Journal of Traditional and Complementary Medicine Vol. 3, No. 2, pp. 95-101

Journal of Traditional and Complementary Medicine
Journal homepage http://www.jtcm.org

Bioconversion of Ginsenosides in the American Ginseng (西洋參 Xī Yáng Shēn) Extraction Residue by Fermentation with Lingzhi (靈芝 Líng Zhī, *Ganoderma Lucidum*)

Bo Yang Hsu[1,§], Chia Hui Chen[1,§], Ting Jang Lu[1], Lucy Sun Hwang[1]

[1]Institute of Food Science and Technology, National Taiwan University, Taipei, 106, Taiwan.
[§]Contributed equally.

ABSTRACT

Ginseng (人參 Rén Shēn) has been widely employed in functional foods and traditional medicines in many Asian countries. Owing to the high consumer demand of ginseng products, a large amount of ginseng residue is generated after extraction of ginseng. However, the ginseng residue still contains many bioactive compounds such as ginsenosides. The objective of this research was to convert ginsenosides in American ginseng (西洋參 Xī Yáng Shēn) extraction residue (AmR) by fermentation with lingzhi (靈芝 Líng Zhī, *Ganoderma lucidum*) and the fermentation products will be used for further hypoglycemic activity research. Thus, this study was primarily focused on the ginsenosides that have been reported to possess hypoglycemic activity. In this study, the changes in seven ginsenoside [Rg1, Re, Rb1, Rc, Rg3(S), compound K (CK), and Rh2(S)] in the products as affected by fermentation were investigated. Our results showed that the levels of ginsenosides, namely, Rg1, Rg3(S), and CK increased, while the other ginsenosides (Re, Rb1, and Rc) decreased during the fermentation process.

Key words: Bioconversion, *Ganoderma lucidum*, Ginseng, Ginsenoside, Lingzhi

INTRODUCTION

Ginseng (人參 Rén Shēn) possesses several beneficial effects in the prevention of diabetes, cancer, and cardiovascular disease,[1-3] and therefore, it is extensively used in different therapeutic and health-promoting preparations. These biological activities in ginseng may be attributed to the presence of bioactive compounds such as ginsenosides, polysaccharides, and flavonoids.[4] Among these bioactive compounds, ginsenosides have been extensively investigated as different ginsenosides possess varying pharmacological and biological activities. For instance, Rg1 and compound K (CK) were shown to stimulate glucose uptake in 3T3-L1 adipocytes,[5] with the former one increasing the performance of learning/memory in a mice model as well.[6] Furthermore, the composition of ginsenosides is the major indicator of ginseng quality and species type as the amount and type of ginsenosides in ginseng vary depending on the species, age, harvest season, growth condition, and processing methods.[4,7,8] The ratio of ginsenoside Rb1/Rg1 in American ginseng (西洋參 Xī Yáng Shēn) was reported to be higher than in Asian ginseng (東洋參 Dōng Yáng Shēn). Despite its potential health benefits, ginsenosides suffer from poor bioavailability. Previous studies have pointed out that the conversion of ginsenoside Rb1 into its deglycosylated metabolite CK could substantially increase both bioavailability and bioactivity,[9,10] with their bioavailability being 0.3%-1.2% and 35.0%, respectively.[11-14] Thus, it is important

Correspondence to:
Dr. Lucy Sun Hwang, Institute of Food Science and Technology, National Taiwan University, Taipei, 106, Taiwan. Tel.: 886-2-23629984; Fax: 886-2-33664113; E-mail: lshwang@ntu.edu.tw

DOI: 10.4103/2225-4110.110416

to convert the major ginsenoside Rb1 into its minor metabolites for improved bioactivity and bioavailability.

Lingzhi (靈芝 Líng Zhī, *Ganoderma lucidum*) is a popular traditional Chinese medicine (TCM) belonging to white rot basidiomycete. Generally, *G. lucidum* is investigated for its pharmacological activities including hyperglycemia, hypertension, immunomodulatory, liver protection, and anti-tumor effects.[15-18] In addition, lignin-modifying enzymes synthesized by *G. lucidum* could degrade lignins, cellulose, and hemi-cellulose.[19] In this study, *G. lucidum* was employed to degrade lignin and cellulose in the cell walls of American ginseng residue (AmR) for bioconversion of ginsenosides. Several bioconversion methods in ginsenosides include heating, acid or alkaline hydrolysis, enzymatic and microbial conversion. Specifically, chemical conversion methods are not environment friendly and may cause poor selectivity and low efficiency, eventually reducing the biological activity of ginsenosides,[20-22] whereas enzymatic conversion method is highly selective and environmentally compatible, especially under mild reaction conditions. However, the enzymes currently employed are not stable enough for their usage in industries.[22] On the other hand, microbial conversion method is more advantageous as it is ecofriendly, economically viable, and can be scaled up for good reproducibility. Consequently, microbial conversion is the most desirable bioconversion method for industrial application.[23]

In many Asian countries, abundant amounts of ginseng residue are produced as waste byproduct every year owing to its large application in manufacturing functional food products. Ultimately, the efficient utilization of ginseng extraction residue for potential application in various fields is a subject of significant interest. Moreover, *G. lucidum* is also a valuable TCM and has been rarely investigated for bioconversion of ginsenosides. In this study, we intend to study the bioconversion of ginsenosides in AmR by *G. lucidum* and determine the changes in ginsenoside composition as affected by fermentation conditions of *G. lucidum* grown on AmR. This study can not only develop a valuable functional food ingredient by combining the benefits of both ginseng and lingzhi, but can also resolve the problem of ginseng waste management.

MATERIALS AND METHODS

Materials

American ginseng extraction residue was supplied by a local food company, while AmR fermentation products were prepared by Dr. Ting-Jang Lu of our institute using *G. lucidum* (BCRC37066) from Bioresource Collection and Research Center, Food Industry Research and Development Institute (Hsinchu City, Taiwan). Ginsenoside standards including Re, Rg1, Rb1, Rc, Rg3(S), and Rh2(S) were purchased from Advantage Chemical Co. Ltd. (Taichung, Taiwan). Ginsenoside standard CK was obtained from Tauto Biotech Ltd. (Shanghai, China), digoxin from Sigma (St. Louis, MO, USA) and organic solvents acetonitrile, ethyl alcohol, and methyl alcohol from Mallinckrodt Baker (NJ, USA).

Instrumentation

Shaking water bath (model 905) was from Hotech (Taipei, Taiwan), rotary evaporator (RE111) from Buchi (Flawil, Switzerland), centrifugal vacuum concentrator (SCV100H) from Savant (Farmingdale, NY, USA), ultrasonic processor (S4000) from Misonix (Farmingdale, NY, USA), and centrifuge (model 2100) from KUBOTA (Tokyo, Japan).

Fermentation method

The fermentation method included two steps. The first step was inoculum preparation. *G. lucidum* was cultivated in malt extract agar with 2% ginseng residue at 25°C for 7 days. Afterward, *G. lucidum* was harvested and then seeded in AmR at 1% w/w (low inoculation), 5% w/w (medium inoculation), and 10% (w/w) (high inoculation) of inoculum quantity for subsequent incubation at 25°C for 4, 8, or 13 days.

Sample preparation

A method based on Wang, *et al*.,[24] was modified for extraction of ginsenosides from unfermented AmR and *G. lucidum* fermented AmR (FAmR). One gram sample was mixed with 20 mL of 80% methanol–water solution at a sample to solvent ratio of 1:20 and shaken for 1 h at 50°C, followed by filtering through a Whatman No. 1 filter paper, concentrating under vacuum at 40°C, and freeze-drying. Next, 50 mg of the extract was dissolved in 1 mL of deionized water and purified by using Sep-Pak C18 Cartridge (Phenomenex, Inc., Torrance, CA, USA) by activating sequentially with 5 mL each of methanol and deionized water. The extract solution (1 mL) was then passed through the C18 Cartridge, washed with 5 mL of deionized water, and finally eluted with 5 mL of methanol. The eluted methanol extract was evaporated to dryness under vacuum, the residue redissolved in 1 mL of methanol, filtered through a 0.2 μm membrane filter (Chrom Tech, Inc., Minnesota, USA), and 10 μL injected into high-performance liquid chromatography (HPLC) for analysis.

HPLC analysis of ginsenosides

For the separation of various ginsenosides in unfermented AmR and FAmR, a method based on Kim *et al*.,[25] was modified and used. A Hitachi HPLC system (Toyko, Japan) consisted of a chromatographic pump (L-7100), autosampler (L-7200), and UV-VIS detector (L-7420). A C18 Atlantis column (4.6 mm ID × 150 mm, 3 μm particle size) from Waters and a binary solvent system of deionized water (A) and acetonitrile (B) was used for separation with the following gradient conditions: 79% A and 21% B initially, increased to 22% B at 6 min, 23% B at 7 min, 24% B at 25 min, 30% B at 30 min, 32% B at 40 min, 50% B at 45 min, 65% B at 60 min, 100% B at 61 min, and maintained until 71 min. The flow rate was maintained at 0.8 mL/min, column temperature at 30°C, and detection wavelength at 203 nm.

Quantification of ginsenosides in ginseng residue and its fermentation products

The quantification of ginsenosides in unfermented AmR and FAmR samples was conducted by incorporating an internal standard into the ginsenoside standard solution and developing standard curves based on the peak area ratio versus the concen-