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Phase separation induced molecular fractionation of gum arabic—Sugar beet pectin systems

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ABSTRACT

This paper investigates the phase separation and phase separation-induced fractionation of gum arabic (GA)/sugar beet pectin (SBP) mixed solutions. A phase diagram, including cloud and binodal curves, was established by visual observation and phase composition analysis. The deviation of the binodal curve from the cloud curve was a result of phase separation-induced fractionation of polydisperse GA and SBP molecules. Fractionation of GA increased the content of arabinogalactan-protein complex (AGP) from ca. 13% to 27%. The fractionated GA (FGA) showed improved emulsifying functionality, whereas the fractionated SBP (FSBP) had a reduced emulsifying functionality. The changes in emulsifying efficiency can be explained by interfacial adsorption behaviors at the oil–water interface as indicated by interfacial tension measurements.

1. Introduction

Phase separation is a fundamental phenomenon associated with biopolymer mixtures. When two biopolymers are similarly charged or when one of them is neutral, they are rarely miscible and the mixture shows a strong tendency to separate into two phases, due to thermodynamic incompatibility (Fang, Li, Inoue, Lundin, & Appelqvist, 2006). The biopolymers are separately enriched into two different phases and, therefore, the phenomenon is referred as to segregative phase separation. Phase separation is important for industrial applications, such as food structural design (Lazaridou & Biliaderis, 2009; Pérez, Wargon, & Pilosof, 2006), multiple emulsion preparation (Kim, Decker, & Julianmcclements, 2006; Perrechil & Cunha, 2012), microencapsulation (Matalanis, Lesmes, Decker, & McClements, 2010), and protein purification and separation (Jara & Pilosof, 2011). Factors that could influence phase separation are complex, including: (1) biopolymer concentrations; (2) biopolymer characteristics such as molecular weight, charge, shape and conformation; (3) external conditions such as ionic strength, temperature, pH and mechanical field (Edelman, van der Linden, de Hoog, & Tromp, 2001; Edelman, van der Linden, & Tromp, 2003; Loret, Schumm, Pudney, Frith, & Fryer, 2005). Thermodynamic incompatibility underlying phase separation largely comes from the entropy of mixing (excluded volume effect) (Matalanis et al., 2010), which again depends on the size and shape of biopolymers. Linear polysaccharides have relatively larger space occupancy, thus a lower phase separation threshold concentration, than globular proteins (Tolstoguzov, 2003).

Biopolymers are polydisperse in nature, and their phase separations are much more complex than ideally predicted for monodisperse systems. Phase separation of polydisperse systems is often accompanied with molecular fractionations, in which fractions of higher molecular weight prefer to stay in their own phases and those of lower molecular weights tends to coexist with the other biopolymers (Edelman, Tromp, & van der Linden, 2003; Loret et al., 2005; van Heukelum et al., 2003).

Sugar beet pectin (SBP) is a polysaccharide produced from the industrial by-product beet sugar pulp. Compared with citrus and apple pectins, SBP has a higher proportion of hairy region and acetyl group in structure, and a higher content of phenolic esters and proteinaceous materials. These characteristics are responsible for its non-gelling and emulsifying properties (Nakauma et al., 2008; Siew & Williams, 2008; Williams et al., 2005).

Gum arabic (GA) is a natural exudate from Acacia trees of the Sahelian region of Africa, and contains branched polysaccharides and proteinaceous material. It is heterogeneous and consists of

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three major fractions, namely, arabinogalactan-protein complex (AGP), arabinogalactan (AG) and glycoprotein (GP). The AG fraction represents about 90% of the total gum and structurally is an oblate ellipsoid with a molar mass of a few hundred thousands. AGP, accounting for 10% of total gum, has a wattle blossom-type structure in which branched polysaccharide blocks (i.e. AG) are linked to a common polypeptide chain. AGP has a molecular mass of several millions and contains about 10% proteins, and is regarded as the active component for emulsification (Aoki, Al-Assaf, Katayama, & Phillips, 2007; Williams & Phillips, 2000). Different modifications including radiation and maturation have been used to enhance the emulsifying property of GA. The basic principle was to induce molecular association between different components to increase molecular weight, particularly by forming more AGP (Aoki, Al-Assaf, et al., 2007).

The present work was designed to study the phase separation and phase separation induced fractionation of the polydisperse GA and SBP, and to evaluate the emulsifying functionality of the fractionated emulsifiers. The knowledge gained in the study identifies a green and effective method for fractionating functional components in polydisperse biopolymers, for example, in increasing the AGP content of GA and so providing a GA with enhanced emulsifying functionality.

2. Experimental

2.1. Materials

Commercial GA (powder SD LOT 110512) and SBP (LOT 10808235) were provided by San-Ei Gen F.F.I., Inc. (Osaka, Japan). GA sample was used without further purification. It has a weight-average molecular weight (M_w) of 840 kDa and a polydispersity (M_w/M_n) of 3.08. SBP solution was purified by centrifugation at 4000 rpm for 35 min and only supernatants used. SBP has $M_w = 410$ kDa and $M_w/M_n = 2.85$. Medium-chain triglyceride (MCT) containing C8 fatty acid and C10 fatty acid at a ratio of 60:40 was obtained from KLK OLEO Ltd., Malaysia. The density of MCT is 0.93 g/ml, and the viscosity at 20 °C is 29 mPa s. All other reagents were of analytical grade.

2.2. Preparation of stock solutions

Stock solutions of GA (20 wt%) and SBP (2 wt%) were prepared by dispersing dry powders in Milli-Q ultra pure water (unless otherwise specified, all the concentrations in the paper are weight percentage concentrations). Sodium azide (0.005%) was added to the stock solutions as a preservative. The solutions were stirred for 24 h at room temperature to ensure complete dissolution of the biopolymers.

2.3. Determination of phase diagram

A cloud curve for GA/SBP mixtures was determined by visual observation. A series of mixed solutions were prepared at a fixed GA concentration (1.0–7.0%) and varying the SBP concentration (0.05–2.5%). The cloud points were identified from the observation of bulk phase separation after the mixtures were left quiescently for 24 h.

The binodal curve of GA/SBP was determined by analyzing phase compositions in the bulk phase separated systems. SBP concentrations in the upper and lower phases were obtained by measuring the concentration of ferulic acid associated with the SBP, according to the procedures reported by Funami et al. (2007, 2011). Ferulic acid was quantified by measuring the absorbance at 310 nm using a TU-1990 UV spectrophotometer (Persee Co., China). From the ferulic acid concentration and relating this to a SBP calibration curve at a series of known concentrations the SBP could be quantified.

GA concentrations in the upper and lower phases were derived using the following equations:

$$C_{\mathrm{GA,U}} = C_{\mathrm{total,U}} - C_{\mathrm{SBP,U}} \qquad (1)$$

$$C_{\mathrm{GA,L}} = C_{\mathrm{total,L}} - C_{\mathrm{SBP,L}} \qquad (2)$$

where $C_{\mathrm{GA,\,U}}$ and $C_{\mathrm{GA,\,L}}$ are GA concentrations in the upper and lower phases respectively; $C_{\mathrm{SBP,\,U}}$ and $C_{\mathrm{SBP,\,L}}$ are SBP concentrations in the upper and lower phases as determined by the UV/vis method; $C_{\mathrm{total,\,U}}$ and $C_{\mathrm{total,\,L}}$ are total biopolymer concentrations in the upper and lower phases, which were as determined from dry residues at 105 °C. The phase compositions are reported as a mean of three measurements.

2.4. Gel permeation chromatography-multiangle laser light scattering (GPC-MALLS)

Phase separation induced fractionation was evaluated by measuring the molecular distribution in the upper and lower phases using GPC-MALLS for GA/SBP mixtures which exhibited phase separation to different extents. This could be varied by controlling GA and SBP concentrations and was characterized by the ratio of the volumes of upper phase to lower phase (V_U/V_L) after bulk phase separation. After appropriate dilution, the samples taken from the upper and lower phases were loaded on to the analytical GPC-MALLS at 25 °C. The system used consisted of a Superose 6 10/300GL column (GE Healthcare, USA), in series with an SPD-10Avp series UV detector (Shimadzu Technologies, Japan) operated at 214 nm, a DAWN HELEOS multiangle light scattering detector (Wyatt Technology Corporation, USA) operated at 658 nm, and an Optilab rEX refractometer (Wyatt Technology Corporation, USA). 0.2 M aqueous NaCl solution filtered through 0.2 μm Millipore filter was used as the eluent, and was delivered at a constant rate of 0.4 ml/min by a Waters 515 HPLC pump (Waters Corporation, USA). Refractive index increment dn/dc values of 0.141 and 0.146 ml/g were used for GA and SBP, respectively (Aoki, Al-Assaf, et al., 2007; Li, Al-Assaf, Fang, & Phillips, 2013).

2.5. Emulsification using GA, FGA, SBP and FSBP

2.5.1. Emulsion preparation

The emulsifying effectiveness of GA and SBP before and after phase separation was compared to evaluate the impact of phase separation induced fractionation, using 4.0% GA and 1.0% SBP. After bulk phase separation, the upper and lower phases were carefully separated using a syringe and then lyophilized to obtain the fractionated SBP (FSBP) and GA (FGA) samples, respectively.

O/W emulsions were prepared using 15% MCT as oil phase and 5.0% GA (FGA) or 1.0% SBP (FSBP) as aqueous phase. 0.13% sodium benzoate was added as a preservative. The mixtures were pre-homogenized with a PT-MR2100 Polytron-type mixer (Kinematica Co., Switzerland) at 26,000 rpm for 3 min. The pre-emulsions were then passed through a high-pressure homogenizer (M-110L, MFIC Co., USA) at 75 MPa to obtain fine emulsions. The samples during homogenizations were maintained in ice bath to minimize possible degradation.

2.5.2. Emulsion characterization

Emulsion particle size and size distribution was measured using the laser diffraction technique and a Mastersizer 2000 (Malvern Instruments, U.K.). Emulsion stability was evaluated by using an acceleration test at 60 °C for a period of 7 days (Aoki, Katayama, et al., 2007; Li, Fang, Al-Assaf, Phillips, & Jiang, 2012). Before each