Potential of nuclear magnetic resonance spectroscopy to measure biochemical changes during post mortem aging of beef hung by Achilles or pelvic suspension prior to rigor

S F Graham[1], T Kennedy[2], A Gordon[2], L J Farmer[2,1], C Elliot[1], B W Moss[2,1]
[1]Queen's University of Belfast, Belfast, United Kingdom, [2]Agri-food & Biosciences Institute, Belfast, United Kingdom
Email: bruce.moss@afbini.gov.uk

Introduction During the aging of beef tenderness increases mainly due to the proteolysis of myofibrillar proteins (Koohmaraie & Geesink, 2006). Increases in amino acids, nucleotides and sugars during the post mortem aging period (Koutsidis *et al.*, 2008) are important in relation to flavour development when the meat is cooked. Although a single extraction procedure can be used for amino acids, nucleotides and sugars, different analytical procedures are required for each type of metabolite. ^{1}H nuclear magnetic resonance (^{1}HNMR) has been shown to be an ideal technique to systematically characterise and quantify metabolites in plants (Graham *et al.*, 2009); and has the potential to characterise a wide range of metabolites thus removing the requirement for different analytical techniques. ^{1}HNMR has been used to investigate the relationship between stress, metabolite profiles and water-holding capacity in pigs (Bertram *et al.*, 2005) but not been applied to beef muscle. The aim of this study was to evaluate the ability of ^{1}HNMR to characterise the changes in amino acids, nucleotides and sugars during the post mortem aging of beef.

Materials and methods Six carcasses were selected at random at a commercial meat plant and one side of each carcass was hung by Achilles tendon (AT) and the other by pelvic suspension (TS). Three days post slaughter the fore rib joint was removed and from the *longissimus dorsi* (LD) four 2cm thick slices were taken, placed in bags and vacuum packed. The samples were stored at 2°C for periods of 3, 7, 14 and 21 days after which time they were blast frozen and stored at -80°C. Metabolites were extracted from a 3 g sample of LD using 0.6M perchloric acid according to the procedure of Aliani & Farmer (2005). The neutralized supernatants were lyophilized, then reconstituted in in 650 µl of 0.1 M phosphate buffer (pH 7.0), in D_2O, containing 1 mM of the internal standard sodium trimethylsilyl-2,2,3,3,-tetradeuteroproprionate (TSP, Sigma Aldrich, UK). Insoluble material was removed by centrifugation (16,000 *g* for 15 min), and 600 µl of the remaining supernatant was transferred to a 5 mm diameter NMR tube.
The spectra were recorded in D_2O on a Brüker AC 300 and 500 (2D (^{1}H/^{13}C) heteronuclear multiple quantum coherence (HMQC) NMR experiments) MHz spectrometer. Thirty-two transients were acquired. Spectral processing was carried out using ACDlabs NMR Processor v9.0 (ACD labs, Toronto, Canada). Baseline correction was performed manually. Data reduction was carried out by manually binning the spectra and measuring the integral for each bin between 0.80 p.p.m. and 8.70 p.p.m.. The region from 4.75 p.p.m. to 5.00 p.p.m., which contained the signal for the water resonance, was excluded. Analysis of variance was undertaken to determine the effect of carcass suspension method and aging on metabolites measured. Principal component analysis (PCA) was also undertaken to evaluate the relationship between metabolites and period of aging.

Results A total of 27 compounds were identified by using a combination of NMR databases, spiking with known pure compounds and 2 dimensional NMR (^{1}H/^{13}C). The metabolites identified included a range of amino acids, adenine nucleotides and sugars. The two major peaks were lactate and creatine. There were 20 peaks in the NMR spectra which could not be identified and require complementary NMR techniques to aid identification. Fructose could not be clearly identified due to its low level relative to adjoining peaks of ribose and glucose at a much higher level.
There was no statistically significant effect of carcass suspension method on any of the metabolites measured. Three components in the PCA explained 71% of the variation (PC1 45%, PC2 19%, PC3 7.5%). Separation according to ageing periods was mainly along principal component 1 (Fig 1) but followed a diagonal vector from low PC1 & PC2 scores (3 days aging) to high PC1 and PC2 scores (day 21). The PCA loadings showed that longer ageing periods were associated with a number of amino acids, particularly; alanine, phenylalanine, isoleucine and valine. The PCA loadings for shorter aging periods were associated with peaks identified as ATP and combination of ATP/ADP/hypoxanthine/inosine, however, the level of these metabolites was low.

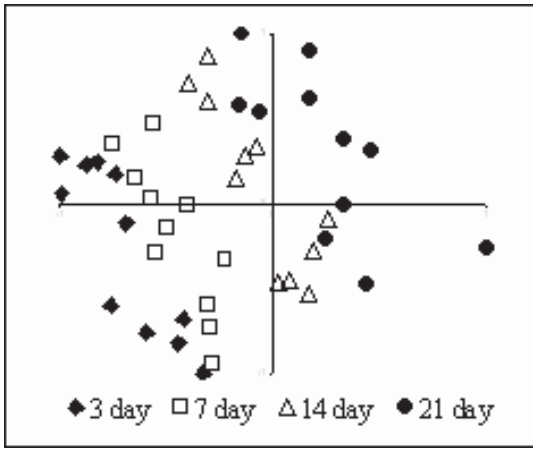

Figure 1 PCA of metabolites by NMR

Conclusion ^{1}HNMR provides a useful analytical technique for the measurement of polar metabolites in the post rigor period. Further work is required to identify unknown peaks and the rate at which metabolites change during aging of meat.

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