Scientific Contributions

Measurement of meat quality using light spectrometry

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Introduction

Methods commonly used to measure meat quality parameters such as pH, cooked meat toughness and water-holding capacity are time-consuming and require either the sample or a portion of it to be destroyed during assessment. As a result, meat quality is rarely assessed in an industrial setting, as the sacrifice of valuable product is seen as untenable. Also, the demand for meat is inelastic and largely perceived to be unaffected by product quality except in the case of extreme quality defects such as cold shortened, dark, firm and dry or pale, soft and exudative meat. Invention of a non-invasive method of assessing meat quality would allow for classification of carcasses or cuts into quality categories, removal of poor quality product from discerning markets, selection of animals on the basis of meat quality and objective substantiation to guarantee product quality (Rosenvold et al. 2009).

Of technologies studied, that of estimating meat quality using light reflectance has been the most promising. Light with wavelengths in the visible (VIS) and near-infrared (NIR) region (400 to 700 and 700 to 2500 nm, respectively) has shown particular promise, giving *r* values between NIR measurements and Warner-Bratzler shear force (WBSF) values greater than 0.8 (Mitsumoto, Maeda, Mitsuhashi & Ozawa 1991; Park, Chen, Hruschka, Shackelford & Koohmaraie 1998). Dian et al. (2008) used VIS and NIR reflectance of peri-renal fat to differentiate the carcasses of pasture-fed and concentrate-fed lambs and Liu et al. (2004) showed that tough and tender chicken breast could be differentiated based upon VIS-NIR spectra. NIR technology exists commercially for the laboratory assessment of meat fat, water and protein content, with laboratory-grade NIR technology best used to assess the chemical composition of minced or ground meat as it is calibrated for a homogenized product (Ripoll et al. 2008; Sierra et al. 2008). Moisture, fat and water have also been successfully predicted in whole pork using VIS-NIR (400 to 1700 nm) (Chan et al. 2002) and fat in whole beef (Rødbotten et al. 2000).

Although Prieto et al. (2009) recently provided an excellent review of the application of NIR spectroscopy to predict meat and meat product quality that focused on the relationship between NIR and muscle chemistry, the current review will focus on the commercial applicability of visible, NIR and infra-red (IR) spectroscopy to determine the quality of whole beef primals, particularly the *m. longissimus thoracis*, which is used for quality grading of beef carcasses in Canada. This review will provide a summary of the state of the technology and its commercialization to date and describe what further research is required for commercialization of this promising technology to occur.

Measurement of colour, pH and water-holding capacity

Although many studies have purported to use NIR spectra, most spectral measurements have generously included visible (VIS) or infrared spectra (IR). Prevolnik et al. (2009) used artificial neural network technology to use VIS-NIR spectra (400 to 2500 nm) to predict pork pH and colour and obtained a prediction model that had at best an R^2 of 0.45 for the test data set. Rosenvold et al. (2009) used VIS-NIR spectra (400 to 1700 nm) to predict whole muscle pH (R^2 = 0.84), pre-rigor glycogen content (R^2 = 0.70), and post-rigor water-holding capacity (R^2 = 0.68). Andrés et al. (2008) found a very strong relationship between VIS-NIR spectra and meat beef pH (R^2 = 0.97), beef lightness (R^2 = 0.82) and beef redness (R^2 = 0.51) but did not see a relationship with cooking loss (R^2 = 0.20). Leroy et al. (2003) also found strong correlations between NIR reflectance (833 to 2500 nm) and L* (R^2_{cv} = 0.83 to 0.85) and a* (R^2_{cv} = 0.73 to 0.75) but the correlation with drip loss (R^2_{cv} = 0.38 to 0.54) was not as strong. Prediction of beef colour by light spectroscopy is poor if the visible wavelength region is not included (Prieto et al. 2008). Myoglobin structural changes are best observed between 450 and 600 nm, with isobestic absorbance of all chemical states at 525 nm (Cox and Hollaway 1977). Light spectroscopy should readily differentiate meat on the basis of pH because the optical properties of skeletal muscle changes with acidity, with the path difference of a depth of fibre increasing as acidity increases (Swatland 2002). Mitsumoto et al. (1991) obtained correlations between NIR spectra (1100 to 2500 nm) and pH ranging from 0.58 to 0.74. Although most studies have incorporated both VIS and NIR light, VIS light alone has been shown to indicate lightness (L*) and drip in pork, with correlation coefficients of 0.82 and 0.76, respectively (Swatland 1998).

Water-holding capacity is an important indicator of meat quality because low water-holding capacity is associated with poor processing functionality. Water-holding capacity has been of most interest as it pertains to pork quality, as the regular occurrence of pale, soft, exudative pork has prompted methods to measure its severity and effect on subsequent meat eating quality. Moisture of meat homogenates can be readily determined by laboratory NIR technology with R^2 greater than 0.7 using a first or second derivative prediction equation (Ripoll et al. 2008; Barlocco et al. 2006); however, the estimation of moisture or water-holding capacity in fresh meat using NIR is generally accepted to be poor with R^2 values ranging from 0.001 to 0.58 (Prieto et al. 2009). Chan et al. (2002) was able to estimate the moisture content and water-holding capacity of whole pork with R^2 values equal to 0.69 and 0.51, respectively.

Estimation of sensory tenderness and Warner-Bratzler shear force

Warner-Bratzler shear force has been used to estimate the toughness of meat since the 1930's as it is less expensive than sensory testing. Although used to replace sensory testing, the relationship between Warner-Bratzler shear force and sensory assessment of toughness is not an R^2 of 1, meaning that they are not perfectly related. As to why the relationship between the two measures is not perfect is unknown, although the machine may be more sensitive than sensory panelists. Raw colour has also been used to estimate beef eating quality with some success with the BeefCam System, a modular addition to the Computer Vision System, which is a video imaging analysis system (Vote et al. 2003; Wyle et al. 2003). The Computer Vision System has only been able to identify tough beef and has not been able to stratify beef by tenderness (Wyle et al. 2003), and Vote et al. (2003) found that up to 36% of carcasses that were certified tender were actually unacceptably tough. Goñi et al. (2007) found that a regression equation including chroma (C) and redness (a*) described 41% of the shear force variation of beef *m. longissimus dorsi* texture index, which was calculated from scores for juiciness, hardness, cohesiveness, and shear force. Wheeler et al. (2002) found that neither the BeefCam nor the colour of beef were as accurate as slice shear force, a method of estimating cooked beef toughness similar to Warner-Bratzler shear force, for estimating the toughness of beef. In fact, in the study of Wheeler et al. (2002), predicting tenderness using a colorimeter was more accurate than using the prototype BeefCam. This is not unexpected because colour has been shown to account for 15 to 23% of the variation in beef eating quality (Wulf and Page 2000).

The use of VIS-NIR may offer a more accurate estimate of sensory toughness than Warner-Bratzler shear force because the absorption spectra of VNIR is related to both the chemical composition of the meat, such as the fat content and myoglobin state, and the optical scattering, which is determined by the physical state of the proteins and the texture of the meat surface (Xia et al. 2007). Xia et al. (2007) found that the scattering coefficients were more strongly related to Warner-Bratzler shear force than the absorption coefficients, suggesting that meat structural properties were more influential than myoglobin chemical state at indicating beef toughness. The potential advantage of VIS-NIR was supported by the work of Yancey et al. (2010), who used VIS-NIR wavelengths to predict WBSF of cooked beef *m. longissimus thoracis.* Yancey et al. (2010) found that the 2nd derivative of the original absorbance data gave the best prediction equation, which had an r of 0.80 to 0.86 (R^2 = 0.64 to 0.74). In fact, these authors found that VIS-NIR predicted consumer overall impression and consumer perception of tenderness more closely than WBSF, implying that VIS-NIR may be the most efficacious measurement of the two because it may capture subtle variations in protein functionality and moisture (Yancey et al. 2010).

Rosenvold et al. (2009) also found that Warner-Bratzler shear force was less sensitive than VIS-NIR in predicting beef toughness. Rosenvold et al. (2009) obtained an R^2 of 0.58 between VIS-NIR (400 to 1700 nm) measurements and Warner-Bratzler shear force values of whole beef strip loin. These authors suggested that the low linear relationship was due to the Warner-Bratzler shear force measurements being less sensitive than the VIS-NIR reflectance (Rosenvold et al. 2009); however, one reason may be that the VIS-NIR measurement was made on the *m. longissimus lumborum* (LL) before the sample for pre-rigor shear force was removed from each LL. Removing a piece of muscle from pre-rigor muscle would have caused significant contracture due to calcium being released from the sarcoplasmic reticulum during cutting. This contraction would have changed the structure of the meat considerably from that on which the VIS-NIR spectral analysis was performed, causing the VIS-NIR to appear to underestimate the toughness. This hypothesis is supported by the results of Rosenvold et al. (1996), which showed that the VIS-NIR underestimated shear force value for the very tough beef, which is what pre-rigor beef would be. Andrés et al. (2008) obtained a similar R^2 of 0.65 between VIS-NIR spectra and Warner-Bratzler shear force, suggesting the sampling irregularity of Rosenvold et al. (2009) modified the linearity of the relationship only slightly. Xia et al. (2007) found a linear relationship between VIS-NIR and Warner-Bratzler shear force similar to that of Rosenvold et al. (2009) as well, finding an R^2 = 0.59 between the two measurements. Liu et al. (2003) found that they could achieve R^2 values between Warner-Bratzler shear force and VIS-NIR spectra (400 – 1100 nm) that ranged from 0.18 to 0.72 depending on the number of days post mortem, which in this instance were 2 and 14 days, respectively. To achieve this increase in relatedness, the data were corrected for multiplicative scatter and the mean centre and the second derivative was used.

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Ripoll et al. (2008) found positive linear relationships between Warner-Bratzler shear force of cooked whole beef strip loin (R^2 = 0.74), sensory tenderness ratings (R^2 = 0.98) and VIS-NIR reflectance (408 – 2492.8 nm) of a minced portion of the strip loin using Partial Least Squares analysis. Relationships between the VIS-NIR spectra of minced beef and the toughness of whole beef are usually poor, but the strong relationships observed in the Ripoll et al. (2008) study most likely indicative of an overwhelming influence of muscle fat content on the toughness of the strip loin muscles studied, and this was substantiated by a strong correlation between intramuscular fat and sensory toughness (Ripoll et al. 2008). Because of this, any estimation of tenderness may need an estimation of marbling to be included so that intramuscular fat content is considered in the prediction equation.

Price et al. (2008) found that VIS-NIR could be used to sort tough beef from tender. These researchers used the Field Spec Pro Jr. (Analytical Spectral Devices Inc., Boulder, Colorado), a unit that operates at wavelengths from 400 to 2500 nm. This unit has a fibre-optic contact probe for transmitting and receiving light to the surface of the meat (Price et al. 2008). The unit has 3 internal detectors: a 512 channel silicon photodiode array, a thermoelectrically-cooled indium gallium arsenide and a thermoelectrically-cooled extended indium gallium arsenide that measure at 350-1000, 1001-1670 and 1671-2500 nm respectively (Price et al. 2008). The light source is a 20 W halogen bulb with a diffuse reflection probe with 35 ° geometry and a measuring area of 1 mm^2 (Price et al. 2008). This unit was highlighted pictorially by Rust et al. (2008) and was able to correctly classify carcasses as tough and examination of a sub-set of the carcasses used in the Rust et al. (2008) study showed that the Field Spec Pro Jr correctly classified 92.9% of the known tough carcasses (Price et al. 2008).

Again, in studies where NIR is being investigated as a predictor of meat toughness, visible light is usually included in the range of spectra used. In fact, in these studies, the effectiveness of visible and NIR reflectance cannot be separated. Bowling et al. (2009), found that visible and NIR light were equally effective at predicting the toughness of whole beef strip loin, but were not additive, meaning that there was no advantage to combining reflectance from the two light regions together for prediction purposes. Visible light alone has been found to be effective at sorting strip loins on the basis of tenderness (Shackelford et al. 2005). In fact, the *BeefCam*, a module that links to the Computer Vision System (CVS), uses the Commission Internationale de l'Eclairage (CIE) *L**, *a** and *b** reflectance, which is within the visible light spectrum, to estimate beef toughness (Bowling et al. 2009).

NIR has also been correlated to beef eating quality measures without the influence of VIS light. Tender beef has been associated with reduced reflectance values in the 1150 to 1300 nm range and from 1400 to 2500 nm (Hildrum et al. 1994; Ripoll et al. 2008). Rust et al. (2008) however found that spectral values beyond 1500 nm were not relevant to beef toughness and formulated their prediction algorithm using spectra from 400 to 1500 nm. Additionally, correlation of NIR spectra from 833 to 2500 nm to Warner-Bratzler shear force was low (R^2_{cv} = 0.12 to 0.25) in the study by Leroy et al (2003), who had a sample size of 184 with a mean shear force of 40.7 $\pm$ 9.5 N on day 8 post mortem, indicating that this NIR region may not be influential in separating carcasses on tenderness that are relatively uniform. Naganathan et al. (2008) also found that spectra from wavelengths greater than 1400 nm were not related to beef toughness but that those from 1000 to 1400 were, with key wavelengths identified at 1074, 1091, 1142, 1176, 1219, 1365, 1395, 1408, and 1462 nm. These authors implicated interference from water as negating the meaningfulness of the wavelengths greater than 1400 nm as that is the region in which water absorbs the most light (Barlocco et al. 2006).

These authors also concluded from supporting literature that light absorption at 1219 nm was due to fat, at 1365 and 1395 nm were due to protein and at 1408 and 1462 nm were due to water. Rødbotten et al. (2000) found correlation (*r*) values between NIR (1100 to 2500 nm) was not strongly correlated to Warner-Bratzler shear force or sensory assessment of beef toughness, achieving *r* values of up to 0.68 for shear force and between 0.34 and 0.51 for sensory. Park et al. (1998) examined NIR from 1100 to 2498 nm and obtained an R^2 value of 0.67 between specific NIR wavelengths and Warner-Bratzler shear force and could predict Warner-Bratzler shear force with an accuracy of 89%. The key wavelengths in the Park et al. (1998) study were 1854, 1688, 1592 and 2140 nm, suggesting that key indicators of beef tenderness may exist at wavelengths greater than 1400 nm.

Byrne et al. (1998) found very strong correlations between NIR spectra from 750 to 1098 nm and 14 day aged Warner-Bratzler shear force, realizing *r* values of 0.83 and 0.89 and 0.45 and 0.67 for 24 and 48 h post mortem readings, respectively. Byrne et al. (1998) had less success predicting sensory tenderness than Warner-Bratzler shear force, observing *r* values of 0.48 and 0.49 and 0.71 and 0.80 for NIR readings made 24 and 48 h post mortem, respectively. These results suggest that the spectra between 750 and 1098 nm may be most indicative of myofibrillar toughness, but not representative of other factors such as juiciness, which can influence the perception of tenderness by a sensory panel.

Because of the strong correlation between marbling, subcutaneous fat and beef tenderness, carcasses with a certain amount of marbling may be able to be guaranteed tender (Ripoll et al. 2008). Yancey et al. (2010) found that cooked *m. longissimus thoracis* was liked the least when the muscle was from carcasses that graded Select than from those graded Low Choice, Top Choice and Prime. Estimation of beef tenderness using light spectroscopy may be important then for adding value to beef from carcasses with decreased marbling (Rust et al. 2008; Shackelford et al. 2005). This ability may become increasingly important as consumer market preferences move from highly marbled to lean beef and recent research has indicated that sorting lean carcasses according to tenderness may be possible using VNIR (Shackelford et al. 2005) .

The relationship between NIR and visible NIR (VNIR) measurements performed early post mortem coincident with grading and the toughness of the meat after ageing is not well understood. Byrne et al. (1998) found that NIR spectra could be used to predict the overall acceptability of beef tenderness whether measured at 24 or 42 hours or 7 or 14 days post mortem. Whether an early measurement is indicative of a quality after ageing or not has yet to be determined and warrants investigation (Park et al. 1998).

The predictability of WBSF by VIS-NIR is limited by the size of the dataset from which the predictive algorithm is drawn and by the amount of variability within the data set, as predictive accuracy increases as data variability increases (Prieto et al. 2009). Also, in most cases, reflectance (R) values are transformed into absorbance values using log (1/R) in order to linearize the relationship between the absorbing substance and the absorption spectrum (Hruschka 2001).

Commercialized Products

ASD Inc., Colorado, a company that specializes in light technology, has commercialized a visible-NIR system (Field Spec Pro Jr, 400 to 2500 nm) that identifies US Select beef carcasses that will produce a tender *longissimus* muscles (Rust et al. 2008). This technology was based upon the research results of Shackelford et al. (2005) and consists of a computer backpack worn by a worker with a laptop strapped in front of the worker. Reflectance of beef rib eye is then measured using a hand-held fibre-optic probe connected to the computer backpack unit. Although an effective unit, it appears unwieldy, heavy and supplying adequate power to the unit in an abattoir environment could be a challenge. Also, the fibre-optic probe must contact the meat surface in order to accurately assess the VNIR spectra, a distinct disadvantage in Canadian federal plants, for which such contact represents potential bacterial contamination.

Another technology, the "Goldfinch", is currently in the final stages of commercialization and it has been successful at guaranteeing that beef rib sections are tender but cannot as yet differentiate the toughness within the tender product category. This technology draws from the results of Naganathan et al. (2008) and Cluff et al. (2008). The "Goldfinch" is a large, rifle-like apparatus that covers the rib eye face and uses NIR hyperspectral images captured between 965 and 1625 nm with a diffuse-flood lighting system. Using this technology, the accuracy of prediction was demonstrated to be 77% using an early prototype (Naganathan et al. 2008). Hyperspectral imaging is a combination of video imaging and light spectroscopy as it provides both spatial and spectral resolution (Naganathan et al. 2008). In this way, a reflectance spectrum can be attached to each pixel of the image and then related to the physical characteristics of the object from which the image was taken. At publication, this technology was using InGaAs sensors, which are not as well developed as CCD sensors that are commonly used in other VNIR systems and they produce 'dead pixels' that require removal prior to analysis of the image (Naganathan et al. 2008). Beef hyperspectral data is then discriminated statistically using canonical methods (Naganathan et al. 2008). This system uses data from key wavelengths identified in Naganathan et al. (2008) that have been correlated to beef quality to identify tough beef in order to guarantee tender product.

The system used by Cluff et al. (2008) was slightly different and incorporated a VIS-NIR light source that was a 250 W quartz tungsten halogen lamp (Oriel Instruments, Stratford, Connecticut). Light output was stabilized with a light intensity controller. The light was focused with a fiber optic cable (600 ìm internal diameter), which produced a beam of light that had a diameter of 1.6 mm. Hyperspectral images were captured using a high performance CCD camera (model C4880-21-24A, Hamamatsu Photonics System, Bridgewater, New Jersey). The camera was cooled to -40 °C to improve signal to noise ratio. The camera produced images that were 256 x 256 pixels with a spectral resolution of 4.54 nm within the 410 to 1086 nm range. Only a narrow 30 x 0.2 mm beam of the meat surface was imaged and so 10 images were acquired every 1 mm over a 10 mm length. Cluff et al. (2008) identified seven wavelengths that were regressed into a prediction equation using stepwise regression techniques: 501, 510, 646, 651, 927, 1005 and 1023. At wavelengths 501 and 510, the full scattering width at half maximal was important whereas at the remaining wavelengths, the slope at the full scattering width at half maximal was important, indicating that the optical scattering of the light was the most important feature for predicting beef toughness. Using this algorithm, these researchers were able to predict Warner-Bratzler shear force values with an R of 0.67.

Future Work

Future iterations of VNIR technology should use polarization, as that enables protein structural differences to be measured such as sarcomere length (Luc et al. 2008), which could further enhance the accuracy of a predictive algorithm. There is also the potential of predicting collagen solubility in whole muscle, which would be of value in separating beef toughened due to animal age (Mitsumoto et al. 1991; Young et al. 1996) and could supplant age estimation using ossification. Regression involving spectral data from 1072 and 1082 nm was shown by Young et al. (1996) to be related to collagen solubility in lamb (r = 0.91) and could potentially be applicable to beef. NIR may also be useful for predicting total collagen content of beef as well, because Mitsumoto et al. (1991) found a correlation between collagen content and NIR reflectance optical density of 0.74, a relationship that was not found by Young et al. (1996) in lamb. The sex of animal studied should also be tightly controlled as sorting data for animal sex as improved correlations between NIR and sensory toughness of beef (Hildrum et al. 1994). Also, post mortem management of beef studied also needs to be controlled as freezing of beef has been found to improve the correlation between NIR and sensory toughness of beef, as freezing may enhance the difference between tough and tender beef (Hildrum et al. 1994).

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