EFFECT OF THE TECHNOLOGICAL PROCESSING ON RESIDUAL ENZYMATIC ACTIVITY OF CATHEPSINS IN TYPICAL ITALIAN HAMS

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Background

The fast pace of modern-day life has reduced meal times, making it simple cooking and easily prepared dishes increasingly necessary. The food that most satisfies present consumer needs, because it is easy to use and nutritional, is dry-cured ham. The growing demand for this product and competition with foreign boned dry-cured hams, has led Italian producers to provide boned dry-cured hams, in addition to traditional dry-cured hams. Typical Italian end ripened hams (12 or 16 months) are hand or machine boned, the cut running along the bone for an easier extraction, and then the ham is mended and vacuum-packed with plastic or aluminum film. These further phases of preparing and packaging could modify, even if minimally, ultimate product features.

Objectives

The present work aims to investigate the possible effects of these additional technological phases on some chemical-physical parameters like proteolysis, ashes and texture, which are important during ripening of typical Italian hams.

Methods

Five samples were taken from each product (pig skeletal muscle, Parma ham, Parma boned ham, San Daniele ham), and the origin from different farms was verified to guarantee the reliability of results.

The first phase of preparation, common to all samples, was the separation of the adipose and connective tissue from the muscular tissue by a lance. After this the moisture and ash contents were determined as an indirect measure of the salt content.

Enzymatic activity measurements were carried out according to [11]. B, B+L and H cathepsin activities were carried out using derivatives of the 7-amino-4-methil coumarin as substrate, because they show fluorescence at used wavelengths. The activity was expressed as μmoli of AMC min^{-1} g ham^{-1}.

Fresh meat and end ripened ham water extracts were filtered through a cut-off 3000 Da membrane and then carried out by FSCE at acid pH, to verify the proteolysis index according to [15].

Finally, samples 13 mm thick, 40 mm wide and parallel to the fibers were cut from the Biceps femoris, Semitendinosus and Semimembranosus muscles to evaluate the Young's modulus of elasticity (E), and particularly the strain, using an Instron Universal Testing Machine. Strain [1] is the change in size or shape of a body in response to the applied force. It is a non-dimensional parameter delineated as a ratio or percentage, and is expressed as the change in relation to the original size or shape ($\Delta L/L$ where L is unstressed length and ΔL is change in length caused by the application of force F).

Results and Discussion

Proteolysis is the most important biochemical process during ham ripening, nevertheless an advanced proteolysis produces the greatest defects in this product. The local moisture and salt content affects the enzymatic activity greatly during ripening; processing, imposed by different geographical area traditions and by scientific knowledge, which has improved the organoleptic and nutritional qualities of this product, affects the balance between proteolysis, moisture and salt content.

The Parma and San Daniele dry-cured hams excel among Italian hams, and HAM PRODUCER UNIONS guarantee their quality by extending controls from fresh meat to end product. Controls are extended to the every production phases because product features depend essentially on two factors: a genetic-environmental factor, concerning meat features and muscular enzymatic endogenous complement, and a technological factor, concerning the moisture and salt content. Table 1. reports the average values of moisture and ash content. The Parma boned ham was found to have a significantly higher moisture content than Parma ham ($P<0.001$) and San Daniele ham ($P<0.01$) and a significantly lower ash content than Parma ham ($P<0.001$) and San Daniele ham ($P<0.01$); instead Parma and San Daniele hams show significant values of moisture content ($P<0.001$) and of ash content ($P<0.01$); those values suggest a greater remaining enzymatic activity of cathepsin in Parma boned ham.

The remaining enzymatic activity of cathepsin in hams was carried out using capillary electrophoresis of less than 3000 Da molecular weight components. Table 2. reports the average values of peak areas. Electropherograms of fresh meat and of three different hams show an unidentified peak, labeled Peak 1, which results lower in hams than in fresh meat. In this case, considering that its components must have a smaller or equal molecular weight than 3000 Da, we can attribute its composition to a peptides mixture which generates free amino acids in time, by endogenous exopeptidase. Indeed, as Peak 1 decreases, amino acid peaks, labeled Tryptophan, Phenylalanine and Tyrosine (Peaks 2,3 and 4 respectively), according to [15], increase. Particularly, peak areas of three aromatic amino acids are very similar in fresh meat, while they change unevenly in hams.

The Protheolysis Index (PI), which is the ratio between average values of aromatic amino acid areas and Peak 1 area, is higher in boned ham than in other hams. Actually Parma and Parma boned hams were found to have a higher aromatic amino acid content, while Peak 1 is lower; this results higher in fresh ham, while there is a smaller amino acid content. The aromatic amino acid peak area change is a result of B, B+L and H cathepsin action, which being protease cysteines [2], are similar to papain or chymopapain, and so they show the principal attachment sites close to aromatic amino acids [3, 4]. It is a known fact that in end ripened hams there is a residual protheolitic activity caused by B, B+L and H cathepsins until 15 months, while D cathepsin activity is almost inexistent after 10 months [5, 6]. Bearing in mind these results and different moisture and ash contents, the residual enzymatic activity of B, B+L and H cathepsin was carried out Table 3. shows these results. Parma boned ham, which shows a greater Tryptophan content, was also found to have a significantly higher B and B+L cathepsin activity, and this supports the greater protheolysis index too. Further validations arise from moisture and salt content which by influencing enzymatic activity, influences the release of free amino acids [5, 6, 7], and from almost identical Tryptophan areas in Parma and San Daniele hams, while cathepsin activities are significantly similar. We can also assign the Tyrosine peak differences to specific D cathepsin action until 10 months. Even though we observe a smaller residual activity of H cathepsins in hams, almost 10 times less than B and B+L cathepsins, differences between this cathepsin activity in three different hams result most significant. Besides we can note that differences between Parma and Parma boned ham are always significant, and that B cathepsin residual activity in Parma boned ham is significantly greater than in Parma and San Daniele hams.

Finally the strain of these hams was investigated, and this showed the lesser compactness of Parma boned ham compared with Parma and San Daniele hams. This result agrees with previous results, supporting chemical differences.

Conclusions

These preliminary results indicated that Parma boned ham manufacturing may favour the resumption of enzymatic activity of cathepsins in the same way as the massage phase during the production process. We cannot observe a higher level of H cathepsin residual activity, thus supposing a different action of this enzyme during protheolysis compared with B and B+L cathepsins. Finally we point out that, even if H cathepsin activity is the smallest, H cathepsin residual activity differences between the three hams are the most significant. Further studies are necessary to describe the observed differences.

Pertinent literature

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Table 1. *Comparison between average values (st. dev.) of moisture content, ash content and B, B+L and H cathepsin residual activities ($\mu mol\ min^{-1}\ g^{-1}$ ham) in Parma, Parma boned and San Daniele end ripened hams.*

	Moisture (%)	Ashes (%)		B	B+L	H
Parma	52.67 ± 1.97^{A}	6.50 ± 0.43^{Aa}		0.298±0.022	0.346±0.015	0.0230±0.0011
San Daniele	54.62 ± 0.53^{aB}	7.25 ± 0.32^{Ab}		0.324±0.006	0.362±0.008	0.0364±0.0015
Parma boned	56.80 ± 1.78^{bB}	5.05 ± 0.37^{B}		0.336±0.006	0.380±0.021	0.0290±0.0016
P – SD	***	***		n.s.	n.s.	***
P – Pb	***	***		**	*	***
SD – Pb	**	***		*	n.s.	***

* *significant differences (P<0.05)*
** *significant differences (P<0.01)*
*** *significant differences (P<0.001)*
n.s. = not significant

Table 2. *Average values of electropherogram peak areas (evaluated as product of height and base at the middle of the height). Peaks 2, 3 and 4 correspond to amino acids Trp, Phe and Tyr respectively.*

	Peak areas (cm^2)				PI
	1	2 (Trp)	3 (Phe)	4 (Tyr)	AA/1
Fresh meat	0.98	0.40	0.36	0.39	1.7
Parma	0.28	1.04	1.44	2.52	17.9
San Daniele	0.47	1.02	1.04	1.56	7.7
Parma boned	0.26	1.75	1.51	2.03	20.3