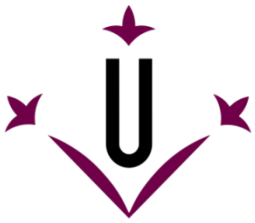

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HIGH-POWER ULTRASOUND AS PRE-TREATMENT IN DIFFERENT STAGES OF SOYMILK MANUFACTURING PROCESS TO INCREASE THE ISOFLAVONE CONTENT

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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/100mL). 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 2^{nd}-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.

Key words: ultrasound, soymilk, isoflavones, response surface methodology, Box-Behnken design

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 [1,2,3]. 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 [3,8,9,10,11].

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, 17, 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 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.

MATERIALS AND METHODS

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.

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 (400W, 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.

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.

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) and regression coefficients obtained.

$$Y = \beta_0 + \sum_{i=3}^{3} \beta_i X_i + \sum_{i=3}^{3} \beta_{ii} X_i^2 + \sum \sum_{i<j=1}^{3} \beta_{ij} X_i X_j \quad \text{(Eq. 1)}$$

where β_0, β_i, β_{ii}, and β_{ij} were the regression coefficients for intercept, linear, quadratic and interaction terms, respectively, and X_i and X_j were the independent variables. The Minitab software was used to generate response surfaces plots while holding a variable constant in the 2nd-order polynomial model. Fitness of the second order model was expressed by the regression coefficient R^2 and its statistical significance was determined by the ANOVA test. Optimal values of the selected US parameters (X_1, X_2, and X_3) to achieve the highest total IC were obtained using the predictive equation of RSM. Finally, soymilk was processed under US optimal conditions and total IC was quantified to compare experimental and predictive data in order to determine the validity of the model.

RESULTS AND DISCUSION

Soybeans used as raw material to elaborate the soymilks for this study were analysed to quantify and characterize their isoflavone content and profile. Obtained results indicated that the isoflavone profile of the soybeans was characterized by Gin, as the most abundant isoflavone (641.0 ± 36.2 µg/g), followed by Din (536.6± 38.1 µg/g), Ge (40.3 ± 3.4 µg/g)

and Da (38.8 ± 4.2 µg/g). As a result, their glucoside and aglycone concentration corresponded to the 93.8 and 6.2 % of the total IC, respectively (Table 2). Total IC (1283.7 ± 86.7 µg/g) of the analysed soybeans, calculated by the sum of individual isoflavones concentration, was within the range reported by other authors (1160 to 3090 µg/g) for soybeans of different cultivars, locations and years [12,29,30]. According to Kim et al. [31] isoflavone concentrations in soybeans are affected by the environment, the cultivar, the genotype, and the interaction between these factors. Moreover, in agreement to the obtained results, other authors have demonstrated that glucosides are the primary isoflavones present in whole soybean [32, 33]. Namely, Jung et al. [13] reported that β-glucosides were the predominant forms of the total isoflavones in soybeans, representing more than 90 %. However, during manufacturing processes of soy-based products the glucoside isoflavones could be converted to aglycones depending on processing conditions [34].

In this regard, Gin and Din concentrations of control soymilk were 2.65 ± 0.23 mg/100 mL and 1.81 ± 0.23 mg/100 mL, respectively; while its content of aglycones was significantly lower, with 1.36 ± 0.04 mg/100 mL for Ge and 1.14 ± 0.02 mg/100 mL for Da. Similarly, Wang and Murphy [12], Prabhakaran and Perera [6], Ishihara et al. [35], and Xu and Chang [36] reported that isoflavone profile of soymilk is characterized by Gin, Din, and in minor concentrations their respective aglycones, Da and Ge; being Gin the major isoflavone present in soy-based products [37]. Total IC of control soymilk was 6.97 ± 0.49 mg/100 mL, with 64 % of glucoside conjugated forms and 36 % of aglycones (Table 2). Usually, the total IC of soy-based products varies between 5 – 2000 mg/100 g [38] and this wide range is mainly related to *a*) the soybean cultivar used for the soymilk extraction and *b*) process operations such as soybean soaking time, soybean:water ratio, milling temperature, the use of enzymes, heat pasteurization parameters and storage conditions, among others [13,39,40].

As seen in table 2, aglycone concentration of control soymilk (36%) was significantly higher than in the raw soybeans (6.2%). It is possible that the different stages of the soymilk manufacturing process led to some changes between conjugated and non-conjugated isoflavone structures, increasing the aglycone proportion in the resultant soymilk. Baú and Ida [41] elucidated that there are different reactions occurring during the manufacturing of soy-based products such as decarboxylation of malonate to acetate, de-esterification of malonate to underivatized glycosides, and generation of aglycones through the breakdown of glycosides bonds. Consistently, Niammuy et al. [42] indicated that isoflavones are generally more susceptible to interconversion reactions than to degradation during processing.

As seen in Figures 1 and 2, the concentration of individual isoflavones significantly changed and the isoflavone profile was shifted towards the aglycone structures in the soymilks obtained from sonicated HSB or SBS at the highest amplitude levels (75 and 100%) compared to the isoflavone profile of the control soymilk. The clearest effect was noticed in Din content of soymilk prepared with US-treated HSB (Fig. 1) which significantly increased up to 134 – 156 %. Also, there was a significant augment (198 %) in the Din concentration of soymilk obtained from SBS sonicated at 75 % of amplitude and 30 °C during 20 min (Fig. 2). Conversely, Gin concentration decreased between 15.9 and 79.5 % or remained with no significant changes in most of the soymilks elaborated with sonicated HSB and SBS. Regarding isoflavone aglycones, the concentration of Da and Ge significantly increased between 90.3 - 131 % and 19.6 – 59 %, respectively, in the resultant soymilks from both sonicated soybean samples (HSB and SBS).

To the best of the authors' knowledge, there is little information available about the effects of US applied as pre-treatment during soymilk manufacturing process. Namely, Fahmi et al. [7] reported that the application of US at different frequencies (35 and 150 kHz) in soybean slurry induced a significant increase in the concentration of glycosides and aglycone

isoflavones on extracted soymilk. They attributed these results to the cavitation phenomenon which cause the permeability of plant tissues. In a different study, Hossain et al. [43] investigated the effects of US treatment in the extraction of antioxidant molecules from marjoram (*Origanum majorana* L.), just like the obtained results, they observed that among the US processing parameters, amplitude showed the highest effect resulting in a greater extraction of phenolic compounds. According to them, higher amplitude levels could have damaged more cell walls releasing the antioxidant molecules, including flavonoids. Otherwise, Jung et al. [13] suggested that high hydrostatic pressure process combined with mild temperature promoted the interconversion of malonyl forms to β-glucosides. Therefore, it might be possible that the cavitation phenomenon occurring during US treatment of the HSB or SBS promoted on one side, the release of isoflavones from intracellular tissues and, on the other, the interconversion of malonyl-Din to Din, and of β-glucosides to Da and Ge, increasing their concentration in the obtained soymilk. Nonetheless, further research is required to identify the specific physical and chemical reactions occurring in soy-based products under US treatment.

As a result of the changes in the concentration of individual isoflavones of soymilks prepared from US-treated HSB or SBS, their total IC (Table 1) was significantly higher compared to that of the control soymilk (6.97 ± 0.49 mg/100 mL), except in the soymilks prepared from sonicated HSB (5.51 ± 0.53 mg/100 mL) at 50% amplitude/60 °C/40 min and SBS (4.1151 ± 0.18 mg/100 mL) at 50% of amplitude/30 °C/40 min. As can be noticed, soymilks prepared from sonicated HSB presented the highest levels of total isoflavones compared to those quantified in the soymilks obtained from US-treated SBS. It is well known that isoflavones, which have a polyphenolic structure, are associated with the interior moiety of native form of globular soy protein [44]. Hence, the cavitation phenomena occurring at the highest amplitude levels (75 and 100%) may cause denaturation and

unfolding of proteins, resulting in the breakdown of isoflavone-protein interactions, increasing their extractability in the sonicated medium. Welti-Chanes et al. [45], Chandrapala et al. [46], Mason and Lorimer [47], Ashokkumar and Mason [48] and Ashokkumar [49] stated that during US application, thousands of bubbles are generated inside the sample and around the sonotrode; depending on the structure characteristics of the sonicated product, being homogeneous or dense fluids, the formed bubbles could move faster or slower and collapse between them at high or low intensity. As a consequence, elevated temperature and pressure are produced in specific points, leading to the disintegration of biological cells and to the formation of microscopic channels, which facilitates the extraction of intracellular phytochemicals [50,51]. Since the processing medium for HSB was distilled water, it could be expected that during sonication the formation of bubbles was easier and faster than in the SBS, which was a dense medium, increasing the intensity of the cavitation and facilitating the isoflavone release from the protein-isoflavone complex. Moreover, the enhanced isoflavone extractability in the soymilk prepared from US-treated HSB could be due to the changes in the structure of the cotyledon surface or epidermal cells of sonicated beans, promoting the isoflavones release in the soaking water during the treatment.

Process conditions optimization

Table 2 shows the total IC of soymilks extracted from all sonicated HSB and SBS. Multiple linear regressions were performed considering data from table 2. Obtained coefficient values of the predictive model (Eq. 1) as well as the results of analysis of variance (ANOVA) are presented in tables 3 and 4, respectively. The *p*-values (<0.05) were used as a tool to evaluate the significance of each coefficient. According to Wu et al. [52], small *p*-values means more significant corresponding coefficient. Hence, as shown in table 3, the linear coefficients (β_2 and β_3) and the interactions terms (β_{12} and β_{23}) for the predictive model of

the IC in soymilk extracted from US-treated HSB, were found to be significant, with a very small *p*-value ($p < 0.05$); while the other terms were not significant. Regarding regression coefficients for the predictive model of IC in soymilk prepared from sonicated SBS, the linear coefficients ($\beta_1, \beta_2, \beta_3$) and the interactions ($\beta_{13}, \beta_{23}$) and quadratic ($\beta_{11}, \beta_{33}$) terms were also significant ($p < 0.05$). To provide a better visualization of the significant factors derived from the statistical analysis, the response surface plots for the effects of *A*, *T* and *t* on the total IC in soymilks extracted from sonicated HSB or SBS are given in Fig. 3a and 3b, respectively. The plots show the effects of two factors on the response (*A*, *t*), while the third factor (*T*) was kept constant at zero level (central point) in both cases. The constructed surface plots for soymilk obtained from sonicated HSB (Fig. 3a) illustrates that amplitude had a key role among US processing parameters. High amplitude levels, independently of the processing time, result in higher isoflavone content.

As seen in Fig. 4, predicted data obtained from the model (Table 3) of the total IC in soymilk prepared from HSB (Fig. 4a) were well-related to the experimental results, conversely to those values resulting from the model of the IC of the soymilk elaborated with SBS (Fig. 4b) which were overestimated compared to the experimental data. According to these results, the determination coefficient (R^2) of both models were 0.91 and 0.69 for total IC of soymilk extracted from sonicated HSB and SBS, respectively. Hereof, the data of the model obtained for the total IC of soymilk prepared with US-treated HSB fitted better than the model for the soymilk prepared from sonicated SBS. In addition, the lack-of-fit test, presented in table 4, for the model of the IC of soymilk from HSB was not significant (p= 0.868), contrary to the lack-of-fit for the model of the IC of soymilk from SBS (p <0.05).

Optimal conditions of the selected US variables were obtained by solving the regression equation (Eq. 1). After calculation, an amplitude of 100 %, temperature of 30 °C and 20 min of treatment time were the best combination of US processing parameters applied in HSB to

obtain the highest total IC in the resultant soymilk (11.38 mg/100 mL). To corroborate the total IC predicted value, US treatment was applied in HSB under optimized conditions obtaining a soymilk with a total IC of 12.8 ± 1.14 mg/100 mL, which clearly showed that the model fitted the experimental data ($R^2 = 0.91$). These results pointed out the suitability of the model employed and the success of RSM-BBD in optimizing US processing parameters to attain the highest isoflavone content in soymilk formulated with sonicated HSB.

CONCLUSIONS

The application of US as pre-treatment in HSB or SBS caused the release of isoflavones from the cell walls of the soy-samples or from the protein-polyphenol complex, augmenting their concentration in the extracted soymilk. Furthermore, cavitation phenomena might induce interconversion between the different isoflavone structures, increasing the glucosides and aglycones content. US treatment applied in HSB induced to a higher concentration of total IC in the resultant soymilk than its application in the SBS. Hence, food matrix has an important influence on the effects of US processing. The experimental results indicated that *A* significantly affected the isoflavone content of the soymilk obtained from sonicated HSB or SBS. Moreover, the statistical analysis based on a BBD showed that an amplitude of 100% at 30 °C during 20 min are the best conditions for an US treatment applied in HSB to obtain a soymilk with high isoflavone concentration; approximately 63 – 84 % higher than the isoflavone content of a soymilk extracted from untreated soybeans. Hence, US is a potential technology which can effectively enhance the isoflavone content of soy-based products. Further studies related with the changes in sensory properties and storage stability are suggested to carry out with the soymilk obtained in this study.

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TABLES

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*)

Table 2. Glucosides and aglycones concentration from total isoflavone content of raw soybeans and soymilks: control, from sonicated hydrated-soybeans (US-HSB) and soybean-slurry (US-SBS)

Table 3. Coefficients values of the predictive model for total isoflavone content of soymilk obtained from sonicated hydrated-soybeans (HSB) or soybean-slurry (SBS)

Table 4. Analysis of variance (ANOVA) for the regression equation

FIGURES

Fig. 1 Individual isoflavone content of soymilk extracted from US-treated hydrated-soybeans. Processing conditions of US-treatments 1 to 15 (X axis) are from the Box Behnken Design (BBD) detailed in table 2. Din: daidzin, Gin: genistin, Da: daidzein, Ge: genistein.

Fig. 2 Individual isoflavone content of soymilk extracted from US-treated soybean-slurry. Processing conditions of US-treatments 1 to 15 (X axis) are from the Box Behnken Design (BBD) detailed in table 2. Din: daidzin, Gin: genistin, Da: daidzein, Ge: genistein.

Fig. 3 Response surface plots of the effects of amplitude and time, on the total isoflavone content of soymilk extracted from sonicated hydrated-soybeans (a) and soybean-slurry (b) at a constant temperature of 45°C

Fig. 4 Scatter plot of the predicted and experimental data of total isoflavone content (TIC) of soymilk extracted from sonicated hydrated-soybeans (a) and soybean-slurry (b).

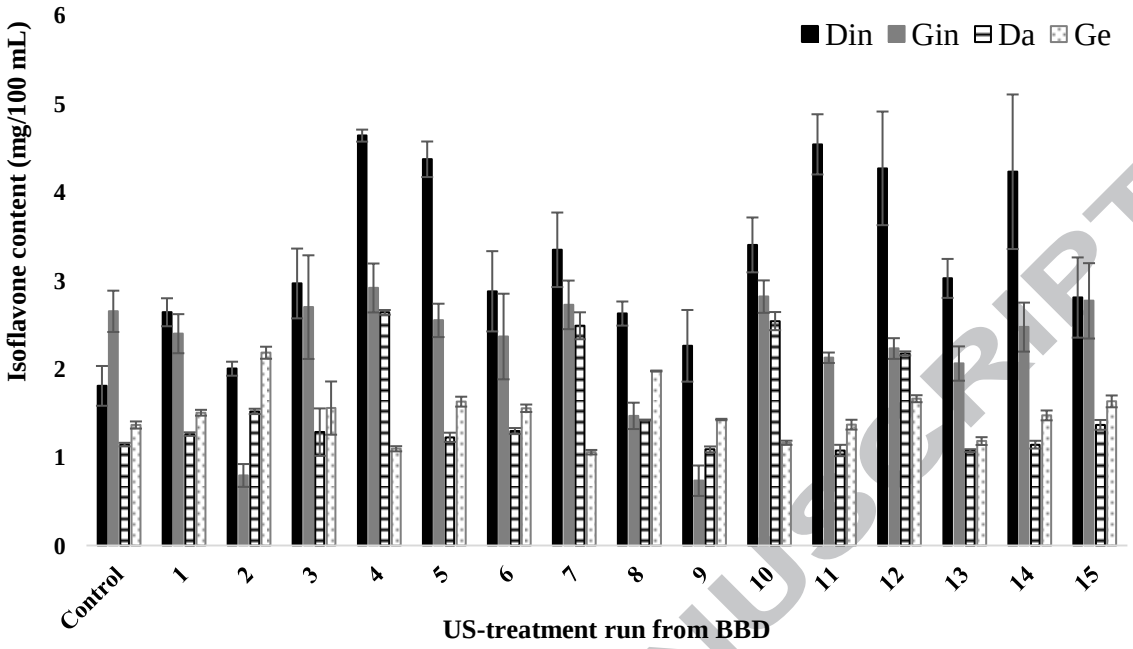

Fig. 1

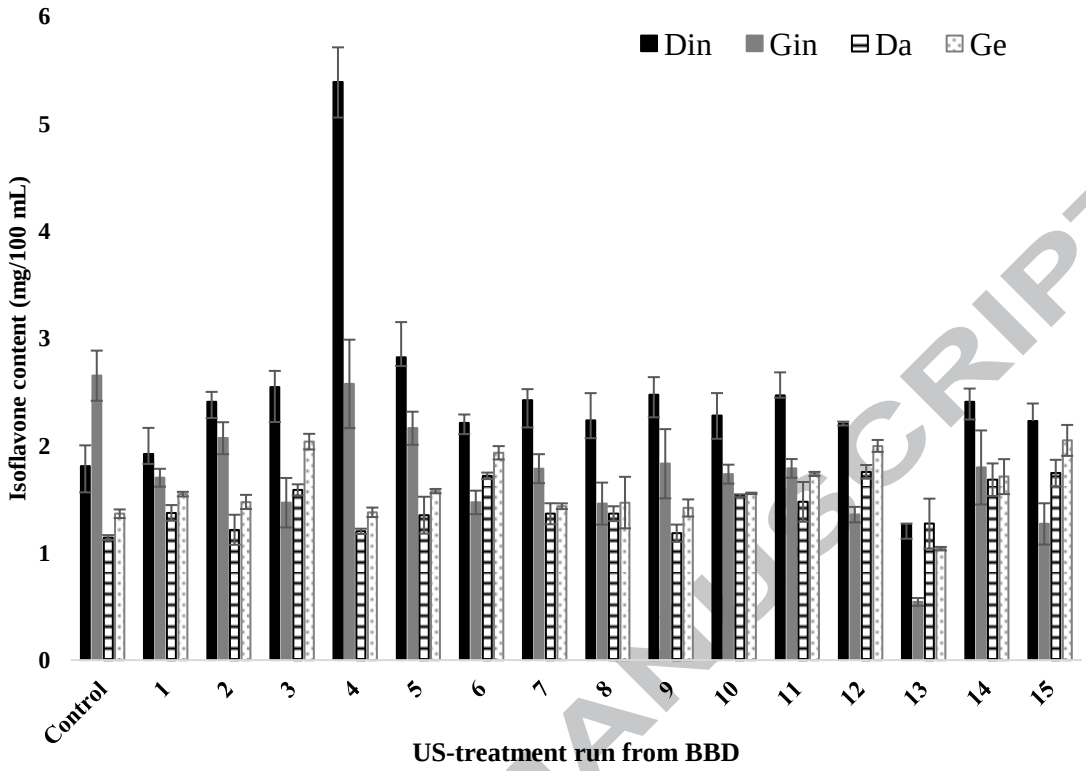

Fig. 2

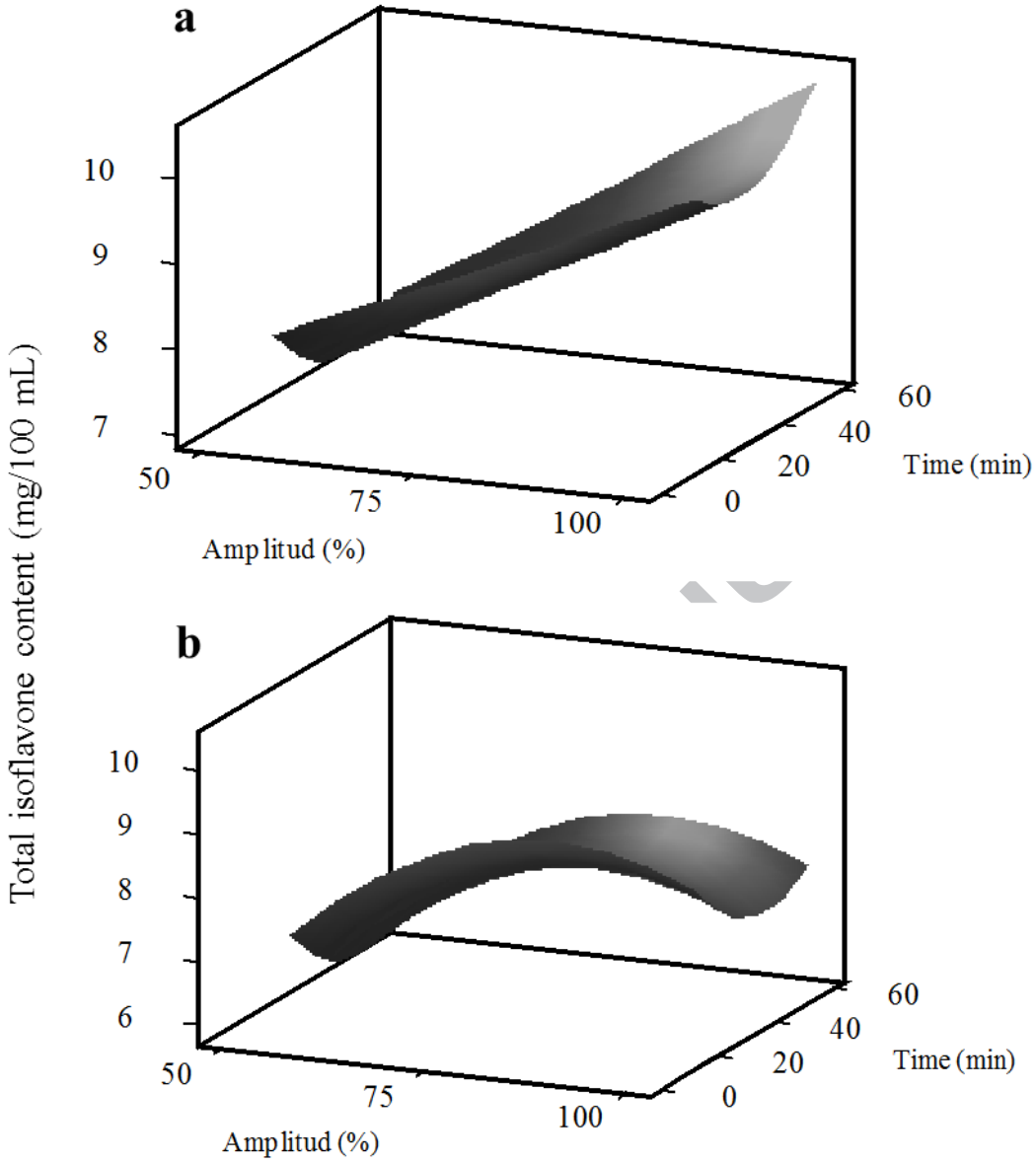
a
10
9
8
7
50
75
100
Amplitud (%)
0
20
40
60
Time (min)
Total isoflavone content (mg/100 mL)
b
10
9
8
7
6
50
75
100
Amplitud (%)
0
20
40
60
Time (min)

Fig. 3

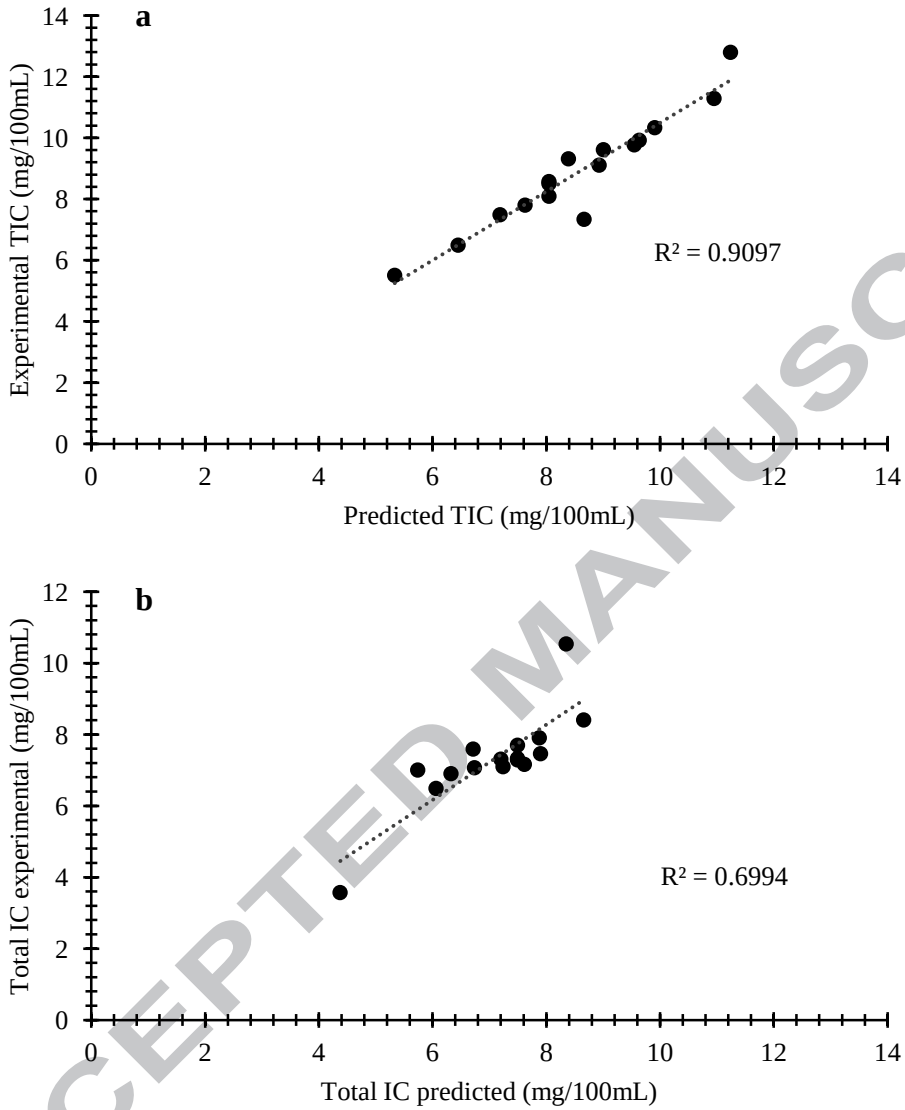

Fig. 4

Table 1.

Treatment	Treatment conditions			TIC (mg/100mL)	
	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

Table 2.

Sample	Glucosides (%)	Aglycones (%)
Raw soybeans	93.8	6.2
Control soymilk	64	36
Soymilk from US-HSB	43-66	33-57
Soymilk from US-SBS	43-75	24-56

Table 3.

Term	HSB		SBS	
	Coefficient	***p-value***	**Coefficient**	***p-value***
β_0	25.5050*	0.000	-10.5342*	0.020
β_1	-0.0506	0.537	0.3963*	0.000
β_2	-0.414*	0.004	0.2960*	0.011
β_3	-0.324*	0.000	-0.2089*	0.004
β_{11}	0.000	0.953	-0.0018*	0.000
β_{22}	0.0005	0.684	-0.0020	0.069
β_{33}	0.0013	0.082	0.0015*	0.014
β_{12}	0.0018*	0.027	-0.0023*	0.001
β_{13}	0.0004	0.444	0.0000	0.919
β_{23}	0.0042*	0.000	0.0016*	0.040
R^2	0.91		0.69	
S	1.122		0.926	

*Significant at 5%

Table 4.

Source	Hydrated-soybean					Soybean-slurry				
	DF	Adj SS	MS	*F-value*	*p-value*	DF	Adj SS	MS	*F-value*	*p-value*
Regression	9	117.2175	13.0242	10.35	0.000	9	60.59	6.7321	7.85	0.000
Linear	3	25.8660	8.6220	6.85	0.001	3	48.21	16.0687	18.74	0.000
Quadratic	3	4.1163	1.3721	1.09	0.362	3	27.91	9.3022	10.85	0.000
Interaction	3	32.3507	10.7836	8.57	0.000	3	15.53	5.1779	6.04	0.001
Residual Error	48	60.3848	1.2580			49	42.01	0.8574		
Lack of Fit	3	0.9518	0.3137	0.24	0.868	3	16.34	5.448	9.76	0.000
Pure Error	45	59.4331	1.3207			46	25.67	0.558		
Total	57	117.602				58	102.6			

DF: degree of freedom, Adj SS: adjusted sum of squares, MS: mean square

HIGHLIGHTS

- Sonication of hydrated-soybeans or slurry increase isoflavone content of soymilk
- Food matrix has an important influence on the effects of ultrasound processing
- Amplitude is the main US parameter affecting the isoflavone content of soymilk