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Data

Changes in Contents of Ginsenoside Due to Boiling Process of *Panax ginseng* C.A. Mayer

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Abstract - The purpose of the study was to determine a method to use fresh white Korean ginseng in the form of higher intake of medicinal components. Decoction was made at 70℃ and 90℃ in different intervals of time. HPLC (DAD) system was employed to monitor the ginsenosides content in the decoctions and the components were identified by comparing the retention time with that of reference compounds. However, decoction made at 70℃ in 72 hrs possessed higher amount of total ginsenosides (209.6 μg/mL) content where considerable amount of bioactive ginsenosides like Rg3, Rb2, Rb1 and Rg1 were accumulated. Overall, it can be concluded that the fresh white Korean ginseng decoction made in 72 hrs at 70℃ would be useful for the health and other medicinal approach of ginseng.

Key words - Decoction, White Korean ginseng, Ginsenoside

Introduction

During the last several decades, great progress has been made on the research of the bioactivity, chemistry and clinical efficacy of ginseng (Li *et al.,* 2010). Ginsenoside is the main pharmacological component of *Panax ginseng*. Among the discovered ginsenosides in white ginseng, Rg1 (protopanaxatriols) is considered as stimulator of central nervous system and involved in adaptogen activity (Ko *et al.,* 2009). Likewise, Rh2 has anti-allergic (Park *et al.,* 2003) and anticancer activity (Guo *et al.,* 2012). Rg3 and compound K are generally not present in white ginseng, but after processing by steaming or drying, these compounds can be generated artificially. These compounds are reported to show antitumor (Keum *et al.,* 2003), antioxidant (Keum *et al.,* 2000), and lowering blood pressure (Kim *et al.,* 1999). Rb1 and Rb2 also reported to have antiviral, antidiabetic activity and anti-hyperlipidemic effect (Yoo *et al.,* 2013; Lee *et al.,* 2011).

Decoction is a traditional method for extraction of chemical components (organic compounds, oils etc) by boiling with water from plant materials like roots, barks, stem, fruits etc. Decoctions can produce liquids with different chemical properties as the temperature, time and preparation varies. In general, people still practice traditional method of consumption of ginseng by boiling with water (decoction). However, there are no clear indications of standardization decoctions of fresh white Korean ginseng which have higher functional ginsenoside content. Standardized decoctions can increase the quality and bioactivity of ginseng. Recently, there was a report of decoctions of white ginseng where the alteration of ginsenosides was observed (Jin *et al.,* 2012). However, their investigation was based on the sulfur-fumigated white ginseng during post harvest handling. Therefore, to the best of our knowledge, no standardization of ginsenoside compound has been reported using fresh white Korean ginseng decoctions. Therefore, the aim of the present study was to develop a decoction method to enhance the functional ginsenosides (Rb1, Rb2, Rg1, Rg3, Rh2, and C-K) in fresh white Korean ginseng.

Materials and Methods

Chemicals and Standards

HPLC grade acetonitrile was purchased from Merck (Germany). All other chemicals and solvents were of analytical

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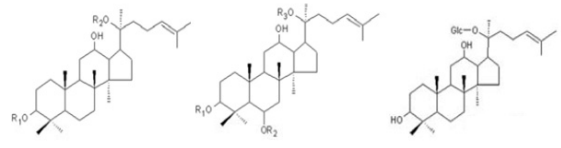
grade. The deionised water was prepared with a Milli-Q water purification system (Millipore, Bedford, MA, USA). The standards reference samples of ginsenosides Rb1, Rb2, Rg1, Rg3, Rh2, and C-K were purchased from Sigma-Aldrich. The standard's stock solutions were prepared by dissolving with methanol (100 μg/mL) and were stored at 4℃ for further analysis.

Collection of ginseng roots and preparation of ginseng decoction extracts

Fresh six years old Korean ginseng root grown in an organic system using lime Sulfur complex at Gyeongi province, South Korea were harvested in the month of October 2011. The roots were ranged from 37.5 g to 50 g in fresh weight. The fresh white Korean ginseng root was accurately weighted (approximately 20 gram each) and refluxed with 200 mL of water for 24, 48, and 72 hrs at 70℃ and 90℃ in a water bath. After cooling, the samples were filtered and used for further experiment.

Ginsenoside analysis by HPLC

HPLC analysis of ginsenosides in the decoctioned extracts was carried out on Agilent 1260 Infinity Quaternary LC System consist of diode array detector (DAD) and 1260 Evaporative Light Scattering detector (ELSD) with auto injector using Eclipse XDB C18 column (150 × 4.6 mm, 3.5 micron Phenomenex. Inc., Torrance, CA, USA). HPLC conditions were as follows: Solvent A (water) and solvent B (acetonitrile) was prepared.



Protopanaxadiol (PPD) Protopanaxatriol (PPT) Compound-K (C-K)

Ginsenosides	R1	R2	R3
Rb1 (PPD)	O-g-g	O-g-g	
Rb2 (PPD)	O-g-g	O-g-Ap	
Rg3 (PPD)	O-g-g	O-H	
Rh2 (PPD)	O-g-g	O-H	
Rg1 (PPT)	O-H	O-g	O-g

Fig. 1. Chemical structure of ginsenosides (Rb1, Rb2, Rg3, Rh2, Rg1 and C-K) used in the experiment. Abbreviations refer to the following: Ap = arabinopyranose, g = glucopyranose.

Gradient elution was used for 0-30 min, 17-21 % B; 30-35 min, 21-23 % B; 35-45 min, 23-25 % B; 45-65 min, 25-40 % B; 65-85 min, 40-65 % B; 85-95 min, 65-75 % B. The flow rate of mobile phase solution was 1.5 mL/min, and detection was carried out at 203 nm. 20 μL of each sample was injected. The compounds were identified in solvents by matching their retention times and spectra with that of the standards and the data were calculated on the basis of the peak area obtained.

Statistical analysis

All data were expressed as the mean value ± standard deviation (SD) of each experimental group (n = 3). The results were processed using Excel 2003 (Microsoft, Redmond, WA, USA).

Results and Discussion

Ginsenosides content at 70℃ decoction extracts

High temperature can change the properties of water thus making the polarity of water closer to those of non-polar compounds. This will enhance the solubility of less polar compounds in water for extraction from different matrices (Toe *et al.,* 2010; Ong *et al.,* 2000). In our research, decoction was made using boiling water which can exude higher amount of photochemical or may cause transformation of glycoside-attached ginsenosides (Fig. 1) to another form in temperature and time dependent manner.

Fig. 2 represents the ginsenoside quantification at 70℃ for 24 hrs treatment. In the given temperature and time, total ginsenoside was calculated as 104.9 μg/mL. The individual compounds like Rb1, Rb2 and Rg1 were also detected and

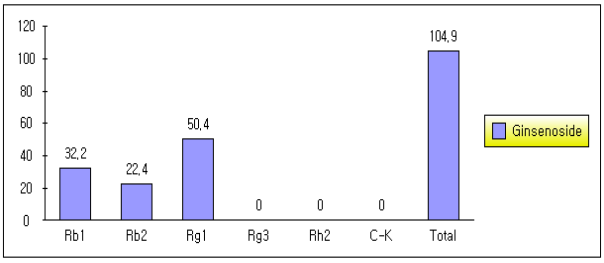


Fig. 2. Ginsenosides (Rb1, Rb2, Rg3, Rh2, Rg1 and C-K) content in the 24 hrs treated decoction at 70℃. The content is expressed in μg/mL.

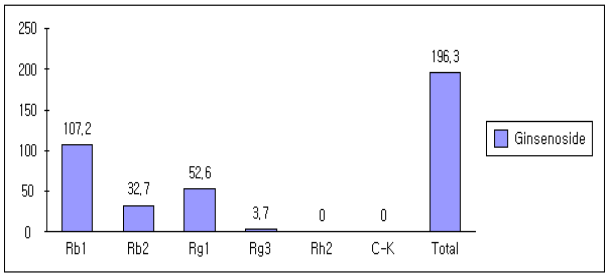


Fig. 3. Ginsenosides (Rb1, Rb2, Rg3, Rh2, Rg1 and C-K) content in the 48 hrs treated decoction at 70℃. The content is expressed in μg/mL.

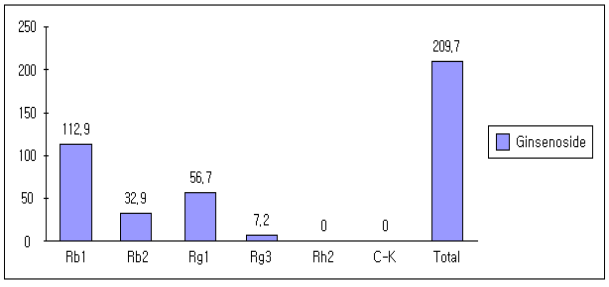


Fig. 4. Ginsenosides (Rb1, Rb2, Rg3, Rh2, Rg1 and C-K) content in the 72 hrs treated decoction at 70℃. The content is expressed in μg/mL.

found to be 32.2, 22.4 and 50.4 μg /mL respectively; however, other ginsenosides Rg3, Rh2 and C-K were not observed. In 48 hrs of treatment at 70℃ (Fig. 3), the ginsenoside Rb1 increased to 107.2 μg/mL followed by Rg1 and Rb2 with 52.6 μg/mL and 32.7 μg/mL respectively. Similarly, the total ginsenoside was also increased to 196.3 μg/mL, which was 1.9 times increase than that of 24 hrs treatment. During the treatment Rg3 ginsenoside was also detected to be 3.7 μg/mL. As the time increased for 72 hrs at 70℃, the Rb1 concentration was accounted to 112.9 μg/mL followed by Rg1 and Rb2 with 56.7 μg/mL and 32.9 μg/mL respectively (Fig. 4). The Rg3 compound was also increased by 1.9 fold as compare to 48 hrs treatments at the same temperature; however, Rh2 and C-K were not detected. In the given treatment, total ginsenoside was increased to 209.7 μg/mL, which was about 2.0 times higher than the treatment at 70℃ for 24 hrs.

Ginsenosides content at 90℃ decoction extracts

The ginsenoside present in decoction extracts at 90℃ for 24 hrs was shown in Fig. 5. The ginsenosides Rb1, Rb2, Rg1 and

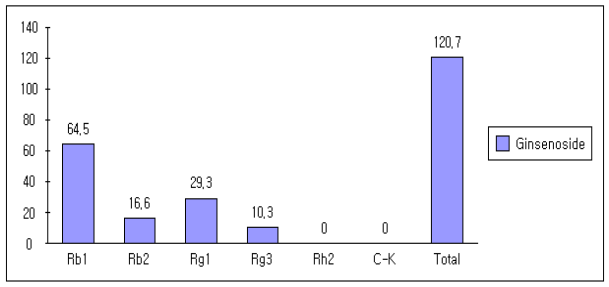


Fig. 5. Ginsenosides (Rb1, Rb2, Rg3, Rh2, Rg1 and C-K) content in the 24 hrs treated decoction at 90℃. The content is expressed in μg/mL.

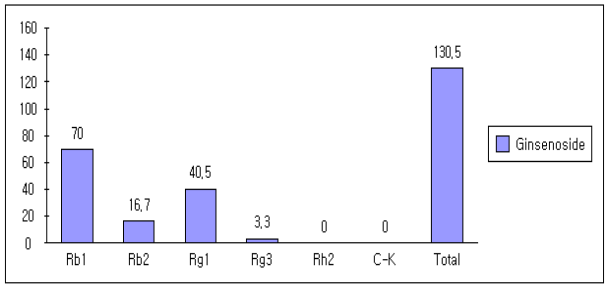


Fig. 6. Ginsenosides (Rb1, Rb2, Rg3, Rh2, Rg1 and C-K) content in the 48 hrs treated decoction at 90℃. The content is expressed in μg/mL.

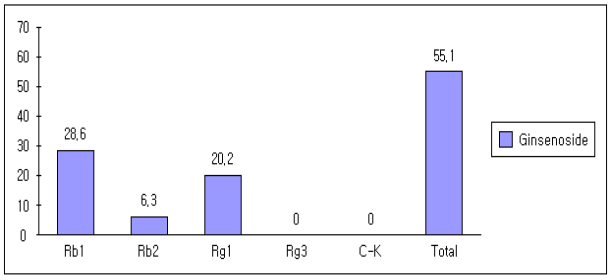


Fig. 7. Ginsenosides (Rb1, Rb2, Rg3, Rh2, Rg1 and C-K) content in the 72 hrs treated decoction at 90℃. The content is expressed in μg/mL.

Rg3 content were 64.5, 16.6, 29.3 and 10.3 μg/mL respectively at the given time and temperature. Comparing to the data of 24 hrs at 70℃, there was an increase in total ginsenoside content to 120.7 μg/mL. However, Rb2 and Rg1 were decreased by 1.3 and 1.7 folds respectively. During the treatment, there was a formation of Rg3 ginsenoside (10.3 μg/mL) which may be formed from the transformation process of other ginsenosides.

The increased amount of Rb1 ginsenoside with 70 μg/mL was shown in the Fig. 6. Similarly, Rg1 also increased 1.4 fold

compared to 24 hrs treatment at the same temperature. In contrast, Rg3 decreased by 3 folds (3.3 μg/mL). The total ginsenoside content was slightly increased to 130.5 μg/mL compared to 24 hrs treatment. When boiling time increased to 72 hrs, the content of Rb1, Rb2 and Rg1 ginsenosides decreased and other ginsenosides viz. Rg3, Rh2 and C-K were not detected (Fig. 7). Comparing to 48 hrs treatments, the Rb1, Rb2 and Rg1 decreased by 2.4, 2.6, and 2.0 folds respectively. Similarly, total ginsenoside also reduced to 55.1 μg/mL, which was 2.4 fold less than that of 48 hrs treatments at 90℃.

Conclusion

Our results highlighted the content of some major ginsenoside compound in fresh white Korean ginseng decoctions in different temperature and time. Among the ginsenoside compounds (Rb1, Rb2, Rg1, Rg3, Rh2 and C-K) used for investigation, Rh2 and C-K were not detected in any of the treatments. Therefore, their availability could be related to the intensity of the reactions such as hydrolysis and oxidation that occur during increased temperatures (Toe *et al.,* 2010). However, Rg3 was detected during the decoction of fresh white Korean ginseng. It has been well known that ginsenosides undergoes transformation by different factors like heat, acid hydrolysis, enzymatic activities (Han *et al.,* 1982; Kim *et al.,* 2007; Cheng *et al.,* 2006; Eom *et al.,* 2009) etc. Therefore, the mechanism involved in the formation for Rg3 ginsenosides in the treatment could be due to hydrolysis, deglycosylation or addition reactions of the many original ginsenosides in white ginseng (Li *et al.,* 2010). Summarizing the overall data, decoction made at 70℃ in 72 hrs possessed higher amount of total ginsenosides (209.7 μg/mL) content where considerable amount of bioactive ginsenosides like Rg3, Rb2, Rb1 and Rg1 were accumulated. Therefore, it can be concluded that the fresh white Korean ginseng decoction made in 72 hrs at 70℃ would be useful for the health and other medicinal approach of ginseng.

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