71st ICoMST
GIRONA - CATALONIA - SPAIN
3rd-8th August - 2025

MUSCLE-SPECIFIC VARIATIONS AND DYNAMICS IN THE DONKEY EXTRACELLULAR MATRIX PROTEINS DURING MEAT AGING

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I. INTRODUCTION

The extracellular matrix (ECM) is a highly dynamic and complex meshwork of proteins that constitute the architectural scaffold for myofibers, mainly composed of collagens, proteoglycans and glycoproteins. Beyond its mechanical and structural role in skeletal muscle, the ECM repertoire also includes a variety of signalling molecules, such as cytokines, chemokines, and growth factors, that respond to different stimuli and stressors to maintain cellular homeostasis [1]. Despite its important role in several post-mortem meat processes, the dynamics of the matrisome, along with other ECM components has not been comprehensively explored. This gap in knowledge limits our ability to fully understand the mechanisms behind meat tenderization and, consequently, to optimize aging processes, especially in different muscles possessing different contractile, metabolic and connective tissue properties [2]. Recently, a study on beef [2], demonstrated muscle-specific variation in proteolysis along of the composition of ECM proteins mainly of collagens and proteoglycans. However, nothing is yet known, or explored using omics on aged donkey meat. In fact, donkey meat although perceived as tough meat has recently attracted growing interest due to its favorable nutritional profile and potential as an alternative red meat [3]. Given the central role of the ECM in muscle architecture and its contribution to meat texture, investigating the post-mortem dynamics of the matrisome in donkey muscles is prerequisite to elucidate and better understand the biochemical mechanisms contributing to muscle-specific variations and texture determination. By characterizing these molecular changes, we aim to provide new insights into the role of the ECM in donkey meat quality development. Therefore, we investigate for the first time the dynamic changes of the ECM during aging in *Semimembranosus* (SM) and *Rectus femoris* (RF) donkey muscles.

II. MATERIALS AND METHODS

Eight male donkeys of about 18 months of age (290 ± 5 kg of average slaughter weight) reared under the same extensive farming and farm were used. The animals were slaughtered under standard conditions [3] and the *Semimembranosus* (SM) and *Rectus femoris* (RF) muscles were sampled at different post-mortem times these being 1, 7, and 14 days and stored at -80°C until proteomics analysis. Total muscle protein extracts were used to prepare the protein bands for shotgun proteomics [4], which were analysed by Sequential window acquisition of all theoretical mass spectra (SWATH-MS) [5]. The SWATH-MS analysis yielded a database of 778 proteins. The proteins were then used to profile the matrisome proteins using a domain-based *in silico* prediction algorithm that uses the gene lists compiled in the Matrisome Project [6]. The online MatrisomeAnalyzeR annotator tool allows the identification and classification of the proteins belonging to the ECM. The identified matrisome proteins were then gathered in a new dataset that was subjected to statistical analysis using a GLM model, considering the fixed effects of muscle (SM and RF), aging time (1, 7, and 14 days) and their interaction along with the random effect of animals. All effects were tested for statistical significance and considered significant if the p-value is < 0.05 (Tukey comparison).

III. RESULTS AND DISCUSSION

From the 778 proteins, around 6.7% (52 proteins) were accounted as matrisome proteins (Figure 1a), from which 20 were matrisome-associated proteins (9 ECM-affiliated proteins, 10 ECM regulators, and 1 secreted factors) and 32 were core matrisome proteins (12 ECM glycoproteins, 12 collagens, and 8 proteoglycans). The detailed list of the proteins within each category is given in Figure 1b and 1c. The ANOVA analysis revealed that 15 proteins of 52 were affected by at least one of the factors. A significant decrease during aging was observed for 9 matrisome proteins (COL1A1, COL3A1, COL4A5, LAMA2, LAMB2, LAMC1, ANXA7, LGALS1, HSPG2), whereas only ANXA6.1 showed a progressive increase over post-mortem time. Five proteins were affected by muscle type (COL4A1, COL4A5, LAMA2, ANXA11, ADAMTS1) showing a greater abundance in SM muscle probably due to its higher collagen content [7]. An interaction effect muscle x aging was also found for 3 proteins (COL1A1, COL1A2 and ADIPOQ). Overall, the core matrisome proteins, such as collagens (n= 5), ECM glycoproteins (n= 4) and ECM-affiliated (n= 4) are those more impacted, thereby can be suggested to play a key role in the post-mortem biochemical processes taking place during donkey meat tenderization. Due to their scaffold role, their dynamic changes over post-mortem time indicates

disorganization and destructuration of the ECM structure, in line with previously proposed mechanisms using traditional meat biochemistry methods [1, 2, 8].

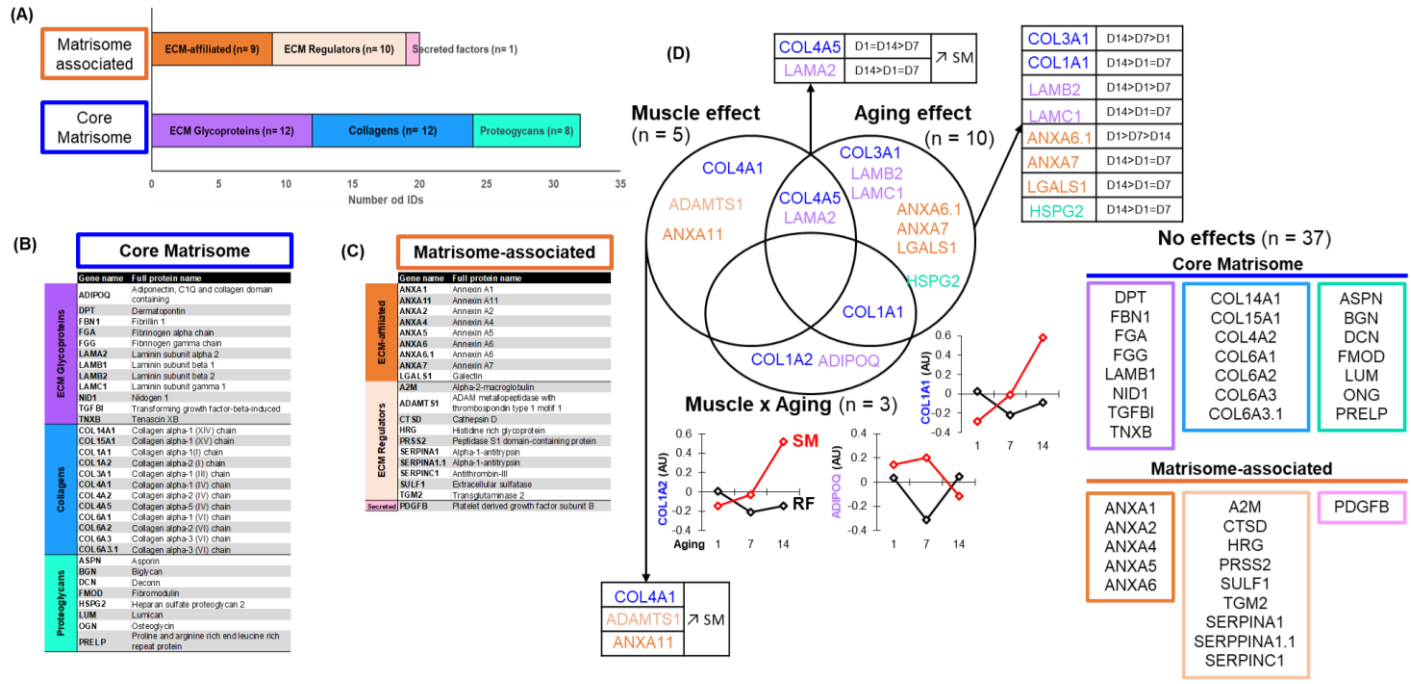


Figure 1. *In-silico* matrisome characterization of the donkey meat proteome from the SWATH-MS database of 778 proteins using Matrisome AnalyzeR. **a**) Category and division distribution of the matrisome (ECM: extracellular matrix) proteins (n = 52) identified in the donkey meat proteome database. Bar represents the number of "core matrisome" and "matrisome-associated" proteins classified according to matrisome categories. **b**) List, gene name and full names of the core matrisome proteins (ECM glycoproteins, collagens and proteoglycans) and **c**) matrisome-associated proteins (ECM-affiliated proteins, ECM regulators and secreted factors); **d**) Venn diagram displaying the ANOVA results on the effects of muscle type (SM and RF), aging time (1, 7 and 14 days) and the muscle x aging interaction on the ECM proteins.

IV. CONCLUSION

The findings evidenced that integrating the matrisome analysis is a new innovative way of studying the post-mortem muscle proteome changes. It further allows an in-depth study of the biochemical processes that occur during post-mortem aging. Data revealed that aging is the main factor impacting the matrisome changes in post-mortem muscle, but muscle-specific changes exist. These can be valuable ECM biomarkers to monitor the mechanisms underlying meat tenderization. The study further revealed that ECM proteins undergo changes during aging and open a new era of their study by combining proteomics and matrisome *in silico* analyses to expand on the role of ECM proteins in meat research as recently proposed [9]. Therefore, in-depth profiling of ECM composition during post-mortem aging may open new possibilities for developing targeted strategies of meat aging and improvement of meat quality outcomes.

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