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RAPID COMMUNICATION

An alternative sequential extraction process for maximal utilization of bioactive components from Korean red ginseng

Guijae Yoo*, Sung Dong Lee, Man-Jin In***, Woo Ik Hwang****, Kwang Seung Lee*****, Eun Sil Lee*****, Dong Chung Kim******,†, and Hee Jeong Chae*,†**

*Department of Food and Biotechnology, Center for Food Function and Safety, Hoseo University, Asan 336-795, Korea
**Department of Food and Nutrition, College of Health Science, Korea University, Seoul 136-703, Korea
***Department of Human Nutrition and Food Science, Chungwoon University, Hongseong 350-701, Korea
****Department of Biochemistry, College of Medicine, Korea University, Seoul 136-701, Korea
*****NongHyup Korea Insam Co., Ltd, Jeungpyeong 122-1, Korea
******Institute of Basic Science, Sungkyunkwan University, Suwon 440-746, Korea
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Abstract−Two types of sequential extraction process (SEP) for the recovery of bioactive components from Korean red ginseng were examined. As a SEP (normal SEP, nSEP), Korean red ginseng was sequentially treated using hot water and n-hexane for the successive extraction of water-soluble and lipid-soluble components. Also by changing the sequential order of solvents, an alternative SEP (reverse SEP, rSEP) in which n-hexane extraction was followed by hot water extraction, was proposed. Regardless of the sequential order of solvents, the recovery yield of acidic polysaccharide (AP) and crude saponin (CS) showed no significant change. On the other hand, in the rSEP, the lipid-soluble fraction was obtained from red ginseng with an enhanced recovery yield, four times higher than that in nSEP. Additionally, from dose-response assays to assess the effects of lipid-soluble components on the proliferation of human hepatoma (HepG2) and breast (MCF-7) cancer cell lines, it was found that hexane extract of red ginseng (HER) in rSEP has higher efficacy than the hexane extract of red ginseng marc (HERM) obtained in nSEP. This strongly suggested that rSEP would be a more attractive industrial process in terms of the efficacy of lipid-soluble extract as well as the recovery yield.

Key words: Sequential Extraction Process, Red Ginseng, Normal SEP, Reverse SEP

INTRODUCTION

Ginseng (Panax ginseng C.A. Meyer) is a well-known herbal medicine that has been used as a natural remedy for thousands of years [1-3]. In particular, Korean red ginseng has been reported to possess anti-diabetes, anti-cancer and anti-hypertension activities [4]. Red ginseng has been known to have higher pharmacological activities than white ginseng due to its higher content of specific ginsenosides such as Rg_3, Rh_2 and Rb_2 [5]. The ginseng industry in Korea has concentrated on utilization of aqueous extracts of red ginseng. Since water-soluble components consisting primarily of ginsenosides and carbohydrates have been recovered for industrial production and commercial use of red ginseng extracts, water-insoluble red ginseng marc has been discarded as a waste even though it still contains lipid-soluble components such as polyacetylenes with an anticancer activity [6,7].

In general, an aqueous extract of red ginseng has been produced by a conventional method of hot water extraction [8,9]. In a process where a lipid-soluble extract is the product of interest, more hydrophobic solvent is required. Additionally, in the case of producing dual-products (both water-soluble and lipid-soluble products), a sequential extraction process (SEP) using hydrophilic and hydrophobic solvents can be adopted. In the present study, SEP, which is denoted as 'normal SEP (nSEP)' including a series of water extraction steps and n-hexane extraction steps, is able to sequentially recover water-soluble and lipid-soluble components in red ginseng. Also, an alternative SEP (reverse SEP, rSEP), in which the sequential order for the use of extraction solvents is reversed: the hexane extraction step is adopted as the first step and the water extraction step as the second step of SEP, is proposed. The recovery yield and biological activity of lipid-soluble components obtained by each sequential extraction process (nSEP and rSEP) for Korea red ginseng were compared in the present study. Based on the data on recovery yield and bioefficacy, a sequence of extraction solvents will be proposed for an industrial process.

†To whom correspondence should be addressed.
E-mail: hjchae@hoseo.edu, kimdc@skku.edu

MATERIALS AND METHODS

1. Sequential Extraction of Red Ginseng

Red ginseng (first grade, six years old) was purchased from Nonghyup Koreainsam Co. (Jeungpyeong, Korea) and milled up to a size of less than 400 μm. In nSEP (Fig. 1(A)), a water extract of red ginseng (WER) was prepared by a conventional manufacturing process used in NongHyup Koreainsam Co. Since it was reported that more than 94% of total saponins were recovered by four repetitions of hot water extraction for 4 h [10], the similar aqueous extraction conditions were applied in the present study. The first aqueous fraction was extracted by combining one part red ginseng powder with nine parts distilled water at 85 °C for 8 h in a shaking water bath,

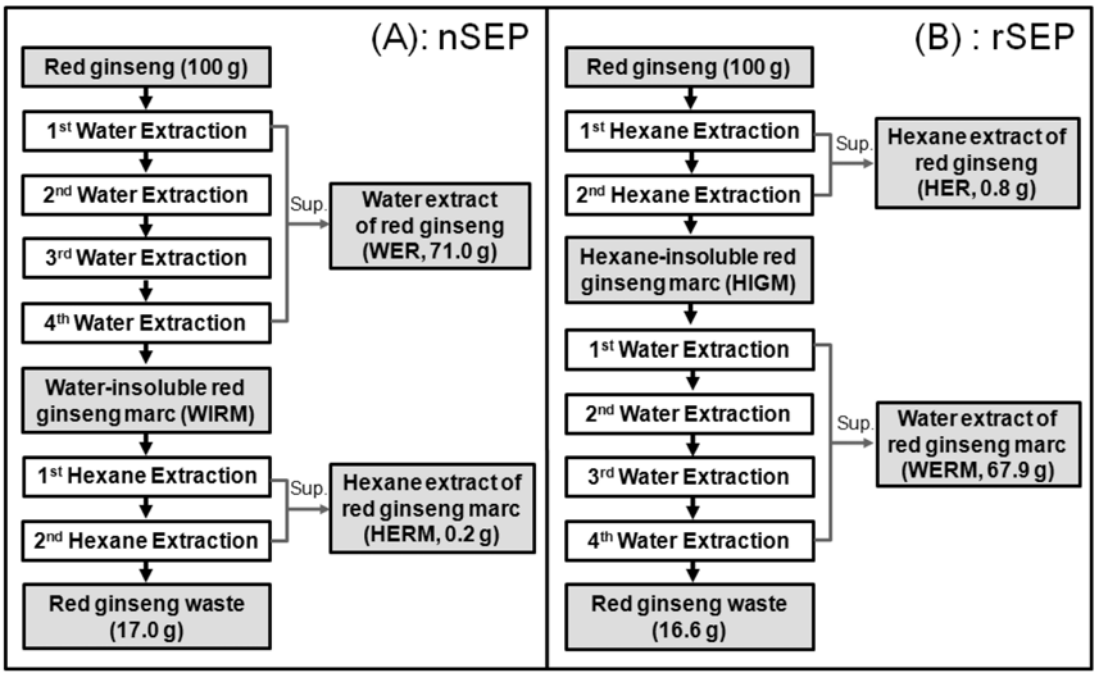

Fig. 1. Two SEPs for the extraction of bioactive components in red ginseng (A: nSEP, B: rSEP). Data are averages of duplicate measurements with spread less than 10%.

and centrifuged at 2,890×g for 20 min. The first supernatant was collected, pellets were re-suspended in eight parts distilled water, and the extraction process was repeated. The second supernatant was collected through centrifugation, pellets were re-suspended in five parts distilled water, and the extraction process was repeated. The third supernatant was collected through centrifugation, pellets were re-suspended in five parts distilled water, and the extraction process was repeated. The forth supernatant was collected through centrifugation. The final pellet as a red ginseng marc (WIRM) was dried for use in the next hexane extraction. Each supernatant as WER was lyophilized. After the above aqueous extraction, lipid-soluble components were recovered by the following n-hexane extraction of dried red ginseng marc (WIRM) powder. The lipid-soluble fraction in red ginseng marc was extracted by combining one part powder with ten parts n-hexane for 4 h at room temperature and then centrifuged. The supernatant was collected, and pellets were then re-suspended in n-hexane of the original volume used. The extraction process was repeated and the next supernatant was collected through centrifugation. Hexane was eliminated from the lipid-soluble extract using a rotary vacuum evaporator (Eyela, Tokyo, Japan).

In rSEP (Fig. 1(B)), lipid-soluble components were first recovered by n-hexane extraction of red ginseng. A hexane extract of red ginseng (HER) was prepared by using the same conditions as described in the hexane extraction of red ginseng marc in nSEP. The following aqueous extraction process with the same conditions as described in the water extraction of red ginseng in nSEP was applied to extract the water-soluble components from the hexane-insoluble red ginseng marc (HIGM) that remained in the hexane extraction process.

2. Analytical Methods for Active Components of Red Ginseng

The carbohydrate content of each extract was determined by the phenol-sulfuric method [11] with glucose as a standard. The acidic polysaccharide content of each extract was analyzed by carbazole-sulfuric acid method [12] with pectin as a standard. The crude saponin content of each extract was determined as described by Kwon et al. [13].

3. *In Vitro* Anti-cancer Activity of Red Ginseng Extracts Based on Cell Culture

Dose-response assays were used to assess the effects on the proliferation of human hepatoma (HepG2) and breast (MCF-7) cancer cells (American Type Culture Collection, Manassas, VA) using a commercial assay kit, Cell Counting Kit-8 (CCK-8, Dojindo Corp., Kumamoto, Japan) following the supplier's instructions [14]. The absorbance of red ginseng extract-treated samples was compared to that of untreated control at 450 nm. The inhibition rate was calculated as follows: inhibition rate (%)=($OD_{control}$–OD_{sample})÷$OD_{control}$ ×100. The GI_{50} value is the concentration of test samples that cause 50% growth inhibition of cancer cells.

RESULTS AND DISCUSSION

1. Recovery Yield of Water-soluble Components in nSEP and rSEP

To investigate the material flow in the process for recovery of red ginseng extracts, mass balances of both SEPs (nSEP and rSEP) are shown in Table 1. From the mass balance of nSEP (Table 1(A)), 71.0 g out of 100 g of red ginseng were recovered as water-soluble components by four repetitions of hot water extraction (recovery yield=71%). In this nSEP, well-known active compounds such as acidic polysaccharide and crude saponin were obtained with recovery yields of 25.7% and 2.52%, respectively. Similarly, through the second step in rSEP (four repetitions of water extraction), about 67.9 g of water-soluble extract including 24.1 g of acidic polysac-

Table 1. Mass balance of SEPs: (A) nSEP and (B) rSEP

A: nSEP		DM (g)	TS (g)	AP (g)	CS (mg)
WER	1st Water-soluble fraction	48.0	37.2	18.8	2179.2
	2nd Water-soluble fraction	15.1	10.4	4.9	280.9
	3rd Water-soluble fraction	5.7	3.1	1.6	53.0
	4th Water-soluble fraction	2.2	0.9	0.4	9.2
	Sub total	71	51.6	25.7	2522.3
HERM	Lipid-soluble fraction	0.2	n.d*	n.d	0.1
Final ginseng waste		17	5.5	2.1	3.2
Total		88.2	57.1	27.8	2525.6
B: rSEP		DM (g)	TS (g)	AP (g)	CS (mg)
HER	Lipid-soluble fraction	0.8	n.d	n.d	0.7
WERM	1st Water-soluble fraction	46.4	34.9	17.3	2037.0
	2nd Water-soluble fraction	13.8	10.0	4.9	265.0
	3rd Water-soluble fraction	5.9	3.0	1.5	67.9
	4th Water-soluble fraction	1.8	0.8	0.4	10.3
	Sub total	68.7	48.7	24.1	2380.9
Final ginseng waste		16.6	5.2	1.7	1.3
Total		85.3	53.9	25.8	2382.2

DM: dry matter, TS: total saccharides, AP: acidic polysaccharides, CS: crude saponin
*: not detected

charide and 2.38 g of crude saponin were recovered (Table 1(B)). Comparing the yield data between nSEP and rSEP for aqueous fractions including acidic polysaccharide and saponin, no significant change was observed. These results indicate that the use of rSEP as well as nSEP could successively recover lipid-soluble and water-soluble components in red ginseng without a significant change of recovery yields for major industrial components such as acidic polysaccharide and saponin. Most saponins are soluble in aqueous solutions and diluted alcohols, but almost insoluble in hexane. Also, acidic polysaccharide is generally soluble in aqueous solutions, but insoluble in hexane. That is, in the presence of hexane, organic acids, saponin, and macromolecules such as water-soluble starch, proteins and acidic polysaccharides could not be extracted [10]. Therefore the total yield of crude saponin extracted from red ginseng was hardly affected by the hexane extraction process.

2. Recovery Yield of Lipid-soluble Components in nSEP and rSEP

From the mass balance of nSEP as shown in Table 1A, approximately 0.2 g of lipid-soluble components as a hexane extract of red ginseng marc (HERM/nSEP) were recovered from the residual red ginseng marc by two repetitions of the n-hexane treatment. In rSEP (Table 1(B)), from the first step (twice-repeated hexane extraction), approximately 0.8 g of the lipid-soluble fraction (HER) was recovered from 100 g of red ginseng. This yield (0.8%) of the lipid-soluble fraction was four times higher than that in nSEP (0.2%). Assuming that the lipid-soluble components in rSEP of red ginseng were fully recovered as HER, only 25% of the lipid-soluble components seemed to have remained as HERM after four repetitions of water extraction in nSEP of red ginseng. Therefore, the yield difference can be explained by the difference of the hexane extraction sequence. This higher recovery yield of the lipid-soluble fraction in rSEP, which is under development as a potential anti-cancer drug substance, is a promising point to bioprocess engineers.

3. Biological Activities of Lipid-soluble Components Obtained by nSEP and rSEP

It is essential to compare the biological activities of both HER/rSEP and HERM/nSEP in order to establish an optimal extraction process for maximal utilization of both lipid-soluble and water-soluble bioactive components from red ginseng.

Cell growth was potently inhibited by HERM/nSEP and HER/rSEP in either cell line in a concentration-dependent manner (Fig. 2). The inhibitory effect of HERM/nSEP was potent in HepG2 (GI_{50} =47.2 μg/mL, Fig. 2(a)) and MCF-7 (GI_{50}=58.4 μg/mL, Fig. 2(b)) cells. The HER/rSEP with GI_{50} values of 19.8 μg/mL in HepG2 (Fig. 2(a)) and 32.3 μg/mL in MCF-7 (Fig. 2(b)) showed higher efficacy than the HERM/nSEP. The results showed that the HERM/nSEP (by-product of water extraction process of red ginseng) as well as the HER/rSEP (the first product of hexane extraction process of red ginseng) possesses anticancer-active components. This is in good agreement with the results of experiments using white ginseng [15]. It is generally expected that rSEP can lead to the maximal recovery of the lipid-soluble fraction with a higher anticancer activity in red ginseng. From these results, it is concluded that rSEP is more desirable than nSEP in terms of efficacy of target extract (lipid-soluble) products as well as the recovery yield. The difference in recovery yields and biological activity of bioactive components obtained from nSEP and rSEP needs to be further discussed in detail.

This is the first trial to compare nSEP and rSEP for maximal utilization of bioactive components in red ginseng. We have shown that

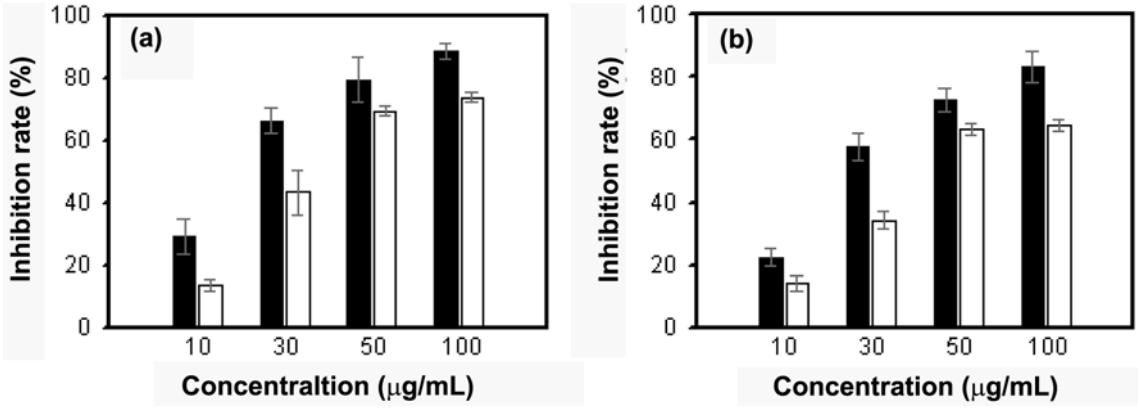

Fig. 2. Effect of HER/rSEP (closed rectangle) and HERM/nSEP (open rectangle) on human hepatoma (a) and breast (b) cancer cell growth. The experiments were repeated three times and the results were given as the means±SE.

while there is no significant difference in the contents of acidic polysaccharide and crude saponin and in the recovery yield of two water extracts recovered from nSEP and rSEP, the recovery yield and bioefficacy of HER/rSEP are higher than that of HERM/nSEP. This strongly suggests that rSEP of red ginseng would be a more attractive industrial process for pharmaceutical and cosmetic products for which lipid-soluble bioactive components are used.

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