Distributions of Saponin Constituents in Some Varieties of Soybean Plant

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Saponins are bioactive substances with physiological activities and bitter taste. We investigated the distributions of saponins in soybean plants. Acetyl-soyasaponins A₁ and A₄ occur only in seed hypocotyls of soybean plants. Soyasaponin I was detected in all organs of the plant. However, soyasaponin I levels in plant showed very heterogenous distributions, for example, stem and main root had very low soyasaponin I levels, but nodule and leaf had higher levels.

It is reported that soybean seeds (the seeds of Glycine max (L.) Merrill) have many kinds of glycosides such as saponins and isoflavonoids. Kitagawa et al. and others have reported the structures of saponins in soybean seeds; soyasaponins I, II, III, IV, V, A₁, A₂, A₃, A₄, A₅ and A₆, and these constituents have many physiological activities. Further, it was suggested that especially acetyl-soyasaponins have more undesirable tastes than non-acetylated constituents.

The saponin distributions in soybean seed have been reported and the saponin levels were higher in hypocotyl than in cotyledon, and especially acetyl-soyasaponins and soyasaponin V occur almost only in hypocotyl. There were little saponins in the seed coat, but no study was attempted to find the saponin distributions in soybean plant organs.

In this paper, we report the distributions of saponin constituents in soybean plant organs.

Materials and Methods

Soybean plants. The varieties of soybean plants used to analyze the saponin composition were Moshidou Gong 503, Keburi, Bonminori, Sakagami 2, T229, and Shakujo, which were grown at the National Institute of Agrobiological Resources, Ministry of Agriculture, Forestry and Fisheries of Japan. Four plants of each variety were harvested in September 1986, before complete maturation.

Extractions and analyses of saponins from soybean plants. Each soybean plant was divided into organs: stem, petiole, branch, leaf, pod shell, immature seed hypocotyl, immature seed cotyledon, main root, lateral root, root hair, and nodule. Then these samples were lyophilized with a freeze dryer (FD-550; Tokyo Rikakikai Co., Ltd.). Each sample was milled, then extracted in screw-capped test tubes with 20 volumes of 70% ethanol for 5 hr at 80°C. After cooling, extracts were used directly in thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) and about their saponin constituents analyzed.

Thin layer chromatography. TLC was done using precoated silica gel plates (Kieselgel 60 F-254; Merck) with chloroform–methanol–water (65:35:10, v/v lower layer). Spots were colored by spraying with 10% sulfuric acid and heating for 10 min at 120°C.

High performance liquid chromatography. The instrument used was a EYELA PLC-20 (Tokyo Rikakikai Co., Ltd.) with a UV detector (205 nm). Injection was done with a Rheodyne injection valve, model 7125. The column
used was a LiChrosorb RP-18 (5 μm, 250 × 4.0 mm i.d.; Merck). Two mobile phases were used: solvent A was acetonitrile–1-propanol–water–acetic acid (32.3:4.2:63.4:0.1, v/v) and solvent B, methanol–1-propanol–water–acetic acid (70.0:6.0:23.9:0.1, v/v). The flow rate was 0.5 ml/min.

Preparation of purified saponin components. The authentic compounds for the quantitative analysis of saponin constituents were isolated as follows. Soybean seed hypocotyls (Miyagishimore and Nakasennan) were milled. Milled samples were extracted with 70% ethanol for 5 hr three times. The combined 70% ethanol extracts were concentrated under reduced pressure, dispersed in butanol–water (1:1, v/v), and left overnight. The upper layer was collected and lyophilized to give a crude glycosides fraction (11 U). Then the 11 U was filtered on Sephadex LH-20 gel; (Pharmacia) and eluted with methanol to separate bisdesmoside- and monodesmoside-type saponin fractions. Each fraction was used in HPLC (LiChrosorb RP-18; 5 μm, 250 × 7.6 mm i.d.; Merck). Mobile phases used to purify saponin constituents were the same compositions as those used for HPLC analyses.

Chemicals. Ethanol used for extraction was of guaranteed grade (Nakarai Chemicals, Ltd.). The solvents for HPLC were specially prepared grade (Nakarai Chemicals, Ltd. or Wako Pure Chemical Industries, Ltd.).

Results

Weight of soybean plant organs

The dry weights of soybean organs are summarized in Table I. These suggested that the dry weights of pods which involve seed and shell were greater than those of any other organs. In particular, in the Keburi variety these were more than 70% of the total weight, but it is likely that these differences were not due to the differences between varieties but due to the extent of maturation of every plant. Sakagami 2, a late-ripening variety showed that weight of pod was small and those of leaf, stem, and branch were large in September.

Qualitative distributions of saponins in soybean plants and immature seeds

At first, to analyze saponin constituents in soybean plant, the 70% ethanol extracts obtained from every organ harvested in 1986 were chromatographed by TLC. Acetylsoyasaponins A1 and A4 were detected in only seed hypocotyls and never in other plant organs (Fig. 1), and these distributions were also confirmed by HPLC. On TLC, bands corresponding to soyasaponin A1, etc. are shown, but were not identified by HPLC (data not shown).

Soyasaponin I was detected in all plant organs by TLC and HPLC (Figs. 1 and 2), and other constituents, e.g., soyasaponins II, and III, were rarely detected.

With regard to saponins, there were little differences on TLC among plant organs, but there were in other constituents, e.g., some constituents with greater Rf values showed very different patterns among organs (Fig. 1).

\[Table\ 1.\  Dry\ Weights\ of\ Soybean\ Plant\ Organs\ (\%)
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<tbody>
<tr>
<td>Leaf</td>
<td>11.1</td>
<td>1.3</td>
<td>17.5</td>
<td>15.0</td>
<td>26.1</td>
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<tr>
<td>Stem</td>
<td>5.1</td>
<td>12.4</td>
<td>10.1</td>
<td>22.2</td>
<td>14.2</td>
<td>26.6</td>
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<tr>
<td>Branch</td>
<td>7.4</td>
<td>7.5</td>
<td>7.9</td>
<td>12.4</td>
<td>4.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Petiole</td>
<td>4.8</td>
<td>0.8</td>
<td>10.5</td>
<td>13.8</td>
<td>5.9</td>
<td>7.3</td>
</tr>
<tr>
<td>Pod</td>
<td>68.4</td>
<td>71.9</td>
<td>42.9</td>
<td>25.7</td>
<td>55.4</td>
<td>32.4</td>
</tr>
<tr>
<td>Root</td>
<td>2.6</td>
<td>6.1</td>
<td>4.9</td>
<td>5.9</td>
<td>5.2</td>
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</tr>
<tr>
<td>Nodule</td>
<td>0.2</td>
<td>0.2</td>
<td>0.9</td>
<td>0.5</td>
<td>0.3</td>
<td>0.5</td>
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</table>

Abbreviations: Mos., Moshidou Gong 503; Keb., Keburi; Bon., Bonminori; Sak., Sakagami 2; Sha., Shakujo.

Fig. 1. TLC Patterns of 70% Ethanol Extracts from Soybean Plant Organs.
The variety used was Keburi. A, acetyl-soyasaponin A1 or A4; B, soyasaponin I or V; cont., crude glycoside fraction and see Materials and Methods. 1, leaf; 2, stem; 3, branch; 4, petiole; 5, hypocotyl; 6, cotyledon; 7, pod shell.
Saponin Distribution in Soybean Plants

Fig. 2. HPLC Patterns of 70% Ethanol Extracts from Soybean Plant Organs.
A, immature hypocotyl of Keburi; B, stem of Sakagami 2; C, leaf of Moshidou Gong 503. I, soyasaponin I; V, soyasaponin V; mobile phase, solvent B.

Fig. 3. Calibration Curve of Soyasaponin I.
Mobile phase, solvent B.

The quantitative differences of saponin distributions were estimated by HPLC.

Soyasaponin I levels in soybean plant organs
To measure soyasaponin I levels with HPLC, a calibration curve was prepared (Fig. 3). A linear relationship between the peak height and soyasaponin I concentration was obtained in the range of 0.02–0.58 mg/ml with a high correlation (r = 0.9999), and recovery of soyasaponin I was 99.6 ± 3.0%. On the basis of this calibration curve, saponin levels were estimated as percentages by weight. Some chromatograms of plant samples are summarized in Fig. 2.

Saponin levels are summarized in Table II. Soybean plant organs were roughly separated into three parts; seed parts, aerial parts, and underground parts. In seed parts, which involved immature seeds, the soyasaponin I level in hypocotyl was the highest, from 0.50 (var. Keburi)–1.90 (T229)% and more than 4-fold those in cotyledon and pod shell; in Keburi it was more than 16-fold. And the soyasaponin I level in hypocotyl was the highest in all organs.

<table>
<thead>
<tr>
<th>Table II. Soyasaponin I Levels in Soybean Plant Organs (%)</th>
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<tr>
<td>-----------------------------------------------------------</td>
</tr>
<tr>
<td>Hypocotyl</td>
</tr>
<tr>
<td>Cotyledon</td>
</tr>
<tr>
<td>Pod shell</td>
</tr>
<tr>
<td>Leaf</td>
</tr>
<tr>
<td>Stem</td>
</tr>
<tr>
<td>Branch</td>
</tr>
<tr>
<td>Petiole</td>
</tr>
<tr>
<td>Main root</td>
</tr>
<tr>
<td>Lateral root</td>
</tr>
<tr>
<td>Root hair</td>
</tr>
<tr>
<td>Nodule</td>
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</table>

* Whole seed.
Abbreviations: see Table I.
of the soybean plants used. Then, in aerial parts, the soyasaponin I level in leaf was higher (0.15 to 1.1%) than any other organs. This level was next to that in seed hypocotyl. The levels in stem, branch, and petiole were less than 0.30% and almost equal to those in cotyledon and pod. In underground parts, the soyasaponin I level decreased in the order, root hairs, lateral root, and main root, and that in nodules was between lateral root and root hairs.

The soyasaponin I levels of Keburi in most organs were lower than any other varieties in most organs, and the levels of Moshidou Gong 503 and Bonminori were next to those of Keburi. These saponin-poor varieties have acetyl-soyasaponin $A_4$ in seed hypocotyls. In contrast, soyasaponin I levels of Sakagami 2, T229, and Shakujo, which have acetyl-soyasaponin $A_1$ instead of $A_4$, were slightly higher.

Acetyl-soyasaponins $A_1$ and $A_4$ and soyasaponin $V$ levels in immature seed organs

Acetyl-soyasaponins $A_1$ and $A_4$ and soyasaponin $V$ were analyzed by HPLC under the same conditions as for soyasaponin I. Acetyl-soyasaponins $A_1$ and $A_4$ and soyasaponin $V$ occurred only in immature hypocotyls of soybean plants. Their levels were measured only in immature seed hypocotyls (Table III).

Hypocotyls of Moshidou Gong 503 and Keburi have acetyl-soyasaponin $A_4$, but Sakagami 2, T229, and Shakujo have acetyl-soyasaponin $A_1$ instead of $A_4$. Shiraiwa et al. found that there were varietal differences of acetyl-soyasaponin type in hypocotyl and that acetyl-soyasaponins $A_1$ and $A_4$ were controlled by codominant allelic alternatives at single locus. Acetyl-soyasaponins $A_1$ or $A_4$ levels were very high (1.20–2.91%) and those of 4 varieties, with an exception of Sakagami 2, were even higher than soyasaponin I levels in immature hypocotyls.

Soyasaponin $V$ levels in these varieties were lower (0.20–0.70%) than acetyl-soyasaponins $A_1$ and $A_4$ and soyasaponin I levels in immature hypocotyls. Further, differences of the levels among varieties were much larger than those of acetyl-soyasaponins $A_1$ and $A_4$.

**Discussion**

To date, several saponins in soybean seeds have been reported and characterized. We considered acetylated saponins as intact constituents at least for saponins with a soyasapogenol A. Acetyl-soyasaponins have some acetyl groups which are concentrated at one terminal sugar (glucose or xylose) of a sugar chain linked to the $C_{22}$ position of soyasapogenol A. In this study, it was shown that these constituents occurred only in seed hypocotyls and that their levels were unusually high (1.2–2.9%), thus these findings coincide with previous reports. Behaviors of these acetyl-soyasaponins during seed maturation or germination are interesting problems. Soyasaponin $V$, which was isolated in haricot bean and hypocotyl of soybean seeds, was also detected but only in immature seed hypocotyls in this study. This distribution was similar to those of acetyl-soyasaponins $A_1$ and $A_4$, but its level was much lower.

In contrast with these constituents, soyasaponin I was detected in all organs of soybean plants, but they were concentrated at some organs: seed hypocotyls (1.9% in T229), leaves (1.1% in Shakujo), and root hairs (0.8% in Sakagami 2). These levels were very high, but lower than acetyl-soyasaponins $A_1$ and $A_4$ levels in hypocotyls. On the other hand, soyasaponin I levels in other organs were much lower; pod shells (0.01%) in Moshidou

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**Table III. Acetyl-soyasaponins and Soyasaponin V Levels in Immature Seeds Hypocotyls (%)**

<table>
<thead>
<tr>
<th></th>
<th>$A_1$</th>
<th>$A_4$</th>
<th>$V$</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moshidou Gong 503</td>
<td>—</td>
<td>2.91</td>
<td>0.42</td>
<td>4.26</td>
</tr>
<tr>
<td>Keburi</td>
<td>—</td>
<td>1.39</td>
<td>0.20</td>
<td>2.09</td>
</tr>
<tr>
<td>Sakagami 2</td>
<td>1.20</td>
<td>—</td>
<td>0.48</td>
<td>3.06</td>
</tr>
<tr>
<td>T229</td>
<td>2.88</td>
<td>—</td>
<td>0.70</td>
<td>5.48</td>
</tr>
<tr>
<td>Shakujo</td>
<td>2.50</td>
<td>—</td>
<td>0.38</td>
<td>4.18</td>
</tr>
</tbody>
</table>

*Abbreviations: $A_1$, acetyl-soyasaponin $A_1$; $A_4$, acetyl-soyasaponin $A_4$; $V$, soyasaponin $V$.***
Gong 503), stems (0.03% in Keburi), and main roots (0.06% in T229) and decreased more than one order. In other words, sotasaponin I levels may be higher in some organs which are physiologically active parts; e.g., hypocotyl, leaf, and root hairs, but lower in other organs which support the plant.

In comparison, ginsenosides showed the highest levels in lateral roots, more than 6%\(^{17}\). Saikosaponin levels, which were rather lower than ginsenosides, showed similar trends, namely from the main root (0.54%) to root hairs (5.58%), and there are reports that these levels have some high negative correlations with root diameters.\(^{18}\) The tendency of saponin levels found in these medicinal plants roots might be also related to saponin levels in soybean plants, although the level in ginseng lateral root was much higher than even those of soybean root hairs which showed the highest levels in soybean roots.

In addition, by investigating physiological roles of saponins in soybeans, we will be able to control these saponin constituents as medicinal components in foodstuffs, and use legume seed foods for human health.

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References

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