LIGHT SCATTERING IN BEEF *LONGISSIMUS* MUSCLE IS MAINLY GENERATED BY SHRINKAGE OF THE MYOFILAMENT LATTICE, MYOFIBRILS & MUSCLE FIBRES.

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I. INTRODUCTION

In beef muscle, myoglobin and haemoglobin are the primary absorbers of light and give rise to red meat colour, but structural elements of the muscle scatter the light and affect the paleness /darkness of the muscle. The structures which generate light scattering are yet to be defined. In pork, pale muscles show more myofilament lattice shrinkage compared to dark muscles [1], and more recently, sarcoplasmic protein denaturation is thought to promote lattice shrinkage [2]. Sarcoplasmic protein binding to myofilaments influences the lattice spacing and is a pH-dependent process [3]. Also, a lower pH of the muscle is known to promote myofilament lattice and muscle fibre shrinkage [4, 5]. These separate observations could assist in elucidating the light scattering mechanism, in regards to pale and dark meat.

We hypothesize that three mechanisms are responsible for the decreased light scattering in dark meat, relative to pale meat, namely (i) a larger lateral separation of thick and thin myofilaments, (ii) decreased optical density of proteins in the I-band and (iii) decreased denaturation of sarcoplasmic proteins.

II. MATERIALS AND METHODS

Beef *longissimus thoracis* muscles were collected 3 to 4 days post-mortem (PM) to ensure the final pH had been achieved. Meat colour was visually assessed using a standard set of colour chips, by a qualified AUS-MEAT grader and allocated to one of 3 meat colour groups (light, medium or dark): 1C (light, n = 7); 2 (medium, n = 7); >3 (dark, n = 5). Muscles were vacuum packed and transported to the laboratory, after which 3 slices (40 mm thick) were cut (10 °C) and used for all analysis including pH, colour, drip loss, sarcomere length and light scattering measurement as detailed in Hughes, et al. [4]. One slice was used for small angle X-ray scattering (SAXS), where myosin-myosin (d1,0) and myosin-actin (d1,1) spacings were measured. Another slice was trimmed, chopped and stored at -80 °C for sarcoplasmic activity assays. Aldolase and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) activities were completed on 1:10 (w/v) sarcoplasmic fractions (0.1M sodium phosphate buffer, pH 7.4) after 2 washes in isotonic buffer (380 mM mannitol + 50 mM potassium acetate buffered to ultimate pH of muscle). Units were calculated in µmol/min and expressed as a percentage of the total of the three washes. One way analysis of variance (ANOVA) was used to analyse the effects of colour treatment using Genstat 16th edition.

III. RESULTS AND DISCUSSION

The light and medium colour groups had similar values for all colorimetric, pH and drip loss measurements (

Table *1*), but dark muscles had lower lightness, redness and yellowness with a higher pH and less drip loss (all $P<0.001$). Dark muscle fibres also had lower global brightness, indicative of less light scattering ($P<0.01$).

Dark samples had a longer distance between myosin-myosin (d1,0) and myosin-actin (d1,1) filaments than paler samples and this complements previous findings in pork [1]. The wider spacing of the myofilaments indicates less shrinkage occurred PM compared to both the light and medium groups. This shrinkage was also evident at the larger length scale, as muscle fibres were also wider ($P<0.01$). The light muscles had undergone most shrinkage of the muscle fibres and were similar to the medium muscle fibres.

Dark muscles had a higher intensity ratio between the 1,1 and 1,0 equatorial diffraction peaks ($P<0.05$). The relative areas under the two 1,1 and 1,0 peaks (peak intensities) provide information about the relative amount of mass associated with the thick and thin filaments. In post-rigor muscle, nearly all the myosin heads are bound to the actin filaments, and so the differences in the intensity ratio is unlikely to arise from different amounts of crossbridge attachment.

Rather, in the darker samples there was some additional mass to the thin filaments, most likely by attachment of other proteins or peptides. Under light microscopy, the dark, higher pH muscles had more Z-line degradation and shorter sarcomere lengths. In addition, these dark muscles had higher GAPDH enzyme activity and this coincides with the added mass of protein decorating the thin filaments, as shown by the SAXS intensity ratio results. Stewart, et al. [3] also observed a higher intensity ratio after sarcoplasmic binding to actin filaments. Our results confirm our hypothesis and indicate the dark muscles have larger spacing of myofilaments, partially due to the extra mass on the thin filaments, which could be a result of increased proteolysis and more active sarcoplasmic proteins bound to the actin filaments. This would alter the diffraction pattern along the sarcomere, whilst contributing to a reduction in light scattering.

Table 1: Effect of light, medium and dark colour groups, as defined by AUS-MEAT colour scores: light 1C; medium 2; dark >3, respectively, on beef *longissimus thoracis* muscle. Measurements for myofilament spacing were conducted using small angle x-ray scattering (SAXS). Global brightness and muscle fibre diameter measurements were conducted using reflectance confocal laser scanning microscopy (rCLSM).

	Light	Medium	Dark	LSD	*P*-value
Lightness (L*)	34.8	34.0	27.4	0.91	<0.001
Redness (a*)	16.6	16.5	10.5	0.72	<0.001
Yellowness (b*)	1.8	1.1	-3.9	0.62	<0.001
pH	5.47	5.52	6.15	0.180	<0.001
Drip loss (%)	5.2	5.7	1.7	1.33	<0.001
rCLSM global brightness	121.0	102.3	78.4	24.72	0.008
Muscle fibre diameter (µm)	65.1	71.0	75.6	6.27	0.009
Myosin- myosin (d1,0) spacing (nm)	36.3	36.7	38.6	1.16	0.002
Myosin- actin (d1,1) spacing (nm)	21.4	21.3	22.6	0.81	0.006
Intensity ratio (d1,1/ d1.0)	2.4	2.9	3.7	0.78	0.015
Sarcomere length (µm)	2.13	2.11	1.89	0.162	0.015
GAPDH activity (% of total)	8.0	11.3	13.4	3.67	0.018
Aldolase activity (% of total)	14.3	15.4	24.2	10.41	0.132

IV. CONCLUSION

Myofilament lattice spacing appears to be central not only to sarcomere length, muscle fibre diameter, pH and drip loss but also colour development and light scattering in post-rigor meat.

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