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Odorants Generated by Thermally Induced Degradation of Phospholipids

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The qualitative and quantitative aroma composition of heated aqueous dispersions of egg phosphatidylcholine (PC) and egg phosphatidylethanolamine (PE) were characterized by aroma extract dilution analysis and isotope dilution assay. On the basis of FD-factors and odor activity values, *trans*-4,5-epoxy-(*E*)-2-decenal was found to be the most potent odorant 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 major odor-active compound in the PE sample. (*E*,*Z*,*Z*)-2,4,7-Tridecatrienal was identified for the first time as an odor-active volatile constituent of heated phospholipids exhibiting a characteristic egg white-like note. Further odorants first reported in thermally treated phospholipids were (*Z*)-2-decenal, (*E*)-2-decenal, and (*E*)-2-undecenal. Differences in the fatty acid composition of PC and PE can only partially explain the quantitative results found in this study, thus suggesting that further parameters may influence the formation of carbonyls from heated aqueous dispersions of phospholipids.

KEYWORDS: Phospholipids; phosphatidylcholine; phosphatidylethanolamine; lecithin; cephalin; key odorants; aroma extract dilution analysis; flavor dilution factor; isotope dilution assay

INTRODUCTION

Phospholipids are essential constituents of cell membranes and widely occur in nature, however, in fairly low amounts compared to triglycerides. Vegetable matter usually contains 0.3−2.5% (dry weight) phospholipids with higher levels present in animal sources (egg 14%, brain 6%, milk 2%) (*1*). 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) and phosphatidylethanolamine (PE, cephalin) as major constituents of the phospholipid fraction (**Figure 1**) (*1*).

Phospholipids are amphiphilic lipids containing a lipophilic and a hydrophilic portion. They are rich in polyunsaturated fatty acids (PUFAs), especially linoleic acid (C18:2), arachidonic acid (C20:4), and other highly unsaturated fatty acids (C22:5, C22:6) (*4*). Due to their emulsifying capacity and water dispersability, phospholipids are the natural emulsifiers in fluid dairy products. Phospholipids are also frequently used in the food industry as emulsifiers and stabilizers, for example, in chocolate production, baked products, shortenings, margarine, instant products, mayonnaise, and low-fat products, as well as in animal feed due to their dietary value based on essential fatty acids (*1*).

Phospholipids have been shown to contribute to the species-specific aroma of cooked meat (*5*). Removing triglycerides only had a small effect on the aroma of cooked beef meat. However, when both triglycerides and phospholipids were removed, a remarkable difference in aroma was observed, i.e. less meaty, indicating that structural phospholipids play a significant role in meat aroma specificity. Phospholipids contribute through lipid-derived odorants generated by thermally induced lipid oxidation and interaction of lipid intermediates with the Maillard reaction, both of which modify the overall aroma of cooked meat (*4*).

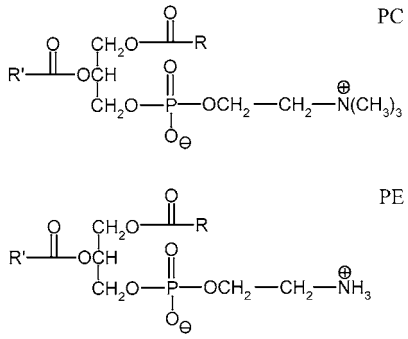

Figure 1. Chemical structures of phosphatidylcholine (PC, lecithin) and phosphatidylethanolamine (PE, cephalin). R represents the fatty acid chain.

Various volatile compounds have been identified in phospholipids, depending on the starting material, preparation conditions, and analytical techniques used. The major volatile compounds found in heated phospholipids were hexanal, nona-

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nal, 2-octenal, 2-decenal, (*E*,*E*)-2,4-decadienal, 1-octen-3-ol, 2-pentylfuran, and others (*6*). The authors also noted significant differences 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. However, no data were reported on the sensory relevance of the volatile degradation products.

Recently, application of aroma extract dilution analysis (*7*) to commercial soybean lecithin led to the identification of more than 60 odor-active volatile constituents (*8*). Most of the potent odorants were lipid degradation or Maillard reaction products. Short-chain fatty acids, 2-heptanone, hexanal, and short-chain branched Strecker aldehydes were the major volatiles found in soybean lecithin. Some of these volatiles in combination with odorants occurring at low concentrations, such as (*E*,*E*)-2,4-decadienal, (*E*)-2-nonenal, and 1-octen-3-one, showed high sensory relevance (*9*).

In this paper we report on the identification and the quantification of the most potent odorants generated in aqueous dispersions of egg PC and egg PE.

MATERIALS AND METHODS

Materials. The following chemicals were commercially available: hexanal (**1**), (*E*)-2-octenal (**4**) (containing traces of the *Z*-isomer), (*E*,*E*)-2,4-nonadienal (**9**) (*E*,*Z*: 5%), 2-(1,1-dimethylethyl)-4-methoxy-phenol (BHA), 2,6-bis(1,1-dimethylethyl)-4-methylphenol (BHT), neutral aluminum oxide (alumina), and methanol (Aldrich/Fluka, Buchs, Switzerland); 1-octen-3-one (**2**) (Oxford, Brackley, UK); (*E*)-2-nonenal (**7**) (Agipal, Paris, France); (*E*,*E*)-2,4-decadienal (**13**) (*E*,*Z*: 5%, **12**) and (*E*)-2-undecenal (**14**) (Fontarom, Cergy Pontoise, France); (*E*)-2-decenal (**11**) (Bedoukian, Danbury, Connecticut, USA); egg phosphatidylcholine (PC, >99%) and egg phosphatidylethanolamine (PE, >99%) (Avanti Polar Lipids, Copenhagen, Denmark); and diethyl ether (Et_2O), hexane, pentane, silica gel 60, sodium chloride (NaCl), anhydrous sodium sulfate, disodium hydrogenphosphate, and sodium dihydrogenphosphate (Merck, Darmstadt, Germany). The following reference compounds were synthesized: (*Z*)-1,5-octadien-3-one (**3**) (*10*), *trans*-4,5-epoxy-(*E*)-2-decenal (**15**) (*11*), (*E*,*Z*,*Z*)-2,4,7-tridecatrienal (**16**) (*12*). (*Z*)-2-Nonenal (**6**) was a gift from Prof. W. Grosch (Technical University of Munich, Germany). The synthesis of the labeled internal standards was reported elsewhere: [5,6-$^2H_2$]-hexanal (***d*-1**) (*13*), [1-$^2H_{1;2}$,2-$^2H_{1;1}$]-1-octen-3-one (***d*-2**) (*10*), [2,3-$^2H_2$]-(*E*)-2-octenal (***d*-4**) (*14*), [2,3-$^2H_2$]-(*E*)-2-nonenal (***d*-7**) (*13*), [3,4-$^2H_2$]-(*E*,*E*)-2,4-nonadienal (***d*-9**) (*13*), [3,4-$^2H_2$]-(*E*,*E*)-2,4-decadienal (***d*-13**) (*13*), [4,5-$^2H_2$]-*trans*-4,5-epoxy-(*E*)-2-decenal (***d*-15**) (*11*), and [4,5,7,8-$^2H_4$]-(*E*,*Z*,*Z*)-2,4,7-tridecatrienal (***d*-16**) (*14*).

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. 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 NaCl and the organic compounds continuously extracted with Et_2O (100 mL, containing 10 mg/L of BHT and BHA 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. Nonvolatile compounds present in the solvent extract were removed by high-vacuum transfer at 10^{-3}−10^{-5} mbar (*15*). The solvent extract was introduced dropwise into the vacuum system via a dropping funnel. Volatile compounds were transferred into two glass traps cooled with liquid nitrogen. The procedure was repeated by adding Et_2O (30 mL) to the residue. The condensates trapped were applied to HVT once again to completely remove nonvolatile lipids. The condensates were combined, dried over anhydrous sodium sulfate, and then concentrated to 2 mL on a Vigreux column (50 × 1 cm) and finally to 0.5 mL with a microdistillation apparatus (*16*).

Table 1. Parameters Used in the Quantification of Phospholipid-Derived Odorants by Isotope Dilution Assay

analyte	internal standard (IS)	selected ions (*m*/*z*)[a] analyte	IS	linearity[b] (r^2)	linear range[b] (ratio analyte/IS)
4	[2,3-$^2H_2$]-(*E*)-2-octenal (***d*-4**)	127	129	0.998	0.05–9.0
7	[2,3-$^2H_2$]-(*E*)-2-nonenal (***d*-7**)	141	143	0.998	0.05–19.0
9	[3,4-$^2H_2$]-(*E*,*E*)-2,4-nonadienal (***d*-9**)	139	141	0.999	0.05–19.0

[a] Chemical ionization was applied, using isobutane as the reagent gas. The ion pairs measured generally were the species $[M + H]^+$. IS = internal standard. [b] Linearity and linear range were obtained from the calibration graphs with use of selected ions (*14*).

Column Chromatography (CC). In the identification of odorants **6**, **15**, and **16**, fractionation and enrichment were achieved by CC. Volatiles isolated from 5 g of heated phospholipid were fractionated at about 10 °C with use of a water-cooled glass column (20 × 1 cm) packed with a slurry of silica gel 60, which was treated according to Esterbauer (*17*). 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 with a Vigreux column and finally with a microdistillation apparatus for GC-O and GC-MS analysis. Odorants **6** and **16** were enriched in fraction F3, odorant **15** in F5.

Gas Chromatography−Olfactometry (GC-O). GC-O was performed with a Carlo Erba Mega 2 gas chromatograph (Fisons Instruments, via Brechbühler, Schlieren, Switzerland) equipped with a cold on-column injector and a flame ionization detector (FID). Helium (80 kPa) was used as the carrier gas. Fused silica capillary columns of low (DB-5), medium (DB-1701), and high (DB-FFAP) polarity were used, all 30 m × 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 × 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 (*13*). Linear retention indices (RI) were calculated according to van den Dool and Kratz (*18*).

Aroma Extract Dilution Analysis (AEDA). The sensory significance of each odorant was evaluated by AEDA and expressed as the flavor dilution (FD) factor (*7, 19, 20*). The FD factors were determined as follows: the original extract was stepwise diluted with Et_2O to obtain dilution factors of 5−500 until no odor-active region could be detected by GC-O, which was performed on the DB-1701 capillary column by injecting 0.5 μL of sample. The FD factors were obtained by two trained assessors injecting the sample twice.

Gas Chromatography−Mass Spectrometry (GC-MS). GC-MS 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 (*13*). 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 (*14*). 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 used for quantification of **1**, **2**, **13**, **15**, and **16** have been reported (*14*), those of **4**, **7**, and **9** are listed in **Table 1**.

Table 2. Identification of Odorants in the Aroma Extract of Heated Aqueous Dispersions of Phosphatidylcholine (PC) and Phosphatidylethanolamine (PE)[a]

no.	compd	retention index (DB-1701)	retention index (DB-5)	odor quality (GC-O on DB-1701)	FD-factor (aroma intensity)[e] PC	FD-factor (aroma intensity)[e] PE
1	hexanal[b]	882	807	green	1 (1)	1 (1)
2	1-octen-3-one[b]	1069	986	mushroom-like, metallic	50 (2)	200 (2)
3	(*Z*)-1,5-octadien-3-one[c]	1087	979	geranium-like, metallic	1 (1–2)	20 (2)
4	(*E*)-2-octenal[b]	1173	1060	fatty, soapy	1 (1)	1 (1)
5	unknown	1212		sweet, fresh note	10 (1)	50 (1)
6	(*Z*)-2-nonenal[b,d]	1260	1151	fatty, soapy	1 (1–2)	1 (1)
7	(*E*)-2-nonenal[b]	1280	1164	fatty, soapy	50 (1)	5 (1)
8	unknown	1293		fishy, creamy		50 (1)
9	(*E*,*E*)-2,4-nonadienal[b]	1355	1218	fatty	5 (1–2)	5 (1–2)
10	(*Z*)-2-decenal[b]	1365	1254	soapy	10 (1)	1 (1)
11	(*E*)-2-decenal[b]	1388	1267	soapy	5 (1–2)	1 (1)
12	(*E*,*Z*)-2,4-decadienal[b]	1436	1299	fatty, soapy	20 (2)	5 (1)
13	(*E*,*E*)-2,4-decadienal[b]	1461	1312	fatty, fried	500 (3)	50 (2)
14	(*E*)-2-undecenal[b]	1487	1369	soapy	100 (2)	20 (2)
15	*trans*-4,5-epoxy-(*E*)-2-decenal[b,d]	1564	1379	metallic, green	100 (3)	100 (3)
16	(*E*,*Z*,*Z*)-2,4,7-tridecatrienal[b,d]	1740	1589	egg white-like	20 (2–3)	50 (2–3)

[a] Egg phospholipid (1 g, PC or PE) dispersed in phosphate buffer (50 mL, 0.5 M, pH 5.6) was reacted in an autoclave for 30 min from 25 to 145 °C. [b] Identification was based on reference compounds (GC-O on two capillary columns) and GC-MS. [c] Identification was based on reference compounds only (GC-O on two capillary columns). The MS signals were too weak for unambiguous identification. [d] MS spectra were obtained after enrichment by column chromatography: odorants **6** and **16** were enriched in F3, odorant **15** in F5. [e] Aroma intensity was perceived at the sniffing port. The intensity was scaled from 1 (weak) to 3 (high). The original aroma extract (FD = 1) was diluted stepwise with Et_2O, i.e., 1:5, 1:10, 1:20, 1:50, 1:100, 1:200, 1:500, 1:1000, until no odor-active region could be detected.

Isotope Dilution Assay (IDA). Defined amounts of labeled internal standard (IS) in solution were added to the reacted samples prior to isolation of volatiles by liquid−liquid extraction and purification by HVT. Calibration curves were obtained by using mixtures of defined amounts of analyte and labeled IS (*21*). As recently described for **15** and ***d*-15** (*11*), nine mixtures composed of the analyte and the corresponding labeled standard were used, i.e. from 0.5 + 9.5, 1 + 9, 2 + 8, 3 + 7, 5 + 5 to 7 + 3, 8 + 2, 9 + 1, 9.5 + 0.5. Calibration curves were established for the seven odorants quantified in this study. The parameters used in the IDA of the odorants are summarized in the literature for **1**, **2**, **13**, **15**, and **16** (*14*) and in **Table 1** for **4**, **7**, and **9**. Samples for establishing the calibration curves and for quantification were injected twice.

RESULTS

Qualitative Aroma Composition. The aroma extract of heated aqueous dispersions of phospholipids revealed the characteristic aroma notes of the thermally treated sample, i.e., fatty, fried, and metallic for PC and fishy, green, and metallic for PE. GC-O of the aroma extracts, used as a screening method for odorants, resulted in 16 odor-active regions (**Table 2**), most of which were common to PC and PE. However, the sensory relevance of these odorants was different for the two samples as indicated by the odor intensities perceived at the sniffing port and the FD factors obtained by AEDA. The fatty-fried smelling odorant **13** was found to be the most odor-active compound in the PC sample. Odorants **2**, **7**, **14**, **15**, and **16** were revealed as additional impact odorants. The aroma quality of all these odorants represented well the overall aroma of the PC sample described as fatty, fried, and metallic. On the other hand, odorant **2** was more pronounced in the PE sample, showing the highest FD factor. Further odor-active compounds with lower sensory relevance were the volatiles **5**, **8**, **13**, **15**, and **16**. The sensory characteristics of these odorants were in good agreement with the overall aroma of the PE sample described as fishy, metallic, and green. On the basis of these results, identification work was focused on odorants having high and medium FD factors.

Fourteen of the sixteen odorants detected by GC-O were identified as shown in **Table 2**. In general, identification was achieved on the basis of GC retention indices on two different capillary columns and sensorial characteristics as well as by comparison of these data with those obtained for the reference compounds (*20*). The identities of odorants **1**, **2**, **4**, **6**, **7**, and **9**−**16** were further confirmed by GC-MS. The concentration of odorant **3** was too low to obtain unambiguous mass spectra. Its identity, however, was supported by co-injection with the corresponding reference compound.

The most intensely smelling odorant in PC was (*E*,*E*)-2,4-decadienal (**13**) with FD = 500, followed by (*E*)-2-undecenal (**14**) and *trans*-4,5-epoxy-(*E*)-2-decenal (**15**). 1-Octen-3-one (**2**) with FD = 200 dominated in the heated PE sample followed by several odorants with lower FD factors (**Table 2**). Most of the odorants have been reported as volatile constituents of phospholipids, e.g. egg or soya lecithins (*6*, *8*). 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.

Because phospholipids are readily oxidized, a control experiment without heat treatment of egg PC was performed. The results shown in **Figure 2A** indicate that some of the odor-active lipid oxidation products were already present in nonheated egg PC, i.e. hexanal (**1**), 1-octen-3-one (**2**), (*E*)-2-nonenal (**7**), (*E*,*E*)-2,4-decadienal (**13**), (*E*)-2-undencenal (**14**), *trans*-4,5-epoxy-(*E*)-2-decenal (**15**), and (*E*,*Z*,*Z*)-2,4,7-tridecatrienal (**16**). However, odor intensities and amounts were significantly lower in the control sample. These compounds might have been formed during production and storage of the phospholipid or during sample preparation. The majority of the volatiles, including odor-active compounds, detected in heated PC were generated during thermal treatment (**Figure 2B**). The major volatiles found in heated PC were the odorants (*E*,*E*)-2,4-decadienal (**13**) followed by hexanal (**1**), (*E*)-2-undecenal (**14**), (*E*)-2-decenal (**11**), (*E*,*Z*)-2,4-decadienal (**12**), (*E*)-2-octenal (**4**), and (*E*)-2-nonenal (**7**), as well as (*E*)-2-heptenal, nonanal, and 2-pentylfuran, which did not smell at the concentration present in the sample.

Quantitative Aroma Composition. 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 disper-

Table 3. Concentrations, Odor Thresholds (OT), and Odor Activity Values (OAV)[a] of Odorants Generated in Heated Aqueous Dispersions of Egg Phosphatidylcholine (PC) and Egg Phosphatidylethanolamine (PE)

odorant	concn (mg/kg phospholipid)		OT	OAV (air; $\times 10^6$)		OT	OAV (oil)	
	PC	PE	(ng/L of air)[b]	PC	PE	(μg/L of oil)[c]	PC	PE
1	60.7 ± 5.0	96.9 ± 7.4	43	1.4	2.3	300	202	323
2	7.4 ± 2.4	1.8 ± 0.1	0.07	106	26	10	740	180
4	20.2 ± 7.5	8.1 ± 0.2	47	0.4	0.2	7000	2.9	1.2
7	5.3 ± 1.4	2.5 ± 0.2	0.1	53	25	900	5.9	2.8
9	1.2 ± 0.2	0.70 ± 0.04	0.4	3.0	1.8	2500	0.5	0.3
13	108.4 ± 28.4	4.8 ± 0.2	0.1	1085	48	180	602	27
15	4.6 ± 1.0	3.4 ± 0.7	0.0015	3070	2270	1.3	3540	2615
16	2.8 ± 0.5	1.7 ± 0.6	0.07	40	24	180	15.6	9.4

[a] Odor activity values were obtained by calculating the ratio of concentration to odor threshold determined in air and oil. [b] Orthonasal thresholds in air were from the literature: **1** (*21*), **2** (*23*), **4** (*21*), **7** (*24*), **9** (*21*), **13** (*27*), **15** (*28*), and **16** (*14*). [c] Orthonasal thresholds in oil were from the literature: **1** (*22*), **2** (*22*), **4** (*27*), **7** (*22*), **9** (*28*), **13** (*22*), **15** (*22*), and **16** (*14*).

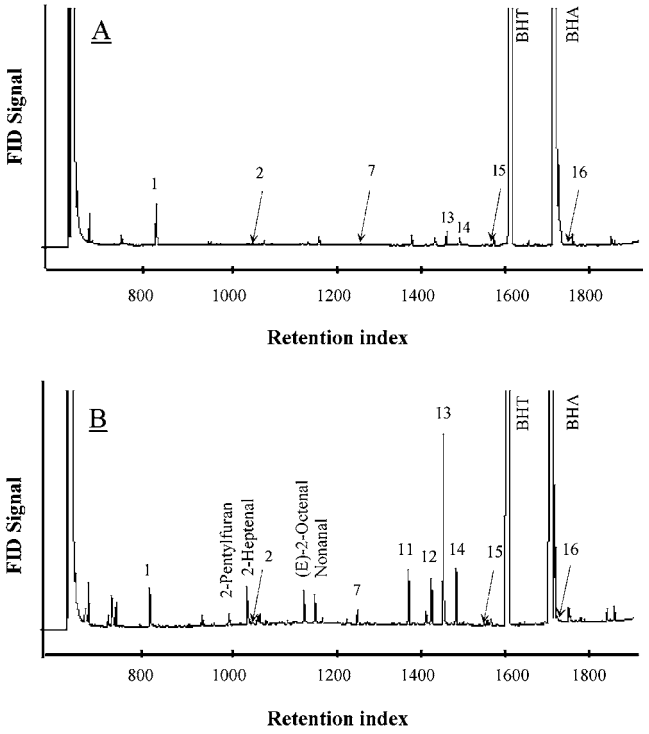

Figure 2. Volatile profiles of an aqueous dispersion of phosphatidylcholine (A) and of a thermally treated aqueous dispersion of phosphatidylcholine (B).

sions. Reliable quantitative data were obtained by IDA in various phospholipid samples for the following odorants: 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 3**, 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** which 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.

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 (*7*). 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 odor activity values (OAV) were calculated by dividing the concentration of an odorant by its orthonasal threshold determined in air and oil to evaluate the sensory contribution of individual compounds to the overall aroma.

As shown in **Table 3**, odorant **15** (metallic) showed the highest OAV in both PC and PE on the basis of odor thresholds determined in air and oil. 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, in particular when considering the threshold value in oil. 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, and 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, and metallic note of PE.

The significant contribution of **15** is due to its extremely low odor thresholds of 1.5 pg/L of air (*26*) and 1.3 μg/L of oil (*21*). Therefore, despite the low amounts found (**Table 3**), 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 100 pg/L of air (*25*) and 180 μg/L of oil (*21*).

DISCUSSION

As shown in **Table 2**, the odor-active compounds generated upon heating phospholipids are all carbonyls belonging to two chemical classes, i.e., aldehydes and vinyl ketones. Hexanal (**1**) was the only odor-active alkanal detected by GC-O, while 1-octen-3-one (**2**) and (*Z*)-1,5-octadien-3-one (**3**) were the two odorous vinyl ketones found. However, the majority of odorants are 2-alkenals such as (*Z*)-2-nonenal (**6**), (*E*)-2-nonenal (**7**), (*Z*)-2-decenal (**10**), (*E*)-2-decenal (**11**), (*E*)-2-undecenal (**14**), and *trans*-4,5-epoxy-(*E*)-2-decenal (**15**). Three alkadienals were found, i.e. (*E*,*E*)-2,4-nonadienal (**9**), (*E*,*Z*)-2,4-decadienal (**12**), and (*E*,*E*)-2,4-decadienal (**13**), as well as one alkatrienal, namely (*E*,*Z*,*Z*)-2,4,7-tridecatrienal (**16**).

In agreement with results reported by Farmer and Mottram (*6*), alkenals and alkadienals dominated in heated aqueous dispersions of PC, whereas vinyl ketones were reported to be more pronounced in the PE sample. However, GC-O allowed the detection of minor volatiles, which significantly contributed to the overall aroma, i.e. 1-octen-3-one (**2**), (*Z*)-1,5-octadien-

Table 4. Potential Precursors and Intermediate Hydroperoxides of Aroma Compounds Formed by Degradation of Phosphatidylcholine and Phosphatidylethanolamine

odorant	precursors	intermediates[a]	refs
hexanal (**1**)	linoleate (C18:2)	13-HPOD	*29*
	arachidonate (C20:4)	15-HPETE	*14*
1-octen-3-one (**2**)	linoleate (C18:2)	10-HPOD	*30*, *31*
	arachidonate (C20:4)	12-HPETE	*32*, *14*
(*Z*)-1,5-octadien-3-one (**3**)	linolenate (C18:3)	10-HPOT	*33*
(*E*)/(*Z*)-2-nonenal (**6**/**7**)	linoleate (C18:2)	9-HPOD	*34*, *30*
	arachidonate (C20:4)	12-HPETE	
(*E*)/(*Z*)-2-decenal (**10**/**11**)	oleate (C18:1)	9-HPOE	*34*, *30*
(*E*,*Z*)/(*E*,*E*)-2,4-decadienal (**12**/**13**)	linoleate (C18:2)	9-HPOD	*35*, *30*
	arachidonate (C20:4)	11-HPETE	*14*
(*E*)-2-undecenal (**14**)	oleate (C18:1)	8-HPOE	*30*, *31*
trans–4,5-epoxy-(*E*)-2-decenal (**15**)	linoleate (C18:2)	13-HPOD[b]	*23*, *36*
	arachidonate (C20:4)	15-HPETE[c]	*14*
(*E*,*Z*,*Z*)-2,4,7-tridecatrienal (**16**)	arachidonate (C20:4)	8-HPETE	*14*

[a] 8-HPETE, 8-hydroperoxy-5,9,11,14-eicosatetraenoic acid; 8-HPOE, 8-hydroperoxy-9-octadecenoic acid; 9-HPOD, 9-hydroperoxy-10,12-octadecadienoic acid; 9-HPOE, 9-hydroperoxy-10-octadecenoic acid; 10-HPOD, 10-hydroperoxy-8,12-octadecadienoic acid; 10-HPOE, 10-hydroperoxy-8-octadecenoic acid; 10-HPOT, 10-hydroxy-8,12,15-octadecatrienoic acid; 11-HPETE, 11-hydroperoxy-5,8,12,14-eicosatetraenoic acid; 11-HPOE, 11-hydroperoxy-9-octadecenoic acid; 12-HPETE, 12-hydroperoxy-5,8,10,14-eicosatetraenoic acid; 13-HPOD, 13-hydroperoxy-9,11-octadecadienoic acid; 15-HPETE, 15-hydroperoxy-5,8,11,13-eicosatetraenoic acid. [b] 13-HPOD via 12,13-epoxy-9-hydroperoxy-10-octadecenoic acid. [c] 15-HPETE via 14,15-epoxy-11-hydroperoxy-5,8,12-eicosatrienoic acid.

3-one (**3**), *trans*-4,5-epoxy-(*E*)-2-decenal (**15**), and the newly identified odorant (*E*,*Z*,*Z*)-2,4,7-tridecatrienal (**16**). Odorants **13**, **7**, **15**, and **2** were also found to develop high FD factors in standardized soybean lecithin (*8*).

The sensory contribution of an odorant depends, among other parameters, on its concentration and threshold value. While the former can accurately be determined, odor thresholds may vary considerably, depending on the solvent used. Therefore, the OAV results in a first estimation on the sensory relevance of individual compounds to the overall aroma, without considering possible interactions. As indicated in **Table 3**, OAV data obtained on the basis of odor thresholds in oil suggest that the mushroom note of **2** and green odor of **1** are more pronounced in lipid-rich food systems. On the contrary, the role of the fatty smelling odorant **13** is less dominant. Odorant **15** (metallic) is, however, the character impact compound in both systems.

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 potential fatty acid precursors and hydroperoxide intermediates of these odorants are summarized in **Table 4**. Their formation mechanisms have been described elsewhere (*14*, *31*, *34*). As reported in the literature (*4*), various unsaturated fatty acids are present in remarkable quantities in egg phospholipids (**Table 5**). These fatty acids are precursors of the odorants detected.

Hexanal (**1**) was found to be the major odor-active volatile degradation product of heated phospholipids with about 100 mg/kg. This is not surprising as it 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 (**12**/**13**) (*37*, *38*). 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 (8-HPETE) (*14*), in analogy to the formation of (*E*,*Z*,*Z*)-2,4,7-decatrienal from linolenic acid (*39*). Odorant **16** has been reported as a volatile constituent of autoxidized arachidonic acid (*32*, *14*) and also tentatively identified in cooked chicken, which is known to be rich in polyunsaturated fatty acids such as C20:4 (*40*).

Table 5. Fatty Acid Composition of Egg Phosphatidylcholine (PC) and Egg Phosphatidylethanolamine (PE)[a]

	fatty acid composition (% total fatty acids)	
fatty acid	egg PC	egg PE
C18:1 (n-9)	29.6	20.1
C18:2 (n-6)	16.2	13.8
C20:3 (n-6)	0.4	0.4
C20:4 (n-6)	3.8	13.7
C22:5 (n-3)	1.1	3.5
C22:6 (n-3)	0.7	2.3
$\sum$ monounsaturated (1 DB)	31.6	21.5
$\sum$ diunsaturated (2 DB)	16.2	13.8
$\sum$ polyunsaturated ($\geq$3 DB)	6.2	20.7

[a] Data were taken from the literature (*4*). DB = double bond.

However, there is apparently no direct relationship between the amounts of (*E*,*E*)-2,4-decadienal (**13**) and (*E*,*Z*,*Z*)-2,4,7-tridecatrienal (**16**), for example, and the concentration of ω-6 fatty acids (e.g. 18:2 and 20:4) in the phospholipid, from which these aldehydes are formed. According to literature data (**Table 5**) (*4*), 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, thus indicating that the fatty acid composition is unlikely to be the only parameter explaining the formation of odorants from aqueous dispersions of phospholipids. Therefore, additional work is required to better understand the factors driving the formation of odor-active carbonyls from phospholipids under cooking conditions.

In conclusion, characterization of the qualitative and quantitative aroma composition of heated phosphatidylcholine and phosphatidylethanolamine by GC-O and IDA techniques revealed the green-metallic smelling *trans*-4,5-epoxy-(*E*)-decenal (**15**) as a character impact odorant. (*E*,*Z*,*Z*)-2,4,7-Tridecatrienal (**16**) was unequivocally identified for the first time as a volatile constituent of heated phospholipids and is most likely a degradation product of arachidonic acid. The aroma properties of the most potent odorants were in good agreement with the overall aroma of heated phospholipids. Our study also suggests that further parameters other than fatty acid composition affect the formation of carbonyls from heated aqueous dispersions of phospholipids. This will be the subject of a future contribution.

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