PROTEOME ANALYSIS OF THE SARCOPLASMIC FRACTION OF CATTLE LONGISSIMUS DORSI MUSCLE

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Abstract – Sarcoplasmic proteins changes were evaluated in 24 young bulls from different breed at four aging time (1, 7, 14, 21d). Proteolysis was investigated by SDS-PAGE and two-dimensional electrophoresis coupled to mass-spectrometry. SDS-PAGE and 2DE showed that many changes in the sarcoplasmic proteins occurred among breeds and during aging. During post-mortem some sarcoplasmic proteins decline in intensity after 21d highlighting that they were susceptible to aging. Proteins identification showed the presence of myosin light chains and tropomyosin proteins during aging, suggesting a degradation of myofibers and a more intense proteolysis especially in Podolian breed.

Key Words – aging, effect of breed, proteolysis

I. INTRODUCTION

A number of factors contribute to *post mortem* tenderization of meat, in particular, many authors [1], [2], [3] indicate that proteolytic degradation of myofibrillar proteins plays an important role in tenderization showing ultrastructural changes in skeletal muscle. Even though, studies in pork reported that the denaturation of sarcoplasmic proteins has an impact on meat quality parameters such as color and water holding capacity [4]. A large number of genetically distinct cattle breed are reared in Italy and this genetic diversity produces meat with many peculiar features. The effect of breed and aging on meat tenderness and on proteolytic pattern of myofibrillar proteins are reported in previous studies [5], while to our knowledge, limited data are available on changes in meat sarcoplasmic proteins among different cattle breeds during aging.

Therefore, in the present study we investigated the effect of breed and aging time on sarcoplasmic proteins changes in *longissimus dorsi* muscle in three breeds with different productive purpose.

II. MATERIALS AND METHODS

Twenty four young bulls of three cattle breeds with different productive purpose, dairy (Friesian), beef (Romagnola x Podolian crossbreed) and rustic (Podolian), were used in this study. Animals were reared intensively during the finishing period and were slaughtered at 19 months of age, according to industrial routines used in Italy and to the EU rule n. 119/1993. 24 h post mortem *Longissimus dorsi* (LD) muscle was removed from each half of the carcass. Each muscle was divided longitudinally into two sections resulting in four samples for each animal and each sample was stored at 2°C under vacuum packaging and analyzed at 1, 7, 14 and 21 d of aging, respectively.

Sarcoplasmic proteins were extracted and resolved by Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in a gradient gel 8-18%. The run was performed in a continuous buffer system using a Protean II xi vertical slab gel unit (Bio-Rad Laboratories, Hercules, CA). Destained gel images were acquired by the Chemi Doc EQ system (Bio-Rad Laboratories, Hercules, CA) using a white light conversion screen and analyzed with the Quantity One software (Bio-Rad Laboratories, Hercules, CA)

Two dimensional separation was performed on a Protean II xi vertical slab gel unit (Bio-Rad Laboratories, Hercules, CA) using SDS-PAGE gradient 8–18%. The destained gels were acquired by the Chemi Doc EQ system (Bio-Rad Laboratories, Hercules, CA) using a white light conversion screen and analyzed with the ImageMaster 2DE Platinum software 5.0 (GE Healthcare, Piscataway, NJ).

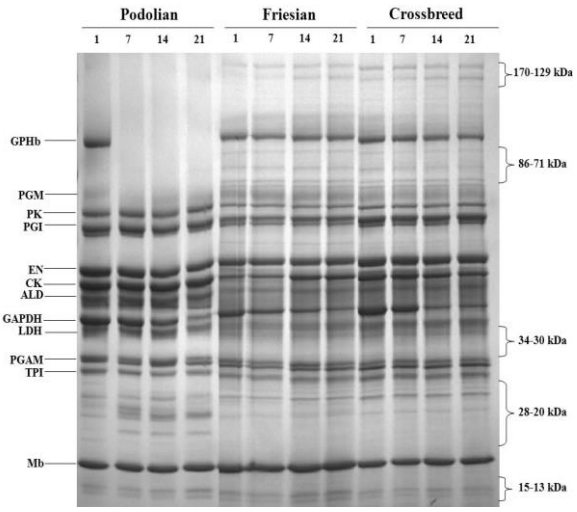
Protein of interest from SDS-PAGE and 2DE preparative gels were destained, digested with trypsin as reported by Giangrande et al. [6]. and analysed using a 6520 Accurate-Mass Q-TOF LC/MS System (Agilent Technologies, Palo Alto, CA, USA) equipped with a 1200 HPLC system and a chip cube (Agilent Technologies).

III. RESULTS AND DISCUSSION

The electrophoretic profile of sarcoplasmic proteins of the three different breeds during aging time is depicted in Figure 1. Breed affected sarcoplasmic protein profiles showing bands not consistent in all breeds. In particular, Podolian meat did not show bands from 170 to 129 kDa and from 86 to 71 kDa, while Glycogen phosphorilase b kinase was present 1 day *post mortem* and disappeared thereafter. Furthermore, Podolian meat showed the highest values ($P<0.001$) of PK, Aldolase, Phosphoglycerate mutase, Triosephosphate isomerase and of 28-20 kDa polypeptides compared to Friesian and Romagnola x Podolian during all the aging time. Conversely, Romagnola x Podolian showed higher value of LDH and 170-129 kDa polypeptides than the other two breeds during aging. The present study highlight that many changes in the sarcoplasmic profile occurred during aging time and among breeds. Through *post mortem* aging, some sarcoplasmic proteins were degraded and declined in intensity. The decrease of band intensities during aging could be the direct result of the loss of solubility of sarcoplasmic proteins due to their denaturation. Previous studies [7] found a decrease of some sarcoplasmic proteins in the soluble fraction, suggesting that this change was due to chemical modification of these proteins. Different mechanism could be responsible for these changes such as isoelectric precipitation due to the pH decline or postmortem degradation [8].

On the contrary, other protein bands increased with aging time in all breeds. In particular, the increase of 34-30 kDa and 28-20 kDa polypeptides could be due both to solubilization of myofibrillar proteins and to the accumulation of their degradation products.

Figure 1. Gradient (8-18%) SDS-PAGE gel of sarcoplasmic proteins from *Longissimus dorsi* of Podolian, Friesian and Crossbreed after 1, 7, 14 and 21 days of aging (GPHb= Glycogen Phosphorylase b; PGM= Phosphoglucomutase; PK= Pyruvate kinase; PGI= Phosphoglucose isomerase; EN= Enolase; CK= Creatine kinase; ALD= Aldolase; GAPDH= Glyceraldeihyde phosphate dehydrogenase; LDH= Lactate dehydrogenase; PGAM= Phosphoglycerate mutase; TPI= Triosephosphate isomerase; Mb= Myoglobin).

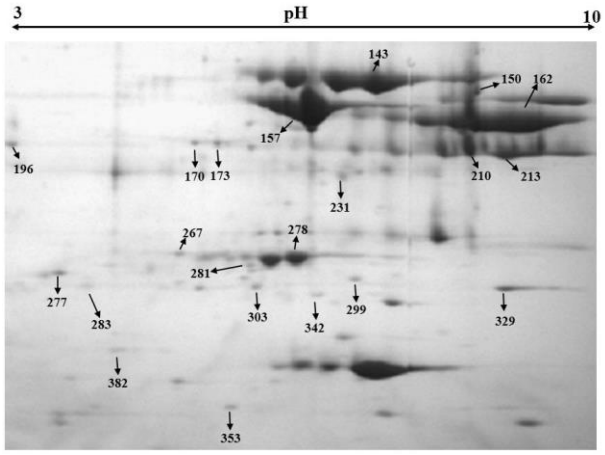


Increased solubility of certain myofibrillar protein during aging time could be a consequence of the weakening of actomyosin complex releasing proteins which were solubilized.

The 2D-PAGE showed that the sarcoplasmic fraction in the three different breeds presented a similar trend. A total of 23 spots that were found to change significantly in intensity and number among breeds and during aging were cut out of preparative gel and identified by LC-MS/MS. Twenty-one spots were successfully identified and annotated in the 2DE gel reference map presented in Figure 2. Changes in abundance and in number were observed for proteins of different functions such as metabolic, stress and structural proteins. Most of the glycolitic enzymes were found in the neutral or basic region of the 2DE gels and were identified in multiple spots isoforms.

Figure 2. Representative 2DE map of the sarcoplasmic proteins identified (spots 143, 170, 173= Beta-enolase; 150= Phosphoglycerate kinase;

157, 281= Creatine kinase M-type; 162, 231= Fructose-bisphosphate aldolase B; 196= Tropomyosin beta chain; 210, 213= Glyceraldehyde 3-phosphate dehydrogenase; 267= Peroxiredoxin-6; 277= Myosin light chain 1 skeletal muscle isoform; 278= Triosephosphate isomerase; 283= Peroxiredoxin-2; 299= Glutathione S-transferase P; 303= Protein DJ-1 (PARK7); 329= Adenylate kinase isoenzyme 1; 342= Superoxide dismutase [Mn] mitochondrial precursor; 353= Histidine triad nucleotide-binding protein; 382= Myosin regulatory light chain 2 skeletal muscle isoform).



Spots of Beta-enolase (EN), Creatine kinase (CK), Fructose-bisphosphate aldolase B (ALD), Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and Triosephosphate dehydrogenase (TPI) decreased in abundance during aging time in all breeds. In particular at 21 days, GAPDH almost disappeared in all breeds, mostly in Podolian cattle, the major decrease of these spots supports findings of our previous note [5] in which an increase of fragments of GAPDH protein on the myofibrillar fraction was observed during aging. This glycolytic enzyme could be modified after exposure to oxidative stress and this can explain its changes in solubility passing from soluble to insoluble aggregates during aging. Other protein spots, involved in the cellular response to stress, changed significantly in abundance, decreasing during aging in all breeds and were identified as Glutatione S-transferase P (GST) and Protein DJ-1. Intensities of 14 spots were significantly affected by the interaction of breed x aging. Protein spots identified as Phosphoglycerate kinase 1 (PGK1), beta-enolase, GAPDH and fragment of ALD and CK, involved in the glycolitic pathway, were higher in Friesian and Romagnola x Podolian at 1 day of aging. The finding that several glycolitic enzymes increase in intensity early after slaughter may suggest an increased rate of glycolysis in these two breeds to support and maintain the ATP production. Only for Podolian cattle 3 spots ascribed at myofibrillar proteins were found in the soluble fraction after 21 days of aging. These proteins were identified as myosin light chain 1 (MLC1), myosin regulatory light chain 2 (MLC2) and tropomyosin beta chain (TPMβ). Consistently with our results, previous studies [9] detected myofibrillar proteins in the sarcoplasmic protein fraction highlighting a relationship between the increase of myofibrillar proteins in the soluble fraction and tenderness.

IV. CONCLUSION

Although sarcoplasmic proteins do not directly impact muscle structures and meat tenderness, the results of this study highlight that changes in the composition of the water-soluble protein fraction of the muscle occur simultaneously to post mortem aging tenderization. The proteomic analysis showed that during muscle aging the solubility of some sarcoplasmic proteins decreased and some myofibrillar proteins were fragmented and released in the soluble fraction

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