Food Chemistry 111 (2008) 592–598

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Isoflavone profiles of soymilk as affected by high-pressure treatments of soymilk and soybeans

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ARTICLE INFO

Article history:
Received 15 November 2007
Received in revised form 31 March 2008
Accepted 9 April 2008

Keywords:
Isoflavone
Soybean
Soymilk
High-pressure processing

ABSTRACT

High hydrostatic pressure was applied to hydrated soybeans (100–700 MPa, 25 °C) and soymilk (400–750 MPa; 25 and 75 °C) to assess its effect on isoflavone content, profile and water-extractability. Neither pressure level nor initial treatment temperature affected soymilk isoflavone content. However, combined pressure and mild thermal treatment modified the isoflavone distribution. At 75 °C, the isoflavone profile shifted from malonylglucosides toward β-glucosides, which was correlated to the effect of adiabatic heating. When pressure was applied to the hydrated soybeans, the soymilk isoflavone concentration varied between 4.32 and 6.06 μmol/g. The content of protein decreased and fat increased in soymilks prepared from pressurized soybeans with increasing pressure level.

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1. Introduction

Isoflavones, which have received considerable attention due to their biological activity over the past 20 years, are phytoestrogens that are present in a concentration of 0.3–0.8% (db) in soybean seeds (USDA-Iowa State University Database on the Isoflavone Content of Foods, 2002). Interest in soy isoflavones is based on data suggesting potential of isoflavones in lowering cholesterol levels, preventing both prostate and breast cancers and attenuating bone loss in postmenopausal women, and alleviating menopausal symptoms (Hendrich & Murphy, 2007; Liu, 2004). Daidzin, genistin and glycitin are the three isoflavone glucosides existing in soybeans and soy-based foods. They can be found as non-conjugated β-glucosides and conjugated malonyl- or acetyl-β-glucosides. In raw soybeans, although the isoflavone forms depend on many seed characteristics such as variety, crop year and growth location, the most predominant ones are malonyl-daidzin and -genistin which together constitute 71–81% of total isoflavones (Charron, Allen, Johnson, Pantalone, & Sams, 2005). The processing operations and conditions applied for production of soy-based products and ingredients determine the final content and profile of isoflavones (Liu, 2004; Murphy et al., 1999; Wang & Murphy, 1994). Extraction in water is the first important processing step in the recovery of isoflavones from soy matrices to produce soymilk, tofu and soy protein isolate (SPI). During SPI and soymilk production isoflavones can be partially retained in the fibre fractions. These losses in the fibre fractions can be minimized by adjusting temperature and pH (Prabhakaran & Perera, 2006; Rickert, Johnson, & Murphy, 2004; Rickert, Meyer, Hu, & Murphy, 2004; Speroni, Milesi, & Anon, 2007). These adjustments should be done carefully as an increase of extracted isoflavone can negatively affect the extractability of protein (Barbosa, Lajolo, & Genovese, 2006). Besides their extractability, defined in this paper as the amount of isoflavones recovered from the starting material into water, other determining parameters accounting for isoflavones' final concentration and prevalence of the different forms are their rate of conversion. The conversions of isoflavones during processing are dictated by both their chemical structure, and other parameters such as pH, temperature, moisture and activity of endogenous β-glucosidases (Ismail & Hayes, 2005).

Soy proteins constitute another critical constituent in soybeans. For decades they have been recognized for their excellent nutritional quality and functionalities as food ingredients. In 1999, the soy protein-cardiovascular health claim was approved by the US Food and Drug Administration and apparently spurred increased interest in soy-based products. This claim has certainly contributed to the steady increase in soymilk consumption in Western countries, which added to their long-standing popularity in East Asian countries, making soymilk a popular beverage around the world. Soymilk production has several thermal treatments, which can negatively affect its nutritional quality, color and sensory attributes (Kwok & Niranjan, 1995) and modify isoflavone distribution. High-pressure processing (HPP), a newly developed food technology, could be an alternative to thermal processing for soymilk production (Kajiyama, Isobe, Uemura, & Nohuchi, 1995; Lakshmanan, de Lamballerie, & Jung, 2006; Zhang, Li, Tatsumi, & Isobe, 2005). The unique effects of HPP are due to

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0308-8146/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2008.04.025

the effect of pressure on non-covalent bonds while leaving the covalent bonds of the food intact, in contrast to the changes occurring during thermal processing. Because of the pressure-induced modification in protein structure and interactions, and the potential for creation of food products with unique properties, the effects of high-pressure processing have been investigated on functionality of soy protein isolates, soy protein gels and tofu (Lakshmanan et al., 2006). There is, however, a lack of data on the effect of high-pressure processing on the stability and interconversion of the health-promoting isoflavones. In addition, little information is available on the interaction between soy isoflavones and proteins and how this interaction modifies isoflavones extractability in water. During soymilk production, about 30% of the total mole mass of isoflavones is lost in the insoluble fraction, the okara (Wang & Murphy, 1996). Protein–polyphenol interactions involve hydrogen bonding, ionic, and hydrophobic interactions (Boye, 1999), which are known to be pressure-sensitive. There is body of evidence that denaturation and modification of physico-chemical characteristics of the two main soy proteins, glycinin and β-conglycinin, occurs during high-pressure processing (Torrezan, Tham, Bell, Frazier, & Cristianini, 2007; Puppo et al., 2004). Isoflavones seem to have an affinity for denaturated soy proteins (Rickert et al., 2004). Therefore under pressure, the unfolding and denaturation of soy protein and/or changes in the polyphenol interaction, could modify isoflavone extractability in water. In addition, considerable changes in the structure of cotyledon surface and epidermal cells of pressurized soybean seeds were observed as well as some release of the soy protein in soaking water surrounding the beans during treatment (Omi, Kato, Ishida, Kato, & Matsuda, 1996). These changes in cotyledon surface and epidermal cells induced by HPP may also affect isoflavone extractability. To the best of our knowledge, no studies have investigated the possible changes in the isoflavone water-extractability as a result of high-pressure processing.

This study reports on the effect of pressure level and initial treatment temperature on the content and profile of isoflavones in soymilk. The potential of using high-pressure processing as a means to modify isoflavone water-extractability was determined, in addition to the composition, viscosity and protein characteristics of soymilk obtained from pressurized soybeans.

2. Materials and methods

2.1. Materials

Vinton 81 cultivar soybeans (*Glycine max* L.) were purchased locally (Pattison Bros., Fayette, IA) and stored in the dark at 4 °C. All reagents were of analytical grade and purchased from Fisher Scientific (Pittsburgh, PA) and Sigma–Aldrich (St. Louis, MO). Acetonitrile and methanol from Fisher were of HPLC-grade.

2.2. Soymilk and soaked soybean preparation

One hundred grams of soybeans were washed to remove dirt and soaked for 12 h at room temperature in tap water. The drained soybeans were weighed to determine the water uptake and then ground at low speed for 1 min with water to yield a dry bean-to-water ratio of 1:8 (w:w) in a 4-L Waring heavy-duty laboratory blender (Torrington, CT). The slurry was filtered through a 100-mesh nylon filter-sack and water was added to reach a dry soybean-to-water ratio of 1:10 (w:w). Finally, the slurry was squeezed manually to separate the insoluble residue, okara, from the filtrate. The soymilk had a pH of 6.6 and a Brix of 6.5° ± 0.2°. Soymilk yield was calculated as the weight (g) of soymilk obtained per 100 g of soybeans.

For pressure treatment of the hydrated soybeans, 28 g of soybeans were soaked as described above and after a water uptake determination, the appropriate amount of water needed to reach a dry bean-to-water ratio of 1:8 (w:w) was added to the soybeans directly in a polyester bag. After HPP treatment, the content of the bag, i.e. pressurized soybeans and water was transferred in a 1-L Waring heavy-duty laboratory blender (Torrington, CT) and the soymilk was prepared as described above.

2.3. Thermal treatment

The soymilk was heated at 95 ± 2 °C for 15 min in a 2-L reaction vessel (model CG-1929-16, ChemGlass, Vineland, NJ) with continuous stirring at 695 rpm (stirrer model BDC 3030, Caframo, Wiarton, Ontario) prior to HPP. It took ~15 min to reach the 95 °C temperature. After the 15 min at 95 °C, the soymilk was immediately cooled down in an ice-water bath.

2.4. High-pressure processing

Soymilk and hydrated soybeans in water were vacuum-packaged in polyester bags (SealPaks, KAPAK, Minneapolis, MN) in a tabletop Multivac machine (Model C 100, Multivac, Kansas City, MO). The samples were pressurized with a Food-Lab 900 high-pressure food processor (Stansted Fluid Power, Stansted, UK). The sample holder was 6.5-cm i.d. and 23-cm height. The rates of pressurization and depressurization were 260 and 500 MPa/min, respectively. Distilled water containing 10% vegetable soybean oil, 0.18% Tween 80, 0.02% Span 80, and 0.1% potassium sorbate was used as the pressure transmitting fluid. In preliminary experiments, the pressures and temperatures of soymilk, pressurization fluid, and vessel were recorded over the entire period using a Stansted fluid power FPG55000 RAP system and a Scan 1000 supervisory control and data acquisition system (Hexatec, Hexham, UK). Both the pressurization fluid and soymilk had the same temperature increase during treatment due to adiabatic heating. Therefore, for practical reasons the temperature was directly measured in the pressurization fluid. The quasi-adiabatic temperature increase upon compression (δ_s) was defined as the ratio of the change of temperature (ΔT, °C) under pressure divided by the pressure (MPa) × 100 (Patazca, Koutchma, & Balasubramaniam, 2007).

The soymilk previously heated at 95 °C was pressurized from 400 to 750 MPa for 10 min at 25 and 75 °C. Soymilk was pre-warmed to the initial temperature of treatment for 10 min in a water bath. The control was pre-warmed in the same conditions but not submitted to any pressure treatment. Immediately after pressure treatment, the samples were cooled to room temperature in an ice-water bath. Aliquots were taken from all soymilk samples and kept at 4 °C for further analyses, and the remaining portions were immediately frozen. The hydrated soybeans were treated at 100, 200, 300, 400, 500, 600, and 700 MPa for 10 min at 25 °C. The control was raw soymilk, i.e. soymilk prepared without thermal or pressure treatment. After soymilk preparation, aliquot of soymilk was immediately frozen. The experiments were conducted in duplicate.

2.5. Moisture, fat and crude protein determination

The moisture content of the samples was determined according to the AOCS (1995) method Ba 2a-38 with slight modifications. One gram of freeze-dried soymilk or 3 g of liquid soymilk were weighed into tared aluminum dishes and dried in a Precision Economy forced-air oven (Thermo Electron, Waltham, MA) for 3 h at 130 °C. Lipid content of freeze-dried soymilk was determined with AACC method 30-25. The freeze-dried soymilk was extracted with petroleum ether with a Goldfish extractor for 4 h. Crude protein

content was measured by the Dumas method using a Rapid NIII nitrogen analyzer (Elementar Americas, Mt. Laurel, NJ) as described by Jung et al. (2003). A factor of 6.25 was used to convert nitrogen to crude protein content.

2.6. Isoflavone content

Isoflavone content of control soymilks, pressurized soymilks and soymilks prepared from pressurized beans was determined from freeze-dried soymilk. Soybeans were ground in a coffee grinder and extracted and analyzed as described for freeze-dried soymilk. Freeze-dried soymilk was ground with pestle and mortar, and approximately 2 g were accurately weighed into a 125-mL screw-capped Erlenmeyer flask. After 10 mL of acetonitrile and 7 mL of Milli-Q system HPLC-grade water (Millipore, Bedford, MA) were added, the flask was capped and stirred for 2 h at room temperature in a rotary shaker (Innova Model 2050, New Brunswick Scientific, Edison, NJ) at 300 rpm. The mixture was vacuum-filtered (No. 42 filter paper, Whatman, Hillsboro, OR), and the filtrate was evaporated to dryness under vacuum at $\leqslant$30 °C. Dry matter was dissolved to a final volume of 10 mL with 80% methanol in water. The sample was filtered through a 0.45-μm polytetrafluoroethylene filter unit (Alltech, Deerfield, IL) and isoflavones were quantified by HPLC according to the method of Murphy et al. (1999). Total isoflavone contents were expressed as μmol/g of dry sample. The total isoflavone recovery (%) was the ratio of the total isoflavone amount (μmol, db) in soymilk divided by the total isoflavone amount in soybeans (μmol, db) multiplied by 100.

2.7. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed on defatted freeze-dried soymilks. Defatting procedure involved extracting 5 g of freeze-dried sample on a stir plate with 25 mL of hexane in the fume hood for 1 h. The mixture was filtered using 41-mesh paper filters and the procedure was repeated until the hexane fraction was clear. The residual hexane was allowed to evaporate overnight and the defatted samples were stored in a desiccator until analyzed. Calibration of the Exstar 6000 Seiko II calorimeter (Seiko Instruments, Torrance, CA) was performed with indium. The freeze-dried soymilk sample (0.1 g) was dissolved in 0.9 mg of 0.05 M Tris–HCl buffer, pH 7.0, and 13 mg of this solution was hermetically sealed in pre-weighed aluminum pans. A pan containing buffer was used as reference. Calorimetric measurements were carried out at a 10 °C/min scan rate from 25 to 110 °C. Enthalpy of thermal denaturation was estimated from the DSC curve. Triplicates were run for each sample. After DSC analysis, pans were punctured on top and samples were dried in a forced-air oven at 130 °C for 3 h to determine their moisture contents. The results were expressed as J/g of dry protein.

2.8. SDS PAGE

Freeze-dried soymilk samples were defatted as described in & 2.7 and prepared at 6 g/L in 0.5 M Tris–HCl, 30% urea, 20% glycerol, 2.5% bromophenol-blue, 2% SDS solution and 2% 2-mercaptoethanol, pH 6.8. Electrophoresis was performed as previously described (Lakshmanan et al., 2006) with a mini Protean III electrophoresis system using 4–20% linear gradient gel (Bio-Rad Laboratories, Hercules, CA).

2.9. Apparent viscosity

Rheological measurements of 6.3 mL of soymilk sample were performed using a Haake RS150 Rheometer (ThermoOrion, Karlsruhe, Germany) equipped with a DG41 sensor system. The system consisted of a cup-and-bell-shaped rotor. The inner and outer cylinders had an outer diameter of 36.0 and 43.4 mm, respectively. The shear rate was increased from 10 to 1500 s^{-1} within 7.5 min. Apparent viscosity was determined at 1500 s^{-1}. Samples were tested a minimum of three times.

2.10. Statistical analysis

Least significant differences (LSD) were calculated at a 5% level using the Statistical Analysis System Version 9.1 (SAS, Cary, NC) software package.

3. Results and discussion

3.1. Isoflavone content and profile in soybeans, raw soymilk and thermal processed soymilk

The total isoflavone concentration in soybean seeds was 4.81 μmol/g or 2130 μg aglucons/g, which was in the same range reported in previous studies (Hou & Chang, 2002; Murphy et al., 1999; Prabhakaran & Perera, 2006; Table 1). Acetyl-β-glucosides were only detected in trace amounts as expected since the acetylated isoflavones production requires dry thermal treatment. The aglucons, daidzein, glycitein and genistein, represented less than 2% of the mole mass, and were considered as negligible. The malonyl-β-glucosides and β-glucosides were the predominant forms, both representing 98.3% of the total concentration. Total daidzein mole content was the highest at 51% of the total isoflavones followed by genistein and glycitein with 41 and 7.5%, respectively. This was very similar to daidzein and genistein, expressed in μmol/g, of Prabhakaran and Perera (2006) and Murphy, Barua, and Hauck (2002).

In this study, several soymilks submitted or not to a thermal treatment were prepared. To study the effect of pressure on isoflavone content and profile in soymilk, the soymilk was submitted to a thermal treatment at 95 °C for 15 min prior to pressure treat-

Table 1
Isoflavone contents of soybeans, control soymilk and heat-treated soymilks (μmol/g, dry basis)

	Glucoside				Malonylglucoside				Aglucon				Total			
	Din	Gin	Glin	Total	MD	MG	MGl	Total	Dein	Gein	Glein	Total	TD	TG	TGl	TI
Soybeans	0.27[a]	0.26[a]	0.12	0.65[a]	2.12[a]	1.71[a]	0.25[a]	4.08[a]	0.05[a]	0.03[a]	ND	0.08[a]	2.44	2.01	0.36	4.81
Raw soymilk	0.29[a]	0.30[a]	0.12	0.71[a]	2.02[a]	1.70[a]	0.25[a]	3.97[a]	0.16[a]	0.12[a]	0.05	0.33[a]	2.47	2.12	0.42	5.01
95 °C Soymilk	0.50[b]	0.59[b]	0.14	1.23[b]	1.55[b]	1.34[b]	0.18[b]	3.07[b]	0.31[b]	0.26[b]	0.07	0.64[b]	2.27	2.20	0.39	4.86
95/75 °C Soymilk	0.51[b]	0.60[b]	0.14	1.25[b]	1.45[b]	1.29[b]	0.18[b]	2.92[b]	0.30[b]	0.26[b]	0.07	0.63[b]	2.36	2.19	0.39	4.94
LSD	0.08	0.06	NDif	0.16	0.39	0.34	0.05	0.80	0.14	0.10	NDif	0.24	NDif	NDif	NDif	NDif

Values in the same column with different letters were significantly different ($p \leqslant 0.05$). Abbreviations: Din: daidzin; Gin: genistin; Glin: glycitin; Dein: daidzein; Gein: genistein; Glein: glycitein; MD: malonyldaidzin; MG: malonylgenistin; MGl: malonylglycitin. TD: total daidzein; TG: total genistein; TGl: total glycitein; TI: total isoflavones. 95 °C Soymilk was heated at 95 °C for 15 min. 95/75 °C Soymilk was heated at 95 °C for 15 min and 75 °C for 10 min. NDif: Not different.

ment. This thermal treatment was chosen to totally inactivate β-glucosidases, known to convert glucosides to free aglucons (Matsuura, Obata, & Fukushima, 1989). No residual β-glucosidase activity, determined by the method of Matsuura and Obata (1993), was found on this thermal-treated soymilk. Effect of pressure on isoflavone of soymilk was investigated at an initial temperature of 25 and 75 °C. The samples were pre-heated at 25 or 75 °C for 10 min, and the controls were prepared in the same conditions but not submitted to HPP. When soymilk was prepared from pressurized soybeans, the control was prepared from soybeans not submitted to any thermal treatment and, therefore, corresponded to a raw soymilk.

The total and individual isoflavone content of raw soymilk and soymilks prepared with thermal treatments is presented in Table 1. For all the samples, the acetyl-β-glucosides concentration represented less than 2% mole mass, and considered negligible, therefore, these data are not shown. There was no statistical difference in the final isoflavone concentration, expressed in μmol/g (dry basis), between the soybeans and different soymilks. The changes in the isoflavone distribution depended on thermal treatment applied. The raw soymilk had the same isoflavones distribution as the soybeans. In soymilk treated at 95 °C for 15 min as compared to soybeans and raw soymilk, the distribution of the individual isoflavones was shifted towards the β-glucoside and aglucon forms at the expense of a decrease of 23% in the malonyl-β-glucoside content. There was an equi-mole conversion from malonylglucosides to β-glucosides and aglucons forms. Small but significant conversion of the β-glucosides to aglucons in 95 °C soymilk compared to raw soymilk can be attributed to the action of endogenous β-glucosidase. For the raw soymilk, aliquot was immediately frozen after grinding/filtration while for the 95 °C soymilk, the enzyme might have been active longer before its thermal inactivation, leading to the conversion of β-glucosides to corresponding aglucons. The additional heating step at 75 °C for 10 min did not further alter the distribution of the different isoflavone forms of this sample compared to the control at 95 °C ($p > 0.05$).

Soymilk was pressurized from 400 MPa, which was identified in our laboratory as the minimum pressure reducing the numbers of spoilage microorganisms in soymilk. Soybeans were treated to modify component extractability and therefore pressurization was applied from 100 MPa.

3.2. Isoflavone content and distribution in pressurized soymilk

There was no significant effect of pressure and initial temperature on the total isoflavone concentration of soymilk pressurized from 400 to 750 MPa at 25 and 75 °C ($p > 0.05$; Table 2). The total isoflavone concentration of pressurized soymilks was not statistically different from their 95 °C control soymilks. While the content of isoflavone was unchanged by the HPP treatment applied alone or combined with a mild thermal treatment, the profile of isoflavone was modified depending on the initial treatment temperature. At initial temperature of 25 °C, the distribution of the isoflavones in soymilk pressurized at 400 MPa was similar to the corresponding control soymilks (Table 1). A pressure increase up to 750 MPa did not affect the isoflavone profile. Samples pressurized at 500 and 600 MPa and 25 °C were not statistically different from the ones treated at 400 and 750 MPa (data not shown). At 75 °C, the three malonyl-β-glucosides and the β-glucosides decreased and increased, respectively, with increasing pressure while the aglucons and acetyl-β-glucosides remained constant ($p > 0.05$). These results suggest that pressure combined with mild temperature promoted the interconversion of the malonyl forms to β-glucosides. Daidzin and genistin forms showed greater interconversion than glycitin. When compared to the 400 MPa, 25 °C, the same pressure treatment at 75 °C converted ~0.30 μmol/g of malonyldaidzin and malonylgenistin while only 0.04 μmol/g of malonylglycitin was converted. At the highest pressure and initial temperature of 75 °C, β-glucosides daidzin and genistin increased by 0.63–0.9 μmol/g from the malonyl esters.

During high-pressure processing, soymilk samples were subjected to a temperature higher than the initial temperature due to adiabatic heating. This increase of temperature was the result of compression heating and depends on the nature of the product, the initial product temperature, and the pressure level (de Heij et al., 2003). When samples were pressurized at 25 °C, the average quasi-adiabatic temperature increase upon compression δ_s was 1.0 °C/100 MPa. The δ_s calculated at each pressure was not affected by pressure level. When initial temperature was 75 °C, the average δ_s was increased to 2.0 °C/100 MPa. The δ_s value calculated at each pressure increased as pressure increased. Similar trends were reported for foods with high water content by Patazca et al. (2007). Therefore, when HPP was applied at an initial temperature of 25 °C, the temperature of soymilk was approximately 30–35 °C for 400–750 MPa treatment, which apparently did not affect isoflavone distribution. When HPP was conducted at 75 °C, the temperature of soymilk ranged from 85 to 90 °C for pressures from 400 MPa to 750 MPa. When profile obtained after 400 MPa treatment at 25 °C was compared to the same soymilk treated at 75 °C, an average of 0.6 μmol/g of total glucoside and total malonylglucoside inter-converted. At the highest pressure, 1.7 μmol/g of total glucoside and malonylglucosides inter-converted. These

Table 2
Isoflavone contents of soymilk processed by high-pressure at 25 and 75 °C (μmol/g, dry basis)

Pressure (MPa)	Glucoside				Malonylglucoside				Aglucon				Total			
	Din	Gin	Glin	Total	MD	MG	MGl	Total	Dein	Gein	Glein	Total	TD	TG	TGl	TI
25 °C[A]																
400	0.50	0.58	0.14	1.22	1.55	1.36	0.18	3.09	0.31	0.26	0.07	0.64	2.39	2.27	0.39	5.06
750	0.55	0.64	0.15	1.34	1.52	1.34	0.18	3.04	0.31	0.26	0.07	0.64	2.43	2.31	0.40	5.14
LSD	NDif	NDif	NDif	NDif	NDif	NDif	NDif	NDif	NDif	NDif	NDif	NDif	NDif	NDif	NDif	NDif
75 °C[B]																
400	0.75[a]	0.88[a]	0.18[a]	1.81[a]	1.18[a]	1.06[a]	0.15[a]	2.39[a]	0.30	0.28	0.07	0.65	2.25	2.28	0.40	4.93
500	0.87[b]	1.03[b]	0.20[ab]	2.10[b]	1.03[b]	0.94[b]	0.13[b]	2.10[b]	0.30	0.25	0.07	0.62	2.23	2.29	0.40	4.92
600	1.04[c]	1.22[c]	0.22[bc]	2.48[c]	0.92[c]	0.85[c]	0.12[c]	1.89[c]	0.31	0.26	0.07	0.64	2.29	2.40	0.41	5.11
700	1.17[d]	1.38[d]	0.24[c]	2.79[d]	0.77[d]	0.71[d]	0.11[d]	1.59[d]	0.31	0.26	0.07	0.64	2.26	2.41	0.42	5.09
750	1.21[d]	1.45[d]	0.24[c]	2.90[d]	0.58[e]	0.55[e]	0.08[e]	1.21[e]	0.30	0.25	0.07	0.62	2.10	2.31	0.39	4.80
LSD	0.09	0.11	0.02	0.21	0.08	0.05	0.01	0.12	NDif	NDif	NDif	NDif	NDif	NDif	NDif	NDif

Values in the same column with different letters were significantly different ($p \leqslant 0.05$). Abbreviations: Din: daidzin; Gin: genistin; Glin: glycitin; Dein: daidzein; Gein: genistein; Glein: glycitein; MD: malonyl-daidzin; MG: malonylgenistin; MGl: malonylglycitin; TD: total daidzein; TG: total genistein; TGl: total glycitein; TI: total isoflavones.
[A] Samples pressurized at 500 and 600 MPa were not statistically different from the ones treated at 400 and 750 MPa, so the data are not shown. NDif: Not different.
[A,B] Soymilks control corresponded to the 95 °C and 95/75 °C treated soymilk (Table 1).

results suggested that high-pressure promoted conversion of malonyl- to β-glucoside isoflavones due to adiabatic heating. More research is needed to precisely calculate the conversion rate constants under high-pressure combined with mild thermal treatment.

3.3. Isoflavone content and distribution in soymilk prepared from pressurized soybeans

The total isoflavone mole recovery in control soymilk was 79% of the 120 μmol originally present in the soybeans. This result suggested some isoflavone loss in the okara, the insoluble fraction, as only small amounts (~1%) are expected to be lost in the soaking water (Jackson et al., 2002; Wang & Murphy, 1996). The total isoflavone recovery was not modified when the beans were pressurized from 100 to 700 MPa prior to soymilk production (data not shown). Pressure level applied to hydrated soybeans has a significant effect on the total isoflavone concentration (μmol/g, db) in soymilk (Table 3). The isoflavone content and profile of the soymilk prepared from the 100 MPa processed soybeans was not significantly different from the control raw soymilk (Tables 1 and 3). The lowest concentration of total isoflavones and total glucoside in soymilk were observed after pressurization of the soybeans at 300 MPa while the highest were obtained for soymilk prepared with 700 MPa processed soybeans. Treatment at 300 MPa decreased the content of isoflavones in the total daidzein, genistein and glycitein by 0.55 μmol/g, 0.30 μmol/g and 0.10 μmol/g, respectively. This decrease can mainly be explained because of significant changes in malonyl-β-glucoside contents. The total aglucon content was stable for pressure up to 400 MPa but progressively decreased from 500 MPa to half at 700 MPa. The three aglucons, daidzein, genistein and glycitein, contributed partially to this decrease. Soymilk prepared from 700 MPa processed soybeans contained only 55% of daidzein and genistein compared to the control with no glycitein detected at this pressure treatment.

The soymilks treated up to 750 MPa at a temperature of 25 °C had similar isoflavone content and distribution than the control as reported above. Therefore, if the assumption that pressure did not modify either isoflavone content or profile in soybeans pressurized at 25 °C is made, the isoflavone profile and content in soymilk obtained from pressurized soybeans could be explained by changes in their water-extractability during soymilk preparation. The increase of extraction yield could be due to an increase of mass transfer by enhancement of solvent penetration into the solid material and release of intracellular product due to disruption of cell walls (Houqin, Ruizhan, & Chanzeng, 2007). There was no apparent alteration in shape, size, or color between pressurized and non-pressurized soybean seeds. This visual observation agrees with Omi et al. (1996) which was carried out on soybeans pressurized up to 700 MPa at 25 °C for 25 min. However, Omi et al. (1996) reported 0.2–0.5% of soy proteins release in the soaked-pressurized water and related it to changes in cotyledon surface and epidermal cells of the soybeans as observed by scanning microscopy. In addition, small changes in the aglucon profile of the soymilk obtained from pressurized beans might be explained on the basis of residual endogenous β-glucosidase activity. We found that β-glucosidase activity in soymilk is stable up to 400 MPa and gradually decrease when pressure is further increased to reach 60% inactivation after 700 MPa treatment (results not shown). Similar decrease of activity of β-glucosidase from strawberry was reported for treatment higher than 500 MPa (Zabetakis, Leclerc, & Kadja, 2000). The decrease in β-glucosidase activity starting at 500 MPa coincided with the decrease in the aglucons content. Observed isoflavone profile changes might also be the result of modification of the isoflavone/protein interactions. Isoflavone profile of soymilk from pressurized hydrated soybeans therefore probably resulted in combined pressure-induced changes in enzymatic, cell structure, protein of the beans and additional investigation will be needed to determine the involvement of the soybean structural modification.

3.4. Characterization of soymilk prepared from pressurized soybeans

The weight of soymilk and its solids, fat, crude protein content, and apparent viscosity varied depending on the pressure level submitted to the hydrated soybeans (Table 4). The yield of soymilk solid decreased from 74.8% to 66.4% for the control and the soymilk obtained from 700 MPa-treated beans, respectively. The protein content varied from 51.7% to 47.7% (db) for the same samples. This decrease represented a total variation of 7.7% in the protein content. Fat content increased with increasing pressure level. Soymilks prepared from 400–700 MPa-treated beans had a 2-fold higher fat content than the control. The storage protein profile of the soymilks was similar to the control regardless of the pressure level applied to the beans (Fig. 1). Thermal properties of soymilks were determined. The control soymilk exhibited two thermal transitions at 76 and 95 °C, which were attributed to β-conglycinin and glycinin, respectively. The enthalpy of glycinin and β-conglycinin from soymilk obtained from pressurized soybeans are summarized in Table 5. Enthalpy of denaturation of glycinin and β-conglycinin of soymilk prepared from control soybeans were 8.24 and 1.51 mJ/mg, respectively, which agrees with results of L'hocine, Boye, and Arcand (2006). When HPP was applied to the soybeans, the native state of the extracted glycinin stayed similar for treatment up to 300 MPa and decreased significantly at 400 MPa while having denaturation enthalpy lower than 0.4 mJ/mg for 600 and

Table 3
Isoflavone contents of soymilk prepared from pressurized beans (μmol/g, dry basis)

Pressure (MPa)[A]	Glucoside				Malonylglucoside				Aglucon				Total			
	Din	Gin	Glin	Total	MD	MG	MGl	Total	Dein	Gein	Glein	Total	TD	TG	TGl	TI
100	0.29^{bc}	0.30^{ab}	0.12^{a}	0.72^{bc}	2.12^{bc}	1.80^{ab}	0.26^{c}	4.17^{bc}	0.15^{b}	0.11^{a}	0.05^{a}	0.31^{a}	2.56^{a}	2.21^{ab}	0.43^{a}	5.20^{bc}
200	0.27^{c}	0.28^{a}	0.12^{a}	0.67^{c}	1.90^{d}	1.65^{b}	0.24^{d}	3.78^{cd}	0.15^{b}	0.11^{a}	0.05^{a}	0.31^{a}	2.32^{b}	2.04^{a}	0.40^{b}	4.76^{cd}
300	0.26^{c}	0.30^{ab}	0.10^{b}	0.66^{c}	1.61^{e}	1.51^{b}	0.19^{e}	3.32^{d}	0.14^{bc}	0.11^{a}	0.04^{ab}	0.30^{a}	2.01^{c}	1.92^{a}	0.34^{c}	4.27^{d}
400	0.36^{ab}	0.36^{abc}	0.14^{c}	0.86^{ab}	2.37^{a}	1.90^{ab}	0.28^{b}	4.55^{ab}	0.16^{ab}	0.11^{a}	0.04^{ab}	0.31^{a}	2.89^{d}	2.37^{ab}	0.46^{d}	5.72^{ab}
500	0.37^{ab}	0.37^{bc}	0.15^{c}	0.89^{ab}	2.26^{ab}	1.82^{ab}	0.31^{a}	4.38^{ab}	0.12^{cd}	0.08^{b}	0.03^{ab}	0.23^{b}	2.75^{ad}	2.26^{ab}	0.49^{e}	5.51^{ab}
600	0.33^{abc}	0.33^{ab}	0.15^{c}	0.81^{bc}	2.28^{ab}	1.82^{ab}	0.30^{a}	4.41^{ab}	0.10^{de}	0.07^{bc}	0.02^{bc}	0.19^{bc}	2.72^{ad}	2.22^{ab}	0.47^{de}	5.41^{ab}
700	0.41^{a}	0.42^{c}	0.17^{d}	1.00^{a}	2.23^{ab}	2.28^{a}	0.31^{a}	4.82^{a}	0.09^{e}	0.06^{c}	0.00^{c}	0.16^{c}	2.74^{ad}	2.76^{b}	0.48^{de}	5.98^{a}
LSD	0.08	0.08	0.02	0.17	0.16	0.54	0.01	0.50	0.03	0.02	0.02	0.06	0.20	0.59	0.03	0.60

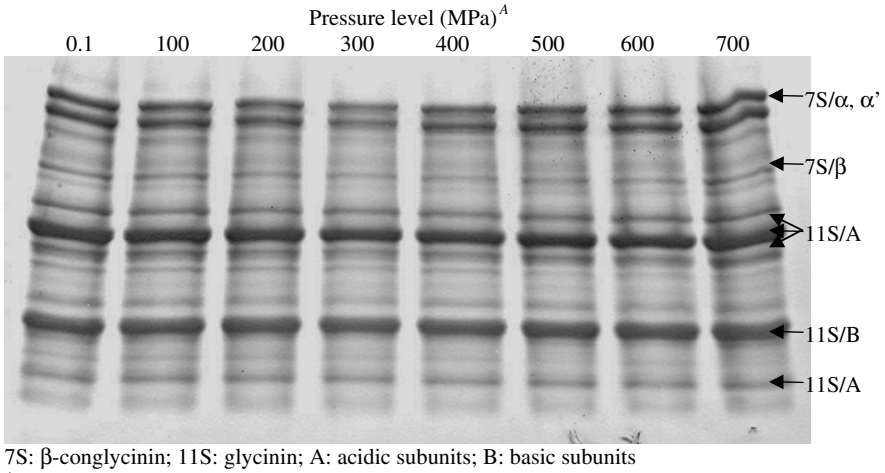
Values in the same column with different letters were significantly different ($p \leqslant 0.05$). Abbreviations: Din: daidzin; Gin: genistin; Glin: glycitin; Dein: daidzein; Gein: genistein; Glein: glycitein; MD: malonyl-daidzin; MG: malonylgenistin; MGl: malonylglycitin; TD: total daidzein; TG: total genistein; TGl: total glycitein; TI: total isoflavones. Soymilk control corresponded to raw soymilk (Table 1).

[A] Pressure level applied to the hydrated beans before soymilk preparation.

Table 4
Weight, proximate analysis and apparent viscosity of soymilk prepared from pressurized soybeans

Pressure (MPa)[A]	Soymilk weight[B] (g)	Solid content (%)	Crude protein (%, db)	Fat content (%, db)	Apparent viscosity
0.1	270[a]	6.96[a]	51.76[a]	11.10[a]	1.47[a]
100	263[ab]	6.93[a]	51.20[a]	13.75[ab]	1.47[a]
200	270[a]	6.95[a]	51.26[a]	14.36[b]	1.55[ae]
300	264[ab]	7.05[a]	51.47[a]	19.33[c]	1.85[c]
400	257[bc]	6.98[a]	49.53[b]	21.81[cd]	1.97[d]
500	252[c]	6.85[a]	48.91[c]	23.04[d]	1.96[d]
600	250[c]	6.57[b]	48.14[d]	22.93[d]	1.67[b]
700	254[bc]	6.58[b]	47.70[d]	23.98[d]	1.52[e]
LSD	10	0.24	0.59	3.21	0.06

Values in the same column with different letters were significantly different ($p < 0.05$).
[A] Pressure level applied to the beans before soymilk preparation.
[B] Soymilk was prepared from 28 g of hydrated processed beans with a soybean-to-water ratio of 1:10.



7S: β-conglycinin; 11S: glycinin; A: acidic subunits; B: basic subunits
[A] Pressure level applied to the hydrated beans before soymilk preparation.

Fig. 1. SDS PAGE profile of soymilk obtained from pressurized soybeans.

Table 5
Enthalpy of denaturation of glycinin and β-conglycinin of soymilk prepared from pressurized beans

Pressure (MPa)[A]	Denaturation enthalpy (mJ/mg of protein)	
	Glycinin	β-Conglycinin
0.1	8.24[a]	1.51[a]
100	8.45[a]	1.28[b]
200	8.25[a]	1.35[b]
300	7.68[b]	<0.4[c]
400	4.70[c]	<0.4[c]
500	0.85[d]	<0.4[c]
600	<0.4[e]	<0.4[c]
700	<0.4[e]	<0.4[c]
LSD	0.18	0.20

Values in the same column with different letters were significantly different ($p < 0.05$). <0.4: denaturation enthalpy was lower than 0.4 mJ/mg of proteins.
[A] Pressure level applied to the beans before soymilk preparation.

700 MPa treatment. β-Conglycinin enthalpy decreased by 15% after 200–300 MPa treatment of the beans while for higher pressures, values were lower than 0.4 mJ/mg. These results suggested unfolding and aggregation of extracted glycinin and β-conglycinin depending on pressure level (Molina, Papadopolou, & Ledward, 2001; Puppo et al., 2004).

Apparent viscosity of control soymilk was 1.5 mPa s, which was in the same range as the ones reported in the study of Lakshmanan et al. (2006). After pressure treatment, soymilk apparent viscosity displayed a bell-shaped curve with highest value obtained after treatment of the beans at 400 and 500 MPa.

Our results have shown that there were no apparent differences with pressure level in extraction rate of individual glycinin and β-conglycinin from pressurized soybeans as peptide profile remained unchanged. The solids and fat content, protein content, and native state of glycinin and β-conglycinin were affected by the HPP pre-treatment. These parameters are known to impact viscosity of soy products (Cheng, Shimizu, & Kimura, 2005; Forster & Ferrier, 1979; Liu, Chang, Li, & Tatsumi, 2004; Toda, Chiba, & Ono, 2007; Wagner, Sorgentini, & Anon, 1992) and variations in these parameters probably contributed to the small but significant change observed in the apparent viscosity of soymilk prepared from pressurized soybeans, yet the mechanism responsible for the observed changes in viscosity is not clear.

4. Conclusions

This is the first study reporting the effect of high-pressure processing on the stability, conversion and water-extractability of soy isoflavones. The pressure alone did not affect the isoflavone content of pressurized soymilk, and the isoflavone profile may be significantly modified due to the adiabatic heating occurring during pressurization combined with mild temperature treatment. The results presented here show that high-pressure processing applied as a pre-treatment of the hydrated beans in soymilk production does not increase the water-extractability of isoflavone during soymilk production and that overall only small changes in isoflavone profile and content occur in these conditions.

Acknowledgements

This journal paper of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA, Project No. 6599, was supported by National Research Initiative Grant No. # 2005-355-0316129 from the USDA Cooperative State Research, Education, and Extension service improving food quality and value (71.1), the Hatch Act, and State of Iowa funds.

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