

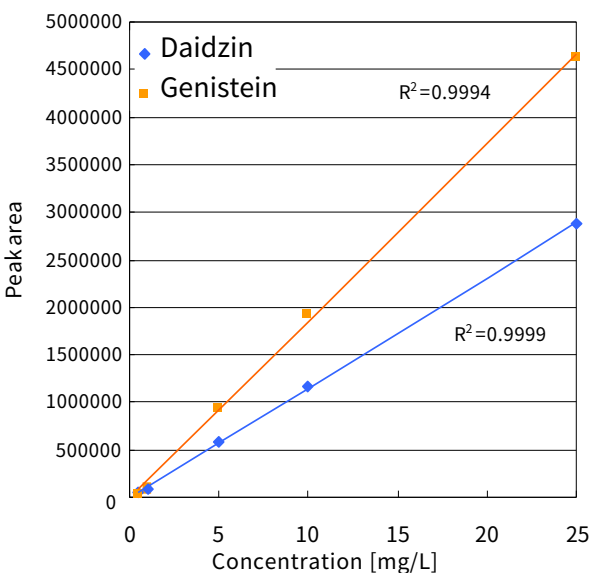
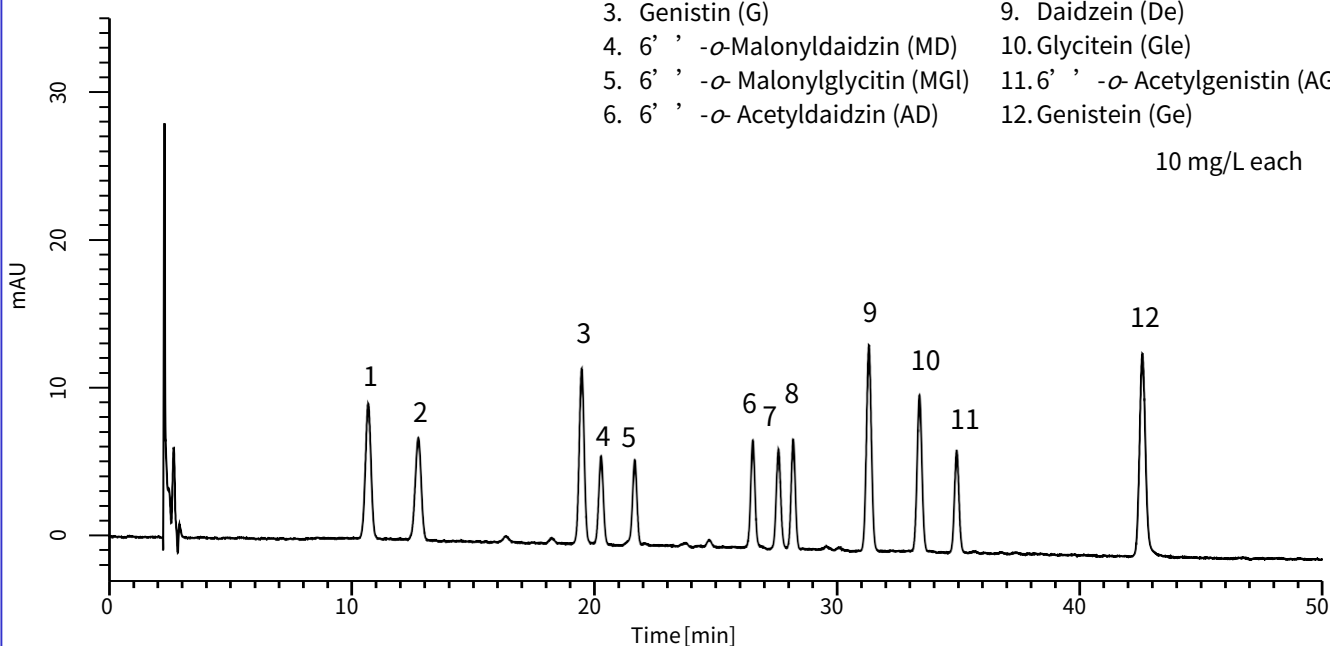
Isoflavones are a type of flavonoid and found in a number of legumes, grains, and, vegetables. Among them, soybean is by far the most concentrated source of isoflavones. As their chemical structures are very similar to those of endogenous estrogens, isoflavones are known to have estrogen-like effect and provide several health benefits.

In this note, a simultaneous determination method for 12 major isoflavones (daidzein, genistein, glycitein, and their glycosides) in soy foods is described. The HPLC separation of isoflavones was achieved by using Inertsil ODS-SP as a separation column.

(K.Suzuki)

A Chromatogram Obtained from Standard Solution

PDA



	R ²
1. D	: 0.9994
2. Gl	: 1.000
3. G	: 1.000
4. MD	: 1.000
5. MGl	: 1.000
6. AD	: 0.9999
7. AGI	: 0.9999
8. MG	: 1.000
9. De	: 0.9999
10. Gle	: 1.000
11. AG	: 1.000
12. Ge	: 0.9999

Conditions

Column : Inertsil ODS-SP
(5 μm, 250 x 4.6 mm I.D.)
Cat.No. 5020-02746

Eluent : A) 0.1 % acetic acid in CH₃CN₃
B) 0.1 % acetic acid in H₂O
A/B = 15/85 – 8 min
– 15/85 – 42 min – 35/65
equilibration 10 min) , v/v
Mixed by a gradient mixer)

Flow rate : 1.5 mL/min

Col. Temp. : 35 °C

Detection : PDA 254 nm

Inj. Vol. : 10 μL

Calibration curves and their correlation coefficients

Sample (liquid)

-1 g

Extraction

- make up to 100 mL with 70 % ethanol aqueous solution
- centrifuge (3500 rpm, 10 min)
- filtrate the supernatant with 0.45 μm membrane filter

HPLC

Sample (solid)

Extraction

- 70 % ethanol aqueous solution 25 mL
- shaking for 30 min
- centrifuge (3500 rpm, 10 min)

Residue

- 70 % ethanol aqueous solution 25mL
- shaking for 30 min
- centrifuge (3500 rpm, 10 min)

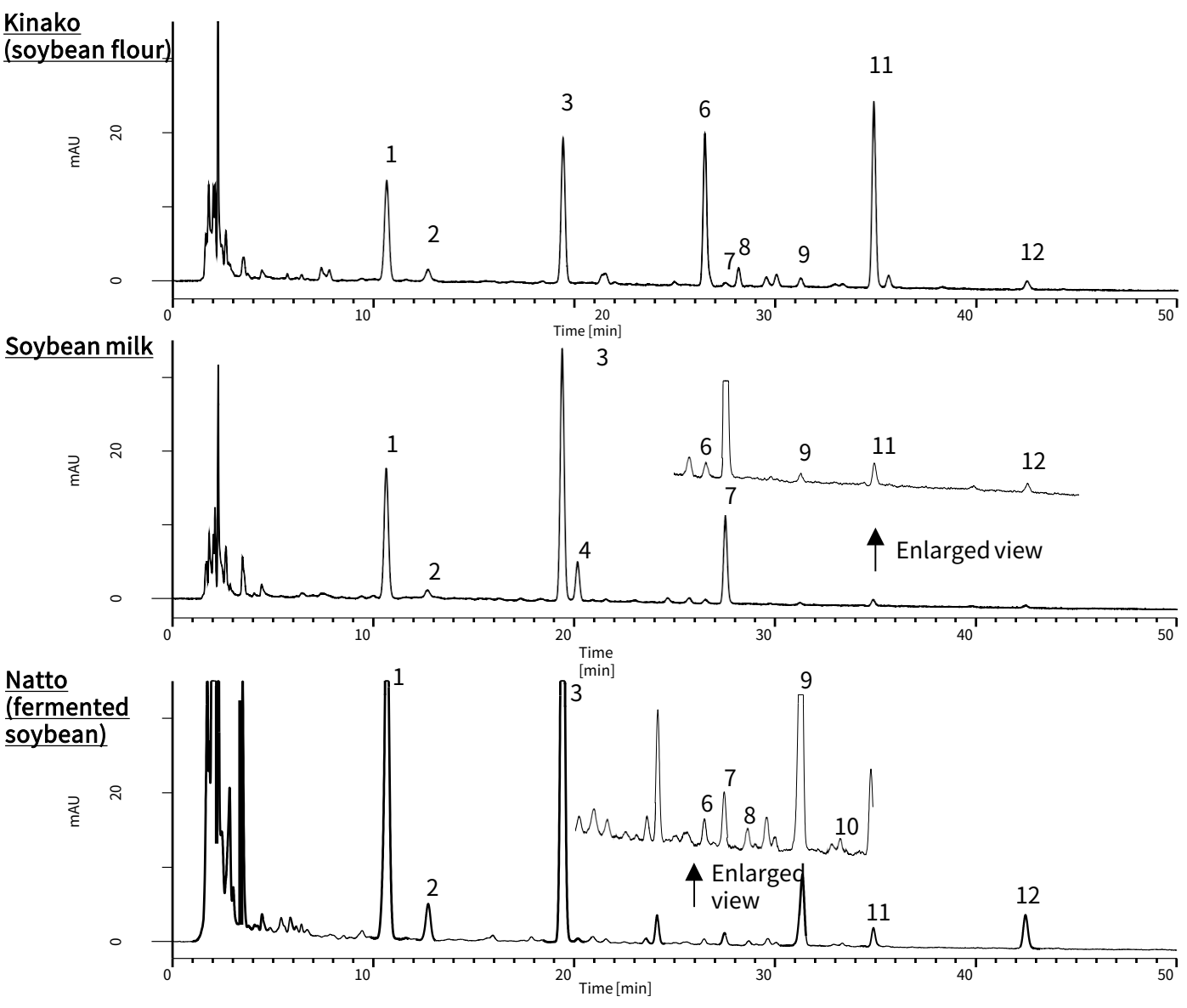
} X
2

Supernatant

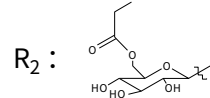
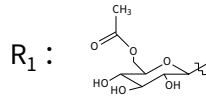
- make up to 100 mL
- filtrate with 0.45 μm membrane filter

HPLC

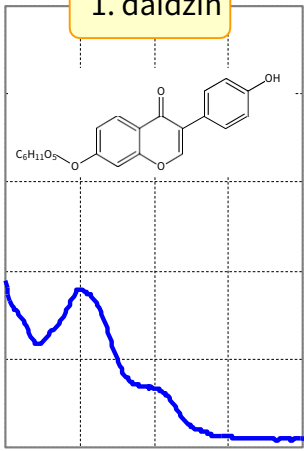
Chromatograms obtained from soy food samples



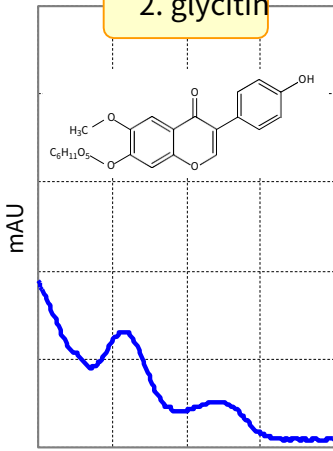
The chemical structures and the absorption spectra



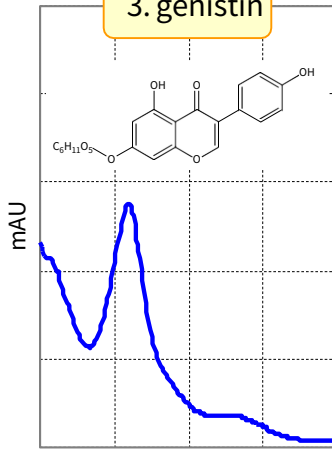
1. daidzin



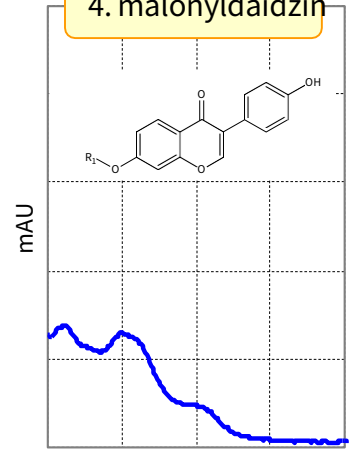
2. glycitin



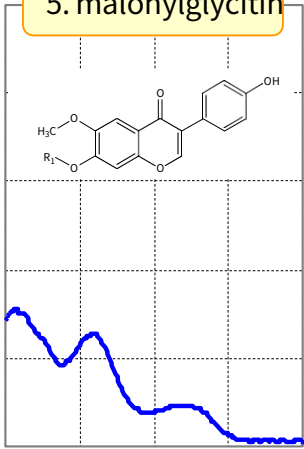
3. genistin



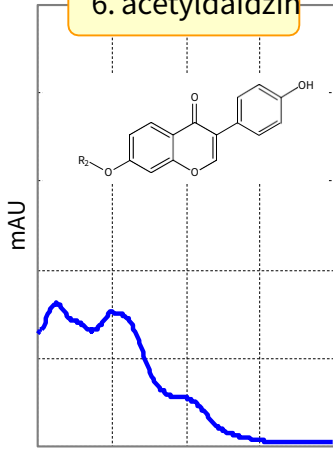
4. malonyldaidzin



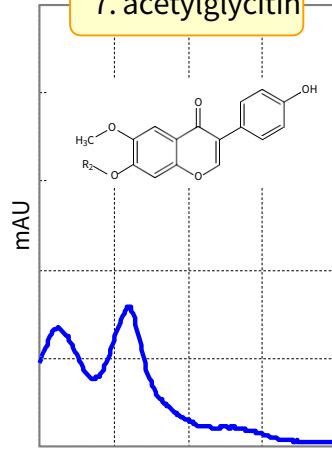
5. malonylglycitin



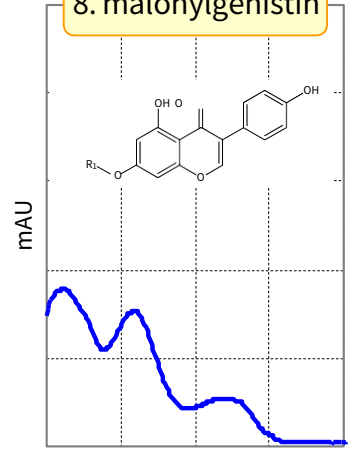
6. acetyldaidzin



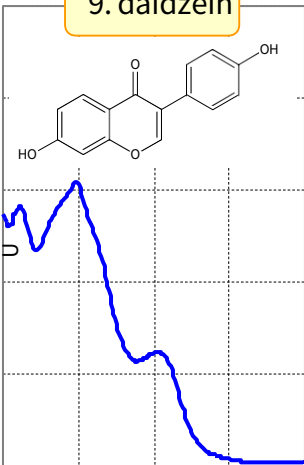
7. acetylglycitin



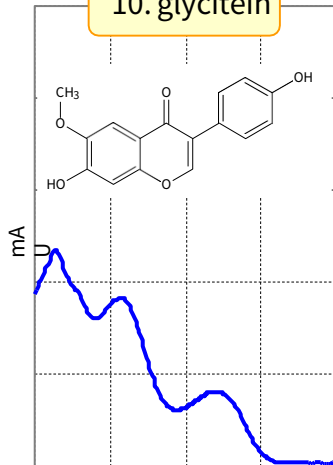
8. malonylgenistin



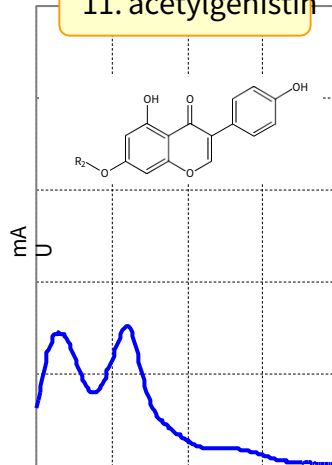
9. daidzein



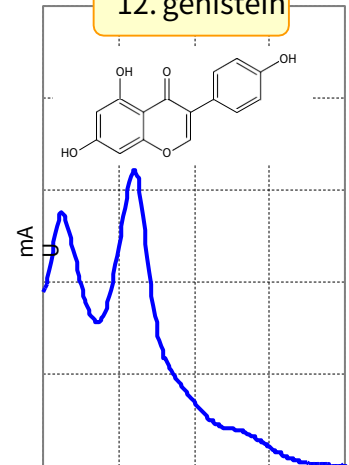
10. glycitein



11. acetylgenistin



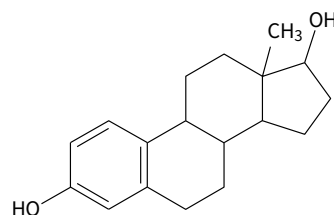
12. genistein



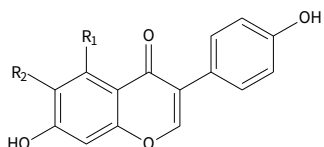
* The absorbance scale of each spectrum was all the same.

Coffee Break

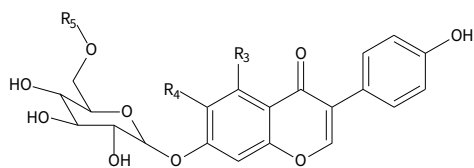
Since isoflavones are similar to estrogens (e.g. estradiol shown on the right) in chemical structure, isoflavones are also classified as phytoestrogen. It is suggested that isoflavones attenuate some estrogen-deficiency diseases such as osteoporosis due to their estrogen-like effect.



Estradiol



Isoflavones



Isoflavone glycosides

	R1	R2
Daidzein	: H	: H
Genistein	: OH	: H
Glycitein	: H	: OCH ₃

	R3	R4	R5
Daidzin	: H	: H	: H
6' -O-Acetyldaidzin	: OH	: H	: H
6' -O-Acetylgenistin	: H	: OCH ₃	: H
6' -O-Acetylglycitin	: H	: H	: COCH ₃
6' -O-Malonyldaidzin	: OH	: H	: COCH ₃
6' -O-Malonylgenistin	: H	: OCH ₃	: COCH ₃
6' -O-Malonylglycitin	: H	: H	: COCH ₂ COOH
6' -O-Succinyldaidzin	: OH	: H	: COCH ₂ COOH
6' -O-Succinylgenistin	: H	: OCH ₃	: COCH ₂ COOH
	: H	: H	: COC ₂ H ₄ COOH
	: H	: H	: COC ₂ H ₄ COOH

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GL Sciences Inc. Japan

22-1 Nishishinjuku 6-chome
Shinjuku-ku, Tokyo
163-1130, Japan

Phone: +81-3-5323-6620
Fax: +81-3-5323-6621
Email: world@glsc.co.jp
Web: www.glsciences.com

GL Sciences Inc. USA

4733 Torrance Blvd. Suite 255
Torrance, CA 90503
USA

Phone: +1-310-265-4424
Fax: +1-310-265-4425
Email: info@glsciencesinc.com
Web: www.glsciencesinc.com

GL Sciences B.V.

Dillenburgstraat 7C
5652AM, Eindhoven
The Netherlands

Phone: +31-40-254-9531
Email: info@glsciences.eu
Web: www.glsciences.eu

GL Sciences (Shanghai) Limited

Tower B, Room 2003
Far East International Plaza
No.317 Xianxia Road, Changning District
Shanghai, China 200051

Phone: +86-21-62782272
Email: contact@glsciences.com.cn
Web: www.glsciences.com.cn



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