INFLUENCE OF pH_{24h} AND MUSCLE CATHEPSIN B ACTIVITY ON PROTEOLYSIS OF PARMA, SERRANO, IBERIAN AND

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Background

Dry-cured ham manufacturing merges very old and traditional practises with new approaches coming from the scientific research. One of the most debated topic in meat processing industry is the role of raw material in affecting properties of final product and its proneness to be manufactured into matured hams of valuable taste and texture. Amount and pattern of low-weight nitrogen molecules released during proteolytic process taking place during ham processing, proved to be closely related to ham taste and texture: an excessive final proteolysis was charged with drawbacks of texture and taste of hams like softness, pastiness and bitter taste development (Arnau 2000; Garcia-Garrido et al., 2000). Among raw material parameters, pH_{24h} and muscle proteolytic enzymes (Banon 1998, Virgili et al. 1998, Schivazappa et al. 2002) are accepted by meat scientists as very promising indicators of fresh pork quality because they are related to proteolysis of end product. Moreover, manufacturing techniques, ageing length and salt amount influence proteolysis values predictable from raw matter pH and proteolytic enzymes; nevertheless, these parameters may be regarded as very effective in affecting final proteolysis, even if different ham types proved to be differently influenced.

The aim of the present study is to evaluate the influence of raw matter pH_{24h} and cathepsin B activity, on the proteolysis index of four different ham types (Serrano, Iberian, Parma and Estero).

A large number of legs for each ham type were tested (m. Semimembranosus) for pH_{24h} and cathepsin B activity; thighs having cathepsin B activity below or over the prefixed values (see Table 1) were processed. According to muscle pH_{24h} values, hams were subgrouped into samples with pH≤5.6 (low pH), 5.6< pH<6.0 (normal pH) and pH≥6.0 (high pH) in agreement with Van der Wal et al. (1988). Thighs underwent manufacturing in Italy (Parma and Estero hams) and in Spain (Serrano and Iberian hams) and the corresponding dry-cured hams (m. Biceps Femoris) were analysed for proteolysis index and salt amount. To focus the effect of meat quality on dry-cured ham features, raw hams allotted to each dry-cured ham type were selected with a very narrow weight range and manufactured in the same plant.

Table 1. Samples selection for cathepsin B activity (as nmol AMC · min⁻¹ · g muscle⁻¹) levels.

Parma 1.46 ^a ± 0.33	Serrano		Estero $1.47^a \pm 0.37$
$ \begin{array}{c c} low & high \\ \leq 1.2^b & \geq 1.6^b \\ n^{\circ}61 & n^{\circ}85 \end{array} $	low high ≤1.5 ^b ≥2.0 ^b n°105 n°105	low high ≤1.5 ^b ≥2.0 ^b n°104 n°103	$ \begin{array}{c cc} low & high \\ \leq 1.2^b & \geq 1.6^b \\ n^{\circ}43 & n^{\circ}45 \end{array} $

^a overall mean ± std. dev. of raw hams

pH measurements were made in the m.. semimembranosus 24h post mortem.

Muscle Cathepsin B activities were assayed 48h post mortem with N-CBZ-Phe-Arg-AMC as described by Barrett (1980).

Proteolysis index (NPN) assay was carried out following the method described by Careri et al. (1993).

Salt amounts were determined following AOAC procedure (1982).

Data were statistically processed by means of the ONE WAY ANALYSIS OF VARIANCE procedure (LSD test) of the statistical package SPSS/PC (version 10.0).

Results and discussion

As displayed in Table 2, weights of raw hams were rather homogeneous, with the exception of Iberian hams. According to manufacturing procedures of each ham type, Iberian hams were the most aged, whereas Estero hams underwent the shortest maturation period. The Italian product was characterised by a low salt amount, accounting for high NPN value of Parma ham, overcoming the Iberian one, though the latter was more aged (18 vs 16 months).

Table 2. Data (mean \pm dev.std.) of selected hams.

to be	Estero	Serrano	Parma	Iberian
Ageing length (months)	9	12	16	10011411
Raw ham weight (kg)	10.0 ± 0.36	11.1 ± 0.36	13.0 ± 0.35	9.5 ± 0.93
NPN ¹	30.3 ± 2.11	31.1 ± 4.2	34.0 ± 3.41	9.3 ± 0.93 32.2 ± 2.32
Salt (%)	4.51 ± 0.41	7.33 ± 0.68	4.73 ± 0.47	6.08 ± 0.73

per cent ratio between soluble nitrogen in 5% trichloroacetic acid and total nitrogen in the meat.

The effects of pH_{24h} and raw ham cathepsin B activity on NPN of dry-cured hams, were evaluated in different ham types (Table 3): hams with a lower cathepsin B activity showed NPN values significantly lower than those of hams with higher activity, with the exception of Iberian hams. The variation of pH_{24h} accounts for differences between NPN of hams grouped according to pH levels: a low pH is associated to high protein breakdown probably for activation and/or release of lysosomial acid protease (O'Halloran et al., 1999). Figure 1 shows the comparison between NPN of dry-cured hams with the same pH_{24h} and different cathepsin B activity levels.

^b fixed values of cathepsin B activity for green ham selection

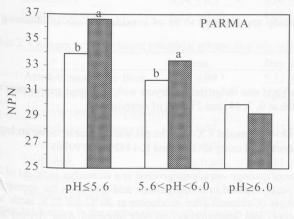
For high and low pH ranges no significant differences were found between samples differing for cathepsin B levels. The strong influence of pH on proteolytic activity (reduction for pH≥6.0 and activation for pH≤5.6) overcomes differences of cathepsin B activity. Only Parma ham, being low-salty, long aged and more heavy showed a remarkable effect of both parameters. Within normal pH range significant differences between NPN of hams are attributable to cathepsin B levels: hence normal pH values retained the differences of enzymatic activity. In case of Iberian ham, showing only a pH effect on NPN, a distinct role of raw matter (Iberian pig vs White pig of other hams) as to proteolytic mechanism may be postulated.

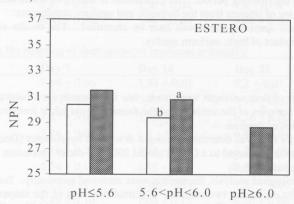
Table 3. Cathepsin B and pH_{24h} effects on NPN of dry-cured ham (means in a row with a different superscript letter differ (P<0.05))

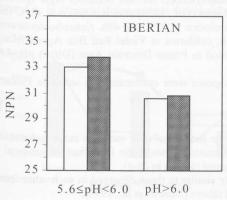
ham	cathepsi	cathepsin B level		pH level		
	low	high	pH≤5.6	5.6 <ph<6.0< th=""><th>pH≥6.0</th></ph<6.0<>	pH≥6.0	
Parma	32.3 ^b	35.3 ^a	35.9 ^a	32.6 ^b	29.7°	
Serrano	30.3 ^b	31.8 ^a	33.5 ^a	28.5 ^b	#	
Iberian	32.1	32.7	#	33.0^{a}	30.5 ^b	
Estero	29.8 ^b	30.9 ^a	31.0^{a}	30.0^{b}	28.7 ^b	

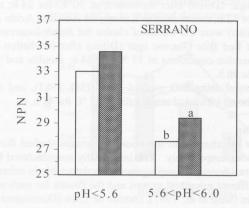
not included because the number of hams falling in this pH range was too small.

Figure 1. Comparison between NPN of dry-cured hams with the same pH_{24h} and different cathepsin B activity levels (\square low; \blacksquare high). Means in each pH category with a different superscript letter differ (P<0.05).









Conclusion

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> Excessive proteolysis proved to be associated with sensory properties of dry-cured hams like bitterness and pastiness. The results obtained suggest that the check of pH_{24h} and cathepsin B activity in raw matter, could be a useful tool for control of final NPN and for improving standardisation of ham quality, mainly for low-salty and long aged hams.

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Acknowledgements

This work was supported with funds from the European Commission (FAIR-CT-97-9517).