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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.

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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:

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

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

where $C_{GA,\,U}$ and $C_{GA,\,L}$ are GA concentrations in the upper and lower phases respectively; $C_{SBP,\,U}$ and $C_{SBP,\,L}$ are SBP concentrations in the upper and lower phases as determined by the UV/vis method; $C_{total,\,U}$ and $C_{total,\,L}$ 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

measurement, the emulsions were shaken gently to keep a homogeneously dispersed state. The refractive indexes for disperse and water phases were 1.520 and 1.330, respectively. Volume weighted average diameters ($D_{4,3}$) obtained from size distribution was used to characterize mean particle size and were reported as average of triplicate measurements.

2.5.3. Zeta potential measurements

The zeta potential ζ of emulsion droplets was measured using a Zetasizer Nano-ZS (Malvern Instruments, U.K.) equipped with a 4 mW He/Ne laser emitting at 633 nm. Electrophoretic mobility U_E of charged particles was obtained by means of laser Doppler velocimetry (LVD) at 17°, which was related to ζ by the Henry equation: (Li, Fang, Al-Assaf, Phillips, Yao, et al., 2012)

$$U_E = \frac{2\varepsilon\zeta f(Ka)}{3\eta} \quad (3)$$

where ε is the dielectric constant and η the viscosity of medium. $f(Ka)$ is the Henry's function which has a value of 1.5 under the Smoluchowski approximation. The emulsions were diluted approximately 200 times using Milli-Q ultra pure water before measurements at 25 °C.

2.6. Interfacial adsorption of GA, FGA, SBP and FSBP

The interfacial tension of GA, FGA, SBP and FSBP adsorbed on to MCT–water interface was measured at 25 °C using a drop profile tensiometer (Teclis Tracker, France). A rising drop of MCT oil was formed at the tip of a syringe needle which was submerged in an optical glass cuvette containing different gum solutions. The gum concentration was 0.4% for GA and FGA, and 0.1% for SBP and FSBP, respectively. The drop profile was recorded by a high speed charge couple device (CCD) camera and was analyzed according to the Laplace equation (Castellani, Al-Assaf, Axelos, Phillips, & Anton, 2010).

3. Results and discussion

3.1. Phase diagram of GA/SBP mixed systems

Fig. 1A shows an example of bulk phase separations of GA/SBP containing 1.0% SBP and various concentrations of GA. After standing quiescently for 24 h, all of the mixtures exhibited a clear phase boundary. The proportion of the lower phase increased with increasing GA concentration, which indicated that the lower phase is the GA-rich phase, with the upper phase being SBP-rich. This can also be confirmed by phase composition analysis and GPC-MALLS measurements. Based on visual observation of bulk phase separations, the cloud points of GA/SBP mixtures were determined (Fig. 1B). GA and SBP are compatible at all mixing ratios only when GA < 1.2% or SBP < 0.05%. The threshold concentration of SBP eliciting incompatibility decreases with increasing GA concentration.

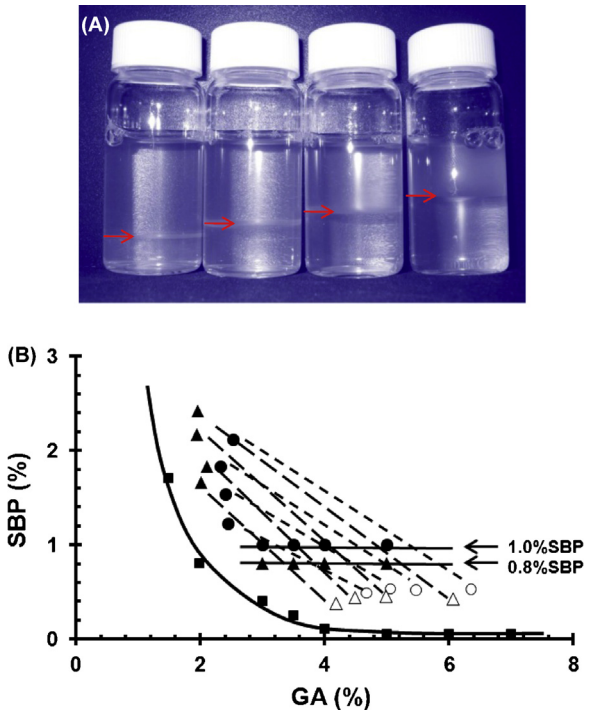

Fig. 1. (A) Example of bulk phase separations of GA/SBP mixtures containing 1.0% SBP and various GA concentrations (from left to right): 3.0%, 3.5%, 4.0% and 5.0%; (B) phase diagram of GA/SBP mixtures at 25 °C: cloud points (■) determined by visual observation and binodal points determined by phase composition analysis for mixed systems with two different SBP starting concentrations: (▲, △) 0.8% SBP and (●, ○) 1.0% SBP. The solid and open symbols represent the upper and lower phase, respectively. The broken lines are tie-lines.

For bulk phase separated systems, we analyzed the compositions of the upper and lower phases and the data was shown in Table 1 for GA/SBP mixtures with two different SBP starting concentrations (0.8% and 1.0%). The upper phase always contains more SBP than the lower phase, and thus is SBP-rich phase. The lower phase is therefore GA-rich phase.

The phase composition data (binodal points) were plotted in Fig. 1B. The binodal determined from the mixtures with 0.8% SBP does not overlap with that determined from the mixtures with 1.0% SBP. The mixed systems with 0.8% SBP seem to have a smaller domain of miscibility than those with 1.0% SBP. It seems then that the phase separation behavior depends on the starting concentrations of the components in the mixtures. Furthermore, none of the binodal points are close to the cloud points. The difference between the binodal and cloud points is too large to be due to measurement error and a similar behavior was observed in poly(ethylene oxide)/dextran, gelatin/dextran and maltodextrin/agarose systems (Edelman, Tromp, et al., 2003; Edelman, van der Linden, et al., 2003; Loret et al., 2005). The phenomenon has been interpreted to be

Table 1
Phase composition and volume determined from different GA/SBP mixtures at 25 °C.

Mixture	Upper phase (SBP-rich phase)			Lower phase (GA-rich phase)		
	GA% (w/w)	SBP% (w/w)	Volume% (v/v)	GA% (w/w)	SBP% (w/w)	Volume% (v/v)
3.0% GA + 1.0% SBP	2.46 ± 0.02	1.23 ± 0.08	78.2 ± 0.00	4.68 ± 0.10	0.49 ± 0.01	21.8 ± 0.00
3.5% GA + 1.0% SBP	2.42 ± 0.03	1.54 ± 0.03	66.4 ± 0.37	5.06 ± 0.06	0.53 ± 0.08	33.6 ± 0.37
4.0% GA + 1.0% SBP	2.33 ± 0.06	1.83 ± 0.09	54.7 ± 0.28	5.48 ± 0.05	0.52 ± 0.05	45.3 ± 0.28
5.0% GA + 1.0% SBP	2.53 ± 0.06	2.12 ± 0.09	43.9 ± 0.19	6.36 ± 0.04	0.53 ± 0.06	56.1 ± 0.19
3.0% GA + 0.8% SBP	2.03 ± 0.01	1.66 ± 0.01	85.2 ± 0.24	4.19 ± 0.06	0.38 ± 0.01	14.8 ± 0.24
3.5% GA + 0.8% SBP	2.12 ± 0.08	1.83 ± 0.05	65.7 ± 0.09	4.49 ± 0.03	0.44 ± 0.05	34.3 ± 0.09
4.0% GA + 0.8% SBP	1.95 ± 0.08	2.17 ± 0.08	51.3 ± 0.00	4.99 ± 0.10	0.45 ± 0.07	48.7 ± 0.00
5.0% GA + 0.8% SBP	1.97 ± 0.03	2.42 ± 0.07	36.5 ± 0.20	6.07 ± 0.02	0.42 ± 0.01	63.5 ± 0.20

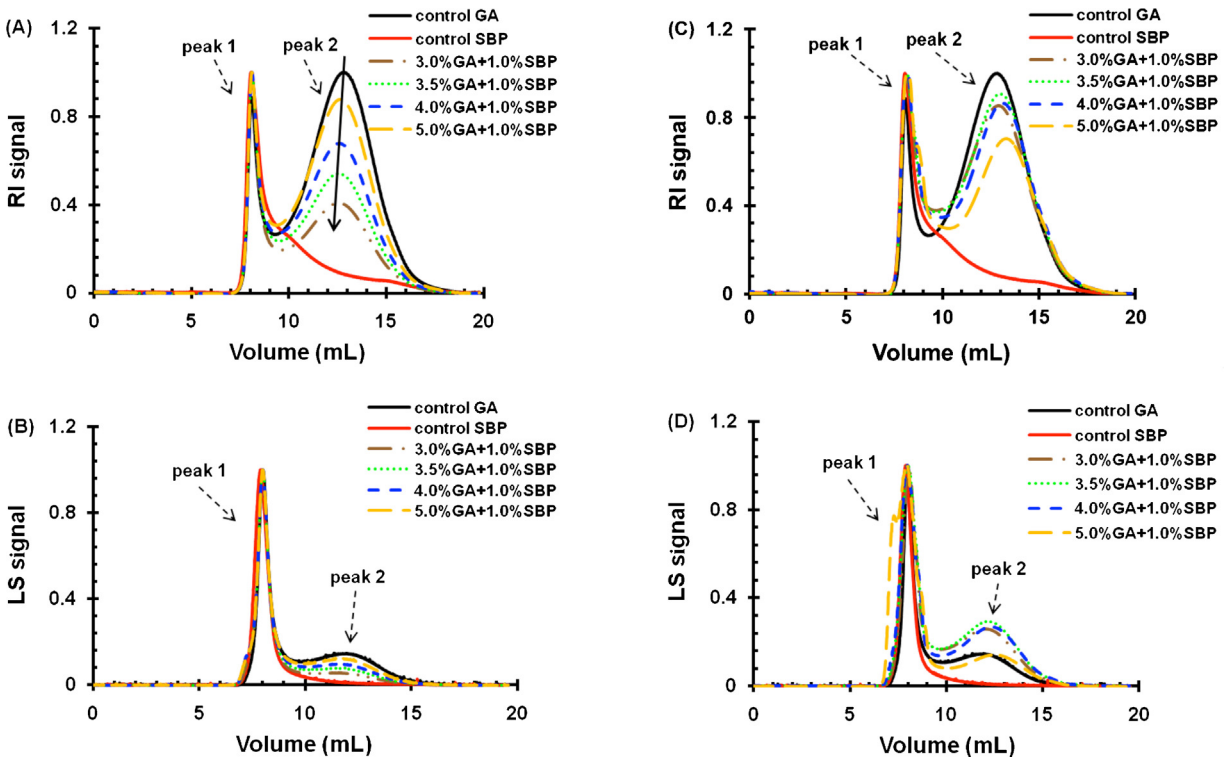

Fig. 2. Comparison of GPC elution profiles of the upper and lower phases of phase separated systems containning 1.0% SBP with different concentrations of GA: (A) RI signal for lower phases; (B) LS signal for lower phases; (C) RI signal for upper phases and (D) LS signal for upper phases. All the signals were normalized to the height of the first peak.

due to a phase separation induced fractionation of polydisperse biopolymers.

It is well known that phase separation is strongly influenced by the molecular weight of biopolymers (Loret et al., 2005). Generally, a higher molecular weight results in a stronger tendency toward phase separation. The GA and SBP samples used in the study have polydispersity index values of 3.08 and 2.85, respectively. It might be expected that the fraction of higher molecular weight would tend to segregate from the other biopolymer, whereas the fraction of lower molecular weight has relatively a higher tolerance to coexistence (Loret et al., 2005). The fractions of different molecular weights certainly do not react in the same way toward phase separation, thereby leading to molecular fractionation during phase separation. Furthermore, GA is well-known to be heterogeneous molecules, consisting of three molecular species: AGP, AG and GP (Aoki, Al-Assaf, et al., 2007; Randall, Phillips, & Williams, 1988). These three components have distinct chemical structures. SBP is also quite complex and polydisperse in structure and it contains a small amount of proteinaceous materials (Nakauma et al., 2008; Siew & Williams, 2008; Williams et al., 2005). The phase separation of GA/SBP should ideally be treated as a multi-component system rather than a classic binary system. The deviation between binodal points and cloud points could be attributed in part to the complex components contained in GA and SBP, in addition to their broad molecular weight distributions (Loret et al., 2005).

3.2. *Phase separation induced molecular fractionation in GA/SBP mixed systems*

As can be seen in Fig. 1A, the extent of phase separation, characterized by V_U/V_L, can be adjusted by varying the mixing ratio of GA to SBP. Here we investigated how the phase separation extent influences molecular fractionation. Fig. 2 shows the refractive index (RI) and light scattering (LS) signals of GPC-MALLS profiles measured for the upper and lower phases of the mixed systems containing 1.0% SBP and different concentrations of GA. The signals for control GA and SBP are also included. For comparison, all the signals were normalized.

The control GA has two distinct peaks both in RI and LS signals, located at 8 mL and 13 mL, respectively. The elution profiles are typical of GA. The first peak can been assigned to AGP and the second to AG and GP (Aoki, Al-Assaf, et al., 2007). The control SBP has one major peak located at more or less the same elution volume as AGP and a negligible tail extending to 17 mL.

For the phase separated systems, the shape of RI and LS profiles of the lower phases (Fig. 2A and B) resembles that of control GA. This is because the composition of lower phases is predominantly GA, and the concentration of GA is more than ten times that of SBP (open symbols in Fig. 1B). The intensity of the peak for AG and GP (the second peak) decreases with decreasing GA concentration when the SBP concentration is fixed, indicating a decreased proportion of the low molecular weight fractions and correspondingly an increased proportion of the high molecular weight fraction AGP. In addition, the combined peak for AG and GP shifts slightly to lower elution volumes with decreasing GA concentration, indicating a molecular fractionation for AG and GP with the higher molecular weight AG and GP retained in the lower phases.

For the upper phases (Fig. 2C and D), the RI and LS profiles are composites of those for control GA and control SBP. It means that both GA and SBP exist in the upper phases in comparable amounts. This is in agreement with the phase diagram in Fig. 1B which shows that the upper phases contain roughly equal concentrations of GA and SBP (solid symbols). At the fixed SBP concentration, the peak for AG and GP (the second peak, Fig. 2C) in general increases with decreasing GA concentration, implying an increased proportion of the lower molecular weight fractions in the upper phases.

The results show that the lower phases are enriched relatively in GA and the upper phase enriched in SBP. Molecular fractionation

Table 2
Molecular parameters of control GA, control SBP, FGA and FSBP as obtained by phase separation.

Mixture	V_U/V_L	M_w of FGA ($\times 10^6$)	M_w of FSBP ($\times 10^6$)	AGP in FGA (%)	M_w of AGP in FGA ($\times 10^6$)
Control GA	N/A	0.84	N/A	12.8	3.09
Control SBP	N/A	N/A	0.41	N/A	N/A
3.0% GA + 1.0% SBP	3.59	1.58	0.43	27.2	3.55
3.5% GA + 1.0% SBP	1.98	1.32	0.38	21.5	3.27
4.0% GA + 1.0% SBP	1.21	1.12	0.37	18.7	3.05
5.0% GA + 1.0% SBP	0.78	0.94	0.42	15.6	2.95
3.0% GA + 0.8% SBP	5.75	1.75	0.45	24.3	3.67
3.5% GA + 0.8% SBP	1.92	1.44	0.40	21.2	3.44
4.0% GA + 0.8% SBP	1.05	1.21	0.38	18.0	3.24
5.0% GA + 0.8% SBP	0.57	0.96	0.36	14.4	2.82

occurs for both of the biopolymers, but is more markedly for GA. We, therefore, designated the lower phases as fractionated GA (FGA) and the upper phases as fractionated SBP (FSBP). GPC-MALLS software was used to calculate molecular parameters of FGA and FSBP. Table 2 gives the molecular parameters together with V_U/V_L for two sets of phase separated systems starting with two different SBP concentrations (0.8% and 1.0%). With increasing V_U/V_L, the M_w of FGA increases relative to that of control GA and nearly doubles. Meanwhile, the AGP content in FGA increases from 12.8% for control GA to a level as high as 27.2%. The M_w of AGP in FGA only increases slightly. Thus the increase in M_w of FGA mainly results from the increased proportion of the high molecular weight fraction AGP, rather than any chemical change or physical aggregation occurring in the samples. With varying V_U/V_L, M_w of FSBP is almost unchanged and is slightly lower than that of control SBP in most systems. This can be attributed to the introduction of low molecular weight fractions AG and GP into the FSBP during phase separation. AG and GP were reported to have molecular weights in the range of 200–473 kDa (Al-Assaf, Phillips, Aoki, & Sasaki, 2007; Renard, Lavenant-Gourgeon, Ralet, & Sanchez, 2006).

The effect of the initial concentration of the phase separation mixture on the molecular fractionation was considered. Fig. 3 plots AGP content in FGA for two sets of phase separated systems with two different SBP starting concentrations. Notably, the AGP content falls along a master curve when plotted against the extent of phase separation extent V_U/V_L, regardless of SBP starting concentration. It would seem then that the molecular fractionation during phase separation is essentially governed by the extent of phase separation. For the GA/SBP system we have used, the AGP content increases with increasing V_U/V_L, and nearly levels off when V_U/V_L is further increased. The maximum relative AGP content in FGA that can be achieved by phase separation-induced fractionation is around 26%. This indicates an upper limit for phase separation induced molecular fraction.

3.3. Emulsifying properties of GA, FGA, SBP and FSBP

The emulsifying properties of FGA and FSBP as produced by phase separation were evaluated by preparing emulsion containing 15% MCT and these were compared with those of control GA and SBP. Fig. 4 illustrates the particle size distribution of the freshly prepared emulsions. Compared with control GA, the emulsion stabilized with 5% FGA exhibited relatively broader distribution yet smaller particle size. The volume-weighted mean diameter $D_{4,3}$ was 0.72 μm for FGA emulsion while that of control GA emulsion was 1.03 μm. This shows that FGA is an enhanced emulsifier than control GA and is more efficient in stabilizing an oil–water interface. It is the AGP which is known to be the main component responsible for the emulsifying activity of gum arabic (Al-Assaf et al., 2007; Dickinson, 2003), due to the presence of both polypeptide and polysaccharide blocks. It is therefore reasonable to attribute the enhanced emulsifying activity of FGA to its higher content of AGP generated by phase separation. It is noteworthy also that the presence of small amount of SBP in FGA (less than 10%) seems not to impair its emulsifying activity.

On the other hand, in contrast, FSBP has an inferior emulsifying activity when compared to the control SBP. The emulsion stabilized with 1% FSBP has a broader particle size distribution and a larger average size. $D_{4,3}$ was 1.61 μm for FSBP emulsion in contrast to that of 0.95 μm for control SBP emulsion. The reduced emulsifying activity of FSBP is possibly due to: (1) the presence of considerable amount of GA in FSBP as shown by the phase composition analysis and in particular to the GA entering into FSBP, which is predominantly composed of the AG and GP fractions. AG and GP fractions have been reported to have no or less emulsifying activity than AGP (Castellani, Al-Assaf, et al., 2010; Jayme, Dunstan, & Gee, 1999); (2) the competitive adsorption of GA and FSBP at the oil–water interface could hinder the formation of interfacial coating around oil droplets, and led to the reducing emulsifying activity (Li et al., 2013; Li, Fang, Al-Assaf, Phillips, & Jiang, 2012).

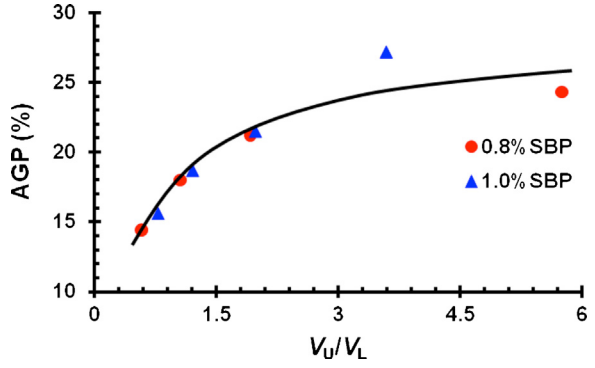

Fig. 3. Plot of AGP content in FGA obtained from the lower phases of GA/SBP mixtures against phase separation extent as characterized by the ratio of the volumes of upper phases to lower phases V_U/V_L: (●) phase separated systems starting with 0.8% SBP; (▲) phase separated systems starting with 1.0% SBP.

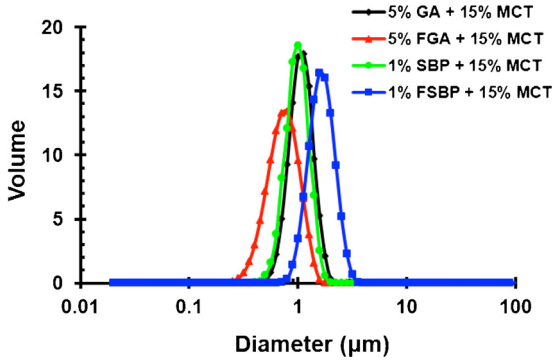

Fig. 4. Droplet size distribution of freshly prepared oil-in-water emulsions containing 15% MCT as oil phase and 5% GA, 5% FGA, 1% SBP or 1% FSBP as emulsifiers.

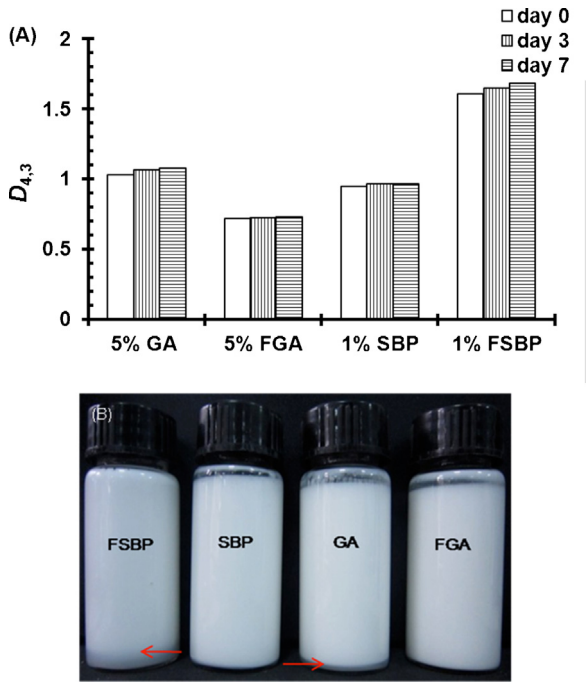

Fig. 5. Stability during storage at 60 °C of the emulsions containning 15% MCT and 5% GA, 5% FGA, 1% SBP or 1% FSBP: (A) change in $D_{4,3}$ with storage time; (B) appearnce of the emulsions after stroage for 7 days at 60 °C.

The emulsion stability was assessed using the acceleration test at elevated temperature. Fig. 5A shows the change of $D_{4,3}$ for the emulsions stabilized with GA, FGA, SBP and FSBP during storage at 60 °C for 7 days. $D_{4,3}$ for the emulsion stabilized with 5% FGA is smaller than that with 5% control GA throughout the evaluation period and shows less development. In contrast to FGA emulsion, a slight tendency of creaming was noticed for GA emulsion on the 7th day of storage (indicated by an arrow in Fig. 5B). Thus FGA can provide better stabilization of oil-in-water emulsions. This can be attributed to the higher content of AGP in FGA which confers a thicker interfacial layer and so providing more effective steric stabilizing effect, due to the larger hydrodynamic size of the AGP fraction (Dickinson, 2003; Dickinson, Galazka, & Anderson, 1991). On the other hand, the emulsion stabilized with 1% FSBP shows larger $D_{4,3}$ vaules than 1% SBP throughout the evaluation period and exhibits more pronounced growth. Creaming was clearly observed in the emulsion stabilized with 1% FSBP on the 7th day of the storage (indicated by an arrow in Fig. 5B). There is therefore reduced stabilizing effect of FSBP compared with SBP. This probably arises from the presence of considerable amount of AG and GP in the FSBP which compete for the interface. The adsorption of AG and GP on to the interface cannot provide comparable steric due to their smaller hydrodynamic size relative to SBP (Li et al., 2009; Nakauma et al., 2008).

Fig. 6 measures the zeta potential ζ of the emulsions stabilized by GA, FGA, SBP and FSBP during storage at 60 °C for 7 days. The fresh GA emulsion (0 day) has a ζ of −48.6 mV, compared with that of −47.1 mV for FGA emulsion. During storage, the GA emulsion, however, displays a more pronounced reduction in the absolute value of ζ relative to the FGA emulsion, indicating less stability. The ζ of GA emulsions are less negative than that of the FGA emulsions after the 3rd day of the storage. The more negative value ζ of FGA emulsions indicates a stronger electrostatic stabilizing effect between emulsion droplets, which can be attributed to the higher content of AGP in FGA, a relatively stronger polyelectrolyte compared to AG and GP (Renard et al., 2006). The ζ of SBP emulsion is more negative than that of FSBP emulsion throughout the storage

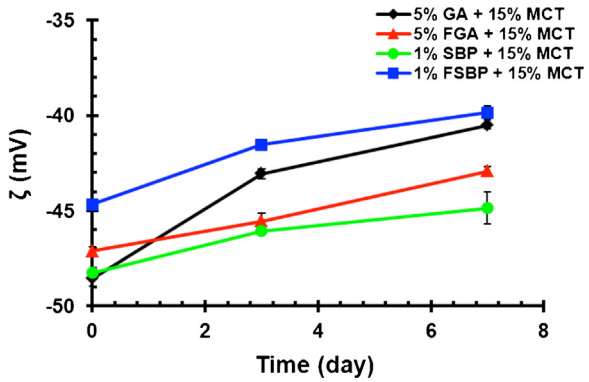

Fig. 6. Change in zeta potential ζ during storage at 60 °C for the emulsions containning 15% MCT and 5% GA, 5% FGA, 1% SBP or 1% FSBP.

period, and these show similar extent of reduction. The less negative ζ of FSBP emulsion results in a weaker electrostatic stabilizing effect, and thus contributes to a less stable emulsion compared to SBP emulsion.

Additionally, we also assessed the viscosity difference between GA/FGA and SBP/FSBP. The steady shear rheological measurements indicated that the viscosity difference bewteen GA and FGA was negligible while FSBP had a lower viscosity than SBP (data not shown). The decrease in viscosity for FSBP should be due to the considerable amount of AG and GP contained therein. The enhanced emulsifying functionality of FGA is therefore not a result of viscosity effect. However for FSBP, the reduction in emulsifying functionality could be linked to the reduction in viscosity.

3.4. Interfacial adsorption of GA, FGA, SBP and FSBP

Emulsification effectiveness is directly influenced by the surface activity of macromolecular emulsifiers. The interfacial properties of GA, FGA, SBP and FSBP were investigated at typical concentrations and the results are shown in Fig. 7. It is significant that although FGA contains a higher proportion of large molecular fraction AGP, it reduces the interfacial tension much more quickly than the control GA. Additionally, FGA can attain a lower interfacial tension than GA. This suggests that FGA has a more surface active component than GA, from both kinetic and thermodynamic points of view, which is in consistent with other reports (Castellani, Al-Assaf, et al., 2010; Castellani, Guibert, et al., 2010). The adsorption of FSBP is more rapid than that of SBP. However, the equilibrium interfacial tension attained by FSBP is higher than that by SBP, indicating that the former is less surface active. The interfacial properties of GA, FGA, SBP and FSBP are consistent with and provide further understanding of their effectiveness in emulsifying and stabilizing MCT emulsions.

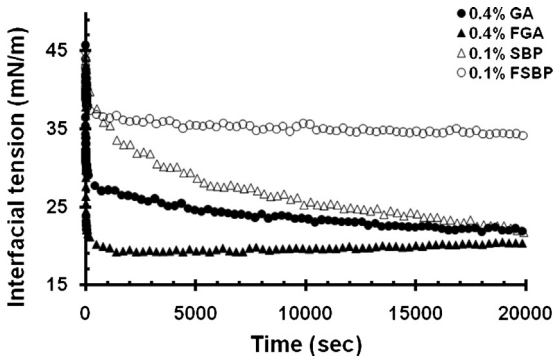

Fig. 7. Linear plot of interfacial tension kinetics of GA, FGA, SBP and FSBP adsorbed on to the MCT–water interface at 25 °C.

4. Conclusion

This study investigates the phase separation and phase separation induced fractionation of polydisperse GA/SBP mixtures. GA undergoes a significant molecular fractionation during segregative phase separation with SBP, resulting in a two-fold increase in AGP content. The fractionation efficiency in producing AGP is controlled by the extent of phase separation, as characterized by the ratio of the phase volumes. After phase separation the fractionated GA showed improved emulsifying functionality, whereas that of the fractionated SBP is reduced. The changes in the emulsifying functionalities described can be explained by the zeta potential and interfacial adsorption measurements. The methods described offer a green and facile method to fractionate polydisperse biopolymers and to concentrate functional components in order to increase their functionality.

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References

Al-Assaf, S., Phillips, G. O., Aoki, H., & Sasaki, Y. (2007). Characterization and properties of *Acacia senegal* (L.) Willd. var. senegal with enhanced properties (Acacia (sen) SUPER GUM™): Part 1 – Controlled maturation of *Acacia senegal* var. senegal to increase viscoelasticity, produce a hydrogel form and convert a poor into a good emulsifier. *Food Hydrocolloids*, *21*(3), 319–328.

Aoki, H., Al-Assaf, S., Katayama, T., & Phillips, G. O. (2007). Characterization and properties of *Acacia senegal* (L.) Willd. var. senegal with enhanced properties (Acacia (sen) SUPER GUM™): Part 2 – Mechanism of the maturation process. *Food Hydrocolloids*, *21*(3), 329–337.

Aoki, H., Katayama, T., Ogasawara, T., Sasaki, Y., Al-Assaf, S., & Phillips, G. O. (2007). Characterization and properties of *Acacia senegal* (L.) Willd. var. senegal with enhanced properties (Acacia (sen) SUPER GUM™): Part 5. Factors affecting the emulsification of *Acacia senegal* and Acacia (sen) SUPER GUM™. *Food Hydrocolloids*, *21*(3), 353–358.

Castellani, O., Al-Assaf, S., Axelos, M., Phillips, G. O., & Anton, M. (2010). Hydrocolloids with emulsifying capacity. Part 2 – Adsorption properties at the n-hexadecane–water interface. *Food Hydrocolloids*, *24*(2–3), 121–130.

Castellani, O., Guibert, D., Al-Assaf, S., Axelos, M., Phillips, G. O., & Anton, M. (2010). Hydrocolloids with emulsifying capacity. Part 1 – Emulsifying properties and interfacial characteristics of conventional (*Acacia senegal* (L.) Willd. var. senegal) and matured (Acacia (sen) SUPER GUM™) *Acacia senegal*. *Food Hydrocolloids*, *24*(2–3), 193–199.

Dickinson, E. (2003). Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food Hydrocolloids*, *17*(1), 25–39.

Dickinson, E., Galazka, V. B., & Anderson, D. M. W. (1991). Emulsifying behaviour of gum arabic. Part 2: Effect of the gum molecular weight on the emulsion droplet-size distribution. *Carbohydrate Polymers*, *14*(4), 385–392.

Edelman, M., Tromp, R., & van der Linden, E. (2003). Phase-separation-induced fractionation in molar mass in aqueous mixtures of gelatin and dextran. *Physical Review E*, *67*(2), 12.

Edelman, M. W., van der Linden, E., de Hoog, E., & Tromp, R. H. (2001). Compatibility of gelatin and dextran in aqueous solution. *Biomacromolecules*, *2*(4), 1148–1154.

Edelman, M. W., van der Linden, E., & Tromp, R. H. (2003). Phase separation of aqueous mixtures of poly(ethylene oxide) and dextran. *Macromolecules*, *36*(20), 7783–7790.

Fang, Y. P., Li, L. B., Inoue, C., Lundin, L., & Appelqvist, I. (2006). Associative and segregative phase separations of gelatin/kappa-carrageenan aqueous mixtures. *Langmuir*, *22*(23), 9532–9537.

Funami, T., Nakauma, M., Ishihara, S., Tanaka, R., Inoue, T., & Phillips, G. O. (2011). Structural modifications of sugar beet pectin and the relationship of structure to functionality. *Food Hydrocolloids*, *25*(2), 221–229.

Funami, T., Zhang, G., Hiroe, M., Noda, S., Nakauma, M., Asai, I., et al. (2007). Effects of the proteinaceous moiety on the emulsifying properties of sugar beet pectin. *Food Hydrocolloids*, *21*(8), 1319–1329.

Jara, F., & Pilosof, A. M. R. (2011). Partitioning of α-lactalbumin and β-lactoglobulin in whey protein concentrate/hydroxypropylmethylcellulose aqueous two-phase systems. *Food Hydrocolloids*, *25*(3), 374–380.

Jayme, M. L., Dunstan, D. E., & Gee, M. L. (1999). Zeta potentials of gum arabic stabilised oil in water emulsions. *Food Hydrocolloids*, *13*(6), 459–465.

Kim, H., Decker, E., & Julianmcclements, D. (2006). Preparation of multiple emulsions based on thermodynamic incompatibility of heat-denatured whey protein and pectin solutions. *Food Hydrocolloids*, *20*(5), 586–595.

Lazaridou, A., & Biliaderis, C. G. (2009). Concurrent phase separation and gelation in mixed oat β-glucans/sodium caseinate and oat β-glucans/pullulan aqueous dispersions. *Food Hydrocolloids*, *23*(3), 886–895.

Li, X., Al-Assaf, S., Fang, Y., & Phillips, G. O. (2013). Competitive adsorption between sugar beet pectin (SBP) and hydroxypropyl methylcellulose (HPMC) at the oil/water interface. *Carbohydrate Polymers*, *91*(2), 573–580.

Li, X., Fang, Y., Al-Assaf, S., Phillips, G. O., & Jiang, F. (2012). Complexation of bovine serum albumin and sugar beet pectin: Stabilising oil-in-water emulsions. *Journal of Colloid and Interface Science*, *388*(1), 103–111.

Li, X., Fang, Y., Al-Assaf, S., Phillips, G. O., Nishinari, K., & Zhang, H. (2009). Rheological study of gum arabic solutions: Interpretation based on molecular self-association. *Food Hydrocolloids*, *23*(8), 2394–2402.

Li, X., Fang, Y., Al-Assaf, S., Phillips, G. O., Yao, X., Zhang, Y., et al. (2012). Complexation of bovine serum albumin and sugar beet pectin: Structural transitions and phase diagram. *Langmuir*, *28*(27), 10164–10176.

Loret, C., Schumm, S., Pudney, P., Frith, W., & Fryer, P. (2005). Phase separation and molecular weight fractionation behaviour of maltodextrin/agarose mixtures. *Food Hydrocolloids*, *19*(3), 557–565.

Matalanis, A., Lesmes, U., Decker, E. A., & McClements, D. J. (2010). Fabrication and characterization of filled hydrogel particles based on sequential segregative and aggregative biopolymer phase separation. *Food Hydrocolloids*, *24*(8), 689–701.

Nakauma, M., Funami, T., Noda, S., Ishihara, S., Al-Assaf, S., Nishinari, K., et al. (2008). Comparison of sugar beet pectin, soybean soluble polysaccharide, and gum arabic as food emulsifiers. 1. Effect of concentration, pH, and salts on the emulsifying properties. *Food Hydrocolloids*, *22*(7), 1254–1267.

Pérez, O. E., Wargon, V., & Pilosof, M. R. (2006). A Gelation and structural characteristics of incompatible whey proteins/hydroxypropylmethylcellulose mixtures. *Food Hydrocolloids*, *20*(7), 966–974.

Perrechil, F. A., & Cunha, R. L. (2012). Development of multiple emulsions based on the repulsive interaction between sodium caseinate and LBG. *Food Hydrocolloids*, *26*(1), 126–134.

Randall, R. C., Phillips, G. O., & Williams, P. A. (1988). The role of the proteinaceous component on the emulsifying properties of gum arabic. *Food Hydrocolloids*, *2*(2), 131–140.

Renard, D., Lavenant-Gourgeon, L., Ralet, M. C., & Sanchez, C. (2006). *Acacia senegal* gum: Continuum of molecular species differing by their protein to sugar ratio, molecular weight, and charges. *Biomacromolecules*, *7*(9), 2637–2649.

Siew, C. K., & Williams, P. A. (2008). Role of protein and ferulic acid in the emulsification properties of sugar beet pectin. *Journal of Agricultural and Food Chemistry*, *56*(11), 4164–4171.

Tolstoguzov, V. (2003). Some thermodynamic considerations in food formulation. *Food Hydrocolloids*, *17*(1), 1–23.

van Heukelum, A., Barkema, G. T., Edelman, M. W., van der Linden, E., de Hoog, E. H. A., & Tromp, R. H. (2003). Fractionation in a phase-separated polydisperse polymer mixture. *Macromolecules*, *36*(17), 6662–6667.

Williams, P. A., & Phillips, G. O. (2000). Gum arabic. In G. O. Phillips, & P. A. Williams (Eds.), *Handbook of hydrocolloids* (Vol. 9) (pp. 155–168). New York: CRC Press.

Williams, P. A., Sayers, C., Viebke, C., Senan, C., Mazoyer, J., & Boulenguer, P. (2005). Elucidation of the emulsification properties of sugar beet pectin. *Journal of Agricultural and Food Chemistry*, *53*(9), 3592–3597.