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EFFECT OF AGEING ON THE TENDERNESS OF ITALIAN HEAVY PIG (M. *Longissimus dorsi*)

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Background
Consumers consider the tenderness of pork meat to be its most important eating quality and most of them are available for paying an higher price for tender meat. People working in pork market believe that the commercial value of domestic heavy pig (slaughtered when nine-month old and over) rely mainly on its suitability for processing into typical dry-cured products, missing the opportunity of improving the sensory traits of cuts undergoing fresh consumption. In order to meet consumer request of more tender and juicy pork a study was carried out to evaluate sensory properties of heavy pig loins.

Objective
The aim of this work was to assess:
- the ageing time needed to improve significantly the tenderness of M. *Longissimus dorsi* of Italian heavy pigs;
- the role of pH and calpain-calpastatin system activity in affecting pork tenderness.

Preliminary data are shown.

Methods
Animals. Twenty pork carcasses, representing a broad array of pork quality (pork weight ranged from 150 to 170kg, ages ranged from 9 to 11 months) were selected whith the aim of covering a representative range of pH and including two samples with pH_{1h} <5.8. Loins (M. *longissimus thoracis* and M. *longissimus lumborum)* were removed from carcasses, deboned 1 h after slaughter and transported to the laboratory. Chilling (4°C) began 3h after slaughtering.
pH measurements. pH values were measured with a pH-meter (WTW mod. 330) equipped with an insertion glass electrode (Hamilton electrode double pore) 1h and 24h post mortem at the last rib of the *M. Longissimus thoracis* (sample 6, fig. 1).
Shear force and cooking loss were determined on the same sample.
Shear force. 3.0 cm thick slices were taken from M. *Longissimus dorsi* at 1, 3, 4, 7 days post-mortem (samples 2, 3, 4 and 5, fig. 1), packaged under vacuum and stored at –18°C until analysis (no longer than 10 days). Samples were thawed overnight at 4°-5°C and cooked in a water bath at 70°C for 1h. Samples were subsequently cooled overnight at 4-5°C before taking core samples (∅=1.3 cm in the direction of muscle fibres). The tenderness was measured using an INSTRON mod. 5565 (Warner-Bratzer shear blade speed: 200 mm/min). The average maximum force (expressed as Newton) of 8-10 measurements per samples is reported as the shear force of the sample. Ageing index was calculated as the decrease of shear force recorded between the 1st and the 4th day of ageing.
Cooking loss (% wt/wt). Cooking loss was determined by removing excess liquid after cooking and weighing the samples again
Calpain and calpastatin measurements. Pre rigor μ-, m-calpain and calpastatin activities were determined in a sample taken at 2h post mortem (sample 1, fig. 1). μ- and m-calpain activities were determined as described by Geesink *et al.*, 1994, in frozen (-18°C) samples. Calpain activities were measured at pH 7.5 against 0.4% casein (Hammarsten) and $CaCl_2$ 0.4%. Calpastatin activity was determined as previously reported (Campanini et al. 1999) in unfrozen samples. Calpastatin activity was calculated from the slope of the linear part of the inhibition curve. For μ-, m-calpain, one unit (U) of activity was defined as the amount of enzyme that catalysed an increase of 0.1 absorbance unit at λ=278 nm in 60 min at 25°C. For calpastatin, one unit of activity was defined as the amount of the inhibitor that inhibited one unit of m-calpain activity. μ- and m-calpain activities are reported as U/g muscle. Calpastatin activity data are reported as U/g muscle.
Statistic. Comparison tests and correlation coefficients were computed by the ONEWAY and CORRELATION procedures of the SPSS-PC (ver. 10.0) package, respectively.

Figure 1: Dissection of the M. *longissimus lumborum* et *thoracis*.

M. *longissimus thoracis*						M. *longissimus lumborum*			
8th rib					last rib				
1	2	3	4	5	6				

Results and discussion. Only samples with regular post-mortem glycolysis (pH_{1h}>5.8) were taken into account for statistical analyses reported into next tables.
Shear force, but not cooking loss, was significantly affected by time of ageing (table 1). Several hypotheses were formulated upon the influence of post mortem ageing on the tenderness of pork (Harrison *et al.*, 1970; Bennet *et al.*, 1973; Feldhusen *et al.*, 1992; Wood *et al.*, 1996); the present investigation led to the conclusion that shear force of loins of Italian heavy pig decreases significantly within 96h post-mortem. Afterwards shear force didn't show significant differences between 96 and 168h. No decrease or even increase of shear force with ageing time was detected for a small number of samples. This behaviour may be attributable to both a negligible myofibril fragmentation and increasing cooking loss leading to higher amount of fibers per area unit.

The rate of pH decline may influence the ageing rate of pork affecting sarcomere length (Honikel K. O. *et al*., 1986), protein denaturation, and activity of the tenderising enzymes (Ertbjerg *et al*. 1999; Koohmaraie *et. al* 1986). Table 2 shows the means, standard deviations and range of the variability of the pH values and enzyme activities. Table 3 shows the correlation coefficients between pH, enzyme activities, shear force and ageing index. pH_{24h} significantly correlates with shear force at 4th day and shear force decrease, while pH_{1h} correlates with shear force at 1st day. Correlations with ultimate pH were higher for shear force values measured at 4th days than at 1st day. A positive relationships between pH_{24h} and shear force decrease from 1st to 4th day (ageing index) was found. This result may be due to the influence of ultimate pH on the muscle proteolytic enzymes activity associated with meat tenderization. The inclusion of samples with $pH_{1h}<5.8$ meant lower correlation coefficients and the lost of some significant relations reported in Table 3; a negligible ageing index at 4th day was found in the samples showing a PSE-type pH drop and a lower ultimate pH, supporting the hypothesis of a more quick post-mortem inactivation of enzymes involved in myofibril fragmentation. Pre rigor m-calpain activity was positively and significantly correlated to ageing index, but not to shear forces at 1st and 4th days. Calpastatin activity was positively correlated to shear force at 1st day. μ-Calpain didn't show significant correlations with any over mentioned variables. The highest correlations were found between the ratio m-calpain/calpastatin and ageing index: the highest the ratio, the highest the tenderness achieved by means of a prolonged ageing time. These findings show that the improvement of tenderness is associated at the same time with ageing time, pH_{1h} and pH_{24h}, and free calpains.

Conclusions

96 h of ageing time improved significantly tenderness of Italian heavy pig loins. pH_{24h} significantly affected the tenderness changes. Loins with regular post-mortem glycolysis ($pH_{1h}>5.8$), achieved higher tenderness and ageing index in association with higher pH_{24h}, showing a main role played by pH during ageing. The ageing index is enhanced by an higher ratio calpain/calpastatin in case of loins with $pH_{1h}>5.8$. These results suggest that an ageing time up to 96 h post-mortem may be a way for improving tenderness of heavy pig loins with $pH_{1h}>5.8$. Inclusion of loins with $pH_{1h}<5.8$ weakened the relationships among over mentioned parameters, so that for these samples a prolonged ageing time post-mortem might not be suitable for tenderness improving.

Table. 1: Effect of ageing time on shear force and cooking loss (mean ± s.d.).

	Ageing time, days post-mortem			
	1	3	4	7
Shear force (N)	43.6 ^{a}±13.2	39.1 ± 9.5	37.0 ^{b}± 9.9	34.2 ^{b}± 9.8
Cooking loss	23.8 ± 5.8	25.3 ± 5.7	25.3 ± 4.7	25.6 ± 6.8

[a] Mean with different superscript letters differ significantly (P<0.05).

Table 2: pH and enzyme activities in pork *longissimus* muscle.

	mean	s. dev.	range
pH_{1h}	6.24	0.19	5.89-6.58
pH_{24h}	5.58	0.08	5.40-5.72
μ-calpain	30.75	17.78	1.60-64.98
m-calpain	14.17	7.28	4.67-29.31
calpastatin	46.17	11.79	29.30-70.13
μ-calpain : calpastatin	0.66	0.33	0.04-1.33
m-calpain : calpastatin	0.29	0.13	0.11-0.54

Table 3: Correlation coefficients between pH, enzyme activities, shear force and ageing index.

	shear force 1 day	shear force 4 days	ageing index (1-4 days)
pH_{1h}	-0.48*	-0.40	-0.15
pH_{24h}	-0.27	-0.63*	0.48*
μ-calpain	0.22	-0.33	0.37
m-calpain	0.19	-0.21	0.58*
calpastatin	0.45*	-0.03	0.35
μ-calpain : calpastatin	0.29	-0.15	0.46*
m-calpain : calpastatin	-0.16	0.05	0.65*

Significance levels: *P<0.05

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