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Characterization of defective textures in dry-cured ham by compositional and HPLC analysis of soluble substances of low-molecular weight

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Abstract

The relationship of normal and defective textures in dry-cured ham to compositional and chromatographic parameters for low-molecular weight substances was examined. Three different types of defective texture, viz. soft, pasty and very pasty, were considered. An analysis of variance of the test results revealed a significant relationship ($P < 0.05$) between the presence of defective textures and both the compositional parameters studied (viz. pH and the moisture, non-protein nitrogen and salt contents) and the chromatographic peaks obtained. A post-hoc analysis based on the Duncan method allowed significantly different ($\alpha = 0.05$) groups to be established on the basis of the different abnormal textures (soft, pasty and very pasty). Finally, a discriminant analysis of the compositional results yielded functions that allowed hams with normal and defective textures to be discriminated. Also, the discriminant analysis of chromatographic peaks provided functions that allowed normal and pasty hams—but not normal and soft hams—to be distinguished, which suggest a differential origin for the texture defects examined.

Keywords: Cured ham; Defective textures; Proteolysis; Compositional parameters; HPLC

1. Introduction

Spanish dry-cured ham is a typically Spanish meat product widely known and appreciated by consumers. The present growth of the ham manufacturing sector and its projections rely on both the increased consumption and popularity of dry-cured ham in Spain, and on a growing foreign market for this product. Hence, special care is exercised to obtain a quality product as homogeneous and acceptable by prospective consumers as possible. Ham quality rests both on some characteristics of the raw material (e.g. genetic factors, pH, endogenous enzyme activity, amount of subcutaneous fat, water-retention capacity) and on technological variables (e.g. the use of controlled salt, temperature and relative humidity levels at the different stages of the curing process), which, in combination, dictate the sensory properties (viz. flavour, odour, colour, texture) of the finished product (Rovira, Ordóñez, & Jaime, 1996).

The presence of some defects in the sensory properties of cured ham dramatically decrease its quality in some cases. One such defect is a pasty texture; pasty ham is exceedingly, soft and sticky, but scarcely elastic, and exhibits anomalous flavours and odours. Not to be confused with a pasty texture, a soft texture in ham is the result of the product not reaching adequate hardness and consistency by the end of the curing process (Parolari, Virgili, & Schivazappa, 1994).

The principal origin of pasty textures in ham is strong proteolytic activity (Feidt, Brun-Bellut, & Dransfield, 1998; Toldrá & Flores, 2000) that can affect proteins in both muscles and connective tissue (Arnau, Guerrero, & Pere, 1997; Virgili, Parolari, Schivazappa, Soresi-Bordini, & Borri, 1995). The underlying degradation is caused by cathepsin B, L, H and D, and, to a lesser extent, by

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calpain (Hortos, Gil, & Sárraga, 1994). The ensuing anomalies can arise both at the early (Parreño, Sárraga, Gil, & Cussó, 1990) and at the late stages of the process where an increased temperature is used. Thus, cathepsin B has been related to the degradation of low-molecular weight protein fractions to free aminoacids (Parreño, Cussó, Gil, & Sárraga, 1994) and to those of actin and myosin (Toldrá, Flores, & Sanz, 1997); also, cathepsin B and L are particularly active at a high temperature (Toldrá, Rico, & Flores, 1993). A high enzyme activity results in uncontrolled hydrolysis and in the potential loss of protein structure, thereby resulting in an anomalous texture or even an undesirable colour in the product. Also, it increases the amounts of free aminoacids (which form tyrosine deposits and "white films"), low-molecular weight compounds (e.g. peptides) and, in general, the non-protein nitrogen (NPN) fraction (Batlle, Aristoy, & Toldrá, 2000; Toldrá, 1992) which is present at increased levels in pasty hams relative to normal hams (García-Garrido, Quiles-Zafra, Tapiador, & Luque de Castro, 1999).

The origin of a pasty texture in ham has also been related to the genetics of the animals from which the raw material is obtained (Parolari et al., 1994), as well as to the amount of salt adsorbed during the salting stage (Martín, Córdoba, Antequera, Timón, & Ventanas, 1998; Toldrá & Etherington, 1998).

The aim of this work was to characterize hams with and without a soft or pasty texture in terms of analytical determinations of pH and the moisture, NaCl and NPN contents, as well as from the chromatographic profiles for low-molecular weight substances as obtained by reversed-phase HPLC. This separation technique is quite expeditious, robust, reproducible, precise, easy to operate and readily adapted for systematic analysis, all of which make it highly suitable for food analysis and hence for the separation of aminoacids, peptides and proteins (Moyá, Flores, Aristoy, & Toldrá, 2001a, 2001b; Flores, Moyá, Aristoy, & Toldrá, 2000; Hansen-Møller, Hinrichesen, & Jacobsen, 1997) on the basis of—essentially—hydrophobicity differences (Regnier & Gooding, 1980).

2. Material and methods

2.1. Analytical methods

2.1.1. Compositional analysis

pH, and the moisture and NaCl contents, were determined using the official methods of analysis issued by the Spanish Ministry of Agriculture, Fisheries and Food (1994).

2.1.2. Non-protein nitrogen

The NPN content was determined using the method of Keresse (1984), modified as follows: an amount of 5 g of sample was mixed with 30 ml of 6 N $HClO_4$ and allowed to stand at 4 °C for 1 h. Then, the mixture was passed through a Whatman no. 6 filter, the filtrate being adjusted to pH 6.00 with 30% (v/v) KOH and made to 100 ml. This solution was allowed to stand at 4 °C for at least 12 h and filtered again through Whatman no. 6 paper. A 20-ml aliquot of this extract was used to determine NPN by the Kjeldahl method, using a digester and a semi-automatic Büchi distillation apparatus.

2.1.3. Reversed-phase HPLC

The chromatographic analysis of the $HClO_4$ extract (Rodríguez-Núñez, Aristoy, & Toldrá, 1995) was performed on a Hewlett-Packard 1100 Series instrument equipped with a diode array detector and a 250×4.6 mm Kromasil C-18 column of 5 μm particle size and 120 Å pore size. The operating conditions were as follows: sample volume 5 μl, mobile phase flow-rate 0.5 ml/min, $\lambda = (215 \pm 2.5)$ nm. Each test was started with a linear gradient from 100% mobile phase (0.1% v/v trifluoroacetic acid, TFA, in ultrapure water) to 60% of the starting mobile phase and 40% of the elution mobile phase (0.1% v/v TFA in acetonitrile) over 40 min.

2.2. Samples

Biceps femoris muscle samples (free of external fat and connective tissue) were taken from cured hams belonging to various batches of n elements from different European suppliers. The hams were previously boned and classified into four groups according to the results of a sensory evaluation conducted by an expert panel in accordance with Spanish standard UNE 87024-2-1996, namely: Group 1, very pasty texture ($n = 9$); Group 2, pasty texture ($n = 20$); Group 3, soft texture ($n = 13$); and Group 4, normal texture ($n = 15$).

Hams with a pasty texture were split into two groups (*viz*. pasty and very pasty hams) on the basis not only of the sensory evaluation, but also of an unexpected pH difference.

2.3. Statistical analysis

Experimental data were statistically processed using the software package SPSS v. 9.0 for Windows. Chromatographic and compositional data were subjected to a separate analysis of variance (ANOVA) and a discriminant analysis (DA).

3. Results and discussion

Table 1 shows the results of the NaCl, moisture, NPN and pH determinations. An analysis of variance (ANOVA) revealed the presence of significant differences

Table 1
Statistics for the compositional parameters studied, grouped according to texture

	N	Mean	Standard deviation	95% Confidence interval for mean	
				Lower bound	Upper bound
% NaCl					
Very pasty	9	12.3	2.8	10.1	14.4
Pasty	20	14.1	1.9	13.2	15.0
Soft	13	14.6	2.1	13.3	15.9
Normal	15	15.2	1.6	14.3	16.1
% Moisture					
Very pasty	9	60.1	4.2	56.9	63.4
Pasty	20	59.4	3.1	57.9	60.8
Soft	13	61.5	2.9	59.7	63.2
Normal	15	56.9	1.3	56.2	57.7
% NNP					
Very pasty	8	4.0	0.4	3.7	4.3
Pasty	20	4.0	0.7	3.7	4.3
Soft	13	3.0	0.6	2.6	3.3
Normal	15	3.3	0.5	3.0	3.5
pH					
Very pasty	9	6.3	0.3	6.1	6.6
Pasty	20	6.0	0.2	6.0	6.1
Soft	13	6.2	0.2	6.0	6.3
Normal	15	6.2	0.1	6.1	6.2

among groups. The data in these two tables allow the following conclusions to be drawn:

1. Hams with a soft texture possess much higher moisture contents than hams with a normal texture. Pasty and very pasty hams also contain more water than do normal hams ($P < 0.005$).
2. The NaCl contents of soft and pasty hams are similar but lower than those of normal hams. In pasty and very pasty hams, pastiness decreases with increasing salt content ($P < 0.05$).
3. Pasty hams possess higher NPN contents than hams of normal texture. Also, soft hams exhibit the lowest contents ($P < 0.001$). The post-hoc analysis (Duncan method) split hams into two categories, viz. normal and soft hams on the one hand, and pasty and very pasty hams on the other.
4. Normal and soft hams exhibit similar pH values. On the other hand, pasty hams have lower values. Surprisingly, very pasty hams exhibit the highest pH values ($P < 0.005$). This parameter allows very pasty hams to be readily distinguished from pasty hams. These results can be ascribed to proteolysis in very pasty hams substantially differing—either in its mechanism or its extent—from that in pasty hams.

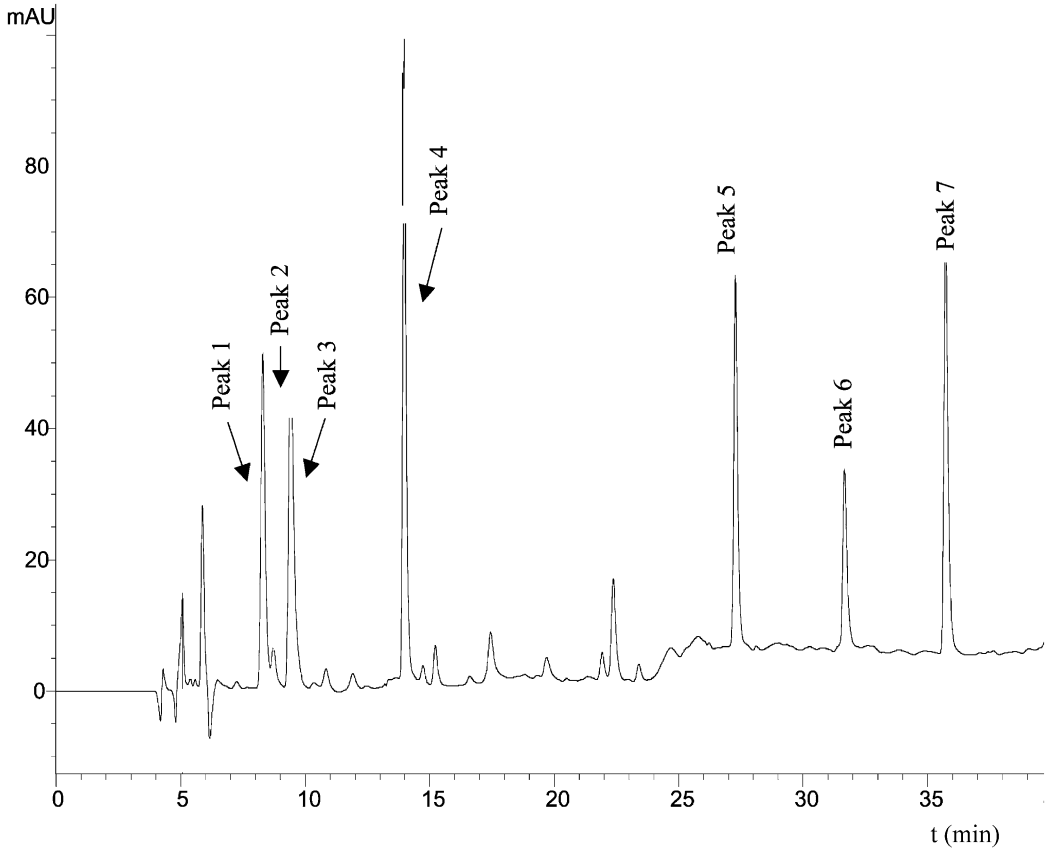

Fig. 1. Chromatographic profile for soluble compounds of low-molecular weight obtained from an extract of *Biceps femoris* muscle.

The chromatographic profiles for the $HClO_4$ extracts were used to select peaks common to all samples with a view to characterizing ham texture. Fig. 1 shows a typical chromatogram obtained under the operating conditions described in the Experimental section. Table 2 shows the results as per cent peak areas for each texture group. An analysis of variance (ANOVA) revealed significant differences among groups ($P < 0.005$). Also, a post-hoc analysis using the Duncan method confirmed the presence of two significantly different groups ($\alpha = 0.05$), namely: one consisting of normal and soft hams, and the other encompassing pasty and very pasty hams. From the per cent area values it follows that:

Table 2
Statistics for the chromatographic results, grouped according to texture

	N	Mean% PEAKS AREA	Standard deviation	95% Confidence interval for mean	
				Lower Bound	Upper Bound
PEAK 1					
Very pasty	9	8.7	1.3	7.7	9.7
Pasty	20	10.6	1.9	9.7	11.5
Soft	13	12.3	1.0	11.6	12.9
Normal	15	11.5	2.5	10.1	12.9
PEAK 2					
Very pasty	9	15	0.2	1.4	1.7
Pasty	20	1.6	0.3	1.5	1.7
Soft	13	2.1	0.2	2.0	2.2
Normal	15	2.1	0.8	1.7	2.6
PEAK 3					
Very pasty	9	14.1	2.2	12.4	15.8
Pasty	20	14.0	2.!	13.0	15.0
Soft	13	17.8	2.1	16.6	19.1
Normal	35	16.6	4.5	14.1	19.3
PEAK 4					
Very pasty	9	15.6	3.3	13.0	38.2
Pasty	20	15.0	1.6	14.3	15.7
Soft	13	18.5	2.5	17.0	20.!
Normal	15	17.9	3.1	16.2	19.6
PEAK 5					
Very pasty	9	11.3	2.2	9.6	12.9
Pasty	20	11.8	1.5	11.1	12.5
Soft	13	9.5	1.1	8.8	10.2
Normal	15	9.7	2.2	8.5	10.9
PEAK 6					
Very pasty	9	5.4	0.6	4.9	5.8
Pasty	20	5.4	0.3	5.2	5.6
Soft	33	4.6	0.5	4.2	4.9
Normal	15	4.5	0.9	4.0	5.0
PEAK 7					
Very pasty	9	12.6	2.0	11.1	14.2
Pasty	20	13.0	1.6	12.2	13.7
Soft	13	30.3	2.0	9.1	11.5
Normal	35	9.5	2.3	8.3	10.8

1. The mean per cent areas for peaks 1–4 are greater in normal and soft hams; those in very pasty hams are the lowest, but significantly different from those in pasty hams. These peaks, which elute first, correspond to hydrophilic compounds of low molecular weight (Toldrá & Aristoy, 1993) and are associated to pleasant flavours (Cliffe & Law, 1990).
2. The mean per cent areas of peaks 5–7 are greater in pasty hams and markedly smaller in normal and soft hams (i.e. these peaks exhibit the opposite trend of the previous ones in this respect). In peaks 6 and 7, normal hams exhibit the smallest areas. These peaks correspond to compounds of an increased hydrophobic character. Compounds of low molecular weight with a relative hydrophobicity $Q > 1400$ kcal/mol exhibit a typical bitter, metallic flavour (Champion & Stanley, 1982; Cliffe, Marks, & Mulholland, 1993; Timón, Barnadiaran, & Ventanas, 1995), which is one of the anomalous features of pasty hams.

The discriminant analysis of the moisture, NaCl and pH data provided two discriminant functions that accounted for 85% of the variance. Fig. 2 shows the scatter diagram obtained from the scores of the two discriminant functions. As can be seen, the first function accounts for 50.4% of the variance and allows normal hams to be distinguished from pasty and soft hams; on the other hand, the second function, which accounts for 34.6% of the variance, discriminates between the different types of anomalous texture.

The data obtained from the selected chromatographic peaks were also subjected to a discriminant analysis that provided two discriminant functions (Fig. 3) jointly accounting for 97.1% of the variance; the first such

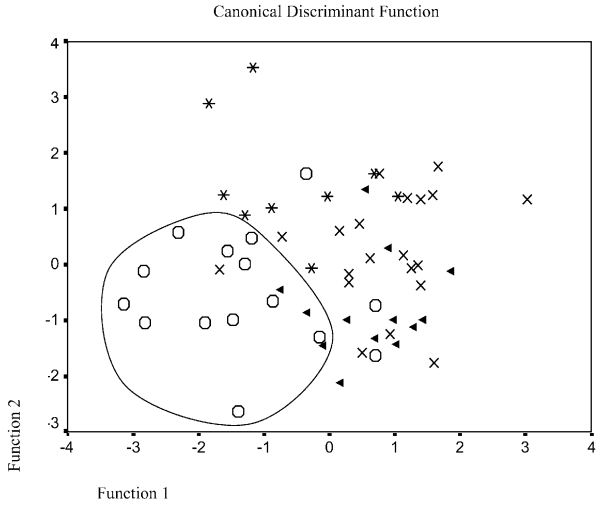

Fig. 2. Scatter graph obtained using the first two discriminant functions derived from the discriminant analysis of the moisture, NaCl and NNP data: ○ Normal; ◀ Soft; **X** Pasty; * Very pasty.

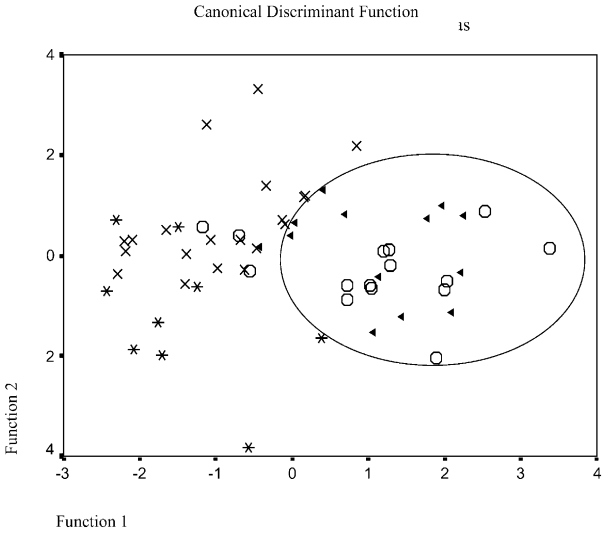

Fig. 3. Scatter graph obtained using the first two discriminant functions derived from the discriminant analysis of the percent peak areas: ○ Normal; ◀ Soft; **X** Pasty; * Very pasty.

function, which accounts for 72.9% of the variance, distinguishes normal and soft hams from pasty hams.

4. Conclusions

In this work, the presence of defective (viz. soft and pasty) textures in Spanish dry-cured ham was related to various physico-chemical parameters. Thus, a pasty texture is clearly related to high NPN and moisture contents, and a low NaCl content; on the other hand, a soft texture is related to a high moisture content—higher than in pasty hams—and to low NPN and NaCl contents.

The analysis of the chromatographic data allowed better discriminating functions to be derived that distinguish normal and soft hams from pasty hams, but not soft and normal hams. This confirms that, unlike a pasty texture, a soft texture is not the result of anomalous proteolysis but, possibly, of inappropriate desiccation. Pasty and very pasty hams both undergo proteolysis during the manufacturing process; however, their marked differences in pH may be the result of differences in the extent of proteolysis and hence in the amounts of basic compounds released.

Based on the results, the liquid chromatography technique is a powerful tool for quality control in the manufacturing of Spanish dry-cured ham. Its combined use with mass spectrometry is the best choice for identifying the compounds that yield the peaks used for discriminating purposes in this work.

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