Research Article

Analysis of oligosaccharides from Panax ginseng by using solid-phase permethylation method combined with ultra-high-performance liquid chromatography-Q-Orbitrap/mass spectrometry

Lele Li1, Li Ma2, Yunlong Guo1, Wenlong Liu1, Yang Wang1,*, Shuying Liu1,**

1 Jilin Ginseng Academy, Changchun University of Chinese Medicine, Changchun, Jilin, China
2 Institute of Mass Spectrometer and Atmospheric Environment, Jinan University, Guangzhou, China

A R T I C L E   I N F O

Article history:
Received 11 May 2019
Received in Revised form 30 July 2019
Accepted 2 August 2019
Available online 9 August 2019

Keywords:
Ginseng
LC-MS
Oligosaccharide
Solid-phase permethylation

A B S T R A C T

Background: The reports about valuable oligosaccharides in ginseng are quite limited. There is an urgent need to develop a practical procedure to detect and analyze ginseng oligosaccharides.

Methods: The oligosaccharide extracts from ginseng were permethylated by solid-phase methylation method and then were analyzed by ultra-high-performance liquid chromatography-Q-Orbitrap/MS. The sequence, linkage, and configuration information of oligosaccharides were determined by using accurate m/z value and tandem mass information. Several standard references were used to further confirm the identification. The oligosaccharide composition in white ginseng and red ginseng was compared using a multivariate statistical analysis method.

Results: The nonreducing oligosaccharide erlose among 12 oligosaccharides identified was reported for the first time in ginseng. In the comparison of the oligosaccharide extracts from white ginseng and red ginseng, a clear separation was observed in the partial least squares-discriminate analysis score plot, indicating the sugar differences in these two kinds of ginseng samples. The glycans with variable importance in the projection value larger than 1.0 were considered to contribute most to the classification. The contents of oligosaccharides in red ginseng were lower than those in white ginseng, and the contents of maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, maltoheptaose, maltooctaose, maltononaose, sucrose, and erlose decreased significantly (p < 0.05) in red ginseng.

Conclusion: A solid-phase methylation method combined with liquid chromatography-tandem mass spectrometry was successfully applied to analyze the oligosaccharides in ginseng extracts, which provides the possibility for holistic evaluation of ginseng oligosaccharides. The comparison of oligosaccharide composition in white ginseng and red ginseng could help understand the differences in pharmacological activities between these two kinds of ginseng samples from the perspective of glycans.

© 2019 The Korean Society of Ginseng. Publishing services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Panax ginseng is a well-known Chinese herbal medicine possessing pharmaceutical activities of antitumor, antioxidant and hypoglycemic properties and so on and has been widely used as an herbal remedy or tonic food in China, Korea, and Japan for thousands of years to adjust the balance of the human body [1–3]. Ginsenosides are essential compounds that are considered to be responsible for most of the pharmaceutical activities of ginseng, and numerous studies have been focused on their structures and bioactivities during the past decades [4,5]. But for the valuable oligosaccharides in ginseng, the reports are quite limited. Several studies showed that ginseng oligosaccharides have various activities, such as antitumor and antioxidant activities [6]. Jiao et al [7] demonstrated that the oligosaccharides isolated from ginseng roots, flowers, and leaves exhibited antioxidant activity in vitro and in vivo. In addition, recent studies showed that ginseng oligosaccharides could exert an immunoregulatory effect. The ginseng oligosaccharides were fractionated into five purified fractions, among which one fraction that contains disaccharides has the highest

* Corresponding author. Jilin Ginseng Academy, Changchun University of Chinese Medicine, Changchun, 130117, China.
** Corresponding author. Jilin Ginseng Academy, Changchun University of Chinese Medicine, Changchun, 130117, China.
E-mail addresses: white-wing@163.com, wangyang@ccucm.edu.cn (Y. Wang), syliu@ciac.ac.cn (S. Liu).

https://doi.org/10.1016/j.jgr.2019.08.001
p1226-8453 e2093-4947/3 – see front matter © 2019 The Korean Society of Ginseng. Publishing services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
immunostimulant effect on lymphocyte proliferation \cite{8,9}. In addition, ginseng oligosaccharides are composed of polymers of 2–14 monosaccharide molecules with low molecular weight ranging from 342 Da to 2,286 Da and have the ability to cross the blood–brain barrier and mediate their effects \cite{10}. The activities of ginseng oligosaccharides should have a close relationship with their chemical characterizations. Thus, there is an urgent need to develop a practical procedure to detect and analyze ginseng oligosaccharides and further to determine their structure–activity relationships.

In herbal markets, ginseng is commercially available as white and red ginseng. White ginseng is usually prepared by air-drying and used to enhance physical fitness and disease resistance, while red ginseng is commonly made by a steaming process and drying, which is used for “boosting yang” and replenishing vital essence with the “warming effect” \cite{10,11}. Recent studies have shown that processing of ginseng alters its chemical profile and may change its properties and pharmacological activities \cite{12,13}. Some works aimed to characterize ginsenosides, amino acids, and polysaccharides in white and red ginseng \cite{10,14}. However, relatively less effort has been devoted to analysis and comparison of oligosaccharides in the two processed ginseng products.

Various analytical tools are now available for oligosaccharide characterization, such as high-field NMR, liquid chromatography (LC), capillary electrophoresis, and mass spectrometry (MS) \cite{15–19}. LC-electrospray ionization MS (LC-ESI/MS) could help resolve isobaric glycan species and reduce the ion suppression compared with directed infusion ESI/MS, which has provided the potential capability to fully define the composition and sequence of complex glycans. Moreover, the permethylated glycan offers improved ionization efficiency and enhanced sensitivity during MS analysis. Compared with traditional permethylation method, the solid-phase permethylation has been considered as an alternative method for traditional liquid-phase methylation, with the advantages of fast reaction time, high throughput, convenience, and high yields of permethylated products for the glycan permethylation \cite{20–23}. Combined with ESI and tandem mass information, permethylated sugar generates the most detailed information for their structure elucidation \cite{24,25}.

We report here an liquid chromatography–tandem mass spectrometry method for the detailed characterization of permethylated oligosaccharides from ginseng sample. The structural analysis of the sample was conducted by LC-ESI/MS combined with an orbitrap analyzer. In addition, we also compared the oligosaccharide composition of white ginseng and red ginseng, which could help to understand the differences in pharmacological activities between these two ginseng samples from the perspective of glycans.

2. Materials and methods

2.1. Chemicals and materials

HPLC-grade acetonitrile was purchased from Tedia (Fairfield, OH, USA). Dimethyl sulfoxide (DMSO), NaOH, NaBH₄, trifluoroacetic acid (TFA), CH₃I, CH₂Cl₂, and acetic acid were obtained from Beijing Shiji (Beijing, China). Ultrapure water was filtered using a Milli-Q device (Millipore, Milford, MA, USA). Red ginseng sample (n = 6) and white ginseng sample (n = 15) were obtained from Wanliang market, Jilin, China, in 2015. The reference standards of maltose, maltotriose, maltohexaose, sucrose, erlose, kestose, and nystose were purchased from Aladdin (Los Angeles, CA, USA).

**Fig. 1.** Total ion current chromatograms of permethylated oligosaccharides. (A) From white ginseng. (B) From red ginseng (Maltose: Peaks 1 and 2, maltotriose: Peaks 4 and 5, maltotetraose: Peaks 6 and 8, maltopentaose: Peaks 9 and 11, maltohexaose: Peaks 12 and 13, maltoheptaose: Peaks 14 and 15, maltooctaose: Peaks 16 and 18, maltononaose: Peaks 19 and 20, sucrose: Peak 3, erlose: Peak 7, kestose: Peak 10, nystose: Peak 17).
2.2. Sample extraction

All ginseng samples were crushed and passed through a 20-mesh sieve. Ten grams of each powdered sample was immersed in 200 mL of water for 1 h and extracted using a magnetic mixer at 15 rpm at 70 °C for 2 h. The extract was centrifuged for 10 min at 6000 rpm. The supernatant was collected, and the residue was extracted with water; this was repeated for 4 times. Then, the supernatants were combined and concentrated to 100 mL. Anhydrous ethanol was slowly added under stirring to the collected solution to adjust the ethanol concentration to 80%. After standing 12 h at 4 °C, the solution was centrifuged for 10 min at 6000 rpm. The supernatant was concentrated to 25 mL, and the ginseng oligosaccharide extract was obtained.

2.3. Preparation of solid-phase permethylation column

NaOH beads were suspended in acetonitrile to prevent atmospheric moisture absorption. Then, these NaOH beads were packed in a 2.1 × 50 mm column. The NaOH-packed column was finally
washed several times with DMSO using a syringe pump at a flow rate of 40 μL/min.

2.4. Sample permethylation

Each of the aforementioned oligosaccharide extract (1 mL) was freeze-dried and added to 2 mL of DMSO; after vortex shocking for 5 min, the solution was filtered through a filter (0.22 μm), and then 50 μL of CH3I was mixed with 450 μL of the filtrate. Sample permethylation was conducted as described previously with modification [9,10]. In brief, 400 μL of this solution was injected into the prepared solid-phase permethylation (SPP) column, followed by washing with 800 μL of DMSO, and the eluent was collected in an centrifuge tube. Afterward, 5% acetic acid solution was added to stop the reaction. Permethylated oligosaccharides were extracted four times with dichloromethane; then, the dichloromethane was evaporated. Residues were dissolved in acetonitrile/water (1:1) for MS analysis.

Maltose, sucrose, maltotriose, kestose, and nystose standards were dissolved in DMSO to obtain stock solutions at approximately 1.0 mg/mL. Permethylated standards were obtained by using the aforementioned method.

2.5. Sample reduction and permethylation

The sample was derivatized by the previously reported procedure with modifications [26,27]: 1 mL of the reducing reagent (20 mg/mL NaBH4, dissolved in 0.02 M NaOH) was added into a 1-mL extract of ginseng sample and left for 12 h at room temperature. The reaction was terminated by the addition of glacial acetic acid, and then, the solvent was freeze-dried. The dried samples were permethylated by the aforementioned method to obtain the reduced-permethylated ginseng oligosaccharides. Maltotriose and maltoheptaose were dissolved in water to obtain stock solutions at approximately 1.0 mg/mL, and the reduced-permethylated maltotriose and maltoheptaose were obtained by using the same methods.

2.6. Sugar composition analysis

The eluate of the permethylated oligosaccharides was collected based on their retention times in chromatogram; then, these eluates were concentrated and lyophilized. The freeze-dried fractionation was hydrolyzed with hydrous methanol containing 2M TFA at 120°C for 1 h. After TFA was removed by evaporation, the hydrolysates were permethylated by using the SPP column and then analyzed by liquid chromatography—mass spectrometry (LC-MS).

2.7. LC-MS analysis

The ultra-high-performance liquid chromatography (UHPLC) was performed on an UltiMate 3000 system (Dionex, Sunnyvale, CA, USA) coupled with Golden C18 column (2.1 × 50 mm, 1.9 μm; Thermo Fisher Scientific, San Jose, CA, USA) maintained at 30°C. Mobile phase A was 0.1% formic acid in water, and mobile phase B was 100% acetonitrile. The proportion of acetonitrile was increased from 10% to 60% (0–5 min), 60–100% (5–10 min), isocratic 100% (10–15 min), and finally adjusted from 100% to 10% (16 min, lasting for 4 min). The injection volume was 2.0 μL for each sample, and the flow rate was 0.3 mL/min.

Mass spectrometric detection was carried out on a Q Exactive Orbitrap MS (Thermo Fisher Scientific) equipped with an ESI source operated in positive-ion mode. The MS source parameters were set.

Fig. 3. Reduced-permethylated maltoheptaose. (A) MS/MS spectrum. (B) Structure.
MS/MS, tandem mass spectrometry.
as follows: sheath gas flow of 40 Arb, aux gas flow of 12 Arb, and sweep gas flow of 1 Arb. The capillary voltage was set to +3.5 kV at the capillary temperature of 333°C and aux gas heater temperature of 317°C. MS data were acquired in positive-ion mode from m/z 200 to 2000 in full-scan mode. The normal collision energy was set to 20–50% for different samples to obtain MS/MS data. Data are recorded and analyzed using the Xcalibur software, version 2.2.42, (Thermo Fisher Scientific).

2.8. Multivariate statistical analysis

Both white ginseng and red ginseng oligosaccharide extracts were permethylated by using the SPP column. The raw data of the nonreduced-permethylated samples were acquired by the LC-MS, and the [M + NH4]⁺ and [M + Na]⁺ ions of permethylated oligosaccharides were extracted from total ion chromatograms, and the chromatographic peaks were integrated. Then, the resulted data set, containing information of oligosaccharides, peak area, and sample code, was generated as a csv file and imported into SIMCA-P software 11.5 (Umetrics, Umea, Sweden) to conduct the multivariate statistical analysis. The Student t test was used to assess the significant difference between groups using Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA). A p-value < 0.05 was considered significant.

3. Results and discussion

3.1. Identification of permethylated oligosaccharides

Each permethylated oligosaccharide extracted from ginseng samples was profiled by the UHPLC-Q-Orbitrap/MS, and the typical base peak intensity chromatogram is illustrated in Fig. 1A. Twenty peaks were observed in the chromatogram, and then, these peaks were tentatively assigned by comparing theoretical exact mass with their measurement mass. The standard references and tandem mass spectra were used to further confirm the results, and more detailed characterization and chemical information of permethylated oligosaccharides are shown in Table 1.

Five oligosaccharides, including maltose, sucrose, maltotriose, kestose, and nystose, were first identified by comparing the retention time and accurate m/z value with standard references. As shown in Fig. 2C, the ammonium adduct and sodium adduct of permethylated maltotriose [M + NH4]⁺ and [M + Na]⁺ ions at m/z 676.38 and 681.33 were observed with high intensity and [M + H]⁺ ions at m/z 659.35 were observed with the weakest intensity; the same results were observed in other oligosaccharides. According to previous studies [24], the ions of [M + Na]⁺ of oligosaccharides could provide more structurally informative fragments for the structure identification; thus, the ions of [M + Na]⁺ of each compound were selected as a precursor ion to perform MS/MS analysis to assign the glycans without the comparison of standard references.

3.2. Identification of reducing saccharides

The chromatogram of permethylated maltotriose is illustrated in Fig. 2; the observed double peak was due to the existence of anomeric hydroxyl, but only one peak could be detected in the chromatogram of its reduced-permethylated products. Based on this difference in the chromatograms, 6 oligosaccharides were considered as reducing sugars. LC-MS analysis showed that these six oligosaccharides were only composed of glucose (Glc) residues, which is consistent with our previous studies [9,28,29]. The full-scan mass spectra of Peak 6 and 8 (Fig. 3A) showed the main

| Compounds          | Formula  | Detected m/z of [M + Na]⁺ | Product ions                                                                 |
|--------------------|----------|--------------------------|-------------------------------------------------------------------------------|
| Maltotetraose      | C₃₆H₇₄O₂₁ | 901.4610                 | \[M + Na]⁺ 348.24, 325.14, 302.14, 279.10, 255.05, 232.00, 208.96, 185.92, 162.88, 139.84, 116.80, 93.76, 70.72, 47.68, 24.64, 11.60, 8.56.  |
| Maltopentaose      | C₄₄H₉₀O₂₆ | 1105.625                 | \[M + Na]⁺ 494.26, 471.22, 448.18, 425.14, 402.10, 379.06, 356.02, 333.00, 310.00, 287.00, 264.00, 241.00, 218.00, 195.00, 172.00, 150.00, 127.00, 104.00, 81.00, 58.00, 35.00, 12.00.  |
| Maltooctoase       | C₃₂H₆₄O₄₁ | 1717.863                 | \[M + Na]⁺ 659.35, 636.31, 613.27, 590.23, 567.19, 544.15, 521.11, 498.07, 475.03, 452.00, 429.00, 406.00, 383.00, 360.00, 337.00, 314.00, 291.00, 268.00, 245.00, 222.00, 200.00, 177.00, 154.00, 131.00, 108.00, 85.00, 62.00, 39.00, 16.00.  |
| Maltononaose       | C₄₆H₉₄O₄₆ | 1921.963                 | \[M + Na]⁺ 878.45, 855.41, 832.37, 809.33, 786.29, 763.25, 740.21, 717.17, 694.13, 671.09, 648.05, 625.01, 602.00, 579.06, 556.02, 533.00, 510.00, 487.00, 464.00, 441.00, 418.00, 395.00, 372.00, 349.00, 326.00, 303.00, 280.00, 257.00, 234.00, 211.00, 188.00, 165.00, 142.00, 119.00, 96.00, 73.00, 50.00, 27.00.  |

MS/MS, tandem mass spectrometry; UHPLC, ultra-high-performance liquid chromatography; MS, mass spectrometry.
ions at m/z 885.43 and m/z 880.48, corresponding to a reduced-permethylated glucose tetramer [Glc4 + Na+] and an ammonium adduct of a reduced-permethylated glucose tetramer, respectively. Additional ions [M + Na+] at m/z 1105.56, 1309.66, 1513.76, 1717.86, and 1921.96 observed in Fig. S1 indicated the presence of reduced-permethylated pentamer, hexamer, heptamer, octamer, and nonamer of glucose in the ginseng extract. Several [M + K+] ions were also observed to be 16 Da higher than the corresponding [M + Na+] ions. Overall, these six oligosaccharides extracted from ginseng roots consisted of glucose oligosaccharides with the degree of polymerization (DP) from 4 to 9.

Subsequently, the reduced-permethylated products of these glucose oligosaccharides were subjected to ESI-MS/MS analysis to determine their glycosidic linkages. Consider Peak 14 and 15 at \( t_R = 7.73 \) and 7.97 min as an example to demonstrate the identification procedure. The double peak and single peak were observed in the chromatograms of its permethylated and reduced-permethylated products, suggesting it was a reducing sugar. The DP of this sugar was calculated to be 7 by using accurate m/z 1513.76 of the reduced-permethylated product. The MS\(^2\) spectrum of this oligosaccharide is depicted in Fig. 3A, the Y-type ions (i.e., m/z 1295.65, 1091.55, 887.45, 683.35, 479.25), the B-type ions (i.e., m/z 1261.60, 1057.50, 853.40, 650.30, 445.20), the Z-type ions (i.e., m/z 1277.63, 1073.54, 869.44, 665.34, 461.24), and the C-type ions (i.e., m/z 1279.61, 1075.51, 871.41, 667.35, 463.21) were detected. The cross-ring cleavage ions, including \(^1\)X-ions at m/z 1323.64, 1119.54, 915.44, 711.34, and 507.24; \(^2\)X-ions at m/z 1351.67, 1147.57, 943.47, 739.37, and 535.27; \(^3\)A-ions at m/z 1335.64, 1131.54, 927.44, 723.34, and 519.24; and \(^3\)A-ions at m/z 1349.66, 1145.56, 941.46, 737.36, and 533.26, were also observed in this spectrum. The presence of specific fragment ions could be used to determine the type of glycosidic linkage. As reported by our group [8], the \( 1\rightarrow6 \) linkage oligosaccharide possesses the ions of \(^0\)X/A-ion series, \(^0\)X/A-ion series, and \(^0\)X/A-ion series; the \( 1\rightarrow4 \) linkage can be confirmed by the presence of \(^0\)X/A-ion series and \(^0\)X/A-ion series, but the absence of \( 0.3\)X/A-ion series; the \( 0.3\)X/A-ion series is the specific diagnostic ion for \( 1\rightarrow3 \) linkage. Based on the aforementioned criteria, the \( 1\rightarrow4 \) linkage was determined in this sugar; thus, this ion was identified as maltoheptaose, and its proposed fragmentation pathway is shown in Fig. 3B. Then, the standard reference was used to further confirm the identification. By means of the aforementioned procedure, a total of six reducing oligosaccharides with DP from 4 to 9 were identified, and their detailed information is shown in Table 2.

### 3.3. Identification of nonreducing trisaccharides

Peak 7 at \( t_R = 7.03 \) and m/z 681.33 showed a single peak in the chromatograms of both the permethylated and reduced-permethylated products, suggesting it should be a nonreducing sugar. This sugar was considered as a trisaccharide on the basis of its accurate mass collection. The chromatogram of the sugar composition analysis of this oligosaccharide is shown in Fig. 4A; a comparison of retention time showed this sugar was composed of glucose and fructose. The peak area ratio of glucose to fructose is approximately 2:1, indicating that the proportion of 2-glucose and 1-fructose exists in this compound. Therefore, the possible structure of this oligosaccharide could be melezitose, gentianose, or erlose. The MS\(^2\) spectrum of this trisaccharide is illustrated in Fig. 4B, where the most intense ion at m/z 463.21 corresponded to the loss of permethylated hexose residues. The product ions at m/z 389.18, 375.16, and 329.16 were formed through cross-ring cleavages. But it is difficult to accurately assign these fragments; therefore, the glycosidic linkages of this trisaccharide were hard to be determined by only using its MS\(^2\) data. By comparing its retention time and fragment pattern with that of permethylated erlose standard, this trisaccharide was finally identified as erlose.

### 3.4. Pattern recognition analysis of oligosaccharides

For the method validation, 10 µL of each permethylated sample was mixed to generate a quality control (QC) sample. The QC sample was analyzed three times at the beginning of the analytical run to ensure system equilibration and then once every ten samples to provide quality assurance for each compound. The reproducibility of the method was evaluated with relative standard deviation of the permethylated oligosaccharides in 6 QC samples. Relative standard deviation% of all 12 oligosaccharides was less...
than 15%, suggesting that the analytical platform was robust with excellent repeatability and stability during the whole sequence.

As shown in Fig. 1, the differences between white ginseng and red ginseng extracts could be observed from their chromatograms, especially for the peak with high intensity, but the visual inspection was insufficient to define the glycan differences between two samples. Therefore, the multivariate statistical analysis was applied to intuitively display the intergroup differences in oligosaccharides. After peak extraction, the data set containing information of oligosaccharides, peak area, and sample code was generated, and partial least squares-discriminate analysis (PLS-DA) model constructed using the data from white ginseng and red ginseng samples is shown in Fig. 5A. All samples were located in the Hotelling T2 (0.95) ellipse, and the clear separation between white ginseng and red ginseng indicates that there are differences in the content of oligosaccharide in white ginseng and red ginseng. The parameters R2Y and Q2 value of 0.966 and 0.959, respectively, were considered to be an excellent fitness and prediction ability of the established PLS-DA model. The permutation test (n = 200) was further conducted to validate the model and avoid overfitting. The values of intercepts, R2 (0.0636) and Q2 (−0.406), were lower than the original values, which indicate great predictability and goodness of fit of the established model.

The oligosaccharide with variable importance in the projection values larger than 1 was highlighted as important for the group separation, and then, these variables were further screened using the student t test to determine whether potential analysis markers were statistically different between two groups. The oligosaccharides with a significant difference (p < 0.05) were kept, and the result of the t test is shown in Fig. 5B.
As illustrated in Fig. 5B, all oligosaccharide content in red ginseng was lower than that in white ginseng, which was consistent with visual chromatogram observation in Fig. 1. Red ginseng is one of the processed products prepared normally by steaming and drying, and the decomposition of ginseng oligosaccharide during processing could lead to the decrease of sugar content. And the decrease of sugar content was also caused by a very typical reaction of a reducing sugar with ammonium such as the Mallard reaction [30]. In addition, the oligosaccharide partially involved in the hydrolysis reaction could lead to a decrease in the content of oligosaccharides in red ginseng [31]. Although it was reported that several polysaccharides are hydrolyzed to oligosaccharides and monosaccharides during the heating and steaming process, which may increase the concentrations of oligosaccharides [10], under the combined effect of the aforementioned factors, the sugar content in the red ginseng was lower than that in the white ginseng.

Good separation and high signal intensity of permethylated saccharides were observed in the LC-MS analysis. The SPP procedure took only 10 min for the derivatization of each sample and showed the advantages of high throughput. The tandem mass spectra of reduced-permethylated products could provide a wealth of information for the structure identification of reducing oligosaccharides. The 1-phenyl-3-methyl-5-pyrazolone (PMP) derivatization method was commonly used in the characterization of glycans, but only reducing saccharides can react with PMP, which makes it impossible for the analysis of nonreducing saccharides in ginseng extracts, which provides the possibility for the potential biological activity/pharmacological activities of those oligosaccharides based on their structures.

4. Conclusions
A SPP method combined with liquid chromatography–tandem mass spectrometry was successfully applied to analyze the oligosaccharides in ginseng extracts, which provides the possibility for holistic evaluation of the oligosaccharides of ginseng and further to determine their structure–activity relationships. Twelve oligosaccharides were characterized in ginseng samples based on the accurate m/z values and tandem mass information, and the identification of several oligosaccharides was further confirmed by using the standard references. The oligosaccharide comparison of white ginseng and red ginseng showed a clear separation in PLS-DA score plots, and the significant different components were highlighted. All oligosaccharides could be detected in both white ginseng and red ginseng samples, but at different concentrations. Therefore, it could be inferred that the oligosaccharides detected in the present experiment are naturally synthesized in the plant, but the differences in content are caused by heating and steaming processes. The results of the comparison could help understand the differences in pharmacological activities between these two ginseng samples from the perspective of glycans.

Acknowledgments
This study was supported by the Natural Science Fund Project of Jilin Province, China (No. 20190201143JC) and National Key R&D Program of the Ministry of Science and Technology (No. 2017YFC1702105).

Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgr.2019.08.001.

References
[1] Liu C, Xiao P. Recent advances on ginseng research in China. J Ethnopharmacol 1992;36:27–38.
[2] Choi J, Kim T, Choi T, Lee MS. Ginseng for health care: a systematic review of randomized controlled trials in Korean literature. Plos One 2013;8:e59918.
[3] Leung KW, Wong AT. Pharmacology of ginsenosides: a literature review. Chin Med 2010;5:20–20.
[4] Wu W, Song F, Guo D, Mi J, Qin Q, Yu Q, Liu S. Mass spectrometry-based approach in ginseng research: a promising way to metabolomics. Curr Anal Chem 2012:8:43–66.
[5] Li L, Wang Y, Xiu Y, Liu S. Chemical differentiation and quantitative analysis of different types of Panax genus stem-leaf based on a UPLC-Q-exactive Orbitrap/MS combined with multivariate statistical analysis approach. J Anal Methods Chem 2018:1–16.
[6] Jiao LL, Kang XY, Li B, Liu Z, Wang MZ, Liu SY. Anti-tumour and immunomodulatory activities of oligosaccharides isolated from Panax ginseng CA Meyer. Int J Biol Macromol 2014;65:229–33.
[7] Jiao L, Li B, Wang M, Liu Z, Zhang X, Liu S. Antioxidant activities of the oligosaccharides from the roots, flowers and leaves of Panax ginseng CA Meyer. Carbohydr Polym 2014;106:293–8.
[8] Wan D, Jiao L, Yang H, Liu S. Structural characterization and immunological activities of the water-soluble oligosaccharides isolated from the Panax ginseng roots. Pflanze 2012;235:1289–97.
[9] Yang H, Shi L, Zhang X, Su R, Wan D, Song F, Li J, Liu S. Identification of structurally closely related monosaccharide and disaccharide isomers by PMI labeling in conjunction with IM-MS/MS. Sci Rep 2016:6:1–9.
[10] Wan JY, Fan Y, Yu QT, Ge YZ, Yan CP, Alojga RN, Li P, Ma ZH, Qi JI. Integrated evaluation of malonyl ginsenosides, amino acids and polysaccharides in fresh and processed ginseng. J Pharmaceut Biomed 2015;107:89–97.
[11] Jia L, Zhao YQ. Current evaluation of the millennium phytomedicine-ginseng (I): etymology, pharmacognosy, phytochemistry, market and regulations. Curr Med Chem 2009;16:2473–84.
[12] Liu Z, Li Y, Li X, Ruan CC, Wang L, Sun CZ. The effects of dynamic changes of malonyl ginsenosides on evaluation and quality control of Panax ginseng CA Meyer. J Pharmaceut Biomed 2012;64:55:56–63.
[13] Xie XY, Luo D, Cheng YJ, Mu JF, Wang YM, Liang QL, Luo GA. Steaming-induced chemical transformations and holistic quality assessment of red ginseng derived from Panax ginseng by means of HPLC-ESI-MS/MS-based multi-component quantification fingerprint. J Agr Food Chem 2012;60:8213–24.
[14] Wu W, Sun L, Zhang Z, Guo Y, Liu S. Profiling and multivariate statistical analysis of Panax ginseng based on ultra-high-performance liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry. J Pharmaceut Biomed 2015;107:141–50.
[15] Munkel F, Wefers F. Fine structures of different dextrans assessed by isolation and characterization of endo-dextranase liberated isomalto-oligosaccharides. Carbohydr Polym 2019;215:296–306.
[16] Choukale R, Kango N. Characterization of a mycelial fructosyltransferase from Aspergillus tamarii NRBC 1228 for efficient synthesis of fructooligo- saccharides. Food Chem 2019:286:434–40.
[17] Martin-Ortiz A, Ruiz-Matute Al, Sanz ML, Moreno FJ, Herrero M. Separation of di- and trisaccharide mixtures by comprehensive two-dimensional liquid chromatography. Application to prebiotic oligosaccharides. Anal. Chim. Acta 2019:1060:125–32.
[18] Brokl M, Hernández-Hernández O, Soria AC, Sanz ML. Evaluation of different operation modes of high performance liquid chromatography for the analysis of complex mixtures of neutral oligosaccharides. J Chromatogr A 2011;1218:7697–703.
[19] Bonen H, Guerrero A, Brownell EA, Smith KC, Barile D, Hagadorn JL, Lebrilla CB, Lusser MM, Matson AP, Sela DA. Human milk oligosaccharide analysis of large sample cohorts using a novel capillary electrophoresis platform. Glycobiology 2018:28:1027–8.
[20] Kang P, Mechref Y, Klouckova I, Novotny MV. Solid-phase permethylation of glycans for mass spectrometric analysis. Rapid Commun Mass Sp 2005:19:3421–8.
[21] Kang P, Mechref Y, Novotny MV. High-throughput solid-phase permethylation of glycans prior to mass spectrometry. Rapid Commun Mass Sp 2008;22:721–34.
[22] Jang K-S, Park H-M, Kim Y-G, Kim B-G, Kim J-H. An on-line platform for the analysis of permethylated oligosaccharides using capillary liquid chromatography-electrospray ionization-tandem mass spectrometry. J Nanosci Nano 2015;15:3962–6.

[23] Kang P, Mechref Y, Kyselova Z, Goetz JA, Novotny MV. Comparative glycomic mapping through quantitative permethylation and stable-isotope labeling. Anal Chem 2007;79:6064–73.

[24] Ashline D, Singh S, Hanneman A, Reinhold V. Congruent strategies for carbohydrate sequencing. 1. Mining structural details by MSn. Anal Chem 2005;77:6250–62.

[25] Lapadula AJ, Hatcher PJ, Hanneman AJ, Ashline DJ, Zhang HL, Reinhold VN. Congruent strategies for carbohydrate sequencing. 3. OSCAR: an algorithm for assigning oligosaccharide topology from MSn data. Anal Chem 2005;77:6271–9.

[26] Ashline D, Singh S, Hanneman A, Reinhold V. Congruent strategies for carbohydrate sequencing. 1. Mining structural details by MSn. Anal Chem 2005;77:6250–62.

[27] Matsuo K. Small-scale, high-throughput method for plant N-glycan preparation for matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis. Anal Biochem 2011;413:200–2.

[28] Wan D, Yang H, Song F, Liu Z, Liu S. Identification of isomeric disaccharides in mixture by the 1-phenyl-3-methyl-5-pyrazolone labeling technique in conjunction with electrospray ionization tandem mass spectrometry. Anal. Chim. Acta 2013;780:36–45.

[29] Wan D, Yang H, Yan C, Song F, Liu Z, Liu S. Differentiation of glucose-containing disaccharides isomers by fragmentation of the deprotonated non-covalent dimers using negative electrospray ionization tandem mass spectrometry. Talanta 2013;115:870–8.

[30] Yamabe N, Lee JG, Lee YJ, Park CH, Kim HY, Park JH, Yokozawa T, Kang KS. The chemical and 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity changes of ginsenosides Rb-1 and rg(1) by maillard reaction. J Ginseng Res 2011;35:60–8.

[31] Yamabe N, Il Song K, Lee W, Han I-H, Lee JH, Ham J, Kim S-N, Park JH, Kang KS. Chemical and free radical-scavenging activity changes of ginsenoside Re by maillard reaction and its possible use as a renoprotective agent. J Ginseng Res 2012;36:256–62.