

## Molecular mechanisms in early post mortem muscle contributing to the development of beef flavour and associated biomarkers as revealed by shotgun proteomics

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**Introduction:** Beef flavour, a complex sensory trait involving aroma and taste of cooked meat products, is regarded as one of the driving factors that affects overall beef palatability (Gagaoua et al., 2018) and, hence, the repeat purchasing decisions by consumers (Kerth and Miller, 2015). Complex biochemical changes, including the production of flavour precursors, lead to beef flavour development with proteins and amino acids playing an important role (Elmore & Mottram, 2009). Recently, proteomics has become a powerful tool for the discovery of candidate biomarkers of beef quality traits (Gagaoua et al., 2021a; 2021b) and holds potential in the unravelling of these complex pathways. The aim of this study was to identify the potential of using shotgun proteomics combined with bioinformatics (Zhu et al. 2021) to elucidate the biological mechanisms related to beef flavour.

**Materials and Methods:** Thirty-four cross breed Limousin-sired bulls from elite Irish beef breed artificial insemination bulls were finished at the Irish Cattle Breeders Federation Tully Progeny Test Centre (Kildare, Ireland). Longissimus thoracis et lumborum (LTL) samples were collected from the 10th rib of each carcass. Trained sensory panellists based on a scale of 1 to 10 evaluated flavour intensity on the 34 LTL samples. Label-free shotgun proteomics was performed on the whole samples exactly as described by Zhu et al. (2021). The data were analysed using multivariate regression to propose regression equations, and Pearson correlations at the level of 5% to relate the proteins with flavour intensity scores. Furthermore, bioinformatics analyses using both ProteINSIDE and Metascape<sup>®</sup> were performed to understand the underpinning biological pathways in the differential proteins.

**Results:** A total of 281 proteins were identified, of which 59 were significantly correlated with beef flavour scores. The proteins belong to 5 molecular pathways: (i) muscle contraction, (ii) energy metabolism, (iii) heat shock proteins, (iv) oxidative stress and (v) regulation of cellular processes and binding. Among the energy metabolism proteins, MDH2 (Malate dehydrogenase) is located in a region on chromosome 25 containing two overlapping Quantitative trait loci (QTL): meat flavour score and fat thickness at the 12th rib. Regarding the optimal regression model, PHKG1 (Phosphorylase kinase), CORO6 (Coronin) and ATP5F1A (ATP synthase subunit alpha) explained 70% of variability in flavour. PHKG1 was positively correlated with beef flavour and CORO6 and ATP5F1A were negative in the regression model. In addition, 16 significant Gene Ontology (GO) terms were identified to be involved in beef flavour development, of which (i) generation of precursor metabolites and energy (GO: 0006091), (ii) muscle contraction (GO: 0006936), (iii) purine ribonucleoside monophosphate metabolic process (GO: 0009167), and (iv) carbohydrate metabolic process (GO: 0005975) were the top enriched terms.

**Conclusion:** Results support the application of proteomics to further our understanding of beef flavour determination, and enabled a regression model of this important beef palatability with high predictive power to be proposed. Moreover, the importance of energy metabolism pathway as a pivotal biological process regulating beef flavour, has been presented.

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