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Oxidative Stability at High Temperatures of Oleyol and Linoleoyl Residues in the Forms of Phosphatidylcholines and Triacylglycerols

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An investigation was carried out into the stability of fatty acyl groups to heat-induced oxidative changes as affected by their chemical environment. The behavior of oleic and linoleic acyl groups when esterified in triacylglycerols (TAGs) and phosphatidylcholines (PCs) was evaluated. The monitoring of the oxidative degradation using liquid chromatography−mass spectrometry (LC−MS) showed that fatty acyl groups are less likely to be oxidized when in the form of PCs than when in the form of TAGs. In addition, oxidation products from PCs were more stable than those from TAGs. This finding strengthens the idea that the choline group in PCs increases the stability of fatty acyl groups to oxidation in comparison to TAGs.

KEYWORDS: Oxidation; phosphatidylcholine; triacylglycerols; oleic acid; linoleic acid

INTRODUCTION

Polyunsaturated fatty acids (PUFAs) play very important roles in many aspects of human health, in particular when it comes to reducing risks of cardiovascular diseases, inflammation, hypertension, allergies, and immune and renal disorders (*6*, *17*). The natural molecular forms of PUFAs are typically triacylglycerols (TAGs) and phospholipids (PLs). PLs can be divided into subclasses according to their polar head, with phosphatidylcholines (PCs) being the predominant one.

In the human diet, TAGs are the major carriers of fatty acids (FAs), with 50−100 g per day for an adult, followed by PLs, with 2−10 g per day (*14*). However, as far as bioavailability is concerned, several studies have shown that PCs are a better carrier of PUFAs than TAGs (*1*, *9*, *23*). Cellular permeability to PUFAs and their intracellular levels are indeed comparatively higher when they are part of PLs (*14*), which suggests that food supplementation with PUFA-rich PLs could enhance essential FA intake.

The bioavailability is an important element to take into account when considering PUFA supplementation, but also as important is the stability of these FAs in the used sources. Very few studies have been performed to evaluate the relative stability of TAGs and PLs. In most instances, the stability of oils that are naturally rich or artificially enriched in PLs is compared to that of oils containing few or no PLs at all (*12*, *18*). One study investigated the relative stability of lipidic fractions from squid and found that the PL fraction was the most stable one (*3*). To our knowledge, however, a comparative investigation of the oxidative stability of pure species of TAGs and PLs is yet to be carried out.

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This study is set out to compare the stability to oxidation of equivalent molecular species of TAGs and PLs. 1-Stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (SOPC) and 1-stearoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (SLPC) were compared, respectively, to 1,3-stearoyl-2-oleoyl-glycerol (SOS) and 1,3-stearoyl-2-linoleoyl-glycerol (SLS) to assess the effect of the molecular environment of the FAs on their resistance to oxidation. These isomers were chosen because it has been shown that in PCs the *sn*-2 position is most of time esterified with an unsaturated FA (*22*). To be able to compare results, the corresponding TAGs were used. Wada and Koizumi showed that the *sn*-2 position of TAGs is most of time more stable toward oxidation that other isomers (*22*). The kinetics of the loss of the precursors (TAG and PL pure species) and the formation of their oxidation products were monitored using liquid chromatography−electrospray ionization−mass spectrometry (LC−ESI−MS) after various heat treatment durations.

MATERIALS AND METHODS

Chemicals and Stock Solutions. *Chemicals*. SOPC and SLPC (99.9%) were purchased from Avanti Polar Lipids (Alabaster, AL). SOS and SLS (99.9%) were purchased from Larodan (Malmö, Sweden). HPLC-grade methanol and analytical-grade formic acid (99−100%) were purchased from VWR (Strasbourg, France). Ultrapure water was produced by a Synergy UV purification system (Millipore, Molsheim, France). Chloroform and aqueous ammonia were of analytical grade and were purchased from Riedel de Haën (Sigma-Aldrich, Seelze, Germany).

Stock Solutions. Stock solutions of 10 mg/mL were prepared by weighing PC and TAG standards into amber vials and dissolving them in chloroform. Aliquots were prepared by introducing 100 μL of each stock solution into a 4 mL amber vial (VWR; vials, 4 mL; height, 44 mm;

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internal diameter, 12 mm). Vials were then dried under nitrogen, closed, and kept at −20 °C until oxidative treatment.

Determination of Oxidation Temperatures. Several temperatures were tested on SOPC and SLPC samples to determine the best conditions for hydroperoxide formation (*7*). All samples were analyzed in triplicate. Both PCs were oxidized in an oven for 30 min at temperatures ranging from 50 to 175 °C. Accuracy of each indicated temperature was ±1 °C. Vials were left open to air during heating. After oxidation, samples were cooled to room temperature. A total of 1 mL of methanol was added to the vial, and then a $^1/_{50}$ dilution was performed to obtain 0.02 mg/mL. Mass analysis was carried on 5 μL of each sample in a 1200 L triple quadrupole mass spectrometer (Varian, Les Ulis, France) fitted with an ESI source using methanol as the mobile phase. High-purity nitrogen, produced by a nitrogen generator (Domnik Hunter, Villefranche-sur-Saône, France), was used as nebulizing gas, set at 0.317 MPa, and as a drying gas, set at 300 °C. Spectral data were acquired in positive mode (single quadrupole analysis), and the *m*/*z* scan range was from 500 to 1100, because only monomeric oxygenated oxidation products were investigated. Neither truncated (for example, volatiles or short-chain aldehydes) nor dimeric products were analyzed. The best temperature of oxidation was defined at the temperature where the percentage of hydroperoxide was the highest for each precursor. The percentage was calculated as follows:

$$\frac{(A_{[M+H+32]})_T}{A_{[M+H]}} \times 100$$

where $(A_{[M+H+32]})_T$ is the area of the peak corresponding to the extracted ion *m*/*z* of the hydroperoxide [M + H + 32] formed at a given temperature (*T*) and $A_{[M+H]}$ is the area of the peak corresponding to the extracted ion *m*/*z* of the precursor [M + H] without oxidative treatment.

Identification of Oxidation Products with LC−ESI−MS. The identification of SOPC and SLPC oxidation products was performed in a previous study (*7*), and the identification of oxidation products of SOS and SLS was performed the same way. The high-performance liquid chromatography (HPLC) system was made of two Prostar 210 solvent delivery modules (Varian), a Prostar 410 autosampler (Varian), and an ESI mass spectrometer as described above. Oxidation products were separated on a reverse-phase C8 Lichrospher RP Select B (Interchim, Montluçon, France) using a linear gradient ranging from 15% ultrapure water in methanol containing 0.1% aqueous ammonia to 100% methanol containing 0.1% aqueous ammonia. The flow rate was set to 1 mL/min through the column and split to derive 0.2 mL/min to the mass spectrometer. Mass spectra were acquired in a *m*/*z* range from 500 to 1100. To confirm oxidation products structures, 10 μL of formic acid (1 M) was added to samples and immediately neutralized with 10 μL of sodium hydroxide (1 M). Samples were then analyzed by LC−ESI−MS as described above. Indeed, by adding formic acid, an epoxy group will be opened and converted into a diol group (*11*), which will result in a retention time (Rt) shift.

Determination of the Starting Material Remaining after Oxidation. To evaluate the impact of oxidation on PCs and TAGs, the percentage of the remaining precursors was determined for each standard at each oxidation time as follows:

$$\text{percentage} = \frac{(\text{area})_t}{(\text{area})_{t_0}} \times 100$$

where $(\text{area})_t$ is the area of the peak corresponding to the ion *m*/*z* of the starting material (PCs or TAGs) after oxidation and $(\text{area})_{t_0}$ is the area of the same ion *m*/*z* without oxidative treatment. Oxidation times were chosen each 15 min, but at the beginning of the kinetics experiments, 5 min oxidation was also sampled. Determined percentages of products were obtained by comparing their signal ratios and not their absolute quantities. All samples were analyzed in triplicate, and results are given as mean ± standard deviation (SD).

Formation of Oxidation Products. Oxidation product formation was also monitored over time. Molecular ions corresponding to each oxidation product were analyzed in single-ion monitoring (SIM) mode (*m*/*z*): $[M+14+H]^+$, $[M+16+H]^+$, $[M+30+H]^+$, $[M+32+H]^+$, and $[M+42+H]^+$, with M representing the precursor. On the basis of areas of the chromatographic peaks, kinetic curves were drawn for the formation of each identified degradation product and for the loss of the precursor. For each oxidation product, kinetics curves were drawn based on peaks areas, to assess their behavior with regard to heating. It is clear that using LC−MS data for drawing kinetics does not allow for a comparison of the oxidation products, in term of abundance, to the others. However, considering each oxidation product separately, kinetics curves bring information about its stability.

Statistical Analysis. Data were subjected to a one-way analysis of variance (ANOVA) using Statgraphics plus 5.1 software (Manugistics, Rockville, MD), with the remaining percentage as the variable. A least significant difference (LSD) test was used for a comparison of the means.

RESULTS

Determination of Optimal Oxidation Temperatures. To determine the optimal oxidative conditions of SOPC and SLPC, the percentage of hydroperoxides [M + H + 32] generated at several temperatures was determined. The temperature allowing for the highest yield of hydroperoxides was different for thermally oxidized SOPC and SLPC. For the same oxidation time (30 min), the production of hydroperoxides was the highest at 125 ± 1 °C for SLPC and at 150 ± 1 °C for SOPC (*7*). This is in accordance with reports showing that free linoleic acid is more sensitive to oxidation than free oleic acid (*17*). Identification of oxidation products was performed in the neat lipids because chloroform was evaporated before oxidation. Several oxidation products were identified for PC (*7*) (**Figure 1**) and TAG species. As expected, di-unsaturated species provided a wider range of oxidation products than mono-unsaturated species. **Table 1** gives all of the identified structures, but the exact positions of the oxygenated groups were not determined. The nature of the identified oxidation products is quite the same, independent of the native lipidic structure, i.e., PCs or TAGs (**Table 1**), and most of these structures have already been identified by other authors, on either PCs (*7*, *15*), TAGs (*5*), or free fatty acids (*10*, *21*, *21*). The formation of each identified product was monitored over time, to evaluate which oxidation products (TAGs or PCs) are the most stable.

Formation of Oxidation Products. *Mono-unsaturated Species.* The formation of oxidation products was monitored for PC and TAG species between 0 and 120 min in the same conditions used for monitoring the loss of the precursor. Kinetics for the formation of oxidation products from SOPC and SOS are given in **Figure 2**. Products from SOPC were detected after 45 min of heating, with 18:0−18:1(OOH)−PC reaching a maximum after 45 min and rapidly declining until it totally disappeared after 90 min (**Figure 2a**). The fact that molecules with hydroperoxide groups tend to decrease let us think that these products are further degraded into secondary oxidation products, such as monomeric products, which are studied here, but also in volatile products, which could be part of another study. Other oxidation products [with a keto group, 18:0−18:1(Ke)−PC; an epoxy group, 18:0−18:0(Ep)−PC; and a hydroxyl group, 18:0−18:1(OH)−PC] reached a maximum after 45−60 min and seemed to stabilize afterward.

As far as SOS oxidation products are concerned, some were detected earlier (after 15 min of heating) in comparison to those generated from SOPC, although they also reached a maximum after 45−60 min (**Figure 2b**). As with SOPC, the amount of 18:0−18:1(OOH)−18:0 peaked after 45 min of heating and then decreased below detection levels. Other oxidation products [18:0−18:0(Ep)−18:0, 18:0−18:1(Ke)−18:0, and 18:0−18:1(Ep)−18:0] behaved in a similar way, which contrasts with SOPC, which appeared to be more or less stable until 120 min.

Di-unsaturated Species. **Figure 3** shows the formation of oxidation products from SLPC and SLS at 125 °C monitored over time. Oxidation products were separated in two groups: the

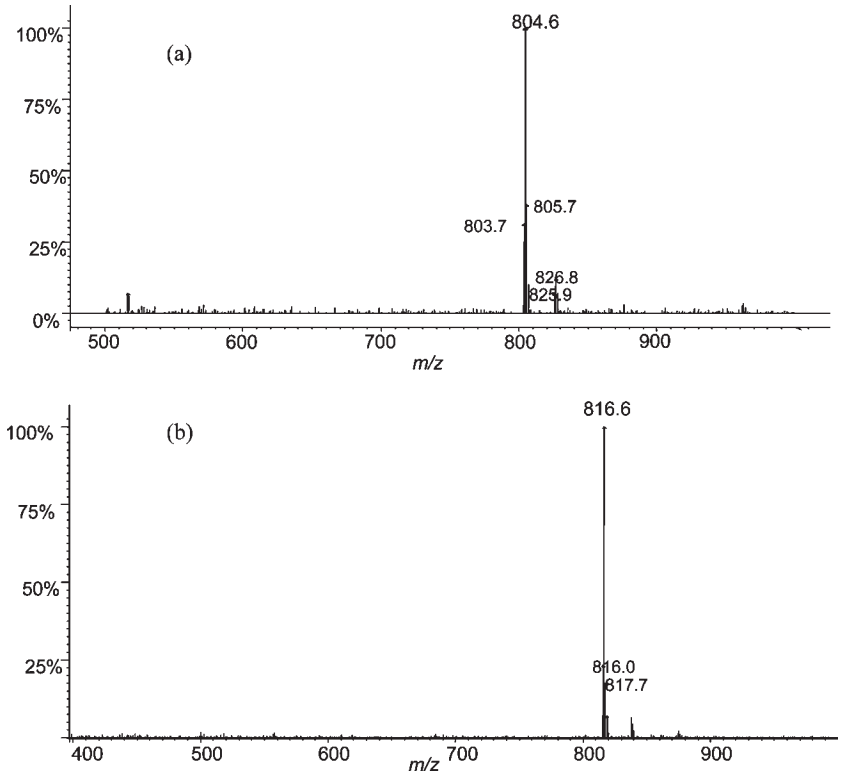

Figure 1. Mass spectra of oxidation products of SOPC (a, *m/z* 804.6) and SLPC (b, *m/z* 816.6).

Table 1. Structures of Identified Oxidation Products from PC and TAG Species

ions *m/z* $[M + H]^+$	retention times	structures	references
		Mono-unsaturated Species	
		SOPC	
788.6	38.7	18:0−18:1−PC	
802.6	29.9	18:0−18:1(Ke)−PC	*7* and *10*
804.6	29.1	18:0−18:1(OH)−PC	*7*
820.6	29.5	18:0−18:1(OOH)−PC	*7*
		SOS	
889.8	53.8	18:0−18:1−18:0	
903.8	45.1	18:0−18:1(Ep)−18:0	*7*
	46.2	18:0−18:1(Ke)−18:0	
905.8	48.8	18:0−18:0(Ep)−18:0	*5* and *7*
921.8	44.2	18:0−18:1(OOH)−18:0	*5* and *7*
		Di-unsaturated Species	
		SLPC	
786.6	37.7	18:0−18:2−PC	
800.6	28.9	18:0−18:2(Ep)−PC	*7* and *15*
		18:0−18:2(Ke)−PC	
802.6	28.3	18:0−18:2(OH)−PC	*7* and *15*
	32.4	18:0−18:1(Ep)−PC	*7*
816.6	24.5	18:0−18:1(Ep,Ke)−PC	*7* and *15*
818.6	27.7	18:0−18:2(OOH)−PC	*7* and *15*
834.6	22.5	18:0−18:1(Ep,OOH)−PC	*7*
		SLS	
887.8	54.1	18:0−18:2−18:0	
901.8	48.9	18:0−18:2(Ep)−18:0	*7*
		18:0−18:2(Ke)−18:0	*7*
903.8	48.5	18:0−18:1(Ep)−18:0	*7*
917.8	44.6	18:0−18:1(Ep,Ke)−18:0	*7*
919.8	44.9	18:0−18:2(OOH)−18:0	*7*
935.8	42.6	18:0−18:1(Ep,OOH)−18:0	*7*

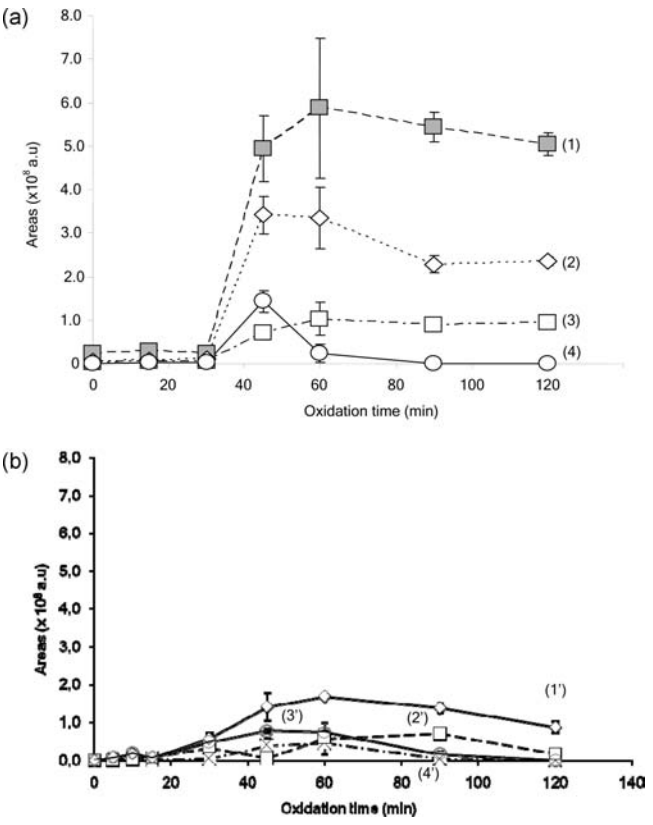

Figure 2. Kinetics of oxidation during heat treatment at 150 °C. (a) Formation of oxidized products from SOPC: (1) 18:0−18:1(Ke)−PC, (2) 18:0−18:0(Ep)−PC, (3) 18:0−18:1(OH)−PC, and (4) 18:0−18:1(OOH)−PC. (b) Formation of oxidized products from SOS: (1′) 18:0−18:0(Ep)−18:0, (2′) 18:0−18:1(Ke)−18:0, (3′) 18:0−18:1(OOH)−18:0, and (4′) 18:0−18:1(Ep)−18:0.

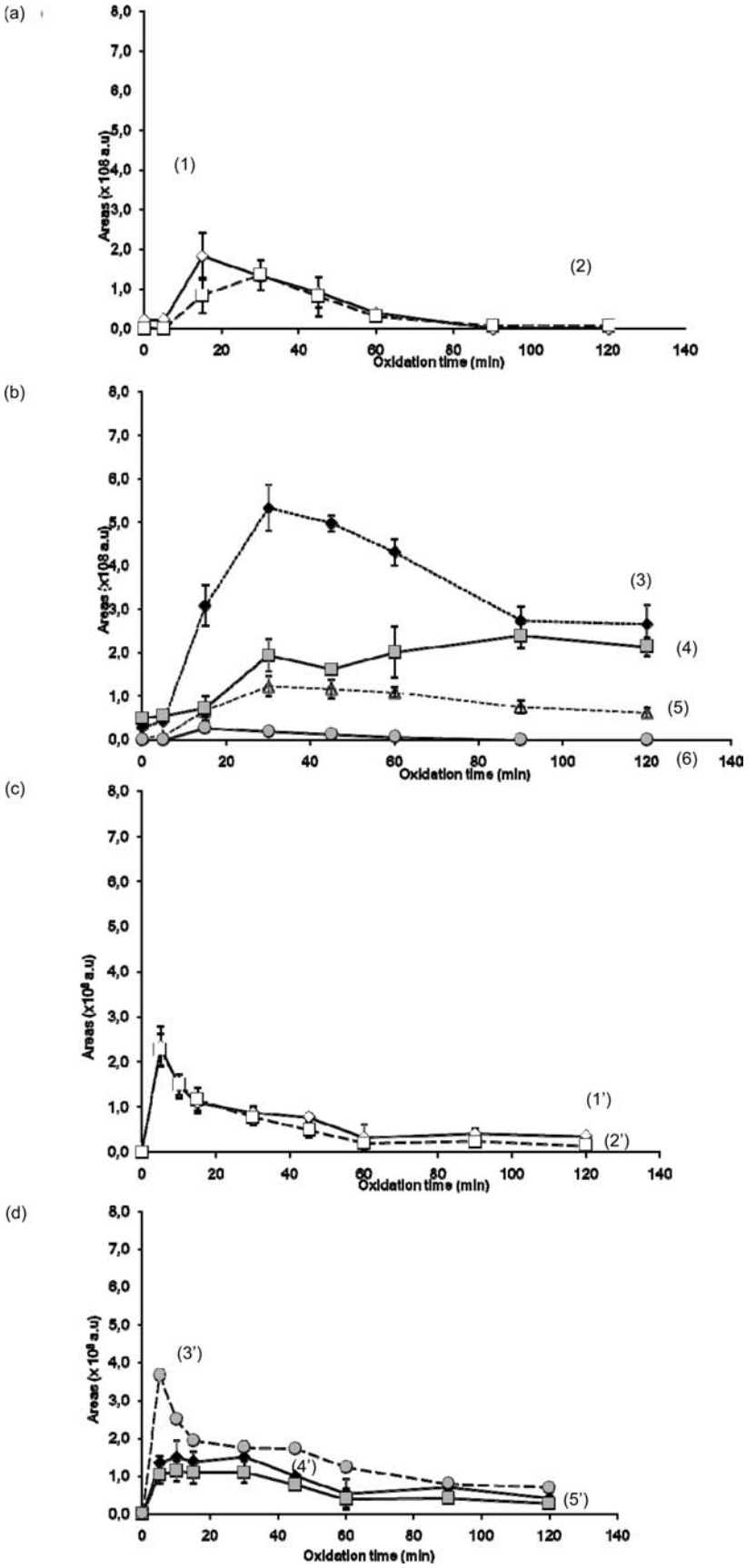

Figure 3. Kinetics of oxidation during heat treatment at 125 °C. (a) Formation of oxidized products from SLPC containing hydroperoxy groups: (1) 18:0−18:2(OOH)−PC and (2) 18:0−18:1(Ep,OOH)−PC. (b) Formation of other oxidized products from SLPC: (3) 18:0−18:2(Ke)−PC + 18:0−18:2(Ep)−PC, (4) 18:0−18:1(Ep)−PC, (5) 18:0−18:2(OH)−PC, and (6) 18:0−18:1(Ep,Ke)−PC. (c) Formation of oxidized products from SLS containing hydroperoxy groups: (1′) 18:0−18:2 (OOH)−18:0 and (2′) 18:0−18:1(Ep,OOH)−18:0. (d) Formation of other oxidized products from SLS: (3′) 18:0−18:1(Ep,Ke)−18:0, (4′) 18:0−18:2(Ep)−18:0 + 18:0−18:2(Ke)−18:0, and (5′) 18:0−18:1(Ep)−18:0.

first with products containing a hydroperoxide group (panels **a** and **c** of **Figure 3**) and the second with other oxidation products resulting from hydroperoxide degradation (panels **b** and **d** of **Figure 3**). Products of the first group obtained from SLPC consisted of [18:0−18:2(OOH)−PC] with a hydroperoxide group and [18:0−18:1(Ep,OOH)−PC] with a hydroperoxide and an epoxy group (**Figure 3a**). These products reached a maximum after 15−30 min of heating, and then their amounts decreased gradually and reached levels below detection limits after 90 min.

Products with the same oxidized groups [18:0−18:2(OOH)−18:0 and 18:0−18:1(Ep,OOH)−18:0] were also identified from SLS (**Figure 3c**). However, in this case, both products reached a maximum much earlier, after only 5 min of heating, before they degraded sharply and stabilized at very small amounts from 60 min.

Kinetics for the formation of oxidation products with no hydroperoxide group are given in panels **b** and **d** of **Figure 3**. As far as SLPC is concerned, five oxidation products were identified [18:0−18:2(Ep)−PC, 18:0−18:2(Ke)−PC, 18:0−18:1(Ep)−PC, 18:0−18:2(OH)−PC, and 18:0−18:1(Ep,Ke)−PC] (**Figure 3b**). These products behaved in the same way as oxidation products from SOPC: the amount of each product reached a maximum after 30 min of oxidation and then decreased and/or stabilized (**Figure 3b**).

SLS is more sensitive to oxidation than SLPC. The four oxidation products detected [18:0−18:2(Ep)−18:0, 18:0−18:2(Ke)−18:0, 18:0−18:1(Ep,Ke)−18:0, and 18:0−18:1(Ep)−18:0] reached a maximum after only 5 min of oxidative treatment and then decreased and stabilized starting at 60 min (**Figure 3d**).

Degradation of Starting Material. The remaining percentages of SOPC and SOS after oxidative treatment at 150 °C were determined as described above. Kinetic investigation showed that, during the first 30 min of oxidation, no significant ($p < 0.05$) decrease in these amounts was observed, for either SOPC or SOS (**Figure 4**). This could correspond to an induction period, because in the following minutes, a slow decrease in relative amounts was noticed but with a marked difference between SOPC and SOS. While in the case of SOS, there was a steady decrease that led to an almost total loss of the precursor after 120 min of oxidation, the decrease in SOPC amounts occurred between 30 and 90 min of oxidation and seemed to have stopped afterward, with 70% of starting material remaining after 120 min.

Figure 5 shows the evolution of the relative amounts of SLPC and SLS after various oxidation times at 125 °C. As opposed to their mono-unsaturated counterparts, the induction period was not observed with SLPC and SLS, probably because it occurred at shorter oxidation times. The decrease in the amounts of starting material was in fact immediate but significantly sharper with SLS than with SLPC ($p < 0.05$). The amount of SLPC seemed to have stabilized after 90 min of oxidation, with 33% of the precursor remaining after 120 min. The loss of SLS on the other hand was steady, such that it could not be detected after 120 min of oxidative treatment.

Results obtained for SLS and SLPC follow the same trend as the results obtained for the mono-unsaturated species. PC species therefore appear to be more resistant to the impact of thermo-oxidation than TAG species.

DISCUSSION

The goal of this study was to compare TAG and PC species in terms of their stability to oxidative treatment and, therefore, to evaluate the interest of supplementing foods with PUFA−PL, as compared to their supplementation with PUFA−TAG. PC and TAG species are both based on a glycerol backbone esterified with two FAs in *sn*-1 and *sn*-2 positions. The *sn*-3 position differs

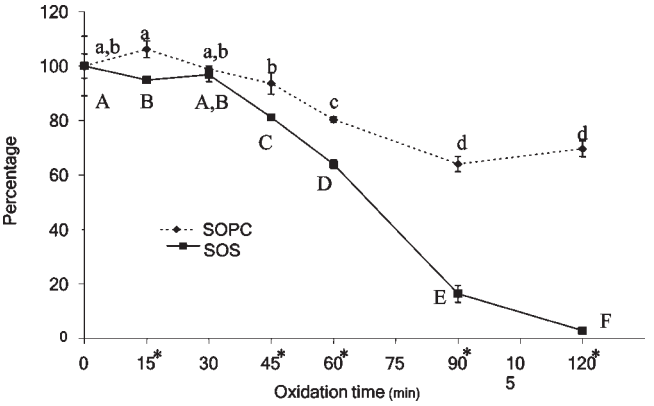

Figure 4. Percentage of intact precursor (SOPC or SOS) remaining after heating at 150 °C. All samples were analyzed in triplicate. Results are given as mean ± SD. For SOPC, means with different letters (a−d) are significantly different ($p < 0.05$). For SOS, means with different letters (A−F) are significantly different ($p < 0.05$). As asterisk indicates that SOPC and SOS remaining percentages are significantly different ($p < 0.05$) at each considered oxidation time.

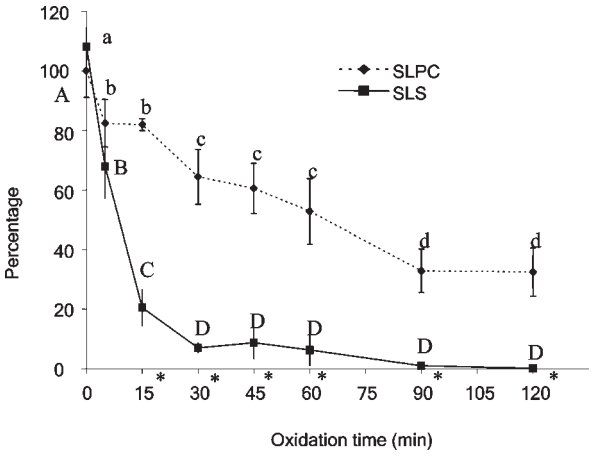

Figure 5. Percentage of intact precursor (SLPC or SLS) remaining after heating at 125 °C. All samples were analyzed in triplicate. Results are given as mean ± SD. For SLPC, means with different letters (a−d) are significantly different ($p < 0.05$). For SLS, means with different letters (A−D) are significantly different ($p < 0.05$). An asterisk indicates that SLPC and SLS remaining percentages are significantly different ($p < 0.05$) at each considered oxidation time.

in that PCs are esterified with a phosphate group containing a choline moiety, whereas TAGs are esterified with a third FA. Our study clearly demonstrates that PC species are more stable than the corresponding TAG species to heat-induced oxidation (**Figures 2** and **3**). Given that the esterified FAs were identical and in the same *sn* position in the investigated PC and TAG species, this difference in behavior is to be ascribed to the presence or absence of the polar head with the amino group (choline group).

Other workers studied the stability of PLs, although not as pure species, but in oils, where PCs and TAGs are mixed together. Song et al. compared the oxidative stability of docosahexaenoic acid (DHA) in oils in the form of ethyl esters, TAGs, or PLs (*18*). They clearly showed that DHA in the PL form was the most resistant to oxidation compared to other forms. Takeuchi et al. showed that the PL fraction was more stable than the TAG fraction in oils containing polyunsaturated FAs (*20*). They showed that the formation of hydroperoxides in a PL-containing

oil was slower when compared to highly purified oil containing no PLs. Results obtained by Moriya et al. (*12*) followed the same trend, because the oil containing the highest quantity of PLs was the most stable with regard to oxidation. Data from these studies are in agreement with our findings, in showing that PCs are more resistant to oxidation than TAGs and, therefore, in suggesting that the polar moiety of PCs has a role to play in their stability to oxidation. The possible mechanisms involved in this stabilization effect will be discussed later. Because all studies about PC or PL stability were performed in oils, these remarks let us think that the oxidation of a mixture composed of PLs and TAGs would follow the same trend, i.e., that PCs are more stable than TAGs, even in a mixture of lipids. A specific involvement of the quaternary amine function of this moiety in the antioxidative activity of PCs has also been suggested (*13*, *16*).

Kinetics of the formation of degradation products formed during the oxidative process was also determined for mono- and di-unsaturated species of PCs and TAGs. According to the well-known mechanisms of lipid oxidation, the hydroperoxides are the first degradation products to be formed (*4*). This could be clearly seen with SOPC, SOS, and SLPC but not with SLS (**Figures 2** and **3**). No lag time was noticed between hydroperoxide formation from SLS and other oxidation products, which can be attributed to the fact that 125 °C was an excessive temperature for SLS because of its higher sensitivity to oxidation (panels **c** and **d** of **Figure 3**). It is well-known that, for high temperatures, the degradation of hydroperoxides is faster than their formation. However, because our aim was to establish a comparison between TAG and PC species, it was necessary to work at the same heating temperature. The higher sensitivity of SLS as compared to SLPC is indicative of the differences between TAGs and PCs with regard to their stability to oxidation. Differences between TAGs and PCs were also noticed in terms of the stability of the generated oxidation products, because products from SOS and SLS appeared to be less stable than those from SOPC and SLPC (**Figures 1** and **2**).

As with the unoxidized PCs, the polar head group is likely to be involved in stabilizing the generated oxidation products from these PLs. The mechanisms involved into this stabilizing effect are not well-known. Antioxidative properties of PLs and their synergism with tocopherols were studied (*2*, *19*), and it was shown that PCs present a smaller synergistic effect with tocopherols than phosphatidylethanolamines (PEs). Nevertheless, PCs used alone present antioxidative properties depending upon their unsaturation degree (*13*, *24*). Although very few explanations were given as to what is behind these properties, it seems that the major antioxidative effect of PCs comes from the direct reduction of hydroperoxides to the corresponding alcohols by the amino group. Thus, radicals are formed to a much lesser extent, which limits the propagation phase of the oxidation process (*17*). This phenomenon would probably be the same with PEs, because their polar head group is able to easily transfer one of its hydrogen to the hydroperoxide group and decompose it in a non-radical way. Another hypothesis was proposed to explain the protective effect of the polar head group, implying the phosphate group. This hypothesis was proposed by Yoshimoto et al. concerning the decomposition of hydrogen peroxide by PCs. They supposed that PCs can lead to the polarization of the oxygen−oxygen bond in the peroxide, which allows for a nucleophilic attack of an oxygen atom of another peroxide group (*25*).

The results presented here support the hypothesis that the presence of a polar head group affects oxidation kinetics of glycerol-based lipids. This study showed in particular that PC species are more resistant to heating than the corresponding TAG species and that the behavior of their respective oxidation products follows the same trend. This aspect is very important when considering food supplementation with PUFAs, because the main part of PUFA supplementation is provided by fish oils, which are rich in TAGs. Therefore, supplementation with PL-rich oils, such as krill oil (*8*), could be more valuable than TAG-rich oils, for either FA bioavailability or stability to oxidation.

ABBREVIATIONS USED

TAG, triacylglycerol; PL, phospholipid; PC, phosphatidylcholine; FA, fatty acid; PUFA, polyunsaturated fatty acid; Rt, retention time; SOPC, 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; SLPC, 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; SOS, 1,3-stearoyl-2-oleoyl-glycerol; SLS, 1,3-stearoyl-2-linoleoyl-glycerol; SIM, single-ion monitoring; Ep, epoxy group; Ke, oxo group; OH, hydroxyl group; OOH, hydroperoxide group; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; DHA, docosahexaenoic acid.

LITERATURE CITED

(1) Amate, L.; Gil, A.; Ramirez, M. Feeding infant piglets formula with long-chain polyunsaturated fatty acids as triacylglycerols or phospholipids influences the distribution of these fatty acids in plasma lipoprotein fractions. *J. Nutr.* **2001**, *131*, 1250–1255.

(2) Bandarra, N. M.; Campos, R. M.; Batista, I.; Nunes, M. L.; Empis, J. M. Antioxidant synergy of α-tocopherol and phospholipids. *J. Am. Oil Chem. Soc.* **1999**, *76*, 905–913.

(3) Cho, S. Y.; Joo, D. S.; Choi, H. G.; Naraand, E.; Miyashita, K. Oxidative stability of lipids from squid tissues. *Fish. Sci.* **2001**, *67*, 738–743.

(4) Frankel, E. N. *Lipid Oxidation*, 2nd ed.; The Oily Press: Bridgwater, U.K., 2005.

(5) Giuffrida, F.; Destaillats, F.; Skibsted, L. H.; Dionisi, F. Structural analysis of hydroperoxy- and epoxy-triacylglycerols by liquid chromatography mass spectrometry. *Chem. Phys. Lipids* **2004**, *131*, 41–49.

(6) Goodnight, S. H., Jr.; Harris, W. S.; Connor, W. E.; Illingworth, D. R. Polyunsaturated fatty acids, hyperlipidemia, and thrombosis. *Arterioscler., Thromb., Vasc. Biol.* **1982**, *2*, 87–113.

(7) Le Grandois, J.; Marchioni, E.; Ennahar, S.; Giuffrida, F.; Bindler, F. Identification and kinetics of oxidized compounds from phosphatidylcholine molecular species. *Food Chem.* **2009**, *119*, 1233–1238.

(8) Le Grandois, J.; Marchioni, E.; Zhao, M.; Giuffrida, F.; Ennahar, S.; Bindler, F. Investigation of natural phosphatidylcholine sources: Separation and identification by liquid chromatography−electrospray ionization−tandem mass spectrometry (LC−ESI−MS^2) of molecular species. *J. Agric. Food Chem.* **2009**, *57*, 6014–6020.

(9) Lemaitre-Delaunay, D.; Pachiaudi, C.; Laville, M.; Pousin, J.; Armstrong, M.; Lagarde, M. Blood compartmental metabolism of docosahexaenoic acid (DHA) in humans after ingestion of a single dose of [^{13}C]DHA in phosphatidylcholine. *J. Lipid Res.* **1999**, *40*, 1867–1874.

(10) Lercker, G.; Bortolomeazzi, R.; Pizzale, L. Thermal degradation of single methyl oleate hydroperoxides obtained by photosensitized oxidation. *J. Am. Oil Chem. Soc.* **1998**, *75*, 1115–1120.

(11) Mori, A. L.; Porzio, M. A.; Schaleger, L. L. Kinetics and mechanism of epoxy ether hydrolysis. I. Mechanism of the hydrolysis of an acyclic hemiacetal intermediate. *J. Am. Oil Chem. Soc.* **1972**, *94*, 5034–5039.

(12) Moriya, H.; Kuniminato, T.; Hosokawa, M.; Fukunaga, K.; Nishiyama, T.; Miyashita, K. Oxidative stability of salmon and herring roe lipids and their dietary effect on plasma cholesterol levels of rats. *Fish. Sci.* **2007**, *73*, 668–674.

(13) Nwosu, C.; Boyd, L.; Sheldon, B. Effect of fatty acid composition of phospholipids on their antioxidant properties and activity index. *J. Am. Oil Chem. Soc.* **1997**, *74*, 293–297.

(14) Parmentier, M.; Sayed Mahmoud, C.; Linder, M.; Fanni, J. Polar lipids: n-3 PUFA carriers for membranes and brain: Nutritional interest and emerging processes. *Ol., Corps Gras, Lipides* **2007**, *14*, 224–229.

(15) Reis, A.; Domingues, M. R. M.; Amado, F. M. L.; Ferrer-Correia, A. J.; Domingues, P. Radical peroxidation of palmitoyl-lineloyl-glycerophosphocholine liposomes: Identification of long-chain oxidised products by liquid chromatography−tandem mass spectrometry. *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.* **2007**, *855*, 186–199.
(16) Saito, H.; Ishihara, K. Antioxidant activity and active sites of phospholipids as antioxidants. *J. Am. Oil Chem. Soc.* **1997**, *74*, 1531–1536.
(17) Shahidi, F. *Nutraceutical and Specialty Lipids and Their Co-products*; CRC Press: Boca Raton, FL: 2006.
(18) Song, J. H.; Inoue, Y.; Miyazawa, T. Oxidative stability of docosahexaenoic acid-containing oils in the form of phospholipids, triacylglycerols, and ethyl esters. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 2085–2088.
(19) Takenaka, A.; Hosokawa, M.; Miyashita, K. Unsaturated phosphatidylethanolamine as effective synergist in combination with α-tocopherol. *J. Oleo Sci.* **2007**, *56*, 511–516.
(20) Takeuchi, M.; Hara, S.; Totani, Y.; Hibino, H.; Tanaka, Y. Autooxidative behavior of polyunsaturated phospholipids. I. Oxidative stability of marine oil containing polyunsaturated phospholipids. *J. Jpn. Oil Chem. Soc.* **1997**, *46*, 175–181.
(21) Velasco, J.; Berdeaux, O.; Marquez-Ruiz, G.; Dobarganes, M. C. Sensitive and accurate quantitation of monoepoxy fatty acids in thermoxidized oils by gas−liquid chromatography. *J. Chromatogr., A* **2002**, *982*, 145–152.
(22) Wada, S; Koizumi, C. Influence of the position of unsaturated fatty acid esterified glycerol on the oxidation rate of triglyceride. *J. Am. Oil Chem. Soc.* **1983**, *60*, 1105–1108.
(23) Wijendran, V.; Huang, M. C.; Diau, G. Y.; Boehm, G.; Nathanielsz, P. W.; Brenna, J. T. Efficacy of dietary arachidonic acid provided as triglyceride or phospholipid as substrates for brain arachidonic acid accretion in baboon neonates. *Pediatr. Res.* **2002**, *51*, 265–272.
(24) Yin, M. C.; Faustman, C. Influence of temperature, pH, and phospholipid composition upon the stability of myoglobin and phospholipid: A liposome model. *J. Agric. Food Chem.* **1993**, *41*, 853–857.
(25) Yoshimoto, M.; Miyazaki, Y.; Umemoto, A.; Walde, P.; Kuboi, R.; Nakao, K. Phosphatidylcholine vesicle-mediated decomposition of hydrogen peroxide. *Langmuir* **2007**, *23*, 9416–9422.

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