Components Responsible for the Undesirable Taste of Soybean Seeds

Kazuyoshi OKUBO, Miyuki IJIMA, Yuji KOBAYASHI, * Masaki YOSHIKOSHI, ** Teiji UCHIDA, *** and Shigemitsu KUDOU****

Faculty of Agriculture, Tohoku University, 1–1 Tsutsumitori, Amamiya-machi, Aoba-ku, Sendai 981, Japan
* Marusanai Co., Ltd., 1 Niki Machi, Arashita, Okazaki 444–21, Japan
** Nestle Co., Ltd., 2–4–5 Azabudai, Minato-ku, Tokyo 106, Japan
*** Kanesa Co., Ltd., 202 Hamada, Tamagawa, Aomori 030, Japan

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The components responsible for undesirable taste in soybeans have been investigated and intensities of undesirable taste were measured by electrophysiological methods. Bitterness and astringency in soybeans were shown to be caused by soybean glycosides such as saponins and isoflavones, and the soybean saponin A group contributes most strongly to the undesirable taste. In the three electrophysiological methods to measure intensity of undesirable tastes, soybean saponins did not induce the membrane potential change of neuroblastoma cells (N-18 clone) and the electrical response of the chorda tympani nerve of rats, and only the electrical response of the glossopharyngeal nerve of the frog was induced by soybean saponins. These results showed that the mechanism of the undesirable tastes caused by soybean saponins was likely to be different from those of basic tastes (sweet, salty, sour, and bitter).

Soybean foods, such as soy milk, have an undesirable taste, usually described as “bitter,” “astringent,” “rough,” and “dry mouth feel.” Such undesirable taste characteristics limit the use of soybeans in new food materials. We have investigated the identity of the soybean components possessing these undesirable taste properties.

Nine phenolic acids from defatted soybean meal, 1) ethyl-α-D-galactopyranoside and t-cryptophan, 2) three oxidized phosphatidylethanolamines, 3)–5) isoflavones, 6) and soyasapogenol 7)–8) have been already reported to contribute to the undesirable taste of soybeans and their product. The tastes of these components were described in various ways including “dry mouth feel,” “rough,” “astringency,” etc. It appeared that all of these words expressed “undesirable feeling” rather than “taste.” We therefore defined these undesirable sensations as DMF (dry mouth feel) activity.

In this paper the characterization of the compounds with DMF activities in soybeans is described and the quantitative measurement of DMF activity is reported.

Materials and Methods

Extraction and fractionation of undesirable taste components from soybeans. After whole soybean seeds were ground, the resulting meal was extracted with a 5-fold volume of 70% aqueous ethanol at 70°C for 5 hr. The residue was filtered, and was extracted a further 3 times under the same conditions. The filtrates were combined and evaporated to dryness in vacuo. The extract was dissolved in methanol and put on a Sephadex LH-20 column (5 × 74 cm). The components were eluted with methanol, fractionated (7.5 g/tube), and examined by TLC.

Preparation of soybean glycosides. Soybean saponins with soyasapogenol A, B, and E as aglycones (i.e., soybean saponin groups A, B, and E 9)–10) were prepared as follows; the 70% ethanol extract of the whole soybean meal was partitioned between water and n-butanol (1:1, v/v). The upper layer was separated, evaporated to dryness, and dissolved in small amount of methanol. After addition of a 9-fold volume of ether, the resulting precipitate was collected by centrifugation, and further fractionated by gel filtration using Sephadex LH-20. A portion of each fraction was hydrolyzed by 2N HCl and the released aglycones examined by TLC. Fractions containing soyasapogenol A, B, and E as aglycones were combined and designated soybean saponin A, B, and E groups, respectively.

Soybean saponins Aa, Ab, Ba, and Bb were isolated by preparative HPLC as described by Shiraiwa et al. 10) Genistin, daidzin, and their aglycones were isolated by the method of Kudou et al. 11) Aglycones of soybean saponins were prepared from acid hydrolysates of their corresponding glycosides.

Thin layer chromatography. Thin layer chromatography was done on Kieselgel 60 F 254 plates (Merck) using chlorormom–methanol–water (65:35:10, v/v lower layer) for the glycoside and benzene–dioxane–acetic acid (100:25:5, v/v) for the aglycone. The components of TLC plate were visualized by spraying with 10% H 2 SO 4 , then heating at 120°C for 10 min.

High performance liquid chromatography. Individual soybean saponins were isolated on a LiChrosorb RP-18 column (5 μm 250 × 7.6 mm; Merck) using methanol–water–propanol–acetic acid (70:0.23:6.0:0.1, v/v) for B group saponins and acetonitrile–1-propanol–water–acetic acid (32.3:42.6:3.4:0.1, v/v) for A group saponins. The instrument used was a Hitachi 655-15 Liquid Chromatograph with RI detector (Erma REC-7520).

Organoleptic assessment. The panelists were university students who had experience in assessing bitterness and astringency. All samples were dissolved or suspended in distilled water and diluted to 10 −4 M or 0.1% solution. A few drops of each sample solution were put on the tongue and the taste characteristics reported.

Threshold value. The stock sample solution (10 −4 M) was diluted stepwise in tenths to a final concentration of 10 −11 M. The tests were initially done with 10 −11 M sample solution and continued until the panelists detected the taste of the sample. This lowest detectable concentration was defined as the threshold value.

Undesirable intensity. Caffeine and naringin were used as the standards of bitterness and astringency, respectively. Solutions of each (0.05% w/v) were compared with 0.05% (w/v) test solutions. The intensities of the stimulating solutions were rated on a four point scale (0, absent; 1, weaker than standard; 2, equivalent to the standard; 3, stronger than standard).

Measurements of DMF activities using neuroblastoma cell. A clonal cell line N-18 cell derived from mouse C-1300 neuroblastoma was used in this study. Culturing of the cell line and fluorescence measurement with Rhodamine 6G were done using the procedure of Miyake et al. 12)

The N-18 cells were maintained in monolayer culture for 7–10 days, removed by treatment with a Ca 2+ - and Mg 2+ -free Ringer solution containing 0.5 mm EDTA and suspended in a Ringer solution. The cells were centrifuged and resuspended in Ringer solution (final concentration
The composition of the Ringer solution was 150 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl₂, and 0.8 mM MgCl₂. The pH of the solution was adjusted to 7.5 with 5 mM HEPES-NaOH buffer.

A small volume of Rhodamine 6G (final concentration 0.3 µM/ml) was added to the cell suspension. After incubation for 20 min at 30°C, the fluorescence of the suspension was measured with a Hitachi FP-2A at 30°C (excitation wavelength 526 nm, emission wavelength 555 nm). Thereafter, the sample (final concentration 10⁻⁴ M) or the Ringer solution as control was added to the cell suspension containing Rhodamine 6G. After incubation for 20 min at 30°C, the fluorescence of the suspension was measured. The intensity of the control was defined as 100 and the increase of the fluorescence was considered to be the intensity of the bitterness stimulus.

Measurement of DMF activity using the glossopharyngeal nerve of the frog. A bullfrog (Rana catesbeiana) weighing about 300 g was anesthetized with an intraperitoneal injection of a 20% (w/v) urethane solution (1.5 ml/g body wt.). The glossopharyngeal nerve on either side was exposed from the surrounding connective tissues and cut near the hyoid bone. To avoid the muscular contraction of the tongue, the hypoglossal nerves of both sides were also cut. The tongue was removed and its anterior portion was fixed with pins on an experimental chamber. The electrical activities of whole glossopharyngeal nerves attached to two silver hook electrodes were amplified with an Extra Cellular Amplifier and integrated with an electronic integrator with a time constant of 0.2 sec (Dai Medical System Co. Type DAP-100E) and displayed on a pen recorder (Fig. 1). When experiments were not in progress, the tongue was always soaked in a low ionic strength solution of 5 mM HEPES-NaOH buffer (pH 7.3) containing 5 mM NaCl. The samples were dissolved in the low ionic strength solution, because the frog responded to the distilled water. Sample solutions were applied to the tongue with a Pasteur pipette, at a flow rate of 3.0 ml/min. Immediately after applying the sample solution, the tongue was rinsed with the low ionic strength solution. About 10 min was interposed between each stimulation experiment. The response to 1 mM quinine sulfate or 0.5 mM CaCl₂ solution was measured at an appropriate interval and used as a standard response to correct the data. The height of the integrated response at the highest peak was used as the magnitude of the response. In the figures are shown the extrapolations of the linear concentration vs. relative peak height plot to the X-axis intercept. This intercept was called the threshold value. The intensity of the response in the standard solution was taken as 1.

Measurement of DMF activity using the chorda tympani nerve of the rat. The method was described by Yoshii et al. An male Wistar albino rat weighing 200—300 g was anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg body wt.), and maintained at a surgical level of anesthesia with supplemental injection of sodium pentobarbital. The animal was subjected to tracheotomy and the chorda tympani nerve was exposed by removing the condyloid and coronoid processes of the mandible and retracting the underlying musculature. The nerve was cut approximately near its entrance to the bulla.

The neural activity of the whole chorda tympani nerve attached to a silver hook electrode was recorded by the procedure described above. The temperature was maintained at 29—31°C. Stimulating solutions were applied to the tongue through a funnel at a flow rate of 20 ml/min. The tongue was rinsed with deionized water; the stimulation experiments were done at appropriately 5-minute intervals.

Results and Discussion

Fractionation of components from whole soybean with undesirable taste

Whole soybean meal was extracted 3 times with 70% ethanol. It was considered that all of components of interest were extracted with 70% ethanol, because the residue lacked undesirable taste completely. The 70% ethanol extract was fractionated by Sephadex LH-20 gel filtration. TLC analysis confirmed that the major components were soybean saponin A group (fractions No. 31—36), B group (No. 42—44), daidzin (No. 86—108), and genistin (No. 126—150). The yield and taste characters of the fractions are summarized in Table 1. All fractions had undesirable tastes (described as bitter, astringent, sweet with aftertaste, etc.). Fractions No. 31—44 (soybean saponins) and No. 86—200 (isoflavones) had much stronger undesirable tastes than the others. Some compounds with undesirable tastes in soybean have already been reported, but these results show that saponins and isoflavones contribute most strongly to undesirable tastes. The taste characteristics of soybean saponins and isoflavones were undesirable, with bitterness and astringency respectively. The soybean saponin A group

<table>
<thead>
<tr>
<th>Fr. No.</th>
<th>Major component</th>
<th>Yield (%)</th>
<th>Taste characters</th>
</tr>
</thead>
<tbody>
<tr>
<td>25—30</td>
<td>Tr.</td>
<td>0.8</td>
<td>Bitter, rough</td>
</tr>
<tr>
<td>31—36</td>
<td>A, lecithin</td>
<td>1.7</td>
<td>Intense bitter, lingering aftertaste</td>
</tr>
<tr>
<td>37—41</td>
<td>A, B+lecithin</td>
<td>5.0</td>
<td>Intense bitter</td>
</tr>
<tr>
<td>42—44</td>
<td>B, unknown</td>
<td>5.7</td>
<td>Bitter</td>
</tr>
<tr>
<td>45—51</td>
<td>B, stachyose</td>
<td>11.4</td>
<td>Rough</td>
</tr>
<tr>
<td>52—53</td>
<td>B, raffinose</td>
<td>3.1</td>
<td>Rough</td>
</tr>
<tr>
<td>54—66</td>
<td>B, sucrose</td>
<td>55.4</td>
<td>Sweet with slight bitter</td>
</tr>
<tr>
<td>67—85</td>
<td>B</td>
<td>4.8</td>
<td>Dry mouth feel</td>
</tr>
<tr>
<td>86—108</td>
<td>Daidzin</td>
<td>3.5</td>
<td>Intense biting taste, astringent</td>
</tr>
<tr>
<td>109—117</td>
<td>Unknown</td>
<td>0.8</td>
<td>Sour with aftertaste</td>
</tr>
<tr>
<td>118—125</td>
<td>Genistin, unknown</td>
<td>1.0</td>
<td>Sour, slight biting taste</td>
</tr>
<tr>
<td>126—150</td>
<td>Genistin</td>
<td>3.6</td>
<td>Metallic, astringent</td>
</tr>
<tr>
<td>151—200</td>
<td>Isoflavone</td>
<td>3.2</td>
<td>Astringent</td>
</tr>
</tbody>
</table>

*Tr. trace. A or B, soybean saponin A or B group.*

Table 1. Major Components, Yield, and Taste Characteristics of Fractions from Whole Soybean Meal by Gel Filtration

![Fig. 1. The Schematic Diagram of Experimental System Used.](image-url)
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(No. 31–36) had the strongest undesirable taste of all the fractions.

The threshold value and the undesirable intensity of soybean glycosides
Soybean saponin A, B, and E groups, and isoflavone glycosides were prepared as described in Materials and Methods, to characterize the tastes of soybean glycosides in detail. The threshold value and the characteristics of isolated glycoside components are shown in Fig. 2. The threshold value of the soybean saponin A group are $10^{-7}$—$10^{-9}$ M, which is the lowest of all glycosides. The taste characteristic was undesirable with intense bitterness. The threshold value of the soyasapogenol A aglycone was higher than those of the saponin A group, and a similar relationship was found for other saponins and their aglycones. The threshold value of daidzin was $10^{-5}$—$10^{-6}$ M, and that of daidzein, its aglycone, was $10^{-6}$ M. The value of genistin ($10^{-2}$ M) was lower than that of genistein ($10^{-4}$ M). This result contrasts with those found for the saponins.

The undesirable intensities of the glycosides are shown in Fig. 3. Soybean saponin solutions were compared with caffeine solution (bitterness), because saponins have an undesirable taste with bitterness; similarly isoflavone component solutions were compared with a naringin solution (stringency). Soybean saponin A group had the strongest undesirable intensity of the soybean glycosides. While soybean saponins showed a tendency to be weaker in undesirable taste as a result of decomposition to their aglycones, the isoflavone glycosides showed the reverse tendency, and were much stronger in taste when decomposed into their aglycone.

Measurements of DMF activities of soybean glycosides using neuoblastoma cell
Since it is known that the membrane potential change of the neuoblastoma cell (N-18 clone) is induced by bitter substances (caffeine, quinine, strychnine, etc.), the possibility of its use as a model for measurements of DMF activities of soybean glycosides was examined. The membrane potential changes by soybean saponin and quinine sulfate solutions were both measured. While the increase of the fluorescence intensity as a result of the induction of the membrane potential change by the addition of quinine sulfate was observed, the change was not induced by the addition of soybean saponins with bitter tastes (Table II). This result implies that the bitterness of soybean saponin may be different from those of bitter substances in ref. 15.

Measurements of DMF activities by the electrical response of the chorda tympani nerve of the rat
It has been reported that the chorda tympani nerve of rats

![Fig. 4. Integrated Response to Various Stimuli of Chorda Tympani Nerve from Rat.](image)

![Fig. 5. Integrated Response to Soybean Saponin of Glossopharyngeal Nerve from Frog.](image)

![Fig. 6. Structures of Soybean Saponins Aa, Ab, Ba, and Bb.](image)

**Table II. Changes in the Fluorescense Intensity**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Relative intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
</tr>
<tr>
<td>Quinine sulfate*</td>
<td>143</td>
</tr>
<tr>
<td>Soybean saponin*</td>
<td>98</td>
</tr>
</tbody>
</table>

* 0.1 mM
the rat responds to the four basic taste stimuli (sweet, salty, sour, and bitter) at the front part of the tongue.\(^\text{16}\)

The patterns of the electrical response of the chorda tympanum nerve after the applications of various stimuli on the tongue of rat are shown in Fig. 4. While the expected electric responses were obtained with sucrose, quinine, NaCl, and NH\(_4\)Cl, those by soybean saponins were not obtained.

**Measurements of DMF activities by the electrical response of the glossoharyngeal nerve of the frog**

It is reported that the glossoharyngeal nerve of the frog perceives stimuli such as temperature and touch at the anterior part of the tongue and that the four basic tastes are perceived at the same location.\(^\text{17}\) After the application of soybean saponin solution to the tongue of the frog, a specific response from the glossoharyngeal nerve was observed (Fig. 5). Although the response of the basic taste solution disappeared after rinsing with the low ionic strength solution, that of soybean saponin continued after such rinsing. This result is consistent with the lingering aftertaste of soybean saponin reported in the organoleptic studies (Table I). It is significant that only the glossoharyngeal nerve responds to the soybean saponin solution and that the chorda tympanum nerve does not respond. So the glossoharyngeal nerve of frog was used for the measurements of DMF activities of soybean glycosides.

**The response intensities of various purified soybean saponins**

A relationship between the peak height of the response (that is, the frequency of the impulses) and the concentration of the stimulating solution has been shown.\(^\text{17}\) An attempt was therefore made to measure the taste intensity of soybean saponin solution using the glossoharyngeal nerve of the frog.

The effects of the isolated saponins Aa, Ab, Ba, and Bb (Fig. 6) on the intensity of the response were examined, and their threshold values measured. The relation between concentration and the response intensities is shown in Fig. 7. The threshold values of A group (saponin Aa and Ab) were lower than that of B group (saponin Ba and Bb). These results are consistent with the results of organoleptic tests. Furthermore, the DMF activity caused by the saponin A group was more intense than that of the B group, especially at the higher concentration. Saponins Aa and Ab were found to have almost equivalent to the threshold values and response magnitude (in concentration from 0.5 to 2.0 \(\text{mm}\))

While soybean saponin Ab is completely acetylated on the terminal sugar moieties of the sugar chain linked to C-22 of soyasapogenol A, soyasaponin A\(_1\) has no such acetyl groups. The effects of the acetyl groups on the electrical response of the glossoharyngeal nerve of the frog were therefore examined. As shown in Fig. 8, the intensities of these two saponin solutions demonstrated a large difference in spite of only a small structural modification. Although their threshold values were almost equal, the DMF activity caused by saponin Ab was much stronger than that of soyasapogenol A\(_1\). Therefore, it is suggested that the increase of the hydrophobic group leads to a much stronger undesirable taste.

The results of the response intensity from the glossoharyngeal nerve of the frog were consistent with those of the organoleptic tests for soybean saponins, while the threshold values from the response intensity were higher than those measured in the organoleptic test.

**The response intensities of isoflavone glycosides**

The intensities of the response with daidzin and genistin were measured. As shown in Fig. 9, the DMF activity of genistin was stronger than that of the daidzin fraction, and the threshold value of genistin was also lower than that of daidzin. This result was not consistent with the findings of the organoleptic test.

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References