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ORIGINAL PAPER

Association Colloids Formed by Multiple Surface Active Minor Components and Their Effect on Lipid Oxidation in Bulk Oil

Ketinun Kittipongpittaya · Atikorn Panya · Leqi Cui · David Julian McClements · Eric Andrew Decker

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Abstract Association colloids formed by surface active minor components play an important role in the oxidative stability of bulk oils. To imitate the formation of nanostructures in refined oils, multiple surface active minor components including phospholipids, free fatty acids, diacylglycerols and sterols were added to stripped corn oil. The critical micelle concentration (CMC) of the mixed components was determined. The impact of mixed minor components at below and above their CMC on oxidative stability of bulk oil and on antioxidant activity of α-tocopherol and Trolox was investigated. The CMC of the mixed surface active components in bulk oil was 20 μmol/kg oil in the presence of 383 ± 2 ppm of water. 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) played an important role on the formation of association colloids since it was the most important component in forming the association colloids as confirmed by CMC and fluorescence probe studies. The association colloids formed by the mixed components showed prooxidative activity in bulk oil as determined by monitoring the formation of lipid hydroperoxide and hexanal. The activity of α-tocopherol or Trolox was not changed by mixed components association colloids. These results suggest that association colloids both physically and chemically impacted the oxidative stability and activity of antioxidants in bulk oil.

Keywords Lipid oxidation · Bulk oil · Minor components · Critical micelle concentration · Reverse micelles

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Introduction

Association colloids are physical structures formed by surface active molecules which self-aggregate in non-polar systems such as bulk oils in the presence of small amounts of water (1). Bulk oils contain not only triacylglycerols, but also a variety of minor components such as free fatty acids, monoacylglycerols, diacylglycerols, phospholipids, sterols, and other polar lipids (2).These types of amphiphilic minor components have been reported to lower interfacial tension in bulk oils (1), suggesting that they are able to concentrate at oil–water interface and act as surfactants and co-surfactants. At concentration above their critical micelle concentrations (CMC), they will self-aggregate and form association colloids. For instance, diacylglycerol monolaurate and diacylglycerol monomyristate at concentration of 5–15 % (by wt) formed reverse rod-like micelles in olive oil [3]. The formation of phospholipid reverse micelles in bulk oil was also studied by using a small angle X-ray scattering technique [4].

These association colloids in bulk oil create oil–water interfaces which physically impact on lipid oxidation. The existence of oil–water interfaces could accelerate lipid oxidation since surface active lipid hydroperoxides and water soluble metal ions are able to migrate to the same location at the oil–water interfaces. This will promote metal promoted lipid hydroperoxides decomposition which leads

to increasing lipid oxidation rates. Moreover, the association colloids could impact the effectiveness of antioxidants since their activities are greatly dependent on the physical locations in heterogeneous food oils [5–7]. For example, the polar paradox theory states that nonpolar antioxidants work well in oil-in-water (O/W) emulsions, whereas, polar antioxidants work better in bulk oils [8]. The existence of physical structures in bulk oil could explain why polar antioxidants have greater efficiency in bulk oil rather than in O/W emulsion as they could localize not in the bulk oil phase but toward the oil–water interface of association colloids where oxidation is supposed to primarily occur [9].

The characteristics of surface active molecules can impact the physical properties of reverse micelles. For example, the curvatures and sizes of the reverse micelles are correspondent to the molecular properties and geometry of the surfactants [10, 11]. In addition, the interfacial properties such as charge and thickness will be dictated by the surface active molecules as has been widely reported in O/W emulsion systems [12]. Bulk oils contain a diversity of surface active minor components which could form complex multi-component association colloids in bulk oils. The physical structures formed by multiple surface active components in bulk oil could greatly impact the activity of both prooxidants and antioxidants and thus the oxidative stability of oil. There are only few studies on the impact of association colloids on antioxidant activity in bulk oil. These studies only use one or two surface active components to form association colloids [9, 13].

Therefore, in this research, we aimed to study the ability of multiple surface active components found naturally in refined oil including free fatty acids, diacylglycerols, phospholipids, and sterols to form association colloids by determining their surface activities and critical micelle concentrations in bulk stripped corn oil. In addition, we investigated the influence of association colloids formed by multiple surface active components on antioxidative activity of α-tocopherol and Trolox (a water soluble derivative of tocopherols) in bulk oil. This study could lead to a better understanding of the mechanisms underlying their antioxidant activity in real bulk oil systems, thus could provide knowledge of how to improve the oxidative stability of oil.

Materials and Methods

Materials

Corn oil was purchased from a local retail store and stored at 4 °C. 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) were acquired from Avanti Polar Lipids, Inc. (Alabaster, AL). N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt (NBD-PE, Cat.No. N-360) was acquired from Invitrogen. Silicic acid (100–200 mesh), activated charcoal (100–400 mesh), 7,7,8,8-tetracyanoquinodimethane (TCNQ), barium chloride, ammonium thiocyanate, iron (II) sulfate heptahydrate, oleic acid, 1,2-dioleoyl-*sn*-glycerol (DAG), stigmasterol, α-tocopherol and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox)were purchased from Sigma-Aldrich Co. (St. Louis, MO). Medium-chain triacylglycerols (MCT, Miglyol) was obtained from Sasol North America Inc. (Houston, TX). Chloroform and *n*-hexane (HPLC grade) were purchased from Fisher Scientific (Fair Lawn, NJ). Deionized water was used in all experiments. Glassware was submerged in 2 M HCl overnight to remove metals, followed by rinsing with deionized water before use.

Methods

Stripped Corn Oil Preparation

Stripped corn oil (SCO) was prepared using column chromatography according to Boon and coworkers (2008) [14]. Briefly, silicic acid (100 g) was washed three times with a total volume of 3 L of distilled water and dried at 110 °C for 20 h. A chromatographic column (3.0 cm internal diameter × 35 cm height) then was packed sequentially with 22.5 g of silicic acid, followed by 5.63 g of activated charcoal and another 22.5 g of silicic acid. Thirty grams of corn oil dissolved in 30 mL of *n*-hexane was passed through the column by eluting with 270 mL of *n*-hexane. The container used to collect the triacylglycerols was held in an ice bath and covered with aluminum foil to retard lipid oxidation during stripping. The solvent present in the stripped oils was removed with a vacuum rotary evaporator (RE 111 Buchi, Flawil, Switzerland) at 37 °C and traces of the remaining solvent were evaporated under a nitrogen stream. The water content of the oil was determined using the Karl Fisher Coulometer (756 KF Coulometer connected to 703 Ti Stand, Metrohm, Herisau, Switzerland). The stripped corn oil was kept at −80 °C for subsequent studies.

Interfacial Tension Measurement of Bulk Oil Containing Multiple Surface Active Components

The surface activity of minor components was determined by interfacial tensiometry using a drop shape analyzer (DSA100, Krüss GmbH, Hamburg, Germany). Minor components (1,000 μmol/kg oil) were mixed in bulk oil (a mixture of stripped corn oil and MCT at 1:3 ratio), which then was loaded into a syringe. A pendant drop of oil was form at the inverted tip of a hypodermic needle (with a diameter of 1.5 mm) that was submerged in double distilled water at room temperature. The tip of the needle was positioned on

an optical bench between a light source and a high speed charge coupled device (CCD) camera. The CCD camera was connected to a video frame-grabber board to record the image onto the hard drive of a computer at a speed of one frame per 1 min. The shape of the pendent drops was determined through numerical analysis of the entire drop shape using the drop shape analysis program supplied by the instrument's manufacturer. The interfacial tension was calculated from the drop shape using the Young–Laplace equation of capillarity [15].This methodology requires accurate determination of solution densities, which were measured using a digital density meter (DMA 35°N; Anton Paar USA, Ashland, VA). The density of bulk oil was 0.9366 g/cm^3 at 20 °C. All interfacial tension measurements were carried out after 10 min.

Determination of the Critical Micelle Concentration of Multiple Surface Active Components in Bulk Oil

The critical micelle concentrations (CMC) of multiple surface active lipids in bulk oils were determined by using the TCNQ solubilization technique [16]. Briefly, the bulk oil was prepared from a mixture of MCT and SCO (3:1, by wt; MCT was used as a non-oxidizable lipid). The CMC of DOPC, DOPE, stigmasterol, oleic acid, and DAG and their mixture at a molar ratio of 3.78:0.67:0.97:0.43:2.25, respectively was determined at concentrations ranging from 1 to 1,000 μmol/kg oil in bulk oil. The ratios of the minor components are similar to those found in refined corn oil [1, 17]. The oil containing surface active components was magnetically stirred for 12 h in a 55 °C incubator room prior to adding 5 mg of TCNQ/5 g oil and mixing for another 5 h. The excess TCNQ was removed by centrifugation at 2,000 *g* for 20 min. The absorbance was measured at 480 nm using a spectrophotometer (Shimadzu 2014, Tokyo, Japan). The CMC was determined as the inflexion point in the semi-log plot of absorbance *versus* surface active lipid concentration [18].

Fluorescence Measurement of Bulk Oil Containing Reverse Micelles and Antioxidants

The surface active fluorescent probe, NBD-PE, isa phospholipid analogue comprised of a fluorescent functional group covalently attached to the choline headgroup. It was incorporated into bulk oil and used to study the surface activity of minor components. The mixture of DOPC, DOPE, stigmasterol, oleic acid, and DAG (molar ratio of 3.78:0.67:0.97:0.43:2.25) in chloroform was added to bulk oil at 100 μmol/kg oil. For antioxidants study, α-tocopherol and Trolox were added into the oil at the same time as mixed components. Chloroform was removed by evaporation under nitrogen at room temperature. The samples were magnetically stirred at the speed of 1,000 rpm for 12 h. Then, NBD-PE was added at concentration of 0.95 μM, this concentration minimized self-quenching by the probe [19]. The samples were stirred for another 5 h in the dark. Steady-state emission spectra of NBD-PE were collected at 22 °C using a PTI spectrofluorometer (PTI, Ontario, Canada). To minimize any reflection of the excitation beam by the cell window and by the underlying liquid surface of the sample into the emission monochromator, measurements were conducted in triangular Suprasil® cuvettes. The emission was observed at 90° to the incident beam, that is, 22.5° with respect to the illuminated cell surface. A 2.0-nm spectral band width for both excitation and emission slits was employed for the NBD-PE excitation at 468 nm. The integration time was 1 s, and the wavelength increment during emission spectrum scanning was 1 nm. The intensity of the spectra were determined as the emission signal intensity (counts per second) measured by means of a photomultiplier.

Samples Preparation for Oxidation Study

Mixtures of surface active minor components including DOPC, DOPE, stigmasterol, oleic acid, and DAG was added to the bulk oil as described above at concentrations below (10 μmol/kg oil) and above (100 μmol/kg oil) the CMC. The samples were magnetically stirred at the speed of 1,000 rpm in a 55 °C incubator room for 12 h. To study antioxidant activity, α-tocopherol and Trolox were added at 10 and 50 μmol/kg oil along with mixed surface active components. Samples (1 mL) were aliquoted into 10 mL GC headspace vials (Supelco), capped with aluminum lids having PTFE/silicone septa and stored at 55 °C in the dark.

Determination of Lipid Oxidation Products in Bulk Oil

Lipid hydroperoxides and hexanal were determined as primary and secondary lipid oxidation products, respectively. The concentration of lipid hydroperoxides and hexanal formation were plotted against time in days. The lag phase which is defined as the time as the first data points that were statistically greater than time 0 values were used to compare the oxidative stability of oils.

Lipid Hydroperoxides Measurement

Lipid hydroperoxides were measured using a method adapted from Shanta and Decker [20]. The bulk oil samples (20 μL) were weighed and dissolved in 2.8 mL of methanol/butanol solution (2:1, v/v). A mixture of 15 μL of 3.94 M ammonium thiocyanate and 15 μL of 0.072 M ferrous solution was used as an indicator. The ferrous solution was obtained from the supernatant of a mixture of one part

of 0.144 M $FeSO_4$ and one part of 0.132 M $BaCl_2$ in 0.4 M HCl. After 20 min of incubation at room temperature, the absorbance of the samples was measured at 510 nm using a spectrophotometer (Genesys 20, Thermospectronic, Waltham, MA). The concentration of hydroperoxides was calculated from a cumene hydroperoxide standard curve.

Headspace Hexanal Measurement

Headspace hexanal was measured using a GC-17A Shimadzu gas chromatograph equipped with an AOC-5000 autosampler (Shimadzu, Kyoto, Japan) [14]. Samples (1 mL) in 10-mL glass vials capped with aluminum caps with PTFE/silicone septa were preheated at 55 °C for 8 min in an autosampler heating block. A solid-phase microextraction (SPME) fiber needle (50/30 µm DVB/Carboxen/PDMS, Supelco, Bellefonte, PA) was injected into the vial for 2 min to absorb volatiles and then was transferred to the injector port (250 °C) for 3 min. The injection port was operated in split mode, and the split ratio was set at 1:5. Volatiles were separated on an Equity-1 column (30 m × 0.32 mm × 1 µm film thickness, Supelco, Bellefonte, PA) at 65 °C for 10 min. The carrier gas was helium set at a flow rate of 15 mL/min. A flame ionization detector was used at a temperature of 250 °C. Hexanal concentrations were determined from peak areas using a hexanal standard curve.

Statistical Analysis

All experiments were conducted in triplicate. Data were presented as means ± standard deviations. Data results were analyzed by analysis of variance (ANOVA) using SPSS 14.0 (SPSS Inc., Chicago, IL). The differences between mean values were compared using Duncan's multiple-range test with significance defined as $p \leq 0.05$.

Results and Discussion

The Surface Activity and Critical Micelle Concentration of Multiple Surface Active Components in Bulk Stripped Corn Oil

Minor components are naturally present in bulk oils and also are generated during refining and storage due to the enzymatic and non-enzymatic hydrolysis, oxidation and thermal degradation reactions [2, 21, 22]. Unlike triacylglycerols, many of these component scan act as amphiphilic surfactants as they contain both hydrophilic and hydrophobic functional groups on their structures. Surface activity of minor components at the oil–water interface can be investigated by measuring interfacial tension (water–oil interface). In this study, we determined interfacial tension of bulk oil containing each minor component including oleic acid, DAG, stigmasterol, DOPC or DOPE at concentration of 1,000 µmol/kg oil.As shown in Fig. 1, all minor components significantly decreased the interfacial tension of bulk oil, suggesting that they were able to concentrate at the oil–water interface and reduce the interfacial free energy [23]. Phospholipids including DOPC and DOPE show relatively strong surface activity compared to oleic acid, DAG, and stigmasterol, by reducing the interfacial tension of the control oil from 22.6 to 9.9 and 10.3 mN/m, respectively. The interfacial tensions of bulk oils containing oleic acid, DAG and stigmasterol were 18.2, 15.8, and 18.5 mN/m, respectively. As reported in several studies, the interfacial tension of the bulk commercial oil is lower than stripped oil due to the presence of surface active compounds and interfacial tension decreases during the frying process due to the generation of surface active components by hydrolysis, oxidation and thermal degradation reactions [1, 24, 25].Gil and Hendel (1995) found that phospholipid at concentration

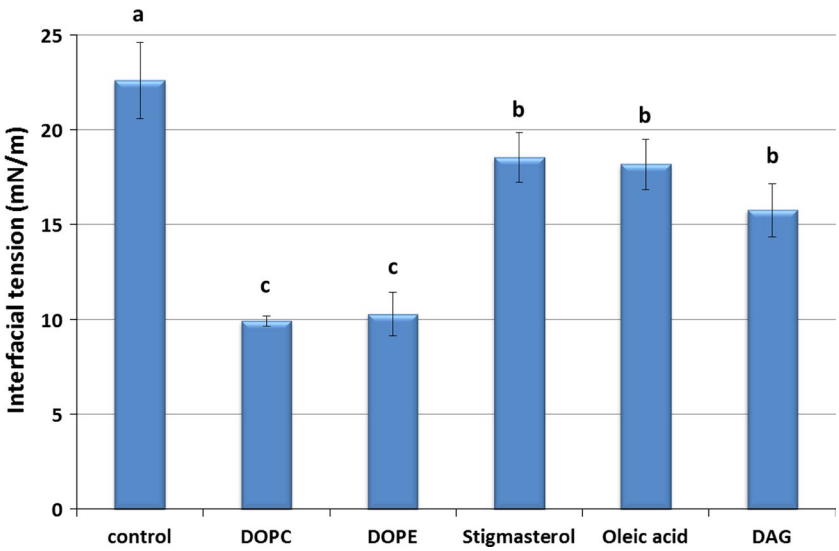

Fig. 1 Interfacial tension of bulk oil containing DOPC, DOPE, stigmasterol, oleic acid, or DAG at 1,000 µmol/kg oil. [a,b,c] Represent significant differences at $p \leq 0.05$

0.2 % w/w lowered the interfacial tension of the soybean oil/water by 42 %, while the addition of DAG and free fatty acid up to 0.1 % w/w did not have impact on the interfacial tension values [25]. However, there are reports suggesting that the presence of DAG and free fatty acids significantly decreased the oil–water interfacial tension [24, 26]. Cercaci and co-workers (2006) found that stigmasterol decreased the interfacial tension of hexadecane/water with increasing concentration of stigmasterol [27].

In the presence of trace amount of water in bulk oils, surface active components tend to aggregate so that the hydrophilic head groups orient toward the water core in order to minimize contact between the hydrophobic environment in bulk oil and the hydrophilic head groups. The concentration at which the surface active molecules start aggregating is defined as a critical micelle concentration (CMC) [28]. In this study, we determined the CMC of surface active minor components by using the TCNQ technique. The absorbance of TCNQ sharply increases upon formation of micelles due to the charge transfer of the TCNQ in the presence of aggregates in the system [16]. The water content in the bulk oil that we used in this study was 383 ± 2 ppm. The CMC of each minor component including DOPC, DOPE, stigmasterol, oleic acid, and DAG was determined by varying the concentration ranging from 1 to 1,000 μmol/kg oil in bulk oil. Figure 2 shows that the CMC of DOPC and DOPE at 55 °C were 40 and 200 μmol/kg oil, respectively. Considering the chemical structures of DOPC and DOPE, it is not surprising that the DOPC had lower CMC than the DOPE since the choline head group of DOPC has higher polarity compared to the ethanolamine group of DOPE, thus this facilitates the DOPC to reside at the oil–water interface better than the DOPE [29]. Moreover, the CMC of phospholipids depends on their degree of hydration. DOPC with higher hydration index of 100 was reported to have lower CMC than the DOPE which possesses the hydration index of 16 [30]. We did not observe a typical CMC inflexion point in the absorbance plots of the oils containing oleic acid, DAG, and stigmasterol in the range of concentrations studied. Our results suggest that although oleic acid, DAG, and stigmasterol were able to concentrate at the oil–water interface according to the interfacial tension results (Fig. 1), they did not form aggregates at the concentrations used in this study.

Furthermore, we investigated the CMC of the mixture of DOPC, DOPE, stigmasterol, oleic acid, and DAG at constant molar ratio of 3.78:0.67:0.97:0.43:2.25, respectively. This ratio was meant to imitate the diverse composition of the major surface active compounds in commercial refined bulk oil [1, 17].Moreover, each component was removed one at a time in order to understand how each component impacts the ability of other surface active molecules to form aggregates. As shown in Fig. 3, the mixed components were able to form association colloids at a concentration of 20 μmol/kg oil at 55 °C which is lower than DOPC alone (Fig. 2).The removal of a component from the mixture did not significantly impact the CMC value with the exception of DOPC as its removal resulted in no formation of association colloids as determined by the TCNQ method. Although, the mixed components without DOPC still contained the DOPE which can form association colloids, the concentration of DOPE in the mixed components was 155 μmol/kg oil which was lower than its CMC (200 μmol/kg oil), thus they did not form aggregates. This

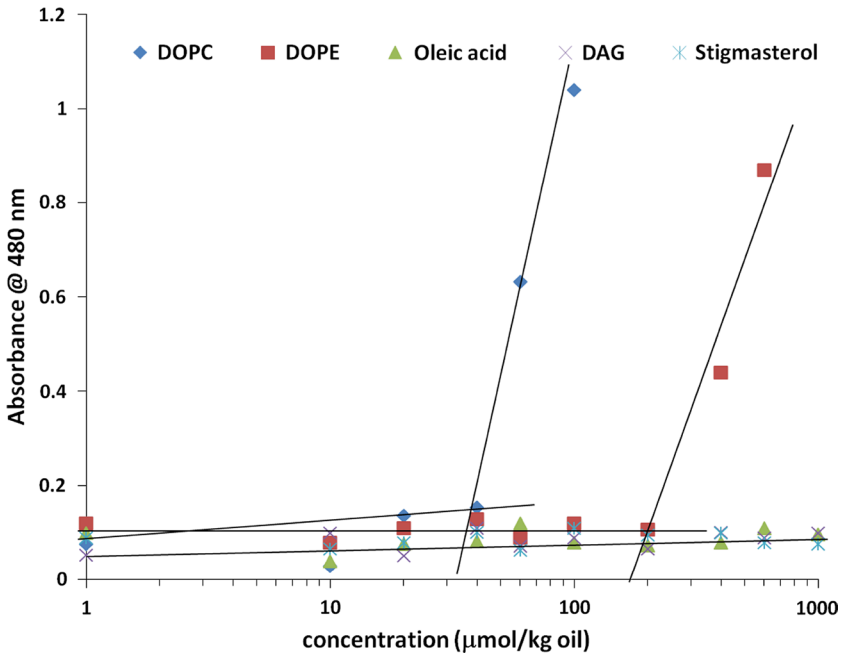

Fig. 2 Critical micelle concentrations of DOPC, DOPE, oleic acid, DAG and stigmasterol in bulk oil at 55 °C. Data points represent means (n = 3) ± standard deviations. Some error bars lie within data points

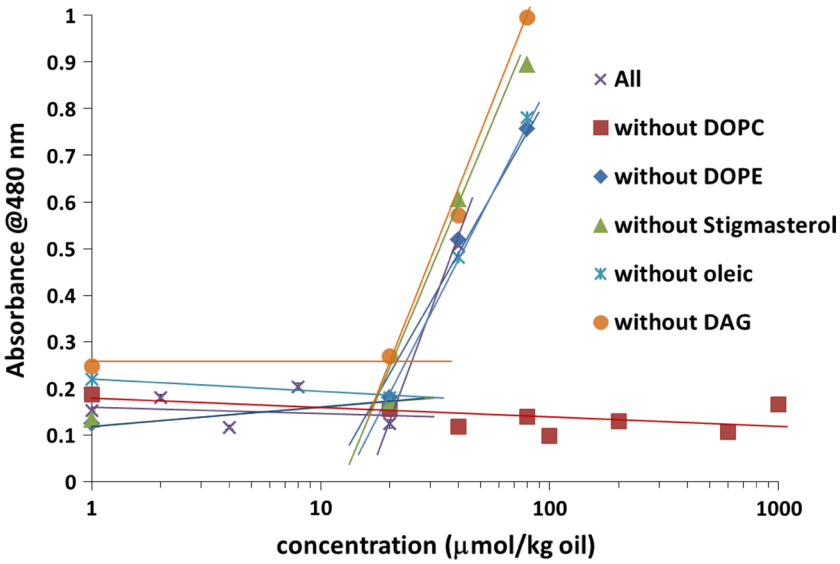

Fig. 3 Critical micelle concentrations of mixed components of DOPC, DOPE, stigmasterol, oleic acid, and DAG (at molar ratio of 3.78:0.67:0.97:0.43:2.25, respectively) in bulk oil at 55° C. Data points represent means (n = 3) ± standard deviations. Some error bars lie within data points

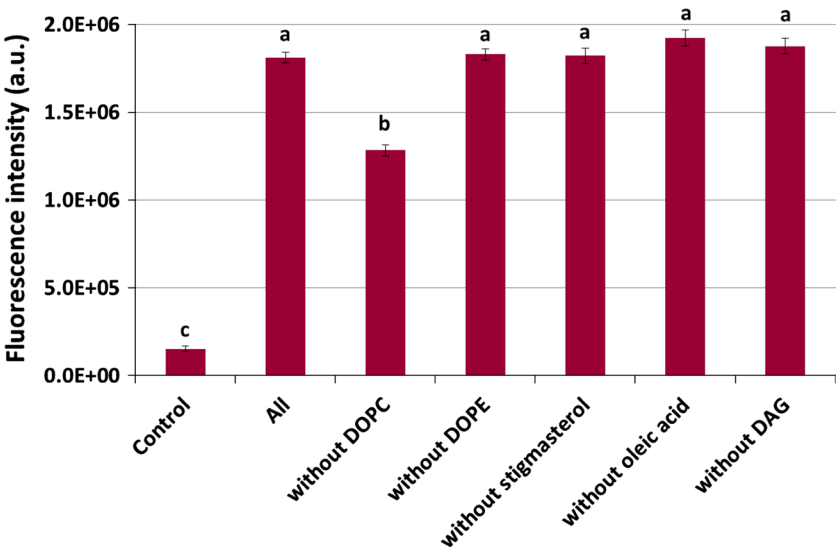

Fig. 4 Fluorescence intensity of NBD-PE in bulk oil containing mixed components of DOPC, DOPE, stigmasterol, oleic acid, and DAG (at molar ratio of 3.78:0.67:0.97:0.43:2.25, respectively) at 100 μmol/kg oil. [a,b,c] Represent significantly different at $p \leq 0.05$

suggests that DOPC plays an important role as a major surfactant responsible for association colloid formation at the concentration of surface active compounds typically found in refined oil. The other components likely acted as co-surfactants since the CMC of the mixed systems was lower than DOPC alone.

To further investigate the surface activity of multiple components at the oil–water interface, we used NBD-PE, which is a fluorescent phosphatidylethanolamine analog grafted with an NBD fluorophore on the head group. The emission fluorescence intensity of NBP-PE was determined and shown in Fig. 4. In the control oil containing only the NBD-PE, the emission fluorescent intensity was relatively low. It has been reported that the exposure of the NBD group to the polar environment caused the fluorescence intensity to decrease [31, 32]. The NBD-PE, same as other phospholipids, preferentially resides at the oil–water interface and orients the hydrophilic head group toward the water core. The imino group and/or the oxygen molecule on the NBD could form H-bonds with water molecules, leading to a decrease in fluorescence intensity [33]. Since the stripped oil contained 383 ± 2 ppm water, it is possible that the probe associated with this water thus decreasing its fluorescence. The addition of 100 μmol/kg oil of mixed components (above the CMC so association colloids were present) caused the fluorescent intensity of NBP-PE to increase. This could be due to the ability of mixed surface active components to compete for the oil water-interface, thus decreasing the NBD-PE/water interaction, and thus leading to the fluorescence intensity increase. This result is in agreement with Chen and coworkers (2011) who observed that the emission fluorescence intensity of

NBD-PE increased in the presence of DOPC in bulk soybean oil [9]. In addition, Chattopadhyay and coworker (2002) found that the fluorescence emission intensity of the NBD-PE increased with decreasing [water]/[surfactant] molar ratio [19].

Compared to the presence of all mixed components, the absence of DOPE, stigmasterol, oleic acid, or DAG did not change the fluorescent intensity of NBP-PE. This is because all these mixtures form association colloids (Fig. 3). However, in bulk oil containing mixed components without DOPC, the fluorescence intensity significantly decreased compared to the oil that contains all mixed components. Again, this supports the CMC results that DOPC is critical in the formation of the association colloids. However, in the absence of DOPC, the fluorescence intensity was still greater than the control even though there were no association colloids. In the absence of DOPC, the DOPE, stigmasterol, oleic acid and DAG could interact with the water without forming association colloids since they are all surface active. The interaction of these compounds with water could prevent the NBD-PE probe from interacting with water thus increasing fluorescence intensity. The ability of the other surface active compounds to out compete NBE-PE for water could be due to their higher surface activity or the much higher concentration (0.95 μmol/kg oil of NBD-PE compared to 100 μmol/kg oil of mixed components).

The Impact of the Association Colloids Formed by Multiple Surface Active Components on Oxidative Stability of Bulk Oil

Lipid oxidation is a major factor causing undesirable flavors and aromas and reducing nutritional values as well as potential safety issues in food oils [29, 34–37]. Minor components that have amphiphilic properties in bulk oil chemically and physically impact lipid oxidation mechanisms [1, 2]. In this study, we investigated the effect of association colloids formed by multiple surface active components on oxidative stability of bulk corn oil. Moreover, the impact of association colloids on antioxidant activity of α-tocopherol (lipid soluble)and Trolox (water soluble analogue of tocopherol) was investigated by measuring the formation of lipid hydroperoxides and hexanal during storage at 55 °C. Figure 5 shows that the addition of mixed components at concentration of 10 μmol/kg oil, which is below the CMC did not appreciably change the lag time of lipid hydroperoxides and hexanal formation compared to the control. However, once multiple components were added at 100 μmol/kg oil, which is above the CMC, the lag time of both lipid hydroperoxides and hexanal formation decreased from 15 to 11 days. This is in consistent with previous reports which revealed that the physical structures formed by surface active components decreased the oxidative stability of bulk oil [4, 38, 39]. Several mechanisms possibly involve in the prooxidative effect of physical structures in bulk oil. For example, the presence of aggregates could reduce the surface tension leading to increasing of oxygen transfer to the oil [29].Chen and coworkers [2012] proposed that the reverse micelles form by DOPC reduced the oxidative stability of oil by attracting the metal ions and lipid hydroperoxides to the oil–water interface resulting in increased lipid oxidation rates [39].

The addition of α-tocopherol at 10 and 50 μmol/kg oil extended the lag time of both lipid hydroperoxides and hexanal formation to 19 and 37 days, respectively, suggesting that α-tocopherol at these concentrations was able to overcome the prooxidant activity of association colloids in this study. We did not observe any impact of the physical structures formed by mixed components on the antioxidative effectiveness of α-tocopherol at both concentrations.

A similar experiment was done to investigate the impact of mixed components on the effectiveness of Trolox as shown in Fig. 6. The addition of Trolox at concentrations of 10 and 50 μmol/kg oil extended the duration of the lag phase of both hydroperoxide and hexanal formation from 15 to 55 and 105 days, respectively. The better antioxidant activity of Trolox compared to tocopherols is usually observed and is postulated to be the higher polarity of Trolox which allows them to concentrate at the oil–water interface, thus to interact more efficiently with surface active lipid substrates [7, 40, 41].Again, the oils containing the combination of Trolox (10 and 50 μmol/kg oil) and mixed components at below and above the CMC had similar lag time compared to the oil containing only Trolox at both concentrations.

To investigate if the association colloids impact the physical location of α-tocopherol and Trolox, we incorporated the NBD-PE probe to the bulk oil containing 100 μmol/kg oil of mixed components of DOPC, DOPE, stigmasterol, oleic acid, and DAG in the presence of either α-tocopherol or Trolox at various concentrations (0, 10, 50 and 100 μmol/kg oil). The emission fluorescence intensity of NBD-PE was measured and is shown in Fig. 7.

Increasing the concentration of α-tocopherol did not impact the fluorescence intensity of NBD-PE, suggesting that α-tocopherol was unlikely to concentrate at the oil–water interface. On the other hand, Trolox caused the fluorescence intensity to decrease with increasing Trolox concentrations. Trolox was not as surface active compared to other surface active components (e.g., at 1,000 μmol/kg oil, Trolox only decreased the interfacial tension of oil–water by 30 % while DOPC decreased the interfacial tension by 56 %). Therefore, instead of competing for the oil–water interface as other surface active compounds did, Trolox could partition into the same location as the NBD-PE at the oil–water interface of the association colloids, thus it could

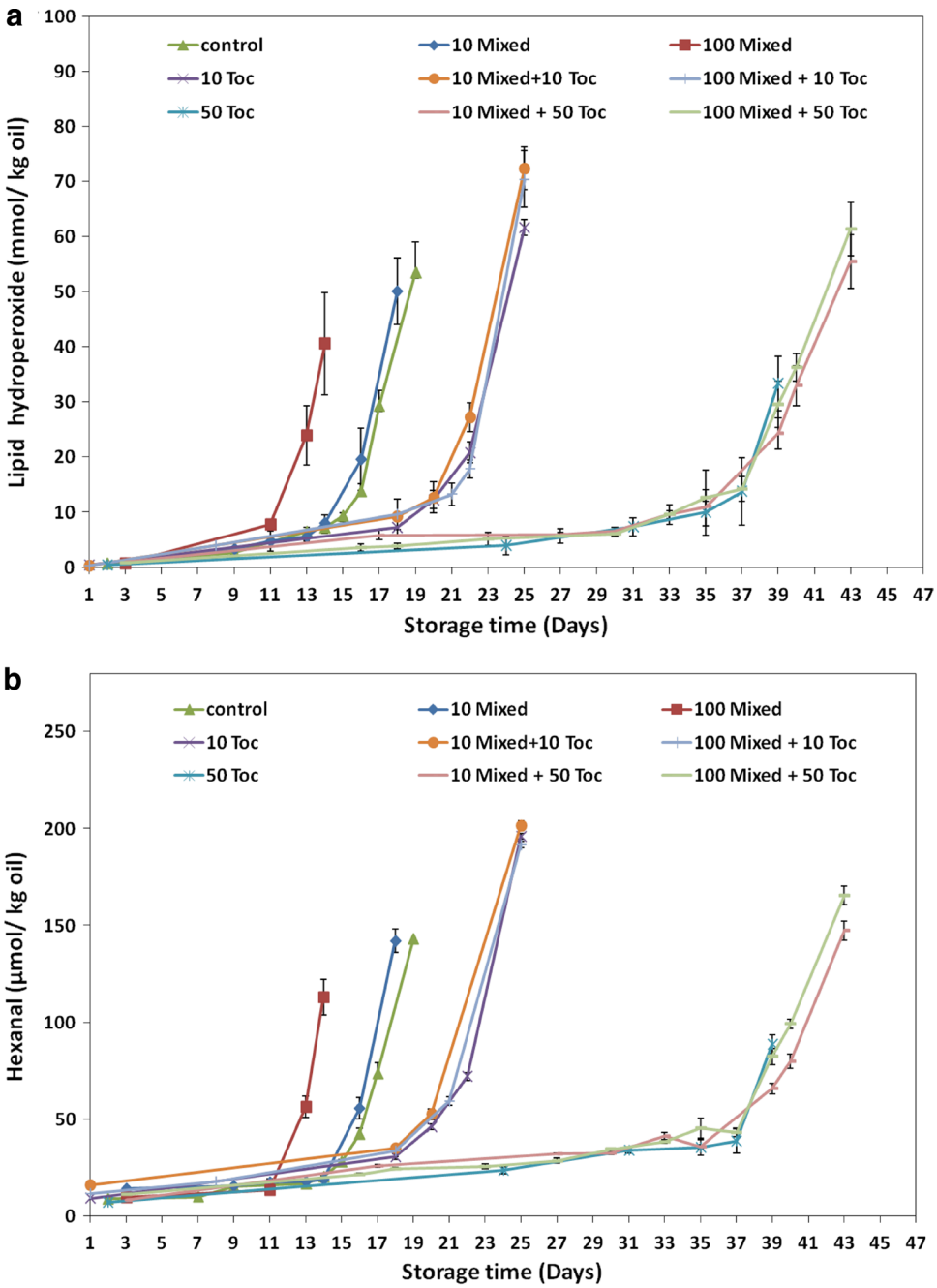

Fig. 5 Formation of lipid hydroperoxides (**a**) and hexanal (**b**) in bulk oil containing mixed components of DOPC, DOPE, stigmasterol, oleic acid, and DAG (at molar ratio of 3.78:0.67:0.97:0.43:2.25, respectively) at 0, 10 and 100 µmol/kg oil in the presence of α-tocopherol at 0, 10 and 50 µmol/kg oil during storage at 55 °C

quench the NBD-PE leading to the observed decrease in fluorescence intensity. This is in agreement with Chen and coworkers (2011) who reported that the fluorescence intensity of NBD-PE decreased as increasing the concentration of Trolox in the bulk oil containing DOPC reverse micelles [9].

Surprisingly, we did not observe that the association colloids influenced the antioxidative activity of both µ-tocopherol and Trolox. In contrast, several studies demonstrated that the physical structures formed by phospholipids enhanced the antioxidant activity of tocopherols and Trolox by facilitating the antioxidant molecules to accumulate at the oil–water where lipid oxidation mainly occurs [9, 42] or by decreasing the iron-promoted tocopherol and Trolox decomposition [39]. Nevertheless, the impact of reverse micelles on the activity of antioxidants could be dependent on several factors including the concentration of antioxidant in the system and the physical and chemical properties of the reverse micelles. Chen and coworkers (2011) found that the effect of DOPC reverse micelles on the activity of antioxidants was varied depending on the antioxidant concentration. They revealed that DOPC

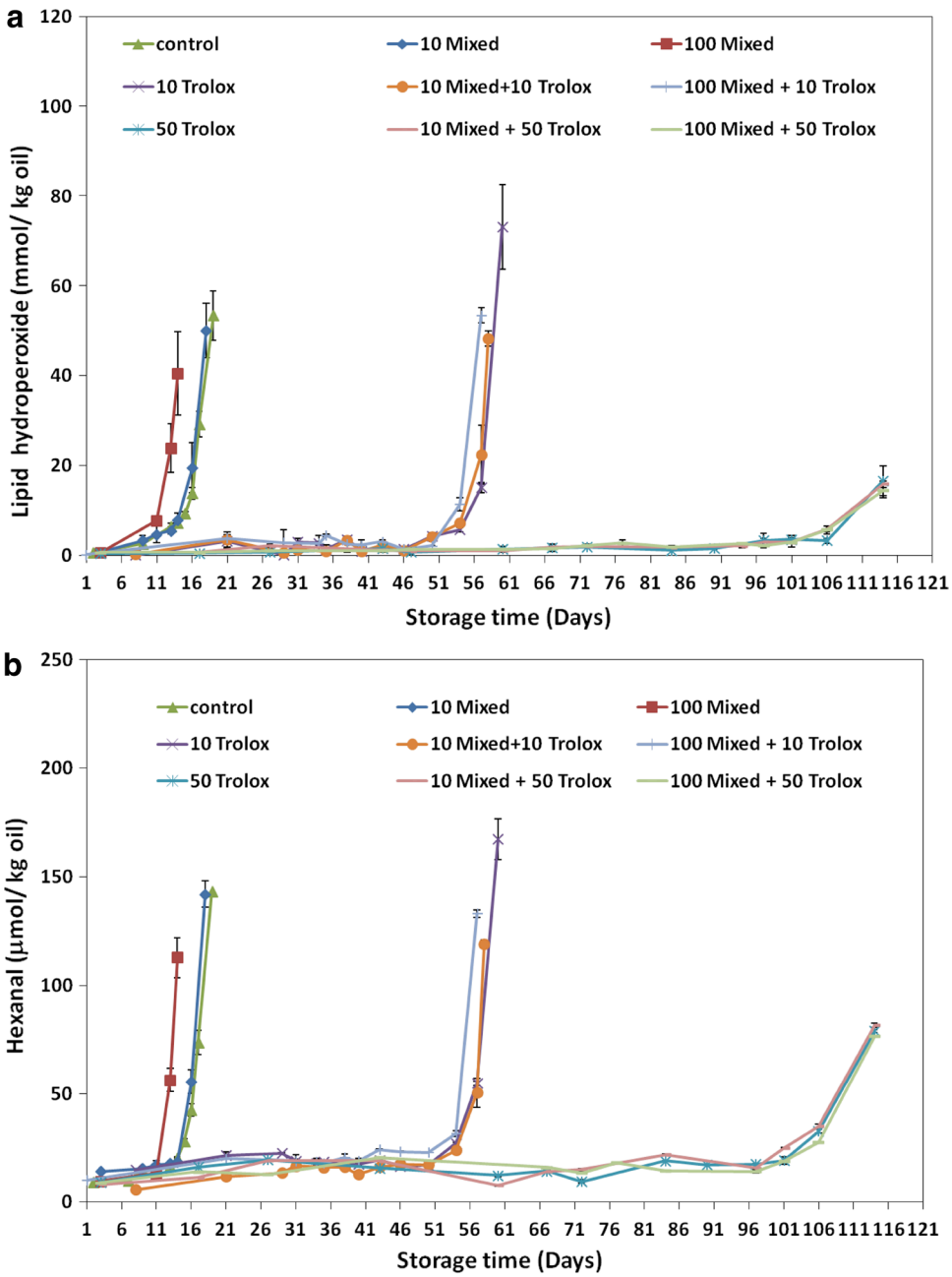

Fig. 6 Formation of lipid hydroperoxides (**a**) and hexanal (**b**) in bulk oil containing mixed components of DOPC, DOPE, stigmasterol, oleic acid, and DAG (at molar ratio of 3.78:0.67:0.97:0.43:2.25, respectively) at 0, 10 and 100 µmol/kg oil in the presence of Trolox at 0, 10 and 50 µmol/kg oil during storage at 55 °C

reverse micelles enhanced the antioxidant effectiveness of low concentrations of α-tocopherol and Trolox (10 µM), while they decreased the antioxidant activity of 100µM of α-tocopherol and Trolox [9].

Moreover, the composition of surface active molecules could affect the physical and chemical properties of the interface. The accessibility of antioxidant to the interface could be governed by the surfactant packing in the association colloid. For example, the surface active molecules could closely pack in the association colloids, thus, limited the accessibility of antioxidant molecules to the interface. This could occur with the mixed components system but not with the DOPC alone. In addition, the individual component of DOPC, DOPE, stigmasterol, oleic acid, and DAG have been reported to affect the oxidative stability of bulk oil differently. Phospholipids exhibit antioxidant activity which is attributed to different mechanisms including the metal chelating property of phosphate group, free radical scavenging ability of the amine group, the formation of Maillard reaction products between phospholipids and oxidation products [43, 44]. Free fatty acids are known as prooxidant which is attributed to their carboxylic groups that accelerate the decomposition of lipid hydroperoxides into free radicals or to their ability to bind metals and make them more

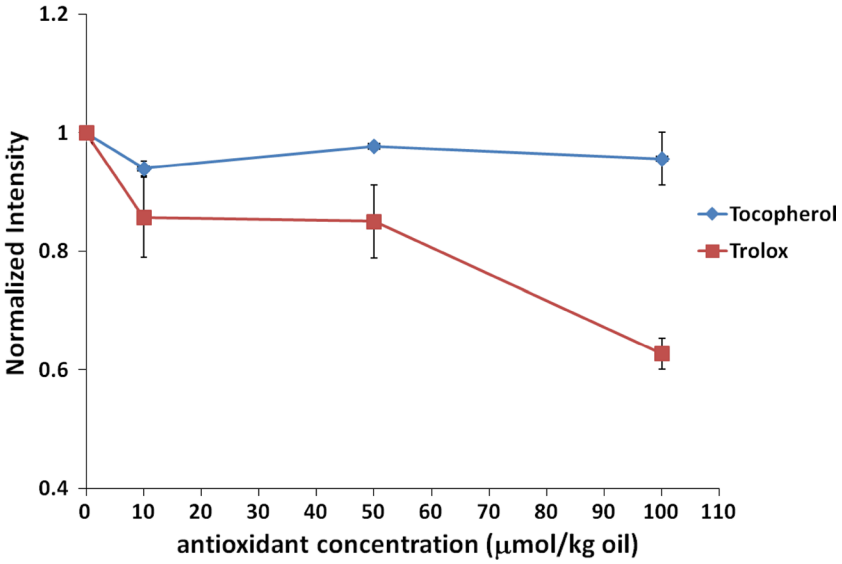

Fig. 7 The normalized fluorescence intensity of NBD-PE in the bulk oil containing 100 μmol/kg oil of mixed components of DOPC, DOPE, stigmasterol, oleic acid, and DAG (at molar ratio of 3.78:0.67:0.97:0.43:2.25, respectively) in the presence of either α-tocopherol or Trolox at 0, 10, 50 and 100 μmol/kg oil

prooxidative [45–47].Prooxidative, antioxidative and neutral effects of DAG have been observed in several studies [13, 48, 49]. Chen and coworkers (2014) revealed that the addition of 0–2.5 % (by wt) of DAG did not significantly impact the oxidative stability of stripped soybean oil incubated at 55 °C and had no effect on the antioxidative activity of 40 μM α-tocopherol [13]. Phytosterols such as stigmasterol exhibited antioxidant activity under high temperature. This is attributed to their ability to donate hydrogen to free radicals, and also to their ability to decrease polymerization under high temperature [50–53]. Thus, the combination of these components not only physically but also chemically impact on the oxidative stability of bulk oil and all these factors together might negate enhancement of the activity of the antioxidants that were observed in other studies.

Conclusions

By determining the interfacial tension of minor components including DOPC, DOPE, stigmasterol, oleic acid, and DAG in bulk oil, we demonstrated that these surface active components were able to concentrate at the oil–water interface in bulk oil and decreased interfacial tension. Among other components, only DOPC and DOPE could form aggregates individually at the CMC of 40 and 200 μmol/kg oil, respectively. The combination of minor components formed association colloids at the CMC as low as 20 μmol/kg oil. The association colloids formed by the mixed components significantly decreased the oxidative stability of bulk stripped corn oil. However, these physical structures did not have an impact on the antioxidative effectiveness of tocopherols and Trolox at 10 and 50 μmol/kg oil. Understanding how these complex structures impact on lipid oxidation and on reactivity of antioxidants could provide a new perspective to improve oxidative stability in bulk oils.

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