A Solubility-Based Separation of Group B Soyasaponins from the Whole Soybean Flour

Hajime KATANO,*† Shota NOBA,* Shu TAIWA,* Taiho KAMBE,** and Masakazu TAKAHASHI*

* Department of Bioscience and Biotechnology, Fukui Prefectural University, Eiheiji, Fukui 910-1195, Japan

** Division of Integrated Life Science, Graduate School of Biostudies, Kyoto University, Kyoto 606-8502, Japan

† To whom correspondence should be addressed.

E-mail: hajime@fpu.ac.jp
Abstract

This note describes a simple and rapid method to separate specific soyasaponins, including the major and multi-functional bioactive species soyasaponin Bb, from the whole soybean flour. The method is based on the difference in solubility of the soyasaponins and other components in methanol, aqueous borax solution, and 1-octanol. First, the whole soybean flour was mixed with methanol to extract hydrophobic components, and the methanol solution was concentrated. Second, the methanol solution was mixed with a larger volume of aqueous borax solution, and the supernatant was acidified to obtain a precipitate. The precipitate was found to be containing mainly four group B soyasaponins. Two of those are non-conjugated molecules, soyasaponin Bb and Bc, and other two are 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one conjugated ones, soyasaponin βg and βa. All of them have two cis-diol groups in their structure. Therefore, although they are fundamentally insoluble in water, they became soluble in the aqueous medium in the form of divalent anionic species by the borate ester formation, and that they were re-precipitated by the hydrolysis. Furthermore, the non-conjugated group B soyasaponins could be separated by washing with 1-octanol to remove highly hydrophobic soyasaponin βg and βa. The solubility-based technique would be useful as pretreatment in purification of these group B soyasaponins.

Keywords: Separation, soyasaponin Bb, soyasaponin Bc, soyasaponin βa, soyasaponin βg, whole soybean flour.
Introduction

Soyasaponins have been a focus of research interest due to their diverse biological activities. Soyasaponin Bb is the most intensively studied type because of its abundance and its significant biological activities.\(^1\)\(^-\)\(^5\) In a previous paper,\(^6\) we presented a rapid and convenient method for the separation of soyasaponin Bb, from a commercially available partially-purified soyasaponin, which contained the target substance at the 10 wt% level. The method was based on the difference in the solubility of soyasaponin Bb and other components in organic-water mixed solvents. The yield was much larger than that by conventional methods, such as extraction and preparative liquid chromatographic techniques. Thus, the solubility-based method is useful to obtain soyasaponin Bb. However, a different operation procedure is required for the separation from other starting material, \textit{e.g.} the whole soybean.

This note describes a solubility-based separation of soyasaponins from easily available resource, the whole soybean. The method involves (i) extracting hydrophobic components into methanol, (ii) dissolving into a borax aqueous solution and re-precipitating to separate the components which formed borate ester. The precipitate was found to be containing mainly soyasaponin Bb, Bc, \(\beta_g\), and \(\beta_a\) (Fig. 1). Thus, soyasaponins having two \textit{cis}-diol groups could be successfully separated from soybean. Furthermore, (iii) by washing the precipitate with 1-octanol, the 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) conjugated soyasaponin \(\beta_a\) and \(\beta_g\) were successfully removed, and the precipitate came to consist of non-conjugated soyasaponin Bb and Bc. At present, each soyasaponin cannot be isolated from the resulting precipitate by such solubility-based method, and the preparative HPLC technique has been applied. However, these findings would be valuable for the sample preparation of this kind of natural products with \textit{cis}-diol groups.
Experimental

Soyasaponin Bb was obtained from Funakoshi Co., and was used as the standard material. Soybean available on the market was homogenized with a household mill, and the resulting flour was used as a starting material. Other chemicals were of reagent grade. The separated samples were characterized by the reversed-phase chromatography with an HPLC system (Thermo, MultiMate). The chromatographic condition was the same as that described in the previous paper.  

Results and Discussion

In the first operation, a methanol extract was prepared as follows. A 5 g quantity of the soybean flour was mixed with 50 mL of methanol with stirring for 30 min at ambient temperature; the mixture was transferred into a centrifugation tube (50 mL) and centrifuged; and the supernatant was transferred into a tall beaker (50 mL). The volume of the supernatant was ca. 40 mL, indicating that ca. 10 mL of the solvent soaked into the soybean powder. The methanol extract was concentrated as follows: the supernatant was heated at 70°C with stirring to evaporate methanol until the volume was decreased to several mL; the mixture was transferred into a centrifugation tube (15 mL) and centrifuged; and the supernatant was transferred into another centrifugation tube (50 mL). The operation is illustrated by sequence (1a) and (1b) in Fig. 2. It is noted that, when the solvent was evaporated completely, the yield of the soyasaponins was decreased significantly. Probably, some components might prevent the re-dissolution of the soyasaponins. Panel (1) in Fig. 3 shows a chromatogram of the supernatant (soln-1), and many elution peaks were observed. Using a standard material, the elution peak for soyasaponin Bb ($t = 20.7$ min; $m/z = 943.52$) could be assigned as indicated in the figure, and the amount in the
methanol extract was estimated to be ca. 1 mg. It is noted that, although the evaporation was carried out under a milder condition, the final result was not changed so much.

We found that soyasaponin Bb is insoluble with water, but becomes soluble in the presence of Na$_2$B$_4$O$_7$. This may be due to the borate ester formation of two cis-diol groups in the galactose and rhamnose moieties. By the acidification, the borate ester would be hydrolyzed, and soyasaponin Bb would be re-precipitated in free acid form. Thus, the second operation was performed as follows (sequence (2a) in Fig. 2). The supernatant was mixed with 40 mL of the 0.02 mol/L Na$_2$B$_4$O$_7$ aqueous solution (pH 9.2), the mixture was centrifuged, and the supernatant was transferred into another centrifugation tube. The supernatant was then mixed with 1 mL of the 5 mol/L HCl solution, the mixture was centrifuged, and the supernatant was removed to obtain a precipitate. The precipitate was transferred into a smaller centrifugation tube as follows (sequence (2b) in Fig. 2). The precipitate was dissolved with 5 mL of the 0.02 mol/L Na$_2$B$_4$O$_7$ solution, the mixture was centrifuged, and the supernatant was transferred into another centrifugation tube (15 mL). Finally, the supernatant was mixed with 1 mL of the 1 mol/L HCl solution, the mixture was centrifuged, and the supernatant was removed to obtain the precipitate.

When the precipitate was mixed with methanol, the mixture was turbid, indicating the contamination of a colloidal substance. In order to remove the substance, the precipitate was treated as follows (sequence (2c)). The precipitate was mixed with 1 mL of methanol, the mixture was centrifuged, and the supernatant was transferred into another centrifugation tube (15 mL). The supernatant was mixed with 8 mL of water and 1 mL of the 1 mol/L HCl solution, the mixture was centrifuged, and the supernatant was removed to obtain the precipitate (ppt-2). The ppt-2 precipitate was dissolved completely in 1 mL of methanol, and the solution gave the chromatogram shown in panel (2) in Fig. 3. Four well-developed elution peaks, including soyasaponin Bb, were observed. The amount of soyasaponin Bb was estimated to be ca. 0.8 mg,
which was smaller than the amount detected in the methanol extract.

The four elution peaks are separated enough from each other, so that the last three eluates could be isolated by the preparative HPLC technique. They gave MS signals of \( m/z = 913.51, 1069.56, \) and 1039.54, and are identified to be soyasaponin Bc, \( \beta_g \), and \( \beta_a \), respectively, as indicated in Panel (2) in Fig. 3. All the four compounds including Bb have two cis-diol groups in rhamnose and galactose (Bb and \( \beta_g \)), or arabinose (Bc and \( \beta_a \)) moieties. They would be soluble in the aqueous borax medium in the form of divalent anionic species by the borate ester formation, and be re-precipitated by the hydrolysis. It is noted that other major soyasaponins Aa, Ab, and Ba, which have only one cis-diol group, were found in the methanol extract, but their peaks were not observed significantly in Panel (2) in the figure.

The reversed-phase chromatogram indicates that DDMP-conjugated group B soyasaponins are more hydrophobic than non-conjugated ones, and that the former may be removed by washing the ppt-2 precipitate with a low-polarity solvent. Thus, as illustrated by sequence (3) in Fig. 2, the ppt-2 precipitate was mixed with 1 mL of 1-octanol, the mixture was centrifuged, and the supernatant was removed to obtain a precipitate (ppt-3). The ppt-3 was dissolved in 1 mL of methanol. Panel (3) in Fig. 3 shows a chromatogram of the ppt-3. Although the amount of soyasaponin Bb was reduced to ca. 0.6 mg, the non-conjugated group B soyasaponins were successfully isolated. The soyasaponin Bb and Bc would be provided by hydrolysis of soyasaponin \( \beta_g \) and \( \beta_a \), respectively.

At present, we cannot find an appropriate solution condition for the solubility-based separation of soyasaponin Bb from the ppt-3. Therefore, soyasaponin Bb has been isolated by the preparative HPLC technique. Thus, several milligrams of soyasaponin Bb with a high purity (96%, Fig. S1) could be prepared from 100 g of soybean. The yield is somewhat less than those by repetition of the preparative HPLC.\(^{9,10}\) However, the present separation method using the borax medium can easily provide the specific group B soyasaponins, and would be useful as
pretreatment in the purification of them.

Acknowledgments

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Supporting Information

Chromatograms for the soyasaponin Bb standard material and the purified product from ppt-3. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci.

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Figure Captions

Fig. 1 Chemical structure of non-conjugated type (Bb, Bc) and DDMP-conjugated type soyasaponin (βa, βg).

Fig. 2 Preparation of (1) the methanol extract (soln-1), (2) the borax solution soluble substance (ppt-2) and (3) the 1-octanol insoluble substance (ppt-3).

Fig. 3 Chromatograms for (1) soln-1, (2) ppt-2, and (3) ppt-3.
Fig. 1  Chemical structure of non-conjugated type (Bb, Bc) and DDMP-conjugated type soyasaponin (βa, βg).
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Graphical Index

Soybean flour

Extraction into MeOH → Treatment with the Na$_2$B$_4$O$_7$ solution

Soyasaponin Bb βg Bc βa

|   | $R_1$   | $R_2$   |
|---|---------|---------|
| Bb | OH      | CH$_3$OH |
| Bc | OH      | H       |
| βg | DDMP    | H       |
| βa | DDMP    | CH$_3$OH |