Physicochemical Properties of Soy Protein Isolates-Acacia Gum Conjugates

Lixia MU, Haifeng ZHAO, Mouming ZHAO, Chun CUI and Liya LIU

College of Light Industry and Food Sciences, South China University of Technology, Guangzhou, P.R. China

Abstract

Mu L., Zhao H., Zhao M., Cui Ch., Liu L. (2011): **Physicochemical properties of soy protein isolates-acacia gum conjugates**. Czech J. Food Sci., **29**: 129–136.

Protein-polysaccharide conjugates were generally prepared by dry-heating. However, it was time-consuming and the sample gained was inhomogeneous. A faster way of preparing protein-polysaccharide conjugates is needed. Accordingly, soy protein isolates (SPI)-Acacia gum (GA) conjugates prepared by the wet-heating method were studied in the present work. Physicochemical properties of SPI-GA conjugates were also determined. The results showed that the wet-heating method could improve the rate of the graft reaction of protein and polysaccharide. The solubility of SPI-GA conjugates was significantly ($P < 0.05$) higher than that of unreacted SPI-GA mixtures and SPI at the same pH values. The emulsion activity index (EAI) of the grafted SPI increased remarkably. Moreover, a significant ($P < 0.05$) improvement on the emulsifying stability index (ESI) was observed and emulsions with a smaller droplet size were obtained. No visible flocculation during extended storage (30 days) was observed. The time course of the development of the graft reaction of SPI with GA was also shown by SDS-PAGE.

Keywords: soy protein isolates; Acacia gum (GA); graft reaction; emulsifying properties; solubility

Abbreviations

SPI – soy protein isolate; GA – Acacia gum; DG – degree of graft; OPA – *ortho*-phthaldialdehyde; SDS – sodium dodecyl sulfate; EAI – emulsifying activity index; ESI – emulsifying stability index

The use of soy proteins as emulsifiers or emulsion stabilisers in food manufacturing is increasing. To expand their application fields, some researchers have found that selecting a protein-polysaccharide conjugates as the emulsifying agent rather than the protein alone can markedly enhance emulsion stabilisation (McNamee *et al*. 1998; Garti 1999; Takano *et al*. 2007). In recent years, polysaccharides are widely used for stabilisation of food emulsions and foams. High molecular weight emulsifiers such as gum arabic, casein, dextran, modified starch, and others, are often used as ingredients to stabilise oil-in-water food emulsions (Ray *et al.* 1995; McNamee *et al*. 1998; Garti 1999).

Protein-polysaccharide graft reaction without any chemicals, have been studied which are based on the Maillard-type reactions between the amino groups of protein and the reducing-end carbonyl groups of saccharide (Kato *et al.* 1988; Wang *et al*. 2006b). Previous researche on protein-polysaccharide graft reactions or Maillard-type reactions have showed a good performance. Generally, protein-polysaccharide conjugates were prepared by dry-heating (Kato *et al.* 1988; Kobayashi *et al*. 1990; Diftis & Kiosseoglou 2003; Wang *et al*. 2006b). Protein-polysaccharide conjugates with increased solubility and improved emulsifying properties may form when SPI-dextran mixtures are dry-heated

Suported by the National Natural Science Foundation of P.R. China, Grant No. 20676044, and the Hi-Tech Research and Development Program of P.R. China, Project No. 2006AA10Z326.

at 60°C for four weeks (Kobayashi *et al*. 1990). An improvement on the emulsifying properties of the mixture, especially oil droplet size reduction and emulsion stabilisation against creaming, could be gained when SPI was mixed with sodium carboxymethyl cellulose followed by dry-heating at 60°C for 5 weeks (Diftis & Kiosseoglou 2003). The preparation of protein-polysaccharide conjugates by dry-heating method was time-consuming and energy-wasting, needing several days or several weeks. Thus, it is necessary to choose a faster method for the protein-polysaccharide conjugates preparation to improve the reaction efficiency and uniformity of the products.

The objective of this study was to find a faster way of preparing more homogeneous SPI-GA conjugates by wet-heating method. The emulsion capability, solubility, and surface hydrophobicity of the grafted SPI were investigated. The droplets size distribution of the emulsion and its creaming behaviour were also measured before and after the graft reaction.

MATERIAL AND METHODS

Materials and chemicals. SPI with protein content of 91.2% was prepared from low-temperature defatted soy flakes (purchased from Yuwang Industrial Co. Ltd., Shandong, China) according to the improved method of Sorgentini and Wagner (1999). GA was obtained from CNI Co. (Philadelphia, USA), the powder composition was (g/100 g of powder): 2.13% protein, 85.27% polysaccharide, 10.59% moisture, 2.01% ash. Soy oil (grade I) 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). Ortho-phthaldialdehyde (OPA) was purchased from Sigma-Aldrich (St. Louis, USA). All other chemicals were of the highest commercial grade and were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

Preparation of the SPI-GA conjugates. SPI and GA (1% for SPI and GA, respectively, w/v) were dispersed in phosphate buffer solution (0.2 mol/l, pH 7.5) at the ratio of 1/1 (w/w), stirred for 1 h, and then heated in a water bath at 80°C for 0–48 hours. The reaction products were centrifuged at 4°C (5000 g × 10 min), dialysed and freeze-dried.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was performed on a discontinuous buffered system by using 12% separating gel (pH 8.8) and 5% stacking gel (pH 6.8) according to the method of Laemmli (1970). The protein samples were dissolved in 0.06M Tris-HCl buffer, containing 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol (2-ME), 25% (v/v) glycerol, and 0.1% (w/v) bromophenol blue, and heated for 5 min in boiling water bath before electrophoresis. Ten microlitres of supernatant were loaded in each lane. After the electrophoresis, the gel was stained in 0.25% Coomassie brilliant blue G-250 for protein and in 0.5% periodate-fuchin solution for carbohydrate (Shepherd *et al*. 2000). The gels were photographed after staining.

Determination of free amino groups. When the solution of SPI and GA mixture (1% for SPI and GA, respectively, w/v) was heated in a water bath at 80°C for 0–48 h, the level of available amino groups was determined by the modified ortho-phthaldialdehyde (OPA) method (Church *et al*. 1983). The OPA reagent was prepared by mixing 40 mg of OPA (dissolved in 1 ml of methanol), 25 ml of 100mM sodium tetraborate, 2.5 ml of 20% (w/w) sodium dodecyl sulfate (SDS), and 100 µl of β-mercaptoethanol, followed by diluting to a final volume of 50 ml with distilled water prior to the experiment. 200 µl of the sample solution was mixed with 4 ml of OPA reagent. After incubation 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). The calibration curve was obtained by using 0.25–2mM L-leucine as the standard. The degree of the graft reaction (DG) was calculated using the following equation:

$$DG = (A_0 - A_t)/A \times 100\% \quad (1)$$

where:

A_0, A_t and A – levels of available amino groups in mixtures, conjugates, and SPI

Protein solubility. Protein solubility was determined by dispersing the samples in distilled water to obtain the final solution of 0.2% (w/w) in protein. The pH values of the protein solution were adjusted from 9 to 3 and the solution was then centrifuged at 12 000 *g* for 20 min (20°C). The content of protein in the resulting solution was determined by the Lowry method (Lowry *et al.* 1951). Percent protein solubility was calculated as follows:

$$\text{Solubility (\%)} = (\text{protein content in the supernatant/initial protein in the sample}) \times 100 \quad (2)$$

Emulsifying properties

EAI and ESI. EAI and ESI indeces were measured following the procedure described by Pearce and Kinsella (1978). The continuous phase (0.2% in protein, w/v) of the individual emulsions was first prepared by vigorous and continuous agitation of SPI-GA samples in phosphate buffer solution (0.2 mol/l, pH 7.5 or 4.0). Oil-in-water (o-w) emulsions were prepared by adding 1 ml of soy oil in 3 ml of SPI-GA solution under continuous agitation and the resulting crude emulsions were homogenised for 1 min using an Ultra-Turrax T25 homogeniser (IKA Labortechnik, Staufen, Germany) equipped with a dispersing tool (S25KG-25F) at 20 000 rpm. Then 50 µl of each emulsion was pipetted into 5 ml of 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 by using the following formula:

$$EAI = 2T\,A_0 \times \text{dilution factor}/c \times \Phi \times L \times 10\,000 \quad (m^2/g) \quad (3)$$

$$ESI = A_0/(A_0 - A_{10}) \times 10 \quad (min) \quad (4)$$

where:

T = 2.303; dilution factor = 1000

c – weight of protein per unit volume (g/ml)

L – width of the optical path (0.01 m)

Φ – oil volumetric fraction (0.25)

Droplets distribution. The continuous phase (2% in protein, w/v) of the respective emulsion was first prepared by vigorous and continuous agitation of protein-polysaccharide sample in distilled water (adjusting pH to 7.5). Oil-in-water emulsion was prepared by adding 20 g of soy oil in 180 g of protein-polysaccharide solution under continuous agitation, and then the resulting crude emulsion was homogenised with a pressure homogeniser (APV Gaulin, Abvertslund, Denmark). Soy oil and protein-polysaccharide fraction dispersions were mixed and homogenised at 30 MPa. Two passes were used to ensure uniform mixing of the oil and protein solutions. The oil droplet size distribution was evaluated with dynamic light scattering using a Malvern Mastersizer 2000 unit (Malvern Instruments Ltd., Malvern, United Kingdom).

Emulsion stability against coalescence was evaluated by determining the average oil droplet size. The stability against creaming was determined visually in the serum separated from the samples stored in glass containers for 0, 1, and 30 days.

Surface hydrophobicity (H_o). H_o of SPI was determined by using 1-anilinonaphthalene-8-sulfonic acid (ANS) as the fluorescence probe in the absence of SDS (Alizadeh-Pasdar & Li-Chan 2000). Protein dispersions were diluted (0.005%, 0.01%, 0.02%, 0.05%, 0.1%, and 0.2%) with phosphate buffer solution (0.2 mol/l, pH 7.5). Then an aliquot of 1-anilinonaphthalene-8-sulfonic acid solution (20 ml, 8.0mM in the same buffer) was added to 4 ml of the sample. Fluorescence intensity was measured with a Hitachi F4500 fluorescence spectrometer (LS55, Maryland, USA), at the wavelengths of 390 nm (excitation) and 470 nm (emission). The initial slope of the plot of the fluorescence intensity versus protein concentration was used as an index of H_o.

Statistical analysis. All the tests were performed in triplicates and the results were given as means ± standard deviations. Duncan's multiple-range test was used to evaluate significant differences ($P < 0.05$) between the results.

RESULTS AND DISCUSSION

SDS-PAGE

A brown colour took place after a certain time of heating, which was based on the graft reaction between the free amino groups of protein and the reducing end carbonyl groups of sugars, the colour intensity increasing thereafter (Guan *et al.* 2006). However, only a light brown colour was found in the present study, which might be due to the differences in colour between both methods.

Figure 1 present the SDS-PAGE patterns of SPI-GA mixtures prepared at different heating times at 80°C. As expected, the results suggest that SPI and GA reaction resulted in the formation of protein-polysaccharide conjugates. The electrophoretic pattern of the high molecular weight fraction in SPI-GA conjugates showed a dense broad band near the boundary between the stacking and separating gels, which suggest that the new constituents with high molecular weights could not enter into the stacking gel. Meanwhile, the intensity of carbohydrate staining was gradually increasing with the extending heating time (Figure 1B), which showed that DG of SPI and GA was also increasing. Similar electrophoretic patterns were

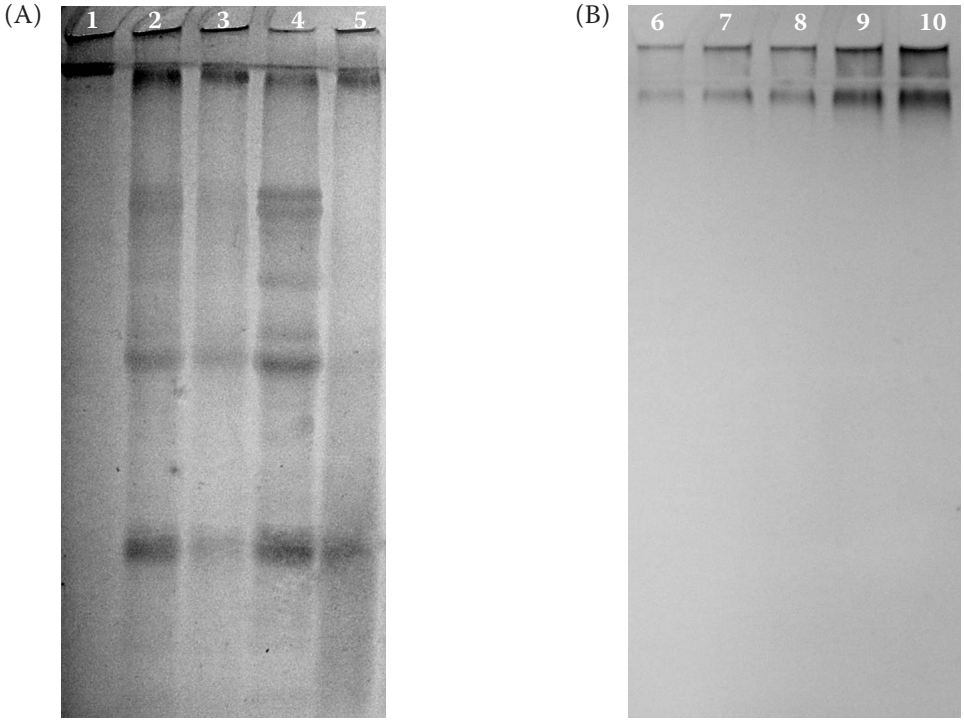

Figure 1. SDS-PAGE patterns for SPI-GA conjugates for various heating times. The gel sheets were stained of with (A) Coomassie brilliant blue G-250 (lane 1 – GA; lane 2 – reaction time 4 h; lane 3 – reaction time 8 h; lane 4 – reaction time 2 h; lane 5 – reaction time 24 h) and (B) periodate-fuchin solution for carbohydrates (lanes 6 to 10 – reaction time 2, 8, 24, 32 h, respectively)

observed by a number of researchers with other protein-polysaccharide hybrids (Kato *et al.* 1993; Difits & Kiosseoglou 2003; Guan *et al.* 2006). Moreover, 7S subunits were disappearing first and then the acidic subunits of 11S vanished with the proceeding of the reaction (Figure 1A). It appears that the protein-polysaccharide interaction was mainly confined to the subunits of 7S and the acidic subunits of the 11S fractions of SPI. The basic subunits of the 11S are less reactive due to their much lower lysine contents (Utsumi *et al.* 1997), which is similar to other SPI-polysaccharide conjugates (Guan *et al.* 2006).

The degree of graft (DG)

DG reflects the protein-polysaccharide reaction degree, while the decrease in free amino groups (such as lysine and arginine) during an extended heating time is due to the graft reaction (Guan *et al.* 2006). As seen from Figure 2, DG increased gradually from 4 h to 32 h and then reached a steady state, suggesting that more and more SPI and GA formed conjugates when heated in a water bath at 80°C.

Protein solubility

Solubility is one of the most important functional properties of proteins, since it has impacts on other functional properties (Sikorski 2001). In many protein-based formulations, for instance emulsions, solubility of the protein is usually required (Hayakawa & Nakai 1985).

Figure 3 shows the changes in solubility of SPI, SPI and GA mixtures, and SPI-GA conjugates at different pH values. The graft reaction between SPI

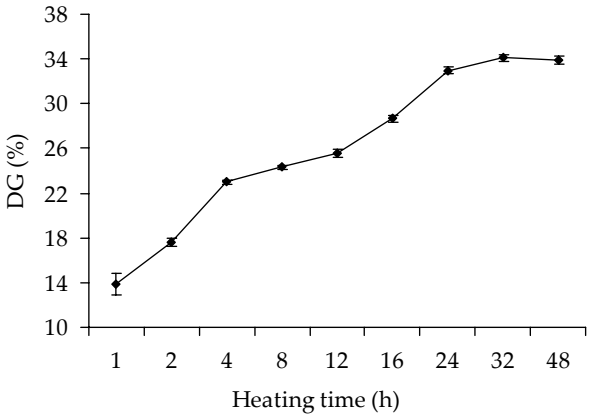

Figure 2. DG of SPI and GA at different reaction time

and GA could remarkably improve the solubility of SPI, especially when pH values were between 3.0 and 5.0. Moreover, the solubility curve of SPI-GA conjugates did not vary rapidly with pH, which indicated that there was no obvious isoelectric point (pI) for SPI-GA conjugates. Generally, the solubility of a protein decreases remarkably at the pH around its pI where the net charge of the protein is about zero and the protein tends to aggregate because of the electrostatic interactions caused by the charge asymmetry of the protein. A covalent protein-polysaccharide hybrid, once formed, does not dissociate, irrespective of the pH or salt concentration. Furthermore, these conjugates may exhibit a higher solubility than simple protein-polysaccharide mixtures (Kobayashi *et al.* 1990). However, the solubility of SPI and GA mixtures was a little higher than that of the individual SPI. This phenomenon may be explained in terms of the addition of GA, itself containing 2.31% proteins, bringing extrinsic proteins, which were difficult to remove by centrifugation (12 000 g × 20 min, 20°C) at these pH values. All these results as mentioned above exhibited that SPI modified by the Graft reaction with GA could significantly ($P < 0.05$) increase solubility in acid pH range.

Surface hydrophobicity (H_o)

The surface hydrophobicity is an index of the protein capacity for intermolecular interaction, and hence an index of its functionality (Wang *et al.* 2006a). Figure 4 shows that the addition of GA increased the surface hydrophobicity. However, the surface hydrophobicity decreased gradually with the increase of DG. Yin *et al.* (2008) found that the heat treatment may lead to the exposure of hydrophobic groups initially buried in the interior of the protein molecules, since proteins with high hydrophobicity would have higher EAI values (as compared with those with low hydrophobicity). The steric structure of SPI changed while the saccharide molecules were linked to the protein by covalent bonds (Guan *et al.* 2006). Although the hydrophobic groups initially buried in the interior of the protein molecules were exposed, the surface hydrophobic environment still decreased with the addition of GA (polysaccharide possessed a lot of hydrophilic groups). The surface hydrophobic environment decreased quickly with the increase of DG.

Emulsifying properties

Emulsions are, from the physicochemical point of view, thermodynamically unstable systems rapidly or slowly separating into two immiscible phases according to the kinetic stability. Mechanisms of physical destabilisation of emulsions include oil droplets size variation processes such as flocculation, and coalescence and particle migration phenomena like sedimentation and creaming (Comas *et al.* 2006). In the present study, emulsifying activity, emulsifying stability, and droplets distribution were measured to study the emulsion properties in detail.

EAI and ESI

The EAI and ESI of SPI and GA graft mixture at different reaction times are shown in Figure 5.

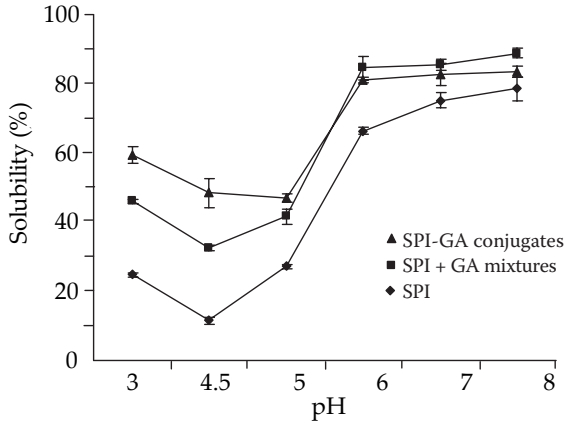

Figure 3. The solubility profiles of SPI, SPI + GA mixtures, and SPI-GA conjugates

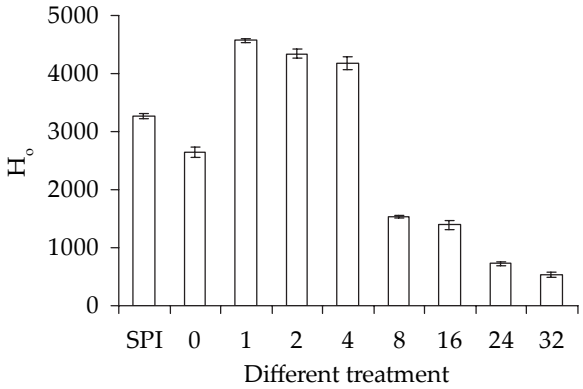

Figure 4. Surface hydrophobicity (H_o) of SPI, SPI and GA mixtures (0 min), SPI-GA conjugates for different reaction time (1, 2, 4, 8, 16, 24, and 32 h)

During extended reaction time, the graft reaction of SPI and GA resulted in a gradual and significant ($P < 0.05$) increase in EAI and reached the highest value after 16 h. These results suggested that graft modification could evidently improve the EAI of SPI. Therefore, the sample grafted for 16 h was chosen for further study. Moreover, ESI of SPI was also improved to some extent (25.17% and 6.28–15.55%, respectively) by mixing and grafting with GA. The EAI of the mixture was higher than those of conjugates, being possibly related with the calculating formula. Where A_{10} of the conjugates is higher, (A_0–A_{10}) is not.

Compared with the control, the EAI of the grafted SPI at neutral and acidic pH values was significantly ($P < 0.05$) increased (Figure 6). The influence of the graft reaction on the EAI at pH 4.0 was more remarkable than that at pH 7.5. EAI of SPI-GA conjugates increased by 58.26% and 82.73% at pH 7.5 and pH 4.0, respectively. This might be mainly attributed to the difference between the solubility of the samples at these pH values. The EAI of all the samples showed higher values at pH 7.5 than were those at pH 4.0. Similar results were also found in other protein-polysaccharide conjugates (Dickinson & Galazka 1991; Kato *et al.* 1993; Diftis & Kiosseoglou 2003). It was concluded that EAI was coincidental with the solubility at these pH values. The solubility of protein is an important prerequisite for the film formation because rapid migration and adsorption on the oil-water interface is critical. A positive correlation between the solubility and emulsifying capacity of proteins has been reported (Felix *et al.* 1990).

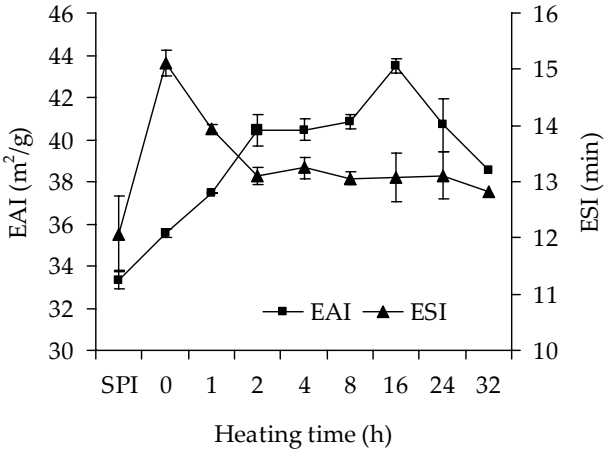

Figure 5. EAI and ESI values of SPI and GA heated for a given time (pH 7.5)

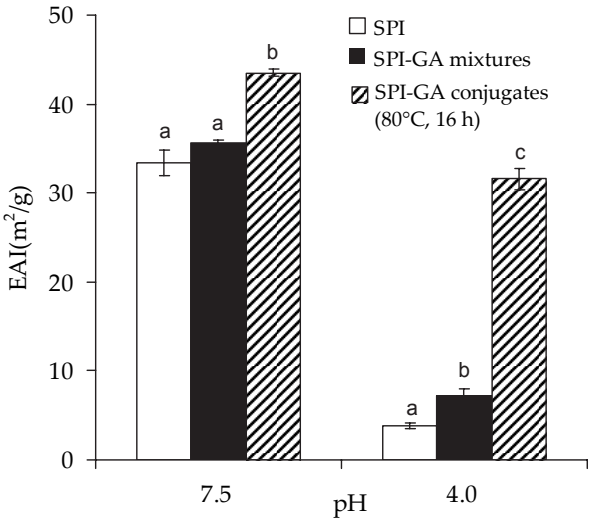

Figure 6. EAI of SPI, SPI and GA mixtures, SPI-GA conjugates (16 h) at pH 7.5 and pH 4.0

Particle distribution and creaming behaviour of emulsions

At present, some researchers believe that the measurement of the emulsifying activity by means of photometry is simple but the results are limited with regard to accuracy (Einhorn-Stoll *et al.* 2002). Therefore, the droplet size distribution of emulsions and flocculation during extended storage were tested.

Figure 7 compared the droplet sizes of emulsions stabilised with SPI, SPI and GA mixtures, and SPI-GA conjugates. When SPI and GA mixtures were heated at 80°C for 16 h, the emulsions of pH 7.5 with the droplet size distributions of SPI-GA mixtures shifted to a lower size as compared to those of the non-treated samples. Similar results could be also observed with wheat protein isolate-pectin conjugates (Neirynck *et al.* 2004). Compared with the average droplet size between SPI-GA conjugates and SPI (d_{43} < 12 μm and 25.7 μm, respectively), SPI-GA conjugates exhibited better emulsifying properties than SPI. Moreover, the droplet size distribution became more homogeneous in the case of SPI and GA mixture.

As can be seen in Figure 8, there was no visible flocculation at pH 7.5 during extended storage period (30 days), which indicated that SPI-GA conjugates could produce efficiently stable emulsions. SPI-GA conjugates in a continuous phase during the formation of oil-in-water emulsions led to an increase in the stability of oil-in-water

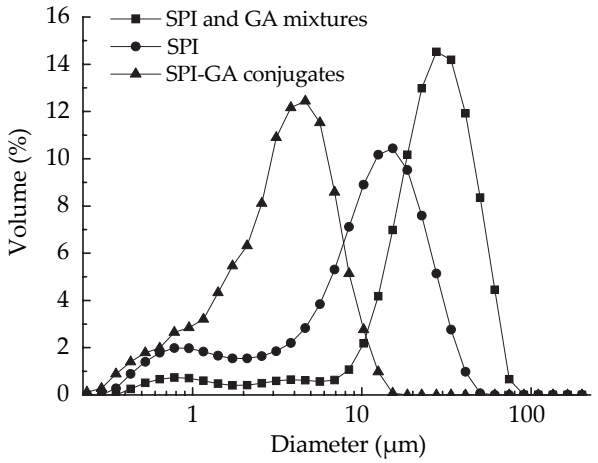

Figure 7. Distribution of oil droplet size for emulsions prepared with SPI, SPI and GA mixtures, SPI-GA conjugates for 16 h, pH 7.5

emulsions. This effect was confirmed by the increased viscosity, creaming stability, and smaller droplet size formation in emulsions (Dickinson & Galazka 1991). However, the main contribution to the improved long term stability is GA in the conjugate providing a more bulky steric stabilising layer around the droplets.

CONCLUSIONS

In conclusion, the wet-heating method was a faster way for the preparation of more homogeneous SPI-GA conjugates. The present study clearly demonstrated that the graft reaction of SPI and GA under wet-heating condition could significantly improve the solubility and emulsifying properties of SPI. The solubility of SPI-GA conjugates was significantly ($P < 0.05$) higher than that of unreacted SPI-GA mixtures or SPI at the same pH values. The emulsion activity index (EAI) of the conjugates increased remarkably. A significant ($P < 0.05$) improvement on the emulsifying stability index (ESI) was observed resulting in emulsions with a lower droplet size. No visible flocculation occurred during extended storage (30 days). Moreover, the solubility and emulsifying properties of SPI were closely correlated in the acid condition. Compared with the dry-heating method, heating in a water bath at 80°C of the mixture of SPI and GA solutions was a more efficient method for shortening the reaction time and lowering the brown colour of the reaction mixtures.

(a) (b) (c)

Figure 8. Creaming behaviour of emulsions prepared with (A) SPI and (B) SPI-GA conjugates after storage for (**a**) 0 day, (**b**) 1 day and (**c**) 30 days at pH 7.5

Acknowledgements. We are grateful to TIC Co. (Philadelphia, USA) for supplying Acacia gum.

References

Alizadeh-Pasdar N., Li-Chan E.C. (2000): Comparison of protein surface hydrophobicity measured at various pH values using three different fluorescent probes. Journal of Agriculture and Food Chemistry, **48**: 328–334.

Church F.C., Swaisgood H.E., Porter D.H., Catignani G.L. (1983). Spectrophotometric assay using *o*-phthaldialdehyde for determination of proteolysis in milk and isolated milk protein. Journal of Dairy Science, **66**: 1219–1227.

Comas D.I., Wagner J.R., Tomás M.C. (2006): Creaming stability of oil in water (O/W) emulsions: Influence of pH on soybean protein-lecithin interaction. Food Hydrocolloids, **20**: 990–996.

Dickinson E., Galazka V.B. (1991): Emulsion stabilization by ionic and covalent complexes of β-lactoglobulin with polysaccharides. Food Hydrocolloids, **5**: 281–296.

Diftis N.G., Kiosseoglou V.B. (2003): Improvement of emulsifying properties of soybean protein isolate by conjugation with carboxymethyl cellulose. Food Chemistry: **81**: 1–6.

Einhorn-Stoll U., Weiss M., Kunzek H. (2002): Influence of the emulsion components and preparation method on the laboratory-scale preparation of o/w emulsions containing different types of dispersed phases and/or emulsifiers. Nahrung/Food, **46**: 294–301.

Felix A., Hill R.A., Diarra B. (1990): *In vitro* and *in vivo* digestibility of soya-bean straw treated with various alkalins. Animal Production, **51**: 47–60.

Garti N. (1999): Hydrocolloids as emulsifying agents for oil-in-water emulsions. Journal of Dispersion Science and Technology, **20**: 327–355.

Guan J.J., Qiu A.Y., Liu X.Y., Hua Y.F., Ma Y.H. (2006): Microwave improvement of soy proteinisolate-saccharide graft reactions. Food Chemistry, **97**: 577–585.

Hayakawa S., Nakai S. (1985): Relationships of hydrophobicity and net charge to the solubility of milk and soy proteins. Journal of Food Science, **50**: 486–491.

Kato A., Murata K., Kobayashi K. (1988): Preparation and characterization of ovalbumin-dextran conjugate having excellent emulsifying properties. Journal of Agricultural and Food Chemistry, **36**: 421–425.

Kato A., Minaki K., Kobayashi K. (1993): Improvement of emulsifying properties of egg white proteins by the attachment of polysaccharide through Maillard reaction in dry state. Journal of Agricultural and Food Chemistry, **41**: 540–543.

Kobayashi K., Kato A., Matsudomi N. (1990): Developments in novel functional food materials hybridization of soy protein to polysaccharides. Nutrition Science of Soy Protein, Japan, **11**: 23–28.

Laemmli U.K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, **227**: 680–685.

Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J. (1951): Protein measurement with the Folin phenol reagent. The Journal of Biological Chemistry, **193**: 265–275.

McNamee B.F., O'Riordan E.D., O'Sullivan M. (1998): Emulsification and microencapsulation properties of gum Arabic. Journal of Agricultural and Food Chemistry, **46**: 4551–4555.

Neirynck N., Van Der Meeren P., Bayarri Gorbe S., Dierckx S., Dewettinck K. (2004): Improved emulsion stabilizing properties of whey protein isolate by conjugation with pectins. Food Hydrocolloids, **18**: 949–957.

Pearce K.N., Kinsella J.E. (1978): Emulsifying properties of proteins: evaluation of turbidimetric technique. Journal of Agricultural and Food Chemistry, **26**: 716–723.

Ray A.K., Bird P.B., Iacobucci A.I., Clark B.C. JR. (1995): Functionality of gum Arabic. Fractionation, characterization and evaluation of gum fractions in citrus oil emulsions and model beverages. Food Hydrocolloids, **9**: 123–131.

Shepherd R., Robertson A., Ofman D. (2000): Dairy glycolconjugate emulsifiers: casein-maltodextrins. Food Hydrocolloids, **14**: 281–286.

Sikorski Z.E. (ed.) (2001): Chemical and Functional properties of Food Proteins. Technomic Publishing Co. Inc., Lancaster-Basel.

Sorgentini D.A., Wagner J.R. (1999): Comparative study of structural characteristics and thermal behavior of whey and isolate soybean proteins. Journal of Food Biochemistry, **23**: 489–507.

Takano K., Hattori M., Yoshida T., Kanuma S., Takahashi K. (2007): Porphyran as a functional modifier of a soybean protein isolate through conjugation by the Maillard reaction. Journal of Agricultural and Food Chemistry, **55**: 5796–5802.

Utsumi S., Matsamura Y., Mori T. (1997): Structure-function relationships of soy proteins. In: Damodaran S., Parat A. (eds): Food Proteins and their Applications. Marcel Dekker, New York: 257–291.

Wang J.S., Zhao M.M, Yang X.Q., Jiang Y.M. (2006a): Improvement on functional properties of wheat gluten by enzymatic hydrolysis and ultrafiltration. Journal of Cereal Science, **44**: 93–100.

Wang J.S., Zhao M.M., Yang X.Q., Jiang Y.M. (2006b): Improvement of emulsifying properties of wheat gluten hydrolysate/λ-carrageenan conjugates. Food Technology and Biotechnology, **44**, 25–32.

Yin S.W., Tang C.H., Cao J.S., Hub ER-K., Wen Q.B., Yang X.Q. (2008): Effects of limited enzymatic hydrolysis with trypsin on the functional properties of hemp (*Cannabis sativa* L.) protein isolate. Food Chemistry, **106**: 1004–1013.

Received for publication July 26, 2009
Accepted after corrections June 3, 2010

Corresponding author:

Prof. Mouming Zhao, South China University of Technology, College of Light Industry and Food Sciences, Guangzhou, P. R. China
tel.: + 86 2087 113 914, e-mail: femmzhao@scut.edu.cn