Oxidation of muscle phospholipids in relation to their fatty acid composition with emphasis on volatile compounds†

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Abstract: The impact of moderate changes of fatty acid (FA) composition of phospholipids (PL) on their oxidative stability and on volatile profiles remains largely unknown. PL of breast muscle of turkeys fed a diet containing 6% tallow, rapeseed oil or soya oil were purified and prepared as liposomes. After 24 h of incubation at 25 °C with iron/ascorbate, oxidation was quantified by measurement of thiobarbituric acid reactive substances (TBA-RS) and volatile compounds. TBA-RS level was the lowest (21.4 nmole eq MDA mg^{-1} PL) in PL from tallow-fed animals but was not significantly different ($P > 0.05$) in soya oil (30.9 nmole eq MDA mg^{-1} PL) and rapeseed oil (30.3 nmole eq MDA mg^{-1} PL) batches. ANOVA did not clearly distinguish between the three groups according to the quantities of individual volatiles except for *Z,E*-2,4-heptadienal. In contrast, principal component analysis (PCA) performed on standardised quantities of volatile compounds distinguished unambiguously the three groups. Axis 1 was positively correlated with volatile compounds arising from oxidation of n-6 fatty acids, and negatively with compounds of n-9 origin. Axis 2 was highly positively correlated with compounds from n-3 origin. Only a few compounds from each origin (n-6, n-3, n-9) had an atypical behaviour. A weak modification of the FA composition of PL led to concomitant modifications of the quantities of volatile compounds generated through oxidation, which were emphasised by multivariate analysis (PCA).
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Keywords: turkey; muscle; dietary fat; phospholipids; oxidation; TBA-RS; volatile compounds; fatty acid composition; multivariate analysis

INTRODUCTION

Poultry meat and especially turkey meat is highly sensitive to oxidative degradation[1] because of its high level of polyunsaturated fatty acids (PUFA),[2] mainly associated with PL.[3,4] Accordingly, enrichment of meat in PUFA increases the oxidative degradation of meat and leads to an increase of TBA reactive substances (TBA-RS)[5–7] and of quantities of volatile compounds, especially hexanal.[8–12] This increase in TBA-RS is correlated to the perception of off-flavour in meat.[13–17] Specific off-flavour perception is also linked to increases in the proportions of n-3 FA in muscle lipids. Accordingly, high levels of n-3 FA in meat leads to the development of fishy flavours.[18,19]

These changes in FA composition of meat are under the influence of the nature of fat added to the diet.[5,6,20–22] They involve changes in FA composition of the triglyceride fraction and, to a lesser extent, changes in FA composition of the phospholipids. As phospholipids are the main precursors for lipid oxidation products in meat,[3] it is of interest to know the influence of dietary modifications of muscle phospholipids on lipid oxidation products, especially on volatile compounds, which have a large sensory significance. Indeed, the oxidation of purified fatty acids in relation to volatile compounds has been widely studied,[23–25] but the relation between the fatty acid composition of complex PL and quantities of volatile compounds remains largely unknown.

This study deals with the effect of moderate modifications of the FA composition of PL, through dietary fat, on their oxidation, and, particularly, on the formation of volatile compounds in a model system. It also indicates some new directions on the choice of pertinent volatiles to evaluate lipid oxidation in meat.

MATERIALS AND METHODS

Animals

Male turkeys of BUT (British United Turkey) strain

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Table 1. Composition of the diets of the turkeys

Ingredient ($g kg^{-1}$)	Diet		
	Starter (0–4 weeks)	Weaner (4–8 weeks)	Finisher (8–16 weeks)
Corn	125	112	181
Wheat	200	250	350
Added fat[a]	60	60	60
Soybean meal	555	484	364
Minerals and vitamin	50.5	86.5	38.5
Trace elements	1	1	1
Vitamin	5	5	5
DL methionine	3	1.5	0.5
Lysine	0.5	–	–

[a] Rapeseed oil, tallow or soya oil.

Table 2. Lipid composition of finisher diets of turkeys

	Fat added to the diet		
	Rapeseed oil	Tallow	Soya oil
Lipid content ($g kg^{-1}$)	83	79	77
Fatty acid composition (% of total FAME)			
16:0	74	223	119
18:0	22	174	35
Others[a]	tr	36	tr
Saturated	96	433	155
16:1 n-9	3	22	3
18:1 n-9	498	336	218
Others[b]	tr	9	tr
Monounsaturated	501	367	221
18:2 n-6	323	183	562
18:3 n-3	80	17	62
Polyunsaturated	403	200	624
n-6/n-3	4.0	10.8	9.1

[a] Others: 14:0, 15:0 and 17:0.
[b] Others: 14:1, 17:1.
tr: trace amounts.

were reared at the Station of Poultry Research (INRA, Nouzilly). The turkeys were fed *ad libitum* a basal diet (Table 1) enriched with $60 g kg^{-1}$ of one of the following refined fat sources: tallow; rapeseed oil, or soya oil. These three fats are commonly used in France in the diet of poultry. Finisher diets contained about $80 g kg^{-1}$ of lipids (Table 2). Their fatty acid composition is given in Table 2. The rapeséed-oil-enriched diet contained the lowest proportion of saturated fatty acids (SFA) and the highest levels of oleic acid and α-linolenic acid. The tallow-enriched diet was characterised by the highest proportion of SFA, a high level of oleic acid and the lowest proportion of polyunsaturated fatty acids (PUFA). The soya-oil-enriched diet exhibited a low level of SFA, and the highest proportion of linoleic acid and consequently of PUFA.

Tissue sampling

After 16 weeks of feeding, 8 animals/batch were bled. Breast (*Pectoralis*) muscle was immediately dissected from the carcasses. Tissues were stored on ice at +4 °C and lipids were extracted the following day.

Preparation of liposomes

Lipids were extracted from turkey breasts according to the procedure of Folch *et al.*[26] Solvents ($CHCl_3$ and CH_3OH) were degassed and cooled to 4 °C before use. Purification of phospholipids was achieved as described previously.[27] Briefly, phospholipids were purified from total lipids on silicic acid columns.[28] Their purity was checked by HPLC coupled to an evaporative light-scattering detector.[29] Dietary fat had no marked effect on phospholipid composition. PL contained from 58 to 63% phosphatidylcholine, 29 to 32% phosphatidylethanolamine, and 2.7 to 6% phosphatidylinositol and phosphatidylserine. The oxidation level of purified phospholipids was low when assessed by conjugated dienes index.[30,31] The fatty acid composition of phospholipids was determined by GLC of fatty acid methyl ester derivatives (FAME) prepared as described by Morrison and Smith.[32]

Liposomes were prepared as described previously.[27] Briefly, the purified phospholipids were first dispersed as multilamellar vesicles in a buffer (PIPES 10 mM, NaCl 0.15 M, pH 6.0), and finally extruded 10 times under nitrogen through two polycarbonate membranes of 0.4 µm porosity.[33] The final lipid concentration was $1 g l^{-1}$. Samples were saturated with filtered air by bubbling at 25 °C for 10 min. Aliquots, from which oxidation catalyst was omitted, were put aside. They were designated as controls and were used to check the initial level of oxidation of the liposomes. After the successive addition of daily prepared equimolar $FeCl_3$ and sodium-ascorbate solutions (final concentration 45 µM), liposomes were kept in a thermostatted oven at 25 °C in the dark. Each experiment was performed on the phospholipids extracted from the 8 birds (8 repetitions) of the three feeding batches.

TBA Reactive Substances (TBA-RS)

TBA-RS were measured according to Buege and Aust[34] from aliquots of 250 µl of liposomes. Then 2 ml of TBA reagent (trichloroacetic acid (TCA) 15%, thiobarbituric acid (TBA) 0.375%, HCl 0.25M) and 750 µl of ultrapure water were added. The reaction mixture was then heated for 15 min at 100 °C. After cooling, samples were centrifuged for 10 min at $1500 \times g$. The absorbance of the supernatant was measured at 532 nm against a blank. Results were expressed in nmoles equivalent malonaldehyde mg^{-1} PL (nmole eq $MDA mg^{-1}$ PL) using the molar extinction coefficient of the TBA-MDA adduct at 532 nm ($1.56 \times 10^5 M^{-1} cm^{-1}$). TBA-RS were measured in triplicate on controls at 0.5, 1, 5 and 24 h after the addition of catalyst.

Analyses of volatile compounds

Volatile compounds were analysed on controls and liposomes incubated for 24 h at 25 °C. They were

extracted by a dynamic headspace method as described previously.[35] Briefly, samples (1 ml) were purged with nitrogen (50 ml min^{-1}) for 30 min at 40 °C, trapped on Tenax and desorbed at 210 °C on a cryo-cooled pre-column (−100 °C). Volatiles were then separated on a non-polar fused silica column (DB5, 30 m × 0.32 mm id, 1 µm film thickness, JW Scientifics, Folsom, CA). Quantification was achieved with a flame ionisation detector using an external standard (nonane). The results were expressed in ng eq nonane mg^{-1} PL.

Statistical analysis

ANOVA analysis

The effect of the diet on TBA-RS, FA composition and quantities of volatile compounds was evaluated by one-way ANOVA. Means of the groups were compared using a Newman–Keuls test. Differences of $P < 0.05$ were considered significant. Treatments were performed with Statgraphics software version 3.0 (Manugistics, Inc. Rockville, MA, USA).

Principal component analysis

Principal component analysis (PCA) is a multidimensional statistical method which optimises the description of the data with a minimum loss of information.[36] It allows correlation between variables and separation between observations. PCA were performed on standardised volatile compound amounts with Unistat Plus software (Uniware, Cergy Pontoise, France). Volatile compounds were variables and repetitions (8 birds × 3 batches) were observations.

Table 3. Fatty acid composition of phospholipids isolated from breasts of turkeys fed different dietary fats

	Dietary fat		
	Rapeseed oil	*Tallow*	*Soya oil*
16:0	202a	197a	222b
18:0	153	151	158
Saturated	356a	352a	382b
16:1n-9	2	2	1
16:1n-7	3	8	3
18:1n-9	185b	205c	133a
18:1n-7	33	24	30
20:1	1	1	tr
22:1	1	tr	tr
Monounsaturated	224b	240c	168a
18:2n-6	182a	234b	222b
20:4n-6	130b	94a	138b
22:4n-6	19a	25b	25b
22:5n-6	4a	6b	8c
n-6	335a	372b	403c
18:3n-3	3	2	2
20:5n-3	14c	7b	4a
22:5n-3	39c	17a	24b
22:6n-3	29c	10a	16b
n-3	85c	36a	47b
Polyunsaturated	420a	408a	450b
LC PUFA	235b	172a	226b
PI	1272b	921a	1183b
n-6/n-3	3.9a	10.5c	8.7b

Results were expressed in % of total FAME. Each value was mean of 8 animals. Within a same row, following different letters were significantly different ($P < 0.05$). LC PUFA: long chain polyunsaturated fatty acids (PUFA minus 18:2 n-6 and 18:3 n-3). PI: peroxidisability index (see text).

RESULTS AND DISCUSSION

Changes in the fatty acid composition of phospholipids due to dietary fat

Phospholipids of breast muscle of the three experimental batches of turkeys contained 35.2 to 38.2% saturated fatty acids, 16.8 to 24.0% monounsaturated fatty acids (MUFA), and 40.8 to 45.0% PUFA (Table 3). PL of the three experimental batches could also be distinguished according to the proportion of some fatty acids. PL of birds fed tallow exhibited the highest proportion of 18:1 n-9 (20.5%), followed by PL of birds fed rapeseed oil (18.5%) and soya oil (133%). Proportions of n-6 PUFA in PL increased in the following order: rapeseed oil (33.5%); tallow (37.2%), and soya oil (40.3%). PL of the three experimental batches were also distinguished according to the proportion of n-3 PUFA: tallow (3.6%); soya oil (4.7%), and rapeseed oil (8.5%). Consequently, the n-6/n-3 ratio ranged from 3.9 for birds fed rapeseed oil to 10.5 for birds fed tallow. Birds fed soya oil possessed intermediate values (8.7). Nevertheless, overall unsaturation (PUFA) was similar in PL of birds fed tallow or rapeseed oil (40.8 and 42.0%, respectively) while in PL of birds fed soya oil, proportion of PUFA reached 45.0%. As the oxidative stability of unsaturated lipids decreases with their increasing degree of unsaturation,[37–39] we include in the composition data proportions of long chain polyunsaturated fatty acid (LC PUFA) and peroxidisability index (PI). This index reflects the relative rate of peroxidation of each fatty acid. It was calculated from the following equation:[40] PI = (% monoenoic × 0.025) + (% dienoic × 1) + (% trienoic × 2) + (% tetraenoic × 4) + (% pentaenoic × 6) + (% hexanenoic × 8). LC PUFA were present in similar proportions in PL of birds fed rapeseed or soya oil (23.5 and 22.6%, respectively), while they were the lowest (17.2%) in PL of birds fed tallow. Accordingly, PI was similar for PL of birds fed vegetable oils ($P < 0.05$) and the lowest for PL of the tallow batch.

Therefore, the nature of the fat added to the diet influenced significantly the fatty acid composition of the phospholipids, especially their n-9, n-6 and n-3 FA relative proportions even if unsaturation did not change in large proportions. This result corroborates previous work on poultry meat.[5,6,21,41,42] The discussion of the metabolic significance of these changes is not the aim of this paper, but two facts must be underlined. First, our results show that large differences in fatty acid composition of the diet lead to smaller differences in fatty acid composition of phospholipids. This may be explained by the need for the cellular membranes to maintain their physical proper-

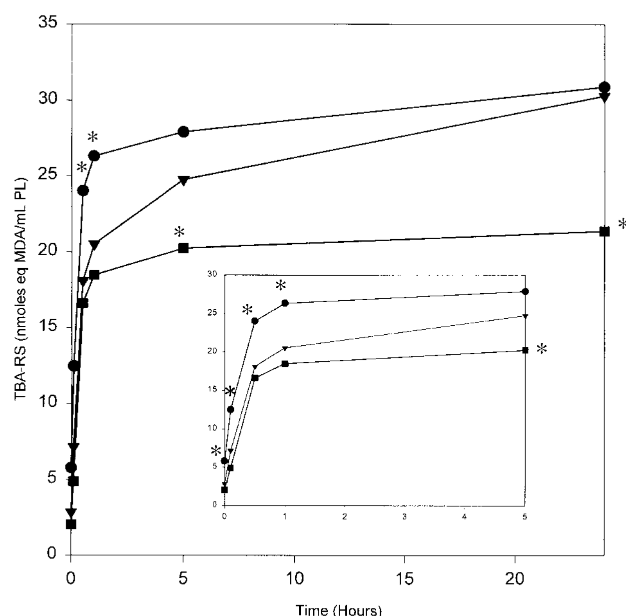

Figure 1. Effect of dietary fat on the oxidation of liposomes as measured by, TBA-RS. Liposomes were prepared from phospholipids of: ▼, rapeseed oil; ●, soya oil; ■, tallow fed turkey breasts ($n = 8$). For a given time, values marked with* were significantly different from the others ($P < 0.05$).

ties and the integrity of cellular functions.[43] Second, in this work, the most obvious changes were found in n-6/n-3 FA ratio, which was very close in diets and muscle PL (Tables 2 and 3).

Phospholipid oxidation

TBA-RS

The shape of the curves for TBA-RS amounts with time were similar to those obtained on the similar model system made up with PL of pork muscle.[27,35] Freshly prepared liposomes from PL of turkeys fed soya oil contained significantly more TBA-RS (5.8 nmoles eq MDA mg^{-1} PL) than the two other batches (2.0 and 2.8 nmoles eq MDA mg^{-1} PL, respectively), and during the first hour of the reaction (Fig 1). Then, while oxidation in liposomes from birds fed tallow levelled off, that of the rapeseed oil batch increased and, after 5 h, reached the level measured in liposomes of soya oil batch. After 24 h at 25 °C, liposomes prepared from birds fed tallow were less oxidised (21.4 nmole eq MDA mg^{-1} PL) than those from fed soya oil (30.9 nmole eq MDA mg^{-1} PL) or rapeseed oil (30.3 nmole eq MDA mg^{-1} PL). This result can be related to the similar proportion of LC PUFA (22.6 and 23.5%), and peroxidisability index of PL of birds fed rapeseed and soya oil (127.2 and 118.3, respectively).

Our observations on TBA-RS produced during oxidation of phospholipids of turkeys fed tallow show similar trends to results obtained with meat from turkeys and others animals fed fats rich in SFA or MUFA and poor in PUFA. Oxidation induced on homogenates of turkey muscle was lowest in the case of birds fed tallow and similar in the case of birds fed rapeseed or soya oil.[44] Olive-oil-fed broilers were found to be less susceptible to oxidation than soybean-oil- or linseed-oil-fed broilers.[5] In the latter, the peroxidisability index of PL were, respectively, 108.5 for PL of broilers fed olive oil, 124.7 for PL of broilers fed soya and 147.7 for those fed linseed oil. Similarly, when tallow was substituted for soya oil in pig diets, the peroxidisability index of muscle PL increased from 76.6 to 95.0 as well as susceptibility of muscle to oxidation, induced by a catalyst or by cooking, and, hence, meat shelf life was reduced.[20]

Our results concerning the similar levels of TBA-RS in liposomes prepared with PL of turkeys fed rapeseed or soya oil after 5 and 24 h of oxidation apparently do not agree with studies showing that TBA numbers in meat or isolated membranes of broilers fed a fat containing large proportions of n-3 FA as linseed oil were higher than the corresponding samples from broilers fed partially hydrogenated soybean oil.[5] Muscle homogenates from broilers fed sunflower oil were also more stable towards oxidation than homogenates from linseed-oil-fed animals.[45] We attribute these apparent discrepancies to the actual fatty acid composition of lipids and especially to their peroxidisability index. Indeed, in our study PL of turkeys fed rapeseed or soya oil exhibited similar PI (127.2 and 118.3), while calculated PI of PL of broilers fed linseed oil or partially hydrogenated soybean oil[5] were 147.7 and 124.7, respectively, and PI of total lipids for broilers fed sunflower or linseed oil[45] were 45.3 and 89.3 and consequently different. Final quantities of TBA-RS correspond to an overall measurement of secondary products of lipid oxidation. In the present study, TBA-RS were in the same order range for soya and rapeseed oil batches in agreement with their PI and were increased according to the increase of peroxidisability index of lipids in other studies.[5,20,45]

Volatile compounds

Forty-seven volatiles were identified in the headspace of the liposomes after 24 h of incubation with the catalyst (Table 4). The same volatiles were generated throughout the oxidation of turkey phospholipids irrespective of the diet. These compounds are typical fatty acid oxidation products.[23–25,35] With respect to the high proportion of n-6 fatty acids in phospholipids, hexanal was by far the major volatile whatever the diet. Its proportion varied from 50.9% of total volatiles for the PL prepared from the rapeseed-oil-fed batch to 60% for PL from the soya-oil-fed batch. Other major compounds arising from n-6 fatty acids were 1-octen-3-ol, 1-octen-3-one and *E*-2-heptenal. FA of n-3 family led to 1-penten-3-one, 1-penten-3-ol and isomers of 2,4-heptadienal as major compounds. FA of n-9 family gave rise mainly to saturated aldehydes such as nonanal.

One-way ANOVAs were performed on the 52 individual variables related to volatile compounds consisting of 47 individual volatiles, the sum of compounds of unknown origin, the sums of com-

Table 4. Volatile compounds identified in the headspace of liposomes prepared from phospholipids of breasts of turkeys fed different dietary fats and incubated 24 h in the dark at 25 °C with $FeCl_3$/AsNa (1/1, 45 μM)

Variable number	Compound	Dietary fat: Rapeseed oil	Tallow	Soya oil
Volatile compounds of unknown origin				
1	undecanal	37b	11a	5a
2	2,4-octadienal	6b	2a	3a
3	*E,Z*-2,4-nonadienal	18b	9a	14ab
4	*E,E*-2,4-nonadienal	36	41	50
5	hexanol	8b	2a	2a
6	3-penten-2-one	7b	3a	2a
7	toluene	9	15	tr
8	xylene	4b	1a	tra
9	2-heptanone	5	5	6
10	nonane	6	4	4
11	benzaldehyde	5a	6a	9b
12	2,3-octanedione	128a	108a	156b
13	3-octen-2-one	14a	11a	33b
14	total unknown	289b	221a	285b
Volatile compounds from n-3 FA				
15	2-butenal	18b	5a	8a
16	*Z*-2-pentenal	23b	18ab	11a
17	*E*-2-pentenal	90b	50a	59a
18	*Z*-2-hexenal	51	26	5
19	*E*-2-hexenal	29b	19a	23a
20	*E,Z*-2,4-heptadienal	151c	70a	108b
21	*E,E*-2,4-heptadienal	68b	33a	39a
22	2,6-nonadienal	7b	3a	2a
23	1-penten-3-ol	102b	36a	30a
24	1-penten-3-one	227b	119a	169a
25	2-ethyl-furan	8b	2a	4a
26	3,5-octadien-2-one	74	51	55
27	total n-3	849b	432a	514a
Volatile compounds of n-6 FA				
28	pentanal	698	590	786
29	hexanal	4848a	4375a	6475b
30	*Z*-2-heptenal	13b	7a	5a
31	*E*-2-heptenal	210	188	236
32	*Z*-2-octenal	45	41	49
33	*E*-2-octenal	371ab	286a	401b
34	*E*-2-nonenal	42b	52b	30a
35	*E,Z*-2,4-decadienal	140	138	141
36	*E,E*-2,4-decadienal	173	157	183
37	pentanol	249	193	241
38	1-octen-3-ol	349b	216a	322b
39	1-octen-3-one	356b	235a	367b
40	2-pentyl-furan	112ab	97.2a	129b
41	total n-6	7607a	6576a	9365b
Volatile compounds from n-9 FA				
42	heptanal	108ab	125b	87a
43	octanal	128	135	92
44	nonanal	267b	367b	139a
45	decanal	48	51	53
46	*E*-2-decenal	58b	52b	28a
47	2-undecenal	36	41	35
48	heptanol	13	12	8
49	octanol	36b	24a	39b
50	heptane	50	37	39
51	total n-9	743b	842b	519a
52	Total volatiles	9483ab	8068a	10679b

Results were means of eight determinations and were expressed inng eq nonanemg^{-1}, PL. In a row following different letters were significantly different ($P < 0.05$). Variable numbers and related chemical names of compounds allowed the identification of number used in Fig 3 (two-dimensional plot of components weights).

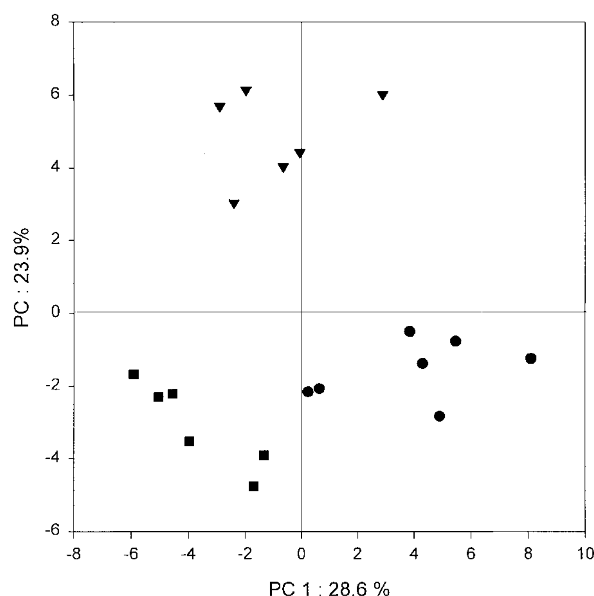

Figure 2. Plot of the observations with respect to the first two principal components (PC): ▼, rapeseed oil; ●, soya oil; ■, tallow fed turkey breasts.

pounds arising from oxidation of n-6, n-3 and n-9 FA and finally the sum of all volatiles. The analysis highlighted a significant effect of the dietary fat ($P < 0.05$) for 35 variables, but comparison of means (Newman–Keuls test) only distinguished the three groups in the case of the quantities of *E,Z*-2,4-heptadienal. For others variables, one of the three groups was alternately different. Consequently, the overall impact of the fatty acid composition of PL on the volatile compounds is difficult to appreciate from this monovariate analysis. Another deficiency of ANOVA is to ignore relationships between variables. To overcome these difficulties, we performed a PCA with the 52 variables previously analysed with ANOVA.

The two first principal components accounted for 52.5% of the variance. On the 1–2 PC map, three groups of observations were unambiguously distinguished according to dietary fat (Fig 2), whereas this distinction was hard to see by ANOVA. This result highlights that the changes in fatty acid composition which were induced by diet entailed modifications of the profiles of volatile compounds formed through lipid oxidation and not only to an overall change of the extent of lipid oxidation as shown by TBA measures. On the 1–2 PC map, observations related to rapeseed oil batch were positively correlated with axis 2 and slightly correlated with axis 1. Conversely, observations of tallow and soya oil batches were negatively correlated with axis 2, and separated on axis 1. These results can be linked to a correlation circle (Fig 3).

Most of the volatile compounds arising from oxidation of n-9 FA (42 to 51) were gathered and slightly correlated to axis 1 and 2. In contrast, nonanal (44) was negatively correlated to axis 1, and octanol (49) was strongly positively correlated to axis 1 and to

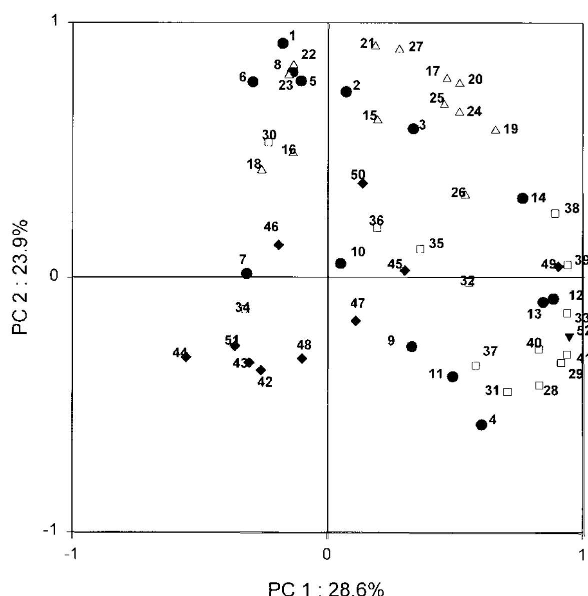

Figure 3. Two-dimensional plot of components weights with respect to the first two, PCs. ●, volatile compounds of unknown origin; △, volatile compounds of n-3, FA; □, volatile compounds of n-6, FA; ◆, volatile compounds of n-9, FA; ▼, total quantity of volatile compounds. Variable number are associated to volatile compounds as indicated in Table 4.

volatile compounds arising from oxidation of n-6 FA such as 1-octen-3-one (39).

Most of the volatile compounds arising from the oxidation of n-6 FA were strongly correlated to axis 1 and slightly to axis 2. *Z*-2-octenal (32), *E,Z*-2,4-decadienal (35) and *E,E*-2,4-decadienal (36), which were not affected by the dietary fat as shown by ANOVA (Table 4), were slightly correlated with axis 1 and 2. *Z*-2-heptenal (30) exhibited an atypical behaviour as it was negatively correlated to axis 1 and positively to axis 2. Finally, *E*-2-nonenal (34) was negatively correlated to axis 1 and close to volatile compounds arising from oxidation of n-9 FA.

Volatile compounds arising from the oxidation of n-3 FA generally had a strong positive correlation to axis 2 and gathered in the same area. Only 3,5-octadien-2-one (26) was slightly correlated to axis 2 and to axis 1 and was opposite to *Z*-2-pentenal (16) and *Z*-2-hexenal (18).

Volatile compounds of unknown origin had heterogeneous behaviour. A first group can be drawn with compounds correlated with volatiles of n-6 origin. It contained 2,3-octanedione (12), 3-octen-2-one (13), benzaldehyde (11), *E,E*-2,4-nonadienal (4), and the total amount of volatile compounds of unknown origin (14). Undecanal (1), 3-penten-2-one (6), xylene (8), hexanol (5), 2,4-octadienal (2) and *E,Z*-2,4-nonadienal (3) made up a second group which was related to volatile compounds arising from oxidation of n-3 FA. Toluene (7) was close to volatile compounds arising from oxidation of n-9 FA. Finally, nonane (10) and 2-heptanone (9) were poorly correlated to axis 1 and 2 and not related with other volatile compounds.

Overall, volatile compounds arising from the oxidation of a series of FA (n-6, n-3, n-9) were well correlated. Some volatile compounds had an atypical behaviour, which remains unexplained.

As a consequence of the distribution of volatile compounds on the correlation circle, volatile compounds arising from oxidation of n-6 FA and nonanal (44) distinguished observations of the soya oil batch from those of tallow batch on axis 1. Observations related to the rapeseed oil batch possessed intermediate position on axis 1 and were clearly distinguished from observations of soya oil and tallow batches on axis 2. This distinction was mainly the consequence of volatile compounds arising from oxidation of n-3 FA. This result agrees with ANOVA (Table 4), and can be directly related to the FA composition (Table 3) of the PL of the three batches.

To summarise these results, PCA shows that slight modifications of FA composition of PL led to sufficient modifications in the profiles of volatiles generated through their oxidation to allow a clear distinction of samples according to the nature of fat added to the diet. The most obvious change was not the formation of specific compounds but rather a balance between the quantities of volatiles originated from the different FA.

Our results can be connected to those obtained by Larick *et al*[46] on meat. They found that a high 18:2 level in the diet led to a higher content of 18:2 in porcine lean tissue and to higher concentrations of aldehydes, especially pentanal and hexanal, in freshly cooked meat. To our knowledge, it is the first time that a direct relationship has been established between the fatty acid composition of phospholipids of natural origin and the amounts of volatiles generated through their induced oxidation. Our results are important regarding olfactive characteristics of volatile compounds and the likely consequences on flavour. For example, compounds generated from n-3 fatty acids possess fishy notes.[7,24,25] Their increased proportions in volatiles from PL of turkeys fed rapeseed oil can lead to changes in meat flavour and eventually to the appearance of off-flavours when meat is not consumed immediately after cooking.[19]

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