

## NMR-based metabolites profiling of wet and dry aged pulsed electric fields treated venison (#462)

Tanyaradzwa Mungure<sup>1</sup>, John E. Birch<sup>1</sup>, Alan Carne<sup>2</sup>, Ian Stewart<sup>3</sup>, Mustafa Farouk<sup>4</sup>, Maryann Staincliffe<sup>4</sup>, Alaa El-Din A. Bekhit<sup>1</sup>

<sup>1</sup> University of Otago, Food Science, Dunedin, New Zealand; <sup>2</sup> University of Otago, Biochemistry, Dunedin, New Zealand; <sup>3</sup> University of Otago, Chemistry, Dunedin, New Zealand; <sup>4</sup> AgResearch NZ Limited, Ruakura Research Centre, Hamilton, New Zealand

### Introduction

Storage of meat under specified conditions (aging) improves its palatability. This improvement is attributed to proteolysis of muscle structural proteins over the aging time and as such changes in the meat metabolites profile. Metabolomics, the study of metabolites, is an emerging technique that analyses small molecular weight compounds (MW<1.5kDa). Metabolomics has recently been utilised to study changes in tenderisation and flavour precursors during storage [1]. Proteolytic processes during aging generate free amino acids, water-soluble sugars, dipeptides, nucleotide derivatives and organic acids. These metabolites contribute to flavour, tenderness, colour stability and overall palatability of red meat products [2].

This study investigated the effects of aging regime (wet vs dry, 65% relative humidity (RH) and 80%RH) and application of pulsed electric fields (PEF) treatment on the metabolites profile of venison. Most studies have focused on the metabolic profile of wet and dry aged beef, but this current research group is the first to investigate and compare the metabolite profiling distribution of wet and dry aged venison using NMR-based metabolomics coupled with multivariate analysis (principal component analysis, PCA).

### Methods

Venison loins (*M. longissimus et lumborum*, LL) were obtained from twelve 2-year-old hinds (average cold carcass weight of 113 ± 6.7 kg and 108 ± 9.8 kg) over 2 slaughter days that were used for 65%RH (n = 6) and for 80%RH (n = 6), respectively. The left and right loins from each carcass were obtained at 24 h post-mortem, processed into blocks of average weight of 318 ± 11.6 g, avoiding any visible fat and connective tissue. The blocks were randomly distributed to wet-aged control, dry-aged control, wet-aged low PEF, dry-aged low PEF, wet-aged high PEF, and dry-aged high PEF. Total specific energy was approximately 1.93 kJ.kg<sup>-1</sup> for LPEF (2.5 kV, 50 Hz and 20 µs) and 70.2 kJ.kg<sup>-1</sup> for HPEF (7.5 kV, 50 Hz and 20 µs). The first set of samples (n = 6) were dry-aged in a chiller at 65%RH for 10 days, vacuum packed and stored for 11 days. The second set (n = 6) were dry-aged at 80%RH for 21 days at 4°C. NMR spectroscopy, metabolite identification, verification, quantitation and processing were conducted as described by Kim et al., (2016).

### Results

Across the two trials, a total 16 of 34 identified (see representative NMR spectrum in figure 1.) metabolites differed significantly between wet and dry-aged venison (p<0.05). Dry-aged samples had higher levels in nine of the

12 identified amino acids than wet-aged samples (p<0.05). These higher concentrations may be attributable to evaporation of water and increased protein hydrolysis during the dry-aging treatment. The sugars, glucose and mannose, and of the alcohols identified, glycerol, were higher in the dry aged samples (p<0.05).

The organic acid and purine derivative lactate and inosine monophosphate were abundant and significantly higher in both trials for wet-aged samples (p<0.05), possibly explaining the elevated sour taste and strong umami attributes in wet compared to dry-aged meat previously reported [3].

PEF treatment and RH were not found to have any effect on the metabolites profile (p > 0.05). The PCA applied on the 65%RH samples showed the first 2 PCs explained 46.58% of the cumulative variance (shown in Figure 2.). PC1 explained 33.61% of the variance and 12.97% by PC2. The PCA model using projection onto two dimensions of PC1 and PC2 showed some clustering according to the aging method. The 80%RH trial (shown in Figure 3.) also showed the same phenomena with a cumulative variance of 40.22% explained. The PC1 explained 30% of the variance and 10.22% by PC2. The aging method had an effect with dry aged in the positive PC 1 (blue circle) and wet as negative PC1 (red circle) in both trials.

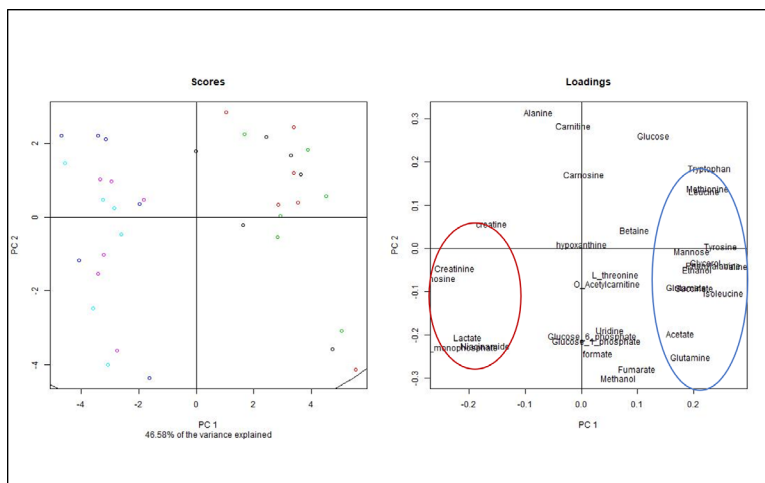
### Conclusion

The metabolomic analysis highlights clear differences in the profiles of the two aging regimes where build-up of characteristic metabolite flavour precursors is attributable to the processing conditions. PEF treatment and RH% do not have any effect on these profiles.

### Reference

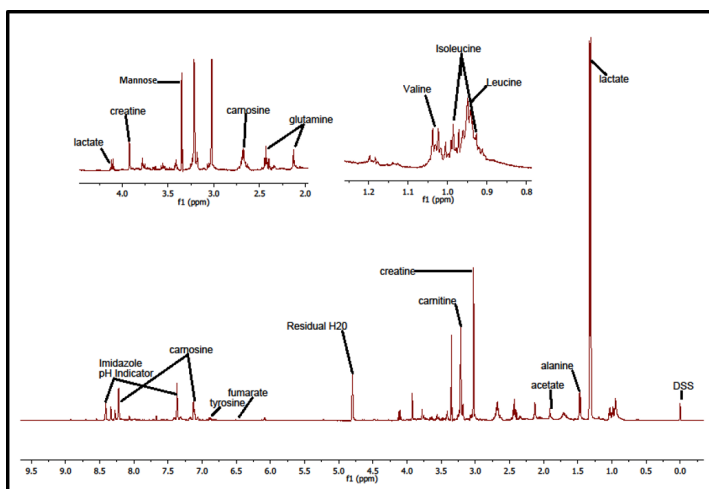
1. Kim, Y. H. B., Kemp, R., & Samuelsson, L. M. (2016). Effects of dry-aging on meat quality attributes and metabolite profiles of beef loins. *Meat Science*, 111, 168-176.
2. Kodani, Y., Miyakawa, T., Komatsu, T., & Tanokura, M. (2017). NMR-based metabolomics for simultaneously evaluating multiple determinants of primary beef quality in Japanese Black cattle. *Scientific reports*, 7(1), 1297.
3. Zhang, J., Yi, Y., Pan, D., Zhou, G., Wang, Y., Dang, Y., He, J., Li, G., & Cao, J. (2019). <sup>1</sup>H NMR-based metabolomics profiling and taste of dry-cured boneless hams during processing. *Food Research International*, 122, 114-122.

## Notes



### PCA score and loading plots of identified metabolites from wet and dry aged venison, 65%RH

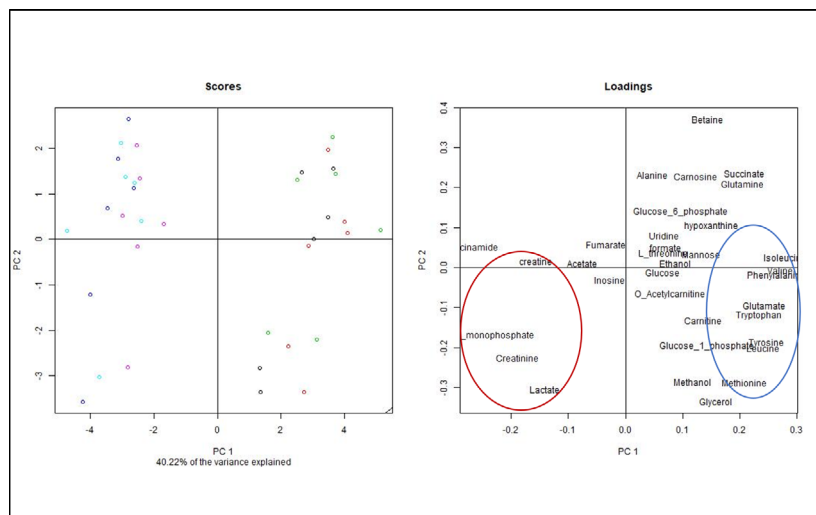
Figure 2. PCA score and loading plots derived from  $^1\text{H}$  NMR spectra of identified metabolites of wet and dry aged venison in 65%RH trial. The two PC's explained 46.58% of the cumulative variance. The PC1 showed some clustering according to aging method



### Representative $^1\text{H}$ NMR spectrum with metabolite chemical shifts

Figure 1.  $^1\text{H}$  NMR spectra with labeled chemical shifts of metabolites from aged venison.

## Notes



### PCA score and loading plots of identified metabolites from wet and dry aged venison, 80% RH

Figure 3. PCA score and loading plots derived from quantitative  $^1\text{H}$  NMR analysis of identified metabolites of wet and dry aged venison in 80%RH trial.

## Notes