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Effects of sonication on the physicochemical and functional properties of walnut protein isolate

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ABSTRACT

The objective of this study was to investigate the impact of high-intensity ultrasound treatment (sonication) on the molecular, physicochemical, and functional properties of walnut protein isolate. Aqueous walnut protein suspensions were sonicated at varying power levels (200, 400 or 600 W) and times (15 or 30 min), and then any alternations in protein structure and properties were determined. SDS-PAGE demonstrated that there were no changes in protein electrophoretic patterns, indicating that sonication did not break covalent bonds. Circular dichroism spectroscopy indicated a small change in protein secondary structure after sonication, with a decrease in α-helix and increase in β-sheet, β-turn, and random coil content. There was an increase in surface free sulfhydryl (SH) groups and a decrease in fluorescence intensity after sonication, indicating that appreciable changes in tertiary structure occurred. Ultrasound reduced the size of the particles in aqueous walnut protein dispersions as confirmed by static light scattering and scanning electron microscopy, suggesting that sonication dissociated protein aggregates. Moreover, the water-solubility (+22%), emulsifying activity index (+26%), and emulsifying stability index (+41%) all increased after sonication. These results suggest that sonication is a valuable tool for improving the functional attributes of walnut proteins.

1. Introduction

Walnuts (*Juglans regia* L.) are widely consumed because of their desirable flavor, nutritional, and health profile (Martinez, Labuckas, Lamarque, & Maestri, 2010; Sze-Tao & Sathe, 2000). China and America are currently the two major walnut-producing countries (Fuentealba et al., 2017). Typically, walnuts contain about 55 to 70% lipids, with about 70% of this fraction consisting of polyunsaturated fatty acids, which have been reported to be important for the potential alleviation of a range of human diseases and disorders (Luo et al., 2017). However, walnut lipids are highly susceptible to oxidation leading to quality deterioration, shelf life reduction, and consumer rejection (Ravai, 1992). Walnuts are also a valuable source of plant proteins, containing about 18 to 24% proteins, which are rich in essential amino acids (Lv et al., 2017). Walnut proteins can be categorized into four major categories, albumin, globulin, prolamin, and glutelin. Walnuts are also rich in other potentially beneficial bioactive components, such as tocopherols and phenolics (Gómez-Caravaca, Verardo, Segura-Carretero, Caboni, & Fernández-Gutiérrez, 2008). For instance, a number of these bioactive components have been reported to have free radical-scavenging capacities and have been shown to protect LDL-cholesterol from oxidative modification *in vitro* (Anderson et al., 2001; Li et al., 2006). In addition, walnuts can provide appreciable amounts of thiamin, riboavin, niacin, vitamin B_6, folic acid, iron, magnesium, potassium, and phosphorus.

Recently, there has been growing interest in the utilization of plant-based proteins as functional ingredients in foods because of consumer demand for "clean labels" (Lima, Lima, Tavares, Costa, & Pierucci, 2014; Swami, Thakor, Haldankar, & Kalse, 2012). Many proteins can be utilized as gelling agents, foaming agents, stabilizers, or emulsifiers in foods (Resendiz-Vazquez et al., 2017). Walnut proteins are a by-product of the production of walnut oil, and are typically used as animal feed or discarded, which is a waste of an important protein resource (Bakkalbasi, Meral, & Dogan, 2016). However, the major category of proteins (≈70%) present in walnuts are glutelins, whose low water-solubility limits their functional performance in many aqueous-based food products. Consequently, new approaches are required to improve the physicochemical attributes of walnut proteins, so that they can be used as functional ingredients in foods.

Ultrasound may be used in the food industry either to monitor the

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properties of foods or to alter their properties. Low-intensity ultrasound is used in analytical instruments that provide information about food properties (Coupland, 2004; McClements, 1997), whereas high-intensity ultrasound is used in processing equipment to modify food properties (Ojha, Mason, O'Donnell, Kerry, & Tiwari, 2017). In general, ultrasonic waves are considered to be pressure waves with frequencies above the range the human ear can perceive *i.e.*, greater than about 20 kHz (Demirdöven & Baysal, 2008). High-intensity ultrasound has previously been used in various food processing operations, including emulsification, extraction, crystallization, depolymerization, fermentation, aging, and microbial deactivation (Castro & Priego-Capote, 2007). The fundamental effects of high-intensity ultrasound on liquid systems are primarily attributed to its ability to induce cavitation and microstreaming currents (Zheng & Sun, 2006). During sonication, small gas bubbles are formed within the fluid that violently collapse, leading to extreme local temperatures (up to 5000 K) and pressures (up to 1000 atm) (Chandrapala, Zisu, Palmer, Kentish, & Ashokkumar, 2011; Gülseren, Güzey, Bruce, & Weiss, 2007). The intense cavitation, turbulence, and shear stresses produced through this process result in physical alterations in material properties (Soria & Villamiel, 2010). Furthermore, highly reactive free radicals can be produced by ultrasonic waves from water ($H_2O \rightarrow \cdot H + \cdot OH$), which may chemically react with and modify other molecules (Gülseren et al., 2007; Hu, Cheung, Pan, & Li-Chan, 2015). Recently, several studies have demonstrated the ability of high-intensity ultrasound to improve the functional properties of biopolymers, such as their solubility, interfacial, emulsifying, foaming, and gelling properties. For instance, the foaming properties of wheat gluten proteins were improved by sonication, which was attributed to alterations in the molecular structure of the proteins by the ultrasonic waves (Zhang, Claver, Zhu, & Zhou, 2011). The solubility and surface hydrophobicity of black-bean protein isolate have been shown to increase after sonication, which was again attributed to changes in their molecular structure (Jiang et al., 2014). The conjugation of peanut proteins with dextran was accelerated by applying high-intensity ultrasonic waves, which led to an appreciable increase in the solubility and emulsifying properties of the peanut proteins (Liu, Zhao, Zhao, Ren, & Yang, 2012). Finally, sonication improved the water holding capacity and gel strength of soy protein isolate, which was again attributed to ultrasonic-induced changes in the molecular structure of these globular proteins (Hu, Fan, et al., 2013). However, to the best of our knowledge, the effect of sonication on the properties of walnut proteins has not been studied previously. It was hypothesized that treatment of walnut proteins with high-intensity ultrasound would alter their molecular, physicochemical, and functional properties, which could lead to greater application of this underutilized proteins in commercial food products.

2. Material and methods

2.1. Materials

A defatted walnut flour was produced using a method described previously (Mao & Hua, 2014). Briefly, walnuts were obtained from a local farmers' market. Peeled walnut kernels were then defatted using petroleum ether at a ratio of 1:6 (*w/v*) for 24 h with constant magnetic stirring. The resulting slurry was vacuum filtered through filter paper, and the filter cake was extracted with petroleum ether again. After the final extraction, the residue was dried at 50 °C, homogenized in a blender to obtain a 40-mesh defatted flour, and then collected in a valve bag and stored at −20 °C until further use.

5, 5-dithiobis (2-nitrobenzoic acid) (DTNB) was purchased from the Aladdin Reagent Co. (Shanghai, China). Bovine serum albumin (BSA), glycine, ethylenediaminetetraacetic acid (EDTA), sodium dodecyl sulphate (SDS), β-mercaptoethanol, and Coomassie brilliant blue G250 were purchased from the Sigma Aldrich Company (St. Louis, MO). Other reagents were obtained from the Yuanye Biotechnology Co. Ltd. (Shanghai, China). All the chemicals used were of analytical grade.

2.2. Preparation of walnut protein isolate

Walnut protein isolate (WNPI) was prepared by modifying a method described previously (Wolf, 1970). The defatted walnut flour was first dispersed in deionized water (1:15 *w/v*) and then adjusted to pH 9.0 using a 0.5 M NaOH solution. The resulting slurry was stirred for 1 h at 45 °C, and then centrifuged at 8000 *g* for 10 min at 4 °C. The precipitate obtained was dispersed in deionized water and extracted twice using the same method. After extraction, the pH of the supernatant was adjusted to 4.5 with 0.5 M HCl solution. The slurry was then stirred for 1 h at room temperature, and centrifuged at 8000 *g* for 10 min at 4 °C. The sediment obtained from this process was dispersed in deionized water. This dispersion was then stirred for 1 h, adjusted to pH 7.0 using 0.5 M NaOH solution, dialyzed for 48 h, and then freeze-dried. The WNPI powders obtained were stored in a valve bag at −20 °C prior to use. The final product was characterized according to AOAC (1990) methods, and found to contain 80.32% (N × 5.3), 0.67%, and 2.13% protein, fat, and ash, respectively.

2.3. Ultrasonic treatment of samples

Before sonication, aqueous walnut protein dispersions (*w/v* 0.5%) were prepared by adding WNPI powder into phosphate buffer solution (10 mM, pH 7.0) and then stirring for 60 min at ambient temperature. An ultrasound processor (XH-300UA, 25 kHz, maximum power: 1200 W, Xianghu Development Co. Ltd., Beijing, China) was used to sonicate 200 mL solutions in a 250-mL quartz glass flask for either 15 or 30 min at a sonication power of 200, 400, or 600 W (pulse duration: 2 s on, 1 s off). The sonication probe had a vibrating titanium tip (1.8 cm), which was immersed into the solutions about 3 cm from the upper surface. The calculated energy density was 5, 10, or 15 J/cm^3 and 10, 20 or 30 J/cm^3 for sonication conditions of 200, 400, or 600 W for 15 and 30 min, respectively (Silva, Gomes, Hubinger, Cunha, & Meireles, 2015). The temperature of all the protein solutions was kept below about 45 °C during sonication by immersing the containers in an ice bath. This temperature was well below the reported thermal denaturation temperature of walnut proteins of 70 °C (Zhao et al., 2017), and should therefore not have caused a large change in their structure. Untreated protein solutions were used as a control.

2.4. Structural modifications of proteins

2.4.1. Molecular weight distribution

The molecular structure of untreated and sonicated walnut proteins was determined using reducing and non-reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), which was performed according to a previously described method (Liu & Xiong, 2000). For the reducing conditions, 100 mL of buffer solution was mixed with 20 mL of 10% SDS, 50 mL deionized water, 5 mL β-mercaptoethanol, 2.5 mL of 4% (*w/v*) bromophenol blue, 12.5 mL 0.5 M Tris–HCl buffer (pH = 6.8), and 10 mL glycerin. A protein sample was then prepared by mixing 400 μL protein (2.5 mg/mL), 400 μL buffer, and 400 μL deionized water together. The mixtures were boiled for 5 min at 95 °C, and 10 μL of the mixture was loaded into each lane. For the non-reducing conditions, β-mercaptoethanol was omitted from the preparation procedure. Using 5% stacking gels and 15% separating gels, images were captured using a Gel image analysis system equipped with Smart View software (FR-980A, Shanghai Furi Science & Technology Co., Ltd., Shanghai China).

2.4.2. Secondary structure

Circular dichroism (CD) was used to analyze changes in the secondary structure of the walnut proteins after sonication. CD spectra (190–260 nm) were collected at ambient temperature on a Jasco J-810