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Changes of intramuscular phospholipids and free fatty acids during the processing of Nanjing dry-cured duck

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Abstract

In this study, changes of intramuscular phospholipids and free fatty acids were tracked during the processing of Nanjing dry-cured duck. Phospholipids were identified and quantified by high performance liquid chromatography combined with UV and evaporative light scattering detectors. The types and quantities of free fatty acids and fatty acids derived from phospholipids were analyzed by capillary gas chromatography. The results showed that raw duck meat had high quantities of phosphatidylethanolamine, and phosphatidylcholine (37.95% and 54.07% of total phospholipids, respectively), which contained high percentages of polysaturated fatty acids. The percentages of total phospholipids, phosphatidylethanolamine, and phosphatidylcholine decreased during processing, with a concomitant increase in quantities of free fatty acids. The lipolysis of phospholipids, especially phosphatidylethanolamine is the main contributor to the increase of free fatty acids.

Keywords: Nanjing dry-cured duck; Phospholipids; Free fatty acids; Lipolysis

1. Introduction

Nanjing dry-cured duck is a well known local delicacy in Nanjing, China and has a history of over 300 years (Li, 1988). The annual production of Nanjing dry-cured duck reaches 4 million (Xiong, 2000). Similar to dry-cured Jinhua ham, dry-cured duck is produced by dry curing, marinating, piling and drying naturally but the period of its production is shorter than that of hams. Dry-cured duck is well accepted by consumers in China and Southeast Asia due to its delicate flavor and texture.

The biochemical changes that occur during the processing of dry-cured duck are directly associated with the final taste of the product. Intramuscular phospholipids and free fatty acids are two important factors in determining the meat flavor. Flavor development in meat and meat products are reported to be associated with phospholipid composition, the extent of lipolysis and oxidation of lipids and free fatty acids during processing (Chizzolini, Novelli, & Zanardi, 1998). Lipolysis and oxidation of phospholipids have been studied in dry-cured ham (Buscailhon, Gandemer, & Monin, 1994; Motilva, Toldrá, Nieto, & Flores, 1993; Wang, 2001), smoked and dried reindeer meat (Sampels, Pickova, & Wiklund, 2004), dry-cured pork loin and pickled pork loin (Hernandez, Navarro, & Toldrá, 1999) and also cooked sardine meat (Jittrepotch, Ushio, & Ohshima, 2006). However, such data are not available

Abbreviations: BF_3, boron trifluoride; RSD, residue standard deviation; UV, ultraviolet; N_2, nitrogen; HPLC, high performance liquid chromatography; GC, gas chromatography; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; PI, phosphatidyl inositol; PS, phosphatidyl serine; SPH, sphingomyelin; LPC, lysophosphatidyl choline.

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for dry-cured duck meat. Therefore, the objective of this study was to evaluate the changes of intramuscular phospholipids and free fatty acids, and their inter-relationships during the processing of Nanjing dry-cured duck.

2. Materials and methods

2.1. Chemicals

Phosphatidyl ethanolamine (PE), phosphatidyl choline (PC), phosphatidyl inositol (PI) phosphatidyl serine (PS), sphingomyelin (SPH) and lysophosphatidyl choline (LPC), fatty acids $C_{14:0}$, $C_{14:1}$, $C_{16:0}$, $C_{17:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{20:4}$, $C_{22:4}$ and $C_{22:6}$ standards were bought from Sigma–Aldrich Chemical Co. (St. Louis, MO); and methanol, *n*-hexane, 2-propanol and acetamide were chromatographic pure grade. Chloroform, acetic acid, diethyl ether, 2,2-dimethoxypropane, BF_3, NH_4Ac, NaCl and $CaCl_2$ were analytic pure grade.

2.2. Procedures for Nanjing dry-cured duck

Forty-two Cherry Valley ducks from a commercial feedlot were slaughtered humanely in a commercial meat processing company (Jiangsu Yurun Food Ltd.). After chilling (2 h), duck carcasses were dry-cured (salt content: 7% of carcass weight, 24 h), marinated in brine (saturated salt solution, 20 h), piled (48 h) and then dried at 17–18 °C in a well-ventilated room for 5, 10 and 15 days, respectively. At the end of each processing stage (including raw), six carcasses were selected for the biochemical analyses.

2.3. Lipid extraction

Duck leg muscles were removed from carcasses and trimmed of all visible subcutaneous fat and connective tissue. Lipids were extracted from muscle samples according to the method of Folch, Lees, and Stanley (1957) with small modifications. Briefly, 3.0 g of muscle sample was homogenized with 60 ml of chloroform/methanol (2/1, V/V) solution at 1500 rpm using an Ultra Turrax (T25, IKA, Germany). The homogenate was allowed to stand for 1 h and then pass through a layer of filter. After that, 0.2-fold its volume of a solution containing 7.3 g l^{-1} NaCl, and 0.5 g l^{-1} $CaCl_2$ was added to the filtrate. The mixture was centrifuged for 15 min at 3000 rpm (Allegra 64R, Beckman, USA) and the lower phase was dried under vacuum on a rotary evaporator (RE-85C, Yarong, China) in a 44 °C water bath and then stored at −20 °C.

2.4. Lipid extract fractionation

Phospholipids were separated from neutral lipids according to the procedure of García, Gibert, and Díaz (1994). Briefly, 20.0 mg of total lipid extract was dissolved in 1.0 ml of chloroform, and 0.5 ml of the solution was transferred into an aminopropyl-silica minicolumn (100MG, VARIAN, USA) that was activated with 1.0 ml of chloroform before transfer. The minicolumn was washed with 2.0 ml of chloroform/2-propanol (2/1, V/V) to remove hydrocarbons, cholesterol esters and triacylglycerols, and then free fatty acids were eluted with 3.0 ml of 2% acetic acid in diethyl ether (W/W). Finally, phospholipids were eluted with 3.0 ml of methanol. The solvent was removed by rotary evaporation and the residue was dissolved in 0.3 ml of mobile phase C solution (hexane/2-propanol/water, 120/80/11, V/V/V) for HPLC analysis.

2.5. Phospholipids identification

The sample was analyzed in an Agilent 1100 HPLC system using a Lichrosorb SI 60-5 silica gel column (5 μm, 250 mm × 4.0 mm i.d) operating at 30 °C. A gradient elution was carried out at a flow rate of 1.0 ml min^{-1} using different ratios of solutions A (*n*-hexane/2-propanol, 3/2, V/V), B (*n*-hexane/2-propanol/25 mmol l^{-1} NH_4Ac, 120/80/11, V/V/V), and C (*n*-hexane/2-propanol/H_2O, 120/80/11, V/V/V). The best separation was obtained using the following gradient: from 0 to 5 min, B was increased from 0% to 50%; from 5 to 30 min, B was increased from 50% to 100%; from 30 to 45 min, B was kept constant at 100%; from 45 to 50 min, C was increased from 0% to 100%; from 50 to 60 min, C was kept constant at 100%; from 60 to 62 min, A was increased from 0% to 100%; from 62 to 70 min, solution A was kept constant at 100%. Chromatographic peaks were detected with a UV detector and ELSD, which were installed in series; the UV absorbance was measured at 205 nm, and the ELSD was run at 70 °C with N_2 at 1.8 l min^{-1} (Wang, Xu, & Xu, 2006).

2.6. Fatty acid composition of phospholipids and free fatty acids

One hundred microlitre of phospholipid or free fatty acids elute was evaporated to remove the solvent. The residue was mixed with 2.0 ml of 14% BF_3/methanol. One hundred microgram per milliliter of heptadecanoic acid was added to the mixture as an internal standard. And then the mixture were methylated at 60 °C for 30 min. Thereafter, 2.0 ml of 2,2-dimethoxypropane was added to remove water that is produced during methylation. After cooling, 1.0 ml of water and 1.0 ml of *n*-hexane were added and shaken for several minutes. The resulting mixture was allowed to stand for 1 h and then the upper organic phase was dried by rotary evaporation under N_2. The residue was dissolved in 0.4 ml of hexane for GC analysis.

The methylated fatty acids were analyzed with a gas chromatograph (GC-14B, Shimadzu, Japan) equipped with a flame ionization detector and a split injector. One point five microlitre of the sample was injected onto a capillary column (CP-Sil 88 for Fame, 50 m × 0.25 mm × 0.20 μm, Varian, USA) containing a non-polar stationary phase (5% phenylmethyl/95% siloxane). The oven temperature

increased from 160 °C to 220 °C at 6 °C/min and maintained for 30 min at 220 °C. The detector temperature was maintained at 280 °C. The carrier gas was nitrogen and its pressure was maintained at 80 KPa. The peaks were identified by comparing their retention times with those of the standards. The relative percentages of fatty acids were determined by the peak areas (Gandemer, 2002; Gandemer, Morvan-mahi, Meynier, & Leperq, 1991).

2.7. Statistical analyses

The changes of lipid composition, fatty acid composition of phospholipids, and free fatty acids were evaluated by one-way analysis of variance techniques where these measurements were as dependent variables and the processing stage as independent variables. And means of the measurements at different processing stages were compared using the Duncan's multiple-range test at the significance level of 0.05. Correlation coefficients among all the variables were evaluated by descriptive analysis of correlations. All statistical analyses were performed by SAS8.12 (SAS Inst. Inc. Cary, NC, 2001).

3. Results and discussion

3.1. Changes of lipid fractions

Table 1 shows the contents of the different lipid fractions (phospholipids, free fatty acids and triacylglycerols) expressed as percentages of the total lipids. The percentages of free fatty acids showed a great increase through the processing of dry-cured duck which is also observed in the dry-cured hams (Buscailhon et al., 1994), dry-cured sausages (Navarro, Nadal, Izquierdo, & Flores, 1997) and dry-cured loins (Hernandez et al., 1999). As the muscle enzyme systems play an important role in the generation of free fatty acids (Motilva, Toldrá, & Flores, 1992), the increase in the amounts of free fatty acids could be the result of the action of lipolytic enzymes.

The total percentage of triacylglycerols was also found to increase significantly at the marinating and drying stages, corresponding to a significant decrease in the total percentage of phospholipids. Buscailhon et al. (1994) did not find any differences in the amounts of glycerides in dry-cured ham, whereas they found a decrease in the amount of phospholipids. Hernandez et al. (1999) observed that either glycerides or phospholipids varied little during the processing of dry-cured loins. The difference between the present study and mentioned literatures is probably due to the different origins of experimental materials and also to the different processing periods.

3.2. Changes of intramuscular phospholipids

The role of intramuscular phospholipids in flavor formation and rancidity of traditional meat products was reviewed by Chizzolini et al. (1998). Figs. 1 and 2 show the changes of intramuscular phospholipids: PE, PI, PS, PC, SPH, LPC through the processing of dry-cured duck. In raw duck meat, phospholipids accounted for 45.72%

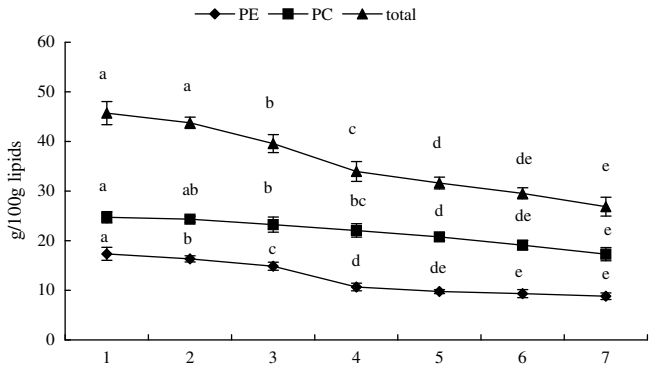

Fig. 1. Changes of PE, PC and total phospholipids through the process of salted duck. 1, raw; 2, dry salted; 3, marinated; 4, piled; 5, dried for 5 days; 6, dried for 10 days; 7, dried for 15 days.

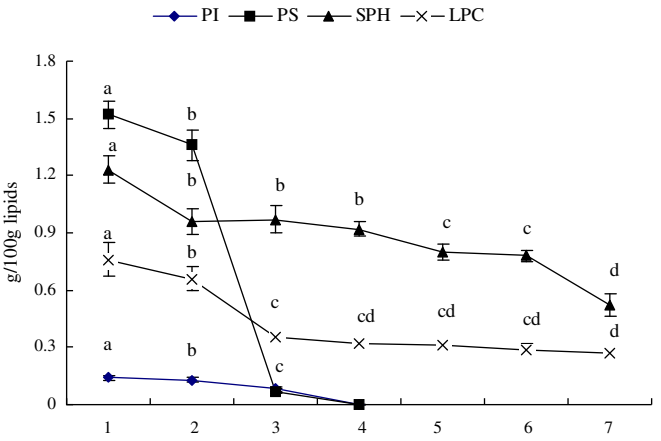

Fig. 2. Changes of PI, PS, SPH and LPC through the process of salted duck. 1, raw; 2, dry salted; 3, marinated; 4, piled; 5, dried for 5 days; 6, dried for 10 days; 7, dried for 15 days.

Table 1
Changes of phospholipids, triacylglycerols, and free fatty acids in leg muscles of Nanjing dry-cured duck at different processing stages (g/100 g lipids)

Items	Raw	Dry curing	Marinating	Piling	Drying, 5d	Drying, 10d	Drying, 15d
Phospholipids	45.72 ± 2.33^{a}	43.76 ± 1.16^{a}	39.56 ± 1.82^{b}	33.95 ± 1.98^{c}	31.63 ± 1.19^{d}	29.53 ± 1.12^{de}	26.85 ± 1.92^{e}
FFA	5.86 ± 0.34^{a}	8.10 ± 0.38^{b}	6.72 ± 0.32^{c}	9.83 ± 0.48^{d}	12.15 ± 0.54^{e}	13.35 ± 0.61^{ef}	13.99 ± 0.63^{f}
Triacylglycerols	48.42 ± 2.74^{a}	48.14 ± 2.33^{a}	53.72 ± 3.89^{b}	56.22 ± 4.17^{bc}	56.22 ± 4.26^{bc}	57.12 ± 4.32^{cd}	59.16 ± 5.12^{d}

^{a,b,c,d,e,f} Means in the same row with different letters differ significantly ($P < 0.05$).

of total lipids. PE and PC accounts for the largest quantities of phospholipids (17.35, 24.72 g/100 g lipids, respectively) and PI the smallest (only 0.14 g/100 g lipids). PS, SPH and LPC lie in the middle and account for 1.52, 1.23 and 0.76 g/100 g lipids, respectively. This is in agreement with the study of Hernandez et al. (1999) who reported that PC accounted for 53.5–60.3% of the phospholipid fraction in pork loin, and 12.1–16.2% for PI + PS.

All of six phospholipids decreased ($P < 0.05$) in quantity during the processing, which indicates that they underwent hydrolysis, especially enzymatic lipolysis (Toldrá, Flores, & Sanz, 1997). For PE, PI and PS, the lipolysis occurred primarily at the marinating and piling stages. In fact, PI and PS had been completely hydrolyzed at piling stage. The quantities of SPH declined at the dry-salting stage and also at the final period of drying stage. The decrease in the quantity of LPC mainly took place at dry-salting and marinating stages. During the processing, the percentage of PE decreased by 50% while that of PC declined by 30%, which indicated that PE was more susceptible to hydrolysis or oxidation than PC. Hernandez et al. (1999) ascribed the greater susceptibility of PE to oxidation to its high content of polyunsaturated fatty acids and plasmalogen, which are very sensitive to hydrolysis. Phospholipases A_1 and A_2 could play an important role in hydrolysis of the major phospholipids in dry-cured duck, where they catalyze the hydrolysis of ester bonds in glycerophospholipids (in positions 1 and 2, respectively), releasing free fatty acids (Arthur & Choy, 1987). In addition, the increase of free fatty acids could also be partially associated with increased activities of acid and neutral lipases due to dehydration and salt diffusion (Vestergaard, Schivazappa, & Virgili, 2000).

3.3. Changes of fatty acids of phospholipids

Table 2 lists the quantities of fatty acids that are composed of phospholipids during the processing of dry-cured duck. In raw duck meat, arachidonic acid ($C_{20:4}$) was the most abundant and accounted for one quarter of the total fatty acids from phospholipids, which is characteristic of fatty acid composition in duck meat (Cobos, Veiga, & Diaz, 2000). Except for arachidonic acid, stearic ($C_{18:0}$), oleic ($C_{18:1}$), and palmitic ($C_{16:0}$) acids were the major fatty acids that comprised of phospholipids (18.89%, 17.32%, and 16.41%, respectively). The total quantity of saturated fatty acids increased greatly, corresponding to a substantial decrease in the total quantity of polyunsaturated fatty acids due to faster hydrolyze through the processing of dry-cured duck. However, monounsaturated fatty acids had a slight ($P > 0.05$) decrease. If each single fatty acid was concerned, a distinct decline ($P < 0.05$) was observed in the percentages of docosatetraenoic ($C_{22:4}$), docosahexaenoic (DHA, $C_{22:6}$), linoleic ($C_{18:2}$) and arachidonic ($C_{20:4}$) acids, concomitant with a marked increase ($P < 0.05$) in myristic and stearic acids. This further indicates that phospholipids composed of polyunsaturated fatty acids are faster hydrolyzed than those comprising of saturated and monounsaturated fatty acids. According to data in Tables 1 and 2, the extent of degradation of different types of fatty acids was calculated as follows: the polyunsaturated fatty acids were degraded in the highest quantity (69.6%), followed by monounsaturated (51.2%) and saturated (7.5%) fatty acid. Yang, Ma, & Qiao (2005) also found such a selective degradation when they studied lipolysis in Xuanwei hams. But other studies found that lipolysis in phospholipid was not specific to fatty acid chain length

Table 2
Fatty acid profiles of phospholipids in leg muscles of Nanjing dry-cured duck at different processing stages (%)

Fatty acids	Raw	Dry-salting	Marinating	Piling	Drying, 5d	Drying, 10d	Drying, 15d
Myristic $C_{14:0}$	3.78 ± 0.21^{a}	4.79 ± 0.25^{b}	5.37 ± 0.35^{bc}	5.78 ± 0.44^{bc}	6.54 ± 0.31^{cd}	7.79 ± 0.26^{de}	8.23 ± 0.31^{e}
Myristoleic $C_{14:1}$	4.79 ± 0.24^{a}	4.54 ± 0.17^{a}	4.32 ± 0.28^{a}	3.78 ± 0.31^{ab}	3.54 ± 0.29^{ab}	3.23 ± 0.40^{b}	3.1 ± 0.34^{b}
Palmitic $C_{16:0}$	16.41 ± 1.58^{a}	16.79 ± 1.39^{a}	17.13 ± 0.85^{ab}	17.83 ± 1.54^{b}	18.12 ± 0.94^{bc}	19.46 ± 1.17^{cd}	19.79 ± 1.37^{d}
Stearic $C_{18:0}$	18.89 ± 0.93^{a}	21.87 ± 1.52^{b}	23.8 ± 1.34^{bc}	25.56 ± 2.37^{c}	29.76 ± 1.69^{d}	31.32 ± 1.53^{de}	33.54 ± 2.19^{e}
Oleic $C_{18:1}$	17.32 ± 1.83^{a}	16.97 ± 1.24^{ab}	16.48 ± 0.64^{ab}	16.29 ± 0.94^{ab}	15.87 ± 1.26^{ab}	15.43 ± 0.76^{b}	15.26 ± 1.11^{b}
Linoleic $C_{18:2}$	7.60 ± 0.41^{a}	6.81 ± 0.37^{ab}	6.27 ± 0.42^{b}	5.83 ± 0.34^{bc}	5.12 ± 0.47^{c}	4.54 ± 0.72^{cd}	4.32 ± 0.35^{d}
Arachidonic $C_{20:4}$	25.14 ± 1.23^{a}	24.01 ± 0.98^{a}	23.79 ± 2.36^{ab}	23.27 ± 2.81^{ab}	20.14 ± 2.81^{b}	17.7 ± 1.25^{c}	15.32 ± 1.12^{d}
Docosatetraenoic $C_{22:4}$	2.79 ± 0.31^{a}	1.48 ± 0.25^{b}	0.74 ± 0.22^{c}	0.23 ± 0.21^{d}	0.37 ± 0.19^{d}	0.24 ± 0.23^{d}	0.21 ± 0.20^{d}
Docosahexaenoic $C_{22:6}$	3.28 ± 0.36^{a}	2.74 ± 0.19^{b}	2.1 ± 0.32^{c}	1.43 ± 0.24^{d}	0.54 ± 0.21^{e}	0.29 ± 0.17^{ef}	0.23 ± 0.21^{f}
ΣSFA	39.08 ± 2.72^{a}	43.45 ± 3.16^{b}	46.3 ± 2.54^{bc}	49.17 ± 4.35^{c}	54.42 ± 2.94^{d}	59.2 ± 2.96^{e}	61.56 ± 3.87^{e}
ΣMUFA	22.11 ± 2.07^{a}	21.51 ± 1.41^{a}	20.8 ± 0.92^{ab}	20.07 ± 1.25^{ab}	19.41 ± 1.57^{b}	18.66 ± 1.16^{b}	18.36 ± 1.45^{b}
ΣPUFA	38.81 ± 2.31^{a}	35.04 ± 1.79^{b}	32.9 ± 3.32^{bc}	30.76 ± 3.6^{c}	26.17 ± 3.68^{d}	22.14 ± 2.37^{e}	20.08 ± 1.88^{e}

[a,b,c,d,e] Means in the same row with different letters differ significantly ($P < 0.05$).

Table 3
Free fatty acids in leg muscles of Nanjing dry-cured duck at different processing stages (mg/g lipids)

Fatty acids	Raw	Dry-salting	Marinating	Piling	Drying, 5d	Drying, 10d	Drying, 15d
Myristic $C_{14:0}$	5.81 ± 0.41^{a}	6.01 ± 0.34^{ab}	5.10 ± 0.46^{c}	6.26 ± 0.51^{ab}	6.39 ± 0.44^{b}	6.51 ± 0.24^{b}	6.69 ± 0.48^{b}
Myristoleic $C_{14:1}$	1.74 ± 0.12^{a}	1.23 ± 0.11^{b}	1.17 ± 0.19^{b}	1.25 ± 0.13^{b}	1.28 ± 0.16^{b}	1.34 ± 0.28^{b}	1.41 ± 0.39^{ab}
Palmitic $C_{16:0}$	4.95 ± 0.37^{a}	9.11 ± 0.42^{b}	8.75 ± 0.56^{b}	12.43 ± 1.07^{c}	18.57 ± 1.25^{d}	21.54 ± 0.98^{e}	22.71 ± 1.98^{e}
Stearic $C_{18:0}$	8.64 ± 0.51^{a}	9.92 ± 0.73^{b}	8.37 ± 0.49^{a}	12.16 ± 1.10^{c}	20.34 ± 1.45^{d}	24.20 ± 2.17^{e}	25.41 ± 1.64^{e}
Oleic $C_{18:1}$	13.58 ± 0.91^{a}	18.26 ± 1.29^{b}	15.48 ± 1.30^{c}	20.38 ± 1.86^{b}	21.02 ± 1.99^{bd}	22.22 ± 1.09^{d}	23.91 ± 2.04^{d}
Linoleic $C_{18:2}$	14.69 ± 1.17^{a}	19.92 ± 1.49^{b}	17.04 ± 1.19^{c}	27.99 ± 1.93^{d}	30.59 ± 2.14^{e}	32.72 ± 2.37^{ef}	33.53 ± 1.58^{f}
Arachidonic $C_{20:4}$	8.09 ± 0.52^{a}	15.18 ± 1.38^{b}	10.05 ± 0.81^{c}	15.95 ± 1.14^{b}	21.40 ± 1.92^{d}	23.67 ± 0.85^{de}	25.19 ± 1.62^{e}
Docosatetraenoic $C_{22:4}$	0.64 ± 0.12^{a}	0.91 ± 0.18^{b}	0.83 ± 0.21^{ab}	1.25 ± 0.46^{b}	1.06 ± 0.37^{b}	0.93 ± 0.10^{b}	0.67 ± 0.21^{a}
Docosahexaenoic $C_{22:6}$	0.43 ± 0.11^{a}	0.45 ± 0.20^{a}	0.41 ± 0.19^{a}	0.62 ± 0.13^{ab}	0.88 ± 0.29^{b}	0.39 ± 0.25^{a}	0.34 ± 0.19^{a}
ΣSFA	19.4 ± 1.14^{a}	25.04 ± 1.84^{b}	22.22 ± 1.17^{c}	30.84 ± 2.68^{d}	45.3 ± 3.41^{e}	52.25 ± 4.16^{f}	54.81 ± 3.42^{f}
ΣMUFA	15.32 ± 0.94^{a}	19.49 ± 1.13^{b}	16.65 ± 0.99^{a}	21.53 ± 1.54^{c}	22.3 ± 1.43^{c}	23.56 ± 1.94^{cd}	25.32 ± 1.73^{d}
ΣPUFA	23.85 ± 1.95^{a}	36.46 ± 2.08^{b}	28.33 ± 1.69^{c}	45.81 ± 2.81^{d}	53.93 ± 3.44^{e}	57.71 ± 2.98^{ef}	59.73 ± 4.11^{f}

^{a,b,c,d,e,f} Means in the same row with different letters differ significantly ($P < 0.05$).

Table 4
Correlation coefficients between the decline of phospholipids and the increase of free fatty acids in leg muscles through the processing of Nanjing dry-cured duck

	$C_{14:0}$	$C_{14:1}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:4}$	$C_{22:4}$	$C_{22:6}$	ΣSFA	ΣMUFA	ΣPUFA	ΣFFA
PE	0.80^{***}	0.08	0.07	0.01	0.58^{**}	0.90^{***}	0.37^{*}	0.74^{***}	0.43^{*}	−0.14	0.32	0.15	0.13
PC	−0.13	0.26	0.64^{**}	0.73^{***}	0.48^{*}	−0.19	0.15	−0.34	0.54^{**}	−0.11	0.55^{**}	0.71^{***}	0.63^{**}
Total	0.37^{*}	0.17	0.60^{**}	0.58^{**}	0.02	0.44^{*}	0.48^{*}	0.29	0.75^{***}	-0.39^{*}	0.70^{**}	0.69^{**}	0.52^{*}

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

or unsaturation during the processing of dry-cured hams (Buscailhon et al., 1994) and pork loins (Hernandez et al., 1999), which maybe results from the different processing methods for different meat products.

3.4. Changes of free fatty acids

Total free fatty acids, whether saturated, monounsaturated or polyunsaturated fatty acids, increased ($P < 0.05$) greatly through the processing of dry-cured duck, except a decline at the marinating stage. This decline could result from the diffusion of fatty acids in the duck meat into the curing solution. However, it was very complex for individual fatty acids due to the oxidation. There was a relatively small ($P < 0.05$) change for the contents of myristic and myristoleic acids, compared with those of palmitic, stearic, oleic, linoleic and arachidonic acids. The docosatetraenoic and docosahexaenoic acids increased slightly before drying but decreased gradually at drying stage (Table 3). Correlation analyses showed that the increase in the content of free fatty acids derived mainly from the lipolysis of phospholipids (Table 4). For example, myristic, oleic, linoleic, and docosatetraenoic acids derived mainly from the degradation of PE. Palmitic, stearic and docosahexaenoic acids originated primary from the degradation of PC. Similar conclusions had been put forward by several other researchers (Buscailhon et al., 1994; Hardy, McGill, & Gustone, 1979; Hernandez et al., 1999; Sharma, Kowale, & Joshi, 1982).

In a summary, the HPLC method combined with UV and ELSD detectors was proven to be an effective method quantifying the phospholipids in the Nanjing dry-cured duck meat. PE and PC were found to be major components of phospholipids in the duck meat. The contents of phospholipids, fatty acids and triacylglycerols varied greatly during the processing of Nanjing dry-cured duck. The lipolysis of phospholipids, especially that of PE was the main contributor to the increase of free fatty acids. This study could be useful to further understand the role of phospholipids in the formation of meat flavor of Nanjing dry-cured duck.

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