Modified phosphatidylcholine with different alkyl chain length and covalently attached caffeic acid affects the physical and oxidative stability of omega-3 delivery 70% oil-in-water emulsions

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Highlights

- Increased amount of modified phosphatidylcholine(PC)s improved physical stability
- Modified PCs enhanced antioxidant activity at the oil-water interface
- Having caffeic acid attached to PC significantly improved oxidative stability
- Modified PC_C16 led to a higher surface protein load compared to PC_C14

1 **Modified phosphatidylcholine with different alkyl chain length and covalently attached**
2 **caffeic acid affects the physical and oxidative stability of omega-3 delivery 70% oil-in-**
3 **water emulsions**

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20 **Abstract**

21 This study investigated the effects of modified phosphatidylcholine (PC) with different alkyl
22 chain lengths (PC_C14 and PC_C16) and covalently attached caffeic acid on the physical
23 and oxidative stability of 70% fish oil-in-water emulsions. High fat emulsions were produced
24 using different amounts of modified PCs in combination with sodium caseinate and soy-PC.
25 Results showed that the physical stability of the emulsions was improved with increasing
26 concentrations of modified PCs, due to their high surface activity. Emulsion stabilized with
27 PC_C14 led to smaller droplets and higher viscosity, whereas PC_C16 had higher protein
28 surface load, which may result in a thicker interfacial layer. Modified PCs enhanced the
29 oxidative stability of the emulsions due to the attachment of caffeic acid to the glycerol
30 backbone of PC, which brings the antioxidant in the vicinity of oil-water interface. PC_C16 led
31 to less formation of primary and secondary oxidation products compared to PC_14 at their
32 equivalent concentrations.

33 **Key words:** modified phospholipids; phosphatidylcholine; sodium caseinate; lipid oxidation;
34 oil-water interface; caffeic acid; high fat delivery emulsions

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1. Introduction

Evidences for health benefits of long chain (LC) omega-3 (n-3) polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) have been increasing in the last decades. Some of the effects include decreasing the risk of cardiovascular diseases, improving brain development in infants, maintaining normal blood pressure and triglyceride levels in the blood, supporting mental health as well as immune system (Calder & Yaqoob, 2012; Song et al., 2016; Cheatham, Colombo, & Carlson, 2006).

LC n-3 PUFAs are mainly available in marine fish and fish products. However, the consumption of these LC n-3 PUFAs is inadequate in most Western countries (EFSA, 2010). Therefore, increasing the consumption of these bioactive compounds has attracted interest from food researchers and industry. Due to easier hydrogen abstraction from the bis-allylic positions, LC n-3 PUFAs are highly prone to oxidation (Frankel, 2012b). Oxidation causes loss of nutritional value and the formation of undesired off-flavors. Therefore, delivery systems have been developed in order to protect LC n-3 PUFAs against lipid oxidation. One of the delivery systems for LC n-3 PUFAs is oil-in-water emulsions. High fat (70%) oil-in-water emulsions were found to be advantageous for enrichment of high fat content food products with LC n-3 PUFAs (Horn et al., 2011; Yesiltas et al., 2018a,b).

Previous studies focused on different ways of improving physical properties of the oil-water interface as well as enhancing the oxidative stability of the oil-in-water emulsion system. The strategies studied include to: i) use phenolipids with various alkyl chain lengths and phenolic compounds (Laguerre et al., 2009; Sørensen et al., 2014; Alemán et al., 2015; Sørensen et

61 al., 2017), ii) incorporate emulsifiers with antioxidant activities (Yesiltas et al., 2019), iii) add
62 free antioxidants in the emulsion along with emulsifiers (Sørensen et al., 2008), or iv) to have
63 emulsifiers modified with various alkyl chains and covalently attached phenolic acids
64 (Anankanbil et al., 2018; Yesiltas et al., 2018b).

65 One of the approaches to limit lipid oxidation whilst ensuring an acceptable physical stability
66 is engineering an interfacial layer with optimal properties to resist oxidation, as oxidation has
67 been claimed to be initiated at the interface (Berton-Carabin et al., 2014). In order to have a
68 bioinspired interface structure, proteins and phospholipids could be used in combination.
69 Such a combination of emulsifiers has been suggested to form an interface structure
70 providing a better coverage of the oil droplets compared to a single emulsifier (Fang and
71 Dalglish, 2016; García-Moreno et al., 2014; Berton-Carabin et al., 2018; Yesiltas et al.,
72 2019), which could provide a good physical barrier for prooxidants` diffusion into the oil
73 phase.

74 Previous studies have focused on the antioxidant activity at the oil-water interface of the oil-in-
75 water emulsions (Laguerre et al., 2013; Berton-Carabin et al., 2014, 2018). However,
76 lipophilicity of the surface active antioxidative compound has an important effect on its
77 antioxidative activity in heterogeneous systems. Based on polar paradox and cut-off effect
78 theories, researchers have focused on finding the most efficient lipophilicity for particular
79 surface active compounds with antioxidant activity in emulsion systems. In the studies
80 conducted using phenolipids with various lipophilicity, it was observed that the most efficient
81 alkyl chain lengths could be system and particular compound dependent (Laguerre et al.,
82 2013; Alemán et al., 2015; Sørensen et al., 2017).

83 Caffeic acid is a commonly used antioxidant with radical scavenging and metal chelating
84 (especially iron) activities and its efficacy has been shown to be dependent on pH, addition of
85 iron and emulsifier type (Gülcin, 2006; Sørensen et al., 2008). The antioxidative properties
86 (radical scavenging and reducing power) of caffeic, ferulic and coumaric acids and their
87 derivatives were reported in a previous study. Caffeic acid and caffeates had the highest
88 radical scavenging and metal chelating activities, whereas the lowest radical scavenging and
89 metal chelating activities were measured for coumaric acid and coumarates (Sørensen et al.,
90 2014). The effect of the alkyl chain length on antioxidant efficacy has also been studied for
91 caffeic acid esters. It was found that the medium alkyl chain length caffeic acid (octyl caffeate)
92 had higher antioxidant activity compared to shorter and longer alkyl chain lengths in 40%
93 soybean oil-in-water emulsions (Costa, Losada-Barreiro, Paica-Martinsa, and Bravo-Díaz,
94 2017). Similar results were obtained when alkyl caffeates were applied in fish oil enriched
95 mayonnaise; short to medium alkyl chain (C4, C8, and C12) caffeic acid were found to be
96 more effective antioxidants (Alemán et al., 2015). Another study carried out with modified
97 diacetyl tartaric acid esters of mono- and diglycerides (DATEM) with covalently attached
98 caffeic acid and C12 or C14 alkyl chain lengths showed that the modified DATEM with C14
99 chain length provided slightly better oxidative stability compared to the modified DATEM with
100 C12 chain length in 70% fish oil-in-water emulsions produced with sodium caseinate (CAS),
101 DATEM and modified DATEMs (Yesiltas et al., 2018b).

102 The aim of this study was to investigate the effect of modified phosphatidylcholine (PC) with
103 covalently attached caffeic acid and different alkyl chain lengths on physical and oxidative
104 stability of 70% fish oil-in-water emulsions stabilized by sodium caseinate, soy PC and

105 modified PCs. Soy PC and modified PC in combination with CAS was used in order to
106 decrease the viscosity of the high fat omega-3 delivery emulsions when compared to
107 emulsions stabilized only with CAS. This will make it easier to apply the delivery emulsions in
108 food systems. It was hypothesized that incorporation of modified PCs in the 70% fish oil-in-
109 water emulsions will lead to a more pronounced reduction in interfacial tension compared to
110 conventional PC with 2 alkyl chains. Thus, this will result in emulsions with enhanced physical
111 stability. Moreover, different chain length (C14 and C16) of modified PCs is expected to have
112 an impact on adsorption of the molecule at the oil-water interface of the emulsions due to the
113 different hydrophilic-lipophilic balance (HLB) values. In addition, modified PCs with covalently
114 attached caffeic acid are expected to enhance oxidative stability of the emulsions compared
115 to physical mixtures of soy PC and free caffeic acids with the strategy of bringing the phenolic
116 compounds with antioxidant activity into the vicinity of the interface taking advantage of the
117 amphiphilicity of the PCs.

118 **2. Materials and Methods**

119 **2.1. Materials**

120 Cod liver oil was donated by Vesteraalens A/S (Sortland, Norway) and kept at -40°C until use.
121 Peroxide value of the cod liver oil was 0.09 ± 0.00 meq peroxides/kg oil. The fatty acid
122 content of the fish oil (% w/w) was as follows: C14:0 (4.0), C16:0 (9.2), C16:1 n-7 (8.3),
123 C18:0 (2.2), C18:1 n-9 (15.8), C18:1 n-7 (4.1), C18:2 n-6 (2.5), C18:3 n-3 (0.2), C20:1 n-9
124 (11.4), C20:5 n-3 (8.8), C22:1 n-11 (5.4), and C22:6 n-3 (11.4). Alpha-, beta-, gamma-, and
125 delta tocopherol contents were 146 ± 7 , 0 ± 0 , 97 ± 2 , 43 ± 0.3 μg toc/g oil, respectively. Sodium

caseinate (Miprodan 30) was provided by Arla Foods Ingredients a.m.b.a (Viby J, Denmark). Protein content of the sodium caseinate was reported as 92% by Arla.

Phosphatidylcholine extracted from soybean (LIPOID S100, soy PC) was donated by Lipoid GmbH, Germany. Peroxide value of phosphatidylcholine was 1.91 meq peroxides/kg sample. It was reported in the analysis of certificate of the LIPOID S100 that 97.1% of the product was phosphatidylcholine (based on dry weight) and contained 0.20% DL- α -Tocopherol, which is an antioxidant. Other lipid molecules were also reported as follows: 1.2% lysophosphatidylcholine, 0.5% N-acyl-phosphatidylethanolamine, $\leq 0.1\%$ phosphatidylethanolamine, $\leq 0.1\%$ phosphatidylinositol, 0.8% non-polar lipids, and 0.3% triglycerides. The fatty acid (% w/w) content of the soy PC (LIPOID S100) was analyzed and found as follows: C14:0 (0.09), C16:0 (12.55), C18:0 (3.77), C18:1 n-9 (8.42), C18:2 n-6 (65.82), C18:3 n-6 (7.11). Caffeic acid was purchased from Sigma Aldrich. Modified PCs (PC_C14 and PC_C16) with different alkyl chains C14 or C16 and caffeic acid were synthesized as described in a previous study (Anankanbil et al., 2018). The fatty acid content (% w/w) of PC_C14 and PC_C16 were C14:0 (99.38%) and C16:0 (98.74%), respectively. All solvents and chemicals used were of analytical grade.

2.2. Experimental design

Table 1 shows the sample codes, descriptions and amounts of ingredients. All emulsion samples include 70% w/w fish oil and 2.8% w/w total emulsifier with a 1.2 CAS: total PC ratio. These values were determined based on a previous study (Yesiltas et al., 2019). Soy PC was replaced by modified PCs in order to obtain various final caffeic acid concentrations, namely

147 360, 1080 and 2160 ppm in the final emulsion. Emulsions produced with soy PC and free
148 caffeic acid were included as controls.

149

150 **2.3. Emulsion preparation, storage and sampling**

151 Aqueous phases were prepared by dissolving emulsifiers (CAS, PC, and modified PCs) in
152 distilled water and stirred overnight at 4°C. Before emulsification, aqueous phases were
153 adjusted to pH 7 using 2M NaOH. Emulsions were produced in 500 g batches using a
154 Stephan Universal mixer (Stephan, UMC5, 1995, Hameln, Germany) as described by Horn et
155 al. (2011). Ferrous (Fe^{2+} , 100 μM) and sodium azide (0.05% w/v) were added into emulsions
156 in order to accelerate lipid oxidation and prevent microbial growth, respectively. All emulsions
157 were divided into 100 mL bottles in approximately 90 g and stored for 12 days at room
158 temperature in darkness. Physical and oxidative stability parameters were assessed at days
159 0, 2, 5, 8 and 12.

160 **2.4. Methods for characterization of emulsions**

161 **2.4.1. Creaming index**

162 Creaming rate was determined on days 1, 5, 8 and 12 in the stored bottles without replicates
163 by the measurement of creaming index. Creaming index (CI) was evaluated using equation 1:

$$164 \quad \text{CI (\%)} = \frac{b}{a} \times 100 \quad (\text{Equation 1})$$

165 where (a) is the height of total emulsion and (b) is the height of aqueous phase which is
166 separated at the bottom of the bottle. Creaming index was presented as in percentage.

2.4.2. Interfacial tension of the emulsifier combinations – pendant drop method

The dynamic interfacial tension of the emulsifiers at the oil-water interface was determined using an automated drop tensiometer OCA20 (DataPhysics Instruments GmbH, Filderstadt, Germany) at 25°C. Aqueous phases of emulsions 1 CAS, 2 PC_com, 5 PC_C14_2160 and 8 PC_C16_2160 (codes explained in Table 1) were prepared as described before and measured in duplicates. The surfactant solution (aqueous phase) was filled into a syringe with a screwed needle. For each measurement, a small drop of the aqueous phase sample solution was generated using the automated syringe into a quartz glass cuvette filled with MCT oil (WITARIX® MCT 60/40, IOI Oleo GmbH, Hamburg, Germany). The image of the drop was recorded with a camera every 10 s for 15 min. The images were transferred to the drop shape analysis software. The calculation of the interfacial tension was based on the shape analysis of a pendant drop according to the Young-Laplace equation, equation 2:

$$\Delta P = \gamma \left(\frac{1}{R_1} + \frac{1}{R_2} \right) \quad (\text{Equation 2})$$

where ΔP (mN/m²) is the pressure difference across the interface, γ (mN/m) is the interfacial tension and R_1 and R_2 (m) are the principal radii of curvature of the pendant drop. Measurements were carried out in duplicate. Changes in the interfacial tension (mN/m) were plotted as a function of time (min) for each aqueous phase solution.

2.4.3. Droplet size

Particle size of the emulsions was performed using laser diffraction in a Mastersizer 2000 (Malvern Instruments, Ltd., Worcestershire, UK) on days 0 and 12 according to the method described by Horn et al. (2011). Results were calculated as the surface weighted (D[3,2]) and

189 volume weighted (D[4,3]) mean diameter, which were calculated based on the equations 3
190 and 4, respectively:

$$191 \quad D[3,2] = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad (\text{Equation 3})$$

$$192 \quad D[4,3] = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \quad (\text{Equation 4})$$

193 where n is the number of droplets with a specific diameter, d is the diameter of the droplet
194 and, i represents the size class of the droplets. Samples were measured in duplicates.

195 **2.4.4. Relative protein content in the aqueous phase and the surface load of** 196 **proteins**

197 Protein content of the aqueous phase was determined based on the method described by
198 Jacobsen, Meyer, & Adler-Nissen (1998). Emulsion sample (~20 g) was centrifuged for 10
199 min at 25,400g and 10°C (Sorvall RC-6 PLUS, Thermo Fisher Scientific, Osterode, Germany;
200 rotor SS-34) to separate the aqueous phase and oil phase. Supernatant (oil phase) was
201 removed by the use of a pipette. The rest was mixed with distilled water (1:1) and then
202 subjected to ultracentrifugation (Beckman Ultracentrifuge L8-60M, Fullerton, CA; rotor 21102)
203 for 1 h at 106,979g and 15 °C. Protein content of the aqueous phase was determined by the
204 Dumas method (Elementar, Mt. Laurel, NJ, USA). Approximately 1 g of aqueous phase was
205 placed in the sample tray and further steps were automated including sample combustion in a
206 chamber at a high temperature (900°C) in the presence of oxygen. Content of crude protein
207 was estimated by using a conversion factor (6.25). Protein content (g) was calculated in the
208 aqueous phase. Measurements were performed in duplicate.

209 The surface load of proteins (Γ , mg/m²) was calculated according to the equation described
210 by Zhu et al. (2018), equation 5:

$$211 \quad \Gamma = \frac{V_C(C_{INI} - C_{SER})}{S V_{OIL}} = \frac{(1-\Phi)d_{32}}{6\Phi} (C_{INI} - C_{SER}) \quad (\text{Equation 5})$$

212 where V_C and V_{OIL} are the volume of the aqueous and oil phase (mL), S is the surface area of
213 the emulsion droplets (m²), Φ is the oil phase volume fraction, C_{INI} is the initial concentration
214 of the protein in the aqueous phase (mg/L), and C_{SER} is the non-adsorbed protein
215 concentration in the aqueous phase after emulsification (mg/L).

216 **2.4.5. Zeta potential**

217 Zeta potential measurements were carried out using Zetasizer Nano 2S (Malvern
218 Instruments, Ltd.) in order to determine the surface charge of the emulsion droplets. Samples
219 were prepared by diluting emulsion samples in distilled water (0.32 g emulsion + 40 g of
220 distilled water) and vortexed before measurement. Samples were placed in DTS-1070
221 disposable folded capillary cell (Malvern Instruments, Ltd., United Kingdom). The zeta
222 potential range was set to -100 to +50 mV and measurements were done at 25°C on the
223 samples collected on day 2. Measurements were done in duplicates.

224 **2.4.6. Apparent viscosity**

225 Apparent viscosity was determined with a stress-controlled rheometer (Stresstech, Reologica
226 Instruments AB, Lund, Sweden) on days 1 and 12. CC25 standard bob cup system in a
227 temperature vessel was used in order to perform the measurements. Emulsions (15 ml) were
228 measured over a shear stress range from 0.0125 to 50 Pa at 25°C. Results were calculated

229 on a specific shear rate (20 s^{-1}) for each emulsion in Pascal second (Pa·s). Samples were
230 measured in duplicate.

231 **2.5. Methods for lipid oxidation measurements of emulsions**

232 **2.5.1. Primary oxidation products—peroxide value (PV)**

233 Primary oxidation products were determined according to the Bligh and Dyer method with
234 slight changes (Bligh & Dyer, 1959). Lipids were extracted using 5 g of emulsion and a
235 reduced amount of solvent (30.0 mL of methanol and chloroform, 1:1). PV was subsequently
236 determined on the lipid extracts by colorimetric determination of iron thiocyanate on a
237 spectrophotometer (Shimadzu, UV mini 1240, Kyoto, Japan) at 500 nm (Shantha and Decker,
238 1994). Measurements were done in duplicates.

239 **2.5.2. Tocopherol content - HPLC**

240 Tocopherol content of emulsions was analyzed by HPLC (Agilent 1100 Series; Column:
241 Waters Spherisorb 3 μm Silica; 4.6×150 mm). Tocopherol analysis was performed according
242 to the official AOCS method (1998) using lipid extracts (Section 2.5.1), which were further
243 evaporated and dissolved in heptane. Measurements were carried out in duplicates.

244 **2.5.3. Secondary oxidation products—Dynamic Head Space GC-MS**

245 Volatile secondary oxidation products were analyzed according to the method described by
246 Yesiltas et al (2018a). Volatile compounds were trapped on Tenax GR tubes. The volatile
247 compounds were separated in a gas chromatograph (Agilent Technologies, 6890N Network
248 GC System, DE, USA) on a 30 m DB 1701 fused silica capillary column (0.25 mm i.d., 1 μm

249 film thickness; Agilent Technologies, J&W GC Columns, CA, USA). Mass-spectrometry
250 (Agilent 5973 Network Mass Selective Detector, Agilent Technologies, 70 eV; mass to charge
251 ratio scan between 30 and 250) was used to analyze individual volatile compounds and MS-
252 library searches (Wiley 138 K, John Wiley and Sons, Hewlett-Packard) was used for the
253 identification. The volatile compounds 2-ethyl-furan, 1-penten-3-one, 1-penten-3-ol, (*E*)-2-
254 pentenal, hexanal, (*E*)-2-hexenal, (*Z*)-4-heptenal, 2-pentyl-furan, (*E*)-2-heptenal,
255 benzaldehyde, (*E,E*)-2,4-heptadienal, nonanal and (*E,Z*)-2,6-nonadienal were analyzed in
256 emulsion samples.

257 **2.6. Statistical analysis and principle component analysis (PCA)**

258 Statgraphics XVII (Statpoint Technologies, Inc., Virginia, USA) was used to carry out the
259 analysis of variance (ANOVA) using Fisher's least significant difference test. The significance
260 was evaluated statistically at the confidence level $1-\alpha = 95\%$.

261 PCA was performed using Latentix 2.12 (LatentiX, Copenhagen, Denmark). It was carried out
262 with the emulsions as objects and creaming, droplet size, zeta potential, viscosity, peroxide
263 value, alpha-tocopherol, and volatile compounds as variables. The data was transformed
264 using the autoscale function in Latentix and the PCA models were calculated.

265 **3. Results and Discussion**

266 **3.1. PCA of all emulsions**

267 Two different PCA models were calculated; one where all time points for each of the variables
268 were included and one where only the last time point (day 12) of the variables was included.
269 The conclusion that could be made from these 2 models was more or less the same. For the

sake of simplicity, only the model calculated with the last time point for the variables is shown in Fig. 1. The first principle component (PC1) and second principle component (PC2) explained 43 and 29% of the variation in the data, respectively. The scores plot showed that the 1_CAS emulsion located differently compared to the rest of the emulsions, indicating that it behaved differently with respect to the physical and oxidative parameters measured. The loadings plot showed that the location of CAS was mostly explained by the high viscosity as well as higher protein content in the aqueous phase (non-adsorbed CAS). Oxidation parameters, PV, tocopherols and volatile compounds were located in the same quadrant and correlated with the following emulsions: 2_PC_com, 3_PC_C14_360, 6_PC_C16_360, and 9_PC_com_caf_360, which were most prone to lipid oxidation.

Emulsion 10_PC_com_caf_2160 was mostly explained by less negative zeta potential, high creaming, low viscosity and low non-adsorbed proteins. Emulsions produced with middle and high concentrations of modified PCs were not described specifically by any of these physical parameters. However, they located far from the oxidative parameters, which indicated that their oxidative stability were superior compared to the rest. In order to obtain a better overall picture of the emulsions according to the physical parameters, see the biplot (Supplementary Fig. 2) for the PCA model calculated only with physical parameters.

It was observed that the emulsions with PC_C14 were closer to viscosity and non-adsorbed CAS compared to PC_C16, whereas emulsions with PC_C16 located closer to zeta potential and creaming. This indicated differences in physical stability. Thus, emulsions containing PC_C14 had higher viscosity, which might be due to the higher amount of non-adsorbed CAS content in the water phase. On the other hand, emulsions containing PC_C16 had higher

protein surface load, creaming, and lower negative zeta potential. Moreover, the oxidation parameters showed that the emulsions with PC_C14 located closer to oxidation parameters (volatiles, PV) compared to PC_C16 emulsions, which confirmed better oxidative stability of the emulsions produced with PC_C16. In order to confirm this interpretation of the PCA model, the original raw data of the physical and oxidative stability parameters were studied in more detail.

3.2. Characterization of emulsions

3.2.1. Creaming Index

Emulsions produced with the combinations of CAS and soy PC were creamed 4-6%, whereas emulsions produced with only CAS did not have any creaming during 12 days of storage (Supplementary Fig. 3a,b). Modified emulsifiers also showed between 2-4% creaming during 12 days of storage. As these emulsions have lower creaming rate than 1 mm/day, they were all considered as stable emulsions (McClements, 1999).

3.2.2. Interfacial tension of the emulsifier combinations

Oil-water interfacial tension of the aqueous phase of the emulsions was measured in order to determine the effects of substituting 45% of the CAS with different PCs. The interfacial tension values of all the samples at 15 min were significantly different from each other ($p < 0.05$). It was observed that 2_PC_com had an oil-water interfacial tension of 11.47 ± 0.35 mN/m, whereas aqueous phase of 1_CAS had an interfacial tension of 8.34 ± 0.02 mN/m (Fig. 2). Thus, including soy PC increased the interfacial tension. However, when 60% of the soy PC in 2_PC_com was substituted with PC_C14 or PC_16, interfacial tension dropped

313 significantly from 11.47 ± 0.35 mN/m to 4.57 ± 0.01 or 3.94 ± 0.04 mN/m, respectively. Thus,
314 although both commercial and modified PC's could self-assemble in the aqueous phase and
315 form micelles, significant differences in interfacial tension values were observed between the
316 different emulsifier combinations. Aqueous phases with PC_C16 showed significantly lower
317 interfacial tension compared to PC_C14, which could be due to the interaction of PC_C16
318 with soy PC and CAS at the interface as well as higher affinity to the oil phase. Low interfacial
319 tension provided by modified PCs implies a superior surface activity, which provided
320 minimized contact area between hydrophobic and hydrophilic regions.

321 **3.2.3. Droplet size**

322 The mean particle diameter ($D[4,3]$) of the emulsions was determined in order to observe the
323 impact of emulsifier types and different concentrations of modified PCs (Table 2). Substituting
324 45% of CAS with soy PC resulted in a significant increase in droplet size from 8.8 ± 0.3 to 17
325 ± 0.2 μm . However, increasing concentration of modified PCs in the emulsions led to a
326 significant decrease in droplet size (Table 2). This could be due to the fact that modified PCs
327 had one alkyl chain instead of 2 alkyl chains compared to soy PC as well as a larger head
328 part as a result of covalently attached caffeic acid in the glycerol backbone, which might have
329 enhanced the surface activity. This was valid for both PC_C14 and PC_C16, which could be
330 attributed to their fast adsorption rates compared to proteins and soy PC, thereby producing
331 smaller oil droplets by inhibiting droplet coalescence during homogenization. Improved
332 performance of lysolecithins compared to conventional PC with 2 fatty acid chains was
333 reported previously by other researchers (Choi et al., 2011; Casado, Martin, Torres, &
334 Reglero, 2012). This could be due to higher HLB values of modified PCs compared to

335 conventional PC (HLB = 8), which provides higher surface activity and better stabilizing ability
336 in oil-in-water emulsions. It has been emphasized that lysolecithins usually disperse better in
337 the aqueous phase which influence their effectiveness (McClements et al, 2017).

338 When the droplet size of the emulsions with the equivalent concentrations of modified PCs
339 was compared, it was observed that PC_C14 provided smaller droplets compared to
340 PC_C16, which indicated faster adsorption of PC_C14 at the oil-water interface compared to
341 PC_C16 at their middle and higher concentrations. At the low concentration of modified PCs,
342 there was no significant effect of chain length observed for droplet sizes.

343 These results are generally consistent with the interfacial tension results, for which it was
344 found that the aqueous phase of 2_PC_com had higher oil-water interfacial tension compared
345 to 1_CAS, whereas addition of modified PCs decreased the interfacial tension significantly.
346 Higher surface activity of modified PCs improved emulsification of smaller droplets before
347 their coalescence. Moreover, it was obvious that the increasing concentration of modified PCs
348 provided significant decrease in droplet size (Table 2). The change in the droplet size with
349 increased concentration of modified PCs can also be seen in the optical microscope images
350 (Supplementary Fig. 4a,b). There was no significant increase in D[4,3] droplet sizes observed
351 during storage except for 5_PC_C14_2160 (Table 2, Supplementary Fig. 5a).

352 **3.2.4. Protein content in the aqueous phase and the surface load of proteins**

353 Protein content in the aqueous phase has several effects on physical and oxidative stability of
354 the emulsions. A previous study showed that viscosity of the emulsions was directly increased
355 by the concentration of CAS in the continuous water phase when the concentration was

between 5-10% in 30% oil-in-water emulsion (Liang et al., 2014). Viscosity of the final emulsion increases with the increasing viscosity of the continuous water phase (Tesch & Schubert, 2002; Yesiltas et al., 2018a). It was observed that the protein content in the water phase was around 2-fold higher for emulsion produced with only CAS compared to the rest of the emulsions, which might explain the significantly higher viscosity of the CAS emulsion (Table 1). On the other hand, proteins (e.g., CAS) inhibit oxidation due to their radical scavenging and metal chelating activities, which allow proteins to deactivate prooxidants either at the oil-water interface or in the aqueous phase (Faraji, McClements, & Decker, 2004; Berton, Ropers, Viau, & Genot, 2011). For these reasons, we have determined the protein content in the aqueous phases of the emulsions. Additionally, we have calculated the protein surface load in order to indirectly infer the adsorption of modified PCs at the interface. Forty to 66% of the protein was non-adsorbed in the aqueous phase, which indicated that the proteins were available in the aqueous phase in concentrations ranging between 2.25 – 6.23 wt%. Non-adsorbed protein of 8_PC_C16_2160 was significantly lower compared to the rest of the emulsions except for 6_PC_C16_1080 and 7_PC_C16_2160 (Table 2). Changing modified PC concentration did not affect the protein content in the aqueous phase significantly neither for PC_C14 nor PC_C16. Given that 5_PC_C14_2160 had significantly more protein content in the water phase compared to 8_PC_C16_2160, PC_C14 showed better surface activity at the oil-water interface, thereby replacing more protein at the oil-water interface. Protein surface load results revealed more information about the adsorbed protein due to the impact of droplet size on the protein surface load. Interestingly, involvement of soy PC led to 4 folds higher protein load at the oil-water interface compared to 1_CAS (see 1_CAS versus 2_PC_com, 9_PC_com_caf_360, and 10_PC_com_caf_2160, Table 2). This could be due to

the interaction between CAS and soy PC, which presumably resulted in the adsorption of larger CAS aggregates and PC multilayers at the interface. This hypothesis was supported by results from another study from our lab, which indicated that emulsions produced with CAS and PC provided thicker interface layer compared to only CAS emulsions and which provided a model for the complex structure of 70% oil-in-water emulsions with CAS and PC as emulsifiers (data submitted for publication). Protein surface load decreased with increasing concentration of modified PCs both for PC_C14 and PC_C16 (Table 2). Emulsions with PC_C14 had lower protein surface load compared to emulsions with PC_C16, which would normally support that PC_C14 was more surface active and replaced more proteins at the interface compared to PC_C16. However, interfacial tension results (Section 3.1.2) indicated that aqueous phase with PC_C16 had lower interfacial tension (4 mN/m) compared to PC_C14 (5 mN/m). This was presumably due to PC_C16's more balanced molecular interactions with soy PC and CAS at the interface, such as forming a densely packed interfacial layer with less permeability, which are not merely related to the surface activity of the molecule. Therefore, these results could be attributed to thicker oil-water interfacial layer formed in the presence of PC_C16, which thereby led to a higher protein surface load compared to PC_C14.

3.2.5. Zeta potential

All the samples had negative zeta potential, which was mainly attributed to the CAS being above its isoelectric point (pH 4.6) as the pH of the emulsion was 7 (O'Kennedy, 2011) (Table 2). Soy PC is a zwitterionic molecule, but it generally gives negative surface charge at neutral conditions (Anankanbil et al., 2018). Modified PCs were also negatively charged due to the

phosphate groups at pH 7. Thus, when these emulsifiers were used alone to emulsify 20% fish oil-in-water emulsions at pH 7, they resulted in a surface charge of -37.2 ± 0.2 , -39.5 ± 0.1 and -50.2 ± 0.1 mV for PC_C14, PC_C16 and soy PC, respectively (Anankanbil et al., 2018). In the current study, it was observed that the substitution of some of the CAS with soy PC or soy PC + modified PCs resulted in less negatively charged lipid droplets.

Free caffeic acid addition did not result in any significant change in surface charge of the emulsions produced with CAS and soy PC (Table 2). Increasing the concentration of modified PCs did not have a clear effect on surface charge. Comparison of the surface charge of the emulsions produced with equivalent concentrations (360 and 1080 ppm caffeic acid) of modified PCs showed that the emulsions with PC_C14 provided more negatively charged particles compared to PC_C16. The determination of the protein content in the aqueous phase and protein load at the interface showed that PC_16 interacted more with CAS and soy PC at the oil-water interface compared to PC_C14. Since CAS is more negatively charged than modified PCs, one would expect that the PC_16 emulsions would have a more negative charge than the emulsion with PC_14. However, adsorption of these emulsifiers does not necessarily result in a monolayer. The overall zeta potential of the oil droplets will be affected by all the emulsifiers involved, e.g., CAS, commercial PC and modified PC as well as their conformation at the oil-water interface depending on their interactions, which might not lead to a straight forward correlation between zeta potential results and emulsifiers used. Therefore, protein surface load results indicated that the interaction between CAS, soy PC and PC_C16 resulted in a thicker interfacial layer, which gave less negative zeta potential due to the higher amount of PC_C16 compared to the amount of PC_C14 when involved in the emulsions.

423 The reason for less negatively charged droplet surfaces for 2_PC_com compared to
424 3_PC_C14_360 and more negatively charged than 7_PC_C16_1080 could also be attributed
425 to higher amount of CAS, soy PC and PC_C16 adsorbed at the oil-water interface and the
426 interaction between emulsifiers forming a thicker interface. Moreover, as there was no
427 significant difference in protein surface load of emulsions produced with PC_C14 and
428 PC_C16 at their equivalent concentrations, different surface charge could be attributed to the
429 content of soy PC and modified PCs. As emulsions with PC_C16 had less negative zeta
430 potential, this indicates the presence of higher amount of soy PC and PC_C16 at the oil-water
431 interface compared to emulsions produced with PC_C14.

432 **3.2.6. Apparent viscosity**

433 Emulsions were non-Newtonian and showed shear thinning behavior. Substitution of 45% of
434 the CAS with soy PC decreased viscosity significantly as expected due to the increase in
435 droplet size (D[4,3], Table 2). Viscosity of the emulsions decreased significantly during
436 storage except for 1_CAS and 9_PC_com_caf_360 (Table 2, Supplementary Fig. 5b).
437 Addition of 2160 ppm free caffeic acid into the CAS + soy PC emulsions resulted in significant
438 decrease in the viscosity as well, which correlated well with the significant increase in the
439 droplet size (D[4,3], Table 2). This negative correlation between droplet size and viscosity
440 could be due to smaller droplets leading to more friction between oil droplets at an expanded
441 surface-to-volume ratio of the dispersed phase. This results in less mobility of the droplets in
442 the emulsion and therefore provides higher viscosity compared to emulsions having larger
443 droplets (Yesiltas et al., 2019). Similar observations regarding decrease in viscosity with the

444 addition of caffeic acid were made for 70% fish oil-in-water emulsions produced with CAS and
445 diacetyl tartaric acid esters of mono- and diglycerides (DATEM) (Yesiltas et al., 2018b).

446 On the other hand, this argument was not valid when looking at the effects of increased
447 modified PCs concentration on viscosity. It was observed that the viscosity did not necessarily
448 increase with decreasing droplet size. This could be due to the effect of protein content in the
449 aqueous phase. As discussed in section 3.1.4, protein content in the aqueous phase might
450 increase the viscosity of the final emulsion when proteins are present in higher
451 concentrations. The concentration of CAS in the aqueous phase (2.25 – 6.23 wt%, Table 2)
452 might have affected the viscosity of the emulsions with modified PCs at their highest and
453 middle concentrations; 4_PC_C14_1080 and 5_PC_C14_2160 had significantly higher
454 viscosity compared to 7_PC_C16_1080 and 8_PC_C16_2160, respectively, whereas this was
455 not the case for the lower concentrations of modified PCs (3_PC_C14_360 and
456 6_PC_C16_360).

457 **3.3. Oxidative stability of emulsions**

458 **3.3.1. Primary oxidation products - peroxide value**

459 Lipid hydroperoxides were analyzed to compare the oxidation rate of the emulsions during 12
460 days of storage (Fig. 3). As expected the use of modified PCs' had an impact on the oxidative
461 stability of high fat fish oil-in-water emulsions. Peroxide value (PV) of 1_CAS was lower
462 compared to 2_PC_com, which showed that substitution of 45% of the CAS with soy PC did
463 not improve the oxidative stability of the 70% fish oil-in-water emulsion in terms of primary
464 oxidation products. The addition of modified PCs low concentrations (see 3_PC_C14_360

465 and 6_PC_C16_360, in Fig. 3) resulted in prooxidant effect when compared to 2_PC_com.
466 Emulsions containing modified PCs had higher PV than CAS + soy PC emulsions, which
467 contained the equivalent amount of caffeic acid but in free form. Thus, the use of modified PC
468 with caffeic acid attached did not reduce the formation of hydroperoxides when added both at
469 low and high concentrations. The beneficial effect of having free caffeic acid in the aqueous
470 phase compared to having caffeic acid covalently attached to the PC could be due to the
471 enhanced ability of free caffeic acid in binding iron in the aqueous phase, which limits metal
472 catalyzed initiation and free radical formation (Frankel, 2012a).

473 Emulsions with PC_C16 had higher PV compared to emulsions with equivalent amount of
474 PC_C14. This could be explained by the faster degradation of hydroperoxides in emulsions
475 with PC_C14 (see section 3.3.3).

476 **3.3.2. Changes in tocopherol content**

477 Alpha-, gamma-, and delta-tocopherol levels in emulsions were in the range of 105.7 ± 0.7 –
478 133.6 ± 3.7 , 72.1 ± 0.1 – 74.2 ± 0.7 , 29.1 ± 0.7 – 30.5 ± 0.3 $\mu\text{g toc /g emulsion}$ at day 0
479 (Supplementary Fig. 6a-c). As the fish oil content was the same in all emulsions, significant
480 differences between tocopherol contents in emulsions were attributed to the consumption of
481 tocopherol during emulsion production at day 0. Emulsion 1_CAS had significantly higher
482 consumption of all types of tocopherols compared to 2_PC_com. When the soy PC was
483 substituted with increasing concentration of modified PCs, alpha tocopherol content
484 decreased significantly, which indicated that the alpha-tocopherols were acting as
485 antioxidants in the presence of modified PCs or there was an interaction between modified

486 PCs and alpha-tocopherols during emulsification. Free caffeic acid addition did not have any
487 significant effect on any tocopherols in the emulsions produced with CAS and soy PC, which
488 indicated that the alpha-tocopherol content was not affected by the presence of free caffeic
489 acid at day 0.

490 The changes in the tocopherol content were followed during 12 days of storage and it was
491 found that the alpha-tocopherol content decreased significantly only for 5_PC_C14_2160 and
492 8_PC_C16_2160, whereas gamma-tocopherol decreased significantly for 5_PC_C14_2160,
493 8_PC_C16_2160, and 10_PC_com_caf_2160 (Supplementary Fig. 6a,c). There was no
494 significant decrease in delta-tocopherol content of the emulsions during 12 days of storage.
495 These results indicated that the total tocopherol content of the emulsions with the highest
496 concentration of caffeic acid decreased significantly, which showed that part of the antioxidant
497 activity was due to tocopherols in these emulsions. However, the decrease in the amount of
498 tocopherol from day 0 to 12 was only around 5µg toc/g sample. It should also be considered
499 that alpha-tocopherols can be regenerated from oxidized tocopherol (e.g., tocopherol
500 quinone) with the proton-donating capacity of the amino group of phospholipids such as
501 phosphatidylethanolamine, phosphatidylserine, and phosphatidylcholine (García-Moreno et
502 al., 2014; Samdani, McClements, & Decker, 2018). Therefore, the antioxidant activity of
503 alpha-tocopherol could be even higher than what was measured due to its possible
504 consumption and regeneration.

505 **3.3.3. Secondary volatile oxidation products – DHS GC-MS**

506 Volatile oxidation products formed in the emulsions showed similar trend in terms of their
507 content during 12 days of storage; therefore, sum of the volatiles was presented in Fig. 4.
508 Development of 1-penten-3-ol, (*E,E*)-2,4-heptadienal, 2-pentenal, and 2-ethylfuran, which
509 were in high concentration compared to rest of the volatile compounds, are also shown in
510 Supplementary Fig. 7a-d. It was observed that 360 ppm caffeic acid provided with modified
511 PCs resulted in a prooxidant effect. On the contrary, physical mixture of caffeic acid and PC
512 (9_PC_com_caf_360) did not cause a prooxidant effect, when compared to 2_PC_com.

513 For both modified PCs, oxidative stability was improved with the increasing amount of
514 modified PCs. All emulsions with modified PC added in concentrations above 360 mg/kg had
515 lower formation of volatile oxidation products than the emulsion with commercial PC as also
516 observed in the PCA plot. Improved oxidative stability of emulsions with modified PCs could
517 be due to the fact that caffeic acid was located at the interface showing antioxidant activity. It
518 could also be due to the larger surfactant head group of modified PCs compared to soy PC,
519 which led to thicker oil-water interfacial layer (Berton-Carabin et al., 2014).

520 Emulsion 8_PC_C16_2160 had better oxidative stability compared to 10_PC_com_caf_2160,
521 which showed that caffeic acid in high concentrations was more efficient when attached to
522 PC_16 than when present in its free form. This could be attributed to different interface
523 structure and composition of the emulsions due to the molecular structure differences
524 between PC and PC_16, which affects the adsorption performance of the molecules and the
525 location of caffeic acid. It is worth paying attention to the significant differences in physical
526 properties of these two emulsions such as droplet size. There has been contradicting results
527 on the impact of droplet size on oxidative stability (Berton-Carabin et al., 2014). Nevertheless,

528 studies which have shown an impact of droplet size have found that small droplets increase
529 lipid oxidation. Hence, the large droplets in the soy PC emulsions would favor decreased
530 oxidation and not increased oxidation as observed here.

531 Results also showed that on the last day of storage, CAS emulsion had higher concentrations
532 of volatile compound than emulsions with PC_C14_2160, PC_C16_1080 and PC_C16_2160
533 ppm.

534 On the other hand, PC_C16 had a lower amount of volatile compounds formed compared to
535 PC_C14 in all concentrations of added modified PCs (Fig. 3), which could be attributed to the
536 interface structure of the emulsions. This could be explained by the interfacial tension results
537 discussed under the section 3.2.2, where PC_C16 was indicated to have a better interaction
538 with CAS and soy PC, thereby forming a thicker interface, higher packing density and less
539 permeability at the oil-water interface compared to PC_C14 (McClements and Decker, 2018).
540 Moreover, as it was discussed in the section 3.2.4, PC_C16 had lower amount of non-
541 adsorbed protein in the aqueous phase compared to PC_C14 at their highest concentration,
542 thereby higher protein surface load, which supported the formation of a thicker interfacial
543 layer for PC_C16 and a better coverage of the oil droplets compared to PC_C14. Higher
544 amount of emulsifiers at the oil-water interface inhibited prooxidant diffusion from aqueous
545 phase to oil phase and brought antioxidant activity (due to the presence of antioxidant
546 emulsifiers) to the interface, thereby increasing oxidative stability.

547 Another reason for higher oxidative stability could be attributed to the diffusion of some of the
548 PC_C16 into the oil phase in low concentrations (e.g., below its critical micelle concentration),

549 due to its high hydrophobicity and thereby acting as a chain-breaking antioxidant in the oil
550 phase. Individual PC_C16 molecules might act as an antioxidant owing to the covalently
551 attached caffeic acid on the head group of the molecule and thereby contribute to the overall
552 increased oxidative stability of the emulsions with PC_C16. Besides, both modified PCs could
553 also have potential hydrophobic interactions with emulsifiers or aggregates in the aqueous
554 phase, which might have improved their antioxidative effects (Shahidi and Zhong, 2011).

555 **4. Conclusion**

556 Soy and modified PCs in combination with CAS, as emulsifiers, decreased the viscosity of
557 70% omega-3 delivery emulsions compared to emulsions stabilized with only CAS. This
558 suggests that the emulsions can potentially be used in wider range of applications in food
559 systems when higher amount of omega-3 polyunsaturated fatty acids are needed. The use of
560 soy PC in combination with CAS decreased physical stability. However, the physical stability
561 was significantly improved when soy PC was partly replaced by modified PCs with C14 or
562 C16 alkyl chain as well as covalently attached caffeic acid due to their high surface activity.
563 The highest oxidative stability was observed for the emulsion with the highest concentration of
564 modified PC with C16 alkyl chain length and covalently attached caffeic acid. This was
565 attributed to its low interfacial tension and ability to interact with CAS and soy PC, which led to
566 thicker interfacial layer and less permeability for prooxidants, thereby resulting in better
567 oxidative stability.

568 **Abbreviations used**

569 **CAS** – sodium caseinate

570 **DHA** – docosohexaenoic acid

571 **DHS** – dynamic head space

572 **EPA** – eicosapentaenoic acid

573 **LC n-3 PUFAs** – Long chain omega-3 polyunsaturated fatty acid

574 **PC** – phosphatidylcholine

575 **PC_C14** – modified PC with covalently attached caffeic acid and C14 alkyl chain

576 **PC_C16** – modified PC with covalently attached caffeic acid and C16 alkyl chain

577 **PCA** – principle component analysis

578 **PV** – peroxide value

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587

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Table 1. Emulsion codes, descriptions and experimental design with the content of emulsions

Emulsion code	Description	Soy PC (%, w/w)	Modified PC (%, w/w)	caffeic acid (ppm)
1 CAS	CAS only	-	-	-
2 PC_com	CAS + com PC	1.27	-	-
3 PC_C14_360	CAS + com PC + mod PC C14	1.15	0.12	360*
4 PC_C14_1080	CAS + com PC + mod PC C14	0.90	0.37	1080*
5 PC_C14_2160	CAS + com PC + mod PC C14	0.52	0.75	2160*
6 PC_C16_360	CAS + com PC + mod PC C16	1.14	0.13	360*
7 PC_C16_1080	CAS + com PC + mod PC C16	0.88	0.39	1080*
8 PC_C16_2160	CAS + com PC + mod PC C16	0.48	0.79	2160*
9 PC_com_caf_360	CAS + com PC + caffeic acid	1.27	-	360
10 PC_com_caf_2160	CAS + com PC + caffeic acid	1.27	-	2160

All the emulsions have 70% (w/w) fish oil, 2.8% (w/w) total emulsifier content and the ratio

between CAS to PC is 1.2, which results in 1.53% (w/w) CAS, except for 1CAS as it includes only CAS (2.8%, w/w). Soy PC was substituted with modified PCs in different ratios in order to obtain different concentrations of caffeic acid in the final emulsion (caffeic acid (ppm)).

*Free caffeic acid was not added in emulsion 3 to 8. Concentration was calculated according to the added modified PC, which had caffeic acid attached to the compound itself.

Table 2. Droplet size, apparent viscosity, zeta potential, protein in the aqueous phase, and protein surface load results of emulsions

Emulsion code	D[3,2] (μm) (Day 1)	D[4,3] (μm) (Day 1)	Apparent viscosity ($\text{mPa}\cdot\text{s}$) at 20 s^{-1} (Day 1)	Zeta potential (mV) (Day 2)	Protein in the aqueous phase (g) (Day 4)	Protein surface load (mg/m^2) (Day 4)
1 CAS	1.5 ± 0.7^b	8.8 ± 0.3^d	2457 ± 11^d	(-) 61.9 ± 1.4^a	1.70 ± 0.01^c	4.35 ± 0.05^a
2 PC_com	10.8 ± 0.2^d	17.0 ± 0.2^g	$923 \pm 14^{c*}$	(-) 52.8 ± 4.4^{cd}	0.95 ± 0.34^b	16.47 ± 9.67^b
3 PC_C14_360	8.3 ± 0.1^c	11.7 ± 0.0^f	$939 \pm 7^{c*}$	(-) 59.3 ± 5.2^{ab}	0.94 ± 0.05^b	12.81 ± 1.13^b
4 PC_C14_1080	1.0 ± 0.0^{ab}	8.0 ± 0.1^c	$841 \pm 9^{b*}$	(-) 56.3 ± 1.4^{bc}	0.91 ± 0.01^b	1.63 ± 0.01^a
5 PC_C14_2160	$0.7 \pm 0.1^{a*}$	$5.0 \pm 0.1^{a*}$	$971 \pm 3^{c*}$	(-) 52.2 ± 1.1^{cd}	0.91 ± 0.01^b	1.13 ± 0.02^a
6 PC_C16_360	$8.0 \pm 0.1^{c*}$	11.9 ± 0.0^f	$943 \pm 36^{c*}$	(-) 52.1 ± 1.2^{cd}	0.82 ± 0.10^{ab}	14.80 ± 2.05^b
7 PC_C16_1080	$1.8 \pm 0.3^{\S}$	$9.5 \pm 0.2^{e*}$	$740 \pm 32^{a*}$	(-) 47.0 ± 3.9^e	0.81 ± 0.06^{ab}	3.40 ± 0.30^a
8 PC_C16_2160	$1.1 \pm 0.3^{\S}$	6.7 ± 0.3^b	$777 \pm 83^{ab*}$	(-) 48.8 ± 1.3^{de}	0.61 ± 0.19^a	2.64 ± 0.54^a
9 PC_com_caf_360	11.1 ± 0.2^d	17.8 ± 0.1^h	$844 \pm 44^{b*}$	(-) 48.8 ± 1.9^{de}	0.96 ± 0.01^b	16.59 ± 0.17^b
10 PC_com_caf_2160	10.9 ± 0.6^d	18.4 ± 0.0^i	$831 \pm 93^{b*}$	(-) 49.4 ± 3.0^{de}	1.00 ± 0.08^b	15.00 ± 2.29^b

*Significant changes happened in droplet size and viscosity during 12 days of storage at

$p < 0.05$.

^{a-f}Letters indicate the significant differences between samples for the same physical parameter.

^{\S}There was only one replicate for these 2 samples.

Figure 1
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Figure 1. PCA scores and loading were plotted using the results of oxidation parameters (PV, tocopherols, volatiles) and physical parameters (D[3,2], D[4,3], viscosity, zeta potential, creaming, non-adsorbed CAS and CAS surface load).

Figure 2
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The oil-water interfacial tension without emulsifiers was 26 mN/m during 15 min for MCT oil/water. Relative standard deviation was lower than 6% in all samples.

Figure 2. Interfacial tension of emulsifier combinations

Figure 3
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Figure 3. Formation of primary oxidation product in the emulsions during 12 days of storage.

Figure 4
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Figure 4. Sum of the volatile secondary oxidation products formed in emulsion samples during 12 days of storage

Supplementary Material

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