A HIGH pH IN BEEF *LONGISSIMUS LUMBORUM* CONTRIBUTES TO A DARK COLOUR BY REDUCING ACHROMATIC LIGHT SCATTERING WITHIN THE MICROSTRUCTURE.

Joanne Hughes[1], Frank Clarke[2], Peter Purslow[3] and Robyn Warner[1,4]

[1] CSIRO Food and Nutrition Flagship, 39 Kessels Road, Coopers Plains, Qld, Australia.

[2]School of Biomolecular and Physical Sciences, Griffith University, Nathan, Qld, Australia.

[3]Facultad de Ciencias Veterinarias, UNCPBA, Tandil, Bs.As, Argentina.

[4]Department of Veterinary and Agricultural Science, The University of Melbourne, Parkville, Vic, Australia.

Abstract – We postulate that beef meat colour is determined not only by the pigment, myoglobin, but also by the microstructure of the muscle cell which influences the light scattering properties of the meat, by a pH-dependent mechanism. 18 samples from light, medium and dark coloured muscles were obtained and reflection confocal scanning laser microscopy (rCSLM) was used to investigate the relationships between pH, fibre fragment width and light scattering. Dark, high ultimate pH (pH_u) muscles had swollen muscle fibres with lower light scattering ($P<0.001$). In addition, muscle fibres from low and high pH_u muscles were homogenised at 5 different pHs, from 5.40 to 6.10. Increasing the homogenising buffer pH induced fibre swelling by 13 to 17% for high and low pH_u muscles respectively and ~25% reduction in light scattering, indicative of a pH-dependent swelling mechanism. Transverse and longitudinal periodicity measurements of refractive zones showed that 'dark' muscles had longer distances between zones in both orientations, indicative of the swollen nature of the myofibrils, compared to 'light' and 'medium' coloured muscles. In conclusion, rCSLM demonstrated that beef meat colour is determined not solely by pigment, but also by the microstructure which determines the magnitude of light scattering by a pH-dependent mechanism.

Key Words – color, muscle fibre, reflectance confocal laser scanning microscopy (rCLSM).

I. INTRODUCTION

Beef meat colour is a fundamental criterion of consumer acceptability and can also determine the value of the carcass. Although meat colour is largely determined by myoglobin, the microstructure of the muscle and the light scattering are also believed to contribute [1]. The microstructure is composed of both the myofilament and myofibril lattices (regular networks) within the cell and also the packing of muscle cells (muscle fibres) themselves. In post-rigor beef muscle, maximum light scattering occurs around pH 5 and declines closer to pH 7 [2]. We postulate that the dominant driver of light scattering is the transverse shrinkage of the muscle fibres and myofibrils, which is dependent on the rate and extent of the pH decline early post mortem (PM). Our hypothesis is that beef meat colour is determined not only by pigments, but also by the microstructure of the muscle which would influence the light scattering properties of the meat, by a pH-dependent mechanism. The aim of this study was to use reflection confocal scanning laser microscopy (rCSLM) to quantify light scattering at a range of pH values.

II. MATERIALS AND METHODS

Beef carcasses, from 3 processing plants (n = 18), were selected at grading to represent 3 different meat colour groups Light, Medium or Dark, (defined by AUS-MEAT colour scores: 1B or 1C; 2 or 3; >3 respectively (1B being the palest and 5 being the darkest)) [3]. At ~30 h PM, a slice (~25mm thick) from the caudal end of the *longissimus lumborum* (LL) was bloomed at 0-4 °C, for exactly 60 min and a Hunterlab Miniscan

EZ 45/0 LAV (light source A, observer angle 10°, aperture size 5 cm) was used to measure triplicate lightness, redness and yellowness; L*, a* and b* values respectively. The ultimate pH (pH_u) of the interior of the muscle was measured using a TPS WP-80 pH meter and probe. Sub-samples (~50 g) for microscopy were frozen using liquid nitrogen and stored at -80 °C.

Muscle fibre fragments were isolated using 380 mM mannitol + 50 mM potassium acetate buffer (±0.3 pH units of the pH_u) to minimise osmotic modification of fibre fragments [4]. In addition, 8 muscles of low (pH_u 5.42±0.01) and high pH_u (pH_u 6.15±0.23) were homogenised at 5 different pH's (5.40, 5.65, 5.80, 5.95 and 6.10).

An Olympus Fluoview™ 1000 CLSM, with the following configuration: numerical aperture (N.A.) 1.35, 60x magnification, aperture 105 µm, 473 nm blue diode laser at 15% (bandwidth 470-545 nm) and photomultiplier tube (PMT) at 400 V (8% offset, 2x gain) was used. For image acquisition (6 longitudinal images/ sample), 1024x1024 resolution at 20 µs/pixel was used. Light scattering or global brightness measurements were the mean greyscale pixel intensity of the image and a fibre fragment width (µm) were all measured using Image J software [5]. Periodicities in longitudinal and transverse orientations of the fibres (3 measurements per image) were measured using Image Pro software [6]. Intensity profiles of the signal were generated and the mean distance from peak to peak was measured (µm).

One way analysis of variance (ANOVA) was carried out either using a treatment structure of colour group and a block structure of meat plant or secondly, for a treatment structure of homogenising buffer pH and a block structure of muscle pH_u.

III. RESULTS AND DISCUSSION

A reflection confocal scanning laser microscopy (rCSLM) method for quantitative determination of the light scattering properties in muscle fibres was successfully developed. Observations through the depth (*z* plane) showed that optimal resolution was given at one third of the depth from the surface of the fibre (the least endomysial interference or over-exposure) and hence this accepted as most appropriate standard imaging depth. Example images of longitudinal 'light' and 'dark' muscle fibre fragments and their relevant individual lightness and pH_u values are displayed in Fig. 1, with the group means summarised in Table 1. The most predominant separation of the data was observed in 'dark' muscles, which had lowest global brightness, L*, a*, b* values and highest pH_u values (all $P<0.001$).

Global brightness values were positively correlated to lightness ($R^2= 0.31$, $P<0.05$) and negatively correlated to pH_u, ($R^2= -0.49$, $P<0.001$).

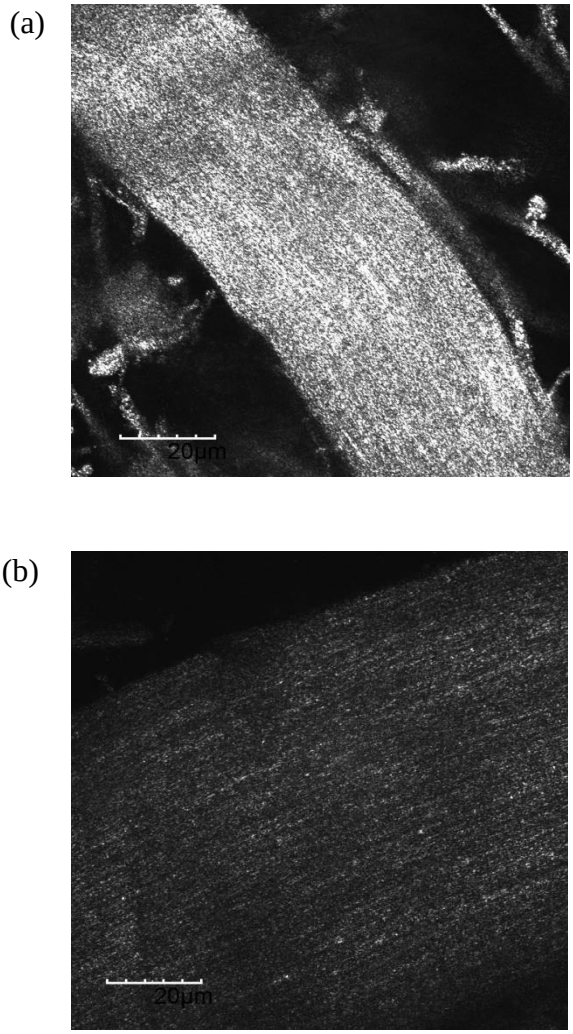


Figure 1. Beef *longissimus lumborum* muscle fibre fragments from (a) 'light' (L* = 35.3, pH_u = 5.42) and (b) 'dark' (L* = 28.3, pH_u = 6.11) muscles. Scale bar 20 µm.

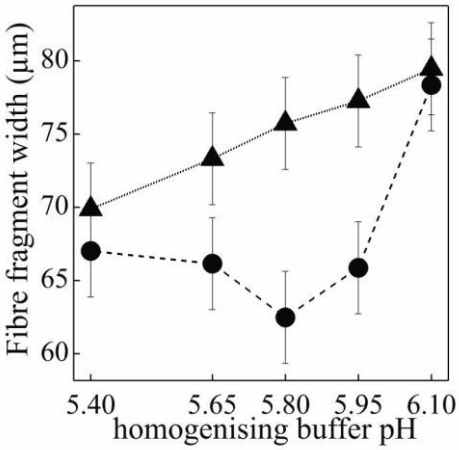
Table 1 Beef *longissimus lumborum* muscle fibre fragments from 'light', 'medium' or 'dark' muscles. Values are ANOVA means and the least significant differences (LSD) and *P*-values from comparison tests are shown (n = 18).

	Light	Medium	Dark	LSD	*P* value
Lightness (L*)	37.5	35.0	29.7	1.80	<0.001
Redness (a*)	29.4	28.5	26.6	1.22	<0.001
Yellowness (b*)	21.6	20.4	18.6	1.42	<0.001
pH_u	5.41	5.42	5.84	0.132	<0.001
Global brightness (mean pixel intensity)	98.5	112.9	77.9	16.10	<0.001
Fibre fragment width (µm)	61.4	60.8	72.3	5.90	<0.001
Longitudinal periodicity (µm)	1.307	1.248	1.431	0.1228	0.014
Transverse periodicity (µm)	1.266	1.182	1.400	0.1180	0.002

'Dark' muscle fibres displayed significantly lower (P<0.001) levels of scattering (global brightness) compared to the 'light' and 'medium' muscle fibres.

The maximum myofibril shrinkage is close to the isoelectric point of the proteins, ~pH 5.3, where we would also expect the maximum shrinkage of muscle fibre volume, which has been previously reported [7]. The periodicity of the refractive zones within the 'dark' muscle fibres were further apart compared to the 'light' or 'medium' coloured muscles, as seen in Table 1. Fewer refractive zones per unit length would reduce light scattering within the structure. The transverse periodicity values were in the range expected for the space between myofibrils (1-2 µm), with 'dark' muscles values being 0.124 to 0.218 µm (9.6 to 15.6 %) larger compared to the lighter, lower pH_u muscles fibres. Together these results suggest the 'light' and 'medium' myofibrils and muscle fibres undergo more shrinkage compared to those of the 'dark' muscle, driven by the lower pH which the proteins are exposed. These changes result in a higher density of scattering refractive zones. Although a* and b* also change, it is these structural changes that dominate L* values.

(a)



(b)

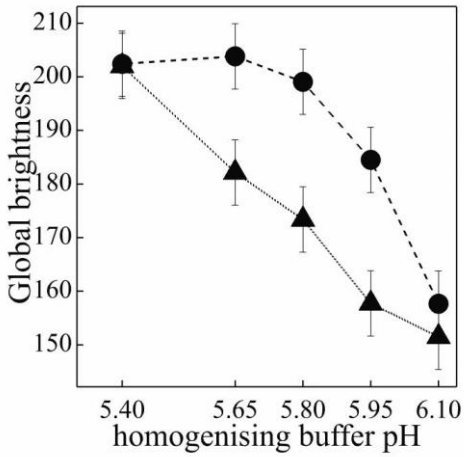


Figure 2. Effect of pH on (a) fibre fragment width or (b) global brightness of beef *longissimus lumborum* muscle extracted from low pH_u (5.42±0.01, circles) or high pH_u (6.15±0.23, triangles) muscles (n = 8). Each point represents mean ± s.e., with both muscle and homogenising buffer pH significantly different (P<0.05) for both variates and the interactions being non-significant (P>0.05).

Reducing the pH environment of the muscle was predicted to increase the light scattering properties of the fibres and consequently within the whole muscle. As homogenising buffer pH was reduced from pH 6.10 to 5.40, muscles displayed a decline in fibre width of 9.6 to 11.3 µm (13.7 to 17.0 %) for high and low pH_u muscle fibres respectively, as shown in Fig. 2(a). This shrinkage of the muscle fibres was accompanied by ~25 % increase in light scattering as measured using global brightness values (Fig. 2b). Global brightness values were generally higher than those for meat in different colour groups, probably due to the freeze-thaw cycle imparted on these samples, which is known to increase lightness and is a limitation of the study. High pH_u muscles showed more of a linear relationship with buffering pH for both fibre width and brightness. We hypothesize both effects are primarily due to the transverse shrinkage; less microstructure shrinkage results in decreased light scatter. In contrast, low pH_u muscles displayed more of a curvilinear relationship, which supports similar findings in other studies [8] and alludes to a more complex mechanism with more permanent modifications to muscle proteins. Factors involved in this complex mechanism are mainly due to integrity of the structural proteins (hydrophobicity, denaturation, surface charge) and their ability to bind to other small molecules, such as water, or more complex sarcoplasmic proteins. Therefore, muscles exposed to low pH conditions have semi-permanent modifications to proteins which prevent them from behaving like muscle fibres which have not been exposed to these conditions. Our results confirm the microstructure of the muscle determines the extent of light scattering and is dependent upon the pH_u achieved during the early PM decline. High pH_u muscle fibres and myofibrils are swollen and have longer distances between refractive zones which limit their ability to scatter light.

IV. CONCLUSION

These findings clarify the mechanism for darkness in high pH_u meat and provide an insight for improving meat lightness. Interventions to reduce the ultimate pH could promote light scattering and thus could increase consumer acceptability and the value of the carcass, bearing in mind the time dependent manner which these events occur early post-mortem.

ACKNOWLEDGEMENTS

The authors and CSIRO acknowledges funding provided by Australian Meat Processor Corporation (AMPC) and matching funds provided from Meat Livestock Australia (MLA), to support the research and development detailed in this publication. The support of the Imaging and Image Analysis Facility at Griffith University and the technical assistance of Vicki Eggleston, Anita Sikes and Janet Stark are also gratefully acknowledged.

REFERENCES

1. Swatland, H. (2008). How pH causes paleness or darkness in chicken breast meat. Meat Science 80: 396-400.
2. Rome, E. (1968). X-ray diffraction studies of the filament lattice of striated muscle in various bathing media. Journal of Molecular Biology 37: 331-344.
3. AUS-MEAT (2005). Handbook of Australian Meat, . In I. King, International Red Meat Manual, (pp 11). South Brisbane, Queensland, Australia: AUS-MEAT, Ltd
4. Winger, R.J., and Pope, C.G. (1981). Osmotic properties of post-rigor beef muscle. Meat Science 5: 355-369.
5. Rasband, W. (Accessed: 2014) ImageJ.Ink, USA National Institutes of Health, http://imagej.nih.gov/ij/index.html
6. MediaCybernetics (Accessed: 2012) Image Pro Premier, 9.1, http://www.mediacy.com
7. April, E.W., Brandt, P.W., and Elliott, G.F. (1972). The myofilament lattice: studies on isolated fibers: II. The Effects of Osmotic Strength, Ionic Concentration, and pH upon the Unit-Cell Volume. The Journal of Cell Biology 53: 53-65.
8. MacDougall, D.B., and Jones, S.J. (1981). Translucency and colour defects of dark-cutting meat and their detection. The problem of dark-cutting in beef. Current topics in veterinary medicine and animal science 10: 328-343.