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Effect of ultrasonic treatment on the rheological property and microstructure of tofu made from different soybean cultivars

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ABSTRACT

Effect of high intensity ultrasound (HIUS) treatment on the rheological property and microstructure of tofu made from yellow and black soybeans was investigated. Raw soymilk was either heated at 95 °C for 10 min, or HIUS treated at 25 and 50 °C for 5, 15, and 30 min, and then was coagulated by adding Glucono-δ-lactone (GDL) to produce tofu. Results showed that HIUS denatured the soy protein and was confirmed by the changes in protein surface hydrophobicity. The HIUS-treated soymilk and the heat-treated soymilk expressed different gelation behaviors, which led to distinct rheological property and microstructure in the final products. The network of tofu prepared with HIUS-treated soymilk was constructed by thicker strands in a loose arrangement. The rheological property and microstructure of HIUS-treated tofu can be modified by changing the sonication time and temperature. The tofu made form soymilk with HIUS at 25 °C for 15 min gave the best rheological property.

Industrial relevance: Tofu is a widely accepted and commonly manufactured soy product in Asia. The worldwide tofu consumption is increasing due to its unique texture and health benefits. However, the thermal process of soymilk during tofu making is time and energy consuming. HIUS is a more efficient and environment-friendly technology that can be commercially applied as an energy and labor saving alternative to the conventional thermal treatment. The rheological property and microstructure are two important parameters for the gel texture evaluation. This research paper presents a comparative study between soymilk treated by heating and by HIUS using yellow soybean and black soybean to produce tofu with unique rheological property. Finding in this research gave the primary knowledge for further application of HIUS on tofu processing.

1. Introduction

The worldwide consumption of soy products, particular in Western countries, is increasing due to the health benefits. Tofu is a gel-like soy protein product and is one of the popular daily foods in Asia. The conventional tofu making procedure involves two heating steps, heating raw soymilk, and heating cooked soymilk during acidification with Glucono-δ-lactone (GDL). The purpose of first heating step is to denature the proteins in soymilk, which is a prerequisite for tofu gel formation. The second heating step is to manage the gelation behavior by controlling the rate of acidification. However, these two soymilk heating steps involving heating, holding, and cooling are time and energy consuming.

High intensity (low-frequency) ultrasound (HIUS) technology currently attracts considerable attention in food industry, since it represents an efficient, environment-friendly, and reliable alternative to improve the food quality and to develop new products with specific functional property (Chemat & Khan, 2011). Several studies have investigated the application of HIUS as a pretreatment to promote the soy protein modification. Jambrak, Lelas, Mason, Krešić, and Badanjak (2009) found that HIUS treatment modified the solubility, emulsifying property, and flow behavior of soy protein concentrates. Tang, Wang, Yang, and Li (2009), Hu et al. (2013a), and Hu, Li-Chan, Wan, Tian, and Pan (2013b) revealed that HIUS treatment significantly changed the gelation property of soy protein isolate (SPI). Some studies further investigated the changes in molecular structure of soy protein after HIUS treatment. Hu et al. (2013c) and Jiang et al. (2014) reported that HIUS treatment for <30 min altered the amount of free sulfhydryl groups, particle sizes, surface hydrophobicity, and secondary structure of soy protein, leading to the SPI dispersion with better solubility and flow behavior. Application of HUIS in tofu making might re-innovate products with unique quality, and take less processing time and cost when comparing with the conventional processes.

Yellow soybean is grown and consumed in the form of grains, sprouts, soymilk, and tofu. The green-cotyledon black soybean has a black seed coat, which contains anthocyanins and higher quantities of isoflavones than the yellow soybean. It is commonly used in foods as soymilk, roasted beans, and soy sauce. The objective of this research was to study the effect of HIUS treatment on the physicochemical property and gelation of tofu made from the yellow and the black soybeans.

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2. Material and methods

2.1. Materials

The two soybean cultivars used in this study were the green-cotyledon black soybean Tainan 3 (T3) and the yellow soybean BB50, which were obtained from the Tainan District Agricultural Research and Extension Station (Tainan, Taiwan), and from the Neco Seeds (Neco Seeds Farms Inc., Garden City, USA), respectively. Glucono-δ-lactone (GDL), 1-anilino-8-naphthalene-sulfonic acid magnesium salt monohydrate (ANS), and 5,5′-Dithiobis-2-nitrobenzoic acid (DTNB) were purchased from Sigma Chemical Co. (St. Louis, USA). Others chemicals were purchased from Panreac Química S.A.U. (Barcelona, Spain). All chemicals were analytical grade.

2.2. HIUS treatment of soymilk

The soymilk for tofu making was prepared at a 1:5 soybean-to-water ratios, which is determined by the protein content of soybean. Soybeans were first washed and soaked in the 2.5-fold distilled water at 25 °C for 8 h. After soaking, an extra 2.5-fold distilled water was added, and the soybeans were ground with water in a food grinder (Mr. Pineapple CL-010, Great Yen Food Grinder Co., Taipei, Taiwan). Raw soymilk was then obtained by filtering the liquefied soybean suspension with the cotton cloth on the top of a 120 mesh screen.

Subsequently, the raw soymilk was treated by the ultrasonic cell disruptor/homogenizer (S-450D, Branson Ultrasonics, Danbury, USA) at the frequency of 20 kHz and the amplitude of 30% for 5, 15, and 30 min (pulse duration of on-time, 1 s and off-time, 1 s). For the ultrasonic treatment, the sample temperature was controlled at 25 or 50 °C by the temperature control system. The raw soymilk was also heated and boiled for 10 min by the conventional heating process as the control for comparison. All the treated soymilks were then immediately cooled down to 4 °C in an ice bath for tofu making and for the gelation behavior analysis. Some of these treated soymilks were freeze-dried into powder for the surface hydrophobicity and the sulfhydryl content analysis.

2.3. Tofu making

Tofu was prepared following the procedure of Liu and Kuo (2011) with some minor modifications. The treated soymilk was mixed with 10% (w/w) GDL solution, and was loaded into a 10 × 8 × 3 cm (length × width × height) container. The container was then heated in a water bath at 80 °C for 30 min. After cooling down to the room temperature, the tofu was stored at 4 °C in the refrigerator for further analysis.

2.4. Composition analysis

The composition analysis of the soybean and the tofu were performed based on the Chinese National Standards (2013). Moisture content was determined after drying the samples at 100 °C in the oven for 6 h. Results were expressed as g of moisture held of 100 g sample. Protein content was determined by micro-Kjeldahl method with the conversion factor of 6.25. Results were expressed as g of proteins of 100 g dried matter. Crude lipid was measured by extraction with petroleum ether. Lipid content was estimated by weighing lipid extract after solvent evaporation. Results were expressed as g of lipids of 100 g dried matter. Ash content was determined by ashing the samples at 550 °C in a furnace for 6 h. Results were expressed as g of ash of 100 g dried matter.

2.5. Surface hydrophobicity and sulfhydryl contents of protein in soymilk

The surface hydrophobicity and sulfhydryl contents of protein in treated soymilk was measured according to the method of Lakshmanan, Lamballerie, and Jung (2006) with slight modifications. The hydrophobic fluorescence probe, ANS, was used to determine the surface hydrophobicity of protein. The fluorescence intensity of sample solution was measured by using the fluorescence spectrometer (Model FP-750, JASCO Inc., Tokyo, Japan) at the excitation and emission wavelengths of 380 nm and 490 nm. Surface hydrophobicity of the protein in soymilk was obtained from the slope of fluorescence intensity versus protein concentration plot.

The surface free sulfhydryl content (SFSH) and total free sulfhydryl content (TFSH) measurements were determined by using the Ellman's reagent (10 mM DTNB). The absorbance was measured at 412 nm wavelength by the UV–Vis spectrophotometer (SP-8001, Metertech, Inc., Kaohsiung, Taiwan). The SFSH and TFSH contents were calculated using a molar extinction coefficient of 13,600 M^{-1} cm^{-1} and were expressed as μmol of SH/g protein.

2.6. Gelation behavior analysis

The treated soymilk of 1 mL was filled into an Eppendorf tube immediately after adding of 10% GDL solution and heating at 80 °C for 30 min in a water bath. After cooling, the tubes were flipped over for the observation of tofu formation. Firm tofu would stay on the top, whereas weak tofu would break down at the bottom. The appearance of tofu was capture by a digital camera (EOS 1000D, Canon Inc., Tokyo, Japan) with a Canon EF-S 18–55 mm f/3.5–5.6 lens.

The controlled stress dynamic rheometer (AR2000ex, TA Instruments, Inc., New Castle, USA) was used to investigate the gelation behavior and the viscoelastic properties of samples. The stress amplitude sweep was performed firstly to ensure that all the measurements were carried out within the linear viscoelastic region (data not shown). From the primary results, the stress amplitude of 10 Pa was chosen for further analysis.

The gelation behavior of tofu was measured by the method of Huang and Kuo (2015). The treated soymilk was loaded onto the plate of rheometer immediately after the addition of GDL. A parallel plate geometry (40-mm diameter, 2-mm gap) was used. The edge of geometry was sealed with Vaseline oil and the geometry was coved by the solvent trap to prevent the moisture loss during the test. Oscillating stress was applied to the sample at the frequency of 1 Hz. The temperature sweep of the sample was from 25 °C to 80 °C at the heating rate of 5 °C/min. The time sweep was then carried out at 80 °C for 30 min. Storage modulus (G′) and loss modulus (G″) were both recorded as a function of time.

The viscoelastic property of tofu was measured based on the procedure of Lee and Kuo (2011) with a few modifications. The treated soymilk with GDL was placed on the plate of rheometer. A parallel plate geometry (40 mm diameter) with a gap of 2 mm was used. The edge of geometry was filled with Vaseline oil and the geometry was coved by the solvent trap. The tofu sample was formed between the geometry and the plate under the following conditions: heated from 25 °C to 80 °C at the rate of 5 °C/min, held at 80 °C for 30 min, cooled down to 4 °C at the same rate, and equilibrated at 4 °C for 1 h. The frequency sweep was then conducted on the sample at 4 °C from 0.01 to 10 Hz.

2.7. Microstructure analysis of tofu

Scanning electron microscopy (SEM, Tabletop Microscope TM-1000, Hitachi High Technologies Co., Tokyo, Japan) was used to examine the microstructure of tofu. Sample preparation for SEM was following the method of Lee and Kuo (2011) with some modifications. Tofu was cut into 5 × 5 × 5 mm cubes. These cubes were rapidly frozen in the liquid nitrogen (− 196 °C) and dehydrated in a freeze drier. Dried samples were then placed on an aluminum stub and were fixed by using double-sided adhesive carbon-tapes. The nature-broken side of each cubic sample was faced up on the tape and sputter-coated with gold.