Interconnected molecular pathways are involved, in a pH-dependent manner, in the concomitant appearance of the 110 and 30 kDa proteolytic fragments during beef tenderization

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Introduction: Aging is an important step allowing to produce high quality tender beef. Previous studies evidenced that the extent of beef tenderisation is related to early post-morem (p-m) pH decline (O'Halloran et al. 1997). A better understanding of those relationships was possible using proteomics, which revealed the main p-m muscle modifications with pivotal role in beef tenderness determination (Ouali et al. 2013; Gagaoua et al. 2021a). In this context we hypothesised that tenderness variability is related to a differential response at the molecular level within individuals during early p-m carcass handling and aging. Thus, the identification of the proteome affected by the biochemical processes during aging by means of LC-MS/MS (the latest mass spectrometry Q Exactive HF-X) would contribute to deeper our understanding of the phenomenon of p-m beef aging.

Materials and Methods: Seventy eight beef cattle were slaughtered at DawnMeats (Ballyhaunis, Republic of Ireland). From these, 12 heifers and 12 steers were selected to fit four pH decline rates (n=3 per group for each gender) based on the time@pH6: fast < 3 h; medium 3–5 h; slow 5–8 h and very slow 8+ h. Samples of M. Longissimus thoracis et lumborum were collected at day 0 being 3h, day 2 (48 h), day 7 and day 14. The proteolytic patterns were examined in all the muscle protein extracts representing the 4 sampling times across the pH decline categories using 1D-SDS-PAGE. An LC-MS/MS approach was then used to identify the proteolytic breakdown products appearing over 14 days p-m, at two important molecular weights, these being the 110 and 30 kDa major proteolytic fragments. Bioinformatics and statistics were performed using several tools: Metascape, ProteINSIDE, String v11 and SAS 9.4.

Results: Both 110 and 30 kDa bands appeared during aging and increased in intensity as a function of p-m time in a pH decline-dependent manner (Gagaoua et al. 2021b). The 110 kDa band appeared as early as 3h p-m and displayed an incremental increase in all groups through to 14 d p-m. From 2 d p-m, this increase in abundance during aging was significantly ($P < 0.001$) influenced by glycolytic rate: fast > or = medium > slow > very slow. The day 2 p-m appearance of the 30 kDa band was most evident for fast glycolysing muscle with little or no evidence of appearance in slow and very slow. For day 7 and 14 p-m the strength of appearance was dependant on glycolysing groups: fast > medium > or = slow > very slow. LC-MS/MS analysis yielded 22 unique proteins for the 110 kDa fragment and 13 for the 30 kDa, with 4 common proteins related to both actin and fibrinogen complex. The Gene Ontology analysis revealed a myriad of biological pathways are influential with many related to proteins involved primarily in muscle contraction and structure. Other pathways were energy metabolism, apoptotic mitochondrial changes, calcium and ion transport, etc. Interestingly, most of the proteins composing the fragments were so far identified as biomarkers of beef tenderness (Gagaoua et al. 2021a).

Conclusion: This study is the first to decipher in beef muscle the proteome and associated pathways which are connected with the appearance of the 110 and 30 kDa bands.

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