Cathepsin D activity as an index of curing time for Spanish dry-cured ham.

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SUMMARY

In this study evolution of cathepsin D activity during the curing process of Spanish dry-cured ham is analyzed. The period between 3 and 7 months of curing is especially emphasized in order to establish a relationship between the African Swine Fever Virus (ASFV) lose of viability and the activity of the enzyme.

INTRODUCTION

The sector of Spanish dry-cured ham represents a very important part of the Spanish agro-industry. It has a competitive substructure and it is prepared to export its products. However, the existence of the ASFV arises as an important barrier to overcome.

The studies made so far show that the ASFV loses its viability at 150-180 days of curing, as it was stressed in a EEC meeting in Brussels, in 1986. Nevertheless, it was also admitted that no clear correlation could have been established between curing time (related to the ASFV lose of viability) and the physico-chemical parameters usually analyzed (a_w, pH, free Nitrogen, free aminoacids, etc.).

Most of the commercialized hams undergo a 3-4 months curing process, so that it would be necessary to find a parameter easy to determine to guarantee a minimum curing time of 6-7 months.

Studies reported by Melo et al. (1974) in country-style ham, and by Toldrà and Etherington (1988), and by our group (Sárraga et al. 1988) in Spanish dry-cured ham, suggested that cathepsin D activity could be a suitable parameter to use as an index of curing time.

This work was included in a wider study on the action of muscular proteases - especially those related with meat tenderization - in the curing process of Spanish dry-cured ham.

MATERIALS AND METHODS

Manufacturing technology

Evolution of cathepsin D activity was studied in three series of hams manufactured according

to three different technologies:

Series A - 4 months curing process

Series B - 8 " " "

Series C - 12 " " "

Series A was analyzed to see whether activity changes were influenced by the curing "per se" or not.

Selection of fresh hams was done by measuring pH, electric conductivity (Quality Meater) and light scattering (Fiber Optic Probe).

Sampling

Taking into consideration the muscular variation in ham, the different rates of salt penetration (Arnau et al., 1987), the effect of salt in proteolytic activity (Sárraga et al., 1989), and the muscle volume, Biceps femoris muscle (internal) and semimembranosus muscle (external) were used in the study. Samples (eighteen hams in each point) were taken every month between 3 and 7 months of curing.

Determination of cathepsin D activity

Muscle samples were trimmed of fat and connective tissue and homogenized in an Ultra-Turrax (15000 rpm, 20s) in cold formiate buffer 250 mM, pH 3.0. The homogenates were centrifuged at 20.000 xg, 20 min at 4ºC and supernatants filtered and used as enzymatic sources. The activity was determined by the method of Anson (1938), modified according to our work conditions, using denatured haemoglobin as substrate.

One enzyme unit was defined as that amount which caused a change in absorbance of 0.001 units per min. Specific activity was given in enzyme units per mg protein.

Protein determination

Protein concentration was determined by the method of Lowry et al. (1951), using bovine serum albumin as standard.

Statistics

A linear model was used for the analysis of cathepsin D activity data between 3 and 7 months of the curing process, with process and time as fixed variables.

Analysis were performed with the statistical Analysis System (SAS, 1985) and the General Linear Models (GLM).

RESULTS

A great number of factors take part in the Spanish dry-cured ham manufacturing technology, some of them inherent to the technology itself (concentration of the curing salt, temperature, etc.), and some others extrinsic to the process, but that influence the quality of the final product, too (age and sex of the animal, ante-mortem and post-mortem treatment, sanitary conditions of the drying room, etc.).

In the present study, selection of the raw material and elaboration of hams were carried out in order to obtain a representative group of hams, with the more usual characteristics found in the Spanish market. Moreover, a very simple methodology for the determination of cathepsin D activity was used since there was a great number of samples to analyze, and taking into account the final aim of the study.

Cathepsin D activity was maintained during the whole curing for processes A, B and C.

The results obtained from the stastistical analysis are summarized on Table 1. No significant effect of the process for the variable cathepsin D specific activity was observed in the muscles studied. However, a high significant effect of time in cathepsin D activity was found: For Biceps femoris muscle activity at 6 months of curing increased significatively in relation to 3, 4, 5 and 7 months of curing. For Semimembranosus muscle, significant differences between activity at 6 months of curing and at 3, 4 and 5 months, but not at 7, were found.

Figure 1 shows cathepsin D activity evolution between 3 and 7 months of curing, for both muscles and both processes B and C.

CONCLUSIONS

1) Evolution of cathepsin D specific activity through the curing was not affected by the manufacturing process.

2) Cathepsin D activity was observed during the whole curing for processes A, B and C.

3) Activity of the enzyme at 6 months of curing was significatively higher in relation to 3, 4 and 5 months for Semimembranosus muscle, and to 3, 4, 5 and 7 months for Biceps femoris muscle.

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Table 1. Least Squares Means (LSM) and Standard Errors (SE) of Cathepsin D specific activity in the Semimembranosus (SM) and Biceps Femoris (BF) muscles of Spanish dry cured hams.

	PROCESS						PROCESS
	A		B		C		
Muscle	LSM	SE	LSM	SE	LSM	SE	
SM	1.071	0.118	1.125	0.067	1.236	0.067	N.S.
BF	1.510	0.166	1.350	0.093	1.337	0.097	N.S.

	TIME										TIME
	3		4		5		6		7		
Muscle	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	
SM	1.078^{b}	0.089	0.932^{b}	0.081	1.059^{b}	0.116	1.494^{a}	0.115	1.155^{ab}	0.115	**
BF	1.016^{bc}	0.126	0.913^{c}	0.114	1.537^{b}	0.160	2.206^{a}	0.166	1.323^{bc}	0.162	***

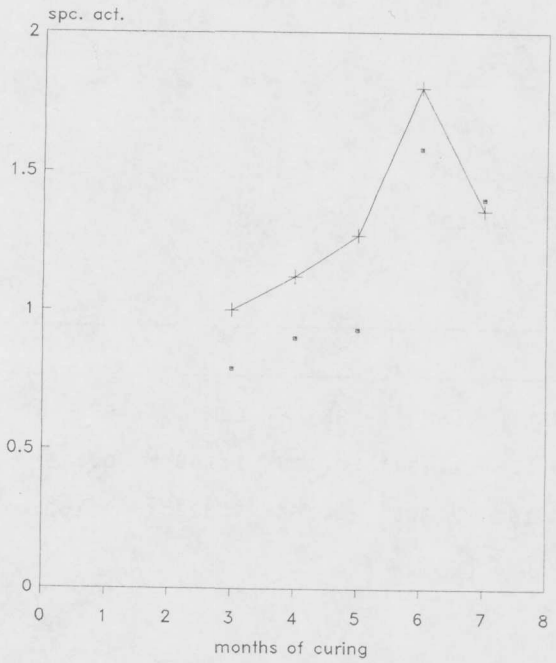
** = P<0.01
*** = P<0.001
NS = Not significative

Means with different superscripts are significantly different at the level of the P<0.05

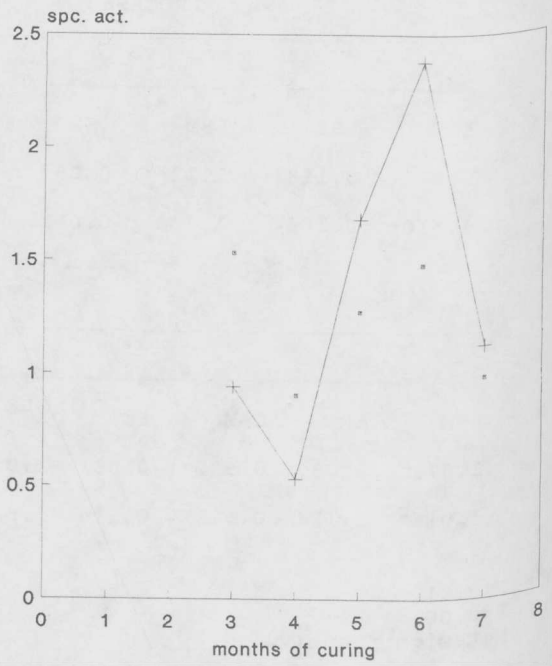
REFERENCES

- Anson, M.L. (1938) The stimation of pepsin, trypsin, papain and cathepsin with haemoglobin J. Gen. Physiol. 22 : 79.
- Arnau, J., Maneja,E., Monfort, J.M. (1987): Estudio de la influencia de la carne PSE en el proceso de curación del jamón. Cárnica 2000. 2ª etapa 48: 77.
- Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J. (1951): Protein measurement with Folin-Phenol reagent. J. Biol. Chem. 193: 265-275.
- Melo, T.S., Blumer, T.N., Swaisgood, H.E., Monroe, R.J. (1974): Catheptic enzyme activity in aged country-style hams as influence by precuring treatment. J. Food Sci. 39: 511-511.
- Sárraga, C., Gil, M., Cussó, R. (1988): Proteinase activities in the spanish ham during the curing process. 14th International Congress of Biochemistry. Czechoslovakia.
- Sárraga, C., Gil, M., Arnau, J., Monfort, J.M., Cussó, R. (1989): Effect of curing salt and phosphate on the activity of porcine muscle proteases. Meat Science 25: 241-249.
- SAS (1985) SAS User's Guide: Basics, Version 5. Edition (1985). SAS Institute INC. Cary, N.C.
- Toldrà, F., Etherington, D.J. (1988): Examination of cathepsins B, D, H and L activities in dry-cured hams. Meat Science 23: 1-7.

Fig.1 Evolution on Cathepsin D during curing



Process B (-+-) BF muscle (-.-) SM muscle



Process C (-+-) BF muscle (-.-) SM muscle