EXAMINATION OF CATHEPSINS B, L AND S ACTIVITIES IN CARSO DRY-CURED HAM, DRIED IN NATURAL AND CONTROLLED ATMOSPHERES

Key words: ham, carso dry-cured ham, enzimic activity, cathepsins

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ABSTRACT

Levels of cathepsins B, L and S were determined in the *Biceps femoris* (BF) and the *Semimembranosus* (SM) muscles of carso dry-cured hams manufactured according to two different drying processes. The influence of manufacturing process on cathepsins B, L and S activities in carso dry-cured ham was found. Hams dried in a controlled atmosphere at a constant air temperature and relative humidity exhibit lower activities of cathepsins B, L and S. The results also show the influence of the water activity on the cathepsins B, L and S activities, particularly in SM muscle. Cathepsin S activity has been measured at all stages of manufacturing. It was found to be higher in a warmer period of drying and in BF muscle than in SM.

BACKGROUND AND OBJECTIVES

Dry-cured ham is a traditional meat product of great commercial value and there is great interest in the different technological and biochemical parameters involved in its manufacture. Several studies have shown intense proteolysis during curing (Sárraga et al., 1993, Parreno et al., 1994). It has been suggested that cathepsins B, D, H and L could be involved in this proteolysis (Toldra & Etherington, 1988). In the present study we have examined the activities of cathepsins B, L and primarily cathepsin S in carso dry-cured ham, produced by two different processes of drying.

MATERIAL AND METHODS

Hams. Twenty-four hams of normal quality (pH_u 5.6 - 5.8) were dressed for carso dry-cured ham production. The process of salting at 0-2°C (20 days), pressuring at 4-5°C (4 days), washing and presalting respectively extended salting at 4-8°C (70 days) was the same for all hams. The two groups of hams were produced by different processes of drying/ripening, which each lasted 9 months. Group I was dried in a curing chamber with an uncontrolled atmosphere, with air temperatures in spring at 10-15°C, in summer 17-24°C and in autumn 14-16°C. Group II was dried in a curing chamber with a controlled atmosphere of air temperature at 14-15°C and relative humidity 70-85%. In both groups, samples of muscles *Biceps femoris* (BF) and *Semimembranosus* (SM) were taken from three fresh hams (t_0), three hams after the process of presalting (t_1), three hams after 208 days (t_2) and three hams after 379 days (t_3) of the production.

Preparation of muscle extracts. Lysosomal enzymes were extracted according to the method of Etherington et al.(1990). Briefly, a portion of ground muscle was homogenised in four parts (w/v) of ice-cold 50 mM sodium acetate buffer, pH 5.0, containing 1 mM EDTA and 2 ml litre^{-1} Triton X 100. The extract was stirred for 1 h at 4°C and then centrifuged (10000 x g). The supernatant was filtered to remove the debris and used as the source of cathepsins.

Assay of enzyme activities. Cathepsin B, L and S were assayed fluorimetrically using fluorescent peptides as substrates. Cathepsin B and L were assayed with the common substrate Z-Phe-Arg-NMec (Sigma) at pH 5.5. Cathepsin B was assayed with Z-Arg-Arg-NMec (Sigma) at pH 6.0 (Barrett & Kirschke, 1981). Cathepsin S was assayed with Z-Phe-Val-Arg-NMec (Sigma) at pH 7,5 (Stoka, 1993).
The protein concentration was determined by the Bio-Rad method described by the manufacturer (Bio-Rad Protein Assay, 1977).
One unit of enzymatic activity was defined as that amount of enzyme that hydrolyses 1 nmol of substrate per minute at 37°C. Specific activities were given in enzyme units per mg of protein.
Water activity (a_w) was determined with a CX-1 apparatus, which measures the humidity above the sample.
Statistical analysis. Results were statistically analysed with SPSS PC+ program. Significance level of effect is described as ***, **, * for $P<0.001$, $P<0.01$ and $P<0.05$.

RESULTS (Table 1)

The specific activity of cathepsins B, B+L and S in both groups (I in II) and during the whole process of production is higher in BF muscles than in SM muscles.

Cathepsin B activity. The specific activity of cathepsin B in BF muscle of hams of group I is higher than in hams of group II, during the whole process of drying and significantly higher at the end of the production (t_3). The same increase in activity is exhibited also in SM muscle after 208 days (t_2),whereas at the end of the production (t_3) the activity is lower in hams of the group I. Hams of both groups in both muscles exhibit the highest specific activity after 208 days (t_2). The activity is significantly higher in hams of the group I.

Cathepsins B+L activity. Both muscles of hams of the group II show significantly the highest specific activity already after the presalting stage (t_1), whereas hams of group I in both muscles exhibit the highest activity only after 208 days (t_2) of the production. As found with cathepsin B, the activity of cathepsins B+L in the BF muscle of hams of group I is higher than the activity of hams of group II during the whole process of drying, and significantly higher at the end of production (t_3).

Cathepsin S activity. Hams from both groups and both muscles show significantly the highest specific activity after 208 days (t_2) of production. As for cathepsins B and B+L, the activity of cathepsin S in the BF muscle of hams of group I is higher than the activity of the hams of group II during the whole process of drying, whereas the activity in the SM muscle of hams of group I is lower at the end of production (t_3).

a_w. Water activity significantly decreases at all stages of production. In both muscles of hams of group I a_w is higher after 208 days (t_2) and lower at the end of the production (t_3), compared with hams of group II. In SM muscle that difference is also significant. During the whole process of production in both groups of hams, a_w is higher in BF muscle than in SM muscle.

CONCLUSIONS

The mode of drying in the production of carso dry-cured ham has a strong influence on the activity of cathepsins B, L and S. Hams dried/ripened in a controlled atmosphere with a lower air temperature and constant relative humidity (group II) exhibit lower activities of cathepsins B, L and S in BF muscle, than hams dried/ripened in a natural atmosphere (group I). The water activity (a_w) influences the activity of cathepsins B, L and S most of all in the SM muscle which is on the opened side of the carso dry-cured ham. At all periods of the production of carso dry-cured ham a higher activity of cathepsin S was found in warmer periods and higher in BF muscle than in SM. We suggest that the high temperature in summer may be important for increasing cathepsin S activity in carso dry-cured ham.

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Table1: Specific activities of cathepsins B, B+L and S in carso dry-cured ham of two groups and two muscles

			BF		SM		
Parameters	*Groups of hams*	*Period of sampling*	$\bar{x}$	SD	$\bar{x}$	SD	*t-values*
cathepsin B	group I	t_0	0,0035	0,000	0,0032	0,000	1,52
		t_1	0,0058	0,001	0,0038	0,001	3,54*
		t_2	0,0080	0,002	0,0050	0,000	2,64
		t_3	0,0067	0,001	0,0034	0,001	5,39**
	group II	t_0	0,0035	0,000	0,0032	0,000	1,52
		t_1	0,0058	0,001	0,0038	0,001	3,54*
		t_2	0,0075	0,002	0,0040	0,000	2,55
		t_3	0,0052	0,001	0,0037	0,001	1,83
cathepsin B+L	group I	t_0	0,0258	0,003	0,0251	0,004	0,24
		t_1	0,0492	0,005	0,0328	0,001	5,09**
		t_2	0,0537	0,020	0,0341	0,003	1,69
		t_3	0,0372	0,001	0,0214	0,002	13,6***
	group II	t_0	0,0258	0,003	0,0251	0,004	0,24
		t_1	0,0492	0,005	0,0328	0,001	5,09**
		t_2	0,0478	0,004	0,0239	0,003	8,35***
		t_3	0,0292	0,004	0,0219	0,004	2,44
cathepsin S	group I	t_0	0,0075	0,007	0,0072	0,005	0,08
		t_1	0,0273	0,005	0,0173	0,000	3,67
		t_2	0,0561	0,008	0,0382	0,014	1,94
		t_3	0,0234	0,002	0,0168	0,001	4,79**
	group II	t_0	0,0075	0,007	0,0072	0,005	0,008
		t_1	0,0273	0,005	0,0173	0,000	3,67
		t_2	0,0492	0,009	0,0293	0,008	2,84*
		t_3	0,0193	0,002	0,0176	0,002	1,16

Mean ($\bar{x}$) and SD data refer to three separate hams.