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Analysis of raw meat to predict proteolysis in Parma ham

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Abstract

Four hundred and thirty-seven pigs (223 purebred Italian Large White, 97 Italian Landrace, and 117 Duroc), were studied to examine the effect of breed on meat quality and assess the possibility of relating proteolysis of dry-cured hams to raw meat quality. The Duroc pigs had intramuscular fat contents and water holding capacities (*M. Semimembranosus*) significantly higher than those of the Large White and Landrace. The latter had a significantly higher pH_{24h} and cathepsin B activities significantly lower than the Duroc breed. The dry-cured hams (*M. Biceps femoris*) from the three breeds were significantly different in proximate composition, proteolysis and weight loss at the end of ageing. Data for green hams (including salt content) were used to compute a model to fit the proteolysis of the corresponding dry-cured hams. The variables included in the model ($R^2 = 0.53$ and $P < 0.01$) were cathepsin B activity of raw ham, pH_{24h}, weight loss after the first salting step, and the salt content of the dry-cured ham. The raw hams with the highest cathepsin B activities, the lowest pH_{24h}, and the highest weight loss after the first salting were those in which greatest proteolysis occured. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Pork; Breed; Dry-cured ham; Proteolysis index

1. Introduction

One of the main goals for the selection of the Italian heavy pig is to find parameters in the raw meat able to predict the quality of dry-cured products made from different muscles. To meet these requirements, the current selection scheme includes the use of indices such as weight loss after the first salting step and the content of visible intramuscular fat (only for the Duroc breed; ANAS, 1995).

The aim is to minimise water loss in such a long aged product as typical Italian dry-cured ham and to estimate the intramuscular fat content (Ruiz-Carrascal, Ventanas, Cava, Andres, & Garcia, 2000), due to its positive effect on the sensory properties of the fresh meat (Fernandez, Monin, Talmant, Mourot, Lebret, 1999a, 1999b). Other parameters of the raw meat, such as pH (Arnau, Gou, & Guerrero, 1994; Arnau, Guerrero, & Sarraga, 1998; Buscailhon, Berdaguè, Gandemer, Touraille, & Monin, 1994; O'Halloran, Ferguson, Egan, & Hwang, 1999; Warner, Kauffman, & Greaser, 1997), endogenous proteolytic activity (Rosell & Toldrà, 1998; Sarraga, Gil, & Garcia-Regueiro, 1993; Toldrà, 1998), ham morphology (Guerrero, Gou, Alonso, & Arnau, 1996; Trombetta et al., 1994), and the amount and quality of covering fat (Lo Fiego, Nanni Costa, & Santoro, 1990), can also influence the quality and analytical character of the cured ham.

Some authors have highlighted proteolysis and endogenous proteolytic activity of the ham as a major factor affecting flavour and texture (Arnau et al., 1998; Parolari, Virgili, & Schivazappa 1994; Rosell & Toldrà, 1998; Sandrolini, 2000; Virgili, Schivappa, Parolari, Soresi Bordini, & Degni, 1998) of long aged dry-cured ham, through the release of free amino acids and peptides in appropriate amounts and ratios (Careri, Mangia, Barbieri, Bolzoni, Virgili, & Parolari, 1993; Rosell & Toldrà 1998; Toscani, Virgili, Corbari, & Calzolari, 2000; Virgili, Parolari, Soresi Bordini, Schivazappa, Cornet, & Monin, 1999). These low-weight nitrogenous molecules, together with moisture, salt, and fat, are responsible for the flavour properties of the lean, to

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which no other ingredient or flavouring other than salt is added (D.O.T. Prosciutto di Parma, 1992). Typical Italian dry-cured ham with different ageing times should fall within a given proteolysis range, i.e. a proteolysis value regarded as too high for a 12-month old ham and responsible for bitter flavour and poor texture, may be perfect for a longer aged dry-cured ham. Good quality dry-cured hams depend on taking into account both the parameters controlling salt diffusion and weight loss (preserving factors) and those affecting the final flavour, such as proteolysis.

Thighs from over 400 pigs of the Large White, Landrace and Duroc purebreds, from ANAS (National Association of Pig Breeders) genetic centres, were assayed post-mortem (*M. Semimembranosus*) for a number of parameters to determine differences between the purebreds and to look for correlations between these parameters measured on green hams and proteolysis in the corresponding dry-cured hams.

2. Materials and methods

Two-hundred and twenty-three, 97, and 117 pigs belonging, respectively, to the Italian Large White (LW), the Italian Landrace (L), and the Duroc (D) breeds, coming from ANAS (National Association of Pig Breeders) genetic centres, were slaughtered on a day every two weeks at a commercial abattoir, for 23 days of slaughtering. After 2 h of lairage, pigs were stunned with 80% CO_2. The pigs were fed with a "quasi ad libitum" regime from 100 days of age to a live weight of approximately 155 kg (between 8 and 9 months). Twenty-four hours post mortem, the following parameters were determined on the trimmed green hams (*M. Semimembranosus*):

1. proximate composition: moisture, protein and intramuscular fat content according to the AOAC (1990) procedures;
2. endopeptidase proteolytic activity of cathepsin B using the fluorimetric method of Schivazappa, Virgili, and Parolari (1992). The results are expressed as nmol AMC$\times$min$^{-1}\times$g protein^{-1};
3. water holding capacity (WHC) determined by the paper press method of Grau and Hamm (1957). The results are expressed as the ratio of meat area to total area.
4. pH_{24h} of *M. Semimembranosus* was measured by insertion of a glass electrode (HANNA FC 200).

Each green ham was labelled with a numbered punch and manufactured following the procedures for Parma ham production (Virgili & Parolari, 1991). All the hams were processed at a single plant.

For each breed, a random procedure was applied to select a representative subgroup of dry-cured hams (77 hams from the LW pigs, 28 from the L pigs, and 31 from the D pigs), to be assayed for the following:

1. weight loss after first salting (per cent difference between the weights of the green trimmed ham and the salted ham) recorded after the first week of salting (Russo, Nanni Costa, LoFiego, & De Grossi, 1991);
2. weight loss at the end of ageing (per cent difference between the weights of the green trimmed ham and the cured ham) after 12 months of ageing.

A section of *M. Biceps femoris* was taken from the centre of 12-month old deboned hams, on the cushion side, at knee level. *M. Semimembranosus*, from the outer part of the ham, was discarded, because it was too dehydrated because of the environment in the curing rooms.

The *Biceps femoris* muscle was analysed for the following:

1. proximate composition: moisture, protein, sodium chloride, and intramuscular fat content according to the AOAC (1990) procedures;
2. proteolysis index, expressed as per cent ratio between nitrogen soluble in 5% trichloroacetic acid and total nitrogen (Careri et al., 1993).

2.1. Statistical analysis

Statistical analysis of the data was carried out using the statistical package SPSS/PC ver.10, running the procedures DESCRIPTIVES, CORRELATE, GENERAL LINEAR MODEL (Least Significant Difference method), RANDOM SAMPLE OF CASES, MULTIPLE REGRESSION ANALYSIS (Stepwise method). In the GENERAL LINEAR MODEL procedure, the model included the fixed effects of breed, sex and day of slaughter.

3. Results and discussion

3.1. Fresh hams

Table 1 shows the results of the analyses made on the 437 thighs (*M. Semimembranosus*). Muscle parameters are in the range commonly reported for the Italian heavy pigs (Chizzolini et al., 1991; Nanni Costa, Dall'Olio, De Grossi, Lo Fiego, & Russo, 1995; Schivazappa et al., 1992). Muscle traits were compared to evaluate differences between the breeds (Table 2). Significant differences were found in proximate composition of muscle, pH_{24h}, WHC and for cathepsin B activity. The D pigs had average intramuscular fat contents and water holding capacities significantly higher than those of the LW and L breeds. These parameters may be

linked as the higher intramuscular fat content means a decrease in moisture content and thus in water lost during compression for WHC assay. The D muscles had lower protein contents yeilding higher cathepsin B activity, since the enzyme activity is expressed on a protein basis (the same parameter expressed on muscle weight basis did not yield significant differences between breeds). The L pigs had a significantly higher pH_{24h} than the other breeds. Moreover, for the L breed, the pH_{24h} showed some variability (C.V.% = 6.1%), with 20% having $pH_{24h} \geqslant 6.0$ and 20% having $pH_{24h} \leqslant 5.5$. Other authors found significant differences in cathepsin B activity between breeds (Armero, Barbosa, Toldrà, Baselga, & Pla, 1999; Flores, Romero, Aristoy, Flores, & Toldrà, 1994) but in these studies, activities were expressed on a muscle weight basis. These authors, who studied proteolytic activity in the muscles of pigs of different breeds from different sires, found differences due to the sire but not between the breeds, suggesting a resonably high level of heritability for cathepsin B. This hypothesis is supported by the results of a study on the coefficient of heritability for cathepsin B in pigs of the LW breed, for which the estimated coefficient fell in the range 0.23–0.28 (Russo et al., 2000).

Table 1
Summary of the analytical parameters of the 437 raw hams (*M. Semimembranosus*)

Variable	Mean	S.D.	C.V. (%)
Live weight (kg)	155.6	10.54	6.77
Intramuscular fat (%)	4.04	1.56	38.96
Protein (%)	22.4	0.84	3.75
Moisture (%)	73.6	1.19	1.60
pH_{24h}	5.66	0.25	4.42
WHC[a]	0.43	0.06	13.95
Cathepsin B[b]	7.14	1.90	26.76

[a] Ratio of meat area to total area.
[b] Expressed as nmol AMC×min^{-1}×g protein^{-1}.

3.2. Dry-cured ham

A representative sample of 136 12-month old dry-cured hams (31 D, 28 L, and 77 LW), was analysed (*M. Biceps femoris*) for proximate composition and proteolysis values. The weight loss after the first salting step was recorded for all the hams of the trial. Data for the 136 dry-cured hams are reported in Table 3. Mean values of the parameters are in agreement with previous studies on processing weight losses (Russo et al., 1991) and on Parma ham composition (Careri et al., 1993; D.O.T. Prosciutto di Parma, 1992; Virgili et al., 1999). The correlation between the weight loss after first salting step and weight loss at the end of ageing was positive and significant ($r = 0.36$ with $P \leqslant 0.01$), supporting the results of Russo et al. (1991). Analytical data for the dry-cured hams of the three breeds, were compared to determine if differences found in the green hams were reflected in the final products, and if variability in dry-cured ham quality may be partly attributable to breed. The results are shown in Table 4. Since the 136 dry-cured hams are a sample of the green hams examined (Table 2), data for the corresponding 136 green hams also are reported.

The final weight loss was significantly higher for the LW hams than the L hams, while the difference to the D hams was not significant. This may be partly explained by the relationships found between intramuscular fat content and pH_{24h} with the final weight loss ($r = -0.26$

Table 2
Effect of breed[a] on the raw meat parameters (Least Square Means ± Residual Standard Deviation)

	D ($n = 117$)		L ($n = 97$)		LW ($n = 223$)	
	LSM	Residual S.D.	LSM	Residual S.D.	LSM	Residual S.D.
Live weight (kg)	155.3	10.9	156.2	11.1	155.6	11.0
Intramuscular fat (%)[b,c,d]	5.33 a	1.31	3.01 b	1.28	3.51 b	1.28
Moisture (%)[c,d]	72.7 b	1.17	73.9 a	1.12	74.0 a	1.12
Protein (%)[b,c,d]	21.9 c	0.64	23.2 a	0.63	22.5 b	0.64
pH_{24h}[b,c]	5.65 b	0.24	5.78 a	0.23	5.63 b	0.24
WHC[b,c,d]	0.46 a	0.05	0.44 b	0.05	0.42 c	0.06
Cathepsin B[b,c]	7.34 a	1.75	6.76 b	1.74	7.00 a,b	1.70

[a] Different letters along the same row indicate significant differences ($P < 0.05$).
[b] Significant effect of day of slaughter ($P < 0.05$).
[c] Significant effect of breed ($P < 0.05$).
[d] Significant effect of sex ($P < 0.05$).

with $P \leqslant 0.05$ and $r = -0.23$ with $P \leqslant 0.05$, respectively). The higher intramuscular fat content of the D hams and the higher pH_{24h} of the L hams (Tables 2 and 4) could lead to a lower final weight loss when compared with LW hams (Guerrero et al., 1996).

In accordance with data for the fresh muscles (*M. Semimembranosus*), the D dry-cured hams (*M. Biceps femoris*) had significantly higher intramuscular fat contents. The lower salt content could also be related to the higher marbling of this meat (Arnau, 1991): since the diffusion of sodium chloride in pork is aided by the water between the myofibrillar proteins (Offer & Knight, 1988), the increased inter- and intramuscular fat contents would be unfavourable to the diffusion of salt in the muscles (the correlation coefficient between intramuscular fat and salt contents of the 136 hams was $r = -0.18$ $P < 0.05$). The cured hams of the D breed were significantly lower in moisture: this may be due to the different fat contents rather than differences in dehydration, as suggested by the moisture/protein ratios, a marker for dehydration, only being significantly lower for the L hams.

Table 3
The weight losses and analytical parameters of the 137 Parma dry-cured hams (*M. Biceps femoris*)

Variable	Mean	S.D.	C.V.%
First salting weight loss (%)	1.93	0.59	30.6
Weight loss at end of ageing (%)	28.5	3.00	10.5
Intramuscular fat (%)	6.59	2.70	41.0
Moisture (%)	60.8	2.23	3.67
Protein (%)	25.9	1.25	4.84
Salt (%)	6.76	0.73	10.8
Proteolysis index[a]	29.4	2.40	8.2

[a] Expressed as grams of soluble nitrogen (after 10% trichloroacetic acid addition)/100g total nitrogen in the meat.

The proteolysis indices for the D and LW hams were significantly higher than those of the L pigs. Previous studies on dry-cured hams attempted to establish which parameters influenced the proteolysis of the cured products, and variables such as pH_{24h} (Arnau et al., 1994; Buscailhon et al., 1994; Gigli, Pacchioli & Barchi, 1993), endogenous proteolytic activity (Virgili, Parolari, Schivazappa, Soresi Bordini & Borri, 1995; Virgili et al., 1998), salt, water activity, and temperature (Flores, Aristoy, & Toldrà, 1997; Toldrà, Rico, & Flores, 1992) were found to affect the process and the pattern of formation of low-weight nitrogenous molecules (Toscani et al., 2000).

In this study, the differences in cathepsin B activity between the three breeds were shown to be statistically significant though not importantly so, both in the entire population examined ($n = 437$) and in the sample selected from the dry-cured hams ($n = 137$). Other significant differences were found between the breeds in terms of pH_{24h}, online intramuscular fat, salt content, and proteolysis of the dry-cured hams at the end of ageing.

The L dry-cured hams had lower proteolysis indices which might be due to the higher pH_{24h} and salt contents and lower cathepsin B activity. It has been suggested that a rapid pH drop post mortem and a low ultimate pH (Kas et al., 1983; O'Halloran et al., 1999) may favour the release of these enzymes from the lysosomes and thus their activity. It should also be noted that

Table 4
Effect of breed[a] on raw and dry-cured ham parameters (Least Square Means ± Residual Standard Deviation)

	D $n = 31$		L $n = 28$		LW $n = 77$	
	LSM	residual S.D.	LSM	residual S.D.	LSM	residual S.D.
Raw ham						
Intramuscular fat (%)[2]	6.21[a]	1.68	3.17[b]	1.55	3.68[b]	1.60
pH_{24h}[2]	5.60[b]	0.27	5.83[a]	0.28	5.61[b]	0.27
WHC[1,2]	0.47[a]	0.05	0.46[a]	0.05	0.43[b]	0.05
Cathepsin B	7.29	1.86	6.31	1.95	7.12	2.00
First salting weight loss[1,2]	2.14[a]	0.38	1.81[b]	0.40	2.16[a]	0.40
Dry-cured ham						
Intramuscular fat (%)[2]	9.08[a]	2.63	5.69[b]	2.70	5.64[b]	2.71
Moisture (%)[1,2]	59.7[b]	2.09	62.0[a]	2.14	61.5[a]	2.15
Protein (%)[1,2]	24.7 c	1.14	25.5[b]	1.16	26.1[a]	1.17
Moisture/Protein[1,2]	2.42[a]	0.12	2.44[a]	0.12	2.36[b]	0.13
Salt (%)[1]	6.50[b]	0.62	6.87[a]	0.64	6.73[a,b]	0.64
Proteolysis index[1,2,3]	30.2[a]	2.18	28.5[b]	2.19	30.0[a]	2.20
Cured ham weight (kg)	9.32	0.97	9.24	0.97	9.16	0.99
Weight loss at end of ageing[1,2]	28.0[b]	3.43	27.5[b]	3.42	29.3[a]	3.52

*Different letters along the same row indicate significant differences ($P < 0.05$).
[1] Significant day of slaughter effect ($P < 0.05$).
[2] Significant breed effect ($P < 0.05$).
[3] Significant sex effect ($P < 0.05$).

Table 5
Correlation coefficients between final proteolysis and the analytical parameters of the raw and cured ham (only parameters correlated with $P<0.05$ are reported)

	Proteolysis index
Raw ham	
pH_{24h}	−0.54*
Cathepsin B	0.46*
First salting weight loss	0.42*
Dry-cured ham	
Weight loss at end of ageing	0.36*
Moisture (%)	0.23**
Protein (%)	−0.18
Salt (%)	−0.44*

*$P<0.05$.
**$P<0.01$.

Table 6
Model computed by means of multiple regression analysis (stepwise method) to predict proteolysis index using both raw matter parameters and salt content (included to take into account its inhibitory effect on cathepsin B)

R multiple = 0.73	F = 35.6
R^2 = 0.53	signif. = 0.000

Variables included in the model

	Stdz. coeff.	Signif.
Salt (%)	−0.35	0.000
Cathepsin B	0.31	0.000
pH_{24h}	−0.28	0.000
First salting weight loss	0.21	0.004

these enzymes are acid proteases and a low pH favours their activity, while an increase in salt concentration and a decrease of a_w inhibits them (Toldrà et al., 1992).

The correlation coefficients found between the final proteolysis of dry-cured hams and the parameters assayed on the green and cured hams are reported in Table 5. As expected, significant relationships were found between pH_{24h}, salt content and final proteolysis, in agreement with the lower proteolysis shown by the L dry-cured hams. Moreover, a positive and significant relationship was found between cathepsin B activity of green ham and final proteolysis (Garcia-Garrido, Quiles-Zafra, Tapiador, & Luque de Castro, 2000; Virgili et al., 1995). Intramuscular fat did not show a significant correlation with proteolysis but it played a significant though moderate role in lowering the final salt content. In this respect, intramuscular fat might indirectly favour proteolysis, and it is negatively related to salt content (Table 5).

In order to take into account possible interactions between the parameters of the green hams (marbling, proteolytic activity, pH_{24h}, WHC, first salting weight loss), they were used in a multiple regression analysis to

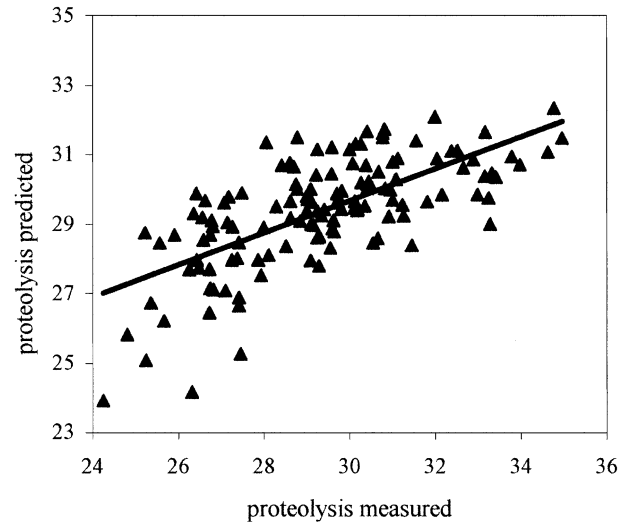

Fig. 1. Relation between the proteolysis measured on the cured hams and the proteolysis predicted by the equation shown in Table 6.

compute a model fitting the proteolysis indices of the dry-cured ham. Since the inhibitory effects of sodium chloride on cathepsin B is well known (Toldrà et al., 1992) and was confirmed by the negative coefficients reported in Table 5, salt content was included in the analysis. Table 6 shows the coefficients of the variables that were significant in the model ($R^2=0.53$); WHC and marbling were discarded as they were of little importance in predicting the final proteolysis of the hams. Fig. 1 shows the relationship between the measured proteolysis indices and those predicted by the equation in Table 6. Among the variables, a similar importance is due to salt, cathepsin B activity and pH_{24h}, followed by the first salting weight loss. The negative sign for salt underlines its inhibitory role.

According to the model, green hams with the highest cathepsin B activity, the lowest pH_{24h}, and the greatest first salting weight loss are more prone to proteolysis, with corresponding impairment of texture, increase in bitter flavour, white surface film formation and tyrosine chalks (Guerrero et al., 1996; Virgili et al., 1995, 1998). Furthermore, a 12-month old Parma ham with a proteolysis index exceeding 31%, is rejected by the tutelary Consortium, and it is regarded as not suitable for the brand. (D.O.T. Prosciutto di Parma, 1992).

4. Conclusions

In this study, the three Italian breeds Large White, Landrace, and Duroc were found to be significantly different in pH_{24h}, WHC, intramuscular fat, cathepsin B activity of the green hams and in proximate composition and proteolysis index of the cured hams. The statistical model computed for predicting final proteolysis showed that the green ham parameters cathepsin B,

pH_{24h}, first salting weight loss, together with salt content, affect the final proteolysis.

Excessive proteolysis of the dry-cured ham and the associated defects (softness and bitter flavour), a phenomenon which is more common in low salt hams, might be avoided by removing from the process green hams with high proteolytic activity, low ultimate pH, and high first salting weight losses. The study should be carried by taking into account those factors, in addition to breed, which, during breeding, and pre- and post-slaughtering handling, may influence the parameters in the raw meat which are important for proteolysis in the dry-cured ham.

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