Differential expression of sarcoplasmic protein in \'Casertana\', \'Calabrese\' and PEN AR LAN Pork

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Differential expression of sarcoplasmic protein in 'Casertana', 'Calabrese' and PEN AR LAN Pork

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Abstract. The characterization of the water soluble fraction of muscle proteins was carried out on 30 samples of meat taken from a pool of muscles (*Semimembranosus, Semitendinosus* and *Biceps femoris*) each representative of the 30 subjects {[20 ancient autochthonous genetic type (AAGT) with 'black' coat [10 '*Casertana*' (CT), 10 '*Apulo Calabrese*' (Calabrese) (CL)] and 10 PEN AR LAN ('white' coat, from cross breeding Large White and Landrace)}. Protein profile was analyzed by two-dimensional gel electrophoresis and MALDI-TOF mass spectrometry. The comparison of 60 two-dimensional maps was performed by Image Master 2D-Platinum software in order to establish the position and relative intensity, expressed as vol %, of each spot for each gel. In the range of our observation, image analysis showed 32 spots common to all samples analyzed; 17 spots of 32 common differed in relative 'abundance' ($P<0.05$). These spots, identified by peptide mass fingerprint, were classified as metabolic, cellular defense and other protein types. The results suggest a further possible use of proteomic approach in the tracing back of traditional food.

Keywords. MALDI-Tof fingerprint – Pig – Sarcoplasmic proteins – Two-dimensional gel electrophoresis.

Expression différentielle des protéines de la fraction soluble dans l'eau pour la viande des TGAA 'Casertana', 'Calabrese' et des hybrides commerciaux PEN AR LAN

***Résumé**. La caractérisation de la fraction soluble des protéines musculaires a été effectuée sur 30 échantillons de viande provenant d'un pool de muscles (*Semimembranosus, Semitendinosus *et* Biceps femoris*) pour chacun des 30 sujets traités {20 de type génétique autochtone ancien (TGAA) à robe 'noire' [10 'Casertana' (CT), 10 'Apulo Calabrese' (Calabrese) (CL)] et 10 PEN AR LAN (à robe 'blanche', d'ascendants Large White et Landrace)}. Le profil protéique a été évalué en utilisant des procédures analytiques telles que l'électrophorèse bidimensionnelle couplée à la spectrométrie de masse MALDI-TOF. La comparaison des 60 cartes a été réalisée avec le software Image Master 2D-Platinum afin de comparer la position et l'intensité relative, exprimée en % vol de chaque spot pour chaque gel. Dans le cadre de l'observation, l'analyse d'image a mis en évidence 32 spots communs à la totalité des échantillons analysés ; 17 spots sur les 32 diffèrent en % vol ($P<0,05$). Ces spots ont été ensuite identifiés par peptide mass fingerprint et classés comme protéines du métabolisme, de défense cellulaire et autres protéines. Les résultats suggèrent une possible utilisation ultérieure de l'approche protéomique pour des études de caractérisation visant, entre autres, à l'analyse de la traçabilité.*

***Mots-clés**. MALDI-Tof fingerprint – Porc – Protéines de la fraction soluble dans l'eau – Électrophorèse bidimensionnelle.*

I – Introduction

Proteomic analysis defines the identity, the structure and the relative abundance of proteins in a given cell type in a specific set of conditions. Proteins are the expression of genetic inheritance which also undergo post-translational modifications (phosphorylation, glycosylation and acetylation). The study of these modifications, by proteomic approach, may assist to differentiate animal species. The proteomic approach can be used for 'molecular

characterization' of raw materials and their products; this 'characterization' can be employed in order to: (i) trace back product; (ii) point out flow chart phases; (iii) characterize possible relationships among protein (and their fragments) 'quantity' and 'quality attributes' of raw materials and their products.

The aim of this contribution was to suggest a proteomic approach to differentiate swine races. Differential analysis of the proteome in relation to the soluble fraction (sarcoplasmic) was carried out on meat samples taken from a pool of muscles (*Semimembranosus, Semitendinosus* and *Biceps femoris*) in pigs.

II – Materials and methods

The study involved 30 meat samples taken from a pool of muscles (*Semimembranosus, Semitendinosus* and *Biceps femoris*) each representative of the 30 subjects {[20 ancient autochthonous genetic type (AAGT) with 'black' coat [10 '*Casertana*' (CT), 10 '*Apulo Calabrese*' (Calabrese) (CL)] and 10 PEN AR LAN ('white' coat, from cross breeding Large White and Landrace)}. The slaughter was carried out in a single establishment and the carcass maturation was carried out in refrigerator at a temperature of 2-4 °C for a period of approximately 72 hours.

The analysis covered a total of 60 samples (2 for each sample) processed in parallel by:

(i) 2D-IPG-SDS-PAGE:

- the first dimension (IEF-IPG) was carried out by Ettan IPGphor II (GE Healthcare) using Immobiline DryStrips gel pH 3-10NL (18 cm) rehydrated with a solution of 8 M Urea, 0.5% CHAPS, 0.2% DTT, 0.5% IPG Buffer.
- the second dimension was carried out in accordance with the procedure of O'Farrell (1975) in polyacrylamide gradient gel electrophoresis (T = 9-18% and C = 2.5%) by using Ettan Twelve System (GE Healthcare).

(ii) Image analysis of two-dimensional maps (2-DGEm):

- was performed by software Image Master 2D-Platinum (GE Healthcare) quantifying in vol % the expression level of each spot; the spots found in common to three swine races were subjected to statistical analysis Student's t test.

(iii) Identification by MS:

- each spot was digested *in situ* with trypsin according to the procedure of Shevchenko *et al.* (1996) and tryptic digests were analyzed with Ettan MALDI-Tof/PRO mass spectrometer (GE Healthcare).

III – Results and discussions

The measurements carried out by MALDI-Tof mass spectrometer allowed us to identify 49 spots (Figure 1, Table 1). Some proteins appeared heterogeneous with differences in mass [MW (kDa)] and / or isoelectric point (pI) and they were identified as: Adenylate kinase, Myoglobin, Peroxiredoxin, Phosphoglycerate mutase, Similar parvalbumin, Transferrin, Triosephosphate isomerase. This heterogeneity could be due to:

(i) Genetic polymorphism (for example single nucleotide polymorphism).

(ii) Post-transcriptional modifications (for example alternative splicing).

(iii) Post-translational modifications (for example glycosylation and phosphorylation).

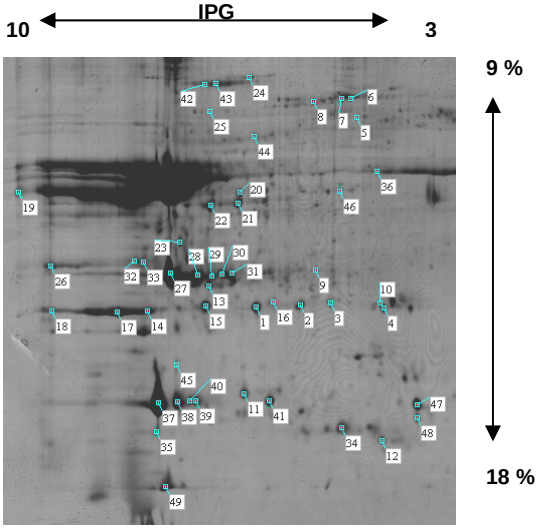

Fig. 1. 'Casertana' AAGT. Two-dimensional map of the sarcoplasmic proteins.

Considering the three breeds, 32 spots (65% of those identified) resulted in common to totality of subjects analyzed. The proteins identified were grouped by protein function as reported in Table 1:

(i) Metabolic protein:

- adenylate kinase (it catalyzes the reversible transfer of terminal phosphate group between ATP and AMP),
- enolase (it is an enzyme involved in the development and regeneration of striated muscle).

We compared 32 common spots of the three swine races analyzed. The differences observed were analyzed by Student's t test (Figure 2; Table 2) and divided in significant (P<0.05-0.001) and near to significant (P<0.20-0.10):

- CL *vs* CT: 14 comparisons out of 32 [44%, 6 of which significant (P<0.05-0.001) and 8 near to significance (P<0.20-0.10)].
- CL *vs* PEN AR LAN: 22 comparison out of 32 [69%, 11 of which significant (P<0.05-0.001) and 11 near to significance (P<0.20-0.10)].
- CT *vs* PEN AR LAN: 16 comparison out of 32 [50%, 12 of which significant (P<0.05-0.001) and 4 near to significance (P<0.20-0.10)].

Table 1. Spots identified by MALDI-Tof mass spectrometry. Red spots were common to all two-dimensional maps

Group	Spot, N	Protein name
Cellular defense proteins	1, 2, 3	DJ-1
	4	Glyoxalase
	5	HSP 60
	6, 7, 8	HSP 70
	9, 10	Peroxiredoxin
	11	Superoxide dismutase
	12	Thioredoxin
Metabolic proteins	13, 14, 15, 16, 17, 18	Adenylate kinase
	19	Aldolase
	20, 21, 22	Enolase
	23, 24	Muscle Creatine kinase
	25, 26	Phosphoglicerate mutase
	27, 28, 29, 30, 31	Triosephosphate isomerase
Transport proteins	32, 33	Carbonic anhydrase
	34	H-FABP
	35	Haemoglobin ß-chain
	36	Haptoglobin
	37, 38, 39, 40, 41	Myoglobin
	42, 43	Transferrin
Indicators of proteolysis	44	Leucine aminopeptidase
	45	Muscle Creatine kinase fragment
Miscellaneous	46	Zinc finger protein
	47, 48	Similar Parvalbumin
	49	Similar to polyubiquitin

The major number of significant and near to significant critical limit comparisons was found in the comparison CL *vs* PEN AR LAN (69%) while the lower number (44%) was found in the CL *vs* CT comparison. It is interesting to note that the major differences in protein expression were detected among race with 'white' coat (PEN AR LAN) and those with 'black' coat (CL and CT). The differences related to the proteins classified by function as:

(i) cellular defense proteins (DJ-1, HSP 70, thioredoxin),

(ii) metabolic proteins (adenylate kinase, enolase, triosephosphate isomerase),

(iii) transport proteins (H-FABP, haemoglobin beta chain, myoglobin),

(iv) proteolysis indicator [leucine aminopeptidase (LAP)],

(v) other proteins (parvalbumin).

Table 2. Differentially expressed spots. Comparison between 'Apulo Calabrese' (Calabrese), 'Casertana' and PEN AR LAN. Identification by MALDI-Tof mass spectrometry

Group	Spot, *N*	Protein name	CL *vs* CT	CL *vs* H	CT *vs* H
Cellular defense proteins	2	DJ-1	<<<	***	***
	3		**	***	***
	4	Glioxalase	<	<<<	
	5	HSP 60	<<<		<<<
	6	HSP 70		<	
	7			<<	**
	8			<<	*
	12	Thioredoxin	**	<<	
Metabolic proteins	13	Adenylate kinase	**	<<<	
	14		<<<		*
	15		<<<	<<<	
	20	Enolase		<<<	<
	21			*	<<<
	22			*	<<<
	23	Muscle creatine kinase	<<<	<<<	
	27	Triosephosphate isomerase	*	*	
Transport proteins	34	H-FABP	<<	***	**
	35	Haemoglobin ß-chain		**	*
	37	Myoglobin		***	**
	38		*	***	**
	39		***	***	**
Indicators of proteolysis	44	Leucine aminopeptidase	<<	<<	*
Miscellaneous	47	Similar Parvalbumin		*	<<

CL = 'Apulo Calabrese' (Calabrese); CT = 'casertana'; H = Pen Ar Lan 'Hybrid'

< P<0.20; << P<0.15; <<< P<0.10; * P<0.05; ** P<0.01; *** P<0.001

Our results highlighted the importance of 'race' for relative quantitative expression (volume, %) of spots considered, as previously showed in sheep and pig by Matassino *et al*. (2010 a,b), and for energy metabolism that may be different depending from coat color ('black' or 'white'). In fact, in the 'white' pig the higher expression of enzymes involved in the glucidic metabolism and the lower expression of myoglobin may point out that muscle cells favour the glycogen catabolism and, so, in order to balance the energy metabolism, they may pre-eminently use glucose rather than fatty acid. The opposite behaviour is observed in 'black' pig in which, an increased expression of myoglobin and H-FABP and a decreased expression of glucose metabolism enzymes would indicate a metabolism mainly based on oxidative chain. This is also confirmed by the predominantly 'oxidative' nature of some muscles of 'black' and 'belted' pigs caused by a higher percentage of Slow Oxidative fibers (SO), as evidenced by previous research (Matassino *et al.*, 1993; Barone *et al.*, 2000, 2005). This would result in a different use of the energy stored by two genetic types of pigs: the 'black' pig would use preferentially energy stored in adipose tissue while the 'white' pig would use energy stored under glycogen form. This difference in the metabolic activity of muscles may be attributed to a different use of the metabolic - energy pathways (glycolysis or oxidative). This differentiation could derive from a diversity of both genetic (for example coat color gene) and hormonal by stress (for example adrenaline) nature; both could influence the structure and the functionality of the muscular fiber.

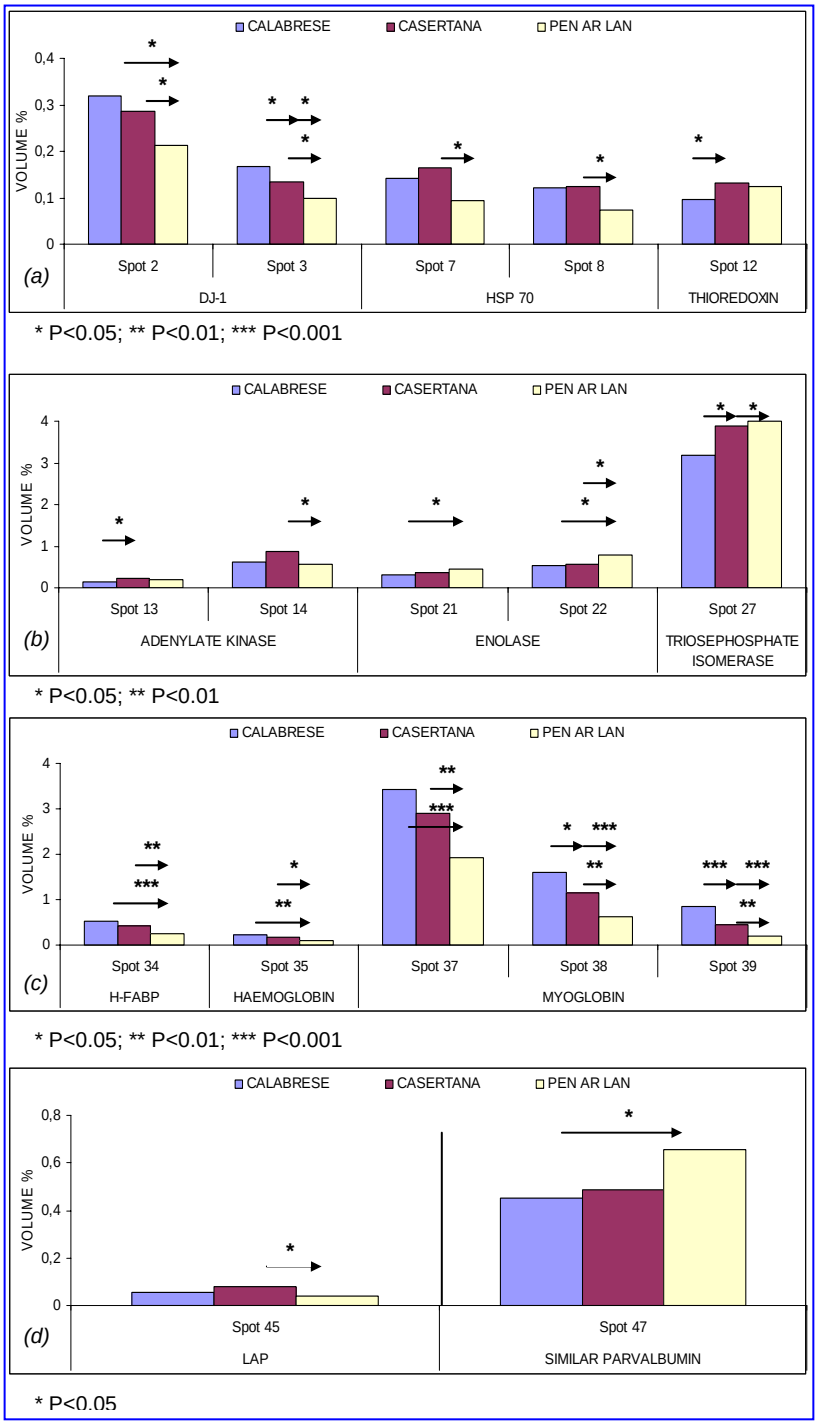

Fig. 2. Spots differentially expressed grouped in: (a) cellular defense proteins, (b) metabolic proteins, (c) transport proteins, (d) indicator of proteolysis and miscellaneous.

This differentiation could derive from a diversity of both genetic (for example coat color gene) and hormonal by stress (for example adrenaline) nature; both could influence the structure and the functionality of the muscular fiber.

These results demonstrated that it was possible to study muscle phisiology not only by fiber composition but by molecular level too.

The increased expression of cellular defense proteins in 'black' pig may suggest a better its response to phenomena of environmental stress. This is in agreement with the greater ability to constructivism (Matassino,1989; Lewontin, 1993) hypothesized for 'black' pig in comparison with 'white' pig, being the former also in a initial phase of anthropic conditioning.

IV – Conclusions

The proteomic approach to the study of sarcoplasmic protein of muscle revealed the differences in energy metabolism between 'black' and 'white' pig. These differences could represent sources of 'molecular characterization' that could allow both to trace back meat of different genetic types and to assess their 'nutritional' and 'extra-nutritional' quality. However, it is necessary to extend sampling and to deepen the study of muscular protein composition.

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