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Plasmalogens in pork: aldehyde composition and changes in aldehyde profile during refrigerated storage of raw and cooked meat

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Abstract

Phospholipids from raw and cooked *m. Longissimus dorsi* from lean and fatty pigs, after 10 days of refrigerated storage at +4 °C, were analyzed for fatty acid and fatty aldehyde profiles. After refrigerated storage of raw meat, the hexadecanal proportion significantly declined ($P<0.05$) while octadecanal and octadecenal proportions increased. In m. *Longissimus dorsi* from lean pigs, percentages of saturated fatty acids (SFA) decreased while the proportions of polyunsaturated fatty acids (PUFA) concomitantly increased ($P<0.05$). The ratio dimethyl acetals/fatty acids from phospholipids (DMA/FA) significantly increased ($P<0.05$) after 10 days of refrigerated storage, being significantly higher in m. *Longissimus dorsi* from lean pigs. The proportions of different aldehydes did not vary after refrigeration of cooked meat, while percentages of PUFA significantly decreased and those from SFA concomitantly increased in both groups ($P<0.05$). The ratio DMA/FA, in cooked samples, significantly decreased ($P<0.001$) and this change was larger in m. *Longissimus dorsi* from lean pigs ($P<0.05$).
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1. Introduction

Plasmalogens are phospholipid subclasses in which the *sn-1* position of the glycerol backbone is linked with a long chain fatty aldehyde via a vinyl–ether bond. In the plasmalogen molecule, the *sn-1* position of glycerol is occupied specifically by a saturated fatty aldehyde: hexadecanal (C16:0-al), octodecanal (C18:0-al) or a monounsaturated fatty aldehyde: octodecenal (C18:1-al), while the *sn-2* position of the glycerol is esterified with a long chain polyunsaturated fatty acid, such as linoleic, linolenic or arachidonic (Ten-Ching Lee, 1998) giving plasmanylglycerophospholipids (1-alkyl-2-acyl glycerophospholipids) and plasmenylglycerophospholipids (alk-1-enyl-2-acyl-glycerophospholipids), respectively. Substances containing a vinyl–ether bond (a double *cis* bond adjacent to an ether bond) exhibit characteristics of plasmalogens: high sensitivity to acids, to the mercury cation and to reactive oxygen substances (ROS) (Nagan & Zoeller, 2001). The proportion of plasmalogens in the total of phospholipids depends on several factors, plasmalogens being around 20% of phospholipids (Nagan & Zoeller, 2001). In muscle, the plasmalogen proportion is between 12% in *m. Longissimus lumborum* of rabbits (Alasnier & Gandemer, 1998) and 20.6% in *m. Longissimus dorsi* of pigs (Morcuende, Estévez, Ruiz, & Cava, 2003).

Research focussed on plasmalogens has increased in human medicine, due to the proposed role that they play as endogenous antioxidants (Morand, Zoeller, & Raetz, 1988; Zoeller, Lake, Nagan, Gaposchkin, Legner, & Lieberthal, 1999) and their implications in the development of several human diseases, such as Alzheimer's, heart disease, myocardial infarct or cellular ageing (Brosche, 1997; Nagan & Zoeller, 2001). Studies on plasmalogens in meat, the fatty aldehyde composition of phospholipids and the factors that affect their occurrence and proportions, are very scarce. The role of plasmalogens in oxidative/antioxidative and lipolytic processes in meat has not yet been described. However, some authors have suggested that plasmalogens, together with a high content of polyunsaturated fatty acids, could favour the incipient rancidity in refrigerated meat (Marmer, Nungesser, & Foglia, 1986).

The Iberian pig is a rustic pig breed, traditionally free-range-reared in the southwest of the Iberian peninsula.

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Muscles from Iberian pigs show higher contents of intramuscular lipids and heme pigments (Estévez, Morcuende, & Cava, 2003) and different enzyme muscle patterns from those of lean pigs as a result of specific genetic characteristics and different productive systems (Rosell & Toldrá, 1998). These differences in the composition of the muscles lead to variation in the susceptibility to oxidative and lipolityc changes during processing or storage (Estévez, Morcuende, Ventanas, & Cava, 2002).

The aim of this work was to analyse the composition of phospholipids from *m. Longissimus dorsi* from lean and fatty pigs and the changes occurring in the fatty acid and fatty aldehyde compositions after refrigerated storage, of raw and cooked samples, in order to shed light on the role of phospholipid plasmalogens in meat.

2. Material and methods

2.1. Animals

For the study, five fatty pigs (Iberian pigs) and five lean pigs (Landrace×Large White) were used. Fatty pigs were free-range-reared, feeding on grass, supplemented with a feed based on cereals, while lean pigs were intensively reared. Fatty and lean pigs were slaughtered at 90 kg live weight (Estévez, Morcuende, & Cava, 2003).

2.2. Sampling

After slaughtering, *m. Longissimus dorsi* were removed from the carcasses and freed of visible fat. Twelve hours post-mortem, *m. Longissimus dorsi* were sliced (1.5 cm thick slices) and used for the experiments, 1 and 2.

2.2.1. Experiment 1

Slices of *m. Longissimus dorsi* were over-wrapped in PVC film, placed on Styrofoam meat trays, and stored at +4 °C under fluorescent light for 10 days.

2.2.2. Experiment 2

Muscle slices were introduced into a plastic bag and cooked in a water bath for 10 min at 80 °C. After cooking, muscle slices were over-wrapped in PVC film and placed on Styrofoam meat trays and stored at +4 °C under fluorescent light for 10 days.

2.3. Methods

2.3.1. Lipid isolation

Intramuscular total lipids from muscles were extracted according to the method described by Bligh and Dyer (1959).

2.3.2. Lipid fractionation

From the extracted lipids, polar lipid (PL) fractions of muscles were isolated using NH_2-aminopropyl solid phase extraction cartridges according to the method developed by Garcia-Regueiro, Gilbert, and Diaz (1994).

2.3.3. Fatty acid analysis

Fatty acid methyl esters (FAMEs) and dimethylacetals (DMA) from fatty acids and fatty aldehydes from the polar lipid fraction were prepared by acidic sterification in presence of sulphuric acid (Cava et al., 1997). FAMEs and DMAs were analysed using a Hewlett Packard, model HP-5890A, gas chromatograph, equipped with a flame ionisation detector (FID). FAMEs and DMAs were separated on a 30 m FFAP-TPA fused-silica column (Hewlett Packard) with an i.d. of 0.53 mm and a 1.0 μm film thickness. The injector and detector were maintained at 230 °C. Column oven temperature was maintained at 225 °C. The carrier gas was nitrogen at a flow rate of 1.8 ml/min. Identification of FAMEs and DMAs was based on retention times of reference compounds (Sigma) and mass spectrometry. Fatty acids and fatty aldehydes from phospholipids were expressed as percentage of total of fatty acids and fatty aldehydes analysed.

2.3.4. Statistical analysis

Data obtained from fatty acid and fatty aldehyde compositions of phospholipids were used as variables. In order to determinate the effect of the source of meat (fatty and lean pigs) and the two different experiments (refrigeration and cooking+refrigeration) on the fatty acid and fatty aldehyde compositions of phospholipids, an analysis of variance together with the interaction was used, following the generalised linear model (GLM) procedure of SPSS software (SPSS, 1999). Tukey's tests were used when ANOVA showed significant differences between treatments. Significance was defined at $P<0.05$. In order to determine the effect of meat source on the increments of dimethylacetals and the ratios DMA/FA, DMA/PUFA and DMA/SFA measured after the different experiments, student *t*-tests for dependant variables were performed.

3. Results and discussion

3.1. *Fatty acids and fatty aldehyde composition of phospholipids from raw* m. Longissimus dorsi

Fatty acid and fatty aldehyde composition of phospholipids and the ratio dimethylacetals/fatty acids (DMA/FA) from *m. Longissimus dorsi* are shown in Tables 1 and 2, respectively.

Regarding fatty acid composition of polar lipids, polyunsaturated fatty acids (PUFA) were the most

Table 1
Fatty acid profiles of polar lipids of raw, refrigerated (10 days at +4 °C) and cooked (80 °C for 10 min, 10 days at +4 °C) *m. Longissimus dorsi* from fatty and lean pigs[a]

	Raw meat (day 0)			Refrigerated meat (day 10)			Cooked refrigerated meat (day 10)			Statistical significance[b]		
	Fatty	Lean	SEM	Fatty	Lean	SEM	Fatty	Lean	SEM	M	E	I
12	0.14a	0.06c	0.02	0.16a	0.11b	0.01	0.10b	0.08bc	0.01	*	ns	ns
14	0.43bc	0.32c	0.06	0.43bc	0.63a	0.04	0.45bc	0.59b	0.03	ns	ns	*
16	19.8b	21.2a	0.26	19.6b	11.7c	1.27	20.7ab	22.6a	0.35	ns	*	*
17	0.37c	0.48bc	0.03	0.59b	1.65a	0.17	0.35c	0.42bc	0.09	*	*	*
18	8.84c	9.65c	0.26	10.3bc	9.36c	0.47	11.5b	13.4a	0.41	ns	*	ns
20	1.03	0.10	0.04	tr	tr	–	0.12	0.07	0.02	ns	ns	ns
Σ SFA	30.6b	31.8b	1.61	31.1b	23.4c	0.69	34.9a	35.1a	0.16	ns	*	**
16:1	1.33c	1.45c	0.10	1.76b	1.97b	0.08	1.86b	2.31a	0.12	ns	*	ns
17:1	0.34b	0.34b	0.03	0.35b	0.10c	0.02	0.46ab	0.58a	0.08	ns	*	ns
18:1	24.3ab	20.5b	1.43	21.9b	15.0c	1.09	26.90a	27.7a	0.17	ns	*	**
20:1	2.13b	0.56c	0.21	0.36d	3.36a	0.46	0.62c	0.46cd	0.07	**	*	*
Σ MUFA	28.1ab	22.8bc	0.32	24.5b	20.4c	1.31	29.8a	31.07a	0.19	ns	*	**
18:2	23.2c	31.3a	1.37	26.1bc	28.1b	0.79	25.2bc	24.2c	0.24	**	ns	**
18:3	1.08bc	0.78c	0.04	1.34b	6.80a	0.88	0.87c	1.18bc	0.13	***	**	**
20:2	2.48a	1.43c	0.06	1.68b	1.78b	0.07	1.16d	1.02d	0.06	ns	*	**
20:4	14.5b	11.8c	0.43	15.4b	19.5a	0.92	7.99d	7.72d	0.07	ns	***	**
Σ PUFA	41.3bc	45.4b	1.51	44.5b	56.2a	1.86	35.2c	34.1c	0.14	ns	***	***

On the same line, means with different letters were statistically different.
[a] Results are expressed as total of fatty acids identified.
[b] Statistical significance in ANOVA test. M: meat source effect, E: experiment effect, I: interaction effect. *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; ns: non significant.

Table 2
Fatty aldehyde profiles from plasmalogens and ratios DMA/FA, DMA/SFA and DMA/PUFA of raw, refrigerated (10 days at +4 °C) and cooked (80 °C for 10 min, 10 days at +4 °C) *m. Longissimus dorsi* from fatty and lean pigs[a]

	Raw meat (day 0)			Refrigerated meat (day 10)			Cooked refrigerated meat (day 10)			Statistical significance[b]		
	Fatty	Lean	SEM	Fatty	Lean	SEM	Fatty	Lean	SEM	M	E	I
C16AL	52.52a	46.23b	1.28	44.40b	39.84c	1.42	52.41a	46.87b	1.32	**	ns	*
C18AL	30.85c	32.35b	0.61	31.65bc	37.60a	1.12	30.70c	32.33b	0.20	*	ns	*
C18:1AL	16.63c	21.43b	0.88	23.95a	22.56ab	1.22	16.89c	20.80b	0.62	**	ns	*
DMA/FA[c]	20.01bc	22.70bc	0.74	28.97b	41.76a	1.85	12.81c	9.92c	0.57	*	***	*
DMA/SFA[d]	69.99c	73.61c	1.04	96.48b	199.62a	2.06	37.48d	29.21e	1.16	**	**	**
DMA/PUFA[e]	54.16b	50.38b	0.83	65.40ab	74.39a	0.62	36.41c	29.41d	1.21	ns	*	*

On the same line, means with different letters were statistically different.
[a] Results are expressed as total of dimethylacetals identified.
[b] Statistical significance in ANOVA test. M: meat source effect, E: experiment effect, I: interaction effect. *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; ns: non significant.
[c] Ratio dimethylacetals/total fatty acids from phospholipids.
[d] Ratio dimethylacetals/saturated fatty acids from phospholipids (C16 + C18).
[e] Ratio dimethylacetals/polyunsaturated fatty acids from phospholipids (C18:2 + C18:3 + C20:2 + C20:4).

abundant fatty acids in polar lipids (41.3–45.3%). Saturated fatty acids (SFA) (30.6–31.8%) and monounsaturated fatty acids (MUFA) (22.8–28.1%) were in lower proportions. *m. Longissimus dorsi* from fatty pigs showed a higher proportion of MUFA and less PUFA than lean pig's *Longissimus dorsi* ($P>0.05$). The fatty acid profile exhibited by lean pigs' *Longissimus dorsi* predispose them to suffer higher oxidation processes during refrigerated storage and cooking, decreasing quality, since off-flavours present in cooked meat (warmed-over flavour) depend on PUFAs content and oxidation (Pearson & Gray, 1983). In general, results agree to those reported by other workers in lean pigs (Buscailhon, Gandemer, & Monin, 1994) and Iberian pigs (Cava et al., 1997).

The analysis of fatty aldehydes from plasmalogen revealed that hexadecanal (C16:0-al) was the most abundant fatty aldehyde, representing 46.2–52.5% of the total fatty aldehydes analysed, followed by octodecanal (C18:0-al) (30.9–32.1%) and octodecenal (C18:1-al) (16.6–21.4%).

Fatty aldehyde profile was significantly affected by the source of meat. *m. Longissimus dorsi* from fatty pigs

exhibited a significant higher proportion of C16:0-al ($P < 0.05$) than *m. Longissimus dorsi* from lean pigs. Inversely, muscle phospholipids from the latter showed a higher percentage of C18:1-al ($P < 0.05$). The ratio DMA/FA was between 20.01 and 22.70%. Our findings are in agreement with results previously described by Alasnier and Gandemer (1998) in *m. Longissimus lumborum* of rabbits and by Morcuende et al. (2003) in *m. Longissimus dorsi* of pigs.

The synthesis of plasmalogens is regulated by the enzyme acyl-CoA reductase and their activity is fairly specific for oleate (C18:1), stearate (C16:0) and palmitate (C16:0). The formation of fatty aldehydes is mediated by a reduction reaction in the presence of NADPH, synthesizing fatty aldehydes that are linked to the *sn-1* position of the glycerol backbone by an ether link (Ten-ching Lee, 1998). A non-specific linking of fatty aldehydes to the plasmalogen could lead to different proportions of fatty aldehydes in the plasmalogen molecule, dependent on the proportion of precursor fatty acids from which fatty aldehydes are synthesized. However, in our study a higher proportion of C18:1-al in *m. Longissimus dorsi* from lean pigs is found, in spite of a higher proportion of oleic acid (C18:1) in the *m. Longissimus dorsi* from fatty pigs (Estévez, Morcuende, & Cava, 2003), in contradiction to the exposed trend. Therefore, results suggest that plasmalogen synthesis is under complex regulation systems in which different variables can take part, as previously described by Snyder, Lee, and Wykle (1985). Consequently, the differences observed between lean and fatty pigs could be due to genetic or breeding system factors that could be related to the activity of enzymatic systems involved in the synthesis of certain plasmalogens.

3.2. Changes in the profiles of fatty acids and fatty aldehydes of phospholipids during refrigerated storage of fresh meat

The percentage of palmitic acid decreased in both groups, 0.15% for fatty pigs and 44.8% for lean pigs, this change being statistically significant only for the latter ($P < 0.05$). Likewise, total SFA followed a similar trend, and the differences between lean and fatty pigs are clearly significant (Table 1). Storage under refrigeration affected oleic acid and MUFA percentages, which significantly decreased in all groups at the end of the refrigeration period ($P < 0.05$). PUFA proportions concomitantly grew in polar lipids against the other fatty acids. This increase depended on the breed, being higher in lean pig muscles than in fatty pig muscles (fatty pig: 11.8% vs. lean pig: 19.3%). These results suggest a possible specific phospholipase activity on the palmitic acid or glycerol backbone position in which this fatty acid is sterified in the phospholipid molecule as has been described in previous papers (Estévez et al., 2002; Morcuende et al., 2003).

Fatty aldehyde profiles underwent strong changes during refrigerated storage at +4 °C for 10 days (Table 1).

The percentage of hexadecanal decreased in the two groups, while the proportion of octodecanal and octodecenal conversely increased after the refrigerated storage (Table 1). These results could be attributable to a specific activity of muscle phospholipase enzyme on C16-al plasmalogens, as previously was described for palmitic acid (C16:0) (Morcuende et al., 2003). However, there are no reported data about muscle enzymes with a specific activity that hydrolyses the ether-link between the hexadecanal and glycerol backbone in the plasmalogen molecule, so muscle phospholipases could have some difficulties in hydrolysing this ether-link. This is supported by the evolution of the ratio DMA/FA from phospholipids. The ratio DMA/FA significantly increased ($P < 0.001$) in lean and fatty pigs' *m. Longissimus dorsi* (from 22.7 to 41.7% and 20.0 to 28.9%, respectively; $P < 0.05$). When analysed, the ratio between DMA and the fatty acids was especially affected by the refrigerated storage (i.e. the SFA), and the increase of this ratio (DMA/SFA) after refrigeration, is statistically significant (lean pigs: from 73.6 to 199.6%; fatty pigs: from 69.9 to 96.5%; $P < 0.05$). These different trends could be attributed to phospholipase activity. Thus, the vinyl–ether bond in fatty aldehydes from plasmalogen phospholipids makes them more susceptible to oxidative reactions than their PUFA ester analogues on which, the former would have a protective role (Marmer et al., 1986). Furthermore, the results suggest that these changes are more pronounced in *m. Longissimus dorsi* from lean pigs. The ratios DMA/FA and DMA/SFA increased 84 and 63%, respectively in lean pigs' *m. Longissimus dorsi*, while in *m. Longissimus dorsi* from Iberian pigs these ratios increased in 33.3 and 30.3% respectively. Some previous papers have suggested a higher phospholipase activity in *m. Longissimus dorsi* from lean pigs than in *m. Longissimus dorsi* from fatty pigs (Rosell & Toldrá, 1998).

3.3. Changes in the profiles of fatty acids and fatty aldehydes of phospholipids during the refrigeration of cooked meat

The fatty acid profile from the polar lipid fraction was clearly affected by cooking and refrigerated storage (Table 1).

The PUFA proportion sharply decreased both in samples from fatty pigs (from 41.2% to 35.2%) and in samples from lean pigs (from 45.3 to 34.1%) these decreases only being statistically significant in the latter ($P < 0.05$). Changes registered for linoleic acid depended on the group studied. *Longissimus dorsi* muscle from fatty pigs showed a decrease of around 8%, while samples from lean pigs suffered a significant reduction, near

23% ($P<0.05$). Consequently, total SFA percentages concomitantly increased in fatty pigs (from 30.6 to 34.9%; $P<0.05$) and lean pigs (from 31.8 to 35.1%); ($P<0.05$). These changes can be attributed to oxidative deterioration, since the high temperature reached during cooking reduces enzyme activity and favour oxidative reactions in meat (Kristensen & Andersen, 2001). In fact, the proportion of susceptible fatty acids the most to oxidation (PUFA) decreased against SFA and MUFA percentages, resulting in a clear opposition to results obtained from changes in the fatty acid profile of refrigerated raw meat.

The effect of cooking and refrigeration on fatty aldehyde profiles from plasmalogens is shown in Table 2.

Cooking did not affect the relative proportion of the different fatty aldehydes in polar lipids. However, the ratio DMA/FA showed opposite behaviour to the behaviour in refrigerated meat. After refrigerated storage of cooked meat, the ratios, DMA/FA and DMA/PUFA, significantly decreased ($P<0.05$) in both groups (fatty pig: 40.2 and 30.8%; lean pig: 56.3 and 41.6%, respectively).

Taking into account that changes in lipid composition of intramuscular fat during refrigeration of cooked meat are principally oxidative changes, the reduction in the ratio DMA/FA supports the hypothesis of the high oxidative susceptibility of plasmalogens. In the presence of singlet oxygen and/or free radicals, plasmalogens markedly delayed the oxidative degradation of intrachain double bonds, probably due to the interaction of vinyl ether double bonds with initiating peroxyl radicals, as well as with products generated by the initial oxidation of PUFA (Brosche, 1997). In this sense, plasmanylglycerophospholipids are rapidly and preferentially destroyed to the corresponding lisoplasmalogen (2 monoacyl-GPL), formic acid and pentadecanal (Morand et al., 1988), these being products that do not propagate the peroxidation of PUFA. This mechanism implies that plasmalogens could act as antioxidant substances, blocking the lipid autoxidation chain, which is attributed to the presence of the vinyl–ether bond (Marmer et al., 1986; Nagan & Zoeller, 2001), avoiding the propagation of peroxidation of PUFA and functioning as 'chain breakers'.

Our results show a decrease in the ratio DMA/FA and DMA/PUFA, being more intense ($P<0.05$) in lean pig muscles (ΔDMA/FA = 56.3%; ΔDMA/PUFA = 41.6%) than in fatty pigs (ΔDMA/FA = 40.1%, ΔDMA/PUFA = 35.3%). These results suggest a higher oxidative activity in the *m. Longissimus dorsi* from lean pigs than in those from fatty pigs, agreeing with the evolution of fatty acid profile of phospholipids during refrigeration of cooked meat. Using the same samples, we have previously reported higher oxidative deterioration in cooked meat from lean pigs than from fatty pigs as measured by TBARS numbers and lipid-derived volatiles (Estévez, Morcuende, Ventanas, & Cava, 2003b). Differences were attributed to a lower PUFA content in subcellular membranes in muscles from Iberian pigs and to a possible higher content of tocopherols in these muscles due to a feeding based on grass (Cava, Ventanas, Tejeda, Ruiz, & Antequera, 2000). Deterioration processes similarly affected the three fatty aldehydes identified. This shows that the process of oxidative deterioration of fatty aldehydes and plasmalogens is very different from the phenomena that affect PUFA. In the former, their susceptibility depends on the vinyl–ether bond, and does not depend on the number of carbon residues and unsaturation, contrary to the fatty acids.

4. Conclusions

Esterified fatty acids in phospholipids are hydrolysed to a greater degree than fatty aldehydes linked to glycerol. This different susceptibility to hydrolytic processes could be attributed to inability of muscle phospholipases to fully hydrolyse the ether-link in plasmalogens. These enzymes seem to have an actuating preference for hexadecanal or the plasmalogen in which this fatty aldehyde is linked and, furthermore, its activity is more intense in lean pigs than in fatty pigs.

Muscle plasmalogens show a high oxidative instability that could lead to a protective effect on polyunsaturated fatty acids, retarding their oxidative deterioration and the development of rancidity in meat, and confirming the proposed role of plasmalogens as protective substances against radical attack on biological structures. Results derived from the study of the evolution of fatty acids and the relation DMA/PUFA after refrigerated storage and cooking show a higher oxidative stability in *m. Longissimus dorsi* from Iberian pigs, deriving a more favourable fatty acid composition.

References

Alasnier, C., & Gandemer, G. (1998). Fatty acid and aldehyde composition of individual phospholipid classes of rabbit skeletal muscles is related to the metabolic type of the fibres. *Meat Science*, *48*, 225–235.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*, 911–917.

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, triglycerides and phospholipids in muscles of the Iberian pig. *Meat Science*, *45*, 263–270.

Cava, R., Ventanas, J., Tejeda, J. F., Ruiz, J., & Antequera, T. (2000). Effect of free-range rearing and alpha-tocopherol and copper supplementation on fatty acid profiles and susceptibility to lipid oxidation of fresh meat from Iberian pigs. *Food Chemistry*, *68*, 51–59.

Estévez, M., Morcuende, D., Ventanas, J., & Cava, R. (2002). Lipolytic changes in meat from three lines of free-range reared Iberian pigs slaughetered at 90 kg live weight and commercial pigs under refrigerated storage. In: *Proceedings of the 48th Congress of Meat Science and Technology*. *Rome, Italy* (pp. 564–566).

Estévez, M., Morcuende, D., & Cava, R. (2003). Physico-chemical characteristics of m. *Longissimus dorsi* from three lines of free-range reared Iberian pigs slaughtered at 90 kg live-weight and commercial pigs: a comparative study. *Meat Science*, *64*, 499–506.

Estévez, M., Morcuende, D., Ventanas, S., & Cava, R. (2003). Analysis of volatiles in meat from Iberian pigs and lean pigs after refrigeration and cooking by using SPME-GC-MS. *Journal of Agricultural and Food Chemistry*, *51*, 3429–3435.

García-Regueiro, J. A., Gilbert, J., & Díaz, I. (1994). Determination of neutral lipids from subcutaneous fat of cured ham by capillary gas chromatography and liquid chromatography. *Journal of Chromatography A*, *667*, 225–233.

Kristensen, L., & Purslow, P. P. (2001). The effect of processing temperature and addition of mono- and di- valent salts on the heme-nonheme-iron ratio in meat. *Food Chemistry*, *73*, 433–439.

Marmer, W. N., Nungesser, E., & Foglia, T. A. (1986). *Lipids*, *21*, 648–651.

Morand, O. H., Zoeller, R. A., & Raetz, C. R. H. (1988). Disappearance of plasmalogens from membranes of animal cells subjected to photosensitized oxidation. *Journal of Biological Chemistry*, *263*, 11597–11606.

Morcuende, D., Estévez, M., Ruiz, J., & Cava, R. (2003). Oxidative and lipolytic deterioration of different muscles from free-range reared Iberian pigs under refrigerated storage. *Meat Science*, *65*, 1157–1164.

Nagan, N., & Zoeller, R. A. (2001). Plasmalogens: biosynthesis and functions. *Progress in Lipid Research*, *40*, 199–299.

Pearson, A. M., & Gray, J. I. (1983). Mechanism responsible for warmed-over flavor in meat. In G. R. Walker, & M. S. Feather (Eds.), *The Maillard reaction in food and nutritian* (pp. 287–300). Washington: Chemical Society Symposium. Series 215.

Rosell, C. M., & Toldrá, F. (1998). Comparison of muscle proteolytic and lipolytic enzyme levels in raw hams from Iberian and white pigs. *Journal of the Science of Food and Agriculture*, *76*, 117–122.

Snyder, F., Lee, T., & Wykle, R. L. (1985). Ether-linked glycerolipids and their bioactive species: enzymes and metabolic regulation. In A. N. Martonosi (Ed.), *The enzymes of biological membranes (Vol. 2)* (pp. 1–58). New York: Plenum Press.

SPSS. (1999). *SPSS Base 10.0*. *User manual: application guide*. Republic of Ireland: SPSS Inc.

Ten-ching, Lee (1998). Biosynthesis and possible biological functions of plasmalogens. *Biochimica and Biophysica Acta*, *1394*, 129–145.

Zoeller, R. A., Lake, A. C., Nagan, N., Gaposchkin, D. P., Legner, M. A., & Lieberthal, W. (1999). Plasmalogens as endogenous antioxidants: somatic cell mutants reveal the importance of the vinyl ether. *Biochemical Journal*, *338*, 768–776.