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ARTICLE

Effect of Ultrasonic Treatment on the Graft Reaction between Soy Protein Isolate and Gum Acacia and on the Physicochemical Properties of Conjugates

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Soy protein isolate (SPI) was grafted with gum acacia (GA) in this work. In comparison with classical heating at high water activity, ultrasound could accelerate the graft reaction between SPI and GA. A degree of graft (DG) of 34 was obtained by ultrasonic treatment for 60 min, whereas 48 h was required with classical heating. Ultrasonic treatment improved the concentration of available free amino groups of SPI. The grafted SPI showed significantly ($p < 0.05$) higher levels of emulsifying activity index, emulsifying stability index, and surface hydrophobicity than native SPI. The droplet size ($D[3,2]$ and $D[4,3]$) of SPI emulsion decreased from 8.3 to 2.3 μm and from 25.7 to 7.1 μm through ultrasound-assisted grafting, respectively. Moreover, SPI−GA conjugates gave emulsions exhibiting more stability against creaming than those prepared with only SPI during ambient temperature storage (20 days). Decreases of lysine and arginine contents during the graft reaction indicated that these two amino acid residues attended the covalent linkage between SPI and GA. The results of secondary structure suggested that grafted SPI had decreased α-helix and β-sheet levels and increased unordered coils level.

KEYWORDS: Soy protein isolate; acacia gum; graft reaction; ultrasonic treatment

INTRODUCTION

Generally, the functional properties of proteins are of key interest to manufacturers of pharmaceutical, food, and cosmetic products. They are influenced by the molecular structure, which determines the inter- and intramolecular interactions, such as formation of covalent and/or noncovalent bonds (*1*). The structure−function relationship of proteins may be altered during processing due to oxidation, glycosylation, hydroxylation, phosphorylation, methylation, and acylation. These reactions depend on the changes in process temperature and mechanical forces, such as high pressure or shear (*2*, *3*).

The use of soy proteins as emulsifiers or emulsion stabilizers in food formulations is increasing, ranging from meat emulsions and baby foods to liquid diet. Recently, some researchers have attempted to improve the functional properties of proteins through protein−polysaccharide graft reactions, which are based on Maillard reactions between the amino groups of proteins and the reducing-end carbonyl groups of polysaccharides (*4*−*10*). Previous research on protein−polysaccharide graft reactions or Maillard-type reactions showed good performance (*4*−*9*). However, protein−polysaccharide graft reaction is time-consuming when using classical heating, such as water baths and heating jackets (*10*). Therefore, it is necessary to find a novel technology to improve the efficiency of graft reactions.

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Application of an ultrasonic technique greatly increases in the food industry both analysis and processing times (*1*, *11*, *12*). The considerable interest in low-frequency ultrasound is due to its promising effects on food processing and preservation, such as high product yield, short processing time, low operating and maintenance costs, improved taste, texture, flavor, and color, and reduced pathogens at low temperature (*13*). Ultrasound is able to produce these effects through the physical, mechanical, and chemical effects of acoustic cavitation. This involves the formation, growth, and violent collapse of small bubbles in liquid as a result of acoustic pressure fluctuation. Cavitation can accelerate chemical reactions, increasing diffusion rates, dispersing aggregates, and breaking down small particles and polymeric materials (*14*).

In this work, soy protein isolate (SPI) was grafted with gum acacia (GA) with the assistance of ultrasonic wave. The emulsion capability, solubility, and surface hydrophobicity of grafted SPI were investigated. The droplet size distribution of emulsion was measured to observe the size change before and after graft reaction. Moreover, the secondary structure and amino acid composition were determined.

MATERIALS AND METHODS

Materials and Chemicals. SPI was prepared from low-temperature defatted soy flakes (purchased from Shandong Yuwang Industrial Co. Ltd.) according to the improved method of Sorgentini and Wagner (*15*). The powder composition of SPI was (g/100 g of powder) 91.2% protein,

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5.6% moisture, 4.5% ash, and 0.5% fat. GA (type SD) was obtained from TIC Gums Co. (Philadelphia, PA). The polysaccharide was solubilized from the exuded pellets from *Acacia senegal* trees and filtered before spray-drying. The powder composition was (g/100 g of powder) 2.13% protein, 85.27% polysaccharide, 10.59% moisture, and 2.01% ash. Soy oil was obtained from a local supermarket and used directly without further purification. Bovine serum albumin (BSA) was purchased from Huamei Biotechnology Co., Ltd. (Luoyang, China). *o*-Phthaldialdehyde (OPA) and L-leucine were obtained from Sigma-Aldrich (St. Louis, MO). All other reagents were of analytical grade.

Preparation of the SPI−GA Conjugates. SPI (1%, w/v) and GA (1%, w/v) were dispersed in phosphate buffer solution (0.2 M, pH 7.5) and then stirred for 1 h in a water bath to reach the required temperature. Then the slurry (150 mL) was treated by an ultrasonic equipment (15 kHz probe, Newpower Ultrasonic Equipment Co., Ltd., Guangzhou, China) for different times (5, 10, 15, 20, 30, 40, or 60 min) at different temepratures (40, 60, 80, or 90 °C) and ultrasonic powers (100, 200, 300, or 400 W). A probe with a vibrating titanium tip of 1.5 cm was immersed in the slurry. The ultrasound irradiation was produced directly from the horn tip. Thereafter, the slurry was cooled to ambient temperature and dialyzed at 4 °C for 24 h. Finally, the samples were freeze-dried and stored at −20 °C. The slurry without ultrasonic treatment was used as control. Furthermore, the same slurry was conducted by classical heating in a water bath at 80 °C for different times. The subsequent procedure was the same to ultrasonic treatment.

Determination of Free Amino Groups. When SPI (0.2% in protein, w/v) was treated by classical heating or ultrasonic treatment for 0, 20, 40, or 60 min, the level of free amino groups was determined by a modified OPA method (*16*). Meanwhile, the levels of free amino groups from reaction mixtures were also determined in the same way. The OPA reagent was prepared by mixing 40 mg of OPA (dissolved in 1 mL of methanol), 25 mL of 100 mM sodium tetraborate, 2.5 mL of 20% (w/w) sodium dodecyl sulfate (SDS), and 100 μL of β-mercaptoethanol and then diluting to a final volume of 50 mL with distilled water. Sample solution (200 mL) was incubated with 4 mL of OPA reagent at 35 °C for 2 min. The absorbance at 340 nm was measured by a Spectrumlab 22PC spectrophotometer (Shanghai Lengguang Technology Co. Ltd., Shanghai, China). A calibration curve was obtained using 0.25−2 mM L-leucine. Degree of graft (DG) was calculated using the equation

$$\mathrm{DG} = (A_0 - A_t)/A \times 100\%$$

where A_0, A_t, and A are the levels of free amino groups in SPI and GA mixtures, conjugates, and SPI, respectively.

Emulsion Activity and Stability. The emulsifying activity index (EAI) and emulsifying stability index (ESI) were measured following a procedure described by Pearce and Kinsella (*17*). The continuous phase (0.2% in protein, w/v) of emulsions was first prepared by vigorous and continuous agitation of samples in phosphate buffer solution (0.2 M, pH 7.5). Oil-in-water (o/w) emulsions were prepared by adding 1 mL of soy oil in 3 mL of protein−polysaccharide solution with continuous agitation, and the resulting crude emulsion was homogenized for 1 min using an Ultra-Turrax T25 homogenizer (IKA Labortechnik, Staufen, Germany) equipped with a dispersing tool (S25KG-25F) at 20000 rpm. Then 50 μL of the emulsions was pipetted into 5 mL of phosphate buffer solution (0.2 M, pH 7.5) with 0.1% SDS. After emulsion formation, the absorbance was measured at 500 nm at 0 (A_0) and 10 min (A_{10}), respectively. EAI and ESI were calculated using the formulas

$$\mathrm{EAI}\ (\mathrm{m^2/g}) = 2TA_0 \times \text{dilution factor}/c \times \Phi \times L \times 10000$$

$$\mathrm{ESI}\ (\mathrm{min}) = A_0/(A_0 - A_{10}) \times 10$$

where $T = 2.303$, dilution factor = 1000, c is the weight of protein per volume (g/mL), L is the width of the optical path (0.01 m), and Φ is the oil volumetric fraction (0.25).

Droplet Size Distribution. The continuous phase (2% for samples, w/w) of emulsions was first prepared by vigorous and continuous agitation of samples in distilled water (adjusting the pH to 7.5). O/w) emulsions were prepared by adding 20 g of soy oil in 180 g of sample with continuous agitation, and then the resulting crude emulsion was homogenized by using a pressure homogenizer (APV Gaulin, Abvertslund, Denmark). Soy oil and sample dispersions were mixed and homogenized at 30 MPa. Two passes were used to ensure uniform mixing of the oil and protein solutions. The oil droplet size distributions were evaluated with dynamic light scattering using a Malvern Mastersizer 2000 unit (Malvern Instruments Ltd., Malvern, U.K.). Measurements were carried out at room temperature, and the volume fraction of emulsion in the diluted deionized water was approximately 1:1000 in all cases. The droplet size distribution of emulsions was measured for different times of ambient temperature storage (0 and 20 days).

Protein Solubility at Different pH Levels. Protein solubility was determined by dispersing the samples in distilled water to obtain a final solution of 0.2% (w/w) in protein. The pH values of the protein solution were adjusted from 9 to 3 and then centrifugated at 12000*g* for 30 min (20 °C). The content of protein for the resulting solution was analyzed according to the Lowry method (*18*).

Surface Hydrophobicity (H_0). H_0 values of samples were determined using 1-anilinonaphthalene-8-sulfonic acid (ANS) as the fluorescence probe in the absence of SDS (*19*). Protein dispersions were diluted (0.005, 0.01, 0.02, 0.05, 0.1, and 0.2%) in phosphate buffer solution (0.2 M, pH 7.5). Then, an aliquot of ANS solution (20 mL, 8.0 mM in the same buffer) was added to 4 mL of sample. Fluorescence intensity was measured with a Hitachi F4500 fluorescence spectrometer (LS55) at wavelengths of 390 nm (excitation) and 470 nm (emission). The initial slope of fluorescence intensity versus protein concentration plot was used as an index of H_0.

Analysis of Amino Acids. SPIs before and after graft were acid hydrolyzed at 110 °C for 24 h in 6 M HCl in vacuum-sealed tubes (*20*). The lysine and arginine levels were determined by an HP1100 system (Agilent Co.).

Analysis of Circular Dichroism Spectrum. An MOS-450 circular dichroism spectrometer equipped with a Peltier element (Bio-Logic Science Instruments, Grenoble, France) was used for circular dichroism analysis. For steady state investigation, spectra were recorded at a protein concentration of 0.1 mg/mL using a 10 mm path length quartz cuvette at room temperature in the far UV (190−250 nm) region. Secondary structure was interpreted by visual assessment of the spectra and calculation of the computer program CDPro (http://lamar.colostate.edu/sreeram/CDPro/main.html). Four secondary structures, α-helix, β-sheet, β-turns, and unordered coil, were calculated.

Statistical Analysis. All of the tests were performed in triplicate, and the results are given as means ± standard deviations. Duncan's multiple-range test was used to evaluate significant differences ($p < 0.05$) between results.

RESULTS AND DISCUSSION

Effect of Temperature and Ultrasonic Power on Graft Reaction. The graft reactions between SPI and GA were conducted at different temperatures and ultrasonic powers. As shown in **Table 1**, positive effects on DG were observed for both temperature and ultrasonic power. Increase of temperature could give high DG values for the Maillard-type reaction between SPI and GA. Brown color was developed after a certain time of heating, which was originated from the Maillard-type reaction. The reaction between free amino groups of proteins and the reducing end carbonyl group of sugars could increase the color intensity (*10*). Too high temperature and ultrasonic power would lead to serious browning of conjugates.

The EAI and ESI values of grafted SPI−-GA obtained at different reaction conditions for 40 min are shown in **Table 1**. Results showed that SPI−GA conjugates gained by different temperatures and ultrasonic powers had different EAI and ESI values. The highest EAI (91.13 $\mathrm{m^2/g}$) was obtained at a temperature of 80 °C and an ultrasonic power of 100 W, whereas the highest ESI (19.42 min) was observed at 80 °C and 200 W. By considering the EAI and ESI values, a temperature of 80 °C and an ultrasonic power of 200 W were chosen for the following expeirments.

Table 1. DG, EAI, and ESI of Grafted SPI−GA at Different Reaction Conditions for 40 min[a]

temperature (°C)	power (W)	DG (%)	EAI (m^2/g)	ESI (min)
40	100	1.75 ± 0.01j	57.13 ± 2.17d	10.23 ± 0.93hi
	200	5.71 ± 1.33i	53.51 ± 0.35e	13.14 ± 1.03def
	300	7.13 ± 0.86i	47.69 ± 0.33g	11.21 ± 1.59gh
	400	11.28 ± 0.49h	38.57 ± 1.28hij	11.17 ± 1.33gh
60	100	7.32 ± 0.37i	40.47 ± 0.75hi	13.45 ± 0.76de
	200	13.14 ± 1.17gh	52.26 ± 3.33ef	15.16 ± 0.08bc
	300	19.03 ± 1.59f	49.77 ± 1.57fg	11.39 ± 0.37gh
	400	23.08 ± 0.93e	40.84 ± 0.53h	10.16 ± 0.43hi
80	100	15.27 ± 1.03g	91.13 ± 4.18a	14.19 ± 1.31cd
	200	33.27 ± 2.06d	88.75 ± 1.36a	19.42 ± 0.52a
	300	37.57 ± 1.33c	73.16 ± 1.26c	15.78 ± 0.18b
	400	43.27 ± 1.07b	76.80 ± 1.47b	12.42 ± 0.74efg
90	100	33.32 ± 0.37d	40.16 ± 0.45hi	12.19 ± 1.07efg
	200	36.14 ± 1.13c	37.43 ± 0.22ij	11.83 ± 0.17fg
	300	44.13 ± 1.59b	36.71 ± 0.05jk	9.06 ± 0.41ij
	400	47.17 ± 2.73a	33.90 ± 1.46k	8.13 ± 0.13j

[a] Results having different letters in one column are significantly different ($p < 0.05$).

Table 2. Levels of Free Amino Groups of SPI after Ultrasonic Treatment and Classical Heating for Different Times

	mM/mL			
treatment	0 min	20 min	40 min	60 min
classical heating	0.86 ± 0.01	0.87 ± 0.02	0.87 ± 0.02	0.87 ± 0.02
ultrasonic treatment	0.86 ± 0.01	0.90 ± 0.01	0.93 ± 0.01	0.98 ± 0.003

Determination of Free Amino Groups. The level of free amino groups was determined for SPI after different treatments. When SPI was heated by classical heating in the absence of polysaccharides, the concentrations of free amino groups were not significantly ($p > 0.05$) changed (**Table 2**). However, when SPI was treated by ultrasonic treatment, the concentrations of free amino groups were increased gradually from 0.86 to 0.98 mM/mL. The increase of free amino groups implied that ultrasonic treatment broke peptide bonds of SPI by hydrolysis. We concluded that ultrasonic treatment was helpful for giving more free amino groups for graft reaction with GA. During ultrasound treatment, a great number of cavitation bubbles are produced, which increase the local temperature and pressure in the surrounding area of collapsing bubble. This leads to unfolding of protein and breaking of peptide bonds by hydrolysis. This treatment induces a decrease in the molecular weight of proteins, resulting in larger areas of proteins exposed to water molecules (*21*). Jambrak et al. (*14*) have also predicted that high-intensity ultrasound leads to an increase of solubility through decreasing protein molecular weight.

Degree of Graft (DG). The graft reactions between SPI and GA were conducted by classical heating and ultrasonic treatment. The DG values at different times are shown in **Table 3**. It was obvious that classical heating took much longer to obtain a specific DG value than ultrasonic treatment. A DG of 34.11 was obtained by ultrasonic treatment for 60 min, whereas a DG of 33.89 was reached by classical heating for 48 h. Moreover, the color of SPI−GA obtained by classical heating was more brown than by ultrasonic treatment, when the same DG was reached (results not shown here).

The thermal, mechanical, and chemical effects of high-intensity ultrasound are attributed to the rapid formation and collapse of cavitational bubbles in liquid at high water activity, which generate intensive shear stress (*22*, *23*). These bubbles collapse in the positive pressure cycle and produce highly turbulent flowing conditions and extremely high pressure and temperature. Transient temperatures of up to 5000 K and pressures up to 1200 bar have been calculated (*24*). The ultrasound was able to support more energy and free amino groups for the process of graft reaction, which were helpful for the Maillard reaction of protein and polysaccharide. Meanwhile, local relative translational motions induced by the ultrasound might allow reactive groups to be brought into closer proximity. During ultrasound treatment, there was rapid molecule movement due to cavitation and unfolding of protein chains, leading to hydroxyl radical development of water hydrolysis (*25*, *26*) and polymerization.

Table 3. DG Values (Percent) of SPI and GA by Ultrasonic Treatment and Classical Heating

SPI−GA conjugates by classical heating		SPI−GA conjugates by ultrasonic treatment	
time (h)	DG	time (min)	DG
4	12.99 ± 3.53	5	2.52 ± 0.42
8	24.33 ± 1.98	10	13.85 ± 0.67
12	25.53 ± 3.35	15	15.11 ± 1.05
16	28.65 ± 1.92	20	17.13 ± 2.49
24	32.96 ± 1.32	30	22.17 ± 0.73
32	34.08 ± 0.24	40	33.27 ± 2.06
48	33.89 ± 0.93	60	34.11 ± 0.73

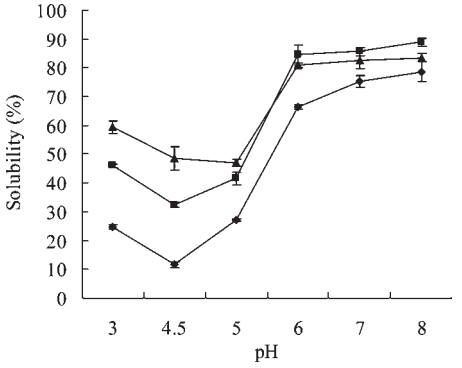

Figure 1. Protein solubility of (◆) SPI, (■) SPI−GA conjugates obtained by classical heating for 24 h, and (▲) SPI−GA conjugates obtained by ultrasonic treatment for 40 min. The curves represent the averages of three replications.

Protein Solubility. Solubility is one of the most important physicochemical properties of proteins. It can influence other functional properties, such as emulsifying and gelling properties (*27*). The pH−solubility profiles of samples are shown in **Figure 1**. The results showed SPI had the lowest solubility at pH 4.5. The solubility of SPI was improved remarkably after grafting with GA. However, SPI−GA conjugates obtained by ultrasonic treatment showed better solubility than those obtained by classical heating. Furthermore, SPI−GA conjugates obtained by different treatment had no obvious isoelectric point.

Generally speaking, the solubility of proteins decreases at pH around its p*I*. At this point, the net charge of proteins is near zero and the proteins tend to aggregate due to the electrostatic interactions caused by the charge asymmetry of the proteins (*28*). Kobayashi et al. (*29*) found that protein−polysaccharide conjugates had more hydrophilic groups than proteins themselves. GA is a water-soluble gum (*30*). The combination of SPI and GA brought SPI remarkable improvement in solubility. Meanwhile, ultrasonic treatment led to unfolding of proteins and breaking of peptide bonds. The exposure of hydrophilic amino acid residues

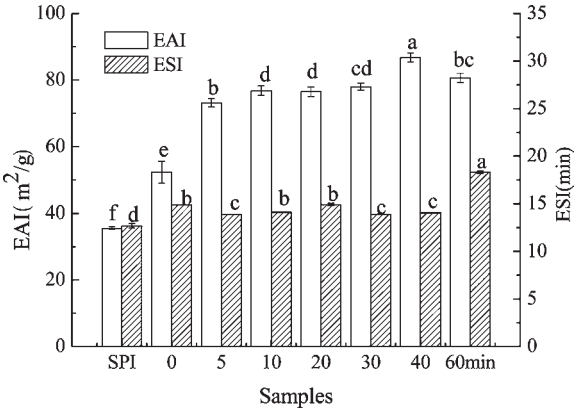

Figure 2. EAI and ESI values of SPI and SPI−GA conjugates at different times of ultrasonic treatment. Different letters (a−f) on the tops of columns indicate significant ($p < 0.05$) differences among samples. 0 represents SPI−GA conjugates obtained by classical heating for 24 h; 5, 10, 20, 30, 40, and 60 min represent the times needed to produce SPI−GA conjugates by ultrasonic treatment at pH 7.5.

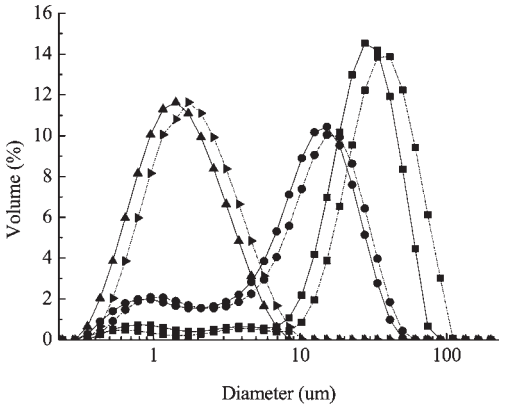

Figure 3. The droplet size distribution of SPI (■) and SPI−GA conjugates obtained by classical heating for 24 h (●) and by ultrasonic treatment for 40 min (▲) during ambient temperature storage (— and · · · · represent ambient temperature storage for 0 and 20 days, respectively).

in the inner site of proteins also contributes to the increase of protein solubility (*21*).

Emulsifying Properties. Emulsions are thermolabile systems from the physicochemical viewpoint, which can be rapidly or slowly separated into two immiscible phases according to the kinetic stability. Physical destabilization mechanisms of emulsions include size variation processes of oil droplet, such as flocculation, coalescence, and particle migration phenomenon (*31*).

The EAI and ESI of grafted SPI−GA at different reaction times of ultrasonic treatment are shown in **Figure 2**. With the extension of reaction time, the grafting product of SPI and GA showed a gradual and significant ($p < 0.05$) increase of EAI and reached the highest value at 40 min. Compared with classical heating, the graft reaction between SPI and GA could significantly ($p < 0.05$) increase the EAI of SPI. EAI of SPI−GA, which was grafted by ultrasonic treatment for 40 min after preheating, was increased by 65.98 and 143.94% as compared with classical heating and SPI, respectively. Moreover, ESI was increased by 23.05 and 44.3% as compared with classical heating and SPI by ultrasonic treatment for 60 min. When treatment by ultrasound for 60 min was used, ESI was higher than that for 40 min, which could be concluded from the calculating formula. A_{10} was not related with ($A_0 - A_{10}$).

GA is confirmed to have good emulsifying capacity and emulsion stabilizing property, which can lower the interfacial tension at the hexadecane−water interface during the early stages of adsorption (*32*). A combination of adsorption ability of protein moiety and the high hydrophilicity of polysaccharides leads to the formation of a strong solvated layer near the oil−water interface, which confers steric stabilization to emulsion oil droplets (*33*). Meanwhile, changes in protein conformation and structure by ultrasonic treatment were predicted to give improvement of emulsifying properties. Ultrasonic treatment made soy proteins exhibit partial unfolding of 7S and 11S fractions and an aggregation of proteins, especially of the 11S fraction. Partial denaturation and a more disordered structure are able to provide a better potential for adsorption at the oil−water interface (*34*). Guzey and Weiss (*35*) have also reported that high-intensity ultrasound treatment improves the emulsifying properties of whey protein isolate. Primary research also showed that SPI−GA conjugates presented good emulsifying properties when treatment by classical heating at 80 °C for 16 h was used (*36*). However, better emulsifying properties were gained by ultrasonic treatment for 40 min.

Droplet Size Distribution. The volume-surface average diameter (D[3,2]) is defined as the diameter of a sphere that has the same volume/surface area ratio as a particle of interest. The mean droplet size is characterized in terms of equivalent volume-weighted moment mean diameter (D[4,3]). The comparative droplet size distributions of SPI and SPI−GA conjugates obtained by different treatments are shown in **Figure 3**. Results showed SPI−GA conjugates gained by ultrasonic treatment resulted in emulsions of pH 7.5, with droplet size distributions shifted to lower values compared to those of SPI−GA conjugates obtained by classical heating and SPI only. D[4,3] values of SPI grafted with GA by ultrasonic treatment decreased from 25.7 to 7.1 μm, whereas that of the SPI−GA conjugates obtained by classical heating was 21.3 μm. Meanwhile, D[3,2] values decreased from 8.3 to 2.3 μm, which was also smaller than 4.3 μm of SPI−GA conjugates obtained by classical heating. Moreover, SPI−GA conjugates gave emulsions more stability against creaming than those prepared with only SPI during storage at ambient temperature (20 days). SPI−GA conjugates obtained by ultrasonic treatment still had smaller values of D[3,2] and D[4,3] during storage at ambient temperature (20 days). Cavitational forces of ultrasound gave SPI partial denaturation and more disordered structure, which were able to provide a better potential to be adsorbed at the oil−water interface (*34*).

Surface Hydrophobicity (H_0). Quantitative analysis of protein surface hydrophobicity is essential for the accurate prediction of functionality (*37*). **Figure 4** shows that preheating before ultrasonic treatment (80 °C for 20 min, ultrasonic treatment for 0 min) increases the surface hydrophobicity of proteins. Ultrasonic treatment also led to an increase of surface hydrophobicity. However, the surface hydrophobicity began to decrease gradually when sample was treated by ultrasound for 15 min. The H_0 value of SPI was noticeably low because the accessibility of ANS to the hydrophobic residues was inhibited. Most of hydrophobic residues were buried in the interior of the compact globular region (*38*). Yin et al. (*39*) have found that heat treatment might lead to the exposure of hydrophobic groups initially buried in the interior of protein molecules. SPI−GA graft mixtures subjected to ultrasonic treatment had markedly higher surface hydrophobicity than SPI, probably due to aggregate dissociation or protein unfolding. Although hydrophobic groups initially buried in the interior part of protein molecules are exposed, the surface hydrophobic environment still decreased with the combination of GA and SPI. The surface hydrophobic environment decreased quickly when SPI was grafted with more GA. The results showed

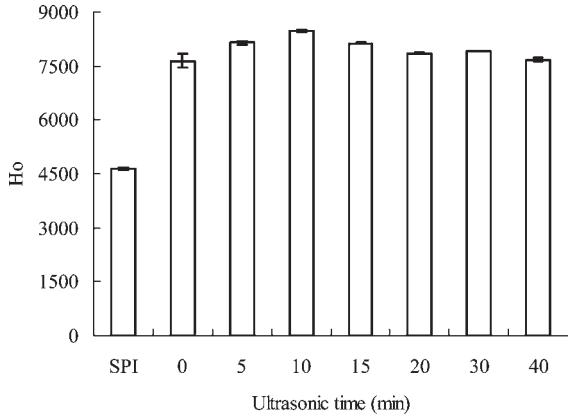

Figure 4. H_0 values of SPI and SPI−GA conjugates by ultrasonic treatment. 0 represents SPI and GA mixtures obtained by preheating for 20 min without ultrasonic treatment; 5, 10, 20, 30, 40, and 60 min represent the times needed to produce SPI−GA conjugates by ultrasonic treatment at pH 7.5.

Table 4. Lysine and Arginine Contents of SPI and SPI−GA Conjugates Obtained by Classical Heating for 24 h and by Ultrasonic Treatment for 40 min

		SPI−GA conjugates	
amino acid	SPI	classical heating	ultrasonic assistance
lysine (%)	6.87	5.94	5.47
arginine (%)	7.78	5.02	5.76

that the steric structure of SPI changed while polysaccharide molecules were linked to the proteins by covalent bonds (*10*).

Analysis of Amino Acids and Circular Dichroism Spectra. It is known that protein and polysaccharide graft reactions were performed by covalent binding between the ε-amino groups of proteins and the reducing-end carbonyl groups of polysaccharides (*6*). **Table 4** shows the contents of lysine and arginine of SPI−GA conjugates obtained by classical heating or ultrasonic treatment after preheating. The contents of lysine and arginine were both decreased after SPI and GA were treated by ultrasound for a given time, which suggested that the free amino groups taking part in the reaction mainly came from lysine and arginine of the proteins. The results showed that SPI−GA conjugates using ultrasonic assistance were formed by covalent binding, which was similar to protein−saccharide graft reactions through other heating methods (*5*, *10*).

The secondary structure of dispersions of SPI and SPI−GA conjugates obtained by classical heating or ultrasonic treatment is shown in **Table 5**. Native dispersions of SPI contained 10.6% α-helix, 39.9% β-sheet, 10.9% β-turn, and 38.6% unordered coils. The secondary structure of dispersions of SPI−GA conjugates obtained by classical treatment changed, which contained 10.3% α-helix, 31.5% β-sheet, 18.3% β-turn, and 40.1% unordered coils. SPI−GA conjugates obtained by ultrasonic treatment were reduced in α-helix (from 10.6 to 2.8%), β-sheet (from 39.9 to 9.3%), and β-turn (from 10.9 to 5.8%) and increased in unordered coils (from 38.6 to 82.2%). Results showed that the attachment of GA increased evidently α-helix and β-sheet distributions, whereas its β-turns and unordered coil increased significantly. Ultrasound-assisted SPI−GA conjugates showed more evident change in the distribution of secondary structure. It proved that unordered structure was dominant in the secondary structure for the grafted SPI. Structure and functionality were highly correlated. Generally, α-helix and β-sheet of proteins are buried in the interior site of polypeptide chains (*40*). The attachment of GA to SPI led to changes in spatial structure and unfolding of protein molecules.

Table 5. Secondary Structure Distribution of SPI and SPI−GA Conjugates Obtained by Classical Heating for 24 h and by Ultrasonic Treatment for 40 min

sample	α-helix (%)	β-sheet (%)	β-turns (%)	unordered (%)
SPI	10.6	39.9	10.9	38.6
SPI−GA conjugates obtained by classical heating	10.3	31.5	18.3	40.1
SPI−GA conjugates obtained by ultrasonic treatment	2.8	9.3	5.8	82.2

Meanwhile, ultrasonic treatment also led to unfolding of proteins and breaking of peptide bonds (*21*). Structural modifications allowing greater conformational flexibility of protein may improve its foaming and emulsifying abilities (*41*). Research has shown that partial denaturation and a more disordered structure make adsorption at the oil−water interface better (*34*, *42*). The increase of unordered structure contributes to evident improvement of emulsifying properties.

In conclusion, ultrasound treatment was confirmed to be an efficient method for forming protein and polysaccharide conjugates. In this work, grafted SPI obtained by ultrasonic treatment led to improved functional properties (e.g., solubility and emulsifying properties) as compared with classical heating and native SPI. Further work on grafting between SPI and polysaccharides with ultrasonic treatment will be conducted to elucidate the grafting mechanism.

ABBREVIATIONS USED

SPI, soy protein isolate; GA, gum acacia; DG, degree of graft; OPA, *o*-phthaldialdehyde; SDS, sodium dodecyl sulfate; EAI, emulsifying activity index; ESI, emulsifying stability index; *D*[3,2], surface-weighted mean diameter; *D*[4,3], weight mean diameter; ANS, 1-anilinonaphthalene-8-sulfonic acid; H_0, surface hydrophobicity.

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