MEAT COMPOSITION AND PROTEOLYTIC AND LIPOLYTIC ENZYME ACTIVITIES IN MUSCLE *LONGISSIMUS DORSI* FROM IBERIAN AND WHITE PIGS

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Background

Lipolysis is involved in the degradation of intramuscular lipids during storage and processing of meat and meat products (Coutron-Gambotti et al., 1999). It generates free fatty acids, which may promote lipid oxidation because free fatty acids, especially polyunsaturated fatty acids, are more sensitive to oxidation than sterified ones (Nawar, 1996). Based on current studies, muscle lipases and phospholipases appear to be responsible for lipolysis in meat and meat products (Motilva et al., 1993). Proteolytic enzymes are implied in meat tenderization and their activity has been associated to the apparition of defective textures in dry-cured hams (García-Garrido et al., 2000).

Objectives

In the present study we have examined the levels of proteolytic, cathepsin B, B+L and H, and lipolytic, acid and neutral lipases, sterases and phospholipases, in fresh *m. Longissimus dorsi* from three Iberian pig lines and commercial pigs in order to determine their activities and the relationship with other compositional traits.

Material and Methods

Samples

For the experiment *m. Longissimus dorsi* from three lines ("Lampiño", "Retinto" and "Torbiscal", n=7 samples by pig line) of free-range reared Iberian pigs (»100kg live weight) and commercial pigs (n=5) were sampled.

Analytical methods

Intramuscular fat isolation and fatty acid profiles

Intramuscular total lipids from muscles were extracted according to the method described by Bligh & Dyer (1959). From the fat extracted, neutral lipids (NL) and polar lipids (PL) fractions of muscles were isolated according to the method developed by Garcia-Regueiro et al., (1994). Fatty acid methyl esters (FAMEs) of neutral and polar lipid fractions were prepared by acidic sterification in presence of sulphuric acid (Cava et al., 1997).

Preparation of muscle extracts for assays of muscle cathepsins.

Two grams of muscle Longissimus dorsi was homogenized in 50 mM sodium citrate buffer, pH 5.0, containing 1 mM EDTA and 0.2% (vol) Triton X-100.

Assay of proteolytic enzyme activities

Cathepsin B, B+L and H were assayed as previously described by Toldrá and Etherington (1988).

Assay of lipolytic enzyme activities

Five grams of muscle were homogenized in 50 mM phosphate buffer, pH 7.5, containing 5 mM EGTA. Enzyme assays were performed as previously described by Motilva et al. (1992).

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 when was significant means were compared by Tukey's test at level of $P < 0.05$.

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 and commercial pig are shown in Table 1. Moisture and protein contents did not differed among pig breeds being comprised between 72.28-73.78% and 23.46-24.80% for moisture and protein contents respectively. However, the intramuscular fat content showed significant differences between Iberian pig lines and commercial pig. Average intramuscular fat content in *m. Longissimus dorsi* from Iberian pig lines were 1.8-2.4 fold-times higher ($p<0.05$) than in commercial pig muscle (3.01g/100g vs 1.41g/100g muscle). No differences were found among Iberian pig lines. Numerous works have shown that Iberian pig muscles contain higher amounts of intramuscular fat than the same muscles from lean pig breeds (Cava et al., 1997; Serra et al., 1998). Fatty acid composition of lipid fractions showed noticeable differences between pig breeds. Neutral lipid fraction in *m. Longissimus dorsi* from Iberian pig lines exhibited significant higher ($p<0.005$) percentages of monounsaturated fatty acids and oleic acid and lower percentages of polyunsaturated fatty acids and linoleic and arachidonic acids than muscles from commercial pigs. Polar lipids from commercial pig contained significant ($p<0.05$) higher percentages of linoleic acid than "Lampiño" Iberian line.

The analysis of residual activities of cathepsins and lipolytic enzymes in *m. Longissimus dorsi* from the three lines of Iberian pigs and commercial pig revealed notable differences in the proteolytic activity caused by genetic factors. The results from the analysis are shown in the Table 3. The analysis of activities of cathepsin B and cathepsin B+L in the *m. Longissimus dorsi* did not show significant differences between pig breeds and within Iberian pig lines. However, some significant differences were found in cathepsin B+L/B and cathepsin H activities. The ratio of the measurements of cathepsin B+L activity and cathepsin B activity alone was calculated to obtain an indication of the contribution of cathepsin L to the activity using a common substrate (Schreurs et al., 1995). Significant differences were obtained in the ratio cathepsin B+L/B, indicating that cathepsin L should have an important contribution for most of the differences observed between pig breeds and Iberian pig lines. In this sense, the activity was significantly higher ($p<0.05$) in "Retinto" (3.73U/g) and "Torbiscal" (4.03U/g) Iberian pig lines than in "Lampiño" (3.17 U/g) Iberian pig line and commercial pig (3.15U/g). Cathepsin H measured activity was significantly higher ($p<0.05$) in the commercial pig (14.50U/g) than in the three lines of Iberian pigs (11.45U/g, 12.12U/g and 11.76U/g, for Lampiño, "Retinto" and "Torbiscal" Iberian pig lines, respectively) that did not significantly differ among them. Results did not agree at all with previous studies carried out by Rossell and Toldrá (1998) on Iberian and white pigs. The reason could be attributed to the different age of animals used in each experiment. In the cited study the age of animals differed in a great extent being Iberian pigs older than white pigs (18-month-old vs 6-month-old).

The analysis of lipolytic enzyme activities showed important differences between breeds and within Iberian pig breed (Table 3). In relation to lipase activities, neutral and acid lipases, measurements showed a significant ($p<0.05$) higher activity of acid lipase in *m. Longissimus dorsi* from Iberian pig lines (0.72 U/g, 0.63 U/g and 0.49 U/g in Lampiño, "Retinto" and Torbiscal, respectively) than in the muscle of white pigs

(0.34 U/g). In this way, activity of acid lipase was more than two-fold times higher in "Lampiño" group than in commercial pig. 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. In relation to neutral lipase activity measurements in *m. Longissimus dorsi* any relationship between enzyme activity and pig breed was observed, however statistical significant differences were found ($p<0.05$). "Torbiscal" (0.63 U/g) Iberian pig line showed the highest activity and "Lampiño" (0.19 U/g) Iberian pig line the lowest, "Retinto" (0.41 U/g) and commercial pig (0.41 U/g) breed showed intermediate activities. Inversely to the pattern showed by acid lipase, the measured activity in commercial pig muscle was doubled the neutral lipase activity found in "Lampiño" Iberian pig line. Concerning acid sterase activity, *m. Longissimus dorsi* from Iberian pigs exhibited higher values than muscle from white pigs, being significant different ($p<0.05$) for the "Retinto" (5.42 U/g) and "Torbiscal" (5.40 U/g) Iberian pig lines, while the activity in "Lampiño" (4.38 U/g) Iberian pig line and white pigs (3.85 U/g) muscles was not statistically different. Phospholipase activity did not show significant ($p>0.05$) differences among groups, being the values very similar for all the groups analysed (0.41 U/g in white pigs and 0.47 U/g, 0.45 U/g and 0.42 U/g in Lampiño, "Retinto" and "Torbiscal" Iberian pig lines, respectively). Results obtained in the present work did not agree with previous results from Rossell and Toldrá (1998) who analysed the content of lipolytic enzyme residual activities in the *m. B. femoris* from Iberian and white pigs and found a lower acid and neutral lipase activity in Iberian pig muscles than in those from white pigs. The lack of agreement with the results of the previous authors could be due to the differences in the age and weight of slaughtering of the animals in the two studies.

Conclusions

Muscles from Iberian pigs show a different enzymatic pattern and chemical composition that could affect their behaviour during refrigerated storage and/or freezing. Both characteristics could affect characteristics like tenderization, lipolysis and lipid oxidation.

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

	Iberian pig lines						Commercial pig	
	'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	3.34^{a}	0.18	3.17^{a}	0.53	2.51^{a}	0.15	1.41^{b}	0.09
Protein	24.34	0.44	23.46	0.36	23.84	0.35	24.80	0.63
Neutral lipids								
C16	26.04	0.30	26.35	0.40	26.25	0.44	24.91	0.32
C18	12.41	0.21	12.59	0.26	12.58	0.26	12.03	0.60
Σ SFA	40.74	0.40	41.16	0.69	41.09	0.74	39.19	0.78
C18:1	46.36^{a}	0.49	44.45^{a}	0.52	44.73^{a}	0.95	38.97^{b}	1.76
Σ MUFA	51.74^{a}	0.53	49.69^{a}	0.52	49.97^{a}	1.03	43.36^{b}	2.03
C18:2	6.11^{b}	0.37	7.11^{b}	0.66	7.23^{b}	0.43	14.23^{a}	1.91
C18:3	0.36^{b}	0.03	$0.45^{a,b}$	0.03	$0.52^{a,b}$	0.04	0.66^{a}	0.14
C20:4	0.89^{b}	0.06	1.39^{b}	0.25	1.03^{b}	0.12	2.27^{a}	0.16
Σ PUFA	7.51^{b}	0.30	9.15^{b}	0.40	8.95^{b}	0.44	17.55^{a}	0.32
Polar lipids								
C16	19.82	1.46	22.35	0.79	23.86	1.34	21.24	0.26
C18	8.84	0.68	9.05	0.30	8.99	0.94	9.65	0.39
Σ SFA	30.62	1.33	32.92	0.92	34.04	2.33	31.82	0.62
C18:1	24.33	1.91	25.21	1.60	23.01	2.68	20.47	2.15
Σ MUFA	28.12	1.50	28.13	1.87	25.64	2.71	22.83	2.39
C18:2	23.18^{b}	1.76	$26.84^{a,b}$	1.55	33.3^{a}	2.84	31.30^{a}	1.50
C18:3	1.08	0.10	0.87	0.05	1.04	0.15	0.78	0.02
C20:4	14.51	2.20	10.01	0.92	12.35	0.80	11.84	0.69
Σ PUFA	41.25	2.45	38.85	2.55	48.22	3.30	45.35	1.84

^{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).

Table 2.- *Means and standard errors (SE) of cathepsins (U/g of muscle) and lipolytic (U/g muscle) activities*

	Iberian pig lines						Commercial pig	
	'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	19.90	1.08	23.19	1.45	23.38	1.52	19.74	1.71
Cathepsin B+L/B	3.17^{b}	0.10	3.73^{a}	0.10	4.03^{a}	0.10	3.15^{b}	0.12
Cathepsin H	11.45^{b}	0.50	12.12^{b}	0.35	11.76^{b}	0.23	14.50^{a}	1.52
Acid lipase	0.72^{a}	0.02	0.63^{b}	0.03	0.49^{c}	0.01	0.34^{d}	0.02
Neutral lipase	0.19^{c}	0.03	0.41^{b}	0.03	0.63^{a}	0.03	0.41^{b}	0.03
Phospholipase	0.47	0.05	0.45	0.02	0.42	0.01	0.41	0.03
Acid sterase	4.38^{b}	0.10	5.42^{a}	0.16	5.40^{a}	0.22	3.85^{b}	0.21

^{a,b,c}: Different letters indicate significant difference ($p<0.05$) between means.