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Soybean ultrasound pre-treatment prior to soaking affects β-glucosidase activity, isoflavone profile and soaking time

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ABSTRACT

Ultrasound may convert conjugated isoflavones into their corresponding aglycones, the best form for absorption in the human body. However, ultrasound may also influence the activity of endogenous β-glucosidase. Therefore, the present work evaluated the effects of soybean ultrasound pre-treatment by applying the Box-Behnken design prior to soaking, a step that is important for industries to prepare certain soy products. Furthermore, a multi-response optimisation is provided. The best conditions for soybean ultrasound pre-treatment were established as temperature, $X_1 = 55\,^{\circ}C$; exposure time, $X_2 = 5$ min and ultrasound intensity, $X_3 = 19.5\ W\ cm^{-2}$. Under these conditions, soybeans with higher contents of aglycones were obtained and β-glucosidase activity was kept as high as possible. A second experiment was conducted and confirmed that ultrasound pre-treatment results in a lower soaking time (2 h) to achieve the highest moisture content, lower hardness as well as increased content of aglycones.

1. Introduction

Soybean isoflavones have been widely studied because of their potential health benefits. Isoflavone intake has been linked to a lower incidence of certain types of cancer and osteoporosis (Villares, Rostagno, García-Lafuente, Guillamón & Martínez, 2011). These flavonoids have also been found to act as antioxidants and anti-inflammatory compounds (Rostagno, Villares, Guillamón, García-Lafuente, & Martínez, 2009; Villares et al., 2011). A human trial conducted by Ye et al. (2012), demonstrated that bioactive compounds from soy germ also alleviate menopausal symptoms. There are several pieces of evidence demonstrating that isoflavones are not well absorbed in their conjugated forms (Larkin, Price, & Astheimer, 2008; Rowland et al., 2003). Thus, to be bioaccessible, soybean isoflavones must be hydrolyzed into their corresponding aglycones (Larkin et al., 2008). Accordingly, procurement of soybean products with greater content of aglycone isoflavones is desirable.

The presence and the concentration of different forms of isoflavones in soybeans may vary among different cultivars. In addition, further processing to obtain different soybean products may also change both their identities and quantities (Wang & Murphy, 1994). Soybean isoflavones exist in four different groups as aglycones (genistein, daidzein, and glycitein), β-glucosides (genistin, daidzin, and glycitin), acetylglucosides (acetylgenistin, acetyldaidzin, and acetylglycitin), and

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malonylglucosides (malonylgenistin, malonyldaidzin, and malonylglycitin). Therefore, there are twelve forms of isoflavones in soybeans. Isoflavones in the β-glucoside form may be converted to aglycones during processing (Fernandez-Lopez, Lamothe, Delample, Denayrolles, & Bennetau-Pelissero, 2016) or by hydrolysis via the action endogenous β-glucosidases (Lima & Ida, 2014).

Soaking of soybean is an important pre-treatment step to obtain several products, such as soymilk, kefir-fermented soymilk, yogurt, germinated soybean, tofu, and tempeh, among others. Soaking of soybeans improves the softness of the product due to water absorption, thus facilitating further processing. Furthermore, Lima, Kurozawa, and Ida (2014) demonstrated that soybean soaking (especially at 55 °C) may enhance the conversion of conjugated isoflavones to their aglycone forms due to the action of their endogenous β-glucosidases.

Consumers are looking for high-quality products, which has been driving the industry to search for safer and more effective processing methods. Ultrasound, an emergent technology, has been considered safe, non-toxic, and environmentally friendly (Yang, Gao, Yang, & Chen, 2015). The process brings about the application of high-frequency sound generated energy (Yeo & Liong, 2013). Cavitation bubbles are generated due to pressure changes caused when high power ultrasound propagates in a liquid. The violent collapse of these microbubbles generates high pressure and temperature regions. The level of energy transmitted to the medium is expressed as ultrasound power (W), ultrasound intensity (W cm^{-2}), or acoustic energy density (W ml^{-1}) (O'Donnell, Tiwari, Bourke, & Cullen, 2010). Ultrasound application may influence chemical, physical, and biological aspects of food. Therefore, its use may have specific benefits depending on each application (Rokhina, Lens, & Virkutyte, 2009).

Ultrasound has been applied to different food products to give uniform heat transfer during cooking and increase mass transfer. It has also been applied during freezing and drying, as well as to generate stable emulsions (Chemat, Huma & Khan, 2011). Furthermore, ultrasound application may enhance the germination rate of soybeans. Additionally, both physical and nutritional properties of germinated soybeans were improved compared to their ungerminated counterparts (Yang et al., 2015). Furthermore, ultrasound-assisted extraction of phenolic compounds from plant foods has been reported (Nipornram, Tochampa, Rattanatraiwong, & Singanusong, 2018); since food phenolics may be linked to the cell wall materials (Shahidi & Yeo, 2016), ultrasound-assisted extraction may actually affect the release of the insoluble-bound fraction of these bioactive phenolics. Furthermore, due to the influence of several ultrasound parameters (e.g. frequency, intensity, and time of application) (Subhedar & Gogate, 2014; Szabó & Csiszár, 2013), a detailed study about their effects on the activity of endogenous enzymes is required. Likewise, considering several response functions, such as the concentration of different aglycones (genistein, daidzein, and glycitein) and enzyme activity, application of response surface methodology (RSM) and careful statistical evaluation for validation are deemed necessary (Granato, Calado, & Jarvis, 2014; Handa, Couto, Vicensoti, Georgetti, & Ida, 2014).

Cavitation, a consequence of ultrasound, may influence endogenous enzymatic activity via different mechanisms, which may occur at the same time as well as separately. Delgado-Povedano and Luque de Castro (2015) summarized the mechanisms involved as follows: (a) thermal effects due to high temperatures generated in cavitation microzones; (b) free radical effects generated due to hydrolysis of water and/or any other solvent system, and (c) mechanical effects created by microstreaming and shock.

Therefore, considering that soybean treatment with ultrasound prior to hydration can enhance the conversion of conjugated isoflavones into their aglycone forms, and influence the activity of endogenous β-glucosidase, this contribution is the first report on the application of ultrasound treatment. The objective of the present study was to evaluate the effects of temperature, time and ultrasound intensity applied to soybean pre-treated with ultrasound, designed to evaluate the effects such a process has on chemical and physical changes that may play an important role on the soybean industry. Two sets of experiments were conducted as follows: (i) The Box Behnken design was applied to optimize the conditions of soybean pre-treatment with ultrasound to achieve the highest content of isoflavones in the aglycone form as well as to enhance the activity of endogenous β-glucosidase to the highest possible level. Finally, a multi-response optimization was carried out. (ii) Soybean samples subjected to ultrasound under optimized conditions were subsequently soaked at a fixed temperature and the soaking time, physical properties, endogenous β-glucosidase activity and isoflavone contents were evaluated.

2. Materials and methods

2.1. Soybean samples and chemicals

Lipoxygenase-free soybean samples [*Glycine Max.* (L.) Merr], cultivar BRS 257 (crop year 2016) were kindly provided by SL Alimentos (Londrina, Paraná, Brazil). On average, the grains showed the width of 5.234 ± 0.321 mm and length of 6.464 ± 0.662 mm. Acetyldaidzin (6″-*O*-acetyldaidzin), acetylgenistin (6″-*O*-acetylgenistin), acetylglycitin (6″-*O*-acetylglycitin), malonyldaidzin (6″-*O*-malonyldaidzin), malonylgenistin (6″-*O*-malonylgenistin) and malonylglycitin (6″-*O*-malonylglycitin) were purchased from Wako Pure (Osaka, Japan). Daidzin, genistin, glycitin, daidzein, genistein, glycitein, and *p*-nitrophenyl β-D-glucopyranoside were purchased from Sigma-Aldrich (St. Louis, MO, USA). The remaining chemicals and solvents were of HPLC or analytical grade.

2.2. Soybean pre-treatment with ultrasound: effects of temperature, time and ultrasound intensity

The soybean samples (50 g) were pre-treated with ultrasound after their mixing with water at 1:1.5 (w/v) and the assays were done following the factorial Box-Behnken design (Box & Behnken, 1960). Independent variables (X_1 = temperature, X_2 = time, and X_3 = ultrasound intensity), as well as their ranges, are shown in Table 1. A total of 16 experiments were conducted in a random manner. Four replicates of the central point (experiments 13–16) were used to evaluate the pure error. The levels of the independent variables for the activity of endogenous β-glucosidase were defined according to the maximum activity of various enzymes subjected to ultrasound treatment (Subhedar & Gogate, 2014; Szabó & Csiszár, 2013; Wang et al., 2012). Soybean samples, in 150 ml beakers, were kept in a water bath (MA127, MARCONI, Piracicaba, Brazil) until the water temperature reached (X_1) 35, 45 or 55 °C after which the samples and the soaking water were transferred to another beaker coupled to a thermostatic water bath (TE-2005, Tecnal, Piracicaba, Brazil). The latter step was conducted to keep constant the internal temperature of the sample (X_1) during the ultrasound treatment. The ultrasound (Q700, QSonica, Newtown, Connecticut, USA) equipped with a low-intensity probe sonicator was operated at 20 kHz and intensities defined according to the experimental planning (X_3 = 6, 15, and 24 W cm^{-2}). After each assay, the samples were cooled down until they reached 25 °C. The water was removed and the samples were frozen, lyophilized (Crhist Alpha 1–4 plus LSC, Christ, Newtown, UK), milled with a grinder (MDR301, Cadence, Navegantes, Brazil) and stored at −22 °C until determination of β-glucosidase activity and the content of different forms of isoflavones.

2.3. Optimised soybean: moisture content, hardness, aglycones concentration and β-glucosidase activity

Soybeans subjected to ultrasound pre-treatment under optimized conditions (kept in their soaking water) were submitted to a hydrothermal treatment process (55 ± 1 °C) for up to 6 h. Every hour, the samples were cooled down in an ice bath until they reached 25 °C. The

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