How pH causes paleness or darkness in chicken breast meat

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Chicken breasts (*Pectoralis*) at a low pH (5.91 ± 0.12, n = 10) were compared with breasts at a high pH (6.36 ± 0.25, n = 10, $P < 0.001$). Low-pH breasts had the highest reflectance ($P < 0.001$ from 400 to 700 nm). High-pH breasts had the greatest transmittance into their depth and across individual muscle fibres ($P < 0.001$). The differences in refractive index between ordinary and extraordinary rays across individual muscle fibres were greater in low-pH than in high-pH breasts ($P < 0.001$). Light at low wavelengths had greater reflectance and lower transmittance than light at long wavelengths ($P < 0.001$). Myofibrillar refraction contributed to differences in light scattering between PSE (pale, soft, exudative) and DFD (dark, firm, dry) chicken meat, as it does in pork and beef.

Keywords: Chicken; PSE; DFD; Optical properties; Birefringence

Introduction

One of the basic tenets of meat science is that accelerated or extended post-mortem glycolysis may cause the development of PSE (Barbut *et al.*, 2007). There are implicit interrelationships between temperature and pH because glycolysis is exothermic, and the effects of pH are severe when a carcass is still near body temperature. The corollary of this tenet is that low glycogen levels at slaughter will prevent the normal postmortem increase in meat reflectance, thus leaving muscles in a DFD state, as in the live animal. Three biophysical mechanisms have been proposed to explain how a low pH causes increased reflectance: (1) denaturation of sarcoplasmic proteins (Bendall & Wismer-Pedersen, 1962), (2) increased surface reflectance from myofibrils (Hamm, 1960), and (3) increased refraction through myofibrils (Swatland, 2004). Their relative importance is unknown, although probably the first mechanism is only important at extremes of low pH and/or high temperature.

Many surveys of meat pH and paleness have been published, but few attempt to explain how a low pH actually increases reflectance. This may be a fundamental mechanism common to all species, but the assumption has not been rigorously tested. The brief experiments reported here recapitulate earlier experiments on the optical properties of pork and beef, but using new material – PSE and DFD poultry meat. The main hypotheses tested are:

(1) meat with a low pH appears pale because it scatters more light back to the observer than meat with a high pH,
(2) meat with a high pH appears dark because it transmits more light into its depth than meat with a low pH,
(3) the effect of pH is detectable at the cellular level across individual muscle fibres, and
(4) pH affects myofibrillar refraction within muscle fibres.

Materials and methods

Samples and pH

Breasts from 6-week, commercial broiler chickens were obtained directly from a primary supplier packed in sealed polystyrene trays for retail display. Samples were kept at 4º C and measured within 7 days of slaughter. Two sets of samples were selected by their subjective appearance, ten pale and ten dark *Pectoralis* muscles. Sample pH was measured with an ion sensitive field effect probe (model 240, IQ Scientific Instruments, San Diego, CA).

Experiment 1: reflectance from bulk meat

Light from a 100 W halogen source with a stabilized transformer (6642A Hewlett-Packard, Palo Alto, CA) was directed into one branch of a bifurcated light guide through a solenoid-activated iris shutter (467225, Carl Zeiss, Oberkochen, Germany). The light-guide (WW100, Guided-Wave, El Dorado Hills, CA) had one branch with six optical fibres connected to the light source, while the other branch connected to the photometer had only one fibre. The six illuminating fibres in the common trunk were arranged in a ring around the single recording fibre. The light returned to the recording fibre from the sample passed through a grating monochromator (Zeiss 474321 with 474346 grating), through a stray light filter (Zeiss 477215) to remove higher-order harmonics, and onto a side-window photomultiplier (Hamamatsu type HTV R 928, 1126 Ichino-Cho, Hamamatsu City, Japan) with S-20 characteristics. The system was standardized (reflectance = 1 from 400 to 700 nm at intervals of 10 nm) against a mirror, hence the reflectance data reported here are much lower than for a system standardized against a diffuse white plate. A mirror was used to match the standardization method in the later experiments, thus removing any possible confusion from using a diffuse standard for reflectance and direct standardization for transmittance. Algorithms for photometry were taken from Swatland (1998).

Experiment 2: transmittance through bulk meat

The transmittance of light through slices of meat was measured using frames to control the thickness of the slice. The outer dimension of each frame was 25 x 52 mm to fit onto a microscope slide. The inner opening of the frame was 19 x 12.5 mm and was filled by the sample. The thickness of each frame was 2mm. Thus, for example, three frames were used to cut a slice of meat 6-mm thick, which was then moved with its supporting frames to a microscope slide. A second microscope slide held down by two weights of 10 g was placed on top to give a controlled depth of meat. The long axes of the muscle fibres were in line with the optical axis of the microscope so that optical measurements were made coaxially with the long axes of muscle fibres (to minimize the effect of perimysial boundary layers). The microscope objective was a Zeiss 1.25, and the lower surface of the slice was illuminated with collimated light (not the converging cone of light from a substage condenser). The microscope was a Zeiss Universal fitted with the same components for spectrophotometry as in the first experiment on bulk meat reflectance. The system was standardized in the same way, but using direct transmittance through an empty sample chamber instead of reflectance from a mirror. This experiment was not replicated because the magnitudes of the differences detected were very large, and there was some urgency to complete all experiments before risk of storage time affecting results.

Experiment 3: transmittance through individual muscle fibres

Pooled samples from breasts of each type (n = 10) meat were macerated in distilled water using a blender to produce a suspension of muscle fibres. Drops of suspended fibres were mounted in a well slide to avoid compression of fibres. Transmittance measurements were made with a long-distance objective (Zeiss LD Epiplan 40, numerical aperture 0.60) and a 0.63-mm measuring aperture covering approximately half the diameter of fibres in the microscope field. Fibre diameter was measured with a calibrated microscope eyepiece micrometer. The system was standardized as in earlier experiments using a clear area to one side of the muscle fibre.

Experiment 4: refractive path difference through individual muscle fibres

The microscope used for the previous experiments was fitted with polarizing optics and a de Sénarmont compensator (Zeiss 473715), which is a fixed quarter-wave plate oriented diagonally in a NW-SE slot below the analyzer to give a N-S orientation of the slow axis (compass points are used for orientation). Using a rotating stage, a muscle fibre was rotated to the extinction position on the SW-NE diagonal and its transmittance was measured at 589 nm using the Zeiss LD EPIPLAN 40 objective and a 0.32-mm measuring aperture to cover at least 10% of the muscle fibre diameter at its deepest point. The de Sénarmont compensator was inserted and the analyzer was rotated until extinction transmittance was obtained again on the photometer. Analyzer degrees were converted to path differences using the calibration factor of the compensator. The path difference is the difference between ordinary and extraordinary rays passing through birefringent muscle fibres. Further information may be obtained from Swatland (1998). Muscle fibre diameters were measured with the eyepiece micrometer.

Results

pH values

The pH values of pale and dark samples, respectively, were 5.91 ± 0.12 and 6.36 ± 0.25 (P < 0.001, n = 20). These values are comparable with those reported by other researchers. For example, Barbut, Zhang and Marcone (2005) reported pH values of 5.72 versus 6.27 for pale and dark chicken meat, respectively. In subjective terms, therefore, the results reported below are for pale, tending to normal meat versus very dark breast meat.

Experiment 1: reflectance from bulk meat

The reflectance of chicken breast meat with a low pH was higher (P < 0.001 from 400 to 700 nm) than for meat with a high pH (Fig. 1). Reflectance spectra showed absorbance in the Soret band at 410 nm but little or no evidence of selective absorbance by myoglobin (around 550 nm). From 460 to 700 nm, therefore, spectra were almost flat showing stronger reflectance at 460 nm than at 700 nm (P < 0.0005). Thus, beyond the Soret band, light at low wavelengths was reflected more than light at high wavelengths.

Experiment 2: transmittance through bulk meat

The transmittance of light through slices of meat followed the expected inverse pattern to reflectance: transmittance was low at 400 nm and high at 700 nm. The differences between high and low wavelengths, and between low-pH (Fig. 2) and high-pH meat (Fig. 3) were very large. Of particular note was transmittance through 2-mm of meat at 700 nm in high-pH meat (Fig. 3) where no attenuation was detected. However, cutting thin slices of soft meat has a high degree of

experimental error and it was no surprise that the spacing of transmittances through progressively thicker slices was irregular.

Experiment 3: transmittance through individual muscle fibres

Spectra were almost linear with no evidence of haemoprotein absorbance in the Soret band (around 410 nm) or myoglobin absorbance peaks (around 550 nm). Transmittance at 700 nm was higher than at 400 nm in both low-pH and high-pH breasts ($P < 0.001$). Low-pH breasts had lower transmittance than high-pH breasts ($P < 0.001$).

No differences in muscle fibre diameters were detected between low-pH and high-pH breasts (732 ± 93 μm versus 667 ± 217 μm, $P > 0.05$). However, this was a very small sample and one would normally expect to find shrunken muscle fibres in PSE meat. Another expectation is that, from the photometric laws, one would expect transmittance to be inversely proportional to path length (muscle fibre diameter). This was detected in low-pH breasts (for example; $r = -0.57$, $P < 0.05$ at 400 nm) but not in high-pH breasts.

Experiment 4: refractive path difference through individual muscle fibres

Path differences between ordinary and extraordinary rays through individual muscle fibres were greater in low-pH than in high-pH breasts (93.1 ± 24.5 nm versus 70.2 ± 20.3 nm, respectively, $P < 0.001$). Again, in this second small sample of measured fibres, no differences in muscle fibre diameters were detected between low-pH and high-pH breasts (674 ± 140 μm versus 639 ± 244 μm, respectively, $P > 0.05$). From the geometry of measuring optical path differences, one would expect path difference to be proportional to muscle fibre diameter (see Fig. 7 of Swatland, 2004). This was detected in both low-pH and high-pH breasts ($r = 0.61$ and $r = 0.72$, respectively, both $P < 0.005$). Applying a correction for muscle fibre diameter, path differences were still greater in low-pH than in high-pH breasts (respectively, 0.139 ± 0.029 nm versus 0.117 ± 0.035 nm path difference per μm fibre diameter, $P < 0.02$).

Discussion

The brief experiments reported here confirm that pH-dependent light scattering in chicken meat exhibits the same features it does in pork and beef, but there are still many unsolved problems in two main areas. The first area concerns scattering when the muscle microstructure is not severely damaged post-mortem – in other words, a system dominated by intact thick and thin myofilaments arranged regularly and separated by clear fluids. A decrease in pH reduces the negative electrostatic repulsion between myofilaments (Millman, 1998) which then move closer together laterally (Diesbourg, Swatland & Millman, 1988) to increase refractive index in the lateral direction. Thus, as in pork (Swatland, 2003), path differences between longitudinal and transverse optical axes are increased in PSE poultry meat (experiment 4). But a high refractive index will have two effects. It will increase reflectance at the refractive index boundary at the myofibrillar surface, as well as increasing scattering from rays refracted through the myofibrils. The challenge is to separate these two effects and quantify them.

Another problem with intact myofilaments separated by clear fluids is there are many levels of boundaries at which reflection might occur: (1) between myofibrils and sarcoplasm, (2) between muscle fibre surfaces and intercellular fluids, and (3) between muscle fasciculi and intercellular exudates. The refractive contribution is more restricted in distribution and is probably dominant when rays pass through myofibrils and are then reflected internally from the far side or strike another

myofibril. This phenomenon is explained by Williamson and Cummins (1983) for refractive inclusions in paint and the results reported here show a similar explanation is applicable to meat.

The second area of factors contributing to scattering is when the microstructure is disrupted *post-mortem* as, for example, by the precipitation of sarcoplasmic proteins (Bendall & Wismer-Pedersen, 1962). Guarnieri *et al*. (2004) examined the microstructure of PSE chicken meat and found a 10% shrinking of muscle fibre diameters with enlargement of endomysial and perimysial spaces. Z-lines were fragmented, there was a loss of A-bands and M-lines, and sarcomeres were supercontracted. Many of these changes may increase scattering without acting through changes in refractive index. Diffraction also contributes to light scattering in muscle (Pollack, 1990) but the effects are obscured by irregularities in the striation patterns of muscle (Huxley, 1990).

In summary, pH-related scattering has a major effect on the translucency of poultry meat causing low-pH meat to appear pale and high-pH meat to appear dark. But what are the implications of this conclusion in practical terms? Uniformity of meat colouration matched to the requirments of a particular market is extremely important in the poultry industry (Fletcher, 2002). Thus, when PSE poultry meat becomes a problem, the solution is most likely to be found in a critical examination of any factor that may have contributed to unusually rapid or extended post-mortem glycolysis, starting with the variables of primary production such as nutrition, and following through to all aspects of transport, slaughter and refrigeration. Thus, for researchers measuring poultry meat colour, no study is complete without some measurement of, and correction for, the rate and extent of pH decline post-mortem. Poultry meat is translucent. If light scattering is weak because the meat has a high pH, then the light path through the tissue will be relatively long and will increase the selective absorbance of light my myoglobin and its derivatives. Conversely, if light scattering is strong because the meat has a low pH, the the light path through the the tissue will be relatively short and will decrease selective absorbance. Thus, every aspect of meat colourimetry depends on the translucency of the samples. Most researchers use colourimeters designed for measuring painted metal surfaces and plastics and are unaware of the optical problems created by translucency.

References

Barbut, S., Sosnicki, A.A., Lonergan, S.M., Knapp, T., Ciobanu, D.C., Gatcliffe, L.J., Huff-Lonergan, E., & Wilson, E.W. (2007). Progress in reducing the pale, soft and exudative (PSE) problem in pork and poultry meat. *Meat Science*, in press.

Barbut, S., Zhang, L., & Marcone, M. (2005). Effects of pale, normal and dark chicken breast meat on microstructure, extractable proteins, and cooking of marinated fillets. *Poultry Science*, 84, 797-802.

Bendall, J.R. & Wismer-Pedersen, J. (1962). Some properties of the fibrillar proteins of normal and watery pork muscle. *Journal of Food Science*, 27, 144-159.

Diesbourg, L., Swatland, H.J. & Millman, B. M. (1988). X-ray diffraction measurements of postmortem changes in the myofilament lattice of pork. *Journal of Animal Science, 66*, 1048-54.

Fletcher, D.L. (2002). Poultry meat quality. *World's Poultry Science Journal,* 58, 131-145.

Hamm, R. (1960). Biochemistry of meat hydration. *Advances in Food Research*, 10, 355-463.

Huxley, A.F. (1990). A theoretical treatment of diffraction of light by a striated muscle fibre. *Proceedings of the Royal Society, Biological Sciences (Series B),*241, 65-71.

Guarnieri, P.D., Soares, A.L., Olivo, R., Schneider, J.P., Macedo, R.M., Ida, E.I., & Shimokomaki, M. (2004). Preslaughter handling with water shower spray inhibits PSE (pale, soft, exudative) broiler meat in a commercial plant. Biochemical and ultrastructural observations. *Journal of Food Biochemistry*, 28, 269-277.

Millman, B.M. (1998). The filament lattice of striated muscle. *Physiological Reviews*, 78, 359-391.

Pollack, G.H. (1990). Optical scattering studies of muscle contraction. In J.K. Foskett & S. Grinstein (Eds) *Noninvasive Techniques in Cell Biology* (pp. 311-326). Wilmington: Wiley-Liss.

Swatland, H.J. (1998). *Computer Operation for Microscope Photometry*. CRC Press, Boca Raton, FL.

Swatland, H.J. (2003). Ellipsometry across isolated muscle fibres indicates a refractive contribution to paleness in pork. *Meat Science*, 63, 463-467.

Swatland, H.J. (2004). Progress in understanding the paleness of meat with a low pH. *South African Journal of Animal Science*, 34, Supplement 2, 1-4.

Williamson, S.J. & Cummins, H.Z. (1983). *Light and Color in Nature and Art.* Pages 363-375. John Wiley, New York.

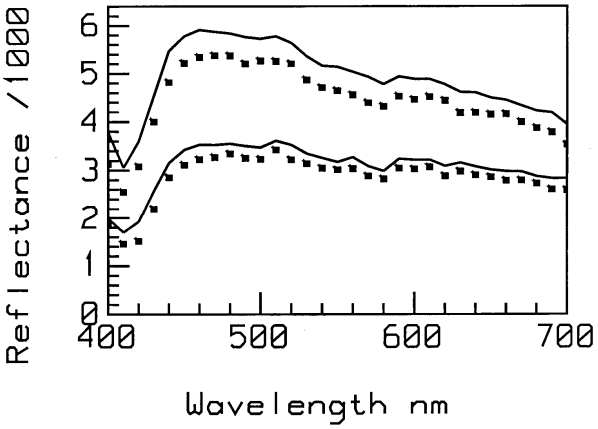

Fig. 1. Fibre-optic reflectance of low-pH (top) and high-pH chicken breasts (bottom). Squares show a standard deviation subtracted from the mean (n = 10).

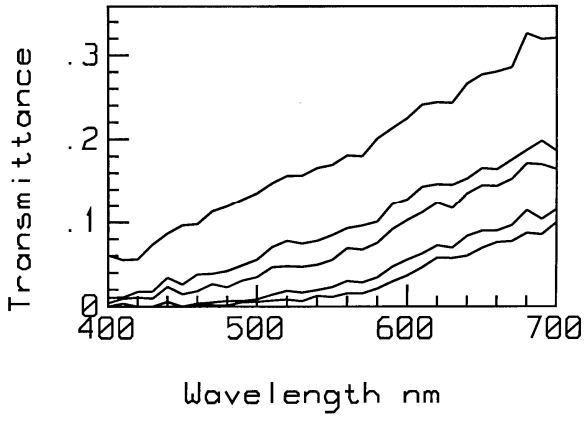

Fig. 2. Transmittance through 2-mm slices of low-pH chicken breast. From top to bottom, slices at thickness 2, 4, 6, 8 and 10 mm.

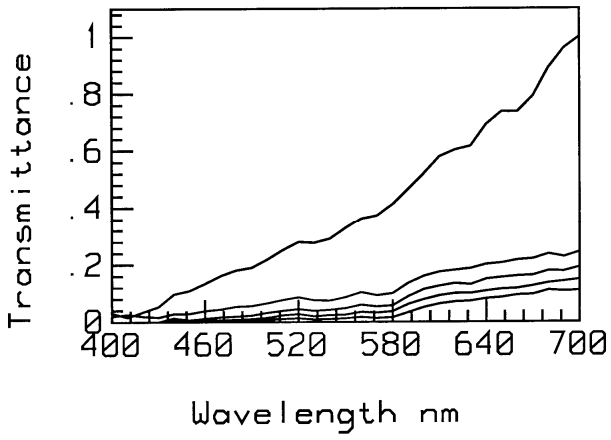

Fig. 3. Transmittance through 2-mm slices of high-pH chicken breast. From top to bottom, slices at thickness 2, 4, 6, 8 and 10 mm.

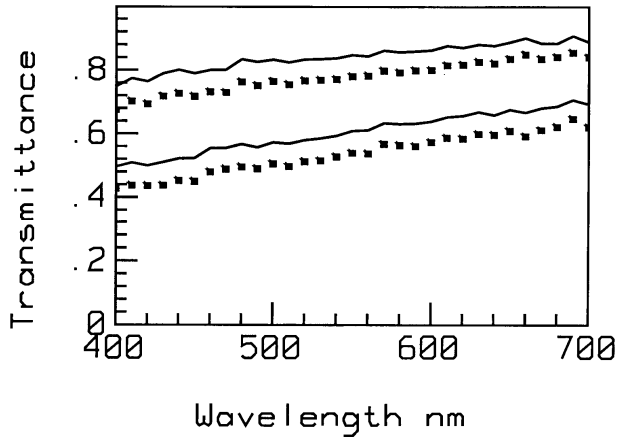

Fig. 4. Transmittance through individual muscle fibres from high-pH (top) and low-pH chicken breasts (bottom). Squares show a standard deviation subtracted from the mean (n = 10).