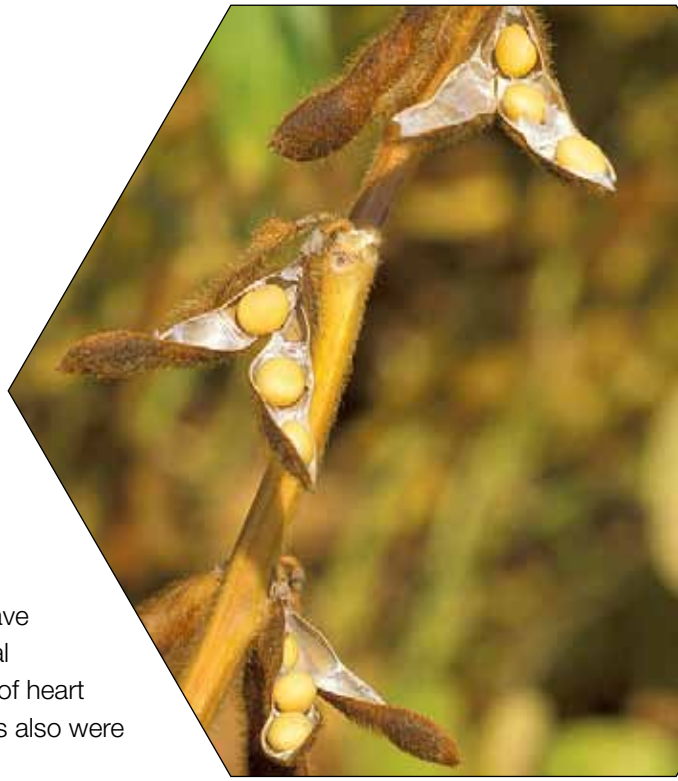
ChromaDex®

Application Note

As published in "The Handbook of Analytical Methods for Dietary Supplements"

0066 - Soy for Isoflavones by HPLC



Botanical Name: *Glycine max L.*

Common Names: Soybean

Parts of Plant Used: Beans

Uses: Prevention of heart disease; possible prevention of osteoporosis and cancer; as a phytoestrogen; beneficial effects on women's health.

Modes of Action:

Three groups of compounds—proteins, isoflavones, and saponins—have been found to have beneficial effects on health. Soy proteins lower total cholesterol and low-density lipoproteins, presumably reducing the risk of heart disease. Soy isoflavones are well-known phytoestrogens. Soy saponins also were reported to lower cholesterol and to prevent cancer.

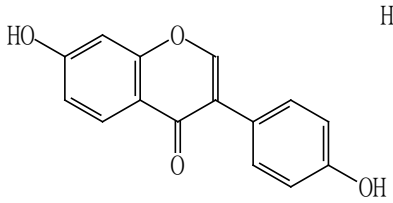
Chemical Markers:

Because soy is a valuable crop, its chemical components have been studied extensively. Soybeans are a good source of protein, lipids, sterols, isoflavones, and saponins. It is generally recognized that there are 12 major isoflavones in different soy products, divided into three groups based on the aglycone moiety, namely, daidzein, genistein, and glycitein. In each group, there are four types: isoflavone aglycone, glycoside, 6"-acetylglycoside, and 6"-malonylglycoside. In nature, 6"-malonylglycoside is the most common form, but it is unstable and will decompose with heat. Isoflavone 6"-acetylglycoside does not exist in nature; it is produced from 6"-malonylglycoside by heat during drying and processing. In addition to these 12 compounds, other isoflavones also were identified in soy products.[1,2]

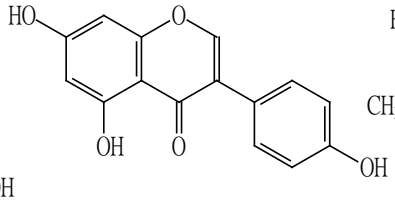
Soybeans also were reported to contain 5% to 6% soyasaponins.[3] The majority of soyasaponins can be divided into two groups (A and B) based on their aglycone structures. Soyasaponins in group A are bidesmosidic saponins with two glycosylation sites on their aglycones (soyasapogenol A). Group B soyasaponins are monodesmosidic saponins, with only one glycosylation site on the aglycones (soyasapogenols B and E). The genuine soyasaponins of group B in the legume are conjugated with 2,3-dihydro-2,5-dihydroxy-6-methyl-4-pyrone (DDMP), which will degrade to non-DDMP counterparts during heat treatment.[4,5] Currently, the isoflavones and soyasaponins are used as marker compounds for quality control of different soy products.

10005 Muirlands Blvd., Suite G, Irvine, CA 92618 | Tel: +1-949-419-0288
Fax: +1-949-419-0294 | sales@chromadex.com | www.chromadex.com

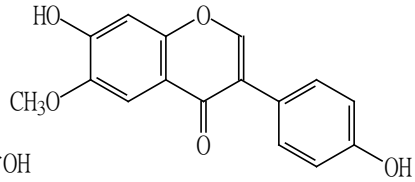
Major Flavonoids in Skullcap



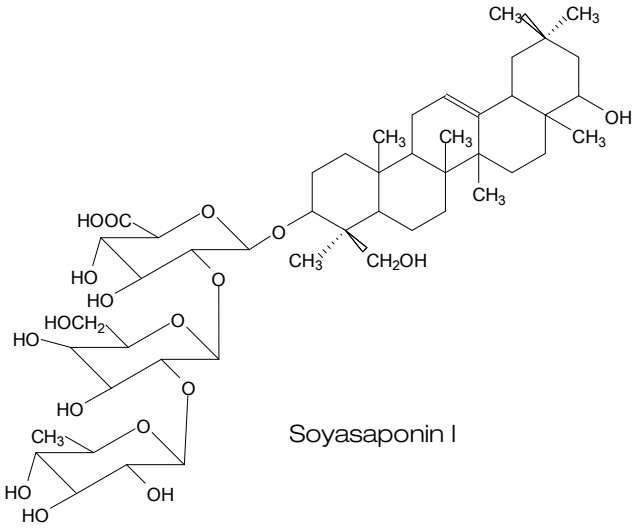
Daidzein



Genistein



Glycitein



Soyasaponin I

Methods of Analysis

Various methods have been used to analyze the isoflavone content in soy products; the most common method is HPLC coupled with UV and MS detection. HPLC is also the method of choice for analysis of soyasaponins.

Method 1:

A method found at www.nsfina.org was used. This method analyzes six isoflavones in various soy products: genistein, genistin, daidzein, daidzin, glycitein, and glycitin.

Sample Preparation:

For soy flours high in protein, accurately weigh about 1 g into a 50-mL centrifuge tube. Add 40 mL of methanol–water (80:20), cap, and then shake or stir in a 65°C water bath for 2 hours. Cool to room temperature, add 3 mL of 2 N sodium hydroxide, and shake at room temperature for 10 minutes. Add 1 mL of glacial acetic acid, swirl to suspend, and dilute to a 50-mL final volume with methanol–water (80:20). Filter the sample through quantitative-grade filter paper into a 250-mL beaker. Transfer the contents of the beaker to a 50-mL centrifuge tube and centrifuge for 10 minutes at 2000 rpm. Transfer 1 mL of supernate to a 1.5-mL microfuge tube and centrifuge for 10 minutes at 8000–10,000 rpm. Transfer the supernate to HPLC sample vials.

For samples high in isoflavones, accurately weigh about 300 mg of sample into a 50-mL centrifuge tube. Add 40 mL of methanol–water (80:20), cap, and shake or stir in a 65°C water bath for 2 hours. Cool to room temperature, and add 3 mL of 2 N sodium hydroxide, and shake for 10 minutes at room temperature. Add 1 mL of glacial acetic acid and swirl to suspend. Dilute to a 50-mL volume with methanol and centrifuge for 10 minutes at 2000 rpm.

For samples with 40% isoflavones, dilute 1 mL of the supernate to a 10-mL final volume with methanol–water (80:20).

For samples with 20% isoflavones, dilute 2 mL of the supernate to a 10-mL final volume with methanol–water (80:20).

Chromatography:

Column: Phenomenex Luna C18(2), 5 µm, 4.6 × 250 mm, or Varian Microsorb MV C18, 5 µm, 4.6 × 250 mm, 100Å.
Mobile phase: Solvent A = water–methanol–10% acetic acid (88:10:2), solvent B = methanol–acetic acid (98:2).
Gradient:

Time (minutes)	%A	%B
0	80	20
2	70	30
10	50	50
16	30	70
16.01	0	100
19	0	100
19.02	80	20
25	80	20

Column temperature: 30°C
Flow rate: 0.8 mL/minute
Injection volume: 10 µL
Detection wavelength: UV at 260 nm

Method 2:

The method of Setchell and Cole[6] was used.

Sample Preparation:

Reflux about 3 to 5 g of powder or liquid sample containing about 0.5 to 2 g of solids with 70 mL of methanol–water (80:20 vol/vol, final ratio) for 1 hour. Cool to room temperature and filter into a 100-mL volumetric flask, wash, and dilute to 100 mL with 80% methanol.

For samples with a high amount of fat, wash the fats out with hexane before sample injection.

Chromatography:

Column: C18, 5 µm, 4.6 × 250 mm.
Mobile phase: Solvent A = water (10 mM ammonium acetate and 0.1% trifluoroacetic acid), solvent B = acetonitrile–water (90:10 vol/vol).
Gradient:

Time (minutes)	%A	%B
0	0	100
2	0	100
24	50	50
29	50	50
34	0	100
40	0	100
25	80	20

Detection wavelength: UV at 260 nm
Flow rate: 1.0 mL/minute
Injection volume: 10 µL

Method 3:

The unpublished method of Mingfu Wang was used.

Sample Preparation:

For a soy 40% isoflavone extract, weigh a 50-mg sample into a 50-mL volumetric flask, add about 35 mL of 70% methanol, sonicate for 10 minutes, and shake for 20 minutes. Allow the flask to cool to room temperature and fill to volume with 70% methanol.

Chromatography:

HPLC column: Phenomenex Prodigy ODS (3), 5 µm, 150 × 3.2 mm.
Mobile phase: Solvent A = water (0.1% formic acid) ,solvent B = acetonitrile.
Gradient: 10%B to 35%B in 40 minutes.
Flow rate: 0.8 mL/minute
Injection volume: 10 µL
Detection wavelength: 255 nm
Column temperature: Ambient

Method 4:

The method of Dalluge et al.[3] can be used to identify soyasaponins and isoflavones simultaneously in soy products.

Sample Preparation:

Extract the soy product with 95% aqueous methanol (vol/vol).

Chromatography:

HPLC column: Zorbax Eclipse XDB-18, 250 × 4.6 mm.
Mobile phase: Solvent A = water (0.05% trifluoroacetic acid vol/vol), solvent B = acetonitrile (0.05% trifluoroacetic acid vol/vol).
Gradient:

Time (minutes)	%A	%B
0	87	13
25	70	30
35	60	40
47	60	40
55	10	90
65	0	100
70	0	100
72	87	13

Flow rate: 0.5 mL/minute
Injection volume: 25 µL
Detector: MS
Column temperature: Ambient

Method 5:

This method of Hu et al.[4] can be used to quantify group B soyasaponins.

Sample Preparation:

Extract about 4 g of a dried, finely ground soy sample or 0.2 to 1 g of concentrated soy ingredients with 100 mL of 70% aqueous ethanol with stirring for 2.5 hours at room temperature. Filter, then evaporate the extraction solution to dryness at less than 30°C. Dissolve the residue in 10 mL of 80% methanol.

Chromatography:

HPLC column: YMC RP-18 YMC-ODS-AM-303, 5 µm, 250 × 4.6 mm.
Mobile phase: Solvent A = 0.05% trifluoroacetic acid, solvent B = acetonitrile with 0.05% trifluoroacetic acid.
Gradient:

Time (minutes)	%A	%B
0	63	37
12	60	40
37	52	48
38	0	100
40	0	100
45	63	37

Column temperature: 30°C
Flow rate: 1 mL/minute
Injection volume: 50 µL
Detection wavelength: 205 nm

Validation Data:

Linearity: Not specified
Accuracy: The percent of recovery for soyasaponin I was between 93.9 and 120.9 for three different products.
Precision: The within-day variation was less than 9.8% and the between-day variation was less than 14.3% for soyasaponin I, II, V, αg, ßg, and ßa.
Selectivity: Peak identification was determined against standards.
Ruggedness: Not specified
Robustness: Not specified
LOD/LOQ: Not specified

References:

1. Hosny M, Rosazza JP. New isoflavone and triterpene glycosides from soybeans. *J Nat Prod.* 2002;65(6):805–13.
2. Gu L, Gu W. Characterisation of soy isoflavones and screening for novel malonyl glycosides using high-performance liquid chromatography-electrospray ionisation-mass spectrometry. *Phytochem Anal.* 2001;12(6):377–82.
3. Dalluge JJ, Eliason E, Frazer S. Simultaneous identification of soyasaponins and isoflavones and quantification of soyasaponin Bb in soy products, using liquid chromatography/electrospray ionization-mass spectrometry. *J Agric Food Chem.* 2003;51(12):3520–4.
4. Hu J, Lee SO, Hendrich S, et al. Quantification of the group B soyasaponins by high-performance liquid chromatography. *J Agric Food Chem.* 2002;50(9):2587–94.
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6. Setchell KD, Cole SJ. Variations in isoflavone levels in soy foods and soy protein isolates and issues related to isoflavone databases and food labeling. *J Agric Food Chem.* 2003;51(14):4146–55.