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Application of small angle X-ray scattering synchrotron technology for measuring ovine meat quality

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ABSTRACT

A small angle X-ray scattering (SAXS) synchrotron was used to evaluate 100 ovine *m. longissimus lumborum*, representing lamb (n = 50) and sheep (n = 50). The diffraction of X-rays gives information on muscle myofibril structure and fat content. The linear relationships between SAXS measures with measures such as, shear force, intramuscular fat content (IMF) and collagen content/solubility, were investigated. A relationship was found between the d-spacing of the actin/myosin fibril spacing (SAX1 and SAX2) and the cross sectional area of the rhombohedral unit cell (Cell area) and shear force after 1 and 5 day ageing. There was a positive relationship between IMF and a SAXS Fat area measure. There was a muscle site effect on SAX1, SAX2 and Cell area, with the cranial site having a larger distance between myofibrils. The potential of SAXS as a powerful research tool to determine not only the structural components of ovine tenderness, but also the fat content related to IMF is evident.

1. Introduction

Meat quality is a generic term that describes the properties and perception of meat, with its most important aspects identified as tenderness, juiciness and flavour (Maltin, Balcerzak, Tilley, & Delday, 2003). Within the Australian lamb industry, consistency and quality of lamb products is often scrutinised and this has prompted a need to guarantee ovine meat eating quality (Russell, McAlister, Ross, & Pethick, 2005). Novel technologies and research that provide information on meat quality aspects, such as tenderness and intramuscular fat content (IMF), can help to increase processing efficiency and ensure a product standard for both retailers and consumers (Lambe et al., 2009). Consumer demand has been a main driver for the adoption of objective meat quality measures instead of the subjective measures of the past (Stanford, Jones, & Price, 1998). These measures routinely applied technologies, for example near infrared reflectance spectroscopy (NIR) (Prieto, Roehe, Lavin, Batten, & Andrés, 2009) and other emerging technologies such as Raman spectroscopy (Schmidt, Scheier, & Hopkins, 2013).

A small angle X-ray scattering beamline (SAXS) works by passing X-ray beams through samples, and when applied to tissue these beams encounter structural obstructions, including collagen or myofibrils, and scatter dependent on their electron density distribution (Goh et al., 2005). Hence, sample position and the angle of the beam can allow different spacing and content information to be gleaned (Goh et al., 2005; Wells et al., 2013). The use of X-ray diffraction to determine muscle structure is not a new concept. Previous researchers have applied X-ray diffraction to muscle cells and produced equatorial diffraction patterns that provide information on the structure and distribution of actin and myosin filaments (Yu, 1969). More recently, Diesbourg, Swatland, and Millman (1988) investigated *post-mortem* changes to the myofilament lattice of pork muscle using a SAXS and found differences in the distances between filaments and suggested water loss (drip loss) as the causal factor. This work was under taken using muscle fixed in glutaraldehyde and to overcome any artefacts from this process impacting on the outcomes, a subsequent study was performed by Irving, Swatland, and Millman (1989). In this latter study muscle samples at 24 h post-mortem were held in chambers at three temperatures. It was established that as sarcomere length decreased the separation of myofilaments increased (Irving et al., 1989) and this was confirmed by the work of Schäfer, Knight, Wess, and Purslow (2000) using fresh

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unfixed *pre-rigor* and *post-rigor* porcine muscle. Other previous studies have used SAXS to measure the structural integrity of leather (specifically that of the collagen fibril structure), along with the structural and physical properties of connective tissue (Goh et al., 2005; Wells et al., 2013). SAXS has also been used in human studies to produce diffraction patterns that differentiate between fat, bone and muscle tissue (Kosanetzky, Knoerr, Harding, & Neitzel, 1987).

These studies support the use of SAXS to provide insight to the structure of the fibrils of actin, myosin and collagen, and potentially provide estimates of the IMF of ovine meat. To the best of our knowledge, the usefulness of SAXS for studying ovine muscle structure as it undergoes protein degradation has not been previously investigated.

This study evaluated the application of SAXS beamline (Australian Synchrotron) to measure ovine meat quality with the aim to compare or relate the muscle characteristics of lamb and sheep (older than lamb) determined using the SAXS beamline with traditional laboratory meat quality measures.

2. Materials and methods

2.1. Carcase selection

A total of 100 ovine carcases that represented lamb (n = 50) and sheep (n = 50) were randomly selected for this study, so that a range of fatness scores (Low, Medium, and High) and slaughter lots were represented. This was to provide variation in fat and tenderness levels of the experimental carcases. Carcases were selected from a commercial abattoir located in Melbourne, Victoria during autumn (March 2014; n = 50) and spring (October 2014; n = 50) seasons and on each occasion muscle samples were collected over 2 consecutive days from an equal number of lamb and sheep carcases.

2.2. Measurements

Carcass weight and GR fat depth were recorded. GR fatness was measured using a GR knife inserted into the left side of each carcase at the lateral surface of the twelfth rib, 110 mm from the midline and was recorded 15 h *post-mortem*. pH was measured using a pH meter/electrode (model TPS WP-80 pH meter with ionode probe IJ44c attached, TPS Pty Ltd., 4 Jamberoo Street, Springwood, Qld. 4127, Australia) calibrated using known standards (pH 4 and 7).

A 400–500 g section of the *m. longissimus lumborum* (LL) (Product identification number HAM 4910; Anonymous, 2005) was removed at 15 h *post-mortem* from the left side of each carcase, taking the LL from the sacral lumbar junction through to the 8th rib. All external subcutaneous fat was removed prior to sub-sampling.

Four individual sample blocks (1 cm^3) were taken on the day of sampling (15 h *post-mortem*) from the cranial (A), medial (B and C) and caudal (D) LL sites and these samples were held chilled (3–4 °C) until measured using the SAXS beamline. For SAXS beamline measures, fresh (36 h *post-mortem*) samples (1 × 1 × 0.2–0.4 cm) were prepared from blocks A–D and mounted with muscle fibres oriented transverse to the beam and running vertically within holes in a metal plate rig and polyimide (Kapton) tape was placed on both exposed sides to keep samples in place and prevent desiccation. These were then analysed using the Australian Synchrotron SAXS/WAXS beamline (Kirby et al., 2013) using a Pilatus2-1M detector with approximately 2–5 × 10^{12} photons/s monochromatic 8.200 and 20.000 keV X-rays and a camera length of 7.33 m to cover q-ranges of 0.0015–0.1 $Å^{-1}$ and 0.003 to 0.3 $Å^{-1}$ respectively. Silver behenate was used to calibrate the q-scale of the instrument, and glassy carbon was used to calibrate scattering intensities per mm of sample thickness. As the beam size (250 × 130 μm horizontal × vertical, full width at half maximum) is too small to provide a representative analysis volume in a single exposure, 110 on-the-fly scans (i.e. frames acquired with the sample continuously moving), with two second exposures in a orthogonal grid (5 × 5 mm area of sample) were taken for each sub-sample (A, B, C or D). These scans were taken at 0.5 mm intervals so 11 per interval to give the 110 exposures, with no overlap of each vertical scan. SAXS image analysis was carried out using the program scatterBrain IDL (V 1.230, Australian Synchrotron Facility). Whilst individual frames can be analysed (e.g. to spatially map structural features), this study instead averaged frames from the whole grid to maximise the volume of analysis for each sub-sample. Blank measurements were also performed on Kapton tape to subtract any significant background intensity.

Data were recorded at 20 keV primarily to determine relative fat content from the intensity of the isotropic diffraction ring at 0.145 $Å^{-1}$. The absolute intensity scaling procedure accounts for total transmitted flux and scales to an absolute intensity (cm^{-1}) using an empirical scattering intensity sample (glassy carbon), but does not normalise for sample thickness, which had to be determined separately. This was done by empirically measuring the X-ray transmission of each ovine meat sample at 20 keV of various thicknesses from 2 to 12 mm measured with a Vernier gauge to determine the linear attenuation coefficient from the Beer–Lambert Law ($T = e^{-\mu t}$) (T = transmission, t = thickness, μ = linear attenuation coefficient).

$$\text{Transmission} = (\text{I transmitted sample}/\text{I incident sample})/(\text{I transmitted Kapton}/\text{I incident Kapton})$$

Relative transmitted flux and/or absolute transmission is easily measured in real time for each exposure on the beamline as transmitted flux is accurately measured by a detector inside the beamstop and incident flux is determined using an upstream foil-scattering detector.

The observed linear attenuation coefficient (0.7 cm^{-1}) was assumed to be constant with meat composition. The thickness of unknown sample was thus determined as:

$$\text{Transmission} = -\ln(\text{thickness})/0.07(\text{cm}).$$

Absolute intensity corrected data was simply normalised by dividing by the sample thickness determined by transmission.

Two dimensional data were radially averaged into a one dimensional form where appropriate and the two major equatorial peaks used to determine the d-spacing of the A-band (1,0) and (1,1) reflections (SAX1 & SAX2 respectively) and the cross sectional area (Cell area) of the rhomboidal unit cell as described by Diesbourg et al. (1988). The area of the unit cell is $[2 \times d(1,0)^2 \div \sqrt{3}]$. For example the myosin–myosin spacing in the Z-band is $2\sqrt{3} \times d(1,0)$. The (2,0) peak of the A-band was also often observable as a shoulder on the high-angle side of the (1,1) peak, but its intensity was often too low to permit analysis in detail. Fat in the samples gave rise to an isotropic diffraction ring at q ~ 0.15 $Å^{-1}$. The net integrated area of this peak in the radially averaged data (above a local polynomial background fit) was used to determine "Fat area", along with a calculation to determine Fat volume.

2.3. Meat quality measures

The intramuscular fat (IMF) content of LL muscle samples was determined using a modified version of AOAC Method 960.39 (AOAC, 2007) for ether-extractable fat. Approximately 40 g of diced wet muscle was collected in 50 ml Falcon tubes and stored at −20 °C until subsequent freeze drying. Prior to freeze drying, the wet muscle weight of the sample was recorded. Each sample was then completely freeze dried using a Dynavac, model FD 600 freeze dryer. The dry muscle weight of the sample was then recorded. After freeze drying the sample was ground using a FOSS Knifetech™ 1095 sample mill (FOSS Pacific, North Ryde, NSW). The IMF content of each sample was then determined using the soxhlet IMF extraction method (Savell, Cross, & Smith, 1986).

The method to determine total and soluble collagen was derived from AOAC method 990.26 (AOAC, 2000) as previously described by Starkey, Geesink, Oddy, and Hopkins (2015). Sarcomere length (SL) and particle size (PS) samples were removed from each LL so that the