RELATIONSHIP BETWEEN PROTEOLYSIS OF RAW PORK HOMOGENATES AND DRY CURED HAMS

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BACKGROUND

Protein breakdown in dry cured ham is the result of muscle proteinase activity. The role of endogenous enzymes in dry cured ham proteolysis has been a source of controversy because proteolytic enzymes have appeared to either improve flavor and texture or couse the development of white film on the cut surface of vacuum-packed sliced ham, the onset of bitter or metallic aftertastes, loss in firmness, in particular for dry cured hams with a low salt content (Arnau et al. 1994, Parolari et al. 1994, Virgili et al. 1995, Virgili 1996).

OBJECTIVES

The aim of this study is to clarify the role of proteolytic enzymes in releasing soluble nitrogen fraction in 5% trichloroacetic acid following raw meat homogenate incubation and at the end of the ageing period of the corresponding dry cured hams. Correlations between the degree of proteolysis of raw and cured thighs will be investigated in order to establish wether the production of soluble nitrogen fraction following raw homogenate incubation could be an early marker of proteolysis of dry cured ham.

METHODS

Samples: Sixteen green hams to be processed into Parma hams as reported by Parolari (1996) were analysed prior to salting and underwent the curing process.

Raw hams: The *biceps femoris* muscle was analysed for protein determination following the AOAC procedures and for proteolytic enzyme activity by fluorimetric assays (Perkin Elmer LS30 fluorimeter). Cathepsin B was assayed at pH 6.0 with N-CBZ-Arg-Arg-AMC, as described by Barrett (1980). Muscle dipeptidylpeptidase activity (DPP) was assayed as described by Blanchard et al. (1993) with the substrates H-Gly-Arg-AMC, H-Lys-Ala-AMC, H-Gly-Pro-AMC. Owing to the higher specificity of these substrates for dipeptidylpeptidase I, II, and IV respectively, the resulting activities are reported as DPPI, DPPII and DPPIV. Muscle aminopeptidase activity was assayed following the methods of Toldrà et al. (1992) with the substrates L-Ala-AMC and L-Arg-AMC. Because of the higher specificity of these substrates for alanyl aminopeptidase and arginyl aminopeptidase, the resultant activities are reported as AAP and RAP respectively. A linear relationship between proteolytic activity and both incubation time and protein concentration was tested foe each assay.

A portion of minced meat was incubated according to the method of Yamashita et al. (1991) modified as follows: 10 g of *biceps femoris* muscle were homogenised with 20 ml of cold water with Polytron. The homogenate was diluted with 60 ml of Mc Ilvaine's buffer (pH =5.8) and incubated for 5 h at 37°C to enhance protein hydrolysis. The proteolytic process was stopped by adding 40 ml of cold 5% (w/v) trichloroacetic acid (TCA). The mixture was stored at 4°C for 18 h to precipitate protein, and filtered. The TCA-soluble nitrogen was assayed with the method of Alson (1938) and considered as a proteolysis index of raw homogenate. A further portion of TCA-soluble nitrogen was analysed for free amino acids (FAA) using an HP 1050 modular HPLC (Virgili 1994).

Dry-cured hams: After 13 months the aged hams were sectioned perpendicular to the bone, at the knee level and the *biceps femoris* muscle was analysed for protein following the AOAC procedures, for FAA in the TCA-soluble fraction as described by Virgili (1994) and for the proteolysis index as previously reported (Careri 1993).

Statistical analyses: Data were analysed statistically with the procedures CORR, ONE-WAY ANOVA and FACTOR of the SPSS-PC package (Norusis 1986).

RESULTS AND DISCUSSION

Table 1 summarises the analytical data of 16 green and dry cured hams. The sum of FAA and the proteolysis index of the dry cured hams were found to be within the normal range of Parma dry cured ham (Virgili 1996). Most proteolytic enzymes are affected by wide variability as shown by the C.V. %, generally exceeding 10%.

Table1: Mean, coefficient of variation (C.V.%), Min., Max. values for variables of 16 green and dry cured hams (*biceps femoris* muscle)

Variable	*Mean*	*C.V.%*	*Min.*	*Max.*
Cathepsin B[a]	6.90	18.7	4.92	9.15
DPP I[a]	1.33	35.3	0.68	2.14
DPP II[a]	0.20	10.0	0.16	0.24
DPP IV[a]	0.47	17.0	0.27	0.56
ALA[b]	0.58	12.1	0.44	0.71
RAP[b]	0.84	32.1	0.58	1.61
FAA sum (raw)[c]	9.17	14.6	6.50	11.4
Proteolysis index (raw)[d]	41.6	12.5	32.7	48.9
FAA sum (cured)[c]	177.5	9.5	146.9	206.2
Proteolysis index (cured)[e]	28.91	6.9	25.67	33.27

a: nmol subst. hydrolysed · min^{-1} · g $proteine^{-1}$

b: µmol subst. hydrolysed · min^{-1} · g $proteine^{-1}$

c: mg per g protein

d: µg of tyrosine · min^{-1} · g $meat^{-1}$

e: g of soluble nitrogen in 10%TCA · 100 g total nitrogen $^{-1}$

Table 2: Variable loadings on the two principal components.

Variable	*PC1*	*PC2*
Cathepsin B	-0.13	0.74
DPP I	-0.15	-0.75
DPP II	0.79	0.01
DPP IV	0.79	0.01
ALA	0.89	-0.03
RAP	0.79	0.04

Significant correlations were found between the FAA sums and the proteolysis indices of green and aged ham (r= 0.55, $p<0.05$ and r=0.60, $p<0.05$ respectively) indicating that the TCA-soluble fraction generated by means of incubation of raw homogenates could be regarded as predictive of meat proneness to proteolysis during ageing.

Principal Component Analysis (PCA) was used in order to simultaneously evaluate the role of the assayed proteolytic activities of green hams. The signs of the loadings (table 2) display the correlation among the variables: cathepsin B and DPP I were opposed to DPP II, DPP IV, ALA and RAP activities along the PC1 (45.2 % of the original variance) whereas cathepsin B is negatively related to DPP I along PC2 (18.4% of the original variance). This variable distribution is in agreement with the results found by Toldrà et. al. (1996), confirming that samples high in cathepsin B are generally low in peptidases.

The corresponding score plot of samples is shown in Figure 1 and each sample is labelled with the sum of FAA given by raw and cured ham. This way of sample labelling shows the role of different proteolytic activities toward the production of FAA. Meat high in proteolysis. This way of sample labelling shows the role of different proteolytic activities toward the production of FAA. Meat high in cathepsin B activity and low in esopeptidase activity (see variable loadings in table 2) are in the upper area of the plane and were

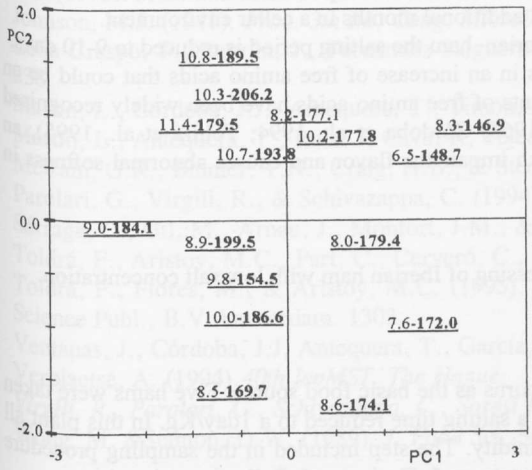

Fig 1: Plot for the samples in the PC1 and PC2 plane. Each sample is labelled with the sum of FAA of raw pork homogenates (normal) and dry cured hams (bold).

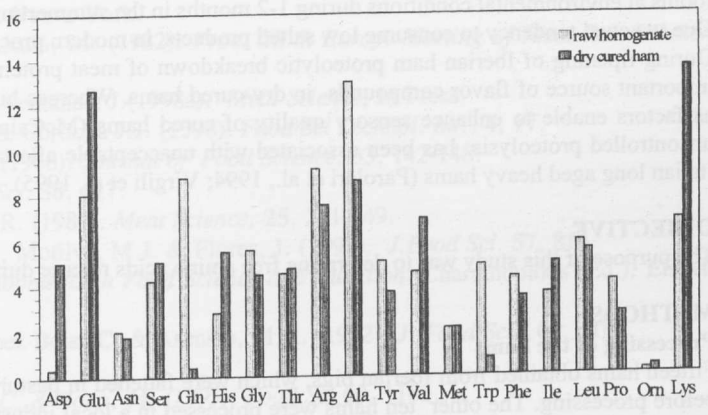

Fig.2: One-way variance analysis of FAA pattern (as percent of total amount) of raw pork homogenate and dry cured ham.

characterised by a higher sum of FAA (both in raw and cured ham) whereas the samples with low cathepsin B and high esopeptidase activity, were placed on the right and characterised by a lower FAA content. The samples with high DPP I activity were placed at the bottom and characterised by an intermediate proteolysis.

This is in agreement with the studies finding that the initial cathepsin B activity is an important indicator for predicting the aged ham proteolysis index (Sarraga et al. 1993,Parolari et al. 1994, Virgili et al. 1995).

The percentage composition of single FAA in green and cured ham was calculated; a comparison between the FAA pattern by means of one-way variance analysis is reported in figure 2. Significant differences ($p<0.05$) were found for most amino acids except metionine and leucine. For aspartic acid, glutammic acid, glutamine, histidine, triptophane, ornitine and lysine the differences were extensive.

CONCLUSIONS

Nitrogen fractions soluble in 5% TCA produced by raw pork homogenates (*biceps femoris* muscle) were found to be significantly ($p<0.05$) correlated with the proteolysis index of the corresponding dry-cured hams. The soluble nitrogen fractions of both raw and cured samples increase strongly in the presence of high cathepsin B activity while a negative relation was obtained with dipeptidyl- and aminoacyl amino peptidase activity of raw muscle. The sums of FAA of raw meat homogenate and that of dry cured ham are significantly correlated, while differences were found in the two patterns of FAA peculiar to product tastes. The existence of a positive and significant correlation between proteolysis of raw meat homogenate and proteolysis of the dry cured ham allows us to use the autolysis of raw meat as a simple prediction method of protein breakdown of the dry cured hams.

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