EVOLUTIVE TENDENCIES OF CATHEPSIN D ACTIVITY ALONG THE MATURATION OF DRY CURED HAM

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INTRODUCTION

We delineated a study in wich a set of dry cured hams was subjected to technological treatments of fabrication a little different (see table) (salted from 6 or 10 days, rest in containers or in suspension, cure at 20°C or dry cold) and afterwards we let that these products evoluted until they accomplished 15 months of preparation (1, 3).

During this period going between 2 and 15 months we determined in each sample and in the two types of muscle (*biceps femoris*, BF, and *semimembranosus*, SM) the activity of cathepsin D (EC 3.4.23.5).

MATERIALS AND METHODS

200 unfrozen pork legs were analysed, divided in groups of 50 and sub-groups of 25 (A-A1-B-B1-C-C1-D-D1). These groups were subjected to diversified squemes of cure (some steps of the fabrication process) outlined in the following table. The determinations of the activity of cathepsin D according to the methodologies referred in 2 and 4.

SOME STEPS OF THE FABRICATION PROCESS

Sub-Group	Salting (days)	Rest	Stabilization
A A1	6	Brushing 14 days	A-B-C-D (chamber 1) 21 days of cold drying 3 days of pre-drying 6 days of smoking 6 days of chilling
B B1	10	Readily rinsed	
C C1	6	Readily rinsed	
D D1	10	Brushing 14 days	A1-B1-C1-D1 (chamber 2) 36 days of cold drying

METHODS:

Determination of protease activity from cathepsin D (2):

The determination was performed using hemoglobin as a substrate and by quantification of acid soluble peptides produced during an incubation period, readings of absorbancy taken at 277 nm with a blank assay containing a specific inhibitor of cathepsin D: isovalerilpepstatin A or pepstatin A.

Preparation of the enzymatic extract:

Homogenization of 5,7143 grams of pieces of muscle in 20 ml of formiate buffer 100 mmol/l at pH 4.50, with an Ultra-turrax T25 at 15.000 rpm for 20 seconds. Centrigugation at 20.000 g (RCF) for 20 minutes. Filtration with Whatman paper nº 1. Supernatant acidified with a solution of formic acid 1 mol/l at pH 3.00 and a new centrifugation at 20.000 g/20 minutes.

Technical procedure:

1.25% (w/v) hemoglobin (Sigma Chemical Co., St. Louis, U.S.A.) in formiate buffer 250 mmol/l, pH 3.00. Incubation during 1 hour at 40.0°C followed by the adition of a solution of trichloroacetic acid 10% (w/v). Each assay, in the presence and in the absence of isovalerilpepstatin, was performed in quadruplicate (4 assays of the blank and 4 assays of the sample).

Validation of the method:

Control assays have been carried out, incubated only with substrate or only with the enzymatic extract, but in the same experimental conditions of the efective determination of proteolytic activity, elucidating the existence of autolysis from the enzymatic extract, of intrinsic proteolysis of the substrate and of possible interferences of the inhibitor employed. The efective value of cathepsin D activity was calculated by the difference between the assays performed in the absence and in the presence of the specific inhibitor. The inter-assay precision was evaluated by the coefficient of variation of the quadruplicate assays, the results yielding values below 10%. The specific activity (A. Esp. abs/h/gp) is the quotient between A. Ens (abs/h) [the A. Ens (abs/h) equals the absorbancy of the assay at 277 nm per hour of incubation] and C. Prot. (g/l) [C. Prot. (g/l) is the concentration of protein in the enzymatic extract].

Determination of the protein contents in the enzymatic extract:

Spectrophometric analysis with BCA (bichoninic acid) at 562 nm - BCA protein assay reagent (Pierce Chemical Co., Rockford, U.S.A.).

RESULTS DISCUSSION

There is a general trend for the activity of cathepsin D to diminish from 2 months until 6-8 months for it to rise afterwards until 15 months.

Biceps femoris Muscle Cathepsin D Specific Activity (A. Esp.)

Techno logical process	2 Months Ham nº	2 Months A. Esp	5 Months Ham nº	5 Months A. Esp	6 Months Ham nº	6 Months A. Esp	8/9 Months Ham nº	8/9 Months A. Esp	9 Months Ham nº	9 Months A. Esp	13 Months Ham nº	13 Months A. Esp	14 Months Ham nº	14 Months A. Esp	15 Months Ham nº	15 Months A. Esp
A	51	26.65	59	10.86	67	15.95	75	10.96	93	6.86	101	10.76	109	16.43	117	15.45
A1	52	21.48	60	11.26	68	13.54	76	13.62	94	11.22	102	13.31	110	25.00	118	10.32
B	53	19.05	61	10.42	69	10.39	77	8.55	95	12.02	103	11.87	111	11.01	119	7.84
B1	54	26.05	62	17.39	70	13.44	78	8.77	96	14.15	104	8.06	112	36.06	120	35.01
C	55	16.84	63	15.84	71	19.73	79	6.29	97	10.36	105	10.33	113	10.05	121	17.89
C1	56	14.29	64	8.84	72	9.92	80	11.25	98	6.86	106	19.67	114	8.50	122	17.82
D	57	19.88	65	24.35	73	14.15	81	29.82	99	5.89	107	16.05	115	13.50	123	6.33
D1	58	16.07	66	21.72	74	33.53	82	18.88	100	14.87	108	10.79	116	13.08	124	23.81

A. Esp = ratio A. Ens (Abs/h) and C. Prot (g/l)

Semimembranosus Muscle Cathepsin D Specific Activity (A. Esp.)

Techno logical process	2 Months Ham nº	2 Months A. Esp	5 Months Ham nº	5 Months A. Esp	6 Months Ham nº	6 Months A. Esp	8/9 Months Ham nº	8/9 Months A. Esp	9 Months Ham nº	9 Months A. Esp	13 Months Ham nº	13 Months A. Esp	14 Months Ham nº	14 Months A. Esp	15 Months Ham nº	15 Months A. Esp
A	51	26.69	59	12.92	67	19.28	75	7.32	93	8.27	101	17.85	109	29.09	117	38.70
A1	52	20.57	60	14.56	68	14.35	76	5.18	94	10.35	102	14.71	110	21.52	118	40.14
B	53	25.60	61	12.71	69	4.73	77	10.88	95	15.34	103	12.87	111	25.12	119	12.62
B1	54	23.11	62	13.04	70	9.08	78	6.50	96	12.94	104	17.72	112	25.21	120	10.70
C	55	21.65	63	12.81	71	3.78	79	6.30	97	8.89	105	14.18	113	10.04	121	16.52
C1	56	15.58	64	6.70	72	5.93	80	11.95	98	5.84	106	25.36	114	13.91	122	11.50
D	57	21.12	65	27.77	73	6.36	81	10.34	99	5.30	107	21.36	115	13.82	123	14.18
D1	58	10.63	66	17.21	74	9.67	82	9.30	100	12.50	108	11.78	116	24.75	124	26.10

A. Esp= ratio A. Ens (Abs/h) and C. Prot (g/l)

CONCLUSIONS

The evolution of the activity from cathepsin D in the muscles *biceps femoris* (BF) and *semimembranosus* (SM) tends to diminish in the hams until 6-8 months of preparation, for afterwards it tends to rise that activity, whichever it was the fabrication technological process assayed.

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