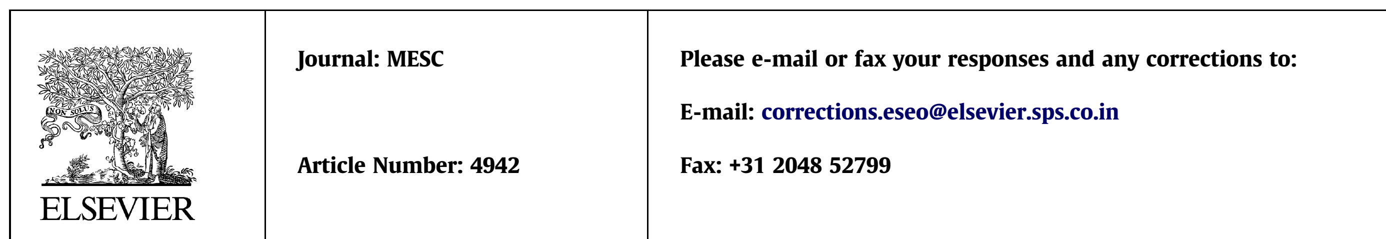
AUTHOR QUERY FORM



ELSEVIER

Journal: MESC

Article Number: 4942

Please e-mail or fax your responses and any corrections to:

E-mail: corrections.eseo@elsevier.sps.co.in

Fax: +31 2048 52799

Dear Author,

Any queries or remarks that have arisen during the processing of your manuscript are listed below and highlighted by flags in the proof. Please check your proof carefully and mark all corrections at the appropriate place in the proof (e.g., by using on-screen annotation in the PDF file) or compile them in a separate list.

For correction or revision of any artwork, please consult http://www.elsevier.com/artworkinstructions.

Articles in Special Issues: Please ensure that the words 'this issue' are added (in the list and text) to any references to other articles in this Special Issue.

Uncited references: References that occur in the reference list but not in the text – please position each reference in the text or delete it from the list.

Missing references: References listed below were noted in the text but are missing from the reference list – please make the list complete or remove the references from the text.

Location in article	Query / remark Please insert your reply or correction at the corresponding line in the proof
Q1	Please note "Bidlingmeyer, Cohen, Tarvin, and Frost (1987) has been changed to "Please note "Bidlingmeyer, Cohen, Tarvin, and Frost (1978) throughout the text. Please check. It has been checked.
Q2	Reference "Martín et al. (1999) is cited in the text but not listed. Please check. "1999" is wrong. "1999" has been changed by "1998", which is cited, in lines 241, 327, 332"
Q3	line 165: "kept" instead of "keep"
Q4	line 202: "centrifugated" instead of "centrifugation"
Q5	line 255: space between "." and "Thus"
Q6	line 328: "cellar" instead of "cellaring"

Electronic file usage

Sometimes we are unable to process the electronic file of your article and/or artwork. If this is the case, we have proceeded by:

☐ Scanning (parts of) your article ☐ Rekeying (parts of) your article ☐ Scanning the artwork

Thank you for your assistance.

Meat Science xxx (2009) xxx–xxx

1

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

2 3 # Influence of pre-cure freezing of Iberian ham on proteolytic changes throughout the ripening process

4 Trinidad Pérez-Palacios [a,*], Jorge Ruiz [a], Jose Manuel Barat [b], María Concepción Aristoy [c], Teresa Antequera [a]

5 [a] Tecnología de los Alimentos, Facultad de Veterinaria, Universidad de Extremadura, Avda. Universidad s/n, 10071 Cáceres, Spain
6 [b] Instituto de Ingeniería de Alimentos para el Desarrollo, Departamento de Tecnología de Alimentos, Universidad Politécnica, Valencia, Spain
7 [c] Instituto de Agroquímica y Tecnología de Alimentos (IATA, CSIC), Burjassot, Valencia, Spain

8

ARTICLE INFO

10 23 11 *Article history:*
12 Received 13 February 2009
13 Received in revised form 4 December 2009
14 Accepted 9 December 2009
15 Available online xxxx

16 *Keywords:*
17 Pre-freezing
18 Proteolysis
19 Amino acids
20 Processing
21 Iberian ham
22

ABSTRACT

This work aimed to investigate the effect of pre-cure freezing Iberian hams on proteolysis phenomena throughout the ripening process. Non-protein nitrogen (NPN), peptide nitrogen (PN) and amino acid nitrogen (AN) as well as amino acid and dipeptide evolution followed the same trend in both refrigerated (R) and pre-cure frozen (F) Iberian hams during processing. At the different stages of ripening, there were no differences in the content of NPN and AN while F dry-cured hams had higher levels of PN than R hams at the final step. This seemed to be more related to the salt content (lower in F than in R hams) than to the pre-cure freezing treatment. Most amino acids and dipeptides detected showed higher concentrations in F than in R Iberian hams at the green stage, being rather similar at the intermediate phases. At the final stage, the effects of pre-cure freezing of Iberian hams were not well defined, higher levels of some amino acids and dipeptides were found in R than in F Iberian hams whereas other amino acids were lower in R than in F hams. 24–34

© 2009 Published by Elsevier Ltd. 35

36

37 38 ## 1. Introduction

39 Dry-cured meat products from Iberian pigs are highly rated by Spanish consumers, because of their unique sensory features, which are a consequence of the characteristics of the raw material and of the particular processing (Ventanas, Ventanas, Ruiz, & Estévez, 2005). Proteolysis has a great influence on the quality characteristics of Iberian hams, being an important source of flavour compounds (free amino acids and small peptides). Moreover, volatile compounds coming from amino acids through the Maillard and Strecker reactions (Ventanas, Estevez, Andrés, & Ruiz, 2008) are considered to be of great importance for the flavour of Iberian ham (Carrapiso, Jurado, Timón, & García, 2002). Such changes in compounds released from proteins are significant, because the overall acceptance of meat products depends to a large extent on their flavour, which is mainly determined by taste and odour compounds (Ruiz, Muriel, & Ventanas, 2002). The proteolysis phenomenon also affects ham texture (Parolari, 1996) and leads to an increase in non-protein nitrogen (NPN) (Córdoba et al., 1994a; Martín, Córdoba, Antequera, Timón, & Ventanas, 1998), free amino acids (Córdoba et al., 1994b; Jurado, García, Timón, & Carrapiso, 2007; Martín, Antequera, Ventanas, Benítez-Donoso, & Córdoba, 2001; Ruiz et al., 1999) and peptide content (Flores, Aristoy, Spanier, & Toldrá, 1997; Martín et al., 2001; Ruiz et al., 1999). Thus, high NPN levels have been found in doughy hams (Ventanas et al., 1998). In addition, the proteolytic processes are highly dependent on the salt content (Martín et al., 1998). 39–63

64 The use of frozen/thawed thighs is not a common strategy within the processing of Iberian ham nowadays (Bermúdez & Córdoba, 2001). Furthermore, some of the regulations for Specific Designation of Origin that protect the production of dry-cured Iberian hams, keep out the procedure of freezing and subsequently thawing raw material before Iberian ham processing (MARM, 2009). On the other hand, there are no scientific studies showing the effect of pre-cure freezing on the quality of the derived Iberian dry-cured meat products. Nevertheless, such a procedure could have technological and economic advantages e.g. processing hams with more homogeneous weight, and avoiding the seasonal availability and the changes in the market price. 64–75

76 Changes in physical (drip loss, texture modifications, and colour), chemical (lipolysis and FA oxidation, protein denaturation and aggregation), and sensory properties of meat could be promoted by freezing. Their extended influence depends on raw meat characteristics, processing and above all the optimum frozen conditions (Carballo & Jiménez, 2001). 76–81

82 However, only a few studies of the effect of freezing on hams are available in the scientific literature (Arnau, Gou, & Guerrero, 1994; Bañón, Cayuela, Granados, & Garrido, 1999; Flores, Soler, Aristoy, & Toldrá, 2006; Motilva, Toldrá, Nadal, & Flores, 1994; Wang, 2001), and none in the case of Iberian hams. 82–86

* Corresponding author. Tel.: +34 927 257123; fax: +34 927 257110.
E-mail address: triny@unex.es (T. Pérez-Palacios).

0309-1740/$ - see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.meatsci.2009.12.015

UNCORRECTED PROOF

Salt penetration is favoured in frozen/thawed hams, due to the higher free water content of such hams (Bañón et al., 1999; Wang, 2001), increasing the amount of solubilised salt on the surface of the ham, which is the main factor regulating its diffusion within the piece (Sorheim & Gumpen, 1986). As a consequence, the salting time of thawed hams should be shorter than that of fresh ones (Bañón et al., 1999; Poma, 1989).

Studies carried out on Serrano hams have shown that the pre-cure freezing treatment seems not to have an influence on colour, sensory features and acceptability scores (Bañón et al., 1999; Motilva et al., 1994), although it increased proteolysis (Bañón et al., 1999; Flores et al., 2006). The lipolytic activity was also more accentuated in hams processed using frozen and subsequently thawed raw material at the beginning of ripening (Flores et al., 2006; Motilva et al., 1994), whereas these differences were not detected in the final stages (Motilva et al., 1994). Pre-cure freezing Serrano hams increased the incidence of white precipitates, formed mainly by tyrosine crystals (Arnau et al., 1994; Bañón et al., 1999).

This work was aimed at studying the influence of pre-cure freezing of Iberian hams on the proteolytic changes and on the evolution of amino acids and dipeptides during the ripening.

2. Material and methods

2.1. Experimental design

Twenty-four hind limbs were obtained from Iberian pigs fattened in confinement and fed with a commercial diet. These hind limbs were divided into two groups, refrigerated (R) and pre-cure frozen (F) hams. The F hams were frozen (at −20 °C) covered with a plastic film and thawed (4 days at 3–4 °C) three months later. The R hams were obtained later from pigs of the same genetic background and fed the same diet as those used for the F hams, but slaughtered two days before the frozen hams were totally thawed. These R hams were kept at 4 °C during 36–48 h until starting their processing. Six hind limbs of each group were used to obtain the data for the green stage. The others were processed to obtain dry-cured hams. Initial weight and pH were 11.15–11.91 kg and 5.76–5.91 for R hams, respectively, and 11.03–11.95 and 5.71–5.93 for F hams. The processing conditions were the same for the two groups of hams, except for the salting time, which were 1 day/kg for the R hams and 0.7 day/kg for the F hams. Before salting, around 300 mg/kg of nitrates and 150 mg/kg of nitrites were applied by brush to both groups of hams. After salting, the salt from the free salt was brushed from the surface and all the hams were processed as follows. They were held at 4–8 °C and 73–75% relative humidity for 70 days (post-salting step). During the drying stage the hams were kept in a room under controlled conditions for 120 days, temperature was increased from 8 to 20 °C, while relative humidity was progressively reduced to 64%. Finally, hams were left to mature for 16 months (cellar stage) at 20–25 °C and relative humidity 55–65%.

2.2. Sampling

Sampling was carried out at the green stage (raw meat) and at the end of each processing step. Samples taken at the end of salting, post-salting and drying steps were obtained by extracting a cylindrical piece of ham (sized 10 × 2.5 cm) using a stainless steel tube with a cutting edge. These samples mainly included the *Biceps femoris* muscle. Samples taken at the beginning and at the end of processing were obtained by dissecting the *B. femoris* muscle of each ham. Samples were vacuum-packaged and kept frozen at −80 °C until analysed.

2.3. Non-protein nitrogen fractions

In order to analyse NPN, amino acid nitrogen (AN) and peptide nitrogen (PN), a muscle extract was prepared following the method described by De Ketelaere, Demeyer, Vandekerckhove, and Vervaeke (1974). Briefly, 5 g of ground sample were mixed with 50 ml of perchloric acid 0.6 N. The mixture was homogenized for 3 min in a Sorval Omnimixer, and subsequently centrifuged (10 min, 5000 rpm) and filtered through Whatman No. 54 filter paper. The residue was rehomogenized with 10 ml of perchloric acid 0.6 N, centrifuged (10 min, 5000 rpm), filtered and the two filtrates pooled. This filtrate was adjusted to pH 6 with potassium hydroxide, chilled, filtered and filled up to 100 ml with distilled water.

Non-protein nitrogen was analysed following the Johnson (1941) method. Muscle extract (0.2 ml) was taken into a tube and dried (1 h, 100 °C). The dried sample was hydrolyzed with sulphuric acid (0.2 ml) on hot sand (120 °C) until it was transparent. Then, it was mixed with distilled water (4.8 ml), Nessler reagent (2 ml) and sodium hydroxide 4 N (3 ml). The mixture was shaken and keep in dark for 10 min. Absorbance was measured at 490 nm on a spectrophotometer (Hitachi U-2000, Tokyo). Concentration of NPN was calculated from a standard curve, which was developed simultaneously with the samples using solutions of ammonic sulphate 0.1 mg N/ml.

Amino acid nitrogen was determined according to Moore and Stein (1954). The muscle extract (10 ml) was mixed with 10 ml of sulphosalicylic acid 10%. The mixture was kept at 0–1 °C during 17 h. After that, it was adjusted to pH 6 with sodium hydroxide 4 N, filtered and made up to 50 ml with distilled water. About 0.5 ml of this solution was mixed with 1.5 ml of ninhydrin reagent. The tubes were shaken, heated for 20 min in boiling water and chilled. Then, 8 ml of *n*-propanol 50% were added to the solution was again shaken and left for 10 min. Absorbance was measured at 570 nm on a spectrophotometer (Hitachi U-2000, Tokyo). Concentration of AN was calculated from a standard curve, which was developed simultaneously with the samples using solutions of leucine 0.1 mg N/ml.

Peptide nitrogen was calculated following the Moore and Stein (1954) method. The 10 ml of hydrochloric acid 6 N were added to 3 ml of the muscle extract. The mixture was kept on hot sand (120 °C) for 24 h. It was adjusted to pH 6 with sodium hydroxide 30%, filtered and filled up to 50 ml with distilled water. The determination of the absorbance and standard curve were the same as for AN. PN was quantified by the difference between the absorbance values obtained for this last solution and those previously determined for AN.

2.4. Amino acid analysis

Amino acid content was determined following the procedure described by Flores et al. (1997). Samples were prepared by homogenizing 5 g of the ground ham, diluted 1:5 with hydrochloric acid 0.1 N, in a Sorval Omnimixer for 8 min and cooled by submerging the extract in ice. The homogenized samples were centrifuged (20 min, 10,000 rpm) and the supernatant material was filtered through glass wool prior to further analyses. About 100 µl of this extract was mixed with norleucine (50 µl), as internal standard, and deproteinized by adding 2.5 V of acetonitrile (Aristoy & Toldrá, 1991) and centrifugation (3 min, 10,000 rpm). Amino acid derivatization was carried out with phenyl isothiocyanate (PITC) according to the method of Bidlingmeyer, Cohen, Tarvin, and Frost (1978). Supernatant (200 µl) was dried, mixed with 15 µl of methanol:sodium acetate 1 M:triethylamine (2:2:1 vol, vol, vol) and dried, repeating this procedure once more. Then, 15 µl of methanol:water:triethylamine:PTIC (7:1:1:1 vol, vol, vol, vol) was added, held for 20 min and then dried. The residue was

Table 1
Non-protein nitrogen (NPN), peptide nitrogen (PN) and amino acid nitrogen (NA) content (expresses as mg/g muscle dry matter) throughout the processing of refrigerated and pre-cure frozen Iberian hams[s].

	NPN			NP			NA		
	R[t]	F[u]	*p*	R[t]	F[u]	*p*	R[t]	F[u]	*p*
Green stage	$4.26^{c} \pm 0.311$	$4.64^{c} \pm 0.434$	0.495	$2.92^{ab} \pm 0.23$	$3.02^{b} \pm 0.35$	0.820	$1.63^{c} \pm 0.160$	$1.68^{c} \pm 0.251$	0.877
Salting	$5.55^{c} \pm 0.377$	$5.08^{b} \pm 0.427$	0.425	$1.82^{b} \pm 0.245$	$2.49^{b} \pm 0.317$	0.135	$1.53^{c} \pm 0.115$	$1.62^{c} \pm 0.190$	0.385
Post-salting	$8.21^{bc} \pm 0.521$	$7.55^{b} \pm 0.816$	0.512	$1.53^{b} \pm 0.316$	$1.87^{b} \pm 0.236$	0.420	$2.32^{c} \pm 0.038$	$2.15^{c} \pm 0.101$	0.156
Drying	$12.42^{b} \pm 2.38$	$15.72^{a} \pm 1.36$	0.24	$2.15^{ab} \pm 0.425$	$2.85^{b} \pm 0.198$	0.175	$4.74^{b} \pm 0.283$	$5.70^{b} \pm 0.273$	0.038
Final stage	$17.49^{a} \pm 1.10$	$17.94^{a} \pm 0.830$	0.75	$3.57^{a} \pm 0.490$	$6.47^{a} \pm 0.478$	0.004	$8.96^{a} \pm 0.417$	$8.70^{a} \pm 0.235$	0.596
p (Evolution)	<0.001	<0.001		<0.001	<0.001		<0.001	<0.001	

[s] Mean values ± standard error of the mean. Means with different superscripts differ significantly throughout ripening ($p < 0.05$).
[t] Refrigerated Iberian hams.
[U] Pre-cure frozen Iberian hams.

dissolved in 200 µl of 0.005 M phosphate buffer, pH 7.4. Amino acid content was determined by high performance liquid chromatography (Hewlett–Packard Model 1050) with a photodiode array detector (254 nm). The solvent system consisted of two eluents: (A) 0.07 M sodium acetate adjusted to pH 6.55 with acetic acid 10% and acetonitrile 2.5%; (B) acetonitrile:water:methanol (45:40:15 vol, vol, vol). The flow rate was 1 ml/min and the solvent gradient was: initial 0% B, 13.5 min linear change to 3% B, 3 min linear change to 3.1% B, 2.5 min linear change to 3.5% B, 2 min to 4.5% B, 3 min to 6% B, 1 min to 6.9% B, 1 min to 8% B, 2 min to 8.8% B, 2.5 min to 9% B, 20 min to 34% B and maintained 10 min at 34% B, then 2 min to 100% B and maintained for 8 min. Identification was based on the retention times of reference compounds (Sigma). Dipeptides anserine and carnosine were also identified supported by standards (Sigma) whereas balenine isolated from pork muscle was used as reference for this dipeptide (Aristoy, Soler, & Toldrá, 2004).

2.5. Statistical analysis

The effect of pre-cure freezing Iberian hams on the non-protein nitrogen fractions and on the profile of amino acid and dipeptides throughout processing were analysed by one-way analysis of variance (ANOVA) using the General Linear Model of SPSS (v.15.0). When a significant effect ($p < 0.05$) was detected, paired comparisons between means were conducted using the Tukey's test.

3. Results and discussion

3.1. Non-protein nitrogen throughout the processing of refrigerated and pre-cure frozen Iberian hams

The NPN content of Iberian hams throughout the process is shown in Table 1. Values of NPN rose during ripening, reaching the highest content at the end of the final stage, indicating the occurrence of proteolysis. These results agree with those obtained by Córdoba et al. (1994a) and Martín et al. (1998), in which two maxima in the daily increase of NPN fraction were found, the first and highest during salting and the second during drying. These authors related the increase in NPN during the drying step to the high temperatures (up to 30 °C) reached during this stage. It has been shown that temperature in the usual range of the drying process (22 and 30 °C) leads to a substantial proteolytic enzyme activities (around 40–50% and 80%, respectively) (Toldrá, Rico, & Flores, 1992b). Toldrá, Cerveró, and Part (1993) indicated that these enzymes showed a maximum activity at around 35 °C. However, NPN values described by Córdoba et al. (1994a) and Martín et al. (1999) in Iberian ham are higher than those observed in this work, which could be due to the lower temperature reached in the present study during the drying stage (up to 20 °C) in comparison with those achieved in the previous studies (25–30 °C). Thus, higher amounts of NPN have been reported in hams ripened at higher temperature (Flores, Bermell, Nieto, & Costell, 1984; Virgili, Parolari, Schivazappa, Soresi Bordini, & Borri, 1995).

The effect of pre-cure freezing did not lead to differences in NPN content throughout processing (Table 1). These results are in agreement with those found by Flores et al. (2006), who did not observe differences between frozen/thawed and traditional fresh processed Serrano hams in the NPN index during the salting and the post-salting stages. On the contrary, Wang (2001) obtained higher NPN content in Taiwanese ham prepared with chilled meat than that made with frozen/thawed meat during the ripening process. Bañón et al. (1999) showed higher levels of NPN/total nitrogen in pre-cured frozen than in refrigerated dry-cured Serrano hams, which were related to protein modifications during freezing, providing a more favourable environment for muscle proteases (Bañón et al., 1999).

It has been reported that salt has a powerful inhibitory effect on proteinases (Sárraga, Gil, Arnau, & Monfort, 1989; Toldrá et al., 1992a). In fact, higher content of NPN has been associated with lower content of salt in Iberian (Martín et al., 1998) and Taiwanese hams (Wang, 2001). However, although the salt content was higher in R than in F Iberian hams of this study at final stage (6.58% vs. 5.21%, respectively), the values of NPN did not show differences between these two groups of hams at the end of the processing. In fact, Córdoba et al. (1994a) considered that salt concentrations in the range 1.5–6% do not markedly affect NPN levels. Other studies have not shown an effect of salt content on NPN generation throughout the processing of hams (Martín et al., 1998; Monin et al., 1997).

3.2. Peptide nitrogen throughout the processing of refrigerated and pre-cure frozen Iberian hams

The PN levels of Iberian hams throughout processing are shown in Table 1. The PN content did not change during the first stages of processing. It mainly increased during the cellar stage, reaching the highest levels at the final stage. Salt strongly inhibits cathepsin activity, but it affects more pronouncedly cathepsins H and D activities, which release large fragments from proteins (Rico, Toldrá, & Flores, 1990; Rico, Toldrá, & Flores, 1991). Nevertheless, these enzymes are very active in the temperature range of 20–30 °C, which is reached at cellar stage (Rico et al., 1991). However, results found in this study are not totally in concordance with those of Martín et al. (1999) who also found an increase in PN values during the drying but not at the cellar stage in which PN levels decreased or remained constant, depending on the processing conditions.

There were significant differences in the PN content between R and F Iberian hams at the final stage (3.57 vs 6.47 mg/g muscle dry matter, respectively), whereas PN levels were very similar to the previous phases. This difference could be explained by the significantly higher salt content in R (6.58%) than F Iberian hams (5.21%). In fact, Martín et al. (1999) related high PN content in Iberian ham

UNCORRECTED PROOF

Please cite this article in press as: Pérez-Palacios, T., et al. Influence of pre-cure freezing of Iberian ham on proteolytic changes throughout the ripening process. *Meat Science* (2009), doi:10.1016/j.meatsci.2009.12.015

with low salt levels. Thus, according to the NPN and PN results of this study, it could be pointed out that minor variation in the levels of salt influenced PN content but not the levels of NPN at the end of the processing of Iberian hams.

The lower salt content in F than in R dry-cured hams could be explained by the salting time (1 day/kg and 0.7 day/kg in R and F thighs, respectively). Commercial ham industries make use of a lower salting time for pre-cure frozen thighs than for refrigerated ones, according to some studies that advise reducing the salting time in frozen–thawed pieces because in these thighs salt diffusion is favoured (Bañón et al., 1999; Poma, 1989). The results of the present work seem to indicate that the salt diffusion is different in Iberian hams than in commercial ones, which Grau, Albarracín, Toldrá, Antequera, and Barat (2007) have related to the higher fat content in Iberian hams.

3.3. Amino acid nitrogen throughout the processing of refrigerated and pre-cure frozen Iberian hams

Table 1 shows the AN content of Iberian hams during the ripening process. The levels of AN were constant from the initial step to the end of the post-salting stage, whereas they increased during the drying and cellar steps, attaining the highest AN content at the end of the processing. Córdoba et al. (1994b) and Martín et al. (1998, 2001) found larger daily increases of AN at the drying stage. Such an increase in AN during cellaring has been only previously found in Iberian hams with high salt content (around 6%) and temperatures close to 25 °C, conditions similar to those found in the hams of this study, but not in hams processed with cellar temperatures below 20 °C and with 4% NaCl (Martín et al., 1998).

Throughout the processing of both R and F Iberian hams in this study, the levels of AN only showed differences at the end of the drying step, being statistically higher ($p = 0.038$) in F (5.82 mg/g muscle dry matter) than in R hams (4.74 mg/g muscle dry matter). However, there were no differences in AN content at the final stage. Virgili et al. (1999) found that free amino acid content was negatively correlated with salt content, which could explain the results of AN content at the drying stage but not at the end of the processing. Thus, it seems that the differences in salt content between R and F Iberian ham were not so large as to influence AN levels during the final step, being more important the process conditions. In fact, Martín et al. (1998) pointed out that temperature is the main parameter regulating AN formation and that high temperature during the cellar stage allows the accumulation of free amino acids only in suitable salted hams.

3.4. Amino acid and dipeptide evolution throughout the processing of refrigerated and pre-cure frozen Iberian hams

Content of free amino acids and dipeptides throughout the processing of Iberian hams in this study is shown in Table 2. Twenty-five peaks were identified in the chromatograms, 22 of them being amino acids and the other three being dipeptides. β-Alanine, taurine and ornithine, which have a non-protein origin, as well as the dipeptides carnosine, anserine and balenine have not been previously reported in Iberian ham (Córdoba et al., 1994b; Jurado et al., 2007; Martín et al., 2001; Ruiz et al., 1999) but they were identified in other types of dry-cured ham (Buscailhon, Gandemer, & Monin, 1994; Flores et al., 1997; Toldrá, Aristoy, & Flores, 2000). Cysteine, a free amino acid coming from proteolysis, was not detected, which agrees with the above cited studies. Levels of most free amino acid detected at the green stage rose significantly throughout the processing, even during the cellar stage, in agreement with Córdoba et al. (1994b). However, other studies showed an increase in free amino acid content between green stage and the end of the drying but no changes during the last step (Jurado et al., 2007; Martín et al., 2001; Ruiz et al., 1999; Toldrá et al., 2000). Several circumstances reduce aminopeptidase activities along the dry-curing process. Salt is an effective inhibitor, while pH has a lower effect due to its narrow range of variation during the process (Flores et al., 1997). The accumulation of free amino acids in the hams also produces a feedback inhibition on aminopeptidases (Flores, Aristoy, & Toldrá, 1998). Enzyme activities are also influenced by moisture and water activity, which diminish as drying progresses, leading to a reduction in the overall proteolytic phenomena (Toldrá et al., 1992b). Dry-cured hams of the present study showed higher moisture content (52.02%) than Iberian hams from the studies cited above, in which amino acid content did not vary during the cellar stage (45–48% of moisture content) (Jurado et al., 2007; Martín et al., 2001). Thus, such higher moisture contents in the hams of the present study could in part explain the progressive increase in amino acids during the cellar step compared to previous studies.

Among amino acids of non-protein origin, ornithine increased above all at the final stages, similarly to findings that at Buscailhon et al. (1994) and Toldrá et al. (2000) described in other dry-cured hams, whereas the content of β-alanine and taurine increased throughout ripening, which is not in agreement with Toldrá et al. (2000), who reported that these amino acids remained constant, nor with Buscailhon et al. (1994), whose results showed that these compounds decreased during the cellar stage. Levels of carnosine, anserine and balenine started to decrease at the post-salting stage and continued diminishing during the rest of processing. Thus, the lowest content of these dipeptides were found at the end of the processing. This result is in concordance with Toldrá et al. (2000) and Buscailhon et al. (1994).

On the other hand, there were significant differences in the content of these amino acids and dipeptides between R and F hams at the end of the different stages of processing, mainly in the initial step, but also in the last phases. Most amino acids and dipeptides showed higher levels in F than in R hams at the green stage. The higher proteolysis rate in F hams could be attributed to an enhanced release of these cathepsins from lysosomes due to the physical change caused by ice crystals (Flores et al., 2006). However, these authors found lower cathepsin activity in the frozen/thawed Serrano hams than in traditional fresh processed ones during the salting and post-salting steps. Khan (1966) also reported that the free amino acid and peptide content increased during frozen storage, suggesting residual activity of cathepsins. Kristensen, Christensen, and Ertbjerg (2006) pointed out that calpain and calpastatin are stable during frozen storage of meat.

At the end of salting and post-salting there were few differences between F and R Iberian hams in amino acid and dipeptide content. However, Flores et al. (2006) found higher concentration of free amino acids in frozen/thawed than in refrigerated hams during salting and post-salting stages of the manufacturing of Serrano hams. At the end of drying, glutamic acid, asparagine, glutamine, leucine and phenylalanine showed higher levels in F than in R hams. These results are in concordance with those of AN content and could be related to the higher salt content in R than in F hams, as it has been explained above. Although there were statistical differences in individual amino acid and dipeptides content between R and F hams at the final stage, the effect of pre-cure freezing does not seem to be well defined. Taurine, arginine, proline, tyrosine, leucine, phenylalanine and tryptophane showed higher levels in F than in R Iberian hams. However glutamine, ornithine and dipeptide balenine showed higher levels in R than in F hams, and no differences were found in the rest of compounds. In contrast to these results, Arnau et al. (1994) did not observe statistical differences in tyrosine content between refrigerated and thawed hams at the end of the ripening in dry-cured Serrano hams. In Taiwanese dry-cured ham, Wang (2001) found a higher free amino acid content in

UNCORRECTED PROOF

Please cite this article in press as: Pérez-Palacios, T., et al. Influence of pre-cure freezing of Iberian ham on proteolytic changes throughout the ripening process. *Meat Science* (2009), doi:10.1016/j.meatsci.2009.12.015

Table 2
Content of amino acid and dipeptide (expresses as mg/100 g muscle dry matter) throughout the processing of refrigerated and pre-cure frozen Iberian hams[s].

	Green stage			Salting			Post-salting			Drying			Final stage			Evolution	
	R[t]	F[u]	*p*	R[t]	F[u]	*p*	R[t]	F[u]	*p*	R[t]	F[u]	*p*	R[t]	F[u]	*p*	*p* (R)	*p* (F)
Asp	6.89^{c} ± 2.72	7.27c ± 1.65	0.807	15.60^{c} ± 5.44	24.89^{bc} ± 9.03	0.202	63.75^{b} ± 2.81	65.19^{bc} ± 10.32	0.828	93.31^{b} ± 23.73	86.26^{b} ± 27.29	0.729	255.75^{a} ± 27.77	236.22^{a} ± 58.78	0.563	<0.001	<0.001
Glu	25.05^{c} ± 0.80	42.10^{d} ± 2.78	<0.001	52.21^{c} ± 9.06	67.29^{d} ± 2.22	0.058	255.29^{b} ± 14.46	177.44^{c} ± 10.32	<0.001	246.57^{b} ± 23.59	300.70^{b} ± 17.91	0.034	371.10^{a} ± 56.25	387.67^{a} ± 56.38	0.716	<0.001	<0.001
Ser	27.64^{c} ± 4.51	38.79^{d} ± 5.14	0.048	54.25^{bc} ± 9.68	64.39^{cd} ± 9.71	0.229	127.33^{bc} ± 3.80	139.08^{bc} ± 21.69	0.327	175.40^{ab} ± 25.82	207.57^{b} ± 23.40	0.185	266.23^{a} ± 95.02	332.11^{a} ± 71.59	0.344	<0.001	<0.001
Asn	10.61^{c} ± 1.98	20.03^{c} ± 1.05	0.001	23.82^{c} ± 4.82	27.62^{bc} ± 2.91	0.247	56.43^{b} ± 5.37	55.21^{abc} ± 8.96	0.823	43.18^{b} ± 6.78	84.07^{ab} ± 9.73	0.002	111.39^{a} ± 13.66	107.57^{a} ± 54.17	0.912	<0.001	<0.001
Gly	38.01^{d} ± 4.84	43.38^{d} ± 5.58	0.230	60.10^{d} ± 10.64	62.72^{cd} ± 7.32	0.713	114.30^{c} ± 7.22	130.67^{bc} ± 16.40	0.117	158.79^{b} ± 18.38	193.58^{b} ± 19.92	0.090	580.66^{a} ± 16.72	622.24^{a} ± 67.87	0.279	<0.001	<0.001
Gln	102.64^{b} ± 9.37	106.81^{bc} ± 14.42	0.645	153.81^{a} ± 25.12	143.88^{a} ± 14.08	0.530	152.17^{a} ± 7.93	142.32^{ab} ± 23.68	0.404	68.77^{c} ± 1.73	101.74^{c} ± 4.56	<0.001	28.86^{d} ± 2.79	22.12^{d} ± 0.79	0.016	<0.001	<0.001
Tau	168.93^{b} ± 22.60	179.06^{bc} ± 24.55	0.566	206.73^{b} ± 74.88	223.76^{bc} ± 21.44	0.724	214.29^{b} ± 18.38	196.25^{bc} ± 31.15	0.357	215.74^{c} ± 10.80	267.00^{b} ± 37.30	0.073	364.22^{a} ± 6.15	451.55^{a} ± 41.88	0.006	<0.001	<0.001
His	16.00^{d} ± 1.80	20.65^{c} ± 3.40	0.044	42.94^{d} ± 6.89	48.44^{c} ± 3.29	0.280	99.93^{c} ± 1.52	113.35^{b} ± 19.06	0.210	134.13^{b} ± 15.44	154.27^{b} ± 31.97	0.381	408.03^{a} ± 24.43	401.49^{a} ± 37.05	0.778	<0.001	<0.001
Thr	21.06^{b} ± 2.38	27.51^{d} ± 3.20	0.018	47.37^{b} ± 9.30	54.62^{cd} ± 7.14	0.293	121.48^{b} ± 4.94	125.40^{c} ± 19.00	0.703	178.34^{b} ± 21.87	230.19^{b} ± 26.72	0.060	375.82^{a} ± 126.83	514.81^{a} ± 67.05	0.090	<0.001	<0.001
Ala	116.61^{c} ± 19.39	122.98^{b} ± 21.96	0.679	137.52^{c} ± 36.46	170.88^{b} ± 16.36	0.222	230.82^{b} ± 7.06	226.63^{b} ± 32.12	0.807	307.78^{b} ± 47.20	368.13^{b} ± 30.53	0.136	1338.96^{a} ± 54.18	1626.94^{a} ± 320.77	0.193	<0.001	<0.001
Arg	30.21^{c} ± 4.17	45.95^{b} ± 5.91	0.005	49.69^{c} ± 16.35	71.22^{b} ± 8.06	0.067	134.90^{b} ± 7.99	157.62^{a} ± 24.66	0.090	175.84^{a} ± 33.26	210.26^{a} ± 28.00	0.242	39.46^{c} ± 18.38	152.29^{a} ± 46.06	0.017	<0.001	<0.001
Pro	16.29^{d} ± 5.79	17.89^{c} ± 1.64	0.614	36.09^{cb} ± 5.46	44.59^{c} ± 9.94	0.244	115.15^{c} ± 14.61	127.22^{c} ± 12.26	0.253	274.65^{b} ± 31.16	294.51^{b} ± 28.84	0.463	1039.75^{a} ± 68.24	1213.00^{a} ± 113.13	0.039	<0.001	<0.001
Tyr	19.91^{d} ± 2.87	35.71^{d} ± 3.50	<0.001	45.23^{d} ± 8.83	49.14^{d} ± 2.81	0.506	118.23^{c} ± 12.37	95.20^{c} ± 13.08	0.030	191.69^{b} ± 26.10	226.12^{b} ± 16.70	0.127	351.54^{a} ± 54.78	455.87^{a} ± 28.90	0.032	<0.001	<0.001
Val	28.50^{c} ± 4.49	41.43^{c} ± 2.71	0.007	56.35^{c} ± 8.06	60.47^{c} ± 6.92	0.538	134.16^{c} ± 4.48	124.58^{c} ± 20.30	0.392	291.39^{b} ± 53.81	306.57^{b} ± 23.69	0.678	1076.41^{b} ± 106.83	1201.18^{a} ± 130.71	0.168	<0.001	<0.001
Met	15.24^{d} ± 3.70	27.47^{c} ± 1.90	0.004	33.67^{d} ± 5.66	37.02^{c} ± 1.73	0.382	67.95^{c} ± 6.37	65.44^{c} ± 9.92	0.685	111.88^{b} ± 15.38	141.71^{b} ± 11.49	0.055	620.60^{a} ± 16.20	598.07^{a} ± 41.32	0.420	<0.001	<0.001
Ile	20.26^{c} ± 1.76	37.40^{c} ± 6.13	0.002	43.94^{c} ± 8.08	46.10^{bc} ± 3.21	0.689	97.39^{bc} ± 6.14	84.66^{bc} ± 5.44	0.026	232.09^{b} ± 46.92	252.17^{b} ± 19.78	0.532	1007.34^{a} ± 120.88	1149.50^{a} ± 155.63	0.179	<0.001	<0.001
Leu	36.29^{d} ± 1.75	68.00^{c} ± 3.03	<0.001	88.51^{d} ± 14.42	92.83^{c} ± 5.19	0.652	225.17^{c} ± 26.46	198.73^{c} ± 5.12	0.093	382.12^{b} ± 55.28	525.40^{b} ± 28.77	0.016	2203.18^{a} ± 49.43	2598.49^{a} ± 245.55	0.044	<0.001	<0.001
Phe	20.98^{d} ± 2.13	43.84^{c} ± 0.93	<0.001	47.65^{cd} ± 9.37	49.06^{c} ± 2.63	0.781	108.46^{c} ± 11.10	118.74^{c} ± 24.99	0.481	245.22^{b} ± 16.55	335.34^{b} ± 46.07	0.033	1398.65^{a} ± 56.74	1627.17^{a} ± 105.31	0.020	<0.001	<0.001
Lys	42.29^{c} ± 10.94	63.10^{c} ± 3.20	0.026	103.09^{c} ± 18.19	117.20^{c} ± 17.42	0.345	227.92^{b} ± 20.46	214.79^{b} ± 43.60	0.599	249.56^{b} ± 44.37	292.07^{b} ± 68.60	0.418	645.17^{a} ± 76.78	602.36^{a} ± 18.63	0.398	<0.001	<0.001
β-Ala	6.97^{b} ± 2.34	7.51^{b} ± 0.40	0.715	8.85^{b} ± 1.36	11.29^{ab} ± 1.32	0.063	9.01^{b} ± 2.11	8.04^{ab} ± 2.01	0.530	9.91^{b} ± 0.68	9.58^{ab} ± 0.17	0.465	13.68^{a} ± 0.54	12.11^{d} ± 2.53	0.213	<0.001	<0.001
Trp	<0.001	<0.001	–	<0.001	<0.001	–	<0.001	<0.001	–	60.60 ± 13.26	74.97 ± 10.00	0.208	226.42 ± 46.81	315.28 ± 10.10	0.025	0002	<0.001
Orn	<0.001	<0.001	–	<0.001	<0.001	–	<0.001	<0.001	–	6.54 ± 2.33	2.80 ± 1.36	0.058	128.02 ± 21.93	99.30 ± 4.22	0.046	<0.001	<0.001
Carnosine	2642.69^{a} ± 340.77	2216.38^{a} ± 353.05	0.110	2654.64^{a} ± 228.42	2641.30^{a} ± 191.19	0.942	1457.79^{b} ± 117.92	1475.16^{b} ± 255.17	0.906	1125.27^{b} ± 87.84	1162.11^{b} ± 358.92	0.872	979.31^{b} ± 117.26	850.58^{b} ± 136.75	0.203	<0.001	<0.001
Anserine	121.45^{ab} ± 13.57	146.83^{a} ± 17.53	0.044	131.85^{a} ± 28.81	153.64^{a} ± 15.58	0.313	86.34^{bc} ± 9.31	79.96^{b} ± 7.96	0.337	69.10^{c} ± 9.68	79.92^{b} ± 33.35	0.618	68.44^{c} ± 10.69	65.75^{b} ± 7.33	0.738	<0.001	<0.001
Balenine	168.29^{b} ± 21.08	205.12^{a} ± 22.94	0.041	224.59^{a} ± 32.77	201.30^{a} ± 8.90	0.301	127.84^{bc} ± 14.26	119.71^{b} ± 10.62	0.377	99.91^{c} ± 15.46	97.02^{bc} ± 26.82	0.863	98.68^{c} ± 4.26	75.54^{c} ± 7.56	0.010	<0.001	<0.001
∑ Amino acid	762.63^{c} ± 12.16	991.10^{c} ± 112.85	0.025	1163.34^{c} ± 250.44	1259.37^{c} ± 77.91	0.597	2539.46^{b} ± 48.93	1869.85^{bc} ± 232.26	0.001	3173.12^{b} ± 642.65	4611.76^{b} ± 655.69	0.085	12165.86^{a} ± 927.29	14001.76^{a} ± 655.69	0.050	<0.001	<0.001
∑ Dipeptide	2741.82^{a} ± 74.15	2360.14^{a} ± 409.70	0.180	2911.65^{a} ± 329.65	2996.24^{ab} ± 209.33	0.727	1673.37^{b} ± 140.13	1674.82^{bc} ± 270.21	0.993	1280.10^{bc} ± 101.24	1294.81^{c} ± 396.92	0.953	1090.61^{c} ± 149.02	956.54^{c} ± 117.51	0.207	<0.001	<0.001

[s] Mean values ± standard error of the mean. Means with different letter superscripts differ significantly throughout ripening ($p < 0.05$).
[t] Refrigerated Iberian hams.
[U] Pre-cure frozen Iberian hams.

Please cite this article in press as: Pérez-Palacios, T., et al. Influence of pre-cure freezing of Iberian ham on proteolytic changes throughout the ripening process. *Meat Science* (2009), doi:10.1016/j.meatsci.2009.12.015

samples from chilled meats than in frozen ones, which the author related to the lower salt content in pre-cure frozen hams.

4. Conclusions

Pre-cure freezing of Iberian hams does not seem to markedly influence the content of NPN, PN and AN in the different stages of the processing. The only significant effect was the higher content of PN in refrigerated than in frozen/thawed hams at the end of the ripening, which seem to be related to the salt level more than to the pre-cure freezing.

On the other hand, pre-cure freezing of Iberian ham seems to influence the content of most free amino acids and dipeptides at the initial stage, being higher in refrigerated than in pre-cure frozen Iberian hams. However, this effect was not that clear in the final stage, where differences in these compounds between Iberian dry-cured hams processed under these two different technologies being did not follow a well defined pattern.

Acknowledgements

Trinidad Pérez-Palacios thanks the "Junta de Extremadura" for the pre-doctoral grant. Authors are grateful to RESTI industry for manufacturing the samples of this research. This study was supported by the "Ministerio de Ciencia y Tecnología. Secretaría de Estado de Política Científica y Tecnológica" proyect entitled "Desarrollo de uAN tecnología basada en el saldo y descongelación en salmuera saturada, con y sin empleo de la impregANción a vacío, para la obtención de jamones curados a partir de perniles congelados" (Ref. PTR1995-0754-OP-03-03).

References

Aristoy, M. C., Soler, C., & Toldrá, F. (2004). A simple, fast and reliable methodology for the analysis of histidine dipeptides as markers of the presence of animal origin proteins in feeds for ruminants. *Food Chemistry, 84*, 485–491.

Aristoy, M. C., & Toldrá, F. (1991). Deproteinization techniques for HPLC amino acid analysis in fresh pork muscle and dry-cured ham. *Journal of Agricultural and Food Chemistry, 39*, 1792–1795.

Arnau, J., Gou, P., & Guerrero, L. (1994). The effects of freezing, meat pH and storage temperature of the formation of white film and tyrosine crystals in dry-cured hams. *Journal of Food and Agricultural, 66*, 279–282.

Bañón, S., Cayuela, J. M., Granados, M. V., & Garrido, M. D. (1999). Pre-cure freezing affects proteolysis in dry-cured hams. *Meat Science, 51*, 11–16.

Bermúdez, E., & Córdoba, G. (2001). El sacrificio del cerdo Ibérico. Manejo ante y post-mortem. Obtención y perfilado del pernil. En Jesús Ventanas Barroso (Ed.), *Tecnología del jamón Ibérico* (Vol. 1, pp. 161–200). Madrid: Mundi-Prensa.

Bidlingmeyer, B. A., Cohen, S. A., Tarvin, L. T., & Frost, B. (1978). A new rapid, high sensitivity analysis of amino acids in food type samples. *Journal Association of Analytical Chemistry, 70*, 241–247.

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

Carballo, J., & Jiménez, F. (2001). Refrigeración y congelación de carne y productos cárnicos. In S. Martin Bejarano (Ed.). *Enciclopedia de la carne y de los productos cárnicos* (Vol. 1, pp. 475–509). Spain: Martin and Macias.

Carrapiso, A. I., Jurado, A., Timón, M. L., & García, C. (2002). Characterization of the most odor-active compounds of Iberian ham headspace. *Journal of Agricultural and Food Chemistry, 50*, 1996–2000.

Córdoba, J. J., Antequera, T., García, C., Ventanas, J., López-Bote, C., & Asensio, M. A. (1994a). Evolution of free amino acids and amines during ripening of Iberian cured ham. *Journal of Agricultural and Food Chemistry, 42*, 2296–2301.

Córdoba, J. J., Antequera, T., Ventanas, J., López-Bote, C., García, C., & Asensio, M. A. (1994b). Hydrolysis and loss of extractability of proteins during ripening of Iberian ham. *Meat Science, 37*, 217–227.

De Ketelaere, A., Demeyer, D., Vandekerckhove, P., & Vervaeke, I. (1974). Stoichiometry of carbohydrate fermentation during dry sausage ripening. *Journal of Food Science, 39*, 297–300.

Flores, M., Aristoy, M. C., Spanier, A. M., & Toldrá, F. (1997). Non-volatile components effects on quality of "serrano" dry cured ham as related to processing time. *Journal of Food Science, 62*, 1235–1239.

Flores, M., Aristoy, M. C., & Toldrá, F. (1998). Feedback inhibition of porcine muscle alanyl and arginyl aminopeptidases in cured meat products. *Journal of Agricultural and Food Chemistry, 46*, 482–4986.

Flores, J., Bermell, S., Nieto, P., & Costell, E. (1984). Cambios químicos en las proteínas del jamón durante los procesos de curado, lento y rápido, y su relación con la calidad. *Revista de Agroquímica y Tecnología de Alimentos, 24*, 503–509.

Flores, M., Soler, C., Aristoy, M. C., & Toldrá, F. (2006). Effect of brine thawing/salting for time reduction in Spanish dry-cured ham manufacturing on proteolysis and lipolysis during salting and post-salting periods. *European Journal of Food Research and Technology, 222*, 509–515.

Grau, R., Albarracín, W., Toldrá, F., Antequera, T., & Barat, J. M. (2007). Study of salting and post-salting stages of fresh and thawed Iberian hams. *Meat Science, 79*, 677–682.

Johnson, M. J. (1941). Isolation and properties of a pure yeast polypeptidase. *Journal of Biological Chemistry, 137*, 575–586.

Jurado, A., García, G., Timón, M. L., & Carrapiso, A. I. (2007). Effect of ripening time and rearing system on amino acid-related flavour compounds of Iberian ham. *Meat Science, 75*, 585–594.

Khan, A. W. (1966). Cryochemistry of animal tissues: Biochemical changes in poultry muscle during freezing and storage. *Cryobiology, 3*, 224–229.

Kristensen, L., Christensen, M., & Ertbjerg, P. (2006). Activities of calpastatin, μ-calpain and m-calpain are stable during frozen storage of meat. *Meat Science, 72*, 116–120.

MARM. (2009). Ministerio de Medio Ambiente, Medio Rural y Marino. Dirección General de Industrias y Mercados Alimentarios. Datos de las DenomiANciones de Origen Protegidas (DOP). Años: 2002–2006. (<http://www.marm.es>).

Martín, L., Antequera, T., Ventanas, J., Benítez-Donoso, R., & Córdoba, J. J. (2001). Free amino acids and other non-volatile compounds formed during processing of Iberian ham. *Meat Science, 59*, 363–368.

Martín, L., Córdoba, J. J., Antequera, T., Timón, M. L., & Ventanas, J. (1998). Effects of salt and temperature on proteolysis during ripening of Iberian ham. *Meat Science, 49*, 145–153.

Monin, G., Marinova, P., Talmant, A., Martin, J. F., Cornet, M., Lanore, D., et al. (1997). Chemical and structural changes in dry-cured hams (Bayonne hams) during processing and effects of the deharing technique. *Meat Science, 47*, 29–47.

Moore, S., & Stein, E. H. (1954). A modified ninhydrin reagent for photometric determination of amino acids and related compounds. *Journal of Biological Chemistry, 47*, 29–47.

Motilva, M. J., Toldrá, F., Nadal, M. I., & Flores, J. (1994). Pre-freezing hams affects lipolysis during dry-curing. *Journal of Food Science, 59*, 303–305.

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

Poma, J. P. (1989). La fabrication du jambon sec: Importante de la congelation de la matiere premiére. *Viandes Produits Carnés, 10*, 179–182.

Rico, E., Toldrá, F., & Flores, J. (1990). Activity of cathepsin D as affected by chemical and physical dry-curing parameters. *Zeitschrift fuer Lebensmittel-Untersuchung und -Forschung, 191*, 20–23.

Rico, E., Toldrá, F., & Flores, J. (1991). Effect of dry curing process parameters on pork muscle cathepsin B, H and L activity. *Zeitschrift fuer Lebensmittel-Untersuchung und -Forschung, 193*, 541–544.

Ruiz, J., García, C., Díaz, M. C., Cava, R., Tejeda, J. F., & Ventanas, J. (1999). Dry cured Iberian ham non-volatile components as affected by the length of the curing process. *Food Research International, 32*, 643–651.

Ruiz, J., Muriel, E., & Ventanas, J. (2002). The flavour of Iberian ham. In F. Toldrá (Ed.), *Research advances in the quality of meat and meat products*. Trivandrum, India: Research Signpost.

Sárraga, C., Gil, M., Arnau, J., & Monfort, J. M. (1989). Effect of curing salt and phosphate on the activity of porcine muscle proteases. *Meat Science, 25*, 241–249.

Sorheim, O., & Gumpen, S. A. (1986). Effects of freezing and thawing of pork on salt diffusion in wet and dry-curing system. In *Proceedings of 32nd European meeting of meat research works* (pp. 295–297). 24–29 August 1986, Ghent, Sweden.

Toldrá, F., Aristoy, M. C., & Flores, M. (2000). Contribution of muscle aminopeptidases to flavor development in dry-cured ham. *Food Research International, 33*, 181–185.

Toldrá, F., Aristoy, M. C., Part, C., Cerveró, C., Rico, E., Motilva, M. J., et al. (1992a). Muscle and adipose tissue aminopeptidase activities in raw and dry-cured ham. *Journal of Food Science, 57*, 816–818.

Toldrá, F., Cerveró, C., & Part, C. (1993). Porcine aminopeptidase activity as affected by curing agents. *Journal of Food Science, 58*, 724–726.

Toldrá, F., Rico, E., & Flores, J. (1992b). Activities of pork muscle proteases in model cured meat systems. *Biochimie, 74*, 291–296.

Ventanas, S., Estevez, M., Andrés, A. I., & Ruiz, J. (2008). Analysis of volatile compounds of Iberian dry-cured loins with different intramuscular fat contents using SPME-DED. *Meat Science, 78*, 172–180.

Ventanas, J., Martín, L., Antequera, T., Timón, M., Córdoba, J. J., & Gázquez, A. et al. (1998). Alcance y consecuencias de la reducción de sal en el jamón. In Excma (Ed.), *IV Encuentro Intersectorial del Cerdo Ibérico* (pp. 193–203). Diputación Provincial de Badajoz, Ilmo. Colegio Oficial de Veterinários de Badajoz.

Ventanas, S., Ventanas, J., Ruiz, J., & Estévez, M. (2005). Iberian pigs for the development of high-quality cured products. *Recent research in development in agricultural and food chemistry* (pp. 27–33). Trivandrum, Kerala, India: Research Singpost.

Virgili, R., Parolari, G., Schivazappa, C., Soresi Bordini, C., & Borri, M. (1995). Sensory and texture quality of dry-cured ham as affected by endogenous cathepsin B activity and muscle composition. *Journal of Food Science, 60*, 1183–1186.

Virgili, R., Parolari, G., Soresi-Bordini, C., Schivazappa, C., Cornet, M., & Monin, G. (1999). Free amino acids and dipeptides in dry cured ham. *Journal of Chromatography, 346*, 413–416.

Wang, F. (2001). Lipolytic and proteolytic properties of dry-cured boneless hams ripened in modified atmospheres. *Meat Science, 59*, 15–22.

UNCORRECTED PROOF