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Proteomics approach for the definition of a molecular identity card of the traditional meat product *fiocco sannita* obtained from ancient autochthonous genetic type (AAGT) *Casertana* pig. Preliminary results[1]

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SUMMARY – The study was carried out on a "typical traditional" product named *fiocco sannita*, obtained from 12 pigs belonging to AAGT *Casertana* and ripened for twelve months. In this product *Semimembranosus*, *Biceps femoris*, and *Semitendinosus* muscles represent about ~ 31, ~ 27 and ~ 42%, respectively. The definition of the muscular protein has been achieved by the proteomics approach. The myofibrillar and sarcoplasmatic proteins were previously fractionated. The profile of the two protein fractions was evaluated, at the end of ripening, by analytical procedures such as 2-DE and MS-MALDI-TOF. Quali-quantitative analysis, within limits of the observation field, highlighted: (i) a remarkable complexity, in addition to the main sarcoplasmatic and miofibrillar proteins, due to some of their multiple isoforms and fragments; (ii) a heterogeneity of two proteins, α-actin and DJ-1; and (iii) a high individual variability. The proteomics approach proved a useful tool for the characterization of this "typical traditional" product.

Key words: *Casertana*, *fiocco sannita*, mass spectrometry, proteolysis, ancient autochthonous genetic type.

RESUME – *"Approche protéomique pour la définition de la carte d'identité moléculaire du produit carné traditionnel* fiocco sannita*, obtenu à partir de porcins de l'ancien type génétique autochtone (TGAA)* Casertana*. Résultats préliminaires". La recherche a été conduite sur un produit "traditionnel typique" dénommé* fiocco sannita *issu de 12 porcs du type génétique autochtone ancien (TGAA)* Casertana*. Le* fiocco sannita *est composé des muscles* Semimembranosus*,* Biceps femoris*, et* Semitendinosus *dans la proportion de ~ 31, ~ 27 et ~ 42%, respectivement. La définition des protéines musculaires a été réalisée selon une approche protéomique. Les protéines de la fraction soluble dans l'eau et de la fraction saline soluble ont été préalablement fractionnées. La définition du profil protéique, après un temps d'affinage de douze mois, a prévu l'emploi de techniques telles que l'électrophorèse bidimensionnelle couplée à la spectrométrie de masse MALDI-TOF qui ont mis en évidence, dans le domaine d'observation : (i) une considérable complexité ; (ii) une hétérogénéité pour deux protéines, α-actine et DJ-1 ; et (iii) une variabilité individuelle élevée. L'étude protéomique s'est révélée un moyen très utile pour la caractérisation de ce produit traditionnel typique.*

Mots-clés : Casertana*,* fiocco sannita*, spectrométrie de masse, protéolyse, type génétique autochtone ancien.*

Introduction

The characterization of products of animal origin represents an essential step for their achievement and safeguard.

The proteomics analysis through very innovative tools, such as two-dimensional electrophoresis (2-DE) at high resolution, image analysis and mass spectrometry MALDI-TOF, allows the characterization of complex patterns of the expressions and turnover of cell proteins. Its application in

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food sector has allowed the individuation and identification of molecular markers (biomarker) and the achievement of a standard map for the characterization of final product. These biomarkers might be used for optimization of meat production and conservation. Moreover, together with other traditional quality parameters (physical, chemical-physical, physiological and chemical), they contribute to the definition of nutritional and extranutritional traits and to the individuation of territorial specificity. The constitution of a bioimages database, from two-dimensional maps of traditional product proteins, was used as collection of fingerprintigs of the product above mentioned.

Fiocco sannita, similarly *prosciutto*, derives from pork leg but, unlike the latter one, has been boned and trimmed in order to obtain the typical shape. From the processing point of view it represents an interesting product because after salting, common to *prosciutto*, it will be conserved into bowels, rigorously swine, this latter tipic of fermented salami. In this case, the microbial flora develops only in the periferic area of the product, as reported by Sarra *et al.* (2004). *Fiocco sannita* was obtained from a AAGT *Casertana*, known since from ancient times and defined by Hoesch (first half of X sec.) "Italian swine pride". For this AAGT, present nowadays mainly in Campania (Italy), a wide recovery programme is in progress and this research is part of this programme, contributing to the achievement of products derived from meat of these pigs.

Materials and methods

The samples were collected from three muscles (*Semimembranosus*, *Biceps femoris*, and *Semitendinosus*) of each *fiocco* and analyzed separately. For the extraction of sarcoplasmatic fraction, muscle tissue (5 g) was homogenized into 20 ml of phosphate 10 mM buffer at pH 7.0; myofibrillar fraction was extracted from homogenized muscular residue, using denaturing and reducing buffer (4% CHAPS, 8 M urea, 65 mM DTT).

The first dimension was performed by Ettan IGPhor II (Amersham-Pharmacia Biotech) using Immobiline DryStrips gel pH 3-10NL (18 cm) for the sarcoplasmatic fraction, and Immobiline DryStrips gel pH 4-7 (18 cm) for miofibrillar one. The two-dimensional electrophoresis gel (2-DE) was performed by an Ettan Twelve System (Amersham-Pharmacia Biotech).

The second dimension was performed according to O'Farrel procedure (1975), on an electrophoretic gel constituted of a gradient of pores (T = 9-18%; C = 2.5).

The two-dimensional map was scanned for image analysis by a Typhoon 9210 (Amersham-Pharmacia Biotech). The spots, detected from image analysis and appropriately selected, were digested *in situ* with tripsina, according to Shevchenko *et al.* (1996), and the triptic digests were analyzed by mass spectrometry MALDI-TOF (Amersham-Pharmacia Biotech).

The peptidic sequencing was performed by using the CAF-PSD methodology (Chemically Assisted Fragmentation – Post Source Decay).

Results and discussion

Figure 1 shows a 2-DE map of the sarcoplasmatic protein fraction in which was highlighted the presence of the myofibrillar protein tropomyosin, as previously reported by di Luccia *et al.* (2005), essentially due to the use of sodium chloride during salting. In this figure emerges the absence of creatin kinase, while other sarcoplasmatic proteins are almost all identifiable (Matassino *et al.*, 2004; di Luccia *et al.*, 2005). In this latter case, 2-DE map is very similar to that one obtained from proteomic study on *prosciutto* ripened.

Figure 2 shows a 2-DE map of the myofibrillar protein fraction, in which is evidenced the deep proteolysis interesting particularly myosin light chains. In the miofibrillar 2-DE map were detected proteins such as: (i) tropomyosins; (ii) fragments of myosin heavy chain; (iii) actin; and (iv) myosin light chain (MLC1 fast and slow, MLC2 fast and slow, MLC3).

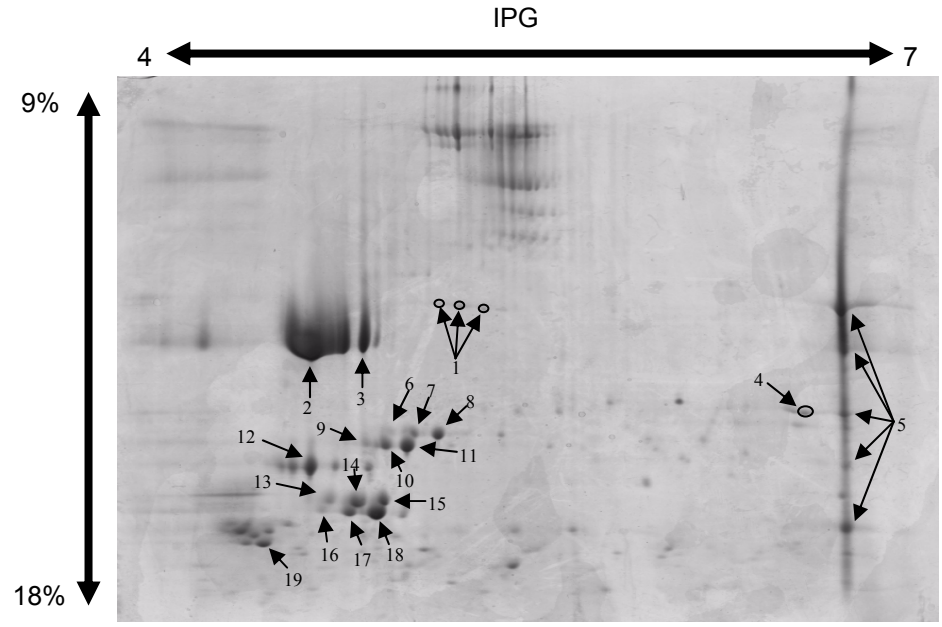

Fig.1. 2-DE map of sarcoplasmatic protein fraction of *fiocco*.

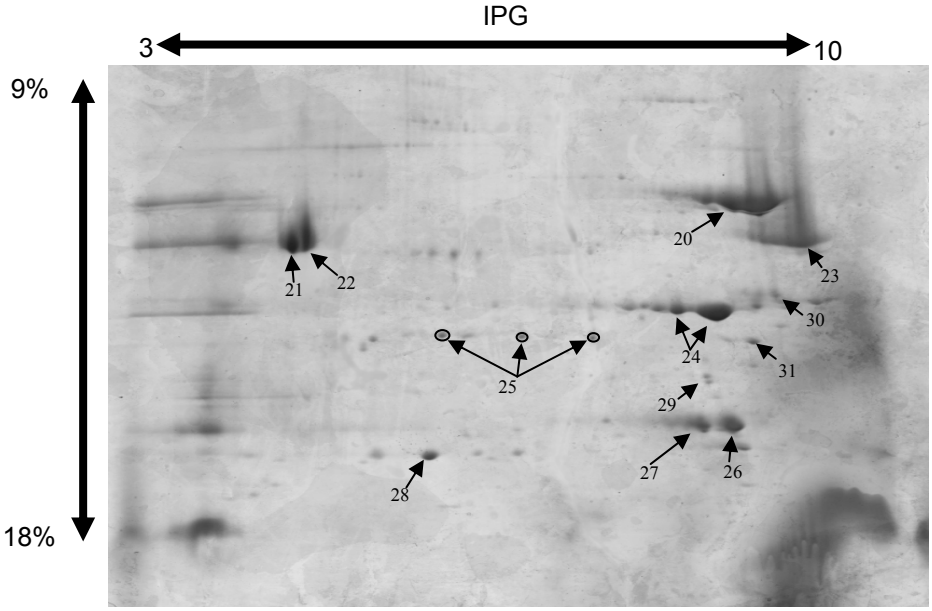

Fig. 2. 2-DE map of miofibrillar protein fraction of *fiocco*.

Table 1 summarizes the peptidic map of identified spots by mass spectrometry MALDI-TOF.

Moreover two-dimensional analysis of the two protein fractions showed a polymorphism for two proteins: (i) actin (myofibrillar protein); and (ii) DJ-1 (sarcoplasmatic protein that would have an important function in neuronal metabolism, with particular regard to Parkinson prevention (Abou-Sleiman *et al.*, 2003).

Both these proteins were identified by mass spectrometry MALDI-TOF and confirmed by peptidic sequencing 1334 Da (Fig. 3) and 1811.85 Da (Fig. 4), respectively.

Table 1. Identification of the protein from pork skeletal muscles by MALDI -TOF mass spectrometry

Spot	Identified protein	Spot	Identified protein
1	Actin	17	Myosin light chain 2 fast
2	β-Tropomyosin	18	Myosin light chain 2 fast
3	α-Tropomyosin	19	Myosin light chain 3
4	Triosephosphate isomerase 1	20	Enolase
5	Fragments of myosin heavy chain	21	β-Tropomyosin
6	Myosin light chain 1 slow	22	α-Tropomyosin
7	Myosin light chain 1 slow	23	Aldolase
8	Myosin light chain 1 slow	24	Triosephosphate isomerase
9	Myosin light chain 1 fast	25	DJ-1
10	Myosin light chain 1 fast	26	Chain A mioglobyn
11	Myosin light chain 1 fast	27	Myoglobin
12	Myosin light chain 2	28	Fatty acid binding-protein
13	Myosin light chain 2 slow	29	Low density lipoprotein
14	Myosin light chain 2 slow	30	Carbonic anhydrase
15	Myosin light chain 2 slow	31	Superoxyde dismutase
16	Myosin light chain 2 fast		

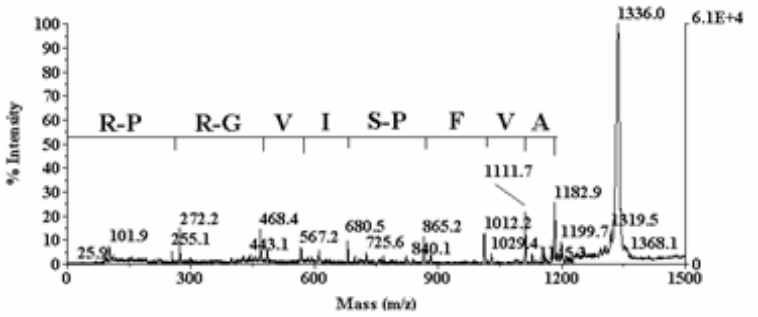

Fig. 3. CAF-PSD spectra of 1334.57 Da peptide from actin parent protein.

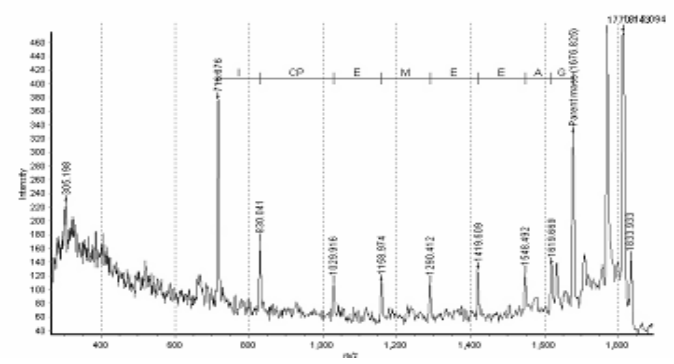

Fig. 4. CAF-PSD spectra of 1811.857 Da peptide from DJ-1 parent protein.

Both the proteins are present into three multiple isoforms that differ each other for the values of isoelectric point. Analysing international literature, at our knowledge, the polymorphism of the DJ-1 and actin would be detected for the first time in pork ripened meat (Matassino *et al.*, 2004, 2005). The heterogeneity observed might be explained considering the spots, related to the two proteins, as

expression of a polymorphism of *loci*, seat of polypeptides encoding DNA segments (genes) or as a result of post-translational modification that originates a different level of phosphorilation.

Moreover, in myofibrillar protein fraction of *Semitendinosus* muscle (Fig. 5), from 137 to 458 spots were detected, only 17 of which matched to the different individual maps; the relative volume (%) of the 17 matched spots highlightes a high variability (24-83) expressed as cv (%).

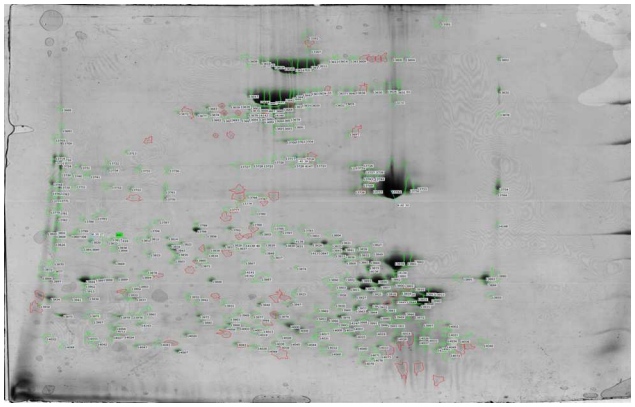

Fig. 5. 2-DE map of myofibrillar protein fraction of semitendinosus muscle.

Conclusions

The polymorphism evidenced by two-dimensional maps for the actins and DJ-1 might be an element of products differentiation from obtained from other genetic types of pork meat.

By image analysis 2-DE standard maps might be obtained, which can give indications for the optimization of the length of ripening, as well as to achieve information for the improvement of the conditions of some steps in the flow diagram. These maps constitute also a term of comparison in order to verify the presence of some typicality and healthy indicators of a product.

Probable discordances between the product map under examination and that one present in a database might be index of factors regarding:

(i) Quality and typicality of product:

- Product of imitation.
- Different conditions of ripening of raw materials processed.
- Origin, due to the traditions of flow diagram of a territory or of a specific geographical area.

(ii) The healthy of the product:

- Anomalous fermentations due to a not desired pollutant or pathogenic bacteric flora.

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