Determination of Phytoestrogenic Compounds of Soybean Sprouts Grown in Antalya, Turkey

Nevzat KONAR

***Abstract*—I**n this study, quantitative identifications of phytoestrogenic compounds, such as free and conjugated isoflavones, lignans, coumestrol and various bioflavonoids, on fresh soybean sprout samples, grown in Antalya Turkey, by a triple quadrupole liquid chromatography-tandem mass spectrorscopy (LC-MS/MS) technique following different pretreatments, such as conventional extraction (CE), acid hydrolysis (AH), enzymatic hydrolysis (EH) and enzymatic and acid hydrolysis (EAH). The obtained data and levels of the identified compounds significantly varied according to the sample preparation method. As example, the most effective sample preparation method for total isoflavone content determination was the CE. It was found that secoisolariciresinol (2446.0 µg/100 g, wet basis, sample prepared by EAH) was the major phytoestrogenic compound in the samples.

***Keywords*—**Soybean sprouts, phytoestrogen, hydrolysis, LC-MS/MS

I. Introduction

Phytoestrogenic compounds are found mainly in legumes, cereals, linseed, other fruits and vegetables clover, and alfalfa sprouts [1]. They naturally present in many plant foods and include isoflavones, coumestans and lignans [2] a group of non-steroidal polyphenolic plant metabolites that induce biological responses and can mimic or modulate the action of endogenous oestrogens, often by binding to oestrogen receptors [3].

Interest in soy foods has increased with consumer awareness of its health benefits, especially with soy-related ingredients being utilized as one of the major sources of high protein fortification [4] and also they contain bioactive compounds as isoflavones which are member of flavonoids.

Depending on their structure, phytoestrogens can be divided into flavonoid and non-flavonoid polyphenols. The main representatives of the flavonoid phytoestrogens are isoflavones. Lignans and coumestans are the main non-flavonoid phytoestrogens [5].

Chromatographic techniques are the principle analysis methods used in phytoestrogenic compound analysis. Before the application of these techniques, samples of the different species underwent sample preparation methods, such as liquid-liquid extraction, methanol extraction, enzymatic hydrolysis, acid hydrolysis, solvent (ethanol or methanol) extraction and enzymatic hydrolysis, solvent extraction and acid hydrolysis, solvent extraction and/or enzymatic hydrolysis and acid hydrolysis [6, 7].

In this study, we analysed the amounts of free and conjugated isoflavones, lignans and, coumestrol in samples of soybean sprouts grown in Antalya Turkey (Glycine max L.) prepared by conventional extraction, acid hydrolysis, enzymatic hydrolysis and also both enzymatic and acid hydrolysis.

II. Materials and Methods

A. Sampling and Sample Preparation

Fresh samples of soybean sprouts grown in Antalya, Turkey region were bought in 1.0 – 1.5 kg amounts from three different local market in Ankara (Turkey) in 2011. All parts of the samples were chopped in a chopper (Fakir, Germany). Subsequently, 100 g of sample was placed in polyurethane bags and kept at -18°C prior to different sample preparation methods.

B. Standards and Reagents

The phytoestrogen standards of ononin (purity 98.0%), genistin (purity ≥95.0%), daidzin (purity ≥95.0%), glycitin (purity ≥95.0%), sissotrin (purity ≥95.0%), biochanin A (purity ≥95.0%), glycitein (purity 97.0%), genistein (purity 98.0%), formononetin (purity ≥99.0%),, daidzein (purity 98.0%), matairesinol (purity ≥85.0%), secoisolariciresinol (purity ≥95.0%), coumestrol (purity ≥95.0%), apigenin (purity ≥95.0%) and quercetin (purity 98.0%) were purchased from Sigma (St. Louis, Mo., USA) and standard of rutin (purity ≥90.0%) was purchased from Wako (Osaka, Japan). 100.0 µg/mL of standards stock solutions were prepared using methanol.

Cellulase (from Aspergillus sp., ≥1000 u/g, Sigma C2605), β-glucosidase (from almond, ≥6 units/mg, Sigma 49290) and β-glucuronidase (from *Helix pomatia*, Type HP2, 100,000 units/mL, Sigma G7017) enzymes were also purchased from Sigma. 1 mg/mL in 0.2 mol/m^3 sodium acetate buffer pH 5 solution of β-glucosidase was prepared and used in the study.

All reagents, solvents and chemicals were of analytical or HPLC grade and were obtained from Sigma or Merck (Darmsadt, Germany).

C. Apparatus

Ultrapure water was prepared using a Milli-Q System (Millipore S.A., Molsheim, France). Additionally, during sample preparation and extraction, the following equipment was

Nevzat KONAR; Author is with the Food Safety Institute of Ankara University, 06110 Diskapi, Ankara, Turkey (phone: +90 312 5961429; fax: +90 312 3178711; e-mail: nevzatkonar@hotmail.com).

used: ultra-centrifuge (Universal 320R, Hettich, Tufflingen, Germany), ultrasonic water bath (LBS2, Falc Instrument, Treviglio, Italy), sample concentrator under nitrogen (EVA-EC1-L 24-16, VLM, Germany) and general laboratory equipment.

D.Conventional Extraction

The conventional extraction performed in this study were based on methods previously developed and used by Konar et al. [6, 7].

E. Acid Hydrolysis

The acid hydrolysis performed in this study were based on methods previously developed and used by Konar et al. [6, 7].

F. Enzymatic Hydrolysis

The enzymatic hydrolysis performed in this study were based on methods previously developed and used by Konar et al. [6, 7].

G. Enzymatic and Acid Hydrolysis

The enzymatic and acid hydrolysis performed in this study were based on methods previously developed and used by Konar et al. [6, 7].

H. LC-MS/MS Conditions

The method of LC-MS/MS applied by Konar et al. [6, 7] was used. The samples were injected into the LC/MS-MS system in triplicate.

H. Statistical Analysis

Quantitative data are expressed as mean. Standard deviations were determined by using SPSS 15.0 (SPSS Inc., Chicago, IL.).

III. Results and Discussion

The quantitative results of LC-MS/MS measurements performed on soybean sprouts are reported in Table 1. The obtained data and levels of the identified compounds significantly varied according to the sample preparation method.

There have been many studies describing the amounts of phytoestrogenic compounds [7] in soy and soy products [8, 9, 10, 11, 12, 13]. Kim et al. [14] stated that soybean sprouts have not a rich content of phytoestrogenic compounds as other soy products. Kuhnle et al. [15] used the LC-MS/MS technique on enzymatically hydrolysed samples to determine that the total isoflavone concentrations are 124,381 µg/100 g for soybean flour (62,125 µg/100 g of genistein, 54,128 µg/100 g of daidzein, and 8,114 µg/100 g of glycitein) and17,544 µg/100 g for cooked soybeans (10,646 µg/100 gof genistein, 5,730 µg/100 g of daidzein, and 1,144 µg/100 g of glycitein). In this study the most effective sample preparation method for total isoflavone content determination was the conventional extraction (CE). By this sample preparation method, total isoflavone content was determined as 536.5 µg/100 g (wet basis) and also glycitein (293.2 µg/100 g) and daidzein (160.8 µg/100 g) were the major isoflavone compounds of the soybean sprout.

Most of the other free- (genistein, bichanin A, formononetin) and –conjugated (genistin, daidzin, glycitin, ononin and sissotrin) isoflavones were quite low for the samples prepared each of methods.

Glycitin, ononin and sissotrin could not be identified in any sample prepared by used different methods whereas coumestrol which as a coumestan was identified all samples prepared by used conventional extraction (CE) (1.50 µg/100 g), enzymatic hydrolysis (EH) (91.7 µg/100 g), acid hydrolysis (AH) (10.4 µg/100 g) and enzymatic and acid hydrolysis (EAH) (37.4 µg/100 g). To identify coumestrol content of soybean sprouts, EH was determined as most efficient sample preparation method in this study.

As coumestrol, the bioflavonoid (rutin, apigenin and quercetin) content of soybean sprout samples were quite low. For example, in samples prepared by AH, any of these compounds could be identified. Amongst the bioflavonoids, quercetin could not be identified in any of the soybean sprout samples. Rutin could not be identified in the samples prepared by used EH and AH. Whereas this compound concentrations in the samples prepared used by CE and EAH were found to be as 20.9 and 37.4 µg/100 g (wet basis), respectively. Apigenin were identified only in samples prepared by EH (28.8 µg/100 g) and (0.60 µg/100 g).

Significant results were obtained in terms of lignan levels in the soybean sprout sample. These results particularly important in terms of secoisolariciresinol amounts, which varied according to sample preparation methods. The amount of this compound varied between 278.6 – 2446.0 µg/100 g (wet basis) for soybean sprout. These results indicated that there were significant differences between the amounts, which were identified according to sample preparation methods. Secoisolariciresionol levels in the sample prepared with EAH were the highest in all samples. Thus, the EAH method should be used for the quantitative identification of secoisolariciresinol in soybean sprout samples. The amount of matairesinol, which is another lignin, was found to be below LOQ or LOD or could not be identified in samples prepared by used EH and EAH. Matairesinol amounts in the samples prepared used by CE and AH were found to be 7.90 and 7.60 µg/100 g (wet basis), respectively.

IV. Conclusion

Soybean sprout could not be an alternative to other soy products depends of phytoestrogenic compound content. Especially, isoflavones were identified at very low levels. Secoisolariciresinol was determined as the most important phytoestrogenic compound in soybean sprout samples grown in Antalya Turkey. Sample preparation method must be chosen as considering targeted compounds.

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Table 1. Phytoestrogenic compound content of soybean sprout sample grown in Antalya, Turkey (µg/100 g, wet weight)

Phytoestrogen	CE	EH	AH	EAH
BiochaninA	6.30 ± 1.10	2.70 ± 0.04	n.d.	n.d.
Daidzein	160.8 ± 4.97	12.3 ± 1.11	52.6 ± 3.33	5.00 ± 0.04
Daidzin	37.5 ± 1.05	n.d.	20.4 ± 2.32	n.d.
Formononetin	n.d.	2.00 ± 0.04	n.d.	1.30 ± 0.02
Genistein	23.5 ± 3.44	28.0 ± 2.31	10.4 ± 1.12	8.30 ± 0.98
Genistin	15.2 ± 0.97	2.50 ± 0.12	7.40 ± 0.07	2.40 ± 0.54
Glycitein	293.2 ± 11.5	23.0 ± 0.13	68.7 ± 2.11	7.80 ± 2.11
Glycitin	n.d.	n.d.	n.d.	n.d.
Ononin	n.d.	n.d.	n.d.	n.d.
Sissotrin	n.d.	n.d.	n.d.	n.d.
Matairesinol	7.90 ± 0.65	n.d.	7.60 ± 0.12	n.d.
Secoisolariciresinol	947.7 ± 14.0	278.6 ± 3.42	364.3 ± 11.4	2446.0 ± 98.9
Apigenin	n.d.	28.8 ± 2.73	n.d.	0.60 ± 0.01
Quercetin	n.d.	n.d.	n.d.	n.d.
Rutin	20.9 ± 1.23	n.d.	n.d.	37.4 ± 2.14
Coumestrol	1.50 ± 0.01	91.7 ± 8.97	10.4 ± 2.21	45.7 ± 3.71
Total isoflavone	536.5	70.3	159.4	24.9
Total lignan	955.6	278.6	371.9	2446.0
Total bioflavonoid	20.9	28.8	0.0	38.0
Total phytoestrogen	1514.5	469.4	541.7	2554.5

CE; conventional extraction, AH; acid hydrolysis, EH; enzymatic hydrolysis, EAH; enzymatic and acid hydrolysis, n.d.; not detected. All data from the samples are the mean ± S.D.of three analyses. Total isoflavones value is the sum of daidzein, genistein, glycitein, biochanin A, formononetin, daidzin, genistin, glycitin, ononin and sissotrin. Total lignans value is the sum of secoisolariciresinol and matairesinol.Total bioflavonoids value is the sum of quercetin, rutin and apigenin.