Application of two-dimensional nuclear magnetic resonance spectroscopy to quality control of ginseng commercial products

Yang, S.Y.; Kim, H.K.; Lefeber, A.W.M.; Erkelens, C.; Angelova, N.S.; Choi, Y.H.; Verpoorte, R.

Application of Two-Dimensional Nuclear Magnetic Resonance Spectroscopy to Quality Control of Ginseng Commercial Products

Shih Ying Yang[1]
Hye Kyong Kim[1]
Alfons W. M. Lefeber[2]
Cornelis Erkelens[2]
Nadezhda Angelova[1]
Young Hae Choi[1]
Robert Verpoorte[1]

Original Paper

Abstract

Ginseng has been used as a powder or a crude extract of the plant roots. The quality control of commercial ginseng preparations is difficult due to the diverse compounds present. Most previous quality control methods using TLC or HPLC-UV (or -MS) cannot be expected to cover a wide range of compounds in the commercial ginseng preparations. In this study, the metabolic fingerprinting of ginseng preparations was performed by ^{1}H-NMR spectroscopy. Although ^{1}H -NMR spectroscopy could provide information about the total profile of the compounds present, low resolution and overlapping signals make it difficult for further identification of each compound. For overcoming the problem two-dimensional *J*-resolved NMR spectra and multivariate data analysis techniques was applied for the analysis. Principal component analysis (PCA) of projected *J*-resolved NMR spectra shows a clear discrimination among those samples by principal component 1 and principal component 3. The loading plot of PC values obtained from all NMR signals indicates that alanine, arginine, choline, fumaric acid, inositol, sucrose as well as ginsenosides are important metabolites to differentiate the preparations from each other. This method allows an efficient discrimination of a ginseng preparation in less than 15 minutes without any prepurification steps.

Key words
Ginseng · metabolic fingerprinting · NMR · *J*-resolved spectrum · principal component analysis

Introduction

Ginseng (Araliaceae) has been used for more than two thousand years as a medicinal herb because of its diverse biological activities. There are many pharmacological effects reported for ginseng. The most important activity is the adaptogenic effect. The activities might be due to a corticosteroid-like action and endocrinological studies have suggested that ginsenosides may augment adrenal steroidogenesis via an indirect action on the pituitary gland [1]. More than 4500 tons are produced per year for commercial purposes at present [2].

There are several classes of compounds reported in ginseng root. They include triterpene saponins, polyacetylenes, sesquiterpenes, polysaccharides, peptidoglycans, nitrogen-containing compounds, fatty acids, carbohydrates, and phenolic compounds. The major constituents are triterpene saponins which are believed to contribute to the pharmacological effects of ginseng. However, there is no report about one single compound explaining the diverse bioactivities of the ginseng although some compounds are thought to be related to the activity. This fact supports the use of ginseng as a powder or an extract of the plant roots. The lack of knowledge on the active principles makes it difficult to control the quality of ginseng preparations. The variability in the different commercial products results in inequivalance

Affiliation
[1] Division of Pharmacognosy, Section Metabolomics, Institute of Biology, Leiden University, Leiden, the Netherlands
[2] Division of NMR, Institute of Chemistry, Leiden University, Leiden, The Netherlands

Correspondence
Young Hae Choi · Division of Pharmacognosy · Section Metabolomics · Institute of Biology · Leiden University · P.O. Box 9502 · 2300 RA Leiden · The Netherlands · Phone: +31-71-527-4471 · Fax: +31-71-527-4511 · E-mail: y.choi@chem.leidenuniv.nl

Received July 4, 2005 · **Accepted** October 7, 2005

Bibliography
Planta Med 2006; 72: 364–369 © Georg Thieme Verlag KG Stuttgart · New York
DOI 10.1055/s-2005-916240 · Published online January 30, 2006
ISSN 0032-0943

of the products, hampering studies on the efficacy of the medicine. Consequently, it is an important issue to define and control the quality of the commercial products of this medicinal plant.

There are a number of reports published about the analysis of ginseng in the past 30 years. Most methods rely on microscopic characteristics, thin-layer chromatography (TLC) [3], high-performance liquid chromatography (HPLC) [4], [5], [6], [7], [8], mass spectrometry (MS) [9], [10], [11], [12], and mass spectrometry connected with different chromatographic instruments; GC/MS, [13], [14], LC/MS/MS [15], [16], [17]. In spite of these efforts, there are still some limitations of these methods. The problem of conventional analytical methods is that they are limited to a certain group of compounds (mainly to ginsenosides). However, other metabolites including phenolics, sugars, and amino acids might also be important as a target of quality control because they can also contribute to the quality. An efficient method based on metabolic fingerprinting to cover a broad range of metabolites is undoubtedly needed for the quality control of ginseng.

The term "metabolome" has been used to describe the observable chemical profile or fingerprint of the metabolites in whole organisms or parts of them [18]. For the metabolic fingerprinting, it is necessary to use analytical methods, which should be fast, reproducible, and stable for a wide array of compounds. Nuclear magnetic resonance (NMR) spectroscopy is one of the techniques that could meet those requirements. Recently, ^{1}H-NMR spectroscopy combined with principal component analysis (PCA) method has been utilized to differentiate several kinds of plants based on metabolomes including *Cannabis* varieties [19], *Strychnos* species [20], and *Ilex* species [21]. However, when applied to the analysis of multicomponents such as in plant materials, one-dimensional ^{1}H-NMR has the problem of overlapping and low resolution of signals. High resolution NMR can solve these problems. Also some two-dimensional NMR methods have been expected to increase the resolution. Recently two-dimensional NMR has been shown to solve these problems to a great extent [22]. Among the possible two-dimensional NMR methods, *J*-resolved spectra considerably increase the resolution of the signals within a short time. Moreover, by projection to a spectral axis, all protons will appear as singlets, simplifying the spectra of complex plant extracts.

In this study, ^{1}H-NMR and *J*-resolved NMR spectroscopic methods coupled with PCA were applied to the metabolomic fingerprinting of three different commercial ginseng preparations and four kinds of ginseng roots. An efficient screening method for quality control of ginseng preparations is reported.

Materials and Methods

Materials

Four-, five-, and six-year-old white ginseng and four-year-old red ginseng roots were obtained from Daedong (Kumsan, Korea). Three different commercial ginseng capsules were purchased from De Tuinen (Alkmaar, The Netherlands), Il Hwa (Seoul, Korea), and Indros B. V. (Almere, The Netherlands). Four samples having different lot numbers were evaluated for each commercial ginseng preparation.

Solvents and chemicals

D_2O (99.0%) and methanol-*d4* (99.8%) was obtained from Cambridge Isotope Laboratories Inc. (Miami, FL, USA). Trimethylsilanepropionic acid sodium salt (TSP) and potassium dihydrogen phosphate (KH_2PO_4) were purchased from Merck (Darmstadt, Germany). NaOD was purchased from Cortec (Paris, France). KH_2PO_4 was added to D_2O as a buffering agent. The pH of the D_2O was adjusted to 6.0 using a 1 N NaOD solution.

Extraction

Two hundred and fifty mg of powdered material from the capsules and ground roots were transferred into a centrifuge tube. Seven hundred and fifty μL of KH_2PO_4 buffer in D_2O (with 0.1% TSP) and 750 μL of methanol-*d4* were added to the tube followed by vortexing for 1 min and sonication for 20 min. The tube was centrifuged at 3000 rpm for 20 min at 25 °C. The extract was transferred into a microtube followed by centrifugation at 13 000 rpm for 1 min. The supernatant (800 μL) was transferred to a 5-mm NMR tube. For verifying recovery of metabolites the first extracts were compared with the second and the third extracts using the same amount of solvents. The first extracts showed more than 92.6(±2.5)% of recovery of metabolites based on total ^{1}H-NMR intensity.

NMR measurements

^{1}H-NMR and *J*-resolved spectra were recorded at 25 °C on a 400 MHz Bruker AV-400 spectrometer operating at a proton NMR frequency of 400.13 MHz. TSP was used as the internal standard. Each ^{1}H-NMR spectrum consisted of 128 scans requiring 10 min acquisition time with the following parameters: 0.25 Hz/point, pulse width (PW) = 90° (6.6 μsec), and relaxation delay (RD) = 5.0 sec. A presaturation sequence was used to suppress the residual water signal with low power selective irradiation at the water frequency during the recycle delay. FIDs were Fourier transformed with LB = 0.3 Hz and the spectra were zero-filled to 32 K points. The window functions have been optimized for the analysis. The resulting spectra were manually phased and baseline corrected, and calibrated to TSP at δ = 0.0, all using XWIN NMR (version 3.5, Bruker). Two-dimensional *J*-resolved ^{1}H-NMR spectra were acquired using 8 scans per 32 increments that were collected into 16 K data points, using spectral widths of 5.208 kHz in F2 (chemical shift axis) and 50 Hz in F1 (spin-spin coupling constant axis). An 1.0 sec relaxation delay was employed, giving a total acquisition time of 14.52 min. Datasets were zero-filled to 512 points in F1 and both dimensions were multiplied by sine-bell functions prior to double complex FT. *J*-resolved spectra tilted by 45°, symmetrized about F1, and then calibrated, all using XWIN NMR (version 3.5, Bruker). Data were exported as the 1D projection (F2 axis) of the 2D *J*-resolved spectra. ^{1}H-^{1}H-correlated spectroscopy (COSY) spectra were acquired with 1.0 sec relaxation delay, 4194 Hz spectral width in both dimensions. The heteronuclear single quantum coherence (HSQC) spectra were obtained with 1.0 sec relaxation delay, 4401 Hz spectral width in F2 and 14 500 Hz in F1. The heteronuclear multiple bond correlation (HMBC) spectra were recorded with the same parameters as the HSQC spectrum.

Data analysis

The ^{1}H-NMR and the *J*-resolved projection spectra were automatically reduced to ASCII files using AMIX (v. 3.7, Bruker Biospin). Spectral intensities were expressed as a percentage of TSP area and reduced to integrated regions of equal width (0.04 ppm) corresponding to the region of δ = 0.40 – 10.00. The region of δ = 4.70 – 5.10 was excluded from the analysis because of the residual signal of water. Principal component analyses (PCA) were performed with the SIMCA-P software (v. 10.0, Umetrics, Umeå, Sweden).

Results and Discussion

It was found that ^{1}H-NMR is a quite attractive method for quality control of plant metabolites, as it allows the recognition of a broad metabolome, detecting diverse groups of compounds such as amino acids, carbohydrates, organic acids, phenolics, and terpenoids. Fig. **1A** shows the ^{1}H-NMR spectrum of the metabolome of a ginseng preparation.

Mono- or disaccharides such as sucrose, fructose, and glucose were identified in the ^{1}H-NMR spectra. Signals at δ = 5.40 (d, *J* = 3.8 Hz), δ = 5.18 (d, *J* = 7.0 Hz), δ = 4.60 (d, *J* = 8.0 Hz), δ = 4.17 (d, *J* = 10.7 Hz), and δ = 4.03 (t, *J* = 8.4 Hz) were assigned to be anomeric protons of sucrose, α-glucose, β-glucose, fructofuran moiety of sucrose, and inositol, respectively. Several signals overlapped with α-glucose and β-glucose were assigned to be anomeric protons of terminal and internal glucose in ginsenosides or in polysaccharides (Fig. **1B**).

In the phenolic region (δ = 5.7 – δ9.0), amino acids, fumaric acid, nucleotides, and phenylpropanoids were detected at low levels. NMR-based plant analysis typically employs one-dimensional ^{1}H-NMR methods to minimize sample acquisition times and hence maximize throughput. However, the spectral congestion of one-dimensional ^{1}H-NMR limits the number of metabolites which can be uniquely identified and quantified. For overcoming this congestion, the *J*-resolved technique was applied to the analysis and definitely improved the resolution of the ^{1}H-NMR spec-

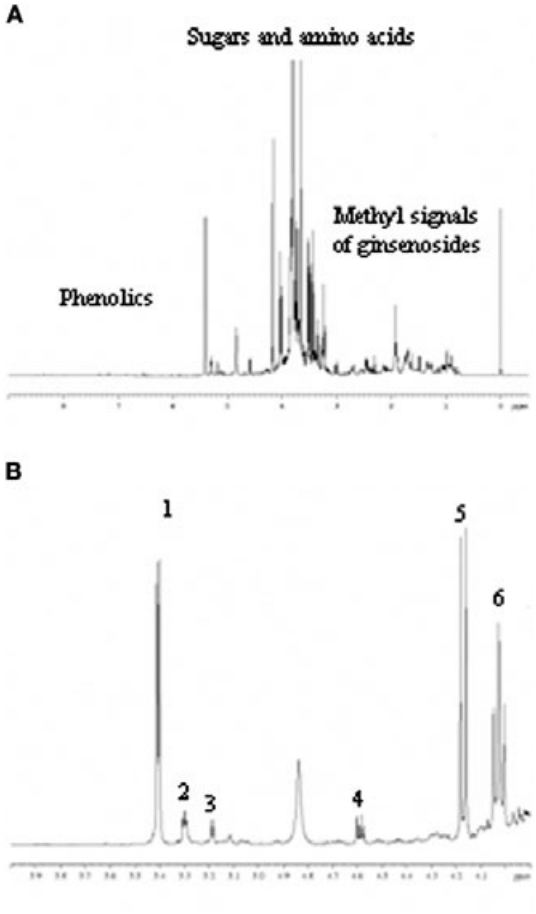

Fig. **1** ^{1}H-NMR spectrum of a ginseng preparation in the range of δ = –0.5 – 9.0 (**A**) and δ = 4.0 – 6.0 (**B**). 1; H-1 of sucrose, 2; H-1 of terminal glucose in ginsenosides, 3; H-1 of α-glucose, 4; H-1 of β-glucose and internal glucose in ginsenosides, 5; H-1 of fructofuran of sucrose, 6; H-2 of inositol.

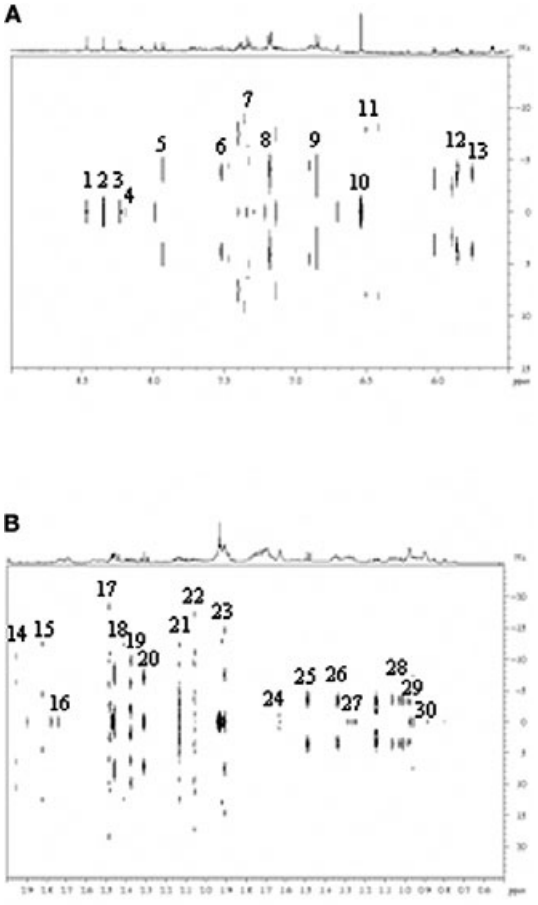

Fig. **2** Two-dimensional *J*-resolved NMR spectrum of a preparation in the range of δ = 6.0 – 9.0 (**A**) and δ = 4.0 – 6.0 (**B**). 1; H-2 of adenosine monophosphate, 2; H-2 of adenosine, 3; H-7 of adenosine monophosphate, 4; H-7 of adenosine, 5; H-6 of uracil, 6; H-6 of cytosine, 7; aromatic protons of phenyl alanine, 8; H-5 and H-9 of tyrosine, 9; H-6 and H-8 of tyrosine, 10 H-2 of fumaric acid, 11; H-8 of phenylpropanoids, 12; H-5 of uracil, 13; H-5 of cytosine, 14; H-3 of malic acid, 15; H-3′ of malic acid, 16; H-2 of succinic acid, 17; H-3 of glutamine, 18; H-3 of glutamic acid, 19; H-3 of proline, 20; H-2 of γ-amino butyric acid, 21; H-2 of glutamine and glutamic acid, 22; H-4 and H-5 of proline, 23; H-3 of arginine, 24; H-26 and H-27 of protopanaxdiol and protopanaxtriol, 25; H-3 of alanine, 26; H-5 of threonine, 27; H-3 and H-21 of protopanaxdiol and protopanaxtriol, 28; H-4 and H-5 of valine, 29; H-5 and H-6 of leucine, 30; H-18, H-19, H-28, H-29, and H-30 of protopanaxdiol and protopanaxtriol.

tra. Fig. **2A** shows the signals obtained from *J*-resolved spectra. Using this spectrum together with the ^{1}H-^{1}H-COSY spectrum adenosine monophosphate at δ = 8.47 (H-2, s) and δ = 8.22 (H-7, s), adenosine at δ = 8.32 (H-2, s) and δ = 8.21 (H-7, s), uracil at δ = 7.92 (H-6, d, J = 8.5 Hz) and δ = 5.84 (H-5, d, J = 8.5 Hz), and cytisine at δ = 7.51 (H-6, d, J = 8.5 Hz) and δ = 5.73 (H-5, d, J = 8.5 Hz) were identified. In addition to these nucleotides, phenylalanine at δ = 7.3 – 7.5 (m); tyrosine at δ = 7.20 (H-6 and H-8, d, J = 8.5 Hz) and at δ = 6.89 (H-5 and H-7, d, J = 8.5 Hz); fumaric acid at δ = 6.56 (H-2, s); and ferulic acid at δ = 6.4 – 6.5 (H-8, d, J = 15.6 Hz) were identified.

Methyl protons of ginsenosides were detected in the crowded region of δ = 0.7 – 1.8. These methyl signals cannot be properly assigned in the ^{1}H-NMR due to the high level of amino acids. The *J*-resolved spectrum shows good resolution of the methyl signals and the interfering amino acid signals (Fig. **2B**). H-26 and H-27 of protopanaxdiol or protopanaxtriol were identified at δ = 1.70 and δ = 1.63 as singlets, respectively. The assignments were confirmed by the HMBC spectrum in which H-26 and H-27 correlated with C-24 at δ = 127.5 and C-25 at δ = 134.4. The clusters of singlets in the region of δ = 1.2 and δ 1.3 were also assigned to be H-21, H-28, and H-29 of ginsenosides because the correlations with oxygen-substituted carbons such as C-3 and C-20 (δ = 80 – 90) are clearly detected in the HMBC spectrum. The other methyl signals of ginsenosides such as H-18, H-19, H-28, H-29, and H-30 were identified as singlets at δ = 0.8 – 1.0. Acetate signals, e. g., from ginsenosides containing acyl sugars were detected at δ = 1.95 (s) having a correlation with δ = 182.1 in the HMBC spectrum.

Several aliphatic amino acids including alanine, γ-aminobutyric acid, arginine, glutamic acid, glutamine, proline, threonine, and valine were identified as major compounds in the ginseng preparations evaluated in this study using *J*-resolved and ^{1}H-^{1}H-COSY spectra (Fig. **2B**). Multiplets at δ = 2.47, δ = 2.45, δ = 2.36, δ = 2.13, δ = 2.04, and δ = 1.91 were assigned to be H-3 of glutamine, H-3 of glutamic acid, H-3 of proline, H-2 of glutamine and glutamic acid, H-4 and H-5 of proline, and H-3 of arginine, respectively. A triplet at δ = 2.32 (J = 7.5 Hz) was assigned to H-2 of γ-aminobutyric acid. Methyl signals of alanine, threonine, valine, and leucine were detected at δ = 1.48 (d, J = 7.2 Hz), δ = 1. 36 (d, J = 7.2 Hz), δ = 1. 04 (d, J = 6.7 Hz), δ = 1. 01 (d, J = 6.7 Hz), δ = 0.98 (d, J = 6.7 Hz), and δ = 0.96 (d, J = 6.7 Hz). *N*-methyl signals of choline were detected at δ = 3.22 (s). Low levels of malic acid at δ = 2.95 (H-3, dd, J = 6.6 Hz, H-4.7 Hz) and δ = 2.81 (H-3′, dd, J = 16.6 Hz, H-4.7 Hz) and succinic acid δ = 2.75 (H-2, s) were also identified in the region of δ 2.5 – 3.0.

After the assignments of the NMR spectra allowing the identification of a wide array of compounds, a chemical classification of seven kinds of ginseng preparations (four kinds of ginseng roots and three kinds of commercial preparations) was performed by multivariate data analysis.

Prior to PCA analysis, projected *J*-resolved spectra were prepared from 2D-*J*-resolved spectra in order to reduce the complexity of ^{1}H-NMR spectra (Fig. **3**). As all protons ideally appear as a singlet in the *J*-resolved spectra using the projected spectrum on the axis of chemical shift (like ^{13}C-NMR spectra showing a fully H de-

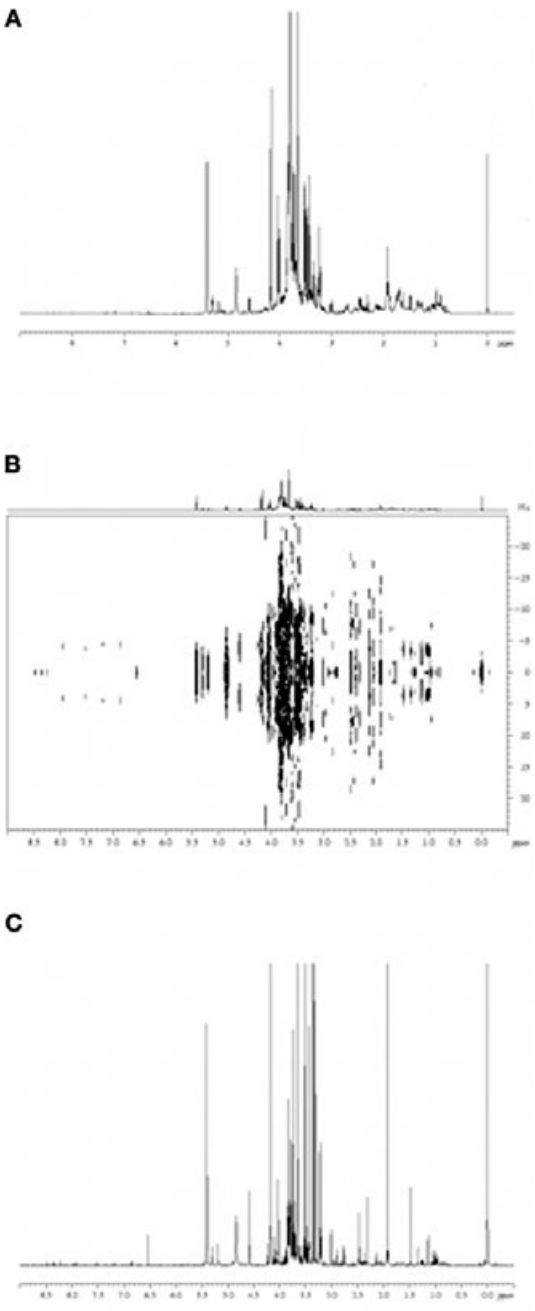

Fig. **3** ^{1}H-NMR (**A**), 2D *J*-resolved (**B**), and projected 1D *J*-resolved (**C**) spectrum of a ginseng preparation in the range of δ = –0.5 – 9.0.

coupled spectrum) it becomes less complex. The degree of spectral complexity in the projected *J*-resolved spectrum is significantly reduced compared to the true 1D dataset. Moreover, this enhanced resolution made it possible to directly compare or match the spectra obtained from different samples. We applied this enhanced resolution obtained from the projected J-resolved spectra to discrimination of the ginseng preparations based on chemical fingerprints obtained from the NMR spectra.

Three kinds of white ginseng roots (4, 5, and 6 years old), 4-year-old red ginseng root, and three kinds of ginseng preparations were analyzed by NMR combined with PCA. For the data set acquired from the analysis, a seventeen-component model explained more than 99.0% of the variance, with the first three

components explaining 80.2%. The commercial samples (1–3) evaluated in study are close to 5- and 6-year-old white ginseng and clearly separated from 4-year-old white and red ginsengs (Fig. **4**).

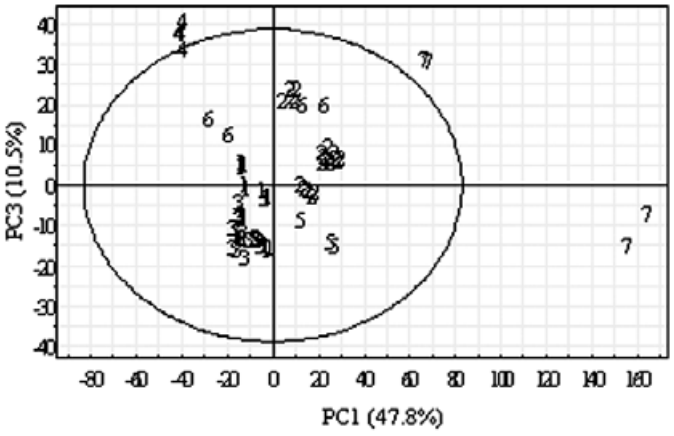

Fig. **4** Score plot of principal component analysis of ginseng preparations using PC1 vs. PC3 (**B**). 1–3; commercial ginseng preparations, 4; 4-year-old white ginseng root, 5; 5-year-old white ginseng root, 6; 6-year-old white ginseng root, 7; 4-year-old red ginseng root.

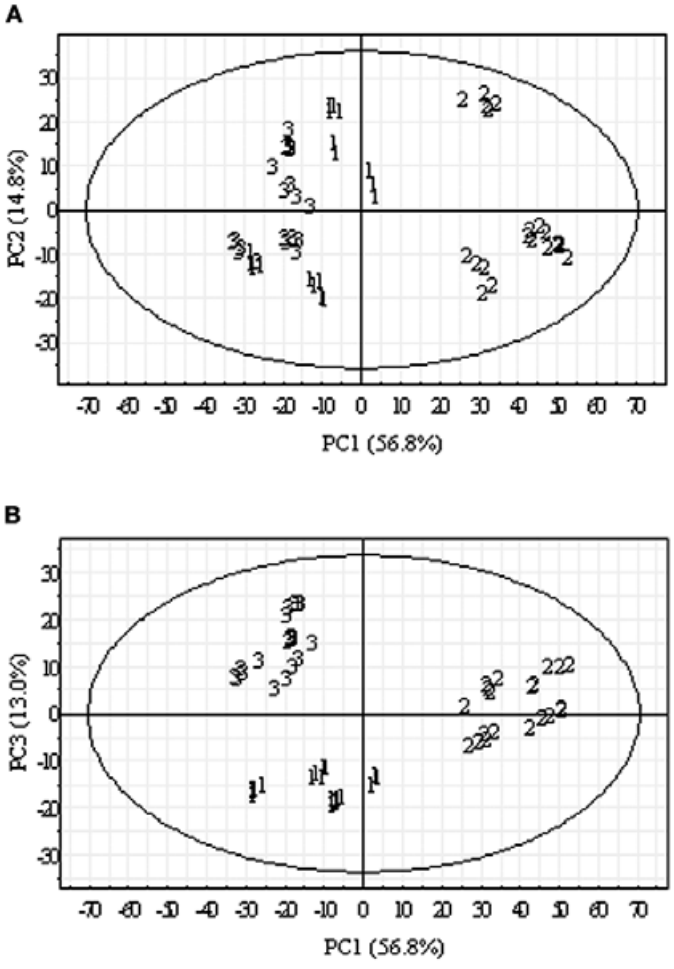

Fig. **5** Score plot of principal component analysis of ginseng preparations using PC1 vs. PC2 (**A**) and PC1 vs. PC3 (**B**). 1–3; commercial ginseng preparations.

However, a large number of ginseng roots from diverse source (e.g., harvesting region and season) are needed in order to draw a final conclusion about the source material of the ginseng commercials. The aim of this study is to evaluate the feasibility of NMR as an alternative quality control method for ginseng. Thus, PCA was performed for only ginseng commercial products in order to investigate the metabolic differentiation of the commercial preparations in more detail.

Examination of the scores plot for PC1 vs. PC2 obtained from the projected *J*-resolved spectra shows that sample 2 is clearly separated from samples 1 and 3 (Fig. **5A**). The separation of sample 2 from the others is influenced by PC1. The high level of alanine, fumaric acid, inositol, and sucrose characterize the sample 2 (Fig. **6A**) while arginine is detected at higher levels in samples 1 and 3. The acetate signal at δ = 1.92 also determines the separation of sample 2. Intriguingly, whereas PC1 influences the separation between different samples, PC2 affects differentiation within samples. Different batches of the same samples are clearly separated by PC2. In the loading plot, alanine, arginine, inositol, and sucrose were found to be the major compounds explaining PC2 (Fig. **6A**). The lack of separation of samples 1 and 3 was solved using another principal component, PC3. In Fig. **5B**, all of products evaluated in this study can be clearly separated by PC1 and PC3. Major NMR signals explaining PC3 were found to be methyl and anomeric signals of ginsenosides (Fig. **6B**). Higher intensities of H-26 and H-27 at δ = 1.70–1.63, H-21, H-28, and H-29 at δ = 1.2–1.3, and H-18, H-19, H-28, H-29, and H-30 in δ = 0.8–1.0 were detected in sample 3. However, in the contrast to the methyl signals of ginsenosides, the anomeric signals are higher in sample 1 compared to sample 3. The anomeric signals at δ = 5.40–4.50 might belong to oligo- or polysaccharides of ginseng. Ginseng contains about 5% of a sugar fraction, which comprises two monosaccharides, D-glucose and D-fructose, two

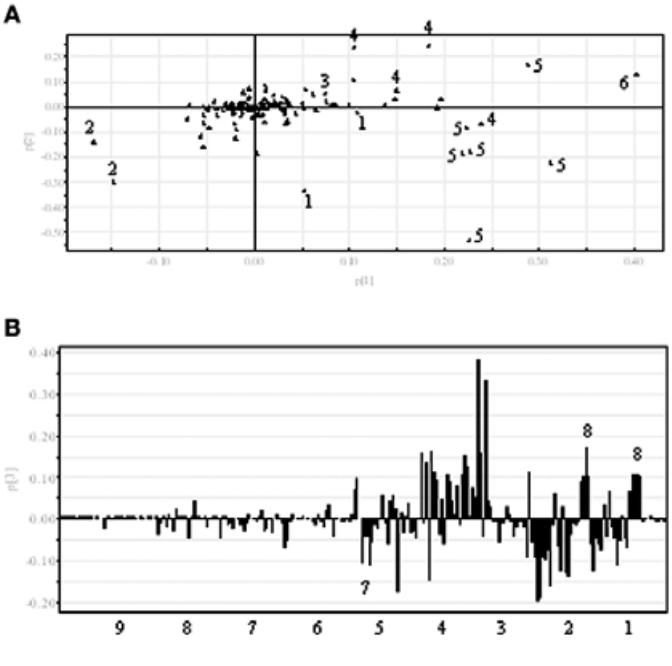

Fig. **6** Loading plot of principal component analysis of ginseng preparations. PC1 and PC2 (**A**) and PC3 (**B**). 1; alanine, 2; arginine, 3; fumaric acid, 4; inositol, 5; sucrose, 6; acetate, 7; anomeric protons of sugars; 8; methyl protons of saponins.

disaccharides, sucrose and maltose, and three trisaccharides, maltosyl-β-D-fructofuranose, *O*-α-D-glucopyranosyl(1→2)-*O*-β-D-fructofuranosyl(1→2)-β-D-fructofuranose, and *O*-α-D-glucopyranosyl(1→6)-*O*-α-D-glucopyranosyl(1→4)-α-D-glucopyranose [23]. In addition to these oligosaccharides, a number of glycans named panaxans have been isolated. Most of these glycans contain the α-1→6 linked D-glucopyranose residue [24], [25]. Based on these previous reports and our PCA results we thus conclude that sample 1 probably contains more carbohydrates and less saponins than sample 3.

In conclusion, ^{1}H-NMR and *J*-resolved NMR spectroscopic methods coupled with PCA were applied to performing the metabolomic fingerprinting of ginseng preparations. It is a highly efficient screening method for quality control of ginseng preparations although there are some limitations such as difficulty to differentiate individual ginsenosides. Among the possible NMR methods *J*-resolved NMR spectra considerably improve the resolution of the complex spectra. Analyzing the spectral data with PCA the samples can be clearly distinguished in the score plot, especially by PC1 and PC3. According to the PCA results obtained in this study, the analysis can be an efficient way to evaluate the difference between different commercial products and to be a fast screening for quality control. It allows determination of the differences of total compounds of commercial preparations. Based on these results, this method combining 2D *J*-resolved NMR spectra with PCA seems very promising for quality control of other herb medicinal products.

References

[1] Grandhi A, Mujumdar AM, Patwardhan BA. Comparative pharmacological investigation of Ashwagandha and Ginseng. J Ethnopharmacol 1994; 44: 131 – 5

[2] Soldati F. *Panax ginseng*: standardization and biological activity. In: Biologically active natural products: pharmaceuticals. Cutler SJ, Cutler HG, editors. New York: CRC Press, 2000: pp 209 – 32

[3] Corthout J, Nassessens S, Apers AJ, Vlietinck AJ. Quantitative determination of ginsenosides from *Panax ginseng* roots and ginseng preparations by thin layer chromatography-densitometry. J Pharm Biomed Anal 1999; 21: 187 – 92

[4] Chuang WC, Sheu SJ. Determination of ginsenosides in ginseng crude extracts by high performance liquid chromatography. J Chromatogr A 1994; 685: 243 – 51

[5] Kwon SW, Han SB, Park HI, Kim JM, Park MK, Park JH. Liquid chromatographic determination of less polar ginsenosides in process ginseng. J Chromatogr A 2001; 921: 335 – 9

[6] Bonfill M, Casals I, Palazon J, Mallol A, Morales C. Improved high performance liquid chromatographic determination of ginsenosides in *Panax ginseng*-based pharmaceuticals using a diol column. Biomed Chromatogr 2002; 16: 68 – 72

[7] Washida D, Kitanaka S. Determination of polyacetylenes and ginsenosides in *Panax* species using high performance liquid chromatography. Chem Pharm Bull 2003; 51: 1314 – 7

[8] Dou DQ, Ren J, Chen Y, Pei YP, Chen YJ. Study on the chemical constituents of the roots of commercial ginseng. Med J China 2003; 28: 522 – 4

[9] Cai ZW, Lee FSC, Wang XR, Yu WJ. A capsule review of recent studies on the application of mass spectrometry in the analysis of Chinese medicinal herbs. J Mass Spectrom 2002; 37: 1013 – 24

[10] Popovich D, Kitts DD. Generation of ginsenosides Rg3 and Rh2 from North American ginseng. Phytochemistry 2004; 65: 337 – 44

[11] Nam MH, Heo EJ, Kim JY, Kim S, Kwon KH, Seo JB et al. Proteome analysis of the responses of *Panax ginseng* C. A. Meyer leaves to high light: use of electrospray ionization quadrupole-time of flight mass spectrometry and expressed sequence tag data. Proteomics 2003; 3: 2351 – 67

[12] Liu SY, Cui M, Liu ZQ, Song FR, Mo WJ. Structural analysis of saponins from medicine herbs using electrospray ionization tandem mass spectrometry. J Am Soc Mass Spectrom 2004; 15: 33 – 41

[13] Cui JF. Identification and quantification of ginsenosides in various commercial ginseng preparations. Eur J Pharm Sci 1995; 3: 77 – 85

[14] Hou D, Zhang W, Hui R. Separation and determination of chemical constituents in the volatile oil of three traditional Chinese crude drugs. J Pharm Biomed Anal 1998; 17: 1423 – 6

[15] Wang XM, Takeo S, Ebenezer A, Gerals KS. Determination of ginsenosides in plant extracts from *Panax ginseng* and *Panax quinquefolius* L. by LC/MS/MS. Anal Chem 1999; 71: 1579 – 84

[16] Cui M, Song F, Zhou Y, Liu Z, Liu S. Rapid identification of saponins in plant extracts by electrospray ionization multi-stage tandem mass spectrometry and liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 2000; 14: 1280 – 6

[17] Harkey MR, Henderson GL, Gershwin ME, Stern JS, Hackman RM. Variability in commercial ginseng products: an analysis of 25 preparations. Am J Clin Nutr 2001; 73: 1101 – 6

[18] Sumner LW, Mendes P, Dixon RA. Plant metabolomics: large-scale phytochemistry in the functional genomics era. Phytochemistry 2003; 62: 718 – 36

[19] Choi YH, Kim HK, Hazekamp A, Erkelens C, Lefeber AWM, Verpoorte R. Metabolomic differentiation of *Cannabis sativa* cultivars using ^{1}H NMR spectroscopy and principal component analysis. J Nat Prod 2004; 67: 953 – 7

[20] Frederich M, Choi YH, Angenot L, Harnischfeger G, Lefeber AWM, Verpoorte R. Metabolomic analysis of *Strychnos nux-vomica*, *Strychnos icaja* and *Strychnos ignatii* extracts by ^{1}H nuclear magnetic resonance spectrometry and multivariate analysis techniques. Phytochemisty 2004; 65: 1993 – 2001

[21] Choi YH, Sertic S, Kim HK, Wilson EG, Michopoulosa F, Lefeber AWM et al. Classification of *Ilex* species based on metabolomic fingerprinting using NMR and multivariate data analysis. J Agric Food Chem 2005; 53: 1237 – 45

[22] Viant MR. Improved method for the acquisition and interpretation of NMR metabolomic data. Biochem Biophys Res Commun 2003; 310: 943 – 8

[23] Shoji J. Recent advances in the chemical studies on ginseng. In: Advances in chinese medicinal materials research. Chang HM, Yeung HW, Tso WW, Koo A, editors. Singapore: World Scientific, 1985: pp 455 – 69

[24] Tomada M, Shimada K, Konno C, Sugiyama K, Hikino H. Validity of oriental medicines, LXXI. Antidiabetes drugs. 2. Partial structure of panaxan A, a hypoglycemic glycan of *Panax ginseng* roots. Planta Med 1984; 50: 436 – 8

[25] Tomada M, Shimada K, Konno C, Hikino H. Validity of oriental medicines, XCIV. Antidiabetes drugs. 12. Structure of panaxan B, a hypoglycemic glycan of *Panax ginseng* roots. Phytochemistry 1985; 24: 2431 – 3