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HR-MAS NMR Technique for Metabolic Profiling of Powdery Ginseng

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Abstract Ginseng is used as a medicinal ingredient. The quality control of species, age, origin and manufacturing process is important. The metabolome of ginseng about quality was studied in many reports. Almost studies carried out the extract of ginseng, however, the reproducibility cannot be obtained using extracted sample. In this study, powdery ginseng samples were analyzed using high resolution-magic angle spinning nuclear magnetic resonance (HR-MAS NMR)-based metabolomics except extraction step. Sample was measured three times using 600 MHz NMR spectrometer equipped with nano probe. Reproducibility can be enhanced using this method and the metabolic profiles of ginseng were identified and quantified.

Keywords Nuclear magnetic resonance (NMR), Metabolomics, High resolution magic angle spinning (HR-MAS), Ginseng

Introduction

Ginseng has been used as a natural medicinal ingredient in East Asia for thousands years.[1] Ginseng is a perennial crop and four to six years old ginseng are used as medicines. Discriminating the age of ginseng is important that the price of ginseng increases as the cultivation age increases. Quality assurance or quality control of species, origins and manufacturing process is also important. Scientific and systematic methods to discriminate the quality are necessary to be settled.

Metabolomics is an emerging and promising field. The end products of metabolic pathway are called metabolites. Metabolites are changed by internal or external variables. Many kinds of factors such as temperature, moisture, nutritional sources, diseases and organism development change the metabolites.[2] Metabolomics is the study of analysis these changes of metabolite profiles in the organisms. Nuclear magnetic resonance (NMR) is one of the most useful tools to detect the metabolic change. NMR-based metabolomics is widely used in many applications such as toxicology, environmental toxicology, forensic and food science including the plant metabolomics.[3,4]

Many studies were conducted to analyze the metabolites of ginseng using NMR spectroscopy.[5-7] However, solvent-extracted samples have a major problem in reproducibility. High resolution-magic angle spinning (HR-MAS) NMR techniques need minimal sample preparation and HR-MAS NMR spectroscopy was introduced to enhance the

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reproducibility in this study. HR-MAS is a powerful NMR technique for investigating of solid or semi-solid samples without any other extraction step.[8] Rapid spinning at the magic angle (54.7˚) reduces the line-broadening effects of powdery samples.[9]

In this study, powdery ginseng samples were measured intactly using HR-MAS NMR spectrometer and analyzed the metabolic profiles.

Experimental Methods

Sample preparation- 10 mg of homogenized powdery ginseng sample (each sample was repeated for three times) was transferred to NMR nano tube (Agilent technologies, USA). 30 µL of D_2O containing 10 mM TSP-d_4 (3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid sodium salt) was transferred to NMR nano tube. This procedure was repeated three times.

NMR experiments- All spectra were acquired at 600.167 MHz Agilent spectrometer operating at ^{1}H frequency and equipped with a 4-mm gHX NanoProbe (Agilent technologies, Santa Clara, CA, USA) at 2,500 Hz of spinning rate. CPMG (Carr-Purcell-Meiboom-Gill) with PRESAT pulse sequence was used for suppression of water and high molecular mass compounds. The spectra were obtained using 1.704 s acquisition time, 1 s relaxation delay and 128 transients. The TSP-d4 peak at 0.00 ppm was used for the reference to calibrate the chemical shift. All spectra were phased and baseline corrected manually. Peak assignment for each sample was processed using Chenomx NMR Suite 7.1 professional (Chenomx Inc., Canada) and the Chenomx 600 MHz library database.

Results

Homogenized powdery ginseng sample was measured using 600 MHz HR-MAS NMR spectrometer at 2,500 Hz of spinning rate and in order to obtain the reproducibility, repeated experiments were conducted three times. Three

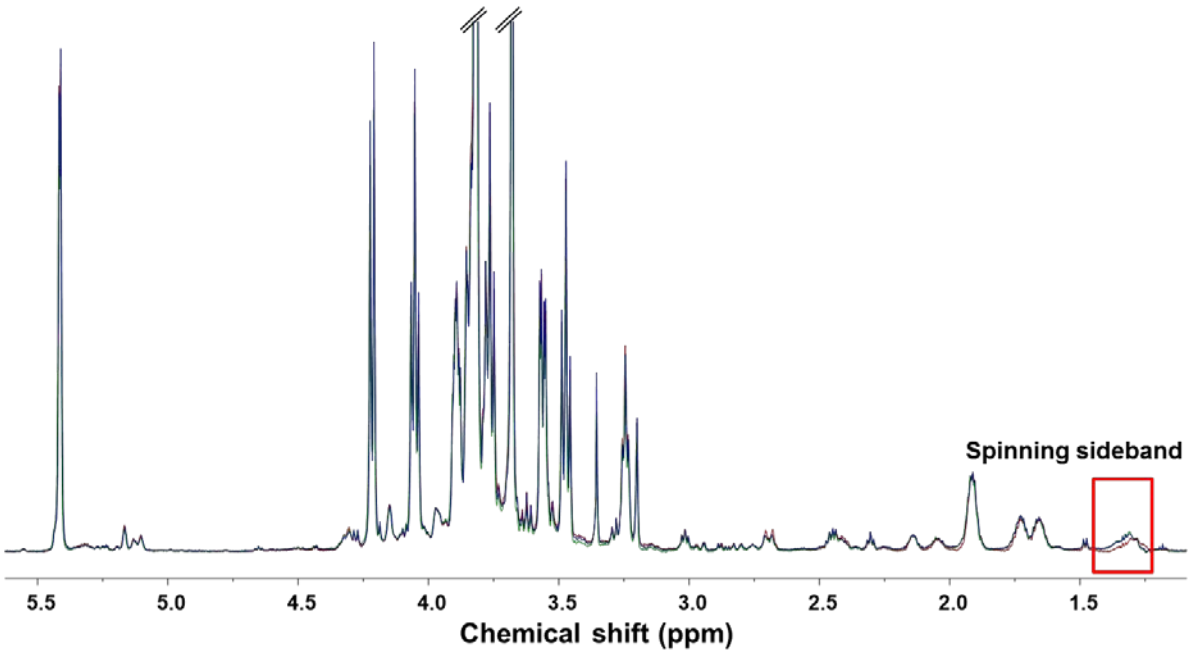

Figure 1. ^{1}H NMR overlapped spectra of three times repeated ginseng samples

spectra were almost overlapped except the spinning side band.(Fig 1)

Metabolites in ginseng sample were identified using Chenomx NMR suite 7.1 professional software with 600 MHz library database. 19 metabolites were identified and quantified.

Discussion

Powdery ginseng samples were analyzed using HR-MAS NMR spectroscopy. To assure the reproducibility of measurement, three replications of one sample were analyzed. Three spectra of each sample were overlapped and compared together. Except the spinning sideband area, all peaks were almost perfectly overlapped. So this method is useful to enhance the reproducibility.

However, spinning side bands were observed using HR-MAS NMR inevitably and they interrupted the analysis of metabolites because they overlapped with some metabolite peaks. So, we increased speed of spinning from 2,000 Hz to 2,500 Hz, and spinning side band moved to lower and higher field which were not affecting the analysis of metabolites.

19 metabolites were confirmed in each sample using Chenomx NMR suite software with 600 MHz library database. 19 metabolites of three samples were quantified using Chenomx NMR suite software and their relative concentrations were calculated. Each metabolite in three samples was compared and their standard errors (S.E.) were calculated to indicate the accuracy. All metabolites show the low standard errors (S.E. <1), therefore, the repeated measurements of 3 samples have the reproducibility. 4-Aminobutyrate (GABA), which is the inhibitory neurotransmitter in the mammalian brain10, amino acids such as alanine, arginine, asparagine, aspartate, glutamate, glutamine, leucine, phenylalanine, tyrosine and valine, organic acids such fumarate and lactate, sugar such as glucose and sucrose, choline, myo-inositol, ethanolamine, and methanol were identified and quantified in the ginseng sample. In the ginseng sample, sucrose was the most abundant metabolite (above the 60 percent of total metabolite concentrations).

Table 1. Relative concentrations of metabolites (%)

	Sample 1	Sample 2	Sample 3	S.E.
4-Aminobutyrate	1.4665	1.6496	1.5574	0.0529
Alanine	0.5675	0.6649	0.6381	0.0290
Arginine	13.4625	13.1604	12.8739	0.1699
Asparagine	1.6535	1.6651	1.6487	0.0049
Aspartate	1.3771	1.3575	1.3502	0.0080
Choline	1.3459	1.4275	1.5564	0.0613
Ethanolamine	0.6576	0.7430	0.5757	0.0483
Fumarate	0.1509	0.1665	0.1570	0.0045
Glucose	1.5172	1.6004	1.8181	0.0897
Glutamate	0.7330	0.6520	0.4103	0.0969
Glutamine	2.7786	2.7965	2.9738	0.0623
Lactate	0.5510	0.5763	0.6614	0.0334
Leucine	0.0265	0.0586	0.0502	0.0096
Methanol	4.7171	5.9947	7.7313	0.8735
Phenylalanine	0.1344	0.1653	0.1421	0.0093
Sucrose	63.7972	61.9657	60.4113	0.9785
Tyrosine	0.2042	0.1542	0.1776	0.0145
Valine	0.0515	0.0968	0.1126	0.0183
myo-Inositol	4.8076	5.1052	5.1541	0.1083

More metabolites should be identified in the ginseng sample, and unknown peak also should be investigated further study. These metabolome profiles of powdery ginseng using HR-MAS NMR based metabolomics will be used for analyzing the quality of ginseng such as species, origin and age.

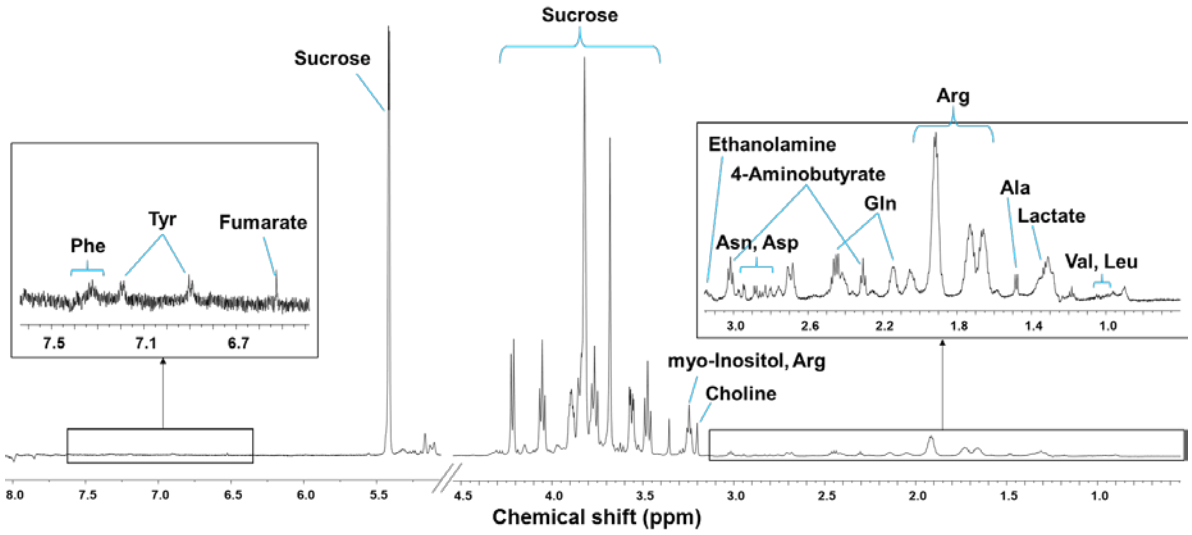

Figure 2. The representative ^{1}H NMR spectrum of ginseng. The abbreviations for the amino acids are: Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartate; Gln, glutamine; Leu, leucine; Phe, phenylalanine; Tyr, tyrosine; Val, valine.

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References

1. M. Y. Jung, B. S. Jeon, and J. Y. Bock, *Food Chem.* **79**, 1 (2002)
2. E. Holmes, H. Tang, Y. Wang, and C. Seger, *Planta. Med*. **72**, 9 (2006)
3. D. Yoon, M. Lee, S. Kim, and S. Kim, *J. Korean Soc. Magn. Reson. Med*. **17**, 1 (2013)
4. D. Yoon, J. Choi, H. Choi, and S. Kim, *J. Korean Soc. Magn. Reson. Med*. **20**, 13 (2016)
5. J. Kang, S. Lee, S. Kang, H.N. Kwon, J. H. Park, S. W. Kwon, and S. Park, *Arch. Pharm. Res*. **31**, 330 (2008)
6. E. J. Lee, R. Shaykhutdinov, A. M. Weljie, H. J. Vogel, P. J. Facchini, S. U. Park, Y. K. Kim, and T. J.

Yang, *J. Agric. Food. Chem.* **57**, 7513 (2009)
7. S. O. Yang, Y. S. Shin, S. H. Hyun, S. Cho, K. H. Bang, D. Lee, S. P. Choi, and H. K. Choi, *J. Pharm. Biomed. Anal*. **58**, 19 (2012)
8. C. Corsaro, D. Mallamace, J. Lojewska, F. Mallamace, L. Pietronero, and M. Missori, *Sci. Rep*. **3**, 2896 (2013)
9. O. Beckonert, M. coen, H. C. Keun, Y. Wang, T. M. D. Ebbels, E. Holmes, J. C. Lindon, and J. K Nicholson, *Nat. Protoc*. **5**, 1019 (2010)
10. M. R. Pears, J. D. Cooper, H. M. Mitchison, R. J. Mortishire-Smith, D. A. Pearce, and J. L. Griffin, *J. Biol. Chem*. **280**, 52 (2005)