Effect of ultrasound assisted extraction upon the Genistin and Daidzin contents of resultant soymilk

Ronak Fahmi · Faramarz Khodaiyan ·
Rezvan Pourahmad · Zahra Emam-Djomeh

Revised: 19 April 2012 / Accepted: 17 May 2012 / Published online: 9 June 2012
© Association of Food Scientists & Technologists (India) 2012

Abstract The purpose of this study was to determine the effect of ultrasound treatment on the contents of daidzin, genistin, and their respective aglycones, daidzein and genistein, in resultant soymilk. Soybean slurry was exposed to ultrasound treatment, filtered, and placed in an ultrasound cleaning bath set with different frequencies (35 and 130 KHz), treatment temperatures (20 and 40 °C), and times (20, 40, and 60 min). Concentrations for these isoflavones were determined using reverse-phase high-performance liquid chromatography. Results indicated that both frequencies significantly ($p < 0.05$) increased isoflavone content (IC), glycosides, and aglycones in extracted soymilk. These results were attributed to induced cavitation, which increases the permeability of plant tissues. However, the frequency of 35 kHz caused a noticeably higher increase in IC than 130 kHz. Results also revealed significant increases in IC with increased sonication time (from 20 to 60 min) and with increased temperature (from 20 to 40 °C).

Keywords Soymilk · Ultrasound · Isoflavones · Daidzin · Genistin · Daidzein · Genistein

Introduction

Recently, soy foods have received much attention for their nutritional and potential health benefits. Among these, soymilk has gained much popularity as a healthful food drink. Soymilk is a traditional oriental food and an aqueous extract of soybeans. It is thought to have been invented in China around 179 to 122 B.C. (Min et al. 2005). The functional properties of soymilk have been well associated with those of its specific bioactive components known as isoflavones. Isoflavones are non-steroidal phytoestrogenic and antioxidative polyphenolic molecules with the potential to protect against hormone-dependent diseases, such as breast cancer, prostate cancer, menopausal symptoms, cardiovascular disease, and osteoporosis (Lee et al. 2005; Rekha and Vijayalakshmi 2010; Villares et al. 2009). Twelve isoflavones have been reported in soybeans: three aglycones (daidzein, genistein, and glycitein) and their respective glucosides and acetyl-, malonyl-, and β -glucosides. However, the major isoflavones are genistin, daidzin, and their corresponding aglycones (genistein and daidzein) (Wang and Murphy 1994).

Ultrasound technology is growing rapidly in the field of research and development of non-thermal food-processing methods. The beneficial use of sound is realized through its chemical, mechanical, or physical effects on the process or product (Suslick 1988). Ultrasound is defined as mechanical waves with a frequency above the threshold of human hearing (16 to 20 kHz). It can be divided into two frequency ranges. High-frequency ultrasound (low-power ultrasound) uses frequencies in the 5–10 MHz range with low intensity levels, typically less than 1 W cm^{-2} . It is mainly used in diagnostic analysis of food materials. Low-frequency

R. Fahmi · R. Pourahmad
Department of Food Science and Technology, Faculty of
Agriculture, Varamin-Pishva branch, Islamic Azad University,
Varamin, Iran

F. Khodaiyan (✉) · Z. Emam-Djomeh
Department of Food Science and Engineering, Faculty of
Agricultural Engineering and Technology, University of Tehran,
Tehran, Iran
e-mail: khodaiyan@ut.ac.ir

ultrasound (high-power ultrasound) uses frequencies in the 20–100 KHz range with much high intensity levels, usually in the range of 10–1000 Wcm⁻² (Barbosa-Cánovas et al. 2005; Jambrak et al. 2009; Mason 1998). Some known applications of high-power ultrasound in the food industry include extraction (release of plant material), inactivation of microorganisms, enzyme inhibition, homogenization, emulsification, filtration, crystallization, and deforming (Vilkhu et al. 2008).

Generally, high-power ultrasound can improve the extraction of intracellular compounds from plant material. It is supposed that this positive effect of ultrasound in the liquid/solid extraction is mainly caused by a phenomenon known as cavitation (Mason and Cordmas 1996; Vinatoru 2001). There have been several reports on the application of ultrasonic treatment in extracting bioactive components from plant materials. Rostagno et al. (2003) found that the efficiency of isoflavone extraction from soybeans was improved by ultrasound treatment. A study by Wang et al. (2008) reported that ultrasonic treatment significantly increased the extraction yields of phenolic compounds from wheat bran.

The purpose of this study is to examine the effect of two ultrasound frequencies (35 and 130 KHz) on the contents of daidzin, genistin, and their respective aglycones, daidzein and genistein, in resultant soymilk.

Materials and methods

Materials

Soybeans (Clark variety) were obtained from local growers and stored at room temperature under dark conditions until they were processed for soymilk. Authentic isoflavone standards of glucosides (genistin, daidzin) and aglycones (genistein, daidzein) were obtained from Sigma–Aldrich (Germany). Spectranalyzed-grade dimethyl sulfoxide (DMSO) and HPLC-grade methanol were obtained from Merck (Germany). All other chemicals used were of analytical grade.

Preparation of soymilk

Soymilk was prepared according to the procedure of Min et al. (2005). Twenty grams of soybeans were washed and soaked in distilled water for 16 h at room temperature. Hydrated beans were drained, rinsed, and ground with boiling water (ratio of soybeans to water was 1:10 on a weight basis) using a Waring blender for 3 min at high speed. Then, produced slurry was filtered through four layers of cheesecloth to separate the soymilk from the residue.

Ultrasound treatment with 35 and 130 KHz ultrasound cleaning baths

Ultrasound treatment was carried out in an ultrasonic cleaning bath (Elma, Model: D-78224 Singen/Htw, Germany; overall dimensions: 340×300×370 mm; internal dimensions: 240×130×150 mm) with temperature and time control. The bath was operated at frequencies of 35 KHz and 130 KHz with maximum input power of 100 W to investigate the effect of ultrasound treatment on tested parameters. One hundred mL of produced soybean slurry was placed in an 250 ml Erlenmeyer conical flask, which was then immersed into the ultrasound bath. Samples were treated for 20, 40, and 60 min at 20 and 40 °C. An ultrasonic transducer was attached to the bottom of the outer surface of the liquid container and the liquid was irradiated with ultrasonic waves from the surface of the liquid container. The suspension in the conical flask was kept at the level of the water in the bath, which was circulated and regulated at constant temperatures to avoid water-temperature increase from ultrasonic exposure. Frequency sweeping is often used to produce a more uniform cavitation field and reduce standing wave zones. After extraction, the slurry was filtered through four layers of cheesecloth to separate the soymilk.

Extraction of isoflavones

The extraction of isoflavones from freeze-dried soymilk samples (-70 °C) was performed in duplicate as described by Barnes et al. (1994) with some modifications. Briefly, 0.5 g of freeze-dried samples was weighed into 15 mL centrifuge tubes, 5 mL of methanol (80 %) was added to each tube, and the tubes were tightly screw-capped. The samples were shaken and heated in a water bath at 60 °C for 2 h. The tubes were centrifuged at 4 °C at 12,000×g for 10 min. An aliquot of the supernatant was filtered through a membrane filter (0.20 μm) (Macherey-Nagel, Germany) and transferred to HPLC vials.

HPLC analysis of isoflavones

Isoflavone analysis was performed based on a method described by Chun et al. (2008). Analyses were carried out using a reverse-phase HPLC system (Knauer, Germany) consisting of an auto-sampler, HPLC pump k-1001, ultraviolet (UV) visible detector K-2600 and reversed-phase C18 column Nucleosil 100 (125 mm×4.0 mm×5.0 μm). The mobile phase consisted of solvents A (0.1 % glacial acetic acid in water) and B (0.1 % glacial acetic acid in methanol). The gradient for solvents A and B was as follows: 85 % A (15 % B) at 0 min, decreasing to 50 % A for 42 min, steady at 50 % A for 3 min, and then increasing to 85 % A for 5 min. A column was equilibrated for 10 min with 85 % A

prior to the next injection. The flow rate was maintained at 0.8 mL/min and the injection volumes of isoflavone standards and samples were set at 20 μ L throughout the run time of 60 min. The UV-visible detector was set at a wavelength of 254 nm to detect glucosides (daidzin and genistin) and aglycones (daidzein and genistein). Single standards were prepared for peak identification. For this aim, standards of isoflavone glucosides and aglycones were dissolved in DMSO and diluted with 80 % methanol. The isoflavones were identified by comparing spectral data and retention times of sample peaks with those of standard references. For each isoflavone, quantification was performed by electronic integration of the chromatographic peak. Isoflavone concentrations were calculated as mg isoflavones/100 mL soymilk.

Statistical analysis

All the experiments were conducted in triplicate independent batch of soy slurry. The mean comparison was carried out with Duncan's multiple range tests using SPSS for Windows version 18.0 (2010). Significant levels were defined using the value $P < 0.05$.

Results and discussion

Figure 1 shows the HPLC trace of the extracted isoflavones from treated soymilk sample. By comparison with standard daidzin, genistin, daidzein, and genistein, these isoflavones were detected at retention times of 6.5, 11.5, 20.7, and 30.8 min, respectively. Other peaks in the chromatogram could be due to the other forms of soy isoflavones, which

Fig. 1 HPLC Chromatogram of isoflavones in an ultra-sonicated soymilk sample

could not be identified in this study owing to the lack of standards.

According to the results, ultrasonic pre-treatment of soybean slurry significantly increased the IC of the extracted soymilks. Ultrasonic enhancement was mainly attributed to the mechanical and chemical effects of cavitation phenomena: shear forces generated by the collapse of cavitation bubbles, as well shock waves, break the biological cell walls and facilitate the release of cell content into the extraction medium. Cell disruption dramatically increases contact areas, leading to a better mass transfer of intracellular isoflavones into the extracted soymilk (Toma et al. 2001; Vinatoru 2001). Moreover, because of isoflavones' polyphenolic nature, they are thought to be associated with the interior moiety of the native form of globular soy protein. These protein-isoflavone interactions may complicate isoflavone extractability (Nufer et al. 2009). Cavitation – specifically, the localized high temperatures generated by the collapse of bubbles – may lead to an easier denaturation and unfolding of the protein. Therefore, a greater proportion of the isoflavone content was released into the sonicated soymilk samples.

Many parameters can influence the effectiveness of ultrasound. The frequency of the ultrasound waves is one of the most important. Figure 2A shows the influence of different ultrasound frequency levels (35 and 130 kHz) on the isoflavone content of soymilk samples. Results illustrated that both frequencies significantly ($P < 0.05$) increased IC, extracting greater amounts of both glucosides and aglycones. However, the lower frequency (35 kHz, higher intensity) produced a noticeably higher increase in the IC, glucosides, and aglycones compared to the higher frequency (130 kHz, lower intensity). The difference is caused by the fact that ultrasound frequency is inversely proportional to the bubble size: lower-frequency ultrasound generates larger cavitation bubbles, in turn resulting in higher temperatures and pressures in the cavitation zone. As the frequency increases, bubble forming becomes more difficult and the cavitation zone becomes less violent (Patist and Bates 2008). Furthermore, higher-frequency ultrasound waves disperse more easily within the solution, reducing the overall intensity of delivered energy (Brennan 2006). The data also showed that the ratio of aglycones to glucosides increased (nearly by 23 %) with the decrease in ultrasound frequency. Because aglycones (genistein and daidzein) are absorbed faster and in greater amounts than their glucosides (genistin and daidzin) in humans, aglycone-rich products may be more effective than glucoside-rich products in preventing chronic diseases such as coronary heart disease and cancer (Izumi et al. 2000; Kano et al. 2006).

Figure 2B shows the influence of the two sonication temperatures (20 and 40 °C) on the isoflavone levels of soymilk samples. The extraction efficiency for IC,

Fig. 2 Effect of ultrasonic frequency (A), temperature (B) and time (C) on the isoflavone content of soymilk. Mean values with different letters are significantly different at $P < 0.05$ ($n=3$). IC: summation of daidzin, genistin, daidzein and genistein

glucosides, and aglycones increased significantly when the temperature was raised from 20 to 40 °C. The higher yields at 40 °C may be attributed to the increase in the number of cavitation bubbles formed and to enhanced mass-transfer rates (Rostagno et al. 2003). In addition, the increased extraction of isoflavones into the treated soymilk could be due to the higher solubility of isoflavones at 40 °C than at 20 °C (Prabhakaran and Perera 2006). Results also showed that the ratio of aglycones to glucosides increased by nearly 19 % with the increase in sonication temperature. This may be due to the high activity of β -glucosidase (glucan endo-1,6- β -glucosidase) at elevated temperatures. According to Matsuura et al. (1995), β -glucosidase possessed the highest activity at 45 °C, and hydrolysis of glucosides achieved its maximum extraction of aglycones at this temperature. Nevertheless, the current study used a maximum sonication temperature of 40 °C, mainly because the nutrients in soymilk would deteriorate at higher temperatures.

Figure 2C shows the influence of sonication time on the isoflavone levels of soymilk samples. Results showed that IC, glucosides, and aglycones from treated soymilk samples increased significantly ($p < 0.05$) with the increase in treatment time from 20 to 60 min which revealed a direct relationship between sonication time and the efficiency of extraction.

Conclusions

The preliminary results of this study showed that ultrasonic pretreatment of soybean slurry significantly improved the isoflavone levels of the resulting soymilk. However, further research work, from both an industrial and an academic viewpoint, is needed in order to expand the findings for food-extraction and processing applications. A wider range of ultrasound frequencies, ultrasound power, treatment temperature, treatment time, and other parameters should be investigated.

Acknowledgments This study was supported by Tehran University. We gratefully acknowledge the Faculty of Food Science and Technology of Tehran University for providing access to equipments.

References

- Barbosa-Canovas GV, Tapia MS, Cano MP (2005) Novel food processing technology. CRC Press, New York
- Barnes S, Kirk M, Coward L (1994) Isoflavones and their conjugates in soy foods: extraction conditions and analysis by HPLC–mass spectrometry. *J Agric Food Chem* 42:2466–2474
- Brennan JG (2006) Food processing handbook. WILEY-VCH Verlag GmbH & Co, KGaA, Weinheim
- Chun J, Kim JS, Kim JH (2008) Enrichment of isoflavone aglycones in soymilk by fermentation with single and mixed cultures of

- Streptococcus infantarius 12 and Weissella sp. 4. Food Chem 109:278–284
- Izumi T, Piskula MK, Osawa S, Obata A, Tobe K, Saito M, Kataoka S, Kubota Y, Kikuchi M (2000) Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. J Nutr 130:1659–1699
- Jambrak AR, Lelas V, Mason TJ, Krešić G, Badanjak M (2009) Physical properties of ultrasound treated soy proteins. J Food Eng 93:386–393
- Kano M, Takayanagi T, Harada K, Sawada S, Ishikawa F (2006) Bioavailability of Isoflavones after Ingestion of Soy Beverages in Healthy Adults. JN 136:2291–2296
- Lee CH, Yang L, Xu JZ, Venus Yeung SY, Huang Y, Chen ZY (2005) Relative antioxidant activity of soybean isoflavones and their glycosides. Food Chem 90:735–741
- Mason TJ (1998) In: Povey MJW, Mason TJ (eds) Power ultrasound in food processing—the way forward. London, Thomson science
- Mason TJ, Cordmas E (1996) Ultrasonic intensification of chemical processing and related operations – a review. T I Chem Eng-Lond 74:511–516
- Matsuura M, Sasaki J, Murao S (1995) Studies on β -glucosidases from soybeans that hydrolyze daidzin and genistin: isolation and characterization of an isozyme. Biosci Biotech Biochem 59(9):1623–1627
- Min S, Yu Y, Martin ST (2005) Effect of soybean varieties and growing locations on the physical and chemical properties of soymilk and tofu. J Food Sci 70(1):8–12
- Nufer KR, Ismail B, Hayes KD (2009) The effects of processing and extraction conditions on content, profile, and stability of isoflavones in a soymilk system. J Agric Food Chem 57:1213–1218
- Patist A, Bates D (2008) Ultrasonic innovations in the food industry: from the laboratory to commercial production. Innov Food Sci Emerg 9:147–154
- Prabhakaran MP, Perera CO (2006) Effect of extraction methods and UHT treatment conditions on the level of isoflavones during soymilk manufacture. Food Chem 99:231–237
- Rekha CR, Vijayalakshmi G (2010) Influence of natural coagulants on isoflavones and antioxidant activity of tofu. J Food Sci Technol 47(4):387–393
- Rostagno MA, Palma M, Barroso CG (2003) Ultrasound-assisted extraction of soy isoflavones. J Chromatogr A 1012:119–128
- Suslick KS (1988) Ultrasound: its chemical, physical, and biological effects. VCH Publishers, New York
- Toma M, Vinatoru M, Paniwnyk L, Mason TJ (2001) Investigation of the effects of ultrasound on vegetal tissues during solvent extraction. Ultrason Sonochem 8(2):137–42
- Vilkhu K, Mawson R, Simons L, Bates D (2008) Applications and opportunities for ultrasound assisted extraction in the food industry -A review. Innov Food Sci Emerg 9:161–169
- Villares A, Rostagno MA, Garcia-Lafuente A, Guillamon E, Martinez JA (2009) Content and profile of isoflavones in soy-based foods as a function of the production process. Food Bioprocess Tech 4:27–38
- Vinatoru M (2001) An overview of the ultrasonically assisted extraction of bioactive principles from herbs. Ultrason Sonochem 8(3):303–313
- Wang H, Murphy PA (1994) Isoflavone content in commercial soybean foods. J Agric Food Chem 42:1666–1673
- Wang J, Sun BG, Cao YP, Tian Y, Li XH (2008) Optimisation of ultrasound assisted extraction of phenolic compounds from wheat bran. Food Chem 106(2):804–10