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Evolution of hydrophobic polypeptides during the ageing of exudative and non-exudative pork meat

V.-Javier Moya, Mónica Flores *, M-Concepción Aristoy, Fidel Toldrá

Instituto de Agroquímica y Tecnología de Alimentos (CSIC), Apt. 73, 46100 Burjassot, Valencia, Spain

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Abstract

Thirty-six carcasses from 6-month-old pigs were classified in different exudative groups based on measurements of pH_{2h}, pH_{24h}, the colour parameter L^* and drip loss. A fraction containing polypeptides between 66 and 21 kDa was analysed by reverse phase chromatography at 2-h post-mortem and the evolution of 8 polypeptide fractions followed during ageing and related to meat quality. Three polypeptide (fractions P2, P3 and P4) at 2-h post-mortem showed significant lowest area values in the dark firm and dry class. During ageing, the higher content of P4 in exudative meats at 8-h post-mortem could be due to activation of the cathepsin system. On the other hand, P3 and P4 increased in DFD meats during the first 96-h post-mortem probably due to higher calpain activity. Few differences in polypeptides were related to meat qualities although they are important as precursors of small peptides and free amino acids.

Keywords: Polypeptides; Meat quality; Ageing and pork meat

1. Introduction

Muscle is a complex mixture of many different compounds. These include sarcoplasmic proteins, myofibrillar contractile proteins, polypeptides, free amino acids and nucleotides. During the ageing process many proteolysis products are released and these products have been studied in relation to meat tenderness in bovine meat (Fritz, Mitchell, Marsh, & Greaser, 1993; Penny & Dransfield, 1979) and to taste and flavour development in pork meat (Aristoy & Toldrá, 1995; Nishimura & Kato 1988; Toldrá & Flores, 1998).

However, one of the main problems in the pork industry is the high incidence of exudative meats that produce loss of protein functionality in processed products and significant weight losses in both fresh and processed meat products. Pale, soft and exudative (PSE) muscles display a rapid postmortem pH decline because of increased rate of glycolysis, a reduction in water holding capacity and altered protein solubility. Other exudative meats have been described as RSE characterised by its normal red colour but being soft and exudative (Cheah, Cheah, & Just 1998; Kauffman, Cassens, Scherer, & Meeker, 1992). The presence of dark, firm and dry (DFD) meat is less relevant to industry. So one of the main objectives of the pork industry is to get meat characterised by a red colour, firmness and a non-exudative appearance (RFN). These different quality classes present different degrees of myofibrillar fragmentation. Also less sarcoplasmic proteins are extracted from exudative muscles probably due to the denaturation resulting in decreased solubility (Boles, Parrish, Huiatt, & Robson, 1992).

The proteolysis products are released by the action of muscle proteinases (calpains, cathepsins and a multicatalytic proteinase complex) that generate intermediate polypeptides (Koohmaraie, Whipple, Kretchmas, Crouse, & Mershmann, 1991; Ouali & Talmant, 1990). Further proteolysis generates small peptides and free amino acids due to the action of exopeptidases including peptidases (dipeptidyl peptidases, tripeptidyl peptidases), aminopeptidases and carboxipeptidases (Blanchard & Mantle, 1996; Nishimura, Rhue, Okitani, & Kato, 1988).

The activity of the pork muscle enzymes depends on muscle metabolism (Flores, Alasnier, Aristoy, Navarro, Gandemer, & Toldrá, 1996), sire type, age and sex

* Corresponding author. Tel.: +34-96-390-00-22; fax: +34-96-363-63-01.

E-mail address: mflores@iata.csic.es (M. Flores).

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(Aristoy & Toldrá, 1998; Armero, Baselga, Aristoy, & Toldrá, 1999; Flores, Romero, Aristoy, Flores, & Toldrá, 1994; Toldrá, Flores, Aristoy, Virgili, & Parolari, 1996). Also, pork meat quality will affect the post-mortem proteolytic system and generate characteristic types and quantities of polypeptides, peptides and amino acids (Flores, Moya, Aristoy, & Toldrá, 2000).

The generation of small peptides (< 10 kDa) during ageing in relation to pork meat quality was studied in another report (Moya, Flores, Aristoy, & Toldrá, 2001). In the present report the aim is to study the polypeptides generated in the different pork meat classes and their evolution during ageing.

2. Materials and methods

2.1. Animals

Thirty-six pork carcasses from 6 month-old pigs (mother: Large White×Landrace, sire: Large White) were obtained from two commercial slaughterhouses. At 2-h post-mortem the *Longissimus dorsi* from the right hand side of the carcass was removed and kept at 4°C. The muscle was aged at 4°C and sampled at 2, 4, 6 and 8 h and at 1, 2, 4 and 7 days, vacuum packaged and stored at −80°C until further analysis.

2.2. Meat quality measurements

The pH was measured at 2 h (pH_{2h}) and 24 h (pH_{24h}) in the muscle *L. dorsi* at the fifth rib level and with a portable Hanna HI 8424 pH-meter (Hanna Instruments, Portugal). The colour, L*, a*, b* coordinates, was measured at 24-h post-mortem with a Hunter Chromameter model Labscan (Hunter, VA, USA). The drip loss (DL) was measured by the method of Warris (1982). Based on these measurements, muscles were classified as PSE, RSE, RFN or DFD as described by Flores, Armero, Aristoy, and Toldrá (1999).

2.3. Polypeptide analysis

The muscle was homogenised with 0.01 N HCl (dilution 1:5) in a Masticator (IUL, Delabo, Barcelona) for 4 min and centrifuged at 10000 ***g*** for 20 min at 4°C. The supernatant was filtered through glass wool and 20 ml of this extract was loaded on an activated Bond Elut Jr C-18 cartridge (Varian, CA, USA) for peptide fractionation. The cartridge was first washed with 20 ml of 0.1% trifluoroacetic acid (TFA) in water and then with 10 ml of 0.1% TFA in 15% acetonitrate (ACN) to remove the more polar small peptides. The fraction containing the hydrophobic polypeptides was then eluted with 10 ml of 0.1% TFA in 50% ACN. This fraction was evaporated under vacuum (35°C) till dry and re-diluted with 300 μl of 0.1% TFA in water. The sample was analysed by reverse phase HPLC (RP-HPLC). 40 μl were injected in a 1050 Hewlett-Packard HPLC (Palo Alto, CA, USA) system with a variable UV detector. The column (4.6×250 mm) used was a Waters Symmetry C-18 (Waters Corporation, MA, USA). The separation was achieved in 50 min at 40°C at a flow rate of 0.9 ml/min by using a step gradient between two solvents: 0.1% TFA in water (solvent A) and 0.085% TFA in water:ACN, 40:60 v/v (solvent B) as follows: initially 30% B to 60% in 42 min and then to 100% B in 8 min. Detection was at 214 nm.

2.4. SDS-PAGE electrophoresis

The molecular weight of the extracted polypeptides was determined by Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) using 12% polyacrylamide gels. The fraction containing hydrophobic polypeptides was denatured by heating at 100°C for 4 min in a solution (1:1) 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% (w/v) bromophenol blue. The elution buffer was 50 mM Tris (Trizma base), pH 8.8, 0.384 glycine and 0.1% SDS. Gels were stained

Table 1
Pork technological parameters among groups[a]

	PSE ($n=14$)		RSE ($n=7$)		RFN ($n=10$)		DFD ($n=5$)	
	M	S.E.	*M*	S.E.	*M*	S.E.	*M*	S.E.
pH_{2h}	5.73c	0.04	5.87bc	0.11	6.13a	0.05	6.00ba	0.04
pH_{24h}	5.53b	0.04	5.30c	0.05	5.49b	0.04	5.83a	0.05
L	52.2a	0.76	46.3b	0.67	46.1b	1.68	44.9b	1.6
DL (%)	7.5b	0.2	8.5a	0.2	4.9c	0.4	2.7d	0.2

[a] PSE, pale, soft and exudative; RSE, red, soft and exudative; RFN, red, firm and non-exudative; DFD, dark, firm and dry; pH_{2h}, pH at 2 h; pH_{24h}, pH at 24 h; L, colour parameter lightness; DL, drip loss. Means in a row with different letters are significantly different ($P < 0.05$).

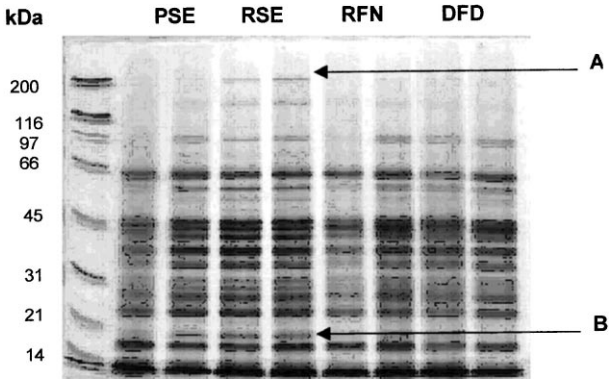

Fig. 1. SDS-polyacrylamide gel electrophoretic profile of the hydrophobic polypeptidic fraction from the different groups at 2-h post-mortem. Arrows show protein bands, A (200 kDa), B (22 kDa).

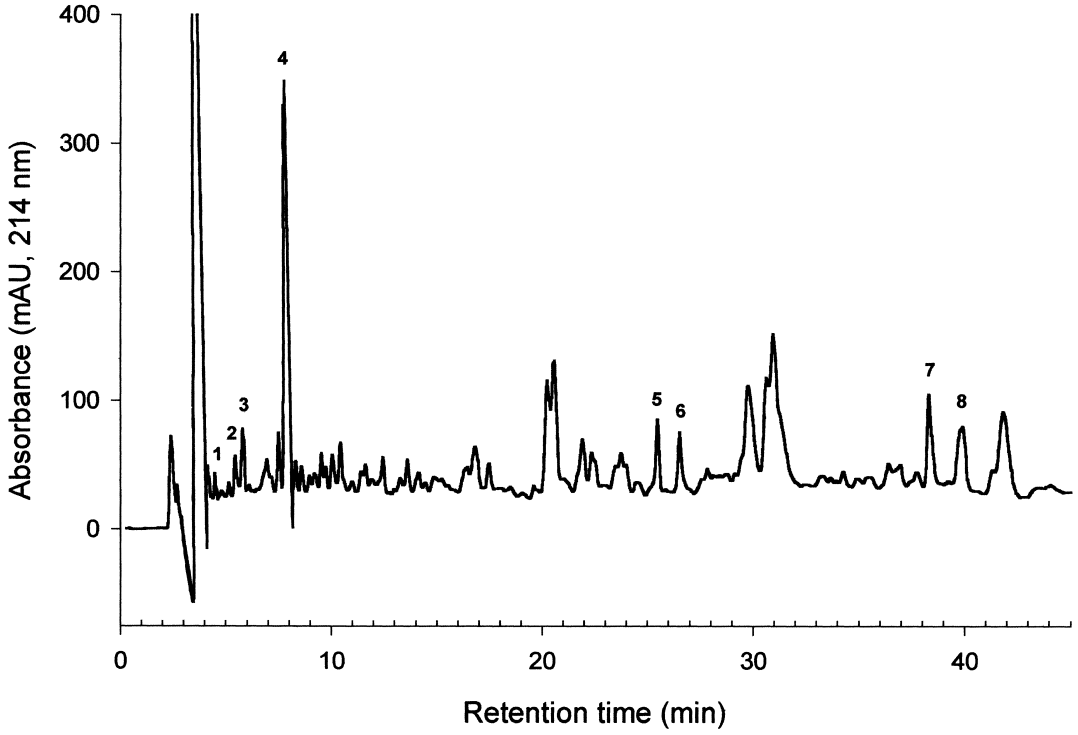

Fig. 2. Reverse-phase chromatogram of the hydrophobic polypeptidic fraction at 2 h post-mortem. Chromatographic conditions were as described in Section 2. Numbered peaks correspond to the studied polypeptide fractions.

Table 2
Comparison of the four groups based on polypeptide fraction areas at 2-h post-mortem time[a]

Fraction	PSE ($n=14$)		RSE ($n=7$)		RFN ($n=10$)		DFD ($n=5$)		P
	M	S.E.	M	S.E.	M	S.E.	M	S.E.	
P2	257.9bc	27.9	336.9ac	64.6	397.4a	51.9	154.8b	48.2	$P<0.05$
P3	310.5a	31.5	258.8ab	28.6	273.5a	59.3	116.7b	20.1	$P<0.06$
P4	2171.0ab	230.9	2544.3a	191.3	1758.7b	229.9	618.8c	108.3	$P<0.05$

[a] PSE, pale, soft and exudative; RSE, red, soft and exudative; RFN, red, firm and non-exudative; DFD, dark, firm and dry. Peak areas in a row with different letters are different at level of significance (P) expressed in the column.

with coomassie brilliant blue R-250 (Laemmli, 1970) and destained overnight. Standard proteins from Biorad (Richmond, CA, USA) were simultaneously run for molecular weight determination.

2.5. Statistical methods

The Fisher's least significant difference (LSD) procedure was used to discriminate among the means of the meat characteristics and polypeptides in the different groups using Statgraphics plus (v 2.0; Steel & Torrie, 1980).

3. Results

Quality classification was based upon pH measured at 2- and 24-h post-mortem, colour parameter L*, and DL. Table 1 shows the mean values of these measurements for each group. The pH_{2h} was useful to distinguish the PSE meat from non-exudative meats (RFN and DFD). The pH_{24h} was enough to discriminate DFD from the other quality classes. Lightness L^* could be used to distinguish PSE from the other qualities. The DL differentiated all four classes but its measurement is obtained only 4 days of post-mortem storage.

The extract containing the most hydrophobic polypeptides was analysed by SDS-PAGE and RP-HPLC. The SDS-PAGE electrophoresis was used to determine the molecular weight of the hydrophobic polypeptides. Fig. 1, shows the SDS-PAGE gel of the different quality classes at 2-h post-mortem. Numerous and similar polypeptide bands were detected between 49 and 21 kDa in all groups. A band at 200 kDa was observed mainly in the RSE class and a fragment of approximately 22 kDa appeared with higher intensity in the RSE class than in the other groups.

RP-HPLC was used to separate the polypeptides contained in the fraction and to quantify their differences between groups. Eight polypeptide fractions (P1 to P8) were selected and analysed at 2 h post-mortem (Fig. 2). The retention times of these eight fractions were 4.5, 5.5, 5.9, 7.9, 25.7, 26.8, 38 and 39.8 min. The peak area of each polypeptide fraction was compared among groups. Only polypeptide fractions P2, P3 and P4 showed significant differences between groups at 2 h post-mortem (Table 2). Most noticeable was the low content in the DFD class of fractions P2, P3 and P4. However, differences between exudative and normal classes were found only in the P4 contents where a significantly higher content was found in the RSE than in the RFN class.

To study the polypeptide fractions during ageing, three samples from each group were analysed at 2, 4, 6, and 8 h and at 1, 2, 4 and 7 days post-mortem by SDS-PAGE and RP-HPLC. On SDS-PAGE gels (data not shown), no major differences were detected in protein profiles between the groups during ageing. The evolution of polypeptide fractions P2, P3 and P4 by RP-HPLC during ageing is shown in Fig. 3. As found at 2-h post-mortem, the content of P2 and P4 to 24 h post-mortem was still lower in the DFD group than in the other groups. P3 and P4 in the DFD group increased until 96 h postmortem but did not change on further ageing. P2 showed similar behaviour during ageing in all groups until 24-h post-mortem but after this time the differences were more marked. P3 showed few differences between groups during the ageing and P4 was lower in the DFD groups until 48-h post-mortem and had a higher content in the exudative groups until 8-h post-mortem.

The evolution of the other polypeptides (P1, P5, P6, P7 and P8) did not show significant differences ($P < 0.05$) at 2-h post-mortem, had similar profiles during ageing with a few exceptions (Fig. 4). Only P6 and P7 showed some differences in behaviour among classes during ageing. The content of P6 in DFD was lower

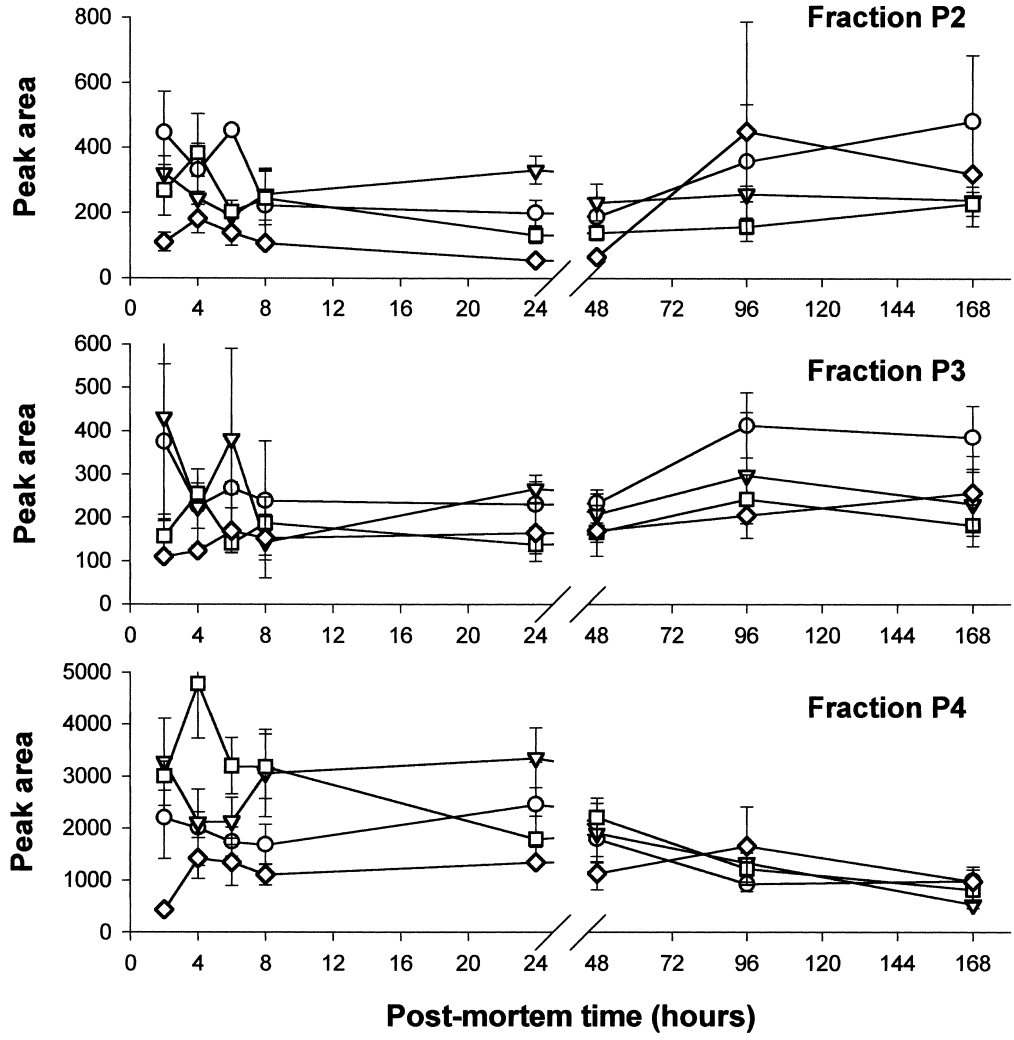

Fig. 3. Evolution of polypeptide fractions 2, 3 and 4 during ageing of the four meat groups. red, firm and non-exudative (○); pale, soft and exudative (▽); red, soft and exudative (□); dark, firm and dry (◇). All values are means±standard errors.

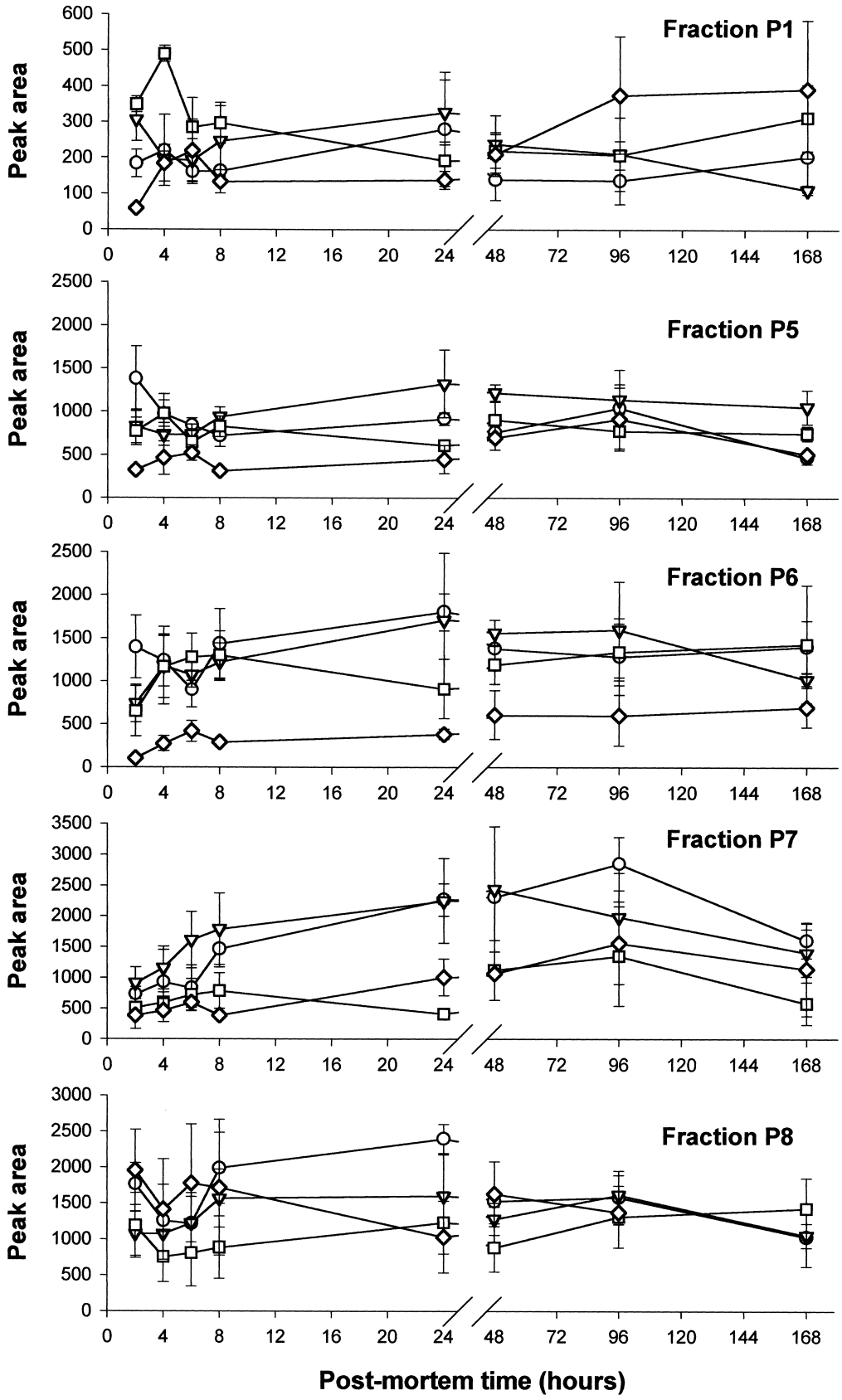

Fig. 4. Evolution of polypeptide fractions 1, 5, 6, 7 and 8 during ageing of the four meat groups: red, firm and non-exudative (○); pale, soft and exudative (▽); red, soft and exudative (□); dark, firm and dry (◇). All values are means±standard errors.

throughout ageing whilst P7 increased in RFN and PSE but its profile was similar between in the other groups.

4. Discussion

Four pork meat qualities, characterised by their pH, lightness and DL, were analysed for their hydrophobic peptides in an extract containing between 15 and 50% acetonitrile. This extract contains a complex mixture of peptides, proteins, mainly glycolytic and mithochondrial enzymes and pigments (McCormick, Reek, & Kropf, 1988). The analysis of hydrophobic polypeptides by SDS-PAGE showed the presence of multiple bands in the range 66 kDa to 20 kDa in all four groups with the 22 kDa band being slightly more marked in the RSE group than in the other groups. These proteins could arise from degradation of sarcoplasmic proteins. Boles et al. (1992) reported that less sarcoplasmic proteins are extracted in exudative pork meat due to denaturation caused by the low pH on the sarcoplasmic proteins. In our case, we did not note differences in solubility.

The study of proteins and polypeptides in meat has mainly focused on the prediction of tenderness (Penny & Dransfield, 1979), for example, the appearance of a 30 kDa fragment. This fragment is related to postmortem tenderisation and shear force measurements and originates from degradation of troponin T (Olson & Parrish, 1977). Also, detection of frozen treatment on beef has been based on a peptide of 15 amino acid residues (Nakai, Nishimura, Shimizu, & Arai, 1995) which, with an additional histidine residue (MW 1734.8 Da), has been proposed as an index of beef tenderness (Stoeva, Byrne, Mullen, Troy, & Voelter, 2000). Moreover, Stoeva et al. (2000) reported two other peptides (MW 1282 and MW 5712), originating from degradation of the sarcoplasmic proteins, glyceraldehyde-3-phosphate dehydrogenase and creatine kinase, respectively, which were also claimed to indicate ageing. Other studies (Spanier & Edwards, 1987; Spanier, Edwards, & Dupuy, 1988) have focused on the contribution of peptides (<5 kDa) to beef flavour. Feidt, Brun-Bellut, & Dransfield (1998) studied the peptides (<10 kDa) generated during goat meat ageing and their interaction with proteinase activity. Thus other studies looked at the changes in polypeptides during the ageing process of meat.

In this work, several polypeptide fractions have been isolated and analysed at 2 hours post-mortem and during the ageing of different pork meat groups to study how postmortem metabolism in the different groups can affect the polypeptide generation. The three polypeptidic peaks (P2, P3 and P4) had the smallest areas for the DFD class at 2-h post-mortem as already shown with smaller peptides (<4 kDa) in high pH goat meats (Feidt et al., 1998) and in high pH pork meat (<10 kDa; Flores et al., 2000). These polypeptides could be the initial stages of the degradation of myofibrillar and sarcoplamic proteins by muscle endopeptidases (cathepsins, calpains and multicatalytic proteinase complex; Koohmaraie, 1994; Toldrá & Flores, 1998). Early action of cathepsins, active at low pH (Toldrá & Flores, 1998) could explain the fact that exudative meats (PSE and RSE) showed higher area values of P4 up to 6-h post-mortem than RFN and DFD classes. O'Halloran, Troy, Buckley and Reville (1997), also showed that the low pH conditions in fast glycolysing beef muscle enhanced the release of cathepsins B and L from the lysosomes at early post-mortem. However, in a recent study, using the same set of samples, no differences in cathepsin activities were detected between pork groups at 2-h post-mortem (Toldrá & Flores, 2000). Also, Beltrán, Jaime, Santolaria, Sañudo, Alberti, and Roncalés (1997) did not find any effect of pH on protease activity at 2-h post-mortem in beef. These results suggest that 2 hours postmortem is too early to establish differences among groups and for this reason it is necessary to study what happens during ageing. During meat ageing the polypeptides in fractions P3 and P4 of the DFD group increased during the first 96-h post-mortem. This fact could be due to the reported higher activity of the calpain system detected at 2-h post-mortem in high pH pork meat (Toldrá & Flores, 2000). A different trend was observed between exudative and non-exudative meats in the polypeptide fraction P4 at 8-h post-mortem although it would be necessary to confirm these results in order to clarify if the different postmortem metabolisms in the groups affects the generation of the polypeptides. In summary, few differences in polypeptides are found among groups but these polypeptides are important as precursors of small peptides and free amino acids in the proteolytical degradation chain.

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