Post-mortem muscle proteome changes in Martina Franca donkey in relation to meat tenderness

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Objectives: This study aimed to evaluate for the first time the effect of different aging times (1, 6 and 14 days) on the tenderization rate and post-mortem muscle protein changes in Martina Franca donkey meat using a gelbased proteomics and bioinformatics ap- proaches.

Materials and Methods: Ten Martina Franca male donkeys reared in the same farm (Apulia region, Italy) were selected for this trial. At about 18 months of age (average slaughter weight of 290 ± 5 kg), the animals were slaughtered according to industrial routines used in Italy and following the European Union regulation rule n. 1099/2009. After 24 hours post-mortem, the *Longissimus thoracis et lumborum* muscle was excised from both sides of the carcasses, divided into three equal-length sections, vacuum packaged, and randomly assigned to aging time at 2°C for 1, 6 and 14 days. At each aging time, Warner-Bratzler shear force (WBSF), texture profile analysis (TPA), myofibrillar fragmentation index (MFI), changes of myofibrillar proteins by means of Two-Dimensional Gel Electrophoresis (2DE) coupled to LC-MS/MS were performed. For bioinformatics analysis, protein-protein interaction on the dif- ferentially abundant proteins during aging was carried out using the STRING database v11.0 (https://string-db.org/), whereas the Metascape® (https://metascape.org/) was used to investigate the pathway analyses and process enrichments.

Results and Discussion: During aging, a progressive and significant decrease in share force ($p < 0.01$), hardness ($p < 0.01$), gummi- ness ($p < 0.01$), and chewiness ($p < 0.01$) values, together with an increase in MFI ($p < 0.001$) were observed highlighting the improvement in donkey meat tenderness, especially in the first week of aging. Proteomics revealed 37 protein spots corresponding to 15 unique gene names to change significantly by increasing aging time. The proteins belong to 3 pathways, these being i) muscle contraction, structure, and associated proteins (n=28 protein spots; myosin-1 "MYH1", myosin-2 "MYH2", actin-alpha 1 "ACTA1", myosin light chain, phosphorylatable "MYLPF", myosin light chain 6B "MYL6B", myosin light chain 1 "MYL1", tro- ponin C2, fast skeletal type "TNNC2", tropomyosin 1 "TPM1", tropomyosin 2 "TPM2"); ii) energy metabolism (n=6 protein spots; ATP synthase subunit d, mitochondrial "ATP5PD", ubiquinol-cytochrome c reductase core protein 1 "UQCRC1", cytochrome c oxidase polypeptide Va "COX5A", glyceraldehyde-3- phosphate dehydrogenase "GAPDH", creatine kinase "CKM"); and iii) cell stress and chaperone (n= 3 protein spots; heat shock 27 kDa protein "HSPB1"). On the basis of protein spot abundances, 8 were down-regulated during aging time, while, 29 were upregulated or appeared only after 14 days of aging. Among these, 2 protein spots of tropomyosin (TPM1 and TPM2) and 6 spots of myosin light chain (MYLPF and MYL1) were highly expressed at day 1 of aging, while, after 14 days of aging, several fragments of structural proteins (n= 20) and also proteins involved in energy metabolisms and cell stress responses (n= 9) are hugely impacted and degraded. These support the significant increase in toughness by ag- ing of donkey meat to achieve WBSF values below 30 N after 14 days of aging. The protein-protein interaction network using the 15 differentially abundant proteins (unique gene names) revealed two major interacting networks, i.e. the striated muscle contraction/filament sliding (n= 9 proteins) and the ATP metabolic processes (n = 4 proteins) which are both linked with the stress response protein HSPB1 (a small heat chock protein known to protect muscle fibers from proteolysis). These findings confirmed the impact of aging on muscle structure and myofibrillar proteins and evidenced that, other pathways, likely energy metabolisms and cell stress responses, are worthy to consider as predictors of the donkey meat tenderness. The comparison of the significantly en- riched GO terms using Metascape® by means of a heatmap on the protein lists of 1 and 14 days revealed 6 enriched term clusters among which "muscle organ development (GO:0007517)" and "muscle contraction (GO: 0006936)" are common pathways. The "regulation of ATPase activity (GO:0043462)" term was specific to the proteome list of early post-mortem muscle (1 day of aging), while the "ATP metabolic process (GO: 0046034)" term, "purine nucleotide metabolic process (GO:0009150)" and regulation of "I-kappaB kinase/NF-kappaB signalling (GO:0043122)" were specific to meat aged for 14 days.

Conclusions: The use of proteomics, for the first time in the case of Martina Franca donkey breed, opens new possibilities in deci- phering the biological mechanisms underpinning the tenderization process of this new source of animal proteins that is not exten- sively characterized. The results of this study will help also in predicting the potential quality of the end-product and in identifying protein biomarkers for monitoring the desirable meat quality traits in donkey.

Key words: Donkey, Tenderness, Proteomics, Myofibrillar proteins, Biological pathways