Food Research International 77 (2015) 704–710

Contents lists available at ScienceDirect

Food Research International

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

The ultrasound-treated soybean seeds improve edibility and nutritional quality of soybean sprouts

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ARTICLE INFO

Article history:
Received 11 November 2014
Received in revised form 11 January 2015
Accepted 11 January 2015
Available online 17 January 2015

Chemical compounds studied in this article:
Gamma-aminobutyric acid (PubChem CID: 119)
Isoflavone (PubChem CID: 72304)
Daidzin (PubChem CID: 107971)
Genistein (PubChem CID: 5280961)
Daidzein (PubChem CID: 5281708)
Genstin (PubChem CID: 5281377)

Keywords:
Ultrasound
Edibility properties
Nutritional quality
Soybean sprouts

ABSTRACT

Limited data are available concerning the physical and nutrient properties of soybean sprouts from the ultrasound-treated seeds. In this study, soybean seeds were treated by ultrasound at different power levels (0 W to 300 W), then germinated for 5 d in darkness. Morphological changes, protein patterns, amino acid contents, gamma-aminobutyric acid (GABA) content, IgE-binding, lipoxygenase isozyme activity, trypsin inhibitor, and isoflavone content of soybean sprouts were quantified. Results showed that ultrasound treatment increased germination rate, sprout length, and GABA content of soybean sprouts. No significant change was observed in protein patterns. The IgE-binding, lipoxygenase isozyme activity, and trypsin inhibitor content of soybean sprouts showed a power-dependent decrease after the seeds were exposed to ultrasound. As for the isoflavone content, daidzin and genstin contents markedly decreased, whereas, daidzein and genistein contents increased compared with those of the untreated sample. The present work indicated that ultrasound treatment will be a novel approach to improve the edibility and nutritional quality of soybean sprouts.

1. Introduction

Due to the increasing consumer demands for high quality food, new safe and effective methods of food processing are being developed. Ultrasound is an example of novel technology in the food industry and is becoming increasingly appreciated (Arzeni et al., 2012). It is generally considered safe, non-toxic, and environmentally friendly, which is a major advantage over other techniques (Awad, Moharram, Shaltout, Asker, & Youssef, 2012). Meanwhile, ultrasound could lead to a reduction of the processing time or decrease the financial costs (Cárcel, García-Pérez, Benedito, & Mulet, 2012). The application of ultrasound is related to many areas, such as enzyme inactivation, crystallization, drying, degassing, extraction, filtration, homogenization, meat tenderization, oxidation and sterilization (De Gennaro, Cavella, Romano, & Masi, 1999; Jambrak, Mason, Lelas, Paniwnyk, & Herceg, 2014; Santacatalina, Garcia-Perez, & Benedito, 2011). Interestingly, ultrasound could also stimulate seed germination, increasing the percentage of germination, and accelerating the growth of plants (Wang et al., 2012). The most common interaction mechanisms involved in this case are the cavitation which causes heat and chemical effects. Moreover, acceleration of the rate of influx or uptake of water into seeds by ultrasound can also be caused by mechanical effects, i.e. shear stress developed by eddies arising from shock waves. Although ultrasound treatment has been applied to stimulate germination in many different types of plant seeds, limited information is available about the effects of ultrasound on the germinated seeds in the view of food science (Goussous, Samarah, Alqudah, & Othman, 2010).

Soybean (*Glycine max* L. Merr.) plays an important role in food consumption worldwide because of its high nutritional quality and physicochemical functions (Natarajan, Luthria, Bae, Lakshman, & Mitra, 2013). Among soybean products, soybean sprouts, which are rich in dietary fiber, various nutrients, and bioactive components, are the valuable dietary supplements in many parts of the world that may promote health and well-being, particularly in rural areas in Asian countries, where seasonal fruits and vegetables are not available all-year-round (Mbithi, Van Camp, Rodriguez, & Huyghebaert, 2001). Germination is considered as an inexpensive and effective technology to improve the nutritional quality of soybean because this process triggers a sequence of metabolic changes (Paucar-Menacho, Berhow, Mandarino, Chang, & Mejia, 2010). It has been demonstrated that

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http://dx.doi.org/10.1016/j.foodres.2015.01.011
0963-9969/

certain undesirable constituents, such as trypsin, chymotrypsin, lipoxygenase activity, phytic acid and oligosaccharides could be eliminated or declined during germination in soybeans (Quinhone & Ida, 2015; Shi, Nam, & Ma, 2010). Moreover, the amounts of vitamins, phytosterols, tocopherols, and isoflavones increase during this metabolic process (Shi et al., 2010).

The aim of this study was to investigate the effect of ultrasound treatment on physical properties and nutritional quality of soybean sprouts. Briefly, soybean seeds were exposed to ultrasound then followed germination, the morphological changes, protein fraction compositions, IgE-binding, anti-nutritional factors, and GABA and isoflavone contents of soybean sprouts were assessed. The technical data would be a strong support for the application of ultrasound as a novel approach to promote the development of soybean sprouts as high quality food.

2. Materials and methods

2.1. Materials

Soybean cultivar 'Dongnong 48' was kindly provided by Dr Li Wenbin (Key Laboratory of Soybean Biology (Ministry of Education), Northeast Agricultural University, China). This cultivar has an average weight of 22.41 g per 100 seeds, and contains 44.53% protein and 19.19% lipids. Linoleic acid, N_{α}-benzoyl-L-arginine 4-nitroanilide hydrochloride, trypsin, and GABA were purchased from Sigma-Aldrich (St. Louis, MO, USA). Bradford dye binding assay was supplied by Bio-Rad (Bio-Rad, Hercules, CA). Daidzin, genistin, daidzein, and genistein were purchased from Tauto Co. (Shanghai, China) with purity ≥99%. All of the other chemicals were of analytical grade.

The sera from eight soybean-allergic patients (with an average age of 24.9 years) were collected from the First Affiliated Hospital of Guangxi Medical University (Nanning, China). Among these eight patients, seven were males and one was female. Their specific IgE levels were 1.45, 1.92, 3.54, 4.53, 4.89, 4.95, 13.59 and 23.71 kU/L, respectively, and quantified using a CAP-fluorescent enzyme immunoassay system (Phadia, Uppsala, Sweden). Among the eight patients, two suffered from asthma, and five manifested urticaria. A serum pool established from eight soybean-allergic patients in equal volumes was used for ELISA.

2.2. Ultrasound treatment and germination of soybean seeds

Soybean seeds were cleaned to completely remove impurities, including broken and diseased seeds. These seeds were then dispersed in distilled water in 500 mL glass flasks (wall thickness = 0.1 cm, height = 11.5 cm, inner diameter = 9.0 cm) for ultrasound treatment. Briefly, the flasks containing samples (100 seeds in 160 mL of water) were put in the center of the ultrasonic bath (Ultrasonic, Model SB-5200DTD, HF-Pk-power 300 W, 40 kHz, internal dimensions: 300 mm × 150 mm × 150 mm, Ningbo, China) filled with 5 L of water. This volume was sufficient to apply ultrasound to the whole of each sample. Subsequently, the seeds were exposed to 100, 200, and 300 W for 30 min at 25 °C. The ultrasonic intensity was 0.35 W cm^{-2}, 0.54 W cm^{-2} and 1 W cm^{-2}, respectively. Only one sample was subjected to each sonication. Ice and chilled water were used during this treatment to prevent excessive temperature rises, whereas water was continually circulated in and out of the ultrasound bath. Afterward these seeds were germinated in a climatic cabinet (model LHP-250H, Shanghai, China) at 30 °C for 5 d in darkness. The seeds were sprinkled with sterile distilled water every 12 h. Germination was performed in triplicate for each sample, 100 seeds per replicate.

2.3. Morphological changes in soybean sprouts

Morphological changes in sprouts were determined as follows: (1) germination rate: the number of germinating seeds divided by the total number of seeds, (2) sprout length: length of sprout was measured from above the root to the base of the cotyledon, (3) root length: length of root was measured from the root to the base of the hypocotyl, (4) fresh weight per sprout: average weight of 30 fresh sprouts, and (5) fresh weight per root: average weight of 30 fresh sprouts. These morphological changes in each sample were determined in triplicate, each sample was then stored at −20 °C until further analysis.

2.4. Analysis of crude protein, lipid, total sugar, and moisture

Crude protein, lipid, and moisture were determined in accordance with the AOAC standard method. Total sugar content in the soybean sprouts was determined on the basis of a previously described protocol with slight modifications (Shi et al., 2010). In brief, 0.5 g of ground dry sprouts was mixed with 10 mL of double distilled water and 1 mL of HCl. After heating for 20 min at 100 °C, the mixture was cooled and diluted with distilled water, and total sugar content was determined using anthrone–sulfuric acid method. Glucose was used as a calibration standard at a linear concentration range of 0 mg/L to 100 mg/L.

2.5. Assessment of protein fraction compositions

2.5.1. Preparation of protein extracts

Sprouts were frozen in liquid nitrogen, and ground immediately to fine powder with mortar and pestle. The powder was defatted with acetone (20 mL/g of flour) for 4 h, shaken, filtered, and air-dried. The powder was then subjected to extraction twice with Tris–HCl buffer (50 mM pH 8.0) containing salt (150 mM NaCl). The extracts (1:10, w/v) were stirred for 3 h at 4 °C, and centrifuged at 9000 *g* for 30 min to remove insoluble materials. The supernatants were dialyzed against distilled H_2O for 24 h at 4 °C by using a dialysis membrane (MWCO, 3500 Da) and freeze-dried. The nitrogen content of the total proteins was determined by Kjeldahl method.

2.5.2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Changes in protein patterns were analyzed by SDS-PAGE in a Bio-Rad Mini protein II system (Bio-Rad, Hercules, CA) with 5% stacking gel and 12% separating gel (Laemmli, 1970). The amount and volume of the protein solutions transferred to each well were approximately 20 μg and 20 μL, respectively, and the gels were stained with Coomassie Brilliant Blue R250. The images were recorded with a Gel Doc 1000/2000 gel documentation system (Bio-Rad, Hercules, CA).

2.5.3. Determination of amino acid

The amino acid composition of each sample was determined after acid hydrolysis was performed. The sample was placed in an ampoule and mixed with 6 M HCl. After sealing the ampoule with nitrogen, the sample was hydrolyzed at 110 °C for 24 h. The hydrolysate was evaporated to remove HCl, filtered, and loaded in an amino acid analyzer (Model S433D, Sykam Corp., Eresing, Germany) for amino acid analysis. Tryptophan was determined by alkaline hydrolysis according to the AOAC standard method. The results were expressed in g/100 g of protein.

2.5.4. Determination of GABA content

The defatted soybean powder was soaked in 70% ethanol for 4 h, evaporated and lyophilized to yield dried powder extract. The GABA content was analyzed by HPLC as described in a previous work with slight modifications (Hyun, Eom, Jeun, Han, & Kim, 2013). In brief, approximately 100 mg of powder extract was dissolved in 1 mL of ethanol–water–triethylamine solution (4:4:2) and then 80 μL of ethanol–water–triethylamine–phenylisothiocyanate solution (6:1:1:1) was added. To induce the formation of phenylisothiocyanate–GABA, the mixture was incubated at room temperature (25 °C) for 30 min. After each sample was filtered using a Millipore membrane (0.22 μm), 10 μL