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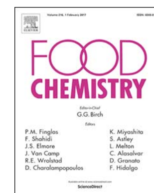

Effects of moisture and temperature during grain storage on the functional properties and isoflavone profile of soy protein concentrate

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ABSTRACT

The objective of this study was evaluate the effects of moisture and temperature during grain storage on the functional properties and isoflavone profile of soy protein concentrate. The protein concentrate was extracted from soybeans on the first day of storage and after 12 months of storage, at 12 and 15% moisture and at temperatures of 11, 18, 25, and 32 °C. The protein concentrate obtained from grains stored at 32 °C showed decreased extraction yield (51.6% with 15% of moisture) and protein solubility (24.8% with 15% of moisture) with increased emulsifying capacity (52.2% with 12% of moisture) and foam formation, compared with sample obtained at the start of the storage period. The isoflavone profile showed a decrease in the concentrations of glycosylated, malonyl, and acetyl isoflavones and a corresponding increase in the concentrations of aglycone isoflavones, with the magnitude of these changes increasing with increasing storage temperature and moisture content.

1. Introduction

Soybeans are one of the main agricultural products produced in the world. The main producers are the United States and Brazil, which represent more than 60% of world production (FAO, 2017). Soybeans are harvested seasonally due to crop characteristics, making storage necessary to satisfy demand for these grains throughout the year. During the storage period, soybeans are susceptible to changes in physicochemical, technological, and nutritional properties. The main factors that affect the quality of these grains during storage include grain moisture, storage time and temperature, relative humidity in the storage environment, and grain quality prior to storage (Kong & Chang, 2013; Ziegler, Marini, et al., 2016; Ziegler, Vanier, et al., 2016).

It is estimated that approximately 60% of processed foods contain some soy ingredients (Liu et al., 2008), due to a protein content of 38% (Ziegler, Marini, et al., 2016). Soy proteins are used in various foods such dairy products, pasta, soups, and a variety of nutritional supplements, due to their excellent functional (Protein solubility, absorption capacity of water and oil, emulsifying capacity and foaming), nutritional, and bioactive properties. These proteins are composed primarily of globulins (~90% by weight), which can be extracted with dilute saline solutions. Glycinin and β-conglycinin are the two major storage globulins. Glycinin, also known as 11S soy protein, has six subunits, each composed of an acidic polypeptide and a basic polypeptide linked by a disulfide bond, while β-conglycinin, a 7S protein, is a glycoprotein that has three subunits (α′, α, and β) (Hou & Chang, 2004).

The quality of major soy products such as tofu and soymilk, which are mainly produced and consumed on the Asian continent, is directly influenced by the quality of the soy protein. Exposure of the soybeans to adverse storage conditions is one of the main causes of reduced protein extractability and structural changes in the soy protein, such as denaturation, glycosylation, strengthening of disulfide bonds, and decreased hydrophobic surface area, which ultimately reduce tofu and soymilk yield (Hou & Chang, 2004; Kong & Chang, 2013).

Soy protein concentrate is rich in isoflavones, which have been cited for their health benefits, including protection against uterine cancer (Eason et al., 2005), relief of menopause symptoms (Nahas et al., 2007), treatment of inflammatory diseases of the airways (Bao et al., 2011), and prevention of oxidative damage (Dixit et al., 2012). In soybeans and their derivatives, isoflavones appear in four distinct chemical forms: malonylglucosides, acetylglucosides, β-glucosides, and aglycones, of which aglycones have the highest bioactivity and bioavailability, and can be absorbed most rapidly (Chen et al., 2013; Lima & Ida, 2014). The changes occurring in the chemical form of these isoflavones during storage are not yet completely elucidated in the literature and deserve to be studied.

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In this context, considering the technological, economic, and nutritional importance of the soybean for numerous foods, and the various factors that can affect the quality of the grains during storage, the objective of this study was to evaluate the effects of grain moisture and storage temperature on the functional properties and isoflavone profile of soy protein concentrate.

2. Material and methods

2.1. Material and sample preparation

The yellow-colored soybeans (*Glycine max* L., cv. BMX Magna RR) used in this study were harvested from the Canguçu countryside (31°23′44″S, 52°41′11″W; 408 m) in the State of Rio Grande do Sul, Brazil. The grains were mechanically harvested when the moisture content was approximately 22%, placed into raffia bags, and immediately transported to the Postharvest, Industrialization and Quality of Grains Laboratory of DCTA-FAEM-UFPel, where the study was carried out. The grains were subjected to artificial drying in an oven dryer (model 400-2ND, Nova Ética, Brazil) set at 38 °C until they achieved 15%, and 12% moisture content; they were subsequently purged using aluminum phosphide to prevent the interference of insects in the experiment. The dried grains were stored in polyethylene bags, which were composed of a 0.2-mm-thick plastic film and had a capacity of 0.9 kg, at temperatures of 11, 18, 25, and 32 °C for 12 mo, in triplicate. The grains were covered with aluminum foil to block light. Every 60 days, the bags were replaced, thus avoiding the accumulation of carbon dioxide generated by the respiration and metabolic processes of the grains and associated microorganisms. This procedure was similar to what occurs during soybean storage in silos (non-hermetic storage), where the carbon dioxide is removed by an aeration process. For analysis, the grains were ground in a laboratory mill (Perten 3100, Perten Instruments, Huddinge, Sweden) equipped with a 35-mesh sieve to obtain flour with uniform particle size and defatted with petroleum ether, continuous extract for 8 h using a Soxhlet extraction apparatus.

2.2. Protein extraction

Protein extraction was performed as described by Liu et al. (2008), with minor modifications. The defatted flour was suspended in distilled water at a ratio of 1:10 (flour:water), and the pH was adjusted to 9.0 with 1 mol/L NaOH. The solution was stirred at room temperature for 2 h and then centrifuged at $3500 \times g$ for 15 min. The supernatant was collected and the precipitate was subjected to further extraction. Fractions of the supernatant were pooled and the pH was adjusted to 4.5 with 1 mol/L HCl. After centrifugation at $3500 \times g$ for 20 min, the supernatant was discarded and the precipitate (protein fraction) was resuspended in distilled water. The pH was adjusted to 7.0 with 1 mol/L NaOH, and the protein was lyophilized. The protein extraction yield was calculated by expressing the yield of lyophilized protein as a percentage of the amount of defatted bran used for extraction.

2.3. Protein, ash, and carbohydrate content of protein concentrate

The protein and ash content of the protein concentrate were determined according to the methodology described by AOAC (2006) and expressed as a percentage. The carbohydrate content of the protein concentrate was determined by taking the difference between the total mass and the mass of the other constituents. This was then expressed as a percentage.

2.4. Protein solubility of protein concentrate

The protein solubility of the protein concentrate was determined as described by Liu et al. (2008), with minor modifications. Sample (0.5 g) was mixed with 25 mL of distilled water in a 50-mL beaker, the pH was adjusted to 7.0, and the mixture was stirred with a magnetic stirrer for 1 h. The samples were then centrifuged at $5000 \times g$ for 15 min using an Eppendorf 5430R centrifuge (Eppendorf AG, Hamburg, Germany). A 1 mL aliquot of the supernatant was collected to determine the crude protein content in the solution by the Kjeldahl method (AOAC, 2006). Protein solubility was calculated by expressing the crude protein content in solution as a percentage of the protein content of the original sample.

2.5. Absorption capacity of water and oil of protein concentrate

The water and oil absorption capacity of the protein concentrate was determined as described by Wani, Sogi, Shivhare, and Gill (2014).

2.6. Emulsifying capacity of protein concentrate

The emulsifying capacity of the protein concentrate was determined as described by Zhao, Liu, Zhao, Ren, and Yang (2011), with minor modifications. Fifteen milliliters of a 0.1% aqueous solution of the protein isolate was prepared, and the pH was adjusted to 7. To this solution, 3 mL of refined soybean oil was added, and the solution was stirred for 60 s in an ULTRA TURRAX homogenizer (IKA, Staufen, Germany) at $15{,}000 \times g$. A 50 μL aliquot of this solution was added to 5 mL of a 0.1% SDS solution and vortex homogenized for 5 s. The absorbance of this solution was then read at 500 nm in a spectrophotometer (Jenway 6705 UV/Vis spectrophotometer, Cole-Parmer, Stone, UK). The emulsifying capacity was calculated according to the following equation:

$$CE(m^2/g) = (2 \times 2.303 \times A \times D)/(c \times \varphi \times \theta \times 10{,}000)$$

where c is the initial protein concentration (g/ml), φ is the optical path length (1 cm), θ is the volume of the fraction of oil used to form the emulsion (0.25), D is the dilution factor (100), and A is the absorbance of the emulsion.

2.7. Formation and stability of foam of protein concentrate

The foaming capacity of the protein concentrate and the foam stability after 30 min was determined as described by Wani et al. (2014), with minor modifications. The sample (0.5 g) was mixed with 25 mL of distilled water with the pH adjusted to 7.0 and shaken in an Ultra Turrax homogenizer at $10{,}000 \times g$ for 60 s. The foaming capacity was expressed as the percentage increase in the volume of the solution after stirring. The foam stability was determined by measuring the volume of the foam after 30 min of rest, and expressed as the percentage decrease in foam volume.

2.8. Thermal properties of soy protein concentrate

Protein denaturation was analyzed by differential scanning calorimetry (DSC) using a Shimadzu DSC-60 (Osaka, Japan). 2.5 mg of sample and 0.75 μL of 0.05 M phosphate-buffered saline (PBS) (pH 7.0) were mixed in aluminum crucibles. The crucibles were sealed and stored for 24 h for complete hydration of the sample before heating. The sample was heated from 20 to 110 °C at 5 °C/min, using an empty crucible as a reference. All tests were performed under a nitrogen atmosphere. The enthalpy of denaturation was expressed in J/g.

2.9. Molecular weight distribution of the protein concentrate

The molecular weight distribution of the protein concentrate was determined as described by Buggenhout, Brijs, and Delcour (2013). Protein was extracted from 10 mg of defatted meal with 10 mL of phosphate buffer (0.05 M, pH 6.8) containing: a) 2% SDS and b) 2% SDS + 1% β-mercaptoethanol. The samples were shaken vigorously, then sonicated for 10 min in an ultrasonic bath. After centrifugation

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