Lipid oxidation products in model food emulsions: do they stay in or leave droplets, that's the question

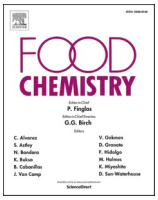
Sten Ten Klooster, Karin Schroën, Claire Berton-Carabin

Food Chemistry 405 (2023) 134992

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Lipid oxidation products in model food emulsions: do they stay in or leave droplets, that's the question

Sten ten Klooster [a,*], Karin Schroën [a], Claire Berton-Carabin [a,b]

[a] Laboratory of Food Process Engineering, Wageningen University, P.O. Box 17, Bornse Weilanden 9, 6708 WG Wageningen, the Netherlands
[b] INRAE, BIA, 44000 Nantes, France

ARTICLE INFO

Keywords:
Emulsions
Lipid oxidation
Hydroperoxides
Transfer
Surfactant micelles

ABSTRACT

Lipid oxidation is a major factor limiting the shelf life of food and other emulsion products. In this work, we explore which lipid oxidation products may transfer between oil droplets in model food emulsions stabilized by excess amounts of surfactant, and whether this affects the overall reaction. No significant differences in concentrations of triglyceride-bound hydroperoxides were found before and after mixing 'clean' oil droplets with pre-oxidized ones. Shorter and more hydrophilic lipid oxidation products, such as 4-hydroperoxy-2-nonenal and 2,4-decadienal, were found to equilibrate between oil droplets within 30 min. Adding exogenous 4-hydroperoxy-2-nonenal to an emulsion led to overall higher lipid oxidation values, although this effect was not systematic nor instantaneous. Therefore, it may be questioned whether transfer and subsequent initiation are always relevant for oxidizing emulsion systems. In future research, this question should be addressed for complex emulsions that are closer to real-life food products.

1. Introduction

Food or biobased products containing polyunsaturated fatty acids (PUFAs) are prone to lipid oxidation, which has a negative impact on the quality of the products (Schaich, 2005). This is particularly marked in oil-in-water (O/W) emulsions, where the large amount of oil–water interface allows contact between PUFAs and water-soluble pro-oxidants such as metal ions (Berton-Carabin, Ropers, & Genot, 2014). Lipid oxidation is therefore not only considered the main factor in determining the shelf life of food emulsions (e.g., mayonnaise), but can also be problematic in other biobased systems (pharmaceuticals [for example mRNA vaccines (Schoenmaker et al., 2021)], cosmetics, etc.). Accordingly, lipid oxidation in emulsions has received a lot of attention in the last two decades.

Besides the chemically complex cascade of reactions that occur during lipid oxidation, it has been proposed that the overall course of the reaction could be affected by transfer of lipid oxidation products between oil droplets, which could be facilitated through surfactant micelles (Laguerre, Bily, Roller, & Birtić, 2017). A well-known mechanism that may point in the direction of such a transfer is compositional ripening; in emulsions containing droplets made of different pure alkanes, complete exchange of the droplets' core materials usually takes place within days. This phenomenon has been reported for emulsions stabilized with surfactants or proteins, usually in excess (McClements, Dungan, German, & Kinsella, 1992; Samtlebe, Yucel, Weiss, & Coupland, 2012; Villeneuve et al., 2021). The exchange of other lipid or lipophilic components between oil droplets has also been studied, as summarized in Table 1. If such a transfer happens in oxidizing food emulsions, this may cause 'clean' (i.e., non-oxidized) oil droplets to become contaminated by lipid oxidation intermediate products from neighboring oxidizing droplets (Laguerre, Tenon, Bily, & Birtić, 2020).

Lipid oxidation intermediates, such as hydroperoxides, can react with metal ions at the oil–water interface, forming reactive peroxyl (LOO*) and alkoxyl (LO*) radicals that rapidly re-initiate the lipid oxidation radical chain reaction (Equations (1)–(4)) (Schaich, 2005). A recent hypothesis suggests that such transfer and 'secondary initiation' pathways are the main mechanisms for propagation of lipid oxidation (Laguerre et al., 2017, 2020).

$$LOOH + Fe^{3+} \rightarrow LOO^{*} + H^{+} + Fe^{2+} \quad (1)$$

$$LOOH + Fe^{2+} \rightarrow LO^{*} + OH^{-} + Fe^{3+} \quad (2)$$

$$LO^{*}/LOO^{*} + LH \rightarrow LOH/LOOH + L^{*} \quad (3)$$

* Corresponding author.
E-mail addresses: sten.tenklooster@wur.nl (S. Klooster), karin.schroen@wur.nl (K. Schroën), claire.berton-carabin@inrae.fr (C. Berton-Carabin).

https://doi.org/10.1016/j.foodchem.2022.134992
Received 14 July 2022; Received in revised form 11 November 2022; Accepted 15 November 2022
Available online 21 November 2022

$$L^{*} + O_2 \rightarrow LOO^{*} \quad (4)$$

Another recent paper showed that a lipophilic fluorescent dye (BODIPY$^{665/676}$), present in medium chain triglyceride (MCT) emulsion droplets, oxidizes faster when oxidizing vegetable oil droplets are present in the same emulsion (Li, McClements, & Decker, 2020). This may suggest that lipid oxidation can spread from oxidizing droplets to initially clean ones. It is, however, unclear which components would be responsible for this.

In the present study, we aim to unravel which lipid oxidation products may transfer between oil droplets in a model food emulsion, and whether transferred molecules would substantially promote lipid oxidation. To do so, an emulsion prepared with high-density, pre-oxidized oil was mixed with an emulsion made with regular (low-density) clean oil. Both emulsions were mixed and incubated. Over time, the droplets that were made with oxidized oil were physically separated from the droplets that were made with clean oil, owing to the density difference between both starting oils. Next, a range of lipid oxidation products were quantified with nuclear magnetic resonance (^{1}H NMR) in the initially clean droplets. In addition, the potential lipid oxidation-initiating effect of the oxidation product 4-hydroperoxy-2-nonenal was investigated.

Table 1
Overview of studies in which the exchange of one or multiple lipid or lipophilic molecules between oil droplets was investigated.

Article	Studied molecule(s) for transfer (% of oil phase)	Oil phase (% of total emulsion)	Continuous phase (% of continuous phase)	Results as interpreted in the papers.
(Nuchi et al., 2002)	4 wt.% peroxidized linoleic acid / methyl linoleate / trilinolein	10 % oil (96 wt% corn oil, 4 wt.% peroxidized lipids)	1 wt% Brij 76, 0.1 mM EDTA	All three types of lipid hydroperoxides were solubilized out of the lipid droplets into the continuous phase containing Brij 76 micelles, in the order of linolenic acid > methyl linoleate ~ trilinolein.
(Li et al., 2020)	1 wt% 2,4-decadienal	0.2 wt% oil (33 % brominated vegetable oil, 66 % MCT oil)	0.14 wt% SDS	This aldehyde equilibrated within 10 min between MCT oil droplets even without surfactant micelles (SDS concentration was 0.5 mM, CMC of SDS is 8 mM).
(Cui, Shen, Gao, Yi, & Chen, 2019)	Hydroperoxides of stripped walnut oil	5 wt% walnut oil	0.25 wt% SDS, with 0 / 0.3 / 0.6 wt% NaCl	Initially, ± 15 % of the hydroperoxides were solubilized by SDS micelles into the aqueous phase, and this increased to 35 % upon increasing the NaCl concentration to 0.3 wt%.
(Raudsepp, Brüggemann, Lenferink, Otto, & Andersen, 2014)	Medium chain triglyceride oil	82 wt% linseed oil / MCT oil	egg yolk (12 wt%), mustard (2 wt%), vinegar (2 wt%), lemon juice (2 wt%)	No mixing of the droplets' contents occurred over 2 d of incubation.
(McClements et al., 1992)	Hexadecane / octadecane	10 wt% hexadecane / octadecane	2 wt% Tween 20	Complete transfer of the oils (all droplets containing 50 % of both oils) in the droplets took over 7 d. The rate of exchange increased with additional surfactant.
(McClements, Dungan, German, & Kinsella, 1993)	Hexadecane / octadecane	10 wt% hexadecane / octadecane	0.5 / 2.5 wt% whey protein isolate / casein	Oil exchange occurred with both proteins and increased with protein concentration, but was incomplete after 29 d.
(Samtlebe et al., 2012)	Tetradecane / eicosane	10 wt% oil	1 / 2 wt% Tween 20	The mass transfer of *n*-tetradecane to *n*-eicosane occurred over the course of several hours. The rate of dissolution increased with the tetradecane droplet to eicosane emulsion ratio and with surfactant concentration.
	Tetradecane / eicosane	10 wt% oil	1 / 2 wt% sodium caseinate	There was some change in the alkane melting peak shape, but no evidence of substantial mass transfer in the time allowed regardless of the concentration of caseinate used.
	Caprylic/capric triacylglycerol / palm stearin	10 wt% oil	1 wt% sodium caseinate	The rate of mass transfer was slower in this triacylglycerol system (after 24 d still no equilibration) than for the hydrocarbon system (see above).
(Richards, Chaiyasit, McClements, & Decker, 2002)	Propyl gallate / tertiary butylhydroquinone / butylated hydroxytoluene	5 wt% hexadecane / olive oil / salmon oil	0.1 mM EDTA, 0.5–3 wt% Brij 700	The nonpolar antioxidant butylated hydroxytoluene was solubilized less than the more polar antioxidants tertiary butylhydroquinone and propyl gallate. Solubilization increased with added Brij 700 (after emulsification).
(Keller, Locquet, & Cuvelier, 2016)	Vanillic acid	30 wt% oil (not emulsified)	2 mmol vanillic acid / kg water, either with or without Tween 40 (0.9 / 3.6 wt%)	3/4 of the vanillic acid partitioned in the aqueous phase and 1/4 in the oil phase for the two-phase non-emulsified system without Tween 40. In the presence of Tween 40, the major part of vanillic acid (90 %) was found in the aqueous phase.
(Raudsepp et al., 2016)	5.7 μM DTBP (di-*tert*-butyl peroxide)	30 wt% stripped sunflower / MCT oil	0.72 wt% Tween 20, sodium acetate-acetic acid buffer (pH = 4.65)	DTBP radical life-times were short; thus, the radical chain reactions progressed only up to ~60 μm from the initiation site. The radical reactions were not able to cross the interfaces and progress to neighbouring oil droplets. The propagation and diffusion of radicals was dependent on the degree of unsaturation of the oil, and the viscosity of the lipid medium.
(Raudsepp, Brüggemann, & Andersen, 2014)	13 mM AMVN (2,2′-azobis(2,4-dimethyl) valeronitrile)	30 wt% stripped sunflower / MCT oil	0.72 wt% Tween 20, sodium acetate-acetic acid buffer (pH = 4.65). Sodium acetate buffer (pH 4.65)	Radicals can be transferred between oil droplets and lipid autoxidation can spread between neighbouring oil droplets.
(Banerjee et al., 2018)	Propagation of lipid oxidation rection	70 wt% cod liver oil	4.7 wt% sodium caseinate	Oxidation reactions readily propagate through the interior of the oil droplet. The oxidation of one droplet does not appear to spread to an adjacent and neighbouring droplet.

2. Materials and methods

2.1. Materials

Rapeseed oil (kindly supplied by Unilever, Wageningen, the Netherlands) and a mixture of brominated oil (Spectrum Chemical, Gardena, USA) and *n*-hexane (Actu-All Chemicals, Oss, the Netherlands) (5:1 v/v) were stripped with alumina powder (MP112 EcoChromet ALUMINA N, Activity: Super I, Biomedicals) to remove impurities and endogenous antioxidants (in particular tocopherols) (Berton, Genot, & Ropers, 2011); hexane was then evaporated from the brominated oil by a continuous flow of nitrogen, while the mixture was stirred at 40 °C, until constant weight was reached. Sodium phosphate monobasic dihydrate and sodium phosphate dibasic dihydrate (Sigma-Aldrich, Zwijndrecht, the Netherlands) were used to make the phosphate buffer (pH 7.0). *n*-Hexane and 2-propanol were obtained from Actu-All Chemicals (Oss, the Netherlands). Deuterated chloroform and dimethylsulfoxide ($CDCl_3$ and DMSO-d_6) were purchased from Euriso-top (Saint-Aubin, France). Ethylenediaminetetraacetic acid calcium disodium salt (EDTA) and Tween 20 were from Sigma Aldrich (Sigma-Aldrich, Zwijndrecht, the Netherlands). 4-Hydroperoxy 2-nonenal (purity ≥ 95 %) was obtained from Cayman Chemical (Ann Arbor, USA), and *trans*, *trans*-2,4-decadienal (purity ≥ 97 %) was obtained from Fisher Scientific (Roskilde, Denmark). Ultrapure water (18.2 MΩ) was used for all experiments and prepared using a Milli-Q system (Millipore Corporation, Billerica, MA, USA).

2.2. Methods

2.2.1. Preparation of the pre-oxidized oil

50 g of stripped rapeseed oil were incubated in a 250-mL bottle with the lid loosely put on the bottle in an oven at 40 °C and stirred for 7 d at 100 rpm. After 7 d, the oxidation level was measured (see section 2.2.5). If the oxidation level was lower than desired (<60 mmol hydroperoxides / kg oil), the incubation was extended, until the desired level was reached. Afterwards, the oxidized oil was collected and stored with a nitrogen blanket at −80 °C until further use. For the independent replicates, the same batch of pre-oxidized oil was used. For the experiments with 0.5 and 2 wt% Tween 20, a different batch of pre-oxidized oil was used. The pre-oxidized rapeseed oil was mixed with stripped brominated oil in a mass ratio of 65:35.

2.2.2. Emulsion preparation, incubation and sample taking

2.2.2.1. Transfer experiment with pre-oxidized oil. Either 0.5 or 2 wt% of Tween 20 was dissolved in a 10-mM phosphate buffer (pH 7.0) and stirred for 15 min at 300 rpm. Next, EDTA (75 mg / kg continuous phase) was added, and the solution was stirred for 15 min. A coarse emulsion was made by adding 10 wt% of either the stripped rapeseed oil (for the clean emulsion) or the mixture of pre-oxidized oil and brominated oil (for the pre-oxidized emulsion) (see section 2.2.1) to the continuous phase, and high-speed stirring was applied at 11,000 rpm for 1 min with a rotor–stator homogenizer (Ultra-turrax IKA T18 basic, Germany) (Fig. 1A & B). To obtain the final emulsion, the coarse emulsions were homogenized by passing them three times through a high pressure M−110Y Microfluidizer (Microfluidics, Massachusetts, USA), equipped with a Y-shaped interaction chamber (F12Y; minimum internal dimension: 75 μm), at 600 bars. The clean emulsion was mixed with the pre-oxidized emulsion in a 1:1 ratio (w/w) (Fig. 1C). Finally, 12 mL of mixed emulsion were added to 20-mL headspace vials, which were treated with a nitrogen blanket to minimize further oxidation. The tubes were rotated horizontally at 2 rpm in a dark oven at 25 °C for up to 14 d.

2.2.2.2. Sample taking procedure for transfer experiments. Samples from the mixed emulsion were taken at carefully selected time points, and the initially clean droplets were separated from the initially oxidized droplets by centrifuging 20 mL of emulsion in 50-mL tubes at 28,000xg for 30 min at 4 °C, which was based on previous research (Li et al., 2020). The liquid in between the thick cream and thick sediment was discarded, and a small amount (~0.2 g) of cream or sediment was transferred with a spatula into 15-mL centrifuge tubes and redispersed in 1.5 mL ultrapure water (Fig. 1D). Next, the samples were treated with a nitrogen blanket and stored at −80 °C until further use (minimally 48 h, maximally 20 d) (Merkx, Hong, Ermacora, & Van Duynhoven, 2018; Ten Klooster et al., 2022).

2.2.2.3. Transfer of exogenously added aldehydes. Tween 20 (2 wt%) was dissolved in a 10-mM phosphate buffer (pH 7.0) and stirred for 15 min at 300 rpm. Next, EDTA (75 mg / kg continuous phase) was added, and the solution was stirred for 15 min. For this set of emulsions, the used oil was either the stripped rapeseed oil for the 'clean' droplets or, for the 'supplemented' droplets, a mix of stripped rapeseed oil (65 wt%) and stripped brominated oil (35 wt%) with 0.2 mmol 4-hydroperoxy-2-nonenal or 2,4-decadienal per kg oil (commercial aldehydes, added exogenously to the oil). The emulsion was prepared as described in 2.2.2.1. The clean emulsion was mixed with the emulsion containing the exogenous aldehyde in a 1:1 ratio (w/w). Sample taking was performed immediately after carefully mixing the two emulsions, and this was done as described under 2.2.2.2.

2.2.2.4. Effect of exogenously added 4-hydroperoxy-2-nonenal on lipid oxidation. Tween 20 (0.5 wt%) was dissolved in ultrapure water and

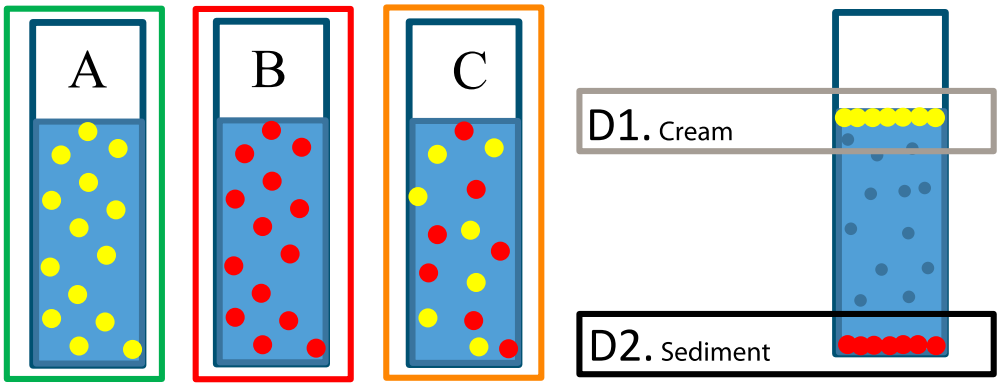


Fig. 1. Schematic representation of the samples that were prepared and analysed for this research. A. indicates the emulsion produced with clean oil, B. the emulsion made with pre-oxidized oil (or spiked with exogenously added aldehydes), C. is the 1:1 ratio (w/w) emulsion of A and B that was incubated. Over time, samples were taken from emulsion C, and this emulsion was centrifuged to collect the cream (D1) containing the initially clean droplets and sediment (D2) containing the pre-oxidized droplets.

stirred for 30 min at 300 rpm. Stripped rapeseed oil was added with or without 0.2 mmol 4-hydroperoxy-2-nonenal / kg oil. A coarse emulsion was then made by mixing the oil (10 wt% of final emulsion) and aqueous phases with a high-speed stirrer at 11,000 rpm for 1 min with a rotor–stator homogenizer (Ultra-turrax IKA T18 basic, Germany). The fine emulsion was prepared by passing the coarse emulsion through a lab scale colloid mill with gap width of 0.32 mm (IKA Magic Lab, Staufen, Germany), operating for 1.5 min at 26,000 rpm, and the colloid mill was cooled with water at 4 °C. Headspace vials (20 mL) containing 2 mL emulsion were rotated horizontally at 2 rpm in a dark oven at 25 °C for up to 14 d.

2.2.3. Droplet size measurement

The oil droplet size of the emulsions was measured by static light scattering (SLS) (Malvern Mastersizer 3000, Malvern Instruments ltd., Malvern, Worcestershire, UK), using a refractive index of 1.465 for the dispersed phase and 1.33 for the dispersant (water); and an absorption index of 0.01. The droplet sizes for all the prepared emulsions remained constant over an incubation period of 14 d (Supplementary Material Figure S1). The droplet size distributions in the creamed layers were always very similar to the droplet size distributions of the clean emulsions (prior to mixing), which confirms that the clean droplets can be effectively isolated from the mixed system (Supplementary Material Figure S2 & S3).

The continuous phase and the smallest oil droplets were separated from the larger oil droplets by centrifuging 2 mL of emulsion at 20,000x*g* for 60 min in a 2-mL Eppendorf tube and collecting ~ 0.3 mL of the subnatant. The size of the colloidal structures present in this subnatant were measured by dynamic light scattering (DLS) (Zetasizer Nano ZS, Malvern Instruments ltd., Malvern, Worcestershire, UK). The refractive index was 1.465 for the dispersed phase, and the absorbance was 0.01.

2.2.4. Lipid extraction

The extraction was performed by adding 8 mL hexane–isopropanol (3:1 v/v) to ± 1.5 mL emulsion and vortexing thoroughly, as previously described (Waraho, McClements, & Decker, 2011). The mixture was centrifuged at 4,000x*g* for 20 min and the upper layer, containing the hexane and fat, was carefully separated from the bottom layer. The hexane was evaporated under a stream of nitrogen at 25 °C until constant weight and the remaining oil was treated with a nitrogen blanket and frozen at −80 °C for a minimum of 48 h and a maximum of 20 d until further measurements, which was based on previous research (Ten Klooster et al., 2022).

2.2.5. Lipid oxidation measured by ^1H NMR

Hydroperoxides (primary oxidation products), aldehydes (secondary oxidation products) and triacylglycerols (as a reference for the total amount of oil) were simultaneously quantified using ^{1}H NMR, with an Advance III 600 MHz spectrometer, equipped with a 5-mm cryo-probe at 295 K, following the method described by Merkx et al. (2018). In brief, extracted oil (as described in 2.2.4) and a mixture of $CDCl_3$/DMSO-d_6 (5:1 v/v) were mixed in a ratio of 1:3 (v/v) and transferred to 5-mm NMR tubes (Bruker, Billerica, Massachusetts, USA). From the recorded single pulse experiment, the glycerol backbone peaks at δ 4.4 pm were used for the quantification of the amount of triacylglycerols. From the band selective pulse, the region between δ 13.0 and 8.0 ppm was selectively excited for the quantification of the lipid oxidation products, following Merkx et al. (2018). The hydroperoxide signals resonate between δ 11.3 and 10.6 ppm, and the aldehydes resonate between δ 9.8 and 9.4 ppm. The calculations, including a factor that accounts for intensity loss during the selective pulse, are described in (Merkx et al., 2018). The data were processed with the Bruker TopSpin 4.0.6 software.

2.3. Experimental design

For each measurement, at least two emulsions were prepared independently. For the experiment on the effect of 4-hydroperoxy-2-nonenal on lipid oxidation, four emulsions were prepared independently per condition investigated. Additionally, per time point, two independently incubated samples from the same emulsion were analyzed for droplet size and lipid oxidation products. Statistical analysis of variance (F-test and T-test) (Microsoft Office Excel 2016) was carried out on experimental lipid oxidation values between different samples. Differences at $p < 0.05$ were considered significant.

3. Results and discussion

3.1. Colloidal structures in the emulsions

The emulsion droplets containing the clean oil, the pre-oxidized oil, and the 1:1 w/w mixture of both had sizes around 0.1–0.8 μm as measured by SLS, and the emulsions were physically stable over incubation (Fig. 2, Supplementary Material Figure S2). We also detected small association colloids with sizes around 25–100 nm in the subnatant of a centrifuged emulsion (Fig. 2). Empty surfactant micelles (with sizes corresponding to the yellow line in Fig. 2) could not be detected in the emulsions' subnatants by DLS, but cryo-TEM did allow us to highlight their presence (unpublished results). This is in agreement with other studies, in which it was shown that empty micelles (and very small oil droplets) could not be detected by DLS due to the presence of larger droplets, which very largely dominate the scattering signal; yet, NMR investigations confirmed the presence of such smaller structures (Awad, Asker, & Romsted, 2018; Law & Britton, 2012). It was previously reported that the subnatant (centrifugation conditions: 35 min 24,000x*g*), referred to as the continuous phase, contained hydroperoxides (Nuchi, Hernandez, McClements, & Decker, 2002). Since physical characterization of the subnatant was not reported in this previous study, this may also have been small droplets with a diameter ≤ 100 nm, instead of oil-free micelles (Nuchi et al., 2002).

3.2. Triglyceride-bound hydroperoxides

Directly after mixing the clean with the oxidized droplets, the clean droplets were isolated by collecting a sample of the creamed phase after centrifugation (Fig. 1). The level of TAG-bound hydroperoxides of the clean emulsion droplets isolated from the mix was found to be unaffected by the mixing ($p > 0.05$) (Supplementary Material Figure S4). This is an indication that: (1) we can properly separate the droplets initially made with clean oil from the mixed emulsion, and (2) that transfer of TAG-bound hydroperoxides is not immediate (or below the detection threshold). For the emulsions prepared with 2.0 wt% Tween 20, some cream samples had a higher hydroperoxide content than the non-mixed clean emulsion, but this slight difference was not statistically significant ($p > 0.05$) (Supplementary Material Figure S4). This may have been caused by the presence of very small pre-oxidized droplets (Fig. 2) that cannot be removed effectively by centrifugation, which results in a relatively large variability in hydroperoxide concentrations (Supplementary Material Figure S4, right panel).

The lipid hydroperoxide content in the mixed emulsion was measured over time for emulsions prepared with 0.5 and 2 wt% Tween 20 (Fig. 3a & b, respectively). The hydroperoxide content in the mixed emulsion and in the pre-oxidized droplets seems to be relatively constant over time, which is due to the incubation conducted in the presence of EDTA and under low amounts of oxygen (headspace contains ~ 0.3 v/v % O_2 after applying the nitrogen blanket). The hydroperoxide content in the initially clean droplets remained close to zero over the incubation for about 10 days, for both the emulsions prepared with 0.5 wt% Tween 20 and with 2 wt% Tween 20 (Fig. 3a & b, respectively). This indicates that barely any (if any at all) hydroperoxides transferred from the pre-oxidized droplets to the clean droplets in the presence of an excess amount of surfactant and under rotation (i.e., no concentration gradients in the samples). Similar conclusions of barely any transfer (if any) of

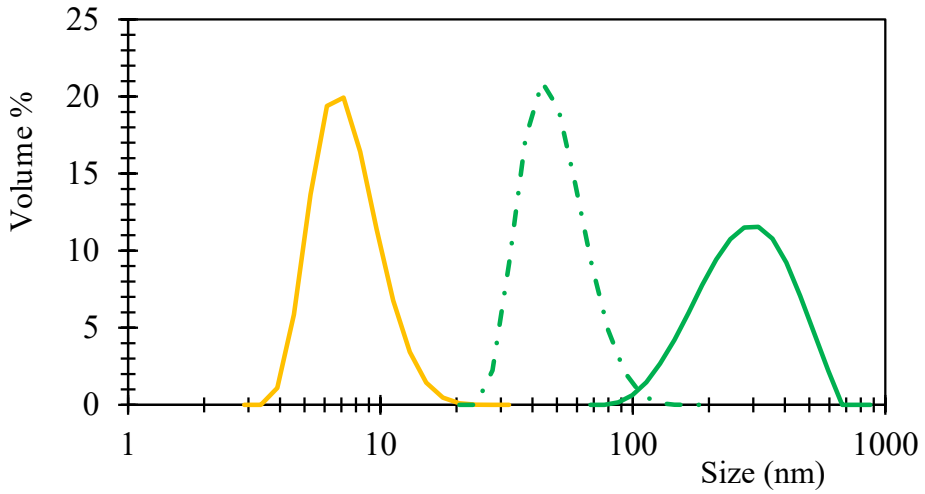


Fig. 2. Droplet size distributions in (from left to right): a 0.25 wt% Tween 20 solution by DLS (yellow solid line); the subnatant collected after centrifugation of an emulsion made with clean rapeseed oil and 2 wt% Tween 20 by DLS (green dotted line); and the whole clean emulsion made with 2 wt% Tween 20 by SLS (solid green line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

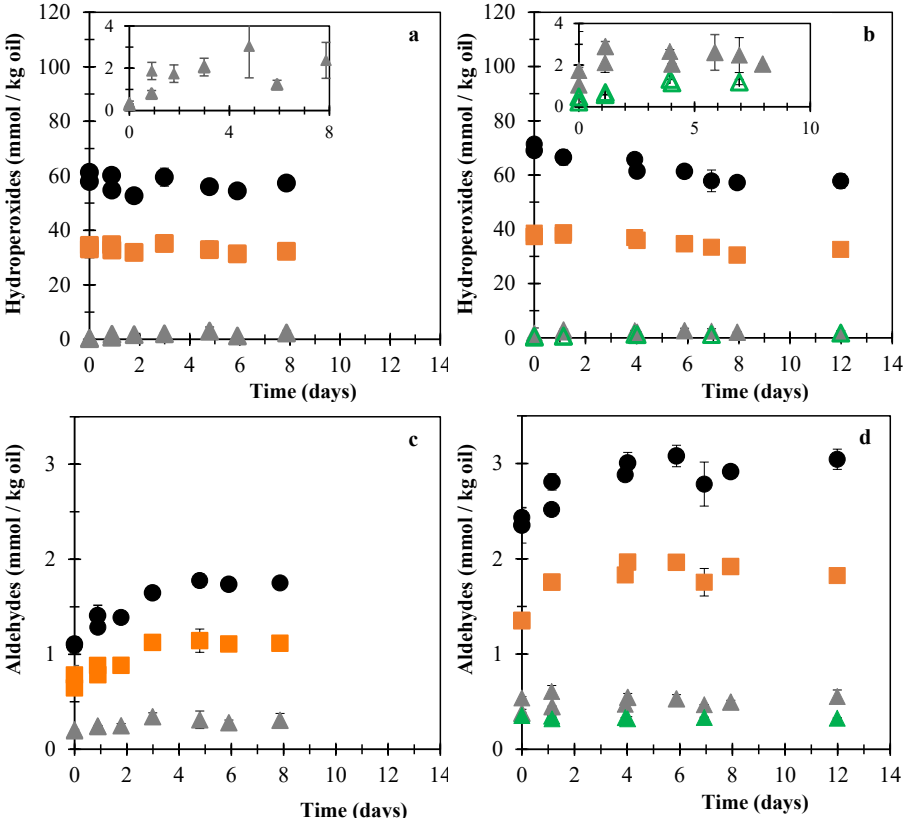


Fig. 3. Hydroperoxide (a & b) and aldehyde (c & d) content over incubation. Symbols correspond to: the (homogeneous) mix of clean and pre-oxidized emulsion droplets (□), the pre-oxidized droplets isolated from the mix (●), the clean droplets isolated from the mix (△), and the clean emulsion incubated separately under the same conditions (△), for the emulsions prepared with 0.5 wt% Tween 20 (a & c) and 2 wt% Tween 20 (b & d). Error bars (sometimes within the marker) denote standard deviations of two independently incubated samples originating from the same emulsion. The outcomes of the independent replicates are shown as separate points.

lipid molecules between O/W emulsion droplets were reported for medium chain triglycerides (Raudsepp, Brüggemann, & Andersen, 2014; Samtlebe et al., 2012). Our results show that the presence of one or multiple hydroperoxide groups on a triglyceride molecule does not make it more prone to transfer, either via collision of droplets or through transfer via the continuous phase, which are two mechanisms that were theoretically proposed (Laguerre et al., 2017).

In contrast, for emulsions containing alkenes, a complete equilibration occurred within 3–6 days (McClements et al., 1992). There is obviously a great difference in solubility in the continuous phase of alkenes compared to long-chain triglycerides. As the latter have almost zero-solubility in water (Wooster, Golding, & Sanguansri, 2008), this most likely hampers any related transfer via solubilization in the continuous phase. Alternatively, transfer may take place through surfactant micelles, although this possibility is largely contingent on the type of lipid (Villeneuve et al., 2021). For instance, the large size of

triglycerides (~3.4 nm) compared to the micelle core (~2 nm) (Villeneuve et al., 2021), may be the reason that these molecules cannot be as easily included in surfactant micelles as, for example, *n*-hexadecane molecules (~1.8 nm) (Coupland et al., 1996). You and coworkers suggested that the reduction of transfer can also be caused by increased hydrophobic interactions between the oil and the targeted molecule (You et al., 2012), which they based on a finding that by increasing the hydrophobic chain length of the surfactant from 4 to 8 groups, the transfer of the surfactant was ~ 600 times slower. Such hydrophobic interactions between triglycerides could also prevent triglycerides (bearing a hydroperoxide group) from transferring to other droplets.

If we zoom in on the hydroperoxide levels in the clean droplets over time (inserts Fig. 3a & b), they increased by ~ 1–3 mmol/kg oil over the first day of incubation, and then remained constant. There are two possible explanations for this result: (1) a transfer of hydroperoxides from the oxidized droplets to the initially clean ones, or (2) *in situ* oxidation of the oil droplets collected in the cream. If this minor increase were due to transfer, we would expect the hydroperoxide content to increase further from 1 to 12 days, but this did not occur. When the clean emulsion droplets were incubated independently (i.e., without being mixed with pre-oxidized ones), under the same conditions, the hydroperoxide content also increased slightly to ~ 1.5 mmol/kg oil (Fig. 3b), which is similar ($p > 0.05$) to the concentration in the cream at t = 7 days and t = 12 days. Moreover, we cannot exclude that the clean droplets in the mixed system oxidize faster than the clean droplets that were incubated separately, which is further addressed in section 3.4.

3.3. Can certain aldehydes transfer to other droplets?

Similarly to hydroperoxides, the aldehyde content was measured in the mixed emulsion, and in the clean and pre-oxidized droplets after separation by centrifugation (Fig. 3c & d, respectively). Please note that the initial aldehyde content was slightly different between the samples made with 0.5 and 2 wt% Tween 20 because a different batch of oxidized oil was used. As was the case for hydroperoxides, the aldehyde content in the initially clean droplets in the mixed emulsion did not seem to increase over incubation time (Fig. 3c & d). The aldehyde content in the mix and in the oxidized droplets from the mix increased slightly over the incubation period, most probably as a result of radicals formed during homogenization that keep propagating the reaction (Serfert, Drusch, & Schwarz, 2009). It was expected that EDTA would prevent secondary oxidation product formation since it chelates pro-oxidant cations such as metal ions, which is why it is recognized as a strong antioxidant (Mcclements & Decker, 2000).

The ^{1}H NMR method allows us to quantify specific secondary oxidation molecules, such as 4-hydro(pero)xyenals and 2-alkenals, with very low quantification thresholds (Guillén & Ruiz, 2004; Merkx et al., 2018). These aldehydes constitute the main part of the total aldehydes in the pre-oxidized oil (Supplementary Material Figure S5). With the ^{1}H NMR method, next to aldehydes such as hexanal, propanal, hexenal, etc., also aldehyde groups that are still attached to a triglyceride (TAG), called oxo-2.5 glycerides, are measured (Hollebrands & Janssen, 2017). The level of 4-hydro(pero)xyenals and 2-alkenals in the clean emulsion at t_0, in the oxidized emulsion droplets at t_0 and in the clean droplets isolated from the mix at t_0 and t = 1 d are shown in Fig. 4a & b. These results indicate that small amounts of 4-hydroperoxyenals and 2-alkenals can transfer from the oxidized droplets to the clean ones, and do so relatively rapidly (<30 min). We performed a similar experiment, but then with pure exogenous commercial aldehydes 4-hydroperoxy-2-nonenal and 2,4-decadienal. These components almost totally equilibrate over the emulsion droplets (Fig. 4c & d), which was in agreement with the publication by Li et al., where it was shown that 2,4-decadienal could rapidly transfer between MCT oil droplets (Li et al., 2020). This indicates that from the pre-oxidized oil, only the low molecular weight oxidation products are expected to transfer, whereas oxo-2.5 glycerides could not transfer, just like the TAG-bound hydroperoxides (section 3.2) (Fig. 4). This finding can probably be explained by the higher water solubility of the low molecular weight oxidation products (Wooster et al., 2008). Alternative explanations are that the latter would fit inside the micelle core (~2 nm) (Villeneuve et al., 2021), or that they have weaker hydrophobic interactions with the oil (You et al., 2012). Lipid oxidation was not shown to progress to neighboring droplets previously (Banerjee, Breitenbach, & Ogilby, 2018; Raudsepp, Brüggemann, Knudsen, & Andersen, 2016), which could be clarified by our finding that only very low amounts of lipid oxidation transfer from oxidized to clean oil droplets (Fig. 4). In contrast, it has been shown to quickly

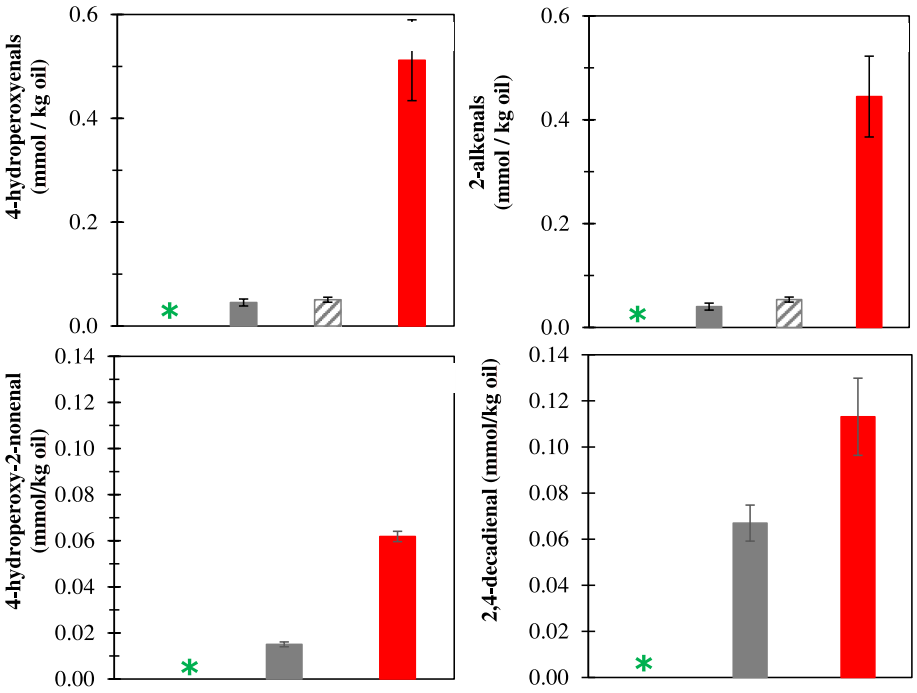


Fig. 4. Specific secondary oxidation product content in emulsion samples produced with 2 wt% Tween 20, where a & b are about the transfer of endogenous oxidation products and c & d about commercial exogenously added aldehydes. The bars with different colours correspond to different emulsion samples: from left to right: the clean emulsion immediately after homogenization (green), the clean droplets re-isolated from the mix immediately after gently mixing (t_0) (filled grey) and after 1 d of incubation (striped grey), and the pre-oxidized emulsion sample immediately after homogenization (filled red). * indicates below detection threshold. Error bars denote standard deviations of two independent replicates that are both measured twice. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

progress within one droplet itself (Banerjee et al., 2018). Yet, there is also experimental evidence that under certain conditions lipid oxidation does progress to neighboring droplets (Li et al., 2020; Raudsepp, Brüggemann et al., 2014). Li and coworkers hypothesized that low amounts of low molecular weight aldehydes may be a possible source of initiation of oxidation (Li et al., 2020), which is discussed in the next section.

3.4. Oxidation re-initiation by lipid oxidation products

Emulsions containing 0.2 mmol commercial 4-hydroperoxy-2-nonenal/kg oil (added exogenously to the oil prior to emulsification) and blank emulsions were incubated and analyzed for hydroperoxide concentrations (Fig. 5). As a general trend, the blank emulsions show lower hydroperoxide levels (which was significantly different at t = 2 d, but insignificant at the other time points, which can be explained by rather large differences between the replicates). Thus, the lipid oxidation-initiating effect that 4-hydroperoxy-2-nonenal may cause does not seem highly systematic or, at least, reproducible. This can be explained by the fact that lipid oxidation is a radical chain reaction, which implies that *if* lipid oxidation is initiated (e.g., by 4-hydroperoxy-2-nonenal), hydroperoxides keep being formed in a cascaded manner (Equations (1)–(4)). This also implies that if lipid oxidation is indeed initiated, e.g., by iron-induced decomposition of 4-hydroperoxy-2-nonenal into alkoxyl and peroxyl radicals (Eqs. (1)–(3)), it may take a few days before the effect on the formation of hydroperoxides becomes pronounced, as also argued in a recently published article (Schroën & Berton-Carabin, 2022). Therefore, it is questionable whether the transfer of lipid oxidation reactive products and subsequent initiation does substantially contribute to propagation of lipid oxidation in emulsions, or whether it is subordinate to the already oxidizing droplets that oxidize further at high rates. This would be in line with experimental findings that lipid oxidation does not progress to neighbouring droplets (Banerjee et al., 2018; Raudsepp et al., 2016). It has also been suggested that certain radicals can transfer between oil droplets and thereby spread lipid oxidation (Raudsepp, Brüggemann et al., 2014). This could speed up spreading of lipid oxidation because these radicals (unlike e.g. 4-hydroperoxy-2-nonenal), can directly propagate lipid oxidation in relatively clean oil droplets without having to be formed into a radical first. Whether a radical present in an oxidizing oil droplet can transfer and initiate lipid oxidation in a clean oil droplet is highly dependent on its lifetime, the time required to transfer from one droplet to another (Laguerre et al., 2017), the degree of unsaturation of the oil and the viscosity of the lipid phase (Raudsepp et al., 2016). Still, whether transfer plays a significant role in overall lipid oxidation is expected to be sample and condition dependent: pro-oxidant effect of (transferred) lipid oxidation products, storage temperature, O_2 availability, oil content, pro-oxidant (metal ion) availability, etc.

4. Conclusion

Starting from a previously published hypothesis that one oil droplet can contaminate neighbouring droplets with lipid oxidation, and thereby propagate this undesired reaction in emulsions (Laguerre et al., 2017, 2020; Villeneuve, Durand, & Decker, 2018), we herein investigate which molecules could play a role in such a scenario. This was done by mixing an emulsion made with pre-oxidized rapeseed oil with an emulsion made with clean rapeseed oil. To the best of our knowledge, we are the first to show that barely any TAG-bound hydroperoxides transfer from one droplet to another (under the applied conditions), if they do at all, even though an excess amount of surfactant was present in the continuous phase and the emulsions were rotated over incubation. It was shown previously that alkadienals can transfer from one MCT oil droplet to another (Li et al., 2020), and our work confirms that such relatively short and hydrophilic molecules, such as 2-alkenals, alkadienals and 4-hydroperoxy-2-alkenals, can equilibrate rapidly (<30 min) over the rapeseed emulsion droplets, although the effect on lipid oxidation is not instantaneous. Therefore, it may be questioned whether the transfer and subsequent initiation are always relevant for oxidizing emulsion systems. It is clear that this highly depends on the emulsion system of interest and the applied conditions, and more research is required to elucidate this further.

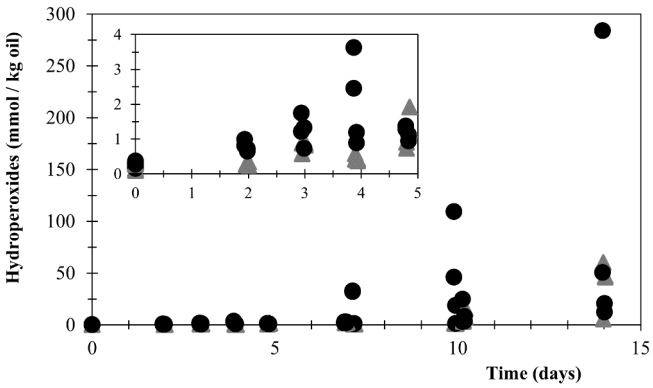


Fig. 5. Hydroperoxide concentration over incubation. Symbol correspond to samples with 0.2 mmol of 4-hydroperoxy-2-nonenal / kg oil (added to the oil prior to emulsification) (●) or without 4-hydroperoxy-2-nonenal (blank) (△). The outcomes of the dependent and independent replicates are both shown as separate points.

CRediT authorship contribution statement

Sten ten Klooster: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **Karin Schroën:** Conceptualization, Investigation, Methodology, Writing – review & editing, Supervision. **Claire Berton-Carabin:** Conceptualization, Investigation, Methodology, Writing – review & editing, Supervision, Funding acquisition.

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.

Acknowledgements

We thank our technicians: Maurice Strubel, Wouter de Groot and Jos Sewalt for their technical assistance regarding the equipment used within this research. We also thank Luuk Adang and Janna Buijs for their preliminary work on this project.

Funding source

This work was supported by the Dutch Research Council (NWO), grant number 731.017.301. NWO had no involvement in the content of this research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.134992.

References

Awad, T. S., Asker, D., & Romsted, L. S. (2018). Evidence of coexisting microemulsion droplets in oil-in-water emulsions revealed by 2D DOSY 1H NMR. *Journal of Colloid and Interface Science, 514*, 83–92.

Banerjee, C., Breitenbach, T., & Ogilby, P. R. (2018). Spatially resolved experiments to monitor the singlet oxygen initiated oxidation of lipid droplets in emulsions. *ChemPhotoChem, 2*(7), 586–595.

Berton, C., Genot, C., & Ropers, M.-H. (2011). Quantification of unadsorbed protein and surfactant emulsifiers in oil-in-water emulsions. *Journal of Colloid and Interface Science, 354*(2), 739–748. https://doi.org/10.1016/j.jcis.2010.11.055

Berton-Carabin, C. C., Ropers, M.-H., & Genot, C. (2014). Lipid Oxidation in Oil-in-Water Emulsions: Involvement of the Interfacial Layer. *Comprehensive Reviews in Food Science and Food Safety, 13*(5), 945–977. https://doi.org/10.1111/1541-4337.12097

Coupland, J. N., Zhu, Z., Wan, H., McClements, D. J., Nawar, W. W., & Chinachoti, P. (1996). Droplet composition affects the rate of oxidation of emulsified ethyl linoleate. *Journal of the American Oil Chemists' Society, 73*(6), 795–901.

Cui, L., Shen, P., Gao, Z., Yi, J., & Chen, B. (2019). New insights into the impact of sodium chloride on the lipid oxidation of oil-in-water emulsions. *Journal of Agricultural and Food Chemistry, 67*(15), 4321–4327.

Guillén, M. D., & Ruiz, A. (2004). Formation of hydroperoxy-and hydroxyalkenals during thermal oxidative degradation of sesame oil monitored by proton NMR. *European Journal of Lipid Science and Technology, 106*(10), 680–687.

Hollebrands, B., & Janssen, H.-G. (2017). Liquid chromatography-atmospheric pressure photo ionization-mass spectrometry analysis of the nonvolatile precursors of rancid smell in mayonnaise. *LC GC Europe, 30*.

Keller, S., Locquet, N., & Cuvelier, M.-E. (2016). Partitioning of vanillic acid in oil-in-water emulsions: Impact of the Tween® 40 emulsifier. *Food Research International, 88*, 61–69.

Laguerre, M., Bily, A., Roller, M., & Birtić, S. (2017). Mass Transport Phenomena in Lipid Oxidation and Antioxidation. *Annual Review of Food Science and Technology, 8*(1), 391–411. https://doi.org/10.1146/annurev-food-030216-025812

Laguerre, M., Tenon, M., Bily, A., & Birtić, S. (2020). Toward a Spatiotemporal Model of Oxidation in Lipid Dispersions: A Hypothesis-Driven Review. *European Journal of Lipid Science and Technology, 122*(3), 1900209.

Law, S. J., & Britton, M. M. (2012). Sizing of reverse micelles in microemulsions using NMR measurements of diffusion. *Langmuir, 28*(32), 11699–11706.

Li, P., McClements, D. J., & Decker, E. A. (2020). Application of flow cytometry as novel technology in studying lipid oxidation and mass transport phenomena in oil-in-water emulsions. *Food Chemistry, 315*, Article 126225.

Mcclements, D. J., & Decker, E. A. (2000). Lipid Oxidation in Oil-in-Water Emulsions: Impact of Molecular Environment on Chemical Reactions in Heterogeneous Food Systems. *Journal of Food Science, 65*(8), 1270–1282. https://doi.org/10.1111/j.1365-2621.2000.tb10596.x

McClements, D. J., Dungan, S. R., German, J. B., & Kinsella, J. E. (1992). Oil exchange between oil-in-water emulsion droplets stabilised with a non-ionic surfactant. *Food Hydrocolloids, 6*(5), 415–422.

McClements, D. J., Dungan, S. R., German, J. B., & Kinsella, J. E. (1993). Evidence of oil exchange between oil-in-water emulsion droplets stabilized by milk proteins. *Journal of Colloid and Interface Science, 156*(2), 425–429.

Merkx, D. W. H., Hong, G. T. S., Ermacora, A., & Van Duynhoven, J. P. M. (2018). Rapid quantitative profiling of lipid oxidation products in a food emulsion by 1H NMR. *Analytical Chemistry, 90*(7), 4863–4870.

Nuchi, C. D., Hernandez, P., McClements, D. J., & Decker, E. A. (2002). Ability of lipid hydroperoxides to partition into surfactant micelles and alter lipid oxidation rates in emulsions. *Journal of Agricultural and Food Chemistry, 50*(19), 5445–5449.

Raudsepp, P., Brüggemann, D. A., & Andersen, M. L. (2014). Evidence for transfer of radicals between oil-in-water emulsion droplets as detected by the probe (E, E)-3, 5-bis (4-phenyl-1, 3-butadienyl)-4, 4-difluoro-4-bora-3a, 4a-diaza-s-indacene, BODIPY665/676. *Journal of Agricultural and Food Chemistry, 62*(51), 12428–12435.

Raudsepp, P., Brüggemann, D. A., Knudsen, J. C., & Andersen, M. L. (2016). Localized lipid autoxidation initiated by two-photon irradiation within single oil droplets in oil-in-water emulsions. *Food Chemistry, 199*, 760–767.

Raudsepp, P., Brüggemann, D. A., Lenferink, A., Otto, C., & Andersen, M. L. (2014). Oxidative stabilization of mixed mayonnaises made with linseed oil and saturated medium-chain triglyceride oil. *Food Chemistry, 152*, 378–385.

Richards, M. P., Chaiyasit, W., McClements, D. J., & Decker, E. A. (2002). Ability of surfactant micelles to alter the partitioning of phenolic antioxidants in oil-in-water emulsions. *Journal of Agricultural and Food Chemistry, 50*(5), 1254–1259.

Samtlebe, M., Yucel, U., Weiss, J., & Coupland, J. N. (2012). Stability of solid lipid nanoparticles in the presence of liquid oil emulsions. *Journal of the American Oil Chemists' Society, 89*(4), 609–617.

Schaich, K. M. (2005). Lipid oxidation: Theoretical aspects. *Bailey's Industrial Oil and Fat Products*.

Schoenmaker, L., Witzigmann, D., Kulkarni, J. A., Verbeke, R., Kersten, G., Jiskoot, W., & Crommelin, D. J. A. (2021). mRNA-lipid nanoparticle COVID-19 vaccines: Structure and stability. *International Journal of Pharmaceutics, 601*, Article 120586.

Schroën, K., & Berton-Carabin, C. C. (2022). A unifying approach to lipid oxidation in emulsions: Modelling and experimental validation. *Food Research International, 111621*.

Serfert, Y., Drusch, S., & Schwarz, K. (2009). Chemical stabilisation of oils rich in long-chain polyunsaturated fatty acids during homogenisation, microencapsulation and storage. *Food Chemistry, 113*(4), 1106–1112.

Ten Klooster, S., Villeneuve, P., Bourlieu-Lacanal, C., Durand, E., Schroën, K., & Berton-Carabin, C. (2022). Alkyl chain length modulates antioxidant activity of gallic acid esters in spray-dried emulsions. *Food Chemistry, 387*, Article 132880.

Villeneuve, P., Bourlieu-Lacanal, C., Durand, E., Lecomte, J., McClements, D. J., & Decker, E. A. (2021). Lipid oxidation in emulsions and bulk oils: A review of the importance of micelles. *Critical Reviews in Food Science and Nutrition*, 1–41.

Villeneuve, P., Durand, E., & Decker, E. A. (2018). The Need for a New Step in the Study of Lipid Oxidation in Heterophasic Systems. *Journal of Agricultural and Food Chemistry, 66*(32), 8433–8434. https://doi.org/10.1021/acs.jafc.8b03603

Waraho, T., McClements, D. J., & Decker, E. A. (2011). Impact of free fatty acid concentration and structure on lipid oxidation in oil-in-water emulsions. *Food Chemistry, 129*(3), 854–859.

Wooster, T. J., Golding, M., & Sanguansri, P. (2008). Impact of oil type on nanoemulsion formation and Ostwald ripening stability. *Langmuir, 24*(22), 12758–12765.

You, Y., Bloomfield, A., Liu, J., Fu, L., Herzon, S. B., & Yan, E. C. Y. (2012). Real-time kinetics of surfactant molecule transfer between emulsion particles probed by in situ second harmonic generation spectroscopy. *Journal of the American Chemical Society, 134*(9), 4264–4268.