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# Preliminary results of a proteomic study on "Fiocco" from Casertana ham at the end of seasoning

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**SUMMARY –** The study is performed on three Fiocchi from "Casertana" castrated males, an autochthonous pig, two of them were full-sibs. The special characteristics of this product at the end of the ripening period is the result of the activities of the muscle endogenous lysosomal enzymes. This work was carried out adopting a proteomic approach, using two dimensional gel electrophoresis and MALDI-TOF mass spectrometry, that evidenced the main proteins of the sarcoplasmic and myofibrillar fraction of *Semimembranosus, Biceps femoris* and *Semitendinosus,* the most representative muscles of Fiocco after 12 months of ripening.

**Keywords:** Pig, autochthonous genetic type, Fiocco, proteomics.

***RESUME –*** *"Résultats préliminaires d'une étude protéomique de Fiocco produit à partir de jambon de porcs Casertana à la fin du séchage". L'étude a été conduite sur trois Fiocchi de porcs mâles castrés appartenant au type génétique autochtone (TGA) "Casertana", deux d'entre eux étant frères germains. Les caractéristiques de ce produit à la fin du séchage sont le résultat de l'activité des enzymes endogènes du muscle. L'approche protéomique a été conduite avec l'électrophorèse bidimensionnelle couplée à la spectrométrie de masse MALDI-TOF. Elle a mis en évidence les principales protéines des fractions sarcoplasmiques et myofibrillaires des muscles,* Semimembranosus, Biceps femoris *et* Semitendinosus*, qui sont les muscles les plus représentatifs du Fiocco après un temps d'affinage de 12 mois.*

***Mots-clés :*** *Porc, type génétique autochtone, Fiocco, analyse protéomique.*

## Introduction

Fiocco is an Italian traditional product from pork leg. It is composed of *Semimembranosus, Biceps femoris* and *Semitendinosus* muscles in proportion of about 31, 27 and 42%, respectively. The raw pork matter was provided by "Casertana", an ancestral black pig reared in Southern Italy.

The object of this work was the identification of polypeptide components of Fiocco after 12 months of seasoning.

## Materials and methods

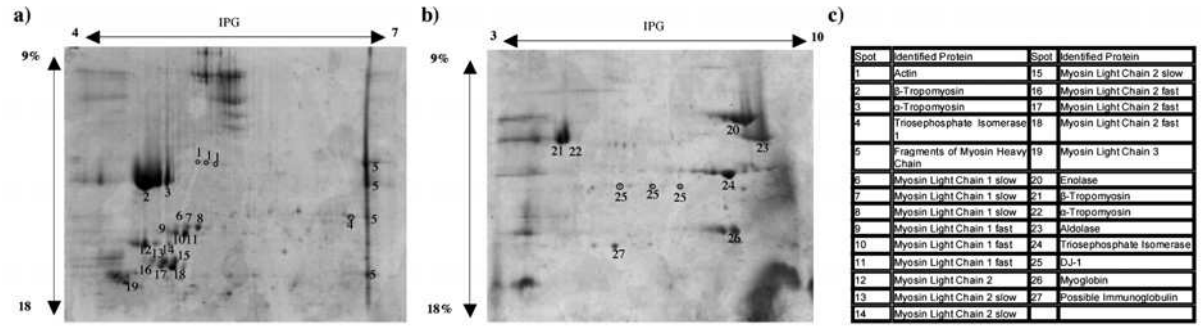
Three muscles (*Semimembranosus, Biceps femoris* and *Semitendinosus*) of each Fiocco per animal were separately analysed. Electrophoresis analysis of each muscle was repeated three times. Sarcoplasmic fraction was extracted by phosphate buffer 10 mM at pH 7.0; myofibrillar fraction was extracted from homogenized muscle residue using a reducing and denaturing buffer (CHAPS 4%, Urea 8 M, DTT 65 mM).

Two dimensional gel electrophoresis (2-DGE) was performed by means of Ettan twelve system (Amersham-Pharmacia Biotech). The first dimension was carried out by isoelectric focusing (IEF) using Immobiline DryStrips gel 3-10 NL (18 cm) for sarcoplasmic fraction and 4-7 (18 cm) for the myofibrillar fraction according to manufacturer's instructions. The second one was realized according

to the procedure of O'Farrell (1975) in pore gradient gel electrophoresis (T=9-18%; C=2.5). Two dimensional maps were acquired for image analyses with a Typhoon 9210 (Amersham-Pharmacia Biotech). Selected spots revealed by image analyses were digested *in situ* with trypsin, according to Shevchenko *et al*. (1996), tryptic digests were analysed by matrix assisted laser desorption ionization-time of flight (MALDI-TOF, Amersham-Pharmacia Biotech) mass spectrometry (MS). Peptide sequencing was realized by using CAF-PSD (Chemically Assisted Fragmentation – Post Source Decay) methodology.

## Results and discussion

The most hydrolysed myofibrillar proteins were identified as myosin light chain. These latter showed major modifications with respect to other myofibrillar proteins. Two-dimensional map of myofibrillar fraction from pork brothers showed a better overlapping with respect to the third sample that is not in relation.




| Spot | Identified Protein | Spot | Identified Protein |
|---|---|---|---|
| 1 | Actin | 15 | Myosin Light Chain 2 slow |
| 2 | ß-Tropomyosin | 16 | Myosin Light Chain 2 fast |
| 3 | ɒ-Tropomyosin | 17 | Myosin Light Chain 2 fast |
| 4 | Triosephosphate Isomerase 1 | 18 | Myosin Light Chain 2 fast |
| 5 | Fragments of Myosin Heavy Chain | 19 | Myosin Light Chain 3 |
| 6 | Myosin Light Chain 1 slow | 20 | Enolase |
| 7 | Myosin Light Chain 1 slow | 21 | ß-Tropomyosin |
| 8 | Myosin Light Chain 1 slow | 22 | ɒ-Tropomyosin |
| 9 | Myosin Light Chain 1 fast | 23 | Aldolase |
| 10 | Myosin Light Chain 1 fast | 24 | Triosephosphate Isomerase |
| 11 | Myosin Light Chain 1 fast | 25 | DJ-1 |
| 12 | Myosin Light Chain 2 | 26 | Myoglobin |
| 13 | Myosin Light Chain 2 slow | 27 | Possible Immunoglobulin |
| 14 | Myosin Light Chain 2 slow | | |

Fig. 1. 2-DGE maps of myofibrillar (a) and sarcoplasmic fraction (b) of *Fiocco sannita* and table of identified proteins (c).

At the end of seasoning were present most of the sarcoplasmic proteins except for the creatine kinase. Among these latter we identified three forms of DJ-1, which were confirmed by peptide sequencing. A such polymorphism in pork product has not been detected so far. The salting step led to the tropomyosin solubilization, a myofibrillar protein, in the sarcoplasmic fraction.

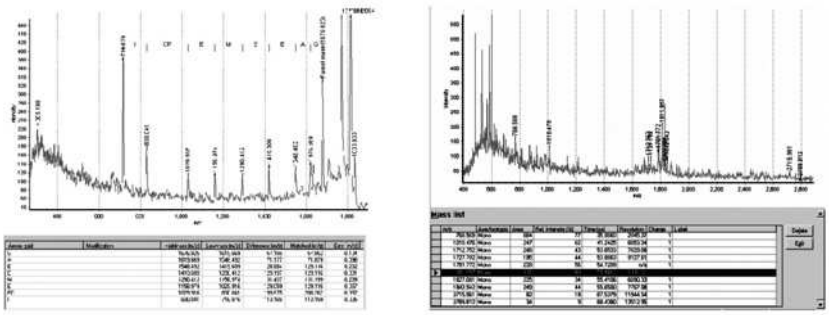


Fig. 2. CAF-PSD spectrum of the peptide with 1811.857 Da selected from peptide mass list of parent protein DJ1.

Therefore we can establish that the proteolysis in the Fiocco occurs essentially by action of muscle endogenous enzymes and that this latter does not hydrolyse the sarcoplasmic proteins as occurs for dry cured ham at ripening end (Di Luccia *et al*., 2004).

The proteolysis products are small peptides and amino acids that contribute to taste and aromas of ripened product (Toldrà *et al*., 1997); nevertheless it has not to be neglected the possible

production of bioactive peptide worked by endogenous muscle enzymes and those encrypted in muscle proteins delivered by digestive enzymes. From an investigation performed on each muscle (*Semimembranosus, Biceps Femoris* and *Semitendinosus*) pre and post seasoning, was obtained 2DGE maps that showed: (i) the presence of the three forms of DJ-1 in the three muscles, which can be used as molecular markers for traceability; (ii) quali-quantitative differences in sarcoplasmic fraction; and (iii) quantitative differences in myofibrillar fraction.

The comparison of these results with those obtained from dry-cured ham of the same animal, in order to reveal possible differences in the ripening phase, is in progress.

On the basis of these preliminary results, we are also investigating enzymatic action of B, L, D and H cathepsins on soluble muscle proteins, known so far, in order to identify generated peptides. Furthermore, we are determining, by quantitative analyses, the importance of different protein fractions: (i) to individuate possible quali-quantitative variations occurring in the ripening phase; (ii) to improve the understanding of proteolytic phenomena; (iii) to define action sites of cathepsins on soluble muscle proteins; and (iv) to reveal the neoformed bioactive peptides in the finished product.

To deep nutritional and extranutritional characteristics of bio-molecules we are performing an *in vitro* study of digestion enzymes with the aim of human bodily, psychic and social welfare.

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