CALPAIN AND LIPOLYTIC ACTIVITY IN POST-MORTEM PORK MEAT AND ITS RELATIONSHIP WITH QUALITY.

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Introduction.

The eating quality property of meat, usually accepted as the most crucial, is tenderness (Wood et al., 1996). Two factors are important in controlling tenderness: the degree of proteolytic breakdown of myofibrillar proteins and the concentration of intramuscular lipids (Wood et al., 1996) being also these two factors related to water holding capacity during processing and palatability. However, meat tenderisation differs greatly among animal species and, within one specie, among muscles probably due to the variability in enzyme levels and anatomical location (Koohmaraie et al., 1991, Ouali and Talmant, 1990).
The prediction of quality on the slaughter line is a difficult process because in this early post-mortem state, some of the biochemical quality properties have not yet been fully expressed. Therefore, the objective of this study was to determine how the post-mortem pork quality affects the activity of proteolytic (calpains and their inhibitor) and lipolytic enzymes present in muscle in order to control the tenderness and the degradation of the intramuscular lipids.

Materials and Methods.

Animals. 12 pork carcasses, representing a broad array of pork quality, were selected from a local slaughterhouse.
Meat quality measurements. The pH was measured at 2h and 24 h in *longissimus dorsi* muscle at fifth rib with a portable pH-meter Crison 506 (Crison Instruments, Alella, Barcelona, Spain). The drip loss (DL) was measured by the method of Warris, (1982).
Calpain and calpastatin measurements. The procedure is based on the method described by Koohmaraie (1990) and Rosell and Toldrá (1996). Preparation of muscle enzyme extracts was done by homogenising 10 g of muscle *longissimus Dorsi* in 30 ml of 50 mM Tris buffer, pH 8.5, with 3 mM EDTA, 1 mM NaN_3, and 10 mM 2-mercaptoethanol. The muscle extract was used for calpains and calpastatin separation after being readjusted to pH 7.5 and then, subjected to anion exchange chromatography (Biosep-DEAE column, 7.5 x 78 mm, Phenomenex, Ca, USA) in a FPLC system. Calpain activity was measured as described by Rosell and Toldrá (1996) using 2 mg/ml casein-fluorescein isothiocyanate as substrate. The reaction mixture was incubated at 30°C for 15 min and the fluorescence measured at 485 nm and 538 nm as excitation and emission wavelengths, respectively, in a Fluoroskan II fluorophotometer (Labsystems, Finland). Four measurements were made for each experimental point. One unit (U) of calpain activity is defined as the amount of enzyme capable of hydrolysing 1 µmol of substrate in 1 hour at 30 °C whereas one unit of calpastatin is the amount of inhibitor inactivating one unit of m-calpain activity.
Muscle lipase and esterase assays. 4 g of muscle *longissimus Dorsi* was homogenised in 20 ml of 50 mM disodium phosphate buffer, pH 7.5, containing 5 mM EGTA. The extract was homogenised and centrifuged, and the supernatant used for lipase and esterase assays. Enzyme measurements were performed by fluorimetric assays as previously described by Motilva et al., (1992) using as substrates 1.5 mM of 4-methylumbelliferyloleate and 4-methylumbelliferylpropionate for lipase and esterase activities, respectively. Reaction mixture of lipase and esterase assays was incubated at 37° C for 20 min and 10 min, respectively. The fluorescence was measured at 355 nm and 460 nm of excitation and emission wavelength, respectively, using a Fluoroskan II fluorophotometer (Labsystems, Finland). Four replicates were performed for each enzyme assay. One unit (U) of lipolytic activity is defined as the amount of enzyme capable of hydrolysing 1 µmol of substrate in 1 hour at 37 °C.

Results and Discussion.

The carcasses were classified based upon pH_{2h}, pH_{24h} and drip loss (DL), into one of the following categories (Table 1); exudative, normal and dark firm dry (DFD). The pH_{2h} and the drip loss were the parameters that better distinguished the exudative quality group .

Table 1. Pork quality classification based on post-mortem measurements.

	pH_{2h}		pH_{24h}		DL	
	LSM	SE	LSM	SE	LSM	SE
Exudative	5.81^{b}	0.07	$5.77^{a,b}$	0.06	6.92^{a}	0.51
Normal	6.32^{a}	0.04	5.71^{b}	0.04	3.45^{b}	0.34
Dark Firm Dry	6.30^{a}	0.08	5.93^{a}	0.08	3.14^{b}	0.63

^{a,b}Means within a column without a common superscript differ ($P < 0.05$)

The normal group was characterised by an intermediate pH_{2h} and pH_{24h}, being significantly different ($P<0.05$) from the DFD group by the pH_{24h}. Kauffman et al., (1993) also indicated that pH_{45min} was indicative of ultimate quality but only with extreme pH_{45min} values. Therefore, it is necessary to focus on other biochemical indicators to predict quality.
Based on this classification we also determined the calpains and their inhibitor, calpastatin, and lipolytic activities at 2 h post-mortem in the different pork quality classes (Table 2). The measurements of µ-calpain, m-calpain and calpastatin were obtained by a previous separation on an anion exchange chromatography column with a NaCl gradient (Figure 1). µ-Calpain eluted at 0.15 M NaCl (peak II in Fig 1), m-calpain at 0.24 M NaCl (peak III in Fig 1) and calpastatin at 0.05 M NaCl (peak I in figure 1). The activity of calpains and their inhibitor was measured in the different pork quality meats although no significant ($P < 0.05$) differences were observed among qualities (Table 2). The DFD pork quality group was higher in m-calpain than the exudative and normal groups but the differences were not significant ($P < 0.05$), probably because of the low number of samples. High pH meat has been associated with an increased tenderness of meat (Tonberg, 1996), probably due to an enhanced calpain activity. Koohmaraie et al., (1991) studied the calpains and their inhibitor in beef, pork and lamb, concluding that the inhibitor concentration in muscle may be the reason for differences observed

in rates of post-mortem tenderization. In our study, the inhibitor concentration was not different in the quality groups and probably, will not be the cause of differences in tenderness. In 1997, Beltrán et al. studied the effect of high post-mortem pH on the beef tenderization process and reported the absence of significant differences in μ- and m-calpain, and calpastatin at 2h post-mortem in bovine *Longissimus thoracicus* muscle in the pH groups (pH<5.8, pH5.8-6.3, pH>6.3). They only found significant differences in m-calpain at 7 d post-mortem, being higher in the pH>6.3 group and well correlated with tenderness and juiciness, two of the main sensory descriptors for meat quality.

The measurement of lipase and esterase activity on the different pork qualities resulted in significant differences ($P < 0.05$) except for acid lipase (Table 2). Neutral lipase was significantly higher ($P < 0.05$) in DFD than in exudative pork meat. Also, the exudative pork meat was significantly lower ($P < 0.05$) in acid and neutral esterase than the normal pork meat. Changes in free fatty acids composition may be relevant to variations in meat flavour and also, influence final meat quality. The activity of muscle lipases and esterases may be of importance in final meat quality because of their role in the release of free fatty acids. The lower esterase and lipase activities in exudative pork meat may be responsible of differences in meat quality among these groups.

Table 2. Calpain system and lipolytic enzymes by pork quality groups.

	Exudative (n=3)		Normal (n=7)		Dark Firm Dry (n=2)	
	LSM	SE	LSM	SE	LSM	SE
Calpain enzymes and inhibitor (U/g of muscle)						
μ-Calpain	0.082	0.009	0.085	0.006	0.089	0.011
m-Calpain	0.167	0.015	0.166	0.009	0.198	0.018
Calpastatin	0.020	0.005	0.028	0.003	0.026	0.006
Lipolytic enzymes (U/g of muscle)						
Acid lipase	0.54	0.04	0.57	0.02	0.58	0.04
Neutral lipase	1.72^{b}	0.14	$1.94^{a,b}$	0.09	2.24^{a}	0.17
Acid esterase	5.37^{b}	0.44	6.59^{a}	0.29	$5.96^{a,b}$	0.53
Neutral esterase	3.36^{b}	0.09	3.67^{a}	0.06	$3.56^{a,b}$	0.11

^{a,b}Means within a row without a common superscript differ significantly ($P < 0.05$)

Conclusion

The exudative pork meat quality can be distinguished by the measurement of neutral lipase and acid and neutral esterases. Lipolytic enzymes may be useful in the pork meat quality classification, although more samples should be analysed in order to confirm these previous results.

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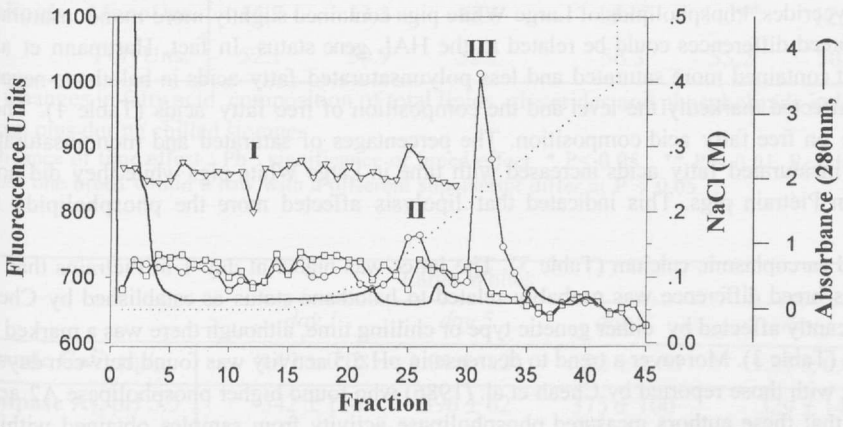

Figure 1. Chromatographic separation of μ-calpain, m-calpain and calpastatin using a Biosep-DEAE column.
—○— Calpastatin, —○— Calpain activity and —○— Blank in presence of EDTA.