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Imaging optical diffuse reflectance in beef muscles for tenderness prediction

J. Ranasinghesagara [a], T.M. Nath [b], S.J. Wells [b], A.D. Weaver [b], D.E. Gerrard [c], G. Yao [a,*]

[a] Department of Biological Engineering, University of Missouri, Columbia, MO 65211, USA
[b] Department of Animal and Range Science, South Dakota State University, Brookings, SD 57007, USA
[c] Department of Animal Poultry Sciences Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

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ABSTRACT

The objective of this study was to investigate the potential of a novel optical reflectance imaging method to predict beef tenderness. Two-dimensional (2D) optical reflectance in beef muscles induced by a point incident light was acquired. A set of five parameters were extracted from each reflectance image to describe quantitatively the reflectance profiles. Two parameters, q and B, were derived by numerically fitting the equi-intensity contours of the reflectance pattern. Two spatial gradients were calculated along the directions parallel and perpendicular to muscle fibers and total scattering intensity was obtained by excluding the specular reflectance. This method was applied to analyze 2D images of optical diffuse reflectance in 336 beef samples obtained from 14 steers in which large variations in tenderness were generated by altering animal genetics, suspension method and aging time as well as utilizing muscles varying in their inherent tenderness. Tenderness was evaluated using Warner–Bratzler shear force (WBSF). The effects of animal breed, muscle, types of suspension, and aging were investigated and results indicate that the scattering intensity measured at 1-d was correlated ($R^2 = 0.50$ at $\lambda = 720$ nm) with 10-d WBSF in *M. longissimus dorsi* muscles; and the q parameters measured at 1-d was correlated ($R^2 = 0.46$ at $\lambda = 720$ nm) with 10-d WBSF in *M. psoas major* muscles. These results show analyzing 2D reflectance images of meat surfaces provides valuable information regarding the physical characteristics of meat that are responsible for beef tenderness.

1. Introduction

Tenderness is considered one of the most important factors in beef eating quality for consumers. However, variation of this trait is the most critical issue facing the beef industry (Dransfield, 1985; Huffman et al., 1996; Roeber et al., 2000; Shackelford et al., 2001). Currently, the majority of carcass value is assigned based on the amount of marbling and physiological maturity of the carcass. These parameters are intended to function as predictors of expected palatability. Unfortunately, while this system is rather quick and efficient, it remains inadequate in determining tenderness, a critical component of palatability, due to the multitude of factors that regulate the tenderness (Campion, Crouse, & Dikeman, 1975). An effective technology that can segregate beef carcasses or cuts, based on predicted tenderness is highly desirable in the beef industry. Among several emerging technologies, optical based methods have the greatest potential for online applications because they are rapid, non-destructive and inexpensive. Much research has been conducted in the area of tenderness prediction using computer vision (Li, Tan, Martz, & Heymann, 1999; Li, Tan, & Shatadal, 2001; Vote, Belk, Tatum, Scanga, & Smith, 2003) and near-infrared spectroscopy or imaging (Liu et al., 2003; Park, Chen, Hruschka, Shackelford, & Koohmaraie, 1998; Shackelford, Wheeler, & Koohmaraie, 2005). A commonality among these methods, as well as the present study, is reliance on optical detection.

Light propagation within meat is strongly modulated by the corresponding optical absorption and scattering properties of the sample. Optical absorption is associated with various chemical components of beef such as myoglobin and its derivatives (Swatland, 1989). Conversely, optical scattering is subject to the effects of muscle structural properties such as sarcomere length and collagen content (Xia, Weaver, Gerrard, & Yao, 2008a, 2008b). As these same meat structural properties are also two primary mechanisms controlling beef tenderness, there has been recent interest in applying optical scattering measurements for beef tenderness characterization (Cluff et al., 2008; Xia, Berg, Lee, & Yao, 2007).

When light is incident at a single point on the sample surface, the scattered photons may exit at locations far away from the incident location. By evaluating the spatial distribution of reflectance/transmission induced by a point incident light, valuable information may be obtained regarding the light scattering process within the muscle tissue. Snyder (1967) and MacDougall (1970) were the first to analyze the diffuse reflectance or transmittance in meat using the Kubelka–Munk model (Kubelka, 1948). Davis and Birth (Birth, Davis, & Townsend, 1978; Davis, Birth, & Townsend, 1978)

* Corresponding author. Tel.: +1 573 882 7044; fax: +1 573 884 7529.
E-mail address: YaoG@missouri.edu (G. Yao).

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further extended this work and derived sample attenuation properties, which were "additive" effects of microstructure-mediated scattering and chromophore absorption as clearly indicated by Swatland (Swatland, 1994; Swatland & Irie, 1992). However, the Kubelka–Munk model is not accurate to describe light transportation in high scattering samples (Loyalka & Riggs, 1995). Using an accurate solution of the optical diffuse equation, Xia Weaver, Gerrard, and Yao (2006), Xia et al. (2008a, 2008b) studied optical properties in beef muscle based on the one-dimensional (1D) surface reflectance measurements. The optical diffuse theory allows complete separation of optical scattering and chromophore absorption. Further, Xia et al. (2007) showed that the optical scattering properties of beef were correlated with Warner–Bratzler shear force (WBSF).

However, all aforementioned studies only examined the 1D intensity profiles of the transmitted/reflected light. In a recent study, Ranasinghesagara and Yao (2007) discovered that two-dimensional (2D) reflectance patterns in skeletal muscle are significantly different from other biological tissues. Using an optical sarcomere model, they further demonstrated that such a unique reflectance profile was caused by the combined effects of optical diffraction by the sarcomere and background tissue scattering in muscle. The 2D measurements clearly captured the strong anisotropic nature of the optical reflectance in muscle and thus may provide a more comprehensive characterization of the sample than the 1D measurement.

To investigate the potential application of 2D reflectance measurements for beef tenderness prediction, this study evaluated 2D reflectance imaging in three muscles across 14 carcasses from two different breed types: *Bos taurus* ($n = 7$) and *Bos indicus* ($n = 7$) subjected to normal or hip suspension, which applies tension on the muscle pre-rigor and induces changes in ultimate sarcomere length. The effects of breed, muscle, type of suspension, and aging on these optical parameters were analyzed. In addition, the potential of applying one or more of these reflectance measurements at 1-d postmortem to predict WBSF of cooked beef measured at 10-d postmortem was investigated.

2. Methods and materials

2.1. Animals and muscle samples

Seven A-maturity *Bos taurus* (Angus-Hereford cross) and seven A-maturity *Bos indicus* (purebred Brahman) steers were harvested at the South Dakota State University meat science laboratory. Simple statistics for carcass and muscle traits are shown in Table 1. *Bos indicus* cattle are generally tougher than *Bos taurus* due to high levels of calpastatin which limits calpain mediated proteolysis (Wheeler, Savell, Cross, Lunt, & Smith, 1990). The right side of each carcass ($n = 14$) was hung from the Achilles tendon (normal suspension, NS) while the left side of each carcass ($n = 14$) was hung from the aitch bone (hip suspension, HS). Hip suspension was utilized as it is known to lengthen the sarcomeres in the *longissimus dorsi* and muscles of the round improving tenderness while it shortens the sarcomeres of the *psoas major*, decreasing tenderness (Hostetler, Landmann, Link, & Fitzhugh, 1970). The above approaches were adopted to induce a large variation in sarcomere length and shear force in the beef samples.

Carcasses were chilled for 24 h at 1 °C. Following the chilling period, the entire *M. psoas major* (PM), and *M. semitendinosus* (ST) were removed from the each side along with a 20.32 cm section of the *M. longissimus dorsi* (*lumborum* portion; LD). The "deep" portion of the ST was removed to reduce variation. Four 3.81 cm steaks and two 2.54 cm steaks were excised from assigned locations on each muscle. The 3.81 cm sections were vacuum packaged and stored at 4 °C for either 1, 4, 7 or 10 d. At the completion of the assigned aging period, samples were analyzed for optical measurements and sarcomere length. The remaining 2.54 cm steaks were vacuum-packaged and stored at 4 °C for either 1 or 10 d and frozen at −20 °C for later analysis of WBSF.

2.2. Optical imaging

Meat samples were positioned so that the long axis of the muscle fibers aligned with the *x*-axis in Fig. 1. A black plate with a thin (150 μm) cover glass window (6663G67, Thomas Scientific, Swedesboro, NJ, USA) was placed on top of the sample to ensure a flat surface. A 100 W Quartz Tungsten Halogen light source was used with a monochromator (MicroHR, Horiba Yvon, Edison, NJ, USA) to produce light of 20 nm spectral bandwidth from 640 nm to 860 nm. The output light from the monochromator was coupled into a 1-mm optical fiber. The transmitted light from the fiber was then projected to the sample surface using an achromatic lens of 3.5 cm focal length. The distance between the lens and the sample was 7.0 cm. The incident light beam was oriented within the *y*–*z* plane and with an oblique incident angle of 46.5° to the *z*-axis. The measured focal spot size was 2 mm in diameter at the sample surface. This incident point was considered the origin of the coordinate system.

A monochrome digital camera (PULNIX 1010, JAI-Pulnix, San Jose, CA, USA) was mounted above the meat sample so that the light incident was at the middle of the image frame. The backscattered reflectance images were transferred to a personal computer via a frame grabber (Meteor-II/Digital, Matrox Inc., Quebec, Canada). The incident fiber, camera and sample mount were enclosed in a black box to block the ambient light throughout the procedure. The acquired images were normalized against the incident light intensity and camera sensitivity at each wavelength. Five continuous images were recorded and averaged to improve the signal-to-noise ratio. After removing the dark image, the reflectance image was stored in a 10 bit 1001×1016 array for further processing.

Table 1
Simple statistics for carcass traits.

Trait	*n*	Minimum	Maximum	Mean	SD
Hot carcass weight (kg)	14	303.7	384.1	335.5	20.9
Adjusted preliminary yield grade	14	2.2	3.8	2.9	0.5
Longissimus area (cm^2)	14	67.7	99.4	83.2	8.4
Kidney, pelvic, and heart fat (%)	14	1.0	3.5	2.0	0.7
USDA yield grade	14	1.5	4.2	2.5	0.7
Marbling score[a]	14	280	620	365	89

[a] 300 = "Slight^{00}," 400 = "Small^{00}," 500 = "Modest^{00}," 600 = "Moderate^{00}," 700 = "Slightly Abundant^{00}."

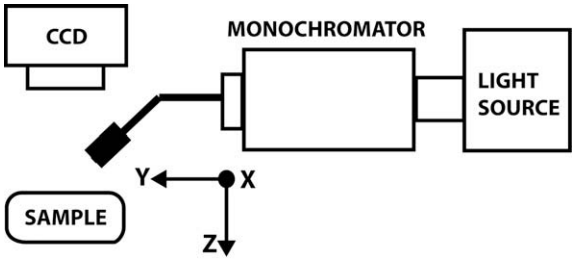

Fig. 1. A schematic showing the experimental setup. The monochromator is coupled to a 100 W Quartz Tungsten Halogen light source. The muscle sample was positioned so that the long axis of the fibers were parallel the *x*-axis. The light beam (2 mm beam diameter and 20 nm spectral bandwidth) was projected to the center of the imaging area via a 1-mm optical fiber.