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

MOLECULAR CHANGES DURING POSTMORTEM WET AGING OF SIX BEEF MUSCLES

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I. INTRODUCTION

Intrinsic biochemical attributes of individual beef muscles can influence overall muscle metabolism [1]. Variations in muscle fiber type composition and subsequent metabolic profile can predispose muscles to certain quality phenotypes [2]. Postmortem biochemical processes such as proteolysis, hydrolysis of lipids, and many others assist in developing tenderness and flavor [3]. Mass spectrometry-based techniques can provide a deeper insight into the metabolic processes that inform final product quality. Based on literature and our understanding of muscle physiology across muscle types, it was hypothesized that changes to the molecular phenotype would be specific to muscles across postmortem aging time. Therefore, this study aimed to evaluate muscle-specific changes during postmortem aging to the metabolome and proteome of six beef muscles.

II. MATERIALS AND METHODS

Fifteen beef carcasses with USDA Moderate marbling scores (600 to 699) and no quality defects were selected from a commercial beef abattoir. From the right side of each carcass, subprimals containing the *Biceps femoris* (BF), *Infraspinatus* (IF), *Longissimus lumborum* (LL), *Psoas major* (PM), *Semitendinosus* (ST), and *Serratus ventralis* (SV) were collected. At 2 and 14 d postmortem, a steak from each muscle with no external fat, visible connective tissue, or accessory muscles was snap frozen in liquid nitrogen, pulverized into a fine powder, and stored at -80°C. Polar and semi-polar metabolites were extracted using 80% methanol. A pooled quality control (QC) sample and extraction blanks were also prepared. Dried extracts were derivatized via methoximation and silylation, separated on an HP-5 capillary column, and ionized via electron ionization. Data were acquired within a mass range of 30 to 600 *m/z*. MS-DIAL (v. 4.92) was utilized for deconvolution, alignment, and annotation (MassBank) of mass spectral features. Feature intensities were normalized by the total ion current and $\log_{10}$ transformed. Within each muscle, data were analyzed by t-tests using MetaboAnalyst v.6.0. Proteins soluble in a low-ionic strength buffer (40 mM Tris-HCl [pH 8.5] and 1 mM EDTA) were extracted, clarified by centrifugation, and standardized to 5 mg/mL [4]. A pooled QC sample of equal volume of all samples and postmortem time points was generated. Extracts were reduced, alkylated, digested with trypsin, and tryptic peptides (200 ng/uL) were spiked with an internal PRTC standard (25 fmol/uL, Thermo Scientific) before analysis. An Orbitrap Astral (Thermo Scientific) coupled to a Vanquish Neo UHPLC (Thermo Scientific) was used for analysis, and data-independent acquisition was used to acquire mass spectral data. Proteome Discoverer (3.2.0.450, Thermo Fisher Scientific) with CHIMERYS (4.0.20-42868eb3, MSAID) was used to process raw data against a *Bos taurus* Uniprot fasta file (26,942 sequences). Within each muscle, data were analyzed using moderated t-tests with the *limma* package in R (4.4.2, R Core Team 2024) to evaluate changes over postmortem aging. Significance for metabolomics was determined at a false discovery rate (FDR) adjusted $P < 0.05$. Significance for proteomic data was Benjamini-Hochberg adjusted $P < 0.05$.

III. RESULTS AND DISCUSSION

Table 1 summarizes the results for metabolomics and proteomics. Generally, wet aging induced differences in the abundance of various metabolites and proteins. Only 2 metabolites were more abundant in 2 d BF, 2,4-hexadienal and methyl 3-ethoxypropanoate, whereas 19 metabolites, predominantly amino acids, were increased in 14 d BF ($P < 0.05$). Twenty-six metabolites were differentially abundant between 2 and 14 d IF, where monosaccharide phosphates were increased in 2 d IF and amino acids, citric acid, and hypoxanthine were increased in 14 d IF ($P < 0.05$). Glucose-1-phosphate and 3-phosphoglycerate were increased in 2 d LL compared to 14 d LL ($P < 0.05$). Conversely, amino acids, carbohydrates, hypoxanthine, and niacinamide were increased in 14 d LL compared to 2 d LL ($P < 0.05$). Of all the muscles, PM showed the least changes in metabolite profile, with only 5 metabolites, lyxose, xylitol, L-methionine, hypoxanthine, and citric acid being increased in 14 d PM ($P < 0.05$). Similar to the IF,

monosaccharide phosphates as well as 3-phosphoglycerate were increased in 2 d ST, whereas amino acids, carbohydrates, 3-aminopropionitrile, hypoxanthine, and niacinamide were increased in 14 d ST ($P < 0.05$). Succinic acid was increased in 2 d SV compared to 14 d SV ($P < 0.05$). Conversely, 14 d SV had increased amino acids, oleic acid, 3-aminopropionitrile, lyxose, glyceric acid, and hypoxanthine ($P < 0.05$). Within the low-ionic strength soluble protein fraction, titin, nebulin, vimentin, and filamin A were more abundant at 14 d versus 2 d postmortem, regardless of muscle ($P < 0.05$). Interestingly, desmin was only more abundant at 14 d versus 2 d postmortem in the PM and IF ($P < 0.05$) and was not different between time points within other muscles. The presence of tryptic peptides of these insoluble structural proteins in a low-ionic strength protein extract suggests proteolytic degradation and the liberation of soluble polypeptides [4].

Table 1. Summary of differentially abundant[1] metabolites and proteins[2] from six beef muscles aged either 2 or 14 d postmortem.

	Metabolites (n = 74)		Proteins (n = 1171)	
Muscle	Increased Postmortem	Decreased Postmortem	Increased Postmortem	Decreased Postmortem
Biceps femoris	19	2	136	101
Infraspinatus	17	7	165	55
Longissimus lumborum	21	2	196	127
Psoas major	5	0	148	86
Semitendinosus	20	4	186	112
Serratus ventralis	11	1	130	59

[1] False discovery rate/Benjamini-Hochberg adjusted $P < 0.05$.
[2] Proteins soluble in low-ionic strength buffer.

IV. CONCLUSION

Generally, amino acid accumulation in tandem with greater abundance of structural proteins such as titin, nebulin, filamin A, and desmin within low-ionic strength extract was observed with postmortem wet aging. Together, these changes are in alignment with proteolytic activity that occurs during beef maturation, leading to a more tender product. While direct comparisons between muscles were not conducted, muscle specific trends were observed where “red” or “intermediate” muscles like the IF, PM, and SV demonstrated fewer differentially abundant metabolites and/or proteins compared to other muscles. These results further highlight that observations in one muscle are not always transferable to another.

ACKNOWLEDGEMENTS

Research coordinated by the National Cattlemen’s Beef Association, a contractor of the Beef Checkoff.

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