Asian-Aust. J. Anim. Sci.
Vol. 22, No. 10 : 1441 - 1446
October 2009

www.ajas.info

Comparative Study of Intramuscular Phospholipid Molecular Species in Traditional Chinese Duck Meat Products*

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ABSTRACT : Composition of intramuscular lipids, phospholipid classes and phospholipid molecular species in traditional Chinese duck meat products was investigated. Free fatty acids and phospholipids were identified and quantified by gas and high performance liquid chromatography, and phospholipid molecular species were determined by mass spectrometry. The results showed that raw duck meat had high quantities of phosphatidylethanolamine and phosphatidylcholine. The percentages of phospholipid classes decreased during three kinds of processing of duck meat products. A selective degradation of phospholipid molecular species with polyunsaturated fatty acids was found in dry-cured duck, but was not found in roasted and water-boiled duck products. (**Key Words :** Duck Meat Products, Phospholipids, Molecular Species)

INTRODUCTION

Traditional Chinese duck meat products (dry-cured duck, roasted duck and water-boiled salted duck) are well accepted by consumers in China and Southeast Asia due to their deliciousness (Liu et al., 2006; Ali, et al., 2007b; Xu et al., 2008). In Nanjing city where these products were originally developed, about thirty million ducks are consumed annually (Liu et al., 2006).

The amount of intramuscular phospholipids in the meat is an important factor (García et al., 1994; Ali, et al., 2007a; Sasaki et al., 2008; Choi, 2009) for the flavor and nutritive quality of fresh, cocked and dry-cured meat products. 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 et al., 1998). Therefore, the research concerning the composition and changes of intramuscular phospholipids is of particular interest. Lipolysis and oxidation of phospholipids have been studied in dry-cured ham (Motilva et al., 1993; Buscailhon et al., 1994), smoked and dried reindeer meat (Sampels et al., 2004), dry-cured pork loin and pickled pork loin (Hernandez et al., 1999), microwave heated soybeans (Takagi et al., 1999), cooked sardine meat (Jittrepotch et al., 2006), and cooked pork meat (Boselli et al., 2008). However, such data are not available for duck meat products. Therefore, the objective of this study was to evaluate the changes of intramuscular lipids and phospholipids, especially phosphatidylcholine and phosphatidylethanolamine which were the main components of phospholipids. This study could be useful to understand changes in phospholipids and the role of phospholipids in the flavor of duck meat products.

* This study was funded by Natural Science Foundation Program of Jiangsu Province (BK2009329) and National Agricultural Transform Program of China (2008GB2C100111).

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Received February 26, 2009; Accepted May 7, 2009

MATERIALS AND METHODS

Chemicals

Phosphatidyl ethanolamine (PE), phosphatidyl choline (PC), phosphatidyl inositol (PI) phosphatidyl serine (PS), sphingomyelin (SPH) and lysophosphatidyl choline (LPC), fatty acids C14:0, C14:1, C16:0, C17:0, C18:0, C18:1, C18:2, C18:3, C20:4, C22:4 and C22:6 standards were bought from Sigma-Aldrich Chemical Co. (St. Louis, MO); and methanol, n-hexane, 2-propanol, acetonitrile and acetamide were chromatographic pure grade purchased from Lingfeng Reagent Co. Ltd. (Shanghai, China). Chloroform, acetic acid, diethyl ether, 2,2-dimethoxypropane, BF_3, NH_4Ac, NaCl and $CaCl_2$ were analytic pure grade.

Processing of traditional Chinese duck meat products

Twenty-four Gaoyou ducks from a commercial feedlot were slaughtered humanely in a commercial meat processing company (Jiangsu Yurun Food Ltd.), each of which was about 2.0 kg. After chilling for 2 h, six ducks each were processed to three kinds of meat products (dry-cured, roasted and water-boiled salted ducks), in accordance with Chinese traditional procedures. Dry-cured ducks were processed following the method of Xu (Xu et al., 2008), roasted ducks and water-boiled salted ducks of Wang (Wang et al., 2009a) and Liu (Liu et al., 2006). Biochemical analyses were performed on each of the three products as well as the raw duck meat.

Intramuscular lipid extraction

Biceps femoris muscles were removed from duck carcasses and trimmed of all visible subcutaneous fat and connective tissue. Total lipids were determined following the ISO recommended method 1443 (ISO, 1973).

Lipids were extracted from muscle samples according to the method of Folch (Folch et al., 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 1,500 rpm using an Ultra Turrax (T25, IKA, Germany). The homogenate was allowed to stand for 1hr and then passed through a layer of filter. After that, 0.2-fold its volume of a solution containing 7.3 g/L NaCl, and 0.5 g/L $CaCl_2$ was added to the filtrate. The mixture was centrifuged for 15 min at 3,000 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.

Free fatty acids and phospholipids purification

Phospholipids were separated from intramuscular lipids according to the procedure of García (García et al., 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 neutral lipids, and then free fatty acids were eluted with 3.0 ml of 2 g/100 g acetic acid in diethyl ether. Finally, phospholipids were eluted with 3.0 ml of methanol, and then were evaporated under N_2 to remove methanol. The method of quantification of free fatty acids was the same as Xu (Xu et al., 2008), determination of phospholipids was as following.

Identification and quantification of phospholipid classes

First, the sample of phospholipids was dissolved in 0.3 ml of mobile phase C (n-hexane/2-propanol/H_2O, 120/80/11, V/V/V) for HPLC analysis. Phospholipids were analyzed in an Agilent 1100 HPLC system (equipped with an autoinjector, HPLC workstation, UV detector, Palo Alto, CA, USA) 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 using different ratios of solutions A (n-hexane/2-propanol, 3/2, V/V), B (n-hexane/2-propanol/25mmol/L NH_4Ac, 120/80/11, V/V/V), and C. The best separation was obtained using the following gradient: from 0 to 5min, 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 an 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 (Wang et al., 2009b). Peaks were identified by comparison with known standards. For the quantitative analysis of phospholipids, a calibration curve for each phospholipid classes was obtained by injecting standard solutions of PC, PE, PC, PI, PS, SPH and LPC at five different concentrations.

Determination of PC and PE molecular species

The sample of PC and PE was prepared by Agilent 1100 HPLC system using a μ-Porasil semi-preparative silica gel column (10 μm, 300 mm×10 mm i.d.) operating at 30°C, with an injection volume of 200 μl. Chromatographic peaks were identified using UV absorbance at 205 nm. The programme of gradient elution was identical to that described in identification and quantification of phospholipid classes, but carried out at a flow rate of 3.0 ml/min. The identification of PC and PE was performed by comparing their retention times with standard samples. PC and PE were collected 5 times from the column outlet and were dried under vacuum with a rotary evaporator at 40°C. Two portions of 0.5 ml n-hexane were used to transfer the residues into a glass conical tube, then the n-hexane was evaporated under N_2 and 0.5 ml chloroform/methanol (1/17.5, V/V) was added. Finally, the samples of PC and PE were kept at -20°C for molecular species analysis.

A reconstructed HPLC-ELSD-MS (HPLC, Waters 2690; ELSD, Alltech 2000; MS, Waters Platform ZMD 4000) system was used to analyze PC and PE molecular species (Wang et al., 2009b). The separation was performed on the HPLC using a Symmetry C_{18} RP column (5 μm, 250×4.6 mm i.d.) operating at 25°C. A gradient elution was carried out using various ratios of solvent A (chloroform/methanol, 1/17.5, V/V) and solvent B (acetonitrile/water: 1/1, V/V).

The elution was begun with solvent A, which was maintained at 80% for 5 min, then increased to 100% in 5 min, and maintained at this level for 45 min. The flow rate was 1.0 ml/min and the injection volume was 5 μl. The HPLC system was coupled in parallel to both an electrospray-ionization mass spectrometer and an evaporative light scattering detector. The HPLC effluent was splitted: 0.25 ml/min entered the MS detector and 0.75 ml/min were delivered to the ELSD. The ELSD was run at 70°C with N_2 at 2.0 L/min. The mass detector was operated in positive ion electrospray ionization mode. The nebulizer gas and desolvation gas were nitrogen. The velocity of the solution entering the MS was 10 μl/min. Typical operating parameters were as follows: capillary voltage 3.3 kV, cone voltage 30 V, source temperature 100°C, desolvation temperature 400°C, gas flow 4.0 L/h, m/z range 200-1,000, and multiplier voltage of 700 V. The relative percentages of molecular species were determined by the peak areas.

Statistical analyses

Results were presented as mean±SD, content of total lipids, phospholipids, neutral lipids, free fatty acids and phospholipid classes were from 6 different samples, molecular species from 4, the value for each sample was the average of two replicate determinations of a single extract. The data of phospholipids, neutral lipids, free fatty acids, phospholipid classes, molecular species of PC and PE in duck meat products were analysed by one-way analysis of variance techniques where these measurements were as dependent variables and the duck meat product as independent variables. And means of the measurements in different duck meat products were compared using the Duncan's multiple-range test at the significance level of 0.05. Statistical analyses were performed by SAS software (version 9.1, Statistical Analysis System Inc., Cary, NC, USA, 2006).

RESULTS AND DISCUSSION

Comparison of lipid fractions

Table 1 shows the contents of the different lipid fractions (phospholipids, free fatty acids and neutral lipids) expressed as percentages of the total lipids in three kinds of duck meat products. The percentages of free fatty acids showed a great increase through the processing of dry-cured and roasted duck ($p<0.05$), but water-boiled salted duck only had smaller increase ($p>0.05$). During the processing of dry-cured duck, as the muscle enzyme systems play an important role in the generation of free fatty acids (Motilva et al., 1993), the increase in the amounts of free fatty acids could be the result of the action of lipolytic enzymes. While the action of lipolytic enzymes in roasted and water-boiled salted duck was limited for the processing temperature was over 85°C. The percentage of neutral lipids was also found to increase significantly in dry-cured and roasted duck ($p<0.05$), corresponding to a significant decrease in the total percentage of phospholipids ($p<0.05$). Buscailhon (Buscailhon et al., 1994) did not find any differences in the amounts of neutral lipids in dry cured ham, whereas they found a decrease in the amount of phospholipids. Bosellin (Bosellin et al., 2008) only found small differences of phospholipids in cooked pork, but Hernandez (Hernandez et al., 1999) observed that neither neutral nor phospholipids varied much during the processing of dry cured loins. The difference between the present study and mentioned literatures was probably due to the different origins of experimental materials and also to the different processing methods.

Comparison of intramuscular phospholipid classes

Table 2 shows the content of intramuscular phospholipid classes in different duck meat products. In raw duck meat, PE and PC accounted for the largest quantities of phospholipids (64.58%, 28.16%, respectively) and PI the smallest (only 0.30%). PS, SPH and LPC accounted for 3.07%, 2.51% and 1.39%, respectively. Water-boiled salted duck had smaller decrease ($p>0.05$) of most phospholipid classes compared to dry-cured and roasted duck. All of six phospholipids markedly decreased ($p<0.05$) at the end of dry-cured duck processing, which indicated that they underwent hydrolysis, especially enzymatic lipolysis (Toldrá et al., 1997), as was also founded by Xu (Xu et al., 2008). Significant differences were found only for the content of SPH after the processing of water-boiled salted duck. Meanwhile, Boselli (Boselli et al., 2008) found no

Table 1. Content of total lipids, phospholipids, neutral lipids, and free fatty acids in biceps femoris muscles of different duck meat products

Lipids[e]	Raw duck	Dry-cured duck	Roasted duck	Water-boiled salted duck
Total lipids (g/100 g wet weight)	2.34±0.27[a]	3.58±0.41[b]	2.73±0.22[c]	2.91±0.18[c]
Phospholipids (g/100 g lipids)	46.73±3.49[a]	26.85±1.92[b]	38.97±3.63[c]	45.30±3.97[a]
Free fatty acids (g/100 g lipids)	5.85±0.42[a]	13.99±0.68[b]	6.95±0.53[c]	6.13±0.37[a]
Neutral (g/100 g lipids)	47.46±2.83[a]	59.08±2.07[b]	54.05±2.42[c]	48.56±2.31[a]

[a-d] Means in the same row with different letters differ significantly ($p<0.05$).

[e] Data expressed as mean±SD (n = 6).

Table 2. Phospholipid classes in biceps femoris muscles of different duck meat products (%)[e]

Phospholipids [f]	Raw duck	Dry-cured duck	Roasted duck	Water-boiled salted duck
PC	64.58±3.14 [a]	71.77±2.68 [b]	69.90±3.03 [a]	65.67±3.49 [a]
PE	28.16±2.06 [a]	25.31±1.37 [b]	26.33±1.62 [a]	26.88±2.13 [a]
PS	3.07±0.12 [a]	0.00±0.00 [b]	0.18±0.01 [c]	2.85±0.15 [a]
PI	0.30±0.02 [a]	0.00±0.00 [b]	0.21±0.01 [c]	0.27±0.02 [a]
SPH	2.51±0.10 [a]	0.94±0.12 [b]	2.49±0.07 [a]	3.16±0.31 [c]
LPC	1.39±0.13 [a]	1.01±0.04 [b]	0.90±0.02 [b]	1.21±0.16 [a]

[a-d] Means in the same row with different letters differ significantly ($p<0.05$).
[e] Data expressed as mean±SD (n = 6).
[f] PE = Phosphatidylethanolamine, PC = Phosphatidylcholine, PI = Phosphatidylinositol. PS = Phosphatidylserine, SPH = Sphingomyelin, LPC = Lysophosphatidylcholine.

significant differences between raw and cooked pork meat in the composition of the different phospholipid classes. These different findings are likely the result of different processing methods for different meat products. During the roasted duck processing, the percentage of PE decreased by 1.83% while that of PC increased 5.32%, which indicated that cooking damaged PE more seriously than PC, that was also reported by Gandemer (Gandemer et al., 1993). After the processing of dry-cured duck, the percentage of PE decreased by 2.85% while that of PC increased 7.19%, which indicated that PE was more susceptible to hydrolysis or oxidation than PC. Hernandez (Hernandez et al., 1999) ascribed the greater susceptibility of PE to oxidation due to its high content of polyunsaturated fatty acids, which are very sensitive to hydrolysis.

Comparison of PC and PE molecular species

Table 3 shows relative percentages of PC and PE molecular species in different duck meat products. In raw duck meat, Major molecular species of PC were PC

Table 3. Molecular species of PC and PE in different duck meat products (%)[e]

Items	$[M+H]^+$ *m/z*	Molecular species [f]	Raw duck	Dry-cured duck	Roasted duck	Water-boiled salted duck
PC	808.7	$C_{16:0}/C_{22:6}$	1.21±0.06 [a]	0.11±0.02 [b]	1.35±0.05 [a]	1.17±0.13 [a]
	810.8	$C_{16:0}/C_{22:4}$	3.21±0.52 [a]	0.32±0.03 [b]	3.62±0.39 [c]	2.85±0.25 [d]
	782.8	$C_{16:0}/C_{20:4}$	10.24±1.97 [a]	7.03±0.62 [b]	9.83±1.66 [a]	10.79±1.10 [a]
	704.8	$C_{16:0}/C_{14:1}$	7.12±0.86 [a]	9.45±0.95 [b]	6.98±0.52 [a]	6.34±0.67 [c]
	730.7	$C_{14:0}/ C_{18:2}$	1.24±0.11 [a]	1.07±0.14 [b]	1.13±0.07 [b]	1.05±0.06 [b]
	760.7	$C_{16:0}/C_{18:1}$	38.42±4.09 [a]	43.57±2.58 [b]	38.36±3.13 [a]	39.17±3.08 [a]
	786.8	$C_{18:0}/C_{18:2}$	27.78±2.43 [a]	26.86±1.57 [a]	26.89±2.54 [a]	26.93±2.62 [a]
	758.7	$C_{16:0}/ C_{18:2}$	0.87±0.15 [a]	0.00±0.00 [b]	0.73±0.11 [a]	0.93±0.06 [a]
	788.8	$C_{18:0}/C_{18:1}$	8.26±1.14 [a]	11.42±1.36 [b]	8.95±0.92 [a]	8.83±0.39 [a]
	756.8	$C_{16:0}/ C_{18:3}$	1.12±0.27 [a]	0.16±0.05 [b]	1.42±0.09 [a]	1.26±0.17 [a]
	784.7	$C_{18:1}/ C_{18:2}$	0.53±0.08 [a]	0.00±0.00 [b]	0.74±0.18 [a]	0.68±0.05 [a]
PE	712.8	$C_{14:0}/C_{20:4}$	2.56±0.25 [a]	1.32±0.14 [b]	2.14±0.07 [c]	2.78±0.11 [a]
	740.8	$C_{14:0}/C_{22:4}$	8.27±1.13 [a]	6.85±0.25 [b]	7.93±0.64 [a]	8.34±1.05 [a]
	716.9	$C_{16:0}/C_{18:2}$	6.35±0.97 [a]	8.53±0.62 [b]	5.97±0.42 [c]	6.67±0.49 [a]
	714.8	$C_{16:0}/C_{18:3}$	0.69±0.08 [a]	0.00±0.00 [b]	0.00±0.00 [b]	0.59±0.10 [a]
	746.8	$C_{18:0}/C_{18:1}$	1.67±0.19 [a]	2.59±0.17 [b]	1.96±0.15 [c]	1.55±0.23 [a]
	768.8	$C_{18:0}/C_{20:4}$	42.18±4.54 [a]	38.96±3.23 [b]	41.55±3.48 [a]	41.95±3.74 [a]
	744.9	$C_{18:0}/C_{18:2}$	31.36±2.12 [a]	35.86±1.19 [b]	32.84±2.06 [a]	31.16±2.85 [a]
	734.8	$C_{14:1}/C_{22:6}$	1.24±0.11 [a]	0.00±0.00 [b]	0.83±0.21 [c]	1.30±0.19 [a]
	742.9	$C_{18:1}/C_{18:2}$	4.85±0.72 [a]	5.89±0.68 [b]	5.84±0.33 [b]	4.94±0.32 [a]
	766.8	$C_{18:1}/C_{20:4}$	0.83±0.15 [ac]	0.00±0.00 [b]	0.93±0.05 [a]	0.72±0.13 [c]

[a-d] Means in the same row with different letters differ significantly ($p<0.05$).
[e] Relative contents are expressed as mean±SD (n = 4).
[f] Fatty acids: $C_{n:m}$ fatty acids (n = carbon number; m = number of double bounds).

$C_{16:0}/C_{18:1}$, PC $C_{18:0}/C_{18:2}$, PC $C_{16:0}/C_{20:4}$ and PC $C_{18:0}/C_{18:1}$ (38.42%, 27.78%, 10.24% and 8.26%, respectively). Major molecular species of PE were PE $C_{18:0}/C_{20:4}$, PE $C_{18:0}/C_{18:2}$, PE $C_{14:0}/C_{22:4}$ and PE $C_{16:0}/C_{18:2}$ (42.18%, 31.36%, 8.27%, 6.35%, respectively). The percentage of molecular species with polyunsaturated fatty acids in PE accounted for 98.33%, while that in PC only 46.2%. They were similar to the pork (Boselli et al., 2008).

Water-boiled salted and roasted duck had smaller decrease ($p>0.05$) of most phospholipid molecular species compared to dry-cured duck. During the processing of dry-cured duck, the percentage of molecular species with polyunsaturated fatty acids (for example PC $C_{16:0}/C_{20:4}$, PE $C_{18:0}/C_{20:4}$) decrease greatly due to hydrolysis, corresponding to a substantial increased in the relative percentage of molecular species with monounsaturated fatty acids (such as PC $C_{16:0}/C_{18:1}$). However, most phospholipids molecular species changed only slightly ($p>0.05$) during the processing of roasted and water-boiled salted duck. According to data in Tables 3, the extent of degradation of PC and PE molecular species during the dry-cured duck processing was as follows: the molecular species with polyunsaturated fatty acids were degraded (PC was 20.89%, and PE was 0.93%), but with monounsaturated fatty acids were increased (PC was 17.94%, and PE was 55.09%). Yang (Yang et al., 2005) also found such a selective degradation when they studied lipolysis in Xuanwei hams. However, it was found that lipolysis in phospholipid was not specific to fatty acid chain length or unsaturation during roasting and water-boiling process, which is in agreement with the study of Boselli (Boselli et al., 2008). The difference between dry-cured duck and cooked duck may be a result of different lipolysis mode due to different processing methods.

CONCLUSION

Composition of intramuscular lipids, phospholipids classes, PC and PE molecular species in three kind of traditional Chinese duck meat products was determined. A significant change in phospholipids classes and molecular species through the processing of dry-cured duck was observed. Results in the present work supported the hypothesis that a selective degradation of the phospholipids molecular species with polyunsaturated fatty acids was found during the dry-cured duck processing, but decrease of phospholipids was not specific to molecular species with polyunsaturated fatty acids through the roasted and water-boiled duck processing. This study could be useful to further understand the role of phospholipids in the formation of meat flavor of traditional Chinese duck meat products.

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