

Physical and Chemical Stability of Gum Arabic-Stabilized Conjugated Linoleic Acid Oil-in-Water Emulsions

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ABSTRACT: Oil-in-water (O/W) emulsions have been used as a delivery system to protect conjugated linoleic acid (CLA), a polyunsaturated fatty acid, from oxidation. Conventional gum arabic (GA) and two matured gum arabic samples (EM2 and EM10) were used as emulsifiers to prepare CLA-in-water emulsions. The emulsions have optimal physical and chemical stability at gum concentrations of 5% for all three gums. Emulsions with higher gum concentrations are more susceptible to lipid oxidation. This is attributed to reduced physical stability at higher gum concentrations because of the coalescence and depletion-induced flocculation of the emulsion droplets. The prooxidants iron and copper intrinsically contained in the gums could also contribute to this instability. Among the three gums, EM10 provides the most effective protection for CLA both physically and chemically, because of its superior interfacial properties over GA and EM2.

KEYWORDS: conjugated linoleic acid, gum arabic, emulsions, stability, oxidation, interfacial properties

■ INTRODUCTION

Conjugated linoleic acid (CLA) comprises a family of positional and geometric isomers of octadecadienoic acid (18:2), which occur naturally in dairy and beef products. Among the isomers, the *cis-9,trans-11* isomer is the principal dietary form of CLA.^{1–3} CLA is considered to have multiple health benefits such as reducing body fat,⁴ lowering serum cholesterol level,⁵ antioxidation activity,⁶ and anticarcinogenic⁷ and immunoenhancing functions.⁸ Interest in incorporating CLA into various food products has grown significantly in recent years.

However, because the susceptibility of polyunsaturated fatty acids to oxidation has restricted their applications in food products, it is important to develop effective protection systems. Here we consider how oil-in-water (O/W) emulsions stabilized with food hydrocolloids can be used as effective delivery system to encapsulate and protect CLA. Previously, hydrocolloids have been used to protect fat-soluble functional factors or flavor components in emulsions. Djordjevic et al. studied the physical and chemical stability of limonene and citral emulsions stabilized by gum arabic (GA), sodium dodecyl sulfate (SDS)–chitosan complex, and whey protein isolate.^{9,10} SDS–chitosan complex and whey protein isolate provided chemically more stable emulsions than GA. Inhibition of flavor oxidation was attributed to the formation of a cationic emulsion droplet interface that could repel prooxidative metals and the ability of protein to scavenge free radicals and chelate prooxidative metals. Charoen et al.¹¹ compared the physical and oxidative stability of rice bran oil-in-water emulsions stabilized by whey protein isolate, modified starch, and GA. Whey protein isolate and modified starch gave more stable emulsions than GA, which was again attributed to the electrical characteristics of interfacial layers formed by the biopolymers. Xu et al. reported that protein–polysaccharide conjugates could

improve the physical stability of β -carotene emulsions and inhibit their oxidation compared with individual proteins or polysaccharides or their mixtures. This was attributed to the formation of a thicker and denser interfacial layer by the protein–polysaccharide conjugates.¹²

GA, a naturally occurring polysaccharide–protein complex, is widely used in the soft drink industry for emulsifying flavor oils (e.g., orange oil) under acidic condition, which is attributed to its well-known property of forming an elastic interfacial film around an oil droplet surface.¹³ GA can stabilize emulsions in both concentrated and diluted forms due to its high solubility, low viscosity, large steric hindrance, and electrostatic interactions and is possibly the most common emulsifier used in the food industry.¹⁴ There are different species of trees producing GA, with about 80% production from *Acacia senegal* and most of the rest from *Acacia seyal*.¹⁵ GA is composed of three components: arabinogalactan protein (AGP), arabinogalactan (AG), and glycoprotein (GP). The high-molecular-weight AGP fraction contains about 10% proteinaceous materials and has now been demonstrated to be responsible for the emulsifying properties of GA.^{16–18} A maturation process was employed to modify GA by heating at an elevated temperature and controlled humidity, resulting in increased fraction of AGP and thus enhanced emulsifying functionality.¹⁶

This study aims to evaluate the suitability of a conventional GA and two matured GA samples (EM2 and EM10) in stabilizing CLA O/W emulsions. The physical and chemical stability of the emulsions is investigated and correlated with the interfacial properties of the macromolecular emulsifiers.

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EXPERIMENTAL PROCEDURES

Materials. Food grade free fatty acid CLA with a purity of 80.2% was purchased from Beijing Health Science and Technology Co. Ltd., China. The remaining components contain 12.5% of oleic acid, and palmitic acid, stearic acid, and linoleic acid account for the rest, which were measured by a gas chromatograph (Varian 3900, USA). Conventional commercial GA and two matured gums (EM2 and EM10) in spray-dried form were provided by San-Ei Gen F.F.I., Japan. The moisture contents of GA, EM2, and EM10 were 8.48, 2.43, and 1.70%, respectively.

Physical Parameters of CLA and Gums. The density of CLA was measured by using the density flask method. Its viscosity was determined using a rotational rheometer (Haake Rheostress 6000). The molecular parameters of GA, EM2, and EM10 were measured using gel permeation chromatography online coupled with multiangle laser light scattering system (GPC-MALLS, Wyatt Technology Corp., USA), and the results are shown in Table 1. The content of metal ions was determined by polarized Zeeman atomic absorption spectrometry (Hitachi Z-2000, Japan).

Table 1. Molecular Parameters of GA, EM2, and EM10 Measured by GPC-MALLS^a

gum	whole fraction	peak 1, AGP		peak 2, AG + GP	
	M_w (g/mol)	fraction (wt %)	M_w (g/mol)	fraction (wt %)	M_w (g/mol)
GA	8.38×10^5	12.8	3.09×10^6	87.2	3.70×10^5
EM2	2.84×10^6	17.9	8.81×10^6	82.1	4.84×10^5
EM10	3.98×10^6	29.4	9.69×10^6	70.6	5.60×10^5

^aGPC-MALLS measurements were carried out at 25 °C using a Superose 6 10/300 GL separation column (GE Healthcare Co., USA) with 0.2 mol/L NaCl as eluent. Peaks 1 and 2 were defined as reported previously.

Interfacial Tension Measurements. The interfacial tensions of GA, EM2, and EM10 adsorbed onto the CLA–water interface were measured at 25 °C using a drop profile tensiometer (Teclis Tracker, France). A pendent drop of gum solutions with a series of concentrations from 0.25 to 5 wt % was formed at the tip of the needle of a syringe whose verticality could be controlled. The needle was submerged in an optical glass cuvette containing CLA, which was located between a light source and a high-speed charge couple device (CCD) camera. The drop profile was recorded by the CCD camera and was analyzed according to the Laplace equation.¹⁹

Emulsion Preparation. Gum solutions were prepared by dispersing gum powders (1, 5, 10, and 15% in the final emulsion) in distilled water and then put on a roller mixer at room temperature overnight to ensure complete hydration. CLA was added to the gum solutions to achieve a final concentration of 15% in the emulsion, and the systems were prehomogenized for 4 min at 26000 rpm using a high-speed blender (Polytron PT 2100, Switzerland). The primary emulsions were further homogenized with a high-pressure homogenizer (Microfluidic M-110L, USA) at 75 MPa for one pass. The homogenizations were carried out in an ice bath to minimize the extent of lipid oxidation.

Particle Size Analysis. The particle size distribution (PSD) of emulsions was measured using a laser diffraction technique (MasterSizer 2000, Malvern Instruments Ltd.). The emulsions were diluted to achieve a laser obscuration slightly above 10% and stirred continuously to avoid multiple scattering effects. The refractive index of sample was 1.52 with an absorption coefficient of 0.01. The particle size is given as the volume-weighted mean diameter ($D_{4,3}$), $D_{4,3} = (\sum n_i d_i^4 / \sum n_i d_i^3)$, where n_i is the number of droplets with diameter d_i . $D_{4,3}$ was reported as the average of triplicates.

Optical Light Microscopy. An optical light microscope (Nikon YS100), equipped with a CCD camera (HV2001UC) and connected to a BT-1600 image particle size analyzer (Dandong Bettersizer

Instrument Ltd., China), was used to visualize the microstructure of emulsions at a magnification of 400.

Oxidative Stability Analysis. To evaluate the oxidative stability of emulsions, a Clark-type oxygen electrode (Chlorolab 2, Hansatech Instruments Ltd., UK) was used to monitor oxygen consumption by the emulsions. Temperature was controlled with a Poly stat refrigerated bath (Cole-Parmer Instrument Co., Vernon Hills, IL, USA). Illumination was provided by a light housing and stabilized power supply (LS2, Hansatech Instruments Ltd.), and the light passed through a fiber optic cable (A8, Hansatech Instruments Ltd.) to the surface of the reaction cuvette. Irradiance was measured with a quantum sensor (QRT1, Hansatech Instruments Ltd.) and controlled by neutral density filters. Emulsions were bubbled with air (about 4 min) to achieve a saturation of oxygen before measurements. Each 200 μ L of the emulsions was transferred into the oxygen electrode chamber and was kept agitated with a magnetic stirrer. The measurements were performed at both 25 and 50 °C with no exposure to light for the CLA emulsions stabilized by GA, EM2 and EM10. The change in oxygen concentration was followed for a period of 6 min, and the oxygen consumption rate was expressed as the linear slope of oxygen concentration versus time.

RESULTS AND DISCUSSION

Physicochemical Properties of CLA and Gums. The density of the CLA used in the study is 0.905 g/mL and its viscosity is 0.05 Pa·s at 25 °C. The molecular parameters of the three types of gum arabic are shown in Table 1. The weight-average molecular weights increased in the order EM10 > EM2 > GA. The content of the AGP fraction also increased in the same order, with the APG fraction of EM10 more than twice that of GA. Meanwhile, the fractions of AG and GP decreased. The changes have been explained by a physical aggregation between AG and GP forming more AGP.¹⁶ As iron and copper are regarded as prooxidative metals for lipid oxidation,²⁰ the levels of the two metals intrinsically contained in the gum samples were determined by atomic absorption. As can be seen in Table 2, the iron and copper contents were more or less the

Table 2. Content of Iron and Copper Metals in GA, EM2, and EM10

	GA	EM2	EM10
Fe (ppm)	8.76	8.82	9.01
Cu (ppm)	1.43	1.40	1.58

same in the three gum samples. EM10 contained only slightly higher levels of iron and copper as compared to GA and EM2. The small difference was presumably introduced by the maturation process.

Adsorption of Gums at the CLA–Water Interface. The adsorption kinetics of GA at the CLA–water interface at various GA concentrations was followed by measuring interfacial tension as a function of time using the drop profile tensiometer (Figure 1). The interfacial tension dropped rapidly at the initial stage of adsorptions and then leveled off after 5000 s. The time to reduce initial interfacial tension by 30% was calculated as onset time t and is listed in Table 3. The onset time t decreased with increasing GA concentration, indicating a more rapid interfacial adsorption at higher gum concentrations. The equilibrium interfacial tension (acquired at 40000 s) for different GA concentrations is given in Table 3. Higher GA concentration is more efficient in reducing interfacial tension, leading to a lower equilibrium value, which is in agreement with previous studies.^{21–24} The results indicated that the adsorption of GA at the CLA–water interface is concentration-dependent.

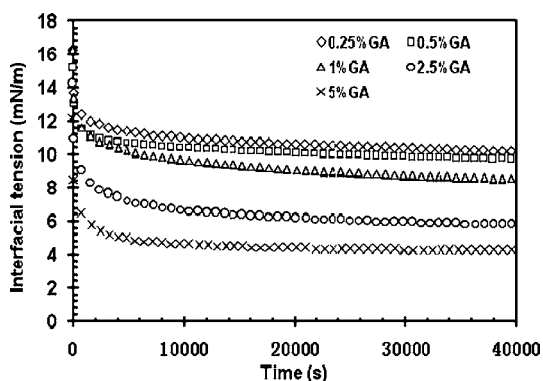


Figure 1. Linear plot of adsorption kinetics of GA with different concentrations at the CLA–water interface at 25 °C.

Table 3. Onset Time t of the Interfacial Kinetics of Different GA Concentrations at the CLA–Water Interface at 25 °C and the Resulting Equilibrium Interfacial Tension at 40000 s

sample	onset time t (s)	equilibrium interfacial tension (mN/m)
0.25% GA	4047	9.92
0.5% GA	3453	9.14
1% GA	1118	8.53
2.5% GA	281	5.81
5% GA	82	4.24

Moreover, the magnitude of the equilibrium interfacial tension achieved by GA at the CLA–water interface is considerably lower than that for most oil–water interfaces, such as the *n*-hexadecane–water interface (ca. 40 mN/m).¹⁹ This has been attributed to the amphiphilic nature of free fatty acids in contrast to their esterified forms or hydrocarbons. Free fatty acids carry both a hydrophobic hydrocarbon tail and a hydrophilic carboxylic acid head and therefore give lower interfacial tension with water.^{25,26}

The adsorption behaviors of GA, EM2, and EM10 are compared in Figure 2, and the corresponding adsorption

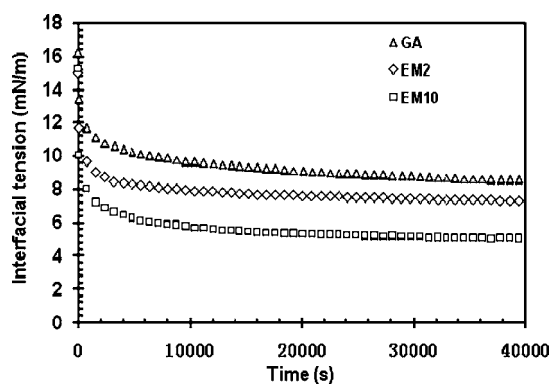


Figure 2. Comparison of adsorption kinetics of GA, EM2, and EM10 (1%) at the CLA–water interface at 25 °C.

characteristics are shown in Table 4. EM10 exhibited the shortest onset time t and the lowest equilibrium interfacial tension when adsorbed at the CLA–water interface. EM10 is, thus, the most surface-active, and the interfacial activity was enhanced by maturation of the gums. The results are in agreement with the study of Castellani et al.,¹⁹ in which EM2 reduced the interfacial tension at the *n*-hexadecane–water

Table 4. Onset Time t and Equilibrium Interfacial Tension of the Adsorptions of GA, EM2, and EM10 at the CLA–Water Interface at 25 °C

sample	onset time t (s)	equilibrium interfacial tension (mN/m)
1% GA	1118	8.53
1% EM2	60	7.30
1% EM10	20	5.05

interface more rapidly and more effectively than unmaturation gum arabic. As aforementioned, gum arabic contains three distinct molecular fractions: AGP, AG, and GP. Castellani et al. found that the GP fraction was the most surface-active, followed by AGP. The AG fraction showed the poorest surface activity and was almost incapable of adsorbing at the oil–water interface.¹⁹ The difference in surface activity could be attributed to different contents of protein in the three fractions, with 50% in GP, 10% in AGP, and trace amount in AG.¹⁹ Because the GP fraction accounts for only ca. 1% of the total gum,^{27,28} the difference in surface activity between GA, EM2, and EM10 should arise from the AGP fraction. As shown in Table 1, maturation led to an increased content of AGP, thus contributing to an enhanced surface activity.

Physical Stability of CLA Emulsions. The optimal concentration of GA to stabilize CLA emulsions was established by following the change of PSD during storage at room temperature (25 °C) and at elevated temperature (60 °C). Figure 3 shows the results for CLA emulsions with 5, 10, and 15% GA. Data are not shown for GA concentrations below 5% because creaming layers are observed even during storage at room temperature. For freshly prepared emulsions (0 days), a monomodal PSD was found for 5% GA, in contrast to bimodal PSDs for 10 and 15% GA. During prolonged storage, the emulsions with 5% GA showed no change in PSD at room temperature (Figure 3A). When stored at 60 °C, negligible change in PSD was observed, and only a very small population of droplets with larger sizes around 8 μm was formed. For the emulsions with 10% GA, the bimodal PSD at room temperature did not change either, but at 60 °C there was a pronounced growth of the larger size population. The emulsions with 15% GA showed the most significant alterations in PSD both at room temperature and at 60 °C. In the bimodal distributions, the smaller size population diminished and the large-size population increased markedly with storage time. Figure 3D plots $D_{4,3}$ against storage time. The initial $D_{4,3}$ values in the emulsions with 5, 10, and 15% GA were 0.95, 1.35, and 1.30 μm , respectively. No or less growth in $D_{4,3}$ occurred for the emulsions with 5% GA during storage. The growth of $D_{4,3}$ became more marked and intense at 10 and 15% GA. These results clearly indicate that the physical stability of CLA emulsions decreases with increasing GA concentration, and 5% GA can be regarded as the optimal concentration for stabilizing CLA emulsions. The slight instability of the emulsion with 5% GA at 60 °C might be linked to chemical deterioration of CLA accelerated at this high temperature due to lipid oxidation, as will be discussed later.

Overall, higher emulsifier concentrations led to finer and more stable emulsions, due to the formation of relatively thicker interfacial layers, which thus conferred stronger steric stabilization.^{11,29} However, excessive free emulsifiers in the aqueous phase can lead to depletion-induced flocculation of emulsion droplets, promoting aggregation, coalescence, and creaming.^{30–34} The reduced stability of CLA emulsions at

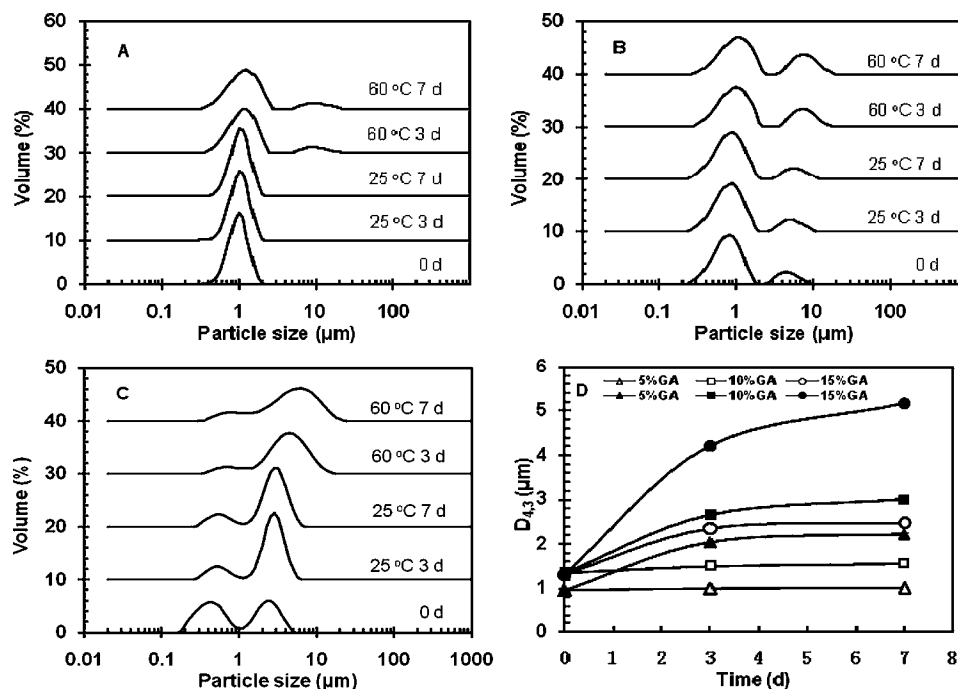


Figure 3. Change of particle size distribution (PSD) of CLA emulsions stabilized by GA at different concentrations during storage at 25 and 60 °C: (A) 5% GA; (B) 10% GA; (C) 15% GA. The plot of volume-weighted mean diameter $D_{4,3}$ against storage time at 25 °C (open symbols) and 60 °C (solid symbols) is shown in panel D: 5% GA (triangle); 10% GA (square); 15% GA (circle).

higher GA concentrations could then be due to the depletion force effect. On the other hand, it cannot be ruled out that the increased viscosity at higher gum concentration possibly also led to reduced emulsion stability. For the higher viscosity of the aqueous phase, more energy is needed to break oil droplets into fine particles, and it is more difficult for GA to diffuse and adsorb onto the CLA–water interface.

Figure 4 shows optical light microscopy images for freshly prepared emulsions with 1, 5, 10, and 15% GA. Emulsification with 1% GA produced a very coarse emulsion (Figure 4A). Emulsification with 5% GA (Figure 4B) gave the finest emulsion, which is consistent with the PSD measurements. Emulsion droplets at 10 and 15% GA (Figure 4C,D) are

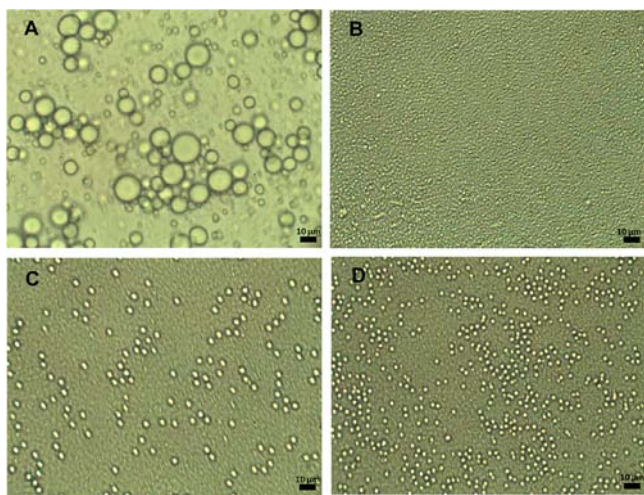


Figure 4. Optical light microscopy images of freshly prepared CLA emulsions stabilized by GA at different concentrations: (A) 1% GA; (B) 5% GA; (C) 10% GA; (D) 15% GA.

inhomogeneous, with a small amount of flocculation discernible. This might be an indication of the presence of strong depletion force effect. Furthermore, the emulsion droplets for 10% GA seem to be larger than those for 15% GA. This is in agreement with the initial $D_{4,3}$ values measured and the PSDs exhibited in Figure 3B,C. This could be explained by a larger extent of droplet coalescence in the emulsion with 10% GA.

For EM2 and EM10, a similar gum concentration dependence of emulsion stability was found. The optimal gum concentration to stabilize CLA emulsions was 5%. Figure 5 compares the growth of $D_{4,3}$ during storage at 60 °C for the emulsions stabilized with GA, EM2, and EM10 and at two different concentrations (5 and 15%). The emulsions stabilized with 5% gums (Figure 5A) showed a lesser extent of droplet size growth in comparison with those stabilized with 15% gums (Figure 5B). All three gums gave a negative correlation of CLA emulsion stability with gum concentration. Comparisons of initial $D_{4,3}$ between GA, EM2, and EM10 at the same concentration indicate that EM10 provided the most physically stable emulsion followed by EM2 and GA. EM10 exhibited the best emulsifying activity, which can be attributed to its highest efficiency in reducing the CLA–water interfacial tension.³⁵ This can overall be explained by the highest content of AGP in EM10, enabling adsorption onto the oil–water interface to form the most elastic interfacial film.

Chemical Stability of CLA Emulsions. The oxidative stability of CLA emulsions stabilized with GA, EM2, and EM10 was evaluated by measuring oxygen consumption using a Clark-type oxygen electrode. Figure 6A illustrates the kinetics of oxygen consumption of CLA emulsions stabilized with 5, 10, and 15% GA at 25 °C without exposure to light. A control experiment is also included, in which oxygen consumption of 5% GA solution without CLA oil phase was measured. The oxygen concentration in the control only slightly decreased with time, indicating negligible oxygen consumption. In

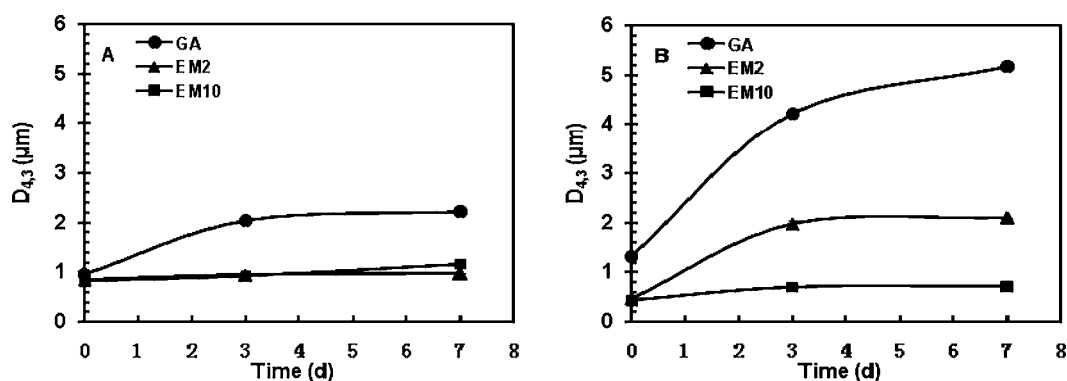


Figure 5. Comparison of the growth of volume-weighted mean diameter $D_{4,3}$ with storage time for CLA emulsions stabilized with GA, EM2, and EM10 at 60 °C. The gum concentrations are 5% (A) and 15% (B), respectively.

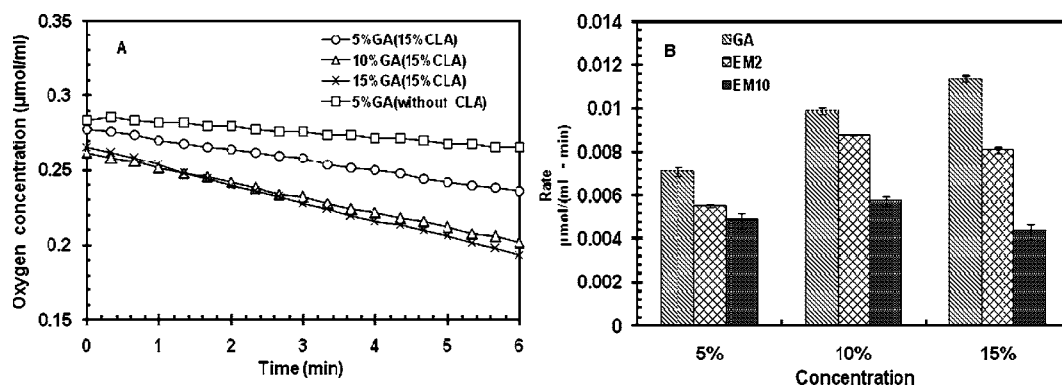


Figure 6. (A) Kinetics of oxygen consumption of CLA emulsions stabilized by GA at different concentrations at 25 °C without exposure to light. (B) Oxygen consumption rate of CLA emulsions stabilized by GA, EM2, and EM10 at different concentrations at 25 °C without exposure to light.

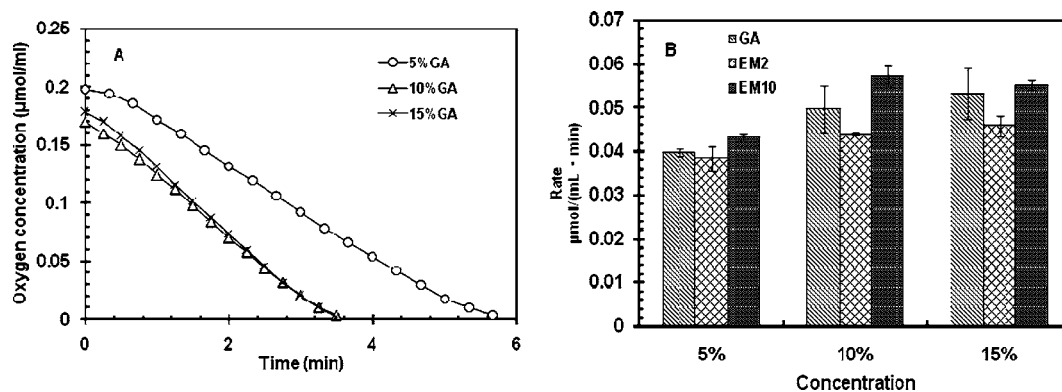


Figure 7. (A) Kinetics of oxygen consumption of CLA emulsions stabilized by GA at different concentrations at 50 °C without exposure to light. (B) Oxygen consumption rate of CLA emulsions stabilized by GA, EM2, and EM10 at different concentrations at 50 °C without exposure to light.

contrast, oxygen concentration in the CLA emulsions decreased significantly. This suggests that considerable oxygen consumption occurred in the CLA emulsions and should arise from the CLA oil phase. Thus, it could be presumed that the oxygen consumption was related to the CLA oxidation that consumed oxygen to form lipid peroxy radicals, in light of the oxidation mechanism reported previously.³⁶

Oxygen consumption rate was calculated from the slopes of the curves of oxygen concentration versus time. Figure 6B compares the oxygen consumption rate for CLA emulsions stabilized with different gums and at different concentrations at 25 °C. With the same type of gums, the oxygen consumption rate is relatively lower at a gum concentration of 5%. At the same gum concentrations, the oxygen consumption rate

decreased in the order GA > EM2 > EM10. Therefore, CLA oxidation was minimal for emulsions stabilized with EM10 and at 5% gum concentration. The optimal conditions for chemical stability seem to coincide with those for physical stability and could be explained from the following aspects: (1) a physically stable and fine emulsion is a prerequisite for the chemical stability of CLA; (2) a lower concentration of gums brings in intrinsic prooxidative metals at lower concentrations, that is, iron and copper (Table 2) and possibly hydroperoxides as well,³⁷ thus less promoting factors for lipid oxidation; (3) lipid oxidation in oil-in-water emulsions is highly dependent on the interaction between lipid hydroperoxides at the oil droplet surface and transition metals present in the aqueous phase, forming free radicals to attack unsaturated fatty acids to

propagate lipid oxidation.^{38,39} Thicker emulsion interfacial layers could provide better physical and chemical barriers for the interaction, retarding lipid oxidation more effectively.^{40–42} EM10 of higher AGP content is envisaged to better stabilize emulsion with thicker viscoelastic interfacial films,¹⁹ providing more effective protection against CLA oxidation.

Figure 7 shows the results for oxygen consumption measurements at an elevated temperature of 50 °C. Clearly the higher temperature promoted CLA oxidation. The oxygen consumption rates measured at 50 °C (Figure 7B) were much larger than the counterparts at 25 °C (Figure 6B). The results corroborate most previous studies on the temperature dependence of lipid oxidation.³⁶ Similar to the results at 25 °C, a gum of concentration of 5% was found to generally give a lower oxygen consumption rate. However, the dependence on gum types changed at 50 °C. The oxygen consumption rate at 50 °C followed the order EM10 > GA > EM2. This indicates that certain attributes associated with EM10 promoted lipid oxidation at higher temperatures. Possible assumptions are (1) EM10 contained slightly more iron and copper than GA and EM2 (Table 2), and the catalyzing effect of these ions for lipid oxidation overrode other factors at the higher temperature;³⁶ (2) gum arabic was found to be capable of binding free fatty acid, which was attributed largely to the hydrophobic interaction between AGP and free fatty acid.²⁷ The increased hydrophobic interaction at the higher temperature and particularly for the EM10 sample of higher AGP content perhaps could increase the activity of CLA at the oil–water interface. Studies showed that free fatty acids at the interface have the ability to promote the diffusion of oxygen into the oil phase and to attract prooxidative metals to accelerate lipid oxidation.⁴³ More surface-active CLA at the higher temperature and for the EM10 sample therefore possibly lead to an accelerated oxidation.

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Notes

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