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Full Length Research Paper

Taste profile characterization of white ginseng by electronic tongue analysis

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We conducted taste profile analysis of white ginseng (*Panax ginseng*) using a taste-sensing system. Taste such as sourness, bitterness, astringent, aftertaste, umami, richness, and saltiness of the four subfractions (*n*-hexane fr. = Pg1; EtOAc fr. = Pg2; $CHCl_3$ fr. = Pg3; *n*-BuOH fr. = Pg4) from white ginseng was checked using an electronic tongue. The bitterness and aftertaste-B of Pg3 were perceived as significantly higher than those of the other subfractions. The sourness of Pg2 had the highest rating compared to that of the other subfractions. The umami of Pg4 was higher than that of the other subfractions, but bitterness was lowest. As a result, the Pg3 subfraction of the white ginseng chloroform fraction showed the largest variation in taste. Medium pressure liquid chromatography of the white ginseng chloroform fraction led to the isolation of two phytosterols, which were identified as β-sitosterol and daucosterol by spectral analysis. Additional study of these compounds on taste should be conducted.

Key words: Electronic tongue, *Panax ginseng*, phytosterol, taste, white ginseng.

INTRODUCTION

Taste is a very important component of eating. Thus, much research has been aimed at determining factors that affect taste during production and processing of foods. Among them, non-volatile substances in meat and meat products may stimulate the taste compounds (Mottram, 1998). Taste is one of the five traditional senses and includes sourness, bitterness, sweetness, saltiness, and umami. Taste refers to the ability to detect the flavor of substances such as foods and poisons. Humans perceive taste through sensory organs called taste buds concentrated on the upper tongue surface. Basic taste contributes to the sensation and flavor of foods in the mouth. Sourness is the taste that detects acidity. The sourness of substances is rated relative to dilute hydrochloric acid (McLaughlin and Margolskee, 1994). Bitterness is the most sensitive of the tastes and is perceived by many to be unpleasant, sharp, or disagreeable. Bitterness is of interest to health researchers, as a large number of natural bitter compounds are toxic. Sweetness is produced by the presence of sugars and is often associated with aldehydes and ketones (Grace et al., 2003). Saltiness is a taste produced primarily by the presence of sodium ions. The saltiness of substances is rated relative to NaCl (McLaughlin and Margolskee, 1994). Umami is an appetitive taste and is described as a savory or meaty taste. Unami taste activation was greater in the post central gyrus (somatosensory cortex) (Smeets et al., 2011).

Amino acids and peptides contribute to the taste of a wide variety of foods. Astringents such as polyphenols have a high affinity for binding with proline-rich proteins in saliva (Hagerman and Butler, 1981). Polyphenols or tannins elicit bitterness and astringency in wines, tea, and fruits (Joslyn and Goldstein, 1964). Wine contains a large

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number of bitter components, such as tyramine and tyrosol. A large number of bitterness components in Gentian and Swertiae herbs are secoiridoid glucosides. Bitter orange peel and Picrasma wood contain a large number of deformed triterpene derivatives. The *Coptis* rhizome and *Phellodendron* bark contain berberine-type alkaloids (Kataoka et al., 2008). Many bioactive constituents, such as polyphenols, vitamins, and amino acids, are present in green tea. Among them, catechins have a broad range of physiological functions and act as the bitterness taste ingredient in green tea (Narukawa et al., 2011).

Tastes such as sourness, bitterness, sweetness, saltiness, and umami have been measured using an electronic tongue (ET). ET is a liquid analysis device that mimics the taste-sensing mechanism and information processing of the gustatory system; it comprises an array of sensors that are specific for liquids and can classify sourness, saltiness, bitterness, and umami taste (Toko, 2000). This type of artificial taste sensor has been used quite extensively to characterize the taste of foods or beverages such as beer, sake, and green tea (Toko, 1998; Tan et al., 2001). However, relatively few applications have been reported in the field of pharmaceutical development, although this is an area that is under examination (Legin et al., 2004). Few reports are available on the usefulness of a taste sensor for predicting the bitterness of a number of medicines (Miyanaga et al., 2002) and the evaluation of bitterness suppression (Uchida et al., 2003).

P. ginseng is a plant widely used in therapeutics and food preparations in Asian countries. *P. ginseng* has been traditionally used as an expensive and precious medicine in Asian countries for more than 2,000 years, so many forms of ginseng products including teas, capsules, tablets, wine, chewing gum, cigarettes, and candy are available in the market (Choi et al., 2011; Nam, 2005; Paul et al., 2005; Chan and Wu, 2006). Many studies have reported the phytochemical constituents and biological activities of *P. ginseng* (Banza et al., 2001; Beveridge et al., 2002; Cho et al., 2010; Jung et al., 2005; Paul et al., 2005; Xu et al., 2009). Ginseng contains many chemical constituents, such as ginsenosides, volatiles, and polysaccharides, which relate to various pharmacological activities (Banza et al., 2001; Xu et al., 2009). Modern chemical and pharmacological studies indicate that multiple components such as flavonoids, saponins and polyacetylenes are bioactive compounds in *P. ginseng*, and that their pharmacological activities include antioxidant, hypotensive, neuroprotective, antibacterial, antitumor, cognitive, sedative, analgesic and anti-stress effects (Jung et al., 2005; Sun et al., 2009).

Most studies focused on investing phytochemical constituents and biological activities of *P. ginseng*. However, no reports are available on the properties of a white gingseng taste profile analysis using an ET. Therefore, the purpose of this study was to describe white gingseng sensory characteristics using an ET.

MATERIALS AND METHODS

Plant materials

The dried and powdered white ginseng samples (*P. ginseng*) were supplied by Korea Food Research Institute, Sungnam, Korea. The white ginseng was collected from Geumsan, Republic of Korea in 2010.

Reagents

Solvents such as *n*-hexane, chloroform ($CHCl_3$), ethyl acetate (EtOAc), and *n*-butanol (*n*-BuOH) (SamChun Pure Chemical Company, Pyeongtaek, Republic of Korea) were used as the medium pressure liquid chromatography (MPLC) mobile phase. First grade solvents such as $CDCl_3$ and pyridine were used as nuclear magnetic resonance (NMR) solutions. All other solvents were analytical grade.

Instruments

Electron ionization mass spectrometry (EI-MS) was conducted with a JEOL JMS-600W (Tokyo, Japan) mass spectrometer and fast atom bombardment mass spectrometry (FAB-MS) was performed with a JEOL JMS-AX505WA mass spectrometer. ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra were recorded with a Bruker AVANCE 500 NMR (Bremen, Germany) spectrometer using TMS as the internal standard. Chemical shifts are reported in parts per million (δ), and coupling constants (*J*) are expressed in hertz. An EYELA rotary evaporator system (Tokyo, Japan) under reflux *in vacuo* was used for evaporation. Thin layer chromatography was conducted with Kiesel gel 60 F_{254} (Art. 5715, Merck Co., Darmstadt, Germany) plates (silica gel, 0.25 mm layer thickness), and compounds were visualized by spraying with 10% H_2SO_4 in MeOH followed by heating to 100°C. Medium pressure liquid chromatography (MPLC) (Biotage, Uppsala, Sweden) and cartridges (KP-SIL, 39 × 225 mm, Biotage) were used to isolate components.

Electronic tongue (ET) apparatus and taste sensors

Taste analyses were performed with the commercially available SA 402B taste-sensing system (Intelligent Sensor Technology Company, Limited., Tokyo, Japan) (Figure 1). The detecting part of the system consists of five sensors whose surface is attached with artificial lipid membranes with different response properties to chemical substances based on their taste (Laureati et al., 2010). The electrode set is attached to a mechanically controlled robot arm. The detecting sensor consists of five electrodes composed of lipid/polymer membranes (Gan and Hu, 2011). The lipids and plasticizers used for the sensor membranes are shown in Table 1. Each lipid was mixed in a test tube containing poly (vinyl chloride) and dioctylphenyl phosphonate as a plasticizer, dissolved in tetrahydrofuran, and dried on a glass plate at 30°C to form a transparent thin film of approximately 200 μm thick. The electrodes consisted of a silver wire with an Ag/AgCl-plated surface and an internal cavity filled with 3.33 M KCl solution. The difference between the electric potentials of the working and reference electrodes was measured as sensor output by means of a high input impedance amplifier connected to a computer. Sensors were

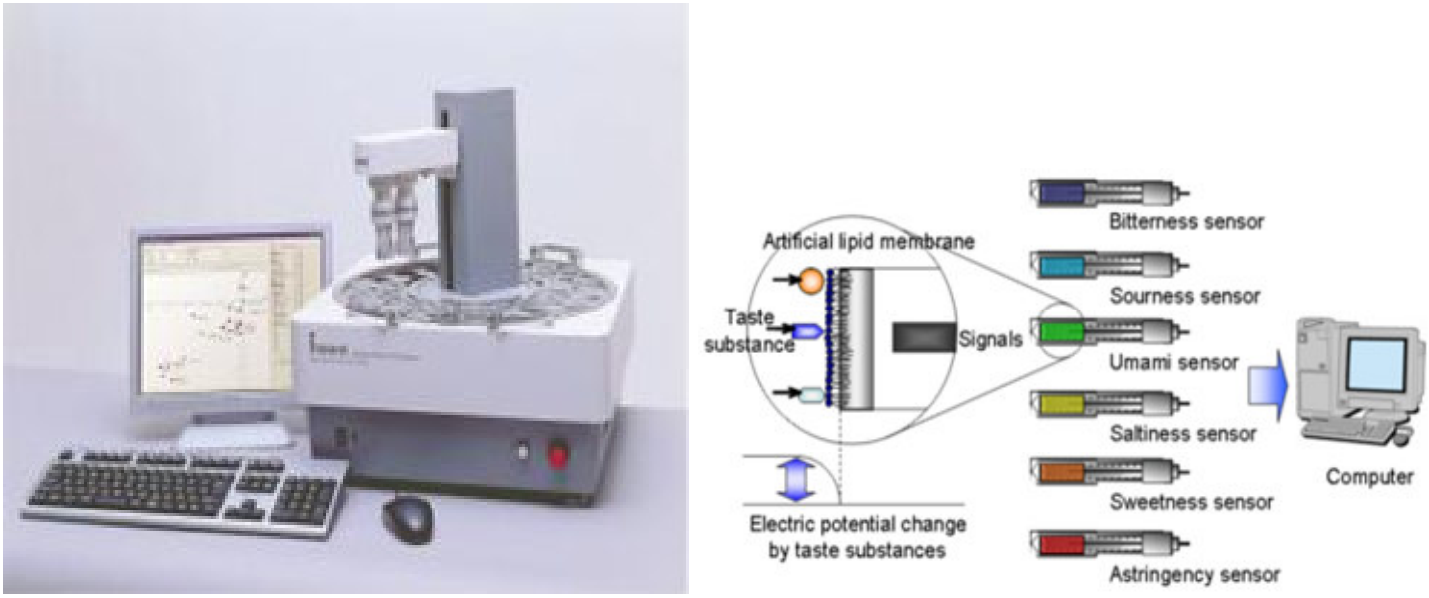


Figure 1. ET apparatus and the taste sensors (taste sensing system SA402B).

Table 1. Lipids and plasticizers used for the sensor membranes.

Sensor	Taste information	Lipid	Plasticizer
AAE	Umami	Trioctylmethyl ammonium chloride Phosphoric acid di(2-ethylhexyl) ester	Di-*n*-octylphenyl phosphonate
CT0	Saltiness	Tetradodecyl ammonium bromide Cetyl alcohol	Di-*n*-octylphenyl phosphonate
CA0	Sourness	Trioctylmethyl ammomium chloride Phosphoric acid di(2-ethylhexyl) ester Oleic acid	Di-*n*-octylphenyl phosphonate
AE1	Astringency	Tetradodecyl ammonium bromide	Di-*n*-octylphenyl phosphonate
C00	Bitterness	Tetradodecyl ammonium bromide	2-Nitrophenyloctyl ether

used to predict taste. This system detects the initial taste and the aftertaste.

Taste analysis sample preparation

Dried and powdered white ginseng (2,000 g) was extracted with EtOH (8 L × 7) under reflux for 3 h. The filtrates were concentrated *in vacuo* to produce EtOH extracts (159 g). The extracts were suspended in distilled water and then partitioned using *n*-hexane (42 g), $CHCl_3$ (26 g), EtOAc (10 g), and *n*-BuOH (24 g). EtOH was employed as an extraction medium during the experiment, as solvents such as *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH could remove various components from the artificial sensor and thereby cause damage to the sensor characteristics. Thus, the residue was filtered after each fraction (fr.) was dissolved in MeOH, and the solvent was removed *in vacuo*. Each fr. Was weighed and dissolved in 30% EtOH at concentrations of 0.6 mg/ml (*n*-hexane fr. = Pg1; EtOAc fr. = Pg2; $CHCl_3$ fr. = Pg3; *n*-BuOH fr. = Pg4).

Taste measurements with the electronic tongue (ET)

The detecting and reference electrodes were first dipped into the reference solution (30 mM potassium chloride and 0.3 mM tartaric acid). Then, the electrodes were dipped for 30 s into the sample solution (*n*-hexane fr., EtOAc fr., $CHCl_3$ fr., and *n*-BuOH fr.). The "relative" sensor outputs were represented by the differences between the potentials of the sample and the reference solution. Electrodes were rinsed with fresh reference solution for 6 s and then dipped into the reference solution again. The difference between the potentials of the reference solution before and after sample measurement was the change in membrane potential corresponding to the ET "aftertaste". Before a new measurement

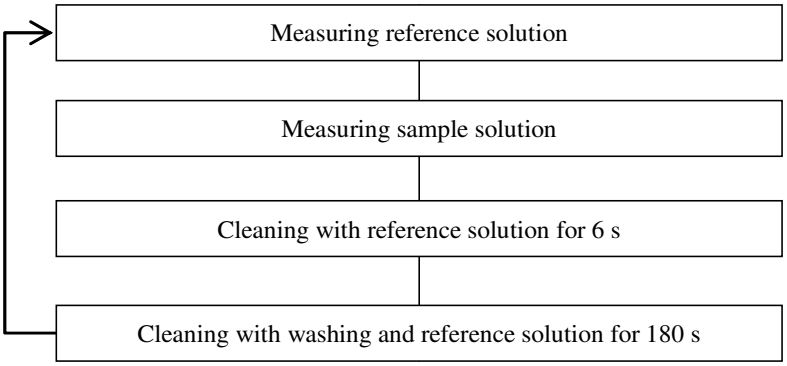


Figure 2. ET measurement procedure.

cycle started, electrodes were rinsed for 90 s with a washing solution and then for 180 s with the reference solution (Figure 2). Each sample fraction was evaluated three times, and the average of the results was used for data analysis. All measurement procedures were conducted at room temperature.

Isolation and identification of the phytochemical components by medium pressure liquid chromatography (MPLC)

A portion of the $CHCl_3$ fr. was separated by MPLC using a stepwise gradient of *n*-hexane-EtOAc and EtOAc-MeOH as the mobile phase. The flow rate was kept at 20.0 ml/min, and the peaks were identified by ultraviolet (UV) absorbance at 254 nm. The $CHCl_3$ fraction yielded 38 sub-fractions. Sub-fr. 6 (*n*-hexane : EtOAc = 85 : 15) led to the isolation of compound **1**. Sub-fr. 18 (EtOAc : MeOH = 9 : 1) led to the isolation of compound **2**.

Compound **1**: White crystals; EI-MS (rel. int., %): *m/z* 414 $[M]^+$ (100), 396 (49.9), 381 (24.3), 329 (28.0), 303 (32.3), 273 (32.7), 255 (69.3), 213 (37.9), 159 (42.9), 145 (45.1); ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$): (Table 2).

Compound **2**: White powder; FAB-MS: *m/z* 577 $[M+H]^+$; ^{1}H-NMR (500 MHz, pyridine) and ^{13}C-NMR (125 MHz, pyridine): (Table 2).

RESULTS AND DISCUSSION

The Pg1-Pg4 frs. obtained by partitioning the white ginseng EtOH extract with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH were subjected to a taste assay, such as sourness, bitterness, astringent, aftertaste, umami, richness, and saltiness. The sensor output profiles for the four white ginseng subfractions could be classified into seven patterns (sourness, bitterness, astringent, aftertaste, umami, richness, and saltiness) (Figure 3).

According to multiple comparison tests, the taste patterns and the taste intensities of the four subfractions are shown in Figure 3. Four frs. were perceived as significantly less salty and most bitter. Among them, the bitterness and aftertaste-B of Pg3 were perceived as significantly higher than those of the other subfractions. The sourness of Pg2 had the highest rating compared to that in the other subfractions. The umami of Pg4 was higher than that of other subfractions, but bitterness was lowest among the fractions. Astringency, aftertaste-A, richness, and saltiness were not significantly different among the four fractions. Aftertastes-A and -B were aftertastes of astringency and bitterness, respectively. Aftertaste is the taste intensity of a food or beverage that is perceived immediately after that food or beverage is removed from the mouth. Unifying feature of aftertaste is that it is perceived after a food or beverage is either swallowed or spat out (Neely and Borg, 1999). Richness is the umami aftertaste (Kobayashi et al., 2010).

Pg3 was the most effective fr. in the taste pattern analysis. Therefore, we isolated and identified the constituents of the Pg3 fr. by medium pressure liquid chromatography (MPLC). Chromatographic separation of Pg3 led to the isolation of compounds **1** and **2** (Figure 4). Compounds **1** and **2** were obtained as white powders. They were identified as phytosterols by MS and ^{1}H- and ^{13}C-NMR spectral analysis. The typical ^{1}H- and ^{13}C-NMR spectra patterns of **1** and **2** showed the existence of a sterol skeleton. The structures of **1** and **2** were elucidated as β-sitosterol (stigmast-5-en-3-ol) and daucosterol (β-sitosterol-3-*O*-glucoside), respectively, by interpretation of spectroscopic data in the literature (Chang et al., 1981; Kim et al., 2008; Park et al., 2009) (Figure 5). β-Sitosterol, a well-known plant sterol, reduces serum cholesterol levels and prevents cardiovascular events by inhibiting cholesterol absorption in the intestines (Umlauf et al., 2004). β-Sitosterol also regulates key molecules involved in inflammation, the immune response, anti-cancer defense, and apoptosis (Bouic, 2002). Daucosterol has an immunoregulatory effect on disseminated candidiasis caused by *Candida albicans* (Lee et al., 2007).

In conclusion, the bitterness and aftertaste-B of Pg3 were perceived as significantly higher than those of other frs. The sourness of Pg2 had the highest rating compared to that in other frs. Umami was higher in Pg4 than that in other frs, but bitterness was lowest. A chromatographic separation of the active Pg3 fr. led to the isolation of β-

Table 2. ^{1}H- and ^{13}C-NMR data for compounds **1** and **2**.

Number	1		2	
	δ_H	δ_C	δ_H	δ_C
1		37.4		37.8
2		29.8		29.4
3	3.52 (m)	72.0		77.6
4	2.27 (m)	39.9		38.4
5		141.1		141.3
6	5.35 (m)	122.2	5.65 (m)	122.3
7		32.0		31.8
8		31.8		30.6
9		50.3		50.1
10		36.6		35.0
11	1.83 (m)	21.2		22.1
12		40.7		40.1
13		42.4		44.0
14		56.9		55.8
15		24.4		22.3
16		28.4		26.2
17		56.2		56.6
18	0.69 (s)	11.9	0.68 (s)	12.7
19	1.00 (s)	19.1	0.75 (s)	13.5
20		36.3		41.6
21	0.92 (d, 6.3)	18.9	1.09 (d. 6.5)	21.9
22		34.1		32.6
23		26.2		24.9
24		46.0		51.9
25		29.3		32.7
26	0.85 (d, 6.5)	19.9	0.90 (d, 6.6)	19.7
27	0.83 (d, 6.6)	19.5	0.88 (d, 6.4)	21.8
28		23.2		23.8
29	0.81 (t, 6.0)	12.1	0.86 (t, 7.8)	13.0
1′		-	5.06 (d, 7.6)	102.8
2′		-		75.9
3′		-		78.9
4′		-		72.3
5′		-		79.1
6′		-		63.5

Chemical shifts are reported in parts per million (δ), and coupling constants (J) are expressed in hertz.

sitosterol and daucosterol.

To the best of our knowledge, the Pg3 of *P. ginseng* was found to demonstrate high bitterness by ET. Therefore, we suggest that β-sitosterol and daucosterol from Pg3 have a possibility for the bitterness substances. It was envisaged that some sample chemical characters could be identified by ET and chromatography. In this regard, further studies should be optimized for the quantitative and qualitative determination of taste compounds in white ginseng, red ginseng, and black ginseng. Different cultivation years and different process would cause a major change in the overall white ginseng metabolic profile. This observation highlights the need to develop new *P. ginseng* products. Industrial applications should be the focus to develop *P. ginseng* products.

Conclusion

Taste profile analysis of white ginseng (*P. ginseng*) using a taste-sensing system was evaluated. Taste such as sourness, bitterness, astringent, aftertaste, umami,

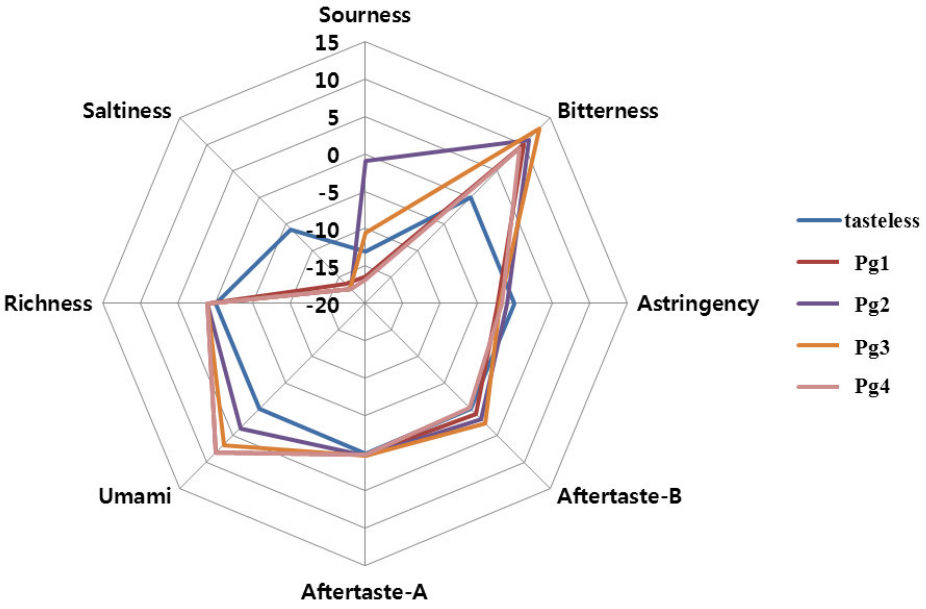


Figure 3. Taste profile patterns of the four white ginseng frs. (Pg1: the *n*-hexane fr.; Pg2: the EtOAc fr.; Pg3: the $CHCl_3$ fr.; Pg4: the *n*-BuOH fr.).

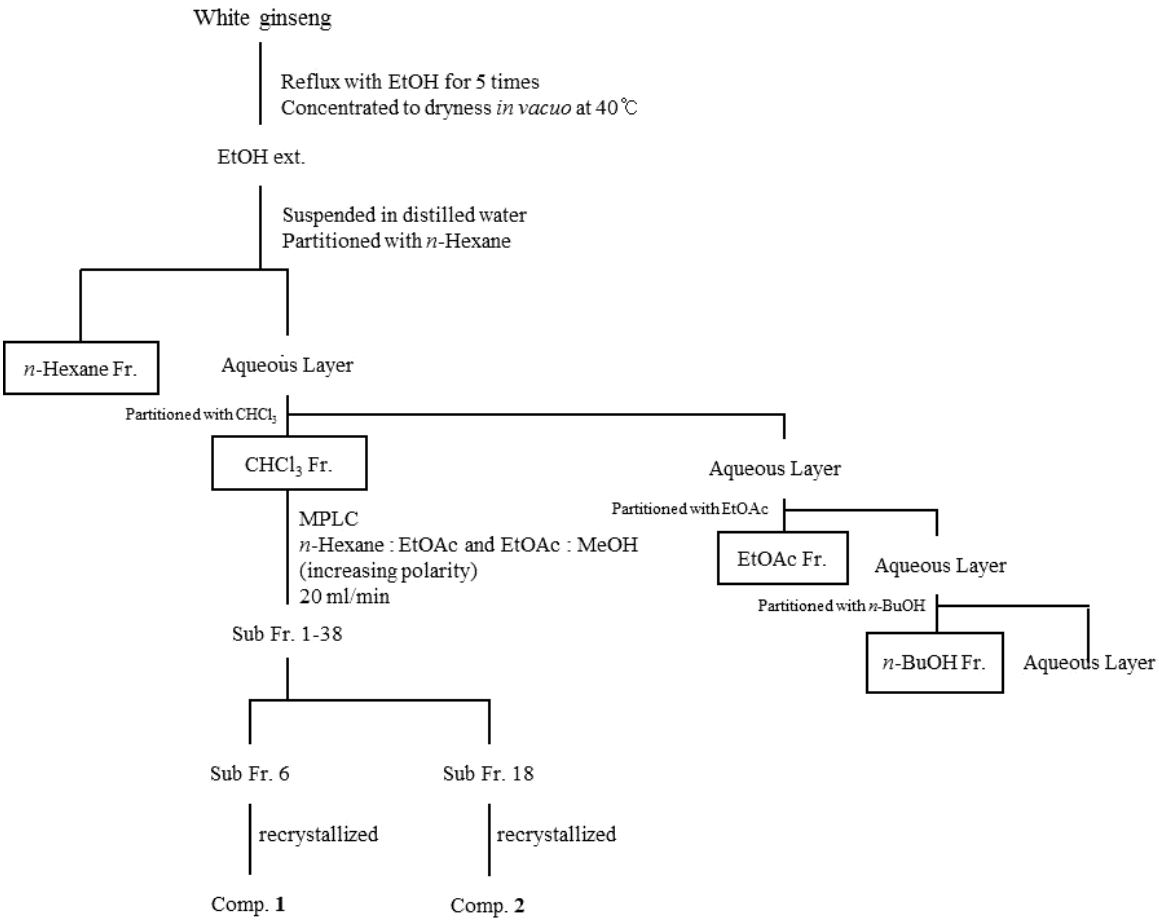
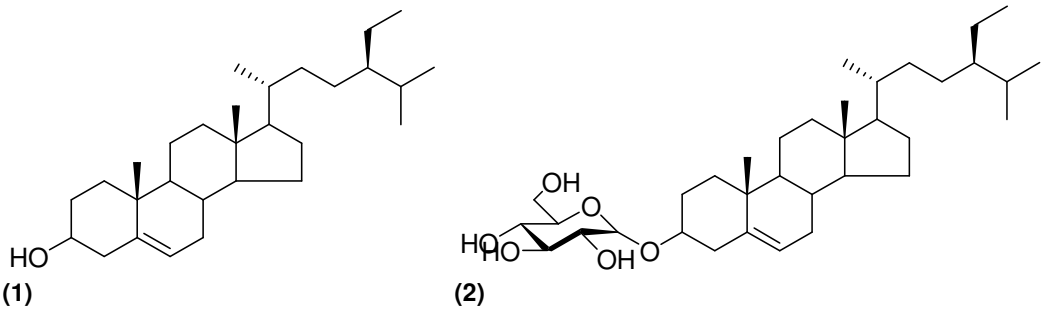


Figure 4. Fractionation and isolation scheme for compounds **1** and **2**.



(1) **(2)**

Figure 5. Structures of compounds **1** and **2**.

richness, and saltiness of the four subfrs. (Pg1, Pg2, Pg3, and Pg4) from white ginseng was checked using an ET. The bitterness and aftertaste-B of Pg3 were perceived as significantly higher than those of the other subfrs. The sourness of Pg2 had the highest rating compared to that of the other subfrs. The umami of Pg4 was higher than that of the other subfrs., but bitterness was lowest. As a result, the Pg3 subfr. of the white ginseng chloroform fr. showed the largest variation in taste. Medium pressure liquid chromatography of the white ginseng chloroform fr. led to the isolation of two phytosterols, which were identified as β-sitosterol and daucosterol by spectral analysis.

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