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Optimization of phytochemicals production from the ginseng by-products using pressurized hot water: Experimental and dynamic modelling

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ABSTRACT

This study investigates the influence of process conditions on pressurized hot water extraction (PHWE) to maximize the bioactive compounds, including total saponins (TS), total phenolic compounds (TP) and total antioxidants (TA), produced from ginseng by-product (GBP). Response surface methodology (RSM) was employed to study the influences of temperature [180–240 °C], pressure [15–45 bar], solid to liquid (S/L) ratio [0.04–0.09 g/mL], agitation speed [100–200 RPM], and reaction time [5–15 min]. Box-Behnken design (BBD) was used with five variables and three levels. The optimal conditions were 207 °C, 43.45 bar, S/L ratio of 0.04 g/mL, agitation speed of 199 RPM, and reaction time of 15 min. The obtained values of TS, TP and TA under these conditions were: 7.12 g Gin Re/100 g, 49.11 mg GAE/g, and 5.31 g TE/100 g, respectively. The optimized ginseng hydrolysate showed good antimicrobial and antihypertensive activities. Homogentisic and gentisic acid were the main phenolic compounds present in the ginseng hydrolysate.

1. Introduction

Ginseng (*Panax ginseng* Meyer) is a half-shade plant in the Araliaceae family. It has been used as a medicinal ingredient in East Asian medicine for centuries. Its root is used in medicine and is widely known as a natural health food [1]. Saponins are one of the key compounds present in ginseng, and nearly 30 types of saponins have been discovered so far [2]. Saponins are collectively known as ginsenosides and have various medicinal properties, such as anticancer, antiaging, antioxidant, and antidiabetic [3]. The phenolic compounds present in ginseng possess various biological properties, such as antioxidant and anticancer properties. More than 10 phenolic compounds, including *p*-hydroxybenzoic, vanillic, caffeic, ferulic, gentisic and syringic acids have been reported in fresh and/or processed ginseng [4].

On the basis of the processing methods, ginseng may be classified into fresh ginseng, red ginseng, and white ginseng [5]. Traditionally, white ginseng is produced by sun drying of fresh ginseng; whereas red ginseng is manufactured by steaming fresh ginseng at 95–100 °C for 2–3 h followed by drying; red ginseng has a longer storage period than white ginseng [6]. In addition, red ginseng is known to have greater pharmaceutical efficacy and functionality than white ginseng because of its high saponin content [7]. During the production process of red ginseng, a fibrous byproduct, called GBP is obtained. In South Korea, Korean red ginseng byproducts (GBPs) are mixed with poultry feed for poultry production purposes [8]. All these GBPs obtained from the processing of fresh, white and red ginseng are not being used optimally for the extraction of useful materials.

In recent years, following the general trend of reusing of agro-industrial and food wastes [9], attempts have been made to add value to GBP. The extraction of polysaccharides [10], recovery of triterpene glycosides from red ginseng marc [11], and production of bioethanol [12] are some of the applications under consideration. However, less attention has been paid toward using GBP as a source of antioxidant and anti-inflammatory compounds [13], and even less attention has been paid toward the possibility of developing an integrated process that combines the extraction of these compounds with some other forms of waste valorization.

Aqueous extracts of ginseng, which contain amino acids, minerals, saponins, and various water-soluble low- and high-molecular weight compounds among others, have been used for centuries as a folk medicine to reduce fatigue and boost the immune system. An extract of the *P. ginseng* species has become increasingly

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desirable owing to its pharmacological activity [14]. The inhibitory effects of aqueous and alcoholic extracts of the *P. ginseng* (root as well as leaves) on the growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Escherichia coli*, the causal agent of intestinal infections in humans were examined using the *in vitro* agar well diffusion method [15]. Kim et al. [16] also found that aqueous ginseng extracts scavenged 40% of hydroxyl radicals at 0.1 mg/mL and completely scavenged superoxide radicals at 2 mg/mL.

Several extraction procedures have been employed previously to extract functional components from ginseng, primarily using different solvents, such as methanol, ethanol and water. To increase extraction yields, processes such as heating, refluxing and sonication have been applied [17]. In the current food processing trends, extraction methods employing "green technology" are preferred because these methods can extract natural ingredients without the need for toxic or chemical solvents [18]. In this context, PHWE is an excellent choice to produce functional compounds from ginseng. In the PHWE process, water in the subcritical phase is employed as a solvent at an elevated temperature under pressure to modify the dielectric constant and to change the polarity of water, thereby selectively extracting different classes of compounds [19,20]. The PHWE technique has been previously used to extract aromatic compounds and to isolate essential oils from plant materials [21]. Therefore, PHWE can be used as a green technology to extract functional compounds from GBP for a safe and rapid analysis [21–24].

The most common approach for process optimization is one-factor-at-a-time, in which the influences of independent variables on responses are investigated one by one, while all other factors are maintained at constant values. This approach can be time-consuming and expensive for certain experiments. Moreover, possible interactions between variables may not be evaluated. To overcome these disadvantages, RSM can be applied [25]. RSM is a collection of statistical and mathematical techniques and is useful for developing, improving and optimizing processes in which the response of interest is influenced by several variables, and the objective is to optimize this response [26]. By analyzing the effects of independent variables, this experimental methodology generates a mathematical model that describes the chemical processes within an experimental range [27].

In this paper, GBP was hydrolyzed using PHWE and the effect of various conditions, such as temperature, pressure, agitation speed, S/L, and reaction time, were investigated. The optimization process was performed by applying RSM for these conditions to improve the yields of TS, TP, and TA from a GBP hydrolysate.

2. Materials and methods

2.1. Chemicals

Ginsenoside Re, vanillin, Folin–Ciocalteu reagent, ABTS$^+$ [2,2-azinobis-(3-ethyl benzothiazoline-6-sulfonic acid)], Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), rutin, pyrogallol, gallic, homogentisic, gentisic, chlorogenic, caffeic, *p*-coumaric, *o*-coumaric acid, ferulic, *t*-cinnamic acids, o-phthalaldehyde (OPA), Captopril, and Hipouril Histidine leucine (HHL) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MI, USA). All reagents used in this study were of analytical or High Performance Liquid Chromatography (HPLC) grade. High-purity nitrogen gas (99%) was supplied by KOSEM (Yangsan, Korea).

2.2. Ginseng by-product (GBP)

GBP used in this work was obtained during the red ginseng production process and it was acquired from Happy Ginseng (haengbokhansam, http://happysam.co.kr/, Chungcheongnam-do, Korea). GBP was washed with distilled water and was then chopped into small pieces before drying in an oven (OF12GW, Jeio-Tech Co., Seoul, Korea) at 40 °C for 4 h. Then, the samples were finely ground using a mechanical blender (PN SMKA-4000 mixer, PN Co., Ltd., Ansan-si, Korea) and were sieved with a 10-mm stainless steel sieving mesh. Samples that passed though the mesh sieve were stored at −20 °C until further use.

2.3. Pressurized hot water extraction (PHWE)

PHWE was performed in a 200 cm^3 batch reactor made of 276 Hastelloy (continuous-type supercritical water system, Phosentech, South Korea) (Fig. S1). GBP and distilled water were loaded into the reactor. The reactor was closed, was purged with nitrogen gas through a valve, and was maintained at its required temperature, pressure, agitation speed, S/L ratio, and reaction time. The temperature and pressure in the reactor were controlled by a temperature controller and a pressure gauge, respectively. The hydrolysate samples from the reactor were collected after reaching the room temperature, filtered using membrane filters (0.45 μm pore size, Fisher Scientific), and stored at 4 °C.

2.4. Determination of total saponins (TS)

The TS content of each GBP hydrolysate was measured as described in a previous work [28] with some modifications. Briefly, the GBP hydrolysates (50 μL) were mixed with vanillin (8% w/v, 0.5 mL) and sulfuric acid (72% w/v, 5 mL). The mixture was incubated at 60 °C for 10 min and cooled in an ice water bath for 15 min; the absorbance measured at 538 nm using a UV mini 1240, Shimadzu Co., Japan. Ginsenoside Re was used as a reference compound and the total saponin content was expressed as grams Ginsenoside Re equivalents per 100 g of fresh weight (g Gin Re/100 g).

2.5. Total phenolic (TP) content

TP content was determined using the Folin–Ciocalteu procedure [29] by adding 75 μL of the GBP hydrolysate extracts (75 μL of water in the blank) to 425 μL of distilled water, 500 μL of the Folin–Ciocalteu reagent, and 500 μL of a sodium carbonate aqueous solution (10% w/v). The mixture was stirred and left in the dark for 60 min before the absorbance was measured at 723 nm using a UV mini 1240, Shimadzu Co., Japan. Gallic acid was used as a reference compound, and TP was expressed as milligram gallic acid equivalents per gram of fresh weight (mg GAE/g) of sample.

2.6. Determination of total antioxidant content (TA)

For the ABTS$^+$ assay, the method described by a previous study [30] was followed. The stock solutions included a 7.4-mM ABTS$^+$ solution and 2.6 mM potassium persulfate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. A 150-μl sample of GBP hydrolysates was reacted with 2850 μl of ABTS$^+$ solution (1 mL ABTS$^+$ solution mixed with 60 mL methanol) for 2 h in the dark. The absorbance was measured against methanol at 734 nm using a UV mini 1240, Shimadzu Co., Japan.

The percentage of inhibition of ABTS$^+$ using GBP hydrolysates was calculated according to the following equation:

$$\text{Inhibition}\% = (1\text{-}A_{\text{testsample}}/A_{\text{blank}}) \times 100, \quad (1)$$

where A_{blank} is the absorbance of the methanolic blank and $A_{\text{testsample}}$ is the absorbance of the hydrolysates. Trolox was used as a reference compound and the results were expressed as grams Trolox equivalents per 100 g of fresh weight (g TE/100 g).