

Enhancement of Biological Activity of Saponin-rich Plants by Subcritical Water Hydrolysis

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Introduction

The *Panax* species plants contain dammarane-type saponins with one to four saccharides combined with a dammarane backbone [1]. Triterpenoid saponins such as ginsenosides, notoginsenosides, and gypenosides are the main bioactive compounds in *Panax ginseng* (Korean ginseng), *Panax notoginseng* (Chinese ginseng), and *Gynostemma pentaphyllum*, respectively. Most of saponins occur in plants and food products as different glycosides. Hydrolysis of some glycosidic bonds of saponins can increase their pharmacological activities.

A common conventional method to hydrolyze glycosides from plants is to use acid, alkali, or enzymes. Acidic and alkali hydrolysis is not suitable for food use due to harsh chemicals and toxic residues in the hydrolysate and may result in side reactions such as epimerization, hydration and hydroxylation, and degradation of the target compounds. Enzyme hydrolysis is not toxic, but requires an enzyme separation process and expensive cost. Furthermore, the conventional hydrolysis methodology is rather tedious and requires a substantial amount of manual work [2].

The most environmentally friendly solvent for extraction and hydrolysis of glycosides is water. However, pure water at ambient condition cannot be used as a hydrolysis catalyst and is not a good solvent for medium and non-polar compounds. Instead, the water at elevated temperatures, i.e. subcritical water at temperatures above 100°C can be used [3]. Subcritical water is the condensed phase between the boiling point (100°C) and critical point temperature of water (374°C). Subcritical water has a large ion product due to the destruction of hydrogen bonds between water molecular at high temperature. Therefore, Subcritical water can act as a hydrolysis catalyst. The addition of CO₂ increases hydrolysis catalytic capacity of subcritical water due to high concentration of hydrogen ion from the dissociation of carbonic acid [4,5]. The conversion yield of rutin (quercetin-3-rhamnosyl glucoside) into isoquercetin (quercetin-3-β-D-glucoside) and quercetin was increased with increasing CO₂ pressure at 140°C due to lower pH of water [2]. Subcritical water extraction is fast and efficient compared to conventional liquid extraction due to higher diffusion rate, lower viscosity, and easy-to-tune properties by simply changing temperature. Less polar compounds such as antioxidative compounds, polycyclic aromatic hydrocarbons, and essential oils have been successfully extracted from various plants using subcritical water at temperatures between 100 and 374°C. Subcritical water has been used as an environmentally friendly technique for the hydrolysis of cellulose, polysaccharides, proteins, and bioactive compounds [6,7].

The humid heat-processing method has been used to produce biologically active compounds by hydrolysis the glycosidic bonds of ginseng saponins for a long time. Heating ginseng produces the minor ginsenosides whose pharmacological activities are higher than those of the major ginsenosides in white ginseng [8-12]. Steaming the ginseng at 100°C for 3 hr produces several new ginsenosides

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(F4, Rg3,5,6, Rk1,2,3, Rs3,4,5) that are not present in white ginseng [13]. Ginsenoside Rg3 has strong vasorelaxation activity and anti-platelet aggregation activity, while ginsenoside Rg5, Rs3 and Rs4 have anti-cancer activity through the induction of apoptosis. Steaming the ginseng at 120°C for 3 hr also increases less polar ginsenosides than the previously known ginsenosides. Thus steaming the ginseng at high temperature can greatly enhance its biological activity [8,9]. Le et al. [11] investigated the effect of various steaming time (2-20 hr at 105°C) on the change in chemical constituents and antiproliferative activity of Vietnamese ginseng, and antiproliferative activity against lung cancer cells was increased in steamed ginseng. Ge et al. [14] treated *Panax notoginseng* (Chinese ginseng) with water at 120°C for 6 hr. The contents of ginsenoside 20(S)-Rg3, 20(S)-Rh1, F2, and compound K were significantly increased by the hydrolysis of ginsenosides Rb1, Rd, Rg1, Re, and notoginsenoside R1 which are the major components in untreated notoginseng. The minor ginsenosides such as ginsenosides F1, F2, Rg3, Rh1, and compound K were also produced by the hydrolysis of glycosides from the major ginsenosides Rg1, Re, Rb1, and notoginsenoside R1 [14]. The less-polar ginsenosides (20(S)-Rg3, 20(R)-Rg3,5, and Rk1 in *P. ginseng* were significantly increased in a temperature-dependent manner. Ginsenoside 20(S)-Rg3 is important bioactive compound in the heat-processed ginseng and associated with the control of diabetic nephropathy [1]. Maillard reaction product, maltol, has a pathological effect associated with diabetic nephropathy. Maillard reaction products in ginseng were increased by heat-processing, and maltol is formed by carbohydrate pyrolysis. The maltol in red ginseng (98 to 100°C) and sun ginseng (120°C) was 4- and 36-fold higher, respectively, than that in white ginseng [1]. Koh et al. [12] suggested that processing conditions of ginseng should be carefully employed to enhance the pharmaceutical activities because processing methods, air-drying or steaming, converted the structure of ginsenosides, resulting in the change in the composition of ginsenosides.

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G. pentaphyllum is also rich in saponins which are called as gypenosides [15]. Gypenoside L and LI, and damulin A and B were generated from several saponins of *G. pentaphyllum* by the hydrolysis during heat processing. The contents of those four dammarane-type saponins were increased with the heating temperatures (110, 120, and 130°C) and processing time (1, 2, 3 hr), and were 6,130, 2,463, 5,877, and 11,902 µg/g in *G. pentaphyllum* heat processed at 130°C for 3 hr, whereas those from the raw plant were below 250 µg/g. Those dammarane-type saponins have stronger anticancer activity than total gypenosides [15]. Piao et al. [16] also reported that steamed *G. pentaphyllum* at high temperature (125°C) showed higher cytotoxicity against non-small cell lung carcinoma cells than the untreated one. The less-polar saponins (gypenoside L and LI) were produced from the polar saponins (gypenoside XLVI and LVI) in heat-processed *G. pentaphyllum* by the loss of the glycosyl moiety, and further dehydration led to the increase of damulin B and damulin A which have strong anti-obesity activity (Wu et al., 2014). Recently Xing et al. [17] reported that a new compound, gypenoside Jh1, was produced from gypenoside LVI and XLVI which are the main saponins of raw *G. pentaphyllum* by the loss of sugar moiety by heat treatment at 125°C for 3 hr.

In this commentary, I have summarized that the pharmaceutical effect of *P. ginseng* and *G. pentaphyllum* can be increased by conversion of the dammarane-type saponin by high pressure thermal processing. However, most of the researches for thermal processing of saponin-rich plants were performed at temperatures of 100 to 130°C for more than three hours. Long time exposure of saponin-rich plants at high temperature can cause the degradation of bioactive compounds. Therefore the treatment of saponin-rich plants for short time (5, 10, 15 min) at higher temperature (140, 150, 160°C) needs to be taken into account. The addition of CO₂ into hot water can also increase the hydrolysis capacity due to high concentration of hydrogen ion in the medium, thereby reducing the reaction time and reducing the formation of degradation products. CO₂ can be removed by simply releasing the pressure of the system [18].

In addition to *P. ginseng* and *G. pentaphyllum*, there are several saponin-rich plants such as *Platycodon grandiflorum*, *Codonopsis lanceolata*, *Dioscorea batatas* etc. Those plants also need to be treated with subcritical water to modify the chemical compositions and improve biological activities. This investigation of new bioactive compounds from saponin-rich plants by subcritical water hydrolysis will be important and may contribute to the development of novel nutraceutical products.

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