

A New Insight into the Formation of Odor Active Carbonyls by  
Thermally-Induced Degradation of Phospholipids in  
Self-Assembly Structures<sup>§</sup>JIANMING LIN,<sup>†</sup> MARTIN E. LESER, JÜRGE LÖLIGER, AND IMRE BLANK\*Nestec Ltd., Nestlé Research Center, Vers-chez-les-Blanc, P.O. Box 44,  
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The role of molecular organization in heated aqueous dispersions of egg phosphatidylcholine (PC) and egg phosphatidylethanolamine (PE) was studied with respect to the formation of key odorants. Evidence was found for the crucial role of self-assembly structures adopted by phospholipid molecules on the quantitative composition of volatile constituents. The concentrations of seven aldehydes and one vinyl ketone were determined by isotope dilution assay in heated aqueous dispersions of PC and PE present in various ratios. Addition of PE to PC drastically decreased the amount of (*E,E*)-2,4-decadienal formed, which cannot be explained by the differences in the fatty acid composition of PC and PE. The free amino group in PE does not explain this phenomenon either, as replacing PE by phosphatidic acid distearylester also reduced the amounts of (*E,E*)-2,4-decadienal. We suggest that the type of self-assembly structure adopted by phospholipids in water significantly influences the reaction yields. However, the mechanisms leading to the preferred formation of phospholipid-derived odorants in a lamellar phase, as compared to the reversed hexagonal phase, remain unknown.

**KEYWORDS:** Phospholipids; phosphatidic acid; phosphatidylcholine; lecithin; phosphatidylethanolamine; cephalin; key odorants; quantification; isotope dilution assay; phase diagram; self-assembly structure; emulsion; molecular organization

## INTRODUCTION

Phospholipids are used in the food industry for different purposes. Because of their amphiphilic nature, they function as emulsifiers and stabilizers, for example, in chocolate, baked products, shortenings, margarine, instant products, mayonnaise, and low fat products (1). In general, oilseeds, cereal germs, egg yolk, and fish are the richest sources of phospholipids (2, 3). Industrial phospholipids come almost entirely from soybeans with phosphatidylcholine (PC, lecithin), phosphatidylethanolamine (PE, cephalin), and phosphatidylinositol (PI) as major constituents of the phospholipid fraction. They are derivatives of phosphatidic acid (PA) and rich in polyunsaturated fatty acids (PUFAs), especially linoleic acid (C18:2), arachidonic acid (C20:4), and other highly unsaturated fatty acids (e.g., C22:5 and C22:6) (4).

These PUFAs are also a suitable source of odorants generated upon thermal treatment. Structural phospholipids have been shown to play a significant role in meat aroma specificity, contributing through (i) lipid-derived odorants generated by thermally induced lipid oxidation and (ii) interaction of lipid intermediates with the Maillard reaction, thus leading to a

modified and species specific overall aroma of cooked meat (4, 5). Various volatile compounds have been identified in heated phospholipids, such as hexanal, (*E*)-2-nonenal, (*E*)- and (*Z*)-2-decenal, (*E,E*)-2,4-decadienal, 1-octen-3-one, (*E*)-2-undecenal, (*E,Z,Z*)-2,4,7-tridecatrinal, and 2-pentylfuran (6, 7). Recently, *trans*-4,5-epoxy-(*E*)-2-decenal was found to be the most potent odorant in heated aqueous dispersions of PC and PE (7). The aroma composition of commercial soybean lecithin has been recently described, with lipid degradation and Maillard reaction products as the most potent odorants (8). In general, higher amounts of several unsaturated aldehydes were found in heated PC, while hexanal and 2-pentylfuran dominated in heated PE (6).

The concentration of (*E,E*)-2,4-decadienal in heated PC was about 20-fold higher as compared to PE, while hexanal was the major odor active compound in the PE sample (7). Interestingly, these quantitative results cannot entirely be explained by the differences in the fatty acid composition of PC and PE: despite lower amounts of linoleic and arachidonic acid in PC (20%) as compared to PE (28%), significantly more (*E,E*)-2,4-decadienal was found in PC (~110 mg/kg) than in PE (~5 mg/kg). These data suggested that additional parameters might influence the formation of carbonyls from heated aqueous dispersions of phospholipids, such as their amphiphilic nature that may define the physical state of the reaction medium. Similar effects have recently been observed in Maillard-generated flavors, showing that the yields of impact odorants

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were significantly higher when the meat flavor precursors ribose and cysteine were heated in a cubic phase as compared to an aqueous solution (9).

In this paper, we report quantitative data of eight odor active compounds generated in aqueous dispersions of phospholipids and discuss the role of reaction medium and structure with respect to the reaction yields.

## MATERIALS AND METHODS

**Materials.** The chemicals were commercially available as follows: hexanal (**1**), (*E*)-2-octenal (**4**; containing traces of the *Z*-isomer), (*E,E*)-2,4-nonadienal (**9**; *E,Z*: 5%), acetyl chloride, methanol (MeOH), 2-(1,1-dimethylethyl)-4-methoxyphenol, 2,6-bis(1,1-dimethylethyl)-4-methylphenol, neutral aluminum oxide (alumina), and dichloromethane ( $H_2O < 0.005\%$ ) (Aldrich/Fluka, Buchs, Switzerland); 1-octen-3-one (**2**; Oxford, Brackley, U.K.); (*E*)-2-nonenal (**7**; Agipal, Paris, France); (*E,E*)-2,4-decadienal (**13**; 95%, *E,Z*: 5%, Fontarom, Cergy Pontoise, France); egg PC (>99%), egg PE (>99%), and distearoyl PA disodium salt (>99%) (Avanti Polar Lipids, Copenhagen, Denmark); diethyl ether ( $Et_2O$ ), benzene, hexane, pentane, potassium carbonate, silica gel 60, disodium hydrogenphosphate, sodium dihydrogenphosphate, anhydrous sodium sulfate, and sodium chloride (Merck, Darmstadt, Germany). *trans*-4,5-Epoxy-(*E*)-2-decenal (**15**) and (*E,Z,Z*)-2,4,7-tridecatrinal (**16**) were synthesized (10, 11). The deuterated internal standards (ISs) used in the quantification experiments were prepared as previously described (10, 12–14).

**Fatty Acid Analysis.** The fatty acid composition of egg PC and egg PE was determined after methylation. The phospholipid (1 mg) was dissolved in methanol/benzene (4/1, v/v, 2 mL). Acetyl chloride (0.2 mL) was added first followed by water (0.1 mL). The mixture was placed in a closed tube and heated at 100 °C in an oven for 1 h. After it was cooled with tap water, an aqueous potassium carbonate solution (6%, w/v, 5 mL) was added for neutralization. The fatty acid methyl esters were extracted with hexane (2 mL) and then centrifuged (1500 rpm, 2 min) for phase separation. An aliquot of the organic phase (1 mL) was taken, and the solvent was removed by a stream of nitrogen. After hexane (1 mL) was added, the sample was ready for gas chromatography (GC) analysis. Separation of the fatty acid methyl esters was achieved on a DB-Wax fused silica capillary column (30 m  $\times$  0.32 mm, 0.25  $\mu$ m film thickness, J&W Sci., Folsom, U.S.A.) using a Hewlett-Packard HP-5890 gas chromatograph with an on-column injector. The oven temperature was programmed as follows: 80 °C, 13.7 °C/min to 135 °C (1 min), 3.0 °C/min to 180 °C (81 min), and 2.5 °C/min to 225 °C (10 min). Identification of fatty acid methyl esters was achieved by comparison with a standard mixture composed of 37 fatty acids.

**Model Reactions and Sample Cleanup.** The solvent of a phospholipid chloroform–methanol solution (2:1, v/v, 10 mL) containing PC (1 g), PE (1 g), and mixtures of PC and PE or PC and PA was evaporated with a stream of nitrogen (7). A phosphate buffer (50 mL, 0.5 M, pH 5.6) was then added, and the mixture was stirred with a magnetic agitator to disperse the phospholipid in water. The sample was heated in a laboratory autoclave for 30 min from room temperature to 145 °C (7). After the reaction, the samples were rapidly cooled to room temperature. Defined amounts of labeled ISs were added and well-mixed with the reaction sample before solvent extraction ( $Et_2O$ , 100 mL) (7). Organic compounds were extracted with dichloromethane (3  $\times$  50 mL) for quantification of **13** and **16** by isotope dilution assay (IDA). The nonvolatile compounds in the organic extract were removed either by high vacuum transfer (HVT) (7) or by column chromatography (CC) described in the following. The volatile solvent extracts obtained after HVT or CC were dried over sodium sulfate and concentrated to 2 mL on a Vigreux column (50  $\times$  1 cm) and finally to 0.5 mL using a microdistillation apparatus (15).

**CC.** For IDA of **13** and **16** in heated aqueous dispersions of phospholipids, fats including the free fatty acids released and the remaining phospholipids were removed by CC using a water-cooled glass column (20 cm  $\times$  1 cm) packed with alumina in pentane/ $Et_2O$  (2:1, v/v). The solvent extracts were concentrated to 5 mL and then

**Table 1.** Concentration of Odorants Generated on Heating an Equimolar Mixture of PC and PE Dispersed in Water

| odorant <sup>a</sup>   | concentration <sup>b</sup><br>(mg/kg phospholipid) |
|--|--|
| hexanal ( <b>1</b> )   | 88.9 $\pm$ 6.2                                     |
| 1-octen-3-one ( <b>2</b> )                                   | 4.0 $\pm$ 0.1                                      |
| ( <i>E</i> )-2-octenal ( <b>4</b> )                          | 4.2 $\pm$ 0.3                                      |
| ( <i>E</i> )-2-nonenal ( <b>7</b> )                          | 1.2 $\pm$ 0.1                                      |
| ( <i>E,E</i> )-2,4-nonadienal ( <b>9</b> )                   | 0.41 $\pm$ 0.03                                    |
| ( <i>E,E</i> )-2,4-decadienal ( <b>13</b> )                  | 5.2 $\pm$ 0.8                                      |
| <i>trans</i> -4,5-epoxy-( <i>E</i> )-2-decenal ( <b>15</b> ) | 1.0 $\pm$ 0.1                                      |
| ( <i>E,Z,Z</i> )-2,4,7-tridecatrinal ( <b>16</b> )           | 0.74 $\pm$ 0.14                                    |

<sup>a</sup> The numbering of odorants is in accordance with that in ref 7. <sup>b</sup> Each sample was prepared in duplicate and injected at least twice.

applied onto a column (20 cm  $\times$  1 cm), which was maintained at  $\sim$ 10 °C by a cooling jacket. Ionic compounds were retained on the column, while carbonyl lipid oxidation products were eluted with pentane/ $Et_2O$  (2/1, v/v, 150 mL).

To simplify the GC-MS chromatogram for quantification, the effluent obtained from CC with alumina was concentrated to 2 mL and then applied to a column (20 cm  $\times$  1 cm, cooled at 10 °C) packed with silica gel 60 in pentane. Elution was performed stepwise with 50 mL of pentane/ $Et_2O$  (98/2, v/v) and 150 mL of pentane/ $Et_2O$  (85/15, v/v). Only the second fraction was collected and concentrated to 0.5 mL for GC-MS analysis.

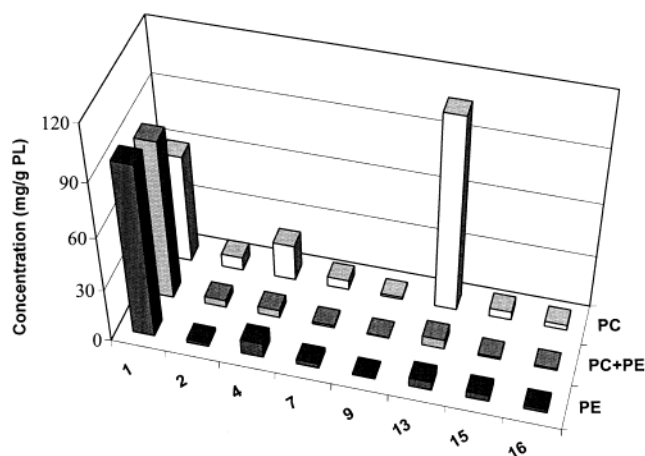
**GC-MS.** Quantitative analysis was performed on a Finnigan SSQ 7000 mass spectrometer (Bremen, Germany) coupled with a HP-5890 gas chromatograph using isobutane as the reagent gas for chemical ionization carried out at 200 eV. Further experimental details have been described previously (14). Quantitative measurements were carried out in full scan or in the selected ion monitoring mode. The characteristic ions used for quantification of **1**, **2**, **4**, **7**, **9**, **13**, **15**, and **16** have been reported (7, 14). Each sample was prepared in duplicate and injected at least twice.

**IDA.** Defined amounts of labeled IS in solution were added to the reacted samples prior to isolation of volatiles (7). Calibration curves were established for the eight odorants quantified in this study using mixtures of defined amounts of analyte and labeled IS (7, 11). The parameters used in the IDA of the odorants are summarized in the literature (7, 11). Samples for establishing the calibration curves and for quantification were injected twice.

## RESULTS

**Formation of Odorants from PC/PE Mixtures.** An aqueous dispersion of equimolar amounts of PC and PE was heated in the same manner as the individual phospholipid samples (7). Eight odorants of the volatile fraction were quantified by using the IDA method. Quantitative characterization revealed **1** ( $\sim$ 90 mg/kg) as the major odor active constituent of this sample (Table 1). The amounts of the remaining odorants varied from 0.4 mg/kg for **9** to  $\sim$ 5 mg/kg for **13**. Surprisingly, the amounts of carbonyl odorants generated in the equimolar mixture of PC and PE (PC/PE 1:1) were very close to the levels generated from PE (Figure 1). As compared to the quantitative data obtained for pure PC and PE (7), PC/PE 1:1 was almost identical with the pure PE sample. The most intriguing difference, however, was the low amount of **13** found in PC/PE 1:1, while this odorant was the major compound in the pure PC sample.

The fatty acid composition of PC and PE used in this study (Table 2) does not explain these findings. Assuming that odorant **13** is mainly formed from linoleic (C18:2) and arachidonic acid (C20:4), high amounts of **13** ( $\sim$ 110 mg/kg) were found in the PC sample containing 20.9% precursors. The amounts of **13** dropped to  $\sim$ 5 mg/kg in PE and PC/PE 1:1 containing 25.4 and 23.2% precursors, respectively. These data confirm the



**Figure 1.** Concentration of eight odorants generated on heating aqueous dispersions of PC, PE, and an equimolar mixture of PC and PE. Compound 1, hexanal; 2, 1-octen-3-one; 4, (*E*)-2-octenal; 7, (*E*)-2-nonenal; 9, (*E,E*)-2,4-nonadienal; 13, (*E,E*)-2,4-decadienal; 15, *trans*-4,5-epoxy-(*E*)-2-decenal; and 16, (*E,Z,Z*)-2,4,7-tridecatrinal. The values for PC and PE were taken from ref 7.

**Table 2.** Fatty Acid Composition of the Egg PC and Egg PE Used in This Study

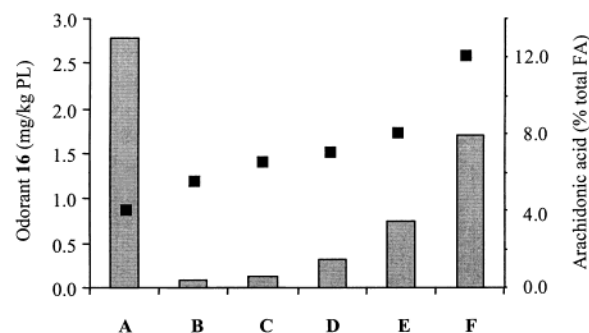
| fatty acid <sup>a</sup>   | fatty acid composition<br>(% total fatty acids) |        |
|---------------------------|---|--------|
|                           | egg PC  | egg PE |
| C14:0                     | 0.2   | 0.1    |
| C16:0                     | 34.3  | 20.6   |
| C16:1                     | 1.5   | 0.6    |
| C17:0                     | 0.2   | 0.3    |
| C18:0                     | 10.5  | 23.3   |
| C18:1 (n-9), <i>cis</i>   | 26.5  | 18.2   |
| C18:1 (n-9), <i>trans</i> | 1.3   | 0.9    |
| C18:2 (n-6)               | 16.7  | 13.2   |
| C18:3 (n-6)               | 0.1   | 0.1    |
| C18:3 (n-3)               | 0.1   | 0.1    |
| C20:2 (n-6)               | 0.2   | 0.2    |
| C20:3 (n-6)               | 0.4   | 0.4    |
| C20:4 (n-6)               | 4.2   | 12.2   |
| C22:5                     | 1.3   | 2.8    |
| C22:6                     | 1.2   | 3.2    |
| minor fatty acids         | 1.3   | 3.8    |
| Σ saturated               | 45.2  | 44.3   |
| Σ monounsaturated (1 DB)  | 29.3  | 19.7   |
| Σ diunsaturated (2 DB)    | 16.9  | 13.4   |
| Σ polyunsaturated (≥3 DB) | 7.5   | 18.8   |

<sup>a</sup> DB = double bond.

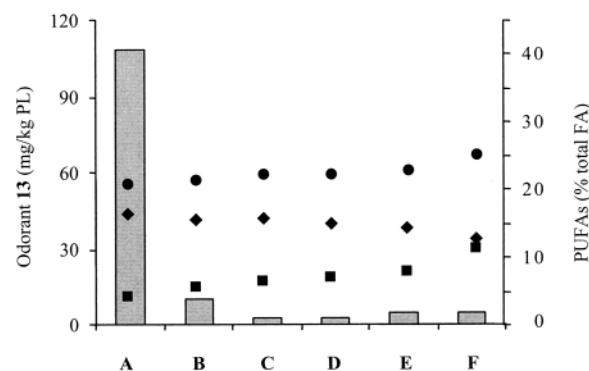
hypothesis that several parameters affect the formation of carbonyls from heated aqueous dispersions of phospholipids. Therefore, additional experiments were carried out to better estimate the role of (i) the fatty acid composition, (ii) the amino group of phospholipids, and (iii) the molecular organization on the formation of odorants from aqueous phospholipid systems upon heating.

**Effect of the Fatty Acid Composition.** The influence of the fatty acid composition of phospholipids on the formation of odor active carbonyls was studied by quantifying the amounts of **13** and **16** in heated aqueous dispersions containing PC and PE in the molar ratios 4:1, 2:1, and 3:2, in addition to the data obtained from pure PC, PE, and PC/PE 1:1.

The amounts of **16** generated in the various phospholipid systems are shown in **Figure 2**, along with the content of its principle precursor arachidonic acid (C20:4). Surprisingly, the



**Figure 2.** Concentration of **16** generated upon heating of aqueous dispersions of PC, PE, and mixtures of PC and PE in various molar ratios (bars, left vertical axis) along with the arachidonic acid content of the phospholipid samples (■, right vertical axis). (A) PC, (B) PC + PE (4:1), (C) PC + PE (2:1), (D) PC + PE (3:2), (E) PC + PE (1:1), and (F) PE.



**Figure 3.** Concentration of **13** generated upon heating of aqueous dispersions of PC, PE, and mixtures of PC and PE in various molar ratios (bars, left vertical axis) along with the content of linoleic acid (◆), arachidonic acid (■), and the sum of both (●) in the phospholipid samples (right vertical axis). (A) PC, (B) PC + PE (4:1), (C) PC + PE (2:1), (D) PC + PE (3:2), (E) PC + PE (1:1), and (F) PE.

concentration of **16** is negatively correlated with the C20:4 content. The highest amounts of **16** were found in pure PC (sample A), even though it contained the lowest level of C20:4 (4.2% of total fatty acids, **Table 2**) among the six phospholipid samples. On the other hand, increasing amounts of C20:4 in samples containing PE led to more **16** generated (**Figure 2**, samples B–E). Finally, the C20:4 content of PE was about three times that of PC (sample A); however, only about half the amount of **16** was formed (sample F).

As shown in **Figure 3**, significantly (>20-fold) higher amounts of **13** were generated from pure PC (sample A) as compared to the other phospholipid mixtures containing PE. Clearly, the presence of PE markedly reduced the concentration of **13**: already, 25% of PE in the phospholipid mixture (sample B) led to a decrease from ~90 mg/kg to less than 10 mg/kg. However, the total amounts of its direct precursors (C18:2, C20:4) increased from samples B to E due to the rising level of PE, which is particularly rich in C20:4 (**Table 2**). The C18:2 content decreased slightly with increasing amounts of PE. This, however, does not explain the drastic decline in **13**. In the samples containing PE, the concentration of **13** decreased with decreasing C18:2 content (samples B and C) and increased slightly with increasing C20:4 content (samples C–F). These data suggest that there is no direct correlation between the amount of **13** and its precursors C18:2 and C20:4.

**Effect of the Polar Moiety.** The role of the polar moiety, in particular the free amino group of PE in reducing the amount of aldehydes by amino-carbonyl reactions, was studied by



**Table 3.** Concentration of **13** Generated on Heating Aqueous Dispersions of PC and Mixtures of PC and PA and PE

| phospholipid<br>(molar ratio) | concentration<br>of <b>13</b> (mg/kg) <sup>a</sup> | precursor content<br>(% total fatty acids) |                    |              |
|-------------------------------|--|--|--------------------|--------------|
|                               |  | C18:2 <sup>b</sup>                         | C20:4 <sup>c</sup> | Σ precursors |
| PC                            | 108.4  | 16.7                                       | 4.2                | 20.9         |
| PC + PA (4:1)                 | 25.4   | 13.4                                       | 3.4                | 16.8         |
| PC + PE (4:1)                 | 10.1   | 16.0                                       | 5.8                | 21.8         |

<sup>a</sup> The concentration is given in mg/kg phospholipid. <sup>b</sup> C18:2, linoleic acid. <sup>c</sup> C20:4, arachidonic acid.

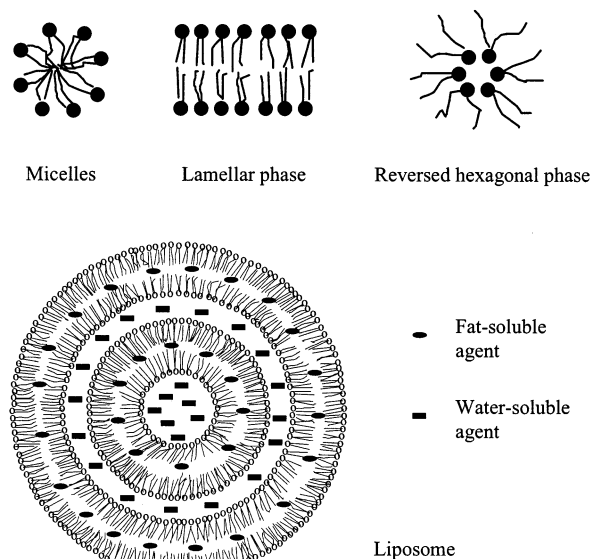
reacting PC and distearoyl PA in the molar ratio 4:1 and determining the concentration of odorant **13**. As shown in **Table 3**, not only PC/PE 4:1 affected the formation of **13** but also the presence of PA; that is, the concentration of **13** dropped from ~110 mg/kg for PC to 10.1 and 25.4 mg/kg for PC/PE 4:1 and PC/PA 4:1, respectively. These data suggest that the reactivity of the free amino group of PE does not sufficiently explain the drastic decrease in **13** by the chemical nature of the polar moiety of the phospholipid, nor the concentration of potential precursors present in the phospholipids.

**Effect of Structure.** When added to water, PC and PE induced significant differences in the appearance of the dispersions obtained, i.e., the reaction medium. While the PC sample was a homogeneous emulsion-like dispersion, the PE sample became a solid block (lump), which did not disperse well into the water (data not shown). This was the most striking difference between the PC sample and those containing PE, which may be linked to the differences found in the chemical composition of the lipid-derived odorants.

## DISCUSSION

This study shows that PC was more efficient than PE in generating carbonyl odorants in aqueous dispersions by thermal treatment. Moreover, when PC was heated in the presence of PE, most of the carbonyl odorants were formed in lower yields. The results obtained also indicate that there is no direct relationship between the amounts of carbonyl odorants, such as **13** and **16**, and the  $\omega$ 6 fatty acid (e.g., 18:2 and 20:4) content of the phospholipids, from which these aldehydes are formed. Several factors may play a role in the formation of odorants by thermal treatment of phospholipids. The content of unsaturated fatty acids is certainly an important parameter, as they are the direct precursors of these odorants. However, the presented experimental results indicate that the polar moiety of phospholipids and, thereby, the molecular organization of the structured fluids, play also a crucial role. On the other side, impurities such as metal traces or antioxidants are unlikely to affect the results obtained, as phospholipids of highest purity were used in this study.

In general, the total unsaturated fatty acid contents of PC and PE are very close, i.e., about 52–54% (**Table 2**). However, PE contains 2–3 times more PUFAs, known to readily oxidize. Surprisingly, the amounts of the odorants **13** and **16** generated from PE were lower than from PC, despite the higher PUFA content in PE. However, as shown in **Figure 2**, a positive correlation between the concentration of **16** and the C20:4 content was found in samples containing PE. This suggests that already small quantities of PE change the reaction system leading to less unsaturated carbonyl odorants. Lower levels of unsaturated carbonyl compounds in PE-containing phospholipid samples were also reported in the literature (6). The authors

**Figure 4.** Structures adopted by amphiphilic phospholipid molecules in aqueous solutions; adapted from refs 19 and 20.

speculated that reactions between aldehydes and the free amino group of PE were responsible for this phenomenon.

Indeed, the most remarkable difference between PC and PE is not the fatty acid composition, but the polar moiety, which can be involved in chemical reactions. For example, the primary amino group of PE may react with either the hydroperoxides or the aldehydes derived from them to give unstable Schiff bases, which may further react to give brown pigments (16–18). As a result, such secondary reactions could contribute to degradation of odorants formed and, thus, affect the final levels of odorants. Therefore, the amino-carbonyl reaction might explain the lower-than-mean levels of carbonyl odorants when PC and PE were heated together in an aqueous dispersion (**Figures 2** and **3**). Interestingly, the amounts of **1** were very pronounced in samples containing PE (**Figure 1**). Possible differences in reactivity between hexanal and unsaturated aldehydes can, however, hardly explain these findings. Furthermore, the amount of **13** was also remarkably decreased when PC was heated in the presence of PA (**Table 3**), which does not contain the free amino group to react with carbonyl odorants.

Phospholipids are amphiphilic molecules and act as emulsifier. They organize spontaneously forming a variety of different self-assembly structures in aqueous solutions, such as micelles, lamellar phase, and reversed hexagonal phase (**Figure 4**) (19, 20). Under certain conditions, the formation of these structures may affect the yields of reaction products and influence the reaction pathways (9). For example, the type of self-assembly structure formed could significantly influence the oxidation of phospholipids, since the differently curved surfactant films are expected to show a different interaction with the reaction products or other components present in the system. Moreover, because chemical reactions strongly depend on the mobility of reactants, the region in which they are solubilized within the self-assembly structure (i.e., aqueous, interfacial, lipophilic chain region) will affect the reaction rate and final composition of the sample (21).

The difference in the appearance of the PC sample as compared to those containing PE is the consequence of the difference in their binary phase behavior. According to the phase diagrams shown in **Figure 5** (22), PC molecules adopt a lamellar phase, while a reversed hexagonal phase is formed in the PE sample under the experimental conditions used (2% in water,

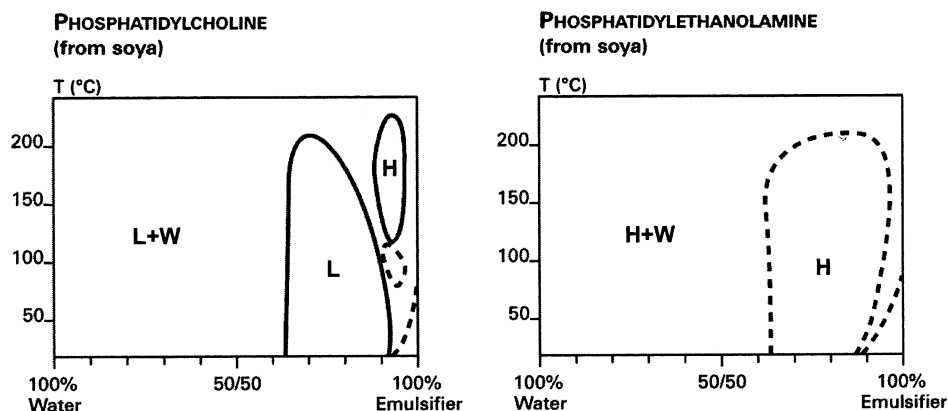


Figure 5. Phase diagrams of PC and PE. L, lamellar phase; L + W, lamellar phase and water; H, reversed hexagonal phase; and H + W, reversed hexagonal phase and water; adapted from ref 22.

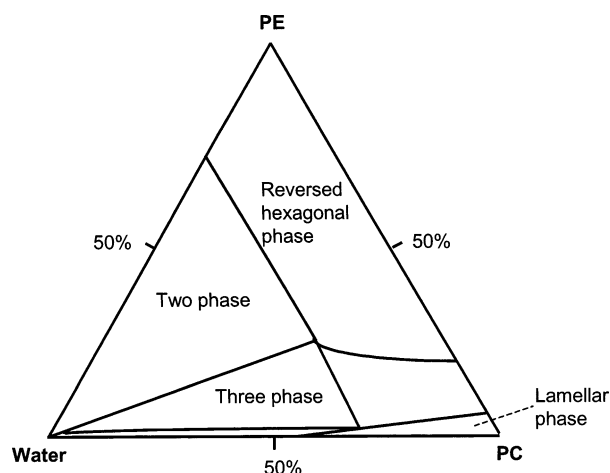


Figure 6. Ternary phase diagram of PC, PE, and water at 20 °C; adapted from ref 19.

25–145 °C). The liquid crystalline lamellar phase is readily dispersible in water forming stable vesicles or liposomes (Figure 4) with the so-called bilayer as basic self-assembly structure, in which the surfactant film has a zero curvature (19). On the contrary, PE molecules form a reverse hexagonal structure, in which the surfactant film is curved toward water. It is not possible to homogeneously disperse molecules organized in a reversed hexagonal phase (23), since this type of self-assembly structure is much more lipophilic, as compared to the lamellar phase, due to the smaller polar moiety (area per headgroup) of the PE molecule. Pieces of such a phase fuse immediately when they come into contact with water, forming nondispersible lumps.

The ternary phase diagram of PC, PE, and water obtained at room temperature is shown in Figure 6 (19), which should also be valid in this study using autoclave conditions (145 °C), since the structure and phase behavior of aqueous dispersions containing pure phospholipid (PE or PC) is not affected by the temperature (Figure 5). As indicated in Figure 6, the reversed hexagonal phase adopted by pure PE is virtually dominating and unchanged until the addition of ~70 wt % of PC. Above 70 wt %, the system separates into a three phase area in which a reversed hexagonal phase is in equilibrium with a lamellar phase and water. This indicates that most probably in all PC/PE phospholipid mixtures used in this study (PC/PE 4:1, 2:1, 3:2, and 1:1) a reversed hexagonal phase was formed. These findings could explain the fact why the formation of both 13 and 16 was drastically reduced when adding PE to the PC. However, the mechanisms explaining why the formation of 13

and 16 is favored in a lamellar over a reversed hexagonal environment remain unclear.

In conclusion, the described experimental findings support the hypothesis that the self-assembly structures adopted by the phospholipid molecules in aqueous media may be an important factor to consider in lipid oxidation (24) and interaction with other reactions such as the Maillard reaction in food systems. Additional work is required to elucidate the exact mechanisms explaining the differences observed in thermally induced odorant formation in the different phospholipid self-assembly structures.

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