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ORIGINAL PAPER

The Influence of Secondary Emulsifiers on Lipid Oxidation within Sodium Caseinate-Stabilized Oil-in-Water Emulsions

Amy Richards · Matt Golding · Chakra Wijesundera · Leif Lundin

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Abstract The effect of protein displacement at the interface by a secondary emulsifier on the oxidative stability of sodium caseinate-stabilized tuna oil-in-water emulsion systems was determined. Emulsions were prepared with a selection of anionic and non-ionic emulsifiers and stored at both 25 and 50 °C with no added prooxidant, and at 4 °C in the presence of ferrous sulfate. The progress of oxidation during storage was monitored through solid phase microextraction headspace analysis. Metal ion catalyzed oxidation was enhanced for the emulsions stabilized with an anionic emulsifier in comparison to emulsion systems stabilized with non-ionic emulsifiers and sodium caseinate alone. The increased oxidation observed for the emulsion with the anionic surfactant is due to electrostatic interactions between divalent metal ions and the negatively charged surfactant at the oil-water interface. The sodium caseinate interfacial layer had little prooxidant effect at the droplet surface, most likely due to the ability of free protein molecules in solution to sequester metal ions, which may have provided some protection against oxidative deterioration.

Keywords Auto-oxidation · Emulsifier · Headspace SPME analysis · Oil-in-water emulsion · Sodium caseinate · Tuna oil

A. Richards (✉) · M. Golding · C. Wijesundera · L. Lundin
CSIRO Food Futures National Research Flagship and CSIRO Food and Nutritional Sciences, 671 Sneydes Road, Werribee, VIC 3030, Australia
e-mail: amy.richards@csiro.au

M. Golding
Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand

Abbreviations

Tween40	Polyoxyethylenesorbitan monopalmitate
SSL	Sodium stearoyl lactylate
MGP	Monoglycerol palmitate
PGE	Polyglycerol ester 55-M
SPME	Solid phase microextraction
GC-FID	Gas chromatography-flame ionization detector
w/w	Weight per weight
TAG	Triacylglycerol
FA	Fatty acids
FAMEs	Fatty acid methyl esters
DHA, 22:6n-3	Docosahexaenoic acid
16:0	Palmitic acid
EPA, 20:5n-3	Eicosapentaenoic acid
18:1n-9	Oleic acid

Introduction

The deterioration of fats and oils can impact on both the sensory characteristics, such as odor and flavor, and the nutritional value of foods. The rate at which oxidative deterioration takes place is dependent upon the storage temperature, the presence of pro- or antioxidants, the chemical characteristics of the fat or oil, and how it is dispersed within the food product [1–3]. Oils are emulsified into oil-in-water emulsion systems within a variety of different products such as milk, mayonnaise, yoghurts and salad dressings. Such emulsions are dispersions of oil droplets within a continuous water phase where the emulsifier is located at the interface and facilitates emulsification through decreasing the interfacial tension of the droplet [3].

Sodium caseinate is commonly used in the food industry to rapidly establish thick, sterically stabilized layers in order to protect the oil droplets against flocculation and coalescence [4]. These tend to be more stable to physical deterioration when compared to those stabilized by whey protein, another dairy protein commonly used in the preparation of emulsion systems. This has been attributed to the physical characteristics of casein, which tends to be more heat stable compared to globular proteins and therefore less likely to undergo conformational changes in structure when a high temperature is applied [4–6]. In addition to physical stabilization, the relatively thick interfacial layer formed by sodium caseinate has been shown to provide a barrier protecting the oil phase from auto-oxidation [1, 3].

Sodium caseinate is also known to protect against auto-oxidation due to its iron chelating properties, where free protein molecules sequester metal ions from within the serum phase [7]. Studies have shown transition metal ion-based catalysis to be the primary cause of lipid oxidation in food emulsions [1]. Transition metals, such as Fe(II), will promote hydroperoxide degradation when located at the emulsion interface because of electrostatic interactions between the droplet surface and the charged metal ion. However, this effect on oxidative stability could be reduced by the presence of bulky proteins or surfactants at the droplet interface [1, 8].

The objective of this study was to observe differences in oxidative stability within binary 10%w/w tuna oil-in-water emulsion systems stabilized with sodium caseinate and a secondary emulsifier at three different temperatures. This is of importance to the food industry as small emulsifiers are commonly used in conjunction with protein to stabilize emulsified food products.

Materials and Methods

Materials

Tuna oil 'HiDHA^{TM} 25S5' was purchased from Nu-Mega Ingredients, Australia. Sodium stearoyl lactylate '45 VEG' (SSL), monoglyceride palmitate 'Dimodan HP-M' (MGP), and polyglycerol ester 'Grindsted PGE 55-M' (PGE) were purchased from Danisco, Australia. Polyoxyethylenesorbitan monopalmitate (Tween40) and sodium azide were purchased from Sigma, Australia. Commercial sodium caseinate was purchased from Fonterra Co-operative, New Zealand.

Preparation of Sodium Caseinate Protein Solution

Sodium caseinate was dispersed in deionized water with stirring for 1 h at room temperature to make a 0.75%w/w solution. The solution was allowed to hydrate at room temperature for a further 3 h and stored at 4 °C overnight for use the following day.

Preparation of Emulsions

Tuna oil was removed from storage (−22 °C) and melted in a water bath at 70 °C. For Tween40 and SSL emulsion systems, oil (20 g) was dispersed in a pre-warmed 0.75%w/w sodium caseinate solution containing 1%w/w (of final emulsion weight) SSL and Tween40, respectively. For PGE and MGP emulsion systems: 1.5%w/w (of final emulsion weight) PGE and MGP was dissolved in the oil phase (20 g) and dispersed in pre-warmed 0.75%w/w sodium caseinate solution. The oil/protein/emulsifier solutions were pre-homogenized using a Silverson rotor stator mixer at 12,000 rpm for 2 min to produce course tuna oil-in-water emulsions (10%w/w tuna oil). The course emulsions were immediately homogenized at 300 bar for five passes using a homogenizer (C5, Avestin) system heated to 70 °C within a water bath. The emulsion was circulated through ice water after the final pass to rapidly drop the temperature of the final collection. After emulsification, sodium azide (0.002%w/w) was added to each sample with stirring to prevent bacterial growth. In an additional step for the 4 °C experiment, 50 μM (final volume) ferrous sulfate was added to each emulsion as a prooxidant at time zero.

Emulsion Particle Size

The particle size distribution of the emulsion droplets were determined immediately after production via laser diffraction using standard optical parameters: dispersant refractive index of 1.330 (water), particle refractive index of 1.456, and particle absorption index of 0.001 (Malvern Mastersizer 2000, Malvern Instruments, Worcestershire, UK). This method measures the angular dependence of the intensity of laser light scattered by the dilute emulsion.

Oxidation Process

Auto-oxidation was performed in the dark at 50, 25, and 4 °C on 2 ml sample aliquots contained within 10 ml capped amber headspace vials. Fresh samples were immediately analyzed for headspace volatiles as time zero. Thirty vials were loaded into the auto sampler (Shimadzu AOC5000, Peltier temperature control) where they were heated or cooled to temperature. All other vials were randomly distributed within a closed oven or refrigerated at temperature to be transferred to the auto sampler as required. Triplicate vials were withdrawn at each time point and immediately analyzed for headspace volatiles.

Headspace Analysis

The sample was pre-heated for 2 min at 50 °C, and the solid phase microextraction (SPME) fiber (carboxen/polydimethyl siloxane/divinyl benzene, 50/30 μm, Supelco, Australia) was inserted into the sample headspace. Heating was continued for a further 15 min. At the end of the extraction time, the fiber was withdrawn and inserted into the GC injector to desorb the compounds onto the GC column. The fiber remained in the injector for 7 min. GC was performed on a VOC fused silica capillary column (60 m, 0.32 mm internal diameter, 1 μm film thickness, Agilent). Helium was used as the carrier gas at a flow rate of 1.0 ml/min. The injector was operated in split mode (50:1), and the temperature was maintained at 230 °C. The oven temperature was increased from 40 to 230 °C at the rate of 22 °C/min where it was held for 12.36 min. The temperature of the detector was 230 °C. The volatile compounds were identified by reference to the retention time of standards. Analysis was performed in triplicate on individual vials for each time point.

Fatty Acid Analysis

Fatty acid (FA) methyl esters were produced as follows: 3 ml of hexane and 1 g of sodium chloride were added to 2 ml emulsion directly in the headspace vial after SPME analysis. The vial was vortexed at 2,000 rpm for 10 min (Heidolph Multi Reax), and 5 drops of ethanol were added to break the surface tension and allow layers to separate. Then 2.5 ml from the top layer was collected and diluted with 4 ml hexane. To this, 200 μl of saturated potassium hydroxide/methanol was added into each solution and vortexed at 2,000 rpm for 10 min. The solution was neutralized with 200 μl 2 M hydrochloric acid-methyl orange solution and allowed to settle for 30 min. The top layer was injected directly into the GC without further concentration. Methylation was conducted in duplicate. A Shimadzu GC-17A and AOC-17 auto injector (Shimadzu) were used, and a 0.1 μl aliquot of sample solution was injected with a split ratio 1:50. The GC was fitted with a BP X70 fused silica capillary column (30 m, 0.25 mm id and 0.25 μm film thickness, SGE) operating with a constant helium pressure of 110 kPa. The GC column temperature was increased from 60 to 170 °C at a rate of 20 °C/min, followed by a further increase at a rate of 1 °C/min to 200 °C. Temperatures of 220 and 250 °C were used for the injector and detector, respectively. FA analysis was conducted on the oil extracted from fresh 10%w/w emulsions. Analysis was performed in triplicate.

Protein Loading at Emulsion Interface

Emulsions were separated into two layers via centrifugation (Beckman-Coulter Optima L-90 K) for 60 min at 30,800 rpm. The separated emulsions were immediately frozen to −22 °C, where the top cream layer was separated from the frozen serum layer. The serum was allowed to return to room temperature before sub-sampling, and 1.5 g of serum was accurately weighed into a clean boat and dried under heat to form a thin protein film for crude protein analysis via Leco. Four replicates of each sample were analyzed. Surface protein was estimated as follows: [total protein] − [serum protein] = [surface protein].

Protein surface coverage was calculated using a depletion method adapted from that described by Tomas et al. [9]. The surface protein concentration was determined after emulsion separation using the equation shown above. Interfacial protein concentration (mg/ml) was determined as the difference between total protein concentration (Ctot) and serum protein concentration (Caq). Emulsion-specific surface area (m^2)/ml) was determined by Malvern Mastersizer. Protein surface coverage (mg/m^2) was calculated according to the equation Ctot—Caq/SSA.

Statistical Analysis

The average value and standard deviation were calculated for each measurement based on triplicate analysis ($n = 3$), with the exception of the protein-loading determination where $n = 4$.

Results and Discussion

Characteristics of Oil Phase and Oil-in-Water Emulsions

Natural antioxidant components present within fresh tuna oil will provide a degree of natural protection towards oxidative deterioration [3]. The tocopherol profile of fresh tuna oil from the same batch used herein is known to contain 1,006 ± 85 μg/g of δ-tocopherol and appreciable amounts of both, α- and γ-tocopherol with 475 ± 4 μg/g and 474 ± 41 μg/g, respectively.

Fatty Acid Composition of the Tuna Oil

Eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) are the main components within tuna oil to undergo oxidation. Both are n-3 FA, and autooxidation of these FAs results in the formation of secondary oxidation products, which contribute to the fishy taste and smell of fish oil (3). The FA composition of the tuna oil is shown in Table 1. The dominant FAs within the fresh tuna oil were DHA and palmitic acid (16:0), with 22.7 ± 0.1% and 20.2 ± 1.2%, respectively. Other major FAs include EPA (5.7 ± 0.1%), oleic acid (18:1n-9,

Table 1 Selected individual fatty acids (% of total fatty acids) of tuna oil determined via GC-FID

	% 16:0	% 18:0	% 18:1n-9	% 20:5n-3	% 22:6n-3
Average	20.2	6.2	12.8	5.7	27.2
SD	1.2	1.0	0.2	0.1	0.5

Standard deviation represents variation between oil extracted from all fresh emulsion systems used within this study

12.8 ± 0.2%) and stearic acid (18:0, 6.2 ± 1.0%). Volatiles produced from the breakdown of n-3 FA include propanal, 1-penten-3-ol, 1-penten-3-one and *t,t*-2,4-heptadienal [3].

Protein Displacement at the Emulsion Interface within Binary Systems

The oil-in-water emulsion systems within this study contained 0.75%w/w sodium caseinate. In order to investigate the effect of protein displacement on oxidative stability within binary emulsion systems, a series of emulsions were prepared whereby interfacial protein was partially displaced by one of: 1% Tween40, an uncharged fatty acid ester of sorbitan polyethoxylate; 1% SSL, an anionic emulsifier produced from lactic acid and FA; 1.5% MGP, a palmitic acid monoacylglycerol; 1.5% PGE, an emulsifier produced from the esterification of polyglycerol with FA. Each emulsion was exposed to auto-oxidation at 4, 25, and 50 °C where the rate of oxidative deterioration was followed by headspace analysis.

Sodium caseinate comprises of a soluble mixture of four different proteins, namely α_{s1}-, α_{s2}-, β-, and κ-casein. These proteins have a strong tendency to associate with each other to form aggregates such as casein micelles and co-exist in equilibrium with free casein molecules [10]. Sodium caseinate molecules rapidly adsorb to the surface of the oil droplet during the emulsification process [11, 12] and form thick interfacial layers in comparison to other protein sources [11, 13]. It is desirable to utilize protein-based emulsifiers within oil-in-water emulsion formulations, as they form relatively stable pre-emulsion systems before the secondary emulsifier of a binary system adsorbs to the interface. However, secondary emulsifiers will displace protein to varying degrees, creating emulsions with different interfacial dynamics. The degree of protein displacement is also dependent upon temperature, where less protein will be present within the aqueous phase at high temperatures compared to low temperatures [14]. Displaced sodium caseinate will remain within the aqueous phase where it has been reported to increase the viscosity of the interface surrounding the droplet, thereby restricting the penetration and mobility of radical initiators into the oil phase [4, 15].

As shown in Table 2, approximately 35% of available protein from the 0.75%w/w sodium caseinate solution used in emulsion preparation was adsorbed to the interface within the primary emulsion system (containing no secondary emulsifier; control) when stored under ambient conditions. In the absence of a secondary emulsifier, there was full surface coverage, and excess protein remained within the aqueous phase. The majority of interfacial protein was displaced in each of the binary systems once the initial pre-emulsion system had been stabilized. This was found to be true irrespective of whether or not the secondary emulsifier was water or oil soluble. The relative concentrations of protein at the interface proportional to surface area can be described in terms of surface coverage (Γ). In the case of the primary emulsion stabilized solely by sodium caseinate, protein surface coverage was determined to be 0.76 mg m^{-2}. This finding is consistent with observations reported elsewhere in the literature for sodium caseinate emulsions of similar composition [16]. With regards to the displacement of protein within the binary systems, surface coverage measurements showed that the highest degree of protein displacement from the interface occurred with the oil-soluble PGE (0.02 mg m^{-2}) followed by the water-soluble Tween40 (0.06 mg m^{-2}). The least amount of displacement occurred with SSL (0.12 mg m^{-2}). The slightly higher interfacial protein concentration observed for the emulsion containing SSL may be due to the fact that SSL is able to form complexes with sodium caseinate in solution [17]. Consequently, the protein may still be co-adsorbed with the more surface active anionic emulsifier.

Table 2 Available protein (% of total) located at the emulsion interface compared to the serum phase under ambient conditions

Emulsion system	% Tuna oil	% Secondary emulsifier	% of available protein (0.75%w/w) at emulsion interface	Surface coverage (mg m^{-2})
Sodium caseinate + Tween40	10.0	1.0	3.3 ± 0.1	0.06
Sodium caseinate + SSL	10.0	1.0	8.0 ± 0.1	0.12
Sodium caseinate + PGE	10.0	1.5	1.3 ± 0.1	0.02
Sodium caseinate + MGP	10.0	1.5	6.7 ± 0.1	0.09
Sodium caseinate	10.0	0.0	34.7 ± 0.1	0.76

Emulsion Droplet Size

In this study, we aimed to create oil-in-water emulsions with similar droplet sizes in order to minimize any surface area effect on the rate of oxidation. Figure 1 shows the particle size distribution for the Tween40/sodium caseinate emulsion system. All final emulsion distributions were monomodal after five passes despite the differences in secondary emulsifier. Emulsion droplet size was similar across all samples (0.14–0.24 μm), with the exception of the binary Tween40/sodium caseinate emulsion system used within the 25 °C study (0.32 μm) and within the sodium caseinate primary emulsion of the 50 °C study (0.32 μm). On the basis of surface effects alone, droplet size would be expected to have a similar affect on oxidation rates as differences in surface area to volume within bulk oil systems. However, it is unlikely the small differences in oil droplet size seen within the present study would significantly affect oxidative deterioration [15, 18]. Hence, it is likely that other factors, such as interfacial thickness and the metal-binding capabilities of the emulsifier, will have a greater effect on oxidation and may be considered the main governing factors contributing towards oxidative stability.

Effect of Interfacial Composition on Lipid Oxidation

The oxidation of n-3 FA into secondary oxidation products within tuna oil emulsions was investigated at three different temperatures: 4, 25, and 50 °C, in order to elucidate if interfacial structure is affecting lipid oxidation.

In order to simulate storage under ambient conditions, the abundance of volatiles developed during the deterioration of the oil phase of each emulsion was measured as a function of time at 25 °C. The results from the GC analysis are presented in Fig. 2 and show a steady increase in propanal concentration as a function of time within the headspace of each emulsion system, with the exception of Tween40, where the abundance of propanal was consistently low throughout the study. The relative concentration of propanal is indicative of the stabilizing influence of each emulsifier surrounding the oil droplet towards autooxidation. Based on a reduction in propanal formation, the order of stability at 25 °C was Tween40 > MGP > PGE > SSL.

The results presented from the propanal analysis indicate that the oxidative stability of an emulsion can be manipulated through changes in emulsifier type and surface charge. The results are in agreement with the findings of Mancuso and co-workers where the anionic SSL emulsion system oxidized at a greater rate than the non-ionic Tween40 emulsion at 25 °C [19]. A similar trend has also been observed for salmon oil-in-water emulsion systems where an anionic surfactant was compared with both a non-ionic and cationic emulsifier [19].

Further, factors such as emulsifier orientation, interfacial structure, and concentration [7, 19–22] have been reported to affect oxidative stability. Within this study the anionic SSL binary emulsion system oxidized at a greater rate than both the non-ionic MGP and PGE systems. This enhanced protection is partly attributed to crystallization of both the PGE and MGP at the interface of the oil droplet.

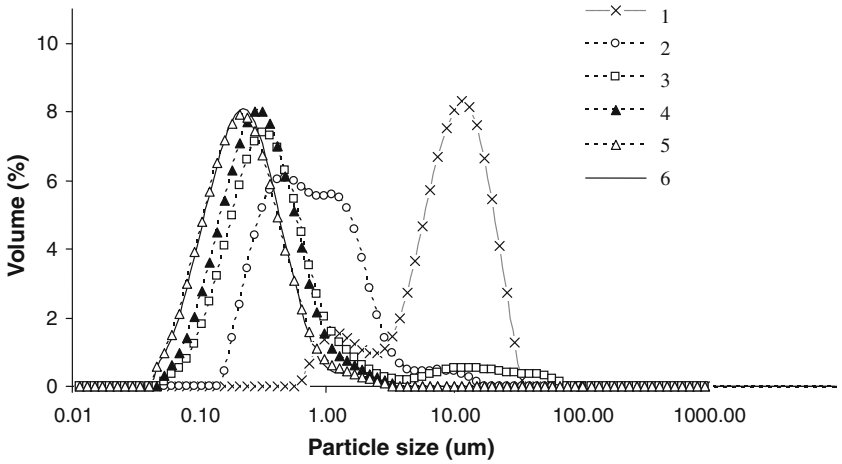

Fig. 1 Particle size distribution of a fresh emulsion system stabilized with Tween40/sodium caseinate after: (1) course homogenization using a Silverson stirrer, and (2–6) 1–5 passes through an Aveston C5 homogenizer, as measured using a laser diffraction instrument (Mastersizer 2000, Malvern Instruments). The 10%w/w tuna-oil-in water emulsion systems with sodium caseinate and Tween40 stabilizers were prepared by passing the emulsion mixture through a homogenizer five times. The course emulsion and emulsion after one pass were multimodal. The emulsion systems after two passes were bimodal. A monomodal system was achieved after three passes through the homogenizer. Additional passes (4–5 passes) achieved a smaller average particle size

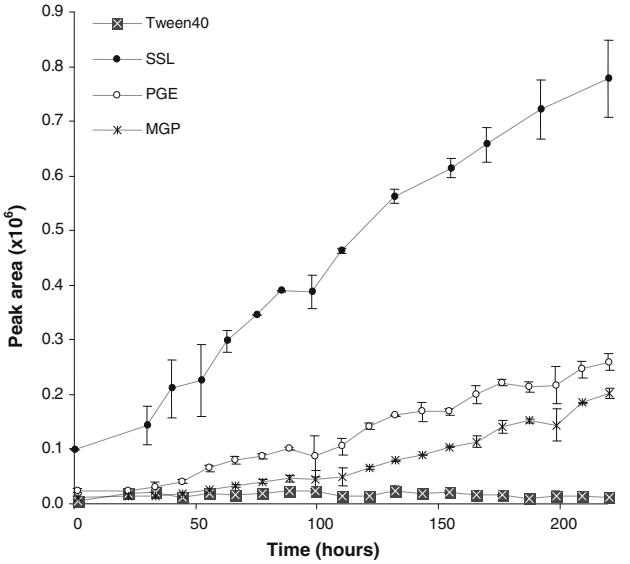

Fig. 2 Development of propanal as measured by SPME GC-FID within four emulsion systems subjected to a storage trial at 25 °C (0–225 h): (1) Tween40/sodium caseinate, (2) SSL/sodium caseinate, (3) PGE/sodium caseinate, and (4) MGP/sodium caseinate. Each data point represents the mean value ($n = 3$) ± the standard deviation

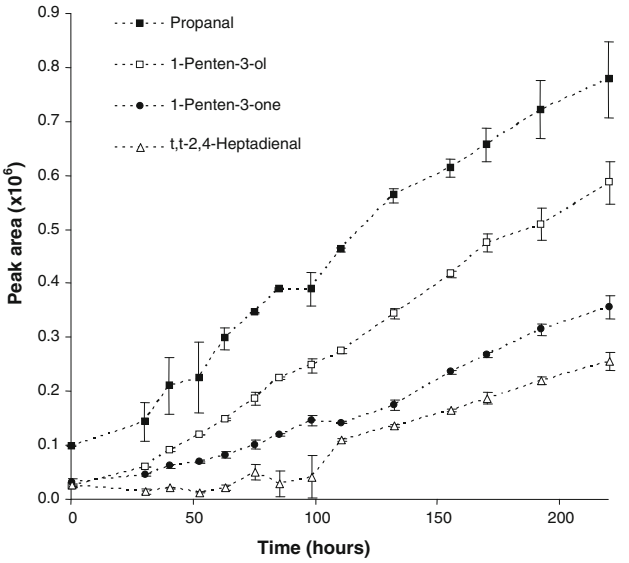

Fig. 3 Development of (1) propanal, (2) 1-penten-3-ol, (3) 1-penten-3-one, and (4) *t,t*-2,4-heptadienal as measured by SPME GC-FID within the emulsion system stabilized with sodium caseinate and SSL, and subjected to a storage trial at 25 °C (0–225 h). Each data point represents the mean value ($n = 3$) ± the standard deviation

Furthermore, the PGE emulsifier is capable of forming hydrated interfacial bi- or multi-layers, depending on concentration, thereby providing an additional barrier to metal ion catalyzed oxidation [23]. The increase in oxidative deterioration over time within the SSL emulsion system is clearly shown through the development of selected headspace markers, namely: propanal, 1-penten-3-ol, 1-penten-3-one, and *t,t*-2,4-heptadienal (Fig. 3).

The experiment was repeated at 50 °C to assess the stability of the same emulsion systems at elevated temperatures. Similar to the previous study, the anionic SSL emulsion system oxidized to a greater extent as measured via propanal formation as a function of time (Fig. 4). From 100 h onwards, the rate of propanal formation within the Tween40 emulsion increased rapidly, approaching levels exhibited within the SSL system. Otherwise, for the most part, the Tween40, PGE, MGP binary systems and sodium caseinate primary emulsion oxidized to a similar degree and rate as each other so that there was no differentiation in stability order among the three nonionic emulsion systems, whereas differences were observed at 25 °C. The difference between the 50 °C study and the 25 °C can be explained through the temperature-dependant changes that occur in interfacial surface chemistry. At lower temperatures, secondary emulsifiers will crystallize and nucleate at the emulsion interface to partially or fully displace the protein layer. At higher temperatures, however, it is believed that the secondary emulsifier will instead be in a molten state, allowing protein molecules from within the serum phase to re-adsorb to the droplet interface. Therefore, the interfacial

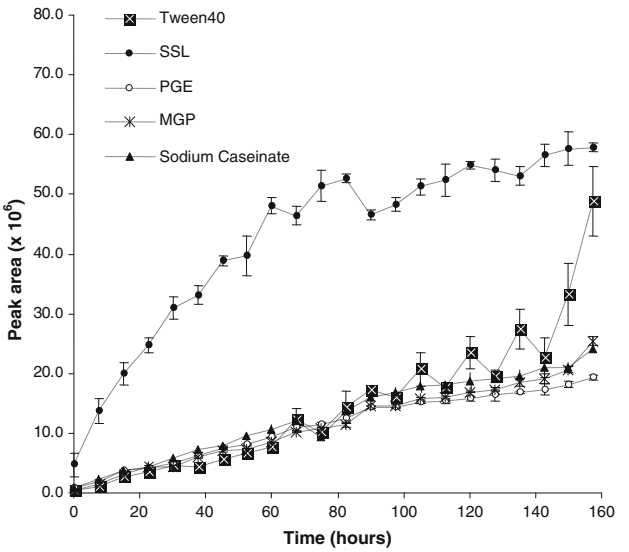

Fig. 4 Development of propanal as measured by SPME GC-FID within four emulsion systems subjected to a storage trial at 50 °C (0–160 h): (1) Tween40/sodium caseinate, (2) SSL/sodium caseinate, (3) PGE/sodium caseinate, and (4) MGP/sodium caseinate. Each data point represents the mean value ($n = 3$) ± the standard deviation

surface chemistry of the emulsions formulated within this study appears to be governed primarily by the ionic nature of the secondary emulsifier at lower temperatures and by the protein at higher temperatures.

Quantitative differences in headspace volatile formation were observed between the 50 °C study and the 25 °C study. Significantly higher levels of both *c,t*-2,4-heptadienal

(not shown) and *t,t*-2,4-heptadienal were detected in the headspace compared to the emulsion systems oxidized at 25 °C (Fig. 5). It is hypothesized that inadequate thermal energy was delivered to the lipid system at 25 °C to overcome the activation energy barriers and degrade larger molecular weight hydoperoxides into indicative aldehydes, such as 2,4-heptadienal. It is also possible 2,4-heptadienal had formed within the emulsion under these conditions, but was not volatile at 25 °C despite the 15-min equilibration period of the sample and SPME fiber at 50 °C immediately prior to GC-FID analysis, hence remaining within the oil phase. Oxidation is known to occur via different mechanisms at lower and higher temperatures, and activation energy requirements are specific for different oil types [24]. The relative concentration of volatiles within the headspace as measured by GC-FID may not necessarily reflect on the sensory characteristics of the food. Some volatile components may be detected within the food matrix by the consumer at lower concentrations than others because of differences in minimum detection threshold values, thereby influencing taste and flavor characteristics to a greater degree.

The oil-in-water emulsions stored under refrigerated conditions (4 °C) were stable to oxidation throughout the life of the study of 420 h (approximately 17 days) in the presence of the 50 μM ferrous sulfate prooxidant, with the exception of the anionic SSL emulsion system where the rate of propanal formation increased steadily over time, similar to both the 25 and 50 °C studies (Fig. 6).

It can be concluded from these results that the effect of metal ion catalysis on oxidative deterioration is minimal

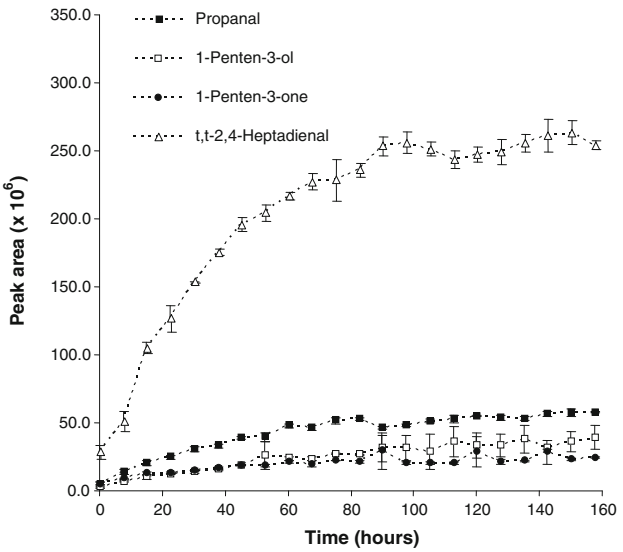

Fig. 5 Development of (1) propanal, (2) 1-penten-3-ol, (3) 1-penten-3-one, and (4) *t,t*-2,4-heptadienal as measured by SPME GC-FID within the emulsion system stabilized with sodium caseinate and SSL, and subjected to a storage trial at 50 °C (0–150 h). Each data point represents the mean value ($n = 3$) ± the standard deviation

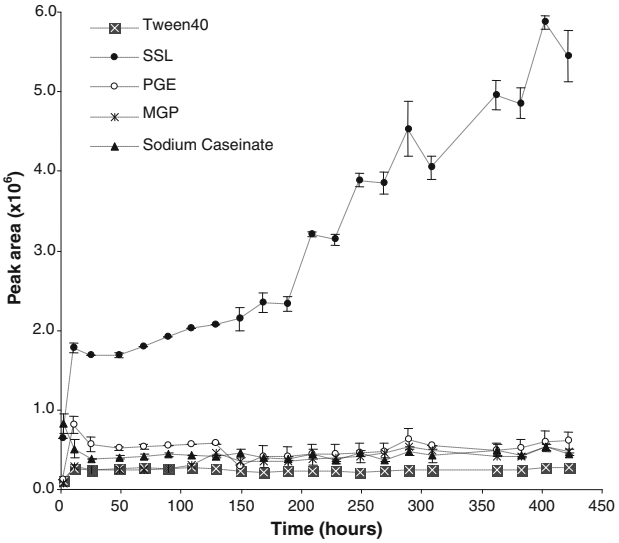

Fig. 6 Development of propanal as measured by SPME GC-FID within four emulsion systems subjected to a storage trial at 4 °C (0–420 h): (1) Tween40/sodium caseinate, (2) SSL/sodium caseinate, (3) PGE/sodium caseinate, (4) MGP/sodium caseinate, and (5) sodium caseinate. Each data point represents the mean value ($n = 3$) ± the standard deviation

under refrigerated conditions for emulsion systems stabilized with non-ionic surfactants commonly used within the food industry. The effectiveness of a prooxidant (and/or antioxidant) is affected by electrostatic attraction/repulsion effects, where the locality of metal ions within the serum phase in relation to the droplet interface influences the rate of metal ion catalyzed oxidation and is much higher in those prepared with anionic emulsifiers compared to non-ionic emulsifiers. In these emulsion systems, electrostatic attraction occurs between the negatively charged oil-water interface and the positively charged transition metal ions, increasing the concentration of ions at the interface [3]. Such metal ions, in particular Fe(II), are naturally present at levels high enough to promote lipid oxidation in many foods and decompose hydro-peroxides into free radicals [8, 19, 25]. In an emulsion system, the metal ions are in the aqueous phase and are orientated at the oil-water interface where they may have an affinity for the hydrated layer around the droplet [3]. In bulk oil systems, however, hydrophilic metal ions are orientated in the air-oil interface to catalyze lipid oxidation.

Conceptual Model

In terms of oxidative stability, the ability of sodium caseinate to sequester metal ions in solution may provide some protection against lipid oxidation, particularly given that only a third of the protein is adsorbed to the droplet surface. Considering that protein is capable of binding metal ions, it could be expected that the sodium caseinate

stabilized interfacial layer would also have a prooxidant effect. This, however, does not appear to be the case (Figs. 4, 6) and may be due to the way in which the protein adsorbs to the oil-water interface. Casein proteins are unusual in that they possess an open, disordered structure, while also having relatively well-defined hydrophobic and hydrophilic regions. According to the train-loop-tail model, on adsorption to the droplet surface hydrophobic regions become tightly bound to the interface, providing a barrier to coalescence, and the hydrophilic regions extend into the aqueous phase, providing electrostatic and/or steric stabilization against flocculation [26, 27].

Negatively charged amino acid residues associated with binding of metal ions, in particular the divalent phosphoserine amino acids (which are prevalent to varying degrees on the α_s- and β-caseins), are mostly associated with hydrophilic regions on the polypeptide chain [12, 28], and as such extend away from the interface into the aqueous phase. Consequently, even if Fe ions become bound to the interfacial protein layer, it is possible that binding will occur at anionic amino acid sites sufficiently extended away from the lipid surface so as to prevent metal ion initiation of the oxidation process from taking place.

As stated earlier, the almost complete protein displacement or preferential adsorption by the non-ionic small molecule emulsifiers was anticipated to result in improved stability with regards to lipid oxidation, based on the concept that an adsorbed non-ionic emulsifier would have little propensity to bind metal ions at the surface of an oil droplet. However, relative to the sodium caseinate-stabilized emulsion system, there appears to be no obvious improvement in oxidative stability based on such changes to interfacial composition.

Conclusion

Metal ion catalyzed oxidation was enhanced in the emulsions stabilized with an anionic emulsifier due to the electrostatic attraction between positively charged metal ions and the negatively charged droplet interface, compared to both the emulsion system stabilized with non-ionic emulsifiers and the primary sodium caseinate control. The results indicate that the oxidative stability of oil-in-water emulsion systems is influenced by the locality of the metal ions contained within the serum phase in relation to the interface, where closer proximity of the metal ions to the oil interface increases lipid oxidation.

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