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The effects of soybean soaking on grain properties and isoflavones loss

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ABSTRACT

The aim of this study was to investigate the effects of soybean soaking on grain properties and isoflavone loss in order to find suitable conditions for this step of soybean processing. A moisture content of 120% (dry basis) was achieved by soaking the soybeans at 55 °C and 70 °C for 3 and 2 h, respectively. Soybeans soaked at temperatures above 25 °C showed no significant difference ($p > 0.05$) in hardness after 1 h of soaking. The contents of total isoflavones and soluble proteins were better preserved at soaking temperatures of 25 °C and 40 °C. The β-glucosidase activity and contents of aglycone and β-glucoside isoflavones were closely related. Soybeans soaked at 55 °C for 5 h had a 6-fold higher aglycone isoflavone content than did whole soybeans (81.4 μg aglycones g^{-1}) without considerable impairment of the aforementioned characteristics; soaking under this condition is therefore recommended before subsequent processing. This work helps to estimate the loss in soluble protein content and the loss and degradation of isoflavones during soybean soaking at different temperatures.

1. Introduction

Soybean soaking is a traditional process that is mainly used to soften the grains and to facilitate their subsequent cooking. In the Illinois process that is used in the production of soymilk, the soybean grains are soaked for 12 h at room temperature (Nelson, Steinberg, & Wei, 1976). Texture changes in the grains result from water absorption, which is affected by the soaking time and temperature (Pan & Tangratanavalee, 2003), and continue throughout the cooking step, with the added effect of the cooking temperature. Lengthy soaking time until reaching equilibrium moisture content in the soaked soybeans is not necessary to promote additional softening of the grains because it does not produce further improvements in cooking rate or cooking quality of the soybeans (Wang, Swain, Hesseltine, & Heath, 1979).

Processes using high temperatures and reduced soaking times can be applied in the preparation of various soy products, such as soymilk, tofu, and tempeh. Soaking temperatures within the range of 40–60 °C can decrease the lipoxygenase activity and improve the digestibility of soybean proteins (Shin, Kim, & Kim, 2013). However, high soaking temperatures increase the mass transfer rate by diffusion, resulting in a significant loss of solids, such as proteins and isoflavones, into the aqueous medium (Pan & Tangratanavalee, 2003).

High levels of protein denaturation may impair the solubility of the soy protein, whose denaturation onset temperatures for β-conglycinin (7S) and glycinin (11S) were observed to be 65–75 °C and 85–95 °C, respectively (Kitabatake, Tahara, & Doi, 1990). The technological properties of soy proteins are dependent on their solubility (Shin et al., 2013). Thus, proper times and temperatures for soybean soaking should be considered to minimise protein insolubilisation.

Soybean isoflavones are bioactive compounds that may benefit human health and can be affected during processing. Isoflavones are divided into four groups: β-glucosides (daidzin, genistin, and glycitin), 6″-*O*-acetylglucosides, 6″-*O*-malonylglucosides and aglycones (daidzein, genistein, and glycitein). Soybean isoflavones have been widely investigated, particularly aglycones, because of their ability to reduce the incidence of breast cancer (Wada et al., 2013) and other diseases. Thus, there is a growing interest in preparing soy products with higher amounts of aglycones, and soybean hydrothermal treatment has been used to favour endogenous β-glucosidase activity in the conversion of β-glucoside isoflavones to aglycones (Lima & Ida, 2014). Therefore, the formation of aglycone isoflavones must be stimulated during soybean soaking without impairing the other aforementioned properties. In this context, the aim of this study was to investigate the effects of soybean soaking time and temperature on the moisture, hardness, soluble proteins, β-glucosidase activity, and isoflavones of the soaked grains and/or

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of the residual solution in order to find a suitable condition for this step of soybean processing.

2. Materials and methods

2.1. Materials and standards

Isoflavone standard solutions were prepared from 6″-*O*-acetylglucosides and 6″-*O*-malonylglucosides (Wako Pure Chemical Industries, Ltd., Osaka, Japan) as well as from β-glucosides and aglycones (Sigma–Aldrich Co., St. Louis, MO, USA). For the soluble protein assay, bovine serum albumin (fraction V, Sigma–Aldrich Co., St. Louis, MO, USA) was used as the protein standard. The β-glucosidase activity was measured using *p*-nitrophenyl-β-D-glucopyranoside (*p*-NPG) (Sigma–Aldrich Co. St. Louis, MO, USA) as the substrate, and the chromogenic reagent *p*-nitrophenol (*p*-NP) (Sigma–Aldrich Co., St. Louis, MO, USA) was used to construct a calibration curve. All the reagents used in the analysis were of analytical grade or liquid chromatography grade.

2.2. Hydrothermal treatment

Approximately 50 g of cleaned and unbroken soybeans [*Glycine max* (L.) Merr.], lipoxygenase-null cultivar BRS 257 (Empresa Brasileira de Pesquisa Agropecuária, Londrina/Paraná, Brazil) from the crop year 2013 was used in a 1:1.5 (g:g, soybean:deionised water) ratio for each soaking assay. This soybean:deionised water ratio was used on the basis of a preliminary study and literature data (Góes-Favoni, Carrão-Panizzi, & Beléia, 2010; Gulati, Chakrabarti, Singh, Duvuuri, & Banerjee, 2010). Glass bottles containing water to soak the whole soybeans were pre-incubated in a thermostatically controlled water bath (Marconi, MA 159, Piracicaba, Brazil) at the required soaking temperature (25, 40, 55, and 70 °C) until reaching thermal equilibrium before the addition of the grains. In addition, the soybeans were also soaked in a 0.1 mol L^{-1} phosphate-citrate buffer solution at pH 6 at a soaking temperature that promoted the highest content of aglycones in the soaked soybean to provide optimal conditions for the β-glucosidase enzyme (Matsuura, Obata, & Fukushima, 1989; Sutil et al., 2008). After soaking for regular time intervals (0, 0.5, 1, 2, 3, 4, 5, 6, and 7 h) at each temperature, the flasks were successively withdrawn from the water baths, and then the soaked soybeans and drained solution were immediately cooled in an ice bath until they reached 25 °C. The assays were performed in closed systems to avoid water evaporation into the environment. The temperature of the soaking medium was continuously monitored with a high accuracy (±0.2 °C) mercury-in-glass thermometer (Incoterm®, Porto Alegre, Brazil) and was maintained at the required level (±1 °C) throughout the soaking period. At the end of each assay, the soaked soybeans were superficially dried at 30 °C for 10 min in a vacuum oven before being weighed. The mass of the residual solution (water not absorbed by the soybeans and compounds leached) was obtained by calculating the difference between the total mass of the system (the sum of the whole soybeans and the mass of the soaking medium) and the mass of the grains superficially dried. A portion of each sample was reserved for the analysis of soluble proteins (residual solution), hardness (soaked soybeans), and moisture content (soaked soybeans), and the remaining samples were frozen, lyophilised (Christ Alpha 2-4 LD plus, Osterode am Harz, Germany), milled (Ika A11 basic, St. Louis, MO, USA) and stored at −22 °C until further analysis.

2.3. Moisture content determination

The moisture content was determined using 2 g of soaked soybeans for each soaking assay. The samples were dried in an oven at 105 °C until reaching a constant weight (AOAC, 2002). The results were expressed as g of H_2O/100 g dry matter (%, dry basis).

2.4. Hardness measurement

The hardness of the soaked soybeans was measured by a penetration test using a TA-XT2i Texture Analyser (Stable Micro Systems, Surrey, UK) equipped with an HDP/MTP multiple pea rig. In this test, 18 soaked soybeans were simultaneously tested by applying a force in the direction perpendicular to the hilum. The settings used were as follows: pre-test speed (2 mm s^{-1}), test speed (2 mm s^{-1}), penetration distance (3.5 mm), and force (0.05 N). The maximum force (N) necessary to penetrate the soaked soybeans (with tegument) was determined as an indicator of hardness.

2.5. Soluble protein content

The soluble protein contents in the soaking residual solution and the soaked soybeans were determined according to the Bradford method (Bradford, 1976) using bovine serum albumin as the protein standard (fraction V; 25–250 μg mL^{-1}). The residual solution from each soaking assay was filtered (nylon membrane, 0.45 μm; Millipore, Billerica, MA, USA) before the protein assay, and when necessary, the samples were diluted with deionised water. Soluble protein extraction from the soaked soybeans was carried out using 0.2 g of lyophilised sample and 10 mL of deionised water, which was kept under continuous rotary agitation (305 rpm) for 1 h at 25 °C. Then, the mixture was centrifuged (4 °C, 8200 × *g*; Centrifuge 5804R e Eppendorf, Hamburg, Germany), and the supernatant was diluted with deionised water and filtered before analysis. The results were expressed as mg of soluble protein leached into the soaking medium or g of soluble protein retained in the soaked soybeans.

2.6. β-Glucosidase activity

The extraction of the β-D-glucoside glucohydrolase (EC 3.2.1.21) enzyme was carried out according to Carrão-Panizzi and Bordignon (2000) using 0.4 g of lyophilised sample and 5 mL of extraction solution. The enzyme activity was assessed using the method of Matsuura and Obata (1993), with minor modifications. The substrate *p*-NPG was diluted with a 0.1 mol L^{-1} phosphate-citrate buffer solution at pH 6. The calibration curve was prepared by varying the concentration of *p*-NP from 0.04 to 0.32 μmol in a 5 mL total reaction volume. One activity unit (AU) was defined as the quantity of enzyme necessary to release 1 μmol of *p*-NP min^{-1} under the experimental conditions.

2.7. Determination of isoflavones

Prior to the analysis of the isoflavones, the samples were defatted with hexane in a 1:10 (g:mL, sample:hexane) ratio by continuous rotary agitation for 1 h at 25 °C, followed by vacuum filtration. The residual solution was only lyophilised. For the isoflavone extraction from the soaked soybeans, 0.3 g of lyophilised sample was used in 6 mL of extraction solution containing ultrapure water, acetone, and ethanol in a 1:1:1 (mL:mL:mL) ratio, as described by Yoshiara, Madeira, Delaroza, Silva, and Ida (2012). The isoflavone extraction from the soaking residual solutions was performed separately with 0.2 g of lyophilised sample in 1.5–2 mL of extraction solution. The separation and quantification of the isoflavones were carried out according to Handa, Couto, Vicensoti, Georgetti, and Ida (2014). External calibrations were prepared from standard solutions (0.1, 0.05, 0.01, 0.005, 0.001, and 0.0005 mg mL^{-1}) of each isoflavone form for quantification. The