



Effect of gum Arabic on stability of oil-in-water emulsion stabilized by flaxseed and soybean protein

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ABSTRACT

The effects of gum Arabic (GA) addition (0–4%, w/w) on stability of oil-in-water emulsion stabilized by flaxseed protein concentrate (FPC) and soybean protein concentrate (SPC) were studied. The result shows that emulsions stabilized by both proteins in the presence of the 2% gum Arabic (w/w) have better stability than its absence, by increasing the emulsion viscosity of the FPC stabilized emulsion and causing competitive adsorption between the GA and SPC layer to give a steric repulsion for the SPC stabilized emulsion, respectively. Then, the influences of ionic strength (0–200 mM NaCl) and temperature (25–95 °C for 20 min) on these emulsions in presence of GA were determined. The GA adsorbed at SPC-stabilized oil–water interface provided stability against NaCl concentration. In presence of GA, the SPC-stabilized emulsions also showed better stability at higher temperatures compared to the FPC-stabilized emulsions due to the denaturation of SPC and competitive adsorption between GA and SPC at higher temperatures.

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1. Introduction

Many food products are in the form of oil-in-water (O/W) emulsions consisting small lipid droplets dispersed in an aqueous phase, such as salad, milk, cream, beverages and desserts (Grigoriev & Miller, 2009; Leal-Calderon, Thivilliers, & Schmitt, 2007; Sikora, Badrie, Deisingh, & Kowalski, 2008). These emulsions are thermodynamically unstable and are prone to destabilization (Dickinson, 1992; Guzey, Kim, & McClements, 2004; McClements, 1999). Destabilization of these emulsions can occur through variety of physicochemical processes such as gravitational separation, flocculation, coalescence, and Ostwald ripening (McClements, 1999). One of the most commonly used methods to improve the stability of O/W emulsions is to utilize emulsifiers (Stauffer, 1999). Emulsifiers are surface-active compounds which adsorb at oil–water interface and facilitate the droplet formation and stabilization by lowering the surface tension. They also prevent droplet aggregation by generating repulsive forces between droplets (Walstra, 1993). In food and pharmaceutical applications, there is an increasing trend of using ‘green’ emulsifiers such as protein and polysaccharides to produce and stabilize emulsions (Dickinson, 1993; Gariti,

1999; Mirhosseini, Tan, Hamid, & Yosuf, 2008; Wang, Li, Wang, & Özkan, 2010; Wang, Li, Wang, Adhikari, & Shi, 2010; White et al., 2008). Generally, proteins are the main emulsifiers and the polysaccharides contribute to the stability of emulsions through their thickening and steric stabilizing characteristics. The interactions between proteins and the carbohydrates can also help stabilizing the emulsions. Several polysaccharides have been used together with proteins to enhance the emulsion stability. The combinations such as whey protein isolate/gum Arabic (Klein, Aserin, Svitov, & Garti, 2010), β -lactoglobulin/pectin (Guzey & McClements, 2007), β -lactoglobulin/ κ -carrageenan (Gu, Regnier, & McClements, 2005), whey protein/chitosan/gum Arabic (Moschakis, Murray, & Biliaderis, 2010) and milk protein products/xanthan (Hemar, Tamehana, Munto, & Singh, 2001) are most frequently used.

Gum Arabic is a complex blend of natural polysaccharides composed of three components differing in molecular size and protein content (Mahendran, Williams, Phillips, & Al-Assaf, 2008; Randall, Phillips, & Williams, 1989). These are commonly referred to as the arabinogalactan–protein (AGP, about 10 wt% of total), arabinogalactan (AG, about 90–99 wt% of total) and glycoprotein (GP, about 1 wt% of total) (Phillips & Williams, 2000). In the gum Arabic obtained from *Acacia senegal* (L.), the molecular weight of AGP and AG are 286 kDa and 1860 kDa, respectively. The three populations of GP have molecular weight of 2670 kDa, 776 kDa and 295 kDa (Renard, Lavenant-Gourgeon, Ralet, & Sanchez, 2006). It has been shown that the protein-containing fraction of this gum adsorbs on

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Nomenclature

η	apparent viscosity, Pa s
η_{∞}	infinite shear rate limiting viscosity, Pa s
$\dot{\gamma}$	shear rate, s^{-1}
K	consistency coefficient in Sisko model, $Pa s^n$
n	flow behavior index of Sisko model, dimensionless
A	absorbance of 500 nm wavelength light of emulsions
T	turbidity of emulsions, m^{-1}
V	dilution factor, dimensionless
l	path length of the cuvette, m
ESI	emulsifying stability index of emulsion, h

the oil–water interface. Therefore, gum Arabic can be used not only as a stabilizer for increasing emulsion viscosity, but it also contributes as emulsifier to create surface active layer at water–oil interface.

Soybean protein has been widely used as a source of high quality proteins in many food products because of its excellent functional properties and low cost. It is preferred in stabilizing O/W emulsions because of the surface active properties of its constituent proteins: 7S and 11S globulins (Palazolo, Mitidieri, & Wangner, 2003). It has been shown that soybean protein decreases the interfacial tension at the oil–water interface and stabilizes the emulsions by forming a physical barrier at the oil–water interface (Molina, Papadopoulos, & Ledward, 2001). It is also reported that the soybean protein possesses better emulsifying properties compared to many other plant proteins (Hettiarachchy & Kalapathy, 1998; Tornberg, Olsson, & Persson, 1997; Zayas, 1997). Recent studies on flaxseed have shown that it contains two protein fractions: a salt-soluble protein with high molecular weight (11–12S globulins) and a water-soluble protein with low molecular weight (1.6–2S globulins) (Madhusudhan & Singh, 1983; Madhusudhan & Singh, 1985; Marcone, Kaduda, & Yada, 1998; Youle & Huang, 1981) with molecular weights of 29.4 kDa and 16 kDa, respectively. These flaxseed proteins are also found to be beneficial to various diseases such as coronary heart disease, kidney disease and cancer (Oomah & Mazza, 2000; Wang et al., 2009; Wang, Li, Wang, & Özkan, 2010). It has also been shown that flaxseed protein concentrate has better water and oil absorption, emulsifying activity and emulsion stability compared to soybean protein (Dev & Quensel, 1988, 1989).

The objective of this study was to investigate the effect of gum Arabic (GA) on the stability of O/W emulsions stabilized by flaxseed protein concentrate (FPC) or soybean protein concentrate (SPC). Furthermore, using the best addition concentration of GA, the effect of environmental stresses such as ionic strength and heating on the emulsion stability was also determined. The study will provide further insights regarding the interaction between GA and these two types of proteins, then benefit for using them in food industrial application.

2. Materials and methods

2.1. Materials

Gum Arabic (GA) was purchased from Xilong Chemicals (Shantou, Guangdong province, China). Soybean protein concentrate was donated by Buer Soybean Protein Corporation (Jilin Province, China). Food grade soybean oil (Fortune, Beijing, China) and flaxseed (Henan province of China) were purchased from local market. Flaxseed protein was extracted using Oomah et al.'s method with some modification (Oomah, Mazza, & Cui, 1994). The crushed flaxseed was defatted using hexane at a ratio of 1:6 for 6 h. The

Table 1

Proximate composition (%) (w/w) of flaxseed protein (FPC) and soybean protein (SPC) concentrates.

Constituent (%) (w/w)	FPC	SPC
Protein	87.25 ± 1.64	88.73 ± 1.36
Fat	2.43 ± 0.44	3.25 ± 0.13
Moisture	5.24 ± 0.10	4.85 ± 0.15
Ash	2.03 ± 0.06	1.78 ± 0.16

These data are the average of the three replicates.

hull was separated from the kernel by screening the tailings using a 0.15 mm sieve. The flaxseed kernel powder obtained by this method contained a negligible quantity of the hull. This powder was subsequently soaked in 0.1 M Tris buffer (pH 8.6 with 0.1 M NaCl) at a seed-to-buffer ratio of 1:16 for 24 h. The extract was passed through double layered cheesecloth to remove large residues. Subsequently, it was centrifuged at 9000 rpm for 20 min using an ultracentrifuge (GL-20G-II, Anke Corporation, Shanghai, China). The pH of the supernatant was adjusted to 4.2 (iso-electric point of flaxseed protein) using 0.1 M HCl. The extract was stored at 4 °C for 16 h to allow the protein to precipitate completely. The precipitate was subsequently centrifuged at 12,000 rpm for 20 min. The pH of this protein concentrate was adjusted to 7.0 by using 0.1 M NaOH and finally freeze-dried (LGJ-18 C, Sihuan Corporation, Beijing, China) to obtain the flaxseed concentrate powder.

The protein content of the concentrates was determined by Kjeldahl method. The fat content was determined by Soxhlet extraction. The moisture and ash contents of the extract were determined by oven drying (105 °C for 24 h and 550 °C for 12 h, respectively). The main composition of SPC and FPC is provided in Table 1.

2.2. Preparation of protein polysaccharide dispersions

Protein and GA dispersions (w/v) were prepared by dissolving the desired amount of SPC, FPC and GA powder in deionized water containing 0.04% (w/v) sodium azide. The sodium azide was used as a protective agent against the microbial growth. These dispersions were stirred overnight to ensure complete dissolution. The pH of all the protein and polysaccharide solutions was adjusted to 7.0 using 0.1 M HCl or 0.1 M NaOH as required.

2.3. Emulsion preparation

Coarse emulsions containing 10% (v/v) soybean oil and 1% (w/v) protein were produced by blending soybean oil (v/v) with protein solutions (v/v) together using an ultra turrax (T25, IKA, Staufen, Germany) at 15,000 rpm for 2 min. Subsequently, the primary emulsions were prepared by passing these coarse emulsions through a homogenizer (NS1001L-PANDA 2 K, Niro Soavi S. P. A., Parma, Italy) at 300 bar pressure for first pass and subsequently at 500 bar pressure for further three passes. The primary emulsions were neutralized using 0.1 M HCl or NaOH. Secondary emulsions were prepared by diluting the primary emulsions with desired amount of gum Arabic solutions. The pH of these secondary emulsions was also brought to 7.0 with the use of 0.1 M HCl or NaOH as required and finally passed through the homogenizer at 200 bar pressure.

The final emulsions containing 0.8% (w/v) protein, 8% (v/v) soybean oil and 0–4% (w/v) GA were stored at 4 °C. Before further analysis and tests, the stored emulsions were brought to ambient temperature by allowing them to stand for 2 h.

2.4. Droplet size and zeta-potential

When the emulsions were aged for 24 h their droplet size and zeta-potential were determined by dynamic light scattering (DLS)

technology. This aging time was chosen as it was shown previously that the emulsions containing biopolymers require 8–10 h to reach their osmotic pressure equilibrium (Dickinson, 1995).

To avoid multiple scattering effects, emulsions were diluted to attain an oil-phase volume fraction of approximately 0.001% using deionized water. The diluted emulsions were poured into a folded capillary cell after stirring them for 10 min. Subsequently, the Z-average diameter of emulsion droplets was measured using a nano laser particle size analyzer (ZS-90, Malvern Instruments, Worcestershire, UK). The zeta-potential of emulsion droplets was determined by measuring the direction and velocity of droplets moving in an applied electric field. Five replicates were made for both the Z-average diameter and zeta-potential values on each sample.

2.5. Rheological properties

After aging the emulsion for 24 h, rheological measurements were carried out using a strain-controlled rheometer (AR2000ex, TA Instruments, Ltd., Crawley, UK). The temperature was maintained at 25 °C during these tests using a water bath connected to a Peltier system (± 0.1 °C). Emulsion viscosity was measured over a shear rate range of 1000–0.01 s⁻¹ with cone geometry. The diameter, cone angle and the gap between the cone and plate were 40 mm, 1° and 26 μ m, respectively. A thin layer of silicone oil was applied on the surface of the samples in order to prevent evaporation.

2.6. Emulsion stability

Emulsion stability can be compared using emulsifying stability index (*ESI*), which is calculated by using turbidity. Turbidity of emulsions was measured using a UV spectrophotometer (TU-1810, Purkinje General Instrument Co., Ltd., China). Emulsions were diluted with 0.1% sodium dodecyl sulfate (SDS) solution to an oil-volume fraction of 0.01–0.2%. The diluted samples were poured in quartz cuvettes with a path length of 0.01 m and all the measurements were carried out at a wavelength of 500 nm. The turbidity was calculated using Eq. (1) given below.

$$T = \frac{2.303 \cdot A \cdot V}{l} \quad (1)$$

where *T* is turbidity of emulsions in m⁻¹, *A* is the observed absorbance, *V* is the dilution factor, and *l* is the pathlength of the cuvette which is 0.01 m.

The emulsifying stability index (*ESI*) was determined using the methods of Pearce and Kinsella (1978), by Eq. (2) given below.

$$ESI = \frac{T_0 \cdot \Delta t}{\Delta T} \quad (2)$$

where *ESI* stands for emulsifying stability index in hour, *T*₀ is the turbidity of emulsion in m⁻¹ determined immediately after it was prepared and ΔT is the value change of turbidity in m⁻¹ in time interval Δt (48 h).

2.7. Effect of environmental conditions on emulsion

Z-average diameter, zeta-potential, turbidity and emulsify stability index (*ESI*) were determined at different environmental conditions such as ionic strength and thermal treatment regimes. An emulsion was prepared as described in Section 2.3 with 8% soybean oil (v/v) and 0.8% protein (w/v) at pH 7 with GA concentration of 2% (w/v).

NaCl stability: The emulsions mentioned above were diluted with an equal volume of NaCl solution to obtain final NaCl concentrations of 0, 50, 100, 150 and 200 mM, respectively. The diluted emulsions were stored at 4 °C before they were used.

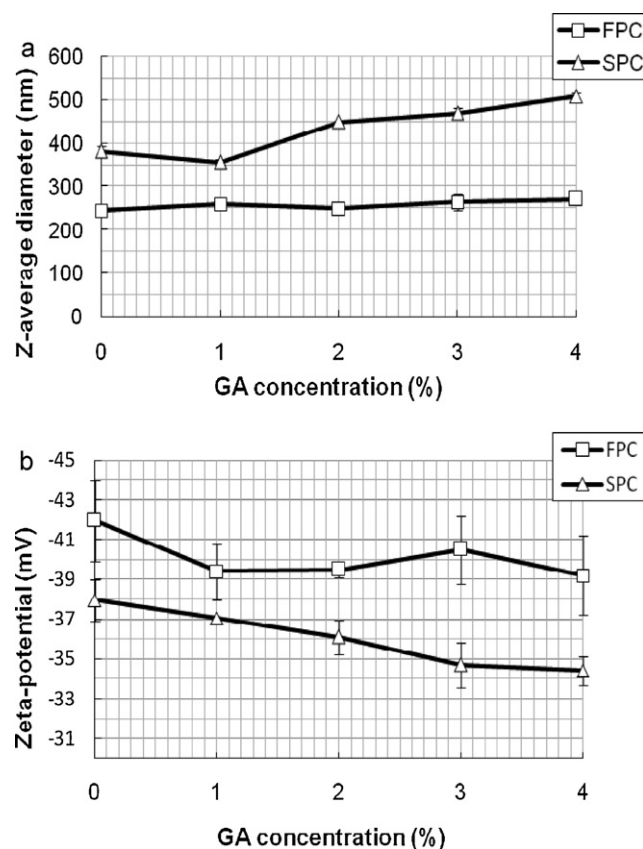


Fig. 1. Droplet Z-average diameter and zeta-potential of FPC- and SPC-stabilized emulsions in the presence of GA: (a) Z-average diameter and (b) zeta-potential. Some of the error bars lie within the symbol.

Thermal treatment: Emulsion samples were diluted with an equal volume of deionized water. The diluted emulsions were transferred into glass cylinders and equilibrated in water baths maintained at 25, 45, 65, 85 and 95 °C for 15 min, respectively. After these treatments the emulsion samples were first cooled down to ambient temperature and finally stored at 4 °C before their use.

2.8. Statistical analysis

All the experiments were carried in triplicate and the average values were recorded. Analysis of variance (ANOVA) was carried out in order to test the significance. The SPSS statistical package (LEAD Technologies, US) was used for this purpose.

3. Result and discussion

3.1. Effect of GA concentration on emulsion properties

3.1.1. Effect on droplet size

The Z-average diameter and size distribution of FPC- and SPC-stabilized emulsions with different GA concentration are shown in Fig. 1a. The figure shows that Z-average diameter of FPC-stabilized emulsion with 0–4% (w/v) GA is in the range of 244.6–270.8 nm while the emulsion stabilized by SPC with 0–4% GA is in the range of 381.7–508.4 nm (Fig. 1a). It can also be observed that the Z-average diameters of SPC-stabilized emulsions increased when the GA concentration exceeded 2% (w/w). Both SPC and GA are repulsive at neutral pH because of their negative charge; hence, the coalescence of the protein and gum is not expected.

In the case of SPC-stabilized emulsions, the increase in the droplet size with concentration (especially >2% GA concentration)

may be due to the competitive adsorption between SPC and GA. The hydrophilic carbohydrate moieties of GA dangle and the hydrophobic polypeptide chains adsorb onto the oil–water interface. This competitive emulsifying activity of GA leads to the creation of less rigid and viscoelastic film around oil droplets which results into larger droplet size. Similar observation of competitive adsorption between GA and protein was reported by Makri and Doxastakis (2006) for emulsions stabilized by legume protein with GA. Padala, Williams, and Phillips (2009) also reported similar findings in emulsions stabilized by egg white protein with GA. Moreover, the presence of gum could lead to an increase in droplet size during homogenization due to their ability to suppress the formation of small eddies during turbulence (Walstra, 1983). However, the amount of GA may be not enough to effectuate the adsorption when its concentration is below 2% (w/w). In the case of FPC-stabilized emulsions, there is no significant difference in Z-average diameter values of droplets when the gum concentration increased from 0 to 4% (w/w) (Fig. 1a). It is probably due to the poorer emulsifying capability of GA compared with FPC. It also suggested that the emulsifying activity capabilities of these three ingredients were FPC > GA > SPC. But with the increase in gum concentration, the Z-average diameter of FPC-stabilized emulsions increased slightly which may be due to some changes in the properties in continuous phase (Walstra, 1983).

Generally, the FPC-stabilized emulsions had much smaller droplet size than the emulsions stabilized by SPC in the same gum concentration. This is consistent with the report of Dev and Quensel (1986, 1988), which suggested that FPC has a stronger emulsifying capability than SPC. It is also consistent with our previous findings on FPC- and SPC-stabilized emulsions in the absence of the gum Arabic (Wang, Li, Wang, Adhikari, et al., 2010).

3.1.2. Effect on zeta-potential

Fig. 1b shows the zeta-potential of emulsion stabilized by both proteins in the presence of GA. As shown in this figure, both the emulsions are negatively charged. Zeta-potential values are in the range of -39.2 to -42.0 mV and -34.4 to -38.0 mV for FPC- and SPC-stabilized emulsions in the presence of GA, respectively. The ANOVA results showed that the concentration of the GA had no significant effect on zeta-potential values of FPC-stabilized emulsion ($p > 0.05$). This can be attributed to two reasons, firstly, there was no interaction between the GA and FPC layer because of which neither coalescence nor competitive adsorption occurred and secondly, the increase in gum concentration in the continuous phase had little effect on zeta-potential of pre-formed emulsion droplets.

However, in the case of SPC-stabilized emulsions, there is a significant decrease in the absolute value of zeta-potential when the concentration of GA is at and above 2% (w/w) (Fig. 1b) ($p < 0.05$). This is contributed by the competitive adsorption mentioned above. The GA solutions generally have a zeta-potential of -23 mV (Charma, 1981; Jayme, Dunstan, & Gee, 1999; Silber & Mizrahi, 1975) which is, in absolute terms, much lower than that of SPC, -38.0 mV (Fig. 1b). Hence, the competitive adsorption of GA leads to the decrease in the absolute value of zeta-potential when the concentration of GA exceeds 2% (w/w).

The absolute zeta-potential value of FPC-stabilized emulsion was much higher than that of SPC-stabilized emulsion in the presence of GA ($p < 0.05$). This may be due to the presence of some specific molecular species in the interfacial layer surrounding the oil droplets which can lead to altered or different charge on the droplet surface (McClements, 2004). According to Henderson–Hasselbach equation, the charge of a protein can be estimated from the sum of the charges of individual amino acid residues. For example, Malhotra and Coupland (2004) showed that the charge of SPC emulsions was dominated by negative contributions from aspartic and glutamic acid beyond its iso-electronic

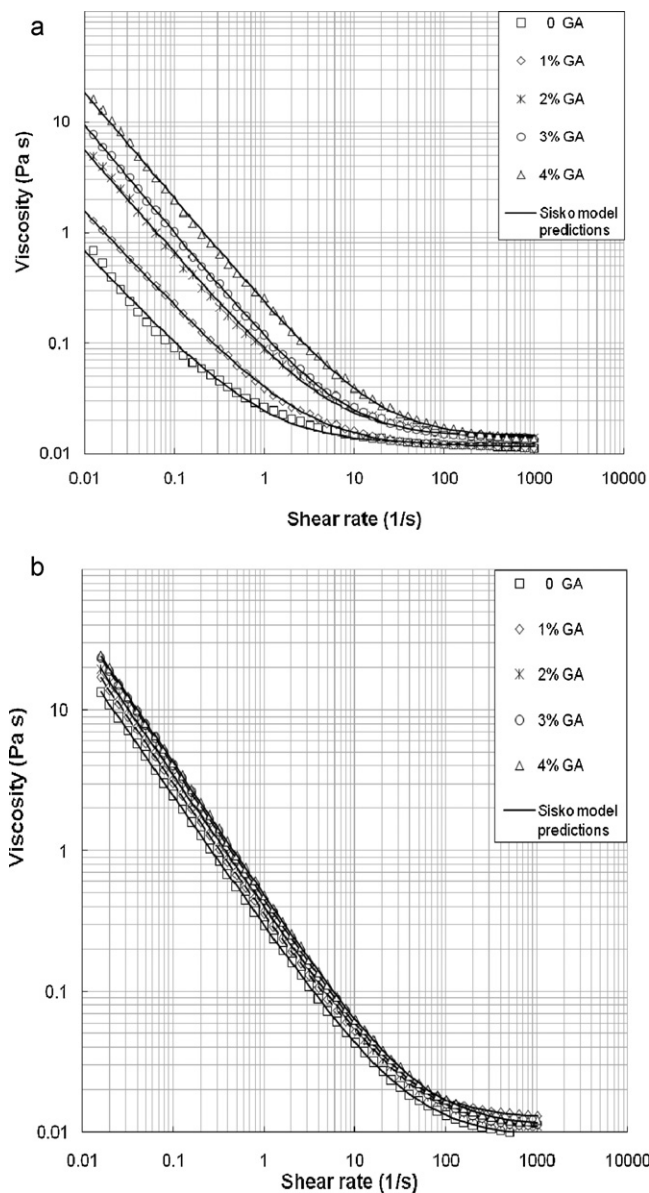


Fig. 2. Apparent viscosity of emulsion in the presence of GA: (a) FPC-stabilized emulsions and (b) SPC-stabilized emulsions.

point. Compared to SPC, FPC is richer in aspartic and glutamic acid (Dev & Quensel, 1986; Friedman & Levin, 1989), hence the FPC-stabilized emulsions exhibited higher absolute zeta potential values at neutral pH compared to SPC-stabilized emulsions. Moreover, greater adsorption of GA in oil–water interface instead of SPC also decreased the droplets zeta-potential absolute values.

3.1.3. Effect on rheological properties

The apparent viscosities of emulsions stabilized by both the proteins with different gum concentration at 25°C are shown in Fig. 2. It can be observed from this figure that all these emulsions exhibit a shear-thinning behavior. And the SPC-stabilized emulsions are more viscous than those stabilized by FPC. The apparent viscosity values of these emulsions are consistent with our earlier research (Wang, Li, Wang, Adhikari, et al., 2010).

ANOVA result shows that gum concentration has a significant effect on flow behavior of FPC-stabilized emulsion ($p < 0.05$), as observed in Fig. 2a. This is because the addition of GA into emulsion would increase the viscosity of emulsion (Jayme et al., 1999).

Table 2

Sisko model parameters for FPC- and SPC-stabilized emulsions in presence of GA.

GA concentration (% w/w)	η_{∞} (Pa s)	K (Pa s ⁿ)	n	R^2
FPC-stabilized emulsions				
0	0.012 ± 0.001 ^a	0.012 ± 0.001 ^a	0.131 ± 0.012 ^a	0.99
1	0.012 ± 0.001 ^a	0.029 ± 0.003 ^b	0.134 ± 0.011 ^a	0.99
2	0.014 ± 0.001 ^a	0.075 ± 0.005 ^c	0.067 ± 0.005 ^b	0.99
3	0.013 ± 0.001 ^a	0.103 ± 0.014 ^d	0.023 ± 0.001 ^c	0.99
4	0.014 ± 0.001 ^a	0.223 ± 0.019 ^e	0.043 ± 0.003 ^d	0.99
SPC-stabilized emulsions				
0	0.009 ± 0.001 ^a	0.288 ± 0.031 ^a	0.073 ± 0.006 ^a	0.99
1	0.013 ± 0.001 ^a	0.346 ± 0.030 ^a	0.061 ± 0.006 ^b	0.99
2	0.010 ± 0.001 ^a	0.389 ± 0.042 ^b	0.056 ± 0.004 ^c	0.99
3	0.010 ± 0.001 ^a	0.442 ± 0.039 ^b	0.044 ± 0.005 ^d	0.99
4	0.011 ± 0.001 ^a	0.472 ± 0.048 ^c	0.049 ± 0.004 ^d	0.99

The data are the average of the three replicates.

Values in the same column of same protein type followed by different letters as superscript are significantly different from each other according to Duncan's tests ($p < 0.05$).

The apparent viscosity of SPC-stabilized emulsions is insensitive to the increase in the gum concentration ($p > 0.05$, Fig. 2b). This may be due to the fact that the viscosity of the SPC-stabilized emulsion is relatively higher and seemingly does not get affected by the addition of the less viscous gum. Fig. 2a shows that FPC-stabilized emulsions in presence of GA have marked shear thinning behavior at low shear rate ($<100 \text{ s}^{-1}$), and Newtonian behavior at high shear rate region ($100\text{--}1000 \text{ s}^{-1}$). However, the SPC-stabilized emulsions with GA exhibit shear thinning behavior at the within entire shear rate range (Fig. 2b).

The three-parameter Sisko model was used to predict the flow behavior of the emulsions with shear thinning behavior at low shear rate and Newtonian behavior at high shear rate region (Sisko, 1958):

$$\eta = \eta_{\infty} + K\dot{\gamma}^{n-1} \quad (3)$$

where η , and η_{∞} are apparent and infinite shear viscosities in Pa s, respectively. K is consistency coefficient in (Pa sⁿ) and n is flow behavior index of shear-thinning fluid. The values for the Sisko model parameters are presented in Table 2. The r -squared (R^2) values between the predicted and the experimental values are higher than 0.99. Furthermore, the average absolute errors between the experimental and predicted values vary between 1.7 and 6.0% in the case of FPC-stabilized emulsions (plus GA) and remain within 1% in the case of SPC-stabilized in the presence of GA. These high R^2 values and very low absolute errors in the predictions suggest that the Sisko model is suitable for the prediction of emulsion viscosity studied in this work.

Generally, for all emulsions stabilized by both FPC and SPC in presence of GA, the gum concentration had no significant effect on the infinite viscosity of the emulsions (Fig. 2a and Table 2).

The consistency coefficient (K) of emulsions stabilized by FPC increased with increase in gum concentration suggesting that a better structured emulsion system was formed, maybe because of increased viscosity and droplets concentration (including the oil-in-water droplets and non-adsorbed GA in continuous phase). Meanwhile, the K value of SPC-stabilized emulsions increased by 1.64 times, i.e. from 0.288 to 0.472, which may be due to stronger interactions between bigger droplets (due to competitive adsorption of GA) and increased droplet concentration.

All the flow behavior indices (n) of the emulsions were much lower than 1.0 indicating highly shear thinning nature of these emulsions. Moreover, n value decreased with increased of GA concentration, which suggested that the stronger shear thinning behavior. It is consistent with the increased slope of shear thinning region at lower shear rate, which could be obviously observed especially in the FPC-stabilized emulsions as Fig. 2a shows.

3.1.4. Effect on emulsion stability

The emulsifying stability index (ESI) values calculated by turbidity of emulsions stabilized by both proteins as function of gum concentration are shown as Fig. 3. In the case of FPC-stabilized emulsions, the addition of GA firstly significantly increased the ESI at a lower gum concentration ($<2\%$, w/w, $p < 0.05$). However, when the concentration of this polysaccharide is increased further, the ESI of FPC-stabilized emulsions decreased. This may be due to the destabilization of emulsions which can be attributed to depletion flocculation. Depletion flocculation occurs when pairs of emulsion droplets come within a distance less than that is occupied by free GA molecules in the continuous phase. The expulsion of the polymer from the gap is associated with an attractive inter-particle force resulting from the tendency of solvent flowing out from the gap under the influence of local osmotic pressure gradient (Napper, 1983). At low GA concentration ($<2\%$, w/w), the entropy loss linked to droplets aggregation outweighs the effects due to depletion thereby the emulsions maintain stability (Fig. 3). Moreover, the viscosity of emulsion increased with the increase in GA concentration (Fig. 2a). The droplets might be immobilized in more viscous aqueous phase, which also contributed to the increase in the emulsion stability. However, when the GA concentration exceeded 2% (w/w), the depletion flocculation became unavoidable. The depletion flocculation increases the concentration or aggregation of the FPC-stabilized emulsion, thus rendering it highly susceptible to destabilization (Fig. 3). The attractive depletion forces exceed the repulsive forces between droplets, causing the droplets to flocculate and cream out rapidly even though the viscosity of emulsion is still increasing.

A wide range of polysaccharides have been reported to be capable of inducing depletion flocculation of protein-stabilized emulsions, such as xanthan gum, gum Arabic, pectin, and car-

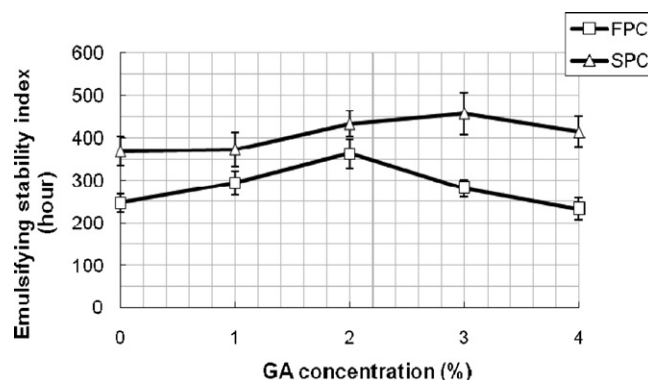


Fig. 3. Emulsifying stability index (ESI) of FPC- and SPC-stabilized emulsions in the presence of GA. Some of the error bars lie within the symbol.

rageenan. Dickison and Paelowsky (1997) reported that the addition of ι -carrageenan in excess beyond the amount required to cover the droplet surface led to the destabilization of emulsions by depletion flocculation. Kemetriades and McClements measured the time-dependence of creaming index of 10% (w/w) soybean O/W emulsions as a function of dextran sulfate concentration. They found that the surplus dextran sulfate could cause droplets to flocculate and cream out rapidly (Kemetriades & McClements, 1999). However, the droplet size of the FPC-stabilized emulsions remained small even during the emulsion destabilization (Fig. 1a). This is because the depletion flocculation is reversible and could have been reversed due to dilution, hence cannot be detected in droplet size determination (Gancz, Alexander, & Corredig, 2006).

In the case of SPC-stabilized emulsions, there is a significant increase in *ESI* when the gum concentration has exceeded 2% (w/w) as shown in Fig. 3. This is mainly due to the adsorption of GA onto the oil–water interface. The adsorption of big molecular weight polysaccharides such as GA increases the emulsion droplet size which then results into a steric repulsion on top of the already existing electrostatic repulsion (Fig. 1b) which enhances the emulsion stability. In a similar note, Gharsallaoui, Yamauchi, Chembin, Cases, and Saurel (2010) reported that the adsorption of pectin onto pea protein-stabilized O/W droplets induced a steric repulsion. Nakamura, Maeda, and Corredig (2006) also reported the steric repulsion was caused by adsorption of soybean soluble polysaccharide onto the oil–water interface. However, the *ESI* of SPC-stabilized emulsion decreased when the GA concentration exceeded 3% (w/w), which can also be attributed to the depletion flocculation resulted by surplus polysaccharide in aqueous phase (Fig. 3).

Since the emulsions stabilized by both the FPC and SPC in the presence of the gum Arabic at 2% (w/w) have good emulsifying stability (Fig. 3), hence the effect of environmental conditions such as NaCl concentration and thermal treatment regimes was investigated using this formulation.

3.2. Effect of NaCl concentration on emulsion properties

3.2.1. Effect on droplet size

Univalent sodium cation is often present in water or can be added into foods as processing aids. These ions are capable of affecting the stability of colloidal dispersions by effectively reducing the negative charge at the surface through electrostatic associations. The Z-average diameter values of emulsions stabilized by FPC and SPC in the presence of GA as a function of NaCl concentration are shown as Fig. 4a. It can be seen from this figure that the NaCl concentration has a significant effect on the size of the emulsion droplets in emulsions stabilized by FPC in the presence of GA ($p < 0.05$). The Z-average value increased from 257.1 to 941.8 nm when NaCl concentration increased from 0 to 200 mM (Fig. 4a). These results can be interpreted in terms of electrostatic interactions between emulsion droplets. The stability of emulsion structure is contributed by electrostatic repulsion in the FPC-stabilized emulsions. The carboxyl ($-\text{COO}^-$) ions are at the periphery of the molecule and are very active in creating an anionic environment (Tan, 1997; Thevenet, 1988). When the emulsion droplets are coated by a protein membrane it is possible for Na^+ ions to neutralize the electrostatic interactions between droplets having oppositely charged groups. This enables the droplets to come closer together and promotes the inter-droplet disulfide bond formation (Israelachvili, 1992; Kim, Choi, Shin, & Moon, 2003).

In our previous study on the effect of NaCl concentration on the stability of emulsion containing 0.5% (w/w) SPC and 5% (v/v) oil, the droplet size varied in the range of 341.8–1007.5 nm within the same NaCl test range (Wang, Li, Wang, Adhikari, et al., 2010). However, in the case of emulsion stabilized by SPC (in presence

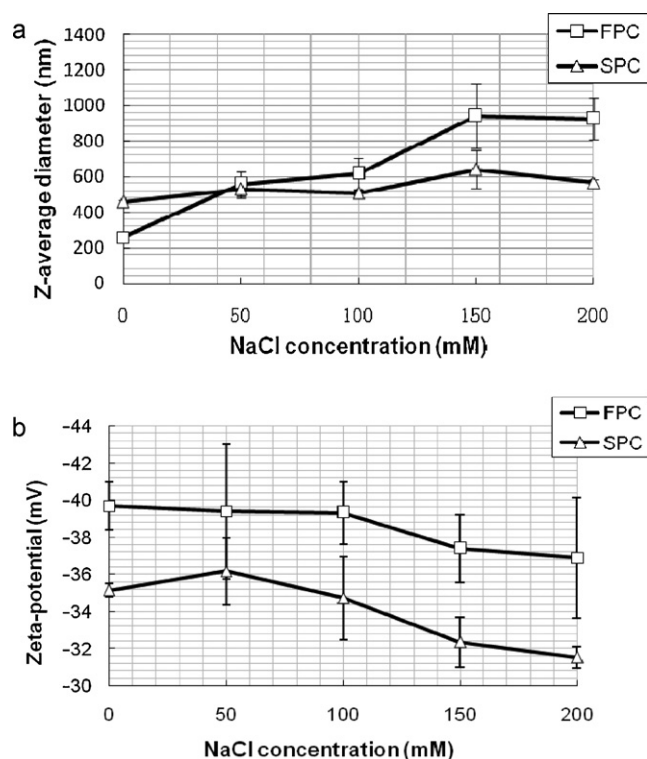


Fig. 4. Droplet Z-average diameter and zeta-potential of FPC- and SPC-stabilized emulsions in the presence of GA as function of NaCl concentration: (a) Z-average diameter and (b) zeta-potential. Some of the error bars lie within the symbol.

of 1% (w/w) GA), the Z-average diameter varied within a relatively smaller range (458.0–641.1 nm). This is because the adsorption of the GA at the oil–water interface occurs during the homogenization. The absorption of GA at the oil–water interface enhanced the steric repulsion in emulsion droplets (in present study) while in our previous study where GA was not used, the emulsion stability was mainly achieved through electrostatic repulsion. With the increase in NaCl concentration, the droplet size also increased due to the screening effect of univalent cation. However, the steric repulsion prevented the emulsion droplets from aggregation and subsequent flocculation and ultimately retarded the emulsion destabilization.

The ANOVA results show that the mean size of the emulsion droplets stabilized by SPC is much lower than the one stabilized by FPC in presence of 1% (w/w) gum. This further suggests that the SPC-stabilized emulsions have a better NaCl resistance than the emulsions stabilized by FPC with 1% (w/w) GA.

3.2.2. Effect on zeta potential

The effect of NaCl concentration on the zeta-potential values of O/W emulsion in the presence of GA is shown in Fig. 4b. The zeta potential values of both FPC- and SPC-stabilized emulsions containing 1% (w/w) gum Arabic remained negative within this NaCl concentration range (0–200 mM). However, the absolute zeta-potential values of FPC- and SPC-stabilized emulsions decreased from 39.7 to 36.9 mV, and 35.2 to 31.5 mV, respectively. This decrease in the magnitude of zeta-potential values with increase in NaCl concentration can be attributed to electrostatic screening effects. Hunter (1986) has suggested that for a spherical droplet with constant surface charge density, zeta-potential varies inversely to ionic strength of aqueous solution surrounding the droplet/particle. The Na^+ is well known for its binding tendency with $-\text{COO}^-$ groups of proteins leading to a decrease in negative charge. The screening effect of NaCl solution in emulsions is observed in previous studies, such as emulsion stabilized

Table 3

Emulsifying stability index (ESI) of FPC- and SPC-stabilized emulsions in the presence of GA as function of NaCl concentration.

Protein	Emulsifying stability index (h)				
	NaCl concentration (mM)				
	0	50	100	150	200
FPC	276.59 ± 25.19 ^a	259.16 ± 20.36 ^b	N.A.	N.A.	N.A.
SPC	477.79 ± 30.25 ^a	191.65 ± 20.24 ^b	142.35 ± 12.33 ^c	N.A.	N.A.

The data are the average of the three replicates.

Values in the same row of same protein type followed by different letters as superscript are significantly different from each other according to Duncan's tests ($p < 0.05$).

N.A. indicates that the emulsions separated within 4 h.

by ovalbumin (Galazka, Dickinson, & Ledward, 2000), whey protein (Pearce & Kinsella, 1978), and even albumin–dextran sulfate stabilized emulsions (Kato, Sato, & Kobayashi, 1989).

In our earlier study without GA, when NaCl concentration varied in the range of 0–200 mM, the absolute zeta potential values decreased from 61.57 to 42.57, and 62.23 to 23.75 mV for emulsions stabilized by FPC and SPC, respectively (Wang, Li, Wang, Adhikari, et al., 2010). However, from the present work, it can be seen that there is a relatively smaller decrease in the magnitude of zeta-potential absolute values in the FPC- and SPC-stabilized emulsions in the presence of GA (39.70–37.40 and 36.17–31.53 mV, respectively). The reason for this lower extent of reduction in zeta potential value is most possibly due to the adsorption of some Na⁺ onto the negatively charged polysaccharide, rather than protein layer. The ANOVA results showed that the droplets of FPC-stabilized emulsions were more negatively charged than the SPC-stabilized ones ($p < 0.05$). This is consistent with our previous study suggesting that there was a stronger electrostatic repulsion in the FPC-stabilized emulsions.

3.2.3. Effect on emulsion stability

The variation of ESI as a function of NaCl concentration is shown in Table 3. For emulsion stabilized by both proteins in the presence of 2% (w/w) GA, the ESI decreased significantly with the increase in salt concentration ($p < 0.05$). At low NaCl concentration (50 mM), there was still a relatively strong electrostatic repulsion between the droplets of FPC-stabilized emulsions which prevented them from coming into close contact. Once the critical salt concentration is reached and exceeded, the electrostatic repulsion is no longer strong enough to overcome the attractive forces (e.g. van der Waals, hydrophobic and attraction resulted by depletion flocculation). However, in the case of SPC-stabilized emulsions containing the GA, there is steric repulsion in addition to the electrostatic repulsion. While the repulsive force decreased with increase in NaCl concentration, the steric repulsion provided by adsorbed GA effectively prohibited the droplets from aggregation. This is the reason why the stability of the SPC-stabilized (with 2% (w/w) GA) emulsion was maintained even when the 100 mM NaCl was added in this emulsion. This result also suggests that the SPC-stabilized emulsions are more resistant to NaCl in the presence of GA than its absence. This result is quite different with our earlier work in the absence of GA, where FPC-stabilized emulsions had a better stability against NaCl compared to those stabilized by SPC (Wang, Li, Wang, Adhikari, et al., 2010). This enhanced stability is mainly due to the steric repulsion caused by the adsorbed GA.

3.3. Effect of thermal treatment on emulsion properties

3.3.1. Effect on droplet size

The Z-average diameter values of emulsions stabilized by both the FPC and SPC (in the presence of GA) as function of thermal treatment are shown in Fig. 5a. It can be seen from this figure that the variation in the emulsions droplets size is relatively small (217.6–257.6 nm for FPC-stabilized emulsions and 458.0–506.7 nm

for SPC-stabilized emulsions, respectively). The ANOVA results indicated that there was no significant difference in droplet size of FPC-stabilized emulsions (with 2% w/w GA) in the temperature range of 25–95 °C ($p > 0.05$). This extent of thermal stability can be attributed to the high denaturation temperature of FPC. According to Li-Chan and Ma (2002), the denaturation temperature of proteins comprising FPC (11–12S) is above 100 °C. However, in the case of SPC-stabilized emulsions (with 2% GA), there is a significant increase in the droplet size when temperature rises above

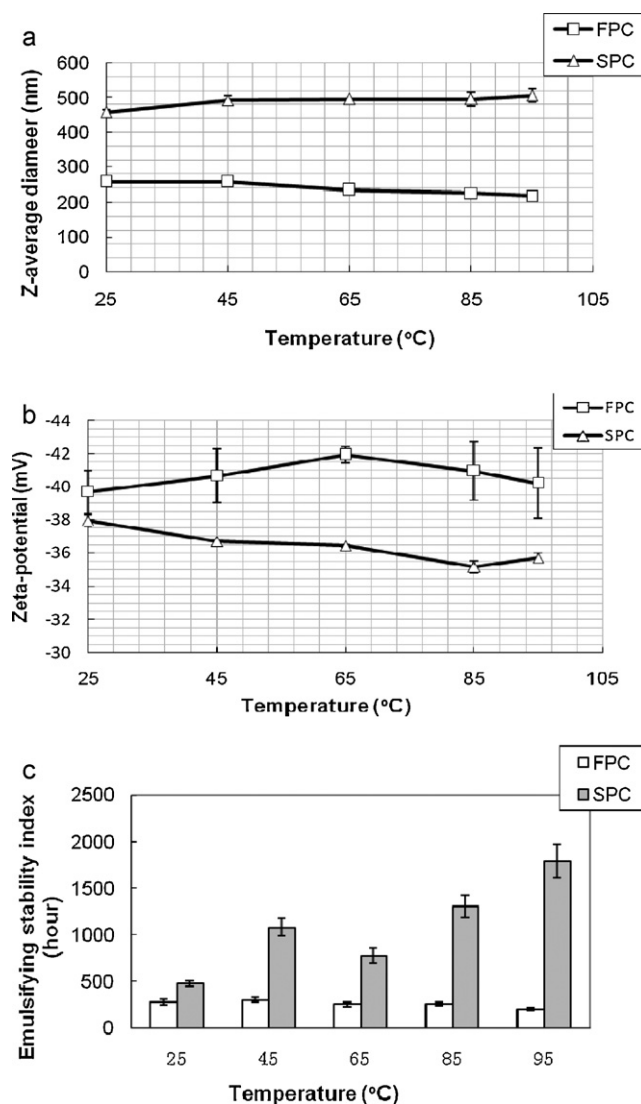


Fig. 5. Droplet Z-average diameters, zeta-potential and emulsifying stability index (ESI) of FPC- and SPC-stabilized emulsion in presence of GA as function of thermal treatment temperature: (a) Z-average diameter, (b) zeta-potential and (c) emulsifying stability index. Some error bars lie within the symbol.

45 °C (Fig. 5a). This could be due to the fact that the increased temperature also promoted the kinetic mobility of the added polysaccharide, enhancing the competitive adsorption between GA and SPC leading to the formation of bigger droplets. In our earlier study we had found that there was a significant increase in the droplet size of SPC-stabilized emulsion in the absence of GA when the temperature was above 75 °C. We also supposed that the denaturation of the adsorbed SPC at the emulsion droplet surface was responsible for the increase in the droplet size. However, in this study, no similar observations were made which also suggests that the GA is adsorbed on the oil–water interface instead of SPC.

3.3.2. Effect on zeta potential

The effect of temperature on zeta-potential of FPC- and SPC-stabilized emulsions in the presence of GA is shown as Fig. 5b. There is no significant difference in the zeta-potential values in FPC-stabilized emulsions (in the presence of gum) when their temperature was raised from 25 to 95 °C ($p > 0.05$). This observation is consistent with the observation made on the emulsion droplet size (Section 3.3.1) suggesting that there is no interaction between droplets and non-adsorbed polysaccharides in the bulk. However, in the case of SPC-stabilized emulsion with GA, the emulsion droplets became less negatively charged when heated ($p < 0.05$). As mentioned above in Section 3.1.2, the adsorbed GA at oil–water interface decreased the absolute zeta-potential values of droplets. At higher temperatures (above 85 °C), the zeta-potential values of SPC-stabilized emulsion (with GA) did not decrease further, which indicated that GA occupied the O/W interface of these emulsions instead of the SPC.

3.3.3. Effect on emulsion stability

The variation of *ESI* indices with the temperature of emulsions stabilized by both the proteins in the presence of GA are shown as Fig. 5c. The *ESI* of FPC-stabilized emulsions consistently show values below 600 h. The *ESI* decreased further with increase in temperature above 45 °C. This may be due to two reasons, firstly, the droplet–droplet interactions become stronger at higher temperatures; and secondly, the un-adsorbed GA (in the bulk) becomes less viscous when the temperature increases both of which favour the destabilization of emulsions. However, in the case of SPC-stabilized emulsions, the lowest *ESI* was observed at 25 °C, suggesting that the interactions between SPC and GA increase with the increase in temperature. As mentioned above, the gum rather than the protein preferentially adsorbs at the oil–water interface. When the temperature increases the competitive adsorption becomes stronger because the constituents of the emulsions become more active and mobile at 45 °C. However, the *ESI* of emulsion decreased instead of increasing when the temperature of the emulsion was further raised to 65 °C. This can be caused by the stronger interactions between droplets at elevated temperatures similar to the FPC-stabilized emulsions. There was also a sharp increase in the *ESI* of SPC-stabilized emulsion above 85 °C. This can be explained from the fact that the SPC-stabilized emulsions exhibit better stability when the SPC is partially denatured (Wang, Li, Wang, Adhikari, et al., 2010). In this study, the fraction of SPC remaining in the continuous aqueous phase after competitive adsorption might have formed a weak gel due to its denaturation. The denatured SPC is capable of increasing the emulsion viscosity which results into higher *ESI* values.

4. Conclusions

This study has investigated the effect of gum Arabic (GA) on the stability of emulsion stabilized by FPC and SPC. Addition of 2% GA to FPC- and SPC-stabilized emulsion would strengthen their stability, by increasing the viscosity for the former and causing

competitive adsorption for the later, respectively. Then influences of environmental stresses (NaCl concentration and thermal processing) on stability of emulsion with 2% GA were also determined. In general, the SPC-stabilized emulsion in the presence of GA had better emulsion stability and better stability against environmental factors such as NaCl concentration and thermal treatments. The stability of SPC-stabilized emulsions in the presence of GA is attributed to the competitive adsorptions between SPC and GA.

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