Effects of ultrasonic treatment on the gel properties of microbial transglutaminase crosslinked soy, whey and soy–whey proteins

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Abstract This paper studied the influences of diverse ultrasonic power treatments on the physico-chemical properties of soy–whey mixed protein induced by microbial transglutaminase. Two groups of 15% (m/v) of protein solution–sole protein (as control group) and mixed protein were prepared and processed under different ultrasonic powers for 30 min. After ultrasonic power treatments, gel properties were significantly increased: under 300 W, the gel hardness of mixed protein reached a maximum of 998.9 g, with its water binding capacity scoring a maximum of 87%. According to the analysis of fluorescence emission spectrum, the fluorescence intensity and maximum absorption peak had changed, for different ultrasonic power treatments had exposed more groups. The Fourier

Transform Infrared Spectroscopy also suggested that ultrasonic power treatments could change the secondary structure of gel samples. The scanning electron microscope demonstrated that the network structure of mixed protein gel displayed more regular and uniform after ultrasonic treatments.

Keywords Soy protein isolate · Whey protein isolate · Ultrasonic · Gel properties · Microbial transglutaminase

Introduction

Proteins are widely used as food ingredients because of their excellent nutritional and functional values (Belicium and Moraru, 2013). Using protein blend, especially animal and plant proteins, is gaining increasing interests in developing new foods. Whey protein, a group of globular protein form milk, had unique functional properties such as emulsification, gelation, thickening, foaming, fat and flavor combination (Bryant and McClements, 1998). On the other hand, soy protein contained two surface active substances, β -conglycinin and glycinin (Palazolo et al., 2011). The food industries often use the emulsification properties of whey protein and soy protein, ranging from meat emulsions and baby foods to liquid diet formulations and cosmetic manufacturers (Demetriades et al., 1997; Floury et al., 2002; Hunt and Dagleish, 1995). Whey protein is a good quality animal protein. It contained 8 essential amino acids, which was close to the proportion of the human body (Marshall, 2004). However, animal protein-derived foods contained harmful substances such as excessive saturated fat and cholesterol that were harmful to the human body (Ono, 2003). Excessive consumption may lead to an increase in body fat and cholesterol which can cause

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cardiovascular diseases. Soy protein isolate (SPI) was one of the most important economic and ready-to-use soy protein products. It was mainly composed of glycinin (11S) and β -conglycinin (7S), accounting for about 70% of the total protein content of SPI (Nishinari et al., 2014; Utsumi and Kinsella, 1985; Wagner and Anon, 1990). Among the plant proteins, only soy protein is well balanced amino acids, while the soy protein lacks methionine, and the soy protein is inferior to animal protein in absorption. However, the puffed protein has been limited due to the poor gelation of Whey protein isolate (WPI) and the poor emulsification of SPI, which seriously restricts the application of mixed protein in food. It would be very valuable to combine soy protein with whey protein to complement each other. The expansion of the application of SPI and WPI in non-food and food fields was unlimited (Qin et al., 2017). Therefore, having a better understanding of the structural and mechanical properties of protein blends (quality plant proteins and premium animal proteins) is critical, which can contribute to understand how these properties could benefit from protein mixture selectively (Jose et al., 2016).

Ultrasonic technology is a non-thermal treatment currently used in food processing. Many studies have showed the significant effects of ultrasound treatment on proteins. Chemat et al. (2011) reported ultrasonic treatment was used in food processing, preservation and extraction. The advantages of using ultrasound for food processing, including: faster energy and mass transfer, more effective mixing and micro-mixing, and reduced thermal and concentration gradients. Arzeni et al. (2012) observed the function of three proteins (Egg white protein, WPI and SPI) was altered significantly under ultrasonic treatment, which was closely related to protein hydrophobicity, particle size, aggregation and denaturation. Qin et al. (2016) reported that the gel properties of wheat gluten (WG) and Soy protein isolate (SPI) mixture were significantly improved by microbial transglutaminase (MTGase) hydrolysis combined with ultrasonic pretreatment. Fourier transform infrared (FTIR) spectroscopy showed that ultrasonic treatment changed the secondary structure of protein in gel. Sui et al. (2017) also found that ultrasonic treatments improved emulsifying properties of soy protein. The nutritional complementarity of soy protein and whey protein blend was that soy protein provided high- arginine and glutamine and whey protein provided highly branched chain amino acids (leucine, isoleucine and valine) (Mingyan, 2012). However, studies on the functional complementarity of soy and whey protein blend treated with ultrasound and MTGase have not been reported.

The aims of this work was to understand the effects of ultrasonic treatment on the physical structure, and water holding properties on soy/whey protein mixture and its

gelation property induced by MTGase. The soy protein and whey protein interactions were also studied using scanning electron microscope SEM and FTIR.

Materials and methods

Materials

Defatted soy flour was kindly provided by Harbin High Tech Co., Ltd. (Heilongjiang, China). Sodium dodecyl sulphate (SDS) from Sigma (St. Louis, MO, USA). All chemicals and reagents were of analytical grade.

Protein sample preparation

SPI and whey protein isolate (WPI) were obtained from Sigma (St. Louis, MO, USA). Protein powder was dispersed in phosphate buffer solution (0.01 M, pH 8.0), stirred (1000 rpm/min) at room temperature for 1 h to make solutions of 15% concentrations. For mixed protein solutions, the single protein solutions were mixed at different ratio with a high shear mixer (Ultra Turrax Model T25, IKA Works Inc., Wilmington, NC) at 5000 rpm for 5 min, then stored overnight at 4 °C.

Ultrasonic treatments

SPI dispersions (30 mL) in 50 mL flat bottom conical flasks were placed in an ice bath and then processed by an ultrasound processor model JY92-IIN (Ningbo Scientz Biotechnology Co. Ltd, Ningbo, Zhejiang, China) with a 0.636 cm diameter titanium probe. Samples were treated at 20 kHz and the ultrasonic treatments were performed at output intensity of 0, 150, 300, and 450 W, (pulse duration of on-time 1 s and off-time 1 s). The ultrasound treated samples were lyophilized and stored in air tight containers at room temperature.

Preparation of MTGase-induced gel

MTGase (Yiming Biological Products Co., Ltd, Jiangsu, China.) was added to the ultrasonic treated protein solutions at the rate of 50 U/g protein. The reaction was carried out in 45 °C water bath for 2 h using HH-4 magnetic stirring constant temperature water bath (Changzhou Saipu Electric Appliance Co., Ltd., Changzhou, Jiangsu, China). The reaction was terminated by heating the solution at 90 °C for 15 min to inactivate MTGase and then cooled down in ice water. The resulting protein gels were stored at 4 °C.

Texture profile analysis

Gel hardness, springiness, cohesiveness, chewiness and gumminess were analyzed using TA-XT2 texture analyzer (Stable Micro Systems Ltd., Godalming, UK) according to the method described by Kaewudom et al. (2013). A flat-surface cylindrical probe (Model P/0.5, 12 mm in diameter) was used and axially compressed penetration rate was set at 10 mm/s.

Water holding capacity (WHC)

The method for determining WHC was modified according to Tang et al.'s method (2011). Five grams of gel was added to a 10 mL centrifuge tube and centrifuged at 4000 rpm for 30 min (TGD-20MC, Mach Co. Ltd, Hunan, China). The supernatant was carefully poured out and weighed. The WHC was calculated using following equation.

$$WHC = ((W_t - W_r)/W_t) \times 100\%$$

where W_t was the total weight (g) of gel sample, and W_r was the weight (g) of the removed supernatant.

Particle size determination

Particle size was determined using a Mastersizer 2000 laser particle analyzer (Mastersizer 2000, Malvern Instrument Co., Ltd., Worcestershire, UK) according to the method of Arzeni et al. (2012). The particles refractive index and dispersion refractive index were regarded as 1.46 and 1.33, respectively. Results were expressed using the volume weighted mean diameter ($D_{[4, 3]}$) (Romero et al., 2009).

Fluorescence measurements

A 0.2 mg/mL protein solution was prepared using 10 mM phosphate buffer at pH 7.0 according to Alvarez et al. (2008) and Shen and Tang (2012), endogenous fluorescence was measured using a F-4500 fluorescence spectrometer (F4500, Hitachi, Tokyo, Japan). The excitation wavelength of the protein solution was 290 nm, the scanning speed was 12,000 nm/min, and emission spectra was recorded between 300 and 460 nm at a constant slit of 2.5 nm and the scan voltage was 450 mV, which was similar with excitation and emission. All the measurements were performed in triplicate.

Fourier transform infrared spectroscopy spectroscopy

FTIR spectroscopy was carried out according to the Zhang et al. (2017), The Freeze-dried samples (2 mg) were mixed with 200 mg KBr powder, then produced by pressing in

KBr windows at room temperature on a Carver press at 5–6 T pressure before measurement. The IR spectra were recorded with 64 scans at 4 cm^{-1} resolution from 4000 to 400 cm^{-1} on 8400S FTIR spectrometer (Shimadzu Corporation, Japan). Analysis of secondary structure changes in the amide I band from 1600 to 1700 cm^{-1} was performed using PeakFit 4.12 software.

SEM

Measurement of the MTGase-induced SPI–WPI gels (Hitachi, Ltd, Tokyo, Japan) was performed with a 5 kV accelerating voltage using an S-3400 scanning electron microscope where morphology of the samples was observed. To guarantee a proper thickness, gold ion sputter was commonly used to cover freeze-dried gel (Hitachi, Ltd, Tokyo, Japan).

Statistical analysis

All the experiments were carried out in triplicate and results were expressed as means $\pm$ standard deviations. Means were compared using one-way ANOVA of SPSS 20 Statistical software followed by Duncan's Multiple Range Test ($P < 0.05$). Origin 9 was used to generate figures.

Results and discussion

Texture profiles

Texture is one of the most essential indicators of protein gel as shown in Table 1. After ultrasonic treatment, the gel hardness of SPI–WPI mixed protein first increased then declined. Under 300 W, it reached a maximum of 998.93 N. As for the control group, the gel hardness of SPI and WPI increased as the power strengthens, hitting a maximum of 777.03 N and 782.13 N, respectively under 450 W. After ultrasonic power treatment, the gel of SPI–WPI mixed protein was prominently harder than that of the controlled group (SPI and WPI) and untreated samples (0 W) ($P < 0.05$). This is due to that the weak bonds among proteins took the dominant position, which contributed to the increase of gel hardness (Buamard and Benjakul, 2018). In addition, strengthening gel hardness could be attributed to more S–S bonds produced by the heat in ultrasonic treatment (Hu et al., 2013a; 2013b). As a result, a more densified, solid, stable and uniform gel network was formed after ultrasonic treatment. Under different ultrasonic power treatments, the springiness and cohesiveness between the SPI–WPI mixed protein and controlled group demonstrated little difference ($P > 0.05$). Gumminess represented the energy used for a semi-solid

Table 1 Effect of different ultrasonic power processing (0, 150, 300 and 450 W) on the textural properties and the water holding capacity (WHC) of MTGase-induced Soy protein isolate–Whey protein isolate

(S–W) mixed protein single Soy protein isolate (SPI) and single Whey protein isolate (WPI) gels

Treatment (W)	Hardness (N)	Springiness (cm)	Cohesiveness	Gumminess (N)	Chewiness (N cm)	WHC (%)
SPI ₀	331.29 ± 19.56 ^g	0.92 ± 0.02 ^c	0.71 ± 0.01 ^c	256.64 ± 17.62 ^h	288.34 ± 7.94 ^f	53.38 ± 4.19 ^h
WPI ₀	402.47 ± 19.89 ^g	0.96 ± 0.01 ^a	0.82 ± 0.01 ^b	273.54 ± 13.75 ^h	260.56 ± 10.17 ^g	69.17 ± 3.89 ^g
S–W ₀	863.32 ± 18.44 ^c	0.96 ± 0.01 ^a	0.85 ± 0.01 ^b	369.07 ± 6.21 ^g	310.86 ± 3.59 ^c	78.05 ± 3.21 ^c
SPI ₁₅₀	600.87 ± 19.56 ^{ef}	0.93 ± 0.01 ^c	0.73 ± 0.01 ^c	358.66 ± 11.85 ^g	312.32 ± 12.37 ^c	69.83 ± 2.67 ^g
WPI ₁₅₀	549.79 ± 11.78 ^f	0.97 ± 0.01 ^a	0.83 ± 0.01 ^b	373.21 ± 21.42 ^g	331.42 ± 6.84 ^{de}	74.84 ± 1.53 ^f
S–W ₁₅₀	838.97 ± 13.29 ^c	0.98 ± 0.01 ^a	0.83 ± 0.01 ^b	425.59 ± 19.44 ^{ef}	345.33 ± 11.22 ^d	81.61 ± 2.52 ^c
SPI ₃₀₀	733.83 ± 10.99 ^d	0.95 ± 0.02 ^b	0.75 ± 0.01 ^c	454.05 ± 21.59 ^{de}	351.78 ± 18.76 ^d	68.77 ± 1.17 ^g
WPI ₃₀₀	642.24 ± 8.25 ^c	0.98 ± 0.01 ^a	0.78 ± 0.01 ^c	547.32 ± 12.50 ^c	413.38 ± 5.87 ^b	77.76 ± 0.55 ^{de}
S–W ₃₀₀	998.93 ± 20.44 ^a	0.97 ± 0.01 ^a	0.90 ± 0.01 ^a	592.73 ± 10.18 ^b	377.2 ± 13.18 ^c	87.11 ± 1.11 ^a
SPI ₄₅₀	777.03 ± 16.65 ^{cd}	0.95 ± 0.01 ^b	0.77 ± 0.02 ^c	492.19 ± 19.83 ^d	402.202 ± 8.96 ^b	80.18 ± 1.46 ^c
WPI ₄₅₀	782.13 ± 11.32 ^{cd}	0.95 ± 0.01 ^b	0.92 ± 0.01 ^a	600.48 ± 20.12 ^b	539.81 ± 22.62 ^a	78.96 ± 1.44 ^{cd}
S–W ₄₅₀	986.71 ± 25.42 ^b	0.96 ± 0.02 ^a	0.89 ± 0.01 ^a	668.51 ± 15.51 ^a	418.15 ± 10.09 ^b	85.64 ± 1.28 ^b

Data represent mean ± standard deviations. Different lowercase letters superscripts in the same column indicate the significant differences of different sample in different ultrasonic power ($P < 0.05$)

food for swallowing. Chewiness represented the energy used for chewing the sample to the point that was able to swallow it (Buamard and Benjakul, 2018). When it came to the gumminess and chewiness, Buamard and Benjakul (2018) reported a growing trend along with the strengthened ultrasonic power in mixed protein and controlled group. Under 450 W, the gumminess of SPI–WPI mixed protein reached a maximum of 668.51 N while the chewiness of WPI gel reached a maximum of 539.81 (N cm). Compared with other power under 300 W, the mixed protein had a higher gel harness, springiness and chewiness. The result again proved that in post-ultrasonic-treatment situation, the interactions among mixed proteins could influence the gel structure and strengthen the gel properties of protein effectively.

Water holding capacity (WHC)

WHC can reflect the roughness of gel internal structure, which is considered as the most essential feature of protein gel. Within the gel system, WHC demonstrates the interactions between water and protein. As shown in Fig. 1 with MTGase crosslinks, different power of ultrasonic treatments could affect the water binding capacity of SPI–WPI protein and controlled group (SPI and WPI). As mixed protein, WHC first rose then declined in accordance with the increasing power, reaching a maximum of 87% under 300 W. When the ultrasonic power was 450 W, the maximum WHC values of SPI and WPI were 80% and 78%, respectively. According to ANOVA, untreated samples (0 W) had significant lower WHC compared with ultrasonic samples, indicating that ultrasonic treatments induced

by MTGase obviously improved WHC of SPI–WPI gel ($P < 0.05$) (Renzetti et al., 2008). This was due to the fact that the particle size of protein mixtures under ultrasonic treatments were reduced, leading to a more densified and uniform gel structure between two types of protein. Stable gel with highly densified pore structure could effectively hold water and enhance texture properties (Wu et al., 2011). Furthermore, after ultrasonic treatments, internal structures of protein mixtures and controlled group were fully exposed and were prone to form disulfide bond under MTGase, which formed a densified and stable three-dimensional structure. Based on Puppo and Anon (1998), protein gel with densified and stable structures had higher WHC than that with normal structure. Moreover, higher temperature during high power ultrasonic pretreatment might produce more S–S bonds. It might enhance the hardness so as to form a more delicate and densified gel network for higher WHC (Hu et al., 2013a; 2013b). As ultrasonic power uprated, WHC might not continue to rise, because excessive power or time might trigger the over-degeneration of protein molecule and the particle size of dispersion could re-aggregate and form larger pores in gel network. It accelerated the release of water molecules and lowered the WHC, which agreed with this experiment (Hu et al., 2013a; 2013b).

Particle size determination

Figure 1 showed the change of the particle size distribution and mean particle size of the control protein (SPI, WPI) and SPI–WPI mixed protein under ultrasonic treatment. Particle size distribution and mean particle size were

Fig. 1 Effect of different ultrasonic power processing (0, 150, 300 and 450 W) for 30 min on the particle size distributions of SPI–WPI mixed protein (A), single SPI (B), and single WPI (C), and de

Brouker mean diameter $D_{[4,3]}$ (D). Means ($n = 3$) between samples without a common letter (a–h) differ significantly ($P < 0.05$)

considered to be pivotal parameters affecting certain structural and physicochemical properties of protein gels including rheology, stability, and chemical reactivity (McClements, 1996). Sui et al. (2017) reported when ultrasonic waves were across protein, the phenomena that particle disruptions were re-coalesced and re-arranged might happen simultaneously.

The particle size distribution was shown in Fig. 1(A–C). Untreated (0 W) samples of SPI–WPI and SPI showed a bimodal particle size distribution. The SPI–WPI and control samples showed a significant decrease in sample particle size with the ultrasonic power increased ($P < 0.05$) as shown in Fig. 1, and the samples presented a unimodal particle size distribution with different peak values just as Jambrak et al. (2009) observed, ultrasonic with probe (20 kHz) for 15 or 30 min revealed that the ultrasonic treatment reduced the size of the soy protein solution particles. For the ultrasonic treatment samples (150 W,

300 W, 450 W) for 30 min it showed that there was a small but significant decrease in the particle size when compared with the untreated (0 w) samples (Figs. 1A, 2B). Sample SPI–WPI 450 W demonstrated smallest particle size and narrowest peak, which indicated its best stability. In terms of the higher ultrasonic power (300, 450 W), the particle size distribution of treated WPI showed no significant decreases (Fig. 1C). Gordon and Pilosof (2010) had the similar results and they found that the size of the WPI gel after ultrasonic treatment was reduced less abrupt after 12.5 min of ultrasonic treatment.

The particle sizes represented as $D_{[4,3]}$ is mean volume diameter (Fig. 1D). The mean particle size of untreated SPI was 381.8 nm. However, it was reduced to 101.6 nm after ultrasonic treatment (450 W). The particle size of untreated WPI was 290.2 nm, which suggested that ultrasonic treatment caused WPI mean particle size decrease to 189.4 nm, but SPI–WPI had a different tendency, the mean particle

Fig. 2 Effect of different ultrasonic power processing (0, 150, 300 and 450 W) on the protein fluorescence spectrum scanning spectra of SPI–WPI mixed protein (A), single SPI (B), and single WPI (C)

size showed a trend of decreasing first and then increasing, and reaching the minimum (154.2 nm) when ultrasonic treatment was 300 W. Arzeni et al. (2012) also obtained

the result that the particle size increased first and then decreased after ultrasonic treated. Hu et al. (2013a; 2013b) suggested that the possible reason was that heat effects caused by prolonged ultrasonic treatment (30–40 min) might result in increasing particle size.

Intrinsic fluorescence emission spectroscopy analysis

The intrinsic fluorescence is depending on environment polarity of specific interactions between tyrosine (Tyr)/tryptophan (Trp) residues or Tyr/Trp. Therefore, it was often used as a sensitive method to characterize changes in proteins and their spatial structure (Zhang et al., 2014). Setting excitation wavelength at 290 nm, the fluorescence emission spectrum of SPI–WPI, SPI and WPI were shown in Fig. 2 respectively. When the ultrasonic power exceeded 300 W, the maximum absorption wavelength (λ_{max}) of SPI–WPI mixtures was 346.6 nm and then maintained to be stable (Fig. 2A). When the power reached 450 W, the maximum absorption wavelength (λ_{max}) of SPI wavelength was 350.6 nm, while that of WPI was 340.6 nm. The maximum fluorescence absorption wavelengths (λ_{max}) of untreated SPI and WPI were 348.6 nm and 339.6 nm, respectively (Fig. 2B, C). The result showed that the peaks of protein fluorescence emission arose red shifts, which indicated that the conformation was changed in treated protein system and fluorophores shifts to a more hydrophobic environment (Dufour et al., 1994). As the ultrasonic power increased, more chromophore exposure in the solvent made the protein interactions easier, thus causing disruption of the microenvironment of amino acids in protein molecules (Pallarès et al., 2004). Zhang et al. (2017) also found that after ultrasonic treated the protein solution, the internal groups of the protein were exposed and the fluorescence intensity increased. Increased the fluorescence intensity could be attribute to the fact that the structure of mixed protein was fully unfolded under high ultrasonic power treatments, and the chromophore within the protein was exposed to the solution, leading to further stretch itself of the tertiary structure of protein. When the ultrasonic power exceeded 450 W, the relative fluorescence intensity reduced significantly, which was because the destructiveness of protein structure resulted in the exposure of abundant chromophores to the solvent (Shen and Tang, 2012).

FTIR spectroscopy

Figure 3 showed FT-IR spectras (4000–400 cm^{-1}) of SPI–WPI, single SPI and single WPI gel samples with MTGase-induced ultrasonic treatment. Zhang et al. (2014) reported the amide I region (1600–1700 cm^{-1}) on the FTIR spectra, which represented primarily the C=O flexing oscillation of

Fig. 3 Effect of different ultrasonic power processing (0, 150, 300 and 450 W) on the FTIR spectra of MTGase-induced SPI–WPI mixed protein (A), single SPI (B), and single WPI (C) gels

the amide groups (coupled to in-plane bending of stretching of the C–N bonds and the N–H). Secondary structure was closely related to the absorption peak, the relationships were as follows: α -helices with a frequency around $1650\text{--}1660\text{ cm}^{-1}$; β -sheet in the frequency region of $1618\text{--}1640\text{ cm}^{-1}$ and $1670\text{--}1690\text{ cm}^{-1}$; $1660\text{--}1670\text{ cm}^{-1}$ and $1690\text{--}1700\text{ cm}^{-1}$ for β -turn; and $1640\text{--}1650\text{ cm}^{-1}$ for random coil (Shen and Tang, 2012).

In this paper, the changes of secondary structure of protein upon ultrasonic treatments were observed. Table 2 showed the proportion of amide I regions within the samples components of the secondary structure. With ultrasonic power increasing, the treated SPI–WPI samples showed a decrease in α -helix (from 24.94% to 17.12%), an increase in β -sheet (from 32.53% to 35.81%) and random coil (from 21.21% to 24.77%). The increase in the α -helix structure content and the decrease in the β -sheet structure content of the treated SPI samples are consistent with the literature (Dufour et al., 1994). The treated WPI samples showed a reduce in α -helix (from 24.06% to 16.15%), an increase in β -turn (from 11.68% to 15.22%) and random coil (From 18.92% to 21.43%). Variations in β -sheet and α -helix content changed the interaction between different proteins, and exposed the hydrophobic site of the protein, thus resulting in an increase in gel viscoelasticity (Zhao et al., 2008). In addition, the increase of β -sheet and β -turn content might be regarded as an important and indispensable factor to contribute to formation of the aggregate system and gels network (Qin et al., 2017). Due to ultrasonic cavitation, the decrease in alpha-helix content may be related to aggregation between SPI and WPI and formation of intermolecular disulfide bonds. Herrero et al. (2011) reported that the formation of disulfide bonds was due to the partial unfolding of globular protein structures (such as whey proteins) and the accelerated polymerization of sulfhydryl groups after adsorption to the interfacial layer (Cui et al., 2013). Moreover, the content of random coil of untreated samples SPI–WPI was increased at the expense of the α -Helix, which was concluded that ultrasonic could change the internal structure of WPI and SPI and had an effect on the interaction between different proteins, thus contributing to the accessibility of proteins and enzymatic cross-linking (Wang et al., 2014). After ultrasonic treatment at 450 W, the α -helix content was significantly reduced. The gel structure formed was disordered and unevenly distributed, and the gel hardness and elasticity were lowered.

Scanning electron microscopy (SEM)

Scanning electron microscopy was used to reveal the effect of different ultrasonic powers on the gel three-dimensional network of SPI, WPI and SPI–WPI induced by MTGase.

Table 2 Effect of different ultrasonic power processing (0, 150, 300 and 450 W) on the secondary structure analysis of MTGase-induced SPI–WPI mixed protein, single SPI, and single WPI gels

Sample	Secondary structure composition (%)			
	α -Helix	β -Sheet	β -Turn	Random coil
S-W ₀	23.94 $\pm$ 0.02 ^a	33.53 $\pm$ 0.01 ^d	22.53 $\pm$ 0.02 ^e	20.21 $\pm$ 0.01 ^d
S-W ₁₅₀	20.12 $\pm$ 0.05 ^b	30.01 $\pm$ 0.02 ^e	27.65 $\pm$ 0.03 ^a	22.23 $\pm$ 0.01 ^c
S-W ₃₀₀	19.1 $\pm$ 0.13 ^c	29.81 $\pm$ 0.11 ^e	26.41 $\pm$ 0.12 ^b	24.68 $\pm$ 0.05 ^a
S-W ₄₅₀	17.12 $\pm$ 0.21 ^d	33.81 $\pm$ 0.12 ^d	26.31 $\pm$ 0.13 ^b	23.77 $\pm$ 0.22 ^b
SPI ₀	10.32 $\pm$ 0.01 ^h	45.15 $\pm$ 0.01 ^b	25.17 $\pm$ 0.02 ^c	19.35 $\pm$ 0.02 ^e
SPI ₁₅₀	12.27 $\pm$ 0.12 ^g	46.44 $\pm$ 0.21 ^a	23.9 $\pm$ 0.11 ^d	17.27 $\pm$ 0.21 ^f
SPI ₃₀₀	13.25 $\pm$ 0.12 ^f	42.32 $\pm$ 0.12 ^c	27.28 $\pm$ 0.05 ^a	17.16 $\pm$ 0.08 ^f
SPI ₄₅₀	15.28 $\pm$ 0.03 ^e	45.39 $\pm$ 0.02 ^b	23.02 $\pm$ 0.04 ^d	16.3 $\pm$ 0.06 ^g
WPI ₀	24.06 $\pm$ 0.12 ^a	45.34 $\pm$ 0.24 ^b	11.68 $\pm$ 0.11 ^h	18.92 $\pm$ 0.21 ^e
WPI ₁₅₀	20.29 $\pm$ 0.01 ^b	46.69 $\pm$ 0.01 ^a	14.03 $\pm$ 0.02 ^g	19.32 $\pm$ 0.02 ^e
WPI ₃₀₀	17.87 $\pm$ 0.11 ^d	46.83 $\pm$ 0.13 ^a	15.61 $\pm$ 0.01 ^f	20.22 $\pm$ 0.21 ^d
WPI ₄₅₀	16.15 $\pm$ 0.11 ^d	47.2 $\pm$ 0.14 ^a	15.22 $\pm$ 0.02 ^f	21.43 $\pm$ 0.01 ^c

Value are mean $\pm$ standard error (n = 3). Different superscripts in the same column indicate the significant differences ($P < 0.05$)

Fig. 4 Effect of different ultrasonic power processing (0, 150, 300 and 450 W) on the SEM images of MTGase-induced SPI–WPI mixed protein (A–D), single SPI (E–H), and single WPI (I–L) gels

Figure 4 demonstrated a set of SEM figures of ultrasonic-treated SPI–WPI, SPI and WPI induced by MTGase. It was observed that the MTGase cross linking higher ultrasonic power gel had a significantly gel network with a more homogeneous denser gel network and smooth structure (Fig. 4C, D, G, H, K, L). Hu et al. (2013a; 2013b) presented that the significant difference of gel networks might be attributed to the decrease of protein particle size resulted by ultrasound cavitation phenomenon. But compared with the SPI and WPI of the two control groups, the MTGase

cross linking SPI–WPI of ultrasonic treatment exposed more protein groups, and mixture interactions could contribute uniform gels which had to have a more stable network with the firm and smaller voids (Fig. 4A–D) (Li et al., 2014). Because the gel formed had a more regular and ordered gel network, these gels could have higher strength and absorb more water. This was showed that the well WHC had higher breaking force and hardness. These results might correspond to Zhu et al. (2014). He reported that added MTGase presented a more homogeneous and

dense network, with smaller and more evenly distributed holes. Jose et al. (2016) showed the consequences of mixing WP and SP for the gel macroscopic properties could be related to changes in microstructure. Ringgenberg et al. (2013) had shown that gel properties were closely related to protein aggregates, and larger aggregates could affect the network structure of the gel. Hence, when the ultrasonic power continued to rise, the protein re-aggregates and the structure of the gel was destroyed to varying extents, so that the gel structure was no longer uniform and densified in terms of microstructure.

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