ELSEVIER

Meat Science 55 (2000) 1–5

MEAT SCIENCE

www.elsevier.com/locate/meatsci

Lipolysis in dry-cured ham maturation

Christian S. Vestergaard, Cristina Schivazappa, Roberta Virgili*

Stazione Sperimentale per l'Industria delle Conserve Alimentari, Viale F. Tanara 31/A, 43100 Parma, Italy

Received 4 February 1999; received in revised form 28 June 1999; accepted 15 July 1999

Abstract

Thirty light Parma hams were tested for muscle lipolytic activity (acid and neutral lipase activity) and free fatty acid (FFA) amounts in *M. semimembranosus* and *biceps femoris*, during progressive phases (0, 3, 6, 10 months) of dry-cured ham manufacturing. No correlation was found between the activities of acid and neutral lipases in fresh *M. semimembranosus*, while during processing the activities were positively related ($p < 0.1$), probably due to effects of muscle composition changes on lipolytic activities. In each processing step tested, acid lipase activities were higher in the *M. semimembranosus* than in the *M. biceps femoris*, and FFA amounts varied accordingly, the only exception being for the very dehydrated 10-month old *M. semimembranosus*, which yielded lower FFA than in the corresponding *M. biceps femoris*. FFAs in the end product correlated positively with acid and neutral lipase activities of green ham, suggesting that FFA production could be influenced by both raw meat properties and muscle composition during processing.

1. Introduction

A common feature of the Mediterranean way of raw ham manufacturing is a dry salting phase at low temperature and a maturing period at higher temperature. A variety of processing practices have been developed throughout centuries using different raw materials, ageing times and manufacturing techniques (Arnau, 1998; Toldrà, Flores, Navarro, Aristoy & Flores, 1997; Virgili, Parolari, Soresi Bordini & Schivazappa, 1997). In the past decades many studies have been carried out to understand the chemical and enzymatic mechanisms taking place during maturing (Buscalihon, Gandemer & Monin, 1994b; Careri, Mangia, Barbieri, Bolzoni, Virgili & Parolari, 1993; Toldrà, Flores & Sans, 1997). These studies have enabled a wide class of chemicals, both volatile and non volatile, to be identified. Among them, the carbonyl compounds from lipid degradation have proved to be relevant to dry-cured ham aroma (Barbieri et al., 1992; Berdagué, Bonnaud, Rousset & Touraille, 1993; Buscalihon, Berdagué & Monin, 1994a; Toldrà, 1998; Ventanas, Cordoba, Antequera, Garcia, Lopez-Bote & Asensio, 1992). Lipid degradation in meat is mainly due to lipolysis of enzymatic origin and to chemical oxidation (Hamilton, 1989). No clear linkage has been demonstrated between lipolysis and the occurrence of oxidation products although some authors have postulated that lipolysis favours oxidation (Nawar, 1996). Based on current studies, muscle lipases and phospholipases appear to be responsible for lipolysis in meat and meat products (Currie & Wolfe, 1977; Flores, Alasnier, Aristoy, Navarro, Gandemer & Toldrà, 1996; Motilva, Toldrà, Nietó & Flores, 1993).

In the pH range from 5.5 to 6.2, most often encountered in dry-cured ham, lipolytic activity on triglycerides is commonly ascribed to lysosomal acid lipase (pH optimum 5.5) (Motilva, Toldrà & Flores, 1992) and to hormone-sensitive lipase (pH optimum 7.0–7.5) (Belfrage, Frederikson, Strälford & Thornqvist, 1984) hydrolysing cholesterol and glycerol esters. The result is, in any case, the formation of di- and monoglycerides, and free fatty acids (FFA).

In this study acid and neutral lipase activities and amounts of FFA were measured in *M. biceps femoris* (BF) and *M. semimembranosus* (SM), i.e. two muscles of similar metabolic type (Flores et al., 1996) exposed to different treatments during ham manufacturing. Our aim was to study lipolytic activity and FAA production as affected by changes in muscle composition throughout processing.

* Corresponding author. Tel. +39-0521-795234; fax: +39-0521-771829.

E-mail address: r.virgili@rsadvnet.if (R. Virgili).

0309-1740/00/$ - see front matter
PII: S0309-1740(99)00095-9

2. Materials and methods

2.1. Sampling

Thirty Italian green hams, with weights ranging from 8 to 10 kg, were submitted to a 10 months ageing process, following the regulations stated for light Parma hams (Gazzetta Ufficiale della Repubblica Italiana, 1970), consisting of the traditional stages of dry-salting for 25 days at 0–2°C, post salting for 15 days at 0–2°C, resting for 60 days at 1–4°C and maturing up to 10th month of processing (Parolari, 1996).

Sampling of green hams was performed 24 h post slaughter, prior to processing. Only the external *M. semimembranosus* (SM) was assayed, because opening of the hams for sampling of the internally situated *M. biceps femoris* (BF), would have made further processing impossible. About 50 g of SM was removed from each leg and samples were immediately analysed for green ham lipolytic enzymes, moisture and protein contents. All hams were identified with a number, dry-salted and matured as previously described.

Sampling of dry-cured hams took place after 3 (end of resting), 6 (end of 1st ageing period) and 10 (end of ageing) months of processing. At each sampling time, 10 dry-cured hams obtained from the original batch of 30 hams, were sectioned perpendicular to the bone at the knee level, and samples were vacuum packed and stored at refrigerate temperature for chemical and enzymatic analyses. BF and SM, trimmed for visible fat and connective tissue, were analysed for proximate composition, water activity, free fatty acids (FFA) and lipolytic enzyme activities.

2.2. Chemical analysis

- Water activity (a_w): Approximately 8 g of roughly cut sample was measured with AQUALAB CX-2 equipment, calibrated with saturated $BaCl_2$ solution with a known a_w of 0.901 (25°C).
- Moisture, salt and protein contents were determined according to AOAC methods (AOAC, 1995). Results were expressed as g per 100 g lean muscle.
- Free fatty acids were analysed according to standards for analysis of lipids, taking into account the possible interference of muscle water soluble acids, (i.e. mainly lactic acid) (NGD, 1976). Results were expressed as percent oleic acid.

2.3. Assay of enzyme activities

2.3.1. Preparation of muscle extracts

5 g (6 g for acid lipases) of minced muscles were homogenised in 50 ml of 0.1 M Tris/HCl buffer, pH 7.0, containing 0.1 M of Na_2EDTA, 0.2% (w/v) Triton X-100, 0.1 μg/ml of pepstatin A and 1 μg/ml of leupeptin-hemisulphate, using a Polytron PT3000 homogenizer (3×15 s, cooling with ice). The homogenates were centrifuged at 5°C and 13 500 *g* for 10 min and then filtered through glass wool. The filtrate was used for enzyme assays.

2.3.2. Assay of lipase activities

Muscle acid and neutral lipase activities were assayed according to current methodologies (Flores et al., 1996) with some modifications, as described below.

2.3.3. Acid lipase

0.1 ml of muscle extract was diluted with 2.8 ml of 0.103 M disodium phosphate/0.049 M citric acid buffer, pH 5.0, containing 0.05% (w/v) Triton X-100 and 0.8 mg/ml bovine serum albumin (BSA). To this mixture 0.1 ml of 1.0 mM 4-methylumbelliferyl-oleate (Sigma) was added as substrate. After incubation at 37°C for 30 min, the reaction was stopped with 0.5 ml of 1 N HCl and the fluorescence was monitored at $\lambda_{ex} = 328$ nm and $\lambda_{em} = 470$ nm.

2.3.4. Neutral lipase

0.1 ml of muscle extract was diluted with 2.8 ml of 0.22 M Tris/HCl buffer, pH 7.5, containing 0.05% (w/v) Triton X-100. To this mixture 0.1 ml of 1.0 mM 4-methylumbelliferyl-oleate (Sigma) was added as substrate. In the slightly alkaline conditions of the neutral lipase assay, BSA caused a very pronounced hydrolysis of the substrate (Jacks & Kircher, 1967; Stead, 1983) and was therefore removed. After incubation at 37°C for 30 min the incubated samples were immediately cooled in ice–water mixture and measured within a minute. The reaction was not stopped with HCl because the BSA removal lowered differences between samples and blanks at low pH. The wavelengths for assaying the released 4-methylumbelliferone, were $\lambda_{ex} = 328$ nm and $\lambda_{em} = 443$ nm in order to measure the maximum of the emission peak at pH 7.5.

Enzymatic activity was expressed as nmol of released 4-methylumbelliferone h^{-1} g $protein^{-1}$. Fluorescence was measured with a Perkin–Elmer LS/30 spectrofluorophotometer

2.3.4.1. Data analysis. One way ANOVA and Pearson correlation coefficients were calculated by the statistical package SPSS-PC (1986).

3. Results

No correlation was found between acid and neutral lipases in the green hams ($r = 0.06$; Fig. 1a), but mid- and fully-matured hams (Fig. 1b–d) exhibited positive

relationships ($p < 0.1$) at all sampling times. The occurrence of a positive relation between acid and neutral lipase activities during processing, could be ascribed to the influence of major changes in muscle composition, mainly a_w decrease resulting from progressive dehydration and salt diffusion (Table 1). This positive correlation could mean that the enzymes could be either activated or inhibited by the same parameters, thus overcoming original activities found in fresh SM (Table 1). Motilva and Toldrà (1993) found lipase activity to be influenced by pH, salt and a_w. It seems therefore that lipid hydrolysis is favoured by the same variables enhancing enzyme activities in vitro, provided the enzyme functionality is not impaired in muscle media (Motilva et al., 1993).

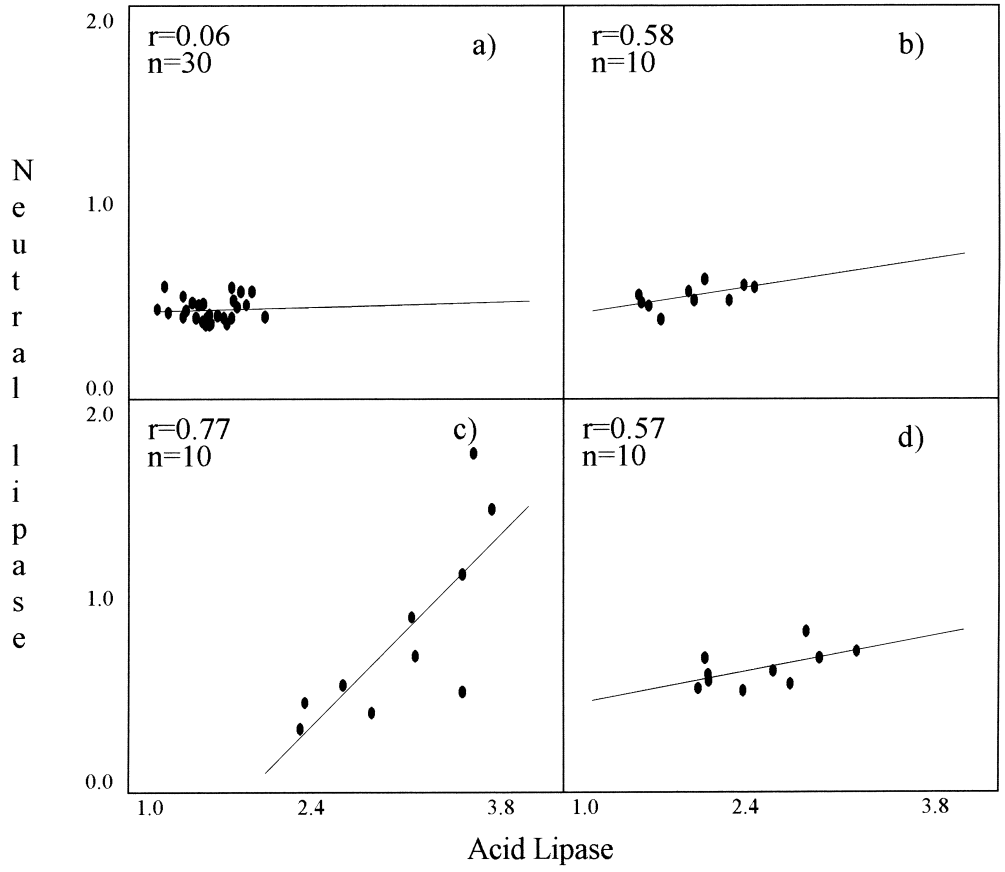

Fig. 1. Relation between SM acid and neutral lipase activities at several steps of manufacturing. The activities were assayed at: (a) before salting (fresh muscle); (b) 3rd month of curing; (c) 6th month of curing; (d) 10th month of curing (end of processing). Activities are expressed as nmol of released 4-methylumbelliferone $\times h^{-1} \times g$ protein^{-1}.

Table 1
Chemical and enzyme activity comparison between BF and SM muscles, at progressive steps of maturing. Means listed in same row, with different letter, mean significant difference ($p < 0.05$) by the test of Duncan between the two muscles for the specified ageing time. Mean values of enzyme activities, protein and moisture in fresh, unsalted SM are reported

	Fresh	Ageing time (months)					
	$n = 30$	3 ($n = 10$)		6 ($n = 10$)		10 ($n = 10$)	
Variables	SM	BF	SM	BF	SM	BF	SM
a_w	–	0.95	0.95	0.94	0.93	0.92	0.91
Moisture	73.5	68.7a	66.2b	66.1a	61.9b	64.2a	56.2b
Protein	21.6	23.0a	26.7b	24.3a	27.8b	25.7a	33.7b
Salt	–	4.48	4.37	4.83a	4.48b	5.47a	4.64b
Acid lipase	1.52	1.29a	1.74b	1.84a	2.86b	1.72a	2.28b
Neutral lipase[a]	0.33	0.79a	0.48b	1.11a	1.40b	0.73a	0.60b
FFA[b]	–	2.70a	4.13b	6.08a	9.07b	12.9a	9.33b

[a] Expressed as nmol of released 4-methylumbelliferone $h^{-1} \times g$ protein^{-1}.
[b] Expressed as percent oleic acid.

In case of muscles of similar metabolic type, like SM and BF (Flores et al., 1996; Hernandez, Navarro & Toldrà, 1998) no significant difference has been found between lipolytic activities in green ham. During Parma ham manufacturing, SM and BF are exposed to very different processing conditions (Virgili, Parolari, Schivazappa, Bordini & Volta, 1995). The outer SM muscle is subjected to salt and dehydration from the beginning of processing, while the internal BF muscle encounters these conditions after several months. Moisture and protein values of both muscles reported in Table 1, show significant differences at each sampling time. To evaluate the influence of changes in muscle composition, lipase activities and total FFAs were measured in both muscles at the same times. The original 30 hams, randomly divided into three groups, were assayed at 3, 6 and 10 months of ageing. The results are reported in Table 1.

During the dry-curing process, a_w values decreased as result of salt diffusion and muscle dehydration and the decrease is more rapid in SM than in BF. The salt content increased with ageing time in both muscles and was lower in the outer SM muscle (Table 1).

Both acid and neutral lipase activities increased throughout manufacturing, showing a maximum at 6 months. This is in agreement with previous findings reporting for Spanish ham an increase of both acid and neutral lipase activity during the first processing months (Motilva et al., 1993).

At all sampling times the activities of acid lipases were significantly higher in SM compared to BF (Table 1). The amount of FFAs in SM and BF muscles parallel the lipolytic activities observed. FFAs increased with ageing time and were higher in SM than in BF up to the 6th month of ageing. The increase in FFAs and acid lipase activity supports the hypothesis of this enzyme being involved in FFA production during ageing. Parameters enhancing acid lipase activity, i.e. a_w reduction and salt increase (Motilva & Toldrà, 1993), would therefore enhance FFA production. SM composition at 6 months of ageing could appear to favour FFA production.

At 10 months of ageing, lipolytic activities decreased in both muscles. The decrease in neutral lipase activity was more pronounced in the highly dehydrated (Table 1) SM muscle. At all sampling times FFAs were significantly different in the two muscles and it is noteworthy that, while FFA values from 6 to 10 months are rather constant for SM, steadily increased in BF. Although no significant difference was found between a_w values of BF and SM at 10 months of ageing, salt increase in the moisture-rich BF muscle seems to be more favourable to FFA generation than dryness of SM muscle.

From Figs. 2a and b, a positive relationship between fresh muscle lipolytic activities and final FFA amount in 10 month-old SM can be observed. Large positive deviation from linearity of sample 4 could ascribed to its high moisture content (58.3% vs an average 55.9% of the other SM samples), supporting that extensive dehydration impairs enzyme functionality hence FFA generation. When this outlying sample was left out, the correlation coefficient increased from 0.54 to 0.70 for acid lipase (Fig. 2a) thereby becoming significant ($p < 0.05$).

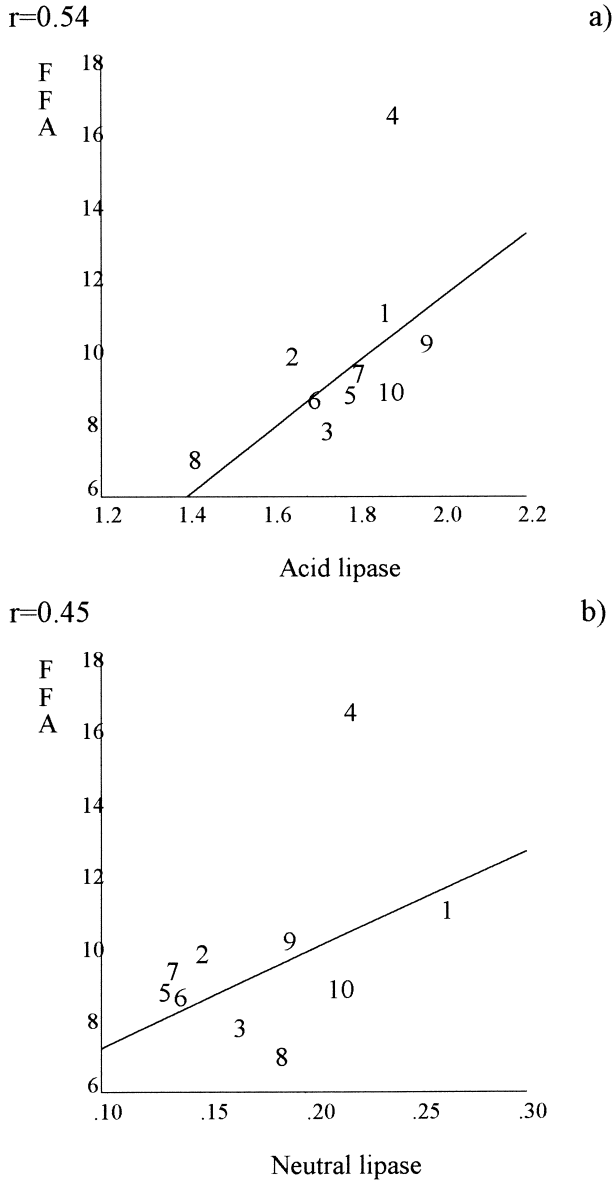

Fig. 2. Dependence between FFA amount (expressed as percent oleic acid) found in 10-month old SM muscles and (a) acid lipase activity in fresh SM, (b) neutral lipase activity in fresh SM. Samples are labelled with progressive numbers.

4. Conclusion

Mild dehydration and salt diffusion proved to be associated with increased activities of both acid and neutral lipases and with extensive production of FFAs

in ham muscles. The stronger dehydration yield in 10-months old SM muscle was paralleled by a drop in recovered lipolityc activities (mainly for neutral lipases) and to a negligible rise of FFAs, compared with their increase in the less dehydrated BF. Because FFAs are known to be associated with flavour in aged meat, there is reason to believe that, in order to enhance dry-cured ham aroma, processing procedures could be improved to optimise dehydration in the late phases of processing.

Acknowledgements

The research was financed by the European Union, TMR research grant no. Fair-CT96-5076.

References

AOAC (Association of Official Analytical Chemists) (1995). *Official methods of analysis* (16th ed.). Washington DC: Association of Official Analytical Chemists.

Arnau, J. (1998). Tecnologia del jamon curado en distintos paises. In *El jamon curado: Tecnologia y analisis de consumo. Simposio Especial — 44th ICoMST* (pp. 9–21). Madrid: Ed. Estrategias Alimentarias S.L.-EUROCARNE.

Barbieri, G., Bolzoni, L., Parolari, G., Virgili, R., Buttini, R., Careri, M., & Mangia, A. (1992). Flavour compounds of dry-cured ham. *Journal of Agriculture and Food Chemistry, 40*, 2389–2394.

Belfrage, P., Frederikson, G., Strälfors, P., & Thornqvist, H. (1984). Adipose tissue lipases. In B. Borgström, & H. Brockerman, *Lipases* (pp. 365–416). Elsevier Science Publishers: Amsterdam.

Berdagué, J-L., Bonnaud, N., Rousset, S., & Touraille, C. (1993). Influence of pig crossbreed on the composition, volatile compound content and flavour of dry-cured ham. *Meat Science, 34*, 119–129.

Buscalihon, S., Berdagué, J-L., & Monin, G. (1994a). Time-related changes in volatile compounds of lean tissue during processing of French dry-cured ham. *Journal of the Science of Food and Agriculture, 63*, 69–75.

Buscalihon, S., Gandemer, G., & Monin, G. (1994b). Time-related changes in intramuscular lipids of French dry-cured ham. *Meat Science, 37*, 245–255.

Careri, M., Mangia, A., Barbieri, G., Bolzoni, L., Virgili, R., & Parolari, G. (1993). Sensory property relationships to chemical data of Italian-type dry-cured ham. *Journal of Food Science, 58*, 968–972.

Currie, R. W., & Wolfe, F. H. (1977). Evidence for differences in post-mortem intramuscular phospholipase activity in several muscle types. *Meat Science, 1*, 185–193.

Flores, M., Alasnier, C., Aristoy, M. C., Navarro, J. L., Gandemer, G., & Toldrà, F. (1996). Activity of aminopeptidases and lipolytic enzymes in five skeletal muscles with various oxidative patterns. *Journal of the Science of Food and Agriculture, 70*, 127–130.

Gazzetta Ufficiale della Repubblica Italiana (1970). Legge no. 506.

Hamilton, R. J. (1989). The chemistry of rancidity in foods. In R. C. Allen, & R. J. Hamilton, *Rancidity in foods* (pp. 1–21). London: Elsevier Applied Sci.

Hernandez, P., Navarro, J. L., & Toldrà, F. (1998). Lipid composition and lipolytic enzyme activities in porcine skeletal muscles with different oxidative pattern. *Meat Science, 49*, 1–10.

Jacks, T. J., & Kircher, H. W. (1967). Fluorimetric assay for the hydrolytic activity of lipase using fatty acyl esters of 4-methylumbelliferone. *Analytical Biochemistry, 21*, 279–285.

Motilva, M. J., & Toldrà, F. (1993). Effect of curing agents and water activity on pork muscle and adipose subcutaneous tissue lipolytic activity. *Lebensmittel Untersuchung und -Forschung, 196*, 228–231.

Motilva, M. J., Toldrà, F., & Flores, J. (1992). Assay of lipase and esterase activities in fresh pork meat and dry-cured ham. *Lebensmittel Untersuchung und -Forschung, 195*, 446–450.

Motilva, M. J., Toldrà, F., Nietó, P., & Flores, J. (1993). Muscle lipolysis phenomena in the processing of dry-cured ham. *Food Chemistry, 48*, 121–125.

Nawar, W. W. (1996). LipidsIn O. R. Fennema, *Food chemistry*, (3rd ed. pp. 139–244). New York: Marcel Dekker Inc.

NGD (1976). *Norme italiane per il controllo dei grassi e derivati* (3rd ed.). Milan, Italy: Stazione Sperimentale per le Industrie degli Oli e dei Grassi (Method no. C11).

Parolari, G. (1996). Review: achievements, needs and perspectives in dry-cured ham technology, the example of Parma ham. *Food Science and Technology International, 2*, 69–78.

SPSS-PC (1986). *Statistical package for social sciences*. IL: SPSS Inc.

Stead, D. (1983). A fluorimetric method for the determination of Pseudomonas fluorescens AR11 lipase in milk. *Journal of Dairy Research, 50*, 491–502.

Toldrà, F. (1998). Proteolysis and lipolysis in flavour development of dry-cured meat products. In *Simposio Especial — 44th ICoMST* (pp. 216–223). Madrid: Ed. Estrategias Alimentarias S.L.-EUROCARNE.

Toldrà, F., Flores, M., Navarro, J. L., Aristoy, M. C., & Flores, J. (1997). New developments in dry-cured ham. In H. Okai, O. Mills, A. M. Spanier, & M. Tamura, *Chemistry of novel foods* (pp. 259–272). Carol Stream, IL: Allured Pub. Co.

Toldrà, F., Flores, M., & Sans, Y. (1997). Dry-cured ham flavour. Enzymatic generation and process influence. *Food Chemistry, 59*, 523–530.

Ventanas, J., Cordoba, J. J., Antequera, T., Garcia, C., Lopez-bote, C., & Asensio, M. A. (1992). Hydrolysis and Maillard reaction during ripening of Iberian ham. *Journal of Food Science, 57*, 813–815.

Virgili, R., Parolari, G., Schivazappa, C., Bordini, C. S., & Volta, R. (1995). Effects of raw material on proteolysis and texture of typical Parma ham. *Industria Conserve, 70*, 21–31.

Virgili, R., Parolari, G., Soresi Bordini, C., & Schivazappa, C. (1997). Sensory and analytical investigations into six types of European hams: Parma, Serrano, Bayonne, Italian country-style, Iberian and Corsican. *Industria Conserve, 72*, 134–143.