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Concentration of soybean isoflavones by nanofiltration and the effects of thermal treatments on the concentrate

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ABSTRACT

An investigation was performed on the profile and the content of isoflavones in the concentrate of aqueous Defatted Soy Flour (DSF) extract obtained by nanofiltration. The effect of thermal treatments on these isoflavones was also evaluated according to a Central Composite Design (CCD 2^k) with varying temperatures (70 to 90 °C) and times (15 to 45 min). Through nanofiltration it was possible to concentrate β-glucosides and malonyl glucosides ($p<0.05$) in aqueous DSF extract but it was not possible to concentrate aglycones ($p>0.05$). The thermal treatments applied on the concentrate showed that the malonyl glucosides were influenced by temperature ($p<0.05$), while the β-glucosides were influenced not only by temperature but also by the time of interaction of the factors investigated ($p<0.05$). Moreover, there was no alteration in the contents ($p>0.05$) of aglycone or total isoflavones.

1. Introduction

Membrane separation processes such as nanofiltration (NF) have been investigated in relation to the concentration of bioactive compounds present in aqueous vegetal extracts (Cassini, Tessaro, Marczak, & Pertile, 2010). One of the reasons for employing these processes is the low temperatures involved, in contrast to most conventional concentration procedures such as evaporation. The use of low temperature is important to maintain the functional properties of bioactive compounds like the isoflavones present in soybeans (Góes-Favoni, Beléia, Carrão-Panizzi, & Mandarino, 2004). Furthermore, NF is an attractive alternative method of concentration since it does not require the use of organic solvents, which are generally used in the extraction of these compounds (Mello, Petrus, & Hubinger, 2010; Xu, Lamb, Layton, & Kumar, 2004). However, NF requires the use of solutions with low lipid contents. According to Saboya and Maubois (2000) and Cuartas-Uribe, Alcaina-Miranda, Soriano-Costa, and Bespia (2007), fat globules tend to accumulate on the membrane surface, thus forming a gel layer, decreasing the permeate flux and resulting in the fouling of the membrane. The importance of removing lipids from the feed was noted by Noordman, Kooiker, Bel, Dekker, and Wesselingh (2003) in the concentration of functional compounds obtained from an aqueous soy flour extract.

Isoflavones are phytoestrogens (Adlercreutz & Mazur, 1997; Góes-Favoni, Carrão-Panizzi, & Beléia, 2010; Lee & Lee, 2009; Ríaz, 1999) that present the following chemical forms: malonyl glucosides (malonyl genistin, malonyl daidzin and malonyl glycitin); β-glucosides (genistin, daidzin and glycitin); acetyl glucosides (acetyl genistin, acetyl daidzin and acetyl glycitin); and aglycones (genistein, daidzein and glycitein) (Genovese, Barbosa, Pinto, & Lajolo, 2007; Ranilla, Genovese, & Lajolo, 2009). These forms have been widely investigated for their beneficial effects on human health (Adlercreutz & Mazur, 1997; Barnes et al., 2006; Chang, 2002). Variations in soybean processing influence the profiles and contents of isoflavones. Wang and Murphy (1994) reported that 98% of the isoflavones in soybeans or in soy products occur as β-glucosides, acetyl glucosides and malonyl glucosides, whereas Barnes, Coward, Kirk, and Sfakianos (1998) state that there is a predominance of conjugated forms such as malonyl, which can be converted into acetyl and β-glucosides during thermal treatment. Fukutake et al. (1996) and Sutil et al. (2008) noted that larger amounts of aglycones are formed at around 50 °C.

According to Eisen, Ungar, and Shimoni (2003), Huang, Liang, and Kwok (2006) and Nufer, Ismail, and Hayes (2009), it is very important to note the transformations and the degradation of isoflavones in the concentrate submitted to different thermal treatments. Thus, the objective of this study was to concentrate the soybean isoflavones of the aqueous extract of DSF by nanofiltration, to evaluate the profile

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and the contents of these compounds in the concentrate and to investigate the influence of different thermal treatments.

2. Material and methods

2.1. Material

Genetically enhanced soybeans of the cultivar BRS 216, which have high protein and isoflavone contents (Carrão-Panizzi et al., 2001) were supplied by Empresa Brasileira de Pesquisa Agropecuária — Centro Nacional de Pesquisa da Soja (Embrapa Soja). The isoflavones were identified and quantified through comparison with the standard curves for daidzin, daidzein (Fujicco Co., Ltd, Tokyo, Japan), genistin, genistein (Sigma Chemicals Co., Ltd, St. Louis, MO, USA.), glycitin, glycitein, malonyl daidzin, malonyl genistin, malonyl glicitin, acetyl daidzin, acetyl genistin and acetyl glycitin (Wako Chemicals, Anaheim, CA, USA). All reagents were of analytical or chromatographic grade.

2.2. Preparation of the aqueous defatted soy flour (DSF) extract

To prepare the aqueous DSF extract, the soybeans were dried in a forced air oven (Model 32O-SE, FANEM®, São Paulo, Brazil) at 40 °C for 24 h. After drying, the soybeans were milled in a knife mill (VIBRAMATT®, São Paulo, SP, Brazil). The flour obtained in this stage was first sieved through a 20-mesh and then a 35-mesh stainless screen (GRANUTEST®, São Paulo, Brazil) and defatted with n-hexane at ambient temperature (around 25 °C). The DSF was mixed with water at a ratio of 1:8 (DSF:water), stored at 50 °C and stirred with an orbital shaker (Model TE-421, TECNAL®, Piracicaba, SP, Brazil) for 15 h. This stage is necessary for the aglycone formation. Additional stirring was performed at room temperature (25 °C) for 5 min with a magnetic shaker (Model Q-261, QUIMIS®, Diadema, SP, Brazil). This mixture underwent two stages of filtration; the first stage was carried out with a nylon filter (80 mesh) (BRASHOLANDA®, Pinhais, PR, Brazil) to remove the larger particles of flour and the second stage was performed using a vacuum filter made of polyamide with a pore size of 7 μm (TEGAPE®, Curitiba, PR Brazil), thus obtaining the aqueous DSF extract.

2.3. Nanofiltration (NF)

The aqueous DSF extract was concentrated through nanofiltration using a tangential flow filtration pilot plant equipped with a poly-viny-lidene difluoride (PVDF) filter in the spiral configuration with molecular weight cut-off (MWCO) ranging between 150 and 300 g mol^{-1} and effective filter area of 0.9 m^2 (GE Osmonics®, Philadelphia, USA). The experiments were carried out in duplicate at 16 ± 2 °C and with a transmembrane pressure of 7 bar until reaching a volume reduction factor (VRF) of 4. The VRF was calculated as the ratio between the initial volume (L) of the aqueous DSF extract used in the feed and the final volume (L) of the concentrate after NF. The permeate flux (J) (L h^{-1} m^{-2}) during NF can be calculated as follows:

$$J = \frac{V_p}{t \times A_p} \quad (1)$$

where V_p (L) is the amount of permeate collected during the period of time t (h) and A_p (m^2) is the permeation surface area of the membrane. The quality of the filtration process was measured based on the isoflavone content present in the concentrate. After each experiment, the equipment was cleaned with alkaline solution (0.1%) according to manufacturer's instructions.

2.4. Experimental design for the thermal treatment of the concentrate

In this study, a Central Composite Design (CCD 2^k) was used to perform the thermal treatment of the concentrate at VRF 4. The independent variables temperature X_1 (°C) and time X_2 (minutes) were analyzed at the three equidistant levels of variation, encoded as −1, 0, and +1. The experimental design consisted of 11 assays; four factorial (combination of levels −1 and +1), four axial (one variable at the level ± α and another one at zero) and three repetitions at the central point (two variables at level zero) (Table 1). Experiments in the center of the design were performed to estimate possible pure errors. Because of systematic errors, all the experiments were carried out at random to minimize the effect of unexplained variability on the responses observed. The dependent variable (response) was the total isoflavone content (μg mL^{-1}).

The levels of the variables and the central point were defined based on data available in the literature on the thermal treatment of soybean products. Some authors (Chien, Hsieh, Kao, & Chen, 2005; Grün et al., 2001; Huang et al., 2006; Toda, Sakamoto, Takayanagi, & Yokotsuka, 2000) have used temperatures of between 70 and 90 °C to evaluate the profile of isoflavones and their kinetic degradation.

2.5. Thermal treatments of the concentrate

The thermal treatments were applied to the concentrate of the aqueous DSF extract in 5 mL glass tubes with 0.9 mm wall thickness, 10 mm internal diameter, and 11 cm in length. These tubes, which contained samples of the concentrate, were closed and then immersed in a thermostated bath (Model Q215M, CALLMEX®, Florianopolis, SC, Brazil) at the temperature of the assay. The temperature was monitored with a thermometer (INCOTERM®, Porto Alegre, RS, Brazil). The experimental design was composed of 11 assays. The thermal treatments were performed for each assay in the experimental design. At the end of each assay, the tubes were immediately immersed in an ice bath to cool the samples. After the thermal treatments, the concentrates were placed into clear conical polypropylene centrifugation tubes (Falcon®) with 50 mL of volume and screw caps with dimensions of 30 mm O.D and 115 mm length. They were then frozen and stored at −18 °C for one week until the determination of isoflavones through high-performance liquid chromatography (HPLC).

2.6. Isoflavone extraction and determination

The extraction of isoflavones and the determination of their components were carried out with samples of the DSF, of the aqueous DSF extract, and of the concentrate before and after thermal treatments,

Table 1
Central Composite Design (CCD) with codified and reals values for two independent variables.

Assay	Codified		Reals	
	X_1[a]	X_2[b]	Temperature (°C)	Time (minutes)
1	−1	−1	70	15
2	+1	−1	90	15
3	−1	+1	70	45
4	+1	+1	90	45
5	−α[c]	0	66	30
6	+α	0	94	30
7	0	−α	80	9
8	0	+α	80	51
9	0	0	80	30
10	0	0	80	30
11	0	0	80	30

[a] X_1 = temperature variable.
[b] X_2 = time of thermal treatment variable.
[c] α = ±1.414 for two independent variables.