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Original Research Article

Spatial and temporal mass spectrometric profiling and imaging of lipid degradation in bovine *M. longissimus dorsi lumborum*

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ABSTRACT

Lipid oxidation plays a critical role in the quality of meat and meat products; however, lipid degradation is generally evaluated at a holistic level, without attention to spatial distribution. Marker lipids were selected based on their relative abundance and characteristic MS fragmentation patterns (10 phospholipids, 2 triglycerides, and cholesterol). These markers were subsequently utilised for temporal and spatial profiling of lipid degradation in bovine *M. longissimus dorsi lumborum* steaks subjected to high (packaged in 80% O_2/20% CO_2 modified atmosphere), atmospheric (oxygen permeable film) and ultra-low (vacuum-packaged) oxygen packaging during storage through matrix-assisted laser desorption/ionisation time-of-flight/time-of-flight (MALDI–TOF/TOF) mass spectrometric imaging. Interestingly, markers showed highly contrasting effects in terms of their oxidative stability over time. The relative abundance of phophatidylcholines generally declined rapidly under high oxygen conditions. In contrast, PC 18:1/18:0 showed high relatively stability to oxidation. Cholesterol also displayed high relative stability. Overall, high oxygen packing was found to result in rapid lipid degradation, while vacuum-packaging significantly mitigated lipid degradation. Oxidative degradation profiles were spatially heterogeneous across meat sub-samples and differences were also observed from the centre and edge of the steaks. This new approach to tracking lipid degradation directly from meat samples offers increasingly precise tracking of modification in meat and other foods.

1. Introduction

Lipid oxidative degradation is a significant factor in the deterioration of muscle food products, with critical correlations to quality, nutritional impact, safety and shelf-life (Cohn, 2002; Kanner, 2007). The most notable deleterious effect that concerns consumers is the development of rancidity and off-flavours which affect the eating experience, while producers and regulators are increasingly concerned about both loss of nutritional value and the postulated links between high relative levels of oxidative degradation products and primary or secondary health effects (Kanner, 2007). In meat, rates and pathways of oxidative degradation are influenced by a variety of factors during production, processing, packaging, storage and retail, including exposure to changing temperatures, oxygen levels and light (Jakobsen and Bertelsen, 2000). For instance, while it is well-known that beef packaged in an atmosphere with a high content of oxygen results in a red colour and an increased shelf-life compared to meat that is packaged with a oxygen permeable polyvinyl chloride film, the high oxygen content also leads to increased lipid oxidation and off-flavour development at the endpoint of display (McMillin, 2008; Clausen et al., 2009; Kim et al., 2010, 2011; Lund et al., 2008).

Control and minimisation of lipid oxidation are therefore key goals in meat and food science. However, evaluation of lipids and lipid degradation products directly within muscle foods has generally been carried out only at the holistic, rather than molecular, level (Demirci Çekiç et al., 2013; McArdle et al., 2013). Furthermore, there has been little assessment of the spatial distribution of oxidation products within meat products, with only a few disparate attempts at differentiating the surface from the interior, and no reported application of mass spectrometric imaging approaches to achieving such spatial information (Arnold et al., 1993; Feldhusen et al., 1995). Most measurements have been performed in homogenised or ground samples (Alvarado et al.,

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2007; Ulu, 2004). Although such holistic approaches enable general analysis of lipids present, all information about the distribution of these compounds is lost in the process. In addition, procedures involving initial extraction of the lipids from the food add significant potential for artefactual modification of the lipids.

Matrix-assisted laser desorption/ionisation (MALDI) is a soft ionisation mass spectrometric technique allowing analysis of biomolecules which are susceptible to fragmentation using harder ionisation methods. Ionisation is induced via laser irradiation in conjunction with the application of a matrix, which protects the sample and facilitates ionisation (Murphy et al., 2009; El-Aneed et al., 2009). Matrix-assisted laser desorption/ionisation mass spectrometric imaging (MALDI–MSI) further exploits the advantages of soft ionisation by allowing analysis directly from tissue samples which have had matrix applied topically. The MALDI laser is rastered across the tissue section, providing a two-dimensional spatial profile of compounds present in the section. This relatively new technique has until recently more typically been applied to medical research, particularly in the evaluation of internal organs (Cornett et al., 2007; Ceuppens et al., 2007; Minerva et al., 2008; Chen et al., 2009; Astigarraga et al., 2008). However, it provides an ideal route to the analysis of meat lipids, which are generally extracted prior to analysis and therefore subject to further modification and unintended preselection. Utilising MSI, these lipids can be observed directly from the meat in their native state, with no chemical extraction or modification required.

We here report the development and application of advanced MALDI–MSI technologies to characterise and resolve the spatial and temporal distribution of selected marker lipids and their oxidative degradation in cross-sections of bovine *M. longissimus dorsi lumborum* steaks. Three different packaging methods, i.e. packaging in high oxygen modified atmosphere, oxygen permeable film, and vacuum-packaging, were applied to create differences in oxidative degradation during the storage period.

2. Materials and methods

All chemicals were supplied by Sigma–Aldrich (St. Louis, MO, USA), unless otherwise stated.

2.1. Sample preparation

The muscle, *M. longissimus dorsi lumborum*, used in this study originated from a grass-fed steer slaughtered at a local abattoir. The day after slaughter, it was excised and transported to the laboratory. pH was measured using a Mettler Toledo pH meter with a combination electrode (Mettler Toledo Inlab 427; Mettler Toledo Inc., Columbus, OH, USA). The muscle was then sliced into 12 10-mm thick samples (steaks) that were cut at right angle to the muscle fibre grain alignment from the anterior end of the muscle. The steaks were then either placed on trays and covered with an oxygen permeable polyvinyl chloride film (*Atmospheric Oxygen*), or placed in oxygen impermeable barrier bags (CRYOVAC Barrier Bab BB7L, Sealed Air, Hamilton, New Zealand) and either vacuum-packaged (*Vacuum*) or packaged in a high oxygen atmosphere (80% O_2/20% CO_2) (*High Oxygen*). After packaging, the steaks were either sub-sampled immediately (1 day *post mortem*), or stored at 4 °C for an additional 2 days (3 days *post mortem*), 7 days (8 days *post mortem*) and 14 days (15 days *post mortem*).

At the end of the specific storage period, four sub-samples were cut from each steak for MALDI–MS analysis in order to evaluate potential lipid degradation differences between the surface and interior of the meat; two sub-samples were cut from the centre and two sub-samples were cut from the edge. The sub-samples were then placed in vials and stored at −80 °C. After the removal of sub-samples for MALDI–MS analysis, the remainder of the steak was homogenised and used for the holistic determination of lipid oxidation.

2.2. Extraction of lipids for MALDI detection methods

Lipids were extracted from 10 g meat samples according to a modified version of the Folch et al. (1957) method, with a ratio of 2:1 (v/v) of chloroform and methanol. The samples were homogenised, sealed from air and were allowed to separate overnight at 4 °C. The organic layer was concentrated by vacuum centrifuge and samples stored at 4 °C.

2.3. Mass spectrometric selection of lipid targets

The extracted lipids were directly analysed using an Ultraflex III matrix-assisted laser desorption/ionisation time-of-flight/time-of-flight (MALDI–TOF/TOF) mass spectrometer (Bruker Daltonics, Bremen, Germany). Two matrices were trialled; an α-cyano-4-hydroxycinnamic acid (CHCA) saturated solution in acetonitrile (ACN):water 1:2 with 0.1% trifluoroacetic acid (TFA), and 9-aminoacridine (9-AA) at 10 mg/mL in 60:40 isopropanol:water. Equal volumes of sample and matrix were mixed and 0.5 μL was applied to an AnchorChip plate (Bruker). Data was collected in both positive and negative modes.

After MS data acquisition, signal lists were extracted from the data using FlexAnalysis software (Bruker).

2.4. Mass spectrometric imaging

Tissue sections 16 μm in thickness were obtained using a Leica CM 1850 cryostat (Leica Microsystems, Nussloch, Germany) and deposited onto ITO coated conductive glass slides (Bruker). The tissue sections were then sprayed with CHCA matrix solution at 10 mg/mL in 70% ACN, 30% H_2O and 0.1% TFA using a Bruker ImagePrep device with a modified CHCA default method allowing approximately 50% residual wetness and 40 s incubation between each spray cycle.

MALDI images were acquired on a Bruker Ultraflex III mass spectrometer using FlexImaging 2.1 (Bruker) in reflectron positive as well as negative ion modes, with a mass range from *m/z* 300 to *m/z* 1660. A laser repetition rate of 200 Hz and a spatial resolution of 200 μm were used and each MS spectrum was obtained on accumulation of 400 consecutive laser shots. Images were normalised using a Y_{mean}/Y_{max} threshold of 0.5. Three independent samples were analysed from each of the three packaging types and location within the steaks (edge and centre), with analysis of each sample performed in triplicate technical repeats.

2.5. Total lipid oxidation

The extent of total lipid oxidation was determined on days 0, 2, 4, 7 and 14 of display using the method described by Bergamo et al. (1998). In brief, approximately 4.5 g of sample was homogenised with 4.75 mL of distilled water and 0.25 mL of ethanolic butylated hydroxy toluene. The homogenised sample was centrifuged (5 min, 10,000 rpm, radius = 11.5 cm; centrifuge 5810R, Eppendorf, Westbury, NY, USA). Five hundred μL of supernatant was mixed with 500 μL of 10% TCA and centrifuged (5 min, 10,000 rpm, radius = 11.5 cm) to remove proteins. Then, 300 μL of supernatant was transferred into 1.5 mL screw cap vials and 700 μL of thiobarbituric acid (TBA) reagent was added. The vials were incubated for 30 min at 90 °C and allowed to cool at room temperature (approximately 22 °C) before injection on the HPLC. The absorption found using HPLC was converted to mg malonaldehyde/kg meat and reported as thiobarbituric acid-reactive