

Investigation into the Physicochemical Stability and Rheological Properties of β -Carotene Emulsion Stabilized by Soybean Soluble Polysaccharides and Chitosan

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In this study, the possibility of producing stable O/W emulsions incorporating β -carotene in oil droplets surrounded by multiple-layer interfacial membranes has been demonstrated. Emulsions were prepared using a two-stage process by homogenization, which relied on the adsorption of chitosan to anionic droplets coated with soybean soluble polysaccharides (SSPS). Results showed that the ζ -potential, particle size, and rheological properties of emulsions were greatly dependent on the chitosan concentration. The electrical charge on the droplets increased from -34 to 58.2 mV as the chitosan concentration was increased from 0 to 2 wt %, which indicated that chitosan adsorbed to the droplet surfaces. The mean particle diameter of the emulsions increased dramatically with the rise of chitosan concentration from 0 to 0.33 wt %, indicating the formation of large aggregated structures. At chitosan concentrations above 0.33 wt %, the mean particle diameter of emulsions decreased and reached a minimum value of $0.79 \mu\text{m}$ at a chitosan concentration of 0.5 wt %. Dynamic oscillatory shear tests indicated that the viscoelastic behavior could be enhanced by the adsorption of chitosan onto the SSPS-coated droplet surfaces. Chitosan concentration had a significant ($p < 0.05$) impact on the stability of β -carotene. The least degradation occurred in the emulsion with chitosan concentration of 0.5%. These results implied that the physicochemical stability of β -carotene emulsions has been improved by the adsorption of chitosan.

KEYWORDS: Soybean soluble polysaccharides; chitosan; β -carotene; multilayer emulsion; stability; rheological properties

INTRODUCTION

In the past decades, great attention has been paid to the carotenoid family, including β -carotene, lycopene, lutein, and astaxanthin, due to their provitamin A activity and antioxidant properties (1, 2). Incorporating these bioactive compounds into food products may offer great health benefits and provide new opportunities for development in the food industry. However, carotenoids are insoluble in water and only slightly soluble in oil at room temperature, which greatly limit their applications. Furthermore, carotenoids from vegetables are probably bound in protein complex or exist as crystal form, which may lead to poor bioavailability (3).

To improve water solubility and bioavailability, carotenoids can be dissolved in the oil phase of oil-in-water emulsions, which can be easily incorporated into food products. Factors responsible for the degradation of carotenoids in emulsions include the composition of emulsion systems and environmental conditions, such as emulsifiers, addition of antioxidants, light, heating, singlet oxygen, and food systems. Recent studies have been focused on the preparation and chemical stability evaluation of carotenoid

emulsions stabilized by various emulsifiers, such as polyglycerol esters of fatty acid (4, 5), Tween series (6, 7), and proteins and hydrocolloids (8, 9). Decker et al. studied the effect of different surfactants (cationic, anionic, and nonionic), oil types, pH, iron, and hydroperoxides on lycopene oxidation in oil-in-water (O/W) emulsions and revealed that the stability of lycopene in O/W emulsions could be improved by altering the emulsion droplet interface and the presence of tocopherols or EDTA (10, 11).

However, accumulated studies suggest that severe degradation of β -carotene in the O/W emulsions occurred at ambient or higher temperatures, especially in the emulsions stabilized by small molecule emulsifiers such as Tween 20 or polyglycerol esters of fatty acid. Proteins that could play the role of antioxidant probably protect β -carotene from degradation (9, 12). However, protein-stabilized emulsions have relatively poor stability to environmental stresses, for example, pH, salt, heating, and freezing (13). On the other hand, polysaccharides provide good stability to environmental stresses but are relatively poor at producing small emulsion droplets or require high concentrations to stabilize the emulsion (14). Therefore, great attention has been paid to the improvement of the stability and functionality of emulsifiers by utilizing their blend (15–17) or protein–polysaccharide Maillard-type conjugates (18, 19).

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Recently, a novel method based on layer-by-layer electrostatic deposition technology was developed to produce O/W emulsions stabilized by multilayered membranes using a two-stage process (20). Studies showed that the multilayered emulsions had good long-term stability at low pH and high NaCl concentration (< 500 mM). Besides, the SDS–chitosan-stabilized emulsions were more effective at retarding the formation of the citral oxidation product, *p*-cymene, than gum arabic (GA)-stabilized emulsions. Limonene degradation and the formation of limonene oxidation products, limonene oxide and carvone, were lower in the SDS–chitosan-stabilized than in the GA-stabilized emulsions at pH 3.0 (21). However, the stability could be affected by the method of preparation; in studies on the dextran sulfate–sodium caseinate- and pea protein isolate–GA complex-stabilized emulsions, it was found that the stability of a mixed emulsion prepared with soluble protein–polysaccharide complexes is greater than that of a bilayer emulsion prepared with the two-step method (22, 23).

To the best of our knowledge, no information is available concerning the effect of multiple layers of emulsifiers on the physicochemical stability of β -carotene in O/W emulsions. Therefore, the purpose of current study was to gain a better understanding of the effect of chitosan adsorption on the physical and rheological properties of O/W emulsions and to evaluate if the degradation of β -carotene could be inhibited using multiple layers of emulsifiers. Soybean soluble polysaccharides (SSPS) and chitosan were used to create multiple layers of emulsifiers. SSPS are acidic polysaccharides containing galacturonic acid, composed of a main rhamnogalacturonan backbone branched by β -1,4-galactan and α -1,3- or α -1,5-arabinan chains. SSPS were preferred as potential stabilizers because the emulsifying properties of SSPS are not affected by pH or ionic strength and lower amounts of SSPS are required to stabilize acidic emulsions compared to those reported for GA and modified starch (24–26). Chitosan, a deacetylated derivative of chitin, has a positive charge in acidic solutions and has received “generally recognized as safe” (GRAS) status for application in foods and beverages (27, 28).

MATERIALS AND METHODS

Materials and Chemicals. β -Carotene suspension (30% by mass β -carotene in sunflower oil) was purchased from Xinchang Pharmaceutical Co., Ltd. (Zhejiang, China). Medium-chain triglyceride (MCT) oil was obtained from Lonza Inc. (Allendale, NJ). SSPS were obtained from Fuji Oil Co. Ltd. (Osaka, Japan). Chitosan with a degree of deacetylation of 95% was obtained from Zhejiang Golden-Shell Biochemical Co., Ltd. (Zhejiang, China). Standard β -carotene (>95% purity) was purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals used were of analytical grade, unless otherwise stated.

Solution Preparation. A stock buffer solution was prepared by dispersing 100 mM acetic acid in distilled water and then adjusting the pH to 3.0 using 1 M HCl. SSPS and chitosan solutions were prepared by dispersing 0.5–8 wt % SSPS and 0–2 wt % chitosan in buffer, respectively. The solutions were stirred overnight at a speed of ~200 rpm to ensure complete dispersion and dissolution. Sodium azide (0.01 wt %) was added as an antimicrobial agent.

Preparation of β -Carotene Emulsions Stabilized by SSPS. Preliminary experiments were carried out to determine the minimum amount of SSPS required to produce emulsions that were relatively stable to droplet aggregation. O/W emulsions were prepared with 10 wt % MCT oil containing β -carotene (0.15 wt % in the final emulsion) as the dispersed phase and aqueous SSPS solutions as the continuous phase. Briefly, β -carotene was first dissolved in MCT oil at 140 °C for several seconds and then mixed with SSPS solutions at a speed of 10000 rpm with a blender to form coarse emulsions, which were further homogenized using a Niro-Soavi Panda two-stage valve homogenizer (Parma, Italy) for three cycles at 60 MPa. Emulsions from the exit of the homogenizer were immediately cooled to room temperature and sampled to measure the droplet size and stability.

Absorption of Chitosan onto the SSPS-Stabilized Emulsions.

Emulsions (4 wt % SSPS, 10 wt % MCT oil, 0.15 wt % β -carotene) were mixed with aqueous chitosan solutions in a high-speed blender followed by one to six cycles at 60 MPa through a two-stage valve homogenizer and then immediately cooled to room temperature and sampled to measure the droplet size, ζ -potential, and stability. For storage tests (4, 30, 50, and 70 °C), the emulsions were kept in screw-capped amber bottles and flushed with nitrogen.

Determination of Droplet Size and Size Distribution. Particle size and size distribution of β -carotene emulsions were determined by dynamic light scattering using a Zetasizer Nano-ZS90 (Malvern Instruments, Worcestershire, U.K.) at a fixed angle of 90°. Emulsions were diluted to a final oil droplet concentration of 0.005 wt % with buffer solution (pH 3.0) prior to each measurement to minimize multiple scattering effects. Results were described as cumulants mean diameter (size, nm) for droplet size and polydispersity index (PDI) for size distribution.

ζ -Potential Measurements. Emulsions were diluted to an oil droplet concentration of approximately 0.005 wt % using buffer solutions (pH 3.0) to avoid multiple scattering effects. Diluted emulsions were injected directly into the chamber of a particle electrophoresis instrument (Nano-ZS90, Malvern Instruments). The ζ -potential was determined by measuring the direction and velocity of droplet movement in a well-defined electric field. All measurements were performed in duplicate with freshly prepared samples, and the ζ -potential measurements were reported as the mean and standard deviation of two separate injections.

Physical Stability Evaluation of Emulsion. The stability of the emulsion was examined with the multisample analytical centrifuge (LUMiSizer, LUM GmbH, Berlin, Germany), which allows the intensity of the transmitted NIR light to be measured as a function of time and position over the entire sample length simultaneously. The instrument simulates comprehensive emulsion processes due to gravitation forces and presents a typical graph for each process: creaming, sedimentation, phase separation. The data are displayed as a function of the radial position, as a distance from the center of rotation (transmission profiles). The shape and progression of the transmission profiles contain information on the kinetics of the separation process and allow particle characterization as well as evaluation of particle–particle interactions (29). It was shown in several papers that the method can be applied at high volume concentrations, too (30% and higher) (30–33). The integration graph shows the percentage of light absorbance per hour, the “creaming rate”. The rate is correlated to the stability of the emulsion; the higher the creaming rate, the lower the stability. The samples in the LUMiSizer were centrifuged at 4000 rpm and 25 °C.

Rheological Behavior of Emulsions. Rheological measurements were performed at 25 ± 0.1 °C, with an AR 1500 rheometer (TA Instruments, West Sussex, U.K.), using a cone and plate geometry (cone diameter = 40 mm, angle = 2°, gap = 0.100 mm). For each measurement, 2.0 mL of the emulsion was carefully deposited over the plateau of the rheometer. After the plateau had been contacted with the cone, the exposed surface of sample was covered with a thin layer of silicone oil to prevent evaporation during the measurement. All samples were allowed to rest for 5 min after loading to allow temperature equilibration and induced stress to relax. Steady-state flow measurements were carried out at 25 ± 0.1 °C in the range of 0–200 s⁻¹ during 5 min, and rheological parameters (shear stress, shear rate, apparent viscosity) were obtained from the software. Experimental flow curves were fitted to the Herschel–Bulkley model, $\sigma = \sigma_0 + k\gamma^n$, where σ is the shear stress (Pa), σ_0 is the yield stress (Pa), γ is the shear rate (s⁻¹), k is the consistency index (Pa s^{*n*}), and n is the flow behavior index (dimensionless). Stress sweep tests (1 Hz at 25 °C) were made to determine the linear viscoelastic region of all samples; a stress value of 1 Pa was chosen for all frequency tests. Oscillatory (dynamic) tests were performed inside the linear viscoelastic region, and the storage modulus (G') and loss modulus (G'') were recorded versus frequency. Frequency sweeps were done from 0.001 to 10 Hz at 1 Pa and at 25 ± 0.1 °C, but in most cases some results had to be discarded due to equipment limitation at low frequencies. TA data analysis software was used to obtain the experimental data (G' , G'' , etc.).

Effects of Storage Temperature on the Chemical Stability of β -Carotene in Emulsions with or without the Adsorption of Chitosan.

Emulsion samples were diluted with buffer solutions (100 mM acetic acid, pH 3.0) to a total oil concentration of 1 wt % (β -carotene 0.015 wt %) and then transferred into screw-capped brown bottles flushed with nitrogen.

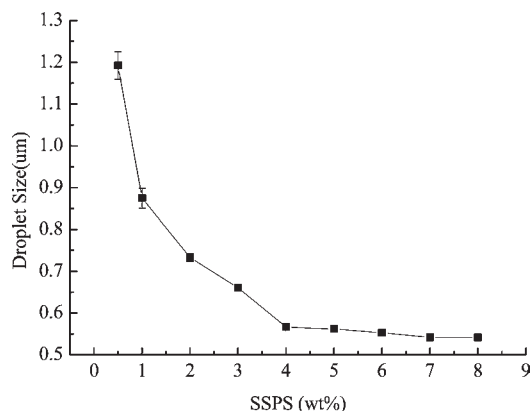


Figure 1. Effects of SSPS concentration on the average droplet size of the emulsions with 10 wt % MCT oil (0.15 wt % β -carotene, 100 mM acetic acid, pH 3.0).

Each emulsion sample was divided into four parts, which were stored at 4, 30, 50, and 70 °C in dark immediately after the dilution. The β -carotene concentration, mean particle diameter, and ζ -potential were determined constantly according to the storage time.

Analysis of β -Carotene Content. The content of β -carotene in the emulsion was determined following the method of Yuan et al. (6). Emulsions were first diluted to an appropriate concentration with distilled water and then extracted with ethanol and *n*-hexane. Absorbance was measured with a Shimadzu UV-1800 UV-vis spectrophotometer at 450 nm. The concentration of β -carotene was obtained by referring to a standard curve of β -carotene prepared under the same condition.

Statistical Analysis. The whole experiment was conducted in duplicate. Data were subjected to analysis of variance (ANOVA) using the software package SPSS 12.0 for Windows (SPSS Inc., Chicago, IL). Means of treatments were separated at the 5% significance level using the LSD method.

RESULTS AND DISCUSSION

Effects of SSPS Concentration on the Stability of β -Carotene Emulsions. The purpose of the experiment was to determine the minimum SSPS concentration required to fully cover the oil-water interface and stabilize the emulsion droplets. If the SSPS concentration was too low, it would not be possible to form stable emulsions; however, if the SSPS concentration was too high, there would be a lot of nonadsorbed SSPS present in the aqueous phase that could interact with the cationic chitosan molecular used to create the multiple-layered interfacial membranes. **Figure 1** shows the effect of SSPS concentration on the average droplet size of β -carotene emulsions. At SSPS concentrations above 4 wt %, the average droplet size distribution was independent of the SSPS concentration. This concentration might be the minimum level necessary to cover the oil droplets. Usually, to avoid the interaction of nonadsorbed SSPS with chitosan, the concentration of nonadsorbed SSPS should be minimized. Therefore, in the following experiments, the effect of SSPS concentration on the stability of emulsions was examined with the multisample analytical centrifuge based on the STEP technology (space time resolved extinction profiles). As shown in **Figure 2**, it was evident that the separation behavior of the emulsions was quite different with low SSPS concentrations. Emulsions with SSPS concentration of 0.5–3 wt % were very unstable, because they exhibited a remarkable increase in the transmission due to creaming and flotation during the centrifugation for 2500 s at 4000 rpm. When the SSPS concentration was above 4%, the emulsion became stable, as there was very little change in the integral transmission. It can be concluded that when the SSPS concentration was too low (< 4 wt %), the oil droplets could not be fully covered. Thus, the emulsions with relatively large particle size (**Figure 1**) were

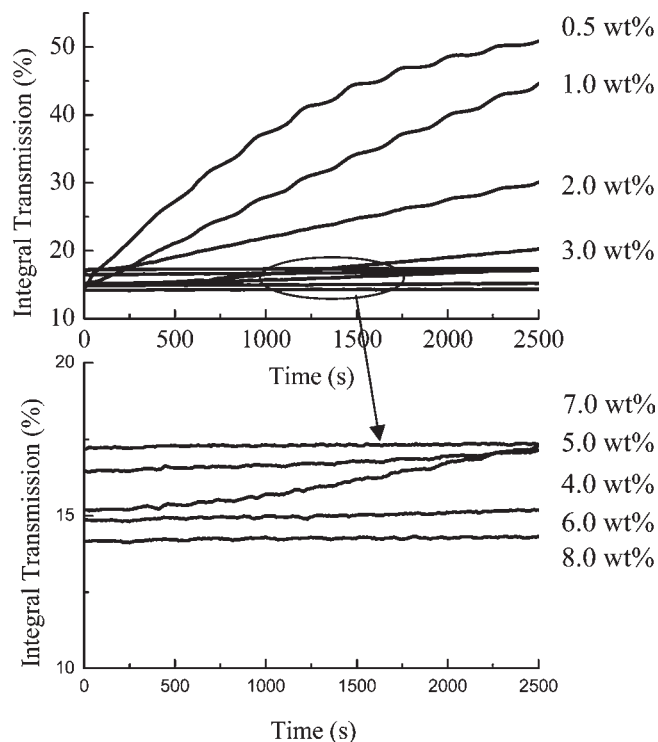


Figure 2. Recorded evolutions of time-dependent integral transmission profiles of β -carotene emulsions with different SSPS concentrations. Profiles were taken every 10 s at 4000 rpm during 2500 s at 25 °C.

unstable to creaming and coalescence (**Figure 2**). Therefore, emulsions stabilized by 4 wt % SSPS were preferred in the subsequent experiments.

Effects of Chitosan Concentration on the Droplet Characteristics of β -Carotene Emulsions. Chitosan is a cationic biopolymer that has many potential applications in the food, cosmetic, and pharmaceutical industries because of its unique nutritional and physicochemical properties (34). Previous studies showed that chitosan can interact with proteins through physical (e.g., electrostatic) or chemical (e.g., Maillard) interactions to form either soluble or insoluble complexes (35–39). Besides, it was demonstrated that the stability of emulsions stabilized by small molecule surfactants (SDS, lecithin) could be improved by the adsorption of chitosan onto the oil droplet surface (40, 41). Therefore, the objective of the following experiments was to determine if the chitosan can be adsorbed to the oil droplet surface of emulsions stabilized by polysaccharides (SSPS). **Figure 3** shows the influence of chitosan concentration (0–1 wt %) on the mean particle diameter and ζ -potential of SSPS-stabilized β -carotene emulsions (10 wt % MCT oil, 0.15 wt % β -carotene, 100 mM acetic acid, pH 3.0). In the absence of chitosan, the net charge on the SSPS coated droplets was about -34 mV. The net charge on the droplets became less negative and changed from negative to positive as the chitosan concentration was increased. Eventually, the net charge on the droplets reached a positive value ($\sim +58$ mV) when the chitosan concentration was 2 wt % (**Figure 3a**). This change in ζ -potential indicated that positively charged chitosan adsorbed onto the surface of negatively charged SSPS-stabilized β -carotene emulsions. The ability of charged polyelectrolytes to adsorb onto the surface of oppositely charged colloidal particles has been well evaluated in the literature (42–44). **Figure 3b** shows the effect of chitosan concentration on the mean particle diameter of SSPS-stabilized emulsions. Preliminary experiments verified that the mean particle diameter of SSPS-stabilized emulsions was about 0.48 μ m and stable to creaming as indicated by the results of

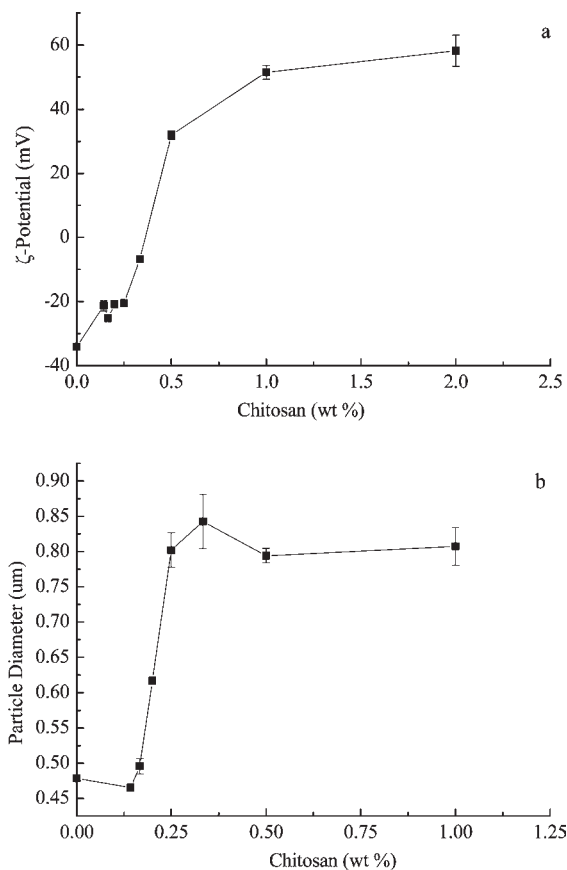


Figure 3. Dependence of (a) particle electrical charge (ζ -potential) and (b) mean particle diameter on the chitosan concentration for SSPS-stabilized β -carotene emulsions.

multisample analytical centrifuge (Figure 2). It was found that at lower chitosan concentrations (0.167 wt %), there was no significant increase of the mean particle diameter (Figure 3b). This indicated that there was little droplet aggregation and that the negative charge of the emulsion droplets was still sufficient to prevent droplet flocculation through electrostatic repulsion (14). When the chitosan concentration was above 0.167 wt %, there was a large increase in mean particle diameter, which was attributed to extensive droplet aggregation. The maximum level of droplet aggregation occurred at a chitosan concentration of about 0.333 wt %, when the net charge on the droplets was about -6.76 mV. When the concentration of chitosan was above 0.333 wt %, the mean particle diameter of the emulsions decreased and reached a minimum value of $0.79 \mu\text{m}$ at a chitosan concentration of 0.5 wt %; further addition of chitosan led to slight increases in particle diameter. Similar phenomena were also found in the preparation of SDS–chitosan–pectin-stabilized multilayer emulsions (45).

Influence of Chitosan Concentration on the Rheological Properties of β -Carotene Emulsions. Figure 4a shows the effect of chitosan concentration on the viscosity of SSPS-stabilized β -carotene emulsions at 25°C and pH 3.0. The viscosity of emulsions decreased with increasing shear rate. As the shear rate sufficiently increases to overcome the Brownian motion, the emulsion droplets become more ordered along the flow field and offer less resistance to flow and hence lower viscosity (46). Emulsions prepared without chitosan had low viscosity ($0.01 \text{ Pa}\cdot\text{s}$ at a shear rate of 10 s^{-1}). It was observed that the addition of low amounts of chitosan increased viscosity slightly; however, a significant increase ($p < 0.05$) in the viscosity was observed when

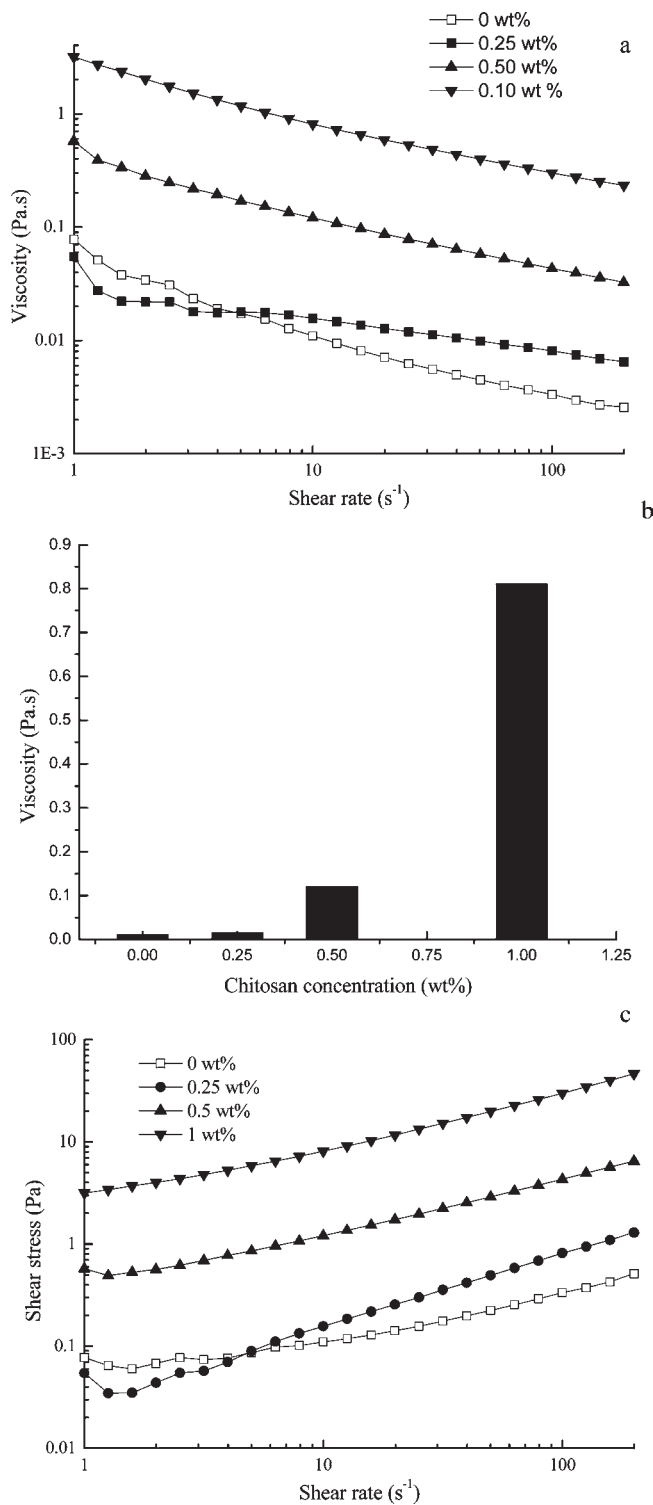


Figure 4. (a, b) Effects of chitosan concentration on the viscosity of SSPS-stabilized β -carotene emulsions (4 wt % SSPS, 10 wt % MCT oil, 0.15 wt % β -carotene, 100 mM acetic acid, pH 3.0); (c) flow curves of SSPS-stabilized β -carotene emulsions (4 wt % SSPS, 10 wt % MCT oil, 0.15 wt % β -carotene, 100 mM acetic acid, pH 3.0) with different chitosan concentrations.

the amount of chitosan was increased from 0.25 to 1 wt % (Figure 4b). The increase in viscosity could be explained by the adsorption of cationic chitosan on the surface of negatively charged SSPS-stabilized emulsions. In fact, at pH 3.0 the chitosan and SSPS molecules carry opposite charges and the complexation was formed between these macromolecules. When the chitosan

Table 1. Rheological Parameters from the Herschel–Bulkley Model for SSPS-Stabilized Emulsions with Different Chitosan Concentrations

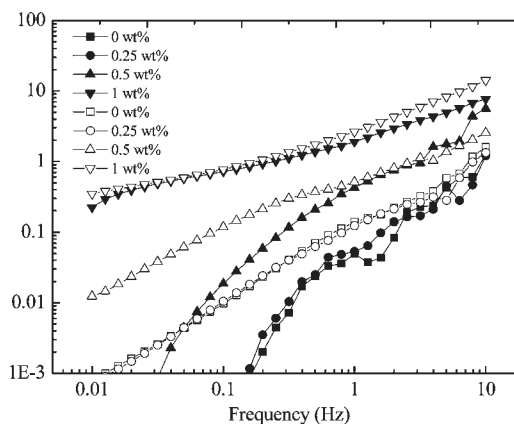
chitosan concn (wt %)	σ_0 (Pa)	K (Pa·s n)	n	R^2
0	0.0549	0.0101	0.7181	0.9987
0.25	-7.411×10^{-3}	0.0335	0.6912	0.9996
0.50	0.2322	0.2335	0.6213	0.9998
1.00	1.870	1.3670	0.6582	0.9999

concentration was low, there was insufficient cationic polyelectrolyte available to coat the anionic droplets, and droplet aggregation occurred, which contributed a slight increase of viscosity at higher shear rates. When the chitosan concentration in the emulsion was above a critical concentration (0.5 wt %), stable emulsions consisting of droplets completely surrounded by the chitosan layer were formed. The attractive force between the anionic SSPS-coated droplets and cationic chitosan promoted the formation of SSPS–chitosan interfaces and obviously increased the viscosity of the system.

Figure 4c shows the flow curves of SSPS-stabilized emulsions with or without the presence of chitosan. For the range of shear rates used in this study, the shear stress showed a practically linear dependence on shear rate. Flow curves were described by the Herschel–Bulkley model, and the parameters are given in **Table 1**. $n < 1.0$ corresponds to shear thinning behavior, $n > 1.0$ corresponds to shear thickening behavior, and $n = 1.0$ corresponds to Newtonian behavior (47). It can be concluded that the SSPS-stabilized emulsions with and without chitosan behaved like a near-Newtonian fluid. Addition of chitosan had no significant effect on flow type. Flow behavior index of emulsions was approximately in the range of 0.6–1.0 both in the absence and in the presence of chitosan, which corresponded to near-Newtonian behavior. The consistency index is an indicator of the viscous nature of the emulsion and was observed to be increased from 0.01 to 1.367 Pa·s n with the rise of chitosan concentration. Shear thinning behavior was associated with the flocculation of fat droplets. This is usually the case with weakly flocculated emulsions or those to which a thickener was added. Flocculation increased the apparent dispersed phase volume and led to the formation of nonspherical aggregates, both factors contributing to increase the emulsion consistency (48). The general tendency of both the consistency index and the flow behavior index was mainly determined by the hydrocolloid concentration: an elevation of chitosan concentration largely increased the viscosity and hence the consistency index of emulsions.

Dynamic frequency sweep tests were performed in the linear viscoelastic range to determine the frequency dependence of the storage modulus (G') and loss modulus (G''). **Figure 5** shows the viscoelastic behavior of SSPS-stabilized emulsions at a chitosan concentration of 0–1%. Both G' and G'' increased with the rise of frequency, and they were almost frequency dependent. At all chitosan concentrations tested, emulsions exhibited a liquid-like behavior at low frequencies where loss modulus (G'') was higher than storage modulus (G'). A gradual increase of the relative values of G' and G'' was observed as the chitosan concentration was increased. This effect was more pronounced when chitosan concentration was above 0.5 wt %. For the emulsion system with a chitosan concentration of 0.5 wt %, G' became greater than G'' at frequencies approximately above 4.0 Hz and the system showed a solid-like behavior. This was also testified by phase angle values ($\tan \delta = G''/G'$). Phase angle values of SSPS-stabilized emulsions in the absence and presence of chitosan were decreased with increasing frequency and found to vary between 89° and 10° within the study frequency range.

The adsorption of chitosan onto the SSPS-stabilized oil droplets promoted the formation of much stronger (more elastic)

**Figure 5.** Dynamic storage modulus and loss modulus as a function of frequency for SSPS-stabilized β -carotene emulsions (4 wt % SSPS, 10 wt % MCT oil, 0.15 wt % β -carotene, 100 mM acetic acid, pH 3.0) with different chitosan concentrations (G' , solid symbols; G'' , open symbols).

structure, and it could be attributed to the formation of a highly flocculated droplet network structure by the SSPS–chitosan interfaces. The multiple layer interfacial complexes were generated by electrostatic deposition of cationic chitosan onto anionic SSPS-coated droplets. It can be concluded that SSPS-stabilized emulsions with the adsorption of chitosan had a viscoelastic property, and it could be characterized as weak gels as a typical behavior of dressings and emulsions (49).

Effects of Storage Temperature on the Chemical Stability of β -Carotene in Emulsions. Due to the highly unsaturated structure, β -carotene is very sensitive to thermal and oxidative degradation during emulsification, processing, and storage. In the series of experiments, the influence of chitosan concentration (0, 0.25, 0.5, 1.0 wt %) on the chemical degradation of β -carotene in SSPS-stabilized emulsions was determined by splitting emulsion samples into four parts, which were stored at 4, 30, 50, and 70 °C in the dark (**Figure 6**), respectively.

Rapid degradation of β -carotene was observed in all emulsion samples stored at 70 °C (**Figure 6a**). Chitosan concentration had a significant ($p < 0.05$) effect on the degradation of β -carotene in multilayer emulsions. After storage of 2 days, about 70, 61, 26, and 20% of β -carotene were degraded in SSPS-stabilized emulsions with chitosan concentrations of 0, 0.25, 0.5, and 1 wt %, respectively. After storage of 1 week at 70 °C, > 96% of β -carotene was lost in emulsions stabilized by SSPS with chitosan concentration of 0 and 0.25 wt %, respectively. Lower concentration of chitosan was found to have little impact on β -carotene stability in SSPS-stabilized emulsions at higher temperatures. This may be due to the severe droplet aggregation when there was not enough cationic chitosan present in the emulsion (**Figure 3**). However, SSPS-stabilized emulsions with chitosan concentration of 0.5 and 1 wt % exhibited 69.3 and 74.2% β -carotene loss, respectively. These results revealed that the degradation of β -carotene was inhibited by the formation of multilayer emulsions. Slower degradation of β -carotene was observed in emulsions during 2 weeks of storage at 50 °C (**Figure 6b**). About 81.8% of β -carotene was lost in SSPS-stabilized emulsions, whereas those with adsorbed chitosan concentrations of 0.25, 0.5, and 1 wt % exhibited 76.2, 37.6, and 50.8% loss, respectively. Therefore, β -carotene degradation was fastest in SSPS-stabilized emulsions without the addition of chitosan, and similar degradation rates were also observed in SSPS-stabilized emulsions with lower chitosan concentrations (0.25 wt %). Higher concentrations of chitosan were effective at inhibiting β -carotene degradation. The degradation of β -carotene during storage of 4 weeks at 30 °C is

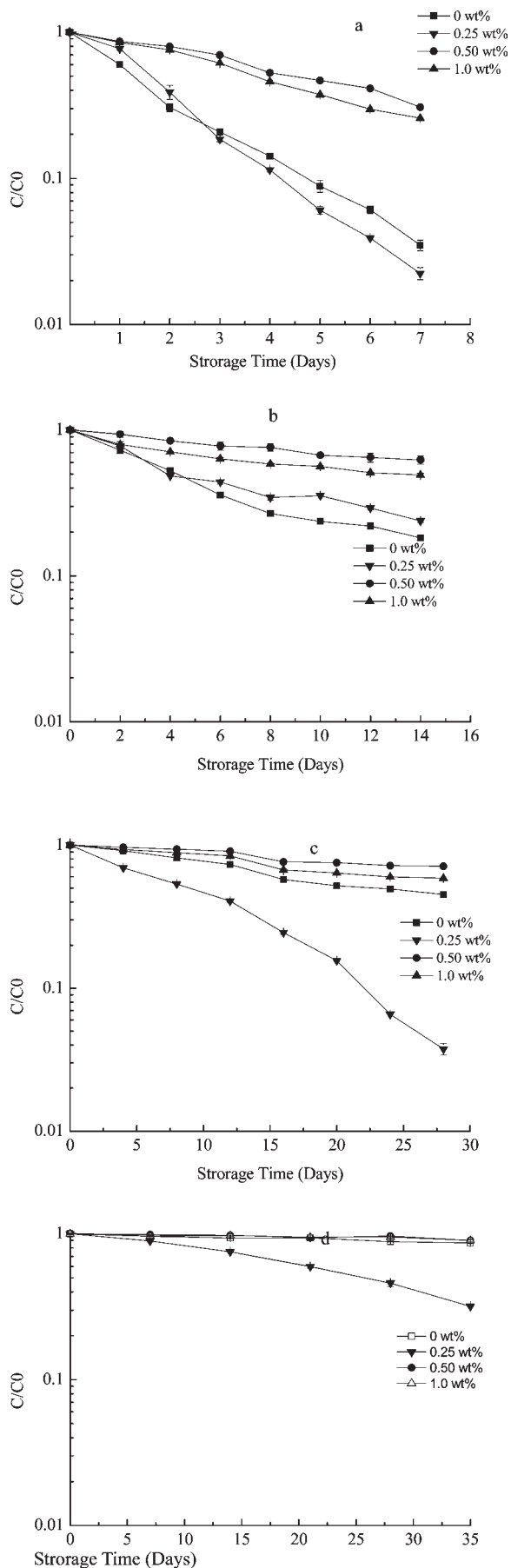


Figure 6. Effects of chitosan concentrations on the degradation of β -carotene in SSPS-stabilized emulsions at different storage temperatures (a, 70 °C; b, 50 °C; c, 30 °C; d, 4 °C).

Table 2. Reaction Rate Constants (k) of β -Carotene Degradation

T (°C)	k (s^{-1}) at a chitosan concentration of			
	0 wt %	0.25 wt %	0.5 wt %	1.0 wt %
4	0.003996 a	0.032507 d	0.002579 a	0.002487 a
30	0.030434 d	0.116037 g	0.013899 b	0.021268 c
50	0.122704 h	0.095966 f	0.043515 e	0.047488 e
70	0.465045 k	0.566665 l	0.164883 i	0.204011 j

illustrated in **Figure 6c**. The degradation rates of β -carotene in SSPS-stabilized emulsions were significantly influenced by the adsorbed chitosan concentration. SSPS-stabilized emulsions with chitosan concentrations of 0, 0.25, 0.5, and 1.0 wt % exhibited 55.0, 96.0, 29.0, and 42.0% β -carotene loss, respectively. As can be found from **Figure 6d**, after storage of 5 weeks at 4 °C, the loss of β -carotene did not significantly differ between the SSPS-stabilized emulsions with chitosan concentrations of 0, 0.5, and 1.0 wt %. The degradation of β -carotene was fastest in SSPS-stabilized emulsions with a chitosan concentration of 0.25 wt %.

To get a better understanding of the effects of chitosan concentration and storage temperature on the degradation of β -carotene in SSPS-stabilized emulsions, the degradation kinetics of β -carotene was developed. Reaction rate constants (k values) were calculated according to the following equation: $C/C_0 = \exp(kt)$ from β -carotene concentration C , initial concentration C_0 , and storage time t . k values are listed in **Table 2**. It was found that degradation of β -carotene in emulsions stabilized by the SSPS–chitosan interface followed first-order kinetics. R^2 values from 0.8934 to 0.9998, which indicated first-order kinetics model, described well the degradation of β -carotene. Results demonstrated that higher temperatures were directly correlated with significant increase of β -carotene loss in all samples. Chitosan concentration had a significant impact on the stability of β -carotene. At temperatures of 30, 50, and 70 °C, the degradation of β -carotene in SSPS-stabilized emulsion samples was faster at a chitosan concentration of 1 wt % than at 0.5 wt %. Severe degradation of β -carotene was detected in the SSPS-stabilized emulsions with adsorbed chitosan concentrations of 0 and 0.25 wt %, and the least degradation occurred in emulsions with a chitosan concentration of 0.5%.

Several possible explanations were involved for the degradation of β -carotene in SSPS-stabilized emulsions with or without the adsorption of chitosan. With the increase of chitosan concentration from 0 to 1 wt %, the mean particle diameter of SSPS-stabilized emulsions increased from 0.48 to 0.81 μm (**Figure 3**). Therefore, SSPS-stabilized emulsions with a chitosan concentration above 0.5 wt % had the largest mean particle diameter, which meant that the β -carotene was encapsulated by a thicker interface. Thus, the diffusion of pro-oxidants or free radical may be limited by the thick coverage of the SSPS–chitosan interface. This was one of the reasons why the β -carotene in SSPS/chitosan-stabilized emulsions had better chemical stability than the β -carotene in SSPS-stabilized emulsion. Besides, it has been shown that chitosan was good in antioxidant properties, especially antioxidant activity and scavenging ability on hydroxyl radicals. Chitosan with higher degrees of N-deacetylation had more amino groups on C2 to enhance their antioxidant properties (50). It was reported that lipid oxidation was reduced by the addition of chitosan (51). Recent studies demonstrated that the adsorption of chitosan onto the oil droplet interface could inhibit the oxidation of fish oil and limonene degradation, which was mainly due to the electrostatic and steric repulsion between the cationic ions and the thick cationic droplet interface (21, 52). In this study, chitosan was used to adsorb onto the SSPS-coated droplet interface and a stronger droplet network structure was formed. It was observed

that the adsorption of chitosan could increase the viscosity of β -carotene emulsions stabilized by SSPS (Figures 4 and 5). Thus, the high retention of β -carotene in SSPS-stabilized emulsions with a chitosan concentration above 0.5 wt % might be due to the formation of the SSPS–chitosan interface complexes, which offered an inhibitory effect on lipid oxidation. The instability of β -carotene in SSPS-stabilized emulsions at low chitosan concentrations was attributed to bridging flocculation, and a similar phenomenon was also demonstrated in other studies (53). However, SSPS-stabilized emulsions with a chitosan concentration of 1 wt % had stronger droplet network structure but lower chemical stability than those with a chitosan concentration of 0.5 wt %. It was reported that depletion flocculation can be promoted when too much free polyelectrolyte is present in the solution (54). Further research needs to clarify the mechanism of the SSPS–chitosan interface on the degradation of β -carotene in emulsions.

In conclusion, it was shown that β -carotene emulsions could be stabilized by SSPS–chitosan interfaces formed by electrostatic deposition of cationic chitosan onto anionic SSPS-coated droplets. The results have provided a more detailed understanding of the rheological and physicochemical properties of multilayer emulsions. All emulsions showed near-Newtonian fluid behavior, and both viscosity and consistency index were increased with the rise of chitosan concentration. The chemical stability of β -carotene emulsions at different temperatures was improved by the formation of SSPS–chitosan interfaces. The least degradation occurred in emulsions with chitosan concentration of 0.5 wt %. This emulsion system could be useful to the food industry for the creation of β -carotene emulsions with improved heat stability and controlled release or triggered release properties. Further research will be carried out to understand the mechanism of SSPS–chitosan interface complexes on the chemical stability of β -carotene and its in vitro release properties.

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