Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 90 (2005) 437–443
Food Chemistry
www.elsevier.com/locate/foodchem

Stereospecific analysis of fresh and dry-cured muscle phospholipids from Iberian pigs

M.E. Muriel, M.T. Antequera, M.J. Petrón, A.I. Andrés, J. Ruiz *

Tecnología de los Alimentos, Facultad de Veterinaria, Universidad de Extremadura, Campus Universitario s/n, 10071 Cáceres, Spain

Received 17 June 2003; received in revised form 6 May 2004; accepted 6 May 2004

Abstract

A stereospecific analysis of phospholipids (PLs) from fresh and dry-cured Iberian loin was carried out in order to study the fatty acid and fatty alcohol composition of *sn*-1 and *sn*-2 positions as well as the changes in the profile of each position during the dry-cured processing. The profiles of fatty acids and alcohols in the *sn*-1 and *sn*-2 positions of PLs from fresh loin showed marked differences. Fatty alcohols were only detected in the *sn*-1 position. Saturated fatty acids (SFA) were located in the *sn*-1 position in a higher proportion ($P < 0.001$) than in the *sn*-2 one, while polyunsaturated fatty acids (PUFA) were mainly occupying the *sn*-2 position ($P < 0.001$). Dry-cured processing led to changes in the profile of each position. Thus, PUFA showed a marked decrease in the *sn*-2 position, monounsaturated fatty acids (MUFA) being the major family in this position in dry-cured loin PLs. Proportions of fatty alcohols in the *sn*-1 position increased during the processing, agreeing with the scarce or absent phospholipase (PLase) A_1 activity against the ether linkage. Moreover, no variation in the calculated amount of plasmalogens was detected between fresh and dry-cured samples, whereas there was a significant decrease ($P < 0.001$) in both total PLs and diacylglycerophospholipids. All these changes are most likely due to lipolytic processes rather than to lipid oxidative phenomena, although the latter could also be implied in the *sn*-2 PUFA decrease.

Keywords: Phospholipids; *sn*-1 and *sn*-2 positions; Dry-cured loin; Plasmalogens

1. Introduction

Principles implied in the regulation of fatty acid distribution in the *sn*-1 and *sn*-2 positions of PLs from different tissues in animals have not yet been totally elucidated (Breckenridge, 1978; Litchfield, 1972; Takahashi, Hirano, Egi, & Zama, 1985). Nevertheless, the *sn*-1 position is usually occupied by SFA while the *sn*-2 is occupied by unsaturated fatty acids (especially long chain PUFA) (Christie, 2003; Nawar, 1996). Moreover, many PLs show an alkyl or alkenyl chain linked to the *sn*-1 position with an ether linkage, instead of an acyl linked with an ester linkage, forming, respectively, 1-(1′-alkyl)-2-acylglycerophospholipids (named alkylacylglycerophospholipids in this work), and 1-(1′-alkenyl)-2-acylglycerophospholipids, in which the double bond of the alkenyl radical, in *cis* configuration, is adjacent to the ether linkage, forming a vinyl-ether linkage. In this latter situation the molecule is called a plasmalogen (Nagan & Zoeller, 2001; Post, Verkleij, Roelofsen, & Op den Kamp, 1988). Fogerty, Whitfield, Svornos, and Ford (1991), found that plasmalogens were between 39% and 47% of total phosphatidylethanolamine and between 10% and 30% of phosphatidylcholine in skeletal bovine, ovine, swine and chicken muscles.

Lipid degradation during storage of fresh meat at refrigeration temperature and during ripening of dry-cured meat products is mainly due to lipolytic phenomena (Alasnier & Gandemer, 2000a). Although both endogenous (muscle) and exogenous (microbial) lipases can be responsible of this lipolysis, in non-minced products, such as dry-cured ham or loin, these processes have been mainly attributed to endogenous lipases (Hernández, Navarro, & Toldrá, 1999; Toldrá, 1998), which in fact remain active during most of the

* Corresponding author. Tel.: +34-927-257122; fax: +34-927-257110.
E-mail address: jruiz@unex.es (J. Ruiz).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.05.007

processing (Motilva, Toldrá, Nieto, & Flores, 1993). Given that free fatty acid composition of both stored fresh meat and cured products resembles the fatty acid profile of PLs, it seems that they are the main substrate for lipolysis in these products (Buscailhon, Gandemer, & Monin, 1994; Martín, Córdoba, Ventanas, & Antequera, 1999; Sklan, Tenne, & Budowski, 1983). Nevertheless, hydrolysis of triacylglycerides in meat and meat products should not be dismissed (Alasnier, David-Briand, & Gandemer, 2000b).

Hydrolysis of lipids during processing could lead to enhanced levels of lipid oxidation, since free fatty acids are more susceptible to lipid oxidation than are esterified ones (Labuza, Tsuyuki, & Karel, 1969; Nawar, 1996). However, recent studies have shown that hydrolysis of PLs does not enhance lipid oxidation in chicken meat, perhaps because free fatty acids remain in the membrane (Zeng, Han, Schlesinger, & Gross, 1998), being therefore protected by the vitamin E accumulated there (Alasnier, Meynier, Viau, & Gandemer, 2000c).

Hydrolysis of PLs in the muscle is carried out by phospholipases (Alasnier & Gandemer, 2000a). More particularly, PLases A_1 and A_2 hydrolyze the ester linkage between the glycerol backbone and the acyl chains in the *sn*-1 and *sn*-2 positions, respectively. There also exist lysophospholipases that hydrolyze the ester linkage of the lysophospholipids formed due to the action of PLases A_1 and A_2. There is, however, scarce information about PLases activity in meat and meat products.

PLases A_2 from different animal sources can be used for structural analysis of PLs, since they hydrolyze the acyl group in the *sn*-2 position. A further separation of the free fatty acids and the lysophospholipids obtained after incubation with the enzyme allows the characterization of both the *sn*-1 and the *sn*-2 position. The more frequently used PLases A_2 are those from snake venom (*Crotalus adamanteus*, *Ophiophagus hannah*) and bee venom (*Apis mellifera*).

There exist several works in the scientific literature dealing with fatty acid composition of fresh and dry-cured loin PLs (Hernández et al., 1999; Muriel, Ruiz, Ventanas, & Antequera, 2002). However, there is no stereospecific study of fresh or dry-cured meat PLs. The present study was aimed to study the distribution of acyl, alkyl and alkenyl chains in the *sn*-1 and *sn*-2 positions of fresh loin PLs from Iberian pigs, as well as the changes in such distribution as a result of dry-cured processing.

2. Materials and methods

2.1. Animals and sampling

Ten pure Iberian pigs, weighing 90 ± 5 kg, were free-reared and fed on acorns and grass during the 60 days previous to slaughter. All animals were slaughtered at 150 ± 5 kg by electrical stunning and exsanguination at a local slaughterhouse. The cranial half of the *Longissimus dorsi* (LD) muscle was taken within the hour following slaughter. A portion of about 100 g was stored at −80 °C until analysis. The rest of the muscle was stored at −20 °C for 2 months and thereafter was processed following the traditional procedure for dry-cured loin elaboration for 90 days. When the processing had finished, a portion of about 100 g was taken and stored at −80 °C until analysis.

2.2. Intramuscular fat extraction

Samples were ground using a commercial grinder immediately before fat extraction. Intramuscular total lipids from fresh and dry-cured loin were extracted according to the method described by Bligh and Dyer (1959).

2.3. Quantification and purification of phospholipids

PLs were quantified by measuring the organic phosphorus in total lipids extracts, following the method described by Barlett (1959). PLs contents were calculated considering that their average phosphorus content is 4% (Wangen, Marion, & Hotchkiss, 1971).

Lipid extracts were separated into lipid classes in NH_2-aminopropyl minicolumns (500 mg), following the method described by Kaluzny, Duncan, Merrit, and Epps (1985) with small modifications by Pinkart, Devereux, and Chapman (1998). Briefly, minicolums were activated with 7.5 ml hexane (3×2.5 ml); 20 mg of total lipids dissolved in 150 μl of hexane:chloroform:methanol (95:3:2; v/v/v) were added to the column. Neutral lipids were eluted with 2×2.5 ml of chloroform, free fatty acids with 2×2.5 ml of diethyl ether:acetic acid (98:2; v/v) and polar lipids were eluted in two fractions, the first one with 2.5 ml methanol:chloroform (6:1; v/v) and the second one with 2.5 ml of 0.05 M sodium acetate in methanol:chloroform (6:1; v/v). The correct separation of lipid classes was ensured by analysing lipid classes by TLC.

2.4. Phospholipid hydrolysis with phospholipase A_2

A stock solution of 1 mg of PLase A_2 from *Apis mellifera* (1225 units/mg; Sigma–Aldrich) in 23.1 ml of 1 M tris buffer (pH 8.9) containing calcium chloride (4 mM) was made up. Hydrolysis was performed by adding 300 μl of the PLase A_2 stock solution to PLs (2–5 mg) in 6 ml of diethyl ether with BHT (0.05%) (Florin-Christensen, Narvaez-Vasquez, Florin-Christensen, & Ryan, 1999). The mixture was incubated for 3 h in a N_2 atmosphere with continuous stirring at 25 °C. Thereafter, it was washed with methanol:chloroform (2:1) and

dried with anhydrous sodium sulfate (Aubourg, Medina, & Pérez-Martín, 1996). The reaction mixture was separated in NH_2-aminopropyl minicolumns (500 mg), following the method described previously with small modifications. Briefly, colums were activated with 3×2.5 ml of hexane. The sample, dissolved in 1 ml of diethylether:acetic acid (98:2; v/v), was added to the column; free fatty acids (position *sn*-2 of the original PL) were eluted with 2×2 ml diethylether:acetic acid (98:2; v/v) and lysophospholipids were eluted in two fractions, the first one with 2.5 ml of 0.05 M sodium acetate in methanol:chloroform (6:1; v/v) and the second one with 2.5 ml methanol. The correct separation of free fatty acids and lysophospholipids was ensured by TLC.

2.5. Fatty acid methyl esters and dimethylacetals preparation and analysis

Methyl esters of acyl chains (FAMEs) and dimethlyacetals of alkenyl chains (DMAs) were prepared by acidic-*trans*-esterification in the presence of sulphuric acid (5% sulphuric acid in methanol) (Cava et al., 1997). FAMEs were analysed by gas chromatography, using a Hewlett–Packard HP-5890A gas chromatograph, equipped with a flame ionisation detector (FID). Separation was carried out on a polyethylene glycol-TPA modified fused silica semicapillary column (30 m long, 0.53 mm id, 1 μm film thickness) maintained at 225 °C. Injector and detector temperatures were 230 °C. Carrier gas was nitrogen at a flow rate of 1.8 $ml\,min^{-1}$. Individual FAME peaks were identified by comparing their retention times with those of standards (Sigma, St Louis).

2.6. Statistical analysis

An individual pig was the experimental unit for analysis of all data. *sn*-1 vs *sn*-2 in fresh and in dry-cured loin and fresh vs dry cured in both the *sn*-1 and the *sn*-2 positions were compared by one way ANOVA using the General Linear Model of SPSS (v. 11.0).

3. Results and discussion

3.1. Stereospecific analysis of phospholipids from fresh loin of Iberian pigs

Methylation of PLs resulted in the formation of fatty acid methyl esters and dimethyl acetals. The latter arise from the formation of an intermediate aldehyde which is in turn formed in acidic conditions from the vinyl-ether linkage of the alkenyl chains of the *sn*-1 position of the glycerophospholipid (Christie, 2003). As a first consequence, alkyl glycerophospholipids (in which the linkage to the glycerol backbone is an ether bond) are not detected when using common methylation methods, since the ether bond is stable to acidic conditions (Christie, 2003). Alkenyl chains linked to the glycerol backbone are formed through a complex biosynthesis route (Nagan & Zoeller, 2001). At any rate, their origin is the replacement of the *sn*-1 fatty acid by a fatty alcohol. In the scientific literature these alkenyl chains are sometimes named fatty aldehydes (Fogerty et al., 1991). However, perhaps fatty alcohol is a more correct terminology, and will therefore be used in this study.

FAMEs and DMAs found in *sn*-1 and *sn*-2 positions of PLs of fresh loins of Iberian pigs are shown in Table 1. Composition of the *sn*-1 position was markedly different from that of the *sn*-2 one. The *sn*-2 position was totally occupied by fatty acids, while the *sn*-1 position showed both fatty acids (75.0%) and fatty alcohols (25.0%). This finding agrees with previous works in which alkyl and alkenyl chains have always been detected only in the *sn*-1 position in PLs from different animal tissues (Khaselev & Murphy, 1999; Nagan & Zoeller, 2001; Post et al., 1988) and with the established biosynthesis route for alkyl and alkenyl glycerophospholipids (Nagan & Zoeller, 2001). DMAs found in significant amounts were those from the hexadecanol (C16ol) (15.6% of total FAMEs and DMAs identified), octadecanol (C18ol) (5.51%) and octadecenol (C18:1ol) (3.87%). These major alcohols agree with those found in other studies in pork and other meats (Cava, Estévez, Ruiz, & Morcuende, 2003; Fogerty et al., 1991; Leseigneur-Meynier & Gandemer, 1991), and levels found are similar to those in previous findings in pork (Cava et al., 1997; Fogerty et al., 1991), but are different from those from other meat sources (Fogerty et al., 1991; Leseigneur-Meynier & Gandemer, 1991).

Oleic acid (C18:1 $n-9$) was the major fatty acid found in the *sn*-1 position (20.5%), followed by palmitic (C16:0) (19.3%), stearic (C18:0) (14.1%) and linoleic acids (C18:2 $n-6$) (10.4%). As a consequence, SFA were the major family found in the *sn*-1 position (37.1%), followed by MUFA (22.83%) and PUFA (15.1%). As far as the *sn*-2 position is concerned, linoleic acid was the major fatty acid (30.8%) followed by oleic (25.8%), palmitic (16.3%) and arachidonic acid (C20:4 $n-6$) (10.1%). Therefore, PUFA was the major family in the *sn*-2 position (43.6%) while MUFA and SFA showed similar levels (28.3% and 28.1%, respectively). Comparing the fatty acid composition of the *sn*-1 and *sn*-2 positions, there were significant differences in most fatty acids detected. SFA showed significantly higher values ($P = 0.008$) in the *sn*-1 position than in the *sn*-2, whereas MUFA and PUFA were higher in the *sn*-2 position than in the *sn*-1 one ($P = 0.035$ and $P < 0.001$, respectively). The latter were almost three times higher in the *sn*-2 position, due to the much higher values of linoleic and arachidonic acids in this position. As a consequence, unsaturation index (UI) and the average length of the

Table 1
Fatty acid and fatty alcohol composition (% of total FAMEs and DMAs detected) in *sn*-1 and *sn*-2 positions of phospholipids of fresh and dry-cured loins from Iberian pigs

	Fresh loin				Dry-cured loin				Fresh vs dry-cured	
	sn-1	*sn*-2	SEM (*sn*-1 vs *sn*-2)	*P* (*sn*-1 vs *sn*-2)	*sn*-1	*sn*-2	SEM (*sn*-1 vs *sn*-2)	*P* (*sn*-1 vs *sn*-2)	*P sn*-1	*P sn*-2
C12:0	1.83	1.31	0.190	0.176	0.67	0.56	0.063	0.367	0.004	0.000
C14:0	1.19	1.30	0.123	0.655	0.68	0.99	0.062	0.007	0.057	0.011
C15:0	0.17	0.10	0.038	0.338	0.14	0.09	0.011	0.006	0.668	0.611
C16ol	15.6	0.00	1.969	0.000	22.2	0.00	2.89	0.000	0.059	–
C16:0	19.3	16.3	1.090	0.169	19.19	18.6	0.629	0.658	0.950	0.046
C16:1 (*n*−7)	1.97	1.42	0.135	0.040	1.60	2.77	0.165	0.000	0.212	0.000
C17:0	0.37	0.26	0.038	0.149	0.35	0.25	0.015	0.000	0.809	0.470
C17:1 (*n*−9)	0.10	0.17	0.040	0.390	0.33	0.22	0.047	0.219	0.076	0.012
C18ol	5.51	0.00	0.668	0.000	8.08	0.00	1.04	0.000	0.026	–
C18:1ol	3.87	0.00	0.470	0.000	4.80	0.00	0.617	0.000	0.173	–
C18:0	14.1	8.48	1.012	0.003	10.2	9.72	0.299	0.401	0.024	0.098
C18:1 (*n*−9)	20.5	25.8	1.17	0.020	17.4	36.2	2.44	0.000	0.271	0.000
C18:2 (*n*−6)	10.4	30.8	2.55	0.000	9.33	20.6	1.47	0.000	0.651	0.000
C18:3 (*n*−3)	0.68	0.75	0.116	0.742	0.41	0.75	0.049	0.000	0.274	0.932
C20:0	0.12	0.54	0.073	0.001	0.13	0.17	0.013	0.199	0.660	0.003
C20:1 (*n*−9)	0.22	0.69	0.083	0.002	0.46	0.93	0.061	0.000	0.011	0.048
C20:3 (*n*−6)	0.30	0.99	0.093	0.000	0.41	0.64	0.040	0.002	0.329	0.000
C20:4 (*n*−6)	3.55	10.1	0.856	0.000	3.13	6.86	0.501	0.000	0.620	0.000
C20:5 (*n*−3)	0.18	0.90	0.090	0.000	0.39	0.52	0.030	0.026	0.025	0.000
C22:6 (*n*−3)	0.03	0.09	0.025	0.238	0.01	0.14	0.021	0.000	0.354	0.334
$\sum$ Fatty acids	75.0	100	3.09	0.000	64.9	100	4.54	0.000	0.055	–
$\sum$ Fatty alcohols	25.0	0.00	3.09	0.000	35.1	0.00	4.54	0.000	0.055	–
$\sum$ alcohols/ $\sum$ acids	0.35	0.00	0.046	0.000	0.60	0.00	0.086	0.000	0.041	–
$\sum$ SFA	37.1	28.3	1.754	0.008	31.41	30.4	0.959	0.606	0.080	0.251
$\sum$ MUFA	22.8	28.1	1.28	0.035	19.8	40.1	2.64	0.000	0.333	0.000
$\sum$ PUFA	15.1	43.6	3.58	0.000	13.7	29.5	2.07	0.000	0.665	0.000
$\sum$ *n*−6	14.2	41.9	3.47	0.000	12.9	28.1	2.00	0.000	0.669	0.000
$\sum$ *n*−3	0.88	1.74	0.151	0.002	0.81	1.40	0.086	0.000	0.748	0.003
n−6/*n*−3	16.6	24.6	1.70	0.015	16.6	20.0	0.774	0.022	0.984	0.012
Unsaturation index (UI)[a]	0.83	1.40	0.082	0.000	0.83	1.16	0.046	0.000	0.941	0.000
Average chain length (ACL)[b]	17.3	17.8	0.068	0.000	17.4	17.7	0.044	0.000	0.731	0.007

[a] UI = $\sum$(% each fatty acid × number of double bonds)/(% total fatty acids).
[b] ACL = $\sum$(% each fatty acid × number of carbons in the chain)/(% total fatty acids).

acyl chain (ALC) were also significantly higher in the *sn*-2 position ($P < 0.001$ for both variables).

This pattern in incorporation of fatty acids and fatty alcohols at each specific position agrees with the general trend established for PLs of different tissues in mammals (Christie, 2003; Gurr & Harwood, 1991) and other living organisms (Aubourg et al., 1996; Jeong, Oshima, & Koizumi, 1991; Yoshida, Abe, Hirakawa, & Takagi, 2001). Nevertheless, there are small differences from what has been observed in other tissues and species. For example, Khaselev and Murphy (1999) found a similar distribution pattern, but found much higher amounts of docosahexaenoic acid (C22:6 *n*−3) in the *sn*-2 position of bovine brain PLs as a result of the high amounts of this fatty acid in the nervous system (Gurr & Harwood, 1991). In muscle PLs from different types of fish and other marine animals, the pattern is similar to that found in the present study (SFA in the *sn*-1 position and PUFA in the *sn*-2) (Aubourg et al., 1996; Bell & Dick, 1991; Jeong et al., 1991) although the amount of PUFA is higher in both positions due to the large quantities of these fatty acids in fish tissues. Yoshida and Takagi (1997) and Yoshida et al. (2001) have also found similar patterns in PLs from soybeans and sesame seeds.

A generally accepted theory (Brockerhoff, Yurkowski, Hoyle, & Ackman, 1964) suggests that there is a preferential esterification of the *sn*-2 position of PLs with PUFA, aimed to protect these (more prone to oxidation) fatty acids against oxidative damage. In fact, Yoshida and Takagi (1997), Yoshida et al. (2001), found that unsaturated fatty acids of the *sn*-2 position of PLs of soybean and sesame seeds were more stable to lipid oxidation that those in the *sn*-1 position during roasting processes.

3.2. Stereospecific analysis of phospholipids in dry-cured loin: Changes during the processing

FAMEs and DMAs found in the *sn*-1 and *sn*-2 positions of PLs of dry-cured loins of Iberian pigs are shown in Table 1. As happened in the fresh samples, DMAs were only found in the *sn*-1 position of PLs of the dry-cured loins. The fatty alcohols found were the same as in the fresh samples. The distribution of fatty acids in the *sn*-1 position in dry-cured loin PLs followed the same pattern as in the fresh samples. SFA was the major family (31.4% of total FAMEs and DMAs detected), followed by MUFA (19.8%) and PUFA (13.7%). On the other hand the *sn*-2 position showed several differences. Thus, MUFA was the major group in this position in dry-cured samples PLs (40.1%), followed by SFA and PUFA (30.4% and 29.5%). Comparing both positions, SFA were equally distributed ($P = 0.606$), while MUFA and PUFA were preferentially located in the *sn*-2 position ($P < 0.001$ for both).

Dry-cured processing led to changes in the proportion of both FAMEs and DMAs in *sn*-1 and *sn*-2 positions of muscle PLs. The proportions of DMAs in the *sn*-1 position with respect to total FAMEs and DMAs detected in this position was 35.1%, higher than that of the fresh samples, and close to showing statistical significance ($P = 0.055$). In fact, the rise in C18ol was significant ($P = 0.026$), whereas the increase in C16ol was close to statistical significance ($P = 0.059$). Moreover, the ratio between fatty alcohols and fatty acids was significantly higher in dry-cured samples ($P = 0.041$). Such an increase in the proportion of fatty alcohols has also been detected during refrigerated storage of pork (Morcuende, Estévez, Ruiz, & Cava, 2003). At the same time, there occurs an overall lipolytic process, as can be observed in the significant ($P < 0.001$) decrease in PLs (around 27%) from fresh to dry-cured loin (Fig. 1). This decrease in PLs has previously been described in dry-cured ham (Buscailhon et al., 1994) and dry-cured loin (Hernández et al., 1999). In the latter study, remaining PLase activity was detected after one month of dry-cured processing. However, enzymes that hydrolyze the vinyl-ether linkage (lysoplasmalogenases) have only been described in liver, heart and brain (Jurkowitz, Harrocks, & Litsky, 1999). Moreover, when comparing the calculated amounts of alkenyl glycerophospholipids (plasmalogens) in fresh and dry-cured samples, there was no variation (1.40 vs 1.43 $g \cdot 100\ g^{-1}$ fat) (Fig. 1), whereas the calculated amount of diacylglycerophospholipids significantly decreased ($P = 0.005$) from fresh to dry-cured samples (4.20 vs 2.64 $g \cdot 100\ g^{-1}$ fat) (Fig. 1). Therefore, the increase in the ratio between fatty alcohols and fatty acids, together with the decrease in total PLs, could be explained by the activity of muscle PLase A_1 against the ester linkage and the absence or

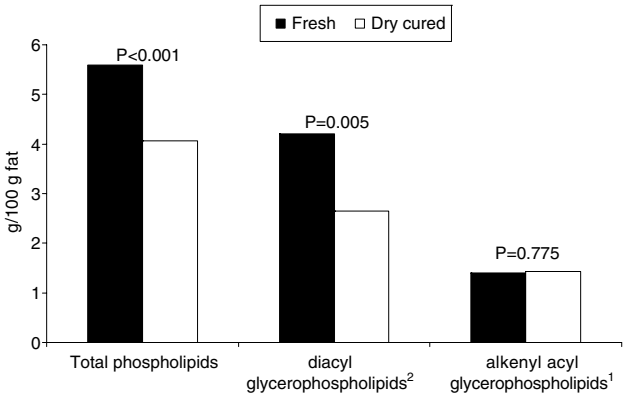

Fig. 1. Amount ($g \cdot 100\ g^{-1}$ fat) of total phospholipids and calculated amount of diacyl glycerophospholipids and alkenyl acyl glycerophospholipids (plasmalogens) in intramuscular fat of fresh and dry-cured loin from Iberian pigs. [1]calculated: Alkenyl acyl glycerophospholipids = (g Pls/100 g fat × % DMAs in *sn*-1)/100. [2] calculated: Diacyl glycerophospholipids = (g Pls/100 g fat − g alkenyl glycerophospholipids/100 g fat).

scarce activity of muscle plasmalogenases. It could be hypothesised that oxidation of fatty acids could be involved in the relative increase of fatty alcohols to fatty acids, since lipid oxidative phenomena throughout the dry-cured processing is intense (Antequera et al., 1992). However, given that vinyl-ether linkages are very sensitive to reactive oxygen species (ROS) (Nagan & Zoeller, 2001, Brosche, 1997), leading to liberation of the alkenyl chain as a free fatty aldehyde (Stadelmann-Ingrand, Favreliere, Fauconneau, Mauco, & Tallineau, 2001), a decrease in fatty acids of PLs due to oxidative processes would probably involve a concomitant decrease in fatty alcohols, which has not been detected in the present study.

Contrary to what was observed in the *sn*-1 position, fatty acids in the *sn*-2 position seemed to be much more affected by chemical and biochemical degradation reactions during the processing. Thus, while SFA did not show any significant variation from fresh to dry-cured samples, there was a significant increase in MUFA (from 28.1% in fresh loin to 40.1% in dry-cured loin) ($P < 0.001$) and a concomitant decrease in PUFA (from 43.6% to 29.5%) ($P < 0.001$). This led to a decrease in the overall unsaturation of the fatty acids in the *sn*-2 position of dry-cured samples PLs ($P < 0.001$) and in the ACL ($P = 0.007$). Individual fatty acids within each group showed similar variations, except for some of the minor fatty acids.

These changes in the fatty acid profile of the *sn*-2 position could be the result of either oxidative or lipolytic phenomena or perhaps both. If the decrease in PUFA and consequent increase in MUFA in the *sn*-2 position of dry-cured sample PLs were caused by lipolysis rather than lipid oxidation, this would imply the existence of a selective PLase A_2 that would hydrolyze the linkage between PUFA and the glycerol backbone in the *sn*-2 position. There is in fact a cytosolic PLase A_2 that preferably hydrolyze PLs with arachidonic acid in the *sn*-2 position (Balsinde, Winstead, & Dennis, 2002; Ficher, 1997; Leslie, 2004). Nevertheless, the PLase A_2 subgroup present in muscle (group IV, subgroup C: $cPLA_2$ gamma) shows a marginal preference. Moreover, Alasnier et al. (2000b) observed unspecific PLase activities in rabbit muscle under refrigerated storage, and Buscailhon et al. (1994) discarded selective lysis of Pls in dry-cured ham.

Lipid oxidation could play an important indirect role in the specific lysis of PUFA from the *sn*-2 position. Van den Berg, Op den Kamp, Lubin, and Kuypers (1993) have observed, in vivo, that PLS oxidation results in conformational changes that alter the membrane structure, making the *sn*-2 position of the PLs more accessible to the action of PLase A_2. Therefore, there would be a preferential hydrolysis of oxidized PLs. Given that PUFA are more prone to oxidation that MUFA or SFA, PLs with a PUFA in the *sn*-2 position would be more liable to get oxidized, and hence, to suffer the action of PLase A_2. This could explain the marked decrease in PUFA of the *sn*-2 position. This is a possible mechanism of cells against oxidative deterioration of membrane PLs, since it would permit the removal of oxidized acyl chains in the *sn*-2 position, allowing the incorporation of a new fatty acid chain and thus, repairing the damaged membranes. In meat products, oxidation of PUFA in the *sn*-2 position may initiate their subsequent hydrolysis by the PLase A_2.

It can be concluded that Iberian pig muscle PLs show different compositions in the *sn*-1 and the *sn*-2 position, the former being preferentially occupied by fatty alcohols and SFA, while the *sn*-2 is mainly linked to PUFA. Dry-cured processing leads to changes in the composition of both positions, probably as a result of lipolysis phenomena. In this sense, the lack of PLase A_1 activity against the vinyl-ether linkage (plasmalogenase)is notable, and this possibly implies PUFA oxidation in a preferential hydrolysis of these fatty acids from the *sn*-2 position by a PLase A_2.

Acknowledgements

The authors are grateful to Ana Galaz and Natividad Hurtado for their technical assistance. AECERIBER is also acknowledged for its help in pig management. This study was supported by the FEDER project "Optimisation of Genetic Evaluation of Iberian swine including quality parameters in both raw meat and processed products" (1FD97-1252-C02-01).

References

Alasnier, C., & Gandemer, G. (2000a). Activities of phospholipases A and lysophospholipases in glycolitic and oxidative skeletal muscles in the rabbit. *Journal of the Science of Food and Agriculture, 80*, 696–702.

Alasnier, C., David-Briand, E., & Gandemer, G. (2000b). Lipolysis in muscle during refrigerated storage as related to metabolic type of the fibres in the rabbit. *Meat Science, 54*, 127–134.

Alasnier, C., Meynier, A., Viau, M., & Gandemer, G. (2000c). Hydrolytic and oxidative changes in the lipids of chicken breast and tight muscles during refrigerated storage. *Journal of Food Science, 65*, 9–14.

Antequera, T., López-Bote, J. J., García, C., Asensio, M. A., Ventanas, J., García-Regueiro, J. A., & Díaz, I. (1992). Lipid oxidative changes in the processing of Iberian pig hams. *Food Chemistry, 45*, 105–110.

Aubourg, S. P., Medina, I., & Pérez-Martín, R. (1996). Polyunsaturated fatty acids in tuna phospholipids: Distribution in the *sn*-2 location and changes during cooking. *Journal of Agriculture and Food Chemistry, 44*, 585–589.

Balsinde, J., Winstead, M. V., & Dennis, E. A. (2002). Phospholipase A_2 regulation of arachidonic acid mobilization. *FEBS Letters, 531*, 2–6.

Barlett, G. R. (1959). Phosphorus assay in column chromatography. *Journal of Biology and Chemistry, 234*, 466–468.

Bell, M. V., & Dick, J. R. (1991). Molecular species composition of the major diacyl glycerophospholipids from muscle, liver, retina and brain of cod (*Gadus morua*). *Lipids, 26*, 565–573.

Bligh, E. G., & Dyer, E. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37*, 911.

Breckenridge, W. (1978). Stereospecific analysis of triacylglycerols. In A. Kuksis (Ed.), *Handbook of lipid research* (Vol. 1, pp. 197–232). New York: Plenum Press.

Brockerhoff, H., Yurkowski, M., Hoyle, R., & Ackman, R. (1964). Fatty acid distribution in lipids of marine plankton. *Journal of Fisheries Research Board Canadian, 21*, 1379–1384.

Brosche, T. (1997). Plasmalogen phospholipids: Facts and theses to their antioxidative qualities. *Archives of gerontology and geriatrics, 25*, 73–81.

Buscailhon, S., Gandemer, G., & Monin, G. (1994). Time-related changes in intramuscular lipids of French dry cured ham. *Meat Science, 37*, 245–255.

Cava, R., Ruiz, J., López-Bote, C., Martín, L., García, C., Ventanas, J., & Antequera, T. (1997). Influence of finishing diet on fatty acid profiles of intramuscular lipids, tryglicerides and phospholipids in muscle of Iberian pig. *Meat Science, 45*, 263–270.

Cava, R., Estévez, M., Ruiz, J., & Morcuende, D. (2003). Physicochemical characteristics of three muscles from free-range reared Iberian pigs slaughtered at 90 kg live weight. *Meat Science, 63*, 533–541.

Christie, W.W., (2003). Lipid library Available (http://www.lipid.co.uk/infores/lipids.html).

Ficher, S. M. (1997). Prostaglandins and cancer. *Frontiers in Bioscience, 2*, 482–500.

Florin-Christensen, J., Narvaez-Vasquez, J., Florin-Christensen, M., & Ryan, C. A. (1999). A method for disinguishing 1-acyl from 2-acyl lysophosphatidylcholines generated in biological systems. *Analytical Biochemistry, 276*, 13–17.

Fogerty, A. C., Whitfield, F. B., Svornos, D., & Ford, G. L. (1991). The composition of the fatty acids and aldehydes of the ethanolamine and choline phospholipids of various meats. *International Journal of Food Science and Technology, 26*, 363–371.

Gurr, M. I., & Harwood, J. L. (1991). *Lipid biochemistry: An introduction.* (4th ed.). London: Chapman & Hall.

Hernández, P., Navarro, J. L., & Toldrá, F. (1999). Lipolitic and oxidative changes in two Spanish pork loin products: Dry-cured loin and pickled-cured loin. *Meat Science, 51*, 123–128.

Jeong, B. Y., Oshima, T., & Koizumi, C. (1991). Molecular species of 1-*O*-alk-1′-enyl-2-acyl-, 1-*O*-2-acyl- and 1,2-diacylglycerophospholipids in Japanese oyster *Crassostrea gigas* (Thunberg). *Lipids, 25*, 624–632.

Jurkowitz, A. S., Harrocks, L. A., & Litsky, M. L. (1999). Identification and characterization of alkenyl hydrolase (lysoplasmalogenase) in microsomes and identification of a plasmalogen-active phospholipase A_2 in cytosol of small intestinal epithelium. *Biochimica et Biophysica Acta, 1437*, 142–156.

Kaluzny, M. A., Duncan, L. A., Merrit, M. V., & Epps, D. E. (1985). Rapid separation of lipid classes in high yield and purity using bonded phase columns. *Journal of Lipid Research, 26*, 135–140.

Khaselev, N., & Murphy, R. C. (1999). Susceptibility of plasmenyl glycerophospphoethanolamine lipids containing arachidonate to oxidative degradation. *Free Radical Biology & Medicine, 26*, 275–284.

Labuza, T. P., Tsuyuki, H., & Karel, M. (1969). Kinetics of linoleate oxidation in model systems. *Journal of the American Oil Chemistry Society, 16*, 409–416.

Leseigneur-Meynier, A., & Gandemer, G. (1991). Lipid composition of pork muscle in relation to the metabolic type of the fibres. *Meat Science, 29*, 229–241.

Leslie, C. C. (2004). Regulation of the specific release of arachidonic acid by cytosolic phospholipase A_2. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 70*, 373–376.

Litchfield, C. (1972). Distribution of fatty acids in natural triglyeride mixtures. In *Analysis of triglycerides* (pp. 233–264). New York: Academic press.

Martín, L., Córdoba, J. J., Ventanas, J., & Antequera, T. (1999). Changes in intramuscular lipids during ripening of Iberian dry-cured ham. *Meat Science, 51*, 129–134.

Morcuende, D., Estévez, M., Ruiz, J., & Cava, R. (2003). Oxidative and lipolytic deterioration of different muscles from free-range reared Iberian pigs slaughtered at 90 kg live weight. *Meat Science, 65*, 1157–1164.

Motilva, M. J., Toldrá, F., Nieto, P., & Flores, J. (1993). Muscle lipolysis phenomena in the processing of dry-cured ham. *Food Chemistry, 48*, 121–125.

Muriel, E., Ruiz, J., Ventanas, J., & Antequera, T. (2002). Free-range rearing increases ($n-3$) polyunsaturated fatty acids of neutral and polar lipids in swine muscles. *Food Chemistry, 78*, 219–225.

Nagan, N., & Zoeller, R. A. (2001). Plasmalogens: Biosynthesis and functions. *Progress in Lipid Research, 40*, 199–229.

Nawar, W. W. (1996). Lipids. In Owen R. Fennema (Ed.), *Food chemistry* (3rd ed., pp. 139–244). New York: Marcel Dekker.

Pinkart, H. C., Devereux, R., & Chapman, P. J. (1998). Rapid separation of microbial lipids using solid phase extraction clumns. *Journal of Microbiological Methods, 34*, 9–15.

Post, J. A., Verkleij, A. J., Roelofsen, B., & Op den Kamp, J. A. F. (1988). Plasmalogen content and distribution in the sarcolemma of cultured neonatal rat myocites. *FEBS Letters, 240*, 78–82.

Sklan, D., Tenne, Z., & Budowski, P. (1983). Simultaneous lipolytic and oxidative changes in turkey meat stored at different temperatures. *Journal of the Science Food and Agriculture, 34*, 93–99.

Stadelmann-Ingrand, S., Favreliere, S., Fauconneau, B., Mauco, G., & Tallineau, C. (2001). Plasmalogen degradation by oxidative stress: Production and dissapearance of specific fatty aldehydes and fatty α-hydroxyaldehydes. *Free Radical Biology and Medicine, 31*, 1262–1271.

Takahashi, K., Hirano, T., Egi, M., & Zama, K. (1985). A mathematical model for the prediction of triglyceride molecular species by HPLC. *Journal of the American Oil Chemistry Society, 62*, 1492–1498.

Toldrá, F. (1998). Proteolysis and lipolysis in flavour development of dry-cured meat products. *Meat Science, 49*, S101–S110.

Van den Berg, J. J. M., Op den Kamp, J. A. F., Lubin, B. H., & Kuypers, F. A. (1993). Conformational changes in oxidized phospholipids and their preferential hydrolysis by phospholipase A_2: A monolayer study. *Biochemistry, 32*, 4962–4967.

Wangen, R. M., Marion, W. W., & Hotchkiss, D. K. (1971). Influence of age on total lipids and phospholipids of turkey muscle. *Journal of Food Science, 36*, 560–562.

Yoshida, H., & Takagi, S. (1997). Microwave roasting and positional distribution of fatty acids of phospholipids in soybean (*Glicine max*, L.). *Journal of the American Oil Chemistry Society, 74*, 915–921.

Yoshida, H., Abe, S., Hirakawa, Y., & Takagi, S. (2001). Roasting effects on fatty acid distributions of triacylglycerols and phospholipids in sesame (*Sesamum indicum*) seeds. *Journal of the Science of Food and Agriculture, 81*, 620–626.

Zeng, Y., Han, X., Schlesinger, P., & Gross, R. W. (1998). Nonesterified fatty acids induce transmembrane monovalent cation flux: Host–guest interactions as determinants of fatty acid – induced ion transport. *Biochemistry, 37*, 9497–9508.