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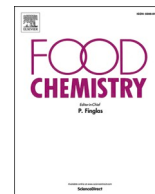
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# Interfacial effects of gallate alkyl esters on physical and oxidative stability of high fat fish oil-in-water emulsions stabilized with sodium caseinate and OSA-modified starch

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## ABSTRACT

Effects of sodium caseinate (SC) and its combination with OSA-modified starch (SC-OS; 1:1) alone and with *n*-alkyl gallates (C0-C18) on the physical and oxidative stability of high-fat fish oil-in-water emulsion were evaluated. SC emulsion contained the smallest droplets and highest viscosity due to the fast adsorption at droplet surfaces. Both emulsions had non-Newtonian and shear-thinning behavior. A lower accumulation of lipid hydroperoxides and volatile compounds was found in SC emulsion due to its better Fe<sup>2+</sup> chelating activity. The incorporated short-chain gallates (G1 > G0 ~ G3) in SC emulsion had a strong synergistic effect against lipid oxidation compared to that of SC-OS emulsion. The better antioxidant efficiency of G1 can be related to its higher partition at the oil–water interface, while G0 and G3 had a higher partition into the aqueous phase. In contrast, G8, G12, and G16 added emulsions indicated higher lipid oxidation due to their internalization inside the oil droplets.

## 1. Introduction

Fish oil contains high concentrations of long-chain omega-3 polyunsaturated fatty acids (LC- $\omega$ 3 PUFA), including eicosapentaenoic acid (EPA) and docosahexaenoic (DHA). Recently, studies have shown that LC- $\omega$ 3 PUFA has several beneficial impacts on human health, including maintaining normal brain function, improving the immune system, reducing cardiovascular diseases, blood triglyceride levels, and blood pressure (Chen, Jayachandran, Bai, & Xu, 2022). Increasing consumer demand for foodstuff containing LC- $\omega$ 3 PUFA has attracted the interest of researchers and the food industry to produce products enriched with fish oil. Lipid oxidation is a significant concern in LC- $\omega$ 3 PUFA enriched food, because it causes loss of nutritional value, generates rancid off-flavors, and reduces consumer acceptance (Yesiltas et al., 2021). Delivery systems (e.g., emulsions) and antioxidants (e.g., phenolipids) are currently used to promote the physical and oxidative stability of fish oil-enriched products. Phenolipids or lipophilized phenolic compounds obtained by esterifying the hydroxylic or a carboxylic group of a phenolic compound with a fatty acid or a fatty alcohol, respectively

(Laguerre et al., 2015). Previous studies focused on various ways to prevent lipid oxidation of low-fat emulsions, including the use of lipophilized antioxidants (Qiu et al., 2017), emulsifiers with antioxidant potential (Tian et al., 2022a), and incorporation of lipophilized antioxidants with emulsifiers (Du et al., 2022). However, only few studies in the literature have reported on high-fat emulsions, especially the effectiveness of combining emulsifiers with phenolipids (Alemán et al., 2015; Yesiltas et al., 2019).

Octenyl succinic anhydride starch (OSA) is obtained from the hydrophobic modification of native starch with octenyl succinic anhydride, and the hydrophobic group of OSA converts it into an amphiphilic compound with suitable surface active properties. The short octenyl succinate side chains bring OSA-starch (OS) molecules to the oil–water interface, and the large amylopectin backbone protects the droplets against flocculation via steric stabilization (Yan et al., 2019). These properties have increased its efficiency in emulsion systems, especially oil-in-water emulsion as an emulsifier and emulsion stabilizer (Zhang et al., 2021). Sodium caseinate (SC) is a milk protein product, which is achieved by the substitution reaction of sodium hydroxide and acid

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casein. SC is an amphiphilic substance with suitable surface active properties, which provides a good physical barrier at the oil–water interface (Yin et al., 2022). Horn, Nielsen and Jacobsen (2012) reported that SC provides a metal chelating activity both at the water phase and in the oil–water interface.

Emulsifiers can affect the particle size, surface charge, thickness, and permeability, and if the optimal emulsifier is used it can minimize the impact of free radicals, oxygen, and pro-oxidants on lipid oxidation of emulsions. In fact, cationic emulsifiers repel cationic transition metals, thereby reducing the lipid oxidation rate (Huang et al., 2022). In contrast, anionic emulsifiers may attract transition metals and increase oxidation rate. Moreover, emulsifiers can form a physical barrier in the interfacial region of the emulsion against pro-oxidants dissolved in the aqueous phase, so thicker interfacial layers, depending on the dimensions and composition of the head and tail groups of the emulsifier, usually provide more protection (Zhu et al., 2022). Very recent evidence has shown that emulsifiers, by reducing interfacial tension, could promote both partitioning and transport of antioxidant molecules at oil–water interfaces, which are the actual initiating sites of lipid oxidation (Park & Choi, 2022; Tian et al., 2022b). Emulsifiers can also form micelles above their critical micelle concentration (CMC), which affects antioxidant reactions (Laguerre et al., 2015).

Selection of an efficient antioxidant is an essential challenge in emulsion-based foods because its efficiency depends on innate potency as a chelating agent or radical scavenger, concentration in the interfacial region, and interaction with other reactants and components (Delfanian, Sahari, Barzegar, & Ahmadi Gavlighi, 2021). The interfacial concentration of antioxidant molecules depends on their distribution between the aqueous, interfacial, and oil phases, which depends on the physicochemical attributes of the antioxidant (e.g., hydrophobicity, and degree of ionization) and environmental conditions (nature of the oil, oil–water ratio, and surfactant concentration, etc.) (Shahidi & Zhong, 2011). Previous results revealed that lipophilization of hydrophilic antioxidants until a critical alkyl chain length improves their interfacial and antioxidative performance in emulsions by better partitioning of the antioxidants at the oil–water interfaces (Laguerre et al., 2009; Oh & Shahidi, 2018). The non-linear trend between antioxidative activity and hydrophobicity is related to the mobility and distribution of lipophilic antioxidants at the oil–water interface affected by polarity and molecular size. In general, the surface-active properties of antioxidants have an important impact on their availability at the reaction sites, which can be improved by grafting a non-reactive alkyl chain and, or incorporation with surfactant molecules (Laguna et al., 2020).

Gallic acid and its alkyl esters are a significant class of phenolic compounds with beneficial biological impacts, including anti-microbial, anti-inflammatory, antitumor, anti-apoptotic, and antioxidant activities (Kosuru, Roy, Das, & Bera, 2018). A limited number of previous studies have shown that gallic acid and its ester derivatives were able to minimize lipid oxidation significantly in the food matrix (Barreiro, Bravo-Diaz, Paiva-Martins, & Romsted, 2013; Phonsatta et al., 2017). The distribution of a homologous series of gallate alkyl esters with increasing lipophilicity was recently evaluated in 10% fish oil-in-water emulsions (Costa et al., 2020) and 1% rapeseed oil-in-water emulsion (da Silveira et al., 2021). Their results showed that in the presence of emulsifiers (Tween 80 and SDS), medium-chain alkyl gallates had more antioxidant activity in low-fat emulsions; the distribution of antioxidants at the interface region was improved without efficiency loss. However, the effect of a homologous series of gallate alkyl esters on the physical properties and oxidative stability of high-fat emulsions has not been studied. Moreover, the impact of emulsifiers on the interfacial performance of lipophilic antioxidants in emulsion systems needs more studies in the future.

Therefore, in this study, we evaluated the impact of the emulsifiers' SC alone or in combination with OS on the antioxidant efficiency of gallate *n*-alkyl esters by measuring the physical properties and the oxidative stability of 70% fish oil-in-water emulsions. A better

understanding of how emulsifiers affect the antioxidant's performance is essential to identify effective compounds that can increase the shelf life of LC- $\omega$ 3 PUFA-enriched foods.

## 2. Materials and methods

### 2.1. Chemicals and samples

Cod liver oil was provided by Vesteraalens A/S (Sortland, Norway) and kept at  $-40^{\circ}\text{C}$  until use. Chemical characteristics of fish oil were as follows: peroxide value  $< 0.1$  meq/kg oil,  $\alpha$ -tocopherol:  $198 \pm 7.4$   $\mu\text{g/g}$  oil,  $\beta$ -tocopherol:  $4 \pm 0.2$   $\mu\text{g/g}$  oil,  $\gamma$ -tocopherol:  $106 \pm 0.4$   $\mu\text{g/g}$  oil, and  $\delta$ -tocopherol:  $39 \pm 0.1$   $\mu\text{g/g}$  oil. The composition of the fatty acid (%w/w) was as follows: C14:0 (3.6), C16:0 (8.8), C16:1n-7 (8.0), C18:0 (2.1), C18:1n-9 (16.5), C18:1n-7 (5.3), C18:2n-6 (1.9), C18:3n-3 (0.9), C20:1n-9 (12.2), C20:5n-3 (8.7), C22:1n-11 (5.6), and C22:6n-3 (12.1). Sodium caseinate (SC, protein content  $\geq 92\%$ ) and octenyl succinic anhydride OSA-modified waxy starch (OS) were obtained from Arla Foods Ingredients amba (Viby J, Denmark) and KMC Amba (Brande, Denmark), respectively. Gallic acid (GO) ( $>99\%$ ), methyl gallate (G1) ( $>98\%$ ), propyl gallate (G3) ( $>98\%$ ), octyl gallate (G8) ( $>99\%$ ), and dodecyl gallate (G12) ( $>99\%$ ) were supplied from Sigma-Aldrich (St. Louis, MI, USA). Stearyl gallate (G18) ( $>97\%$ ) was supplied from Tokyo Chemical Industry (TCI, Portland, OR, USA). Tocopherol standards and external standards used in the identification of volatile oxidation compounds were supplied by Sigma-Aldrich. Other chemicals and HPLC-grade solvents applied in the analyses were obtained from Sigma-Aldrich and Lab-Scan (Dublin, Ireland).

### 2.2. Emulsion preparation and sampling

SC and OSA-starch were mixed in imidazole acetate buffer solution (10 mmol/L, pH 7.0) and stirred overnight by a magnetic stirrer at 500 rpm at  $4^{\circ}\text{C}$  for preparation of the aqueous phases (see Table 1). About 1.2 mM gallic acid (GO) and its *n*-alkyl esters (G1, G3, G8, G12, and G18) were diluted in acetone (1–3 mL) and then incorporated in the oil phase.

**Table 1**

Experimental design including emulsion code and amount of emulsifiers and gallates in the emulsions.

Emulsion code	Description	SC (% w,w)	OS (% w,w)	Gallates (mM)
SC	Sodium caseinate only	2.8	–	–
SC-G0	SC with added gallic acid	2.8	–	0.84
SC-G1	SC with added methyl gallate	2.8	–	0.84
SC-G3	SC with added propyl gallate	2.8	–	0.84
SC-G8	SC with added octyl gallate	2.8	–	0.84
SC-G12	SC with added dodecyl gallate	2.8	–	0.84
SC-G18	SC with added stearyl gallate	2.8	–	0.84
SC-OS	50% SC was replaced by OSA-starch	1.4	1.4	–
SC-OS-G0	SC-OS with added gallic acid	1.4	1.4	0.84
SC-OS-G1	SC-OS with added methyl gallate	1.4	1.4	0.84
SC-OS-G3	SC-OS with added propyl gallate	1.4	1.4	0.84
SC-OS-G8	SC-OS with added octyl gallate	1.4	1.4	0.84
SC-OS-G12	SC-OS with added dodecyl gallate	1.4	1.4	0.84
SC-OS-G18	SC-OS with added stearyl gallate	1.4	1.4	0.84

All the emulsions have 70% (w/w) fish oil.

Our previous studies showed that the optimal concentration of alkyl gallates with maximum efficiency in oil is 1.2 mM (Delfanian, Sahari, Barzegar, & Ahmadi, 2021). Hence, this concentration was used in oil, which is equal to 0.84 mM in the emulsion. Finally, high-fat O/W emulsions (500 g) were prepared by a Stephan Universal mixer (Stephan, UMC5, Hameln, Germany) as described by Yesiltas et al. (2019). To prevent microbial growth and accelerate lipid oxidation, 0.05% sodium azide and 50  $\mu\text{M}$  ferrous (7.6 ppm) was added to the emulsions, respectively. Each emulsion was divided into six equal parts in 100 mL blue cap bottles (56  $\times$  105 mm, 83 g per bottle) with equal headspace, so that there was one bottle for each sampling day. Emulsions were stored at 25 °C for 28 days, and sampling was done on days 0, 2, 7, 14, 21, and 28. Changes in lipid oxidation of emulsions were monitored at all sampling points, and physical changes in the first and last days of storage. Samples for lipid oxidation measurements were frozen at -40 °C immediately after sampling until further analysis.

### 2.3. Emulsions characterization

#### 2.3.1. Droplet size distribution

The droplet size distribution of emulsions was determined on days 0 and 28 by laser light scattering using a Mastersizer 2000 laser diffractometer (Malvern Instruments Ltd., Worcestershire, UK). Briefly, 1 g of emulsion sample was mixed in 5 g SDS buffer (10 mM) and sonicated for 15 min in a water bath at 4 °C. Emulsions droplet size measurements were carried out in duplicates in laser obscuration value 10–20% at 25 °C. The refractive index of water and sunflower oil was 1.33 and 1.47, respectively. Surface-weighted ( $d_{3,2}$ ) and volume-weighted ( $d_{4,3}$ ) mean diameters of the emulsions were determined using Eqs. (1) and (2), respectively (Horn et al., 2011):

$$d_{3,2} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad (1)$$

$$d_{4,3} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \quad (2)$$

#### 2.3.2. $\zeta$ -Potential

$\zeta$ -Potential of emulsions was measured using Zetasizer Nano 2S (Malvern Instrument, Ltd.) based on electrophoretic mobility. To avoid multiple scattering, 0.032 g of emulsion samples were diluted in 40 mL of deionized water and vortexed for 10 s.  $\zeta$ -Potential ranges were set to -100 to + 50 mV, and analysis were performed on days 0 and 28 at 25 °C (Yesiltas et al., 2021). Measurements were done in duplicates.

#### 2.3.3. Intrinsic viscosity

The viscosity of the emulsions was examined using a DHR-2 dynamic shear rheometer (Discovery Hybrid Rheometer, TA Instruments, New Castle, DE, USA) equipped with a cup and rotor on days 0 and 28. The shear flow properties of emulsion samples (35 g) were determined over a range of shear rates 1 to 100  $\text{s}^{-1}$  (Casanova et al., 2020). The curves were fitted using the Herschel-Bulkley model as follows:

$$\tau = \tau_0 + k\dot{\gamma}^n \quad (3)$$

where  $\tau_0$  (Pa) is the yield stress,  $\tau$  (Pa) is the shear stress,  $n$  is the flow behavior index,  $\dot{\gamma}$  ( $\text{s}^{-1}$ ) is the shear rate,  $k$  ( $\text{Pa}\cdot\text{s}^n$ ) is the consistency coefficient, and  $R^2$  shows the degree of fit. Measurements were done in triplicates.

#### 2.3.4. Creaming index

The creaming index of emulsions was determined in duplicates on days 2, 7, 14, 21, and 28 in the 100 mL bottles by measurement of the height of the serum layer ( $H_s$ ) and the total height of the emulsion ( $H_E$ ). The creaming index (CI) was determined using Eq. (4) (Horn et al., 2011):

$$\text{CI} (\%) = 100 \times \left(\frac{H_s}{H_E}\right) \quad (4)$$

### 2.4. Oil stability analysis

#### 2.4.1. Peroxide value (PV)

Freezing of the samples before analyses resulted in phase separation of the emulsion, which enabled extraction of the oil phase by centrifugation at 2500g at 5 °C for 10 min (Yesiltas et al., 2021). The PV of the oil samples was measured based on the method of Shantha and Decker (1994) by colorimetric measurement of the iron-thiocyanate complex at 500 nm. PV analysis of oil samples was carried out in duplicates.

#### 2.4.2. Tocopherol content-HPLC

The contents of  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherols in the oil phase of emulsions were measured by HPLC (Agilent 1100 Series; Column: Waters Spherisorb 3  $\mu\text{m}$  Silica; 4.6  $\times$  150 mm) according to Official AOCS method (1998). Tocopherol homologues mixture (10 mg/L) was added to a stock solution and used for quantification. Analysis was done in duplicates, and data are given in  $\mu\text{g}$  toc/g oil.

#### 2.4.3. Secondary oxidation products-dynamic headspace GC-MS

Volatile compounds were analyzed on days 0, 2, 7, 14, 21, and 28 according to the method of Thomsen et al. (2016). The frozen emulsion sample (4 g) was dissolved in 10 mL distilled water, and volatile compounds were extracted during heating in a water bath at 60 °C for 30 min with 150 mL/min nitrogen flow. Volatile compounds were collected on Tenax GR tubes and separated in a gas chromatograph (Agilent Technologies, 6890 N Network GC System, DE, USA) on a DB1701 column (30 m  $\times$  ID 0.25 mm  $\times$  1  $\mu\text{m}$  film thickness, J&W Scientific, Folsom, CA, USA). The oven temperature program employed for the analysis of volatile compounds was 45 °C kept for 5 min, increasing at 1.5 °C/min to 55 °C, 2.5 °C/min to 90 °C, and at 12 °C/min to 220 °C, and finally kept for 4 min. Each volatile compound was analyzed by Mass spectrometry (Agilent 5973 Network Mass Selective Detector, Agilent Technologies) and identified by MS-library searches (Wiley 138 K, John Wiley and Sons, Hewlett-Packard). The quantification of volatile compounds was carried out using calibration curves prepared from different concentrations of external standards. Analysis was performed in triplicates, and data are given in ng/g emulsion.

### 2.5. Statistical analysis

In this study, SPSS software 19 (SPSS Inc., Chicago, IL) was applied for statistical analysis of data (mean  $\pm$  SD) using one-way analysis of variance, followed by Duncan's test at  $P < 0.05$ .

## 3. Results and discussion

### 3.1. Physical characterization of emulsions

#### 3.1.1. Droplet size

The effect of the types of emulsifiers and antioxidants on emulsifying properties was measured by the surface-weighted ( $d_{3,2}$ ) and volume-weighted ( $d_{4,3}$ ) mean diameters (Table 2). The droplet size (both in  $d_{3,2}$  and  $d_{4,3}$  mean diameters) of the emulsions increased significantly after 28 days of storage. There was no significant difference between  $d_{3,2}$  values for the fresh emulsion samples stabilized by SC and the complex of SC-OS ( $P > 0.05$ ). However,  $d_{4,3}$  value revealed that the droplet size of the SC emulsion was significantly smaller than that of the emulsion in which OSA-starch was added to the formula, which could be attributed to the fast diffusion and adsorption of SC at droplet surfaces. SC is a flexible protein without disulfide bonds. Its conformational change during adsorption exposes the hydrophobic cluster and leads to the formation of small oil droplets (Yesiltas et al., 2019). The hydrophobic interaction of OSA-starch with SC probably led to slower adsorption on the droplet surfaces and increased droplet size by forming thick interfacial layers (Thaiwong & Thaiudom, 2021). Moreover, the combined use of SC and OSA could have led to formation of non-uniform

**Table 2**  
Droplet size and  $\zeta$ -potential of fresh and peroxidized emulsions.

Emulsion code	$d_{3,2}$ ( $\mu\text{m}$ )		$d_{4,3}$ ( $\mu\text{m}$ )		$\zeta$ -potential (mV)	
	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28
SC	0.80 $\pm$ 0.03 <sup>a</sup>	0.83 $\pm$ 0.06 <sup>a</sup>	7.37 $\pm$ 0.01 <sup>bc</sup>	8.47 $\pm$ 0.04 <sup>c</sup>	-54.4 $\pm$ 1.2 <sup>cde</sup>	-55.8 $\pm$ 1.1 <sup>ab</sup>
SC-G0	0.80 $\pm$ 0.06 <sup>a</sup>	0.83 $\pm$ 0.04 <sup>a</sup>	7.26 $\pm$ 0.02 <sup>a</sup>	8.27 $\pm$ 0.09 <sup>ab</sup>	-54.1 $\pm$ 2.1 <sup>bcde</sup>	-54.6 $\pm$ 1.2 <sup>a</sup>
SC-G1	0.80 $\pm$ 0.07 <sup>a</sup>	0.84 $\pm$ 0.03 <sup>a</sup>	7.52 $\pm$ 0.06 <sup>d</sup>	8.42 $\pm$ 0.07 <sup>c</sup>	-56.0 $\pm$ 2.1 <sup>de</sup>	-57.7 $\pm$ 0.6 <sup>ab</sup>
SC-G3	0.81 $\pm$ 0.01 <sup>a</sup>	0.84 $\pm$ 0.06 <sup>a</sup>	7.74 $\pm$ 0.03 <sup>e</sup>	8.34 $\pm$ 0.01 <sup>abc</sup>	-56.1 $\pm$ 1.7 <sup>de</sup>	-58.3 $\pm$ 1.3 <sup>bcd</sup>
SC-G8	0.81 $\pm$ 0.02 <sup>a</sup>	0.84 $\pm$ 0.03 <sup>a</sup>	7.27 $\pm$ 0.05 <sup>a</sup>	8.46 $\pm$ 0.18 <sup>c</sup>	-55.7 $\pm$ 1.6 <sup>de</sup>	-56.8 $\pm$ 2.7 <sup>ab</sup>
SC-G12	0.81 $\pm$ 0.01 <sup>a</sup>	0.83 $\pm$ 0.05 <sup>a</sup>	7.44 $\pm$ 0.06 <sup>cd</sup>	8.23 $\pm$ 0.05 <sup>a</sup>	-54.3 $\pm$ 2.4 <sup>cde</sup>	-55.5 $\pm$ 1.1 <sup>ab</sup>
SC-G18	0.81 $\pm$ 0.05 <sup>a</sup>	0.84 $\pm$ 0.04 <sup>a</sup>	7.32 $\pm$ 0.07 <sup>ab</sup>	8.39 $\pm$ 0.04 <sup>bc</sup>	-53.9 $\pm$ 1.5 <sup>abcde</sup>	-57.4 $\pm$ 0.9 <sup>abc</sup>
SC-OS	0.81 $\pm$ 0.06 <sup>a</sup>	0.83 $\pm$ 0.03 <sup>a</sup>	8.51 $\pm$ 0.04 <sup>h</sup>	9.79 $\pm$ 0.06 <sup>ef</sup>	-50.5 $\pm$ 1.1 <sup>ab</sup>	-63.5 $\pm$ 0.4 <sup>f</sup>
SC-OS-G0	0.81 $\pm$ 0.06 <sup>a</sup>	0.83 $\pm$ 0.03 <sup>a</sup>	8.33 $\pm$ 0.05 <sup>f</sup>	9.61 $\pm$ 0.05 <sup>d</sup>	-52.9 $\pm$ 1.7 <sup>abcde</sup>	-61.1 $\pm$ 0.7 <sup>ef</sup>
SC-OS-G1	0.80 $\pm$ 0.03 <sup>a</sup>	0.82 $\pm$ 0.04 <sup>a</sup>	8.52 $\pm$ 0.07 <sup>h</sup>	9.73 $\pm$ 0.07 <sup>def</sup>	-51.1 $\pm$ 1.8 <sup>abc</sup>	-61.8 $\pm$ 1.4 <sup>ef</sup>
SC-OS-G3	0.81 $\pm$ 0.01 <sup>a</sup>	0.83 $\pm$ 0.02 <sup>a</sup>	8.45 $\pm$ 0.05 <sup>gh</sup>	9.68 $\pm$ 0.08 <sup>de</sup>	-52.3 $\pm$ 3.2 <sup>abcd</sup>	-60.3 $\pm$ 1.3 <sup>de</sup>
SC-OS-G8	0.80 $\pm$ 0.03 <sup>a</sup>	0.83 $\pm$ 0.03 <sup>a</sup>	8.39 $\pm$ 0.07 <sup>fg</sup>	9.75 $\pm$ 0.09 <sup>def</sup>	-52.4 $\pm$ 1.7 <sup>abcd</sup>	-61.5 $\pm$ 1.1 <sup>ef</sup>
SC-OS-G12	0.81 $\pm$ 0.02 <sup>a</sup>	0.82 $\pm$ 0.03 <sup>a</sup>	8.41 $\pm$ 0.06 <sup>fg</sup>	9.86 $\pm$ 0.07 <sup>f</sup>	-51.5 $\pm$ 2.4 <sup>abc</sup>	-60.9 $\pm$ 1.2 <sup>def</sup>
SC-OS-G18	0.81 $\pm$ 0.05 <sup>a</sup>	0.84 $\pm$ 0.04 <sup>a</sup>	8.52 $\pm$ 0.04 <sup>h</sup>	9.74 $\pm$ 0.09 <sup>def</sup>	-50.1 $\pm$ 2.4 <sup>a</sup>	-60.1 $\pm$ 2.1 <sup>cde</sup>

Means  $\pm$  SD (standard deviation) within a column with the same lowercase letters are not significantly different at  $P < 0.05$ .

There was a significant difference ( $P < 0.05$ ) between  $d_{4,3}$  and  $\zeta$ -potential of SC-OS emulsion samples on days 0 and 28.

interfacial layers, and thereby increased the coalescence of droplets. This was supported by the finding that during 28 days of storage, the droplet size of SC-OS emulsion samples increased significantly. However, droplet sizes also increased in SC emulsions during storage, although the increase in general was smaller than in SC-OS emulsions. Overall, the results showed that the ability of OSA starch to lower interfacial tension was not as good as that of SC, and thus it was less effective in making small droplets.

The incorporation of antioxidants had no impact on the droplet size of emulsion samples. Our findings were in accordance with the results of da Silveira et al. (2021) and Yin et al. (2022) that reported the loading of gallate alkyl esters and resveratrol did not affect the size of fresh emulsions. Amphiphilic antioxidants are designed to increase surface activity and adsorb at oil droplet surfaces. Nevertheless, due to the steric effects in the molecule or lack of electrically charged groups and much lower concentration compared to surfactants, phenolipids do not facilitate the formation and physical stability of emulsions (McClements & Decker, 2018).

### 3.1.2. $\zeta$ -Potential

$\zeta$ -Potential of emulsions droplets was negative due to molecular charges of OSA-starch and SC at pH 7 (Table 2). The negative charges of surfactant-coated droplets cause electrostatic repulsion and droplet resistance to aggregation or coalescence, thereby increasing the emulsion stability (Li et al., 2020).  $\zeta$ -Potential of SC-stabilized emulsion was -54.4 mV, while substitution of some of the SC with OSA-starch resulted in less negatively charged lipid droplets (-50.5 mV). A more negative  $\zeta$ -value for SC emulsion indicates greater electrostatic repulsion between oil droplets, which could reduce aggregation or coalescence. The surface charge of SC is more negative than that of OSA-starch. Moreover, it is assumed that the interaction between SC and OSA-starch led to the formation of a thicker interfacial layer and, thereby decreased the  $\zeta$ -potential (Thaiwong & Thaiudom, 2021). The addition of gallate alkyl esters resulted in more negatively charged oil droplets. This could be attributed to the presence of a higher concentration of antioxidants at the interface. In addition, increasing the alkyl chain length of antioxidants did not show a significant effect on the  $\zeta$ -potential of the oil droplets. This was in agreement with the findings of Yesiltas et al. (2018) who reported that addition of caffeic acid caused more negatively charged droplets in DATEM-stabilized emulsion.  $\zeta$ -potential of all emulsions increased after 28 days of storage (Table 2), which could be attributed to the oxidation of amino acid residues on the surface of the protein (Mehr & Koocheki, 2020). Li et al. (2022) observed that oxidation of the main emulsion components increased the absolute values of  $\zeta$ -potential of the emulsion stabilized by rice bran protein.  $\zeta$ -potential changes for emulsion droplets stabilized with SC were the lowest, while the largest changes in  $\zeta$ -value was obtained for emulsion prepared with SC-OS complex. This could be related to the non-uniform interfacial layers created by the SC-OS complex, which accelerated the possibility of lipid and protein oxidation at the oil droplets surface.

### 3.1.3. Intrinsic viscosity and physical stability

The flow behavior curves of emulsions stabilized with SC and SC-OS complex showed that the emulsions were non-Newtonian and had shear-thinning behavior (Fig. S1). The flow behavior of emulsions was fitted with different rheological models such as Power law, Herschel-Bulkley, and Bingham models and it was found that the Herschel-Bulkley model had the highest correlation coefficient (Table 3). The correlation coefficient of Power law and Bingham models was  $< 0.8$  (data not show). The  $k$ -value revealed that the viscosity of the SC emulsion was higher than the SC-OS emulsion. This could be related to the smaller particle size of the SC emulsion compared to the SC-OS emulsion. These findings were in accordance with the results of Consoli et al. (2018) that reported a lower viscosity was obtained by replacing some SC with corn starch. The viscosity of both emulsions increased significantly after 28 days of storage. According to Wang, Sun and Xia (2023), high flocculation of droplets led to form a 3D diametric network (gel-like), which is the main reason for the increase in viscosity of high-fat emulsions.

Our observations confirmed that all emulsions were stable against creaming during 28 days of storage. In fact, the emulsifiers covered the oil droplets and increased their physical stability with strong electrostatic and steric repulsion. In addition, it is expected that SC emulsion had higher creaming stability during longer storage due to the higher  $k$ -

**Table 3**  
Fitting parameters of the Herschel-Bulkley equation for emulsions.

Emulsion code	$\tau_0$	$k$ (Pa·s <sup><i>n</i></sup> )	$n$	$R^2$
Day 0				
CS	32.47 $\pm$ 0.03 <sup>b</sup>	32.17 $\pm$ 0.02 <sup>b</sup>	0.27 $\pm$ 0.01 <sup>c</sup>	0.98
CS-OS	31.66 $\pm$ 0.02 <sup>a</sup>	31.38 $\pm$ 0.03 <sup>a</sup>	0.23 $\pm$ 0.01 <sup>b</sup>	0.99
Day 28				
CS	36.58 $\pm$ 0.03 <sup>d</sup>	35.81 $\pm$ 0.02 <sup>d</sup>	0.21 $\pm$ 0.00 <sup>a</sup>	0.99
CS-OS	34.75 $\pm$ 0.03 <sup>c</sup>	33.89 $\pm$ 0.04 <sup>c</sup>	0.20 $\pm$ 0.01 <sup>a</sup>	0.99

Means  $\pm$  SD (standard deviation) within a column with the same lowercase letters are not significantly different at  $P < 0.05$ .

value and smaller particle size (Hebshy, Buffa, Juan, Blasco-Moreno, & Trujillo, 2017). Yesiltas et al. (2019) reported that increasing the amount of SC increases the creaming stability of O/W emulsion due to its ability to form a stable interfacial layer with a negative surface charge (at pH 7), which reduces droplet coalescence.

### 3.2. Oxidative stability of emulsions

#### 3.2.1. Changes in peroxide value

Lipid hydroperoxides are surface-active compounds that accumulate at the water–oil interface. Amphiphilic antioxidants can delay lipid oxidation by positioning at the oil–water interfaces and donating hydrogen atoms to free radicals (McClements & Decker, 2018).

Peroxide value (PV) was measured to compare the oxidation rate of O/W emulsion samples emulsified with SC and SC-OSA and addition of gallate alkyl esters (Fig. 1). PV of SC emulsion (4.7 meq/kg oil) was lower than the SC-OSA emulsion (6.0 meq/kg oil) during 28 days of storage, which indicated that the substitution of 50% of the SC with OSA-starch could not reduce the hydroperoxides formation in O/W emulsion. The better performance of SC in reducing the oxidation rate of emulsion could be attributed to its ability to bind transition metal ions both at the interface and in the water phase (Elias, Kellerby, & Decker, 2008). It is expected that most of the iron is present in the aqueous phase

and at the interface as iron has a higher solubility in water than in oil. In addition, the interaction between SC and OSA-starch reduced the  $Fe^{2+}$  chelating activity and accelerated the oxidation rate by probably forming nonuniform and permeable interfacial layers. Yesiltas et al. (2019) observed that partly replacing sodium caseinate with phosphatidylcholine resulted in a pro-oxidant effect on lipid oxidation of O/W emulsion. The addition of gallate alkyl esters delayed the oxidation rate in all emulsions (Fig. 1). However, their antioxidant activities were influenced by the length of the alkyl chain and the nature of the emulsifier. Moreover, different emulsifiers did not change the order of antioxidant efficiency of alkyl gallate esters; although we have already observed that the same phenolipids can have different effects in different emulsion systems (Sørensen, Villeneuve, & Jacobsen, 2017). In both SC and SC-OSA emulsions, short-chain alkyl gallates (G0, G1, and G3) had better antioxidant activity than longer-chain gallates (G8, G12, and G18) during 28 days of storage. This result is supported by research findings of Delfanian, Sahari, Barzegar and Ahmadi Gavlighi (2021) that reported higher concentration of G0, G1, and G3 at the oil–water interface with a higher antioxidant efficiency compared to longer chain gallates found in higher concentration in the oil phase. The occurrence of non-linear behavior (cut-off effect) in the antioxidant capacity of the homologous series of phenolipids due to the increase in the alkyl chain length has already been observed by other authors (Panya et al., 2012;

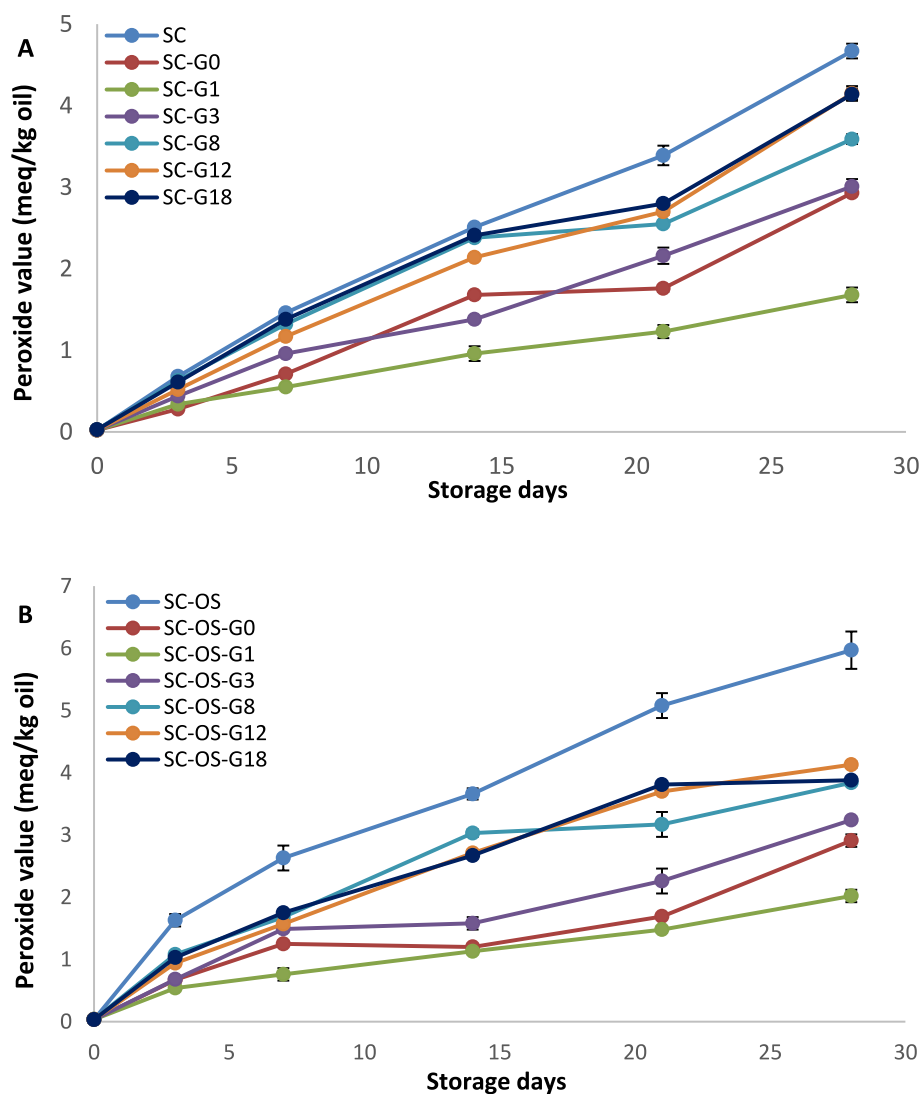


Fig. 1. Changes in peroxide value (meq/kg oil) of emulsion samples during 28 days of storage at 25 °C; (A) Emulsion stabilized with sodium caseinate; (B) emulsions stabilized with sodium caseinate-OSA starch.

Alemán et al., 2015). Incorporation of G1 in SC and SC-OS emulsions showed better inhibitory activity against the formation of lipid hydroperoxides compared to G0 and other lipophilic gallate alkyl esters. This could be related to the higher partition of G1 at the interface, which increased its diffusibility toward the oxidation sites. The stronger interfacial activity of G1 is affected by the hydrophobic interaction of the methoxy group, which increases its ability to be located at the interface (Velasco, Dobarganes, & Márquez-Ruiz, 2004). Negative charges of SC and SC-OS emulsion droplets at pH 7.0 cause electrostatic repulsion of gallic acid as molecules with a negative charge and a lower tendency to form hydrogen bonds. Thus, the lower antioxidant efficiency of G0 can be related to its higher partition into the aqueous phase. These results were in accordance with the findings of Asnaashari, Farhoosh and Sharif (2014), who reported that G1 had higher inhibitory activity in soy protein-stabilized O/W emulsion compared to G0. Different molecular interactions between emulsifiers and G8, G12, and G18, probably increased their partition in the oil phase and reduced their action at the interface. This is further supported by previous studies that observed interactions between long-chain phenolipids and surfactants reduced their concentration and antioxidant efficacy at the interfaces (González et al., 2015; Wang et al., 2020). For example, Wang et al. (2020) reported that the incorporation of G3 accompanied by Tween 20 in the O/W emulsion was more effective in delaying lipid oxidation compared to G8 and G12.

### 3.2.2. Changes in tocopherol content

Changes in homologous series of tocopherols in SC and SC-OS emulsions during 28 days of storage are shown in Fig. 2 and Supplementary Fig. 2. The contents of  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherol in fresh SC emulsion was  $196.5 \pm 4.7$ ,  $5.2 \pm 0.4$ ,  $122.6 \pm 2.8$ , and  $44.7 \pm 1.2$   $\mu\text{g}$  toc/g emulsion and in SC-OS emulsion were  $179.2 \pm 0.7$ ,  $4.1 \pm 0.2$ ,  $117.5 \pm 0.4$ , and  $38.1 \pm 0.1$   $\mu\text{g}$  toc/g emulsion, respectively. The content of tocopherols in fresh emulsion was significantly lower when 50% of SC was substituted with OSA-starch, which indicated that tocopherols acted as an antioxidant or interacted with OSA-starch during emulsification.  $\beta$ -Tocopherol contents in both SC and SC-OS emulsions were low, so its changes were not considered.  $\delta$ -Tocopherol contents of both emulsions did not change significantly from day 0 to 28 ( $P > 0.05$ ). Our

results revealed that only  $\alpha$ - and  $\gamma$ -tocopherols participated in lipid oxidation reactions of emulsions. The percentage changes of  $\alpha$ - and  $\gamma$ -tocopherols in the SC emulsion after 28 days of storage were 39.28 and 27.87%, while their changes in the SC-OS emulsion were 46.92 and 38.46%, respectively. Therefore, the consumption of  $\alpha$ - and  $\gamma$ -tocopherol in SC emulsion was significantly lower than that of SC-OS emulsion during storage, which showed a lower oxidation rate in the SC emulsion.  $\alpha$ - and  $\gamma$ -tocopherol contents in the fresh emulsions were not affected by the addition of gallate alkyl esters (Fig. 2). Moreover, their content in the presence of gallate alkyl esters did not change significantly during the whole storage period ( $P > 0.05$ ). This could be related to the fact that  $\alpha$ - and  $\gamma$ -tocopherols were regenerated by gallate alkyl esters or were not consumed as an antioxidant (Qiu et al., 2017).

### 3.2.3. Changes in volatile compounds

The quantified volatile compounds during storage of all emulsions were 1-penten-3-ol, 1-penten-3-one, pentanal, (*E*)-2-pentenal, 1-pentanol, 2-penten-1-ol, heptanal, (*E,E*)-2,4-heptadienal, 2,3-pentanedione, (*E,E*)-2,6-nonadienal, (*E*)-2-hexenal and 2-ethylfuran (Supplementary Fig. 3). 1-penten-3-ol, 1-penten-3-one, (*E,E*)-2,4-heptadienal and (*E,E*)-2,6-nonadienal are decomposition products of EPA and DHA (LC- $\omega$ 3 PUFA), which have been detected as potent odorants in fish oil enriched-foods (Venkateshwarlu, Let, Meyer, & Jacobsen, 2004a). Moreover, 1-penten-3-ol, 1-penten-3-one, and (*E,E*)-2,6-nonadienal are markers for metallic and fishy off-flavors in fish oil-enriched products (Venkateshwarlu, Let, Meyer, & Jacobsen, 2004b). Fig. 3 shows the formation of 1-penten-3-ol, 1-penten-3-one, (*E,E*)-2,4-heptadienal and (*E,E*)-2,6-nonadienal in SC and SC-OS emulsions with/without gallate alkyl esters during 28 days of storage. The concentration of volatile compounds in both emulsions was as follows: 1-penten-3-ol > (*E,E*)-2,4-heptadienal > (*E,E*)-2,6-nonadienal > 1-penten-3-one. Similar to PV results, SC emulsion had significantly lower contents of volatile compounds compared to SC-OS emulsion during storage, although the difference in the values of volatile compounds was much higher than that of PV. This might be due to the formation of a more stable interfacial layer with a better coverage of the oil droplets, which is expected to limit the diffusion of pro-oxidants (e.g. metal ions) from the water phase to the oil phase. However, the antioxidant activity of SC present in the

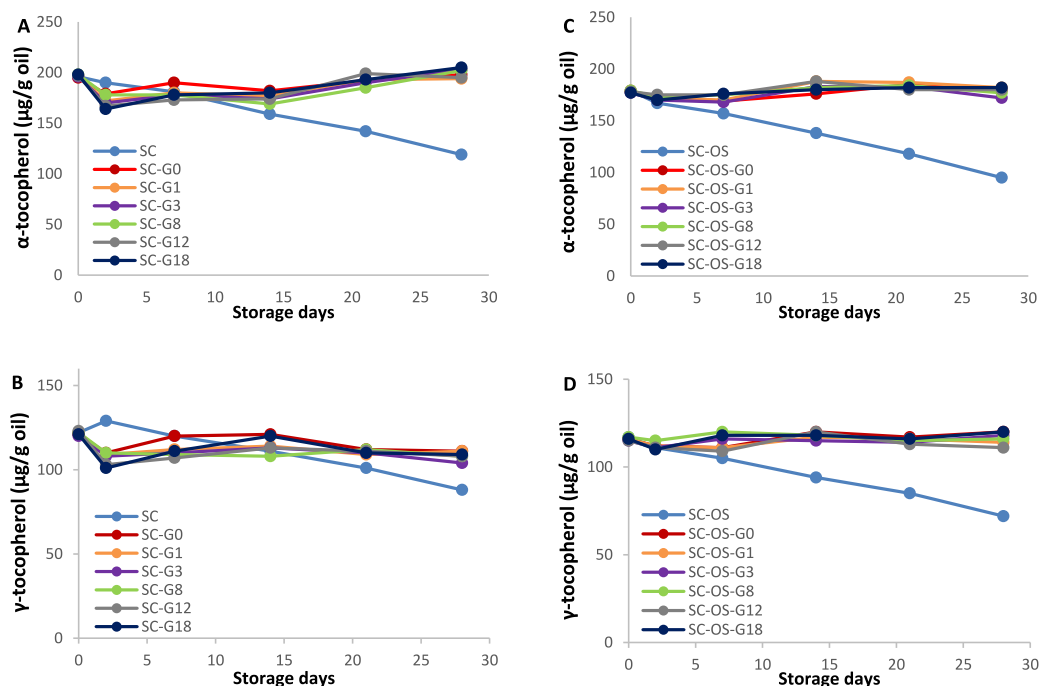


Fig. 2. Changes in  $\alpha$ -tocopherol (A-B) and  $\gamma$ -tocopherol (C-D) of emulsion samples during 28 days of storage at 25 °C.

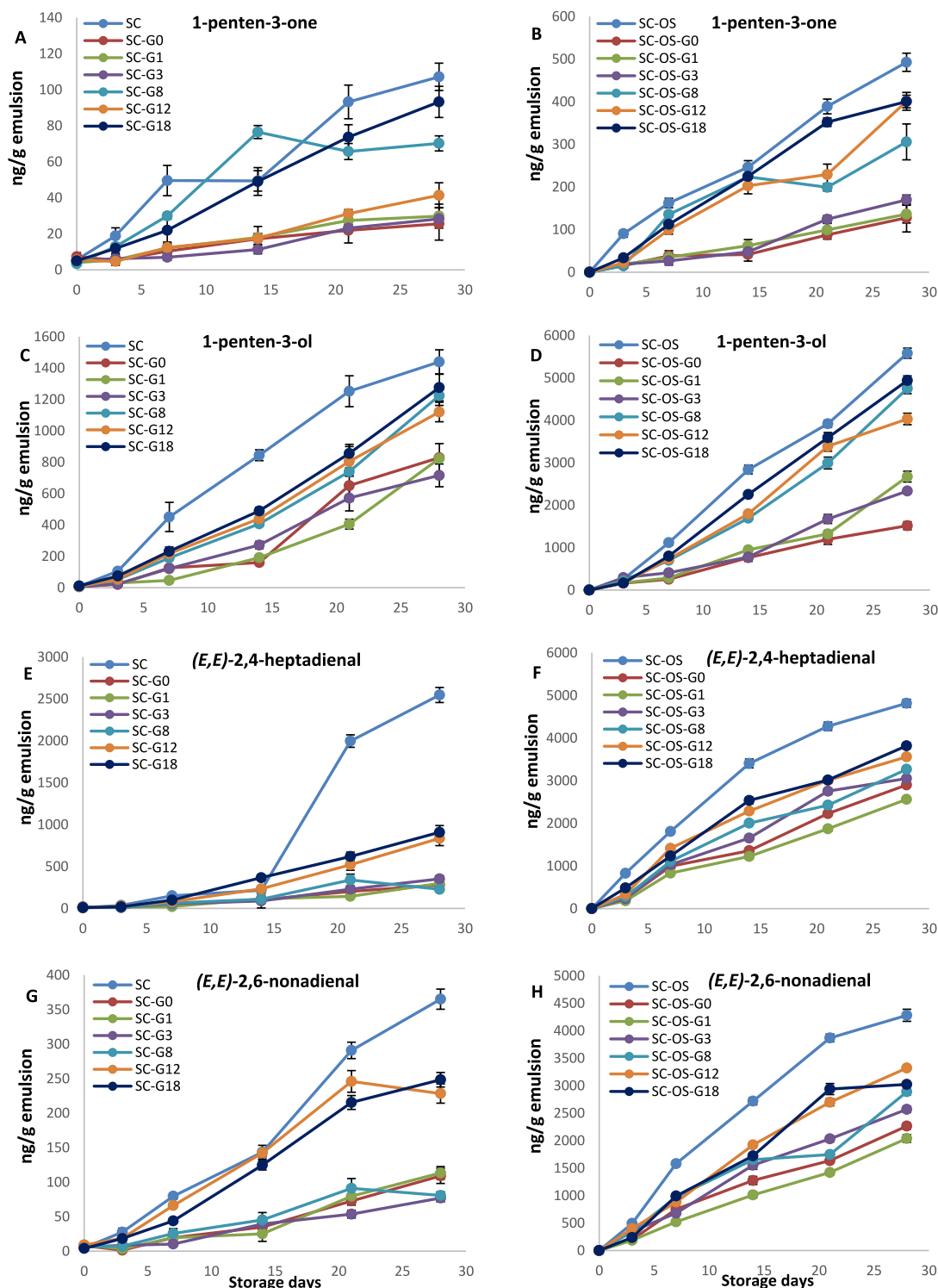


Fig. 3. Secondary volatile oxidation products (ng/g) formed in emulsion samples during 28 days of storage at 25 °C.

water phase and SC adsorbed at the interface may have decreased the formation of volatile compounds in emulsions. This was in line with our previous study that indicated partly replacing SC with phosphatidylcholine increased the concentration of volatile compounds (Yesiltas et al., 2019).

The concentration of 1-penten-3-one in SC emulsion decreased with the addition of gallates, but G12 and G18 showed a weak inhibitory effect compared to other gallates (Fig. 3A). 1-penten-3-one content in SC-G0, SC-G1, SC-G3, and SC-G8 was very low and did not increase until

day 7. After that, the concentration of 1-penten-3-one in these samples increased slightly with no significant difference until day 21 ( $P > 0.05$ ). Overall, a strong inhibition effect was observed by G0, G1 and G3 with no significant differences against the formation of 1-penten-3-one in SC emulsion. According to Fig. 3B, 1-penten-3-one content in the SC-OS emulsion increased dramatically after day 3. The increase of both 1-penten-3-one and PV in SC and SC-OS emulsions showed that lipid oxidation progressed after day 3. SC-OS emulsion containing gallates had significantly lower content of 1-penten-3-one during storage



compared to the control emulsion ( $P < 0.05$ ). G8, G12 and G18 did not lead to a higher reduction of 1-penten-3-one content in SC-OS emulsion until day 14. On day 21, SC-OS-G8 and SC-OS-G12 had a lower concentration of 1-penten-3-one than SC-OS-G18, while its level in SC-OS-G8 was lower than SC-OS-G12 and SC-OS-G18 on day 28. Addition of G0, G1 and G3 to SC-OS emulsion had a better inhibitory effect on reduction of 1-penten-3-one content than G8, G12 and G18. The concentration of 1-penten-3-one in SC-OS-G0, SC-OS-G1 and SC-OS-G3 had no significant differences until day 14, but its amount in SC-OS-G3 increased more from day 14 to day 28 than SC-OS-G0 and SC-OS-G1.

The content of 1-penten-3-ol in SC emulsion samples significantly increased after day 3, especially for SC-G8, SC-G12 and SC-G18 (Fig. 3C). Inhibitory effect of gallates against the formation of 1-penten-3-ol in SC emulsion was different from 1-penten-3-one. The SC-G1 showed a lower concentration of 1-penten-3-ol until day 21; however, the SC-G0 had a 1-penten-3-ol content significantly lower than that of the SC-G1 on day 28. As can be seen in Fig. 3D, 1-penten-3-ol content in SC-OS-G0, SC-OS-G1 and SC-OS-G3 was lower than that of other samples during the whole storage period. Although no significant difference was observed in 1-penten-3-ol content of SC-OS-G0, SC-OS-G1 and SC-OS-G3 until day 14, but SC-OS-G0 had lower 1-penten-3-ol content from day 14 to day 28. In general, 1-penten-3-ol content in both SC and SC-OS emulsions containing medium and long chain gallates was higher than the emulsions containing short chain.

The content of (*E,E*)-2,4-heptadienal in SC emulsion containing G0, G1, G3 and G8 with no significant difference was lower than that of SC emulsion containing G12 and G18 until day 14 (Fig. 3E). On day 21, G8 showed a weak inhibitory effect against (*E,E*)-2,4-heptadienal formation compared to G0, G1 and G3. After day 21, SC emulsion containing G0 and G1 with no significant difference had a lower concentration of (*E,E*)-2,4-heptadienal than the other emulsions. In contrast, for SC-OS emulsion containing gallate alkyl esters the ranking order of the concentration of (*E,E*)-2,4-heptadienal was as follows: G18 > G12 > G8 > G3 > G0 > G1 (Fig. 3F). However, similar to SC emulsions, all SC-OS emulsions containing gallate alkyl esters had a significantly lower concentration than the emulsion without antioxidant addition.

(*E,E*)-2,6-nonadienal content in SC emulsion containing G12 and G18 was higher than the other gallates during the whole storage period (Fig. 3G), suggesting a weak inhibitory effect exerted by these lipophilic antioxidants. G0, G1, G3, and G8 had almost similar antioxidant efficiency until day 14, but on days 21 and 28, G1 presented a stronger effect on the reduction of (*E,E*)-2,6-nonadienal concentration. In contrast, the concentration of (*E,E*)-2,6-nonadienal in SC-OS emulsion increased steadily during the whole storage period after a lag phase of 3 days (Fig. 3H). However, (*E,E*)-2,6-nonadienal content in SC-OS-G1 emulsion followed by SC-OS-G0 emulsion was significantly lower than other samples during 28 days of storage.

In general, the addition of gallate alkyl esters to SC emulsion was more efficient in reducing the content of volatile compounds compared to SC-OS emulsion, which is related to the synergistic effect of SC on the inhibitory capacity of gallates. This could be attributed to the higher partition or localization of gallates at the interface as affected by SC, which increased its diffusibility toward the oxidation sites by further reducing the interfacial tension. The most effective gallates added to both emulsions in terms of less formation of volatile compounds were those with short chain length, whereas the further increase in length of the alkyl chain led to the collapse in their inhibitory capacity. Our results were in accordance with the research findings of Qiu et al. (2017) and Costa et al. (2020), implying the more inhibitory activity of short-chain esterified ferulic acid and gallic acid against the formation of volatile oxidation products in oil-in-water emulsion. In general, the interfacial performance of G1 was better than that G0 and G3 due to the higher partition at the interface, thereby significantly decreased the concentration of volatile products in oil-in-water emulsion. The lower antioxidative efficiency of G0 and G3 can be related to their higher partition into the aqueous phase (da Silveira et al., 2021). However, free

gallates in the aqueous phase can limit the initiation of metal catalysis and free radical formation in emulsions (Hu et al., 2022). According to Sørensen et al. (2012), lipophilization of phenolic compounds beyond the critical chain length reduces their mobility by internalization and self-aggregation. A significant amount of G8, G12, and G18 was probably internalized inside the oil droplets due to their high hydrophobicity, leading to lower antioxidant efficiency in emulsions.

#### 4. Conclusions

In summary, compared to the SC, emulsions containing SC-OS not only increased the droplet size more evidently, but also presumably formed non-uniform layers, thereby intensifying lipid oxidation. SC with better interfacial activity was more effective in delaying the lipid oxidation of O/W emulsion, which was evident by the lower peroxide values and formation of volatile compounds. However, the use of SC alone or combined use of SC-OS emulsifiers did not change the order of antioxidative efficiency of the gallate alkyl esters evaluated in the emulsions. The addition of short-chain gallates was more effective than medium- to long-chain gallates in preventing lipid oxidation of emulsions, which most likely was due to the location of the antioxidant molecules in the systems. The interaction of SC emulsifier and short-chain alkyl gallates (especially G1) had a synergistic effect on the interfacial and antioxidant activities among other gallate alkyl esters in fish oil-in-water emulsion. SC modified the interfacial performance of G1 in the emulsion matrix by affecting their solubility and partitioning, thereby forming a stable delivery system for omega-3 PUFAs. Overall, our findings offer significant insight into the role of emulsifiers on the interfacial performance of lipophilic antioxidants, which contributes to multiple potential applications aimed at developing a wide variety of LC- $\omega$ 3 PUFA-enriched foods and beverages.

#### CRediT authorship contribution statement

**Mojtaba Delfanian:** Methodology, Validation, Investigation, Formal analysis, Data curation, Writing – original draft, Visualization. **Betül Yesiltas:** Supervision, Conceptualization, Writing – review & editing. **Ann-Dorit Moltke Sørensen:** Supervision, Conceptualization, Writing – review & editing. **Mohammad Ali Sahari:** Supervision, Project administration, Writing – review & editing. **Mohsen Barzegar:** Writing – review & editing. **Hassan Ahmadi Gavlighi:** Writing – review & editing. **Charlotte Jacobsen:** Supervision, Conceptualization, Writing – review & editing, Resources.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

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#### Appendix A. Supplementary data

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