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Behavior of functional compounds during freeze concentration of tofu whey

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ABSTRACT

The liquid waste generated from tofu production, denominated as Tofu Whey (TW), contains important amounts of isoflavones that can be evaluated by various separation techniques. In this work TW was concentrated by falling-film freeze concentration from 1.9 to 15.5 °Brix, up to levels of 208 mg of isoflavones per 100 g solids. The levels of isoflavones, proteins, sugars, calcium and magnesium were determined both in the ice and in the concentrate obtained. It was found by rheological characterization that TW shows a Newtonian behavior at concentrations between 1.9 and 15.5 °Brix with freezing points of −0.6 to −2.7 °C.

1. Introduction

Tofu is the main processed soybean product in the world and the TW, the liquid that oozes out of soybean curd during processing, is therefore an important soybean processing by-product. This represents an environmental problem for direct disposal as the TW deteriorates very quickly because of its high water content and its high content of nutritious substances for bacteria. Although TW contains high quantities of beneficial nutrients, most tofu by-products are used as animal feed, fertilizer or simply disposed of as liquid waste (Matemu et al., 2009). Jackson et al. (2002) reported that this effluent contains soluble salts and carbohydrates (oligosaccharides) and also significant levels of isoflavones resulting from the dissolution of these in the water during processing. According to Wang and Murphy (1996), the loss of isoflavones in the liquid waste during the tofu processing reaches 44%.

Isoflavones are a group of naturally occurring hetero cyclic phenols, called phytoestrogens, found mainly in soybean. Isoflavones have been claimed to perform several health-promoting functions. Kim et al. (2005) reported that these health claims place soybean products into a select category of functional foods that possess good overall nutritional values apart from the specific health benefits. The benefits provided by the isoflavones and oligosaccharides from soybeans include positive effects on patients with the following diseases: cancer of breast, prostate, and colon (Espinosa-Martos et al., 2006; Ounis et al., 2008; Kennedy, 1993; Nagata et al., 2007), cardiovascular diseases (Sacks et al., 2006), osteoporosis (Taku et al., 2011) and menopausal symptoms (Taku et al., 2010). Oldoni et al. (2011) reported that a dietary consumption of foods and food additives containing isoflavone phytoestrogens have been associated with several beneficial properties to human health, such as prevention of coronary heart disease and osteoporosis; and reduction of menopausal symptoms.

Soybean contains 12 different phytoestrogens, divided into the following four chemical forms: aglycones (genistein, daidzein and glycitein), β-glucosides (genistin, daidzin and glycitin), malonyl glucosides (6″-0-malonilgenistin, 6″-0-malonildaidzin and 6″-0-malonilglycitin) and acetyl glucosides (6″-0-acetilgenistin, 6″-0-acetildaidzin and 6″-0-acetil glycitin). However, Shao et al. (2009) reported that it has been established that the bioavailability of isoflavones can be influenced by their chemical form. Already, Jackson et al. (2002) shown that some isoflavones could be lost during the processing steps, as obtention of soy products. Since some studies have shown that the chemical form and the content of isoflavones may depend of process used, Rostagno et al. (2005) highlights the importance of studies about the influence of processing and behavior of isoflavones aimed at industrial use.

The composition and properties of TW make it a reusable effluent with potential applications (Espinosa-Martos et al., 2006). The management of this waste with high water content represents an economic problem because of the high transportation costs for its disposal, treatment and/or use. The concentration of TW thus becomes a necessary first step for its waste management and evaluation. In principle one could use evaporation as concentration process, but unfortunately this can cause damage to heat sensitive components or loss of volatile compounds (Bakshi and Johnson,

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1983), so that alternative concentration processes are needed. One possible option is the use of freeze concentration, a process operated at low temperatures, which favors a good retention of thermosensible components.

The literature usually indicates that the concentration of isoflavones in soy foods and soy ingredients may vary with the genetics of soybean cultivars and climatic conditions during the cultivation. In addition, processing techniques also may affect the concentration of isoflavones. Reports in the literature usually indicate that isoflavones are rather stable compounds affected by heat only with regard to their specific conjugated form and there is little evidence for thermal degradation of these compounds. The change of the isoflavone aglycones in soymilk during heat processing is of particular interest, because there are indications that the aglycones, especially genistein, show the greatest biological activity (Huang et al., 2006). Thus, the use of low temperature is important to maintain the functional properties of the isoflavones during the concentration process (Góes-Favoni et al., 2004). Freeze concentration, as solution for separations of most the substances from the solution, has been studied from year ago. This would be a good alternative for treatment of wastewater the food and chemical industrially. This method require small amount of power compared with the high temperature technology such as evaporations.

By falling-film freeze concentration technology tofu industry can recover soluble solids in the effluents, the TW volume can be reduced and in this way its environmental management can be improved (Belén et al., 2012). The aim of this study was to identify the effect of falling-film freeze concentration of TW on the content of isoflavones, proteins, sugars, calcium and magnesium in both the ice and concentrate obtained. Furthermore, the freezing point and rheological behavior of the TW during the steps of freeze concentration was determined.

2. Materials and methods

2.1. Samples of Tofu Whey (TW)

Fresh TW was supplied by NATURSOY SL, Alimentos Naturales and Biológicos (Castellterçol, Barcelona-Spain). 840 kg of fresh TW, at an initial concentration of 1.9 °Brix, were freeze concentrated. The TW freeze-concentration process was carried out in three stages maintaining the average flow rate at $1 \pm 0.2\ L\ s^{-1}$ to ensure good contact between the evaporator plates and the fluid being concentrated. The equipment used in this study was described in detail by Sánchez et al. (2010) and is shown in Fig. 1. Freeze concentrated samples of TW (6.0–15.5 °Brix) and ice were obtained from the test developed by Belén et al. (2012), according to Fig. 2. Samples of concentrate and ice were collected after each step of freeze concentration.

For each freeze concentration test, the following samples were analyzed: fresh TW in stage 0, the final concentrates (CF1, CF2, and CF3) and ice fractions (I1, I2, and I3) for stages 1, 2 and 3 respectively. Analyses included the determination of concentration of proteins, sugars, calcium, magnesium and isoflavones. Moreover, the freezing point depression and the rheological behavior were determined for fresh TW, CF1, CF2 and CF3. The soluble solid concentration in each concentrates was measured with an Atago refractometer (model DBX-55; Barcelona, Spain), and in accordance with Belén et al. (2012), they were equal to 1.9, 6.0 and 11.0 and 15.5 °Brix to TW, CF1, CF2 and CF3, respectively.

2.2. Physicochemical analysis

The protein content ($g\ 100\ g^{-1}$) in the various samples was determined by Kjeldahl analysis ($N \times 5.70$) in an automatic Kjeldahl distillation equipment (Pro-Nitro II-measuring range 1–140 mg of nitrogen), according to AOAC (2005). Following Sánchez et al. (2010), with modifications, the total sugar content ($g\ L^{-1}$) were determined through High Performance Liquid Chromatography (HPLC, Beckman, San Ramon, USA) equipped with two Beckman 110B pumps; injector Hewlett Packard Series 1100; refractive index detector Beckman 156; Hewlett Packard ChemStation software; and Phenomenex Luna NH2 100 Å (250 × 4.6 mm) column, 5 μm particles. The mobile phase used was acetonitrile: water (75:22); while the flow rate was 1.2 mL min^{-1} and the injection volume was equal to 20 μL. All these analyses were carried out in triplicate.

The concentration of calcium and magnesium in the various samples was measure by flame atomic adsorption, according to AOAC (2005). The equipment used was supplied by Varian, model spectra AA110. The references used for calcium and magnesium were solutions of 1–10–25–100 mg L^{-1} and 1–5–10–20 mg L^{-1} respectably. 10 mL of liquid was used both for the samples and for the references; and 100 μL of strontium chloride ($SrCl_2$) was added at 200 g L^{-1}. The wavelengths used were 422.7 nm and 285.2 nm for calcium and magnesium, respectively.

2.3. Isoflavone extraction and determination

Twenty milliliter of each sample were lyophilized firstly (freeze-dryer; CRYODOS-45, Telstar, Barcelona, Spain), in triplicate. The extraction of isoflavones and the determination of their components were carried out with samples dried in accordance with methodology proposed by Carrão-Panizzi et al. (2002), with modifications. One hundred of lyophilized samples were transferred to a 10 mL test tube, into which 4 mL of an extracting solution (70% ethanol and 0.1% acetic acid) were added. The test tubes with the samples and the extracting solution were stirred in a Vortex (Model MA162, MARCONI®, Piracicaba, SP, Brazil). The extraction was realized during 1 h at 25 °C, with stirring at each 15 min. The test tubes were then placed into an ultrasound bath (Model USC5000, UNIQUE®, Indaiatuba, SP, Brazil) and left for 30 min. A 1.5 mL aliquot of this extract was transferred to a refrigerated microcentrifuge (dimensions of 31 × 60 × 25 cm, 35 kg, Model 5417R 230 V/50 Hz, EPENDORFF®, São Paulo, Brazil) and centrifuged at 20,800*g* for 15 min at 5 °C. The supernatant was filtered through 0.45 μm filters (MILLIPORE®, Billerica, MA, USA) and 20 μL was used to separate and quantify the isoflavones through chromatography. The separation and the quantification of the isoflavones were performed using HPLC, as proposed by Berhow (2002), with a photodiode array detector (Model 996) and an automatic sample injector (Model 717 Plus), both manufactured by WATERS® (Milford, USA). In this stage, a reverse phase column (YMC-Pack ODS-AM, C18, S-5 μm, diameter of 250 × 4.6 mm) was used. For the separation of isoflavones, the binary linear gradient system was used and the mobile phases were: (a) methanol containing 0.025% trifluoroacetic acid (TFA) (Phase A) and (b) ultrapure water (MILLIPORE®, Billerica, MA, USA) containing 0.025% TFA (Phase B). The initial condition of the gradient was 20% in Phase A, reaching 90% in 35 min, followed by cleaning of the column with 100% of Phase A for 5 min and subsequently return to 20%, retaining these conditions for up to 60 min. The mobile phase flow was of 1 mL min^{-1} and the temperature during the analysis was 25 °C. For the isoflavone detection, the wavelength of the detector was adjusted to 254 nm. The software used to control the equipment and the data acquisition was Millennium 32 (version 3.05.01) (GCLC® Toronto, Pickering, ON, Canada). For the identification and quantification of the peaks corresponding to each one of the isoflavones, calibration curves with linear regression based on the peak areas were used. These calibration curves were constructed with external standards of daidzin, daidzein, genistin,