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Dry cured Iberian ham non-volatile components as affected by the length of the curing process

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Abstract

Free amino acids and peptides from 10 dry-cured Iberian hams ripened for two different processing times, a prolonged traditional one (600 days) and a shortened process (420 days), were analysed by reverse-phase HPLC. Most amino acids decreased from day 420 to day 600, whereas total peptide content and some of the individual peptides increased during this period. When analysing peptide extracts by HPLC coupled to an APCI-MS detector, peptides found showed a MW between 189 and 317. Partial least-squares regression of chemical and sensory data indicated that saltiness was related to salt content, whereas bitterness was related to some late-eluting peptides.

Keywords: Dry-cured ham; Free amino acids; Peptides; Ripening time; Taste; PLS; 2- and 3-Methylbutanal

1. Introduction

Amino acids, peptides, inorganic salts and nucleotides are the main taste compounds in meat products (MacLeod, 1986). Nucleotides decrease to undetectable levels during the curing process in sausages (Mateo, Domínguez, Aguirrezábal & Zumalacárregui, 1996) and Parma hams (Kohata, Numata, Kawaguchi, Nakamura & Arakawa, 1992). Therefore, in dry-cured products that undergo a longer curing process, such as Iberian hams, the only taste compounds with a certain importance should be amino acids, peptides and sodium chloride (Córdoba, Antequera, García, Ventanas, López-Bote & Asensio, 1994; Flores, Aristoy, Spanier & Toldrá, 1997).

The protein hydrolysis that takes place during the ripening of dry-cured hams is mainly due to endogenous proteolytic activity, produced by cathepsins, calpains and aminopeptidases (Virgili, Schivazappa, Parolari, Soresi, Bordini & Degni, 1998). However, proteolysis occasioned by the presence of bacteria, yeast and moulds in Iberian ham should not be dismissed (Rodríguez, Núñez, Córdoba, Bermúdez & Asensio, 1998).

Regardless of whether the proteolysis has a microbial or an endogenous origin, it leads to an increase in non-protein nitrogen (Martín, Antequera, Ruiz, Cava, Tejeda & Córdoba, 1998; Ventanas, Córdoba, Antequera, García, López-Bote & Asensio, 1992), free amino acid (Buscailhon, Saccani, Virgili & Bordini, 1994; Cordoba et al., 1994; Flores et al., 1997; Schivazzappa, Saccani, Virgili & Bordini, 1995), and peptide content (Flores et al., 1997; Martín, Antequera, Córdoba, Timón & Ventanas, 1998). Such changes in nitrogen compounds are very important, because the overall acceptance of meat products depends to a large extent on their flavour, which is mainly determined by taste and odour compounds (Ramaranthan & Rubin, 1994). In this sense, Flores et al. (1997) have detected a strong influence of peptides and amino acids on the final flavour of "Serrano" dry-cured hams. This is not strange since it is well known that amino acids and peptides contribute to the taste of a wide variety of foods (Kato, Rhue & Nishimura, 1989). Furthermore, some amino acids and peptides

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have been shown to intensify, modify or mask the flavours of certain foods (Maga, 1994).

Dry-cured Iberian ham is a meat product mainly produced in the southwest of Spain. The meat characteristics of Iberian pigs (marbling, fat composition, antioxidant status) along with the prolonged curing process (about twice as long as in any other type of ham) produce a dry-cured ham with special features and a variety of flavour tones that make this the most valuable meat product in Spain, with excellent consumer acceptance. Increasingly hams are being ripened using shorter curing processes than traditionally used, in order to reduce costs, although this leads to a reduction in flavour intensity (Ruiz, Ventanas, Cava, Timón & García, 1998). In order to determine which chemical changes are involved in such decrease of flavour, the present work studies the effect of a shortened processing on non-volatile compounds and its relationship with taste characteristics.

2. Material and methods

2.1. Experimental design

Ten hams obtained from Iberian pigs (140–145 kg live weight), which had been fattened on the traditional extensive production system where acorn and pasture are the main food source, were processed into cured hams according to the traditional method (Córdoba et al., 1994). During the first stages of processing all hams were treated the same, but in the last phase (the ripening of the hams in a cellar) one group was matured for 9 months (420 days of total processing time) (420d) and the other for 15 months, as in the traditional method, the total time of the processing being 600 days (600d). Hams were bone-in 6.0 ± 0.4 kg weight. These times of processing correspond to the minimum and the maximum required for Iberian hams of this weight by the Specific Designation of Origin (SDO) ''Dehesa de Extremadura'' (Diario Oficial de Extremadura, 1990).

2.2. Sampling

Ham slices were taken from three different depths in the front and the back of each ham using anatomical references to sample exactly the same muscles in all hams. Slices from each location were used for chemical and sensory analysis. Samples for chemical analysis were frozen at $-80°C$ until needed, whereas sensory analysis was performed immediately on the fresh samples.

2.3. Sensory analysis

Hams were assessed by a trained panel of 14 members, using a descriptive analysis method (García, Ventanas, Antequera, Ruiz, Cava & Alvarez, 1996) for 20 different attributes. Panelists were trained and had participated in sensory evaluation of dry-cured ham for two years. Sample preparation and serving testing procedures followed guidelines described elsewhere (Ruiz et al., 1998). Twenty traits about sensory characteristic of Iberian ham were evaluated by the 14 panelists using an unstructured 10 cm line, ranging from less (0 cm) to more (10 cm). In the present work only taste (saltiness, sweetness, bitterness) characteristics were considered.

2.4. Chemical analyses

Moisture was determined following the ISO recommended method 1442 (ISO, 1973).

Salt content was estimated as chlorides, which were extracted with water–ethanol (60:40 v/v) and quantified by the Carpentier–Volhard method (AOAC, 1984).

Amino acids were determined following the procedure described by Cordoba et al. (1994). As an internal standard 10 μl of a solution of norleucine (10 mg/ml) was added to samples before homogeneization. For deproteinization, a 10 g sample was homogenized in a Sorvall omnimixer with 5% sulfosalicylic acid for 1 min. The homogenates were maintained at 2°C for 17 h. They were then centrifuged at 15 300 *g* for 10 min and filtered through Whatman No. 54 paper. After the pH of the filtrates was adjusted to 6 with 4 N NaOH, amino acid derivatization was carried out with phenyl isothiocyanate (PITC) according to a modified method used by Yang and Sepúlveda (1985). Fifty μl of filtrate was mixed with 200 μl of PITC solution (ethanol–water–triethylamine–PITC 7:1:2:1) for 10 min, dried in a Speedvac (Savant Instruments) set at 1800 mTorr and low drying time (no heating) for 20 min, and reconstituted in 500 μl of 0.5 M sodium phosphate buffer, pH 7.4 and 5% acetonitrile for analysis. The PITC derivates were detected on a Beckman liquid chromatograph equipped with two pumps (Model 110B) and an UV detector (Model 166). The column was a Supelcosil LC-18 containing octadecyldimethylsilyl, 25×4.6 mm (5 μm particle size) from Supelco (Bellafonte, PA, USA). The temperature was controlled at 35°C. The eluents used were (A) 0.03 M sodium acetate and 0.05% triethylamine, pH 6.80, and (B) 90:10 acetonitrile–water. To achieve the amino acid separation, the flow rate was set to 1 ml/min and the following gradient was performed: initial 3.2% B for 0.5 min, linear change to 4.5% B in 5 min, linear change to 10% B in 9.5 min, linear change to 19% B in 7 min, linear change to 27% B in 10 min, linear change to 99% B in 5 min, wash with this percentage of B for 15 min and equilibrate at 3.2% B. The detection was carried out at 254 nm. 20 μl of standard or sample was injected into the system. For amino acids identification, solutions (1 mg/ml) of standard amino acids (Sigma Chemical Co., St. Louis, MO, no LAA-21),

including L-Ala, L-Arg HCl, L-Asn, L-Asp, L-Cys, L-Glu, L-Gln, Gly, L-His HCl, Pro (4-OH), L-Ile, L-Leu, L-Lys HCl, L-Met, L-Phe, L-Pro, L-Ser, L-Thr, L-Trp, L-Tyr and L-Val, were used. The concentration of different amino acids was calculated from the standard curves of the pure amino acids prepared and derivatized simultaneously with the samples and run under identical conditions.

Peptides were analysed following the procedure described by Martín, Antequera, Córdoba et al. (1998). A 10 g sample was deproteinized with perchloric acid 0.6 N by homogenizing in a Sorvall omnimixer. The homogenates were then centrifuged at 15 300 *g* for 10 min and filtered through Whatman No. 54 paper. The pH of the filtrates was then adjusted to 6 with potassium hydroxide and they were filtered again through Whatman No. 54 paper. Twenty μl of filtrate were injected into the Beckman liquid chromatograph cited previously. The column was a Supelcosil LC-18 containing octadecyldimethylsilyl, 25 cm×4.6 mm (5 μm particle size) from Supelco (Bellafonte, PA, USA). The temperature was controlled at 35°C. The eluents used were (A) water HPLC grade and (B) acetonitrile with 0.01% of trifluoroacetic acid. To achieve the peptide separation, the flow rate was set to 0.5 ml/min and the following gradient was performed: initial of 0.5% B, linear change to 9% B for 5 min, linear change to 22.6% B for 10 min and equilibrate at 0.5% for 15 min.

Some of the extracts were analysed by high-performance liquid chromatography coupled to an atmospheric pressure chemical ionization interface mass spectrometry detector (HPLC–APCI–MS). Chromatographic conditions used were the same as cited previously but on a Hewlett-Packard HP-1100 HPLC. MS detection was performed on a Finnigan LCQ equipped with an APCI interface. Positive ions were detected under the following conditions: vaporizer temperature 450°C, sheath gas flow rate 60 arbitrary units, auxiliary gas flow rate 20 arbitrary units, discharge current 5 μA, capillary temperature 150°C, capillary voltage 0 V, tube lens offset 0 V. Full scan data (between 50 and 2000 m/z) were acquired and processed using Navigator v. 1.1 sp1 software.

Volatile compounds were analysed by dynamic headspace coupled to GC–MS, following the procedure described by Ruiz, Ventanas, Cava, Andrés and García (1999). About 20 g of frozen ham slices were ground in a domestic blender, and 9.00 g weighed into a dynamic headspace vial. The volatile compounds were isolated by the dynamic headspace technique and adsorbed on a capillary Tenax trap, using an automatised dynamic headspace apparatus (Chrompack, Middelburg, The Netherlands). The sample was thermostated at 40°C for 10 min, and subsequently, during 40 min the volatile substances were purged with purified Helium (grade He > 99.999%, flow rate 10 ml/min) and adsorbed on a capillary Tenax trap held at −120°C with liquid nitrogen. The compounds were thermally desorbed into the gas chromatograph (Hewlett–Packard 5890 series II) by quickly heating at 250°C for 5 min. The separation was performed on a 5% phenyl–methyl Silicone (HP-5) bonded phase fused silica capillary column (Hewlett-Packard, 50 m×0.32 mm id, film thickness 1.05 μm), operating at 6 psi of column head pressure. Oven program was: 35°C, 15 min, 4°C min^{-1} to 200°C, 20°C min^{-1} to 250°C. The transfer line to the mass spectrometer (MS) was maintained at 280°C. The mass spectra were obtained using a mass selective detector (Hewlett-Packard HP-5971 A) by electronic impact at 70 eV, a multiplier voltage of 1756 V and collecting data at a rate of 1 scan s^{-1} over the m/z range of 30 to 300. Compounds were tentatively identified by comparing their mass spectra with those contained in the NIST/EPA/NIH and Wiley libraries and by comparison of Kovats indices with those reported in the literature by Kondjoyan and Berdagué (1996) and Acree and Arn (1997).

2.5. Statistical analyses

The effect of processing time (420 and 600 days) and slice location on free amino acid and peptide contents was carried out by analysis of variance, using the GLM procedure (SAS, 1997). Principal component analysis (PCA) was performed using results from the amino acid, peptides and selected identified volatile compounds as variables in the Unscrambler software (CAMO A/S, 1996). The amino acid, peptide, moisture and salt contents data together with the sensory results

Table 1
Amino acid concentrations in dry-cured Iberian hams underwent a shortened (420 days) and a traditional (600 days) processing[a]

	420 days	600 days	*P*
Asp	135.98 ± 8.77	109.04 ± 5.65	0.0046
Glu	805.09 ± 41.36	551.47 ± 19.08	0.0010
Ser	169.62 ± 9.90	136.96 ± 4.72	0.0060
Asn-Gly	169.61 ± 9.90	115.90 ± 4.18	0.0001
Gln	322.18 ± 23.04	276.84 ± 9.96	0.0045
His	167.82 ± 12.56	110.84 ± 6.60	0.0010
Thr	186.60 ± 1.85	139.89 ± 11.15	0.0003
Ala	351.50 ± 24.19	229.43 ± 13.92	0.0001
Arg	335.31 ± 43.44	234.50 ± 25.66	0.0396
Pro	197.14 ± 10.50	136.37 ± 7.53	0.0001
Tyr	189.23 ± 20.49	150.41 ± 13.17	0.0524
Val	212.08 ± 7.904	152.24 ± 10.02	0.0001
Met	126.25 ± 10.10	100.29 ± 4.85	0.0019
Ile	172.01 ± 15.26	134.19 ± 5.63	0.0004
Leu	237.70 ± 7.18	203.07 ± 7.88	0.0014
Phe	143.31 ± 5.29	153.57 ± 80.00	0.1809
Trp	44.11 ± 3.74	62.58 ± 10.10	0.0946
Lys	553.18 ± 28.07	380.48 ± 13.40	0.0001
Total	4512.68 ± 195.02	3378.12 ± 90.86	0.0001

[a] Means of five hams analysed at six different slice locations (mg of free amino acid/100 g dry matter) ± S.E.M.

were analysed by partial least square regression analysis (PLS2) using the Unscrambler (CAMO A/S, 1996).

3. Results and discussion

The effect of shorten the curing process on the concentration of free amino acids in dry-cured ham is shown in Table 1. Contents of free amino acids are similar to those obtained by Cordoba et al. (1994) studying Iberian hams. However, some amino acids showed considerably higher values than those reported in "Serrano" dry cured ham (Aristoy & Toldrá, 1991), Parma ham (Schivazappa et al., 1995) and French ham (Buscailhon et al., 1994), especially in the case of the 420 day hams. The high content of some free amino acids probably contribute to the distinct flavour of Iberian ham.

There was a reduction in most amino acid contents from the short to the long processing, except for phenylalanine and tryptophan, which slightly increased but not to a significant extent. Some studies dealing with free amino acids in dry-cured ham have shown an increase throughout the ripening process (Flores et al., 1997; Schivazappa et al., 1995). However, Buscailhon et al. (1994) studying French hams, found a decrease in all amino acids determined and in amino acid nitrogen during the ripening from the day 179 to the end of the processing (273 days). A similar trend was observed by Flores, Bermell, Nieto and Costell (1984) and Astiasaran, Cid, Melgar and Bello, (1989) studying "Serrano" dry-cured hams. At any rate, results from these studies are hardly directly comparable with those from the present one, since processing time in Iberian ham is almost twice as long as that of French, Parma or "Serrano" hams.

Regarding Iberian hams, Martín, Antequera, Ruiz et al. (1998) have found a slight decrease or no change in amino acid nitrogen during the last steps of processing, depending on the ripening conditions and the salt content. However, Cordoba et al. (1994) found an increase in free amino acids from the middle of the last step of the processing to the end of the processing, although the daily increase in such final step was much lower than in the previous stages.

Whether free amino acid content increases or decreases depends on the ratio between free amino acid formation and degradation. The former is mainly due to the activity of aminopeptidase enzymes, while the later is principally related to the formation of volatile compounds from the amino acids (Buscailhon et al., 1994). With regards to aminopeptidases, they were reported to be active during the whole processing of "Serrano" dry-cured hams, but there was a significant reduction in their activities (Toldrá et al., 1992). There are no studies about aminopeptidases activities in a product with such a long processing as Iberian ham. Nevertheless, they should exhibit a reduction with respect to those reported by the cited study due to the inhibitory effect of the higher concentration of salt produced by the dehydration, and due to the proteolysis of the enzymes themselves (Buscailhon et al., 1994).

As far as volatile formation from amino acid is concerned, in a previous publication dealing with the

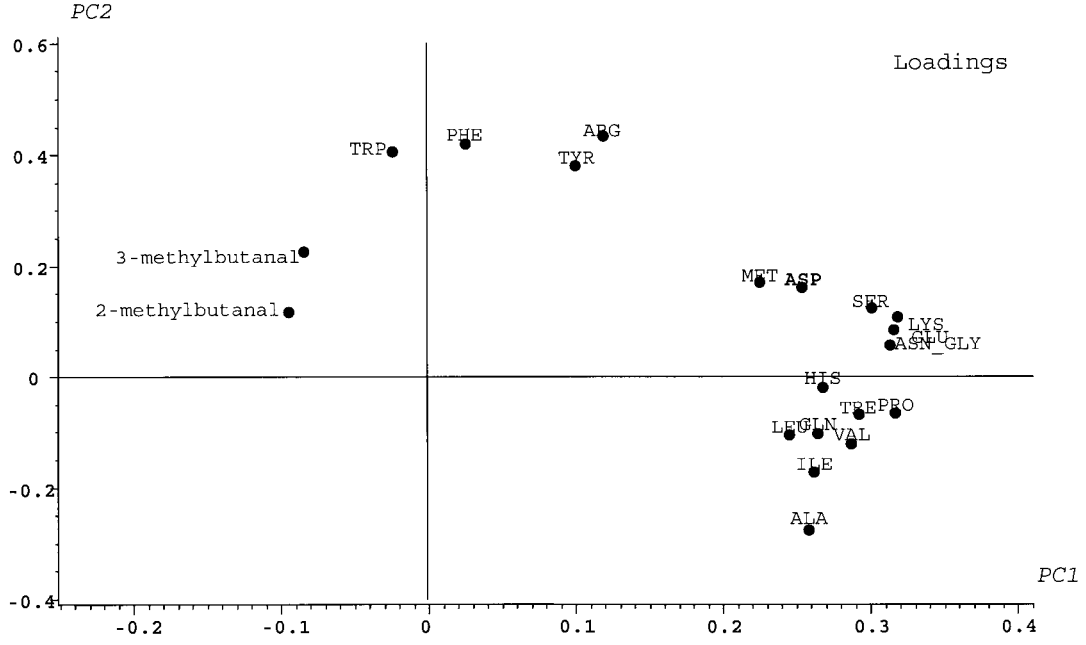

Fig. 1. Loading plot after principal component analysis of dry-cured Iberian ham amino acids and 2- and 3-methylbutanal.

variations in volatile compounds throughout the ripening of the hams, carried out on the same ham samples, we have reported an increase in some volatiles with amino acidic origin, such as 2- and 3-methylbutanal (Ruiz et al., 1999). Furthermore, performing a PCA with the amino acid and the volatile content, an inverse correlation between most amino acids and the mentioned branched aldehydes was found (data not shown), while the rest of volatile were placed near the origin. In order to create a better model, we performed the PCA with the amino acids and only these two volatile compounds. The loadings of this PCA are shown in Fig. 1. The first and second principal components (PC) explained 52 and 12% of the variation of the data, respectively. Free amino acids and 2- and 3-methylbutanal showed an inverse correlation, since all amino acids detected except for tryptophan, phenylalanine, arginine and tyrosine had high values in the first PC, while the two branched aldehydes showed negative ones. Thus, it seems that, from day 420 to the end of the ripening, the rate of amino acid formation was lower than the rate of degradation. This was reflected in the negative correlation showed by 2- and 3-methylbutanal and free amino acids in the PCA.

Variations in free amino acid contents among slice locations were much lower than between the two processes (data not shown). However differences did not follow any pattern. Such variations between locations could be produced by differences between proteolytic enzyme activities of different muscles (Flores, Alasnier, Aristroy, Navarro, Gandemer & Toldrá, 1996).

3.1. Peptides

A total of 15 peaks corresponding to peptides were detected using reverse-phase HPLC (Table 2). The method used to analyse the peptides of the perchloric acid soluble fractions did not allow the determination of their amino acid composition. However, the chromatographic conditions were adjusted to elute all free amino acids in the first part of the chromatogram, and successively, compounds with a higher hydrophobicity. Therefore, late eluting peptides should have a higher hydrophobicity. A similar tendency has been previously observed in dry-cured ham extracts (Aristoy & Toldrá, 1995; Kohata et al., 1992) and cheese extracts (Cliffe & Law, 1990; Cliffe, Marks & Mulholland, 1993; Furtula, Nakai, Amantea & Laleye, 1994) using reverse phase-HPLC. In fact, when the extracts were analysed by HPLC–APCI–MS, only small peptides weighing between 189 and 317 MW were found. Flores et al. (1997) have found larger peptides while studying proteolysis in dry-cured ham. The lack of larger peptides in the present study could be due to the procedure used to prepare the extracts. Although the selected method is the common procedure to obtain the extract for analysing peptide nitrogen (Martín, Antequera, Ruiz, 1998), it seems that the use of perchloric acid 0.6 N to deproteinize the samples, may produce the precipitation of large peptides.

Contrary to the trends observed in the free amino acid contents, hams ripened during 600 days had a higher chromatographic area of most of the peptides detected than those ripened for 420 days (Table 2). Such increase was statistically significant in five of them (peaks eluted in positions 1, 4, 8 9 and 14) and in the total chromatographic area. Therefore, it seems that, after 420 days of processing, peptide generation occurred at a higher rate than peptide hydrolysis in most of peptides detected.

Other authors have also detected an increase in peptide chromatographic area throughout the ripening of the hams (Aristoy & Toldrá, 1995; Møller, Hinrichsen & Jacobson, 1997). In some of these experiments, in which amino acid content was determined, the enhancement in peptide content occurred in parallel with an increase in amino acid content, reflecting an overall proteolysis activity.

In the present study it seems that proteolytic phenomena from day 420 to day 600 were not very intense, since (1) the increase in peptide content was lower than the reported in other hams, and (2) the rate of amino acid degradation was higher than the amino acid formation, whereas other studies have reported an amino acid content increase until the end of the ripening. Such a low proteolysis could be due to the longer curing process underwent by Iberian hams, to the ripening conditions, and to the differences in salt and moisture content. In this sense, several studies have proved the effect of all these factors on different proteolytic

Table 2
Area recovery of peaks corresponding to the peptides in dry-cured Iberian hams underwent a shortened (420 days) and a traditional (600 days) processing[a]

	420 days	600 days	*P*
Peak 1	6.03 ± 0.20	6.89 ± 0.21	0.0061
Peak 2	2.96 ± 0.12	2.90 ± 0.12	0.7557
Peak 3	0.89 ± 0.05	1.01 ± 0.06	0.1038
Peak 4	22.81 ± 1.35	30.03 ± 2.10	0.0054
Peak 5	22.12 ± 0.71	22.90 ± 0.95	0.5204
Peak 6	0.94 ± 0.08	1.56 ± 0.75	0.5486
Peak 7	1.21 ± 0.10	2.53 ± 0.74	0.0810
Peak 8	40.73 ± 1.28	44.74 ± 1.30	0.0404
Peak 9	0.79 ± 0.09	1.47 ± 0.10	0.0001
Peak 10	2.12 ± 0.22	3.47 ± 0.72	0.0857
Peak 11	0.35 ± 0.03	0.33 ± 0.04	0.77336
Peak 12	0.16 ± 0.02	0.17 ± 0.02	0.9316
Peak 13	0.60 ± 0.06	0.52 ± 0.07	0.4085
Peak 14	0.94 ± 0.12	1.66 ± 0.20	0.0037
Peak 15	33.15 ± 1.12	35.94 ± 1.21	0.1041
Total	135.81 ± 4.41	156.11 ± 4.60	0.0310

[a] Means of five hams analysed at six different slice locations (area peak/g de muestra) ± S.E.M.

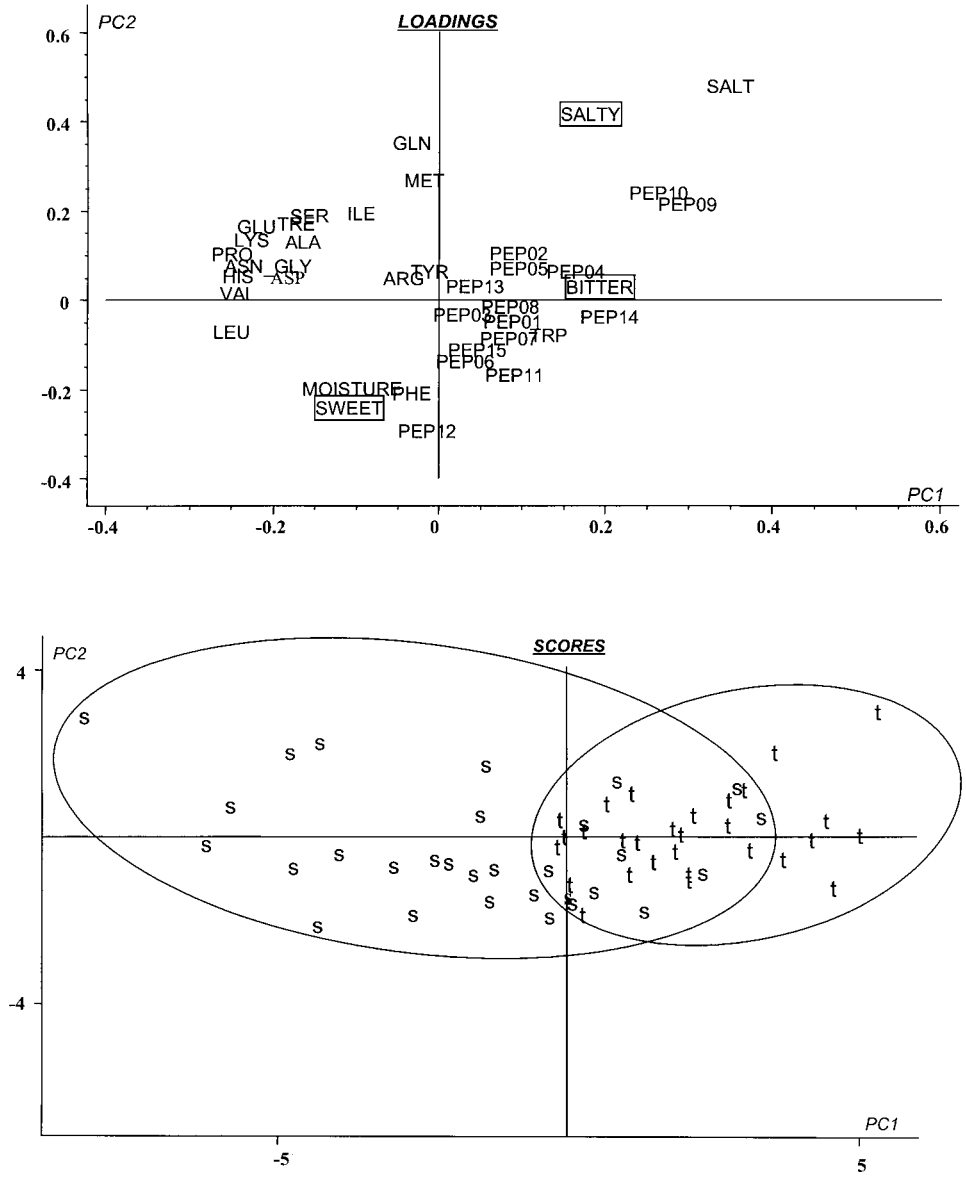

Fig. 2. Loading/score plots of principal components 1 and 2 from PLS regression on sensory and non-volatile components data. In the score plot "t" designates traditional processing, whereas "s" designates shortened processing time.

enzymes activities of pork muscles (Rico, Toldrá & Flores, 1991; Sárraga, Gil, Arnau & Monfort, 1989; Toldrá et al., 1992; Sárraga, Gil & García-Reguero, 1993).

3.2. Relationship between sensory analysis, amino acids and peptides

Figs. 2 and 3 show the loading and score plots of a PLS regression of the free amino acid, the peptide, the salt and the moisture contents, and the taste sensory results. Totally the four first PCs of the PLS analysis explained 51% of the variation in the chemical data and 41% of the variation in the sensory data. Salty taste was mainly determined by chloride content and was inversely correlated with moisture content, following the tendency observed in Parma ham by Careri, Mangia, Barbieri, Bolzoni, Virgili and Parolari, (1995). However, these authors have detected an even closer relationship with free glutamic acid. In the present study no influence of free amino acids on salty taste was detected.

Bitter notes correlated with some peaks of the peptides, especially with those eluted at the end of the chromatographic process (Fig. 3). This is not strange, since according to the chromatographic conditions, the longer the retention time, the higher the hydrophobicity of the molecule eluted. In this sense, Henriksen and Stahnke (1997) studying dried sausages have shown that bitterness of protein breakdown products is related to hydrophobicity. Moreover, using reverse-phase HPLC to study peptides in dry-cured ham (Aristoy & Toldrá,

1995) and in cheese (Cliffe & Law, 1990; Cliffe et al., 1993), a relationship between late-eluting peaks and bitterness has been found. The trend found in the present work agrees with these observations. In a recent paper by Virgili et al. (1998) a link between bitter taste in dry-cured hams and nonprotein nitrogen and some amino acids was revealed. Peptide content was not quantified in the cited work. Nevertheless, hams with lower bitter taste showed higher aminopeptidase activities. It can be deduced that these high aminopeptidase activity hams had lower peptide content, since these enzymes cleave peptides into amino acids.

Regarding sweetness, it was negatively correlated with salt content, and positively with moisture content and most free amino acids (Figs. 2 and 3). Sweet notes are not easily perceived in hams and, therefore, we think that sweetness was perceived as an absence of saltiness, with a certain influence of amino acids exhibiting sweet taste. Strangely, sweetness seemed to be positively influenced by phenyl alanine and peak 12. The former is a bitter taste amino acid and the later was a late-eluting peptide, and as exposed above, it was supposed to be a bitter taste peptide.

Concerning the score plots, hams that underwent the shortened process were characterised by being sweeter, more likely due to the lower salt and the higher free amino acid contents. On the other hand, long processed hams were characterised by higher values of saltiness and, at a lower extent, bitterness, probably because of their higher salt and peptide contents.

From the results of this work, it can be concluded that ripening times shorter than the traditional lead to

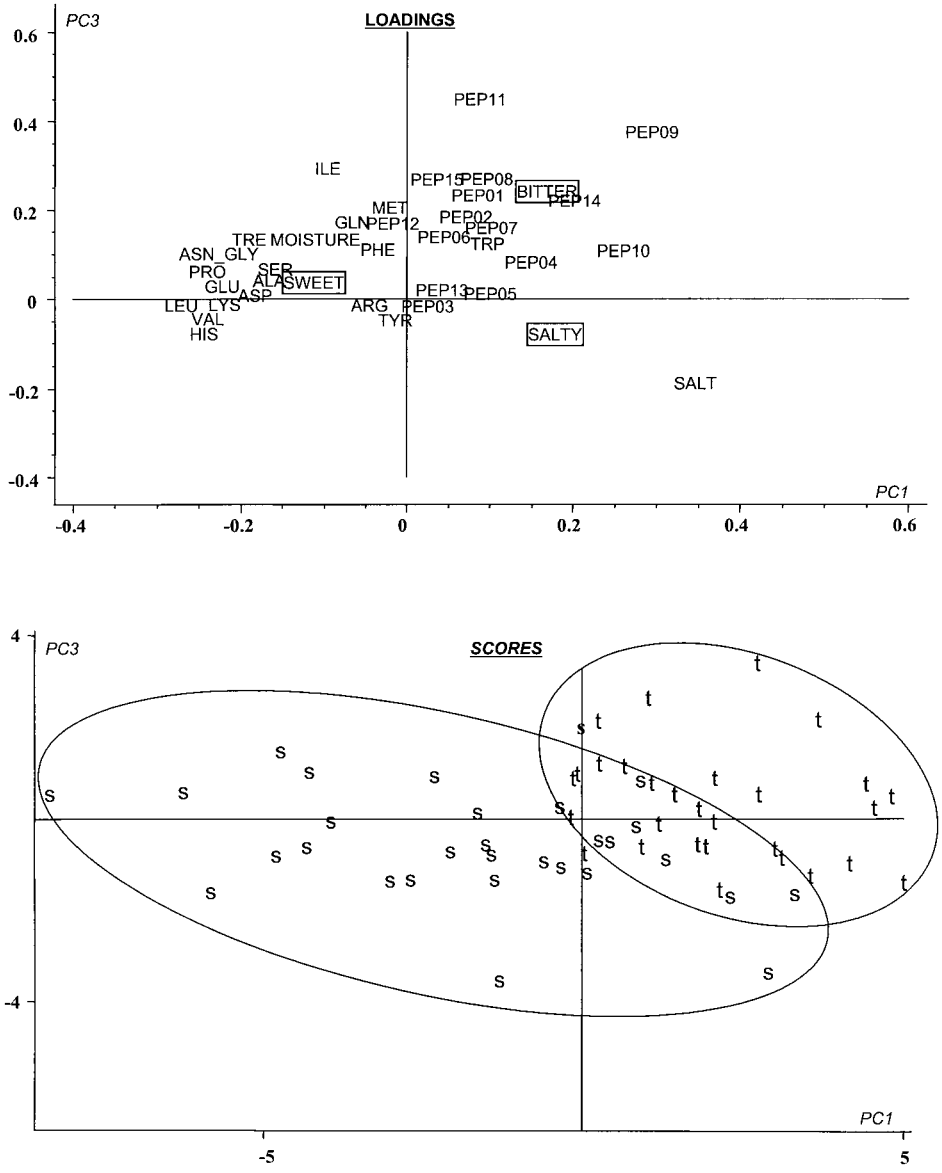

Fig. 3. Loading/score plots of principal components 1 and 3 from PLS regression on sensory and non-volatile components data. In the score plot "t" designates traditional processing, whereas "s" designates shortened processing time.

dry-cured Iberian hams with a slightly higher amino acid and a lower peptide content. In addition, it seems clear that amino acid degradation in dry-cured hams is associated to formation of volatile compounds, such as 2- and 3-methylbutanal.

References

Acree, T., & Arn, H. (1997). *Flavornet. Gas chromatography–olfactometry (GCO) of natural products*. Cornell University. http://www.nysaes.cornell.edu/flavornet/.

AOAC (1984). *Official methods of analysis. Method 24010*. (3rd ed.). Washington, DC: AOAC.

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.

Aristoy, M. C., & Toldrá, F. (1995). Isolation of flavor peptides from raw pork meat and dry-cured ham. In G. Charalambous, *Food flavors: generation, analysis and process influence* (pp. 1323–1344). London: Elsevier Science.

Astiasaran, I., Cid, C., Melgar, J., & Bello, J. (1989). Modifications of the nitrogen fractions in white pork ham during curing. *Revista Española de Ciencia y Tecnología e los Alimentos, 29*, 99–106.

Buscailhon, S., Monin, G., Cornet, M., & Bousset, J. (1994). Time-related changes in nitrogen fractions and free amino acids of lean tissue of French dry-cured ham. *Meat Science, 37*, 449–456.

CAMO A/S (1996). *The unscrambler. User's guide, version 6.11*. Trondheim, Norway.

Careri, M., Mangia, A., Barbieri, G., Bolzoni, L., Virgili, R., & Parolari, G. (1995). Sensory property relationships to chemical data of Italian dry-cured ham. *Journal of Food Science, 58*, 968–972.

Cliffe, A. J., & Law, B. A. (1990). Peptide composition of enzyme-treated Cheddar cheese slurries, determined by reverse phase high performance liquid chromatography. *Food Chemistry, 36*, 73–80.

Cliffe, A. J., Marks, J. M., & Mulholland, F. (1993). Isolation and characterization of non-volatile flavours from cheese: peptide profile of flavour fractions from Cheddar cheese, determined by reverse-phase high-performance liquid chromatography. *International Dairy Journal, 3*, 379–387.

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

Diario Oficial de Extremadura (1990). *Reglamento de la Denominaciòn de Origen "Jamones and Paletas Dehesa de Extremadura" y de su Consejo Regulador* (p. 1). DOE extraordinario no2, 30-5-90.

Flores, J., Bermell, S., Nieto, P., & Costell, E. (1989). Cambios químicos en las proteínas del jamón durante los procesos de curado, lento y rápido, y su relación con la calidad. *Revista Española de Ciencia y Tecnologoía de los Alimentos, 24*, 503–509.

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

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.

Furtula, V., Nakai, S., Amantea, G. F., & Laleye, L. (1984). Reverse phase HPLC analysis of cheeses samples aged by a fast-ripening process. *Journal of Food Science, 59*, 528–532.

García, C., Ventanas, Antequera, T., Ruiz, J., Cava, R., & Alvarez, P. (1996). Measuring sensorial quality of Iberian ham by Rasch model. *Journal of Food Quality, 19*, 397–412.

Henriksen, A. P., & Stahnke, L. H. (1997). Sensory and chromatographic evaluations of water soluble fractions from dried sausages. *Journal of Agricultural and Food Chemistry, 45*, 2679–2684.

ISO (1973). *Meat and meat product-determination of moisture content method 1442*. Geneva: ISO.

Kato, H., Rhue, M. R., & Nishimura, T. (1989). Role of free amino acids and peptides in food taste. In R. T. Teranishi, R. G. Buttery, & F. Shahidi, *Flavor chemistry. Trends and developments* (pp. 158–174). Washington, DC: American Chemical Society.

Kohata, H., Numata, M., Kawaguchi, M., Nakamura, T., & Arakawa, N. (1992). The effect of salt composition on taste development in prosciutto. In: *Proceedings of the 38th ICoMST* (pp. 1271–1274). Clermont-Ferrand, France.

Kondjoyan N., & Berdagué, J. L. (1996). *A compilation of relative retention indices for the analysis of aromatic compounds*. Laboratoire Flaveur, Station de recherches sur la Viande, INRA THEIX.

MacLeod, G. (1996). The scientific and technological basis of meat flavours. In G. G. Birch, & M. G. Lindley, *Developments in food flavours* (pp. 191–223). London: Elsevier.

Maga, J. A. (1994). Umami flavour of meat. In F. Shahidi, *Flavor of meat and meat products* (pp. 98–115). London: Blackie Academic & Professional.

Martín, L., Antequera, T., Córdoba, J. J., Timón, M. L., & Ventanas, J. (1998) Formation of non-volatile flavor compounds in Iberian dry-cured ham during processing. In *Proceedings of the 44th ICoMST* (pp. 1008–1009), Barcelona.

Martín, L., Antequera, L., Ruiz, J., Cava, R., Tejeda, J. F., & Córdoba, J. J. (1998). Influence of processing conditions of Iberian ham on proteolysis during ripening. *Food Science and Technology International, 4*, 17–22.

Mateo, J., Domínguez, M. C., Aguirrezábal, M. M., & Zumalacárregui (1996). Taste compounds in chorizo and their changes during ripening. *Meat Science, 44*, 2455–2540.

Møller, J. H., Hinrichsen, L., & Jacobsen, T. (1997). Evaluation of peptides generated in Italian-style dry-cured ham during processing. *Journal of Agricultural and Food Chemistry, 45*, 3123–3128.

Ramarathnam, N., & Rubin, L. J. (1994). *The flavour of cured meat*. In: F. Shahidi, *Flavour of meat and meat products*. (pp. 174–198). London: Blackie Academic & Professional.

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

Rodríguez, M., Núñez, F., Córdoba, J. J., Bermúdez, E., & Asensio, M. A. (1998). Evaluation of proteolytic activity of microorganisms isolated from dry cured ham. *Journal of Applied Microbiology, 85*, 905–912.

Ruiz, J., Ventanas, J., Cava, R., Andrés, A. I., & García, C. (1999). Volatile compounds of dry-cured Iberian ham as affected by the length of the curing process. *Meat Science, 52*, 19–27.

Ruiz, J., Ventanas, J., Cava, R., Timón, M. L., & García, C. (1998). Sensory characteristics of Iberian ham: influence of processing time and slice location. *Food Research International, 31*, 53–58.

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.

Sárraga, C., Gil, M., & García-Regueiro, J. A. (1993). Comparison of calpain and cathepsin (B, L and D) activities during dry-cured ham processing from heavy and light Large White pigs. *Journal of the Science of Food and Agriculture, 62*, 71–75.

Schivazappa, C., Saccani, G., Virgili, R., & Bordini, C. S. (1995). Changes in free amino acids during dry-curing of typical Italian raw ham. *Industria Conserve, 70*, 377–385.

SAS (1997). *SAS user's guide: statistics*. Cary, NC: SAS Institute Inc.

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

Ventanas, J., Córdoba, J. J., Antequera, T., García, C., López-Bote, C., & Asensio, M. A. (1992). Hydrolysis and Maillard reactions during ripening of Iberian ham. *Journal of Food Science, 57*, 813–815.

Virgili, R., Schivazappa, C, Parolari, G., Soresi Bordini, C., & Degni, M. (1998). Protesase in fresh pork muscle and their influence on bitter taste formation in dry-cured ham. *Journal of Food Biochemistry, 22*, 53–63.

Yang, C., & Sepulveda, F. (1985). Separation of phenylthiocarbamyl amino acids by high-performance liquid chromatography on Spherisorb octadecylsilane columns. *Journal of Chromatography, 346*, 413–416.