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Effects of ultrasound on the structure and physical properties of black bean protein isolates

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ABSTRACT

In this study, our aim was to compare the effects of low-frequency (20 kHz) ultrasonication applied at various powers (150, 300, or 450 W) and for different durations (12 or 24 min) on the functional and structural properties of black-bean protein isolate (BBPI) dispersions. In SDS-PAGE analysis, we detected no marked changes in protein electrophoretic patterns. However, secondary-structure analysis performed using circular dichroism indicated that all samples except Sample E (300 W, 24 min) showed a decrease in the α-helix proportion and an increase in β-sheets content in the BBPI after ultrasonic treatment. Moreover, emission-fluorescence spectra revealed that the tertiary structure of black-bean proteins changed after ultrasonic treatment, and scanning electron microscopy of ultrasonicated BBPI samples showed that BBPI microstructure had changed and it contained larger aggregates when compared with the untreated BBPI sample. When medium-power ultrasonication was applied for 24 min, the particle size was minimized and the absolute zeta potential was maximized. Surface hydrophobicity and protein solubility of the BBPI dispersions were enhanced after ultrasonication, which increased the destruction of internal hydrophobic interactions of protein molecules and accelerated the molecular motion of proteins to cause protein aggregation. However, medium-power ultrasound treatment disrupted BBPI dispersions into small soluble protein aggregates by means of cavitation forces that induced increases in surface hydrophobicity and solubility. High-power ultrasound treatment caused a restructuring of BBPI aggregates, which led to an increase of particle size but a decrease in the absolute zeta potential.

Introduction

The protein content of black beans is higher than that of soybean and even that of meat, eggs, or milk. Moreover, black beans are an optimal source of high-quality protein and contain a favorable balance of amino acids, and they are nutritionally rich not only in calories, isoflavones, vitamin E, saponins, carotenoids, and anthocyanins (Lee, Hung, & Chou, 2008), but also in polyphenols (Takahashi et al., 2005; Xu & Chang, 2008). The health benefits of black beans have been described in Compendium of Materia Medica, an ancient Chinese botanical encyclopedia (Li, 1990), and because of these benefits and their use in traditional medicine, black beans have been widely consumed for centuries. For example, in Japan and Korea, black beans are used as medicinal food because their seed coat is rich in antioxidants, particularly anthocyanins (Díaz-Batalla, Widholm, Fahey, Castaño-Tostado, & Paredes-López, 2006; Heimler, Vignolini, Dini, & Romani, 2005; Inagaki, Morimura, Shigematsu, Kidal, & Akutagama, 2005; K.-G. Lee & Shibamoto, 2000; Madhujith & Shahidi, 2005). Black bean seed-coat extract has been used in the traditional Chinese medicine "Kokuzui" for centuries, and a recent study revealed that this extract can prevent obesity and diabetes by enhancing energy expenditure and suppressing inflammation (Kanamoto et al., 2011). Although black beans exert numerous beneficial physiological effects, they have been examined in only two studies to date (Ko, Kwon, & Song, 1998; Yamai et al., 2003), and no study has described black-bean proteins.

In recent years, ultrasound technology, which currently attracts considerable attention, has been extensively used in the processing of food in both liquid and solid media (Chandrapala, Oliver, Kentish, & Ashokkumar, 2012a). Ultrasound comprises mechanical waves that vary in pressure or density and are of frequencies above the human hearing threshold (approximately 18 kHz) (Mason, 1998). Ultrasound

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can be divided into two frequency ranges: high frequency (100 kHz–1 MHz, power < 1 W cm^{-2}) and low frequency (16–100 kHz, power 10–1000 W cm^{-2}) (Soria & Villamiel, 2010). The effects of ultrasound on liquid systems are mainly related to cavitation, heating, dynamic agitation, shear stresses, and turbulence (Knorr, Zenker, Heinz, & Lee, 2004; O'donnell et al., 2010). Ultrasound plays key roles in food technology, such as in processing, preservation, and extraction. Currently, highly reproducible food processing can be completed in seconds by using ultrasound, which can reduce the processing cost, simplify manipulation, produce a purer final product, and consume less time and energy when compared with conventional processes (Chemat & Khan, 2011). Moreover, ultrasound technology can be used for ensuring food quality and safety (Chandrapala, Oliver, Kentish, & Ashokkumar, 2012b). Numerous studies have investigated the application of ultrasound during protein chemical reactions or as a pretreatment to promote subsequent protein modification. For instance, Mu et al. (2010) reported that the use of ultrasound can substantially increase protein yields and reduce the overall cost of producing soy protein from flakes. Certain studies investigated the changes in molecular structure after high-intensity ultrasound (HIUS) treatment, which induced alterations in free sulfhydryl groups, particle sizes, surface hydrophobicity, and secondary structures of proteins (Gülseren et al., 2007) and an increase in intramolecular mobility and surface activity (Güzey et al., 2006). Similarly, Hu et al. (2013) reported that ultrasonic treatment resulted in partial unfolding and reduction of intermolecular interactions of soy proteins, as demonstrated by increases in free sulfhydryl groups and surface hydrophobicity, and this led to improved solubility and fluid characteristics of soy protein isolate (SPI) dispersions. Furthermore, following lyophilization, ultrasonic-treated SPIs formed larger aggregates in the dry state than untreated SPIs did. HIUS modifies the functional properties of proteins, and these changes are considered to be closely related to molecular modifications; however, HIUS mainly causes an increase in hydrophobicity and a variation in particle size (Arzeni et al., 2012). Chen, Chen, Ren, and Zhao (2011) reported that ultrasound increased the enzymatic hydrolysis of proteins. Similarly, ultrasound treatment markedly changed the conductivity and rheological properties and increased the solubility, specific surface area, and emulsifying activity index values of soy-protein concentrates (Jambrak, Lelas, Mason, Krešić, & Badanjak, 2009), as well as the heat-induced gelling ability of commercial SPI (Tang, Wang, Yang, & Li, 2009). HIUS pretreatment of SPI samples profoundly influenced the gel characteristics of glucono-δ-lactone-induced SPI gels (GISGs), and it changed the SH-group content, hydrogen bonds, and hydrophobic interactions of the final GISGs (Hu, Li-Chan, Wan, Tian, & Pan, 2013). Furthermore, examination of the effects of HIUS on egg-white protein functionalities showed that whereas emulsion stability increased after sonication, the foam capacity and stability decreased together with a reduction in bulk viscosity (Arzeni et al., 2012).

In this study, we investigated how varying the conditions of ultrasound treatment affects the functional and structural properties of black-bean proteins; thus, we used low, medium, and high ultrasound power (150, 300, and 450 W, respectively) for short and long durations (12 and 24 min). Our objective was to determine the effects of ultrasound power and treatment time and to determine which of these variables maximally affects the functional and structural properties of the proteins.

Materials and methods

Materials

We obtained black beans from Hei Long Jiang Agriculture Company Limited (Hei Long Jiang, China). For extracting proteins, a slurry was prepared using defatted black-bean powder and 10-fold volume of water adjusted to pH 8.0 using 2 mol/L NaOH. The solution was subjected to alkaline extraction with magnetic stirring for 3 h and then centrifuged at 10,000 ×*g* for 30 min. The pH of the supernatant was adjusted to 4.5 using 2 mol/L HCl, and then the supernatant was centrifuged at 10,000 ×*g* for 30 min. The precipitate obtained was dialyzed against deionized water for 48 h at 4 °C, neutralized to pH 7.0 using 2 mol/L NaOH, frozen using liquid nitrogen, and then freeze-dried. The final BBPI product had a protein content of 84.72% ± 1.15%, as determined using the Kjeldahl method (N × 5.8) (Speroni, Añón, & De Lamballerie, 2010).

Ultrasound treatment of samples

BBPI dispersions (10.0%, w/v) were prepared by adding BBPI powder to distilled water, stirring the mixture gently for 2 h, and then maintaining it at 4 °C overnight. An ultrasound processor (NingBo Scientz Biotechnology Co. Ltd., Ningbo, China) equipped with a 0.636-cm-diameter titanium probe was used to sonicate 100 mL of BBPI dispersions in 150-mL flat-bottomed conical flasks, which were immersed in an ice-water bath. Samples were placed in the ice-water bath for 60 min and maintained at a temperature below 2 °C, and then treated at 20 kHz at various levels of power output (0, 150, 300, 450 W) for 12 and 24 min (pulse duration: on-time, 4 s; off-time, 2 s) (Table 1). We added ice and stirred the ice-water bath every 5 min to maintain the low temperature. After ultrasound treatment, all samples were lyophilized and stored at room temperature in airtight containers until analysis.

Determination of ultrasound power and intensity

Ultrasonic energy, which is considered a mechanical energy, is partly lost in the form of heat when ultrasound passes through a medium (Thompson & Doraiswamy, 1999). Ultrasonic treatment of a liquid produces heat; therefore, after recording the change in the liquid's temperature as a function of time, the acoustic power can be estimated using the equation: $P = M \times Cp \times (dT / dt)$, where P is power (in W); M is the mass of the sonicated liquid (g); Cp is the specific heat of the medium at a constant pressure, which is dependent upon the composition and volume of the medium ($J \cdot K^{-1}$); and dT/dt is the slope at the origin of the curve of a plot of temperature against time (Margulis & Margulis, 2003). In this study, we used water to estimate the acoustic power, and performed calorimetry to measure ultrasonic intensity (expressed in W cm^{-2}) by using a thermocouple (model TASI-8530, Suzhou, China). Ultrasonic treatment with the 20-kHz probe at power outputs of 150, 300, and 450 W generated ultrasonic intensities of 72–78, 96–104, and 112–120 W cm^{-2}, respectively.

Circular dichroism (CD) spectrum measurement

We performed CD spectroscopy in the far-ultraviolet region to examine the influence of ultrasonication on protein secondary structure. Protein samples were weighed and dissolved in a 0.01 mol/L phosphate buffer (pH 7.0) to a concentration of 0.4 mg protein/mL. Then centrifuged at 12,000 ×*g* for 20 min at 20 °C to remove any insoluble residues. The final concentration of proteins used for CD analysis was 0.2 $mg \cdot mL^{-1}$. A Jasco-815 CD spectrometer (Jasco Co., Japan) was used for scanning between 200 and 250 nm, and the experiments were conducted at 20 °C. The optical length of the sample cell was 1 mm, the sensitivity was 100 mdeg/cm, the scan speed was 100 nm/min, and the resolution was 0.1 nm. Each scan was performed five times, and the mean was calculated from the experimental data.

Table 1
Ultrasonic treatment parameters of black bean protein.

	A	B	C	D	E	F	G
Ultrasonic intensity (W)	0	150	150	300	300	450	450
Ultrasonic time (min)	0	12	24	12	24	12	24