

【DESCRIPTION】**【Invention Title】**

METHOD FOR PREPARING EXTRACT FRACTION REINFORCED
WITH GINSENOSESIDES RG1 OR RB1 FROM GINSENG

【Technical Field】

The present invention relates to a method for preparing an extract fraction reinforced with specific saponin components from ginseng. More particularly, the present invention relates to a method for preparing an extract fraction reinforced with ginsenoside Rg1 known to have anti-fatigue functions OR ginsenoside Rb1 known to have sedative activity, among saponin components of ginseng.

【Background Art】

Panax ginseng C.A. Mayer (hereinafter, abbreviate to 'ginseng') is a generic ginseng plant of the Arliaceae family. *Panax ginseng* has been used for medicinal purposes in China since B.C. and has been employed for medicinal purposes or as a trade item in Korea since the era of the Three Kingdoms. *Panax ginseng* is now widely used for preparation of oriental medicines or health supplements used for a variety of applications.

Ginseng generally includes about 3 to 6% of

saponin-like materials called ginsenosides as species-specific ingredients and the ginsenosides are major physiological active materials and may be classified into panaxadiol (PD), panaxatriol (PT) and oleanane based ginsenosides. About 33 species of ginsenosides have recently been reported.

PD based saponin and PT based saponin have different functions in vivo. PD based saponin including ginsenoside Rb1 as a representative example, is known to exhibit inhibitory action on the central nervous system, in turn accomplishing tranquilization, neuroleptic, analgesic, anti-convulsive and/or hypotensive effects, influence upon papaverine content, or the like. On the other hand, PT based saponin including ginsenoside Rg1 as a representative example, is known to excitedly react to the central nervous system, thus exhibiting anti-fatigue activity. Therefore, it is assumed that, if saponin components in ginseng having opposing activities are separated or reinforced, pharmacological activity of ginseng may be more efficiently attained. However, most recent studies into ginsenosides are directed to a method for increasing contents of trace ingredients present in ginseng (US Patent NO. 7,371,416), use of ginsenosides for treatment of particular diseases (WO 01/056585), or the like. However, research and investigations into

techniques to improve efficacies of ginsenosides Rg1 and Rb1 showing opposing activities, respectively, have not been sufficient.

5 Korean Patent No. 620,107 discloses a process for production of ginsenoside Rg2 as a specific component of ginseng saponin by treating a water extract or an alcohol extract of ginseng using lactic acid. Korean Patents Nos. 517,128 and 192,678 disclose variation in yield of saponin depending upon alcohol concentration or
10 extraction temperature in consideration of thermal stability when the saponin is extracted from ginseng. However, all of these techniques adopt petroleum ether as an extraction solvent, thus being unsuitable for foods or medical use. In addition, the foregoing arts neither
15 disclosed nor suggested methods for preparing an extract fraction reinforced with ginsenoside Rg1 or Rb1.

Moreover, although Korean Patent No. 0,444,394 describes a method for preparation of an extract having high saponin content through adsorption using an
20 adsorption resin, a process of obtaining an extract fraction reinforced with specific saponin components is not disclosed or suggested therein.

【Disclosure】

25 【Technical Problem】

PD based saponin and PT based saponin present in ginseng generally show opposing activities. Therefore, according to a relative ratio of PD based saponin to PT based saponin, the ginseng may express different activities *in vivo*. Also, with regard to a composition including a ginseng extract, there are disadvantages such that: different activities may be imparted depending upon the ratio of ginsenoside Rb1 to Rg1; and, due to opposing activities of ginsenosides Rb1 and Rg1, effects of respective saponin components obtained from a ginseng extract may not be efficiently attained. Furthermore, health drinks usually consumed by normal healthy individuals without particular diseases requiring professional management of medical personnel, entail a problem such that some ingredients having undesirable effects may be taken without consciousness.

Therefore, in order to overcome disadvantages of the ginseng saponin extract described above, the present inventors have extensively studied a method for preparation of ginseng saponin extract fractions having high ratio of specific saponin components (Rb1 or Rg1) of ginseng as well as increased content thereof and, as a result, have developed a method of sufficiently extracting saponin from ginseng and a method of obtaining a fraction reinforced with specific saponin components

(Rb1 or Rg1) from the foregoing, thereby completing the present invention.

Accordingly, an object of the present invention is to provide a method for preparing an extract fraction reinforced with ginsenoside Rg1 or Rb1 from ginseng.

Another object of the present invention is to provide a functional beverage composition with reinforced anti-fatigue effects of ginseng saponin, including an extract fraction reinforced with ginsenoside Rg1 obtained from ginseng.

Still another object of the present invention is to provide a functional beverage composition with reinforced sedative activity of ginseng saponin, including an extract fraction reinforced with ginsenoside Rb1 obtained from ginseng.

【Technical Solution】

In order to achieve the objects described above, the present invention provides a method for preparing an extract fraction reinforced with ginsenoside Rg1 from ginseng, which includes:

concentrating an alcohol extract of ginseng, diluting the concentrated extract in distilled water, and then, adding the diluted extract to an adsorption resin in order to adsorb the extract to the adsorption resin;

passing distilled water through the adsorption resin and then eluting and removing unadsorbed ingredients; and

adding 30 to 40 v/v% alcohol to the adsorption resin to obtain an eluate.

The present invention also provides a method for preparing an extract fraction reinforced with ginsenoside Rb1 from ginseng, which includes:

concentrating an alcohol extract of ginseng, diluting the concentrated extract in distilled water and then adding the diluted extract to an adsorption resin in order to adsorb the extract to the adsorption resin;

passing distilled water through the adsorption resin and then eluting and removing unadsorbed ingredients; and

adding 50 to 80 v/v% alcohol to the adsorption resin to obtain an eluate.

The extract fraction prepared according to the present invention, which is reinforced with ginsenoside Rg1 or Rb1, may be added to a functional drink as an active ingredient.

Accordingly, the present invention may provide a functional drink composition having reinforced anti-fatigue effects of ginseng saponin, which includes an extract fraction reinforced with ginsenoside Rg1 prepared

by the preparation method described above.

Alternatively, the present invention may also provide a functional drink composition having reinforced sedative activity of ginseng saponin, which includes an extract fraction reinforced with ginsenoside Rb1 prepared by the preparation method described above.

Hereinafter, the present invention will be described in detail.

The present inventors have found that, after removing non-saponin components from an alcohol extract of ginseng using an adsorption resin, an extract reinforced with ginsenoside Rg1 may be obtained through elution using 30 to 40 v/v% alcohol, while an extract reinforced with ginsenoside Rb1 may be obtained through elution using 50 to 80 v/v% alcohol. As a result, a ginseng extract reinforced with ginsenoside Rg1 or Rb1 was obtained.

Accordingly, in an aspect of the present invention, there is provided a method for preparing an extract fraction reinforced with ginsenoside Rg1 from ginseng, comprising:

concentrating an alcohol extract of ginseng, diluting the concentrated extract in distilled water and then adding the diluted extract to an adsorption resin to adsorb the extract to the adsorption resin;

passing distilled water through the adsorption resin and then eluting and removing unadsorbed ingredients; and

5 adding 30 to 40 v/v% alcohol to the adsorption resin to obtain an eluate.

In another aspect of the present invention, there is provided a method for preparing an extract fraction reinforced with ginsenoside Rb1 from ginseng, comprising:

10 concentrating an alcohol extract of ginseng, diluting the concentrated extract in distilled water and then adding the diluted extract to an adsorption resin to adsorb the extract to the adsorption resin;

15 passing distilled water through the adsorption resin and then eluting and removing unadsorbed ingredients; and

adding 50 to 80 v/v% alcohol to the adsorption resin to obtain an eluate.

20 The alcohol extract of ginseng may include any extract obtained by treating ginseng with alcohol, preferably, an extract having relatively high contents of ginsenosides Rg1 and Rb1. The extract having relatively high contents of ginsenosides Rg1 and Rb1 may be obtained by adding 60 to 80% alcohol to ginseng and agitating the mixture at 40 to 80°C for 24 to 36 hours to obtain an
25 extract. As a result of extensive studies by the present

inventors, the foregoing method has been developed and may be utilized as an extraction process yielding high concentrations of ginsenosides Rg1 and Rb1. If the extraction temperature exceeds 80°C, thermal stability of ginsenosides Rg1 and Rb1 is reduced, causing a decrease in contents of ginsenosides Rg1 and Rb1. On the other hand, when the extraction temperature is less than 40°C, extraction efficiency may be deteriorated. When extraction time exceeds the above range and too long, stability of ginsenosides and/or economic advantages may be reduced. On the other hand, if extraction time is too short, extraction efficiency may be deteriorated. More preferably, an alcohol extract is obtained by adding 65 to 75 v/v% alcohol to ginseng and agitating the same at 65 to 75°C for 24 to 36 hours to conduct extraction. Most preferably, an alcohol extract is obtained by adding 70 v/v% alcohol to ginseng and agitating the same at 70°C for 24 hours to perform extraction. The extract used herein may be prepared by repeating extraction at least three times and mixing the resultant extracts.

Then, the obtained alcohol extract of ginseng is subjected to heating and concentration. In this regard, concentration may be conducted to reach a range of 70 to 90 brix, most preferably, to about 80 brix.

Next, after adding water to the concentrated

alcohol extract to dilute the extract, the diluted extract may be introduced into an adsorption resin to be adsorbed thereto. Dilution extent with water is not particularly limited, provided that a concentrate of the alcohol extract may react with overall adsorption resin to allow saponin to be adsorbed thereto. Preferably, about 4 to 6 fold water and, more preferably, about 5 fold water the weight of the concentrated extract may be added to the concentrated extract to dilute the same. The adsorption resin may be Diaion HP-20 (Mitsubishi, Japan) and used in a packed column form to implement adsorption and fractionation.

Following this, after passing distilled water through the adsorption resin, a process of eluting and removing unadsorbed ingredients may be executed. According to this process, other components present in the alcohol extract of ginseng except for saponin may be eluted and removed. An amount of distilled water used to remove unadsorbed components is determined such that non-saponin components can be sufficiently eluted and removed, without being particularly limited. For instance, distilled water in an amount of about 5 times or more the capacity of the adsorption resin may be used. Preferably, additional non-saponin components may be eluted and removed by adding about 20 v/v% alcohol.

When non-saponin components are removed from the adsorption resin, saponin may be eluted using alcohol at different concentrations to thereby yield a saponin fraction. For ginsenoside Rg1, using 30 to 40 v/v% alcohol may elute the adsorption resin, in turn yielding an extract fraction reinforced with ginsenoside Rg1. Likewise, for ginsenoside Rb1, using 50 to 80 v/v% alcohol may elute the adsorption resin, in turn yielding an extract fraction reinforced with ginsenoside Rb1. The extract fraction reinforced with ginsenoside Rg1 may have a relative ratio of ginsenoside Rg1 to ginsenoside Rb1 of at least 0.5. On the other hand, the extract fraction reinforced with ginsenoside Rb1 may have a relative ratio of ginsenoside Rb1 to ginsenoside Rg1 of at least 2.

The ginseng used to prepare the ginseng extract described above may include any one containing saponin Rb1 or Rg1, for example, selected from a group consisting of white ginseng, undried ginseng, red ginseng, Tae-guk ginseng, black ginseng, puffing ginseng or enzyme-treated ginseng, or concentrates thereof.

【Advantageous Effects】

As apparent from the foregoing description, according to the method of the present invention, an extract fraction having reinforced ratio of ginsenoside

Rg1 to ginsenoside Rb1, as well as increased contents thereof, may be prepared from saponin components of ginseng. Moreover, a functional food reinforced with saponin components of ginseng prepared by the method according to the present invention, may be provided.

【Brief Description of the Drawings】

The above and other objects, features and other advantages of the present invention will be more clearly understood from the following detailed description, taken in conjunction with the accompanying drawings, in which:

FIG. 1 is an HPLC chromatogram of an alcohol extract obtained by extracting tail ginseng at 70°C for 24 hours using 70 v/v% alcohol;

FIG. 2 is an HPLC chromatogram of a fraction reinforced with ginsenoside Rg1, which is obtained by extracting tail ginseng at 70°C for 24 hours using 70 v/v% alcohol to obtain an alcohol extract, adsorbing the alcohol extract to Diaion HP-20 adsorption resin and eluting the adsorbed extract using 30 to 40 v/v% alcohol;

FIG. 3 is an HPLC chromatogram of a fraction reinforced with ginsenoside Rb1, which is obtained by extracting tail ginseng at 70°C for 24 hours using 70

v/v% alcohol to obtain an alcohol extract, adsorbing the alcohol extract to Diaion HP-20 adsorption resin and eluting the adsorbed extract using 50 to 80 v/v% alcohol;

5 FIG. 4 is an HPLC chromatogram of a fraction obtained by extracting tail ginseng at 70°C for 24 hours using 70 v/v% alcohol to obtain an alcohol extract, adsorbing the alcohol extract to Diaion HP-20 adsorption resin and eluting the adsorbed extract using
10 10 to 20 v/v% alcohol; and

FIG. 5 is an HPLC chromatogram of a fraction obtained by extracting tail ginseng at 70°C for 24 hours using 70 v/v% alcohol to obtain an alcohol extract, adsorbing the alcohol extract to Diaion HP-20
15 adsorption resin and eluting the adsorbed extract using 80 to 100 v/v% alcohol.

【Mode for Invention】

Hereinafter, preferred embodiments and examples of
20 the present invention will be described in detail. However, these examples are given for the purpose of illustration and are not intended to limit the invention.

**EXPERIMENTAL EXAMPLE 1 - Preparation of alcohol
25 extract of ginseng**

After adding about 15 fold (that is, 15 kg) 70 (v/v)% alcohol to 1kg of dried tail ginseng, the solution was extracted three times at 70°C for 24 hours each time, followed by filtration. Alternatively, using 60% and 80% alcohol, respectively, alcohol extracts of ginseng were prepared by the same procedures as described above.

For each of the prepared alcohol extracts obtained by the foregoing methods, contents of ginsenosides Rg1 and Rb1 were measured using HPLC. Measured results are shown in the following Table 1.

TABLE 1

Extraction solvent	Rg1 (mg/g)	Rb1 (mg/g)	Sum of contents of specific ginsenosides containing Rg1 and Rb1 (Rc, Rd, Re, Rb2) (mg/g)	Yield of extract relative to raw material (%)
50 v/v% alcohol	5.3	14.5	62.6	50
60 v/v% alcohol	6.9	20.97	71.1	45
70 v/v% alcohol	6.8	20.18	72.5	45
80 v/v% alcohol	6.38	18.02	63.9	34
90 v/v% alcohol	6.21	16.45	62.1	25

As apparent from the results, it was found that extracts obtained using 60 to 80 v/v% alcohol generally have high total content of ginsenosides Rg1 and Rb1, especially, when using 60 to 70 v/v% alcohol, the extract has the highest total content of ginsenosides Rg1 and Rb1.

EXPERIMENTAL EXAMPLE 2 - Preparation of ginseng extract using 70 v/v% alcohol

An alcohol extract of ginseng was prepared by the same procedures as described in Example 1, except that the alcohol concentration was set to 70 v/v% and the alcohol extract of ginseng was prepared while varying extraction conditions as shown in the following Table 2. Then, contents of ginsenosides Rg1 and Rb1 were measured through HPLC. Measured results are shown in Table 2.

TABLE 2

Extraction conditions	Rg1 (mg/g)	Rb1 (mg/g)	Extraction conditions	Rg1 (mg/g)	Rb1 (mg/g)
40°C - 3hr	6.023	9.928	70°C - 3hr	6.118	11.098
40°C - 6hr	5.976	10.182	70°C - 6hr	6.500	13.001
40°C - 10hr	6.204	10.799	70°C - 10hr	6.581	16.127
40°C - 24hr	6.329	11.572	70°C - 24hr	6.845	20.186
50°C - 3hr	6.136	10.698	70°C - 30hr	6.751	20.904
50°C - 6hr	6.012	10.881	70°C - 36hr	6.650	21.405
50°C - 10hr	6.068	11.404	80°C - 3hr	6.015	14.957
50°C - 24hr	6.454	13.607	80°C - 6hr	6.040	15.598
60°C - 3hr	6.014	10.852	80°C - 10hr	6.176	17.254
60°C - 6hr	6.064	11.718	80°C - 24hr	5.773	17.841
60°C - 10hr	6.054	12.744	80°C - 30hr	5.415	16.776
60°C - 24hr	6.622	16.588	80°C - 36hr	5.209	15.836

According to results of the foregoing experiments, it was confirmed that, if extraction is performed using 70 v/v% alcohol, contents of ginsenosides Rg1 and Rb1 vary slightly depending upon temperature conditions and, especially, Rb1 content is noticeably increased at 70°C. In addition, it was found that a total content of ginsenosides Rg1 and Rb1 is the highest at 70°C.

With regard to treatment time, it was found that, when treating for 24 hours or more, contents of ginsenosides Rg1 and Rb1 are remarkably increased. Although, in the case of treating at 80°C, the contents of ginsenosides Rg1 and Rb1 are decreased as treatment time increases, this may seem to be due to temperature instability.

It can be seen that an alcohol extract of ginseng is preferably prepared through treatment at 70°C for 24 hours and HPLC results of the alcohol extract obtained through treatment at 70°C for 24 hours are shown in FIG. 1.

EXAMPLE 1 - Process for adsorption of Diaion HP-20 adsorption resin

The alcohol extract of ginseng obtained through extraction at 70°C for 24 hours in Experimental Example 1, was heated and concentrated to 80 brix. Then, after adding water in an amount of 5 times the weight of the formed concentrate to sufficiently dilute the concentrate, the diluted extract was passed through Diaion HP-20 adsorption resin, thus enabling a saponin component to be adsorbed to the resin. Next, unadsorbed ingredients were removed by continuously flowing distilled water in an amount of about 5 times the capacity of the resin through the adsorption resin.

Following this, after passing 20 v/v% alcohol in an amount of about 5 times the capacity of the resin through the adsorption resin to remove non-saponin components, an alcohol concentration was regulated to obtain a desired ginsenoside fraction. By using alcohol, elution began at an alcohol concentration of 30 v/v% and continued until the alcohol concentration reached 40 v/v%, resulting in a fraction reinforced with ginsenoside Rg1. Alternatively, elution using alcohol began at an alcohol concentration of 50 v/v% and continued until the alcohol concentration reached 80 v/v%, in turn obtaining a fraction reinforced with ginsenoside Rb1. From these fractions, contents of ginsenosides Rg1 and Rb1 were respectively measured through HPLC. The measurement results are shown in Tables 3 and 4.

In addition, after using 30 to 40 v/v%, 50 to 80 v/v%, 10 to 20 v/v%, and 80 to 100 v/v% alcohol, respectively, to conduct elution, contents of ginsenosides were measured through HPLC. The measurement results are shown in FIGS. 2, 3, 4 and 5. From results shown in FIGS. 2 to 5, it can be seen that a fraction reinforced with ginsenoside Rg1 may be obtained by elution using 30 to 40 v/v% alcohol, while yielding a fraction reinforced with ginsenoside Rb1 when elution is performed using 50 to 80 v/v% alcohol.

TABLE 3

	Rg1 content
Content of ginsenoside in ginseng extract	0.7 wt.%
Ginsenoside Rg1 reinforced fraction	12 wt.% or more

TABLE 4

	Rb1 content
Content of ginsenoside in ginseng extract	2.0 wt.%
Ginsenoside Rb1 reinforced fraction	18 wt.% or more

5 As understood from the foregoing results, an extract fraction reinforced with ginsenoside Rg1 or Rb1 may be obtained by elution using an adsorption resin.

【CLAIMS】

5 **【Claim 1】** A method for preparing an extract fraction reinforced with ginsenoside Rg1 from ginseng, the method comprising:

 concentrating an alcohol extract of ginseng, diluting the concentrated extract in distilled water, and then, adding the diluted extract to an adsorption resin in order to adsorb the extract to the adsorption resin;

10 passing distilled water through the adsorption resin and then eluting and removing unadsorbed ingredients; and

 adding 30 to 40 v/v% alcohol to the adsorption resin to obtain an eluate.

15 **【Claim 2】** A method for preparing an extract fraction reinforced with ginsenoside Rb1 from ginseng, the method comprising:

20 concentrating an alcohol extract of ginseng, diluting the concentrated extract in distilled water, and then, adding the diluted extract to an adsorption resin in order to adsorb the extract to the adsorption resin;

25 passing distilled water through the adsorption resin and then eluting and removing unadsorbed ingredients; and

adding 50 to 80 v/v% alcohol to the adsorption resin to obtain an eluate.

5 **【Claim 3】** The method according to claim 1 or 2, wherein the alcohol extract of ginseng is prepared by adding 60 to 80 v/v% alcohol to ginseng and agitating the mixture at 65 to 75°C for 24 to 36 hours to conduct extraction.

10 **【Claim 4】** The method according to claim 1 or 2, wherein the alcohol extract of ginseng is concentrated to 70 to 90 brix.

15 **【Claim 5】** The method according to claim 1 or 2, wherein the adsorption resin is Diaion HP-20.