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The Effective Preparation of Protopanaxadiol Saponin Enriched Fraction from Ginseng using the Ultrafiltration

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Abstract – In this study, edible protopanaxadiol saponin enriched fraction were prepared by ultrafiltration (UF). Ginseng extract was prepared from mixtures of ginseng main root and rootlet (root: rootlet = 4 : 6). UF system was used the four-piston Diaphragm pump equipped with 5 kDa pore size Hydrosart Cassette made by regenerated cellulose acetate (CA) or 3 kDa pore size Hollow Fiber cartridge made by polyethersulfone (PES). Total ginsenoside contents of concentrated fraction by UF system was found to higher, compared to before those of untreated method. Especially, processing of UF showed the increase of PPD-type ginsenoside, while PPT-type ginsenoside was gradually decreased by both 3 kDa and 5 kDa membrane. After removal of 80% water by the 5 kDa Hydrosart Cassette and by 3 kDa Hollow Fiber cartridge, ginsenoside Rb1 content was higher 37.2 mg/g and 25.3 mg/g than 20.8 mg/g in untreated process. The ratio of Rb1 to Rg1 (Rb1/Rg1) and PPD- to PPT- type ginsenoside (PPD/PPT) were higher in inner fluid of ginseng extract after UF by 3 kDa cartridge (47.1 and 23.5, respectively) and 5 kDa Cassette (25.3 and 11.9, respectively) than those of before UF (5.7 and 3.7, respectively). PPD-type ginsenoside enriched fraction by UF system could be developed as a new ginseng material in food and cosmetic industrials.

Keywords – *Panax ginseng,* Ultrafiltration, Protopanaxadiol ginsenoside

Introduction

The roots of *Panax ginseng* C. A. Meyer (Araliaceae) has been used as a traditional herbal medicine in Korea, China, Japan and other Asian countries.[1] Ginseng contains various pharmacological components such as ginsenosides (ginseng saponins), polyacetylenes, polyphenolic compounds, and acidic polysaccharides. Recently, ginseng has been found to contain more than 40 ginsenosides, which are glycosides that contain an aglycone with a dammarane skeleton, classified in to protopanaxadiol-type (PPD-type) saponins of ginsenoside Rb_1, Rb_2, Rc and Rd and protopanaxatriol-type (PPT-type) saponins of ginsenoside Re and Rg_1.[2-4] It has been generally regarded that ginseng play a synergistic or antagonistic effects on each other in a concerted mamer. Ginsenoside Rg1 has been reported to stimulate the central nervous system, whereas ginsenoside Rb1 and Rc exhibit sedative effects on the central nervous system.[5] Generally, ginseng total saponin (GTS) was divided into PPD- and PPT-type saponin by *n*-butanol extraction method, followed by Diaion-HP 20 anion exchange resin chromatography.[6,7] However, these methods are inappropriate for edible the preparation of healthy food material because of solvent use and its high cost.

Ultrafiltration (UF), a novel and powerful pressure driven separation technology, has been widely used in food industry to concentrate or fractionate protein and aqueous solution.[8-10] UF is a convective process that uses anisotropic semipermeable membranes to separate macromolecular substances and solvents primarily on the basis of molecular size. It is particularly appropriate for the concentration, fractionation, purification, and desalting of biologically active molecules.[11,12] Liu *et al.* reported the efficient separation of lower molecular weight oligosaccharide from the root of *Panax ginseng* by ultrafiltration.[12] Zhang *et al*. also reported the elimination effect of bacterial endotoxins and the transmittance of *Panax notoginseng* saponins by ultrafiltration membranes of different cut-off molecular weight and different materials.[11]

In this study, the cost effective separating method of edible protopanaxadiol saponin enriched fraction using

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the ultrafiltration was established for immune-enhancing agent related with anti-influenza virus.

Experimental

Materials – The red ginseng (*Panax ginseng* C. A. Meyer) safely with the systems of Good Agricultural Practices (GAP) ginseng production and rootlet used in this experiment were purchased from local market Geumsaninsam cooperative association (Geumsan, Korea) and Wooshin industrial Co., LTD (Geumsan, Korea), respectively. The ginseng specimen was deposited in the International Ginseng and Herb Research Institute (No.; GS201105, GS201203). Ginsenoside standards were purchased from Ambo Institute (DaeJeon, Korea). The acetonitrile and methanol were HPLC grade (Fisher Scientific, USA). All other chemicals were of analytical grade.

Apparatus for ultrafiltration – The instruments used for ultrafiltration were FlexStand benchtop pilot hollow fiber system (GE Healthcare, U.K.) with Hollow Fiber cartridge (Model; UFP-3-C-75, molecular weight cut off (MWCO); 3 kDa, membrane area; 6 m^2, GE Healthcare, U.K.) (Fig. 1A) and Sartocon® Slice Cross Filtration system (Satorius Stedim Biotech GmbH, Germany) with Hydrosart Cassette (MWCO; 5 kDa, membrane area; 0.5 m^2, Satorius Stedim Biotech GmbH, Germany) (Fig. 1B). Both filtration systems were equipped with the four-piston Diaphragm pump (Satorius Stedim Biotech GmbH, Germany).

Ginsenoside content analysis by ginseng root and rootlet mixture rate – Four-year-old ginseng roots and rootlets were used in this work. In order to analyze ginsenoside content by mixture rate of ginseng root and rootlet, mixture rate were divided into six groups as follows: R10 (root 100%), R8L2 (root 80%, rootlet 20%), R6L4 (root 60%, rootlet 40%), R4L6 (root 40%, rootlet 60%), R2L8 (root 20%, rootlet 80%), and L10 (rootlet 100%). Ten gram of ginseng mixture was extracted with 120 mL of water at 80 °C for 6 h using a round-bottom flask fitted with a cooling condenser. Each extraction was repeated three times. The obtained water extract was concentrated and dried *in vacuo* for ginsenoside content analysis and yield of extract.

Preparation of panaxadiol saponin fraction by ultrafiltration – Five hundreds gram of R4L6 (root 40%, rootlet 60%) was extracted with 5 L of water at 80 °C three times, for 6 h each. The whole water extract was filtered through Sartopure PP2 filter Cartridges (pore size 20 um, Satorius Stedim Biotech GmbH, Germany). Ginseng water extract was concentrated up to 20% of extraction volume by ultrafiltration system with Hollow Fiber cartridge (pore size; 3 kDa, membrane area; 6 m^2) and Hydrosart Cassette (pore 5 kDa, membrane area; 0.5 m^2). Concentrated ginseng extract was further dried in freeze dryer for preparation of panaxadiol saponin enhanced fraction.

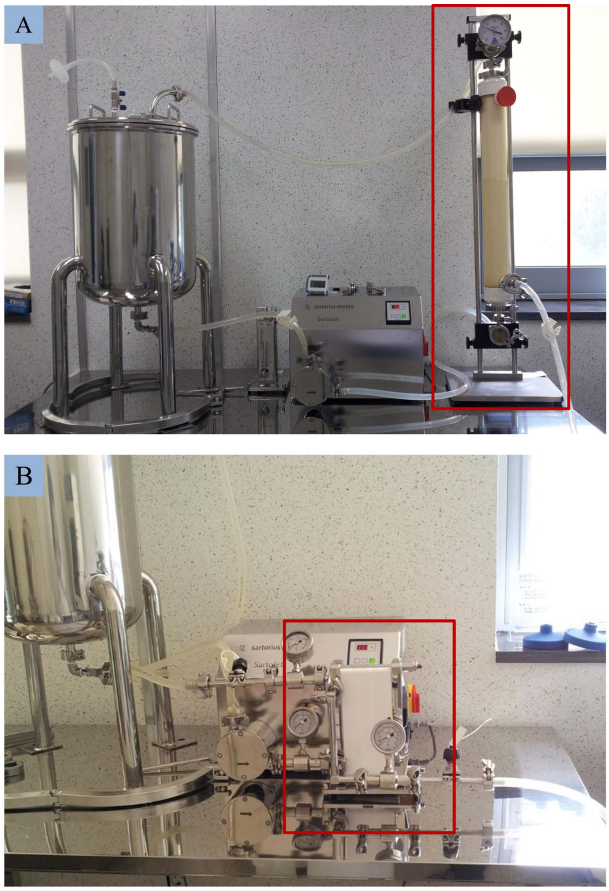

Fig. 1. Ultrafiltration system. A; FlexStand benchtop pilot hollow fiber system, B; Sartocon® Slice Cross Filtration system. Instruments in the rectangle are Hollow Fiber Cartridge (A) and Hydrosart cassette (B), respectively.

High performance liquid chromatography (HPLC) analysis of ginsenoside – Ten mg of ginseng extract powder was melted by 1 mL of methanol (MeOH) and filtered out by 0.45 μm membrane filter after extraction of ultrasonic waves for 2 h, then analyzed in HPLC. The HPLC system was Waters 1525 (Waters, USA) with PDA detector (Water, 2998). Waters Xbridge™ C18 column (250 mm × 4.6 mm, 5 μm, Waters, USA) was also used. The detection wavelength, flow rate, injection volume, and column oven temperature were set at 203 nm, 1.0 mL/min, 20 μL, and 40 °C. The mobile phase consisted of purified water (A) and acetonitrile (B) using the following gradient program: 0 min 18% B, 0 - 42 min 24% B, 42 -

46 min 29% B, 46 - 75 min 40% B, 75 - 100 min 65% B, 100 - 135 min 85% B, and 135 - 180 min 18% B.

Results and Discussion

Extraction yield depending upon the mixture ratio of ginseng root and rootlet – The yields of water extract of four-year-old red ginseng roots (R10) and rootlets (L10) are shown in Fig. 2. Water extraction yield in red ginseng main roots was 54.0%. It was much higher than 29.9% in red ginseng fine roots. The higher content of ginseng main root in ginseng mixture was the higher extraction yield of ginseng mixture. When the portion of L10 in ginseng mixture decreased 20%, the extraction yield decreased approximately 5%. It means that the ginseng main root contains higher soluble carbohydrates content than those of rootlets.[13]

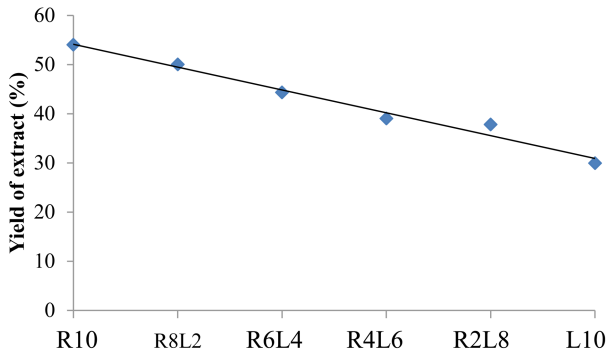

Fig. 2. Yield of extract by red ginseng mixture rate. R10; root 100%, R8L2; root 80%, rootlet 20%, R6L4; root 60%, rootlet 40%, R4L6; root 40%, rootlet 60%, R2L8; root 20%, rootlet 80%, L10; rootlet 100%.

Ginsenoside content by ginseng mixture rate – As shown in Table 1, the contents of ginsenoside, including PPD-type ginsenoside such as Rb1, Rb2, Rb3, Rc, Rd, Rg3, Rk1, and Rg5 and PPT-type ginsenoside such as Rg1, Re and Rf, were measured in ginseng mixture. The total ginsenosides contents of R10, R8L2, R6L4, R4L6, R2L8, and L10 were 16.28, 40.22, 53.09, 78.50, 80.16, and 91.31 mg/g, respectively. The total ginsenoside in L10 was 5.6 times higher than that in R10. The higher content of rootlet in ginseng mixture was the higher content of total ginsenoside in ginseng mixture extract. The ratio of PPD- to PPT-type ginsenoside (PPD/PPT) of R10 was 2.11, while that of L10 was 4.37. Ginsenoside Rb1/Rg1 in L10 (4.37) was 2.3 times higher than that of R10 (1.87). It means that increase of PPD-type ginsenoside content in L10 was higher than that of PPT-type ginsenoside content. These results accorded with report that the contents of total ginsenosides on 6 years old ginseng cultured in the field were high in order of main root, lateral root and fine roots, and content of ginsenoside in fine roots was 3.2 times higher than that in main root.[14]

Yield of inner fluid after ultrafiltration – For the consideration of the extraction yield and ginsenoside content, the R4L6 was selected from among the six mixtures of ginseng main root and rootlet. The water extract of R4L6 was pre-filtered through filter cartridges, pore size 20 μm. Filtrated ginseng water extract was concentrated up to 20% of extraction volume by ultrafil-

Table 1. Ginsenoside content by red ginseng mixture rate. R10; root 100%, R8L2; root 80%, rootlet 20%, R6L4; root 60%, rootlet 40%, R4L6; root 40%, rootlet 60%, R2L8; root 20%, rootlet 80%, L10; rootlet 100%

Ginsenoside	R10	R8L2	R6L4	R4L6	R2L8	L10
Rg1	2.37	2.11	2.97	2.91	3.00	3.04
Re	1.82	5.07	6.48	9.25	9.55	10.79
Rf	1.04	1.70	2.33	2.66	2.93	3.19
Rb1	4.42	10.88	15.18	23.23	23.16	26.86
Rc	2.66	9.12	11.46	17.35	18.20	20.97
Rb2	1.40	4.94	5.76	10.36	10.87	12.09
Rb3	0.15	0.47	0.51	1.45	1.49	1.74
Rd	0.65	2.35	4.23	7.14	6.55	7.93
Rg3(s)	0.47	0.91	1.14	1.16	1.26	1.29
Rg3(r)	0.47	0.75	0.92	0.77	0.75	0.85
Rk1	0.28	0.91	0.83	0.91	0.99	1.08
Rg5	0.56	1.01	1.29	1.31	1.42	1.48
Total	16.28	40.22	53.09	78.50	80.16	91.31
PPD/PPT	2.11	3.53	3.51	4.30	4.18	4.37
Rb1/Rg1	1.87	5.17	5.11	7.97	7.73	8.84

Table 2. Yield of inner fluid by ultrafiltration rate of ginseng water extract

Ultrafiltration (%)	Yield of inner fluid (%)	
	3 kDa	5 kDa
0	100.0	100.0
10	95.1	95.0
20	89.2	89.3
30	83.2	82.8
40	77.2	76.9
50	70.9	70.8
60	65.7	64.6
70	59.8	59.9
80	54.9	54.1

tration system. As show in Table 2, the concentrated inner fluid fraction by ultrafiltration rate was divided into 9 steps; 0, 10, 20, 30, 40, 50, 60, 70, and 80%. The more increase of ultrafiltration rate, was the lower yield of inner fluid fraction of ginseng water extract. It is consider that hydrophilic low molecular compounds such as sugar, organic acid, and amino acid were removed convectively with the water by ultrafiltration. However, the yields of inner fluid by membrane filter pore cutoff size of 3 kDa and 5 kDa were not different from each other. When ginseng water extract was concentrated 50% by ultrafiltration system, the yields of inner fluid fraction by 3 kDa and 5 kDa pore size membrane were 70.9 and 70.8%, respectively. The yields of inner fluids removed 80% water of extraction volume was decreased to 54.9% (3 kDa pore size) and 54.1% (5 kDa pore size), compared to not filtered water ginseng extract.

Change of ginsenoside content of inner fluid after ultrafiltration – Ginsenoside composition after ultrafiltration was compared between 5 kDa pore size Hydrosart Cassette made by regenerated cellulose acetate (CA) and 3 kDa Hollow Fiber cartridge made by polyethersulfone (PES) as shown in Fig. 3. Total ginsenoside contents of inner fluid after ultrafiltration showed high, compared to before ultrafiltration. Processing of ultrafiltration showed the increase of PPD-type ginsenoside such as ginsenoside Rb1, Rc, Rb2, and Rd, while PPT-type ginsenoside such as ginsenoside Re and Rg1 was gradually decreased by both 3 kDa and 5 kDa membrane (Fig. 3, Fig. 5 - 6). After removal of 80% water of extraction volume by the 5 kDa Hydrosart Cassette, ginsenoside Rb1 content in inner fluid of ginseng extract was high (37.2 mg/g), compared to those by 3 kDa Hollow Fiber cartridge (25.3 mg/g) and control (20.8 mg/g). However, ginsenoside Rb1 content by removal of 70% water of extraction volume was not

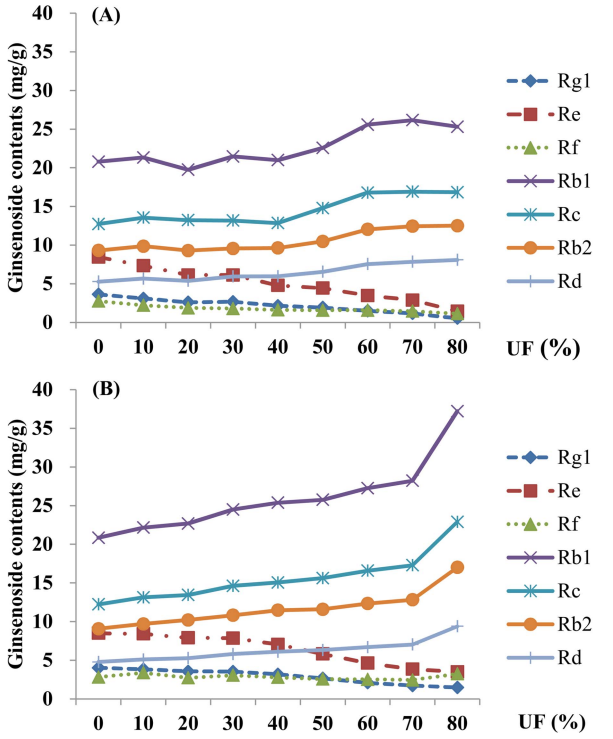

Fig. 3. Change of ginsenside content by ultrafiltration rate of ginseng water extract. (A); 3 kDa Cartridge, (B); 5 kDa Cassette, UF; ultrafiltration.

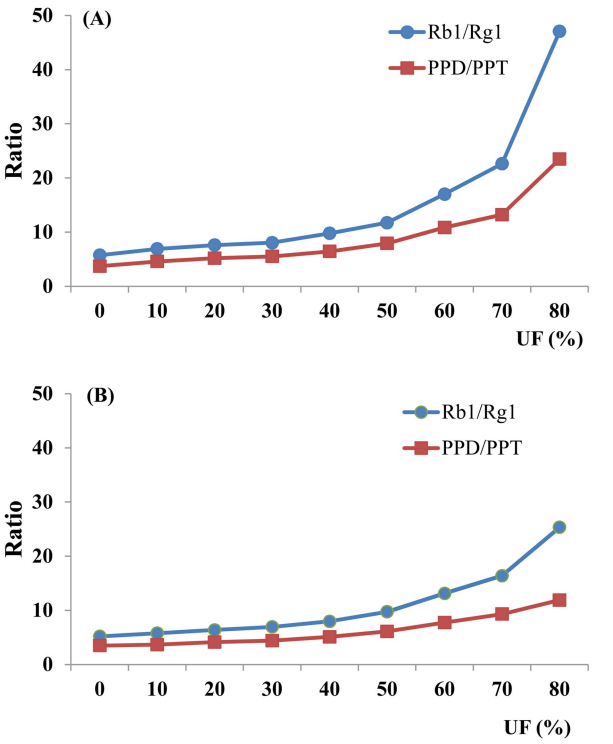

Fig. 4. The ratio of Rb1 to Rg1 and PPD type ginsenoside to PPT type ginsenoside by ultrafiltration rate of ginseng water extract. (A); 3 kDa Cartridge, (B); 5 kDa Cassette, UF; ultrafiltration.

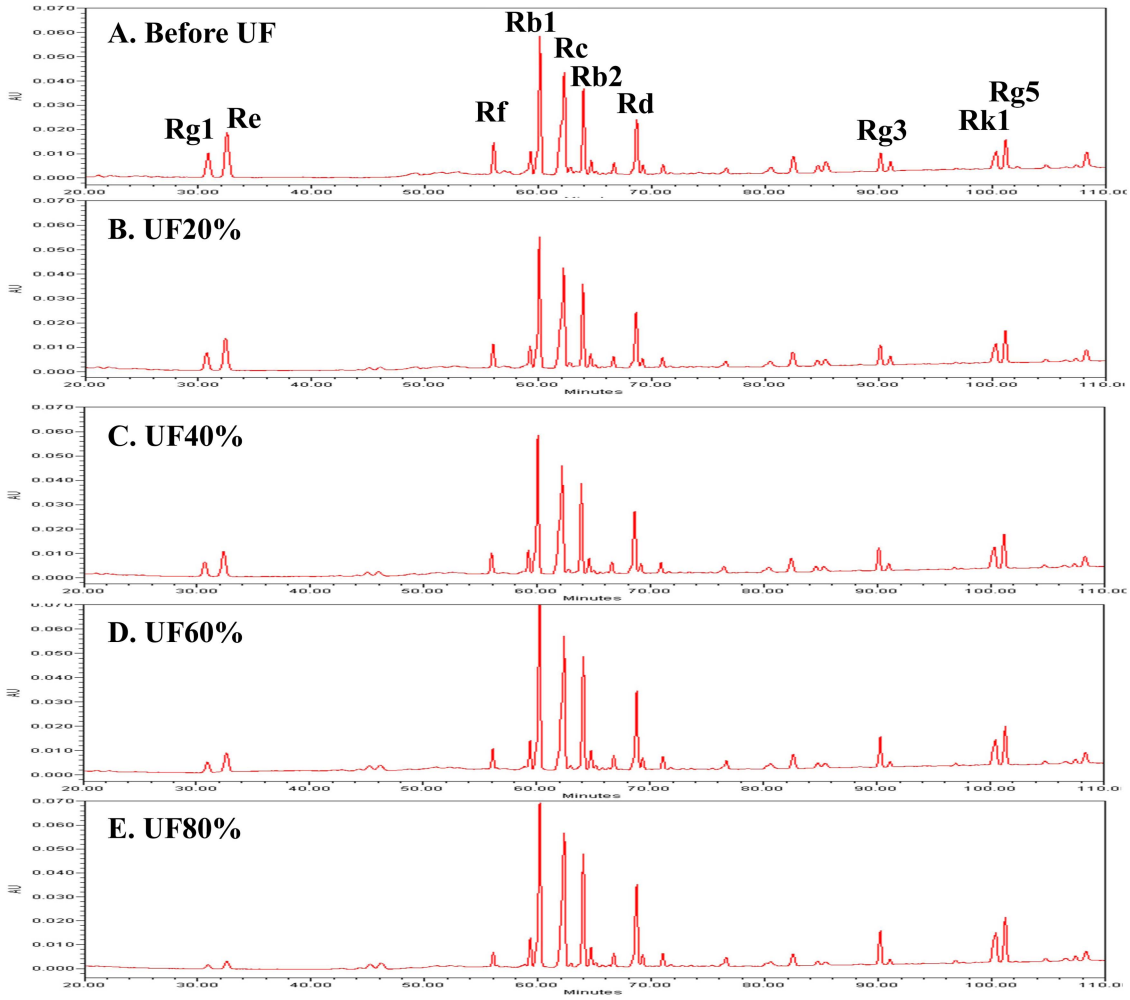

Fig. 5. HPLC chromatogram of ginseng water extract after ultrafiltration (3 kDa). UF; ultrafiltration.

different between 5 kDa Hydrosart Cassette (28.2 mg/g) and 3 kDa Hollow Fiber cartridge (26.2 mg/g). PPD-type ginsenoside content was also not different between 5 kDa and 3 kDa pore size, while PPT-type ginsenoside content was rapidly decrease by 3 kDa Hollow Fiber cartridge. Therefore, the ratio of Rb1 to Rg1 (Rb1/Rg1) and PPD- to PPT- type ginsenoside (PPD/PPT) were higher in inner fluid of ginseng extract by 3 kDa Hollow Fiber cartridge than those by 5 kDa Hydrosart Cassette (Fig. 4). Generally, ultrafiltration is a technique for separating dissolved molecules in solution on the basis of size which means that molecules larger than the membrane pore size rating will be retained at the surface of the membrane. The molecular weight (MW) of ginsenoside was below 1,110 though the molecular weight of PPD-type ginsenoside such as Rb1 (MW 1109.29), Rb2 (MW 1079.27), Rc (MW 1079.27), and Rd (MW 947.15) was slightly larger than those of PPT-type ginsenoside such as Re (MW 947.14) and Rg1 (MW 801.01). The ginsenoside molecular size is smaller than pore size of membrane filters used for these experiments. Nevertheless, PPD-type ginsenosides did not pass through the membrane with 3 kDa or 5 kDa pore size. Only PPT-type ginsenosides, Re and Rg1 slowly passed through the membrane in continuous solution flow system.

During sequential ultrafiltration, smaller suspended particles and dissolved macromolecules pass through the membranes, while bigger molecules are rejected.[15] The most common ultrafiltration membrane filters are Cellulose acetate (CA) and Polyethersulfone (PES) membrane.[16,17] They were hydrophilic making them suitable for aqueous media and exhibit very low protein binding capacity. CA was one of the first membrane polymer that has been used as both reverse osmosis and ultrafiltration membranes.[17]

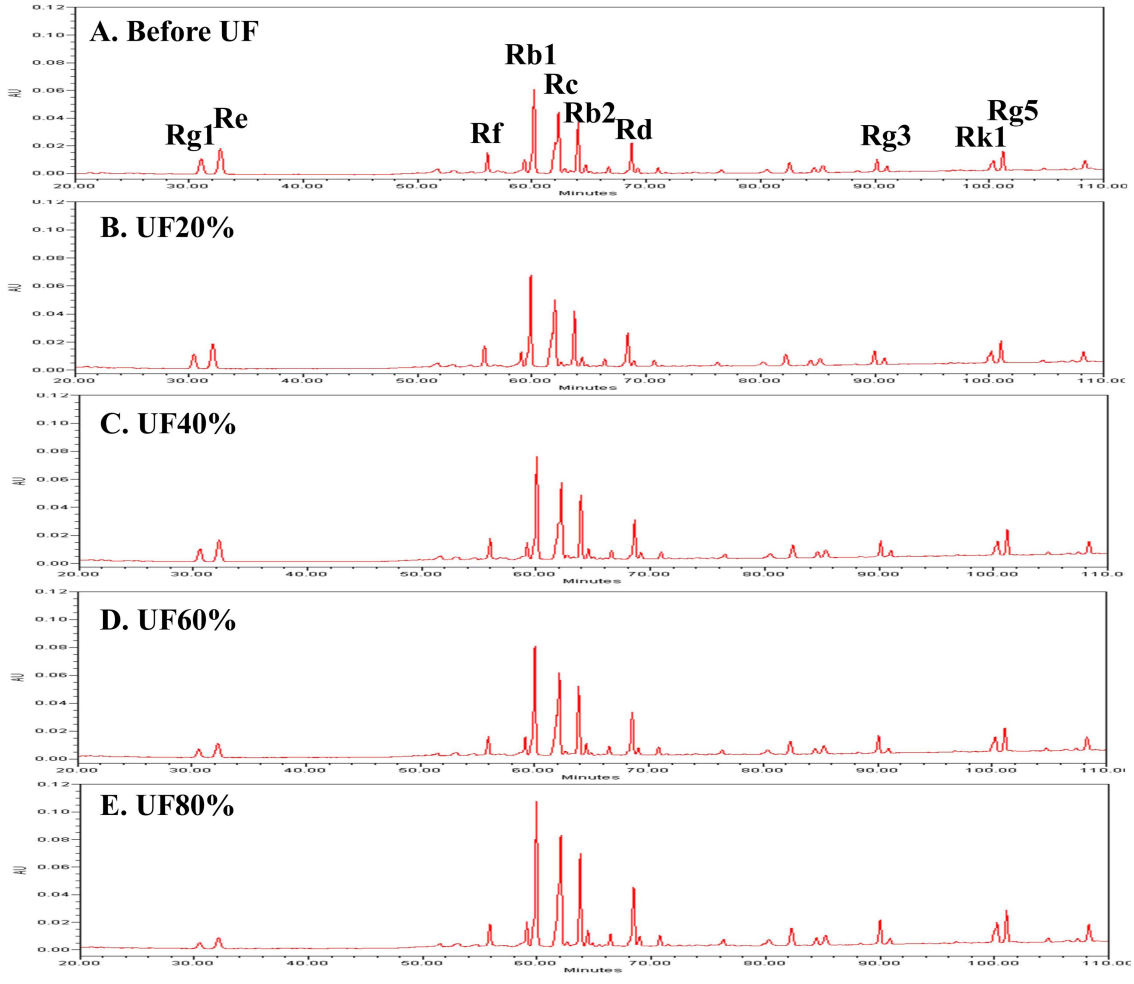

Fig. 6. HPLC chromatogram of ginseng water extract after ultrafiltration (5 kDa). UF; ultrafiltration.

Hydrophilicity and permeability of CA may be increased by sulfonation.[18] Generally, sulfuric acid is used for sulfonation since it avoids degradation and cross-linking reactions.[19] Hydrophilic CA and PES help make the membrane impermeable to hydrophobic substances. PPD-type ginsenoside in aqueous solution is more hydrophobic than PPT-type ginsenoside. Therefore, hydrophilic CA and PES membrane could not permit to pass easily PPD-type ginsenoside.

In this study, we established the effective isolation method of protopanaxadiol saponin fraction using the ultrafiltration and PPD-type ginsenoside enriched fraction by ultrafiltration could be developed as a new functional ginseng materials.

Acknowledgments

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