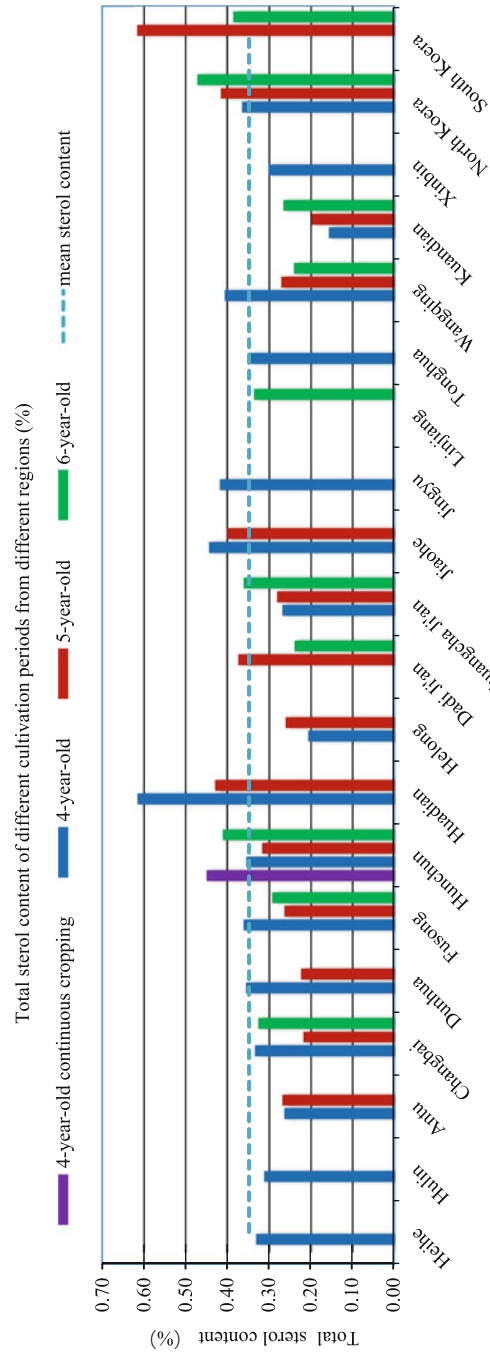


Fig. 11.4 Column diagram of total sterols of ginseng from different regions with different cultivation ages

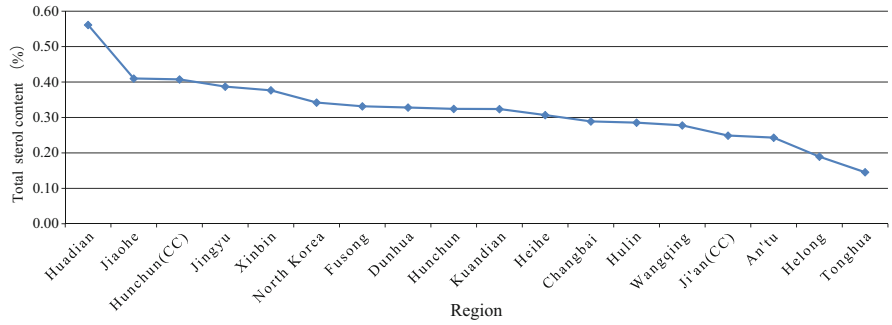


Fig. 11.5 Sequenced total sterol contents of 4-year-old ginseng

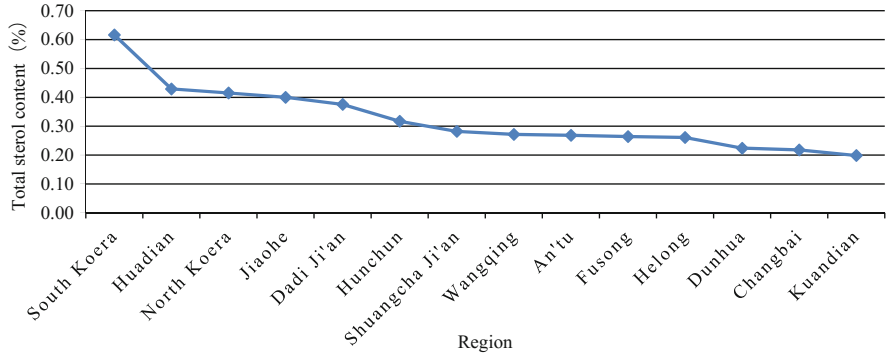


Fig. 11.6 Sequenced total sterol contents of 5-year-old ginseng

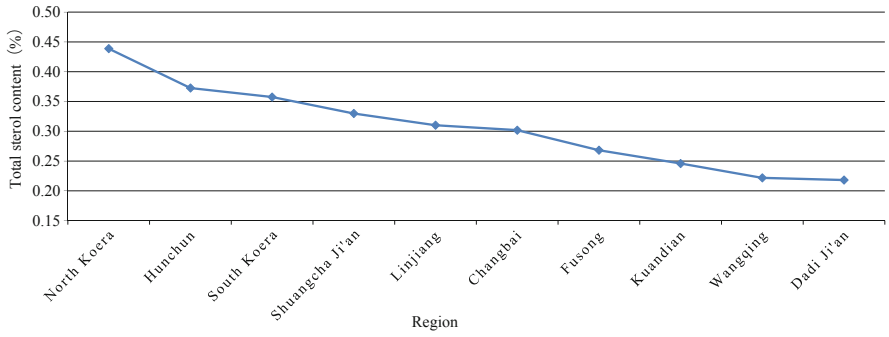


Fig. 11.7 Sequenced total sterol contents of 6-year-old ginseng

Table 11.7 Contents of stigmasterol in ginseng from different regions with different cultivation period (%)

No	Region	Cultivation period (year)	Content of stigmasterol (%)		
			Parallel sample 1	Parallel sample 2	Average value
1	Heihe	4	0.0093	0.0081	0.0087
2	Hulin	4	0.0108	0.0110	0.0109
3	Antu	4	0.0296	0.0356	0.0326
4		5	0.0306	0.0337	0.0321
5	Changbai	4	0.0279	0.0282	0.0281
6		5	0.0051	0.0073	0.0062
7		6	0.0269	0.0236	0.0253
8	Dunhua	4	0.0109	0.0094	0.0101
9		5	0.0088	0.0059	0.0073
10	Fusong	4	0.0105	0.0157	0.0131
11		5	0.0386	0.0272	0.0329
12		6	0.0110	0.0143	0.0126
13	Hunchun	4(CC)	0.0222	0.0222	0.0222
14		4	0.0287	0.0266	0.0277
15		5	0.0395	0.0275	0.0335
16		6	0.0143	0.0128	0.0136
17	Huadian	4	0.0128	0.0099	0.0114
18		5	0.0199	0.0199	0.0199
19	Helong	4	0.0143	0.0173	0.0158
20		5	0.0270	0.0139	0.0204
21	Dadi Ji'an	5	0.0088	0.0112	0.0100
22		6	0.0292	0.0292	0.0292
23	Shuangcha Ji'an	4	0.0084	0.0100	0.0092
24		5	0.0212	0.0213	0.0212
25		6	0.0105	0.0251	0.0178
26	Jiaohe	4	0.0031	0.0122	0.0076
27		5	0.0221	0.0292	0.0257
28	Jingyu	4	0.0110	0.0120	0.0115
29		5	0.0187	0.0201	0.0194
30	Linjiang	5	0.0192	0.0193	0.0191
31		6	0.0099	0.0240	0.0170
32	Tonghua	4	0.0088	0.0111	0.0100
33		5	0.0189	0.0196	0.0193
34	Wangqing	4	0.0177	0.0177	0.0177
35		5	0.0249	0.0110	0.0179
36		6	0.0119	0.0160	0.0140
37	Kuandian	4	0.0083	0.0083	0.0083
38		5	0.0081	0.0050	0.0066
39		6	0.0070	0.0078	0.0074
40	Xinbin	4	0.0082	0.0107	0.0095
41	North Korea	4	0.0098	0.0099	0.0099
42		5	0.0151	0.0128	0.0139
43		6	0.0086	0.0073	0.0079
44	South Korea	5	0.0234	0.0193	0.0213
45		6	0.0201	0.0206	0.0203

Table 11.8 Contents of β-sitosterol in ginseng from different regions with different cultivation period (%)

No	Region	Cultivation period (year)	Content of stigmasterol (%)		
			Parallel sample 1	Parallel sample 2	Average value
1	Heihe	4	0.0195	0.0173	0.0184
2	Hulin	4	0.0191	0.0225	0.0208
3	Antu	4	0.0563	0.0685	0.0624
4		5	0.0656	0.0726	0.0691
5	Changbai	4	0.0556	0.0556	0.0556
6		5	0.0205	0.0139	0.0172
7		6	0.0595	0.0439	0.0517
8	Dunhua	4	0.0245	0.0214	0.0230
9		5	0.0201	0.0250	0.0225
10	Fusog	4	0.0224	0.0299	0.0261
11		5	0.0709	0.0489	0.0599
12		6	0.0173	0.0238	0.0205
13	Hunchun	4(CC)	0.0485	0.0485	0.0485
14		4	0.0604	0.0668	0.0636
15		5	0.0955	0.0702	0.0828
16		6	0.0278	0.0230	0.0254
17	Huadian	4	0.0172	0.0131	0.0151
18		5	0.0281	0.0281	0.0281
19	Helong	4	0.0397	0.0319	0.0358
20		5	0.0714	0.0482	0.0598
21	Dadi Ji'an	5	0.0267	0.0257	0.0262
22		6	0.0662	0.0662	0.0662
23	Shuangcha Ji'an	4	0.0153	0.0187	0.0170
24		5	0.0436	0.0442	0.0439
25		6	0.0197	0.0408	0.0302
26	Jiaohe	4	0.0271	0.0313	0.0292
27		5	0.0562	0.0701	0.0632
28	Jingyu	4	0.0262	0.0263	0.0263
29		5	0.0430	0.0450	0.0440
30	Linjiang	5	0.0460	0.0420	0.0430
31		6	0.0156	0.0424	0.0290
32	Tonghua	4	0.0129	0.0153	0.0141
33		5	0.0430	0.0420	0.0420
34	Wangqing	4	0.0353	0.0353	0.0353
35		5	0.0567	0.0229	0.0398
36		6	0.0243	0.0329	0.0286
37	Kuandian	4	0.0168	0.0162	0.0165
38	Kuandian	5	0.0141	0.0080	0.0110
39		6	0.0145	0.0149	0.0147
40	Xinbin	4	0.0129	0.0159	0.0144
41	North Korea	4	0.0212	0.0218	0.0215
42		5	0.0385	0.0295	0.0340
43		6	0.0173	0.0139	0.0156
44	South Korea	5	0.0606	0.0640	0.0623
45		6	0.0587	0.0582	0.0585

The high-to-low sample content in different regions between stigmasterol and β-sitosterol is almost the same.

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Appendix: Detailed Information of Ginseng Sample Collection

In the world, the main producing countries of ginseng are China, North Korea, South Korea, and Russia. Chinese ginseng is mainly distributed in the 40~45° north latitude and 117.5~134° east longitude in the Changbai Mountain area.

In this book, a total of 45 samples of fresh ginseng are collected from 20 ginseng producing areas of different ages in Jilin Province, Heilongjiang Province, Liaoning Province, North Korea, and South Korea.

The details of the ginseng samples are shown in Table A.1 and Fig. A.1, respectively.

Table A.1 Information of sampling positions

No.	Abbrev.	Age (year)	Collecting spot	Longitude (E°)	Latitude (N°)	Altitude (m)
1	HH04	4	Heihe, Heilongjiang	127.53	50.25	122
2	HHL04	4	Hulin, Heilongjiang	132.94	45.76	84
3	AT04	4	Antu, Yanbian, Jilin	128.55	42.93	604
4	AT05	5	Antu, Yanbian, Jilin	128.56	42.94	604
5	CB04	4	Malugou, Changbai, Jilin	128.21	41.44	727
6	CB05	5	Erdaogang, Changbai, Baishan, Jilin	128.04	41.59	1228
7	CB06	6	Shisidaogou, Changbai, Baishan, Jilin	127.93	41.45	642
8	DH04	4	Dunhua, Yanbian, Jilin	128.23	43.37	505
9	DH05	5	Dunhua, Yanbian, Jilin	128.24	43.38	505
10	FS04	4	Quanyang, Fusong, Baishan, Jilin	127.54	42.35	731
11	FS05	5	Beigang, Fusong, Baishan, Jilin	127.54	42.43	746
12	FS06	6	Beigang, Fusong, Baishan, Jilin	127.54	42.43	746
13	HC04 continuous cropping	4	Erdaogoutun, Hunchun, Yanbian, Jilin, continuous cropping	130.62	43.20	286
14	HC04	4	Erdaogoutun, Hunchun, Yanbian, Jilin	130.62	43.20	286
15	HC05	5	Erdaogoutun, Hunchun, Yanbian, Jilin	130.62	43.20	286
16	HC06	6	Erdaogoutun, Hunchun, Yanbian, Jilin	130.62	43.20	286
17	HD04	4	Erdaodianzi, Huadian, Jilin	127.15	43.15	283
18	HD05	5	Erdaodianzi, Huadian, Jilin	127.15	43.15	283
19	HL04	4	Qingxing, Nanping, Helong, Yanbian, Jilin	128.97	42.37	831
20	HL05	5	Qingxing, Nanping, Helong, Yanbian, Jilin	128.97	42.37	857
21	JADD05	5	Dadi Ginseng Field, Ji' an, Jilin	126.19	41.13	180

(continued)

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Table A.1 (continued)

No.	Abbrev.	Age (year)	Collecting spot	Longitude (E°)	Latitude (N°)	Altitude (m)
22	JADD06	6	Dadi Ginseng Field, Ji' an, Jilin	126.19	41.13	180
23	JASC04	4	Shuangcha Ginseng Field, Ji' an, Jilin	125.93	41.17	531
24	JASC05	5	Shuangcha Ginseng Field, Ji' an, Jilin	125.93	41.17	531
25	JASC06	6	Shuangcha Ginseng Field, Ji' an, Jilin	125.93	41.17	531
26	JH04	4	Xishan, Maanshan, Jiaohe, Jilin	127.47	43.75	372
27	JH05	5	Xishan, Maanshan, Jiaohe, Jilin	127.47	43.75	371
28	JYDS04	4	Dingshan, Jingyu, Baishan, Jilin	126.81	42.38	543
29	JYDS05	5	Dingshan, Jingyu, Baishan, Jilin	126.81	42.38	543
30	LJ05	5	Huashu, Linjiang, Jilin	127.22	41.98	767
31	LJ06	6	Huashu, Linjiang, Jilin	127.22	41.98	767
32	TH04	4	Bawang, Tonghua, Jilin	125.69	41.37	336
33	TH05	5	Bawang, Tonghua, Jilin	125.69	41.37	336
34	WQ04	4	Fuxing, Wangqing, Yangbian, Jilin	130.42	43.40	674
35	WQ05	5	Fuxing, Wangqing, Yangbian, Jilin	130.42	43.33	704
36	WQ06	6	Fuxing, Wangqing, Yangbian, Jilin	130.42	43.33	704
37	LNKD04	4	Kuandian, Dandong, Liaoning	124.78	40.73	284
38	LNKD05	5	Kuandian, Dandong, Liaoning	124.78	40.73	284
39	LNKD06	6	Kuandian, Dandong, Liaoning	124.78	40.73	284
40	LNXB04	4	Xinbin, Fushun, Liaoning	125.04	41.73	335
41	CX04	4	Kaesong, North Korea	126.59	37.94	216
42	CX05	5	Kaesong, North Korea	126.59	37.94	216
43	CX06	6	Kaesong, North Korea	126.59	37.94	216
44	HG05	5	Geushan, South Korea	127.49	36.11	170
45	HG06	6	Geushan, South Korea	127.49	36.11	170

Fig. A.1 Distribution map of ginseng sample collection