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THE EFFECT OF HIGH-PRESSURE PRE-TREATMENT AND DRY AGING ON THE METABOLOMIC PROFILE OF BOVINE MUSCLE

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I. INTRODUCTION

Dry aging involves the storage of unwrapped muscle under controlled refrigerated conditions for prolonged periods of time (> 28 days), allowing proteolysis and lipid oxidation to occur, along with moisture loss via evaporation of water from the surface of the muscle, leading to the development of a signature beef flavor [1]. The impact of high pressure processing (HPP) before dry aging has been found to disrupt the structure of muscle protein and alter proteolytic activity [2]. Therefore, the aim of the current study was to investigate the impact of HPP pre-treatment, followed by 28 days of dry aging, on the metabolomics profile of bovine *Longissimus thoracis et lumborum* muscle.

II. MATERIALS AND METHODS

Bovine *Longissimus thoracis et lumborum* muscle (LTL; left and right) was collected from 4 bulls, 72 h post-mortem. Each LTL was divided into 4 sections. Sections were randomly assigned to two different treatments (dry aging (DA) or dry aging with a high pressure pre-treatment (HPPDA)), at four different dry aging times (0/ 14/ 21/ 28 days). Sections assigned for HPPDA were treated with 200 MPa (20 min, 8 °C). Dry aging was carried out in dry aging chambers for up to 28 days (2 °C, 80% relative humidity, air flow 0.5-2.0 m/sec). At each aging time point, samples were collected, snap frozen and stored at -80 °C until further analysis. One gram of sample was added to 5 ml of HPLC-grade methanol, vortexed for 2 min and homogenised (Ultraturrax for 2 x 30 sec) and treated in an ultrasound bath (25 kHz, 30 min, room temperature). Homogenates were stored at -20 °C for 1 h and transferred to Eppendorf tubes where they were centrifuged (2,000*g* x 5 min), filtered (0.2 µm PTFE filter) and transferred into HPLC vials. Pooled quality control (QC) samples were prepared. Untargeted metabolomic analysis was performed using UPLC-Q-TOF/MS (Agilent 1290 Infinity II UHPLC and Agilent 6545 Q-TOF/MS) with an ACQUITY UPLC BEH C18 1.7 µm (2.1 mm x 150 mm) column (WATERS Corporation). Mobile phases used were A) water and 0.1% formic acid and B) acetonitrile and 0.1% formic acid, using a gradient over 45 min. Dual AJS electrospray ionisation in positive mode was used to detect metabolites. QC samples were injected every six samples to examine the system stability. Raw mass spectrometry data (.d) was converted to .mzXML format using ProteoWizard (version 3.0.2 (64 bit)). The pre-processing of LC-MS data (peak alignment, retention time correction and peak extraction) was carried out using the XCMS package (version 4.0.2) in RStudio (version 4.3.2). Data was log transformed (normalisation) and centered for fold change and PCA analysis. Pareto scaling was applied prior to discriminant analysis (PLS-DA and OPLS-DA). Significant differential metabolites were those with a VIP score (variable importance in projection (OPLS-DA)) > 1 and P (paired t-test) < 0.05. Fold – change (FC) was calculated by comparing the mean of metabolites between treatments (DA/HPPDA) and different time points (D0/D14/D21/D28). Metabolites that increased with time or treatment had a FC > 0.5 and P < 0.05 while metabolites that decreased with time or treatment had a FC < - 0.5 and P < 0.05. Metabolites were tentatively identified using MassHunter software packages B.07.00 (Agilent Technologies) by comparing the observed accurate mass data and MS/MS spectra with the accurate mass data and spectra of the METLIN Metabolomics and Tandem MS Database (Scripps Center for Metabolomics).

III. RESULTS AND DISCUSSION

Principal component analysis (PCA) was performed as an initial quality control step, revealing that QC samples were clustered tightly together, indicating reliable and stable analysis (Figure 1). PLS-DA was conducted to assess class separation among sample groups, from which three distinct clusters emerged (Figure 2). DA and HPPDA treated samples overlapped at day 0, suggesting that HPP did not have an immediate effect on the metabolomic profile of bovine muscle. Over time, two clusters emerged, comprising of DA and HPPDA samples with the HPP pretreatment

having a significant effect on the profiles of bovine muscle that were aged for 14, 21 and 28 days. HPP and dry aging time induced increases and decreases in metabolite abundance. When analyzing the effects of dry aging time on DA and HPPDA samples (Figure 3), the majority of changes occurred between 0 and 14 days of dry aging.

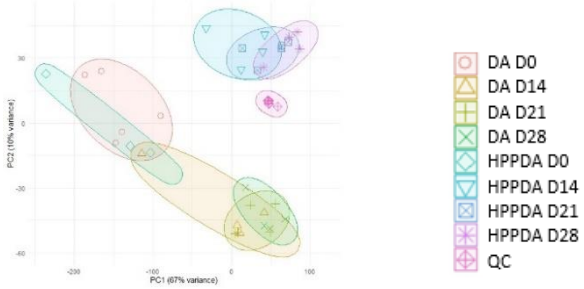


Figure 1. PCA plot of different sample groups

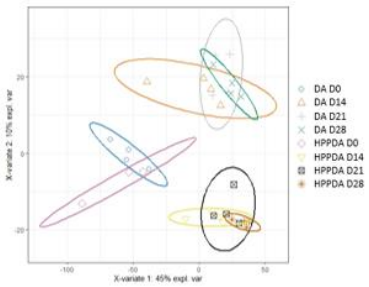


Figure 2. PLS-DA of different sample groups

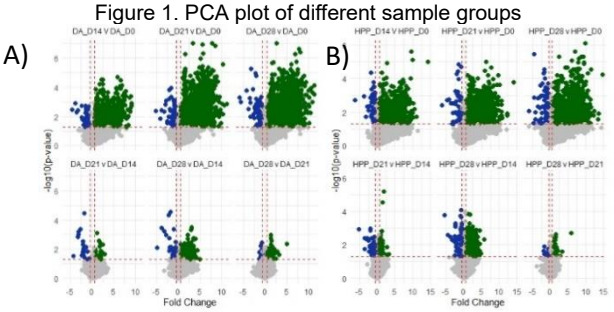


Figure 3. Volcano plots illustrating effects of aging in A) DA and B) HPPDA samples (increased=green, decreased=blue)

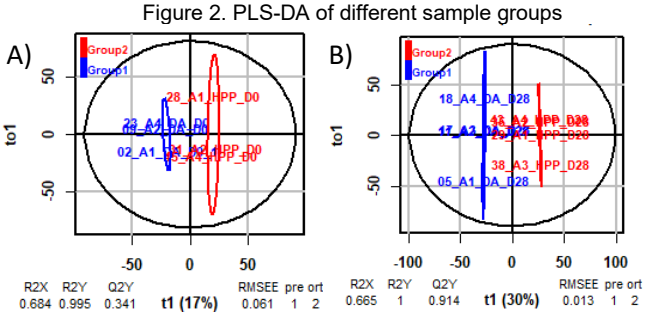


Figure 4. OPLS-DA model distinguishing A) D0 and B) D28 between DA and HPPDA treatments

OPLS-DA enhanced group separation by removing unrelated variation and identifying metabolites responsible for group differentiation. Using OPLS-DA, it was possible to discriminate, with good predictability, DA and HPPDA samples at D0 and D28 (Figure 4), however, it was possible to differentiate between all sampling time points between and within treatments (DA/HPPDA). Significant differential metabolites (VIP > 1, P < 0.05) distinguishing DA from HPPDA samples included peptides and phospholipids, where HPP appeared to increase their concentrations, potentially due to altered proteolytic enzyme activity, tissue disruption and increased extractability. Significant differential metabolites distinguishing aging time for both treatments included peptides, nucleotides (as well as phospholipids for HPPDA samples). Peptides and phospholipids increased over time, while nucleotides either increased or decreased (IMP decreased over time, conversely to inosine and hypoxanthine). These changes may occur due to proteolysis, energy metabolism and increased extractability. The metabolite groups found to be different between treatment and aging groups play a role in the flavor profile of bovine muscle.

IV. CONCLUSION

Dry aging time and high pressure pre-treatment induced changes to the metabolomic profile of bovine muscle, especially on the concentration of peptides, phospholipids and nucleotides. These differential abundances allowed for the discrimination between DA and HPPDA samples and the four aging times, regardless the pre-treatment. These metabolites may act as precursors to flavor compounds in beef. Further investigations are warranted to determine the effect of HPP on the flavor of dry aged bovine meat.

ACKNOWLEDGEMENTS

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