Volatile Compounds of Oxidized Pork Phospholipids

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ABSTRACT: Volatile compounds from oxidized pork muscle phospholipids (PL) were analyzed by a purge-and-trap method. Total volatile compounds were highly correlated with thiobarbituric acid-reactive substances, mainly as a consequence of alkanals. Major compounds of the 32 identified substances were alkanals (6023 ng nonane equivalents/mg PL), followed by 2-alkenals (514 ng nonane eq/mg PL) and 2,4-alkadienals (368 ng nonane eq/mg PL). Hexanal (4850 ng nonane eq/mg PL) was the major compound from the oxidation of n-6 fatty acids (mainly linoleic and arachidonic acid). Volatile compounds from the oxidation of n-3 fatty acids were only minor and included 2,4-heptadienal (45 ng nonane eq/mg PL) and 2-pentenal and 2-hexenal (49 ng nonane eq/mg PL). Finally, nonanal, a degradation compound from oleic acid, was present at a low level (200 ng nonane eq/mg PL) and remained constant during oxidation, which confirmed that monounsaturated fatty acids were stable toward metal-catalyzed oxidation. With the exception of ester compounds, identified volatiles were qualitatively similar to those obtained in simpler systems, such as fatty acids or vegetable oils. Quantitatively, the volatile compound composition reflected the fatty acid composition of PL.
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KEY WORDS: Kinetic, meat, metal-catalyzed, oxidation, phospholipids, TBA-reactive substances, volatile compounds.

Lipid oxidation plays a major role in the quality of lipid-containing foods, such as meat (1,2), by producing volatile compounds that contribute both to the typical flavor of meat and to the development of off-flavors (3–5). Phospholipids (PL) oxidize faster than triglycerides as a result of the level of unsaturation (3).

During lipid oxidation, unsaturated fatty acids react with molecular oxygen *via* a free-radical chain mechanism to form fatty acid hydroperoxides (6). Although these products are colorless, tasteless, and odorless, they degrade to form low-molecular-weight compounds with distinctive odors and flavors (7,8). Volatile compounds are directly related to the structure of hydroperoxides, and their formation depends on the nature of catalysis (enzymes, metals, light, heat). In meat, catalysis is mainly nonenzymic and associated with iron-containing compounds (9,10). Hydroperoxides of unsaturated fatty acids, such as oleic, linoleic, linolenic and arachidonic acid, consist mainly of monohydroperoxides (11–13). Their degradation and the subsequent formation of volatiles have been investigated and reviewed (13–17). However, extensive studies on oxidation of unsaturated lipids have mainly focused on free or esterified fatty acids, and there is a lack of knowledge on the kinetics of oxidation of complex PL mixtures, especially as related to the formation of volatile compounds.

The aim of this work was to analyze and quantify the volatile compounds produced during the $FeCl_3$-sodium ascorbate-catalyzed oxidation of PL extracted from pork muscle.

MATERIALS AND METHODS

Preparation of liposomes. Lipids were extracted from pork longissimus dorsi according to the procedure of Folch *et al.* (18). PL were purified from total lipids on a silicic acid column (19). Their purity was checked by high-performance liquid chromatography (20). The fatty acid and aldehyde composition of PL was determined by gas–liquid chromatography of fatty acid methyl ester or dimethyl acetal derivatives prepared as described by Berry *et al.* (21).

After PL were poured into cap-lockable tubes, chloroform was evaporated under a stream of nitrogen (purity >99.5%) and an appropriate volume of degassed buffer solution [PIPES (piperazine *N*-*N*′-bis-2-ethanesulfonate) 10 mM, pH = 6.0, NaCl 0.15 M] was added. Tubes were kept 15 h at 4°C in the dark under N_2 to ensure the hydration of PL polar heads. The PL suspension was then vortexed for 10 min to obtain multilamellar vesicles (MLV). The resulting suspension was extruded 10 times with nitrogen through two polycarbonate membranes of 0.4 μm pore size (22). The final lipid concentration was 1 g/L. Samples were saturated with filtered-air bubbling at 25°C for 10 min. One preparation, in which catalyst addition was omitted, was designed as a control to check the initial level of oxidation. After the addition of an equimolar mixture of $FeCl_3$-sodium ascorbate to a final concentration in liposomes equal to 45 μM, preparations were kept in a thermostated oven at 25°C in the dark. Aliquots were analyzed just after catalyst injection (T0), after 5 h (T5), and after 22 h of incubation (T22). Each experiment was performed eight times.

Thiobarbituric acid-reactive substances (TBA-RS). The TBA-RS were measured according to Buege and Aust (23) from an aliquot of 250 μg of PL. Two mL of reagent

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(trichloroacetic acid 15%, TBA 0.375%, HCl 0.25 N) and 750 µL of ultrapure water were added. The reaction mixture was then heated for 15 min at 100°C, then cooled and centrifuged for 10 min at 1500 × *g*. The absorbance at 532 nm was measured on the supernatant against a blank with a double-beam ultraviolet-visible spectrophotometer (Lambda 12, Perkin-Elmer, Überlingen, Germany). Results were expressed in absorbance units.

Analysis of volatile compounds. Concentration of volatile compounds. A purge-and-trap concentrator (Model LSC 2000, Tekmar Co., Cincinnati, OH), equipped with a built-in-pocket heater and a capillary interface for cryofocusing, was connected to a gas chromatograph (HP5890; Hewlett-Packard, Ltd., Böblingen, Germany). The volatiles of an aliquot of each sample (1 mL) were purged at 40°C with nitrogen at 50 mL/min for 30 min and swept into a porous polymer adsorbent, Tenax (60/80 mesh, 30 cm × 0.32 mm i.d., Anspec Co., Ann Arbor, MI), which was kept at room temperature. They were then thermally desorbed by heating the trap at 210°C for 2 min and back-flushed through a transfer line to the capillary interface above the gas chromatograph injector. Back-flushed volatiles were cryofocused at −100°C with liquid nitrogen and then rapidly heated to 210°C for injection into the gas chromatograph. Additional parameters were, in the order of automation sequence: prepurge, 5 min at 20°C; preheat, 5 min at 40°C; dry purge, 5 min; desorption preheat to 205°C; bake, 20 min at 225°C.

Separation of volatile compounds by gas chromatography (GC). A Hewlett-Packard 5890A gas chromatograph, equipped with a flame-ionization detector (FID), was used with a fused-silica capillary column, DB-5 (30 m × 0.32 mm i.d., 1.0 µm film thickness) from J&W Scientific (Folsom, CA). The detector temperature was set at 250°C. The carrier gas was hydrogen and set at 5 psi. The oven temperature was programmed from 40°C, after an initial hold for 5 min, to 180°C at a 3°C/min rate and then held for 5 min.

Quantification of volatile compounds. Volatile compounds were quantitated with nonane (Alltech Associates Inc., Deerfield, IL) as a external standard. Aliquots of nonane solutions in ethanol were added to the PIPES buffer. One mL of the mixture was transferred in the purge-and-trap vessel to give final nonane quantities varying from 0 to 25.5 µg. Extraction and chromatography of each solution was performed as described above. Each nonane concentration was analyzed in triplicate. The response of the FID was linear between 0 and 10 µg. Above this concentration, the area tended to an asymptote as the result of detector saturation. The slope of the regression was 2065.37, and the regression coefficient was 0.982. Results were expressed in ng nonane equivalents/mg PL (ng nonane eq/mg PL).

Identification of volatile compounds by GC–mass spectrometry (MS). A Hewlett-Packard 5890 gas chromatograph, interfaced to a purge-and-trap concentrator and a 5971A mass selective detector (Hewlett-Packard), was used with a DB-5 column (60 m × 0.32 mm i.d., 1 µm film thickness). The electron impact ionization was set at 70 eV, and the source temperature at 180°C. Mass spectra, scanned between mass/charge 33 and 300, were recorded on a computer (Vectra QS20; Hewlett-Packard, , Grenoble, France) and identified by comparison of spectra with those of available libraries (NBS, NIST, TNO and INRA Mass).

RESULTS AND DISCUSSION

PL composition. PL classes and fatty acid composition of purified PL are reported in Table 1. Major PL were phosphatidylcholine (58.9%) and phosphatidylethanolamine (25.2%). Total PL of pork longissimus dorsi contained approximately 8% fatty aldehydes. The fatty acid composition was characterized as follows: 34.8% saturated fatty acids, 21.3% monounsaturated fatty acids (mainly as oleic acid) and 44.0% polyunsaturated fatty acids (PUFA). Among PUFA, n-6 fatty acids accounted for 41.3% and n-3 represented only 2.7%. These results agree with the literature (20).

Volatile compounds in the control. The average quantity of volatile compounds was approximately 800 ng nonane eq/mg PL (Table 2). Major volatiles were hexanal, nonanal, and heptanal, which accounted, respectively, for 212, 195, and 108

TABLE 1
Phospholipid and Fatty Acid Composition of Purified Pork Phospholipids (in %)

Phospholipid composition	(%)
Cardiolipin	2.7
Phosphatidylethanolamine	25.2
Phosphatidylinositol and phosphatidylserine	8.5
Phosphatidylcholine	58.9
Sphingomyelin	1.8
Fatty acid composition	**(% of total fatty acids)**
16:0	18.0
18:0	16.4
Saturated	34.7
16:1 n-9	0.3
18:1 n-9	16.8
20:1 n-9	0.2
n-9	17.3
16:1n-7	1.1
18:1 n-7	2.8
Monounsaturated	21.3
18:2 n-6	28.0
20:2 n-6	0.6
20:3 n-6	1.3
20:4 n-6	10.0
22:4 n-6	1.1
22:5 n-6	0.2
n-6	41.3
18:3 n-3	0.4
20:5 n-3	0.4
22:5 n-3	1.1
22:6 n-3	0.8
n-3	2.7
PUFA	44.0

ng nonane eq/mg PL. Hexanal arises from the oxidation of n-6 fatty acids, nonanal and heptanal from n-9 fatty acids (10). Some 2-alkenals, 2-heptenal and 2-octenal, were present at low levels (18 and 14 ng nonane eq/mg PL). Other minor compounds were 2,4-decadienal (26 ng nonane eq/mg PL), pentanol (22 ng nonane eq/mg PL), 1-octen-3-ol (15 ng nonane eq/mg PL), and 2,3-octanedione (12 ng nonane eq/mg PL). The initial level of volatiles from n-9 origin could be the result of *in vivo* β-oxidation.

TABLE 2
Quantities of Individual Volatiles from Oxidation of Pork Phospholipids[a]

Compound	Control	Time[b] (h) 0	5	22
Pentanal	31[c]	105[c]	368[b]	716[a]
Hexanal	212[d]	975[c]	3277[b]	4850[a]
Heptanal	108	156	101	90
Octanal	38	78	74	86
Nonanal	195	282	241	242
Decanal	51[a]	61[a]	49[b]	38[b]
Total alkanals	636[d]	1657[c]	4109[b]	6023[a]
t-2-Pentenal	2[c]	5[c]	21[b]	33[a]
t-2-Hexenal	2[c]	3[c]	10[b]	16[a]
t-2-Heptenal	18[d]	47[c]	140[b]	192[a]
t-2-Octenal	14[b]	53[b]	185[a]	229[a]
t-2-Nonenal	8	10	9	15
t-2-Decenal	8[b]	14[b]	31[a]	29[a]
Total 2-alkenals	53[c]	132[c]	396[b]	514[a]
t,c-2,4-Heptadienal	0[b]	8[b]	31[a]	35[a]
t,t-2,4-Heptadienal	0[b]	1[b]	8[a]	10[a]
t,c-2,4-Nonadienal	0[b]	3[b]	9[a]	12[a]
t,t-2,4-Nonadienal	0[c]	1[c]	13[b]	42[a]
t,c-2,4-Decadienal	12[b]	32[b]	91[a]	117[a]
t,t-2,4-Decadienal	14[b]	39[b]	145[a]	152[a]
Total 2,4-alkadienals	30[b]	83[b]	297[a]	368[a]
Pentanol	22	14	27	48
Hexanol	3[b]	4[b]	6[b]	46[a]
Heptanol + benzaldehyde	0[b]	5[b]	5[b]	11[a]
1-Octen-3-ol	15[c]	62[b]	161[a]	164[a]
Octanol + 3,5-octadien-2-one	1[b]	11[b]	5[b]	1[a]
Total alcohols	39[c]	96[c]	204[b]	270[a]
Toluene	4[c]	28[c]	119[b]	192[a]
2-Heptanone	3[c]	6b[c]	12[a,b]	15[a]
2,3-Octanedione	12[c]	36[c]	164[b]	238[a]
2-Pentylfuran	6[c]	10[c]	28[b]	59[a]
3-Octen-2-one	2[c]	8[b,c]	17[b]	28[a]
Tridecane	5	12	13	12
Tetradecane	6[b]	11[b]	22[a]	25[a]
Total miscellaneous	43[d]	111[c]	375[b]	568[a]
Total	796[d]	2079[c]	5381[b]	7743[a]

[a]Results are expressed in ng nonane equivalents/mg phospholipid. Each value is the mean of eight determinations. Within the rows, the means superscripted with different letters differed significantly ($P < 0.05$).
[b]Time after addition of catalyst.

Evolution of volatile compounds during oxidation. Thirty-two compounds were identified, including six alkanals, six 2-alkenals, six 2,4-alkadienals, five alcohols, and nine miscellaneous (Table 2). Shapes of the curves of volatile compounds and TBA-RS vs. time were similar (Fig. 1A) in agreement with a previous study of liposomes (24). The rate of oxidation was high during the first minutes of the reaction and leveled off after 5 h. The quantities of total volatiles were eight times higher after 22 h of incubation in the presence of a catalyst than in the control. The linear relationship between TBA-RS and total volatiles ($r^2 = 0.996$) agreed with previous studies on meat products (25,26), although the exact meaning of the TBA test has been the subject of numerous conflicts (1,2,6).

Relationship between the evolution of selected volatiles. Data from the literature for probable precursors of some volatiles as well as their odor thresholds of perception are listed in Table 3. Major compounds, such as hexanal, pentanal or *t*-2-octenal, arise from the oxidation of n-6 fatty acids, which is consistent with our data on fatty acid composition of pork PL (Table 1) and with data in the literature (20). Total volatiles and hexanal were highly correlated ($r^2 = 0.989$), as

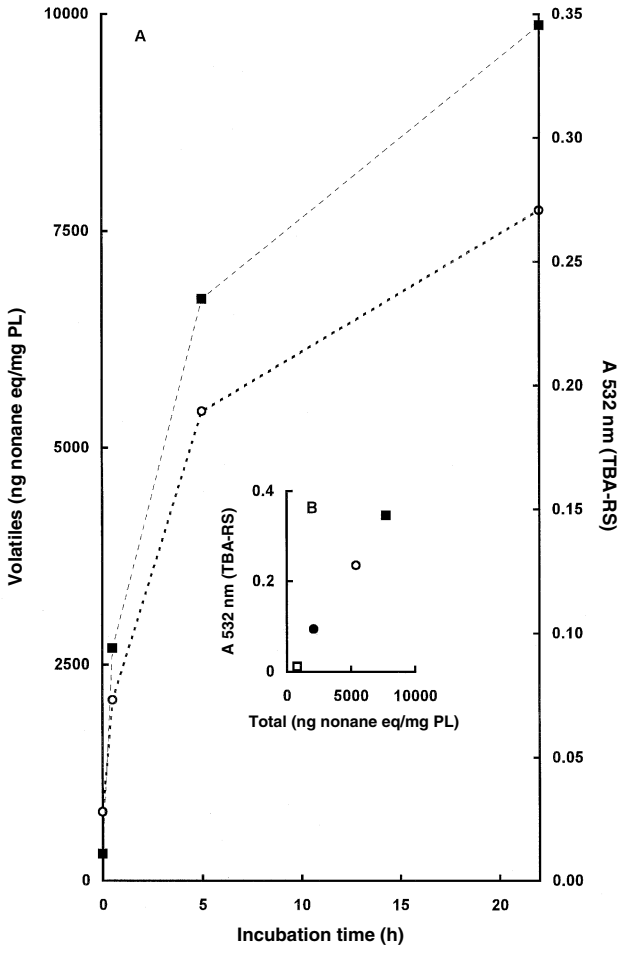

FIG. 1. (A) Evolution during oxidation of phospholipids (PL) of thiobarbituric acid-reactive substances (TBA-RS) (■) and total quantity of volatiles (○) as a function of incubation time. (B) Evolution of TBA-RS vs. total quantity of volatiles. Control (□), T0 (●), T5 (○), and T22 (■).

TABLE 3
Possible Origin and Odor Threshold of the Volatiles of Oxidized Pork Phospholipids

Volatile compound	Fatty acid precursor[a]	Possible origin[b]	Threshold[c]
Pentanal	n-6	13-HPOD, 15-HPETE	21.9 ppb
Hexanal	n-6	9-HPOD, 12-HPOD, 13-HPOD, 14-HPETE, 15-HPETE	58 ppb
Heptanal	n-6, n-9	11-HPOE	23 ppb
Octanal	n-9	10-HPOE, 11-HPOE	7 ppb
Nonanal	n-9	9-HPOE, 10-HPOE	13 ppb
Decanal	n-9	8-HPOE	6 ppb
t-2-Pentenal	n-3	13-HPOT	
t-2-Hexenal	n-3	12-HPOT, 13-HPOT	13 ppm
t-2-Heptenal	n-6	12-HPOD, 14-HPETE	63 ppb
t-2-Octenal	n-6	9-HPOD	10.7 ppb
t-2-Nonenal	n-6	9-HPOD, 10-HPOD, 11-HPETE, 12-HPETE	0.9 ppb
t-2-Decenal	n-9	9-HPOE	2 ppb
t,c-2,4-Heptadienal	n-3		
t,t-2,4-Heptadienal	n-3	12-HPOT	19 ppb
t,c-2,4-Nonadienal	n-6		
t,t-2,4-Nonadienal	n-6		0.2 ppb
t,c-2,4-Decadienal	n-6		
t,t-2,4-Decadienal	n-6	9-HPOD, 11-HPETE	0.2 ppb
Pentanol	n-6	13-HPOD, 15-HPETE	1.6 ppm
Hexanol			0.19 ppm
Heptanol	n-9	11-HPOE	0.1 ppm
1-Octen-3-ol	n-6	10-HPOD, 12HPETE	16 ppb
Octanol	n-9	10-HPOE	
2-Heptanone			676 ppb
2-Pentyl-furan	n-6	10-HPOD, 11-HPETE	91 ppb

[a]From Grosch (13).
[b]Adapted from Frankel (16) and Grosch (13,17).
[c]From Ref. 27. HPOE, hydroperoxyoctadecaenoic; HPOD, hydroperoxyoctadecadienoic; HPOT, hydroperoxyoctadecatrienoic; HPETE, hydroperoxyeicosatetraenoic.

expected, because hexanal accounted for nearly two-thirds of the total volatiles. Because of these results, we examined the evolution of major volatiles, chosen for their olfactory properties and/or for the nature of their precursors, vs. hexanal.

n-6 Compounds vs. hexanal. A comparison of *t*-2-heptenal vs. hexanal quantities is shown in Figure 2A. This alkenal possesses a low odor threshold of 63 ppb (27) and is described as putty, fatty, bitter-almond (13). Quantities of hexanal and *t*-2-heptenal were linearly correlated ($r^2 = 0.949$). Identification of *t*-2-heptenal in the early stage of oxidation indicated that the hydroperoxides 12-hydroperoxyoctadecadienoic (12-HPOD) and 14-hydroperoxyeicosatetraenoic (14-HPETE) of linoleic acid and arachidonic acid were formed. This result suggested that oxidation, catalyzed by iron-ascorbate, generated hydroperoxides in both the inner and outer positions of the 1,4-pentadienic system. The similar evolution of both compounds suggests that they arose from similar chemical reactions. Effectively, hexanal is formed from the homolytic decomposition of 9-HPOD, 12-HPOD and 13-HPOD and from 14-HPETE and 15-HPETE, while *t*-2-heptenal is formed from 12-HPOD and 14-HPETE only. The latter can explain the lower quantity of 2-heptenal as compared to hexanal.

1-Octen-3-ol has a characteristic odor of mushroom and an odor threshold of 16 ppb (13). Its quantitative evolution vs. hexanal is shown in Figure 2B. The linear correlation coefficient was only 0.842. The quantity of 1-octen-3-ol tended to level off after 5 h of oxidation, while the quantity of hexanal increased steadily throughout the oxidation. Accordingly, the two volatiles originate from different hydroperoxides: precursors of 1-octen-3-ol are 10-HPOD and 12-HPETE, whereas hexanal could be formed from at least five different hydroperoxides (13,17). Differences observed between evolution of 1-octen-3-ol and *t*-2-heptenal can be tentatively related to their pathway of formation. Thus, *t*-2-heptenal is formed directly from β-scission of 12-HPOD or 14-HPETE, whereas 1-octen-3-ol is formed *via* a multistep decomposition of hydroperoxides that involves an intermediate reactant, such as OH°, R°, or ROO° (13,17). During oxidation, the concentration of oxygen or free radicals can limit the decomposition.

2,4-Decadienal has a low odor threshold of 0.2 ppb, and its odor is described as deep-fried, fried (13). The evolution of *c,t*-2,4-decadienal and *t,t*-2,4-decadienal vs. hexanal is presented in Figure 2C. The evolution of the quantities of these two compounds was poorly correlated ($r^2 = 0.655$). The

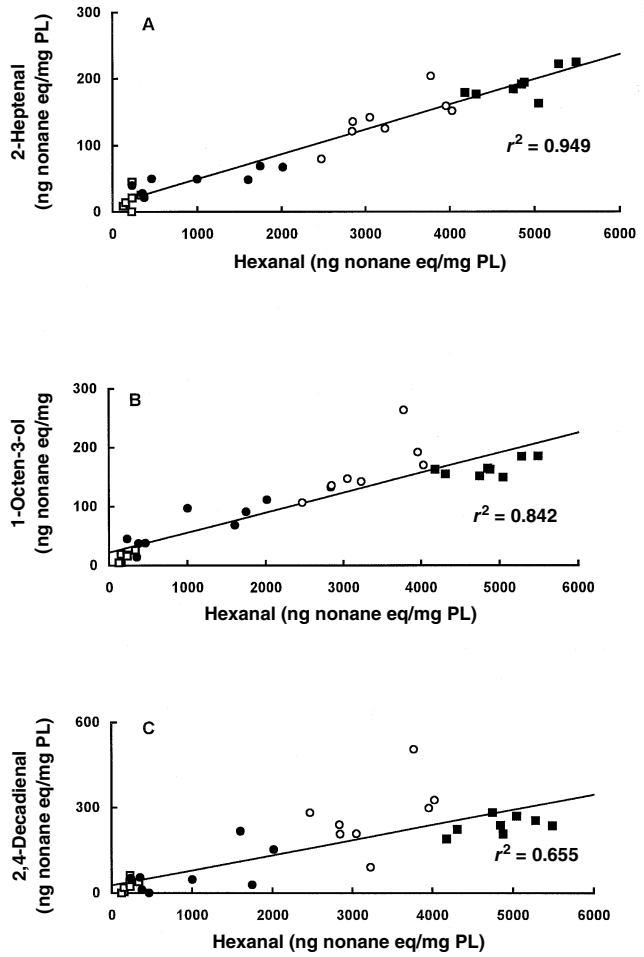

FIG. 2. Evolution of volatiles derived from the oxidation of n-6 fatty acids. (A) *t*-2-Heptenal vs. hexanal. (B) 1-Octen-3-ol vs. hexanal. (C) Two isomers of 2,4-decadienal vs. hexanal. For legend and abbreviation, see Figure 1.

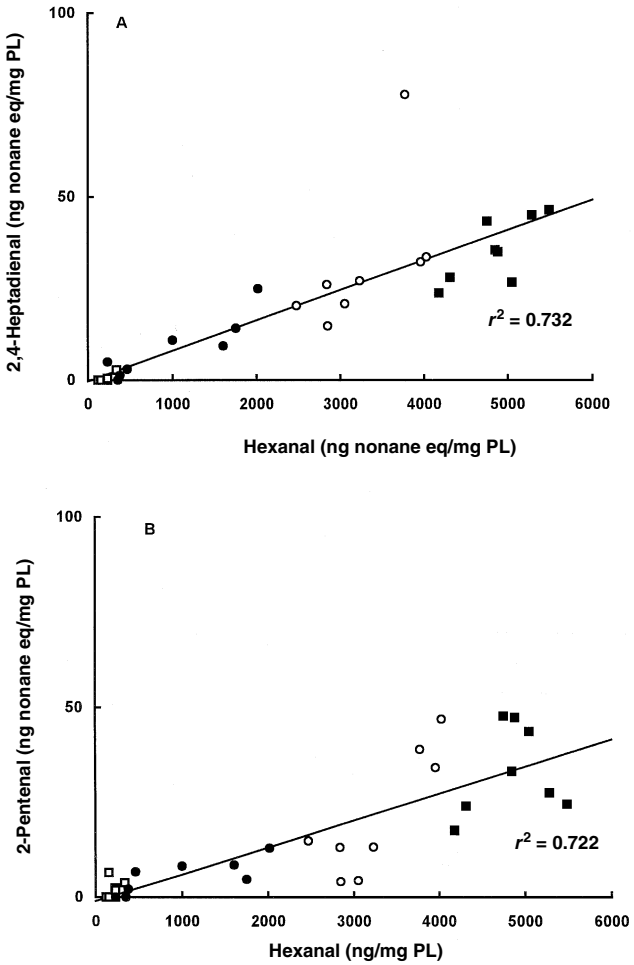

FIG. 3. Evolution of volatiles derived from the oxidation of n-3 fatty acids. (A) 2,4-Heptadienal vs. hexanal. (B) *t*-2-Pentenal vs. hexanal. For legend and abbreviation, see Figure 1.

quantities of both isomers of 2,4-decadienal leveled off after 5 h of oxidation. This result may be attributed to possible autoxidation according to a mechanism proposed by Schieberle and Grosch (28). 2,4-Decadienal is formed by a β-scission of 9-HPOD or 11-HPETE, then a peroxyl radical is postulated to react with decadienal and, depending on the site of addition, to form hexanal or 2-octenal. Similar reactions have been proposed for 2,4-heptadienal (29).

n-3 Compounds vs. hexanal. Compounds generated from the oxidation of n-3 fatty acids were present in small quantities (94 ng nonane eq/mg PL). This result agreed with the fatty acid composition of pork PL, which contained less than 3% n-3 fatty acids. 2,4-Heptadienal and hexanal were not highly correlated (r^2 = 0.732) (Fig. 3A). Similar results were found for *t*-2-pentenal (r^2 = 0.722) (Fig. 3B).

n-9 Compounds vs. hexanal. Nonanal, a major compound formed from the oxidation of n-9 fatty acid (namely, oleic acid), arose from the decomposition of 9- and 10-HPOE (hydroperoxyoctadecaenoic). The evolution of nonanal vs. hexanal is presented in Figure 4. The nonanal quantity remained steady throughout the oxidation, while the quantity of hexanal increased regularly. Consequently, the evolution of nonanal and hexanal was not correlated (r^2 = 0.003). This indicates that, under our experimental conditions, n-9 fatty acids are stable toward oxidation catalyzed by $FeCl_3$-ascor-

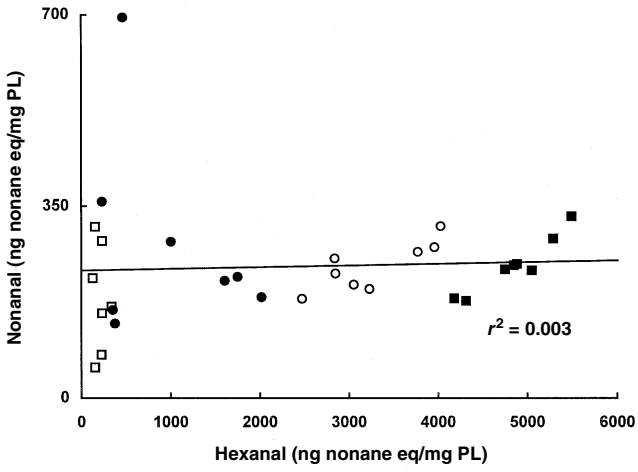

FIG. 4. Evolution of volatiles derived from the oxidation of n-9 fatty acids, nonanal vs. hexanal. For legend and abbreviation, see Figure 1.

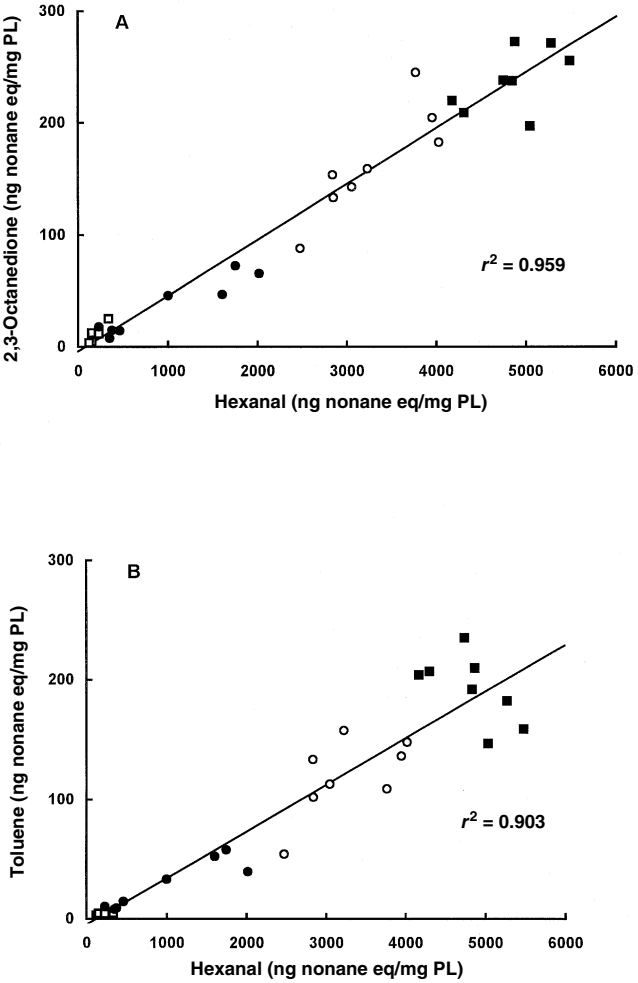

FIG. 5. Evolution of volatiles derived from unknown origin. (A) 2,3-Octanedione vs. hexanal. (B) Toluene vs. hexanal. For abbreviation and legend, see Figure 1.

bate. Other compounds formed from n-9 fatty acid oxidation (heptanal, octanal, decanal) exhibited similar evolution (data not shown).

Compounds of unknown origin vs. hexanal. 2,3-Octanedione, a compound of unknown origin, has been identified in the volatiles of beef (30) and among the volatiles produced during the autoxidation of methyl arachidonate (31). Furthermore, 2,3-octanedione was correlated with TBA number and with the appearance of meat flavor deterioration (26). Variations of 2,3-octanedione quantity vs. hexanal were linearly correlated ($r^2 = 0.96$) (Fig. 5A). Although the exact structure of the precursor of 2,3-octanedione was not elucidated, the strong relationship between this compound and hexanal suggests that they arose from comparable or similar mechanisms, involving n-6 fatty acid oxidation.

Toluene, a hazardous compound that is often associated with contamination from packaging and animal feeding, correlated highly with hexanal ($r^2 = 0.90$) (Fig. 5B). This result established that, under our experimental conditions, toluene was formed throughout the lipid oxidation. Nevertheless, the precursors and mechanisms remain to be elucidated, even if an origin from n-6 fatty acid is strongly suggested.

The composition of volatiles that arise from the metal-catalyzed oxidation of PL of pork muscle was in agreement with their fatty acid composition. Our results were qualitatively similar to those of free fatty acids or vegetable oil, with the exception that we have not identified ester compounds in our model systems. The esterification of fatty acid molecules on phosphoglycerides can be an explanation of this observation. In our model system, we confirmed that evolution of TBA-RS and total volatile compounds are highly correlated, mainly as a consequence of the formation of alkanals. Detailed studies of volatile compounds and correlation between evolution of selected compounds can be of interest with regard to the nature of the hydroperoxides and to their degradation during metal-catalyzed oxidation.

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