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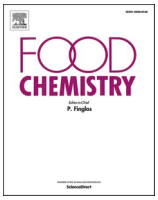

Evaluation of antioxidant efficacy of quercetin encapsulated in micelles, mixed micelles, or liposomes in oil-in-water emulsions

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ABSTRACT

Quercetin was encapsulated in polyoxyethylene laurylether (Brij 35®) micelles, 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) liposomes and Brij 35®/DMPC mixed micelles. These obtained formulations were then physically characterized for their particle size, morphology, physical stability and polydispersity index. All quercetin formulations were evaluated for their antioxidant efficiency in two oil-in-water emulsion systems and compared to the one of free quercetin. In short-term assays, results showed that among the tested formulations, quercetin liposomal form offered superior antioxidant protection, in comparison with polymer micelles or mixed micelles form or free quercetin. These results were attributed to a better synergistic effect of liposomal quercetin formulation with tocopherols present in the oil phase of the emulsion. However, in long-term assay, the loaded quercetin showed chemical degradation over the long term in the liposomal form, thus limiting its long-term antioxidant effectiveness in food emulsions.

1. Introduction

Lipid oxidation in food products leads to the loss of liposoluble vitamins and unsaturated fatty acids, development of off flavors, and the formation of novel oxidized chemical species that may be harmful for human health (Skibsted, 2010). In details, three main mechanisms can be found in lipid oxidation namely photooxidation, which happens when light activates a sensitizer, enzymatic oxidation though the action of lipoxygenases, and autooxidation. While the first two are typically managed in food products by adequate UV barrier packaging and heat treatment, autooxidation is still a problem, due to its complex pathway, which typically involves radical species, and the fact that a multitude of oxidation products are generated (Schröder et al., 2020). In food, most of the lipids are produced in the form of emulsions, mainly oil-in-water emulsions, that can be found, for instance, in a variety of drinks, dairy products, mayonnaises, dressing or infant formula. These emulsions are made up of oil droplets that are dispersed in a continuous aqueous phase and stabilized by molecules that are surface-active and adsorb at the oil-water interface. In emulsions, it is now well known that the oil-water interface is important since it is where pro-oxidants, oxygen, and oxidizable lipids come into contact (Hennebelle et al., 2024). Moreover, various others factors influence oxidation kinetics in emulsions such as the lipid droplet size, the type of used emulsifier, the interface physical-chemical properties and the presence of antioxidant molecules (McClements & Decker, 2000; Villeneuve et al., 2023). Regarding the latter, most antioxidants used in the food industry are phenolic compounds that are known for their radical scavenging action (through H donation or electron transfer mechanism) or their ability to chelate pro-

Abbreviation: AAPH, 2,2′-azobis(2-amidino-propane) dihydrochloride; CAT, Conjugated Autoxidizable Triene assay (CAT assay); CIS, Cold Injection System; DHS, Dynamic Head Space; DLS, Dynamic Light Scattering; DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphocholine; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FAME, Fatty Acid Methyl Esters; GC, Gas Chromatography; GC–MS, Gas Chromatography-Mass Spectrometry; HPLC, High Pressure Liquid Chromatography; PBS, Phosphate Buffer Solution; PDI, PolyDispersity Index; PV, Peroxide Value; Q-Mixed micelles, Quercetin formulated in Mixed Micelles; Q-Liposomes, Quercetin formulated in Liposome (DMPC); TDU, Thermal Desorption Unit.

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oxidant metals (Decker et al., 2010). However, in emulsions, the efficiency of a given antioxidant is not only governed by its chemical reactivity but also by its partitioning in the system and more specifically, its ability to locate at the oil-water interface (Laguerre et al., 2015; Villeneuve et al., 2023; Bravo-Diaz, 2023). In addition, recent studies indicate that a key factor to evaluate when addressing the oxidative stability of oil-in-water emulsions is how oxidation spreads between lipid droplets (Decker et al., 2017; Hennebelle et al., 2024; Laguerre et al., 2017; Villeneuve et al., 2023). In fact, current theories suggest that the diffusion of some primary oxidation compounds, such as hydroperoxides, and secondary ones, such as aldehydes, may be responsible for the transmission of "oxidation information" from one droplet to another (Klooster et al., 2023). Although the exact mechanism of such transport is still unknown, hypotheses propose that polymer micelles could act as carriers of oxidative species. In that context, some studies suggest that the formulation of some antioxidants could favor their activity in emulsion through an antioxidant reservoir effect that would induce their location at the interface or by enhancing their capacity to limit oxidation diffusion in between droplets (Hennebelle et al., 2024; Villeneuve et al., 2023). For example, Kiralan et al. (2014) observed that the formulation of tocopherol into Tween 20 micelles could be used as reservoir of antioxidants that may either replace oxidized tocopherols in the emulsion droplets or that could directly scavenge reactive oxygen species (ROS) located in the continuous phase of the emulsion. Similarly, Inchingolo et al. (2021) observed that sodium dodecyl sulfate at concentrations above its CMC, lead to a marked increase of the partitioning of α-tocopherol in the aqueous phase, suggesting that polymer micelles were solubilizing α-tocopherol out of the emulsion droplet, resulting in a better oxidative stability of the emulsion.

More complex organized structures, such as niosomes or liposomes, are also recognized for their ability to impact the antioxidant activity of molecules. For instance, Feng et al. (2017) formulated catechin and epigallocatechin laurates into gemini dodecyl O-glucoside-based niosomes and found that such assemblies had higher antioxidant efficiency than the non-formulated molecules to protect liposomes from oxidation. On the contrary, using α-tocopherol as antioxidant, others observed that its formulation into Tween 60 based niosomes limits its antioxidant effect in emulsion (Olbińska et al., 2023). These studies suggest that the formulation of an antioxidant into micellar or liposomal vesicles may have an effect on its resulting efficiency in emulsions oxidative stability.

In that context, the present study aimed at evaluating the capacity of quercetin formulated into micelles, mixed micelles or liposomes to protect unsaturated lipids from oxidation in oil-in-water emulsions. Quercetin is one of the most prominent flavonoid antioxidants (Bonina et al., 1996; Hatahet et al., 2016) which is known for its radical scavenging activity (Dangles, 2012; Dufour et al., 2007) and its numerous biological activities as anti-inflammatory (Lin et al., 2012) or anti-tumor compound (Zhang et al., 2012). Its effects as antioxidant for food emulsions are well documented either when used as single antioxidant (Belhaj et al., 2010; Di Mattia et al., 2009; Noon et al., 2020), within natural plant extracts (Martinović et al., 2020; Valerga et al., 2012) or in synergy with tocopherols (Bayram & Decker, 2023; Zhang et al., 2023; Miquel Becker et al., 2007. Due to its poor aqueous solubility limiting its bioavailability, a lot of research work have been carried out to develop delivery systems for quercetin (Kandemir et al., 2022; Wadhwa et al., 2022; Wang et al., 2016). For example, for topical delivery (Hatahet et al., 2016) to improve skin penetration, quercetin has been formulated in nanoemulsions (Fasolo et al., 2009; Scalia et al., 2013), liposomes (Caddeo et al., 2014; Liu et al., 2013; Manca et al., 2014), niosomes (Elmi et al., 2021; Javani et al., 2021), nanoparticles (Wu et al., 2008) or chitosan formulations (Tan et al., 2011). Our group recently demonstrated that the encapsulation of quercetin into lipid nanocapsules increased its apparent water solubility by more than 5000-fold. This encapsulation did not alter its antiradical activity as measured by the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and proved to be efficient in the protection of THP-1 cells from oxidative stress (Hatahet et al., 2017). This study was further pursued with the comparison of lipid nanocapsules, liposomes and Smartcrystals® containing Tween 80 as material for encapsulation of quercetin (Hatahet et al., 2018). All quercetin formulations showed similar activity results compared to crude quercetin indicating the preservation of quercetin antioxidant ability upon formulation. As an alternative to poly(ethylene glycol), we also investigated the potential of polyoxazolines (C_{16}POx) based mixed micelles and lipid nanocapsules for encapsulation of quercetin and its resulting antioxidant activity in topical delivery (Simon et al., 2019; Simon et al., 2020). Mixed micelles, also called polymer/lipid hybrid, are made of polyoxazolines as polymer and phosphatidylcholine as lipid entangled to create an hydrophobic core. Quercetin was efficiently encapsulated in this core and resulted in a decrease of quercetin impact on cell viability while its antioxidant activity remained unchanged.

In this study, we incorporated quercetin into polymer micelles, mixed micelles or liposomes, and then compared its antioxidant activity in free form with these formulations. The aim was to determine whether encapsulating quercetin in these vesicles could enhance its ability to protect unsaturated lipids from oxidation in emulsions. We first analyzed the physical properties of the quercetin-loaded vesicles, focusing on hydrodynamic diameter, polydispersity index (PDI), morphology, physical stability, quercetin loading, and encapsulation efficiency. Then, the quercetin-loaded formulations were tested in a fast-track oil-in-water emulsion assay, with and without the presence of endogenous tocopherols. This initial approach allowed us to identify the most effective formulated system. The selected formulation was then assessed in a long-term oil-in-water emulsion stability test by quantifying primary and secondary markers of lipid oxidation as well as tocopherols consumption.

2. Materials

Quercetin (purity >95 % HPLC), phosphate buffer solution (PBS at 150 mM, pH 7.2), Brij 35® (polyoxyethylene(23)laurylether, MW = 1198 g.mol^{-1}, CMC value from supplier 92 μM), α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol, ammonium thiocyanate, iron (II) chloride, cumene hydroperoxide, 3-heptanol, phenolphthalein, phosphoric acid were all purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). Acetone, acetonitrile, butanol, chloroform, 1,4-dioxane, ethanol, hexane, isooctane, isopropanol, methanol, were all HPLC or analytical grade solvents and purchased from Sigma-Aldrich (Saint Quentin Fallavier, France).

1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) was kindly given by Lipoid (GmbH). Trolox (97 %) was obtained from Accros Organic (Geel, Belgium). 2,2′-azobis(2-amidino-propane) dihydrochloride (AAPH) was purchased from Wako Chemical (Neuss, Germany).

Refined Rapeseed (*Brassica napus L.*) oil was obtained from local supermarkets in Montpellier (France) and used without further purification. The oil quality was as follows: Peroxide value 1.3 meq 0_2/kg oil, α-tocopherol: 510 mg/kg oil and γ-tocopherol: 630 mg/kg oil. Fatty acid composition was as follows: C16:0, 4.4 %; C16:1 n-7, 0.2 %; C18:0, 1.8 %; C18:1 n-9, 62.3 %; C18:2 n-6, 19.7 %; C18:3 n-3, 9.9 %; C20:0, 0.5 %; C20:1 n-9, 1.2 %. Tung oil (*Aleurites fordii)* was purchased from Sigma-Aldrich (Saint Quentin Fallavier, France) with the following characteristics: Peroxide value 1.52 meq 0_2/kg oil, α-tocopherol: 12 mg/kg oil and β-tocopherol: 113 mg/kg oil. Fatty acid composition was as follows: C16:0, 1.9 %; C18:0, 2.1 %; C18:1 n-9, 4.9 %; C18:2 n-6, 6.7 %; α-eleostearic acid (Δ9c, Δ11t, Δ13t), 79.8 %.

3. Methods

3.1. Formulation of Brij 35® micelles loaded with quercetin

The Brij 35® polymer micelles were obtained by simple polymer dissolution in phosphate buffer saline at 150 mM at a concentration of

11.5 g.L^{-1} and quercetin was added at a concentration of 20 μg.mL^{-1}. The solution was left under magnetic stirring for one hour in the dark and then filtered through acetate cellulose filters of 0.65 μm.

3.2. Formulation of mixed micelles (Brij 35 and DMPC) loaded with quercetin

The mixed micelles were formulated based on our previous publication (Simon et al., 2019) using the thin film method. Briefly, quercetin, DMPC and Brij 35® were solubilized in a mixture of chloroform: acetone (molar ratio 1:1) with the molar ratio 4:80:1 respectively. The solution was placed in rotavapor under vacuum at 40 °C for 30 min to remove the solvent and obtain a thin film. Then, 10 mL of filtered PBS were added and left in rotavapor under rotation in the dark at a speed of 150 rpm for 1 h at 40 °C for film hydration. The mixed micelles solution was diluted by 3-fold in PBS.

Lastly, using the high-pressure homogenizer process of 5 cycles at 10000 PSI (Microfluidics LV1, USA), the mixed micelles loaded with quercetin (Q-Mixed micelles) were obtained. The formulation was centrifuged (Sigma 2 K15, Bioblock Sc.) at 10,000 rpm for 10 min and then filtered through acetate cellulose filters of 0.65 μm.

3.3. Formulation of DMPC liposomes loaded with quercetin

Liposomes were formulated using the ethanol injection process as previously published (Simon et al., 2023). The same protocol was carried out but a different quercetin loading was targeted for the antioxidant measurement with Conjugated Autoxidizable Triene (CAT) assay or in rapeseed oil-in-water emulsions as described below.

3.3.1. For conjugated autoxidizable triene (CAT) assay experiment

A solution of 2 mL of absolute ethanol at 40 °C was prepared with DMPC at 25 g.L^{-1} and quercetin at 0.1 g.L^{-1}. This mixture was then injected with a glass syringe and needle of 0.4 mm diameter into 10 mL PBS solution at 40 °C under stirring with a propeller with a diameter of 3 cm at 650 rpm. The quercetin loaded liposomes (Q-Liposomes) was left under stirring for 1 h at 40 °C. The samples were centrifuged (Sigma 2 K15 Bioblock Sc. at 10,000 rpm for 10 min and then filtered through acetate cellulose filters of 0.65 μm.

3.3.2. For rapeseed oil-in-water emulsion experiments

The same protocol was performed but with an absolute ethanol solution with DMPC at 50 g.L^{-1} and quercetin at 4 g.L^{-1}. Then, the liposomes were concentrated using Amicon® filters (MWCO 50 kDa, regenerate cellulose, Merck Millipore). 500 μL of the liposomes were placed onto the upper compartment and centrifuged at 2000 *g* for 30 min (Sigma 2 K15 Bioblock Sc.). The liposomes were withdrawn with a reverse spin at 1000 g for 2 min. The procedure was repeated twice to reach the targeted quercetin concentration of 0.9 g.L^{-1} (equivalent to 3000 μM).

3.4. Dynamic light scattering (DLS)

All formulations or emulsions (CAT emulsions and rapeseed oil-in-water emulsions) were characterized in terms of size (by intensity) and dispersity using Zetasizer NanoZS apparatus (Malvern Instruments, UK) equipped with a He—Ne laser (632.8 nm) to provide hydrodynamic diameter and polydispersity index (PDI). Measurements were conducted without dilution and at 25 °C at scattering angle 173°. All the results were an average of three independent measurements.

3.5. Transmission electron microscopy

Transmission electron microscopy was performed on TEM Jeol 1200 EXII (Jeol. Ltbd, Tokyo, Japan) associated with a camera Jeol 2 K/2 K. The formulations were diluted by 1.5 in PBS and deposited on grids type Cu formvar carbon and negatively stained with an aqueous uranyl acetate solution. TEM images were taken at a 60 k magnification.

3.6. X-ray diffraction

The solid state of the materials presented in this work was characterized using a Bruker D2 Phaser diffractometer (Bruker, MA, USA) and the monochromatic Cu Kα1 radiation (λα = 1.54184 Å, 30 kV and 10 mA). The angular range of data recording was 5–70° 2θ, with a stepwise size of 0.02° and a speed of 0.2 s counting time per step, using LYNXEYE XE-T detector (Bruker, MA, USA).

3.7. HPLC analysis

3.7.1. HPLC analysis for quercetin quantification

Solution were centrifuged at 10,000 rpm for 10 min (Sigma 2 K15 Bioblock Scientific) to remove the potential free quercetin. High Pressure Liquid Chromatography (HPLC) analysis of quercetin was performed on LC 2010AHT apparatus (Shimadzu, Kyoto, Japan) using a Prontosil C18 column (120–5-C18H 5.0 μm, 250 × 4.0 mm). The detection was achieved using a UV–vis detector (Shimadzu, Kyoto, Japan) at 368 nm (Simon et al., 2019). Acetonitrile/phosphoric acid at 0.2 % *v*/v and pH = 1.9 (40/60 % *v*/v) was used as mobile phase. The calibration curves were performed with solutions of quercetin in methanol from 0.1 to 40 μg.mL^{-1} and from 10 to 200 μg.mL^{-1} both with a good linearity (r^2 = 0.999). The flow rate was 1 mL.min^{-1} with an oven temperature of 40 °C and injected volume of 10 μL and analysis of 10 min.

3.7.2. HPLC analysis for tocopherol isoforms

The lipid extract (around 10 mg) was weighted in 2 mL vials, solubilized in hexane (1 mL) and analyzed by HPLC (Ultimate 3000 Thermo Fisher Scientific, Waltham, USA) and fluorescence detection set at 290–330 nm for excitation and emission respectively (FL3000 Fluorescence detector, Thermo Separation products, USA). Separation was carried out on a Silica column (Kinetex 5 μm, 250 mm length [L] x 4.6 mm internal diameter, Phenomenex, Le Pecq, France). Elution was performed in isocratic conditions with hexane/1,4-dioxane (97:3 v/v) as mobile phase. Injection was set at 100 μL, column temperature was maintained at 25 °C and flow rate at 1.3 mL.min^{-1}. Quantifications were carried out for α-tocopherol, β-tocopherol, γ-tocopherol, and δ-tocopherol. Calibration curves were realized with standard solutions of each tocopherol isoform. Analysis were made in triplicate and results were calculated and expressed in mg/kg of oil.

3.8. Encapsulation efficiency

Encapsulation efficiency (EE) was calculated with the following equation:

$$EE = \frac{amount\ of\ encapsulated\ quercetin}{amount\ of\ quercetin\ initially\ loaded} \times 100$$

3.9. Evaluation of antioxidant activity with the conjugated autoxidizable triene (CAT) assay

For the CAT assay, all formulations freshly prepared were diluted to the final quercetin concentration of 64 ng.mL^{-1} (0.212 μM.mL^{-1}). Similarly, quercetin was either evaluated alone (unloaded into vesicles) at the same concentration or, at the same concentration, in the presence of unloaded Brij 35® micelles, mixed micelles or DMPC liposomes. Blank control was made with PBS only.

The method, originally developed by (Laguerre et al., 2008), was slightly modified according to the following procedure:

3.9.1. Preparation of stripped tung oil

Prior to its use in the CAT assay, tung oil was stripped from its native tocopherols by passing 25 mL hexane solution of Tung oil (200 mg. mL^{-1}) through an alumina column. Complete removal of tocopherols was checked by HPLC (see section 3.7.2).

3.9.2. Preparation and physical characterization of tung oil emulsions

40 mg of tung oil (crude or stripped) were added to 200 mL of PBS solution (pH 7.2) containing 70 μM Brij 35®. Afterward, the mixture was homogenized using a high-speed blender (Janke & Kunkel, Staufen, Germany) at 15,000 rpm for 90 s. Then, the coarse emulsion was processed through a high-pressure homogenizer APV-1000 (SPX FLOW, Charlotte, North Carolina, USA) at 400 bars (350 bars +50 bars, 5 passes) to produce nano-emulsions. The particle size distribution of the emulsion was measured by DLS. All prepared emulsions had similar droplet sizes (mean diameter = 200 ± 75 nm) and were physically stable over the course of the experiment.

3.9.3. CAT assay procedure

Stock solutions of encapsulated quercetin (Brij35 micelles, Brij35/DMPC mixed micelles, or DMPC liposomes) were prepared at an isoconcentration of quercetin (0.2 μg/mL, 0.661 μM) in PBS buffer (pH 7.2). Subsequently, 50 μL of Tung oil-in-PBS emulsions, with varying tested formulations (135 μL for crude oil or 67.5 μL for stripped oil), were transferred into a UV-star-96 well microplate (Greiner, Frickenhausen, Germany). For free quercetin (unloaded quercetin) and free quercetin in the presence of blank carriers, a stock solution of quercetin was prepared in ethanol, and 10 μL were added to the microplate. For proper comparison, control wells with 10 μL ethanol were also prepared. Then, 15 μL of freshly prepared AAPH solution in PBS (1.3 mM) were added to each well using a multichannel micropipette, resulting in a final solution volume of 210 μL per well. The final concentrations were 0.05 $mg.mL^{-1}$ in Tung oil, 17.5 μM in Brij35, 1 mM in AAPH, and an isoconcentration of quercetin (128 $ng.mL^{-1}$ or 64 $ng.mL^{-1}$). Reaction kinetics were monitored immediately after AAPH addition by recording the absorbance decrease at 273 nm using an Infinite 200 PRO microplate reader (Tecan, Gröedig, Austria) with Magellan software. Measurements were taken every minute for 9 h at 37 °C, with 5 s of agitation before each reading.

3.10. Evaluation of antioxidant efficiency in rapeseed oil-in-water emulsion

3.10.1. Evaluation of physical and chemical stability of quercetin-loaded liposomes (Q-Liposomes)

The Q-Liposomes prepared for the experiments on rapeseed oil-in-water emulsions were evaluated in regards to their physical and chemical stability. Q-Liposomes with the same dilution factors corresponding to the one of the emulsions were placed at 4, 25 and 37 °C for a month with measurement of physical stability with size and PDI (section 3.4.) and chemical stability with quercetin quantification (section 3.7.1). Three independent formulations were investigated for stability.

3.10.2. Preparation of emulsion

A rapeseed oil-in-water emulsion was prepared by homogenization of 5 % wt rapeseed oil with 95 % aqueous phase (*w*/w). The aqueous phase consisted of phosphate buffer solution (10 mM, pH 7.0) and Brij35 at a 10 mM concentration in the final emulsion. A coarse emulsion was prepared using an Ultra-Turrax IKA (Janke & Kunkel IKA-Labortechnik, Staufen, Germany) for 2 min at 7000 rpm. The resulting coarse emulsion was then homogenized with an APV-1000 (SPX FLOW, Charlotte, North Carolina, USA) at an operating pressure of 400 bars (350 + 50 bars) for 5 cycles. Between each pass, the emulsion was collected in a beaker submerged in an ice-water bath to keep the temperature at ≃25 °C. Quercetin samples (Q-Liposomes, free quercetin or free quercetin in the presence of Blank DMPC Liposomes -B-Liposomes)~~)~~ were introduced in the aqueous phase of the emulsions after the homogenization step (i.e. post-homogenization) to give a 50 μM final isoconcentration of quercetin. Control emulsions with ethanol were also evaluated in the absence of quercetin and DMPC. To induce lipid oxidation, $FeCl_2$ (5 ppm) was added to each sample and the emulsions (15 × 60 mL) were stored in 70 mL closed amber glass bottles at 25 °C for 28 days, under gentle orbital stirring (200 rpm) using an IKA KS 4000 i-control incubator (IKA, Staufen, Germany). Samples were collected at 0, 2, 4, 7, 10, 14, 21, 24 and 28 days for analysis. Three replicates for each antioxidant formulation were carried out.

The evaluation of lipid oxidation in the emulsions was carried out by monitoring the formation of both primary (lipid hydroperoxides), secondary (volatiles) oxidation products and loss of tocopherols content by HPLC (section 3.7.1).

3.10.3. Measurement of peroxide value (PV)

PV was determined according to the following procedure. Briefly, 1 mL of emulsion samples were mixed with 4 mL of isooctane/isopropanol solution (3:1 *v*/v) and vortexed three times for 10 s. Then, an aliquot of supernatant (200 μL) was added to (1800 μL) methanol/butanol mixture (3:7 v/v) and 260 μL are introduced in the microplate well. Then, 2.5 μL of aqueous ammonium thiocyanate (3.94 $mol.L^{-1}$) and 2.5 μL of ferrous solution (0.144 $mol.L^{-1}$) were added to give a final volume of 265 μL in the microplate UV star 96 well COC F-bottom (Greiner Bio-One, Courtaboeuf, France). Each microplate was first incubated at 25 °C for 10 min with a stirring of 1000 rpm in PHMP-4 microplate Thermoshaker (Grant instruments ltd Shepreth, Cambridge, England) and then placed in an Infinite M200 microplate reader (Tecan, Gröedig, Austria). Absorbances were measured at 500 nm. Data acquisition was made using Magellan software (Tecan). PV were determined using a standard calibration curve of cumene hydroperoxide and were expressed as meq O_2/kg oil.

3.10.4. Measurement of volatile compounds

Volatile oxidation products were analyzed according to the following procedure. Samples (2 mL) were withdrawn from the emulsion and placed into 10 mL headspace vials and then quantified by Dynamic Headspace (DHS) (Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany) combined with GC–MS (7890B/MSD 5977, Agilent Technologies, Palo Alto, CA, USA). DHS was used in the following conditions: incubation at 60 °C for 5 min; trapping for 12 min (using Tenax TA type trap at 40 °C with 300 mL of nitrogen at a flow rate of 25 $mL.min^{-1}$, under stirring at 500 rpm); drying phase of 200 mL of nitrogen at 50 °C at a flow rate of 100 $mL.min^{-1}$. For the Thermal Desorption Unit (TDU), a non-fractionated injection at 30 °C followed by a ramp to 300 °C at 120 °C.min^{-1} was performed for 7 min. In the Cold Injection System (CIS) at 2 °C, a ramp to 300 °C at 12 °C/s followed by a plateau for 5 min was conducted. GC–MS was equipped with a Gerstel robot and a DB-Wax UI column (J&W Scientific, Folsom, CA, USA) having the following characteristics: 60 m length, 0.25 mm inner diameter, 0.25 μm film thickness. Hydrogen was used as carrier gas at a flow rate of 2.2 $mL.min^{-1}$ Elution was performed with the following temperature program: isothermal at 40 °C for 5 min, then 3 °C.min^{-1} from 40 °C to 150 °C, followed by 15 °C.min^{-1} to 280 °C. The mass spectrum was recorded in EI+ mode (70 eV) between 40 and 350 Da. Temperatures of the analyzer and the source were set at 150 °C and 250 °C, respectively. Volatile quantification was carried out for the following volatile compounds: pentanal, hexanal, 2,4-heptadienal, 2-pentanal, 2-ethylfuran, using 3-heptanol as internal standard. The calibration curves were prepared from a stock standard solution containing these volatile compounds. Quantification results obtained were processed with Mass Hunter software version 11.1 (Agilent Technologies, Palo Alto, 262 CA, USA) using a quantitative method based on quantifier and qualifier ions specific to each standard. Peak identification was carried out by comparing the mass spectra with those in the NIST 2020 database (National Institute of Standard Technology). Analysis was performed in duplicate, and results were expressed in ng/g.

Table 1
Physicochemical features of blank (B) and quercetin loaded (Q) formulations in terms of hydrodynamic diameter, PDI (mean ± SE), quercetin loading (mean ± SE) and encapsulation efficiency ($n = 3$).

	Hydrodynamic diameter (nm)	PDI	Quercetin concentration (μg.mL^{-1})	Encapsulation efficiency (%)
B-Brij 35® micelles	9.3 ± 0.1	0.12 ± 0.06		
Q-Brij 35® micelles	9.5 ± 0.1	0.13 ± 0.06	2.6 ± 0.3	13
B-Mixed micelles	92.5 ± 5.5	0.25 ± 0.03		
Q- Mixed micelles	111.2 ± 2.6	0.26 ± 0.02	38.5 ± 1.8	77
B-Liposomes	85.2 ± 3.8	0.17 ± 0.04		
Q-Liposomes	87.9 ± 4.4	0.15 ± 0.02	13.1 ± 0.9	65

For tocopherols loss measurements by HPLC, 1 mL of sample were withdrawn from the emulsions and added to a 4 mL isooctane/2-propanol solution (1:1 *v*/v) and then vortexed (3 × 10 s) and then centrifugated for 2 min à 2000 g. The lipid phase was then recovered and analyzed by HPLC according to the procedure described in section 3.7.1

3.11. Fatty acid profiles of rapeseed and tung oils

Fatty acid profiles of rapeseed and tung oil were determined by GC (Agilent 8860 GC, Agilent Technologies, Santa Clara, USA) with the following procedure. Briefly, 500 μL sodium methylate solution with phenolphthalein were added to 6 mg of lipid sample. After heating at 65 °C for 10 min, chlorhydric methanol (500 μL) were added. The mixture was again heated at 65 °C for 10 min and hexane (1 mL) and water (1 mL) were added. After a centrifugation at 1500 rpm for 5 min with a Rotina 380R (Hettich, Westphalie, Germany), the upper phase was collected and analyzed by GC. GC analysis conditions were as follows: Split injector (1/20 ratio), capillary column (CP-Sil 88 Varian) (50 m × 0.25 mm with 0.2 μm film thickness; Helium (1 mL.min^{-1}) was used as carrier gas. Fatty acid methyl esters (FAME) were analyzed by flame ionization detector and Agilent software (Agilent Technologies, Santa Clara, USA). The column temperature started from 150 °C, with a rise of 5 °C.min^{-1} to 225 °C and was maintained at 225 °C for 10 min. The injector and detector temperatures were 250 and 270 °C, respectively. FAME were identified using an external standards of methyl esters mixture.

3.12. Statistical analysis

Results are presented as mean ± SD. Statistical analysis were performed using the statistics software R (version 3.5–1). Statistical differences were evaluated using a one-way ANOVA test and considered significant for $P < 0.05$.

4. Results and discussion

In oil-in-water emulsions, the efficiency of a given antioxidant to protect lipids from oxidation is not only governed by its chemical reactivity (radical scavenging properties) but also by its physico-chemistry and more specifically, its capacity to locate at the oil/water interface where oxidation reactions occur (Laguerre et al., 2015). In that context, various studies aimed at chemically modify antioxidants in order to adjust their polarity and allow them to locate at the interface for an optimized antioxidant effect (Hennebelle et al., 2024). Others investigated the potential of formulating antioxidants in various careers that would favor their partitioning at the Oil/Water interface. For instance, Schröder et al. (2020) used Pickering particles as interfacial reservoirs of antioxidants (α-Tocopherol or carnosic acid) and showed that both tested antioxidants were more effective when loaded within such particles than when solubilized in the oil droplet interior. Feng et al. (2017) loaded catechin and epigallocatechin laurates into niosomes made with dodecyl O-glucoside and found that, once formulated into such carriers antioxidant had higher antioxidant efficiency than the non-formulated molecules to protect liposomes from oxidation. On the contrary, others observed that the niosomal (Tween 60) formulation of α-tocopherol limits its antioxidant effect in emulsion (Olbińska et al., 2023). These studies suggest that the formulation of an antioxidant into different vesicles has effect on its antioxidant efficiency in emulsions. Therefore, in the present study we evaluated how the formulation of quercetin into micelles, mixed micelles or liposomes could impact its efficacy as an antioxidant in oil-in-water emulsions.

4.1. Screening of formulations

Three formulations with different morphologies and sizes were explored for incorporating quercetin: micelles, mixed micelles, and liposomes. Brij 35® (C12(PEO)23) emulsifier and DMPC phospholipid (1,2-dimyristoyl-sn-glycero-3-phosphocholine) were chosen. Brij 35® was selected for its minimal surface charge impact, and suitability for aligning the experimental design (CAT assay and rapeseed emulsified

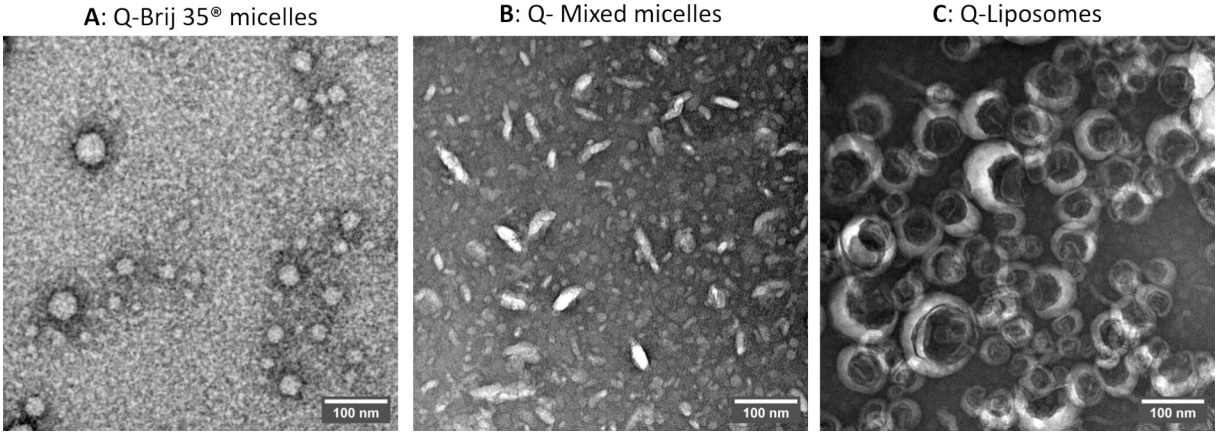

Fig. 1. TEM images of Q-Brij 35® micelles (A), Q-mixed micelles (B) and Q-liposomes (C).

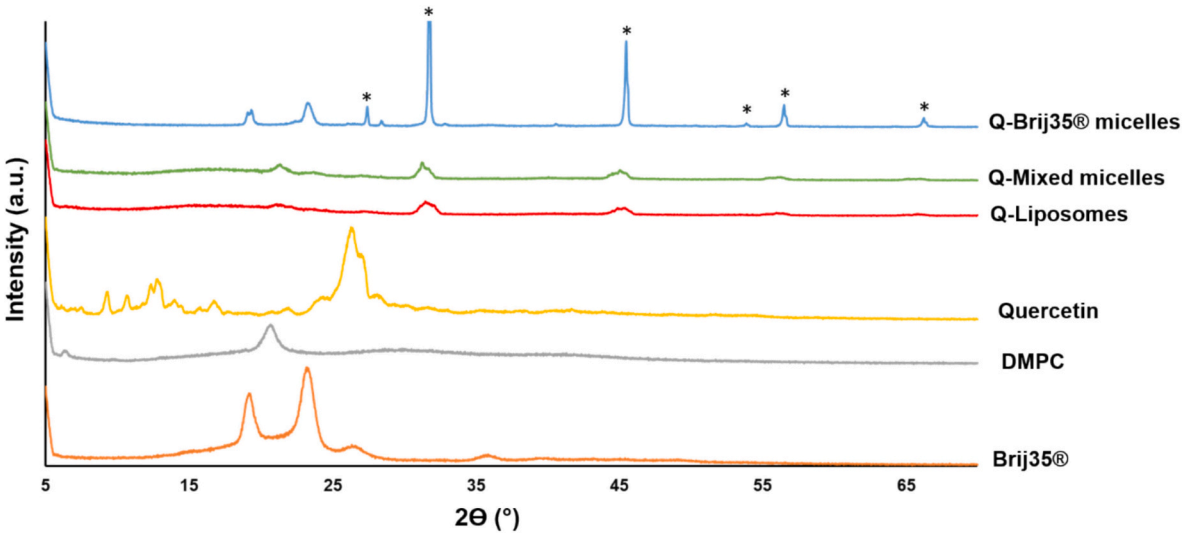

Fig. 2. XRD patterns of Brij35®, phospholipids DMPC, quercetin, Q-Liposomes, Q-Mixed micelles and Q-Brij35® micelles (peaks marked with * were found to correspond to halite (NaCl).

systems). Additionally, Brij 35® and DMPC share similar alkyl chain lengths (12 and 14 carbons, respectively), leading to comparable interfacial fluidity behavior. The formulation characteristics in terms of size, dispersity and quercetin loading were specified in Table 1. Formulations without quercetin were referred as blank (B), while those containing quercetin were labeled (Q). The formulation morphology was also evaluated by TEM imaging in Fig. 1. Brij35® micelles were formed at concentration 100 times above the CMC (11.5 $g.L^{-1}$) with a typical spherical micelle morphology (Fig. 1A). They were able to load quercetin at a concentration of 2.6 ± 0.3 $\mu g.mL^{-1}$ (Q-Micelles) without modification of hydrodynamic diameter (9.5 ± 0.1 nm) or PDI (0.13 ± 0.06) compared to unloaded ones (B-Micelles) (Table 1). Adding DMPC to elaborate mixed micelles with the molar ratio 4:80:1 of quercetin, DMPC, Brij35® induced a size increase and dispersion but higher drug loading than Brij35® micelles. Thus, Q-Mixed micelles presented a hydrodynamic diameter of 111.2 ± 2.6 nm with a PDI of 0.26 ± 0.02 and a quercetin concentration of 38.5 ± 1.8 $\mu g.mL^{-1}$ (Table 1). The TEM image of the Q-Mixed micelles in Fig. 1B revealed different morphologies with mainly disks and some vesicles obtained. Compared to the mixed micelles previously published in Simon et al., 2019 the molar ratio of quercetin, DMPC and polymer was modified from 5:40:1 to 4:80:1. The important raise of phospholipids molar ratio led to a change of morphology from spherical micelles to disk. Also looking at the size, the mixed micelles size distribution obtained in Simon et al., 2019 was

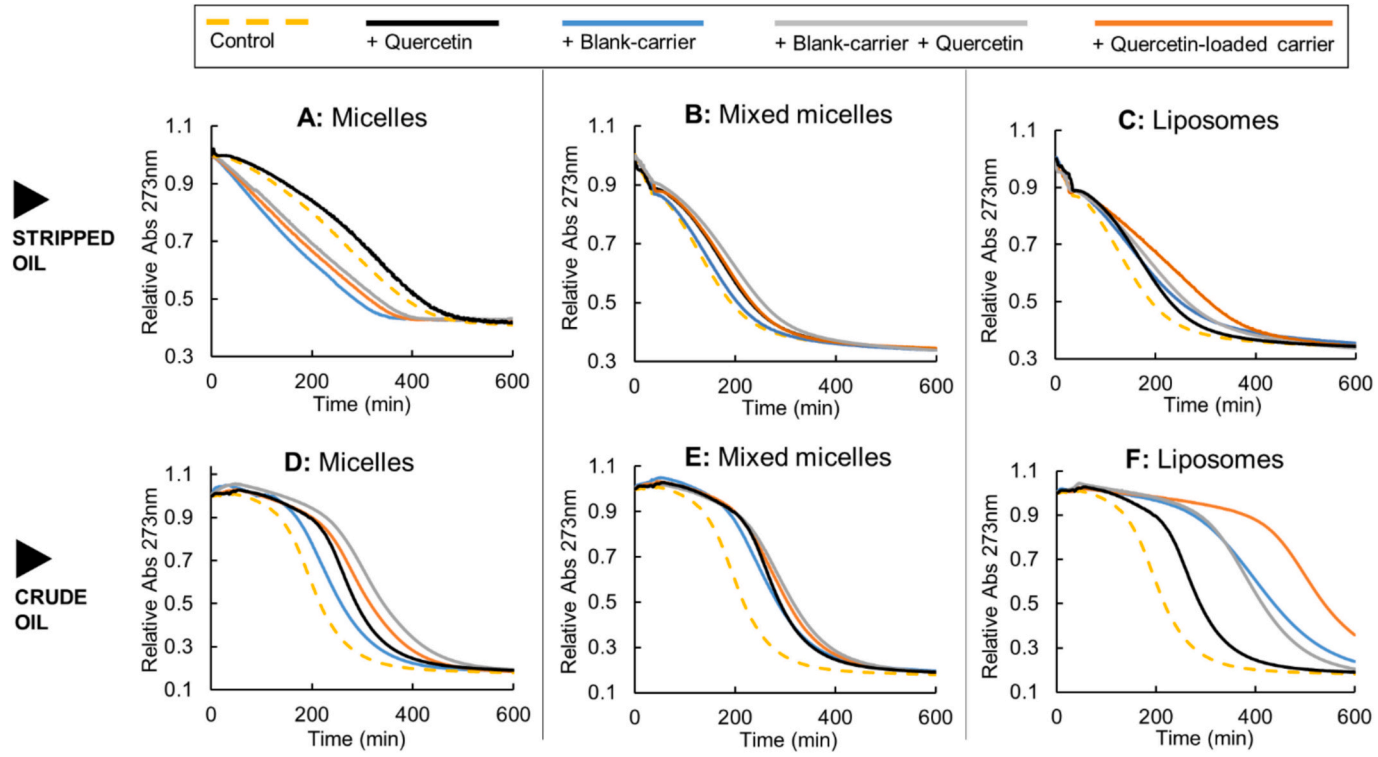

Fig. 3. Results of CAT assay experiments with micelles (Brij 35), mixed micelles (Brij 35/DMPC) and liposomes (DMPC) on stripped (ABC) and crude (unstripped) Tung oil (DEF) (n = 3).

expressed by number with a hydrodynamic diameter of 18 ± 2 nm (B-Mixed micelles) and 19 ± 2 nm (Q-Mixed micelles). Thus, to compare with the publication of reference, our B-Mixed micelles size distribution was measured in number with 27.9 ± 1.1 nm and Q-Mixed micelles at 27.0 ± 0.7 nm which is a slight increase due to the molar ratio modification. Lastly, liposomes of DMPC were obtained by ethanol injection process and allowed a quercetin loading of 13.1 ± 0.9 μg.mL^{-1} for a size of 87.9 ± 4.4 nm with a PDI of 0.15 ± 0.02 (Table 1) and spherical vesicles morphology (Fig. 1C).

Overall, all three formulations were considered monodisperse with a PDI value below 0.3. The size of the three formulations agreed with the literature. The highest quercetin loading was achieved with mixed micelles as previously observed with the work from Hatahet et al. (2018), on DPPC liposomes reaching a loading of 2.6 ± 0.1 % compared to mixed micelles of phosphatidylcholine and polyoxazolines of 3.6 ± 0.2 % (Simon et al., 2019).

The solid-state form of the quercetin in the different formulations was also investigated by powder X-ray diffraction (Fig. 2). The diffraction patterns of the formulations revealed that the quercetin is in amorphous state, since no crystalline peak was distinguished compared to the pure quercetin showing a crystalline peak at 26°. This amorphization of quercetin confirmed its effective encapsulation in the three formulations.

4.2. Evaluation and selection of the most interesting antioxidant formulation using a fast-track oil-in-water emulsion assay (CAT assay)

All quercetin formulations were first assessed for their antioxidant capacity in emulsions with the CAT assay (Laguerre et al., 2008) using an emulsion of stripped Tung oil stabilized by Brij 35® polymer below its CMC. Lipid oxidation was induced by the AAPH hydrophilic azo-initiator. Quercetin alone or either formulated in Brij 35® micelles, Brij:DMPC mixed micelles or DMPC liposomes were added post emulsification and compared for their antioxidant efficiency at iso-concentration of quercetin (64 ng.mL^{-1}). Unloaded formulations (blank carriers) were also evaluated. In the case of Brij 35® micelles (Fig. 3A), for both B-Micelles or Q-Micelles, a pro-oxidant effect was observed in comparison with the control experiment that corresponds to the Tung oil emulsion alone (absence of quercetin and carriers). When quercetin was used alone, a slight antioxidant effect was observed, however, the concomitant addition of B-Micelles with unloaded quercetin resulted in the same pro-oxidant effect as the one observed for B-Micelles alone. Overall, these results suggest that the presence of Brij 35® micelles in the emulsion accelerates lipid oxidation kinetics. Different studies evaluated the influence of micelles on oxidative stability of oil-in-water emulsions and contradictory results were obtained (Villeneuve et al., 2023). Indeed, micelles may modulate lipid oxidation kinetics by different ways including the transport and relocation of antioxidants, prooxidants or lipid oxidation products. Consequently, depending of the type of emulsion, the nature of the antioxidants and the nature of the present pro-oxidants, micelles may either boost or delay lipid oxidation in emulsions. In our present experiments, one can conclude that Brij 35® micelles favor lipid oxidation kinetics and that their loading with quercetin does not counteract this effect. A different behavior was observed in the case of Brij/DMPC mixed micelles (Fig. 3B). Here, B-mixed micelles did not show any prooxidant effect as oxidation kinetic was very comparable to the one obtained with the control emulsion. Here again a slight antioxidant effect was observed for quercetin added alone but this effect was not enhanced with Q-Mixed micelles. Finally, for the experiment with DMPC liposomes (Fig. 3C), results showed that the addition of B-Liposomes could slightly delay lipid oxidation in comparison with the control emulsion. The potential antioxidant effect of phospholipids is known and may be attributed to various mechanisms including prooxidant metal chelation, synergism with tocopherols, or ability to react with aldehydes secondary oxidation compounds and form Maillard type reaction products with potential antioxidant activity (Cui & Decker, 2016). In our specific case, as tocopherols were removed during stripping of the oil and as metal prooxidant effect in PBS buffer is very limited, such synergistic effect or chelating activity are unlikely. Therefore, the apparent antioxidant effect of liposomes may be attributed to their capacity to react or entrap lipid oxidation products and consequently limit the diffusion of lipid oxidation through mass transfer phenomena (Laguerre et al., 2017). However, if we consider the fact that the equilibrium of the system is reached more quickly than the reaction timescale in the presence of micelles, this might not be the case with liposomes. Indeed, a clear difference was observed when quercetin was formulated into liposomes, suggesting that the dynamics within the liposomal system differ significantly from those with micelles. However, one must not exclude the fact that liposomes may have interacted with the hydrophilic AAPH azo initiator that was used in the CAT assay and therefore indirectly hampered oxidation induced by this initiator (Evans et al., 2019). For instance, an interaction between liposomes and AAPH, could alter its half-life or, more importantly, the release of radicals near the liposomes, explaining the greater efficiency when quercetin is encapsulated in such vesicles. Nevertheless, in our experiments, we could still observe that quercetin formulated in liposomes resulted in a better antioxidant efficiency then quercetin alone or quercetin in the presence of blank liposomes.

The same CAT assay experiments were then reproduced this time using unstripped Tung oil in order to keep present the native tocopherols found in this oil (≈ 250 ppm). Moreover, the concentration of quercetin in the emulsion was doubled in comparison with the stripped oil emulsion in order to better distinguish the different oxidation kinetics through an improved antioxidant effect. Interestingly, the prooxidant effect of Brij micelles in the case of stripped Tung oil emulsion (Fig. 3A) was not found when the oil was not depleted in its native tocopherols (Fig. 3D). Indeed, in the presence of tocopherols in the oil, the addition of B-Micelles slightly improved its oxidative resistance. This result can be correlated with the ones from Kiralan et al. (2014) who observed the polymer micelles could bring tocopherols to the continuous phase of an emulsion resulting in a better antioxidant efficiency.

When comparing the efficiency of quercetin alone with quercetin formulated in the presence of micelles (either encapsulated or external), our results show that in the presence of tocopherols, these formulations do not significantly influence the early-stage kinetics of lipid oxidation. However, both appear to slow down the kinetics during the propagation phase. This suggests that quercetin may distribute rapidly in the presence of micelles, faster than its reactivity, and encapsulating quercetin in micelles could enhance its interaction with tocopherols. This might occur by positioning quercetin at the oil/water interface of the emulsion or through co-micellization of tocopherols and quercetin within the micelles. This hypothesis is yet to be confirmed. When considering the experiments with mixed micelles with unstripped Tung oil (Fig. 3E), the addition of B-Mixed micelles in the emulsion improved its oxidative resistance in comparison with the control emulsion. Here again, this could be attributed to a potential interaction of such mixed micelles with the native tocopherols present in the oil. However, similarly to the experiments ran on stripped oil, the formulation of quercetin into these mixed micelles did not improve its antioxidant efficacy in comparison with quercetin alone. Finally, concerning liposomes, the experiments with unstripped Tung oil (Fig. 3F) confirmed their important contribution to the oxidative stability of the emulsion, as even B-Liposomes were more efficient than quercetin added alone. In this case, one can envisage some potential synergistic effects of phospholipids and tocopherols that would favor the antioxidant protection of the oil. Surprisingly when the emulsion was supplemented in both quercetin alone and B-Liposomes, no additive effect was observed. On the contrary, when quercetin was formulated into liposomes, a strong enhancement of its antioxidant efficiency was found in comparison with quercetin added alone in the system. Here again, the formulation of the antioxidant into liposomes may have contribute to favor its location at the interfacial area, favoring its synergistic effect with native tocopherols.

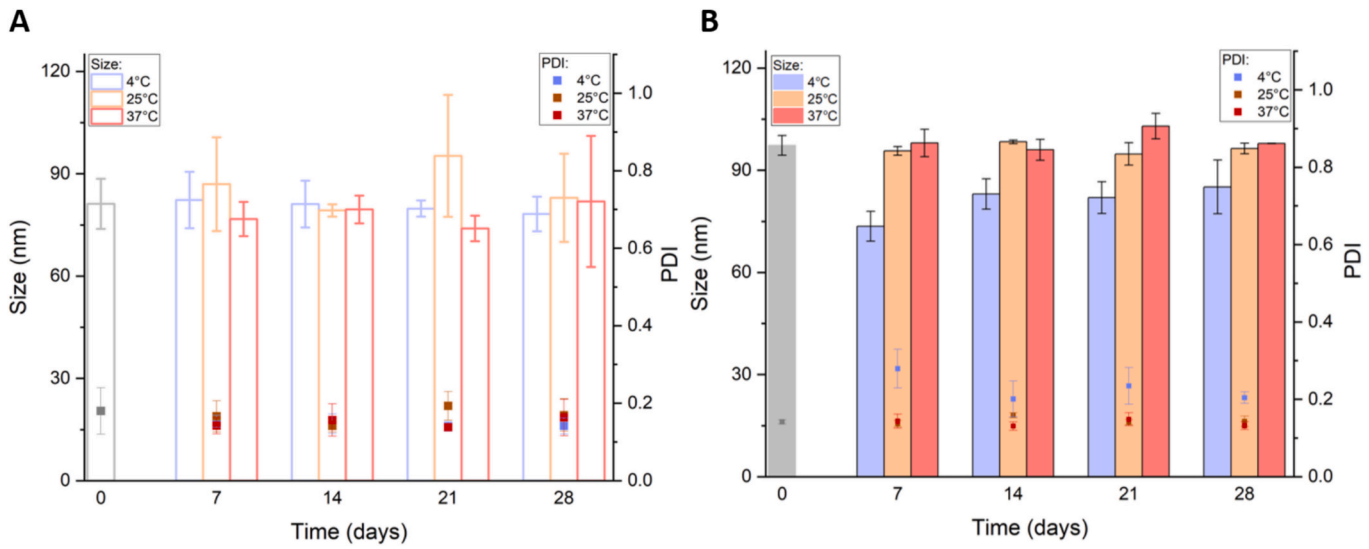

Fig. 4. Physical stability of A) B-Liposomes and B) Q-Liposomes with size (bars) and PDI (dots) for a month at 4, 25 and 37 °C (size mean ± SD, n = 3).

Table 2
Chemical stability of quercetin loaded in liposomes over 28 days at 4, 25 and 37 °C.

Time (days)	0	14	28
4 °C		329.0 μg.mL^{-1}	366.3 μg.mL^{-1}
25 °C	899.3 μg.mL^{-1}	71.7 μg.mL^{-1}	12.3 μg.mL^{-1}
37 °C		0.0	0.0

4.3. Evaluation of quercetin liposomal formulation on the oxidative stability of rapeseed oil-in-water emulsions

According to the results obtained with the CAT assay, the formulation of quercetin into liposomes was further evaluated for its antioxidant efficiency to protect a rapeseed oil-in-water emulsion consisting of 5 % wt unstripped rapeseed oil in 95 % aqueous phase (*w*/w) in phosphate buffer solution (10 mM, pH 7.0) and stabilized by Brij35® polymer at a 10 mM concentration in the final emulsion. For this experiment, the required quercetin concentration was set at 3000 μM in liposomes to be then diluted to a final concentration in emulsion at 50 μM. Therefore, the formulation was modified to reach this goal with a DMPC concentration of 50 g.L^{-1} and quercetin of 0.4 g.L^{-1}. Using ultrafiltration, the solution was concentrated twice to reach the desired concentration. The resulting B-Liposomes presented a hydrodynamic diameter of 81.2 ± 7.1 nm with PDI of 0.18 ± 0.06 and Q-Liposomes of 97.3 ± 2.9 nm with PDI of 0.14 ± 0.01. A stability study was also conducted to ensure the physical and chemical stability of liposomes. The physical stability reflected by the size and PDI at 4, 25 and 37 °C over a month is illustrated in Fig. 4 with B-Liposomes (Fig. 4A) and Q-Liposomes (Fig. 4B) once diluted to their final concentration. Overall for both B-Liposomes and Q-Liposomes, the hydrodynamic diameter and the PDI remained constant over the 28 days for each temperature tested showing that DMPC liposome either unloaded or loaded in quercetin remained physically stable through the duration of the storage.

Regarding the chemical stability of the Q-Liposomes, as indicated in Table 2 at 4 °C the quercetin concentration in liposomes dispersed in PBS dropped to 40 % after 14 days and remained after 28 days. At 25 °C, the quercetin was almost all degraded with 8 % remaining after 14 days and 2 % after 28 days. The quercetin was fully degraded at 37 °C after 14 days due to the quercetin thermal degradation as previously described (Kellil et al., 2021; Zhou & Sadik, 2008). This was considered for the study. Therefore, the oxidative test was conducted at 25 °C for a month.

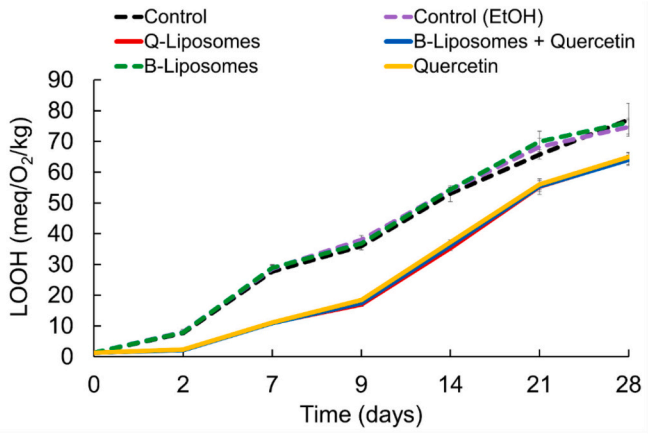

Fig. 5. Kinetics of peroxide formation (LOOH meq/O_2/kg) in rapeseed oil-in-water emulsions (5 % rapeseed oil in PBS (10 mM, pH 7.0) and Brij 35 (10 mM)) in the presence of blank liposomes (B-Liposomes), quercetin loaded liposomes (Q-Liposomes), quercetin alone (quercetin) and quercetin + B-Liposome.

The lipid oxidation kinetics were assessed by measuring the formation of primary oxidation compounds (hydroperoxides), secondary oxidation compounds (volatile aldehydes: hexanal, pentanal, 1,2-heptadienal, 2 pentenal and 2-ethyfuran) and the loss of tocopherol isoforms (α and γ isoforms). Concerning the kinetics of peroxide formation (Fig. 5), the addition of B-Liposomes in the emulsion did not show any impact on oxidative resistance in comparison with the control. This result did not confirm the one obtained in the CAT assay where B-Liposomes were shown to have an antioxidant effect (Fig. 3F). The main difference in the two emulsion systems relies in the different ways lipid oxidation was induced. In the CAT assay, oxidation was induced by free radicals produced in the continuous phase by the degradation of AAPH azo initiator, whereas in the 5 % rapeseed oil-in-water emulsion, no radical initiator was used. The absence of any antioxidant effect of the B-Liposomes in this latter system, could confirm our hypothesis that in the CAT assay, liposomes may have interfered with AAPH (or its degradation radical products). This would modulate the flow of produced radicals and contributes to a delay in oxidation kinetic that would lead to an erroneous interpretation of the potential antioxidant effect of the B-Liposomes. The emulsions supplemented with either quercetin alone,

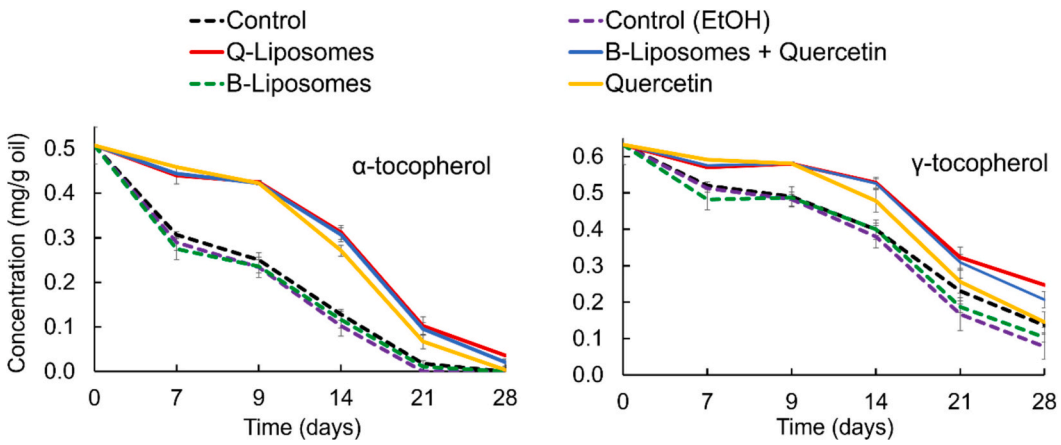

Fig. 6. Kinetics of tocopherol loss (α and γ) in rapeseed oil-in-water emulsions (5 % rapeseed oil in PBS (10 mM, pH 7.0) and Brij 35 (10 mM)) in the presence of blank DMPC liposomes (B-Liposomes), quercetin loaded liposomes (Q-Liposomes), quercetin alone and quercetin + B-Liposome.

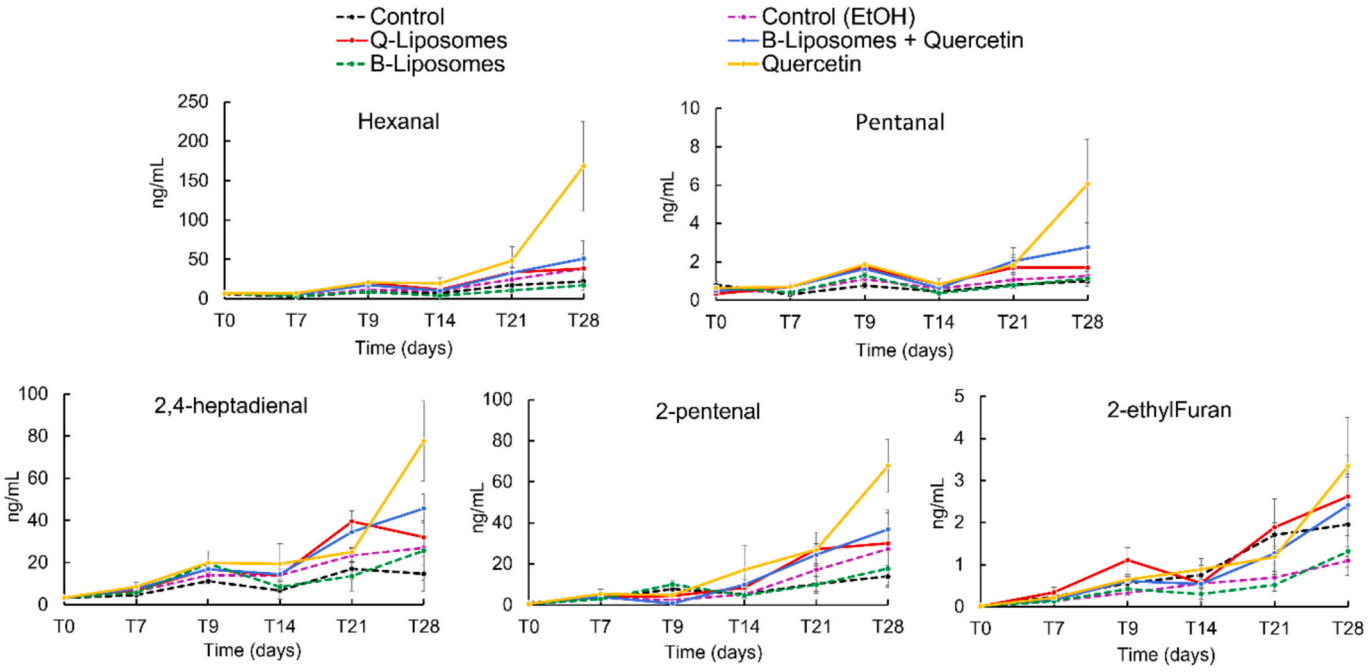

Fig. 7. Kinetics of volatile compounds formation (hexanal, pentanal, 2,4-heptadienal, 2-pentenal, 2-ethylfuran) in rapeseed oil-in-water emulsions (5 % rapeseed oil in PBS (10 mM, pH 7.0) and Brij 35 (10 mM)) in the presence of blank DMPC liposomes (B-liposomes), quercetin loaded liposomes (Q-liposomes), quercetin alone and quercetin + B-liposome.

quercetin + B-Liposomes or Q-Liposomes showed lower peroxide contents than the one of the controls, all along the storage period. However, the loading of quercetin into liposomes is not more efficient in terms of antioxidant effect than quercetin alone. In contrast with the CAT assay that was carried out over a few hours period, the present experiments were made for days (1 month). Therefore, one must consider the chemical degradation kinetics of quercetin that were previously estimated (Table 2), which demonstrated that only 8 % of quercetin was not degraded after 14 days. As both curves corresponding to quercetin alone or Q-Liposomes were almost stacking, one can conclude that the formulation of quercetin into liposome did not preserve it from degradation over time and did not significantly enhance its antioxidant effect in such type of emulsions.

It is also worth noting that between days 0 and 7, quercetin delays oxidation (with lower PV values and a more pronounced lag phase during the initiation), which also results in a lower consumption of tocopherols (Fig. 6) (higher tocopherol levels and almost no consumption when quercetin is present). However, there is no significant influence from quercetin being formulated in vesicles, likely because the release and/or equilibrium between phases is faster than the oxidation reaction suggesting no major effect on the localization of quercetin. After 7 days, the oxidation kinetics (propagation phase) become identical among all formulation samples. However, there appears to be a different effect on LOOH oxidation as volatile levels are slightly higher when quercetin is present (Fig. 7), with a smaller difference in PV values at the plateau phase. This could be attributed to the presence of various reducing agents, potentially arising from oxidized quercetin, or a preferential rerouting of secondary oxidation pathways in the presence of quercetin. For instance, this may involve the reduction of LOO°/LO° to LOOH/LOH with α/β scissions instead of the LOO°/LO° addition or cyclization, which may preferentially form epoxides rather than aldehydes.

5. Conclusion

The antioxidant efficacy of quercetin encapsulated in different delivery systems, namely Brij 35® micelles, DMPC Liposomes and Brij 35® /DMPC mixed micelles was evaluated in oil-in-water emulsions and compared to the one of free quercetin. In short-term assays, our results tend to show that liposomal formulation of quercetin provides the most effective protection against lipid oxidation, in comparison with the antioxidant effect observed for free quercetin or quercetin encapsulated in Brij 35® micelles or Brij/DMPC mixed micelles. We attributed this optimized activity of liposomal formulation of quercetin to its better location at the oil-water interface. Such interfacial location could favor the synergistic interactions of quercetin with naturally present tocopherols. In contrast, the results obtained with blank Brij 35 micelles suggest a potential prooxidant effect in emulsions, as the micelles may act as carriers of lipid oxidation products, a phenomenon that would accelerate the spread of oxidation within the emulsion in between lipid droplets. The formulation of quercetin within mixed micelles showed no significant antioxidant improvement suggesting that the structural arrangement of the mixed micelles might hinder the positioning of quercetin at the emulsion interface, limiting its effectiveness. However, in long-term assay, the observed results were different. Indeed, while liposomal formulation of quercetin did result in enhanced antioxidant efficacy in short-term assays, the chemical stability of quercetin remained a limiting factor over extended period. Degradation studies indicated significant losses of quercetin within liposomes, especially at elevated temperatures, which could compromise their long-term utility in applications requiring extended antioxidant stability. As a base for perspectives, while liposomal formulation may have enhanced the partitioning of quercetin at the interface and accordingly, its synergism with tocopherols, the present study highlights the importance of formulation considerations to protect quercetin's integrity, perhaps through adjustments to the liposome composition or the addition of stabilizing agents. Overall, these results show the influence of formulation on antioxidant effectiveness in emulsified food systems. However, a study regarding the appropriate choice of antioxidant to be encapsulated and the influence of the type of formulation on the resulting antioxidant efficacy remains to be further investigated.

CRediT authorship contribution statement

Laurianne Simon: Writing – review & editing, Validation, Methodology, Investigation, Formal analysis. **Erwann Durand:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis. **Christophe Dorandeu:** Writing – review & editing, Validation, Methodology, Formal analysis. **Bruno Baréa:** Writing – review & editing, Validation, Methodology, Formal analysis. **Noelia M. Sanchez-Ballester:** Formal analysis, Investigation, Methodology, Validation, Writing – original draft. **Sylvie Begu:** Writing – review & editing, Supervision, Investigation. **Pierre Villeneuve:** Writing – review & editing, Supervision, Investigation, Conceptualization.

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.

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Data availability

Data will be made available on request.

References

Bayram, I., & Decker, E. A. (2023). Underlying mechanisms of synergistic antioxidant interactions during lipid oxidation. *Trends in Food Science and Technology, 133*, 219–223. https://doi.org/10.1016/j.tifs.2023.02.003

Belhaj, N., Arab-Tehrany, E., & Linder, M. (2010). Oxidative kinetics of salmon oil in bulk and in nanoemulsion stabilized by marine lecithin. *Process Biochemistry, 45*, 187–195. https://doi.org/10.1016/j.procbio.2009.09.005

Bonina, F., Lanza, M., Montenegro, L., Puglisi, C., Tomainon, A., Trombetta, D., … Saija, A. (1996). Flavonoids as potential protective agents against photo-oxidative skin damage. *International Journal of Pharmaceutics, 145*, 87–94. https://doi.org/10.1016/S0378-5173(96)04728-X

Bravo-Diaz, C. (2023). Advances in the control of lipid peroxidation in oil-in-water emulsions: Kinetic approaches. *Critical Reviews in Food Science and Nutrition, 63*, 6252–6284. https://doi.org/10.1080/10408398.2022.2029827

Caddeo, C., Diez-Sales, O., Pons, R., Fernandez-Busquets, X., Fadda, A. M., & Manconi, M. (2014). Topical anti-inflammatory potential of quercetin in lipid-based nanosystems: In vivo and in vitro evaluation. *Pharmaceutical Research, 31*, 959–968. https://doi.org/10.1007/s11095-013-1215-0

Cui, L., & Decker, E. A. (2016). Phospholipids in foods: Prooxidants or antioxidants? *Journal of the Science of Food and Agriculture, 96*, 18–31. https://doi.org/10.1002/jsfa.7320

Dangles, O. (2012). Antioxidant activity of plant phenols: Chemical mechanisms and biological significance. *Current Organic Chemistry, 16*, 692–714. doi:10.2174/138527212799957995.

Decker, E. A., Chen, B., Panya, A., & Elias, R. J. (2010). Understanding antioxidant mechanisms in preventing oxidation in foods. In E. A. Decker (Ed.), *Oxidation in foods and beverages and antioxidant applications* (pp. 225–248). Woodhead Publishing. https://doi.org/10.1533/9780857090447.2.225.

Decker, E. A., McClements, D. J., Bourlieu-Lacanal, C., Durand, E., Figueroa-Espinoza, M. C., Lecomte, J., & Villeneuve, P. (2017). Hurdles in predicting antioxidant efficacy in oil-in-water emulsions. *Trends in Food Science and Technology, 67*, 183–194. https://doi.org/10.1016/j.tifs.2017.07.001

Di Mattia, C. D., Sacchetti, G., Mastrocola, D., & Pittia, P. (2009). Effect of phenolic antioxidants on the dispersion state and chemical stability of olive oil O/W emulsions. *Food Research International, 42*, 1163–1170. https://doi.org/10.1016/j.foodres.2009.05.017

Dufour, C., Loonis, M., & Dangles, O. (2007). Inhibition of the peroxidation of linoleic acid by the flavonoid quercetin within their complex with human serum albumin. *Free Radical Biology and Medecine, 43*, 241–252. https://doi.org/10.1016/j.freeradbiomed.2007.04.009

Elmi, N., Ghanbarzadeh, B., Ayaseh, A., Sahraee, S., Khakbaz Heshmati, M., Hoseini, M., & Pezeshki, A. (2021). Physical properties and stability of quercetin loaded niosomes: Stabilizing effects of phytosterol and polyethylene glycol in orange juice model. *Journal of Food Engineering, 296*, Article 110463. https://doi.org/10.1016/j.jfoodeng.2020.110463

Evans, K. O., Compton, D. L., Kim, S., & Appell, M. (2019). Charged phospholipid effects on AAPH oxidation assay as determined using liposomes. *Chemistry and Physics of Lipids, 220*, 49–56. https://doi.org/10.1016/j.chemphyslip.2019.02.004. ISSN 0009-3084.

Fasolo, D., Bassani, V. L., & Teixeira, H. F. (2009). Development of topical nanoemulsions containing quercetin and 3-O-methylquercetin. *Pharmazie, 64*, 726–730. https://doi.org/10.1691/ph.2009.8357

Feng, J., Lin, C., Wang, H., & Liu, S. (2017). Gemini dodecyl O-glucoside-based vesicles as nanocarriers for catechin laurate. *Journal of Functional Foods, 32*, 256–265. https://doi.org/10.1016/j.jff.2017.03.005

Hatahet, T., Morille, M., Hommoss, A., Devoisselle, J. M., Müller, R. H., & Bégu, S. (2016). Quercetin topical application, from conventional dosage forms to nanodosage forms. *European Journal of Pharmaceutics and Biopharmaceutics, 108*, 41–53. https://doi.org/10.1016/j.ejpb.2016.08.011

Hatahet, T., Morille, M., Hommoss, A., Devoisselle, J. M., Müller, R. H., & Bégu, S. (2018). Liposomes, lipid nanocapsules and smartCrystals®: A comparative study for an effective quercetin delivery to the skin. *International Journal of Pharmaceutics, 542*, 176–185. https://doi.org/10.1016/j.ijpharm.2018.03.019

Hatahet, T., Morille, M., Shamseddin, A., Aubert-Pouëssel, A., Devoisselle, J. M., & Bégu, S. (2017). Dermal quercetin lipid nanocapsules: Influence of the formulation on antioxidant activity and cellular protection against hydrogen peroxide. *International Journal of Pharmaceutics, 518*, 167–176. https://doi.org/10.1016/j.ijpharm.2016.12.043

Hennebelle, M., Villeneuve, P., Durand, E., Lecomte, J., van Duynhoven, J., Meynier, A., Yesiltas, B., Jacobsen, C., & Berton-Carabin, C. (2024). Lipid oxidation in emulsions: New insights from the past two decades. *Progress in Lipid Research, 94*, Article 101275. https://doi.org/10.1016/j.plipres.2024.101275

Inchingolo, R., Bayram, I., Sibel Uluata, S., Kiralan, S., Rodriguez-Estrada, M. T., Julian McClements, D., & Decker, E. A. (2021). Ability of Sodium Dodecyl Sulfate (SDS) Micelles to Increase the Antioxidant Activity of α-Tocopherol. *Journal of Agricultural and Food Chemistry, 69*(20), 5702–5708. https://doi.org/10.1021/acs.jafc.1c01199

Javani, R., Sadat Hashemi, F., Ghanbarzadeh, B., & Hamishehkar, H. (2021). Quercetin-loaded niosomal nanoparticles prepared by the thin-layer hydration method: Formulation development, colloidal stability, and structural properties. *LWT, 141*, Article 110865. https://doi.org/10.1016/j.lwt.2021.110865

Kandemir, K., Tomas, M., McClements, D. J., & Capanoglu, E. (2022). Recent advances on the improvement of quercetin bioavailability. *Trends in Food Science and Technology, 119*, 192–200. https://doi.org/10.1016/j.tifs.2021.11.032

Kellil, A., Grigorakis, S., Loupassaki, S., & Makris, D. P. (2021). Empirical kinetic modelling and mechanisms of quercetin thermal degradation in aqueous model

systems: Effect of pH and addition of antioxidants. *Applied Sciences, 11*, 2579. https://doi.org/10.3390/app11062579

Kiralan, S. S., Doğu-Baykut, E., Kittipongpittaya, K., McClements, D. J., & Decker, E. A. (2014). Increased antioxidant efficacy of tocopherols by surfactant Solubilization in oil-in-water emulsions. *Journal of Agricultural and Food Chemistry, 62*, 10561–10566. https://doi.org/10.1021/jf503115j

Klooster, S. T., Schroën, K., & Berton-Carabin, C. (2023). Lipid oxidation products in model food emulsions: Do they stay in or leave droplets, that's the question. *Food Chemistry, 405*, 134992. https://doi.org/10.1016/j.foodchem.2022.134992

Laguerre, M., Bayrasy, C., Panya, A., Weiss, J., McClements, D. J., Lecomte, J., Decker, E. A., & Villeneuve, P. (2015). What makes good antioxidants in lipid-based systems? The next theories beyond the polar paradox. *Critical Reviews in Food Science and Nutrition, 55*, 183–201. https://doi.org/10.1080/10408398.2011.650335

Laguerre, M., Bily, A., Roller, M., & Birtić, S. (2017). Mass transport phenomena in lipid oxidation and antioxidation. *Annual Reviews in Food Science and Technology, 8*, 391–411. https://doi.org/10.1146/annurev-food-030216-025812

Laguerre, M., López-Giraldo, L. J., Lecomte, J., Baréa, B., Cambon, E., Tchobo, F. P., … Villeneuve, P. (2008). Conjugated autoxidizable triene (CAT) assay: A novel spectrophotometric method for determination of antioxidant capacity using triacylglycerol as ultraviolet probe. *Analytical Biochemistry, 380*, 282–290. https://doi.org/10.1016/j.ab.2008.06.006

Lin, C. F., Leu, Y. L., Al-Suwayeh, S. A., Ku, M. C., Hwang, T. L., & Fang, J. Y. (2012). Anti-inflammatory activity and percutaneous absorption of quercetin and its polymethoxylated compound and glycosides: The relationships to chemical structures. *European Journal of Pharmaceutical Sciences, 47*, 857–864. https://doi.org/10.1016/j.ejps.2012.04.024

Liu, D., Hu, H., Lin, Z., Chen, D., Zhu, Y., Hou, S., & Shi, X. (2013). Quercetin deformable liposome: Preparation and efficacy against ultraviolet B induced skin damages in vitro and in vivo. *Journal of Photochemistry and Photobiology B: Biology, 127*, 8–17. https://doi.org/10.1016/j.jphotobiol.2013.07.014

Manca, M. L., Castangia, I., Caddeo, C., Pando, D., Escribano, E., Valenti, D., Lampis, S., Zaru, M., Fadda, A. M., & Manconi, M. (2014). Improvement of quercetin protective effect against oxidative stress skin damages by incorporation in nanovesicles. *Colloids and Surfaces B: Biointerfaces, 123*, 566–574. https://doi.org/10.1016/j.colsurfb.2014.09.059

Martinović, N., Polak, T., Poklar Ulrih, N., & Abramovič, H. (2020). Mustard seed: Phenolic composition and effects on lipid oxidation in oil, oil-in-water emulsion and Oleogel. *Industrial Crops and Products, 156*, Article 11285. https://doi.org/10.1016/j.indcrop.2020.112851

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*, 1270–1282. https://doi.org/10.1111/j.1365-2621.2000.tb10596.x

Miquel Becker, E., Ntouma, G., & Skibsted, L. H. (2007). Synergism and antagonism between quercetin and other chain-breaking antioxidants in lipid systems of increasing structural organization. *Food Chemistry, 103*, 1288–1296. https://doi.org/10.1016/j.foodchem.2006.10.034

Noon, J., Mills, T. B., & Norton, I. T. (2020). The use of natural antioxidants to combat lipid oxidation in O/W emulsions. *Journal of Food Engineering, 281*, Article 110006. https://doi.org/10.1016/j.jfoodeng.2020.110006

Olbińska, E., Trela-Makowej, A., Larysz, W., Orzechowska, A., & Szymańska, R. (2023). The effect of α-tocopherol incorporated into different carriers on the oxidative stability of oil in water (O/W) emulsions. *Colloids and Surfaces B: Biointerfaces, 230*, Article 113536. https://doi.org/10.1016/j.colsurfb.2023.113536

Scalia, S., Franceschinis, E., Bertelli, D., & Iannuccelli, V. (2013). Comparative evaluation of the effect of permeation enhancers, lipid nanoparticles and colloidal silica on in vivo human skin penetration of quercetin. *Skin Pharmacology and Physiology, 26*, 57–67. https://doi.org/10.1159/000345210

Schröder, A., Laguerre, M., Sprakel, J., Schroën, K., & Berton-Carabin, C. C. (2020). Pickering particles as interfacial reservoirs of antioxidants. *Journal of Colloid and Interface Science, 575*, 489–498. https://doi.org/10.1016/j.jcis.2020.04.069

Simon, L., De Taddeo, M., Coeurvolan, A., Colpaert, M., Richard, J., Devoisselle, J. M., Morille, M., Marcotte, N., Begu, S., & Lapinte, V. (2023). Various lipid anchors on amphiphilic polyoxazolines to reach efficient intracellular delivery. *International Journal of Pharmaceutics, 642*, Article 123103. https://doi.org/10.1016/j.ijpharm.2023.123103

Simon, L., Lapinte, V., Lionnard, L., Marcotte, N., Morille, M., Aouacheria, A., Kissa, K., Devoisselle, J. M., & Bégu, S. (2020). Polyoxazolines based lipid nanocapsules for topical delivery of antioxidants. *International Journal of Pharmaceutics, 579*, Article 119126. https://doi.org/10.1016/j.ijpharm.2020.119126

Simon, L., Vincent, M., Le Saux, S., Lapinte, V., Marcotte, N., Morille, M., Dorandeu, C., Devoisselle, J. M., & Bégu, S. (2019). Polyoxazolines based mixed micelles as PEG free formulations for an effective quercetin antioxidant topical delivery. *International Journal of Pharmaceutics, 570*, Article 118516. https://doi.org/10.1016/j.ijpharm.2019.118516

Skibsted, L. H. (2010). Understanding Mechanisms of Oxidation and Antioxidant Activity. *Oxidation in Foods and Beverages and Antioxidant Applications* (pp. 3–35). Woodhead Publishing Series in Food Science, Technology and Nutrition.

Tan, Q., Liu, W., Guo, C., & Zhai, G. (2011). Preparation and evaluation of quercetin-loaded lecithin-chitosan nanoparticles for topical delivery. *International Journal of Nanomedicine, 6*, 1621–1630. https://doi.org/10.2147/IJN.S22411

Valerga, J., Reta, M., & Lanari, M. C. (2012). Polyphenol input to the antioxidant activity of yerba mate (Ilex paraguariensis) extracts. *LWT - Food Science and Technology, 45*, 28–35. https://doi.org/10.1016/j.lwt.2011.07.022

Villeneuve, P., Bourlieu-Lacanal, C., Durand, E., Lecomte, J., McClements, D. J., & Decker, E. A. (2023). Lipid oxidation in emulsions and bulk oils: A review of the importance of micelles. *Critical Reviews in Food Science and Nutrition, 63*, 4687–4727. https://doi.org/10.1080/10408398.2021.2006138

Wadhwa, K., Kadian, V., Puri, V., Yogeshvar Bhardwaj, B., Sharma, A., Pahwa, R., Rao, R., Gupta, M., & Singh, I. (2022). New insights into quercetin nanoformulations for topical delivery. *Phytomedicine Plus, 2*, Article 100257. https://doi.org/10.1016/j.phyplu.2022.100257

Wang, W., Sun, C., Mao, L., Ma, P., Liu, F., Yang, J., & Gao, Y. (2016). The biological activities, chemical stability, metabolism and delivery systems of quercetin: A review. *Trends in Food Science and Technology, 56*, 21–38. https://doi.org/10.1016/j.tifs.2016.07.004

Wu, T. H., Yen, F. L., Lin, L. T., Tsai, T. R., Lin, C. C., & Cham, T. M. (2008). Preparation, physicochemical characterization, and antioxidant effects of quercetin nanoparticles. *International Journal of Pharmaceutics, 346*, 160–168. https://doi.org/10.1016/j.ijpharm.2007.06.036

Zhang, H., Zhang, M., Yu, L., Zhao, Y., He, N., & Yang, X. (2012). Antitumor activities of quercetin and quercetin-5′,8-disulfonate in human colon and breast cancer cell lines. *Food and Chemical Toxicology, 50*, 1589–1599. https://doi.org/10.1016/j.fct.2012.01.025

Zhang, Y., Wang, X., Zeng, Q., Deng, Y., Xie, P., Zhang, C., & Huang, L. (2023). A new insight into synergistic effects between endogenous phenolic compounds additive and α-tocopherol for the stability of olive oil. *Food Chemistry, 427*, Article 136667. https://doi.org/10.1016/j.foodchem.2023.136667

Zhou, A., & Sadik, O. A. (2008). Comparative analysis of quercetin oxidation by electrochemical, enzymatic, autoxidation, and free radical generation techniques: A mechanistic study. *Journal of Agricultural and Food Chemistry, 56*, 12081–12091. https://doi.org/10.1021/jf802413v