M-CALPAIN CONTRIBUTION TO DRY-CURING PROCESS

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OBJECTIVE

The aim of the present work is to determine the potential contribution of the calpains to dry-curing process and the effect of the curing agents and process parameters on these enzymes.

MATERIAL AND METHODS

Muscle extract: Samples were obtained from Iberian pigs (12 month old). Extracts were prepared from muscle *Biceps femoris*) taken from raw meat and different stages of the curing process (10, 20, 45 days), following the method reported by Koohmaraie (1990).

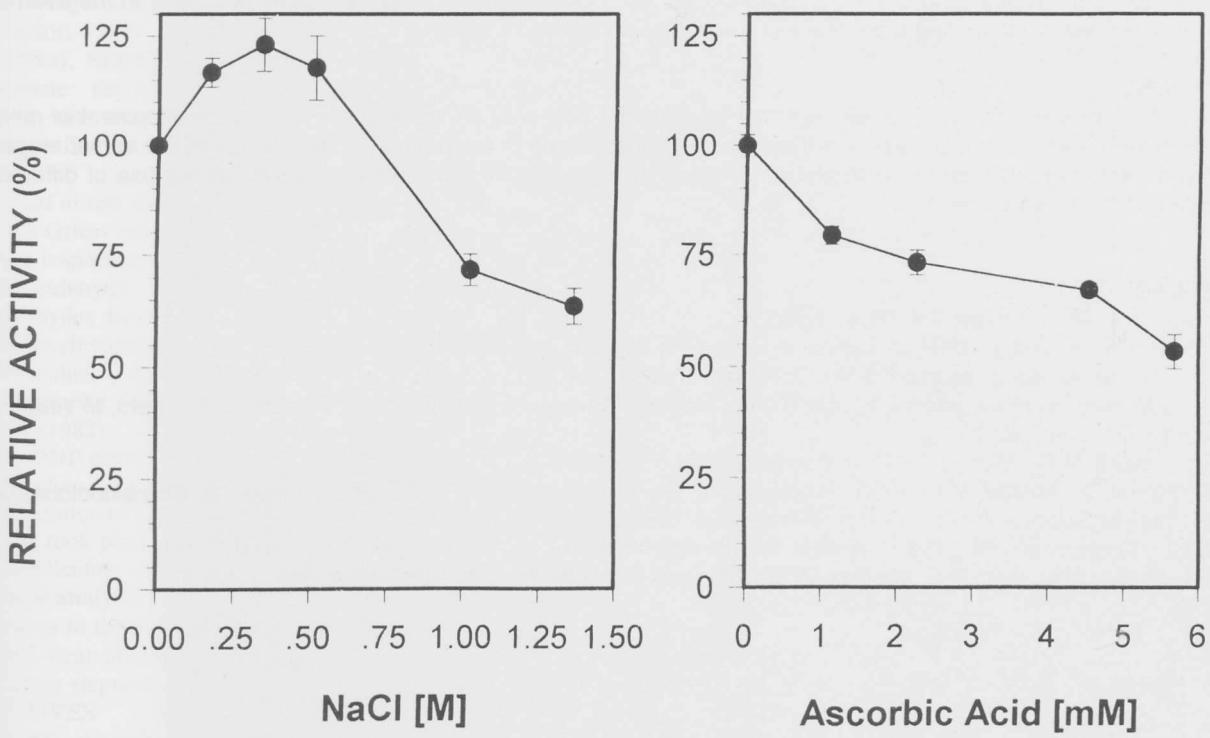
Preparation of calcium dependent proteases: The separation of both calpains and their specific inhibitor, calpastatin, were performed on a DEAE-Sephacel column (Wheeler and Koohmaraie, 1992).

Assays of enzyme activity: All the assays were carried out with m-calpain, due to its greater stability compared to that of μ-calpain. Enzyme activity was measured by using casein-fluorescein isothiocyanate (FITC-casein) as specific substrate (Wolfe et al, 1989). Vertical bars represent the mean of four replicates ± sem.

Effect of curing agents on m-calpain activity: The effect of different curing agents (sodium chloride, sodium nitrate and ascorbic acid) was determined by addition of those agents on the reaction medium, testing several final concentrations in the range usually found during the curing process.

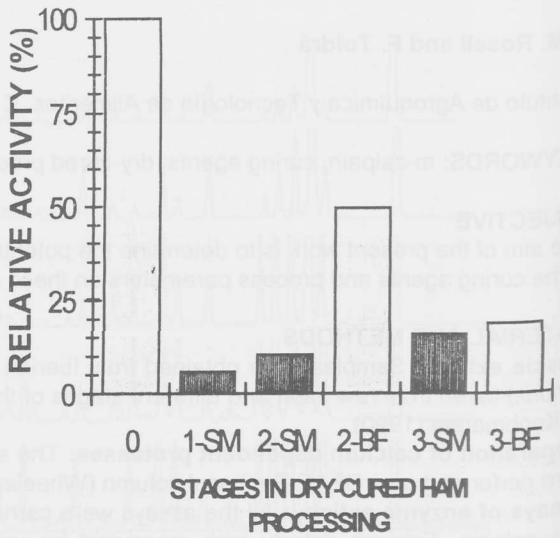
Simulation of different stages of curing process: Four different combinations of curing agents, pH and water activity were made to simulate four distinct stages (Table I): stage 0: would correspond to raw meat, approximately 2 days after slaughter; stage 1 would represent *Semimembranosus* muscle after salting; stage 2 and 3 would represent two different steps (early and end) of the curing agent diffusion during the post-salting stage. Experimental conditions of each simulated stage are detailed in Table Y.

EFFECT OF CURING AGENTS ON M-CALPAIN ACTIVITY



SIMULATION OF DIFFERENT STAGES OF DRY CURING PROCESS IN *BICEPS FEMORIS* AND *SEMIMEMBRANOSUS*

STAGE	TISSUE	Aw (%)	pH	NaCl (mM)	$NaNO_3$ (mM)	Ascorbic Acid (mM)
0 (2d)	SM, BF	99	5.8	--	--	--
1 (10d)	SM	98	5.8	140	5.0	3.0
2 (20d)	SM	97	5.8	100	4.0	2.3
	BF	99	5.8	20	0.6	0.3
3 (2m)	SM	97	5.8	75	1.2	1.5
	BF	97	5.9	35	1.0	1.2



SM: *Semimembranosus* muscle
BF: *Biceps femoris*

RESULTS AND DISCUSSION

Salt, the main component of the curing mixture, up to 0.5M concentration promotes a slight activation of m-calpain, but onwards inhibits the calpain activity (retained 63% of its initial activity at 1.4M NaCl) (Sárraga et al, 1989). No effect was observed with the addition of sodium nitrate, whereas ascorbic acid inhibits the m-calpain activity at all the assayed concentrations, as was previously observed with other enzymes (Rico et al, 1991; Toldrá et al, 1993).

The combined effect of both the curing agents and processing parameters is analyzed by a simulation of the conditions in different stages of the curing process. A dramatic decrease of the enzyme activity is observed in the stage 1, where NaCl and ascorbic acid produce a great inhibition. As the process goes forward (stages 2 and 3) the concentrations of both agents decrease in the *Semimembranosus* muscle, due to the inner diffusion, occurring an increase of the enzyme activity (Toldrá, 1992; Toldrá et al, 1995). Simultaneously, an increase of the curing agents is produced in the internal muscles, *Biceps femoris*, promoting a decrease of the m-calpain activity.

CONCLUSIONS

In the light of the presented results, it appears that calpains not only play an important role in the development of meat tenderness during post mortem storage, but they could also participate in the proteolytic events during the earlier stages of curing processes. In addition, the proteolysis promoted by calpain activity could be controlled by the use of different concentrations of the curing agents.

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