Chapter 2

Formation of Odor-Active Carbonyls in Self-Assembly Structures of Phospholipids

I. Blank[1], J. Lin[2], M. E. Leser[1], and J. Löliger[1]

[1]Nestec Ltd., Nestlé Research Center, Vers-chez-les-Blanc, 1000 Lausanne 26, Switzerland
[2]Current address: Firmenich Inc., P.O. Box 5880, Princeton, NJ 08543

The formation of eight odor-active compounds generated from heated aqueous dispersions of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) was studied. trans-4,5-Epoxy-(E)-2-decenal was found to be the most potent odorant on the basis of flavor dilution factors and odor activity values, followed by (E,E)-2,4-decadienal, 1-octen-3-one, and hexanal. The amount of (E,E)-2,4-decadienal in PC was about 20-fold higher compared to PE, while hexanal was the most abundant odor-active compound in the PE sample. Differences in the fatty acid composition of the phospholipids and the free amino group of PE can only partially explain the quantitative results found. It is suggested that the type of self-assembly structure adopted by phospholipid molecules in water is significantly influencing the reaction yields, thus playing a crucial role for the final quantitative composition of volatile constituents.

Introduction

Due to their amphiphilic nature, phospholipids are used as emulsifiers and stabilizers in food products, such as 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 (Figure 1). 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 (*4, 5*). The major volatile compounds found in heated phospholipids were hexanal, nonanal, 2-octenal, 2-decenal, (*E,E*)-2,4-decadienal, 1-octen-3-ol, 2-pentylfuran, and others (*6*). Significant differences were found between PC and PE on the basis of GC peak areas, in particular higher amounts of several unsaturated aldehydes in PC, while hexanal and 2-pentylfuran dominated in PE. The aroma composition of commercial soybean lecithin has been recently described, with lipid degradation and Maillard reaction products as the most potent odorants (*7*). (*E,E*)-2,4-Decadienal, (*E*)-2-nonenal, and 1-octen-3-one showed high sensory relevance (*8*).

In this contribution 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.
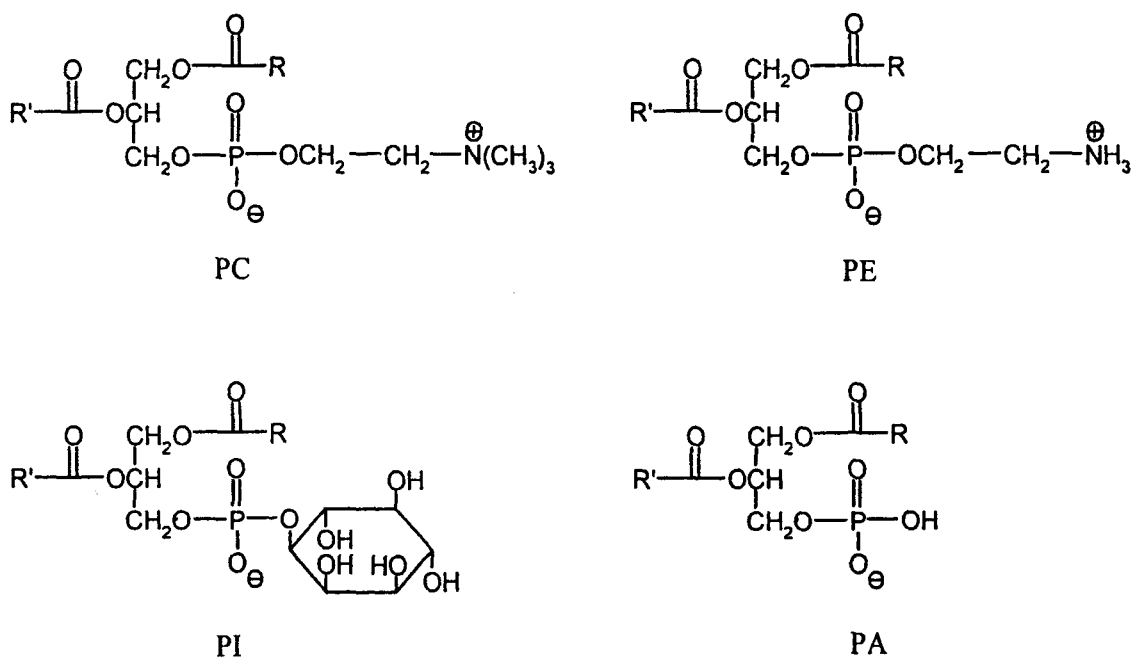

Figure 1. Chemical structures of phosphatidylcholine (PC, lecithin), phosphatidylethanolamine (PE, cephalin), phosphatidylinositol (PI), and phosphatidic acid (PA). The rest R represents the fatty acid chain (in the case of PA, the rest R was stearic acid).

Experimental

Materials

The following chemicals were commercially available: hexanal **1**, (*E*)-2-octenal **4**, (*E*,*E*)-2,4-nonadienal **9** (*E*,*Z*: 5%), 2-(1,1-dimethylethyl)-4-methoxyphenol (BHA), 2,6-di-*tert*-butyl-4-methylphenol (BHT), neutral aluminum oxide, methanol, (Aldrich/Fluka, Buchs, Switzerland); 1-octen-3-one **2** (Oxford, Brackley, UK); (*E*)-2-nonenal **7** (traces of *Z*-isomer **6**) (Agipal, Paris, France); (*E*,*E*)-2,4-decadienal **13** (*E*,*Z*: 5%, **12**), (*E*)-2-undecenal **14** (Fontarom, Cergy Pontoise, France); (*E*)-2-decenal **11** (Bedoukian, Danbury, Connecticut, USA); egg phosphatidylcholine (PC, >99%), egg phosphatidylethanolamine (PE, >99%) and distearoyl phosphatidic acid disodium salt (PA, >99%) (Avanti Polar Lipids, Copenhagen, Denmark); diethyl ether (Et$_2$O), hexane, pentane, silica gel 60, sodium chloride, anhydrous sodium sulfate (Merck, Darmstadt, Germany).

The following reference compounds and deuterated internal standards were synthesized: (*Z*)-1,5-octadien-3-one **3** (*9*), *trans*-4,5-epoxy-(*E*)-2-decenal **15** (*10*), (*E*,*Z*,*Z*)-2,4,7-tridecatrienal **16** (*11*), [5,6-^{2}H$_2$]-hexanal (*d*-1) (*12*), [1-^{2}H$_{1;2}$,2-^{2}H$_{1;1}$]-1-octen-3-one (*d*-2) (*9*), [2,3-^{2}H$_2$]-(*E*)-2-octenal (*d*-4) (*13*), [2,3-^{2}H$_2$]-(*E*)-2-nonenal (*d*-7) (*12*), [3,4-^{2}H$_2$]-(*E*,*E*)-2,4-nonadienal (*d*-9) (*12*), [3,4-^{2}H$_2$]-(*E*,*E*)-2,4-decadienal (*d*-13) (*12*), [4,5-^{2}H$_2$]-*trans*-4,5-epoxy-(*E*)-2-decenal (*d*-15) (*10*), [4,5,7,8-^{2}H$_4$]-(*E*,*Z*,*Z*)-2,4,7-tridecatrienal (*d*-16) (*13*).

Sample Preparation and Cleanup

Model Reactions. The solvent of a phospholipid chloroform-methanol solution (2:1, v/v, 10 mL) containing 1 g of egg PC or egg PE was evaporated with a stream of nitrogen. A phosphate buffer (50 mL, 0.5 M, pH 5.6) was then added and the mixture stirred magnetically to disperse the phospholipid. The sample was heated in a laboratory autoclave (Berghof, Eningen, Germany) for 30 min from room temperature to 145 °C, reaching the final temperature in 12 min with an average heating rate of 10 °C/min (Figure 2). After the reaction, the samples were rapidly cooled to room temperature with ice water.

Isolation of Volatile Compounds. For identification of odorants, the cooled reaction mixture was saturated with salt and the organic compounds continuously extracted with Et$_2$O (diethyl ether; 100 mL, containing 10 mg/L BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisole) as antioxidants) for 15 h using a liquid-liquid extractor. In the quantification experiments, defined amounts of labeled internal standards were added and mixed with the reaction mixtures before solvent extraction. Non-volatile compounds present in the solvent extract were removed by high vacuum transfer

(HVT) at 10^{-3} - 10^{-5} mbar (*14*). The condensates obtained by HVT were combined, dried over anhydrous sodium sulfate, and concentrated to 0.5 mL.

Column Chromatography (CC). Identification of odorants 6, 15, and 16 was achieved by fractionation at about 10 °C using a water-cooled glass column (20 x 1 cm) packed with a slurry of silica gel 60 (*14*). Elution was performed with 25 mL of each of the following pentane/Et_2O mixtures (v/v): 98/2 (fraction F1), 95/5 (fraction F2), 90/10 (fraction F3), 80/20 (fraction F4), and 50/50 (fraction F5). Each fraction was concentrated to 0.2 mL for analytical characterization. Odorants 6 and 16 were enriched in fraction F3, odorant 15 in F5.
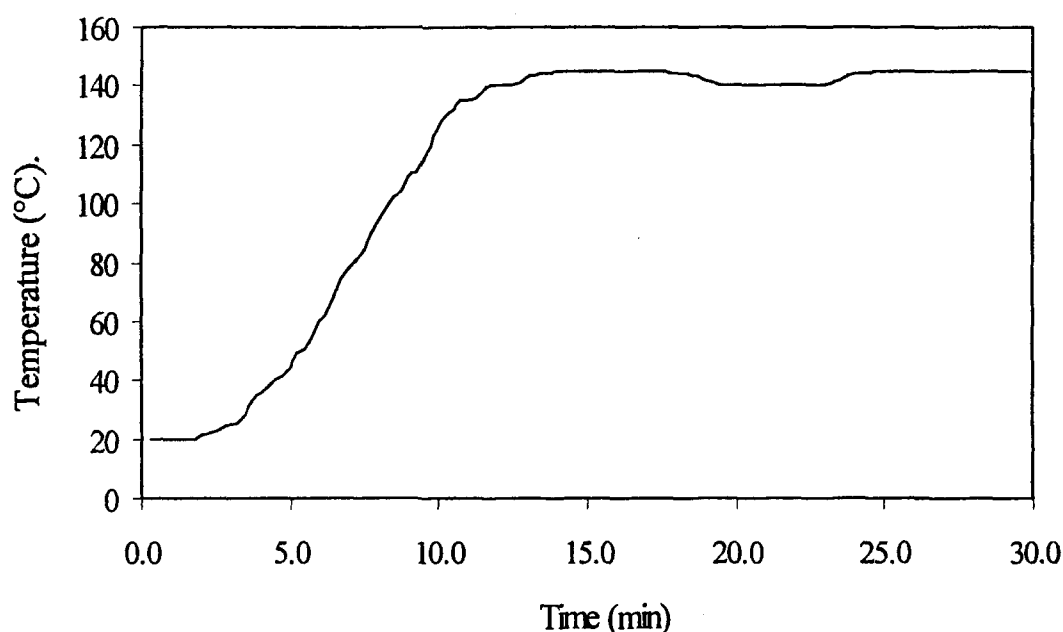

Figure 2. Temperature program for heating the model systems containing aqueous dispersions of phospholipids using a laboratory autoclave.

Chromatographic Techniques

Gas Chromatography-Olfactometry (GC-O). This was performed with a Carlo Erba Mega 2 gas chromatograph (Fisons Instruments, Schlieren, Switzerland) equipped with a cold on-column injector and a flame ionization detector (FID). Helium (80 kPa) was used as carrier gas. Fused silica capillary columns of low (DB-5) and medium (DB-1701) polarity were used, all 30 m x 0.32 mm with a film thickness of 0.25 μm (J&W Scientific, Folsom, CA). A splitter (Gerstel, Mülheim, Germany) was attached to the end of the capillary column to split the effluent 1:1 into the FID and sniffing port, both held at 230 °C, using deactivated and uncoated fused silica capillaries (50 cm x 0.32 mm).

The splitter was flushed with nitrogen (5 mL/min) to accelerate the gas flow. Just prior to the sniffing port, the GC effluent was mixed with humidified air (10 mL/min). Chromatographic conditions were used as described earlier (*12*). Linear retention indices (RI) were calculated according to van den Dool and Kratz (*15*).

Gas Chromatography - Mass Spectrometry (GC-MS). This was performed on a Finnigan MAT 8430 mass spectrometer (Bremen, Germany). Electron ionization (EI) mass spectra were generated at 70 eV. Chemical ionization (MS-CI) was performed at 150 eV with ammonia as the reagent gas. Further details of the GC-MS system and chromatographic conditions have been described previously (*12*). Relative abundances of the ions are given in percent.

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

Results and Discussion

Odor-Active Compounds

The overall odor quality of heated aqueous dispersions of phospholipids was described as fatty, fried, metallic for PC and fishy, green, metallic for PE, which were very close to the overall odor of the corresponding aroma extracts. The GC chromatograms of heated PC and PE were similar and revealed several peaks present in both samples. As shown in Figure 3, the major volatile found in heated PC was compound **13**. The chromatogram of a control PC sample showed only traces of volatile compounds, thus indicating that most of them were generated upon heating.

GC-O was used as a screening method, resulting in sixteen odor-active regions, most of which were common to PC and PE (Figure 3). However, the sensory relevance of these odorants was different for the two samples as indicated by the FD-factors (Table 2). The fatty-fried smelling compound **13** was found to be the most potent odorant in the PC sample (FD= 500), followed by odorants **2, 7, 14, 15** and **16**. The aroma quality of all these odorants represented well the overall aroma of the PC sample (fatty, fried, metallic). On the other hand, odorant **2** was more pronounced in the PE sample, showing the highest FD factor (FD= 200). Further odor-active compounds with lower sensory

relevance were the volatiles **13**, **15** and **16**. The sensory characteristics of these odorants were in good agreement with the overall aroma of the PE sample (fishy, metallic, green).
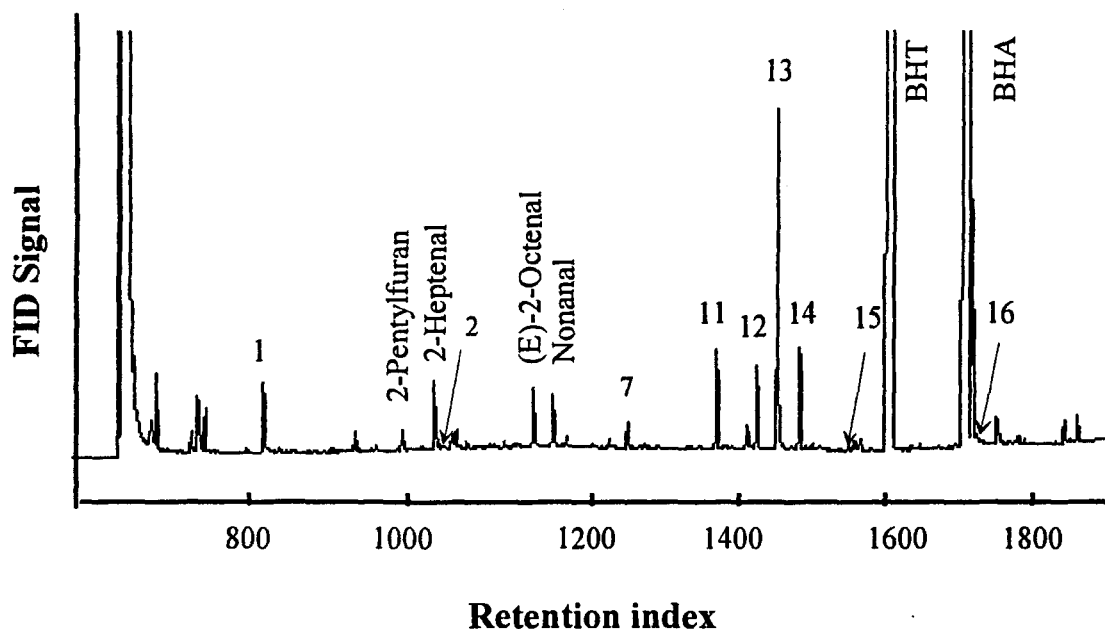

Figure 3. Volatile profile of a thermally treated aqueous dispersion of phosphatidylcholine.

Based on GC-O results, identification work was focused on odorants having high and medium FD-factors. The most intensely smelling odorant in PC was (*E,E*)-2,4-decadienal **13** (FD= 500), followed by (*E*)-2-undecenal **14** and *trans*-4,5-epoxy-(*E*)-2-decenal **15**. 1-Octen-3-one **2** (FD= 200) dominated in the PE sample followed by several odorants with lower FD-factors (Table I). Most of the odorants have been reported as volatile constituents of phospholipids, e.g. egg or soy lecithins (*6*, *7*). However, (*E*)/(*Z*)-2-decenal **10/11**, (*E*)-2-undecenal **14**, and (*E,Z,Z*)-2,4,7-tridecatrienal **16** were identified for the first time in heated phospholipids.

Two odorants with FD= 50 that were more pronounced in the heated PE sample could not be identified due to the low concentration levels, i.e. odorant **5** (sweet) and odorant **8** (fishy). The major odorless volatile compounds generated in heated PC were (*E*)-2-heptenal, nonanal, and 2-pentylfuran (Figure 3), which did not smell at the concentration present in the sample.

Table I. Odorants Identified in the Aroma Extract of Heated Aqueous Dispersions of PC and PE

Odorant	Retention index		Odor quality	FD factor[d]	
	OV1701	SE-54	(GC-O)	PC	PE
Hexanal[a] 1	882	807	Green	1	1
1-Octen-3-one[a] 2	1069	986	Mushroom-like	50	200
(Z)-1,5-Octadien-3-one[b] 3	1087	979	Geranium-like	1	20
(E)-2-Octenal[a] 4	1173	1060	Fatty, soapy	1	1
(Z)-2-Nonenal[a,c] 6	1260	1151	Fatty, soapy	1	1
(E)-2-Nonenal[c] 7	1280	1164	Fatty, soapy	50	5
(E,E)-2,4-Nonadienal[a] 9	1355	1218	Fatty	5	5
(Z)-2-Decenal[a] 10	1365	1254	Soapy	10	1
(E)-2-Decenal[a] 11	1388	1267	Soapy	5	1
(E,Z)-2,4-Decadienal[a] 12	1436	1299	Fatty, soapy	20	5
(E,E)-2,4-Decadienal[a] 13	1461	1312	Fatty, fried	500	50
(E)-2-Undecenal[a] 14	1487	1369	Soapy	100	20
trans-4,5-Epoxy-(E)-2-decenal[a,c] 15	1564	1379	Metallic, green	100	100
(E,Z,Z)-2,4,7-Tridecatrienal[a,c] 16	1740	1589	Egg white-like	20	50

[a] Identification was based on reference compounds by GC-O and GC-MS. [b] Identification was based on reference compounds by GC-O only. The MS signals were too weak for unambiguous identification. [c] MS spectra were obtained after enrichment by column chromatography. [d] The original aroma extract having a flavor dilution (FD) factor of 1 was diluted stepwise with Et_2O until no odor-active region could be detected.

Formation of Selected Odorants from PC and PE

Quantitative Results. Eight compounds were selected for quantification according to their sensory relevance (FD factor) and chemical class to compare the potential of PC and PE in generating odorants when heated in aqueous dispersions (Figure 4), i.e. hexanal 1, 1-octen-3-one 2, (E)-2-octenal 4, (E)-2-noneal 7, (E,E)-2,4-nonadienal 9, (E,E)-2,4-decadienal 13, trans-4,5-epoxy-(E)-2-decenal 15, and (E,Z,Z)-2,4,7-tridecatrienal 16. As shown in Table II, concentrations varied from <1 mg/kg for 7 to about 100 mg/kg for 1 and 13. In the PC sample, 13 and 1 were the two most abundant odorants, followed by 4, 2, and 7. The amount of 1 generated in the PE sample was significantly higher (~100 mg/kg) than that of the other compounds, such as 4 and 13 for example (5-8 mg/kg).

In general, PC was more efficient in generating odorants, except of 1 that was preferably formed in PE. The most remarkable difference was found for the

fatty smelling odorant **13**, the quantity of which was more than 20-fold higher in the PC sample. For the other odorants, the difference was less significant, *i.e.* about 2- to 5-fold. However, PE generated 1.5-fold more of the green smelling odorant **1** compared to PC.
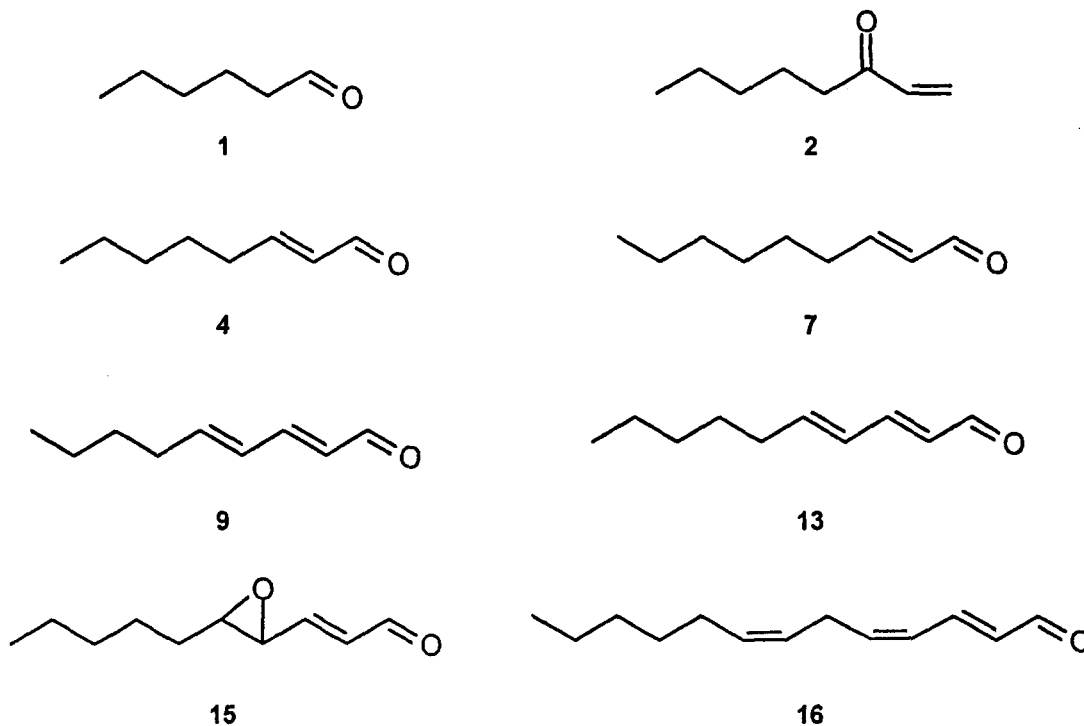

Figure 4. Chemical structure of the odorants quantified in aqueous dispersions of PC and PE.

Table II. Concentrations of Odorants Generated in Heated Aqueous Dispersions of PC, PE, and an Equimolar Mixture of PC and PE[a]

Odorant	*PC*	*PE*	*PC + PE*
1	60.7 ± 5.0	96.9 ± 7.4	88.9 ± 6.2
2	7.4 ± 2.4	1.8 ± 0.1	4.0 ± 0.1
4	20.2 ± 7.5	8.1 ± 0.2	4.2 ± 0.3
7	5.3 ± 1.4	2.5 ± 0.2	1.2 ± 0.1
9	1.2 ± 0.2	0.70 ± 0.04	0.41 ± 0.03
13	108.4 ± 28.4	4.8 ± 0.2	5.2 ± 0.8
15	4.6 ± 1.0	3.4 ± 0.7	1.0 ± 0.1
16	2.8 ± 0.5	1.7 ± 0.6	0.74 ± 0.14

[a] Concentration in mg/kg phospholipid.

Odor Activity Values (OAV). The sensory relevance of the phospholipid-derived carbonyls was estimated on the basis of OAV defined as the ratio of concentration to threshold value *(16)*. As the concentrations of most of the volatile compounds identified in this study were above their odor thresholds, they likely contribute to the overall aroma of heated PC and PE. The OAV values were calculated by dividing the concentration of an odorant by its orthonasal threshold determined in oil *(14)*.

As shown in Figure 5, odorant **15** (metallic) showed the highest OAV in both PC and PE. In PC, odorant **13** (fatty) and **2** (mushroom-like) were further important volatile compounds. In PE, odorant **1** (green) showed high OAV in addition to **2**, whereas the role of **13** was less pronounced. These odorants can be seen as the character impact compounds of heated phospholipids imparting metallic and fatty notes, which is in good agreement with the overall aroma of the heated samples. The fatty, fried, metallic note of heated PC is mainly imparted by odorants **13** and **15**, whereas odorants **1** and **2**, were additionally important for the fishy, green, metallic note of PE.
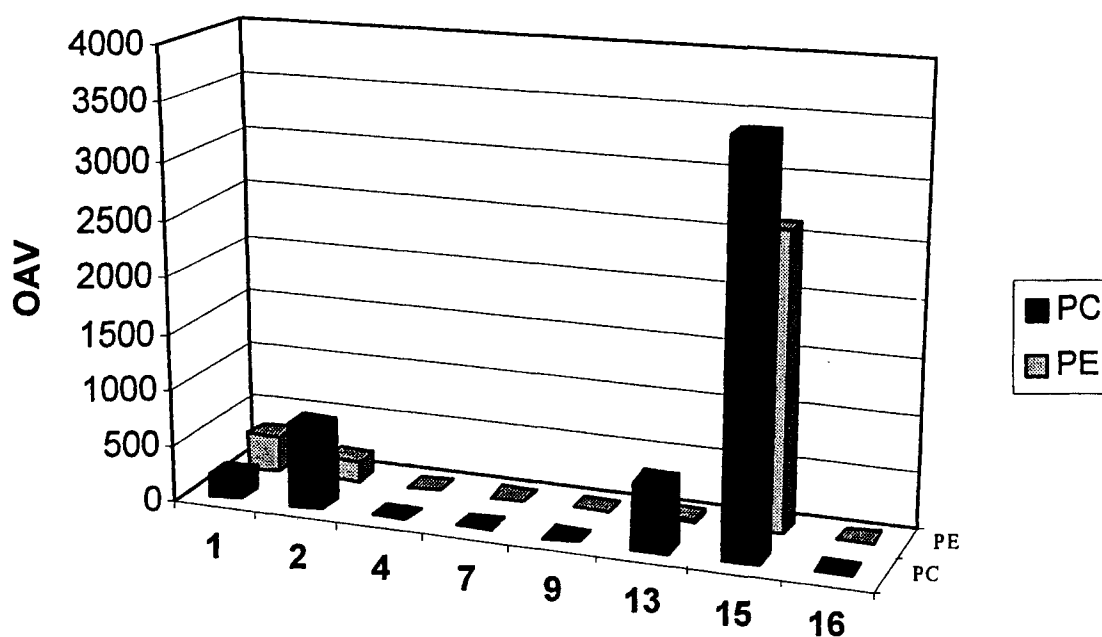

Figure 5. Odor Activity Values (OAV) of Odorants Generated in Heated Aqueous Dispersions of PC and PE.

The significant contribution of **15** is due to its extremely low odor threshold of 1.3 μg/L oil *(17)*. Therefore, despite the low amounts found (Table 2), its sensory contribution is pronounced. On the contrary, the sensory relevance of **13** is mainly due to its high concentration, in particular in heated PC, in combination with a moderately low threshold value of 180 μg/L oil *(18)*.

Precursors of Odorants. The compounds reported in this study are well-known lipid-degradation products of unsaturated fatty acids, such as oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), and arachidonic acid (C20:4) for example. The formation mechanisms have been described elsewhere (*19, 20*). Hexanal 1 can be generated from various fatty acids, such as C18:2 and C20:4. In addition, 1 is also known as a secondary autoxidation product of 2,4-decadienal 13 (*21, 22*). Linoleic and arachidonic acids are also direct precursors of odorant 13. The formation of the newly identified (*E,Z,Z*)-2,4,7-tridecatrienal 16 in phospholipids can be explained by β-cleavage of the corresponding 8-hydroperoxy-5,9,11,14-eicosatetraenoic acid (*13*).

However, there is apparently no direct relationship between the amounts of 13 and 16 for example and the concentration of ω-6 fatty acids (*e.g.* C18:2 and C20:4) in the phospholipid, from which these aldehydes are formed. According to literature data (*4, 14*), the content of C20:4 in egg PE is several times higher than that in egg PC, while more 16 was generated in PC than in PE (Table III), thus indicating that the fatty acid composition is unlikely to be the only parameter explaining the formation of odorants from aqueous dispersions of phospholipids.

Table III. Fatty Acid Composition of Egg Phosphatidylcholine (PC) and Egg Phosphatidylethanolamine (PE)[a]

Fatty acid	*Fatty acid composition (% total fatty acids)*	
	egg PC	*egg PE*
C18:1 (n-9)	26.5	18.2
C18:2 (n-6)	16.7	13.2
C20:4 (n-6)	4.2	12.2
Σ Monounsaturated (1 DB)	29.3	17.7
Σ Diunsaturated (2 DB)	16.9	13.4
Σ Polyunsaturated (≥3 DB)	7.5	18.8

[a] Data were taken from the literature (*14*). DB= double bond.

Factors Affecting the Degradation of Phospholipids

Model Reactions Containing PC and PE. Quantitative characterization of an aqueous dispersion of equimolar amounts of PC and PE revealed hexanal 1 (~90 mg/kg) as the major odor-active constituent (Table II). The amounts of the remaining odorants varied from 0.4 mg/kg for (*E,E*)-2,4-nonadienal 9 to ~5 mg/kg for (*E,E*)-2,4-decadienal 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 (Table 2). Compared to the quantitative data obtained with pure phospholipids, PC/PE 1:1 was almost identical with pure PE. The most intriguing difference, however, was the low amount of **13** found in PC/PE 1:1, while it was the major compound in pure PC.

Again, the fatty acid composition of PC and PE 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** (~110 mg/kg) were found in the PC sample containing 20.9 % precursors. The amounts of **13** dropped to ~5 mg/kg in PE and PC/PE 1:1 containing of 25.4 % and 23.2 % precursors, respectively. These data confirm the hypothesis that several parameters affect the formation of carbonyls from heated aqueous dispersions of phospholipids.

Fatty Acid Composition. Surprisingly, the concentration of **16** is negatively correlated with the C20:4 content when comparing samples of pure PC and PE/PC mixtures (Figure 6). The highest amounts of **16** were found in pure PC (sample A), even though it contained the lowest level of C20:4 (4.2 %, Table III). Addition of PE to PC reduced the amounts of **16** (samples B-E). However, increasing PE in the PE/PC mixtures, and thus increasing the content of C20:4, led to more **16** generated. Finally, the C20:4 content of PE was about 3 times that of PC, however only about half the amount of **16** was formed (sample F).
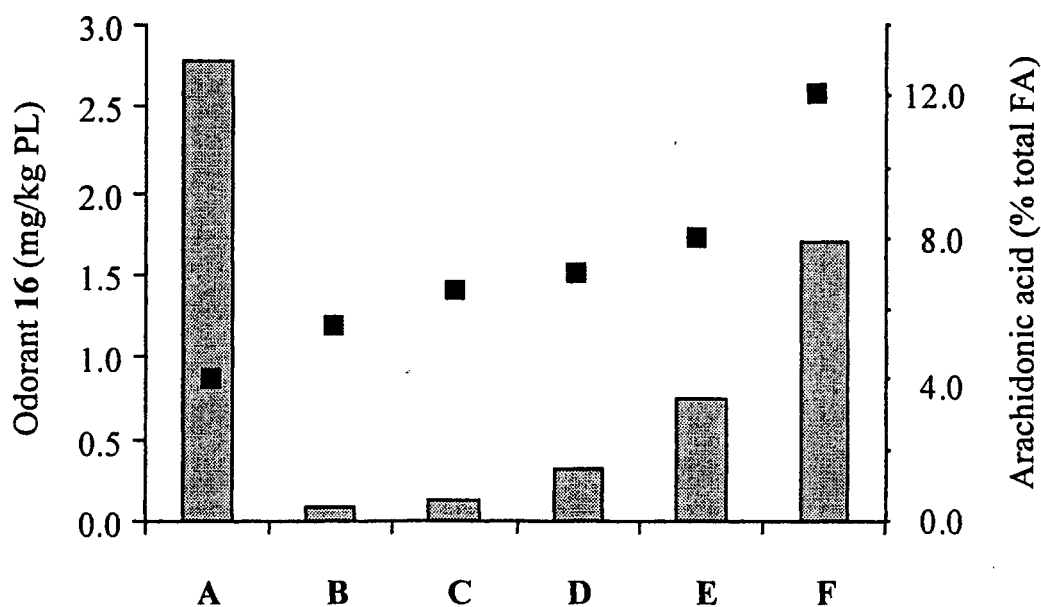

Figure 6. Concentration of (E,Z,Z)-2,4,7-tridecatrienal *16* (bars, left vertical axis) generated upon heating of aqueous dispersions of phosphatidylcholine (PC), phosphatidylethanolamine (PE), and mixtures of PC and PE in various molar ratios along with the arachidonic acid content (■, right vertical axis) of the phospholipid samples. A, PC; B, PC+PE (4:1); C, PC+PE (2:1); D, PC+PE (3:2); E, PC+PE (1:1); F, PE.

As shown in Figure 7, significantly (>20 fold) higher amounts of **13** were generated from pure PC (sample A) 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 with the rising level of PE, which is particularly rich in C20:4 (Table 3). 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.
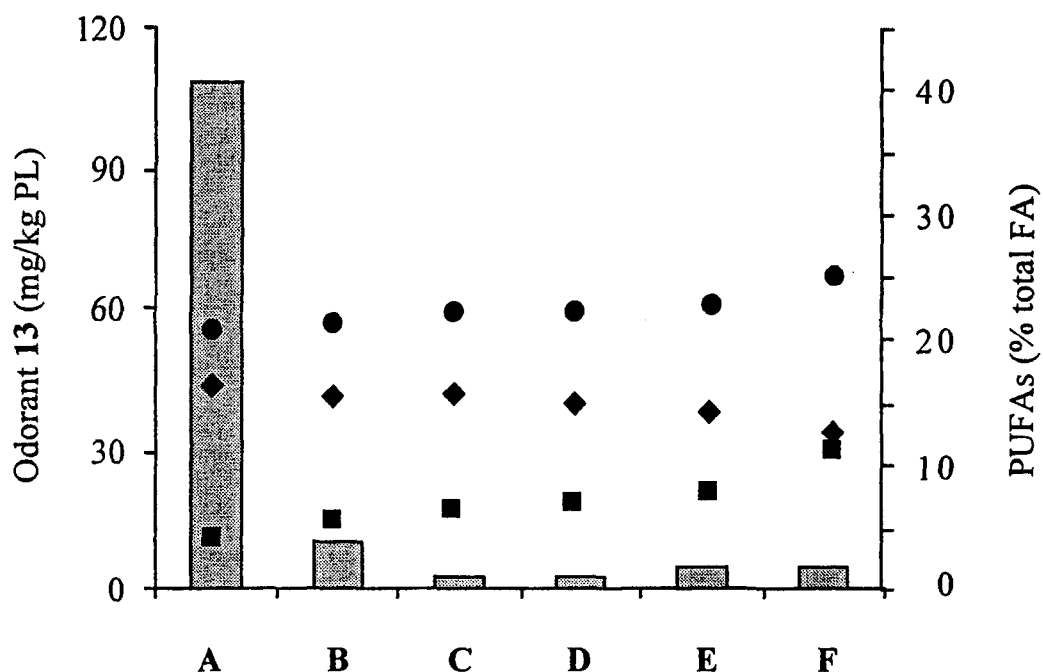

Figure 7. Concentration of (E,E)-2,4-decadienal 13 (bars, left vertical axis) generated upon heating of aqueous dispersions of phosphatidylcholine (PC), phosphatidylethanolamine (PE), and mixtures of PC and PE in various molar ratios along with the content of linoleic acid (◆), arachidonic acid (■), 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); F, PE.

The total unsaturated fatty acid contents of PC and PE are very close, *i.e.* about 52-54% (*14*). 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 6, a positive correlation between the concentration of 16 and 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 speculated that reactions between aldehydes and the free amino group of PE were responsible for this phenomenon.

Polar Moiety. 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 (23, 24). As a result, such secondary reactions could contribute to degradation of odorants formed and, thus, affect the final levels of odorants.

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 reacting PC and distearoyl phosphatidic acid (PA) in the molar ratio 4:1 and determining the concentration of odorant 13. As shown in Table IV, not only PC/PE 4:1 affected the formation of 13 but also the presence of PA, *i.e.* the concentration of 13 dropped from ~110 mg/kg for PC to 10.1 and 25.4 mg/kg for the 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.

Table IV. Concentration of (*E,E*)-2,4-Decadienal (13) Generated on Heating Aqueous Dispersions of PC and Mixtures of PC and PA or PE

Phospholipid (molar ratio)	Concentration 13 (mg/kg)[a]	Precursor content (% total fatty acids)		
		C18:2[b]	C20:4[c]	Σ
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

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

Molecular Organization. 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 and reversed hexagonal phases (25, 26). 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. This was the most striking difference between the PC sample and those containing PE, which may partially explain the differences found in the chemical composition of the lipid-derived odorants.

The difference in the appearance of the PC sample compared to those containing PE is the consequence of their binary phase behavior. According to the phase diagrams (Figure 8) (27), 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, 25-145 °C). The liquid crystalline lamellar phase is readily dispersible in water forming stable vesicles or liposomes with the so-called bilayer as basic self-assembly structure, in which the surfactant film has a zero curvature (25). On the contrary, PE molecules form a reverse hexagonal structure, in which the surfactant film is curved towards water. It is not possible to homogeneously disperse molecules organized in a reversed hexagonal phase (28), since this type of self-assembly structure is much more lipophilic, 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 non-dispersible lumps.
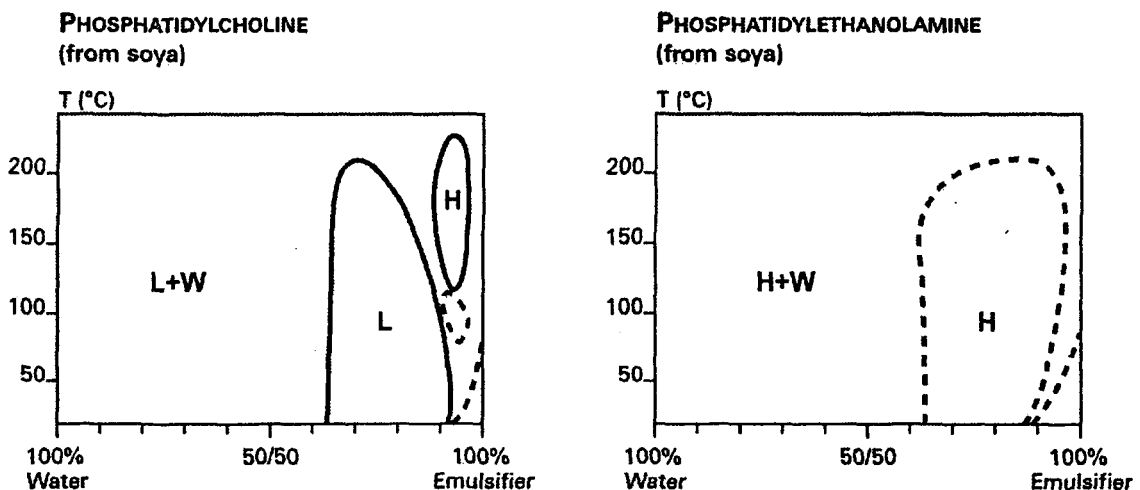

Figure 8. Phase diagrams of PC and PE. L, lamellar phase; L+W, lamellar phase and water; H, reversed hexagonal phase; H+W, reversed hexagonal phase and water. Adapted from ref. 28.

The ternary phase diagram of PC, PE and water obtained at room temperature is shown in Figure 9 (25), 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 8). The reversed hexagonal phase adopted by pure PE (Figure 6) 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 both the formation of **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 remains unclear.
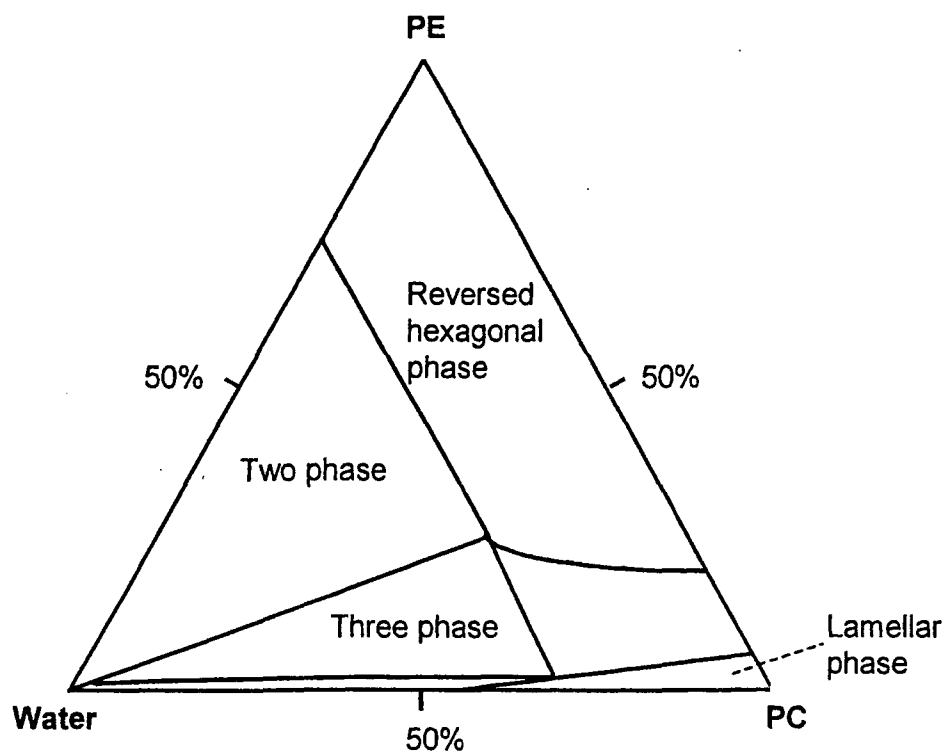

Figure 9. Ternary phase diagram of phosphatidylcholine (PC), phosphatidylethanolamine (PE), and water at 20 °C. Adapted from ref. 25.

In conclusion, our 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. Additional work is required to elucidate the exact mechanisms explaining the differences observed in thermally-induced odorant formation in phospholipid self-assembly structures.

References

1. Gunstone, F. D. In *Structured and Modified Lipids*; Gunstone, F. D., Ed.; Marcel Dekker: New York, 2001; pp 241-250.
2. Cherry, J. P.; Kramer, W. H. In *Lecithins: Sources, Manufacture, and Uses*; Szuhaj, B. F.; Ed.; AOCS Press: Champaign, Il, 1989; pp 16-31.
3. Kuksis, A. In *Lecithins: Sources, Manufacture, and Uses*; Szuhaj, B. F., Ed.; AOCS Press: Champaign, IL, 1989; pp 32-71.
4. Farmer, L. J.; Mottram, D. S. *J. Sci. Food Agric.* **1990**, *53*, 505-525.
5. Mottram, D. S.; Edwards, R. A. *J. Sci. Food Agric.* **1983**, *34*, 517-522.
6. Farmer, L. J.; Mottram, D. S. *J. Sci. Food Agric.* **1992**, *60*, 489-497.
7. Stephan, A.; Steinhart, H. *J. Agric. Food Chem.* **1999**, *47*, 2854-2859.
8. Stephan, A.; Steinhart, H. *J. Agric. Food Chem.* **1999**, *47*, 4357-4364.
9. Lin, J.; Welti, H. D.; Arce Vera, F.; Fay, L. B.; Blank, I. *J. Agric. Food Chem.* **1999**, *47*, 2822-2829.
10. Lin, J.; Fay, L. B.; Welti, D. H.; Blank, I. *Lipids* **1999**, *34*, 1117-1126.
11. Blank, I.; Lin, J.; Arce Vera, F.; Welti, H. D.; Fay, L. B. *J. Agric. Food Chem.* **2001**, *49*, 2959-2965.
12. Lin, J.; Welti, D. H.; Arce Vera, F.; Fay, L. B.; Blank, I. *J. Agric. Food Chem.* **1999**, *47*, 2813-2821.
13. Lin, J.; Fay, L. B.; Welti, H. D.; Blank, I. *Lipids* **2001**, *36*, 749-756.
14. Lin, J.; Blank, I. *J. Agric. Food Chem.* **2003**, *51*, 4364-4369.
15. van den Dool, H.; Kratz, P. *J. Chromatogr.* **1963**, *11*, 463-471.
16. Grosch, W. *Flav. Fragr. J.* **1994**, *9*, 147-158.
17. Guth, H.; Grosch, W. *Lebensm. Wiss. Technol.* **1990**, *23*, 513-522.
18. Hall, G.; Anderson, J. *Lebensm. Wiss. Technol.* **1983**, *16*, 354-361.
19. Grosch, W. In *Autoxidation of Unsaturated Lipids*; Chan, H. W.-S., Ed.; Academic Press: London, 1987; pp. 95-139.
20. Frankel, E. N.; Neff, W. E.; Selke, E. *Lipids* **1981**, *16*, 279-285.
21. Matthews, R. F.; Scanlan, R.A.; Libbey, L. M. *J. Am. Oil Chem. Soc.* **1971**, *48*, 745-747.
22. Schieberle, P.; and Grosch, W. *J. Am. Oil Chem. Soc.* **1981**, *58*, 602-607.
23. Pokorny, J.; Tai, P. T.; Janicek, G. *Z. Lebensm. Unters. Forsch.* **1973**, *153*, 322-325.
24. Tai, P. T.; Pokorny, J.; Janicek, G. *Z. Lebensm. Unters. Forsch.* **1974**, *156*, 257-262.
25. Bergenstahl, B. A. In *Food Polymers, Gels and Colloids*; Dickinson, E., Ed.; Special Publication No. 82, The Royal Society of Chemistry at Norwich: Cambridge, 1991; pp 123-131.
26. Hawker, N. *Food Tech. Europe* **1994**, *1(5)*, 44-46.
27. Bergenstahl, B. A.; Claesson, P. M. In *Food Emulsions*; Marsson, K., Friberg, S. E., Eds.; Marcel Dekker: New York, NY, 1990; pp 41-96.
28. Larsson, K. In *Lipids - Molecular Organization, Physical Functions and Technical Applications*. The Oily Press Ltd.: Dundee, 1994; pp 107-113.