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High-power ultrasound as pre-treatment in different stages of soymilk manufacturing process to increase the isoflavone content

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ABSTRACT

Ultrasound (US) was applied as a pre-treatment in hydrated soybeans (HSB) and soybean slurry (SBS) during soymilk elaboration process to evaluate the feasibility of increasing the isoflavone content (IC) in the resultant soymilk. A predictive model and optimum US processing conditions were obtained by response surface methodology (RSM) using a three-level-three-factor Box-Behnken statistical design (BBD) in which US amplitude (50, 75, and 100%), temperature (30, 45, and 60 °C), and time (20, 40, and 60 min) were selected as independent variables. Most of the US treatments applied in the HSB or SBS caused a significant increase (3–62%) in the total IC of the obtained soymilks over the control soymilk (6.97 mg/100 mL). However, the IC of the resultant soymilks from sonicated HSB (11.38 mg/100 mL) was significantly higher than that in soymilk prepared from US-treated SBS (8.66 mg/100 mL). Experimental data were fitted into a 2nd-order-polynomial model and processing parameters were optimized (100% amplitude, 30 °C, 20 min) to get the highest predicted and experimental IC, 11.38 and 12.8 mg/100 mL, respectively. These results indicated that US is a potential technology that could be implemented during soymilk manufacturing processing as pre-treatment of HSB to obtain soymilk with high isoflavone content and consequently better functionality.

1. Introduction

Soymilk is a colloidal dispersion resulting from the aqueous extraction of soybeans. Its production involves different stages: *i*) selection of soybeans, *ii*) soaking in water, *iii*) wet-milling to get a slurry, *iv*) filtration to separate soymilk from solid residue (okara), and *v*) thermal treatment to inactivate lipoxygenase and trypsin inhibitors. As a result, a homogeneous liquid with milk-like characteristics is obtained [1–3]. It is well known that processing conditions affects its physicochemical and sensory characteristics, as well as nutritional and bioactive composition [2]. Though its beany flavor and astringent aftertaste, soymilk is appreciated among consumers because of its high quality proteins, lactose-free attributes, bioactive compounds profile and health-related benefits [4]. Hence, it comprises one of the two largest segments of the soy-based products market along with soy-based snack bars [5].

Among the bioactive compounds of soymilk, isoflavones are a group of naturally occurring non-steroidal phytoestrogenic and antioxidative polyphenolic molecules [6]. These compounds have been related to some of the pharmacological and antioxidant properties of soymilk [6,7]. The interest in soy isoflavones is based on epidemiological studies suggesting the direct correlation of isoflavone intake and the decreases in the incidence of different type of hormone dependent cancer (breast and prostate), cardiovascular diseases, bone loss, lowering cholesterol levels and alleviating menopause symptoms in women [3,8–11].

There are twelve isoflavone structures reported for soybeans, three aglycones and their respective malonyl-, acetyl- and β-glucosides [6,12]. Jung et al. [13] and Kao et al. [14] observed that processing conditions during soymilk extraction can impact these structures, affecting their concentration and modifying their profile, especially in the conversion to non-conjugated forms. Cederroth and Nef [15] explained that, among the different isoflavone structures, aglycones are those biological active because only they are absorbed by the intestinal tract. Thus, soy-based products with high aglycone concentration may be more effective than glucoside-rich products in preventing chronic disorders. In this regard, some alternatives to enhance isoflavone content, specially aglycones, in soymilk have been developed such as: hydrothermal treatment of soybeans [16–18], soybean fermentation with fungi [19], addition of soy germ, soy protein isolate or bifidobacteria to soymilk [20], and application of novel technologies during soymilk

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processing [2]. Results from these studies corroborate the importance of the selection of raw material and processing conditions to produce soymilk rich in biological active isoflavones.

Currently, emerging technologies, such as ultrasound (US), have been recognized as potential methods to improve the extraction of intracellular compounds from plant materials [21] and the increase of antioxidant and bioactive molecules [7,22,23]. Essentially, US treatment is based on the formation of acoustic waves with high frequency that can be propagated in gases, liquids or solids, generating a cavitation phenomenon [24]. The shear forces and shock waves produced during cavitation break the biological cell walls and facilitate the release of cell content in to the medium. Amplitude (*A*), temperature (*T*), and time (*t*) have been identified as the most influential parameters of US processing to improve the extraction of intracellular compounds from plant-based foods. Hence, their optimal combination could lead to the best processing conditions for achieving the highest bioactive compounds concentration in sonicated products.

In this regard, response surface methodology (RSM) is a statistical tool used for optimizing processing conditions. It has been successfully applied to develop and improve biochemical and biotechnological processes in food systems, including extraction of phenolic compounds from berries, anthocyanins from black currants, and vitamin E from wheat germ, among others [25,26]. Therefore, the aim of this work was to apply US technology in two different stages of soymilk processing: *a*) hydrated-soybeans (HSB) and *b*) soybean-slurry (SBS) to evaluate its effect on the isoflavone profile and total isoflavone content of resultant soymilks.

2. Materials and methods

2.1. Soymilk preparation

Soybeans (*Glycine* max) were purchased at Monterrey (N.L., Mexico) in a local market and stored in darkness at room temperature until they were used. Soymilk (SM) was prepared according to the procedure established by Yeo and Liong [27]. Briefly, soybeans (100 g) were washed with running water and soaked during 16 h at room temperature in 300 mL of distilled water. Then, hydrated soybeans (HSB) were drained, rinsed, and wet-milled with 400 mL of distilled water (Vita-mix Corp., OH, USA) for 3 min. Obtained slurry (SMS) was filtered through four layers-cheesecloth, separating the okara from the SM. The resultant SM was batch pasteurized in a water bath at 60 °C for 30 min, to inactivate lipoxygenase enzyme, and immediately cooled down to 5 ± 1 °C using an ice bath.

2.2. US treatment

US treatments [*A* (50, 75, 100%), *T* (30, 45, 60 °C), and *t* (20, 40, 60 min)] were applied in two different stages of the soymilk extraction process: *a)* HSB (4:1, water:soybeans proportion) and *b)* SBS. Sonication was performed using an ultrasonic processor (Hielscher Inc., USA, Inc. Ringwood, N.J.) model UP400S (400 W, 24 kHz, 100 µm) with a 22 mm diameter titanium probe which was submerged 3 cm in to the soy samples (HSB or SBS). A double walled vessel of 1000 mL was used as a treatment chamber. Temperature was controlled with a thermostatic bath (Lauda Wobser Gmb & Co., Germany) and monitored through the whole processing with a thermocouple attached to the treatment chamber.

2.3. Isoflavone extraction, identification and quantification

Isoflavones were extracted, identified and quantified according to Luthria et al. [28], with some variations. A portion of 0.5 g of freeze-dried soymilk (0.024 mm Hg and -52 °C, Virtis FM 25 EL-85, SP Scientific freeze dryer group, Warminster, PA, USA) was weighted in 50 mL-centrifuge tubes and mixed with 10 mL of methanol (80%) and shaken for 1 min (VWR Digital Vortex Mixer, USA). Tubes were immediately placed in a US bath (Branson 2510, Branson Ultrasonic Corporation, CT, USA) at room temperature during 15 min and then centrifuged for 10 min at 10000*g* and 4 °C. Supernatant was decanted into a 50 mL flask and the residue was re-extracted once with 10 mL of methanol solution (80%). Supernatants were combined and filtered through Whatman paper (No.1), and collected in a 20 mL vial. The extract was concentrated using a Rocket evaporator (Genevac Ltd, Suffolk, UK). The residue was diluted with 1 mL of a methanol (HPLC grade) water solution (80:20 v/v), passed through a 0.20 µm PVDF filter and placed in glass vials. All the extracts were stored at -20 °C until chromatographic analysis.

Isoflavone identification was conducted in an HPLC system (1200 series, Agilent Technologies, Inc., Santa Clara, CA, USA) with a diode array detector. Separation of the isoflavones was achieved using a reversed phase C18 column (XDB Eclipse, Agilent Technologies, CA, USA). The mobile phase consisted of solvent A (0.1% v/v formic acid in water) and solvent B (100% acetonitrile). Gradient elution was as follow: 0 min-0% B, 8 min-10% B, 16 min-35% B, 26 min-90% B, 36 min-100% B. The column was equilibrated for 10 min with 15% B and washed at 100% B for 5 min prior to the next injection. Column temperature was controlled at 30 °C and the flow rate was maintained at 0.4 mL/min. Injection volume of isoflavone standards and samples was set at 1.0 µL. Each isoflavone was identified by comparison of its UV–vis spectra and retention time with that of the reference standard (Sigma Aldrich, Munich, Germany): Daidzein (Da), Genistein (Ge), Daidzin (Din), and Genistin (Gin). Quantification was done by the integration of the peak areas. Data were compared to calibration curves of each standard and results were expressed as mg of isoflavone/100 mL of soymilk.

2.4. Experimental design and statistical analysis

US parameters: *A* (50, 75, 100%), *T* (30, 45, 60 °C), and *t* (20, 40, 60 min) were selected as independent variables (X_1, X_2, X_3, respectively) for the experimental design using a RSM with three-level-three-factor Box-Behnken design (BBD) which consisted in 15 experimental runs (Table 1). All trials were conducted in duplicate in each soy sample (HSB and SBS). Total isoflavone content (IC), calculated by the sum of Din, Gin, Da, and Ge concentrations of the resultant soymilks, was selected as response variable. A Minitab software (Minitab Release 14.1) was used to generate the experimental design and statistical analysis. Experimental data were fitted to a 2nd-order polynomial model (Eq. (1)

Table 1
Box-Behnken design and experimental values of total isoflavone content (TIC) in soymilk extracted from hydrated-soybean (HSB) and soybean-slurry (SBS) treated by different combinations of ultrasound processing variables: amplitude (*A*), temperature (*T*) and time (*t*).

Treatment conditions				TIC (mg/100 mL)	
Treatment	*A* (%)	*T* (°C)	*t* (min)	HSB	SBS
1	50	45	20	7.8 ± 0.41	6.54 ± 0.21
2	75	60	20	6.5 ± 0.26	7.17 ± 0.33
3	75	45	40	8.58 ± 0.76	7.7 ± 0.18
4	75	30	20	11.3 ± 0.80	10.54 ± 0.48
5	100	45	20	9.77 ± 1.17	7.91 ± 0.51
6	75	45	40	8.1 ± 0.84	7.33 ± 0.16
7	100	60	40	9.62 ± 0.92	7.01 ± 0.26
8	50	45	60	7.49 ± 0.31	6.49 ± 0.52
9	50	60	40	5.51 ± 0.53	6.91 ± 0.65
10	100	30	40	9.92 ± 0.61	7.1 ± 0.29
11	75	60	60	9.11 ± 0.91	7.46 ± 0.32
12	100	45	60	10.34 ± 1.42	7.31 ± 0.18
13	50	30	40	7.34 ± 1.90	4.11 ± 0.18
14	75	30	60	9.00 ± 0.54	7.59 ± 0.70
15	75	45	40	8.58 ± 0.87	7.29 ± 0.63

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