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Lipolysis in dry-cured ham: Influence of salt content and processing conditions

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Abstract

Fatty acid composition of neutral lipids (NLs), polar lipids (PLs) and free fatty acids (FFA) from the intramuscular fat of *Semimembranosus* and *Biceps femoris* muscles was analysed in 46 Iberian dry-cured hams processed with different amounts of salt (6% high salt batch – HS vs. 3% low salt batch – LS w/w) and different processing systems (traditional – T vs. modified – M).

Total amounts of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in NLs decreased in similar proportions during processing of the hams as well as SFA, MUFA and PUFA in the PL fraction, whereas the amounts of SFA, MUFA and PUFA of FFAs significantly increased in *Semimembranosus* and *Biceps femoris* muscles. The amount of total fatty acids (TFA), from NLs and PLs, decreased in both muscles throughout the processing. Such a decline was more intense in HS hams than in LS ones, which could be a sign of a promoting effect of sodium chloride on lipolysis. However, the increase in FFA content throughout processing was not more intense in HS hams. Processing conditions studied in this work did not affect the changes in the fatty acid content of each fraction.

Keywords: Lipolysis; Dry-cured ham; Salt; Processing temperature

1. Introduction

Lipolysis is one of the main degradation mechanisms affecting lipids during the processing of dry-cured ham (Martin, Antequera, Cordoba, Timon, & Ventanas, 1998). Muscle lipases and phospholipases appear to be responsible for lipolysis in meat and meat products (Motilva, Toldra, Nieto, & Flores, 1993), resulting in the formation of free fatty acids (FFA) (Coutron-Gambotti & Gandemer, 1999; Motilva et al., 1993). Some authors claim that such an increase in free fatty acid content leads to a higher susceptibility to lipid oxidation (Ansorena, Zapelena, Astiasarán, & Bello, 1998; Coutron-Gambotti & Gandemer, 1999), which in turn leads to formation of a great number of flavour volatiles (Andres, Cava, & Ruiz, 2002; Ruiz, Ventanas, Cava, Andres, & Garcia, 1999). However, other researchers have hypothesized that free fatty acids remain in the membrane, where they are protected against oxidation (Gandemer, 2002). Both neutral lipids (NLs) and polar lipids (PLs) contribute to FFA generation. However, PLs seem to be the main substrates for lipolysis in dry-cured hams (Buscailhon, Gandemer, & Monin, 1994; Flores, Nieto, Bermell, & Alberola, 1987; Martin, Cordoba, Ventanas, & Antequera, 1999).

Very little is known about the effects of technological factors on the development of lipolysis phenomena in dry-cured ham. Temperature seems to enhance lipolysis since, during the stages in which the temperature rises, the amount of FFA increases (Antequera et al., 1993; Martin et al., 1999). The effect of salt on lipolysis has not been elucidated yet. Several studies have shown a positive effect of salt on lipolysis (Motilva & Toldra, 1993; Vestegaard, Schivazappa, & Virgili, 2000) whereas other

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authors have found no effect (Coutron-Gambotti, Gandemer, Rousset, Maestrini, & Casabianca, 1999).

In the past two decades, salt content in Iberian dry-cured ham has been considerably reduced, due to consumer demand for less salty meat products (Guerrero et al., 1998) and due to health recommendations (Morgan, Aubert, & Brunner, 2001). Processing conditions have been adapted to a lower amount of salt (Andres & Ruiz, 2001) in order to avoid technological risks (Baldini, Campanini, Pezzani, & Palmia, 1984) and negative implications on sensorial quality (Arnau, Guerrero, & Sarraga, 1998). However, the influences of reducing salt content and changes in processing conditions on lipolysis in Iberian dry-cured ham remain unknown. Thus, the main aim of this work was to determine the effect of two levels of salt and two different processing systems on the changes in the composition of major muscle lipid fractions (NLs, PLs and FFA) throughout the processing of Iberian dry-cured ham.

2. Material and methods

2.1. Experimental design

Forty-six thighs were obtained from 23 Iberian × Duroc pigs with similar genetics that had been reared in the same rearing system. Six of them were sampled and analysed (raw ham – R), and the rest were processed into dry-cured Iberian hams. Hams were placed on shelves in a cold room held at 1–3 °C and salted by individual addition of a controlled amount of salt in the lean part of the raw ham. Two different salt levels were considered: a group of 20 hams was salted with 6% of salt (w/w) high salt batch (HS), whereas the other 20 hams were salted by adding 3% of salt (w/w) low salt batch (LS). Salting was completed when there was no visible salt on the ham surface. After completion of salting, all hams were held at 2–5 °C and 85–75% relative humidity for 60 days (post-salting phase).

Table 1
Fatty acid composition (expressed as mg/100 g DM) of NLs from intramuscular fat of *Semimembranosus* muscle throughout the ripening of dry-cured Iberian hams with either 6% HS or 3% LS salt (w/w) and processed in a traditional (T) or a modified (M) system

	Salt	Processing	Sampling						SEM	P_{time}
			R ($n = 6$)	PS ($n = 5$)	EPS ($n = 5$)	D ($n = 5$)	ED ($n = 5$)	EC ($n = 6$)		
SFA		T				3287^{1}	3224^{1}	2165^{1}	304	ns
	HS			$3733^{1/12}$	$1444^{1/12}$					
		M				4612^{12}	1917^{12}	1080^{2}	370	*
			$3577^{1/12/1/1}$							
		T				4517^{1}	3977^{1}	2048^{1}	351	ns
	LS			$2313^{1/1}$	$2178^{1/1}$					
		M				4756^{1}	5426^{1}	2555^{1}	555	ns
		$P_{Salt/Processing/Interaction}$	–/–/–	ns/–/–	ns/–/–	ns/ns/ns	ns/ns/ns	ns/ns/ns		
MUFA		T				4155^{1}	3314^{1}	2412^{1}	373	ns
	HS			$5289^{1/12}$	$2045^{1/2}$					
		M				6665^{1}	2399^{2}	1319^{2}	511	**
			$4884^{1/12/1/1}$							
		T				6875^{1}	5469^{1}	2483^{1}	579	ns
	LS			$3060^{1/1}$	$3828^{1/1}$					
		M				7581^{1}	1854^{1}	3301^{1}	925	ns
		$P_{Salt/Processing/Interaction}$	–	ns/–/–	ns/–/–	ns/ns/ns	*/ns/ns	ns/ns/ns		
PUFA		T				371^{1}	972^{123}	477^{3}	79	ns
	HS			$569^{1/12}$	$236^{1/23}$					
		M				615^{1}	315^{1}	208^{1}	44	**
			$584^{1/12/1/1}$							
		T				667^{1}	1712^{1}	444^{1}	177	ns
	LS			$259^{1/1}$	$412^{1/1}$					
		M				614^{1}	812^{1}	357^{1}	69	ns
		$P_{Salt/Processing/Interaction}$	–	*/–/–	ns/–/–	ns/ns/ns	ns/ns/ns	ns/ns/ns		

SFA, total amount of saturated fatty acids; MUFA, total amount of monounsaturated fatty acids; PUFA, total amount of polyunsaturated fatty acids.

R, raw ham = 0 days; PS, post-salting = 102 days; EPS, end of post-salting = 138 days; D, drying = 173 days; ED, end of drying = 203 days; EC, end of cellar = 415 days.

Significant levels: ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$.

[1–3]Different superscripts within the same batch between different stages mean significant differences ($P < 0.05$).

Temperature was thereafter increased from 5 to 20 °C at 0.25 °C/day during 60 days, while relative humidity was progressively reduced to 65%. Before the beginning of the drying stage, HS and LS batches were divided into a further two groups, each one following different processing thereafter. Half the hams of each salt level followed a process which tried to mimic the temperature evolution of the traditional processing. This group (traditional – T) was processed at a maximum temperature of 28 ± 2 °C during the drying stage (77 days), followed by a cellar phase (212 days) at 15.5 ± 0.5 °C. The other group of hams was ripened following a modified processing (modified – M), in which the temperature was kept constant (19 ± 1 °C) during both the drying stage (77 days) and the cellar stage (137 days). Both processes took 415 days and hams were (bone-in) 7.3 ± 0.5 kg in weight. In the R hams and at the end of cellar (EC), *Semimembranosus* and *Biceps femoris* muscles were dissected from the hams whereas, in the rest of stages, samples were obtained by extraction of a cylinder, sized 10×2.5 cm, using a stainless steel tube with a cutting edge. These samples mainly involved *Semimembranosus* and *Biceps femoris* muscles, which were perfectly identifiable. Samples were vacuum-packaged and kept frozen at −80 °C until analysed.

2.2. Lipid analysis

Lipids were extracted with chloroform/methanol (2:1) according to the method of Folch, Lees, and Sloane-Stanley (1957) and dried under nitrogen. NL, PL and FFA fractions from intramuscular fat were separated using NH_2-aminopropyl minicolumns, following the method described by Kaluzny, Duncan, Merrit, and Epps (1985). Fatty acid methyl esters (FAMEs) of both fractions were prepared by acidic-trans-esterification in the presence of sulphuric acid (5% sulphuric acid in methanol (Sandler & Karo, 1992)). FAMEs were

Table 2
Fatty acid composition (expressed as mg/100 g DM) of NLs from intramuscular fat of *Biceps femoris* muscle throughout the ripening of dry-cured Iberian hams with either 6% HS or 3% LS salt (w/w) and processed in a traditional (T) or a modified (M) system

	Salt	Processing	Sampling						SEM	P_{time}
			R ($n = 6$)	PS ($n = 5$)	EPS ($n = 5$)	D ($n = 5$)	ED ($n = 5$)	EC ($n = 6$)		
SFA		T				6454^{12}	7693^{12}	2989^{2}	693	ns
	HS			$8215^{1/1}$	$4638^{12/1}$					
		M				7550^{1}	8147^{1}	3824^{1}	692	ns
			$8367^{1/1/1/1}$							
		T				8287^{1}	12286^{1}	5978^{1}	786	ns
	LS			$6746^{1/1}$	$5019^{1/1}$					
		M				7991^{1}	6713^{1}	3899^{1}	812	ns
		$P_{Salt/Processing/Interaction}$	−/−/−	ns/−/−	ns/−/−	ns/ns/ns	ns/ns/ns	ns/ns/ns		
MUFA		T				8976^{123}	11013^{12}	3798^{3}	894	*
	HS			$12275^{1/1}$	$5986^{23/12}$					
		M				8932^{12}	6675^{12}	4739^{2}	816	*
			$10688^{12/12/12/12}$							
		T				10851^{12}	13607^{12}	7342^{2}	839	*
	LS			$15478^{1/1}$	$7220^{2/2}$					
		M				9706^{12}	9898^{12}	5055^{2}	873	*
		$P_{Salt/Processing/Interaction}$	−/−/−	ns/−/−	ns/−/−	ns/ns/ns	ns/ns/ns	ns/ns/ns		
PUFA		T				710^{12}	768^{12}	393^{2}	62	**
	HS			$933^{1/1}$	$442^{2/1}$					
		M				655^{1}	76^{1}	475^{1}	65	*
			$982^{1/1/1/1}$							
		T			$655^{1/1}$	896^{1}	957^{1}	625^{1}	55	ns
	LS			$976^{1/1}$						
		M				1088^{1}	774^{1}	424^{1}	71	ns
		$P_{Salt/Processing/Interaction}$	−/−/−	ns/−/−	ns/−/−	ns/ns/ns	ns/ns/ns	ns/ns/ns		

SFA, total amount of saturated fatty acids; MUFA, total amount of monounsaturated fatty acids; PUFA, total amount of polyunsaturated fatty acids.

R, raw ham = 0 days; PS, post-salting = 102 days; EPS, end of post-salting = 138 days; D, drying = 173 days; ED, end of drying = 203 days; EC, end of cellar = 415 days.

Significant levels: ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$.

[1–3]Different superscripts within the same batch between different stages mean significant differences ($P < 0.05$).

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 i.d., 1 μm film thickness) maintained at 225 °C. Injector and

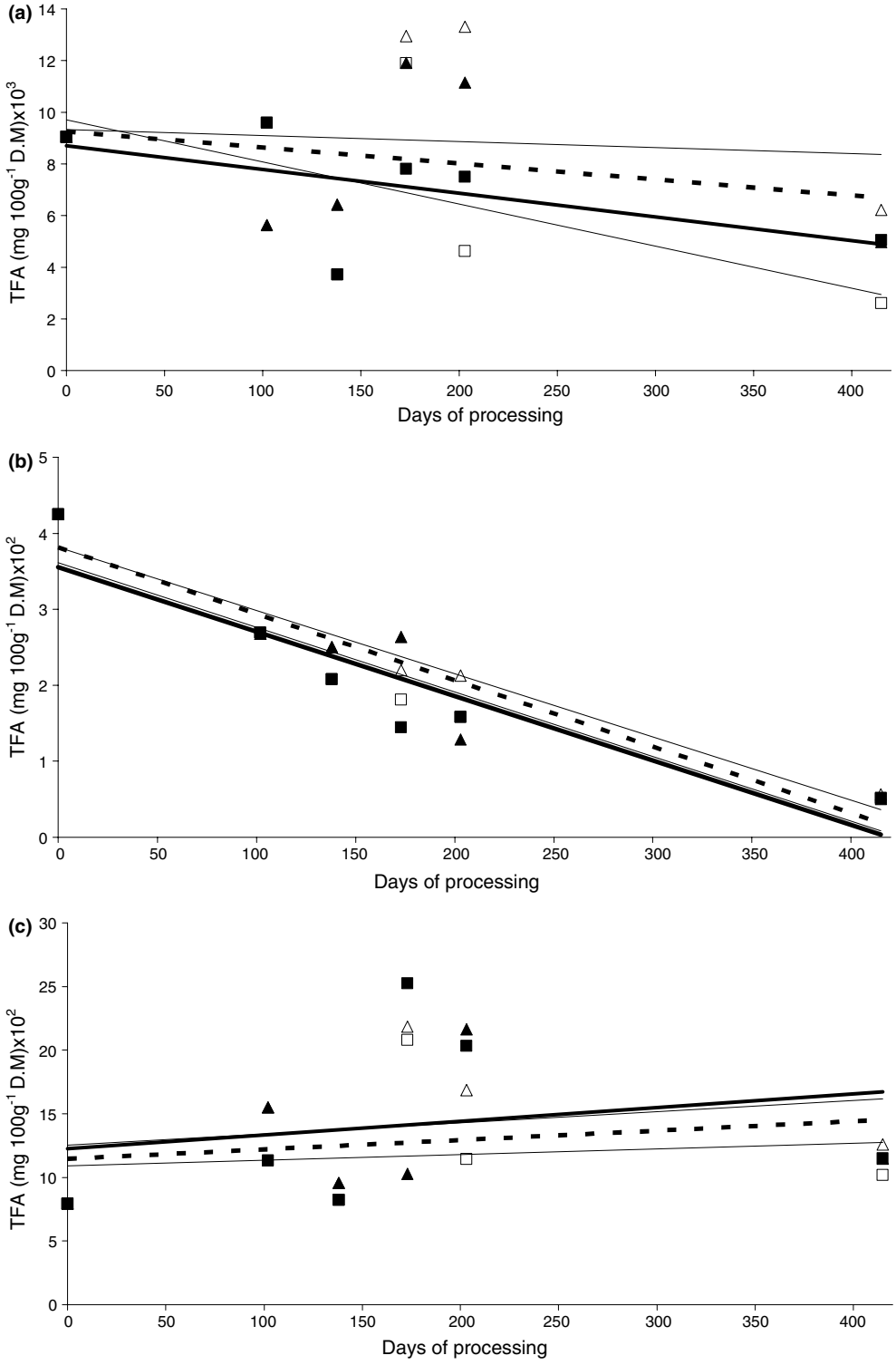

Fig. 1. Evolution of total fatty acids of neutral lipids (a) polar lipids (b) and free fatty acids (c) fractions of *Semimembranosus* muscle throughout the processing of dry-cured Iberian hams with either 6% HS or 3% LS salt (w/w) and processed in a traditional (T) or a modified (M) system. Values of total fatty acid content in each stage are represented as figures (HS–HT batch: ■; HS–LT batch: □; LS–HT batch: ▲; LS–LS batch: △). Evolution trends of fatty acids throughout processing are represented as lines: (HS–HT batch: thick continuous line; HS–LT batch: thin continuous line; LS–HT batch: thick discontinuous line; LS–LS batch: thin discontinuous line).

detector temperatures were 230 °C. Carrier gas was nitrogen at a flow rate of 1.8 ml/min. Individual FAME peaks were identified by comparing their retention times with those of reference compounds (Sigma, St Louis). Tridecanoic acid (Sigma, St. Louis) was used as internal standard. Standard curves for quantification were

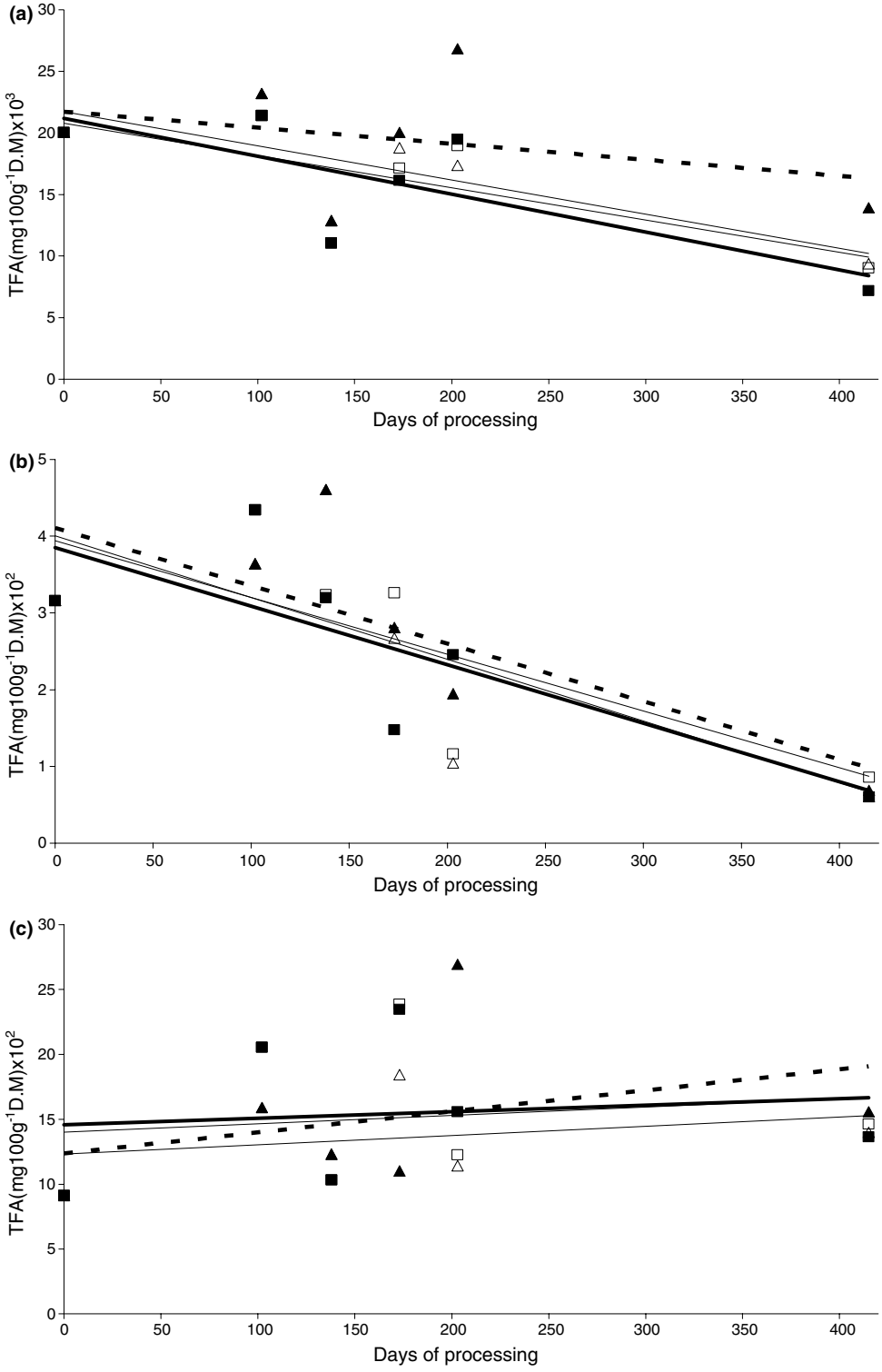

Fig. 2. Evolution of total fatty acid of neutral lipids (a) polar lipids (b) and free fatty acids (c) fractions of *Biceps femoris* muscle throughout the processing of dry-cured Iberian hams with either 6% HS or 3% LS salt (w/w) and processed in a traditional (T) or a modified (M) system. Values of total fatty acid content in each stage are represented as figures (HS–HT batch: ■; HS–LT batch: □; LS–HT batch: ▲; LS–LS batch: △). Evolution trends of fatty acids throughout processing are represented as lines: (HS–HT batch: thick continuous line; HS–LT batch: thin continuous line; LS–HT batch: thick discontinuous line; LS–LS batch: tin discontinuous line).

obtained for all fatty acids analysed under the conditions earlier described. Results were expressed as mg of selected fatty acid 100 g^{-1} of dry matter (DM).

2.3. Statistical analyses

The effects of the amount of added salt (6% vs. 3%) and processing conditions (traditional vs. modified) on fatty acid composition of lipid fractions within each stage were analysed by a two-way analysis of variance together with their interaction (salt × processing), using the general linear model procedure (SPSS 10.0). The effect of processing time on the composition of lipid fractions was also analysed by a one-way analysis of variance using the general linear model procedure (SPSS 10.0). The Tukey's test was used at the 5% level to make comparisons between sample means when pertinent.

3. Results and discussion

Tables 1 and 2 show the total amounts of saturated (SFA), monounsaturared (MUFA) and polyunsaturated (PUFA) fatty acids (expressed as mg/100 g DM) in the NL fraction of *Semimembranosus* and *Biceps femoris* muscles, respectively, from hams with either 6% HS or 3% LS salt (w/w) and processed under a traditional (T) or a modified (M) system. MUFA were the major fatty acids in NL of both muscles, followed by SFA and PUFA. These results are in agreement with previous findings (Andres et al., 2001; Martin et al., 1999). SFA, MUFA and PUFA in NL decreased in a similar manner during processing, final amounts ranging from ≈30% to 65% of the initial quantities determined in R ham. In contrast to this observation, Antequera et al. (1993) observed a preferential decline of certain unsaturated triglycerides during processing of dry-cured Iberian ham.

Table 3
Fatty acid composition (expressed as mg/100 g DM) of PLs from intramuscular fat of *Semimembranosus* muscle throughout the ripening of dry-cured Iberian hams with either 6% HS or 3% LS salt (w/w) and processed in a traditional (T) or a modified (M) system

	Salt	Processing	Sampling						SEM	P_{time}
			R ($n = 6$)	PS ($n = 5$)	EPS ($n = 5$)	D ($n = 5$)	ED ($n = 5$)	EC ($n = 6$)		
SFA		T				44b^{23}	68^{23}	17^{3}	9	***
	HS			91$^{12/12}$	89$^{12/12}$					
		M				62ab^{23}	69^{23}	18^{3}	9	***
			145^{1}							
		T				88a^{12}	54^{23}	18^{3}	9	***
	LS			83$^{2/123}$	90$^{12/12}$					
		M				68ab^{23}	85^{123}	21^{3}	9	**
		$P_{Salt/Processing/Interaction}$	–/–/–	ns/–/–	ns/–/–	*/ns/ns	ns/ns/ns	ns/ns/ns		
MUFA		T				38^{2}	39^{2}	17^{2}	6	**
	HS			56$^{12/12}$	36$^{2/3}$					
		M				48^{3}	30^{3}	17^{3}	9	**
			105^{1}							
		T				81^{12}	35^{12}	17^{2}	9	**
	LS			62$^{12/12}$	84$^{12/12}$					
		M				60^{12}	56^{12}	21^{2}	9	ns
		$P_{Salt/Processing/Interaction}$	–/–/–	ns/–/–	ns/–/–	*/ns/ns	ns/ns/ns	ns/*/ns		
PUFA		T				62^{34}	52^{34}	18^{4}	10	***
	HS			122$^{12/12}$	84$^{23/23}$					
		M				72^{234}	59^{4}	16^{4}	11	***
			175^{1}							
		T				95^{23}	40^{45}	15^{5}	11	***
	LS			123$^{2/2}$	76$^{34/2}$					
		M				92^{2}	72^{2}	15^{3}	11	***
		$P_{Salt/Processing/Interaction}$	–/–/–	ns/–/–	ns/–/–	ns/ns/ns	ns/ns/ns	ns/ns/ns		

SFA, total amount of saturated fatty acids; MUFA, total amount of monounsaturated fatty acids; PUFA, total amount of polyunsaturated fatty acids.

R, raw ham = 0 days; PS, post-salting = 102 days; EPS, end of post-salting = 138 days; D, drying = 173 days; ED, end of drying = 203 days; EC, end of cellar = 415 days.

Significant levels: ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

a–b: Different letters within the same stage mean significant differences between batches ($P < 0.05$). [1–3]Different superscripts within the same batch between different stages mean significant differences ($P < 0.05$).

Figs. 1 and 2 show the evolution trend of the amount of total fatty acids (TFA) from NL of intramuscular fat in *Semimembranosus* and *Biceps femoris* muscle, respectively. The amount of total fatty acids from NL decreased in both muscles throughout the processing (from 9 to 2.6–6.2 mg/100 g DM in *Semimembranosus* muscle and from 20 to 7.1–13.9 mg/100 g DM in *Biceps femoris* muscle). A decrease in fatty acids of NL has also been observed in previous works on Iberian ham (Antequera et al., 1993) and other types of dry-cured ham (Alasnier & Gandemer, 1998). Such a decrease has been mainly linked to lipolysis phenomena, giving rise to a parallel increase in FFA amount during processing. In fact, in Iberian ham, the contribution of fatty acids from NLs to FFA is especially important since the proportion of NLs in this breed is higher than in other pig breeds (80–90% of total intramuscular fat) (Antequera et al., 1993). Moreover, it has been well established that lipases remain total or partially active throughout ripening of dry-cured ham (Motilva et al., 1993; Toldra, 2002; Vestegaard et al., 2000). Direct oxidation of fatty acids from NL fraction could also contribute to the detected decrease. However, a very low susceptibility to lipid oxidation of NLs is well documented (Nawar, 1996; Wilson, Pearson, & Shorland, 1976) because of the lower degree of unsaturation of NL fatty acids and their location in adipocytes (Gray & Pearson, 1987). Furthermore, if oxidation was a significant cause of the decrease in fatty acids from NL, such a decrease would be more marked in PUFA than in MUFA or SFA. However, in our work, a similar decrease, for all families, was detected.

Salt content did not promote significant differences in SFA, MUFA and PUFA contents of NL in any of the studied stages (Tables 1 and 2 for *Semimembranosus* and *Biceps femoris* muscles, respectively). However, the

Table 4
Fatty acid composition (expressed as mg/100 g DM) of PLs from intramuscular fat of *Biceps femoris* muscle throughout the ripening of dry-cured Iberian hams with either 6% HS or 3% LS salt (w/w) and processed in a traditional (T) or a modified (M) system

	Salt	Processing	Sampling						SEM	P_{time}
			R ($n = 6$)	PS ($n = 5$)	EPS ($n = 5$)	D ($n = 5$)	ED ($n = 5$)	EC ($n = 6$)		
SFA	HS	T	$122^{12/1/1/12}$	$142^{1/1}$	$109^{1/23}$	$48b^{34}$	72^{234}	21^{4}	10	***
		M				108^{23}	51^{4}	32^{4}	12	ns
	LS	T		$122^{1/12}$	$154^{1/1}$	104^{12}	79^{3}	35^{3}	12	ns
		M				94^{12}	50^{12}	23^{2}	13	*
		$P_{Salt/Processing/Interaction}$	–/–/–	ns/–/–	ns/–/–	ns/ns/ns	ns/ns/ns	ns/ns/ns		
MUFA	HS	T	$75^{123/123/12/12}$	$121^{1/1}$	$49^{23/23}$	44^{23}	88^{12}	19ab^{3}	8	**
		M				85^{12}	25^{3}	30a^{3}	12	ns
	LS	T		$108^{12/12}$	$137^{1/1}$	85^{12}	58^{12}	15b^{2}	12	ns
		M				77^{12}	24^{12}	22ab^{2}	12	*
		$P_{Salt/Processing/Interaction}$	–/–/–	ns/–/–	ns/–/–	ns/ns/ns	ns/*/ns	ns/*/ns		
PUFA	HS	T	$119^{123/12/1/1}$	$171^{1/1}$	$162^{12/1}$	55^{34}	86^{234}	20^{4}	13	***
		M				132^{1}	41^{23}	23^{3}	16	ns
	LS	T		$134^{1/1}$	$170^{1/1}$	92^{1}	58^{1}	19^{1}	16	ns
		M				96^{1}	31^{1}	16^{1}	17	ns
		$P_{Salt/Processing/Interaction}$	–/–/–	ns/–/–	ns/–/–	ns/ns/ns	ns/*/ns	ns/ns/ns		

SFA, total amount of saturated fatty acids; MUFA, total amount of monounsaturated fatty acids; PUFA, total amount of polyunsaturated fatty acids.

R, raw ham = 0 days; PS, post-salting = 102 days; EPS, end of post-salting = 138 days; D, drying = 173 days; ED, end of drying = 203 days; EC, end of cellar = 415 days.

Significant levels: ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

a–b: Different letters within the same stage mean significant differences between batches ($P < 0.05$). [1–3]Different superscripts within the same batch between different stages mean significant differences ($P < 0.05$).

decrease in total fatty acid of NLs throughout the processing was more marked in hams with a higher salt content, as is shown in Figs. 1 and 2 for *Semimembranosus* and *Biceps femoris* muscles, respectively. This trend shows a promoting effect of salt on lipolysis of TGs. This result is consistent with that reported by other authors in other types of ham (Motilva & Toldra, 1993). However, other authors did not find any effect of salt content on triglyceride degradation (Coutron-Gambotti et al., 1999). Such an effect of salt on total fatty acids from NLs could also be due to a prooxidant effect of sodium chloride, which could lead to a greater degradation of fatty acids from this fraction. However, as previously explained, if such an effect were true, the decrease in PUFA would be more marked than in SFA, due to their higher susceptibility to oxidation (Igene, Pearson, Dugan, & Price, 1980).

Tables 3 and 4 show the total amounts of SFA, MUFA and PUFA (expressed as mg/100 g DM) of PL from *Semimembranosus* and *Biceps femoris* muscle, respectively. PUFA were the major fatty acids in PL, which is consistent with results of previous works on Iberian dry-cured ham (Martin et al., 1999). SFA, MUFA and PUFA contents decreased in a similar extent (75–90%) during processing and more markedly in *Semimembranosus* muscle than in *Biceps femoris* muscle. This is not in accordance with previous investigations in which PUFA reduction in the PL fraction was more intense than these of SFA and MUFA (Martin et al., 1999).

Figs. 1 and 2 show the evolution of total fatty acids of intramuscular PLs from *Semimembranosus* and *Biceps femoris* muscles. Total fatty acids from the PL fraction decreased from 4.2 to 0.5 mg/100 g DM in the *Semimembranosus* muscle and from 3 to 0.6–0.8 mg/100 g DM in the *Biceps femoris* muscle. A decrease in total fatty acids from PLs during the ripening has been shown in previous works on different types of dry-cured ham

Table 5
Free Fatty acid composition (expressed as mg/100 g DM) of intramuscular fat from *Semimembranosus* muscle throughout the ripening of dry-cured Iberian hams with either 6% HS or 3% LS salt (w/w) and processed in a traditional (T) or a modified (M) system

	Salt	Processing	Sampling						SEM	P_{time}
			R ($n = 6$)	PS ($n = 5$)	EPS ($n = 5$)	D ($n = 5$)	ED ($n = 5$)	EC ($n = 6$)		
SFA		T				898^{1}	743^{12}	504^{23}	51	***
	HS			$399^{23/2}$	$315^{3/2}$					
		M				752^{1}	421^{2}	389^{2}	37	**
			$293^{3/2/2/2}$							
		T				382^{12}	782^{1}	516^{2}	47	*
	LS			$554^{12/12}$	$366^{12/12}$					
		M				745^{1}	643^{12}	491^{2}	57	ns
		$P_{Salt/Processing/Interaction}$	–/–/–	ns/–/–	ns/–/–	**/ns/ns	ns/ns/ns	ns/ns/ns		
MUFA		T				667^{1}	638a^{1}	358^{2}	41	***
	HS			$299^{2/12}$	$179^{2/2}$					
		M				605^{1}	284b^{2}	293^{12}	37	**
			207^{2}							
		T				282^{2}	665a^{1}	320^{2}	38	***
	LS			$342^{2/12}$	$213^{2/2}$					
		M				612^{1}	486ab^{12}	414^{12}	40	**
		$P_{Salt/Processing/Interaction}$	–/–/–	ns/–/–	ns/–/–	*/ns/ns	ns/**/ns	ns/ns/ns		
PUFA		T				962^{1}	653^{12}	289^{2}	58	*
	HS			$435^{5/2}$	$331^{2/2}$					
		M				724^{1}	440^{12}	337^{2}	39	*
			$297^{2/2/1/1}$							
		T				366^{1}	718^{1}	311^{1}	52	*
	LS			$654^{1/1}$	$378^{/1}$					
		M				828^{1}	557^{1}	355^{1}	65	ns
		$P_{Salt/Processing/Interaction}$	–/–/–	ns/–/–	ns/–/–	*/ns/ns	ns/*/ns	ns/ns/ns		

SFA, total amount of saturated fatty acids; MUFA, total amount of monounsaturated fatty acids; PUFA, total amount of polyunsaturated fatty acids.

R, raw ham = 0 days; PS, post-salting = 102 days; EPS, end of post-salting = 138 days; D, drying = 173 days; ED, end of drying = 203 days; EC, end of cellar = 415 days.

Significant levels: ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

a–b: Different letters within the same stage mean significant differences between batches ($P < 0.05$). [1–3]Different superscripts within the same batch between different stages mean significant differences ($P < 0.05$).

(Antequera et al., 1993; Buscailhon et al., 1994; Flores et al., 1987; Martin et al., 1999). This is most likely due to the activity of lipolytic enzymes, namely phospholipases and lysophopholipases (Alasnier & Gandemer, 2000; Buscailhon et al., 1994 Toldra, 2002). Oxidation could also play a role in PL degradation. In fact, the high oxidation susceptibility of PLs compared to NLs (Igene et al., 1980) is well established, due to their location in membranes close to heme pigments and oxidant systems and due to their high PUFA content (Gray & Pearson, 1987). A higher decrease in PUFA content than MUFA of SFA should be expectable if oxidation were a major cause of PL degradation. However, SFA, MUFA and PUFA contents decreased to similar extents, suggesting that the decrease in fatty acid of PL is more likely due to lipolysis rather than to lipid oxidation.

As shown in Figs. 1 and 2, *Semimembranosus* and *Biceps femoris* muscles from hams with a higher salt content showed a more marked decrease in the amount of PL total fatty acids than those from less salted hams throughout the processing, this trend being more evident in the *Semimembranosus* muscle. As explained for NL, this suggests a promoting effect of sodium chloride on lipolysis of PL at the concentrations studied in this work. In fact, previous studies have shown a similar effect (Coutron-Gambotti et al., 1999; Motilva & Toldra, 1993).

The amounts of SFA, MUFA and PUFA of the FFA fraction significantly increased during processing in *Semimembranosus* and *Biceps femoris* muscles (Tables 5 and 6). Previous works observed that PUFA of FFA fractions decreased during processing, presumably due to their high susceptibility to oxidation, whereas SFA and MUFA remained stable or increased (Martin et al., 1999). As mentioned above, a decrease in PUFA during processing did not occur in this work though there was a less intense rise in PUFA than in MUFA or SFA.

Table 6
Free fatty acid composition (expressed as mg/100 g DM) of PLs from intramuscular fat of *Biceps femoris* muscle throughout the ripening of dry-cured Iberian hams with either 6% HS or 3% LS salt (w/w) and processed in a traditional (T) or a modified (M) system

	Salt	Processing	Sampling						SEM	P_{time}
			R ($n=6$)	PS ($n=5$)	EPS ($n=5$)	D ($n=5$)	ED ($n=5$)	EC ($n=6$)		
SFA		T				817a^{1}	525ab^{12}	499^{12}	47	**
	HS			772$^{1/12}$	375$^{2/3}$					
		M				820a^{1}	428b^{23}	562^{123}	46	**
			331$^{2/3/2/2}$							
		T				405b^{2}	980a^{1}	568^{2}	54	**
	LS			566$^{2/12}$	472$^{2/12}$					
		M				633ab^{1}	425b^{12}	532^{12}	32	*
		$P_{Salt/Processing/Interaction}$	–/–/–			ns/**/ns	ns/*/ns	ns/ns/ns		
MUFA		T				792^{1}	591^{12}	487^{12}	54	**
	HS			645$^{12/12}$	289$^{2/2}$					
		M				789^{1}	360^{2}	514^{12}	49	**
			286^{2}							
		T				371^{2}	1153^{1}	534^{12}	81	**
	LS			458$^{2/12}$	322$^{2/2}$					
		M				609^{1}	404^{12}	543^{12}	32	**
		$P_{Salt/Processing/Interaction}$	–/–/–	ns		ns/ns/ns	ns/*/ns	ns/ns/ns		
PUFA		T				738^{1}	443^{123}	378ab^{23}	41	**
	HS			639$^{12/12}$	367$^{23/3}$					
		M				778^{1}	439^{23}	387ab^{23}	39	***
			296$^{3/3/1/1}$							
		T				327^{1}	562^{1}	456^{1}	34	ns
	LS			567$^{1/1}$	438$^{1/1}$					
		M				602^{1}	313^{1}	324b^{1}	40	ns
		$P_{Salt/Processing/Interaction}$	–/–/–			ns/**/ns	ns/ns/ns	ns/ns/*		

SFA, total amount of saturated fatty acids; MUFA, total amount of monounsaturated fatty acids; PUFA, total amount of polyunsaturated fatty acids.

R, raw ham = 0 days; PS, post-salting = 102 days; EPS, end of post-salting = 138 days; D, drying = 173 days; ED, end of drying = 203 days; EC, end of cellar = 415 days.

Significant levels: ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

a–b: Different letters within the same stage mean significant differences between batches ($P < 0.05$). $^{1-3}$Different superscripts within the same batch between different stages mean significant differences ($P < 0.05$).

Figs. 1 and 2 show the evolution of total intramuscular FFA in *Semimembranosus* and *Biceps femoris* muscles, respectively. In *Semimembranosus* muscle total FFA increased from 7 to 10–12 mg/100 g DM while in *Biceps femoris* total FFA increased from 9 to 13–15 mg/100 g DM. The increase in free fatty acids observed in this work agrees with previous results reported for Iberian dry-cured ham (Antequera et al., 1993) and other types of ham (Motilva et al., 1993; Vestegaard et al., 2000). Either NLs or PLs contribute to FFA generation. Given that results from this study show a promoting lipolysis effect of salt, a higher increase in FFA content was expected in samples from more salted hams. However, such a tendency was not observed. In this sense, it is worth mentioning that while liberation of FFA is taking place, oxidation of these FFA could be occurring at the same time. Therefore, the amount of FFA depends on the equilibrium between lipolysis and oxidation phenomena.

Differences in temperature between studied processing systems in this work (28 ± 2 °C vs. 19 ± 1 °C at the drying stage and 15.5 ± 0.5 °C vs. 19 ± 1 °C at the cellar stage) were found to have no effect on the evolution of fatty acid composition of the studied lipid fractions. There is some evidence of a positive effect of temperature on lipolysis phenomena in Iberian ham, since some authors found a boost in the amount of FFA during the stages of processing in which temperature rises (Antequera et al., 1993; Martin et al., 1998). It could be that differences in temperature between the two studied processing conditions in the present work were not enough to cause significant variations in the activity of lipolytic enzymes.

4. Conclusions

The overall lipolysis that takes place in dry-cured hams during ripening affects PLs more intensely than NLs. Salt seems to have a slight promoting effect on these phenomena, at least at concentrations below 6%. Using different temperatures during the drying stage in the processing of Iberian dry-cured ham (at the levels that can be found in the industry for this type of ham) has no effect on the changes affecting the amount of fatty acids of the different lipid fractions.

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References

Alasnier, C. & Gandemer, G. (1998). Lipases et esterases endogènes et exogènes: leur rôle dans la maturation des produits à base de viande crue. Viandes et Produits Carnées. In *Proceedings of the VII Journées des sciences du muscles et technologies de la viande* (pp. 30–38). Rodez.

Alasnier, C., & Gandemer, G. (2000). Activities of phospholipases A and lysophospholipases in glycolytic and oxidative skeletal muscles in the rabbit. *Journal of the Science of Food and Agriculture, 80*(6), 698–702.

Andres, A. I., Cava, R., & Ruiz, J. (2002). Monitoring volatile compounds during dry cured ham ripening by solid-phase microextraction coupled to a new direct-extraction device. *Journal of Chromatography A, 963*(1–2), 83–88.

Andres, A. I., & Ruiz, J. (2001). Tecnología del salazonado del jamón Ibérico. In J. Ventanas (Ed.), *Tecnología del Jamón Ibérico* (pp. 227–253). Madrid: Mundi-Prensa.

Andres, A. I., Cava, R., Mayoral, A. I., Tejeda, J. F., Morcuende, D., & Ruiz, J. (2001). Oxidative stability and fatty acid composition of pig muscles as affected by rearing system, crossbreeding and metabolic type of muscle fibre. *Meat Science, 59*, 39–47.

Antequera, T., Cordoba, J. J., Martin, L., Garcia, C., Bermudez, M. E., & Ventanas, J. (1993). Free fatty acids during the ripening of Iberian ham. *Revista Española de Ciencia y Tecnología de los Alimentos, 33*(2), 197–208.

Ansorena, D., Zapelena, M. J., Astiasarán, I., & Bello, J. (1998). Addition of palatase M (lipase from Rhizomucor miehei) to dry fermented sausages: Consequences on lipid fraction and study of the further oxidation process by CG–MS. *Journal of Agricultural and Food Chemistry, 46*, 3244–3248.

Arnau, J., Guerrero, L., & Sarraga, C. (1998). The effect of green ham pH and NaCl concentration on cathepsin activities and sensory characteristics of dry cured hams. *Journal of the Science of Food and Agriculture, 77*, 387–392.

Baldini, P., Campanini, M., Pezzani, G., & Palmia, F. (1984). Réduction de la quantité de chlorure de sodium employé dans les produits séchés. *Viandes Produits Carnés, 5*(3), 83–88.

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

Coutron-Gambotti, C., & Gandemer, G. (1999). Lipolysis and oxidation in subcutaneous adipose tissue during dry cured ham processing. *Food Chemistry, 64*(1), 95–101.

Coutron-Gambotti, C., Gandemer, G., Rousset, S., Maestrini, O., & Casabianca, F. (1999). Reducing salt content of dry cured ham: Effect on lipid composition and sensory attributes. *Food Chemistry, 64*, 13–19.

Flores, J., Nieto, P., Bermell, S., & Alberola, J. (1987). Cambios en los ácidos grasos de los lípidos del jamón durante el proceso de curado. I. Magro de jamón. *Revista de Agroquímica y Tecnología de los Alimentos, 27*(4), 599–605.

Folch, J., Lees, M., & Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry, 226*, 497–509.

Gandemer, G. (2002). Lipids in muscles and adipose tissues, changes during processing and sensory properties of meat products. *Meat Science, 62*, 309–321.

Gray, J. I., & Pearson, A. M. (1987). Rancidity and warmed-over flavour. In A. M. Pearson & T. R. Dutson (Eds.), *Advances in Meat Research: Vol. 3. Restructured meat and poultry products* (pp. 221–269). New York: Van Nostrand Reinhold Company.

Guerrero, L., Gelabert, J., Guardia, M. D., Gou, P., Arnau, J., Shepherd, R., & Sparks, P. (1998). Consumer attitude towards low salt meat products. *Food Science and Technology International, 4*, 263–275.

Igene, J. O., Pearson, A. M., Dugan, L. R., & Price, J. F. (1980). Role of triglycerides and phospholipids on development of rancidity in model meat systems during frozen storage. *Food Chemistry, 5*, 263–276.

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.

Martin, L., Antequera, T., Cordoba, J. J., Timon, M. L., & Ventanas, J. (1998). Effects of salt and temperature in proteolysis during ripening of Iberian ham. *Meat Science, 49*(2), 145–153.

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

Morgan, T., Aubert, J.-F., & Brunner, H. (2001). Interaction between sodium intake, angiotensin II, and blood pressure as a cause of cardiac hypertrophy. *American Journal of Hypertension, 14*(9), 914–920.

Motilva, M. J., & Toldra, F. (1993). Effect of curing agents and water activity on pork muscle and adipose subcutaneous tissue lipolytic activity. *Zeitschrift für Lebensmittel-Untersuchung und-Forschung, 196*, 228–232.

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

Nawar, W. W. (1996). Lipids. In O. R. Fennema (Ed.), *Food Chemistry* (pp. 139–244). New York: Marcel Dekker.

Ruiz, J., Ventanas, J., Cava, R., Andres, A. I., & Garcia, C. (1999). Volatile compounds of dry cured Iberian ham as affected by the length of the curing process. *Meat Science, 52*, 19–27.

Sandler, S. R., & Karo, W. (1992). *Sourcebook of advanced organic laboratory preparations*. San Diego: Academic Press.

Toldra, F. (2002). Characterization of lipolysis and other enzymatic reactions. In F. Todra (Ed.), *Dry-cured meat products* (pp. 135–152). Trumbull (USA): Food & Nutrition Press Inc.

Vestegaard, C. S., Schivazappa, C., & Virgili, R. (2000). Lipolysis in dry cured ham maturation. *Meat Science, 55*, 1–5.

Wilson, B. R., Pearson, A. M., & Shorland, F. B. (1976). Effect of total lipids and phospholipids on warmed-over flavor in red and white muscle from several species as measured by thiobarbituric acid analysis. *Journal of Agricultural and Food Chemistry, 24*, 1–6.