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Postmortem meat quality and sex affect textural properties and protein breakdown of dry-cured ham

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Abstract

Texture measurements by instrumental texture profile analysis (TPA) and protein degradation analysis by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) were performed on 30 dry-cured hams resulting from four different postmortem meat qualities categories (PSE, RSE, RFN and DFD). The main differences were observed in dry-cured hams from PSE and RFN meat qualities. Penetration force (80%), hardness, springiness, cohesiveness and chewiness, were significantly lower ($P < 0.05$) in PSE than in RFN quality classes. The rate of the ripening process was affected as a higher proteolysis and absence of fragments at 150 and 85 KDa in PSE in relation to RFN classes, and with an intermediate proteolysis of RSE and DFD classes. The effect of sex was observed as a significant ($P < 0.05$) low hardness in the hams obtained from female pigs. The duration of the ripening, for a better uniformity in dry-cured ham production, should be adapted to the initial pH and to drip loss parameters of the raw material.

1. Introduction

Texture characteristics of foods constitute one of the main sensory attributes perceived by consumers. Instrumental texture profile analysis (TPA) is a good tool to assess textural properties of food. The knowledge of physical sense and the correct calculation of the parameters allow a good interpretation of results (Pons and Fiszman, 1996). Much research has been published on fresh meat texture but only a very few reports on dry-cured meat products (Parolari et al., 1994; Monin et al., 1997).

Proteolysis of muscle structural proteins is the main biochemical mechanism responsible for the progressive meat tenderization during dry-curing. The enzymes mainly involved in this process are proteinases (cathepsins B, D, H and L and, to a less extent, calpains) (Toldrá et al., 1993) and exopeptidases (peptidases and aminopeptidases) (Toldrá and Flores, 1998). Sensory characteristics of dry-cured ham are strongly affected by these enzymatic reactions although, its activity levels depend on the properties of raw ham such as age, crossbreeding, processing conditions (temperature, time and water activity) and curing agents (mainly salt) (Toldrá et al., 1997; Toldrá and Flores, 1998).

Tenderness is an appreciated and desirable characteristic of dry-cured ham but an excessive degree of proteolysis can result in lower meat texture that can affect consumer acceptability. The reasons for an abnormal intense post-mortem proteolysis are not known. An increment in the addition of added salt to prevent this effect during ageing was proposed by Virgili et al. (1995), in view of its powerful inhibitory effect against cathepsins (Rico et al., 1990, 1991; Toldrá et al., 1992a). However, dry-cured hams are usually characterised by a high sodium chloride content. An additional increase of sodium chloride would not be accepted by consumers due to both sensory impact and healthy reasons. As many factors affect ham tenderness, our objective was to study how the post-mortem meat quality and sex affect protein degradation and texture of ham in order to obtain dry-cured hams with standardised sensory quality.

2. Materials and methods

2.1. Materials

Thirty hams, taken from 6-month-old pigs (Landrace (sire) × crossbred sow (Large white × Landrace)) from a

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local factory, were submitted to a 12 month dry-curing process, consisting in the typical stages of salting, post-salting, and ripening-drying (Toldrá et al., 1997). A 2 kg portion was cut from the centre part of each ham, perpendicular to the bones, and containing the following muscles: *Biceps femoris*, *Semimembranosus*, and *Semitendinosus*. The samples had been previously classified by sex and class (RFN, RSE, PSE o DFD) depending on the postmortem meat quality attributes (pH, L_u and drip loss) (Kauffman et al., 1993; Warner et al., 1993, 1997; van Laack et al., 1994; Cheah et al., 1998). The pH was measured at 2 and 24 h, in *Semimembranosus* muscle, with a portable pH-meter Crison 506 (Crison Instruments, Alella, Barcelona, Spain). The color, L*, a*, b* coordinates, was measured on the *Longissimus thoracis* at the last rib (L_u, a_u, and b_u) at 24 h postmortem with a Minolta Chromameter (Minolta, Camera Co., Osaka, Japan). The drip loss (DL) was measured in *Semimembranosus* muscle by the method of Warris (1982). Postmortem meat quality was classified as PSE ($pH_{2\ h} \leqslant 5.8$; L-value $\geqslant 50$; DL $\geqslant 6\%$), RSE ($pH_{2\ h} \leqslant 5.8$; L-value 44–50; DL $\geqslant 6\%$), RFN or normal ($pH_{2\ h} > 5.8$; $pH_{24\ h} < 6.0$; L-value 44–50; DL < 6%) and DFD ($pH_{24\ h} > 6.0$; L-value $\leqslant 44$; DL $\leqslant 4\%$). The number of hams selected for the different categories was 4 PSE (3 female and 1 male), 7 RSE (2 female and 5 male), 13 RFN (7 female and 6 male) and 6 DFD (4 female and 2 male). Moisture content of dry-cured hams was determined after dehydration at 100°C to a constant weight (ISO, 1973). The intramuscular fat content (IMF) in *Semimembranosus* muscle was analysed by the method of Folch et al. (1957).

2.2. Electrophoresis

The procedure was based on the method described by Toldrá et al. (1992b) with slight modifications. The proteins were extracted from 0.1 g of minced muscle *Biceps femoris*, with no visible fat or connective tissue, and homogenised in 1 ml of 50 mM Tris buffer, pH 6.8, containing 8 M urea, 2 M thiourea, 75 mM dithiothreitol, 3% (w/v) SDS and 0.05% bromophenol blue by using a minipolytron PT 1200 (Kinematica, Switzerland) homogeniser. The extract was centrifuged at 14 000 rpm during 20 min at 4°C. The supernatant was filtered through glass wool and heated at 100°C for 4 min and used for electrophoresis. Protein concentration in the sample was measured by the method of Bradford (1976) using bovine serum albumin as standard. The relative molecular mass was determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using 10% polyacrylamide gels (Laemli, 1970). In each lane 12 μg of protein were injected. The proteins in the gels were stained for 2 h with Coomassie Brilliant Blue R-250, (0.5 g litre−1) in aqueous methanol (50%) and acetic acid (10%), and destained with a solution of aqueous methanol (10%) and acetic acid (7.5%). Standard proteins (BioRad, Richmond, CA, USA) were simultaneously run for protein identification.

2.3. Texture analysis

Texture measurements in form of texture profile analysis (TPA) (Bourne, 1978; Henry et al., 1971) of the obtained samples were performed, at room temperature, with a TA-XT2 Texture Analyser (Stable Micro Systems, Godalming, UK) with a 5 kg load cell. The Texture Expert, version 1.11 computer program by Stable Micro System was used for data collection and calculation. The samples, 2 cm cube *Biceps femoris* dry-cured muscle, were kept in a sodium chloride saturated atmosphere until measured. Each sample was compressed axially in two consecutive cycles of 20% compression with a flat plunger 75 mm in diameter (SMS-P/75), with 5 s between cycles. The cross-head moved at a constant speed of 1 mm/s. From the TPA curves, the following texture parameters were measured: hardness, springiness, cohesiveness, adhesiveness and chewiness. In this case gumminess was omitted because, according to Szczesniak (1995), it is a characteristic parameter for semisolid samples only, and should not be used on the same product along with chewiness. Hardness was defined by peak force during first compression cycle. Cohesiveness was calculated as the ratio of the area under the second curve to the area under the first curve. Springiness was defined as a ratio of the time recorded between the start of the second area and the second probe reversal to the time recorded between the start of the first area and the first probe reversal. Chewiness was obtained by multiplying hardness, cohesiveness and springiness. The adhesiveness was the negative area under the curve obtained between cycles and expressed in g × s as indicated by software.

Another type of test was performed to assess resistance to penetration which is an index of hardness during mastication. One compression cycle of 80% was used to measure hardness which was defined as the maximum force (g) required to achieve the given deformation of the sample by a 10 mm diameter cylinder plunger (SMS-P/10) at a constant speed of 1 mm/s.

2.4. Statistical analysis

Statistical analysis was performed by using the software package Statgraphic plus. At least six replication were done for TPA and three for chemical analysis. The statistic analysis Fisher's least significant difference (LSD) procedure was applied to discriminate among the means of textural properties and chemical parameters.

3. Results

The hams were classified in four quality classes based upon pH, L_u and DL measured on the carcasses at post-mortem time. The moisture and intramuscular fat content (IMF) was also measured in the dry-cured hams (Table 1). Moisture content of dry-cured hams was only significant different ($P < 0.05$) by sex, being higher in females than in males, but there were not differences by quality classes. The IMF content in *Semimembranosus* muscle was significantly lower ($P < 0.05$) in RSE than in RFN quality classes, although there were no differences between sex.

3.1. Texture analysis

The textural properties of the hams were analysed after 12 month of dry-curing processing. A penetration test (80%) was done resulting in an absence of myofibrils breakdown. From the shape of the curves, one can infer that up to approximately 50% penetration the fibers were softly compacted. From this value, the slope of the curves rose more rapidly. The dry-cured ham resistance to penetration was significant different ($P < 0.05$) in the quality classes (see Hardness 80% in Table 2). Dry-cured hams from normal quality meat (RFN) gave a higher significant ($P < 0.05$) resistance than dry-cured hams from PSE meats. Dry-cured hams from RSE and DFD quality classes resulted with lower resistance values than RFN class but the difference was not significant ($P < 0.05$). The effect of sex on resistance to penetration was also significant ($P < 0.05$), being higher in males than in females (see Table 2).

TPA of dry-cured hams resulted in important differences among quality classes. The TPA of a PSE dry-cured ham is shown in Fig. 1. The asymmetry in the curves represents the lack of instantaneous recovery of the samples that was only detected in exudative ones (PSE and RSE). In dry-cured hams from normal quality meat (RFN), the TPA did not show an initial lower compression force (as can be seen in Fig. 1 for PSE), i.e. an absence of change in slope which could be attributed to firmer samples. The TPA explained five textural parameters; hardness 20%, springiness, cohesiveness, chewiness and adhesiveness. The hardness 20% obtained from the TPA was not significant among the different quality classes (see Table 2), probably because this parameter takes into account the humidity content in the sample instead of showing the proteolysis of myofibrilar structure. The sex showed a significant effect on hardness at 20% as also happened with moisture content. Dry-cured hams resulted with low springiness

Table 1
Moisture and intramuscular fat content (IMF) of dry-cured hams. Least square means (LSM) and standard errors (SE) by initial quality class and sex

	Quality class								Sex			
	PSE ($n=4$)		RSE ($n=7$)		RFN ($n=13$)		DFD ($n=6$)		Female ($n=16$)		Male ($n=14$)	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LS	MSE
Moisture	53.4	1.2	56.1	0.9	55.9	0.6	55.8	0.9	57.4[a]	0.6	53.2[b]	0.7
IMF	2.4[a,b]	0.3	1.7[b]	0.2	2.5[a]	0.2	2.1[a,b]	0.2	2.1	0.1	2.3	0.2

[a,b] Means within a row without a common superscript differ significantly ($P < 0.05$).

Table 2
Dry-cured ham textural properties. Least square means (LSM) and standard errors (SE) by initial quality class and by sex corrected by moisture content

	Quality class								Sex			
	PSE ($n=4$)		RSE ($n=7$)		RFN ($n=13$)		DFD ($n=6$)		Female ($n=16$)		Male ($n=14$)	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Penetration test												
Hardness (g) 80%	10841[b]	1428	11749[a,b]	1010	13950[a]	725	11481[a,b]	1077	10311[b]	772	13699[a]	950
Texture profile analysis												
Hardness (g) 20%	1028	298	1483	211	1661	151	1575	225	1181[b]	161	1692[a]	198
Springiness	0.72[b]	0.05	0.82[a,b]	0.03	0.84[a]	0.02	0.82[a,b]	0.04	0.80	0.02	0.80	0.03
Cohesiveness	0.61[b]	0.04	0.68[a,b]	0.02	0.70[a]	0.02	0.68[a,b]	0.03	0.65	0.02	0.68	0.02
Chewiness	398[b]	250	930[a,b]	177	1031[a]	127	938[a,b]	189	685	135	965	166
Adhesiveness	−9.2	2.6	−9.5	1.8	−9.1	1.3	−5.2	1.9	−9.7	1.4	−6.8	1.7

[a,b] Means within a row without a common superscript differ significantly ($P < 0.05$).

values (Table 2) and after the first compression the hams did not recover their initial height, typical characteristic of viscoelastic materials. The cohesiveness values far from one (Table 2) are also indicative of the absence of sample recovery after the first compression knowing that the test was performed to a degree of compression that avoided breakdown of fibres. In the second compression cycle the equipment did not find the sample at the same height so recording a smaller area than in the first cycle. The chewiness is a complementary parameter to hardness, as the same profile detected; a lower significant ($P < 0.05$) chewiness in PSE than in RFN quality classes was found. The main differences among quality classes were obtained in springiness, cohesiveness and chewiness, where these parameters were significantly lower in PSE than in RFN quality classes. The sex effect on the textural TPA parameters was not significant ($P < 0.05$) except for hardness 20% that was higher in males (Table 2).

3.2. Proteolysis in dry-cured ham

The protein extraction procedure ensured that all muscle proteins were retained and that no soluble fragments produced during dry-cured processing were lost by washing steps. Dry-cured hams suffered an intense proteolysis as can be seen in Fig. 2. This is in accordance with previous results (Toldrá et al., 1993). After 1 year of dry-cured processing, the myosin heavy band (MHC, 200 KDa) and α-actinin almost disappeared in the hams from different quality classes, and two fragments appeared at 150 and 85 KDa. Other myofibrilar

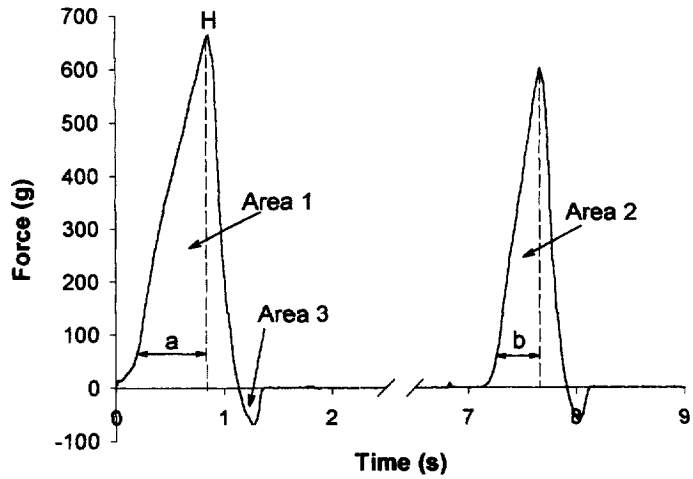

Fig. 1. Typical instrumental texture profile analysis (TPA) of a dry-cured ham from initial PSE quality class. H is hardness, Area 2/Area 1 is cohesiveness, Area 3 is adhesiveness, b/a is springiness.

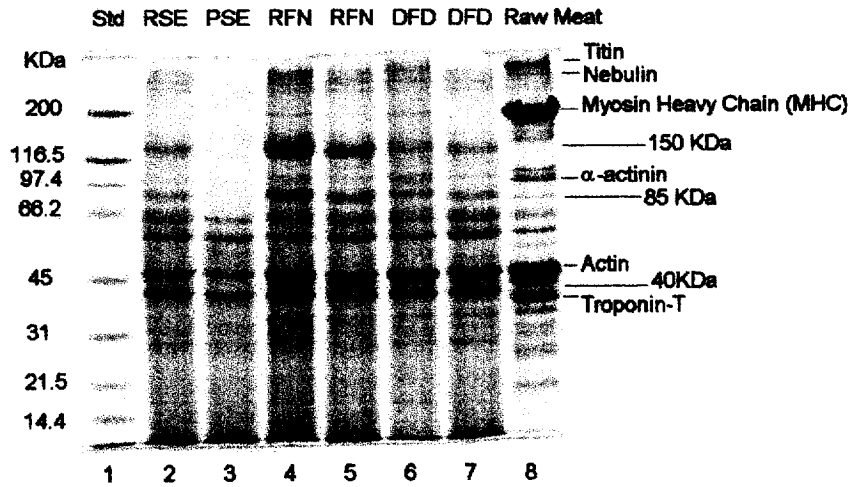

Fig. 2. SDS polyacrylamide gel patterns showing the differences in protein bands among dry-cured ham samples from PSE, RSE, RFN and DFD quality classes. Lane 1 are standards, lane 2 from RSE, lane 3 from PSE, lanes 4 and 5 from RFN and lanes 6 and 7 from DFD dry-cured ham samples. Lane 8 from a RFN raw meat sample.

proteins that disappeared in all classes were: titin (T), nebulin (N) and troponin T. These decreases may be the result of enzyme proteolysis. However, actin did not show major changes. An increase of breakdown products at 40 KDa and below 14.4 KDa was also apparent. Dry-cured hams from the exudative meat quality class (PSE) showed a higher proteolysis than normal class (RFN), detected by the complete disappearance of MHC and fragments at 150 and 85 KDa. On the other hand, in RSE quality class the fragments at 150 and 85 KDa, were observed but at a lower intensity than in RFN samples where these fragments were very well defined. Dry-cured hams from DFD quality meat resulted with an intermediate proteolysis pattern (Fig. 2) similar to that in RSE hams.

4. Discussion

Post-mortem meat quality is one of the main standardisation problems in the dry-cured ham industry due to the lack of uniformity in hams produced under the same conditions. The pork meat classification has been modified in the last few years by the definition of a new fourth quality group (RSE: Red, Soft and Exudative) (Kauffman et al., 1993; Warner et al., 1993, 1997; Cheah et al., 1998). The RSE class was characterised by a low pH, high exudation but with normal red colour.

Proteolysis of key myofibrilar and associated proteins are responsible of meat tenderization. The function of these proteins is to maintain the structural integrity of myofibrils (Koohmaraie, 1996). In the last years, many works have been published dealing with the effect of ultimate pH on meat tenderness (Tornberg, 1996; Koohmaraie, 1996; Watanabe et al., 1996) although, few works have studied the dry-cured ham texture (Parolari et al., 1994; Virgili et al., 1995; Monin et al., 1997).

The differences in hardness and chewiness found among the quality groups in dry-cured ham may be due to the degradation of the myofibrilar structure (Monin et al., 1997) although, the changes in water and salt content may also affect the texture. Dry-cured hams obtained from all meat qualities resulted in similar final moisture contents as also reported by Buscailhon et al. (1994). Only the sex effect was significant being the moisture content higher in females than in males. The sex effect was also remarkable on hardness where the dry-cured hams from females resulted with lower values. The lower hardness and chewiness in PSE than in RFN classes indicates that proteolysis tends to be more active in the former group. Lysosomal enzymes, such as cathepsin B and L, have been recovered even after 15 month of dry-cured processing while cathepsin D was restricted to the first few months of processing (Toldrá et al., 1993). The lower pH in PSE hams will be optimal for these enzymes. Although, as reported by Watanabe et al. (1996), the main impact of low pH on the ageing process will be in the rate at which tenderness develops, since after a period of time, the tenderness becomes equivalent at all values of ultimate pH. In dry-cured ham, the rate of the ripening process is affected as we detected higher proteolysis in PSE than in RFN classes, being the RSE and DFD classes in the middle.

Buscailhon et al. (1994) studied the effect of initial pH on the sensory texture of French dry-cured ham. They compared two groups, low and high pH, observing that the low pH group had a higher proteolysis and texture characteristics; higher firmness and dryness and lower mellowness and fat aroma than the high pH group, in a total ripening time of 9 months. Although, the texture parameters, firm, dry and mellow were not defined, their results were opposite to what happened in Spanish dry-cured ham, where the low pH group (PSE) was characterised by a lower hardness, chewiness, springiness and cohesiveness than the normal class (RFN). However, the effect of post-mortem meat quality on texture parameters was not due to differences in the degree of drying among classes, since moisture content was similar among groups, at the time of the texture analysis, as reported by Buscailhon et al. (1994).

From the proteolysis pattern obtained by SDS-PAGE the same profile as with texture parameters was detected. The PSE hams were excessively proteolysed as shown by the disappearance of 150 and 85 KDa fragments. These two fragments may be very valuable as potential markers for an optimum texture. Similar fragments were observed by Toldrá et al. (1992b, 1993) and Monin et al. (1997). The 150 KDa fragment could result from the MHC degradation (Schwartz and Bird, 1977, 1979), although the fragment at 85 KDa may result from sarcoplasmic degradation as was shown by Toldrá et al. (1993).

The excessive proteolysis of dry-cured ham can produce an inferior meat texture, giving abnormal softness, defined by consumers as mushy and unacceptable (Parolari et al., 1994; Virgili et al., 1995). Parolari et al. (1994), suggested that the use of raw ham with a controlled enzyme activity, specially cathepsin B, would improve the texture quality of the final product. From our results, not only the control of the enzyme activity from the raw material is necessary but also, the duration of the ripening should be adapted to the initial pH and drip loss characteristics of the hams, in order to have a good uniformity of sensory quality in dry-cured ham production.

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