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1 **Improvement of the oxidative stability of camelina oil by enrichment with phospholipid-quercetin** 2 **formulations**

3 Maria del Pilar GARCIA-MENDOZA, Faber Ariel ESPINOSA-PARDO, Raphaëlle SAVOIRE*,
4 Christelle HARSCOAT-SCHIAVO, Maud CANSELL, Pascale SUBRA-PATERNAULT
5 *Univ. Bordeaux, CNRS, Bordeaux INP, CBMN, UMR 5248, F-33600, Pessac, France.*

6 **Corresponding Author: Tel.: +33(0)540006879*

7 *E-mail addresses:* maria-del-pilar.garcia-mendoza@u-bordeaux.fr (M.d.P. Garcia-Mendoza), faber-
8 ariel.espinosa-pardo@u-bordeaux.fr (F.A. Espinosa-Pardo), raphaelle.savoire@enscbp.fr, (R. Savoire),
9 christelle.harscoat-schiavo@u-bordeaux.fr (C. Harscoat-Schiavo), mcansell@enscbp.fr (M. Cansell),
10 p.subra-paternault@cbmn.u-bordeaux.fr (P. Subra-Paternault).

11 **Abstract**

12 Camelina oil (Coil) contains 50–60 % of polyunsaturated fatty acids which are susceptible to oxidation.
13 In this work, addition of phospholipids (0-20 mg/g) was assessed to improve the solubility of quercetin
14 in Coil and enhance its oxidative stability. Results showed that the solubility of quercetin in Coil
15 increased up to 7.7-fold by phospholipid addition. The solubility of quercetin in Coil was correlated to
16 the phospholipid concentration and reached a maximum value of 1 298 µg/g. The addition of
17 phospholipid-quercetin formulations increased the Coil stability, measured at 60 °C, from 24 h up to
18 115 h. Coil saturated with only quercetin (168 µg/g) did not significantly increase Coil stability,
19 whereas phospholipids alone extended the oxidation lag time up to 40 h. This work successfully
20 developed a solvent-free method for improving the solubility of quercetin in Coil and enhance its
21 oxidative stability.

22 **Keywords:** Bulk oil, phosphatidylcholine, antioxidants, solubility, micelles, lipid oxidation.

1. Introduction

Edible plant oils are mainly composed of triglycerides (95-99 %). The fatty acids (FA) esterified in this lipid species are characteristic of the oil natural resources and determine the physical and chemical properties of the oil. Polyunsaturated fatty acids (PUFAs) and, especially those of the n-3 series, have major nutritional interests. Among them, α -linolenic acid (18:3 n-3, ALA) is an essential fatty acid in the diet of humans and is the principal n-3 PUFA present in the western diet. The major dietary sources of ALA are plant oils. For example, ALA accounts for 10 % of total FA in rapeseed oil and soybean oil, and up to 60 % in flaxseed oil. Camelina oil (Coil) is obtained from *Camelina sativa* (L.); a cruciferous oilseed plant less known but presenting an interesting FA composition. Indeed, Coil contains about 50–60 % of PUFAs among which 35–40 % comprise ALA (Moslavac et al., 2014).

Nevertheless, the presence of PUFAs with two or more double bonds favors oxidative reactions in the oil. This alteration is responsible for the development of unpleasant flavors and odors thereby turning products to unsuitable for consumption. Moreover, these changes affect the nutritional quality of food due to the degradation of fat-soluble vitamins and fatty acids. The delay of lipid oxidation can be accomplished by the addition of natural or synthetic antioxidants which maintain quality and extend oil shelf life by protecting them from rancidity. Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are common and effective synthetic antioxidants for fat food protection, however these additives are associated with liver damage and carcinogenesis (Szydłowska-Czerniak, Rabiej, Kyselka, Dragoun, & Filip, 2018). The protective effect of polyphenols against lipid oxidation has been widely reported in literature (Ramadan, 2012). Among polyphenols, flavonoids are natural phenolic derivatives characterized by a common benzo-pyrone structure. Particularly, quercetin (3, 3', 4', 5, 7-pentahydroxyflavone) (Fig. 1) is an abundant flavonoid mostly found in natural sources as onions, apples, tea, red wine that exerts many health benefits due to its antioxidant, anti-inflammatory, anticarcinogenic, cardioprotective, neuroprotective and antihypertensive effects (Xu et al., 2016; Zhang

et al., 2016). The antioxidant mechanism of quercetin is particularly related to its capacity to scavenge reactive species (Ramadan, 2012).

On the other hand, the enrichment of oily systems with phenolic compounds may be limited by their low stability during processing and their poor solubility in oils and fats due to the multi-ring and polar nature of polyphenols (Ghanbarzadeh, Babazadeh, & Hamishehkar, 2016). This reduces their effectiveness to protect oils and fats against lipid oxidation (Nikoo, Regenstein, & Ahmadi Gavlighi, 2018; Szydlowska-Czerniak, Rabiej, Kyselka, Dragoun, & Filip, 2018). In aqueous systems, the use of vesicular carriers or delivery systems of polyphenols made with phospholipids such as liposomes is common (Ghanbarzadeh, Babazadeh, & Hamishehkar, 2016). In oils, the solubility of phenolics can also be improved by the addition of lecithin (Suárez, Romero, Ramo, & Motilva, 2011), a co-enrichment that could moreover increase the stability of polyunsaturated oils. For example, Ramadan (2012) found that increasing the solubility of quercetin through its formulation with soy lecithin resulted in an improved antioxidant effect on triolein and sunflower oil. Suárez, Romero, Ramo, & Motilva (2011) showed that the addition of lecithin improved the dispersion and stabilization of phenolic extracts from olive cake in olive oil and thus enhanced the oxidative stability of the enriched oils. Chen, Zhang, Wu, & Weng (2015) reported a high antioxidant activity of an epigallocatechin gallate-phospholipid complex against oxidation of soybean oil. Similarly, Nikoo, Regenstein, & Ahmadi Gavlighi, (2018) also stated that for instance, both solubility and antioxidant activity of epigallocatechin gallate in lipids might be enhanced by means of carrier systems or esterification with aliphatic acyl derivatives which modifies conveniently the physical properties of the epigallocatechin gallate. Quercetin antioxidant effect on bulk oils has been reported previously by several authors. Huber, Vasantha Rupasinghe, & Shahidi (2009) found a higher effectiveness of quercetin (100 to 1 000 μM) for inhibiting lipid oxidation in bulk fish oil and in oil-in-water emulsion systems, when compared with the effect of BHT and α -tocopherol, respectively.

To the best of our knowledge, there are no studies on the combined influence of various quercetin and phospholipid formulations on the oxidation of non-stripped virgin camelina oil. Therefore, in the current work, we aimed at the assessment of the oxidative stability of Coil enriched with several formulations of phosphatidylcholine-quercetin by monitoring the conjugated dienes formation under accelerated conditions of heating. The use of phospholipids as promoter of quercetin solubility in oils (and not in water) is also a novelty. The enrichment levels were varied in relation to the enhancement of quercetin solubility in Coil with the increasing phospholipid concentration, a solubility study that has not been reported in literature so far. In addition, some data regarding quercetin solubility in sunflower oil has been also provided as supplementary information. The enrichment method was also original compared to literature since we developed a solvent-free route that suppressed the use of any organic solvent.

Fig. 1. Molecular structure of quercetin

2. Materials and methods

2.1 Materials

Cold-pressed camelina (*Camelina sativa* L.) oil was provided by Olead (Pessac, France). Refined sunflower oil (Bouton d'or-Geproc, Monaco, France) (chemical composition is shown in

88 supplementary data, Table 1A) was purchased in a local market in Bordeaux. Table 1 presents the
 89 chemical characterization of Coil determined by ITERG (Institut des Corps Gras, Canéjan, France).
 90 Fatty acid composition was determined according to NF EN ISO 12966.

91 **Table 1.** Chemical composition of Coil

Fatty acids Composition (%)	
16:0	5.7
18:0	2.4
18:1 n-9	13.8
18:2 n-6	17.3
18:3n-3	37.5
20:0	1.4
20:1	13.7
20:2	2.1
20:3	1.7
22:1	2.9
Σ SFA	9.9
Σ MUFA	31.1
Σ PUFA	58.7
PUFA n-3	37.5
PUFA n-6	21.2
Vitamin E (mg/kg)	
α-tocopherol	29.0
β-tocopherol	<2
γ-tocopherol	685.5
δ-tocopherol	11.9
Total Vitamin E	726.4
Phospholipids (g/100g)	
< 0.35	
Metals (mg/kg)	
Copper	<0,005
Iron	1.28
Sterols (mg/kg)	
4824	
Total Phenolic Content (mg GAE/kg)	
30 ± 2.6	

92 Σ SFA: total saturated fatty acids, Σ MUFA: total monounsaturated fatty acids, Σ PUFA: total
 93 polyunsaturated fatty acids, GAE: gallic acid equivalents

2.1.1 Chemicals and reagents

Quercetin (≥ 95 % HPLC, solid, molecular weight: 302.24 g/mol, PubChem CID: 5280343) and potassium iodide were purchased from Sigma Aldrich Chemical Co. (St. Quentin Fallavier, France). Lipoid P100, soybean phospholipids (PL), was purchased from Lipoid GmbH (Grasse, France) and comprised 95.5 % of phosphatidylcholine. The molecular weight of phosphatidylcholine was considered of 775.04 g/mol according to Wang, Xu, Lan, Yang, & Wang (2015). Absolute ethanol (analytical grade), methanol (HPLC grade), chloroform (reagent grade), hexane (96 %, reagent grade) and sodium thiosulfate (0.01 N) were purchased from Scharlab (Barcelona, Spain). Orthophosphoric acid (85 %) was provided by Alfa Aesar (Karlsruhe, Germany), glacial acetic acid by JT Baker (Illkirch, France) and Iotec (iodine indicator) by VWR Chemicals (Leuven, Belgium). The 7,7,8,8 - Tetracyanoquinodimethane was purchased from Merck KGaA (Darmstadt, Germany).

2.2 Experimental methods

2.2.1 Determination of the solubility of quercetin in Coil

Over-saturated solutions of quercetin ($> 5\,000\ \mu\text{g/g}$) in Coil (CQ*) were prepared. The solutions were stirred for 3 h at room temperature followed by sonication for 5 min in an ultrasonic bath (Model FB15049, Fisherbrand, Germany) and again stirred for 5 min, then suspensions were left for settling overnight at room temperature. The supernatants were transferred to Falcon graduated tubes of 15 mL and centrifuged (Rotanta 460 RF, Hettich, Germany) at 13 300 g for 10 min. The supernatants from the first centrifugation were submitted to a second centrifugation at same conditions. The supernatants from the second centrifugation were then filtered on 0.2 μm PTFE syringe filter (Rotilabo-syringe filters, Carl Roth, Germany). Finally, the filtrates were diluted in pure Coil, and the quantification of quercetin was carried out by High Performance Liquid Chromatography (HPLC) analysis (see section 2.2.4).

2.2.2 Solubility of quercetin in Coil enriched with phospholipids

200 mg of PL were added to 10 g of Coil (20 mg PL/g oil) (CQL20*) in sealed amber flasks. The amount of PL added to Coil was the maximum that could be solubilized in the oil. The sample was then over saturated with quercetin and put under nitrogen flux to avoid oxidation reactions. Resulting suspension was stirred at 35 °C for 1 h using a digital magnetic hotplate stirrer with temperature control (Model C-MAG HS 7, Ika®, Germany), then sonicated (ultrasonic bath Model FB15049, Fisherbrand, Germany) for 10 min and stirred again for 4 h at 35 °C. Finally, the sample was left for settling overnight at room temperature. The supernatant was filtered, diluted, and analyzed by HPLC (see section 2.2.4). The solubility of quercetin in Coil containing 5, 10 and 15 mg of PL/g oil was also assessed.

2.2.3 Enrichment of Coil with PL-quercetin formulations

In order to assess the effect of enriching Coil with various phospholipid-quercetin formulations on the oxidative stability, different amounts of PL (5-20 mg/g oil; 5.9-23.4 mM) and quercetin (220-2 000 µg/g oil; 662-6022 µM) were directly added to sealed amber flasks containing 30 g of Coil. Coil enriched with only PL were also prepared (5-20 mg/g oil) in order to study the influence of only phospholipids on the oxidation of Coil. After the addition of PL or PL+quercetin, the samples were stirred at 35 °C for 1 h using a digital magnetic hotplate stirrer with temperature control (Model C-MAG HS 7, Ika®, Germany), then sonicated (ultrasonic bath Model FB15049, Fisherbrand, Germany) for 10 min, to destroy agglomerates and, stirred again for 4 h at 35 °C. The enrichment process was performed in duplicate. Enriched Coils prepared at saturated concentration of quercetin were filtrated (0.2 µm PTFE syringe filter, Rotilabo-syringe filters, Carl Roth, Germany) before analysis for removing excess quercetin, whereas Coils enriched with quercetin at concentrations below its saturation were directly oxidized without filtration.

2.2.4 High Performance Liquid Chromatography analysis for quercetin quantification

Oils containing quercetin were analyzed following the method described by Xu et al. (2016) with some modifications. An Agilent 1260 high performance liquid chromatograph (Agilent Technologies, Santa Clara, USA) equipped with a quaternary pump solvent delivery system, an automatic injector, and a diode array detector Agilent Infinity G1315D was used. The sample injection volume was adjusted from 2 to 10 μ L and the chromatographic separation was carried out on a C18-column (Eclipse XDB-C18 3.5 μ m, 4.6 x 100 mm, Agilent Technologies France, Les Ulis, France) maintained at 30 °C. The mobile phase consisted of HPLC grade methanol (Solvent A) and water with 0.03 % phosphoric acid (Solvent B) at a flow rate of 1 mL/min. The elution program was as follows: 0 to 5 min of 50 % A, 5 to 8 min of 100 % A and 8 to 12 min of 50 % A. Quercetin was quantified at 370 nm using a calibration curve obtained from standard solutions in absolute ethanol. The calibration curve ranged from 0.02 to 0.2 μ g of quercetin ($R^2 = 0.999$). HPLC analyses were run in duplicate and results were expressed in μ g quercetin/g oil.

2.2.5 Assessment of the oxidative stability of Coil enriched with PL-quercetin formulations

The samples of enriched and non-enriched Coil were submitted to accelerated oxidation through the Schaal oven test. For that, 7 g aliquots of sample were placed in 40 mL sealed amber flasks and heated at 60 °C in a laboratory drying oven (DRY-Line, VWR, UK) for 6 to 240 h. At each specific time, one amber flask was got out of the oven and stored at -80 °C until analysis. It was previously verified that enough oxygen was available in the sealed amber flask for oil oxidation. This fact was verified by means of the comparison of the oxidation rate of oils placed in unsealed versus sealed amber flasks. Samples were obtained in duplicate and analyzed for their concentration in remaining quercetin and their conjugated diene (CD) concentrations. CD is a simple method for measuring the

162 primary oxidation products and highly correlated to peroxide value (PV) (Wanasundara, Shahidi, &
163 Jablonski, 1995).

164 For CD analysis, approximately 0.05 g of Coil were weighed in a 10 mL amber round bottom
165 flask which was further gauged with hexane, stirred, and reserved. This solution was diluted in hexane
166 in a hermetic vial to avoid solvent evaporation, then stirred and transferred to quartz cuvettes for
167 reading at 233 nm in a spectrophotometer (Model UH5300, Hitachi, Japan) using pure hexane as blank.
168 CD concentration was expressed as specific absorbance (A.U. 233 nm/mg/mL) normalizing the optical
169 density by the mass of lipids (mg) per unit volume of solution (mL). PV was determined by titration of
170 iodine with a standard solution of sodium thiosulfate 0.01 N and it was expressed in milliequivalents of
171 active oxygen/kg of oil.

172 **2.2.6 Determination of Critical Micelle Concentration (CMC)**

173 The self-assembly property of phospholipids in Coil was investigated using the 7,7,8,8 -
174 Tetracyanoquinodimethane (TCNQ) solubilization method. The principle of the TCNQ method is that
175 the micelles solubilize the TCNQ dye leading to a rapid change of color around the CMC (Lehtinen et
176 al., 2017).

177 Firstly, 70 g of a stock solution of Coil enriched with PL at 20 mg/g oil was prepared. Samples
178 of 10 g of Coil ranging a concentration of 4 to 26 000 $\mu\text{mol PL/kg oil}$ (0.003 to 20 mg PL/g oil) were
179 prepared from the stock solution by appropriate dilution with pure Coil. Then, 10 mg of TCNQ were
180 added to each sample followed by 1 h stirring at 55 °C into a thermostatic water bath and centrifugation
181 at 800 g for 20 min at room temperature. The supernatant was removed, and its absorbance was read at
182 480 nm with a spectrophotometer (Model UH5300, Hitachi, Japan) using quartz cuvettes. Pure Coil
183 was used as a blank. The CMC was estimated by the inflection point in the curve plotting absorbance as
184 a function of PL concentration (semilog plot). The determination of the CMC of PL was also assessed

185 in Coil saturated with quercetin (168 $\mu\text{g/g}$ oil) in order to verify any influence of quercetin on the
186 CMC.

187 **2.3 Statistical analysis**

188 Results were expressed as mean $\pm$ standard deviation (SD) and were statistically evaluated using
189 the Software Minitab 17® (Minitab Inc.). The significant differences ($p < 0.05$) were analyzed through
190 the Tukey's test.

191 **3. Results and discussion**

192 Camelina oil was chosen for its high amount in ALA (17.3 %) (Table 1) whose intake is
193 fundamental for the prevention of many diseases. It is worth noting that the oil is naturally protected
194 from oxidative reaction by the presence of 726 mg/kg of Vitamin E mainly represented by γ -tocopherol
195 (Table 1).

196 **3.1 Solubility of quercetin in enriched and non-enriched Coil**

197 Table 2 summarizes the solubility of quercetin in Coil enriched with PL and in the non-enriched
198 Coil. The solubility of quercetin in Coil without the addition of PL was estimated in
199 $168 \pm 6 \mu\text{g quercetin/g}$, whereas in sunflower oil was $390 \pm 28 \mu\text{g quercetin/g}$ (supplementary data,
200 Table 2A). Similar ranges of solubility of quercetin in plant oils have been previously reported by
201 Chen, Zou, Liu, & McClements (2016) for corn oil ($< 217 \mu\text{g/g}$) and by Rich, Buchweitz, Winterbone,
202 Kroon, & Wilde (2017) for olive oil ($\sim 328 \mu\text{g/g}$). Differences in the solubility of quercetin in oils may
203 be affected by their different fatty acid composition and also by the content of endogenous compounds
204 and water, which play a key role in the formation of hydrogen bonding and van der Waals interactions
205 (Kim, Vinueza, Kelley, & Park, 2018). It is worth remembering that sunflower oil is a refined oil
206 depleted of several minor compounds and mainly composed of fatty acids with 18 carbon chain length

207 (supplementary data, Table 1A), whereas Coil is a virgin oil with chain lengths of 18 and 20 carbons
208 (this latter in less proportion) (Table 1).

209 On the other hand, the enrichment of Coil with phospholipids allowed a significant
210 improvement of quercetin solubility (Table 2). Compared with the system free of phospholipid, the
211 solubility of quercetin in Coil increased 7.7-fold (CQ* vs CQL20*) and in sunflower oil increased 2-
212 fold (supplementary data, Table 2A). The presence of phosphatidylcholine significantly enhanced the
213 limited solubility of quercetin in Coil (i.e. 168 µg quercetin/g without PL against 1 298 µg quercetin/g
214 with 20 mg PL/g). In addition, a significant correlation between quercetin solubility and concentration
215 of PL in Coil (CQL5*, CQL10*, CQL15*, CQL20*; Table 2) was also observed (Pearson correlation
216 $R^2 = 0.98$, $p \leq 0.05$). This behavior can be explained by the interaction between phospholipids and the
217 five -OH groups of the phenolic rings of quercetin which are implicated in the formation of hydrogen
218 bonds and/or van der Waals interactions. Nasibullin, Nikitina, Afanas'eva, Nasibullin, & Spirikhin
219 (2002) studied the interaction of phosphatidylcholine with quercetin by NMR and highlighted the
220 ability of quercetin and phosphatidylcholine to form chain structures linked by hydrogen bonds. In
221 addition, Nasibullin, Nikitina, Afanas'eva, Nasibullin, & Spirikhin (2002) stated that PL and quercetin
222 interact via hydrogen bonding between the OH group of ring A (Fig. 1) and the phosphate group of
223 phosphatidylcholine. On the other hand, the antioxidant activity of quercetin is related to the 3-OH
224 group of the AC-ring and to the catechol group in the B ring (Fig. 1). Therefore, the interaction of
225 quercetin with PL might not hamper its antioxidant capacity since different OH groups are involved.

226 The presence of hydroxyl groups denotes the preference of quercetin for polar domains,
227 whereas its phenolic aromatic rings could be involved in hydrophobic interactions as evidenced by the
228 quercetin affinity to lipid component-hydrophobic environments (Pawlikowska-Pawlega et al., 2007).
229 In a phospholipid membrane system, the chromophore of quercetin was located in the polar head group
230 region rather than in the hydrophobic zone of the lipid bilayer (Pawlikowska-Pawlega et al., 2007).

231 Ratty, Sunamoto, & Das (1988), demonstrated that flavonoid aglycones could interact directly with the
 232 polar head group of phospholipids on the surface of the membranes.

233 The interactions between phospholipids and phenolics are the basis of the fabrication of
 234 phenolic-phospholipid complexes leading to an enhanced solubility in aqueous medium. However, the
 235 ability of phospholipids to enhance the solubility of bioactive compounds in other media is rarely
 236 quantified except for chloroform. Zhang et al. (2016) and Xu et al. (2016) observed a significant
 237 increase of the solubility of quercetin in water (13-fold and 3-fold, respectively) and in chloroform
 238 (more than 700-fold) (Xu et al., 2016) when quercetin was complexed with phospholipids. Moreover,
 239 the physical mixture of quercetin and soy lecithin also significantly increased the solubility of quercetin
 240 in chloroform (14-fold), and to a lesser extent in water (1.3-fold) (Xu et al., 2016). Similarly, Freag,
 241 Saleh, & Abdallah (2018) attributed the increase of solubility of celastrol in aqueous medium to the
 242 solubilizing effect of the phospholipids when celastrol was complexed or only mixed with soy
 243 phosphatidylcholine.

244

245 **Table 2.** Solubility of quercetin in Coil enriched or not with PL

ID Sample	mg PL/g Coil	Solubility (μg quercetin/g Coil) ^a
CQ*	0	168 $\pm$ 6
CQL5*	5	253 $\pm$ 4
CQL10*	10	530 $\pm$ 10
CQL15*	15	981 $\pm$ 70
CQL20*	20	1 298 $\pm$ 84

246 ^aData expressed as mean values and standard deviations

247 **3.2 CMC measurement of PL in bulk Coil in the presence of quercetin**

248 The interactions between quercetin and phospholipid could lead to molecular complexes or to
 249 supramolecular structures. CMC measurements allow to investigate the potential micelle formation and
 250 the concentration at which this formation occurs. In our case, CMC would reveal the formation of

reverse micelles of phospholipids with the two hydrophobic FA chains pointing towards the oil phase and the polar head groups (phosphoric based esters) pointing into the hydrophilic core of the micelles (Kittipongpittaya, Panya, McClements, & Decker, 2014). Indeed, phospholipid self-assembly into such colloidal structures has been reported in bulk oils (Kittipongpittaya, Panya, McClements, & Decker, 2014). Although the water content of Coil was not measured, it naturally may contain 200–2 000 ppm of water, which plays a key role in the formation of colloidal structures since it affords an oil-water interface where phospholipids could associate into micelles. Moreover, exogenous water was indirectly added to Coil by its enrichment with PL, since Lipoid P100 contains 1-2 % of water.

After performing a numerical analysis of data to determine the intercepting of the two slopes that provided the highest R^2 ($R^2 > 0.83$), the CMC of PL (mainly phosphatidylcholine) in Coil was found to be around 79 $\mu\text{mol phospholipid/kg oil}$ (Fig. 2). This value is in the same range as that found for dioleoylphosphatidylcholine in corn oil (65 $\mu\text{mol/kg oil}$) (Laguerre et al., 2011), but lower than that reported for phosphatidylcholine in stripped peanut oil (122 $\mu\text{mol/kg oil}$) (Zhao, Wang, Zhang, Zhao, & Yang, 2020). These variations may account for differences in the FA chain length of the phospholipids, the presence of minor endogenous compounds (such as free fatty acids, partially hydrolyzed triglycerides that are still present in crude oils), and the content of endogenous water in oils.

Fig. 2. Determination of the CMC by TCNQ probe of PL alone and PL + quercetin

The CMC of phospholipid slightly increased to 98 μmol PL/kg oil with the addition of quercetin at the saturated concentration of 168 μg/g (0.016 % wt) (Fig. 2). However, accounting for the model numerical errors, it is difficult to conclude about the significance of a 20 μmol PL/kg oil difference. Some authors have reported that the addition of components to bulk oils, at certain concentrations, may modify the CMC of phospholipids, but it consists mostly in surface-active components. Lehtinen et al. (2017) observed for instance that only the addition of oleic acid above 5 % (wt) significantly increased the CMC of lecithin in rapeseed oil, whereas Kittipongpittaya et al. (2014) showed that the increase of the CMC of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) started at oleic acid concentration above 0.5 % (wt). It was stated that, because of its surface-active capacity, oleic acid could reside at the oil/water interface, acting therefore as a co-surfactant that alters the assembly properties of the DOPC reverse micelle. From that perspective, the influence of quercetin concentrations higher than 168 μg/g on the CMC of PL could not be assessed because of the turbidity of the system that prevented accurate TCNQ absorbance measurements.

282 In the way to understand the possible organization between quercetin and phosphatidylcholine,
283 some authors (Laguerre et al., 2009) suggested that the phospholipid reverse micelles formed above the
284 CMC may act as carriers for phenolic compounds due to the presence of co-micelles which transfer the
285 phenolic compounds to the water phase. The enrichment of Coil carried out in this study has been done
286 at significantly higher concentrations (5 to 20 mg/g or 6 451 to 25 805 $\mu\text{mol/kg}$) than the CMC of PL in
287 Coil (79 $\mu\text{mol/kg}$), therefore, closed proximity between quercetin and phosphatidylcholine is plausible.

288 3.3 Oxidative stability of enriched and non-enriched Coil

289 Autoxidation of oils and fats implies a set of reactions of the unsaturated fatty acids with
290 oxygen. Among the numerous degradation products formed, hydroperoxides are associated to the
291 primary oxidation products that are involved in propagation reactions. These chemical species are
292 classically quantified by the peroxide value (PV), and the Codex Alimentarius fixes the PV of virgin
293 oils and refined oils above which the oils are no more edible. However, PV measurement is rather time
294 consuming, uses large volumes of organic solvents and needs quite high amount of raw materials. In
295 addition, chloroform used in PV analysis is more harmful than hexane. For these reasons, quantification
296 of CD is also used as a measurement of primary oxidation products especially since CD and PV are
297 correlated. In this work, a linear correlation between CD and PV of pure Coil oxidized at 60 °C was
298 also found and it was given by the equation: $\text{PV (m}_{\text{eq}}\text{O}_2/\text{kg}) = (\text{CD (A.U. 233 nm/mg/mL)} - 0.1127) /$
299 $0.0108, (R^2 = 0.99)$. From this linear regression, we deduced that a value of CD of 0.2747 (A.U. 233
300 nm/mg/mL) corresponded to a PV of 15 $\text{m}_{\text{eq}}\text{O}_2/\text{kg}$ which is the highest PV allowed from the Codex
301 Alimentarius for virgin oils. In the following, we defined the oxidation lag time as the time needed for
302 the different enriched Coils to reach a CD of 0.2747 under accelerated conditions of oxidation (60 °C,
303 in the dark, with no limitation of oxygen).

3.3.1 Influence of PL on oxidative stability of Coil

The enrichment of Coil with only phospholipids significantly improved the oxidative stability of Coil, as can be observed in Fig. 3. More precisely, PL concentrations from 5 to 20 mg PL/g increased the oxidation lag time from 24 ± 1 h to 40 ± 2 h (Fig. 3). Nevertheless, the protective effect against lipid oxidation was not correlated with the concentration of phospholipids in Coil since the maximum protective effect was already achieved at 5 mg/g Coil. Sugino et al. (1997) also reported a decrease in the autoxidation of a DHA-rich oil by the addition of egg yolk phospholipids. The protective effect was dose-dependent (in a range of 0.1-5 %) for the egg yolk lipids that contained 31 % of phospholipids. Lyberg, Fasoli, & Adlercreutz (2005) and Nwosu, Boyd, & Sheldon (1997) reported the protective effect of phosphatidylcholine against lipid oxidation of DHA and salmon oil, when phospholipids were added at a concentration about 10 mg/g and 5 mg/g, respectively.

Fig. 3. Oxidation lag times of enriched and non-enriched Coil shown as (A) 3D-plot or (B) table for better interpretation. *Samples saturated with quercetin

The mechanism related to oxidative stability due to phospholipids in oil systems is complex since phosphatidylcholine has been reported to have an antioxidant, prooxidant, or no effect on oxidation reactions (Cui & Decker, 2016). In this work, the addition of PL to Coil was found to exert a protective effect. The antioxidant properties of the phospholipids are mainly related to their polar head group: (i) the phosphoryl group, negatively charged, is able to chelate metals especially iron and copper, thus limiting their reactions with PUFA (Cui & Decker, 2016; Koga & Terao, 1995). This property may be of importance in our case because Coil contained 1.28 mg of metals/kg; (ii) the amine function of phosphatidylcholine could directly interact with free radicals and block the propagation reactions (Henna Lu, Nielsen, Timm-Heinrich, & Jacobsen, 2011); (iii) the amino group of phosphatidylcholine could facilitate the transport of hydrogen or electron to α -tocopherol, favoring its regeneration and thus, delaying the formation of hydroperoxides (Budilarto & Kamal-Eldin, 2015). Moreover, Cui & Decker (2016) has also reported the ability of phospholipids to improve the accessibility of the functional group of tocopherols to the site where iron-dependent oxidation reactions take place. Koga & Terao (1995) stated that exogenous phospholipids enhanced the antiradical activity of Vitamin E in a model bulk oil exposed to a water-soluble radical initiator. They attributed to phospholipids the capacity to improve the accessibility of tocopherol towards the aqueous environment, where the initiating oxidation reactions take place. Since Coil naturally contains tocopherols (Table 1), a synergic effect between phospholipids and tocopherols could not be ruled out.

3.3.2 Influence of PL-quercetin formulations on oxidative stability of enriched Coil

Addition of quercetin to Coil at saturated concentration (168 $\mu\text{g/g}$) in absence of phospholipids, did not significantly protect the oil against lipid oxidation (Fig. 3), whereas sunflower oil enriched with quercetin (230 $\mu\text{g/g}$) exhibited a lower rate of CD formation than non-enriched oil (supplementary data, Fig. 1A). The poor protective effect of quercetin in Coil was unexpected considering that for instance, the addition of quercetin dihydrate in a range of 0.25 to 2 $\mu\text{mol/g}$ oil (84.6 to 676.6 $\mu\text{g/g}$) significantly

increased the induction period of high oleic sunflower oil (Becker, Ntouma, & Skibsted, 2007). On the other hand, authors such as Lee, Gim, Kim, & Lee (2017) reported a prooxidant effect of quercetin incorporated to non-stripped soybean oil at a concentration of 0.4 mM (~132 µg quercetin/g oil). Frenzel & Steffen-Heins (2015) reported that quercetin in a concentration ranging from 750 to 4500 µM (226 to 1 360 µg/mL) was not able to significantly reduce the free radical formation during oxidation of unsaturated soy phosphatidylcholine liposomes. They attributed this behavior to: (i) different solubilization sites for quercetin and lipid radicals, so that both reactants were not close enough to interact, and (ii) the low radical-scavenging activity of quercetin in the presence of α -tocopherol. Although some comparisons related to the effect of quercetin against lipid oxidation have been given above, it is also important to highlight that the antioxidant activity of a molecule is significantly influenced by the lipid system in which this molecule is acting (bulk oils, emulsions, etc.) (Laguette et al., 2009) and by the presence and nature of association colloids (Laguette et al., 2011).

Enriching Coil at a low and fixed concentration of quercetin (220 µg/g) while varying the concentration of PL from 10 to 20 mg/g was also evaluated. Addition of quercetin along with PL enhanced the protective effect against lipid oxidation exhibited by PL alone since enriched oil with quercetin (220 µg/g) extended the oil shelf life from 40 ± 2 h to 60 ± 3 h, regardless of the phospholipid concentration (Fig. 3 and supplementary data Fig. 2A). These results were consistent with what was observed and discussed in section 3.3.1 where Coil enriched with only PL at 5, 10, 15 and 20 mg/g exhibited the same protective effect.

On the other hand, a different behavior was obtained when the effect of quercetin concentration was evaluated in enriched Coil at a fixed PL concentration (either 5, 10, 15 or 20 mg/g oil). Fig. 3 shows that the increase in quercetin concentration led to higher oxidation lag times whatever the concentration of PL (5, 10, 15 or 20 mg/g oil). More generally, combination of phospholipids and quercetin led to an enhanced antioxidant activity against lipid oxidation. This result is consistent with

367 Ramadan (2012) who reported that increases in concentration of quercetin in phenolipid formulations
368 resulted in an increase of its antioxidative potential. Phospholipids may act as carriers of polyphenols
369 and thus improve the antioxidant activity of these compounds (Ghanbarzadeh et al., 2016). In this
370 sense, in presence of phospholipids, quercetin exhibited a higher ability to interact with free radicals by
371 means of the hydroxyl groups. The hydroxyl groups become donators of H-atom to peroxy radicals to
372 stabilize them and delay lipid oxidation. The incorporation of phospholipids to Coil allowed to provide
373 quercetin-phospholipid interactions so that it increased not only the quercetin solubility but also the
374 oxidative stability of Coil.

375 Fig. 3 (and supplementary data Fig. 2A) also evidenced that the combination of both
376 phosphatidylcholine and quercetin led to an enhanced antioxidant activity against lipid oxidation when
377 compared to the individual components. Our results are consistent with the greater oxidative stability
378 observed in sunflower oil and triolein enriched with lecithin-quercetin formulations compared with oils
379 enriched only with phospholipids or quercetin (Ramadan, 2012).

380 The highest phospholipid and quercetin concentration (20 mg/g and 1 298 μ g/g, respectively) in
381 Coil provided the maximum protective effect corresponded to a lag time of 115 ± 7 h, a value similar to
382 that observed simultaneously for other samples enriched also with a mass ratio of ~1:20 (i.e. 1 molecule
383 of quercetin for 8 molecules of phosphatidylcholine) (5 mg PL/g and 253 μ g quercetin/g, 10 mg PL/g
384 and 530 μ g quercetin/g, and 15 mg PL/g and 981 μ g quercetin/g) (Fig. 3). All these samples correspond
385 to oils saturated with quercetin at various concentrations of PL.

386 The effectiveness of antioxidants in oils is influenced by numerous parameters: the structure of
387 the bioactive molecules, the solubilization site, the concentration, mechanism of action as well as by the
388 composition of the oil phase. The microenvironment formed by surface-active compounds such as
389 phospholipids is recognized as the active site of lipid oxidation, therefore oxidation cannot be explained
390 merely by chemical reactions but also by considering molecular positions in space (Budilarto & Kamal-

Eldin, 2015). In the schematic representation of the hydrophilic and hydrophobic antioxidant distribution in bulk oil (Budilarto & Kamal-Eldin, 2015), the hydrophobic antioxidants are diluted in the bulk oil whereas surfactant and hydrophilic antioxidants are located at the oil-water interface of the micelles/association colloids. Quercetin is usually described as a hydrophobic molecule because of its poor solubility in water (from 3.8 to 970 $\mu\text{g/g}$; Abraham & Acree, 2014) though it stands in the same range than the solubility of quercetin in Coil measured in this work (168 $\mu\text{g/g}$). Its predicted octanol-water partition coefficient ($\log P$) of 1.5 (PubChem) indicated some hydrophobicity and, Van Dijk, Driessen, & Recourt (2000) estimated the ratio of quercetin partitioning between water and olive oil to 53 %. Therefore, quercetin can be considered as neither fully lipophilic nor hydrophilic. In this context, quercetin added at its solubility level to pure Coil is probably dispersed in the oil or associated to endogenous surfactants like free fatty acids, phospholipids, among others. We have shown that the addition of phospholipids influenced the quercetin solubility in oil. Our hypothesis is that the solubilizing effect of phospholipids drives the excess of quercetin molecules close to the water-oil interface generated by the PL micelles. The hypothesis is based on the assumption that in bulk oils, quercetin is interacting with the polar part of phospholipids as it does in aqueous liposome suspension (Ghanbarzadeh et al., 2016). The hypothesis is also sustained by the fact that the planar configuration of quercetin favors its intercalation into organized structures of phosphatidylcholine (Van Dijk, Driessen, & Recourt, 2000). Consequently, quercetin molecules are located at the proximity of the polar heads of phospholipids, in close contact with the aqueous microenvironment recognized as being the active site of lipid oxidation. According to Frankel, Huang, Kanner, & German (1994) the antioxidant potential is strongly associated to the effective concentration of the compound at the location where oil oxidation begins. Therefore, the improved accessibility of the antioxidant to the aqueous microenvironment present in the oil, due to the surface-active PL, was expected to enhance the antioxidant activity of the bioactive compound, which was actually observed.

3.4 Monitoring of quercetin concentration during oxidation

The quercetin concentration was monitored for Coil saturated with quercetin at different PL concentrations (0, 5, 10, 15 and 20 mg/g oil) (samples labeled with * in Fig. 3). The evolution of the remaining quercetin during oxidation is shown in Fig. 4A for Coil enriched with only quercetin at 168 µg/g. The remaining quercetin in Coil without PL showed a deep decrease during the first 120 h of oxidation, with 70 % of quercetin consumed at this point. The consumption of quercetin offered only a slight protection to the oil against oxidation. Authors such as Palma, Robert, Holgado, Velasco, & Márquez-Ruiz (2017) reported that the consumption of quercetin in bulk methyl linoleate was correlated with the ability of the flavonoid to protect the model oil. On the other hand, and as mentioned before, the effectiveness of an antioxidant for preventing oil oxidation depends on the solubilization site, the composition of the lipid system and the interaction with other endogenous antioxidants. Goupy, Vulcain, Caris-Veyrat, & Dangles (2007) for instance observed that quercetin was partially spared by tocopherols, based on the experimental evidence that residual quercetin concentration in presence of tocopherols was generally higher than that found in its absence.

In Coil saturated with quercetin at various PL concentrations (5, 10, 15 and 20 mg/g oil), higher quercetin concentrations remained during oil oxidation in comparison with CQ* sample (Fig. 4B, and supplementary data Fig. 3A). The addition of PL to Coil from 5 to 20 mg/g allowed to introduce increasing amounts of quercetin in the oil and therefore, maintained a reservoir of quercetin during accelerated oxidation. This may have favored a higher Coil oxidative stability along with the already discussed protection effect exhibited by phospholipids against lipid oxidation.

Fig. 4. Time course formation of CD and remaining quercetin in Coil saturated with quercetin at (A) 168 µg/g and 0 mg of PL/g oil and at (B) 1 298 µg/g and 20 mg of PL/g oil

Koga & Terao (1995) also observed that phospholipids, in presence of exogenous vitamin E antioxidant, could retard the oxidation of bulk oil comprising methyl linoleate and methyl laurate exposed to a water-soluble radical initiator and simultaneously, delayed the consumption of vitamin E in the oil. In this sense, it can be stated that consumption of an antioxidant compound in a bulk oil and its protective effect depend on several factors and, the system investigated in this work was quite complex since the non-stripped Camelina oil already contained endogenous antioxidants as tocopherols and prooxidants like metals. Because of the addition of several formulations of PL and quercetin, the studied system contained several species active on the lipids oxidation either by acting on molecular organizations and/or by chemical effects through radical scavenging that can moreover interact with each other.

4. Conclusion

Quercetin alone exhibited a poor solubility in camelina oil that provided a low protective effect against lipid oxidation. The low solubility of quercetin in Coil was successfully enhanced by the enrichment with phosphatidylcholine by a solvent-free route. In addition, the enrichment of Coil by PL-quercetin formulations allowed to increase the oil stability from 24 h up to 115 h under accelerated

conditions of heating. The protective effect of PL and quercetin was probably determined by a more specific synergism than the additivity of both components since several combinations provided the same retarded oxidation. It is suggested that quercetin, by interacting with the polar head of phosphatidylcholine, participates to the micellar water-oil microenvironment where the oil oxidation takes place. However, this new structuration needs to be evidenced, by small-angle X-ray scattering for instance. This work opens up potential new applications for phospholipid-quercetin formulations in the food, cosmetic and pharmaceutical industries, since it was demonstrated that the poor solubility of quercetin in oils can be overcome along with a benefit of oil stability by means of a commercial phospholipid.

Declarations of interest

The authors declare that they have no conflicts of interest.

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Appendix

Supplementary material related to this article can be found, in the online version, at doi:

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594

Table 1A. Chemical composition of sunflower oil

Fatty acids Composition (%)	
16:0	6.6
18:0	3.3
18:1 n-9	30.8
18:2 n-6	57.2
18:3n-3	0.1
20:0	0.2
20:1	0.2
20:2	<0,05
20:3	<0,05
22:1	<0,05
Σ SFA	11.1
Σ MUFA	31.1
Σ PUFA	57.2
PUFA n-3	0.1
PUFA n-6	57.2
Vitamin E (mg/kg)	
α-tocopherol	651.5
β-tocopherol	21.8
γ-tocopherol	3.7
δ-tocopherol	<2
Total Vitamin E	677.0
Phospholipids (g/100g)	
< 0.35	
Metals (mg/kg)	
Copper (mg/kg)	<0,005
Iron (mg/kg)	0.009
Sterols (mg/kg)	
3340	
Total Phenolic Content (mg GAE/kg)	
33.1 ± 1.9	

Σ SFA: total saturated fatty acids, Σ MUFA: total monounsaturated fatty acids, Σ PUFA: total polyunsaturated fatty acids

Table 2A. Solubility of quercetin in sunflower oil enriched or not with PL

ID Sample	mg PL/g oil	Solubility (μg quercetin/g oil)
SQ	0	390 ± 28
SQL20	20	792 ± 22

Data expressed as mean values and standard deviations

Fig. 1A. Monitoring of CD formation over Schaal oven test of pure and enriched sunflower oil

Fig. 2A. Monitoring of CD formation over Schaal oven test of pure and enriched Coil

Fig. 3A. Monitoring of remaining quercetin in Coil saturated with quercetin at various PL concentrations

Table 1

Table 1. Chemical composition of Coil

Fatty acids Composition (%)	
16:0	5.7
18:0	2.4
18:1 n-9	13.8
18:2 n-6	17.3
18:3n-3	37.5
20:0	1.4
20:1	13.7
20:2	2.1
20:3	1.7
22:1	2.9
Σ SFA	9.9
Σ MUFA	31.1
Σ PUFA	58.7
PUFA n-3	37.5
PUFA n-6	21.2
Vitamin E (mg/kg)	
α-tocopherol	29.0
β-tocopherol	<2
γ-tocopherol	685.5
δ-tocopherol	11.9
Total Vitamin E	726.4
Phospholipids (g/100g)	
< 0.35	
Metals (mg/kg)	
Copper	<0,005
Iron	1.28
Sterols (mg/kg)	

4824
Total Phenolic Content (mg GAE/kg)
30 ± 2.6

Σ SFA: total saturated fatty acids, Σ MUFA: total monounsaturated fatty acids, Σ PUFA: total polyunsaturated fatty acids, GAE: gallic acid equivalents

Table 2. Solubility of quercetin in Coil enriched or not with PL

ID Sample	mg PL/g Coil	Solubility (µg quercetin/g Coil) ^a
CQ*	0	168 ± 6
CQL5*	5	253 ± 4
CQL10*	10	530 ± 10
CQL15*	15	981 ± 70
CQL20*	20	1 298 ± 84

^a Data expressed as mean values and standard deviations

Figure 1

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Fig. 1. Molecular structure of quercetin

Fig. 2. Determination of the CMC by TCNQ probe of PL alone and PL + quercetin

Fig. 3. Oxidation lag times of enriched and non-enriched Coil shown as (A) 3D-plot or (B) table for better interpretation. *Samples saturated with quercetin

Fig. 4. Time course formation of CD and remaining quercetin in Coil saturated with quercetin at (A) 168 µg/g and 0 mg of PL/g oil and at (B) 1298 µg/g and 20 mg of PL/g oil