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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.

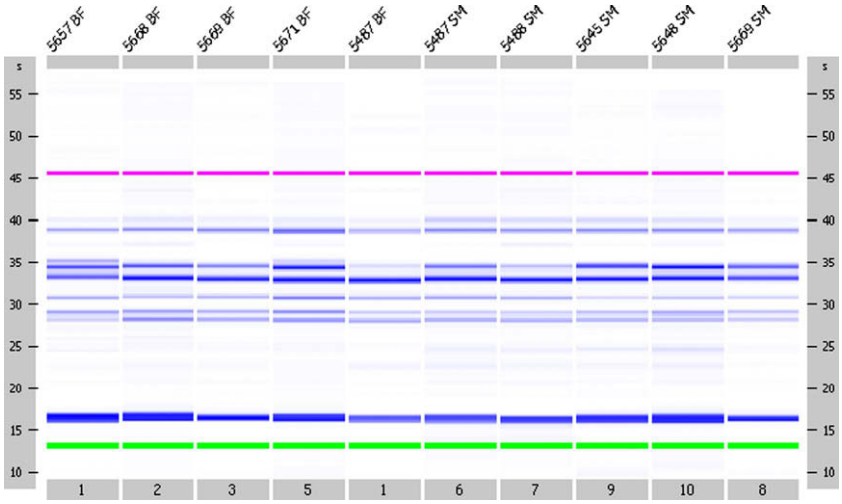


**Fig. 1.** Software-derived gel-like image analogous to the conventional SDS–PAGE gel.