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Glycinin-gum arabic complex formation: Turbidity measurement and charge neutralization analysis

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ABSTRACT

The interaction between glycinin and anionic polysaccharides has gained considerable attention recently because of its scientific impact on the stability of acid soymilk systems. In this study, the formation of glycinin/gum arabic complexes driven by electrostatic interactions was investigated. Turbidity titrations at different glycinin/gum arabic ratios were conducted and critical pH values ($pH_{\varphi 1}$) where insoluble complexes began forming were determined firstly. The corresponding $pH_{\varphi 1}$ values at glycinin/gum arabic ratios of 1:4, 1:2, 1:1, 2:1, 4:1 and 8:1 were 2.85, 3.25, 3.70, 4.40, 4.85 and 5.35, respectively. Afterwards, electromobilities for glycinin and gum arabic at the pH values between 4.1 and 2.6 were measured, and charge densities (ZN) for glycinin and gum arabic were calculated based on the soft particle analysis theory. Further analysis indicated that the product of glycinin/gum arabic ratio (ρ) and ZN ratio of glycinin/gum arabic was approximate 1 at any $pH_{\varphi 1}$ values. It was revealed that charge neutralization was achieved when glycinin/gum arabic insoluble complexes began forming. NaCl displayed multiple effects on glycinin/gum arabic complex formation according to turbidity and compositional analysis. The present study could provide basic guidance in acid soymilk designing.

1. Introduction

Complex formation between an anionic polysaccharide and a protein is a widely known phenomenon in food processing (Turgeon, Beaulieu, Schmitt, & Sanchez, 2003). Arising from electrostatic interaction, the oppositely charged proteins and polysaccharides form a complex. The complexes can be either soluble or insoluble. The insoluble complexes concentrate in complex drops, leading to a phase separation of the mixture into two liquid layers: a biopolymer-rich phase and a solvent-rich phase (de Kruif & Tuinier, 2001; Schmitt & Turgeon, 2011). In recent years, protein/polysaccharide complex formation has attracted considerable interest due to its potential applications in food industry, such as food products stabilization (Laurent & Boulenguer, 2003), fat replacement (Le Révérend, Norton, Cox, & Spyropoulos, 2010), microencapsulation of food ingredient (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007), emulsification and foam stabilization (Dickinson, 2009; Miquelim, Lannes, & Mezzenga, 2010), protein separation and purification (Xu, Mazzawi, Chen, Sun, & Dubin, 2011), and enzyme immobilization and recovery (Xia, Mattison, Romano, Dubin, & Muhoberac, 1997).

Current work in this field has reported various parameters influencing the complex formation, such as pH, biopolymer mixing ratio and ionic strength (Ye, 2008). Two critical pH values are reported along the complexing phenomenon using turbidity titrations: pH_c corresponding to the initial formation of the soluble complexes and $pH_{\varphi 1}$ corresponding to the beginning of macroscopic phase separation. Other parameters such as biopolymer molecular weight and flexibility (Stone & Nickerson, 2012), charge density (Sperber, Cohen Stuart, Schols, Voragen, & Norde, 2010) and temperature (Liu et al., 2010) are also reported to impact the complex formation. Apart from influencing factors, charge neutralization of macro ions when complexes formed has attracted interest in food research area (Ducel, Saulnier, Richard, & Boury, 2005; Weinbreck, Nieuwenhuijse, Robijn, & de Kruif, 2003b). In our previous work (Dong et al., 2013), we identified that 1:1 charge ratio of soy proteins and gum arabic was fulfilled when soy protein/gum arabic insoluble complexes began forming.

Glycinin, one of the main components in soy proteins, is a hexameric protein composed of an acidic (about 40 kDa) and a basic (about 20 kDa) polypeptide linking by a single disulfide bond. The functional properties of glycinin have been extensively studied. The results reveal that glycinin has specific functional properties because of the existence of disulfide bond and sulfhydryl, such as heat gelling properties. Therefore, it can be used as special food ingredients to improve food texture properties and taste. Several studies have reported the electrostatic interaction between glycinin and polysaccharides, such as glycinin/chitosan complexing (Yuan et al., 2013), glycinin/high-methoxyl pectin

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interactions (Lam, Paulsen, & Corredig, 2008). However, no theoretical studies were performed on glycinin/gum arabic mixtures, especially about charge neutralization. Gum arabic is a negatively charged polyelectrolyte which has high solubility and low viscosity at high concentrations. It can be dissolved easily and quickly in water at room temperature, making it apply more widely in food preparation. Moreover, gum arabic has good emulsifying and microencapsulating properties (Williams, 2012).

The aim of this study was to advance our understanding on charge neutralization of biopolymer molecules when glycinin/gum arabic complex formed. In this paperwork, the soft particle analysis theory was firstly introduced to glycinin/gum arabic systems to investigate the electrostatic interaction between them. The model is a validated model that assumes an ion-penetrable polymer layer around the core particle and can reduce or eliminate the effect of counterions in the electrophoretic mobility when assessing the electrical charge of colloidal particles. So far, this model has been successfully applied in studying bacterial surface properties and proteins and polysaccharides charge density in the layer (Ducel et al., 2005). Our work will give a detailed understanding for glycinin/gum arabic complex formation and provide useful information on the functional use of glycinin through protein-polysaccharide complexing, such as acid soymilk stabilization, and also expand the application of soft particle analysis theory in macromolecular substances, especially in proteins and polysaccharides molecules.

2. Materials and methods

2.1. Materials

Hexane defatted and flush desolventized soy flake, provided by Shandong Wonderful Industrial & Commercial Co. Ltd. (Dongying, Shandong, China), had a protein content of 52.4% (dry basis) and nitrogen solubility index (NSI) of 85%.

Powder gum arabic (Instant gum AA) was a gift from the Tianjin Jebsen Specialty Chemicals Co. Ltd. (Tianjin, China). The compositions (w/v) of gum arabic used in this study were 2.2% protein, 10.7% moisture and 3.3% ash, and nearly lipid-free. Carbohydrate content (83.8%) was calculated on the basis of percent differential from 100%, including 16% glucuronic acid and 55% neutral sugars. The arabinose, galactose and rhamnose content were 31%, 37% and 14%, respectively. The average molecular weight (Mw) of gum arabic, as determined by size exclusion chromatography (SEC-HPLC), was 327,000. SEC-HPLC was performed with TSK-G 5000 column (Tosoh, Tokyo, Japan). The separation was carried out at 25 °C using $NaNO_3$ (0.1 mol/L) as eluent (a flow rate of 1.0 mL/min).

2.2. Preparation of glycinin

Glycinin was prepared with a modified method of Nagano, Hirotsuka, Mori, Kohyama, and Nishinari (1992). Briefly, the protein was extracted by making slurry with 15-fold volumes of deionized water and adjusted to pH 7.5 with 2 mol/L NaOH at room temperature under stirring for 1 h. Then, the slurry was filtered to remove solids and the filtrate was centrifuged (HITACHI, Japan; 9500 rpm, 30 min, 4 °C). After dry sodium bisulfite (SBS) being added to the supernatant (0.98 g/L), the pH was adjusted to 6.4 with 1 mol/L HCl. The mixture was stored at 4 °C overnight, and then centrifuged (HITACHI, Japan; 6500 rpm, 20 min, 4 °C). After being washed with distilled water, the protein precipitate was resuspended in distilled water and re-solubilized by adjusting pH to 8.0 with 2 mol/L NaOH. Small quantity of insoluble substances was removed by centrifugation (HITACHI, Japan; 9500 rpm, 30 min). Afterwards, protein solution was dialyzed and freeze dried.

2.3. Glycinin and gum arabic solutions preparation

Glycinin and gum arabic solutions were prepared by dispersing about 0.15 g biopolymer powder in 100 mL distilled water under gentle stirring at room temperature for 2 h and left overnight at 4 °C. Simultaneously, 0.02% (w/v) NaN_3 was added to inhibit bacteria growth. Afterwards, glycinin and gum arabic solutions were centrifuged (HITACHI, Japan; 19,000 rpm, 30 min, 4 °C). Then, mixtures of glycinin and gum arabic were prepared by adding glycinin solution to gum arabic solution at desired ratios, with distilled water to achieve a total biopolymer concentration of 0.1% (w/v, C_p).

2.4. Zeta potential measurement

The zeta potential of glycinin and gum arabic in solution were determined at different pH values (25.0 ± 0.1 °C), using Zetasizer Nano ZS instrument (Malvern, British).

2.5. Turbidity analysis during acid titration

Turbidity titration during acidification was measured using a UV–vis spectrophotometer (Shimadzu, Japan) at 600 nm. Titrations were carried out at room temperature and pH (±0.01 pH units) was monitored with a Mettler Toledo Delta 320 pH meter which had been carefully calibrated. Turbidity titrations were also conducted by varying glycinin/gum arabic mixing ratio (1:4–8:1, w/w) and NaCl concentration (0–100 mmol/L).

2.6. Measurement of the electrophoretic mobility

Charge densities of glycinin and gum arabic (ZN, mol/L) were determined by Ohshima's soft particle theory (Ohshima, 1995; Ohshima, Nakamura, & Kondo, 1992). The electrophoretic mobilities of glycinin and gum arabic in solution were determined at different salt concentrations (0.02, 0.04, 0.06, 0.08 and 0.10 mol/L NaCl) at 25.0 ± 0.1 °C, using Zetasizer Nano ZS instrument (Malvern, British). Electrophoretic mobilities were plotted versus salt concentration, and were fitted with a model in which ZN (mol/L) and 1/λ (nm) were as the adjustable parameters.

The electrophoretic mobility (μ) of a soft particle is expressed as follows:

$$\mu = \frac{\varepsilon_0 \varepsilon_r}{\eta} \times \frac{\psi_0/K_m + \psi_{DON}/\lambda}{1/K_m + 1/\lambda} + \frac{eZN}{\eta\lambda^2}$$

Here, μ is the electrophoretic mobility, ε_0 is the permittivity of vacuum, ε_r is the relative permittivity of the medium, η is the viscosity, e is the elementary charge, K_m is the Debye–Hückel parameter of the surface region, ψ_{DON} is Donnan potential, and ψ_0 is the potential at the boundary between the surface region and solution.

2.7. Compositional analysis

The glycinin/gum arabic samples at different NaCl concentration were prepared (C_p = 0.1%). After acidification to the desired pH value, the mixtures were centrifuged at 8000 rpm (HITACHI, Japan). The dense phase was collected for protein and polysaccharide content measurement using Kjeldahl method (AOAC, 2000) and phenol–sulphuric colorimetric method (Dubois et al., 1956) respectively. The data were shown on dry basis.

2.8. Statistical analysis

All the measurements were performed in three times. Statistical significance analysis ($p \le 0.05$) was determined using the SAS statistical