Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 88 (2004) 25–33
Food Chemistry
www.elsevier.com/locate/foodchem

Composition and proteolytic and lipolytic enzyme activities in muscle *Longissimus dorsi* from Iberian pigs and industrial genotype pigs

Ramón Cava [a,*], José Manuel Ferrer [b], Mario Estévez [a], David Morcuende [a], Fidel Toldrá [b]

[a] *Tecnología de los Alimentos, Facultad de Veterinaria, Universidad de Extremadura, Cáceres, Spain*
[b] *Instituto de Agroquímica y Tecnología de Alimentos (CSIC), Apt. 73, 46100 Burjassot, Valencia, Spain*

Received 18 July 2003; accepted 18 July 2003

Abstract

In this study, composition and fatty acid profiles of intramuscular lipid fractions, proteolytic and lipolytic muscle enzyme activities in muscle *Longissimus dorsi* have been determined in three lines of Iberian pigs (''*Lampiño*'', ''*Retinto*'' and ''*Torbiscal*'') reared under the free-range system and slaughtered at 90kg live weight and industrial genotype pigs (IGP) (Large White-Landrace × Large White) reared intensively. Intramuscular fat content was significantly higher ($p < 0.05$) in Iberian pig lines (IPL) than in IGP. Neutral lipids of IPL showed higher ($p < 0.05$) percentages of palmitic, oleic, total saturated fatty acids and monounsaturated fatty acids and lower ($p < 0.05$) percentages of polyunsaturated acids and linoleic acid in neutral and polar lipids than IGP. No statistical differences were found in cathepsin B or B + L or phospholipase residual activities between IPL and IGP, while cathepsin H residual activity was significantly ($p > 0.05$) higher in IGP than in IPL. The ratio cathepsin B + L/B was significantly lower ($p < 0.05$) in IGP and ''*Lampiño*'' line than in ''*Retinto*'' and ''*Torbiscal*'' lines. Acid lipase residual activity showed a significantly ($p < 0.05$) higher activity in IPL than in IGP. The activity of neutral lipase was significantly higher ($p < 0.05$) in ''*Torbiscal*'' line and lower in the ''*Lampiño*'' line while the ''*Retinto*'' line and IGP showed intermediate activities. Acid esterase activity was higher ($p < 0.05$) in ''*Retinto*'' and''*Torbiscal*'' lines than in IGP and the ''*Lampiño*'' line. The different enzyme patterns in fresh meat may explain the wide proteolysis and lipolysis variations usually observed in the processing of dry-cured ham.

Keywords: Enzyme activities; Lipase; Phospholipase; Cathepsin; Pig; Fatty acid; Lipids

1. Introduction

In recent years consumers have started to demand other types of meats, different from those typically produced under intensive production schemes. These new models of meat production, commonly imply the use of non-selected pig breeds, with both productive and meat characteristics very different from those of industrial pig genotypes and feeding based on natural resources (Cava, Estévez, Ruiz, & Morcuende, 2003; Johsäll, Johansson, & Lundström, 2001; Norgaard & Bennett, 1995). Previous studies have shown that muscles from rustic pig breeds, in contrast to muscles from selected pig breeds, contain higher amounts of intramuscular lipids and heme pigments (Cava et al., 2003; Morales, Pérez, Baucells, Mourot, & Casa, 2002; Serra et al., 1998) and different muscle enzyme residual activities lipase, phospholipase and cathepsin activities (Rosell & Toldrá, 1998; Toldrá, Flores, Aristoy, Virgili, & Parolari, 1996). Additionally, dietary fat differences induce alterations in the fatty acid profiles of total fat and lipid fractions that have important consequences for the oxidative stability of meat (Cava, Ruiz, Tejeda, Ventanas, & Antequera, 2000; Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). Both fatty acid composition of lipid fractions and enzyme activity pools lipolytic and proteolytic- of muscles are directly related to the development of the particular

* Corresponding author. Tel.: +34-927-257-169; fax: +34-927-257-110.
E-mail address: rcava@unex.es (R. Cava).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2003.07.037

characteristics of raw, cured, stored and cooked meat (Cava, Ruiz, Ventanas, & Antequera, 1999; Estévez, Morcuende, Ventanas, & Cava, 2003; García-Garrido, Quiles-Zafra, Tapiador, & Luque de Castro, 2000; Miller, Shackleford, Hayden, & Reagan, 1990; Toldrá, Flores, & Aristoy, 1995).

Lipolysis, based on the action of muscle lipases, esterases and phospholipases, is responsible for the degradation of intramuscular lipids during storage and processing of meat and meat products (Coutron-Gambotti & Gandemer, 1999; Currie & Wolfe, 1977; Flores, Alasnier, Aristoy, Navarro, Gandemer, & Toldrá, 1996; Motilva, Toldrá, Nieto, & Flores, 1993; Sklan, Tenne, & Budowski, 1983). Lipolysis generates free fatty acids, which may promote lipid oxidation and contribute to the generation of compounds responsible, in part, for the flavour of meat and meat products (Nawar, 1996; Toldrá & Flores, 1998; Vestergaard, Schivazappa, & Virgili, 2000). Moreover, muscle protein breakdown in raw meat leads to an increase in the proteolysis index and content of free aminoacids. Proteolysis during the processing of dry-cured meat products mainly depends on muscle lysosomal proteinases (Quali, 1992; Toldrá, 1998). The differences in the activity of the proteolytic enzymes may affect important aspects of meat quality, such as tenderness and contribute to the improvement of meat taste by generation of peptides and aminoacids and meat flavour precursors (Aristoy & Toldrá, 1995; Essen-Gustavsson & Fjelkner-Modig, 1985; Mottram, 1998).

Little is known, however, about the proteolytic and lipolytic activities in muscles from light-weight Iberian pigs and the possible differences in enzymatic pools when compared with muscles from IGP. In the present study, we have examined the composition, fatty acid profile and levels of proteolytic, cathepsin B, B + L and H, and lipolytic, acid and neutral lipases, esterases and phospholipases, in fresh *m. Longissimus dorsi* from three typical Iberian pig lines, reared under free-range systems, and industrial genotype pigs, reared under the intensive system, in order to determine the influence of genetic factors and the relationship with other compositional traits.

2. Materials and methods

2.1. Samples

Twenty-one Iberian pigs (~50 kg live weight) from three different lines: "*Lampiño*" ($n = 7$), "*Torbiscal*" ($n = 7$) and "*Retinto*" ($n = 7$), commonly produced in the South-West of Spain and belonging to Iberian pig pure breed selection schemes, were free-range reared during late spring and early summer seasons, being feed on grass and a concentrate feed based on cereals without incorporation of any animal source of protein or fat. Feed analysis (AOAC, 1990) showed a protein content of 16.2 g/100 g dry matter (d.m.) and a fat content of 2.68 g/100 g d.m. Fatty acid composition of feed (expressed as percentage of total fatty acids identified) was as follows: palmitic acid (C16): 14.2%; stearic acid (C18): 3.13%; oleic acid (C18:1, n-9): 51.3%, linoleic acid (C18:2, n-6): 26.6% and linolenic acid (C18:3, n-3): 1.65%. Pigs were stunned and slaughtered at the end of the fattening period at a live weight of ~90 kg and an age of ~7 months. Samples from industrial genotype pigs (IGP) (Large White-Landrace × Large White) ($n = 5$) were procured in the same slaughterhouse. IGP were reared in an intensive system and fed on a commercial concentrate feed and slaughtered at a live weight of ~90 kg and an age of ~4 months. Mm. *Biceps femoris* were dissected, freed of visible fat, vacuum-packaged and kept frozen at 85 °C until analysed.

2.2. Analytical methods

2.2.1. Intramuscular fat isolation and fatty acid profiles

Intramuscular total lipids from muscles were extracted according to the method described by Bligh and Dyer (1959). From the fat extracted, neutral lipid (NL) and polar lipid (PL) fractions of muscles were isolated according to the method developed by Garcia-Regueiro, Gilbert, and Diaz (1994). Fatty acid methyl esters (FAMEs) of free fatty acids, neutral and polar lipid fractions were prepared by acidic esterification in the presence of sulphuric acid (Cava et al., 1997). FAMEs were analysed using a Hewlett–Packard, mod. HP-5890A, gas chromatograph, equipped with a flame ionisation detector (FID). FAMEs 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 220 °C. The carrier gas was nitrogen at a flow rate of 1.8 ml/min. Identification of FAMEs was based on retention times of reference compounds (Sigma).

2.2.2. Assay of cathepsin activity

Two grammes of muscle *Longissimus dorsi* were homogenized in 20 ml of 50 mM sodium citrate buffer, pH 5.0, containing 1 mM EDTA and 0.2% (vol) Triton X-100. The extract was homogenised (three times at 27000 rpm for 10 s with cooling on ice) with a polytron (Kinematica, Switzerland), and centrifuged at 12000 g for 20 min at 4 °C.

Cathepsin B, B + L and H were assayed, as previously described by Toldrá and Etherington (1998), using N-CBZ-l-arginyl-l-arginine 7-amido-4-methyl-coumarin (Z-Arg-Arg-AMC) at pH 6.0, N-CBZ-l-phenylalanyl-l-arginine 7-amido-4-methyl-coumarin (Z-Phe-Arg-AMC) at pH 6.0 and l-arginine 7-amido-4-methyl-coumarin

(Arg-AMC) at pH 6.8, respectively, as fluorescent substrates. For each assay, 50 ml of extract was diluted with 250 ml of reaction buffer (40 mM sodium phosphate at the different pH levels, containing 0.4 mM EDTA, 10mM cysteine, and 0.05 mM substrate) and then incubated for 20 min at 37 °C. Four replicates were measured for each experimental point. The fluorescence was measured at an excitation wavelength of 355 nm and an emission wavelength of 460 nm by using a Fluoroskan II multiscanning fluorimeter (Labsystems, Finland). One unit (U) of proteolytic activity is defined as the amount of enzyme capable of hydrolysing 1 nmol of substrate per minute at 37 °C.

2.2.3. Assay of lipolytic enzyme activity

Five grammes of muscle were homogenized in 25 ml of 50 mM phosphate buffer, pH 7.5, containing 5 mM EGTA by using a polytron homogeniser (three bursts of 10 s each at 27,000 rpm, with ice cooling). The homogenate was centrifuged at 10,000*g* for 20 min and the resulting supernatant filtered through glass wool and used for further enzyme assays.

Enzyme assays were performed as previously described by Motilva, Toldrá, and Flores (1992) with slight modifications. The reaction mixture consisted of 50 ml of enzyme extract and 250 ml of reaction medium containing 1.5 mM of the specific substrate. Lipase and esterase activities were determined using 4-methylumbelliferyl oleate and 4-methylumbelliferyl propionate (Sigma Chemical Co., St. Louis, MO) as substrates, respectively. Acid activities were assayed in 0.1 M citric acid/0.2 M disodium phosphate containing 0.8 mg ml^{-1} of bovine serum albumin (BSA) and 0.5 mg ml^{-1} Triton X-100 at pH 5, with the addition of 150 mM sodium fluoride when measuring the acid phospholipase activity. Neutral activities were assessed in 0.1 M citric acid/0.2 M disodium phosphate containing 6 mg ml^{-1} bovine serum albumin (BSA) and 0.012 mg ml^{-1} of heparin at pH 7.5. In all cases, the reaction medium was incubated at 37 °C and fluorescence was periodically monitored at λ excitation = 355 nm and λ emission = 460 nm by using a Fluoroskan II multiscanning fluorimeter (Labsystems, Finland). One unit (U) of activity was defined as the amount of enzyme hydrolysing 1 μmol of substrate per hour at 37 °C. Four replicates were measured for each experimental point.

2.3. Statistical analysis

The effect of pig breed on studied parameters was assessed by analysis of variance (ANOVA) using the General Linear Model of SPSS 10.0 (SPSS, 1999) and significant means were established by Tukey's test at level of $p < 0.05$. Principal component analyses were performed with the SPSS Advanced Models of SPSS 10.0 software package.

3. Results and discussion

Moisture, intramuscular fat and protein contents and the fatty acid profiles of neutral, polar and free fatty acid fractions of the intramuscular fat of the *m. Longissimus dorsi* from Iberian pig lines (IPL) and industrial genotype pigs (IGP) are shown in Table 1.

Moisture content, ranging from 72.28% to 73.78%, and protein content, ranging from 23.5% to 24.8%, did not differ among pig breeds. However, the intramuscular fat content showed significant differences between IPL and IGP. Average intramuscular fat content in *m. Longissimus dorsi* from IPL was 1.8–2.4 fold-times higher ($p < 0.05$) than in industrial genotype pig muscle (3.01 g/100 g vs 1.41 g/100 g muscle). No differences were found among Iberian pig lines although the "*Torbiscal*" line showed a trend towards less intramuscular fat than the other two lines ("*Lampiño*": 3.34 g/100 g d.m.; "*Retinto*": 3.17 g/100 g and "*Torbiscal*": 2.51 g/100 g muscle). This is in accordance with previous works showing higher contents of intramuscular fat in Iberian pig muscles than the same muscles from lean pig breeds (Cava et al., 1997; Cava et al., 2000; Serra et al., 1998).

Fatty acid composition of lipid fractions showed noticeable differences among pig breeds. Neutral lipid fraction in m. *Longissimus dorsi* from IPL exhibited significantly higher ($p < 0.05$) percentages of monounsaturated fatty acids (51.5%, 49.7%, 50.0% and 43.4% for "*Lampiño*", "*Retinto*", "*Torbiscal*" and IGP, respectively) and oleic acid (46.4%, 44.5%, 44.4% and 39.0%, for "*Lampiño*", "*Retinto*", "*Torbiscal*" and IGP, respectively) and lower percentages of polyunsaturated fatty acids (7.51%, 9.15%, 8.95% and 17.6%, for "*Lampiño*", "*Retinto*", "*Torbiscal*" and IGP, respectively) and linoleic (6.11%, 7.11%, 7.23% and 14.2%, for "*Lampiño*", "*Retinto*", "*Torbiscal*" and IGP, respectively) and arachidonic acids (0.89%, 1.39%, 1.03% and 2.27%, for "*Lampiño*", "*Retinto*", "*Torbiscal*" and IGP, respectively) than muscles from IGP. In the polar lipid fraction, there were no great differences in fatty acid composition between IPL and IGP. Polar lipids from IGP contained significantly ($p < 0.05$) higher percentages of linoleic acid than "*Lampiño*" line (31.3% vs 23.2%, for IGP and "*Lampiño*" line, respectively). The other two lines ("*Retinto*" and "*Torbiscal*") of Iberian pigs did not show differences in the percentage of linoleic acid with respect IGP. The study of dimethyl acetals from polar lipid fractions showed a significant difference in C16-al and C18:1-al proportions between groups. The percentages of hexadecanal (C16-al) were the opposite to that of octadecenal (C18:1-al), C16-al being statistically significantly ($p < 0.05$) higher and C18:1-al significantly lower ($p < 0.05$) in IGP than in the three IPL. Free fatty acids did not exhibit a clear trend as reported for neutral and polar lipids fractions. IGP showed significantly ($p < 0.05$) higher percentages of C16 (26.0% vs

Table 1
Means and standard errors (SE) of moisture, intramuscular fat and protein contents and selected fatty acid composition (% methyl esters identified) of neutral, polar and free fatty acid fractions of intramuscular fat

	Iberian pig lines						Industrial genotype pigs	
	'*Lampiño*'		'*Retinto*'		'*Torbiscal*'			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Moisture	72.28	0.50	73.37	0.29	73.65	0.31	73.78	0.63
IMF	3.34a	0.18	3.17a	0.53	2.51a	0.15	1.41b	0.09
Protein	24.3	0.44	23.5	0.36	23.8	0.35	24.8	0.63
Neutral lipids								
C16	26.0	0.30	26.4	0.40	26.3	0.44	24.9	0.32
C18	12.4	0.21	12.6	0.26	12.6	0.26	12.0	0.60
ΣSFA	40.7	0.40	41.2	0.69	41.1	0.74	39.2	0.78
C18:1	46.4a	0.49	44.5a	0.52	44.7a	0.95	39.0b	1.76
ΣMUFA	51.7a	0.53	49.7a	0.52	50.0a	1.03	43.4b	2.03
C18:2	6.11b	0.37	7.11b	0.66	7.23b	0.43	14.23a	1.91
C18:3	0.36b	0.03	0.45a,b	0.03	0.52a,b	0.04	0.66a	0.14
C20:4	0.89b	0.06	1.39b	0.25	1.03b	0.12	2.27a	0.16
ΣPUFA	7.51b	0.30	9.15b	0.40	8.95b	0.44	17.6a	0.32
Polar lipids								
C16	19.82	1.46	22.4	0.79	23.9	1.34	21.2	0.26
C18	8.84	0.68	9.05	0.30	8.99	0.94	9.65	0.39
ΣSFA	30.6	1.33	32.9	0.92	34.0	2.33	31.8	0.62
C18:1	24.3	1.91	25.2	1.60	23.0	2.68	20.5	2.15
ΣMUFA	28.1	1.50	28.1	1.87	25.6	2.71	22.8	2.39
C18:2	23.2b	1.76	26.8a,b	1.55	33.3a	2.84	31.3a	1.50
C18:3	1.08	0.10	0.87	0.05	1.04	0.15	0.78	0.02
C20:4	14.5	2.20	10.0	0.92	12.4	0.80	11.8	0.69
ΣPUFA	41.3	2.45	38.9	2.55	48.2	3.30	45.4	1.84
C16-al	52.5a	1.22	54.2a	1.40	51.6a	0.75	46.2b	0.36
C18-al	30.9	0.84	29.7	0.98	30.4	0.75	32.4	0.55
C181-al	16.6b	0.67	16.1b	0.56	18.0b	0.61	21.4a	0.51
DMA/FA	20.0	1.73	18.80	1.64	24.0	1.08	22.7	1.02
Free fatty acids								
C16	22.9a,b	0.70	22.0b	0.97	20.9b	1.12	26.0a	1.00
C18	12.6a,b	0.30	13.3a	0.73	11.6a,b	0.59	10.8b	0.50
ΣSFA	38.5a,b	0.46	38.8a,b	0.70	34.9b	1.47	39.2a	1.33
C18:1	39.4	1.58	40.2	1.63	36.3	2.55	37.1	1.02
ΣMUFA	45.9	1.29	46.0	1.68	40.3	2.78	42.1	0.75
C18:2	11.1a,b	0.66	11.7a,b	1.23	9.40b	1.33	14.7a	1.31
C18:3	1.07a,b	0.09	0.65b	0.09	1.99a	0.90	0.84b	0.11
C20:4	3.09	0.36	2.45	0.43	3.18	0.55	2.87	0.52
ΣPUFA	15.8	1.11	15.3	1.72	13.9	1.84	18.9	1.79

a,b,c – Different letters indicate significant difference ($p < 0.05$) between means.

SFA: saturated fatty acids (C14, C16, C17, C18 and C20); MUFA: monounsaturated fatty acids (C16:1, C17:1, C18:1, C20:1); PUFA: polyunsaturated fatty acids (C18:2, C18:3, C20:2, C20:4); C16-al: hexadecanal; C18-al: octadecanal; C18:1-al: octadecenal; DMA/FA: dimethyl acetals/fatty acids ratio.

20.9%), saturated fatty acids (39.2% vs 34.9%) and C18:2 (14.7% vs 9.40%) than the "*Torbiscal*" Iberian pig line. The other two lines of Iberian pigs ("*Lampiño*" and "*Retinto*") showed intermediate percentages between IGP and the "*Torbiscal*" line that were not statistically significant.

It is well known that fatty acid composition of tissues and lipid fractions in pigs is directly related to dietary fatty acid composition (Miller et al., 1990), so differences found in this experiment could be attributable to differences in fatty acid composition of feeds administered to pigs during the fattening period. In relation to fatty acid profiles, the higher contents of polyunsaturated fatty acids in intramuscular lipids and mainly their higher proportions in polar lipids of commercial genotype pigs, make their meat more susceptible to oxidative stress (Morrissey et al., 1998) and therefore to the development of rancid flavour after culinary preparation or refrigerated and frozen storage. This fact has been recently confirmed by Estévez et al. (2003) who evaluated the volatile compound profiles of meats from Iberian and commercial genotype pigs. They found higher

Table 2
Comparison between cathepsin activities in three Iberian pig lines and Industrial genotype pigs

	Iberian pig lines						Industrial genotype pigs	
	'*Lampiño*'		'*Retinto*'		'*Torbiscal*'			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Cathepsin B	6.31	0.39	6.21	0.33	5.83	0.46	6.28	0.52
Cathepsin B + L	19.9	1.08	23.2	1.45	23.4	1.52	19.7	1.71
Cathepsin B + L/B	3.17b	0.10	3.73a	0.10	4.03a	0.10	3.15b	0.12
Cathepsin H	11.5b	0.50	12.1b	0.35	11.8b	0.23	14.5a	1.52

Results expressed as units per g of muscle.
a,b,c – Different letters indicate significant difference ($p < 0.05$) between means.

relative amounts of certain compounds related to off-flavour in refrigerated and cooked and refrigerated meat from the commercial genotype pigs.

The analysis of residual activities of lysosomal proteases in *m. Longissimus dorsi* from lines of Iberian pigs and IGP revealed important differences in the proteolytic activities caused by genetic factors. The results are shown in Table 2.

The activities of cathepsin B and cathepsin B + L did not show significant differences between pig breeds or even within Iberian pig lines. In the case of cathepsin B, the values were around 5.8–6.31 U/g while for cathepsin B + L they were 19.7–23.4 U/g. However, some significant differences were found in the ratio of the measurements of cathepsin B + L activity and cathepsin B activity. This ratio was calculated to obtain an indication of the contribution of cathepsin L to the activity using a common substrate (Schreurs, Schreurs, van der Heide, Leenstra, & Witt, 1995). Significant differences were obtained in the ratio cathepsin B + L/B, indicating that cathepsin L should make an important contribution to most of the differences observed between pig breeds and Iberian pig lines. In this sense, the activity was significantly higher ($p < 0.05$) in the "*Retinto*" (3.73 U/g) and "*Torbiscal*" (4.03 U/g) IPL than in the "*Lampiño*" (3.17 U/g) Iberian pig line and IGP (3.15 U/g). The activity of cathepsin H was significantly higher ($p < 0.05$) in the IGP (14.5 U/g) than in the three lines of Iberian pigs (11.5 U/g, 12.1 U/g and 11.8 U/g, for "*Lampiño*", "*Retinto*" and"*Torbiscal*" Iberian pig lines, respectively). No significant differences were found among them. Results agree in part with previous studies which reported that muscle cathepsin activity is related to breed (Armero, Barbosa, Toldrá, Baselga, & Pla, 1999; Flores, Romero, Aristoy, Flores, & Toldrá, 1994; Rosell & Toldrá, 1998; Toldrá et al., 1996). However, some findings, (those related to cathepsin B and B + L activities) are not in agreement with results described by Rosell and Toldrá (1998) in a study in which lysosomal protease activities were assessed in muscles from Iberian and white pigs (Large-White × Landrace × Duroc). These authors found significantly higher activities of cathepsins in industrial genotype pigs than in Iberian pigs although the lack of agreement may be due to the different age of animals used in each experiment (18-month-old in that study *vs* 7-month-old in our study). In this sense, Sárraga, Gil, and García-Regueiro (1993) and Toldrá et al. (1996) reported higher cathepsin B and B + L in 7 month-old pigs than in 11 months old pigs, differences that are in accordance with the role of lysosomal enzymes in the regulation of protein turnover (Goll, 1991), that decrease with the animal age. On the other hand, the higher activity of cathepsin H in IGP is related to the evidence that leaner genotypes show higher cathepsin H activity than poorer conformation genotypes (Armero et al., 1999; Rosell & Toldrá, 1998). On the basis of the results obtained, it could be proposed that meat from IGP would be more prone to proteolysis than meat from IPL, as would be reflected in different generation rates of peptides and aminoacids, as well as proteolysis-derived volatile compounds and, thus, sensible differences in sensory properties.

The analysis of lipolytic enzyme activities showed important differences (Table 3). Acid lipase showed a significantly ($p < 0.05$) higher activity in IPL (0.72 U/g, 0.63 U/g and 0.49 U/g in "*Lampiño*", "*Retinto*" and "*Torbiscal*", respectively) than in the muscle of IGP (0.34 U/g). In this way, activity of acid lipase was more than twice higher in the "*Lampiño*" line than in IGP. Also, the statistical analysis of acid lipase activity denoted a significant ($p < 0.05$) difference among the three lines of Iberian pig studied, that increased in the order "*Torbiscal*" > "*Retinto*" > "*Lampiño*". A close relationship could be noticed between acid lipase residual activity and intramuscular fat content, the higher intramuscular fat content, the higher was the acid lipase residual activity (Tables 1 and 3). In relation to neutral lipase activity, no relationship between enzyme activity and pig breed was observed. However, statistically significant differences were found ($p < 0.05$). The "*Torbiscal*" (0.63 U/g) Iberian pig line showed the highest activity and "*Lampiño*" (0.19 U/g) Iberian pig lines the lowest. "*Retinto*" (0.41 U/g) and IGP (0.41 U/g) breeds shown intermediate activities. Inversely to the pattern showed by acid lipase, the measured activity in IGP muscle doubled the neutral lipase activity found in the

Table 3
Comparison between lipolytic enzyme activities in three Iberian pig lines and Industrial genotype pigs

	Iberian pig lines						Industrial genotype pigs	
	'*Lampiño*'		'*Retinto*'		'*Torbiscal*'			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Acid lipase	0.72a	0.02	0.63b	0.03	0.49c	0.01	0.34d	0.02
Neutral lipase	0.19c	0.03	0.41b	0.03	0.63a	0.03	0.41b	0.03
Phospholipase	0.47	0.05	0.45	0.02	0.42	0.01	0.41	0.03
Acid sterase	4.38b	0.10	5.42a	0.16	5.40a	0.22	3.85b	0.21

Results expressed as units per g of muscle.
a,b,c – Different letters indicate significant difference ($p < 0.05$) between means.

''*Lampiño*'' Iberian pig line. Concerning acid esterase activity, Iberian pigs exhibited higher values than muscle from IGP, and significantly different ($p < 0.05$) from the ''*Retinto*'' (5.42 U/g) and ''*Torbiscal*'' (5.40 U/g) Iberian pig lines, while the activities in the ''*Lampiño*'' (4.38 U/g) Iberian pig line and IGP (3.85 U/g) muscles were not statistically different. Phospholipase activity did not show significant ($p > 0.05$) differences among groups, the values being very similar for all the analysed groups (0.41 U/g in IGP and 0.47, 0.45 and 0.42 U/g in ''*Lampiño*'', ''*Retinto*'' and ''*Torbiscal*'' Iberian pig lines, respectively). Results obtained in the present work did not agree at all with previous results from Rosell and Toldrá (1998), who assayed the lipolytic enzyme residual activities in the *m. B. femoris* from Iberian and lean pigs and found a lower acid lipase activity in Iberian pig muscles than in those from IGP although this could be due to the differences in the age and weight of slaughtering of the animals in the two studies, as previously commented upon. These results show the important effects of slaughtering age and weight on the lipolytic and proteolytic enzyme patterns in *m. Longissimus dorsi* from heavy and light-weight Iberian pigs and industrial genotype pigs. Lower age and slaughtering weight lead to more similar enzyme muscle patterns in Iberian pigs and industrial genotype pigs, in contrast with results described when muscles from heavy-weight Iberian pigs and industrial genotype pigs are compared. In recent years, in Spain, there is an increasing interest in meat from light-weight Iberian pigs for fresh consumption and these differences in the muscle enzyme pattern in light-weight Iberian pigs and industrial genotype pigs could be responsible, among other factors, such as fatty acid profiles and phospholipids contents, for a different formations of flavour compounds in meat during refrigerated storage, as previously reported Estévez et al. (2003) that could affect the sensory quality.

A principal component analysis (PCA) was carried out to determine the relationships between the different studied traits. Tables 1–3 show the means and standard errors for the variables used in the analysis. PCA of these variables resulted in three significant factors that accounted for 54.5% of the variability, increasing to 63.1% when the fourth component is computed (Table 4).

Fig. 1 shows the score plot of the different variables (coefficients of the eigenvectors) for the first two principal components (PC#1 and PC#2). In this plot it is possible to distinguish a group of variables, composed of C20:4 from TG, octadecenal, polyunsaturated fatty acids from TG and C18:2 from TG, in the positive axis and of C18:1 and monounsaturated acids from TG, IMF, hexadecanal and acid lipase in the negative part of the first principal component (PC#1), far from the origin. This group explains an important part of the variation. C16, C18 and total saturated fatty acids from PL are located on the second PC (PC#2), far from the origin, explaining an independent cause of variation, not related to the C20:4 from PL. The distribution of the data on the two first PC variables (Fig. 2) shows two great separate groups of points. The *m. Longissimus dorsi* from IGP is confined to right along the PC#1, to the plane area corresponding to high values of cathepsin H, polyunsaturated fatty acids, C18:2 and C20:4 from TG, whereas the *m. Longissimus dorsi* from Iberian pigs are grouped in the left with high values of total monounsaturated fatty acids and C18:1 from TG, intramuscular fat and acid lipase activity. The two breeds may be distinguished by principal component analysis since

Table 4
Results of the principal component analysis

Component	Eigenvalue	Cumulative eigenvalue	Total variance explained (%)	Cumulative variance explained (%)
#1	11.23	11.23	27.38	27.38
#2	6.58	17.81	16.05	43.43
#3	4.55	22.35	11.09	54.51
#4	3.50	25.85	8.54	63.06

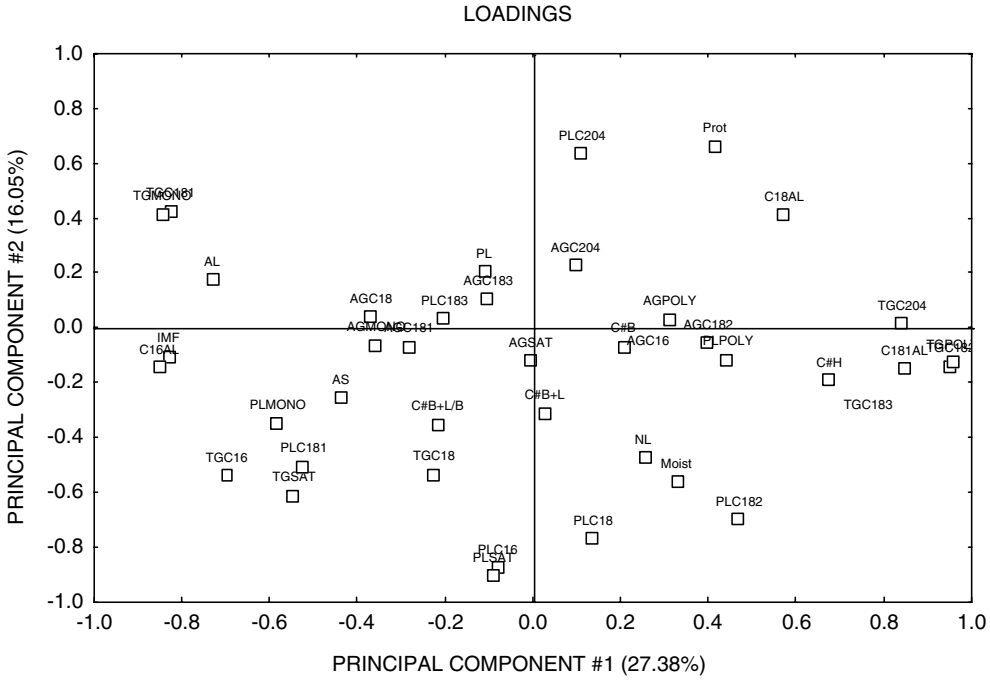

Fig. 1. Loadings plot after principal component analysis of the variables in the plane defined by the two first principal components (PC#1 and PC#2).

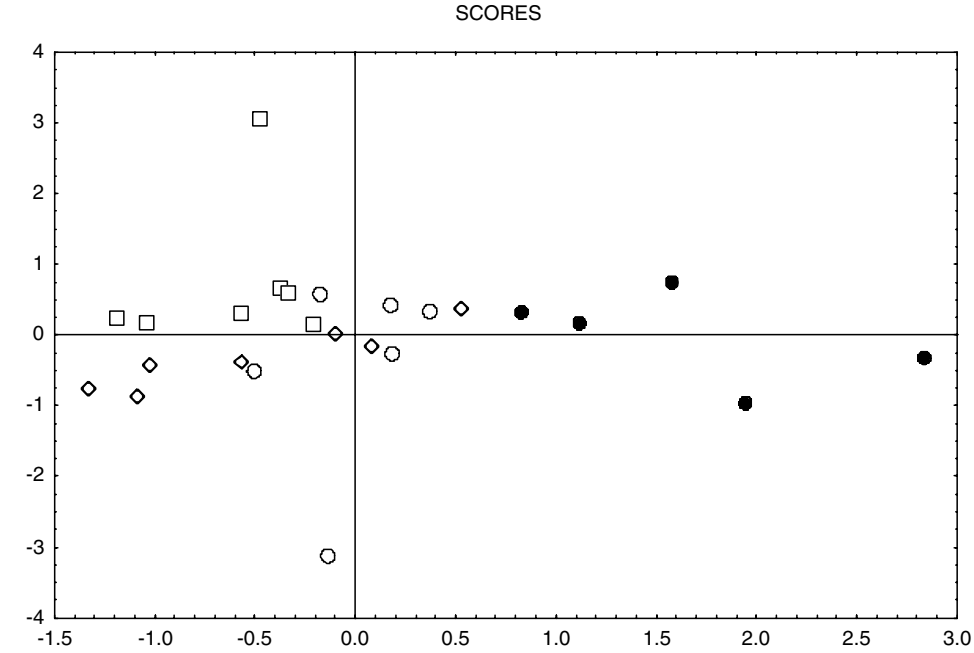

Fig. 2. Scores plot after principal component analysis of the individuals in the plane defined by the two first principal components (PC#1 and PC#2). Key: ●: industrial genotype pigs; □: Iberian pig line "*Lampiño*"; ○: Iberian pig line "*Retinto*"; ◇: Iberian pig line "*Torbiscal*".

each one presents a different pattern, based on the meat composition and enzyme activities.

In summary, although feed is the main factor affecting the fatty acid composition of intramuscular fat and lipid fractions in the assayed pigs, some other parameters, such as proteolytic and lipolytic enzyme pools, which are under the control of the genetic factors of the breed and are hard to manipulate to obtain desirable meat traits, have shown important differences. Small differences have been found among IPL that could be

related to the different origins of the "*Lampiño*", "*Retinto*" and "*Torbiscal*" Iberian pig lines. The interaction between chemical composition and enzyme activities might explain the usual differences found in proteolysis and lipolysis during the processing of dry-cured meat products, such as Iberian dry-cured ham and its influence on the development of sensory characteristics of the fresh meat or cooked meat.

Acknowledgements

Work was funded by a grant from the I Plan Regional de Investigación. Junta de Extremadura (IPRD99001, "*Establecimiento de parámetros predictores de la calidad de carne fresca de cerdo Ibérico y Alentejano destinada a la elaboración de productos cárnicos curados y al consumo en fresco*"). The authors are grateful to AECERIBER and Miss Elena Diéguez and Mr Pedro Cañuelo for providing us with the animals. The I3P-CSIC contract to JM Ferrer is fully acknowledged. We would like to express many thanks to Miss Inmaculada Linares for her excellent laboratory assistance.

References

AOAC (1990). *Official methods of analysis*, Vol. 2, 15th ed. Association of Official Analytical Chemists. Arlington, VA.

Aristoy, M.-C., & Toldrá, F. (1995). Isolation of flavor peptides from raw pork meat and dry-cured ham. In G. Charalambous (Ed.), *Recent developments in food science and nutrition* (pp. 1323–1344). Amsterdam: Elsevier.

Armero, E., Barbosa, J. A., Toldrá, F., Baselga, M., & Pla, M. (1999). Effects of the terminal sire type and sex on pork muscle cathepsins (B, B + L and H), cysteine proteinase inhibitors and lipolytic enzyme activities. *Meat Science, 51*, 185–189.

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.

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., Ruiz, J., Ventanas, J., & Antequera, T. (1999). Oxidative and lipolytic changes during ripening of Iberian hams as affected by feeding regime: Extensive feeding and α-tocopheryl acetate supplementation. *Meat Science, 52*, 165–172.

Cava, R., Ruiz, J., Tejeda, J. F., Ventanas, 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.

Cava, R., Estévez, M., Ruiz, J., & Morcuende, D. (2003). Physicochemical characteristics of three muscles from free-range Iberian pigs slaughtered at 90 kg live weight. *Meat Science, 63*, 531–541.

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

Currie, R. W., & Wolfe, F. H. (1977). Evidence for differences in post-mortem intramuscular phospholipase activity in several muscle type. *Meat Science, 1*, 185–193.

Essen-Gustavsson, B., & Fjelkner-Modig, S. (1985). Skeletal muscle characteristics in different breeds of pigs in relation to sensory properties of meat. *Meat Science, 13*, 33–47.

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–GG–MS. *Journal of Agricultural and Food Chemistry, 51*, 3429–3435.

Flores, M., Romero, J., Aristoy, M.-C., Flores, J., & Toldrá, F. (1994). Differences in muscle proteolytic activities among pork breed types. *Sciences des Aliments, 14*, 469–474.

Flores, M., Alasnier, C., Aristoy, M.-C., Navarro, J. L., Gandemer, G., & Toldrá, F. (1996). Activity of aminopeptidase and lipolytic enzymes in five skeletal muscles with various oxidative patterns. *Journal of the Science of Food and Agriculture, 70*, 127–130.

García-Garrido, J. A., Quiles-Zafra, R., Tapiador, J., & Luque de Castro, M. D. (2000). Activity of cathepsin B, D, H and L in Spanish dry-cured ham of normal and defective texture. *Meat Science, 56*, 1–6.

Garcia-Regueiro, J. A., Gilbert, J., & Diaz, 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.

Goll, D. E. (1991). Role of proteinases and protein turnover in muscle growth and meat quality. *Muscle Biochemical Reciprocal Meat Conference Proceedings, 44*, 25.

Johsäll, A., Johansson, L., & Lundström, K. (2001). Sensory quality and cooking loss of ham muscle (M.*biceps femoris*) from pigs reared indoors and outdoors. *Meat Science, 57*, 245–250.

Miller, M. F., Shackleford, S. D., Hayden, K. D., & Reagan, J. O. (1990). Determination of the alteration in fatty acid profile, sensory characteristics and carcass traits of swine fed on elevated levels of monounsaturated fat in the diet. *Journal of Animal Science, 68*, 1624–1631.

Morales, J., Pérez, J. F., Baucells, M. D., Mourot, J., & Casa, J. (2002). Comparative digestibility and lipogenic activity in Landrace and Iberian finishing pigs fed ad libitum corn- and corn-sorghum-acorn-based diets. *Livestock Production Science, 77*, 195–205.

Morrissey, P. A. ., Sheehy, P. J. A., Galvin, K., Kerry, J. P., & Buckley, D. J. (1998). Lipid stability in meat and meat products. *Meat Science, 49*, S73–S86.

Motilva, M. J., Toldrá, F., & Flores, J. (1992). Assay of lipase and sterase activities in fresh pork meat and dry-cured ham. *Zeitschrift f ür Lebensmittel Untersuchung und-Forschung, 195*, 446–450.

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

Mottram, D. S. (1998). Flavour formation in meat and meat products: A review. *Food Chemistry, 62*, 415–424.

Nawar, W. W. (1996). Lipids. In O. R. Fennema (Ed.), *Food chemistry* (3rd ed., pp. 225–319). New York: Marcel Dekker.

Norgaard, N. H., Bennett, R. M. (1995). Free range pigs in Denmark obtain impressive results. In *Proceedings of the 10th International Farm Management Congress*, (pp. 30–34). University of Reading, UK, 10–15 July.

Quali, A. (1992). Proteolytic and physicochemical mechanisms involved in meat texture development. *Biochimie, 74*, 251–265.

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.

Sárraga, C., Gil, M., & García-Regueiro, J. A. (1993). Comparison of calpain and cathepsin (B, L and D) activities during dry-cured ham processing from heavy and light Large-White pigs. *Journal of the Science of Food and Agriculture, 62*, 71–75.

Schreurs, F. J. G., Schreurs, F. J. G., van der Heide, Leenstra, F. R., & Witt, W. (1995). Endogenous proteolytic enzymes in chicken muscles. Differences among strains with different growth rates and protein efficiencies. *Poultry Science, 67*, 413–416.

Serra, X., Gil, F., Perez-Enciso, M., Oliver, M. A., Vazquez, J. M., Gispert, M., Díaz, I., Moreno, F., Latorre, R., & Noguera, J. L. (1998). A comparison of carcass, meat quality and histochemical characteristics of Iberian (Guadyerbas line) and Landrace pigs. *Livestock Production Science, 56*, 215223.

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

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

Toldrá, F., Flores, M., & Aristoy, M.-C. (1995). Enzyme generation of free aminoacids and its nutritional significance in processed pork meat. In G. Charalambous (Ed.), *Recent developments in food science and nutrition* (pp. 1303–1322). Amsterdam: Elsevier.

Toldrá, F., Flores, M., Aristoy, M.-C., Virgili, R., & Parolari, G. (1996). Pattern of muscle proteolytic and lipolytic enzymes from light and heavy pigs. *Journal Science of Food and Agriculture, 71*, 124–128.

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

Toldrá, F., & Etherington, D. J. (1998). Examination fo cathepsins B, D, H and L activities in dry-cured hams. *Meat Science, 23*, 1–7.

Toldrá, F., & Flores, M. (1998). The role of muscle proteases and lipases in flavor development during the processing of dry-cured ham. *CRC Critical Reviews in Food Science and Nutrition, 38*, 331–352.

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