Ultrasonics Sonochemistry 29 (2016) 380–387

Contents lists available at ScienceDirect

Ultrasonics Sonochemistry

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

Effect of high intensity ultrasound on transglutaminase-catalyzed soy protein isolate cold set gel

Peipei Zhang [a,b,1], Tan Hu [a,b,1], Shaolong Feng [c], Qi Xu [a,b], Ting Zheng [a,b], Moxi Zhou [a,b], Xueqi Chu [a,b], Xingjian Huang [a,b], Xiaonan Lu [c], Siyi Pan [a,b], Eunice C.Y. Li-Chan [c], Hao Hu [a,b,*]

[a] College of Food Science and Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, PR China
[b] Key Laboratory of Environment Correlative Dietology, Huazhong Agricultural University, Ministry of Education, PR China
[c] The University of British Columbia, Faculty of Land and Food Systems, Food Nutrition and Health Program, 2205 East Mall, Vancouver, British Columbia V6T 1Z4, Canada

ARTICLE INFO

Article history:
Received 9 August 2015
Received in revised form 20 October 2015
Accepted 22 October 2015
Available online 23 October 2015

Keywords:
High intensity ultrasound
Transglutaminase
Cold gel
Soy protein isolate
Gelation property

ABSTRACT

The effects of high intensity ultrasound (HIU, 105–110 W/cm^2 for 5 or 40 min) pre-treatment of soy protein isolate (SPI) on the physicochemical properties of ensuing transglutaminase-catalyzed soy protein isolate cold set gel (TSCG) were investigated in this study. The gel strength of TSCG increased remarkably from 34.5 to 207.1 g for TSCG produced from SPI with 40 min HIU pre-treatment. Moreover, gel yield and water holding capacity also increased after HIU pre-treatments. Scanning electron microscopy showed that HIU of SPI resulted in a more uniform and denser microstructure of TSCG. The content of free sulfhydryl (SH) groups was higher in HIU TSCG than non-HIU TSG, even though greater decrease of the SH groups present in HIU treated SPI was observed when the TSCG was formed, suggesting the involvement of disulfide bonds in gel formation. Protein solubility of TSCG in both denaturing and non-denaturing solvents was higher after HIU pretreatment, and changes in hydrophobic amino acid residues as well as in polypeptide backbone conformation and secondary structure of TSCG were demonstrated by Raman spectroscopy. These results suggest that increased inter-molecular ε-(γ-glutamyl) lysine isopeptide bonds, disulfide bonds and hydrophobic interactions might have contributed to the HIU TSCG gel network. In conclusion, HIU changed physicochemical and structural properties of SPI, producing better substrates for TGase. The resulting TSCG network structure was formed with greater involvement of covalent and non-covalent interactions between SPI molecules and aggregates than in the TSCG from non-HIU SPI.

1. Introduction

Currently, the consumption of soybean food is increasing because of the high nutrient value of soy protein and the reported benefits of soy protein associated with isoflavones for lowering cholesterol as well as lowering the risk of coronary heart disease, obesity, type 2 diabetes, high systolic and diastolic blood pressure and certain types of cancer [1,2]. Soy protein isolate (SPI), an important byproduct of the soybean oil industry [3], has at least 90% protein (dry basis) and is widely used in food industry due to its excellent functional properties such as gelation ability, emulsifying ability, water holding capacity and oil-holding capacity [4]. Tofu is an example of soybean food which is made by using soy protein's gelation ability. A recent study showed that soy protein tofu hydrogels had potential to be used as controlled delivery devices for nutraceutical compounds [5]. However, the process of making traditional soy protein tofu hydrogels involves two steps: firstly, heating soy protein at neutral pH to dissociate soy protein so as to expose the functional groups (such as hydrophobic group) from the interior of the molecule to the surface [6,7]. The denatured soy protein is negatively charge at neutral pH. Protons induced by coagulants (such as $CaSO_4$ and glucono-δ-lactone) can neutralize the net charge of the soy protein and hydrophobic interactions of the neutralized protein becomes more predominant and induce aggregation [7]. Therefore, in the second step, coagulants are added and heating is usually applied to form soy protein tofu hydrogels. [8]. In food industry, there are many heat sensitive nutraceutical components, such as vitamin B_1 [9] and probiotics [10], which will lose activity under high temperature. However, from the process of soy protein tofu hydrogels making, it can be observed that heating is usually applied in the second step, which of course, limits soy protein tofu hydrogels' application as

* Corresponding author at: College of Food Science and Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, PR China.
E-mail address: huhao@mail.hzau.edu.cn (H. Hu).
[1] Co-first authors. The authors Peipei Zhang and Tan Hu contributed equally to this work.

http://dx.doi.org/10.1016/j.ultsonch.2015.10.014
1350-4177/

nutraceutical component carrier to provide heat-sensitive nutraceutical components. Interestingly, soy protein can form gels at cold temperature under certain conditions, which are called cold-set soy protein gels [4].

One type of cold-set soy protein gels is catalyzed by transglutaminase (EC 2.3.2.13, protein-glutamine γ-glutamyl transferase or TGase). TGase catalyzes an acyl transfer reaction between the γ-carboxyamide group of a peptide-bound glutaminyl residue and a variety of primary amines. When the amine-containing substrate is the ε-amino group of a peptide-bound lysyl residue, the resulting ε-(γ-glutamyl) lysine isopeptide bonds can lead to intermolecular covalent crosslinking of the peptide chains [11,12]. The 7S (β-conglycinin) and 11S (glycinin) fractions are the principal components accounting for 65–80% of the total soy proteins. The 7S fraction, mainly β-conglycinin, is consisted of subunits α′, α and β, while the 11S fraction, mainly glycinin, is consisted of polypeptides A and B [13]. Tang and colleagues [14,15] used TGase to form cold-set tofu and found that a majority of the subunits of β-conglycinin and the acidic subunits of glycinin in SPI were cross-linked by TGase.

High intensity ultrasound (HIU) (16–100 kHz, power in the range 10–1000 W cm^{-2}) has extensively been used in food industry [16–20]. Recent studies showed that HIU can be used to alter physicochemical properties of soy protein [13,21–24]. Our previous studies found that HIU could result in partial unfolding and reduction of intermolecular interactions as demonstrated by decreases in particle size as well as increases in free sulfhydryl groups and surface hydrophobicity, leading to improved solubility of SPI dispersions [25]. Moreover, we also showed that HIU of SPI increased the gelation property of heated gels formed by $CaSO_4$ [26] and glucono-δ-lactone [27]. The above information suggests the possibility of using HIU to increase the gelation property of SPI-TGase cold set gel. In this regard, Tang and colleagues [28] reported that HIU can lead to an increase in the G' property of transglutaminase-catalyzed soy protein isolate cold set gel (TSCG). However, the gelation enhancing mechanism was not well understood and to the best of our knowledge, little is known about the effects of HIU on the structural and physical properties of TSCG. Therefore, in this study, we investigated the structural and physical properties of TSCG as a function of HIU (105–110 W/cm^2 for 0–40 min), in order to provide some fundamental information on how HIU of SPI may influence TSCG properties, which may expand the applications of HIU in soy protein industry.

2. Material and methods

2.1. Materials

Commercial soy protein isolate (protein content > 90% as measured by the Kjeldahl method) was a gift from Yuwang Company (Shandong, China). Transglutaminase (Activa TI; 100 units of enzyme activity per gram of powdered preparation) was a gift from Ajinomoto North America, Inc. (Itasca, IL, USA). Protein content assay kit based on the Lowry method was obtained from the Labaide Company (Shanghai, China). Tris-base was bought from the Dow Chemical Company (Michigan, USA) while 5, 5′-dithiobis-(2-nitrobenzoic acid) (DTNB), glycine and sodium dodecyl sulfate (SDS) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All reagents were analytical grade.

2.2. High-intensity ultrasound treatment of soy protein isolate

HIU treatments were carried out according to our previous works [26]. SPI samples were dissolved in distilled water in 100 ml flat bottom conical flasks (10%, w/v) stirring at room temperature for 2 h. An ultrasound processor model JY92-2D (Ning Bo Scientz Biotechnology Co. Ltd, Ningbo, Zhejiang, China) with a 0.636 cm diameter titanium probe was used to sonicate 100 mL SPI dispersions. During the sonication process, the sonication probe was maintained at 1 cm under the surface of SPI dispersions and the flat bottom conical flasks were kept in an ice water bath to maintain the sample temperature between 14–20 °C. Samples were treated at 20 kHz at 400 W for 0, 5 or 40 min (pulse duration of on-time 5 s and off-time 1 s to avoid the probe damage). The HIU time differences were chosen based on the physicochemical (e.g. particle size, SH groups and surface hydrophobicity) changes of SPI (Yuwang Company Shandong, China) after HIU treatments at 20 kHz at 400 W according to our previous work [26]. After the ultrasonic treatments, SPI samples were freeze dried, and then stored in air tight containers until analyzed. The ultrasonic intensity, which was analyzed according to Hu, Fan et al. [27] using the method of Jambrak et al. [22] was 105–110 W cm^{-2} in this study.

2.3. Formation of TGase catalyzed SPI cold set gels (TSCG)

The gels were formed in cylindrical glass bottles (2.5 cm in diameter) by adding 9.1 mL of pH 7.5 0.05 M Tris–HCl buffer (containing 0.01% NaN_3) into 0.9 g HIU treated or non-HIU treated freeze-dried SPI powder, stirring for 1 h at room temperature, and then adding 0.9 mL TGase solution (20 units/mL solution prepared as 2 g Activa TI in 10 mL of pH 7.5 0.05 M Tris–HCl buffer containing 0.01% NaN_3) with rapid stirring for 10 s. The final concentrations of TGase and SPI were 20 units/g SPI and 9% (w/v), respectively. The mixtures were incubated at 37 ± 1 °C for 4 h and then kept overnight at 4 °C before the gels were used for further analysis.

2.4. Scanning electron microscopy (SEM)

The morphology of the TSCGs network was observed with a SEM (JSM–6390 LV, Japan) at an accelerating voltage of 15 kV. In preparation for analysis by SEM, the samples were critical point dried and coated with gold/palladium an argon atmosphere using a Balzers evaporator (model SCD 050, Baltec Lichtenstein, Austria) [27].

2.5. Determination of free sulfhydryl (SH) content

The free sulfhydryl (SH) contents of the samples were determined using Ellman's reagent (DTNB) according to the method described by Shimada & Cheftel [29,30], with some modifications. TSCG samples were dispersed in buffer B (0.086 M Tris, 0.09 M glycine, and 4 mM Na_2EDTA, pH 8.0) to reach the protein concentration of 2 mg/mL. The mixtures were homogenized (POLYTRON® PT 2100, Littau-Lucerne Switzerland) at 15,000 rpm for 1 min, then centrifuged at 20,000g for 15 min at 4 °C. To a 3-mL aliquot of the supernatant was added 0.03 mL of Ellman's reagent solution (4 mg of DTNB/mL of buffer B). After the solution was rapidly mixed and allowed to stand at 20 °C for 15 min, the absorbance was read at 412 nm. Buffer B was used instead of protein solutions as a reagent blank. A molar extinction coefficient of 1.36×10^{-4} M^{-1} cm^{-1} was used for calculating micromoles of SH/g of protein.

2.6. Determination of TSCG solubility

The protein solubility of TSCG in different solvents was determined by modification of the methods described by Shimada et al. [29,30]. TSCG samples were dispersed in various solvents to reach the protein concentration of 2 mg/mL. The solvents were prepared as follows: DW, deionized water at pH 8.0 (adjusted by