Characterization and stability of bioactive compounds from soybean meal

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ABSTRACT

In the present study we characterized soybeans and soybean meals, which is the high protein co-product of soybean oil extraction, produced at industrial or laboratory scale. Additionally, for the purpose of future metabolic and bioactivity *in vivo* studies, we conducted, for the first time, a 6-month stability study of soybean meal dry extracts. Soybean meal extracts were obtained either by ethanol or a ternary solvent mixture composed of water, ethanol and ethyl acetate (40:40:20). Soybean meals presented 43% higher protein content, from 29% to 101% higher bioactive compounds contents and 52% higher anti-oxidant capacity than soybeans. High moisture thermal procedure employed during soybean meal processing led to a 13-fold increase in aglycone isoflavones contents, which could affect the bioavail-ability of isoflavones in this residue. In addition to a 29-fold higher extraction yield, bioactive compounds showed higher stability in ternary solvent mixture extracts in comparison to ethanol, independently of the sample or storage conditions. We concluded that dry soybean meal extracts are suitable materials for performing long-term *in vivo* studies, as these extracts were stable when stored at room temperature unprotected from light for 180 days.

1. Introduction

Differently from Asian countries, soybean-based foods are not widely consumed in Western countries, where soybean is most commonly crushed and the oil is extracted and refined into food-grade oil for frying and cooking. The co-product from this process is a high-protein meal, known as soybean meal, mainly used as animal feed. It may also be used for the production of soy protein isolates and concentrates, which in turn are used as ingredients in meat and bakery products, beverages, soups, infant formulas and other food products (Aguiar et al. 2012), thus aggregating economic value to this residue.

In addition to its content of high-quality protein, soybean meal contains high contents of bioactive compounds, mainly isoflavones (Kao & Chen, 2006; Kao, Chien, & Chen, 2008) and soyasaponins (Wang, Wang, Lu, Kao, & Chen, 2009). These bioactive compounds from soybeans and its products are associated with lower preva-lence of a number of chronic diseases, including cardiovascular diseases and certain types of cancer (Georgetti, Casagrande, Souza, Oliveira, & Fonseca,, 2008).

Soybean meal may be used as a source of various bioactive compounds for metabolic and bioactivity studies. However, papers have focused on soybean meal as a source of isoflavones (Wang et al., 2009), while soyasaponins are usually overlooked, even though this class of bioactive compounds may occur in higher amounts than isoflavones (Kao & Chen, 2006). Bioactivity studies investigating the chronic effects of isoflavones and soyasaponins isolated from soybean meal in animals and humans should consider the stability of these compounds during the whole inter-vention period, since it is known that isoflavones and soyasaponins contents and/or profile may be affected by exposure to high tem-peratures, oxygen and UV-light (Georgetti et al., 2008; Rostagno, Palma, & Barroso, 2005). However, to the best of our knowledge, the stability of bioactive compounds in soybean meal extracts has not been yet investigated.

Therefore, the aim of this work was to chemically characterize soybean meal samples, with an emphasis on isoflavones and soyasaponins, in order to promote its use as a food ingredient and thus widen high added-value applications of this residue. Addi-tionally, we aimed to investigate, for the first time, the stability of

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isoflavones and soyasaponins in dry extracts obtained from soybean meal under different storage conditions, for future use in long-term bioactivity studies.

2. Materials and methods

2.1. Standards and chemicals

Folin-Ciocalteau reagent, gallic acid, 2,2′-azino-bis-(2-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-tris(2-pyridyl)-S-triazine (TPTZ), potassium persulfate, fluorescein, 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) and (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Sigma–Aldrich (St. Louis, MO). Sodium carbonate, vanillin and aluminum chloride were purchased from Spectrum Chemical Manufacturing Corp. (Gardena, CA). Iron (II) sulfate was purchased from Merck (Darmstadt, Germany). Daidzin, glycitin, genistin, daidzein, glycitein, genistein, soyasapogenol B and soyasaponins B-I, B-II and B-III standards were purchased from Apin Chemicals® (Abingdon, UK). Saponin standard from soybean was purchased from Wako Pure Chemical Industries (Osaka, JP). All solvents were HPLC grade from Tedia (Fairfield, OH). Milli-Q water (Millipore, Bedford, MA) was used throughout the experiments.

2.2. Samples

Two sets of samples comprised of soybeans and soybean meal were investigated. In both sets, samples were from the same batch. One set was donated by a soybean oil crush industry, while the other was produced in our laboratory using commercial soybeans purchased in local markets.

Lab-scale soybean meal production was analog to industrial processing, with adaptations. Seeds were cracked and the hulls separated using an air stream. Dehulled soybeans were wet and incubated at 60 °C for 1 h in an oven. The sample was occasionally water-sprayed to keep the beans wet. The seeds were then ground in a domestic processor and dried for 5 h at 60 °C in an oven. After drying, the sample was additionally ground in an analytical mill (IKA A11 basic S1) in order to maximize oil extraction. 20 g of sample was placed in a cellulose cartridge (33 × 94 mm) and the extraction procedure was conducted in a Soxhlet extractor for 7 h using n-hexane. The meal was left in a fume hood until dry and stored at room temperature.

2.3. Proximate composition

Moisture, ash, proteins and lipids were determined by the official methods of the AOAC (2000). Total carbohydrates were determined by difference. Each sample was analyzed in triplicate.

2.4. Bioactive compounds analysis

Soybean and soybean meal samples were extracted with a ternary solvent mixture (water:ethanol:ethyl acetate − 40:40:20). Briefly, 2 g of sample were extracted with 10 mL of the ternary mixture in a vortex for 2 min. After centrifugation for 10 min at 1690 × g, the supernatant was collected and the residue was re-extracted twice as described above. The extraction procedure for each sample was conducted in duplicate. These extracts were analyzed for the determination of phenolic compounds, flavonoids and saponins by spectrophotometric assays, and isoflavones and soyasaponins by LC-DAD-MS.

2.4.1. Spectrophotometric assays

Total phenolic content was determined by the Folin-Ciocalteau reagent assay, as described by Singleton, Orthofer, and Lamuela-Raventós (1999). Quantification was performed using a gallic acid calibration curve. Results were expressed as mg of gallic acid equivalents per g on a dry weight basis (dwb) (mg GAE/g).

Total flavonoid content was determined by the spectrophotometric assay described by Taie, El-Mergawi, and Radwan (2008). Quantification was performed using a genistein calibration curve. Results were expressed as mg of genistein equivalents per g dwb (mg GE/g).

Total saponin content was determined by the vanillin-sulfuric acid assay, as described by Shiau et al. (2009). Quantification was performed using a calibration curve prepared with a commercial mixture of saponins obtained from soybeans. Results were expressed as mg of total saponins per g dwb.

The antioxidant capacity of the extracts was determined by FRAP (Ferric Reducing Ability of Plasma) and TEAC (Trolox Equivalent Antioxidant Capacity) assays. The FRAP assay was performed according to Benzie and Strain (1996) with slight modifications. The TEAC assay was performed according to Re, Pellegrini, and Proteggente (1999) with slight modifications.

Each sample was independently analyzed by each assay in triplicate.

2.4.2. Isoflavones and soyasaponins

Isoflavones and soyasaponins were simultaneously analyzed by LC-DAD-MS as described by Fonseca, Villar, Donangelo, and Perrone (2014). The LC system (Shimadzu, Kyoto, Japan) comprised an LC-10ADvp quaternary pump, a CTO-10ASvp column oven, an 8125 manual injector (Rheodyne) with a 20 μL loop and an SPD-M10Avp diode array detector (DAD). This LC system was coupled to an LC–MS 2010 mass spectrometer (MS) (Shimadzu, Kyoto, Japan) equipped with an electrospray ion source. Chromatographic separation was achieved using a Kromasil® C18 column (150 × 2.1 mm, 5 μm, 100 Å) maintained at a constant temperature of 40 °C. The LC two-phase mobile system consisted of a gradient of water (eluent A) and acetonitrile (eluent B), both added with 0.3 g/100 mL formic acid, with a constant flow rate of 0.3 mL/min. Prior to injection, the column was equilibrated with 15% B. After injection, this proportion was modified to 23% B in 1 min, kept constant until 23 min and increased to 50% B until the end of the 35 min run. Twenty min intervals were used to re-equilibrate the column with 15% B. Extracts were filtered through a 0.45 μm PTFE filter unit.

Isoflavones and soyasaponins were monitored by DAD between 190 and 370 nm and by MS using positive ionization, with a nebulizer gas (N_2) flow of 3.0 L/min, operated in the single ion monitoring (SIM) mode. Identification of aglycone and glycosylated isoflavones was performed by comparison with retention time and pseudomolecular ion of the respective standard. Identification of soyasaponins was performed by comparison with retention time and the most abundant ion of the respective standard ($[M+H]^+$ for soyasaponin B-I and $[M\text{-sugar-}H_2O + H]^+$ for both soyasaponins B-II and B-III). Identification of compounds for which there were no commercial standards available (malonylglucosilated and acetylglucosilated isoflavones) was performed by the pseudomolecular ion in the MS (Supplementary Figure 1A–E).

Quantification was performed by external standardization. Isoflavones were quantified by their DAD peak areas at 250 nm. The contents of malonylglucosilated and acetylglucosilated isoflavones were determined from the calibration curve of the corresponding β-glucosylated isoflavone. Soyasaponins (B-I, B-II and B-III) were quantified by their DAD peak areas at 195 nm. Soyasaponins B-II and B-III were quantified together, as it was not possible to chromatographically separate these compounds. Data were acquired by