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Soymilk processing with higher isoflavone aglycone content

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ABSTRACT

The objective of this work was to optimise conversion of β-glucoside isoflavones to aglycones in soymilk processing and to evaluate their thermal stability. The soymilk was assessed for time and temperature effects of incubating supplied to central composite design 2^2. The response functions were investigated for β-glucosidase activity and isoflavone contents. The β-glucosidase activity was reduced through soybean slurry incubation at approximately 64 °C and 3.42 h. Maximum conversion of β-glucosides into isoflavone aglycones involved soaking the soybeans at 5 °C for 14 h at a 1:3 ratio (soybean:water, w:v), homogenising them at a 1:8 ratio (soybean soaked:water, w:v) and incubation at 50 °C for 2.7 h. In evaluation of thermal stability of isoflavones in soymilk at 97 °C for 25 min, the daidzein and genistein aglycone contents were maintained, the glycitein and β-glucosides contents increased and the malonylglucoside content decreased.

1. Introduction

Soybeans are appreciated by consumers because of their high protein content (Liu, 2004) and their bioactive compounds, such as isoflavones, saponins and oligosaccharides. Soybean isoflavones have been widely investigated because they provide benefits to human health associated with the prevention and treatment of some disease types such as cancer, cardiovascular disease, bone loss and relieving the symptoms of menopause in women (Atteritano et al., 2009; Chan et al., 2007; Liu, 2004). Isoflavones in soybeans occur in four distinct chemical structures known as aglycones (daidzein, genistein and glycitein), β-glucosides (daidzin, genistin and glicitin), 6″-*O*-acetylglucosides (acetyldaidzin, acetylgenistin and acetylglicitin) and 6″-*O*-malonylglucosides (malonyldaidzin, malonylgenistin and malonylglicitin) (Liu, 2004; Wang, 2008). In soybeans, aglycone isoflavones are absent or only present at low concentrations (Paucar-Menacho, Berhow, Mandarino, Chang, & Mejia, 2010). Isoflavone aglycones have greater beneficial effects on human health than other types of isoflavones (Messina & Messina, 2000). Isoflavones can be converted to different conjugates, which has a significant effect on their bioavailability. Aglycone forms are absorbed more easily than glycosylated conjugates because their low molecular weight improves diffusion (Xu, Wang, Murphy, & Hendrich, 2000). The aglycones in soymilk are more quickly absorbed than the glycosylated forms (Kano, Takayanagi, Harada, Sawada, & Ishikawa, 2006).

Isoflavones can be converted into aglycones through the action of β-glucosidase, which catalyses the hydrolysis of β-glycosides (Wang & Murphy, 1994). β-Glucosidase is naturally present in soybeans and may also be produced by various microorganisms (Yeon, Kim, Kim, & Oh, 2012). Considering the benefits of isoflavones, and the ability to hydrolyse β-glycosides into aglycones, there is growing interest in enriching soy products with these aglycones (Chen et al., 2013). Thus, some studies seek alternatives to enrich soy products with aglycones, such as: hydrothermal treatment of soybeans to increase the β-glucosidase activity (Góes-Favoni, Carrão-Panizzi, & Beleia, 2010; Lima & Ida, 2014; Sutil et al., 2008); production of soy protein isolate with changes in temperature, pH and ionic strength processing to increase isoflavone extraction (Barbosa, Lajolo, & Genovese, 2006); soybean meal fermentation with fungi (Handa, Couto, Vicensoti, Georgetti, & Ida,

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2014); soymilk fermentation with lactic acid bacteria (Chun, Kim, & Kim, 2008); soybean extract enriched with immobilized β-glucosidase (Chen et al., 2013); and addition of soy germ, soy protein isolate and bifidobacteria in soymilk (Tsangalis, Ashton, Stojanovska, Wilcox, & Shah, 2004).

Soymilk is a turbid colloidal dispersion that contains part of the original soybean component (Nik, Tosh, Woodrow, Poysa, & Corredig, 2009). Soymilk is traditionally obtained after soybean grains are soaked, the water is drained, and the residue is mixed, filtered and heat-treated (Liu, 2004). Processing conditions can affect the content and composition of isoflavones, especially in the conversion to non-conjugated forms (Jung, Murphy, & Sala, 2008; Kao, Lu, Hsieh, & Chen, 2004). Isoflavone content and conversion during processing is directly affected by the chemical structures and other parameters, such as the soybean cultivar, pH, temperature and time of soaking and thermal treatment, soybean:water proportion, filtration and factors affecting the β-glucosidase activity (Chun et al., 2008; Ismail & Hayes, 2005; Jung et al., 2008). Few investigators have proposed modifications in the process in order to increase aglycone content by increasing endogenous β-glucosidase activity.

Therefore, considering the importance of isoflavone aglycones and the possibility of obtaining soymilk with high aglycone contents by modifying some processing stages, the objective of this work was to optimise the conversion of β-glucoside isoflavones to aglycones in soymilk processing and evaluate their thermal stability.

2. Materials and methods

2.1. Materials

The soymilk was prepared from soybean variety BRS 257 2011/2012, which is lipoxygenase-free, and was donated by the Sementes Paraná company (Mauá da Serra city, Parana State, Brazil).

For Ultra High Performance Liquid Chromatography (UHPLC) and the construction of calibration curves, the following standard isoflavones were used: 6″-*O*-acetylglucosides and 6″-*O*-malonylglucosides (Wako Pure Chemical Industries, Ltd., Osaka, Japan), β-glucosides and aglycones (Sigma–Aldrich Co., St. Louis, EUA). The substrate p-nitrophenyl-β-D-glucopyranoside (p-NPG) (Sigma–Aldrich Co., St. Louis, USA) was used to determine the β-glucosidase activity. The reagents used in the analysis were of analytical grade or specific for liquid chromatography.

2.2. The effects of temperature and time on β-glucosidase activity and the conversion of β-glucoside isoflavones to aglycones during soymilk processing

For soymilk processing, the soybeans that had been screened and washed were soaked at a 1:3 ratio (w:v; soybeans:water) at 5 °C for 14 h. The soybeans were drained and rinsed with water and added to distilled water at a ratio of 1:8 (w:v; soybeans soaked:water). The material was homogenised in a blender (Philco All In One, São Paulo, BR) for 2 min at 25 °C to obtain the slurry.

To evaluate the temperature (X_1) and time (X_2) effects on the β-glucosidase activity and conversion of β-glucoside isoflavones to aglycones in the soymilk preparation, a central composite design 2^2 (CCD) was applied with 4 axial points and three replicates at the centre point, for a total of 11 randomised experiments. Table 1 presents the code and the real levels of independent variables X_1 (temperature, °C) and X_2 (time, h). For each assay, a glass containing 150 ml of slurry was incubated in a bath with orbital agitation at different temperatures (X_1 = 36, 40, 50, 60 and 64 °C) and times (X_2 = 0.58, 1, 2, 3 and 3.42 h) according to the CCD (Table 1). After incubating, the slurry was rapidly cooled in an ice bath, filtered through cloth (150 mesh) and soymilk was obtained. In parallel to the assays, a soymilk control (C) was collected in triplicate, and it was not treated with process variables X_1 (temperature) and X_2 (time). The soymilk was frozen (−26 °C) and lyophilised to evaluate the β-glucosidase activity and isoflavones content. The response functions investigated in soymilk were β-GLU (β-glucosidase activity) and the contents of isoflavones DAI (daidzin), GEN (genistin), MDAI (malonyldaidzin), MGLI (EE), MGEN (malonylgenistin), ADAI (daidzein), AGLI (glycitein) and AGEN (genistein). Using the response functions, regression analyses were performed to evaluate the effects of the independent variables (x_1 and x_2) and their interactions. An analysis of variance (ANOVA) and regression coefficient of determination (R^2) were performed to check the fit of the model to the experimental data. The function response was adjusted according to the model as follows: $Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 + e$, where Y = the function response, x_1 e x_2 = the levels of coded variables, β = estimated coefficients on the response surface and e = pure error. The response surfaces and desirability were analysed to assess the β-glucosidase activity and maximum conversion of β-glucoside isoflavones to aglycones. Calculations, graphs for the construction of the response surfaces and desirability were analysed using Statistic 7.0 software. The proposed model was valid after soymilk processing, in triplicate, at the temperature and time conditions with the maximum conversion of β-glucoside isoflavones to aglycones. The total aglycone content in soymilk was quantified and compared with the response function estimated by the model with Student's *t*-test ($p < 0.05$).

2.3. Evaluating the thermal stability of isoflavones from optimised soymilk

The thermal stability of isoflavones was evaluated after optimised soymilk reached at 97 ± 2 °C for 0, 5, 10, 15, 20 and 25 min. Glass vials with semi-open cover containing 200 ml of optimised soymilk that was collected in triplicates after thermal treatment were rapidly cooled on ice, frozen and lyophilised to quantify the isoflavone forms. The results were submitted to an analysis of variance (ANOVA) and Tukey's test ($p < 0.05$).

2.4. β-Glucosidase activity

β-Glucosidase was extracted from 200 mg of soymilk that was lyophilised and added to 3 ml of citrate buffer 0.05 mol l^{-1} (pH 4.5) containing NaCl 0.1 mol l^{-1} and shaken every 15 min for 1 h at 25 °C (Carrão-Panizzi & Bordignon, 2000). Enzyme activity analysis was performed according to Matsuura and Obata (1993), with minor modifications as proposed by Lima and Ida (2014). One activity unit (AU) was defined as the quantity of enzyme necessary to release 1 μmol of p-nitrophenol per min under the experimental conditions. The results were expressed in AU at g of sample on a dry basis (AU g^{-1}).

2.5. Isoflavone determinations

The lyophilised soymilk samples were defatted with hexane (1:10; w:v) at 25 °C for 1 h by continuous rotary agitation (150 rpm), and the isoflavone extraction was performed with 300 mg of sample and 6.0 ml of an extraction solution containing ultra-pure water, acetone and ethanol (1:1:1, v/v/v) (Yoshiara, Madeira, Delaroza, Silva, & Ida, 2012) at 25 °C for 1 h and agitated by vortex for 15 min each. The mixture was then placed in an ultrasonic bath at 25 °C for 15 min, centrifuged at 794 g at 4 °C for