An Improved Method for Basic Hydrolysis of Isoflavone Malonylglucosides and Quality Evaluation of Chinese Soy Materials

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Basic hydrolysis procedure is often included in the sample preparation in order to quantify malonylglucosides or acetylglucosides of soy materials. However, it is preferable not to use NaOH as a hydrolytic reagent considering the effect of its alkalinity on the successive injection to HPLC and low acidity of soy isoflavones. This paper presents an improved method for basic hydrolysis using ammonia as a hydrolytic reagent without the additional neutralization step. Moreover, by means of HPLC and LC-MS methods, a systematic quality evaluation of natural soy materials from Chinese markets were established and discussed, inclusive of soybeans, black soybeans, defatted soy flours, as well as the distribution of isoflavones in the seed coat, hypocotyl and cotyledon. The results indicate that HPLC profiling patterns of originating various isoflavone constituents of Chinese soybeans was similar to those of Japanese ones, and those of Chinese black soybeans was similar to those of American ones. The average content level of total soy isoflavones of Chinese soybeans and black soybeans were a little lower than that of American and Japanese ones. Additionally, the thorough analysis for Semen Sojae Praeparatum, a Chinese herbal medicine made from fermented black soybeans or soybeans was done for the first time and the characteristic of its HPLC profiling patterns shows the higher content of isoflavone glucosides and aglycones than those of natural soy materials.

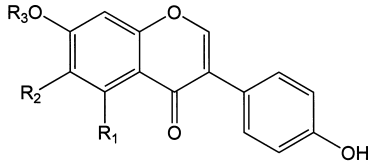
Key words soy isoflavone; basic hydrolysis; Chinese soy material; Semen Sojae Praeparatum; malonylglucosides; HPLC

Soy isoflavones show extensive pharmacological activities, such as the antioxidation,[1)] the regulation of lipid metabolism,[2)] the reduction of blood glucose,[3)] the prevention of bone loss[4)] and cancer initiation,[5)] *etc*. Such activities have led to the worldwide development and application of various soy foods or its supplements. Naturally occurring soy isoflavones mainly include three aglycones, daidzein (DAE), glycitein (GLE) and genistein (GEE), their glucosides, daidzin (DAI), glycitin (GLI) and genistin (GEI), and their malonylglucosides, 6″-*O*-malonyl-7-*O*-*β*-D-daidzin (Mal-DAI), 6″-*O*-malonyl-7-*O*-*β*-D-glycitin (Mal-GLI) and 6″-*O*-malonyl-7-*O*-*β*-D-genistin (Mal-GEI), while three acetylglucosides, 6″-*O*-acetyl-7-*O*-*β*-D-daidzin (Ac-DAI), 6″-*O*-acetyl-7-*O*-*β*-D-glycitin (Ac-GLI) and 6″-*O*-acetyl-7-*O*-*β*-D-genistin (Ac-GEI) are the products of degradation of three above-mentioned malonylglucosides (Fig. 1). Among them, DAE and GEE have been of great interests in the pharmaceutical field because of both different pharmacological activities. Malonylglucosides of soy isoflavones also show antioxidant properties,[6)] and for natural soy materials, they are at a much higher content level than aglycones and glucosides. Some analytical studies on soy isoflavonoids were published, including the extractive methods with the acidic or basic hydrolysis procedures,[7—9)] the isoflavones composition of soybeans from American and Japanese markets,[10)] the determination of soy isoflavones by HPLC, LC-UV, capillary electrophoresis or LC-MS methods, *etc*.[11—14)] A basic hydrolysis procedure was used in the sample preparation for quantification of malonylglycosides or acetylglycosides of soy isoflavones in Delmonte's recent report.[8)] This paper presents an improved method for the basic hydrolysis of malonylglucosides and acetylgluconsides of soy isoflavones, in which ammonia was selected as the hydrolytic reagent instead of NaOH. The HPLC profiling patterns of isoflavone constituents of soy materials collected from Chinese markets, including yellow soybeans, black soybeans, defatted soy flakes and a traditional Chinese medicine Semen Sojae Praeparatum, as well as the characteristics of the distribution of isoflavones in the seed coat, hypocotyl and cotyledon were studied and discussed as well.

Experimental

Materials Reference standards of daidzin, glycitin, genistin, daidzein, glycitein and genistein were purchased from Wako Pure Chemical Industry, Ltd. Japan. Their purity determined by HPLC method was 99.2% (DAI), 98.4% (GLI), 98.3% (GEI), 99.6% (DAE), 99.7% (GLE) and 99.6% (GEE), respectively.

Twelve batches of soy materials were directly collected from the Chinese markets. For the study of the distribution of isoflavones, the samples of the seed coat, hypocotyl and cotyledon were prepared by separating them from



Compounds	R_1	R_2	R_3
DAE	H	H	H
GLE	H	OCH_3	H
GEE	OH	H	H
DAI	H	H	7-*O*-ß-D-glucoside
GLI	H	OCH_3	7-*O*-ß-D-glucoside
GEI	OH	H	7-*O*-ß-D-glucoside
Ac-DAI	H	H	6"-*O*-acetyl-7-*O*-ß-D-glucoside
Ac-GLI	H	OCH_3	6"-*O*-acetyl-7-*O*-ß-D-glucoside
Ac-GEI	OH	H	6"-*O*-acetyl-7-*O*-ß-D-glucoside
Mal-DAI	H	H	6"-*O*-malonyl-7-*O*-ß-D-glucoside
Mal-GLI	H	OCH_3	6"-*O*-malonyl-7-*O*-ß-D-glucoside
Mal-GEI	OH	H	6"-*O*-malonyl-7-*O*-ß-D-glucoside

Fig. 1. Chemical Structures of Soy Isoflavones

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the samples of soybeans and black soybeans. Among four batches of defatted soy flours, sample #21, provided by Laboratory of Chemical Engineering, Shenyang Pharmaceutical University, was a byproduct after the extraction of soybean oils using moist-heat treated soybeans, the others were the ones using normal soybeans. The details for the samples were given in Table 2.

Extraction and Hydrohysis The extraction procedure followed Restagno's method.[7] 1.0 g samples passed through 500 μm mesh sieve (0.1 g powdered hypocotyl ones) was ultrasonically double-extracted, for 20 min and with 20 ml of MeOH–H_2O (75 : 25, v/v) once. The mixtures were centrifuged at 3500 rpm for 5 min. To combined supernatants and washing liquid, MeOH–H_2O (75 : 25, v/v) was added to adjust the volume to 50 ml, and the solution was used as the soy isoflavone extracts.

To 10 ml of each soy isoflavone extract, MeOH–H_2O (75 : 25, v/v) was added to adjust the volume to 25 ml, and then filtered through a 0.45 μm membrane filter. The filtrate was used as the unhydrolyzed test solution.

To 10 ml of each soy isoflavone extract, 10 ml of the solution of MeOH–NH_4OH (25—28% in H_2O)–H_2O (75 : 20 : 5, v/v) were added and refluxed for 2 h. The cooled solution was evaporated to dryness *in vacuo*. The residue was dissolved in 25 ml of MeOH–H_2O (75 : 25, v/v), and filtered through a 0.45 μm membrane filter. The filtrate was used as the base-hydrolyzed test solution.

To 10 ml of each soy isoflavone extract, 10 ml of the solution of MeOH–HCl (36—38% in H_2O) (60 : 40, v/v) were added, and refluxed for 2 h. The cooled solution was adjusted to its pH equal to 6 with NH_4OH (25—28% in H_2O), and evaporated to dryness *in vacuo*. The residue was dissolved in 25 ml of MeOH–H_2O (75 : 25, v/v), and filtered through a 0.45 μm membrane filter. The filtrate was used as the acid-hydrolyzed test solution.

HPLC and LC-MS Conditions for the Determination and Identification of Isoflavones Shimadzu LC-2010A_{HT} HPLC system equipped with CLASS-VP workstation (Shimadzu Co., Ltd., Japan) was used for quantitative determinations. HPLC analyses were performed on a Kromasil C_{18} column (4.6×250 mm, 5 μm, Tianjin Scientific Instruments Co., Ltd., China) at 40 °C. The mobile phase consisted of a gradient system of solution A, 0.5% glacial acetic acid in water, and solution B, 0.5% glacial acetic acid in methanol, at the flow rate of 1.0 ml/min. The gradient program was as follow: isocratic at solution A–B (70 : 30, v/v) for 10 min, followed by linear gradient to solution A–B (60 : 40, v/v) in 20 min, and isocratic at solution A–B (60 : 40, v/v) for 10 min, finally linear gradient to solution A–B (40 : 60, v/v) in 20 min. The injection volume was 25 μl, and the detective wavelength was set at 260 nm.

Waters HPLC system (Waters Co., Milford, MA, U.S.A.) equipped with model 2996 photodiode array detector was used to obtain the UV spectra of the marker compounds. LC-MS identification of malonylglucosides and acetylglucosides of soy isoflavones was carried out on a Shimadzu QP8000α LC-MS spectrometer equipped with CLASS-8000 workstation (Shimadzu Co., Ltd., Japan) because their reference standards were not available. The mass spectrometer was operated under the following conditions: LC-MS-ESI with positive ion mode, electrospray voltage at 4.0 kV, capillary temperature at 250 °C, flow rate of dry air at 4.5 l/min. The ESI-Mass spectra were acquired in the *m*/*z* range of 50—600.

Data Analysis The peak areas of DAI, GLI, GEI, DAE, GLE, and GEE together with those of 6 external standard solutions obtained from parallel injection were used to calculate the amount of compounds present in the samples. The total content of DAE, GLE, and GEE were calculated by their peak areas in the acid-hydrolyzed samples. Mal-DAI, Mal-GLI, Mal-GEI, Ac-DAI, Ac-GLI and Ac-GEI were transformed into their corresponding glucosides by basic hydrolysis, or all the glycosides were transformed into their corresponding aglycones by acidic one. Their concentrations were obtained by a comparison between the responses of their hydrolysates in the unhydrolyzed test solutions and those in the basic hydrolyzed ones or the acid hydrolyzed ones with the correction in molecular mass. For example, the concentrations of Mal-DAI by basic or acidic hydrolysis were calculated using the equations A or B, respectively.

$$C_{\text{Mal–DAI}} = \left(C_{\text{DAIb}} - C_{\text{DAIu}}\right) \times \frac{M_{\text{Mal–DAI}}}{M_{\text{DAI}}} \quad \text{(A)}$$

$$C_{\text{Mal–DAI}} = \left(C_{\text{DAEa}} - C_{\text{DAEu}} - C_{\text{DAIu}}\right) \times \frac{M_{\text{DAE}}}{M_{\text{DAI}}} \times \frac{M_{\text{Mal–DAI}}}{M_{\text{DAE}}} \quad \text{(B)}$$

where $C_{\text{Mal-DAI}}$ is the concentration of Mal-DAI in the unhydrolyzed test solution, C_{DAIu} and C_{DAIb} are the concentrations of DAI in the unhydrolyzed one and the base-hydrolyzed one, C_{DAEu} and C_{DAEa} are those of DAE in the unhydrolyzed one and the acid-hydrolyzed one, respectively, and M represents the molecular mass of the marker compounds.

Results and Discussion

HPLC-UV and LC-MS Identification of Soy Isoflavones Figure 2 shows the HPLC chromatograms of the methanolic extracts of one of hypocotyl samples (sample #17) and one of defatted soy flour samples (sample #21), as well as a methanolic solution of six reference standards tested. All the marker compounds could be satisfactorily separated. The UV spectra of the reference standards were given by the photodiode array detector, and the maximum absorption was at 248.9 nm for DAI and DAE, 257.2 nm for GLI and GLE, 259.6 nm for GEI and GEE. The retention times of DAI, GLI, GEI, DAE, GLE, and GEE were 12.1, 14.8, 20.2, 37.9, 44.0, 50.5 min, respectively, confirmed by co-chromatographic procedures. The peaks of Mal-DAI, Mal-GLI, Mal-GEI in the extract of sample #17 and those of Ac-DAI, Ac-GLI, Ac-GEI in the extract of sample #21 were successively identified by HPLC-ESI-MS according to their characteristic masses with MH^+ values of 503, 533, 519, 459, 489 and 475 (the right in Fig. 2) as well as their UV absorbance spectra with the maximum absorption at 248.9, 257.2 and 259.6 nm (the middle in Fig. 2). Their retention times were 26.5, 29.8, 34.8, 33.2, 36.6 and 46.1 min, respectively.

Optimization of Basic and Acidic Hydrolysis There have been some reports on the basic and acidic hydrolysis procedures for the quantitation of malonylglucosides and acetylglucosides in the absence of their reference substances.[8,11,15] In the present study, acid hydrolysis was conducted according to Franke's method.[15] Ammonia solution was selected as the basic hydrolytic reagent instead of NaOH solution[8] because it has advantage over the latter in consideration of the effect of the strong alkalinity of NaOH on the successive injection to HPLC system and the low acidity of isoflavones. Furthermore, no additional neutralization step was necessary since ammonia is readily removed during evaporation. As shown in Table 1 and Fig. 4 (k and j), the results indicated that hydrolysis using ammonia solution was able to convert malonylglucosides or acetylglucosides into their respective glucosides. Finally, 2.6 mol/l ammonia solution, 4.7 mol/l hydrochloric acid and refluxing for 2 h were found to be the optimum hydrolytic conditions. Figure 3 illustrates the difference of the products of soy isoflavone glycoside by the both kinds of hydrolyses.

Method Validation For six reference standards, good linear calibration curves for unhydrolzed and base-hydrolyzed samples were obtained as follows: $Y=9.530\times10^7X-2.541\times10^3$ for DAI in the concentration range of 6.52×10^{-4}—1.30×10^{-2} mg/ml, $Y=9.502\times10^7X-8.559\times10^2$ for GLI in that of 4.28×10^{-4}—8.56×10^{-3} mg/ml, $Y=1.367\times10^8X+1.254\times10^2$ for GEI in that of 5.76×10^{-4}—1.15×10^{-2} mg/ml, $Y=1.423\times10^8X-5.521\times10^2$ for DAE in that of 5.40×10^{-5}—8.64×10^{-4} mg/ml, $Y=1.272\times10^8X-2.835\times10^3$ for GLE in that of 4.16×10^{-5}—6.66×10^{-4} mg/ml and $Y=1.997\times10^8X+5.241\times10^2$ for GEE in that of 3.10×10^{-5}—4.96×10^{-4} mg/ml. Because the acidic hydrolysis of soy materials resulted in the great increase in the amount of aglycones, additional calibration curves for the acid-hydrolyzed samples were obtained as follows: $Y=1.424$

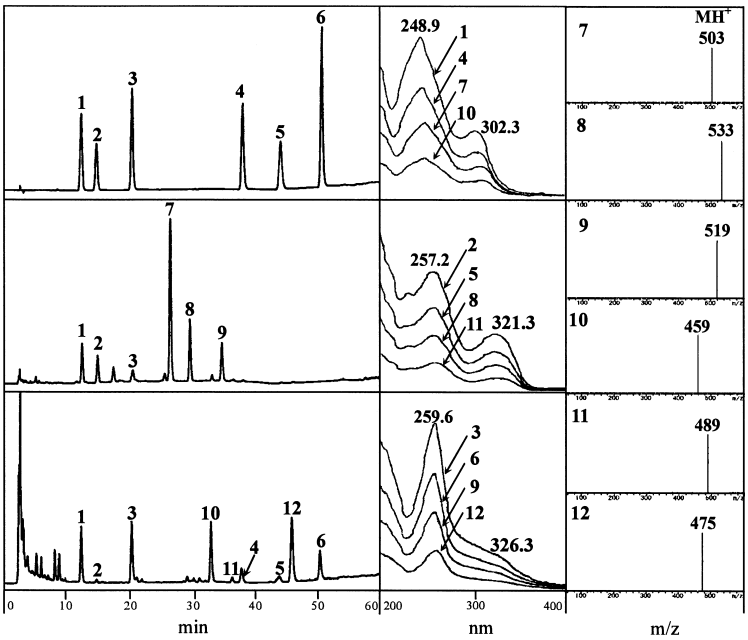

Fig. 2. HPLC Chromatograms (the Left) of Standard Solution as Well as Methanolic Solutions of Soy Materials, UV Spectra (the Middle) of Marker Compounds and Mass Spectra (the Right) of Malonyl and Acetyl Isoflavone Glycosides

The upper on the left indicates the standard solution containing DAI (1), GLI (2), GEI (3), DAE (4), GLE (5), GEE (6), while the middle and the lower on the same line indicate the methanolic extracts of the hypocotyl (sample #17) and the defatted soy flour (sample #21), respectively. The numbers from 7 to 12 indicate the marker compounds, Mal-DAI, Mal-GLI, Mal-GEI, Ac-DAI, Ac-GLI and Ac-GEI, respectively. The UV spectra were obtained with the photodiode array detector. For HPLC and LC-MS conditions, see Experimental.

Table 1. Optimization of Basic or Acidic Hydrolytic Procedures[a] (Peak Area/mg)

Hydrolytic reagents /reflux time	DAI	GLI	GEI	Mal-			Ac-			DAE	GLE	GEE
				DAI	GLI	GEI	DAI	GLI	GEI			
Uunhydrolysis	163339	42868	277376	89389	28802	151054	29504	7853	43300	9856	n.d.	9946
1.3 mol/l NH_3/1 h	256090	61999	423476	13269	4329	37699	tr.	tr.	tr.	10327	n.d.	10211
2.6 mol/l NH_3/1 h	294193	80687	477760	8525	n.d.	20474	n.d.	n.d.	n.d.	10388	n.d.	10991
2.6 mol/l NH_3/2 h	311418	87081	526570	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	11202	n.d.	10778
2.3 mol/l HCl/1 h	115239	29705	153729	26808	10756	53953	tr.	tr.	tr.	158178	23648	159002
4.7 mol/l HCl/1 h	32384	tr.	50340	18166	n.d.	tr.	n.d.	n.d.	n.d.	245644	67513	532229
4.7 mol/l HCl/2 h	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	295967	66739	586626

a) Sample #18, a defatted soybean flour, was used in the experiment. n.d., not detectable (≤limit of detection). tr., trace (>limit of detection, and <minimum of the linear range), as described in the section of method validation.

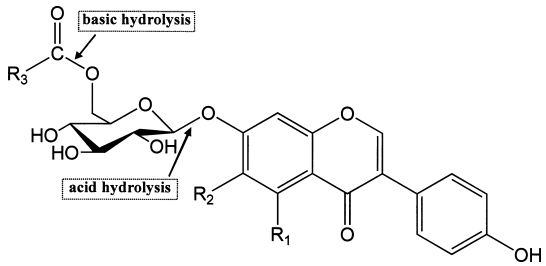

Fig. 3. Basic and Acid Hydrolytic Modes of Soy Isoflavone Glucosides

$\times 10^8 X - 1.869 \times 10^3$ for DAE in the concentration range of 5.40×10^{-4}—1.08×10^{-2} mg/ml, $Y = 1.293 \times 10^8 X - 2.498 \times 10^3$ for GLE in that of 4.16×10^{-4}—8.32×10^{-3} mg/ml and $Y = 2.050 \times 10^8 X + 1.260 \times 10^3$ for GEE in that of 6.20×10^{-4}—1.24×10^{-2} mg/ml. The r values were more than 0.9995 for the six reference standards. The recovery data was obtained from the comparison of the responses between the test samples and the spiked samples. The recoveries of the marker compounds ranged from 96.1 to 101.2% for the unhydrolyzed samples, from 91.4 to 98.3% for the base-hydrolyzed ones and from 93.6 to 99.1% for the acid-hydrolyzed ones. The limit of detection was 9.8 ng/ml for DAI, 6.4 ng/ml for GLI, 8.6 ng/ml for GEI, 16.2 ng/ml for DAE, 20.8 ng/ml for GLE, and 9.3 ng/ml for GEE. The relative standard deviations found for the analysis of samples ranged from 0.7 to 2.0%.

Quantification of Soy Isoflavones of Chinese Soy Materials Studies on the analysis of isoflavones of soy materials, foods or supplements have been reported. For example, Kudou *et al.*[16] and Wang *et al.*[10,11] investigated the contents of isoflavones of Japanese and American soybeans in details. But Chinese soy materials have not been systematically investigated yet. Thus, the hydrolytic and analytical procedures described above were applied to the samples collected from Chinese markets. They included yellow soybeans, black soybeans, the seed coats, hypocotyls and cotyledons separated from the seeds of soybeans or black soybeans, defatted soybean flours and Semen Sojae Praeparatum, a traditional Chi-

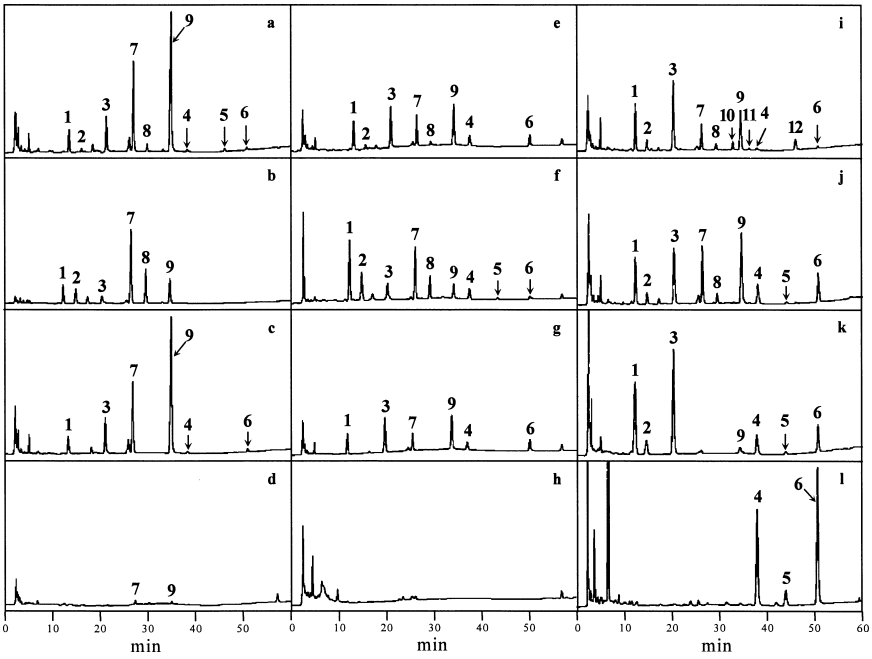

Fig. 4. HPLC Chromatograms of Methanolic Solutions of Soy Materials and Hydrolyzed Sample Solutions

The a, b, c and d indicate the soybean (sample #1) and its cotyledon (sample #2), hypocotyls (sample #3) and seed coat (sample #4), while e, f, g and h indicate the black soybean (sample #13) and its cotyledon (sample #14), hypocotyls (sample #15) and seed coat (sample #16), respectively. The i and j indicate defatted soybean flour (sample #18) and Semen Sojae Praeparatum (sample #23), while the k and l indicate basic-hydrolyzed and acid-hydrolyzed solution of sample #23. The weight of hypocotyl samples (#3 and #15) used in the analyses was 0.1 g, and that of other ones was 1.0 g. For HPLC conditions, see Experimental.

nese medicine made from the fermented black soybean or yellow soybean. Their representative HPLC chromatograms and analytical results were shown in Fig. 4 and Table 2.

There is a significant difference in the HPLC profiling patterns of isoflavones of different soy materials. For black soybean samples, the content ratios between Mal-DAI and DAI and between Mal-GEI and GEI were both near to 1 for black soybean samples, while for soybean ones, the ratios were both more than 3. The results demonstrate that the HPLC profiles of Chinese black soybeans are similar to American soybeans and that those of Chinese soybeans are similar to Japanese soybeans according to Wang and Murphy's report.[10] The distributions of isoflavones in the seed coat, hypocotyl and cotyledon were quite different from each other as well. The weight ratios between the seed coat, prumule, cotyledon and whole seed of soybeans were about 7%, 3% and 90%, respectively, which was determined using 50 g of soybean samples. In comparison with the soybean samples, the basic characteristic of HPLC profiling pattern of cotyledon ones was the absence of the peak of GLE, GLI and Mal-GLI, while that of the hypocotyl ones was the predominant peaks of GLI and Mal-GLI. That of seed coat ones was trace or the absence of all the soy isoflavones. The above results were in agreement with the data from Kudou's report.[16] The similar patterns were shown in the samples of black soybeans. As far as total isoflavones, the content level of Chinese natural soybean was a little lower than American and Japanese ones.[10]

Among four samples of defatted soy flour, sample #21 is a byproduct derived from the extrusion processing with the heat-treated soybeans. It showed the characteristics of higher content of acetylglucosides without the presence of malonylglucosides, while the rest samples contained both. Such results on the changes in malonylglucosides during the processing were demonstrated in the previous reports.[9,17]

The last group of soy materials used in the study is Semen Sojae Praeparatum, a Chinese herbal medicine made from the fermented black or yellow soybeans with the decoction of Folium Mori (mulberry leaf) and Herba Artemisiae Annuae (herb of sweet wormwood). It is described to be clinically effective for getting rid of vexation and expelling the exogenous evils from the body surface.[18] Such effects may be related to the activities of soy isoflavones in relieving postmenopausal symptoms, such as hot flash.[19] It was found that the characteristic of the HPLC profiling pattern of this herbal medicine was the predominant peaks of glucosides and aglycones. The contents of glucosides and aglycones varied only a little, being 0.50—0.59 mg/g for DAI, 0.14—0.17 mg/g for GLI, 0.44—0.58 mg/g for GEI, 0.20—0.25 mg/g for DAE, 0.03—0.06 mg/g for GLE and 0.15—0.17 mg/g for GEE. However, those of malonylglucosides varied markedly, being 0.12—0.75 mg/g for Mal-DAI, 0.03—0.14 mg/g for Mal-GLI, 0.11—0.64 mg/g for Mal-GEI. The acetylglycosides were trace in them. The results suggest that the fermentation process may result in the significant difference in the content of malonylglycosides now that Semen Sojae Praeparatum itself is a kind of fermented soybean product. And a standardized processing practice, thus, should be essential for the quality control of this herbal medicine.

Finally, the analytical results for the samples obtained by basic hydrolysis were similar to those of obtained by acidic hydrolysis, as shown in Table 2, which means both hydrolytic procedures are suitable for the determination of malonylglucosides and acetylglucosides. In comparison with both methods, the basic hydrolyzed procedure may be more accurate and reliable due to the fact that it produces no changes in aglycones, while the acid hydrolyzed one may be simpler and cheaper in the analytical practice due to the need for the ref-

Table 2. The Content (mg/g) of Isoflavones of Soy Materials from Chinese Markets

	Sample items	Production area /collection date	Glucosides			Malonylglucosides (base/acid)[a)]			Acetylglucosides (base/acid)			Aglycones			Total[b)]		
			DAI	GLI	GEI	Mal-DAI	Mal-GLI	Mal-GEI	Ac-DAI	Ac-GLI	Ac-GEI	DAE	GLE	GEE	DAE	GLE	GEE
1	Soybean	Heilongjiang/Oct. 2004	0.24	0.04	0.28	1.08/1.15	0.11/0.11	1.48/1.48	n.d./n.d.	n.d./n.d.	n.d./n.d.	0.02	tr.	0.02	0.70	0.12	0.85
2	Cotyledon		0.21	n.d.	0.28	0.88/0.91	n.d./n.d.	1.38/1.11	n.d./n.d.	n.d./n.d.	n.d./n.d.	0.02	n.d.	0.02	0.48	n.d.	0.82
3	Hypocotyl		2.00	1.66	0.66	9.02/9.61	4.10/3.78	2.16/1.97	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d.	n.d.	n.d.	7.27	3.26	1.35
4	Seed coat		n.d.	n.d.	n.d.	0.05/0.05	n.d./n.d.	0.05/0.05	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d.	n.d.	n.d.	tr.	n.d.	tr.
5	Soybean	Shandong/Nov. 2004	0.13	0.04	0.20	0.98/0.99	0.15/0.14	1.42/1.17	n.d./n.d.	n.d./n.d.	n.d./n.d.	0.01	tr.	0.01	0.69	0.07	0.71
6	Cotyledon		0.11	n.d.	0.20	0.74/0.79	n.d./n.d.	1.51/1.30	n.d./n.d.	n.d./n.d.	n.d./n.d.	0.01	n.d.	0.01	0.60	n.d.	0.77
7	Hypocotyl		2.10	1.73	0.66	9.35/9.70	4.18/4.67	2.25/2.02	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d.	n.d.	n.d.	6.19	3.59	1.47
8	Seed coat		n.d.	n.d.	n.d.	0.06/0.06	n.d./n.d.	0.05/0.05	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d.	n.d.	n.d.	tr.	n.d.	tr.
9	Soybean	Beijing/Feb. 2005	0.23	0.05	0.27	0.67/0.74	0.13/0.14	0.93/0.97	n.d./n.d.	n.d./n.d.	n.d./n.d.	tr.	tr.	0.01	0.50	0.11	0.45
10	Cotyledon		0.17	n.d.	0.24	0.46/0.49	n.d./n.d.	0.79/0.70	n.d./n.d.	n.d./n.d.	n.d./n.d.	tr.	n.d.	0.01	0.35	n.d.	0.50
11	Hypocotyl		2.66	2.37	0.79	7.43/7.71	4.35/4.77	1.94/1.84	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d.	n.d.	n.d.	5.57	4.06	1.45
12	Seed coat		n.d.	n.d.	n.d.	tr./tr.	n.d./n.d.	tr./tr.	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d.	n.d.	n.d.	tr.	n.d.	tr.
13	Black soybean	Liaoning/May. 2006	0.34	0.07	0.33	0.40/0.41	0.08/0.08	0.36/0.38	n.d./n.d.	n.d./n.d.	n.d./n.d.	0.09	n.d.	0.06	0.50	0.05	0.46
14	Cotyledon		0.22	n.d.	0.29	0.27/0.29	n.d./n.d.	0.27/0.27	n.d./n.d.	n.d./n.d.	n.d./n.d.	0.07	n.d.	0.06	0.35	n.d.	0.35
15	Hypocotyl		6.48	3.22	1.50	8.42/8.38	2.66/2.62	1.11/1.14	n.d./n.d.	n.d./n.d.	n.d./n.d.	0.97	0.20	0.56	8.30	3.49	1.57
16	Seed coat		n.d.	n.d.	n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
17	Hypocotyl	Heilongjiang/Sep. 2001	2.35	1.89	0.61	13.9/14.6	5.50/5.84	2.49/2.31	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d.	n.d.	n.d.	8.83	4.33	1.58
18	Defatted Soy floour	Jilin/Sep. 2004	0.52	0.14	0.59	0.42/0.38	0.11/0.10	0.57/0.54	tr./tr.	tr./tr.	tr./tr.	0.02	tr.	0.02	0.55	0.15	0.62
19		Liaoning/Apr. 2005	0.18	0.07	0.23	0.43/0.43	0.12/0.14	0.43/0.47	n.d./n.d.	n.d./n.d.	n.d./n.d.	0.01	tr.	0.01	0.34	0.12	0.41
20			0.23	0.10	0.30	0.41/0.41	0.14/0.17	0.61/0.52	n.d./n.d.	n.d./n.d.	n.d./n.d.	0.03	tr.	0.03	0.37	0.16	0.50
21			0.39	0.02	0.32	n.d./n.d.	n.d./n.d.	n.d./n.d.	0.51/0.52	0.05/0.06	0.53/0.46	0.09	0.05	0.11	0.59	0.10	0.55
22	Semen	Shenyang/Dec. 2005	0.59	0.17	0.58	0.12/0.14	0.03/0.03	0.11/0.12	tr./tr.	tr./tr.	tr./tr.	0.24	0.06	0.16	0.62	0.19	0.51
23	Sojae	Shenyang/Feb. 2005	0.50	0.17	0.44	0.23/0.23	0.06/0.06	0.16/0.20	tr./tr.	tr./tr.	tr./tr.	0.25	0.04	0.16	0.92	0.20	0.82
24	Praeparatum	Tianjin/Dec. 2005	0.53	0.14	0.48	0.75/0.81	0.14/0.15	0.64/0.65	tr./tr.	tr./tr.	tr./tr.	0.20	0.03	0.17	0.67	0.19	0.54

a) Basic/acid indicate the results of the samples treated with basic or acid hydrolysis procedures. *b*) The results of total aglycone content were obtained by the analysis of acid-hydrolyzed samples. n.d. and tr. are the same as both in Table 1.

erence standards of only three aglycones.

Conclusion

From the present study, we conclude that reliable hydrolytic methods as well as the efficiency of modern HPLC separation techniques were developed for the quantification of soy isoflavones of different soy materials. The results indicate that for the determination of malonylglucosides and acetylglucosieds, using ammonia as the basic hydrolytic reagent is more practical and advantageous than NaOH that was used in previous studies. The systematic quality evaluation of Chinese soy materials demonstrates the different characteristics of HPLC profiling patterns of isoflavones of yellow and black soybeans, defatted soy flours and Semen Sojae Praeparatum, the differences in the distribution of soy isoflavones of seed coat, hypocotyl and cotyledon, and a little lower content level of total isoflavones of Chinese natural soy materials than that of American and Japanese soybeans.

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