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Time course of peptide fingerprints in *semimembranosus* and *biceps femoris* muscles during Bayonne ham processing

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ABSTRACT

The aim of this work was to define reliable markers of muscle and processing time in dry-cured ham using a rapid, precise semi quantitative method for the protein fraction soluble in low ionic strength buffer. For this purpose protein labchip Agilent was used to separate proteins and peptides and accurately determine their molecular weights and concentrations electrophoretically. In this way the protein fingerprinting of dry-cured ham at different process times was characterised, together with targets and products of proteolysis. In addition, the comparison of all the electrophoregrams indicated muscle and dry-curing process markers.

1. Introduction

Bayonne ham enjoys EU Protected Geographical Indication (PGI) status. This certification requires professional processors to comply with specifications that provide the consumer with a finished product of optimal quality, in particular as regards texture. Controlling texture in the finished product is important for both organoleptic and technical quality. Defective texture generates considerable losses when the ham is sliced, and often reduces its value.

Many studies have been conducted on the texture of dry-cured ham, and the role of proteolysis during curing has been demonstrated (Arnau, Guerrero, & Sarraga, 1998; Parolari, Virgili, & Schivazappa, 1994; Rosell & Toldra, 1998; Virgili, Schivazappa, Parolari, Soresi Bordini, & Degni, 1998). Physical and chemical conditions, such as a_w and salt content, which change in time, and the action of enzymes, are involved in this relationship between proteolysis and texture.

A distinction is made between the outer and inner parts of the ham, which are represented respectively by the *semimembranosus* and *biceps femoris* muscles. During the dry-curing process the two muscles are subjected to different conditions. The *semimembranosus* muscle is an external muscle; it has a high NaCl content in the first stages of the process, and its water content falls rapidly. Conversely, the *biceps femoris* is an internal muscle with a lower NaCl content and higher water content. This implies greater proteolytic activity in the *biceps femoris* muscle, affecting its texture (Parolari et al., 1994; Rosell & Toldra, 1998; Virgili, Parolari, Schivazappa, Soresi Bordini, & Borri, 1995; Virgili et al., 1998). The aim of this study was to characterise the proteolysis and define muscle protein and processing stage markers. Electrophoresis methods are classically used to monitor proteolysis or define markers. The method used here was the Protein LabChip Agilent method. It allows a rapid separation and qualitative and semi quantitative assay of proteins and protein fragments. It has been used to identify wheat variety and quality type (Uthayakumaran, Batey, & Wregley, 2005), to address the rheological properties of wheat flour (Chanvrier, Uthayakumaran, & Lillford, 2007) and for tear analysis (Mann & Tighe, 2007). To our knowledge, this method has not been applied to the characterisation of a meat product.

2. Materials and methods

2.1. Origin of hams and sampling

The study was based on a total of 22 pigs that had been fed a cereal-based diet (60–80%), slaughtered at the Lahontan abattoir, and selected to meet the processing specifications of PGI Bayonne

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ham (a ham weight average of 10.0 kg, a fat cover average of 16 mm and a semi membranosus pH of 5.8). The processing of Bayonne hams, lasting nine months, was carried out at the Pyragena experimental station using the following sequence: salting for 12 days (temperature 3 °C, relative humidity,RH 85% < RH < 95%), curing for 10 weeks (1–4 °C, 70% < RH < 80%), drying for 10 weeks (14 °C, 68% < RH < 76%) grease covering and ripening for 16 weeks (18 °C, RH = 70%) (Robert, Basly, & Dutertre, 2005).

Ham samples taken at different processing steps from the two main muscles in a cut of one ham were used. Sampling was carried out at 12 weeks (before the drying step) on 6 hams from 6 pigs, at 16 weeks (after drying) on 6 hams from 6 pigs, and at 9 months (at the end of ripening) on 10 hams from 10 pigs. Analysis by the LabChip Agilent method was performed on the *biceps femoris* (internal muscle reference) and the *semimembranosus* (external muscle reference).

2.2. Extraction of soluble proteins

The proteins in the soluble fraction of dry-cured ham were extracted from 150 mg of homogenised ham in a buffer solution consisting of KCl 50 mM, tris 20 mM, $MgCl_2$ 4 mM and EDTA 2 mM, pH 7, with a w/v ratio of 1/8. After centrifuging for 10 min at 10,000 *g* the supernatant was recovered. It contained the proteins of the soluble fraction of the dry-cured ham.

2.3. Bioanalyser lab-on-chip methodology

The results were obtained on a 2100 Bioanalyser using the Protein 80 Plus LabChip kit, which allows the separation of proteins in the 5–80 kDa range. The LabChip kits and reagents were obtained from Agilent Technologies GmbH (Waldbronn, Germany). These micro-fabricated chips can analyse ten 4 μl sample wells in less than 30 min. The chips consists of two glass layers bonded together; the 'run' channels are etched into one layer and are pressure-filled with a sieving mixture and a fluorescent dye. Detection is based on laser-induced fluorescence of an intercalating dye, which interacts with the protein/SDS complex. The 2100 Bioanalyser contains 16 high-voltage power supplies connected to a platinium electrode; the pin electrodes touch the individual chip wells and form an electric circuit. The analytes are separated electrophoretically and detected by their fluorescence (670–700 nm). These data are then translated into individual electrophoregrams that are presented against migration time in seconds and fluorescence units (FU). Each LabChip kit contains a standard molecular weight ladder well with a lower limit marker at 6 kDa and an upper limit marker at 95 kDa for internal calibration.

2.4. Sample preparation

A pre-treatment is required before analysis. This involved adding 4% of the analyte (protein from soluble fraction) to 2 μl of sample buffer (with 3.5% vol. β-mercaptoethanol reducing agent). This solution was then placed in a water bath at 95–100 °C for 5 min. A further 84 μl of deionised water was added to the sample prior to loading onto the chip. The use of a disulphide bond reducing agent in combination with sodium dodecyl sulphate (<7%) essentially denatures the protein into its unfolded conformation with a resultant net negative charge. The sample buffer contains upper and lower marker standards identical to those in the ladder, and is thus incorporated into each unknown sample for direct comparison against the ladder standard. All the chips were prepared and set up according to the protocol provided with each LabChip kit.

2.5. Data output

The software allows modes of data display: either as a gel-like image or an electrophoregram for each analyte and an accompanying importable data table. SDS–PAGE gelscans are commonly presented and are widely accepted as a way to communicate protein molecular weight sizing results. Fig. 1 presents a software-derived gel-like image analogous to the conventional SDS–PAGE gel. The leftmost lane of the gel represents the ladder marker bands. The gel also shows the results of 10 different ham samples in lanes 1–10. The green bands at the top of the gel correspond respectively to the lower and upper marker internal standards. A crucial advantage of this virtual alternative is that it avoids all the cumbersome post-electrophoresis procedural steps required by SDS–PAGE analysis including staining, destaining and storage. Importantly, it also obviates further image analysis equipment. The software can also translate each test sample into separate electrophoregrams converting each protein band separation and intensity parameters into individual sizing peaks. This format offers a comparatively excellent visual aid for result interpretation. In all the electrophoregrams presented here, each peak is given its molecular mass (kDa), but peak height, area, relative concentration and percentage of overall protein content can also be displayed.

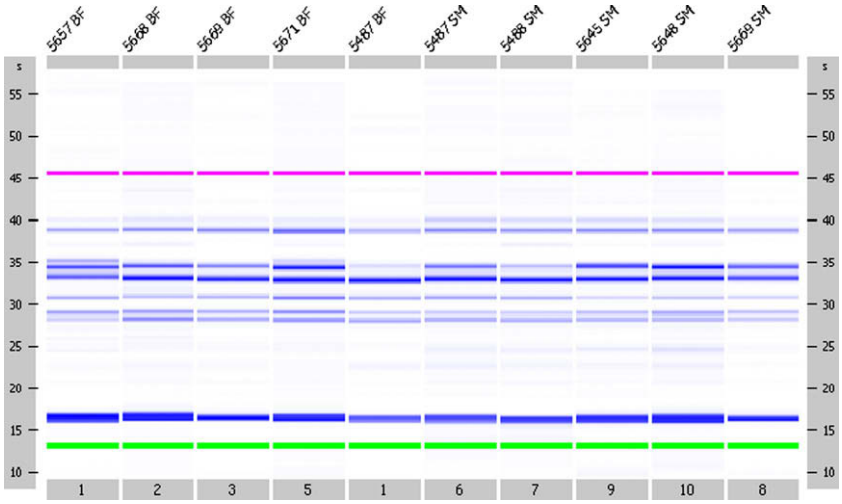

Fig. 1. Software-derived gel-like image analogous to the conventional SDS–PAGE gel.

2.6. Physical and chemical analyses

Physical and chemical variables were measured on the *semimembranosus* and *biceps femoris* muscles at 12 and 16 weeks and at 9 months of processing. Water content was measured according to French Standard NF V 04–401 of April 2001. Chloride content was determined according to Standard NF V 04–405.

2.7. Statistical analyses

Statistical analyses were carried out on relative concentrations of the 21 peaks and on the results of physical and chemical analyses. Principal component analysis, discriminant factor analysis and *t*-test for paired samples were used.

3. Results and discussion

3.1. Electrophoresis profile

A total of 25 peaks could be defined, with molecular weights between 17 and 74.7 kDa. After a first analysis, based on the signal-to-noise ratio of the peaks relative to the baseline and after checking for the presence of the peaks in all the profiles, four peaks were eliminated. It was then necessary to number the peaks consistently in all the peptide fingerprints so as to compare the proteolytic fingerprints of each muscle and each processing time. The statistical analyses were thus carried out on 21 peaks (Table 1).

This method has great potential resolution but it is not coupled with mass spectrometry. So, comparison of the molecular weights of the peaks obtained by this method with the literature (Di Luccia et al., 2005; Larrea, Hernando, Quiles, Lluch, & Perez-Munuera, 2006) offered an approach to identification (Table 2). However, a peak defined by this separation method does not necessarily correspond to a single protein. Indeed as soon as separation is based on molecular weight, one peak can correspond to a set of proteins which differed by isoelectrophoretic point and/or protein fragments produced by proteolysis. The results must therefore be analysed in terms of peaks rather than proteins.

Table 2
Assignment of peaks by comparison with literature (Di Luccia *et al.*, 2005; Larrea et al., 2006).

	Assignment
17.0 kDa	Myoglobin
17.8 kDa	Proteolysis products
18.7 kDa	Unassigned
20.2 kDa	Unassigned
22.3 kDa	Myokinase
23.4 kDa	Unassigned
25.1 kDa	Unassigned
26.4 kDa	Unassigned
27.4 kDa	Phosphoglycerate mutase
31.1 kDa	Unassigned
32.4 kDa	Unassigned
35.5 kDa	Proteolysis products
36.3 kDa	Unassigned
39.0 kDa	Proteolysis products
41.9 kDa	Aldolase
43.9 kDa	Phosphate creatine kinase
45.8 kDa	Enolase
52.6 kDa	Phosphoglycerate kinase
58.4 kDa	Pyruvate kinase
62.8 kDa	Phosphoglucomutase
74.7 kDa	Unassigned

Analysis of all the electrophoresis profiles revealed a certain number of similarities, but also differences in the processing time course of the hams or between the two muscles. Thus some peaks were common to all the profiles, irrespective of the muscle and the process time. For some of them it was possible to suggest protein identification, for others not. These were peaks of molecular weight 25.1 kDa (unassigned), 27.4 kDa (phosphoglycerate mutase), 32.4 kDa (unassigned), 43.9 kDa (phosphate creatine kinase), 58.4 kDa (pyruvate kinase), 62.8 kDa (phosphoglucomutase + proteolysis products) and 74.7 kDa (which again could not be identified by comparison with the literature). Although these peaks were present in all the profiles, their concentrations varied according to the muscle or process time (Fig. 2). Conversely, the disappearance or appearance of a peak in the course of processing enables us to define one or more targets of the proteolysis or the accumulation of proteolysis products, respectively. It is also possi-

Table 1
Protein concentration (μg/mg of dry-cured ham) at 12 and 16 weeks and 9 months, in *semi membranosus* (SM) and *biceps femoris* (BF) muscles.

Peak kDa	12 Weeks				16 Weeks				9 Months			
	Biceps femoris		*Semi membranosus*		*Biceps femoris*		*Semi membranosus*		*Biceps femoris*		*Semi membranosus*	
	Mean $n = 6$	s.d.	Mean $n = 6$	s.d.	Mean $n = 6$	s.d.	Mean $n = 6$	s.d.	Mean $n = 10$	s.d.	Mean $n = 10$	s.d.
17	35.4	11.9	21.5	12.8	4.3	2.8	12.7	10.7	16.3	10.0	9.7	6.8
17.8	N.D.		N.D.		N.D.		N.D.		7.8	2.1	65.5	54.0
18.7	N.D.		N.D.		N.D.		N.D.		N.D.		6.6	3.6
20.2	1.4	1.7	N.D.		2.4	1.3	1.0	0.2	3.2	2.5	4.1	4.9
22.3	10.9	6.0	5.1	1.0	13.6	4.0	17.9	7.6	4.7	3.4	N.D.	
23.4	12.0	3.9	10.3	1.9	N.D.		N.D.		56.3	36.0	3.9	0.8
25.1	46.3	9.3	54.8	12.6	61.7	2.1	83.3	14.7	125.5	10.4	96.8	36.6
26.4	64.9	23.9	21.1	9.9	36.8	5.3	N.D.		82.0	7.0	N.D.	
27.4	96.2	24.2	54.2	20.3	88.6	10.4	94.0	9.2	89.1	29.8	119.9	25.2
31.1	4.4	0.8	N.D.		N.D.		N.D.		83.4	19.6	N.D.	
32.4	84.2	8.9	121.5	32.9	121.1	10.9	144.7	15.0	112.3	11.1	61.3	35.6
35.5	N.D.		N.D.		N.D.		1.1	0.6	7.0	6.6	21.5	3.1
36.3	3.60	0.1	1.1	0.3	2.55	1.25	N.D.		3.2	0.6	N.D.	
39.0	N.D.		135.3	79.3	297.8	29.7	302.7	10.2	304.0	53.0	305.7	55.5
41.9	192.1	16.6	254.7	82.4	N.D.		74.9	27.0	234.4	2.3	N.D.	
43.9	156.0	9.9	196.8	70.5	196.6	79.7	70.6	5.8	180.3	79.0	209.3	59.5
45.8	238.2	23.6	216.4	70.7	N.D.		N.D.		N.D.		N.D.	
52.6	3.1	3.0	0.6	0.4	2.0	0.7	1.3	0.4	7.6	5.7	38.7	58.5
58.4	61.7	9.3	103.9	19.6	105.3	11.6	122.7	11.8	101.5	13.9	79.4	44.0
62.8	19.7	6.1	10.7	4.4	14.7	4.4	22.6	9.9	21.7	6.1	26.8	26.9
74.7	2.3	0.8	1.8	0.6	3.9	0.8	2.8	1.2	2.4	0.8	4.3	5.8

N.D. = not detected.

ble to discriminate among different processing times or muscles according to the height of one or more peaks.

3.2. Protein targets

In the *biceps femoris* and *semimembranosus* muscles the peak with molecular weight 45.8 kDa (enolase) was present only at 12 weeks. The disappearance of the enolase from the electrophoresis profiles of the soluble fraction at 16 weeks indicates that it may be a proteolysis target. Its marked drop in the course of processing, and near-disappearance at the end of ripening has been demonstrated in the *biceps femoris* and *semimembranosus* muscles (Larrea et al., 2006; Monin et al., 1997) and in the overall ham cut (Buscailhon et al., 1994; Picariello et al., 2006). Basso et al. (2004) have shown that enolase disappears in the first steps of dried sausage processing, which takes, altogether one month. According to these results, the hydrolysis of the enolase may be due to the action of bacterial enzymes and not to the action of endogenous enzymes during the processing of dry-cured ham. However, the presence of enolase at 12 weeks processing are consistent with earlier studies (Larrea et al., 2006; Monin et al., 1997). Its disappearance from the protein fingerprints thus seems to be due to the action of endogenous enzymes, probably of the cathepsin family. However, the disappearance of certain peaks from the peptide fingerprint of the soluble fraction can also indicate a change in the solubility of proteins during processing, making them insoluble at low ionic strength.

The study of proteolytic profiles in the two muscles indicates that certain peaks decreased in height between two processing steps and then increased, or conversely increased and then decreased. Thus the peaks of molecular weight 17 kDa (myoglobin) and 41.9 kDa (aldolase) could be defined as proteolysis targets in the course of drying in *biceps femoris*. Although their concentrations increased during ripening, it is difficult to determine whether this was due solely to the accumulation of proteolysis products, or whether the proteolysis was masked and continued right to the end of the dry-curing process. The peak of molecular weight 22.3 kDa (myokinase) emerged as a protease target during ripening in the *biceps femoris* and *semimembranosus* muscles. However, as in the previous case, the increase in peak concentration between 12 and 16 weeks may be due to an accumulation of products that masks the hydrolysis of the protein.

3.3. Proteolysis products

The protein fingerprints of the *biceps femoris* muscle showed the appearance of a peak of molecular weight 35.5 kDa at the end of ripening, and in the case of the *semimembranosus* muscle a weak peak was seen at the end of drying. The peak of molecular weight 39 kDa appeared only in the *biceps femoris* muscle. It was detected in the measurements made at 16 weeks and 9 months of ripening. An earlier study on the soluble protein fraction using SDS-PAGE (Larrea et al., 2006), showed the presence of two bands at 35.4 kDa and 39.7 kDa in fresh meat. Their concentrations increased to reach a maximum at the end of dry-curing. However, others (Di Luccia et al., 2005) using mass spectrometry found that the concentrations glyceraldehyde 3-phosphate dehydrogenase (35.7 kDa) and fructose biphosphate aldolase (39.3 kDa) fell markedly at the end of ripening. Although several authors have demonstrated the complete hydrolysis by bacterial enzymes of certain proteins such as glyceraldehyde 3-phosphate dehydrogenase in only a few days (Fadda, Vignolo, Holgado, & Oliver, 1998; Martin, Cordoba, Rodriguez, Nunez, & Asensio, 2001; Santos et al., 2001; Sanz et al., 1999), the activity of endogenous enzymes caused the reduction of these target proteins only after 12 months ripening in dry-cured ham. In the present case the absence of these peaks in the electrophoresis profiles at 12 weeks suggests an accumulation of proteolysis products. However, the lack of data on fresh meat rules out any firm conclusion on this point. These peaks have thus not been assigned. Therefore we can say that during drying and ripening there is an accumulation in both muscles of products with molecular weights 35.5 kDa and 39 kDa. During the dry-curing process the myofibrillar fraction undergoes intense proteolysis that generates many protein fragments. This effect, coupled to the solubilisation of the myofibrillar proteins by the action of salt, causes myofibrillar fragments to appear in the low ionic strength soluble fraction (Di Luccia et al., 2005).

3.4. Muscle protein markers

The discrimination of electrophoretic profiles of each muscle by means of specific peaks was examined at the end of ripening. A discriminant factor analysis carried out on all the peaks classified 92.5% of the protein peaks of the *semimembranosus* and *biceps femoris* muscles on the basis of the four peaks of molecular weight 23.4 kDa (unassigned), 32.4 kDa (triose phosphate isomerase), 62.8 kDa (phosphoglucomutase + proteolysis products) and 74.7 kDa (unassigned). The electrophoresis profile of the *biceps femoris* muscle was characterised by peaks of 23.4 kDa and 32.4 kDa becoming higher and peaks of 62.8 kDa and 74.7 kDa lower. Conversely, the electrophoresis profile of the *semimembranosus* muscle was characterised by peaks of 23.4 kDa and 32.4 kDa becoming lower and peaks of 62.8 kDa and 74.7 kDa higher.

3.5. Process stage markers

All the electrophoresis profiles of the *biceps femoris* muscle could be classified in terms of the peaks with molecular weights

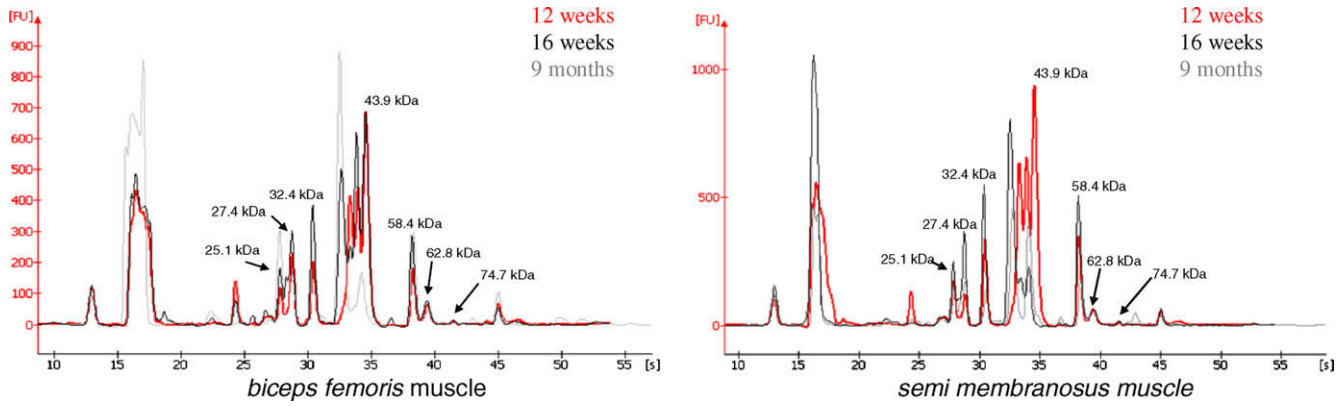

Fig. 2. *Semimembranosus* and *biceps femoris* muscles protein fingerprinting of dry-cured hams at 12 and 16 weeks and 9 months, obtained by the LabChip Agilent method.

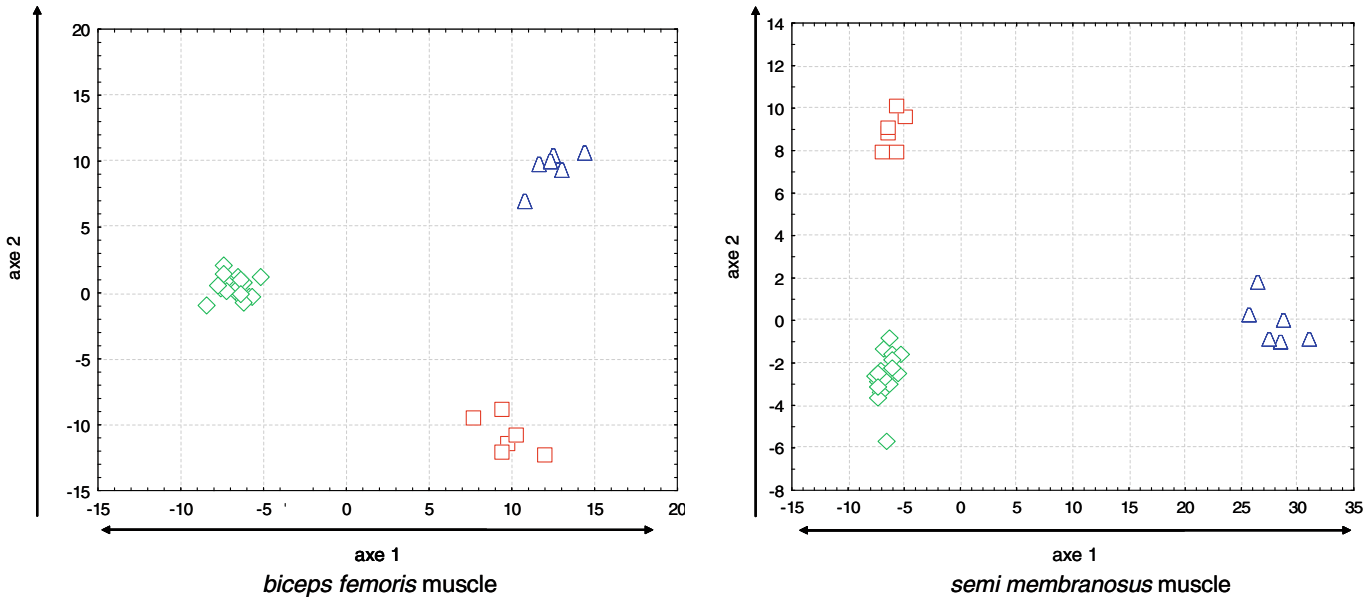

Fig. 3. Discriminant factor analysis: projection of *biceps femoris* and *semi membranosus* muscles fingerprinting at 12 and 16 weeks (respectively in blue and red) and 9 months (in green).

25.1 kDa (triose phosphate isomerase), 39 kDa (proteolysis products), 41.9 kDa (aldolase) and 45.8 kDa (enolase). Discriminant factor analysis indicated that two axes separated the three groups (12 weeks, 16 weeks and 9 months) (Fig. 3). The first axis, contrasting the peaks of 41.9 kDa and 45.8 kDa with those of 25.1 kDa and 39 kDa, separated the samples taken at 9 months from those taken at 12 and 16 weeks. The second axis was positively correlated with the peak of 41.9 kDa and negatively with the peaks of 36.3 kDa and 39 kDa, and separated the samples taken at 12 weeks from those taken at 16 weeks. The concentrations of the peaks of 25.1 kDa and 39 kDa were higher at 9 months, whereas the peaks of 41.9 kDa and 45.8 kDa were lower. At 12 weeks, the proteolysis profiles were characterised by lower peaks at 36.3 kDa and higher ones at 41.9 kDa than those measured at 16 weeks.

Likewise, all the electrophoretic profiles of the *semimembranosus* muscle could be classified using discriminating factor analysis (Fig. 3), on the basis of peaks with molecular weights 22.3 kDa (myokinase), 32.4 kDa (triose phosphate isomerase), 39 kDa (proteolysis products), 41.9 kDa (aldolase), 45.8 kDa (enolase) and 62.8 kDa (phosphoglucomutase + products). The first axis was negatively correlated with the peak of 39 kDa and positively correlated with the peaks of 41.9 kDa and 45.8 kDa. It separated the samples taken at 12 weeks from those taken at 16 weeks and 9 months. The second axis was correlated positively with the peaks of 22.3 kDa and 32.4 kDa and negatively with the peak of 62.8 kDa, and separated the samples taken at 16 weeks from those taken at 9 months. At 12 weeks the peak of 39 kDa was low, in contrast to the peaks of 41.9 kDa and 45.8 kDa. The profiles at 16 weeks, in contrast to those at 9 months, were characterised by higher peaks of 22.3 kDa and 32.4 kDa and a lower peak of 62.8 kDa. They were also defined, like those at 9 months, by a higher peak of 39 kDa and lower peaks of 41.9 and 45.8 kDa.

The use of certain discriminating peaks to classify samples highlighted a major difference between the two muscles. In the *biceps femoris* muscle the classification of profiles according to processing time was first made between the profiles at 12 and 16 weeks and those at 9 months. In the *semimembranosus* muscle the profiles at 16 weeks and 9 months were firstly separated from the profiles at 12 weeks. This contrast is due to the different localisations of the two muscles in the ham, which results in different biochemical reaction rates. The *biceps femoris* muscle is located deeper in the ham than the *semimembranosus* muscle. The salting is done in contact with the *semimembranosus* muscle whereas the *biceps femoris* muscle is located under the fat and rind: water and salt transfer thus take longer than in the *semimembranosus* muscle. Physical and chemical variables such as water activity and salt content, which affect the action of enzymes, have a slower influence on the extent of proteolysis in the *biceps femoris* muscle (Morales, Guerrero, Serra, & Gou, 2007; Parolari et al., 1994; Virgili et al., 1995). Thus the results (Fig. 4) show that the *semimembranosus* muscle had a significantly lower water content than the *biceps femoris* muscle, at all processing times. There was no change between 12 and 16 weeks. On the other hand, in both muscles the water content fell markedly at the end of processing. The chloride content was also measured (Fig. 5) in the *semimembranosus* and *biceps femoris* muscles at the three processing times. It was similar in the two muscles at the first two times, but was significantly higher at 9 months, more so in the *biceps femoris* than in the *semimembranosus* muscle. Most of the salt diffusion seemed to have taken place by 12 weeks, i.e. at the end of the settling stage. The differences observed at the end of processing may have been due to the drying of the product, which concentrates some of the salt present.

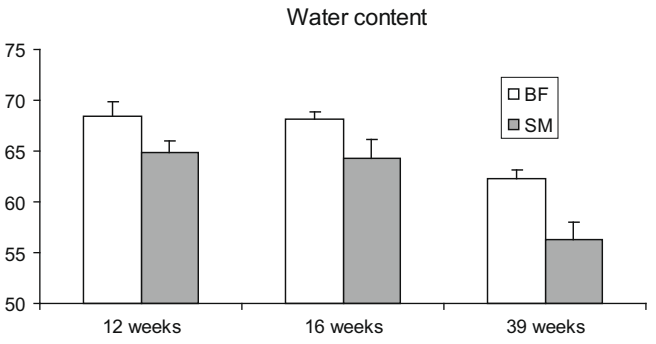

Fig. 4. Water content in biceps femoris (BF) and semimembranosus (SM) muscle during dry-cured ham processing.

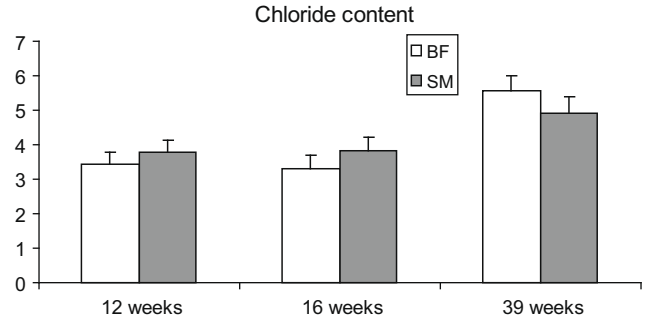

Fig. 5. Chloride content in *biceps femoris* (BF) and *semimembranosus* (SM) muscle during dry-cured ham processing.

4. Conclusion

The Protein LabChip Agilent method, which needs only small amounts of sample and reagents, is rapid and very accurate. The separation of proteins by this method yielded results in terms of molecular weight and concentrations of peptides and proteins in the soluble protein fraction of Bayonne ham.

The application of the Protein LabChip Agilent method to the study of proteolysis during the dry-curing of Bayonne ham (i) established the peptide fingerprints of the *semimembranosus* and *biceps femoris* muscles and showed their changes in the course of processing, (ii) characterised Bayonne ham according to the muscle and processing in both muscles and (iii) defined the targets and products of proteolysis. The result clearly show the possibility to discriminate between the profiles observed at the end of different processing steps. Further studies are needed to determine the accuracy of the technique on shorter processing intervals. One could expect that thanks to the definition of biological markers, this method could identify the authentication of hams in terms of processing time, a criterion incorporated in the specifications that processors have to meet.

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References

Arnau, J., Guerrero, L., & Sarraga, C. (1998). The effect of green ham pH and NaCl concentration on cathepsin activities and the sensory characteristics of dry-cured hams. *Journal of the Science of Food and Agriculture*, 387–392.

Basso, A. L., Picariello, G., Coppola, R., Tremonte, P., Spagna Musso, S., & Di Luccia, A. (2004). Proteolytic activity of *Lactobacillus sakei*, *Lactobacillus farminisand*, *Lactobacillus plantarum* sarcoplasmic proteins of pork lean. *Journal of Food Biochemistry*, 195–212.

Buscailhon, S., Berdagué, J. L., Bousset, J., Cornet, M., Gandemer, G., Touraille, C., et al. (1994). *Meat Science, 37*(2), 229–243.

Chanvrier, H., Uthayakumaran, S., & Lillford, P. (2007). Rheological properties of wheat flour processed at low levels hydration: Influence of starch and gluten. *Journal of Cereal Science*, 263–274.

Di Luccia, A., Picariello, G., Cacace, G., Scaloni, A., Faccia, M., Liuzzi, V., et al. (2005). Proteomic analysis of water-soluble and myofibrillar protein changes occuring in dry-cured ham. *Meat Science, 69*(3), 479–491.

Fadda, S., Vignolo, G., Holgado, A. P. R., & Oliver, G. (1998). Proteolytic activity of Lactobacillus strains isolated from dry fermented sausages on muscle sarcoplasmic proteins. *Meat Science, 49*(1), 11–18.

Larrea, V., Hernando, I., Quiles, A., Lluch, M. A., & Perez-Munuera, I. (2006). Changes in protein during Teruel dry-cured ham processing. *Meat Science, 74*(3), 586–593.

Mann, A. M., & Tighe, B. J. (2007). Tear analysis and lens-tear interactions. Part I: Protein fingerprinting with microfluidic technology. *Contact Lens Anterior Eye, 30*(3), 163–173.

Martin, A., Cordoba, J. J., Rodriguez, M. M., Nunez, F., & Asensio, M. A. (2001). Evaluation of microbial proteolysis in meat products by capillary electrophoresis. *Journal of Applied Microbiology*, 163–171.

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 dehairing techniques. *Meat Science, 47*(1/2), 29–47.

Morales, R., Guerrero, L., Serra, X., & Gou, P. (2007). Instrumental evaluation of defective texture in dry-cured hams. *Meat Science, 76*(3), 536–542.

Parolari, G., Virgili, R., & Schivazappa, C. (1994). Relationship between cathepsin B activity and compositional parameters in dry-cured hams of normal and defective texture. *Meat Science, 38*(1), 117–122.

Picariello, G., De Martino, A., Mamone, G., Ferranti, P., Addeo, F., Faccia, M., et al. (2006). Proteomic analysis of muscle sarcoplasmic proteins using AUT-PAGE/SDS-PAGE as two-dimensional gel electrophoresis. *Journal of Chromatography B Analytical Technology Biomedical Life Science, 833*(1), 101–108.

Robert, N., Basly, S., & Dutertre, C. (2005). Un affinage plus long améliore ses qualités sensorielles. *Viandes et produits Carnés, 24*(6), 201–204.

Rosell, C. M., & Toldra, F. (1998). Comparison of muscle proteolytic and lipolytic enzyme levels in raw hams from Iberian and white pigs. *Journal of the Science of Food and Agriculture*, 117–122.

Santos, N. N., Santos-Mendoça, R. C., Sanz, Y., Bolumar, T., Aristoy, M. C., & Toldra, F. (2001). Hydrolysis of pork muscle sarcoplasmic proteins by Debaromyces hansenii. *International Journal of Food Microbiology*, 199–206.

Sanz, Y., Fadda, S., Vignolo, G., Aristoy, M. C., Oliver, G., & Toldra, F. (1999). Hydrolysis of muscle myofibrillar proteins by Lactobacillus curvatus and Lactobacillus sake. *International Journal of Food Microbiology*, 115–125.

Uthayakumaran, S., Batey, I. L., & Wregley, C. W. (2005). On-the-spot identification of grain variety and wheat-quality type by Lab-on-a-chip electrophoresis. *Journal of Cereal Science*, 371–374.

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*, 1183–1186.

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