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Full Length Research Paper

The evaluation of contents of nine ginsenoside monomers in four commercial ginseng by reverse phase high performance liquid chromatography (RP-HPLC)

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Nine ginsenoside monomers (Rg1, Rg2, Rf, Re, Rb1, Rb2, Rb3, Rc) were simultaneously determined by reverse phase high performance liquid chromatography (RP-HPLC) to assay chemical components in white ginseng, red ginseng, sugar ginseng and fresh ginseng. The results demonstrated that four different processing ginseng contained the same type of ginsenosides (Rg1, Rg2, Rf, Re, Rb1, Rb2, Rb3, Rc), but the content of each ginsenoside monomer varied significantly (P<0.05). The quality of those four different types of ginseng was evaluated according to the total content of nine ginsenoside monomers, and the results showed that white ginseng was the best, followed by sugar and fresh ginseng was the worst.

Key words: *Panax ginseng*, reverse phase high performance liquid chromatography (RP-HPLC), ginsenoside monomer.

INTRODUCTION

Ginseng, the dried root and rhizoma of *Panax ginseng* CA Meyer (Pharmacopoeia Committee of People's Republic of China, 2010) has been used as a tonic remedy for two thousand years (Anoja et al., 1999; Yijun et al., 2011). People respectfully regard it as the king of Chinese herbs. Ginseng not only in China, but also in northeast Asian and western countries remains a leading sales figure as one of the herbal supplements (Courtney et al., 2010, 2009).

At present, in Chinese market there are four species of commercial ginseng named white ginseng, red ginseng, sugar ginseng and fresh ginseng made by different processing methods. The cleansing ginseng root becomes white ginseng by insolation. Red ginseng is obtained from the cleansing fresh ginseng processed by steaming followed by insolation or baking. Sugar ginseng is the fresh ginseng made by the process of blanching, pricking, sugar soaking and drying. Fresh ginseng is the whole and cleaned ginseng dealt with preservative treatment and vacuum packaging. The fresh ginseng used in this experiment was preserved in alcohol.

The principal active components separated from ginseng are triterpenoid saponins known as ginsenosides (Dewir et al., 2010; Ying et al., 2010) with the effects of enhancing immunity (Xiaoming et al., 2010), antioxygen (Kwok et al., 2010), neuroprotective effect (Naval et al.,2007), anti-cancer(Lee et al.,2009) and so on. Thus, the content levels of ginseng saponins become main standards to evaluate the quality of commercial ginseng. Therefore, in present study we determine the contents of nine ginsenosides (Rg1, Re, Rf, Rg2, Rb1, Rc, Rb2, Rb3, Rd) monomers which have been more studied and have stronger pharmacological activities in the 4 kinds of ginseng by RP-HPLC in order to provide a basis and

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reference to evaluate the quality of different processing ginseng.

MATERIALS AND METHODS

Determination materials, white ginseng, red ginseng, sugar ginseng and fresh ginseng were all four years old and bought from Changchun ginseng market of Jilin province in China. Nine ginsenosides (Rg1(201022,98.54%), Re (201035,98.43%), Rf (201007,98.67%), Rg2 (201023,98.44%), Rb1 (201058,98.72%), Rc (201069,98.29%), Rb2 (201078,98.36%), Rb3 (201029,98.75%) and Rd (201018,98.47%)) standards were purchased from Jilin university. The acetonitrile and methanol were chromatographic grade (Fisher Scientific USA) and deionized water (Wahaha China).

HPLC conditions

High performance liquid chromatography Shimadzu, LC-2010A with reverse phase column C18, 5μm (4.6 mm × 150 mm) and Autosampler LC-2010A was used for analysis. The gradient elution system consisted of water (A) and acetonitrile (B). Separation was achieved using the following gradient: 0 min (18% A), 24 min(22% A), 26 min (26% A), 30 min (32% A),50 min (33.5% A),55 min (38% A),65 min(38%A).The column temperature was set at 35°C. The flow rate was 1 ml/min. The UV detection wavelength was 203 nm. The injection volume was 20 μl.The mean values of three replicates were calculated.

Control solution preparation

Standard ginsenosides (Rg1, Re, Rf, Rg2, Rb1, Rc, Rb2, Rb3 and Rd) were accurately weighed (10.5、10.8、9.7、10.7、9.8、9.5、10.5、10.7、10.1 mg) respectively and mixed, then dissolved with methanol in 5 ml flask. Nine ginsenosides concentration in the blended standards reserve solution were: 2.1, 2.16, 1.94, 2.14, 1.96, 1.9, 2.1, 2.14 and 2.02 mg/ml, respectively. Nine ginsenosides monomers standards reserve solution concentration were 1.01, 0.98, 1.05, 1.02, 1.08, 0.98, 0.99, 1.01 and 1.08 mg/ml, respectively.

Sample preparation for HPLC analysis

Four commercial ginseng samples test, 1.0 g of powder (40 mesh) was weighed accurately and refluxed for 4 h in moderate ether in a soxhlet extractor. After discarded ether and added moderate methanol, the sample solution was refluxed for 4 h and discarded methanol. Then, added chromatographic grade methanol dissolve and metered volume to 10 ml, the sample solution was obtained by filtering the supernate with a nylon filter membrane (0.45 μm) prior to the HPLC analysis.

Statistics

Excel and SPSS 11.7 software were used for calculations of data, drawing diagrams and statistical analysis.

RESULTS AND ANALYSIS

Species of ginsenoside monomers in four different commercial ginseng

Figure 1 indicated the chromatograms of standard ginsenosides and ginseng samples obtained by using above HPLC conditions. As shown in Figure 1, nine known ginsenosides were all found in four ginseng samples, besides, four ginseng samples contained different unknown ginsenosides before ginsenoside Rg1, and unknown ginsenoside species were four, five, three, four in white ginseng, red ginseng, sugar ginseng and fresh ginseng, respectively. Red ginseng contained one less kind of unknown ginsenoside than the other three ginseng samples before the Rf, and sugar ginseng and fresh ginseng contained one more kind of unknown ginsenoside than white ginseng and red ginseng after ginsenoside Rb3.

Standard curve of mixed ginsenoside standards

The standard curve was mass (μg) according to ginsenosides injected and peak areas. Ginsenosides Rg1, Re, Rf, Rb1, Rg2, Rc, Rb2, Rb3 and Rd were detected in 0.5 ~ 40 μg by HPLC with good linear correlation. Regression equations of ginsenoside monomer were shown in Table 1.

Determination of ginsenosides contents in different ginseng samples

As shown in Figure 2, Tables 1 and 2, nine known ginsenosides and their sum of white ginseng were the highest in the four ginseng productions, while fresh ginseng contained the lowest, and nine known ginsenosides and their sum of sugar ginseng were higher than that of red ginseng.

As shown in Table 1, the content of ginsenoside Rg1 in white ginseng was significantly higher than that of fresh ginseng ($P < 0.05$) with no significant difference among the other three ginseng samples ($P > 0.05$). Ginsenosides Re and Rb2 of white ginseng were significantly higher than that of red ginseng and sugar ginseng, and Re and Rb2 of sugar ginseng and red ginseng were significantly higher than that of fresh ginseng ($P < 0.05$) with no significant difference among others ($P > 0.05$). Rb1and Rc of white ginseng were significantly higher than that of red ginseng, and the two kinds of ginsenosides in sugar ginseng were significantly higher than in fresh ginseng ($P < 0.05$) with no significant difference among others ($P > 0.05$). Rg2 and Rb3 in white ginseng were significantly higher than that of red ginseng and sugar ginseng and that in red ginseng and sugar ginseng were significantly higher than in fresh ginseng ($P < 0.05$), while no significant difference between red ginseng and sugar ginseng ($P > 0.05$). The contents of ginsenosides Rf and Rd showed no significant difference among them ($P > 0.05$). As shown in Table 2, the total content of ginsenosides Rg1 and Re in white ginseng was significantly higher than that in red

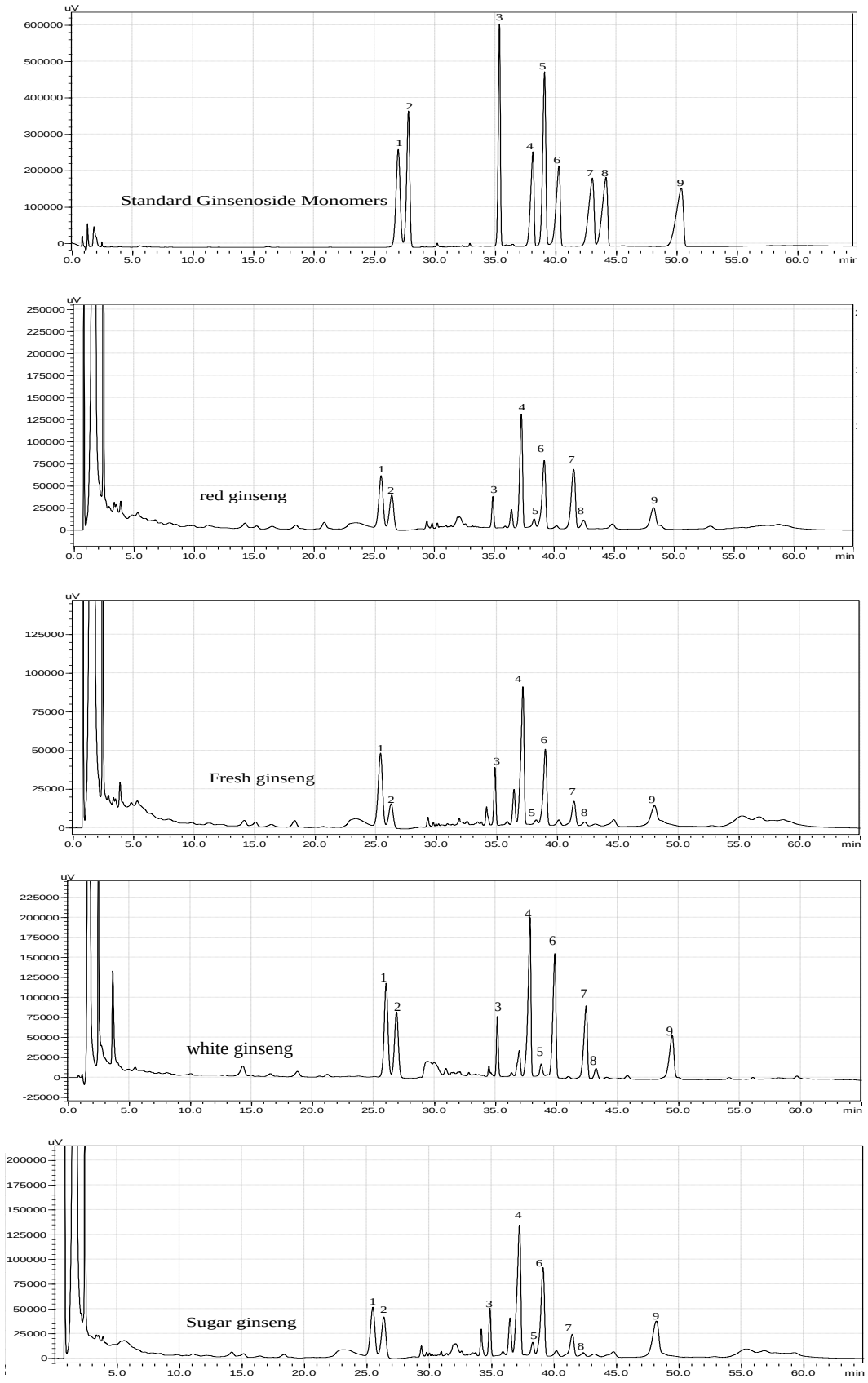

Figure 1. The HPLC of standard Ginsenoside Monomers and ginseng samples. 1:Rg1, 2: Re, 3: Rf, Rb1, 5: Rg2, 6: Rc, 7:Rb2, 8:Rb3, 9:Rd.

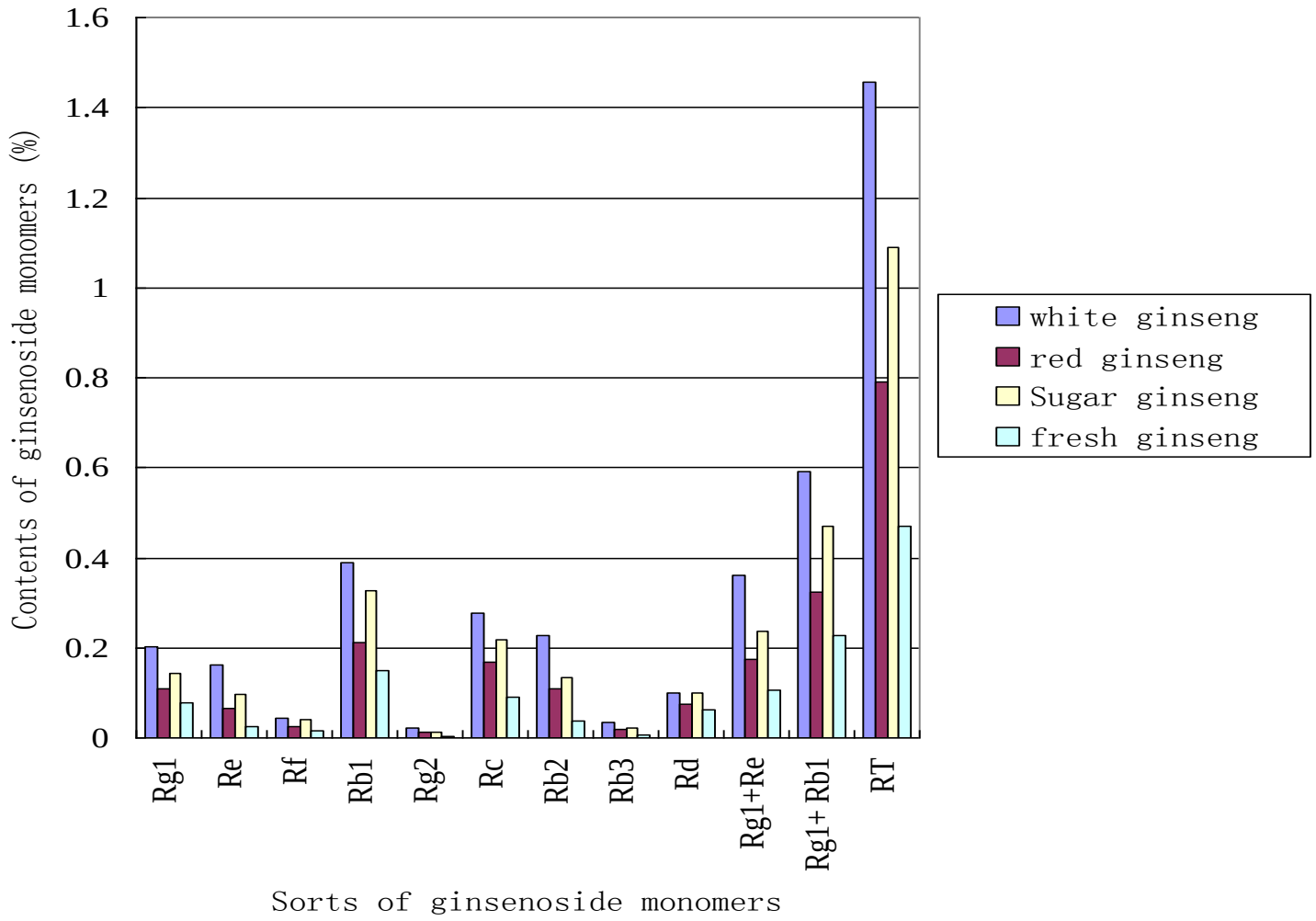

Figure 2. The comparison of contents of nine ginsenoside monomers among four commercial ginsengs (%). RT: Rg1+Re+Rf+Rb1+Rg2+Rc+Rb2+Rb3+Rd.

ginseng and fresh ginseng ($P < 0.05$) while showed no significant difference among others ($P > 0.05$). Total contents of both nine saponins and ginsenosides Rb1 and Rg1 in white ginseng were significantly higher than of red ginseng and fresh ginseng, and that in sugar ginseng were significantly higher than in fresh ginseng ($P < 0.05$), and others showed no significantly difference ($P > 0.05$). The content of the same ginsenoside in four ginseng samples varied greatly, and the quality of different ginseng was inconsistent (Tables 1 and 2). The sequence of nine single ginsenoside content in different ginseng was all as Rb1, Rc , Rb2, Rg1, Re, Rd, Rf, Rb3, Rg2 (Tables 1 and 2, Figure 2).

There were significant differences of some kinds of ginsenosides among the same kind ginseng products which came from different factories or different sources of raw materials, and the coefficient of variation was between 0.0430 and 0.4485.

DISCUSSION

Quality evaluation of different commercial ginseng products

The total content of ginsenoside Rg1 and Re was not less than 0.30% and the content of ginsenoside Rb1 was not less than 0.2% in white ginseng; the content of ginsenoside Rg1 and Re was not less than 0.25% and the content of ginsenoside Rb1 was not less than 0.2% in red ginseng regulated in Chinese Pharmacopoeia 2010 (Chinese Pharmacopoeia, 2010).The content of ginsenoside Rg1 and Rb1 was not less than 0.20% and 0.1% respectively in white ginseng regulated in USP-30-NF25 (USP30-NF25, 2007) However, the total content of Rg1 and Rb1 was not less than 0.40% in white ginseng regulated in European Pharmacopoeia (European Pharmacopoeia 6.0, 2008). In this study we found that

Table 1. The comparison of contents of nine ginsenoside monomers among four commercial ginseng (%).Different letter represent the significant difference at p < 0.05.nine-banded armadillo.

Sorts of sample	Rg1	Re	Rf	Rb1	Rg2	Rc	Rb2	Rb3	Rd
White ginseng (n=6)	0.2020±0.0496^{b}	0.1605±0.0178^{c}	0.0434±0.0083^{a}	0.3889±0.0472^{c}	0.0216±0.0018^{c}	0.2783±0.0206^{c}	0.2278±0.0098^{c}	0.0339±0.0015^{c}	0.0991±0.0095^{a}
Max values	0.3510	0.2114	0.0657	0.4817	0.0295	0.3157	0.2498	0.0370	0.1265
Min values	0.0786	0.1086	0.0198	0.2396	0.0165	0.2130	0.1975	0.0290	0.0709
Coefficient of variation	0.2455	0.1109	0.1912	0.1214	0.0833	0.0740	0.0430	0.0442	0.0959
Red ginseng (n=8)	0.1103±0.0342^{ab}	0.0642±0.0221^{ab}	0.0253±0.0088^{a}	0.2122±0.0576^{ab}	0.0117±0.0033^{b}	0.1672±0.0426^{ab}	0.1098±0.0402^{ab}	0.0176±0.0053^{b}	0.0738±0.0106^{a}
Max values	0.2377	0.1512	0.0476	0.4130	0.0258	0.3063	0.2855	0.0404	0.1386
Min values	0.0162	0.0049	0.0006	0.0603	0.0033	0.0473	0.0232	0.0053	0.04515
Coefficient of variation	0.3101	0.3442	0.3478	0.2714	0.2821	0.2548	0.3661	0.3011	0.1436
Sugar ginseng (n=8)	0.1419±0.0143^{ab}	0.0952±0.0131^{b}	0.0396±0.0069^{a}	0.3276±0.0393^{bc}	0.0127±0.0026^{b}	0.2172±0.0286^{bc}	0.1350±0.0128^{b}	0.0203±0.0020^{b}	0.1004±0.0219^{a}
Max values	0.2038	0.1576	0.0667	0.5085	0.0244	0.3420	0.1885	0.0301	0.2236
Min values	0.0992	0.0730	0.0200	0.2480	0.0061	0.1279	0.0926	0.0165	0.0522
Coefficient of variation	0.1008	0.1376	0.1742	0.1200	0.2047	0.1317	0.0948	0.0985	0.2181
Fresh ginseng (n=6)	0.0789±0.0346^{a}	0.0258±0.0100^{a}	0.0167±0.0103^{a}	0.1479±0.0522^{a}	0.0029±0.0013^{a}	0.0901±0.0255^{a}	0.0375±0.0087^{a}	0.0076±0.0012^{a}	0.0625±0.0080^{a}
Max values	0.1927	0.0585	0.0494	0.3159	0.0067	0.1859	0.0654	0.0120	0.0986
Min values	0.0195	0.0077	0.0001	0.0599	0.0001	0.0400	0.0190	0.0054	0.0432
Coefficient of variation	0.4385	0.3876	0.6168	0.3529	0.4483	0.2830	0.2320	0.1579	0.1280

Note: Different lowercase represents there is significant difference among the data in a row ($P < 0.05$) ; same lowercase represents there is not significant difference among the data in a row ($P > 0.05$).

the quality of different types of commercial ginseng varied greatly, and only white ginseng could meet the requirement on ginsenoside Rg1 and Re; all of them had reached the requirement on ginsenoside Rb1 regulated by those three pharmacopoeias except fresh ginseng; The total content of ginsenoside Rg1 and Rb1 in White ginseng and sugar ginseng exceeded the requirement in Chinese Pharmacopoeia 2010, USP-30-NF25 and European pharmacopoeia. Summed up the content of every ginsenoside monomer, the quality of white ginseng was the best followed by sugar ginseng. However, parts of ginsenoside monomers degraded in red ginseng owing to high-temperature processing, and the diffusion of ethanol lead to the loss of ginsenoside monomers in fresh ginseng. Thus, the processing of different ginseng products need to be improved in order to upgrade the quality of ginseng products. This study provided the basis and reference for the quality control of ginseng.

Quality evaluation of ginseng products with same processing

At present, the highest and lowest values of the ginsenoside monomer content of the same processing ginseng were quite different. Main reasons were as followed: Firstly, there were no standard processing technology, uniform processing technology and consistency processing conditions (An et al., 2011; Kim et al.,2007; Park et al., 2005; Sun et al., 2009). Secondly, the non-standard harvest (Yang et al., 2012), less strict control of selection process before processing and the difference among raw ginseng materials (Shi et al., 2011) resulted in essential differences.

Thirdly, it also related to the origin, variety and planting ways of raw ginseng materials (Lee et al., 2011; Sun et al., 2011). It is thus clear that, great attention need to be paid to the normalization of ginseng cultivation, harvesting and processing in order to ensure the stability and consistency of ginseng products, this study provided the basis and reference for the quality control of ginseng products.

Table 2. The comparison of additive contents of ginsenoside monomers among four commercial ginseng (%). Different letter represent the significant difference at p<0.05.

Sorts of sample	Rg1+ Re	Rg1+ Rb1	Rg1+ Re+Rf+ Rb1+Rg2+Rc+Rb2+Rb3+Rd
White ginseng (n=6)	0.3626±0.0583^{b}	0.5909±0.0932^{c}	1.4556±0.1491^{c}
Max values	0.5138	0.8304	1.7651
Min values	0.1872	0.3211	0.9905
Coefficient of variation	0.1608	0.1577	0.1024
Red ginseng (n=8)	0.1745±0.0562^{a}	0.3225±0.0918^{ab}	0.7922±0.2186^{ab}
Max values	0.3889	0.6507	1.6203
Min values	0.0211	0.0765	0.2499
Coefficient of variation	0.3221	0.2847	0.2759
Sugar ginseng (n=8)	0.2371±0.0264^{ab}	0.4694±0.0517^{bc}	1.0897±0.1132^{bc}
Max values	0.3614	0.7123	1.6063
Min values	0.1765	0.3548	0.8078
Coefficient of variation	0.1113	0.1101	0.1039
Fresh ginseng (n=6)	0.1047±0.0446^{a}	0.2268±0.0868^{a}	0.4699±0.1470^{a}
Max values	0.2512	0.5086	0.9624
Min values	0.0291	0.0886	0.2076
Coefficient of variation	0.4260	0.3827	0.3128

Note: Different lowercase represents there is significant difference among the data in a row ($P < 0.05$) ; same lowercase represents there is no significant difference among the data in a row ($P > 0.05$).

Determination of ginsenosides

There were many ways to determine the content of ginsenosides, but some ways were not precise enough, and some were not reasonable in economy (Morinaga et al., 2006; Kim et al., 2008). What's more, in recent years the content of one to four kinds of ginsenoside monomers were determined using different chromatographic conditions in most papers, and there was no report on detecting these nine kinds of ginsenosides simultaneously using the same chromatographic conditions. In our study we used the HPLC chromatographic conditions as follows: The number of theoretical plates of ginsenosides Rg1, Re, Rf, Rg2, Rb1, Rc, Rb2, Rb3 and Rd were 9321.99, 54217.18, 195750.60, 106588.70, 111779.10, 82908.96, 62362.63, 61650.84 and 45867.14, respectively; The separation factors of ginsenosides Rg1, Re, Rf, Rg2, Rb1, Rc, Rb2, Rb3 and Rd were 0.72, 0.94, 10.08, 1.32, 1.09, 1.09, 1.21, 1.07 and 1.37, respectively. Tailing factors of ginsenosides Rg1, Re, Rf, Rg2**,** Rb1, Rc, Rb2, Rb3 and Rd were 0.83, 0.85, 0.92, 0.82, 0.78, 0.82, 0.68, 0.67 and 0.75, respectively. RSD of precision was between 0.46% and 0.87%. RSD of stability was less than 0.45%. RSD of reproducibility were 1.21, 1.34, 1.63, 1.32, 1.36, 1.17, 1.14, 1.48 and 1.26%, respectively. Recovery was between 98.22 and 100.11%. RSD of recovery was between 0.7 and 2.6%. In this study, the RP-HPLC method which was accurate and reliable was firstly used to detect the content of nine ginsenoside monomers simultaneously which had been more studied and had obvious pharmacological activities for the first time. This study is expected to provide a new basis and reference for the quality assessment of ginseng products.

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