Report

For

Isoflavone compositions, antioxidant activities and total phenolic contents of soybean and soy products

Prepared by

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Summary

In this period of time, we have 1) selected representative soy products from the market, including fresh frozen edamame, soybean, tofu, soybean oil, soy yoghurt and soy milk samples; 2) successfully evaluated the isoflavone profiles of soy products; 3) determined the antioxidant capacities of soy products using ABTS; 4) investigated the total phenolic content of selected soy products.

The current study was conducted by triplicate using separate extraction process for each soy sample by three times individually to provide a much more reliable observation and evaluation. The results of the study were summarized as follows.

1) To evaluate the nutritional values of soy foods, representative soy products, including two fresh frozen edamame (with/without husk), two soybean, four tofu (extra soft, soft, firm, extra firm), three soybean oil, three soy yoghurt and three soy milk samples were selected in this study.
2) Solid soy products (fresh frozen edamame, soybean and tofu) were extracted with 50% acetone or 70% ethanol; liquid soy products (soybean oil, soy yoghurt and soy milk) were extracted with different ratios of acetonitrile.
3) The chemical compositions of three representative soy isoflavones, including daidzein, genistein and glycitein, were determined based on HPLC and standard compounds.
4) For the antioxidant activities of soy product extracts, soybean and tofu samples represented greater activities than other samples on generally. Total phenolic contents represented similar trends as the antioxidant results.

Detail information about experimental results is listed in the following sections.

Table of Contents

Project progress

In this period of time, we finished preliminary study 1) selected representative soy products from the market, including fresh frozen edamame, soybean, tofu, soybean oil, soy yoghurt and soy milk samples; 2) successfully evaluated the isoflavone profiles of soy products; 3) determined the antioxidant capacities of soy products using ABTS; 4) investigated the total phenolic content of selected soy products.

1. Sample selection and preparation

To evaluate the nutritional values of soy foods, representative soy products, including two fresh edamame (with/without husk), two soybean, four tofu (extra soft, soft, firm, extra firm), three soybean oil, three soy yoghurt and three soy milk samples were selected in this study.

Solid soy products (fresh frozen edamame, soybean and tofu) were extracted with 50% acetone or 70% ethanol. These two methods were selected based on our previous experiences, and tested and compared for their effectiveness in extracting the selected solid soy product samples, respectively.

For solid soy products, method 1) 2 gram of blended soy product samples were accurately weighed, extracted with 8 mL of 50% acetone; method 2) 2 gram of blended soy product samples were accurately weight, extracted by 8 mL of 70% ethanol. All experiments were performed in triplicate.

Liquid soy products (soybean oil, soy yoghurt and soy milk) were extracted with different ratios of acetonitrile. Briefly, 1:3 (300 µL sample: 900µL Acetonitrile) and 1:5 (200 µl sample : 1,000 µL Acetonitrile) were prepared in triplicate, vortexed, then centrifuged for 5 minutes at 12,000 rpm. Supernatants were collected for further analysis.

2. Isoflavone compositions of soy products

Optimized condition for HPLC

The HPLC-UV was utilized to determine the chemical compositions of three major soy isoflavones in all the soy product extracts. A Shimadzu HPLC-UV system was used coupled with a luna C-18 column, 4.6 mm inner diameter × 250 mm and 3.5 µm particle size. HPLC grade water with 0.1% formic acid (v/v) was used as solvent A, and methanol with 0.1% formic acid (v/v) was used as solvent B. The elution was carried out at 10% of solvent B at the beginning, increasing via linear gradient to 100% B at 15 min; and the post-run time for re-equilibration was 10 min. The injection volume was 10 µL, flow rate was 1 mL/min, and the oven temperature was 40 °C. The UV wavelength was 280 nm.

Results

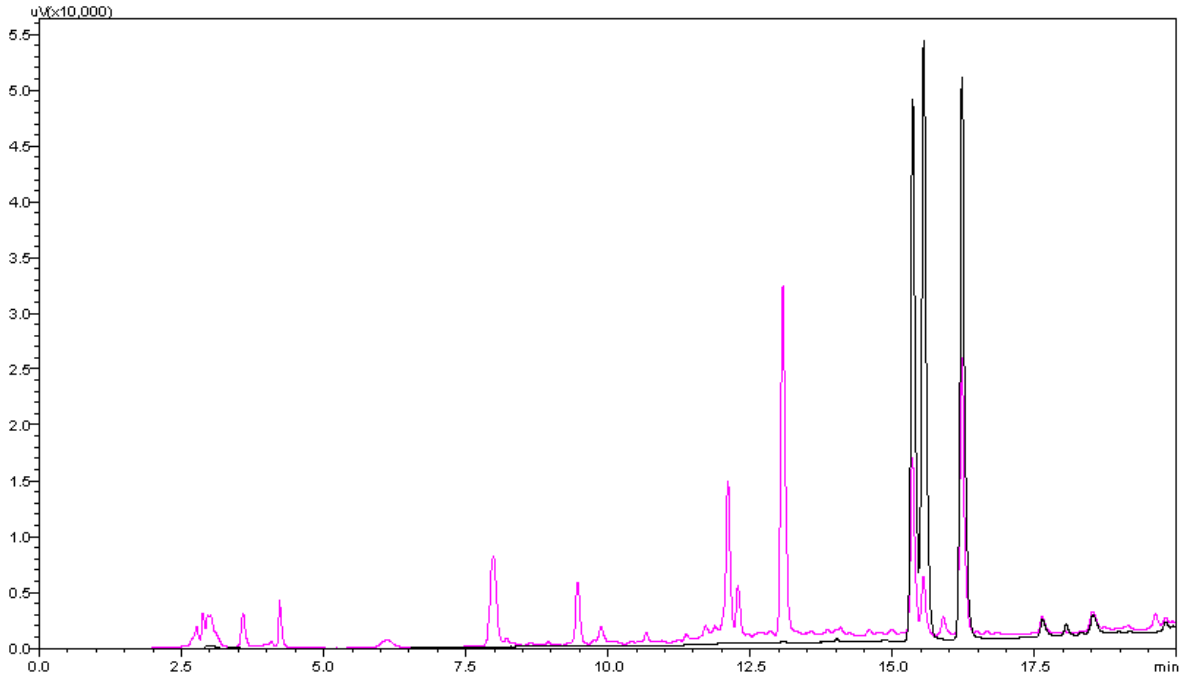

Fig. 1. Representative HPLC chromatogram of isoflavone (black) and tofu samples (pink).

Representative HPLC chromatograms of standard compounds and soy products were showed in **Fig.1**. As the results, different soy products represented different isoflavone compostions. The isoflavone concentrations in fresh frozen edamame and soybean oil samples were lower than the limit of detection. While in the soybean samples, the concentrations of daidzein, genistein and glycitein were 30-100, 4-10 and 30-70 μg/g soybean, respectively. For the tofu samples, the concentrations of daidzein, genistein and glycitein were 2.58-20.98, 13.54-52.50 and 12.31-40.68 μg/g tofu sample, respectively. The amounts of daidzein, genistein and glycitein in soy yoghurt samples were 14.00-14.34, 11.50-11.80 and 12.53-13.03 μg/g sample weight, respectively. For soymilk samples, the concentrations of daidzein, genistein and glycitein were 8.76-10.02, 3-9.06 and 7.56-10.62 μg/mL soymilk, resepectively.

In all the soy products, soybean represented greatest amounts of isoflavone contents, followed with tofu, soy yoghurt and soy milk samples. These results indicated the concentrations of isoflaoves in soy products, but the results might be re-considered or measured again by the dry weight of soy samples, since the water contented in some fresh soy products might significantly change the results. No isoflavone was detected in fresh frozen edamame or soybean oil samples. The isoflavones were metabolized during the later growth stage of soybean, so these compounds were not existed in the early stage of soybean, which was also known as edamame samples. On the other hand, isoflavones were polar compounds, which made them difficult to be existed in the nonpolar oil samples. Besides, 70% ethanol could extract greater amounts of daidzein and glycitein from soy products, whereas 50% acetone could extract more genistein.

3. Antioxidant activities of the soy products

The antioxidant activity is one of the most important bioactivities. Intake of antioxidants is closely linked with the reduced incidence of several chronic diseases. To estimate the availability of free radical elimination components in different soy products, ABTs cation radicals scavenging capacity (ABTS) was utilized in this study.

To measure the ability of antioxidants to scavenge ABTs cation radicals, the decolorization assay ABTs radical scavenging capacity assay was used. Compared to enzymatic methods that also used to test ABTs radical scavenging capacity, this chemical method eliminates the potential interference during radical generation caused by enzyme inhibition and is simpler to be conducted. All the results are expressed as micromoles of trolox equivalents (TE) per g of soy product samples.

Table 1. Antioxidant activity of solid soy products

Sample ID	μmol trolox equivalent/g	
	50% acetone extracts	70% ethanol extracts
Extra soft tofu	0.18 ± 0.04	0.12 ± 0.02
Soft tofu	0.10 ± 0.00	ND*
Firm tofu	0.10 ± 0.01	0.07 ± 0.02
Extra from tofu	0.13 ± 0.08	0.12 ± 0.07
Dry soybean	ND*	ND*
Fresh frozen edamame	ND*	0.23 ± 0.06

*ND represented not detectable.

Table 2. Antioxidant activity of liquid soy products

Sample ID	μmol trolox equivalent/mL		
	50% acetone extracts	70% ethanol extracts	Acetonitrile
Soybean oil	0.07 ± 0.03	0.09 ± 0.03	／
Soy yoghurt	／	／	0.08 ± 0.03
Soy milk	／	／	0.03 ± 0.01

／represented not extracted with this method.

The antioxidant activities of solid soy products were showed in **Table 1** and **Table 2**. Different soy products showed various capacities in scavenging ABTS radical. For solid samples, fresh prozen edamame extracted by 70% ethanol represented the greatest antioxidant activity (0.23 μmol trolox equivalent/g sample), followed by the extra soft tofu extracted by 50% acetone (0.18 μmol trolox equivalent/g sample). None detection of antioxidant activity in soybean samples might because of the mistake during experiment processing. For liquid soy products, all the samples showed almost similar antioxidant activities.

4. Total phenolic content (TPC) of the soy products

Total phenolic content (TPC) of selected vegetables were performed using our previous published method involving Folin-Ciocalteu reagent and gallic acid as standard.

Table 3. Total phenolic contents of solid soy products

Sample ID	mg GAE equivalent/g	
	50% acetone extracts	70% ethanol extracts
Extra soft tofu	0.29 ± 0.05	0.41 ± 0.01
Soft tofu	0.40 ± 0.10	0.23 ± 0.00
Firm tofu	0.50 ± 0.20	0.17 ± 0.01
Extra from tofu	0.22 ± 0.00	0.20 ± 0.04
Dry soybean	1.40 ± 0.20	3.95 ± 0.67
Fresh frozen edamame	0.65 ± 0.15	2.00 ± 0.50

Table 4. Total phenolic contents of liquid soy products

Sample ID	mg GAE equivalent/mL		
	50% acetone extracts	70% ethanol extracts	Acetonitrile
Soybean oil	ND	ND	／
Soy yoghurt	／	／	1.49 ± 0.42
Soy milk	／	／	1.59 ± 0.16

*ND represented not detectable.／represented not extracted with this method.

The total phenolic contents of soy products were showed in **Table 3** and **4**. For solid soy samples, soybean and edamame samples showed greater TPC values compared with the tofu products; this might due to the lower water contented in bean samples, especially for the dry soybean samples. In liquid soy products, no phenolic compounds could be detected from soybean oil samples due to the polarity of oil. Soy yoghurt and soymilk samples showed relatively greater but almost similar TPC values, because their chemical profiles are almost similar.