Ultrasonics Sonochemistry 31 (2016) 590–597

Contents lists available at ScienceDirect

Ultrasonics Sonochemistry

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

Transglutaminase-induced gelation properties of soy protein isolate and wheat gluten mixtures with high intensity ultrasonic pretreatment

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ARTICLE INFO

Article history:
Received 17 December 2015
Received in revised form 5 February 2016
Accepted 6 February 2016
Available online 6 February 2016

Keywords:
High intensity ultrasound
Microbial transglutaminase
Soy protein isolate
Wheat gluten
Gel properties

ABSTRACT

Soy protein isolate (SPI) and wheat gluten (WG) are widely used in commercial food applications in Asia for their nutritional value and functional properties. However, individually each exhibits poor gelation. In this study, we examined the microbial transglutaminase (MTGase)-induced gelation properties of SPI and WG mixtures with high intensity ultrasonic pretreatment. Ultrasonic treatment reduced the particle size of SPI/WG molecules, which led to improvements in surface hydrophobicity (H_o) and free sulfhydryl (SH) group content. However, MTGase crosslinking facilitated the formation of disulfide bonds, markedly decreasing the content of free SH groups. Ultrasonic treatment improved the gel strength, water holding capacity, and storage modulus and resulted in denser and more homogeneous networks of MTGase-induced SPI/WG gels. In addition, ultrasonic treatment changed the secondary structure of the gel samples as determined by Fourier transform infrared spectroscopic analysis, with a reduction in α-helices and β-turns and an increase in β-sheets and random coils. Thus, ultrasound is useful in facilitating the gelation properties of MTGase-induced SPI/WG gels and might expand their utilization in the food protein gelation industry.

1. Introduction

Soy protein isolate (SPI) and wheat gluten (WG) are widely used as important ingredients in food applications in Asia for their abundant nutritional value and functional properties. SPI is one of the most significant economic and readily obtainable soy protein products [1]. It mainly consists of glycinin (11S) and β-conglycinin (7S), which account for approximately 70% of the total protein content of SPI [2]. WG is a commercially important by-product of the wheat starch industry that is currently utilized as a purposeful protein additive in the bakery field because of its desirable rheological and structure-enhancing properties [3,4]. The gelling properties of SPI and WG are used in a wide range of industrial foodstuff, including meat products, tofu, and dairy products [5–10]. Nevertheless, the gelling properties of these vital ingredients are poor.

Microbial transglutaminase (MTGase) has been widely used to improve the gelation and texture properties of food proteins including soybean, wheat, pea, whey, casein, myofibrillar, and myosin [11–14]. MTGase is a calcium-independent enzyme that catalyzes the acyl transfer reaction between the γ-hydroxylamine groups of glutamine residues (Gln) and ε-amino groups of lysine residues (Lys), wherein a covalent ε-(γ-glutamyl)lysine isopeptide bond is formed, leading to inter- or intramolecular crosslinking [7,10,15,16]. The rheological properties of SPI gel is improved by the addition of MTGase, forming a denser, more homogeneous gelation network [17]. Agyare et al. [4] found that MTGase treatment improves protein solubility and leads to the formation of high-molecular-weight polypeptides during WG gelation. However, SPI contains ample Lys but lacks Gln, and WG is rich in Gln but low in Lys. Therefore, a combination of SPI and WG catalyzed by MTGase might enhance the formation of covalent ε-(γ-glutamyl)lysine bonds. However, the effects of SPI and WG mixtures on the gelation properties mediated by MTGase have not yet been investigated. In addition, the dense structure of SPI and WG that sequesters Gln and Lys groups inside the molecule functions to the disadvantage of the MTGase crosslinking reaction. Therefore, appropriate pretreatment might enhance structural unfolding and expose residues at the protein surface, accelerating subsequent enzymatic reactions [8,10,18,19].

The development of ultrasound technology in the food industry is recently generating much attention worldwide. Ultrasound technology utilizes mechanical waves at a frequency above the criticality of human hearing (>16 kHz) [20,21]. High intensity ultrasound (frequency in the range 16–100 kHz, 10–100 W/cm^2 of power) is

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http://dx.doi.org/10.1016/j.ultsonch.2016.02.010
1350-4177/

used to disrupt physical material or promote chemical reactions in food to impact the overall properties [22]. The effects of ultrasound on liquid systems are mainly related to acoustic cavitation phenomenon [3], wherein cavitation bubbles are rapidly formed and violently collapse during sonication [23]. The bubble–bubble coalescence results in the generation of extreme temperatures and pressure that leads to high shear stress waves and turbulence in the cavitation area [24]; together, these elements associate to affect the ultrasound treated system [25].

Numerous studies have investigated the effect of ultrasound on protein alteration. For example, Hu et al. [5,6] found that ultrasonic pretreatment improved the SPI gelation properties induced by glucono-δ-lactone (GDL) and $CaSO_4$, respectively. Similarly, ultrasound was shown to significantly form soluble aggregates from insoluble precipitates of SPI, improving its heat-induced gelling characteristics [2]. Nguyen and Anema [26] reported that the ultrasound treatment of skim milk had a profound effect on the gelation ability of acid-induced gels. In addition, Arzeni et al. [8] examined ultrasound changed the functionality of SPI, whey protein concentrate, and egg white protein and demonstrated that these changes are closely related to particle size modification, hydrophobicity increase, and molecular denaturation and aggregation.

Our previous study showed that the gel strength, WHC, and storage modulus (G') values of MTGase-induced SPI/WG gels were significantly improved with the increasing microwave power, and lead to a denser and more homogeneous microstructures after microwave pretreatment [27]. Therefore, it is possible that ultrasonic pretreatment may have analogical function on the SPI/WG gels induced by MTGase. Moreover, the objective of this study was to explore the influence of high intensity ultrasonic pretreatments on the physical and structural properties of SPI/WG mixtures and the resulting SPI/WG gels induced by MTGase. The results of this research might facilitate the application of ultrasound toward complicated food protein systems.

2. Materials and methods

2.1. Materials

SPI (>90% protein) was obtained from Kunhua Reagent Company, Henan, China. WG (>75% protein) was purchased from Ruifuxiang Reagent Company, Anhui, China. MTGase was purchased from Yiming Biological Products Co., Ltd, Jiangsu, China. 5, 5′-Dithiobis-(2-nitrobenzoic acid) (DTNB), glycine, Tris, bovine serum albumin (BSA), 1-anilino-8-naphthalene-sulfonate (ANS), and Coomassie brilliant blue G-250 were obtained from Solarbio Science & Technology Co., Ltd, Beijing, China. Other chemicals were provided by Sinopharm Chemical Reagent Co., Ltd, Shanghai, China. All reagents were analytical grade.

2.2. High intensity ultrasound treatment of SPI/WG solutions

The solutions (11.0%, 11 g protein/100 mL of water) were prepared by adding SPI/WG (10:1, w/w) flour into distilled water, followed by gentle agitation for 1 h at room temperature (i.e. 25 °C). The solutions were sonicated in flat bottom conical flasks equipped with a cooling jacket below 25 °C using a 40 kHz, 300 W Ultrasonic horn model SB-5200DT (NingBo Scientz Biotechnology Co., Ltd., Ningbo, Zhejiang China) for 0, 10, 20, 30, or 40 min. After ultrasound treatments, samples were lyophilized and then stored in dry containers at room temperature until use.

2.3. Ultrasound power and intensity measurements

Ultrasound power is considered as mechanical energy that would be partially lost in the form of a thermal effect when ultrasound waves pass through an intermediary [28,29]. Thus, the ultrasonic irradiation produces heat in liquid systems. Taking the temperature as a variable of time results in evaluation of the acoustic power by the equation [5]:

$$P = m \times c_p \times (dT/dt) \quad (1)$$

where m is the mass of the sonicated liquid (g), c_p is the heat capacity of the sonicated liquid (J/(g K)), and dT/dt is the slope of the curve. Acoustic power is indicated in watts per unit zone of the sonicated liquid (W/cm^2). In this study, the c_p of water is 4.2×10^3 J/(g K); acoustic power is 91–102 W/cm^2.

2.4. Determination of particle size

The lyophilized samples were dissolved in deionized water (10.0%, 0.1 g protein/10 mL of water) and stirred for 2 h at 25 °C. The particle size of the samples was determined by light scattering using a Mastersizer 2000 laser particle analyzer (Malvern Instruments Ltd., Malvern, UK) as described by Arzeni et al. [8]. The pump speed, refractive index and absorption parameter were set at 1800 r/min, 1.333, and 0.001, respectively. Particle size was measured as the average and standard deviation of 5 readings of the volume-surface mean diameter (D_{32}) and volume-mean diameter (D_{43}).

2.5. Determination of surface hydrophobicity (H_o)

Surface hydrophobicity was measured using the fluorescence probe 1-anilino-8-naphthalene-sulfonate (ANS) according to the method described by Hayakawa and Nakai [30]. The samples were diluted with phosphate buffer (1 mg/mL in 0.01 M buffer, pH 7) and centrifuged at 8000 r/min for 20 min at 4 °C (Hitachi Ltd., Tokyo, Japan). The protein concentration in the supernatant was diluted with the range of 0.1–0.0005 mg/mL according to Bradford method. Then, 60 μL of ANS (8.0 mM in phosphate buffer), was added to 3 mL of diluted sample and the relative fluorescence intensity (RFI) was determined using a FC Multiskan (Thermo Fisher Scientific Inc., Waltham, MA, USA) at 365 nm (excitation wavelength) and 484 nm (emission wavelength). Surface hydrophobicity was indicated as the initial slope of the plot of RFI as protein concentration.

2.6. Preparation of MTGase-induced SPI/WG gels

MTGase (30 U/g protein, 0.33 g MTGase/11 g protein) was added to the ultrasound treated samples (Section 2.2), and the pH of the solution was adjusted to 7.5. The dispersions were incubated using an HH-4 oscillating constant temperature water bath (Changzhou Guohua Electric Appliance Co., Ltd., Changzhou, Jiangsu, China) at 40 °C for 2 h, then heated at 90 °C for 10 min to inactivate the MTGase. MTGase-induced SPI/WG gels were held at 4 °C overnight before being subjected to gel strength, water holding content (WHC), free SH groups, Fourier transform infrared (FTIR) spectra, rheological properties and scanning electron microscopy (SEM) analyses.

2.7. Determination of free sulfhydryl (SH) contents

The free SH contents of the ultrasound treated proteins (Section 2.2) and MTGase-induced SPI/WG gels (Section 2.6) were measured according to Ellman's reaction with modifications as described by Shimada and Cheftel [31] and Hu et al. [5]. Samples were dissolved in Tris-glycine buffer (pH 8.0, 0.086 M Tris, 0.09 M glycine, 4 mM Na_2EDTA) and the sample dispersions adjusted to 0.2% (0.1 g sample/50 mL Tris-glycine buffer). The