DISCRIMINATION OF BEEF DARK CUTTERS USING VISIBLE AND NEAR INFRARED SPECTROSCOPY

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Abstract – This study examined the potential of visible and near infrared reflectance spectroscopy (VIS-NIRS) to segregate dark cutters from normal beef. One hundred and twenty beef carcass sides were selected from a slaughter plant by experienced graders according to their carcass grade: 60 A grade carcasses (normal) and 60 B4 grade carcasses (dark cutters). At approximately 48 h post mortem, a 2.5-cm thick steak (at ~7/8th thoracic vertebrae) was removed, vacuum packaged and frozen at -25°C until spectra collection. After thawing overnight at 2°C, VIS-NIR spectra were collected using a portable LabSpec®4 spectrometer (350-2500 nm) on intact steaks at the laboratory prior to oxygenation (non-bloomed samples) and following 20 min of exposure to atmospheric oxygen (bloomed samples). Partial least squares discriminant analysis correctly classified 95% of the nonbloomed beef samples from both A and B4 grade carcasses, and 88% of the bloomed samples from both grading categories. Further work remains to be carried out to develop robust VIS-NIRS models to be implemented on-line in the abattoir, where portable equipment applied directly on the carcass could objectively assist in dark-cutting carcass segregation.

Introduction

Dark-cutting beef colour is an important and well researched meat quality issue caused by metabolic processes. Dark cutters, often referred to as dark, firm and dry (DFD) meat, theoretically all belong to a group of cattle that have experienced prolonged stress prior to slaughter induced by numerous factors (Murray 1989), such as fluctuations or extreme weather conditions, management prior to slaughter, fighting, mounting to re-establish social hierarchy and use of aggressive implantation. Under those stress conditions, glycogen stores are depleted prior to slaughter, reducing the available post mortem glycogen in muscle that prevents normal post mortem glycolysis and limits pH decline (Lawrie and Ledward 2006). As a consequence, dark cutters result in an abnormally high post mortem pH (≥ 6.0), a glycolytic potential of less than 100 µmol of glycogen/g of muscle (Wulf et al. 2002), a greater water holding capacity and a characteristic and visually unappealing dark red to black colour that is discriminated against by the retail trade and consumers (MacDougall and Rhodes 1972). In addition, dark cutters are more susceptible to bacterial spoilage (Newton and Gill 1981), show a reduced beef flavour (Dransfield 1981) and often seem more tender (Viljoen et al. 2002).

In Canada, dark cutters (carcasses graded as B4) are distinguished at the time of grading by the excessively dark colour of the rib-eye using a visual colour chit developed by the Canadian Beef Grading Agency (CBGA 2010). Dark-cutting carcasses, which would otherwise qualify for the Canada A grade series, are heavily discounted. Although the percentage of youthful graded carcasses across Canada that grade Canada B4 is relatively low (1.2% or 26,815 carcasses in 2012, CanFax 2013) the impact is anything but negligible; the lost retail value from dark cutters is estimated to cost in excess of $8.5M (based on a $0.40 per lb discount on an 820 lb heifer carcasss; CanFax Research Services 2012).

Various researchers have defined dark-cutting beef as having ultimate pH in excess of 5.8-6.2 measured at 24 or 48 h post mortem (Murray 1989; Page et al. 2001). Given the known range in pH, this parameter could be used as a further sorting tool for dark cutter carcasses. However, the increased concerns regarding hazard analysis and critical control points (glass electrodes, penetrating musculature, appropriate cleaning between muscles) and operational difficulties in operating a pH meter continuously in a cooler environment (slow to calibrate and read, space restrictions) limit this option. Hence, a reliable and operationally practical method that objectively assists in discriminating dark cutters from normal beef is needed.

Near infrared spectroscopy (NIRS) is a sensitive, fast, and non-destructive technology, with minimum or no sample preparation, neither requiring reagents nor producing waste, which provides information about the molecular bonds of organic compounds and tissue ultra-structure in a scanned sample (Downey and Hildrum, 2004). NIRS has been successfully used for quantitative estimation of major chemical constituents in meat and also for classification purposes (Prieto et al. 2009). However, to the best of our knowledge, there are no studies testing this technology to discriminate dark cutters from normal beef. Therefore, the aim of the present study was to examine the potential of visible (VIS) and NIR spectroscopy to objectively assist in segregating dark-cutting from normal carcasses.

Materials and Methods

A. Sample collection

Over three collection weeks, 120 left beef carcass sides (n = 24, 48 and 48 carcasses per week, respectively) were selected from a commercial slaughter plant in Alberta, Canada, by experienced graders. The carcasses selected each week were balanced by carcass grade: in total 60 A grade carcasses (normal: Canada AAA, Canada AA and Canada A) and 60 B4 grade carcasses (dark cutters), using a visual colour chit developed by the Canadian Beef Grading Agency (CBGA, 2010) and applied by certified beef graders. At 48 h post mortem, rib-eyes were removed from the carcass, tagged, vacuum packaged in polyethylene bags and transported under refrigerated conditions to the Lacombe Research Centre, Agriculture and Agri-Food Canada (Lacombe, Alberta, Canada), where they were held overnight at 2°C. Then, rib-eyes were removed from packaging, labeled, and denuded. A 2.5-cm thick steak (approximately at the 7-8th thoracic vertebrae, ~23 cm anterior from the grade site) was removed from the 120 rib-eyes, labeled, vacuum packaged and frozen at -25°C until VIS-NIR spectra collection.

C. VIS-NIR spectra collection

The steaks were randomly thawed overnight at 2°C balanced by their carcass grade, to allow NIR spectra collection during four consecutive days. A portable LabSpec®4 Standard-Res spectrometer (Analytical Spectral Device-ASD Inc., Boulder, CO, USA) equipped with an ASD fibre-optic high intensity contact probe (21 mm window diameter) was used to scan intact steaks at the laboratory prior to oxygenation (non-bloomed samples) and following 20 min of exposure to atmospheric oxygen (bloomed samples) (Figure 1). The spectrometer scanned 50 times per reading (~5 s) over the VIS-NIR range (350-2500 nm) in reflectance mode, and spectra were averaged by the equipment software. The data were interpolated to produce measurements in 1 nm steps, resulting in a diffuse reflectance spectrum of 2151 data points. Absorbance data were stored as log (1/*R*), where *R* was the energy reflected. Nine spectra per steak were collected to increase the area of muscle scanned and reduce the sampling error (Downey and Hildrum 2004), and then averaged. Instrument control and initial spectral manipulation were performed with the Indico™ Pro software package (Analytical Spectral Device-ASD Inc., Boulder, CO, USA).

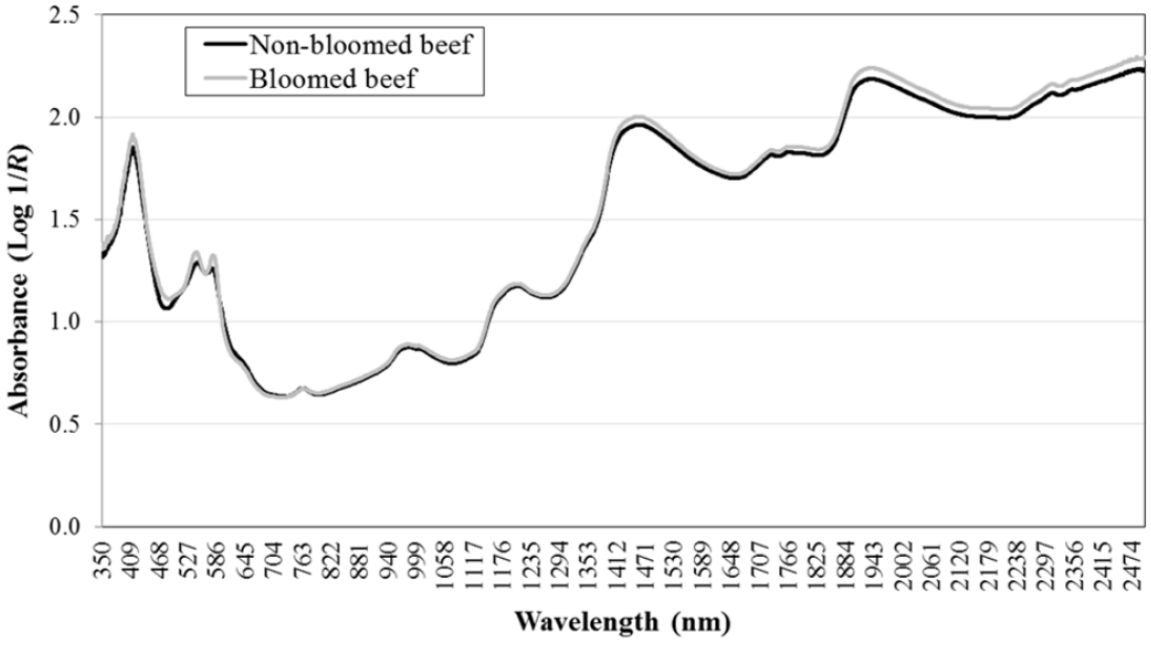

Figure 1. Average VIS-NIR spectra (n = 120) of non-bloomed and bloomed beef samples.

E. Statistical analysis

Principal component analysis (PCA) was performed to decompose and compress the data matrix. Partial least squares discriminant analysis (PLS2-DA, Naes et al. 2002) was applied to classify samples into each of the carcass grades studied (A and B4). This model seeks to correlate spectral variations (X) with defined classes (Y), attempting to maximize the covariance between the two types of variables for group differences and ignoring variance within a class. In this type of approach, Y is a dummy matrix with arbitrary numbers assigned to the different classes to be distinguished (A grade carcasses = 1, B4 grade carcasses = 2). According to this equation, a sample was classified as meat belonging to a specific category (A or B4) if the predicted value was within ±0.5 of the dummy value. The accuracy of the models obtained was evaluated using the percentage of correctly classified samples. Cross-validation (leave one-out) was performed to validate calibrations and to restrict the number of PLS terms incorporated in the regression, to prevent over-fitting. Spectral data management and PLS2-DA were performed by means of The Unscrambler® software (version 10.2, Camo, Trondheim, Norway).

Results and Discussion

When the VIS-NIR spectra were collected on non-bloomed beef samples, the regression model developed using a PLS2-DA and including 4 PLS terms correctly classified 95% of the beef samples from both A and B4 grade carcasses (Table 1). Similar results were observed when the calibration model was cross-validated, where only 5% of the beef samples from both carcass grades were misclassified. Regarding the spectra collection on bloomed samples, the discrimination model including 3 PLS terms showed a decrease of 7 and 2% in the number of correctly-classified beef samples in the calibration set from both A and B4 grade carcasses, respectively, compared to that observed for non-bloomed samples. With regard to the validation, 12% of misclassified beef samples from both grading categories were found.

Table 1. Carcass grade discrimination results based on VIS-NIR spectra collected on non-bloomed and bloomed beef samples.

			Classified (%)			
			Calibration		Cross-Validation	
Analysis mode	PLS terms	Carcass grade	A	B4	A	B4
Non-bloomed	4	A	**94.9**	5.1	**94.9**	5.1
		B4	5.1	**94.9**	5.1	**94.9**
Bloomed	3	A	**88.1**	11.9	**88.1**	11.9
		B4	6.8	**93.2**	11.9	**88.1**

PLS terms: partial least square terms.

Because the LabSpec®4 instrument is provided with the VIS region, changes in the colour of the samples during blooming could have been reflected in the collected spectra. Indeed, in Figure 1, a small but interesting amount of variability amongst spectral absorption from non-bloomed and bloomed samples was detected in the regions at 548 and 580 nm, which could be explained by different redox states of myoglobin (Cozzolino et al. 2000). Nevertheless, the lower accuracy found in the discrimination of the bloomed samples might suggest that the colour changes, due to the exposure to atmospheric oxygen, did not occur at the same rate for all the samples within each carcass grade, hence making segregation of dark cutters from normal beef on bloomed samples more difficult.

Since the musculature from dark cutters is often referred to as DFD (dark, firm and dry), the successful VIS-NIRS performance in the discrimination of dark cutters from normal beef could be due to the information related to the colour, provided by the VIS region, and the structure of the muscle (i.e., the fibre arrangement of the muscle) and water content, obtained from the NIR region. Indeed, in Figure 2, differences between average spectra of non-bloomed samples from A and B4 grade carcasses were observed in both VIS and NIR regions due to the redox states of myoglobin (548, 580 and 762 nm; Cozzolino et al. 2000) and the absorption of O-H bonds of water (890, 970, 1450 and 1940 nm; Shenk et al. 1992), respectively.

Additionally, dark cutters are assumed to have a glycolytic potential of less than 100 µmol of glycogen/g of muscle (Wulf et al. 2002). Because glycogen is a multi-branched polysaccharide of glucose, the molecular bonds of this organic compound absorb energy in the NIR region. Hence, the different content of glycogen could be another reason for NIRS to successfully segregate dark cutters.

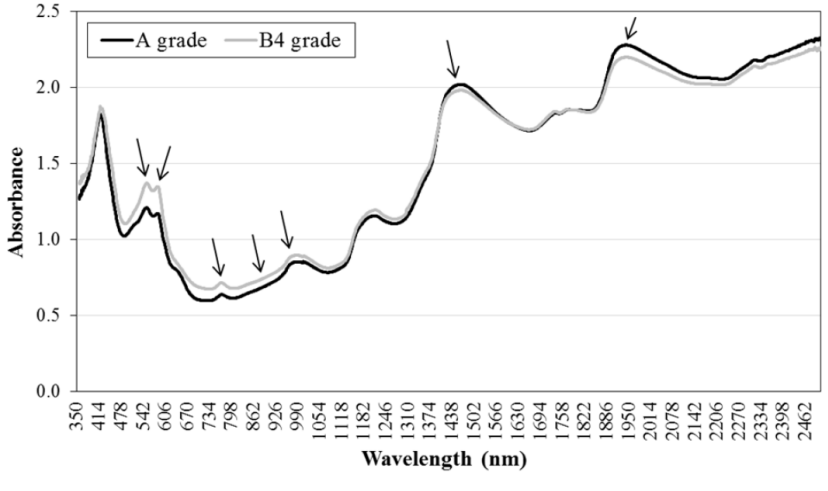

Figure 2. Average VIS-NIR spectra of non-bloomed samples from A grade (n = 60) and B4 grade (n = 60) carcasses.

Conclusion

VIS-NIRS technology has the potential to objectively assist in segregating dark cutters (B4 grade carcasses) from normal beef (A grade carcasses). Partial least squares discriminant analysis based on VIS-NIR spectra correctly classified 95% of the non-bloomed beef samples from both A and B4 grade carcasses. The portable LabSpec®4 could offer advantages over the at-line high-resolution monochromators, chiefly its ease of use and portability. Nevertheless, this device needs to be further tested for on-line applications in the abattoir, where portable equipment applied directly on the carcass may objectively assist in segregating dark-cutting carcasses for marketing purposes.

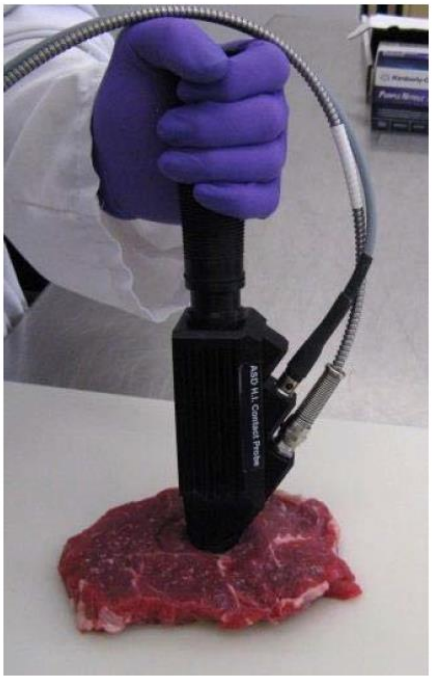

Figure 3. Collection of VIS-NIR spectra on intact beef samples using a portable LabSpec®4 spectrometer equipped with a fibre-optic high intensity contact probe.

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