Antioxidant, lipolytic and proteolytic enzyme activities in pork meat from different genotypes

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Abstract

Oxidative processes in meat lead to meat quality deterioration. Meat has endogenous antioxidants and prooxidants, but information on factors influencing the activity of antioxidant enzymes in meat is limited. Lipolytic and proteolytic enzymes are involved in important aspects of meat quality. Our objective was to find differences between five different genotypes on the activity of antioxidant, lipolytic and proteolytic enzymes in meat. Forty *Psoas major* muscles of females of five different pig genotypes were used, Pietrain, Landrace, Large-White, lberian, and lberian×Duroc. Pre slaughter conditions were similar for all the genotypes. After slaughter, muscles were vacuum packed and frozen at −20 °C until required. Differences between genotypes were found for the activity of catalase and SOD, while GSH-Px showed no differences. The highest differences between breeds were found for the lberian breed where catalase had the highest activity. Catalase activity also showed differences between the white pigs, with large values for LR and lower activities in P. There were no differences in neutral lipase activities between the different genotypes while acid lipase and phospholipase showed significant differences. The activities of cathepsin B and H were significantly lower for Iberian pigs compared with other breeds except LR, while the ratio of cathepsin B+L/cathepsin B was higher in Iberian. The differences between genotypes found in enzyme activities suggest some genetic effects on the antioxidant, lipolytic and proteolitytic activity of pork meat.

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Keywords: Pig; Genetic; Meat; Antioxidant enzymes; Lipolytic enzymes; Cathepsins

1. Introduction

Many studies have reported the influence of breed on growth, carcass composition of pigs (Blasco et al., 1994) and pork quality (Oliver, Gispert, & Diestre, 1993; Oliver et al., 1994), but only a few studies of the influence of genetic type on the enzymatic activity of pork meat have been reported.

Oxidative processes in meat lead to quality deterioration. Meat has endogenous antioxidants and prooxidants and living cells have several mechanisms of protection against oxidative processes, including antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px). Catalase and glutathione peroxidase (GSH-Px) are considered the major peroxide-removing enzymes located in the cytosol (Chan & Decker, 1994; Decker & Xu, 1998; Halliwell, Murcia, Chirico & Okezie, 1995). SOD plays an important role in protecting against damage by the superoxide anion radical (Chan & Decker, 1994).

Information on factors influencing the activity of antioxidant enzymes in meat is limited. Antioxidant enzyme activities differ between meat of different species (Pradhan, Rhee, & Hernández, 2000) and muscle type (Hernández, Park, & Rhee, 2002). Antioxidant enzyme activity could also vary between animals of a single species. Therefore, variations in the activity of these enzymes between different genetic types could lead to differences in oxidative stability of the meat.

Muscle lipases and phospholipases contribute to the hydrolysis of the lipid fraction releasing free fatty acids and related compounds. Differences in the activity of these enzymes could result in different concentration of flavour precursors and, consequently, differences in flavour

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development (Toldrá & Flores, 1998). Cathepsins are involved in structural and biochemical changes that take place during post-mortem storage of meat (Koohmaraie, 1988). Several authors have established a relationship between the activity of proteolitic enzymes and defective texture in dry-cured hams (García-Garrido, Quiles-Zafra, Tapiador, & Luque de Castro, 2000; Parolari, Virgili, & Schivazzappa, 1994). Differences in the levels of these enzymes result in differences in sensory properties of pig meat.

The objective of this experiment was to study the effect of pig genetic type on the activity of antioxidant, lipolytic and proteolytic enzymes in fresh pork.

2. Material and methods

2.1. Animals and meat samples

Forty *Psoas major* of females of five different pig genetic types were used in this experiment, Pietrain (P), Large-White (LW), Landrace (LR), Iberian "*Torbiscal*" (I), and Iberian "*Lampiño*"×Duroc (I×D).

All animals were fed a commercial compound feed. P and LW lines were slaughtered at 6 months while LR, I and I×D were slaughtered at 12 months. Pre slaughter conditions were similar for all genotypes. The resting and fasting period was one day with drinking water available ad libitum, the pigs were slaughtered in accordance with Spanish regulations by stunning electronarcosis. The carcasses were rapidly chilled and the *Psoas major* muscle removed. The muscles were vacuum packed and frozen at −20 °C until required.

2.2. Intramuscular fat content

Intramuscular fat content was evaluated by ether extraction on Sohxtex (AOAC, 1990).

2.3. Assays of antioxidant enzyme activities

A 5-g muscle sample was homogenized in 25 ml of phosphate buffer (0.05 M, pH 7) and centrifuged at 4 °C for 2 min at $7000 \times g$. The supernatant fraction was filtered through glass wool and used to determine catalase, GSH-Px and SOD activities.

Catalase activity assay was performed as described by Aebi (1983) and Mei, Crum, and Decker (1994). The supernatant (0.1 ml) was reacted at room temperature (∼22 °C) with 2.9 ml of 11 mM H_2O_2 in phosphate buffer, and the reaction (H_2O_2 loss) was monitored by measuring the absorbance at 240 nm during the initial 30 s. One unit (U) of catalase was defined as the amount of extract needed to decompose 1 μmole of H_2O_2 per min.

GSH-Px activity was determined by measuring the oxidation of NADPH at 22 °C (DeVore & Greene, 1982; Günzler & Flohe, 1985). The assay medium (3 ml) consisted of 1 mM reduced glutathione, 0.15 mM NADPH, 0.15 mM H_2O_2, 40 mM potassium phosphate buffer (pH 7), 0.5 mM EDTA, 1 mM NaN_3, 1.5 units of glutathione reductase, and 300 μl of muscle extract. Absorbance at 340 nm was recorded over 3 min. An extinction coefficient of 6300 M^{-1} cm^{-1} was used for calculation of NADPH concentration. One unit of GSH-Px was defined as the amount of extract required to oxidize 1 μmole of NADPH per min at 22 °C.

Superoxide dismutase activity (SOD) was determined using the NADH oxidation method of Paoletti and Mocali (1990). The reaction mixture contained 75 mM triethanolamine–HCl buffer (pH 7.4), 0.28 mM NADH, EDTA-$MnCl_2$ (2.3 mM and 1.17 mM, respectively), 0.9 mM mercaptoethanol and 0.1 ml muscle extract which had been diluted 50-fold in 0.05 M phosphate buffer (pH 7.0). Absorbance at 340 nm was determined every 10 min for a total of 20 min. One unit of SOD activity was defined as the amount of extract required to inhibit the rate of NADH oxidation by the control (no SOD) by 50%. All the antioxidant enzyme assays were performed at 25 °C.

2.4. Assays of lipase activities

Five grams of muscle were homogenised in 25 ml 50 mM phosphate buffer, pH 7.5, containing 5 mM EGTA. The homogenate was centrifuged at $10\,000g$ for 20 min, and the resulting supernatant filtered though glass wool and used for further enzyme assays.

Acid lipase, acid phospholipase and neutral lipase were assayed as previously described Motilva, Toldrá, and Flores (1992) with slight modifications, using 4-methylumbelliferyloleate as fluorescent substrate. The reaction mixture consist of 50 μl of enzyme extract and 250 μl of reaction medium containing 1.5 mM of the specific substrate. Acid activities were assayed in 0.1 M citric acid/0.2 M disodium phosphate containing 0.8 mg ml^{-1} bovine serum albumin and 0.5 mg ml^{-1} Triton X-100 at pH 5.0, with the addition of 150 mM sodium fluoride when measuring the acid phospholipase activity. Reaction mixtures for the lipase assays with fluorimetric substrates were incubated at 37 °C for 20 min. The fluorescence was measured at an excitation wavelength of 355 nm and an emission wavelength of 460 nm. On unit of lipolytic activity is defined as the amount of enzyme capable of hydrolysing 1 μmol of substrate in 1 h at 37 °C.

2.5. Assays of cathepsin activities

Two grams of muscle were homogenised in 25 ml 50 mM sodium citrate buffer, pH 5.0, containing 1 mM EDTA and 0.2% (v/v) Triton X-100. The homogenate was centrifuged at $10\,000g$ for 20 min, and the resulting

supernatant, filtered through glass wool was used for cathepsin activity assays.

Cathepsin B, B + L and H were assayed as previously described by Toldrá and Etherington (1988), using N-CBZ-L-arginyl-7-amido-4-methylcoumarin, N-CBZ-L-phenylalanyl-L-arginine-7-amido-4-methylcoumarin, both at pH 6.0, and L-arginine-7-amido-4-methylcoumarin at pH 6.8 as specific fluorimetric substrates of cathepsin B, B + L and H, respectively. The reaction mixture consisted of 50 µl of enzyme extract and 250 µl of reaction buffer, 40 mM sodium phosphate at different pH's, containing 0.4 mM EDTA, 10 mM cysteine, and 0.05 mM of the specific substrate. The reaction mixtures with fluorimetric substrates were incubated at 37 °C and fluorescence was monitored at an excitation wavelength of 355 nm and an emission wavelength of 460 nm. One unit of cathepsin activity was defined as the amount of enzyme hydrolysing 1 µmol of substrate in 1 h at 37 °C.

2.6. Statistical analysis

Least square means were computed using the general Linear Model (GLM) Procedure of SAS (1997) program. The different genetic types were compared using the "*t*" test.

3. Results and discussion

Intramuscular fat contents of the different genotypes are shown in Table 1. Iberian breeds had higher contents while there were no differences between the commercial pig lines. Several studies have reported the high intramuscular fat contents in Iberian breeds (Antequera, García, López, Ventanas, Asensio, & Córdoba, 1994; Serra et al., 1998).

The activity of the enzymes during post mortem processes can be influenced by pre-slaughter treatments, but these were similar for all the genotypes. Large differences between genotypes were found in the activities of catalase and SOD, while GSH-Px showed no differences between the genotypes (Table 2). The highest differences between genotypes were found for the Iberian and Iberian×Duroc breeds compared with the other pigs. In

addition, catalase activity varied between the other pigs, with large values for LR and lower activities in P and LW. Iberian×Duroc breed had the highest value for SOD. Previous studies have indicated that endogenous antioxidant enzymes, especially catalase, could potentially delay the onset of oxidative rancidity in stored meat (Mei et al., 1994; Pradhan et al., 2000). There is limited information about the influence of genotype on antioxidant enzyme activities. Sárraga, Carreras, and García-Regueiro (2002), found higher GSH-Px activity in PSE pork meat than in normal meat, and Daun, Johansson, Onning, and Akesson (2001) found that RN phenotype had no effect on GSH-Px activity but there is no information about how different genetic types influence the activity of antioxidant enzymes. In our experiment, the differences between genetic types suggest some genetic determination for the antioxidant activity of pork meat.

Very little is known about the post-mortem activities of lipolytic enzymes in skeletal muscles. Table 3 shows the values of lipolytic activities measured in pork meat from different genotypes. No effect of age was found for the activity of lipolytic enzymes. There were no differences for neutral lipase activity between the different genotypes. The activity of acid phospholipase showed small differences, being higher in P and lower in LR. In relation to acid lipase, Iberian pigs showed the highest activity. Similar results were found by Cava, Ferrer, Estévez, Morcuende, and Toldrá (2002) when they compared the activity of acid lipase in Iberian (*Torbiscal*) and commercial pigs (crossbreeds not identified). Armero, Barbosa, Toldrá, Baselga, and Pla (1999) found small differences for acid lipase activities due to the terminal sire type, showing the Belgium Landrace had lower activity than the Danish Duroc. Our results show significant differences ($P < 0.05$) between LR and LW for acid lipase activity.

Lipolytic enzymes contribute to the hydrolysis of the lipid fraction and consequently to flavour development. These enzymes remain active during refrigerated or frozen storage (Hernández, Navarro, & Toldrá, 1999; Motilva et al., 1992), curing (Motilva, Toldra, Nieto, & Flores, 1993). Comparisons between hams from Iberian and other pigs reveal higher levels of free fatty acids in the Iberian hams (Antequera et al., 1994) which is in agreement with the higher acid lipase activity found in our experiment in Iberian pigs (I).

Large differences in proteolytic enzyme activities among the genotypes were found (Table 4). Iberian pigs (I) had lower activities for cathepsin B than P, LW and I×D, and no differences were found between I and LR. Cathepsin H activity was lowest in I (I and LR differ with $P < 0.1$). Rosell and Toldrá (1998) also reported lower activities of cathepsin B and H in Iberian compared with other pigs. Differences among the white pigs were found in their cathepsin activities, with a tendency

Table 1
Intramuscular fat content (%) of *Psoas mayor* muscle from different pig genotypes

P		LW		LR		I×D		I	
LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
1.32 b	0.06	1.24 b	0.05	1.22 b	0.05	2.81 a	0.07	2.79 a	0.07

LSM: least square means. SE: standard error. Means with different letters, within a row, differ significantly, $P < 0.05$. P: Pietrain (P); LW: Large-White; LR: Landrace; I: Iberian "*Torbiscal*"; and I×D: Iberian "*Lampiño*"×Duroc.

Table 2
Antioxidant enzymes activities (U/g of muscle) from the different pig genotypes

	P		LW		LR		I×D		I	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Catalase	187 c	18	217 c	17	294 b	17	324 ab	24	381 a	24
GSH-Px	0.177	0.022	0.199	0.021	0.176	0.021	0.210	0.029	0.227	0.029
SOD	657 b	39	636 b	37	637 b	37	928 a	53	739 b	53

LSM: least square means. SE: standard error. Means with different letters within a row, differ significantly, $P < 0.05$. P: Pietrain (P); LW: Large-White; LR: Landrace; I: Iberian "*Torbiscal*"; and I×D: Iberian "*Lampiño*"×Duroc. GSH-Px: glutathione peroxidase; SOD: superoxide dismutase.

Table 3
Lipolytic enzyme activities (U/g of muscle) from different pig genotypes

(U/g)	P		LW		LR		I×D		I	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
AL	0.119 bc	0.007	0.123 ab	0.006	0.107 c	0.004	0.120 bc	0.006	0.138 a	0.006
NL	1.41	0.18	1.28	0.14	1.12	0.11	1.57	0.16	1.02	0.18
PL	0.072 a	0.006	0.053 cb	0.005	0.045 c	0.004	0.064 ab	0.005	0.067 ab	0.006

LSM: least square means. SE: standard error. Means with different letters within a row, differ significantly, $P < 0.05$. P: Pietrain (P); LW: Large-White; LR: Landrace; I: Iberian "*Torbiscal*"; and I×D: Iberian "*Lampiño*"×Duroc. AL: acid lipase; NL: neutral lipase; PL: acid phopholipase.

Table 4
Cathepsin activities (U/g of muscle) from different pig genotypes

(U/g)	P		LW		LR		I×D		I	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
B	0.400 ab	0.050	0.415 a	0.035	0.306 bc	0.027	0.395 ab	0.039	0.238 c	0.039
B+L	1.12	0.15	1.43	0.11	1.36	0.08	1.66	0.12	1.21	0.12
B+L / B	2.77 d	0.45	3.50 cd	0.32	4.65 ab	0.25	4.21 bc	0.35	5.28 a	0.35
H	0.781ab	0.052	0.670 bc	0.043	0.628 cd	0.033	0.801 a	0.047	0.515 d	0.047

LSM: least square means. SE: standard error. Means with different letters within a row, differ significantly, $P < 0.05$. P: Pietrain (P); LW: Large-White; LR: Landrace; I: Iberian "*Torbiscal*"; and I×D: Iberian "*Lampiño*"×Duroc. B: cathepsin B; B+L: catepsin B+L; B+L/B: catepsin B+L/catepsin B; H: catepsin H.

to lower values for LR than P and LW. No differences were found in cathepsin B+L activities. The ratios of the activities of cathepsin B+L and cathepsin B was calculated to give an indication of the contribution of cathepsin L to the activity using a common substrate (Schreurs, van der Heide, Leenstra, & Wit, 1995). Conversely to cathepsin B, significant differences were found in this ratio with higher activities in I compared with P, LW and I×D, no differences were found between I and LR. Russo et al. (2000) found a moderate heritability (0.23–0.28, although with high standard errors) for the activity of cathepsin B, suggesting the possible use of cathepsin B activity as a selection criterion for genetic improvement of meat quality.

The usual slaughter age for Iberian pigs is 12 months compared with the 6 month for other pigs. Rosell and Toldrá (1998) attributed the differences found in cathepsin activity between Iberian and other pigs to the different ages. In our experiment, the differences in age do not seem to be the cause since Iberian had lower activities of cathepsin B and H than I×D ($P < 0.05$), and L ($P < 0.1$), and the animals of these breeds were slaughtered at the same age. Also, P and LW animals were slaughtered at 6 month of age and animals from LW had lower cathepsin H activities than P.

Our results suggest a genetic component in the variability of antioxidant, lipolytic and proteolytic enzyme activities. Further research should be done so this information can be used in genetic programs in order to improve meat quality.

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