BEEF DARK CUTTERS DISCRIMINATION BY 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 left beef carcass sides were selected from a commercial slaughter plant by experienced graders according to their carcass grade: 60 normal and 60 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 on intact steaks prior to oxygenation (non-bloomed samples) and following 20 min of exposure to atmospheric oxygen (bloomed samples), using a portable LabSpec®4 spectrometer (350-2500 nm) at the laboratory. Partial least squares discriminant analysis correctly classified 95% of the non-bloomed beef samples from both normal and dark cutter carcasses, and 88 and 93% of the bloomed samples from normal and dark cutter carcasses, respectively. 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.

I. 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 [1], 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 [2]. 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 [3], 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 [4]. In addition, dark cutters are more susceptible to bacterial spoilage [5], show a reduced beef flavour [6] and often seem more tender [7].

In Canada, dark cutters 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 [8], and are heavily discounted.

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 [1,9]. 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 [10]. NIRS has been successfully used for quantitative estimation of major chemical constituents in meat and also for classification purposes [11]. 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.

II. 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 (60 normal and 60 dark cutters in total) using a visual colour chit developed by the Canadian Beef Grading Agency [8] 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.

B. 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 [10], 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).

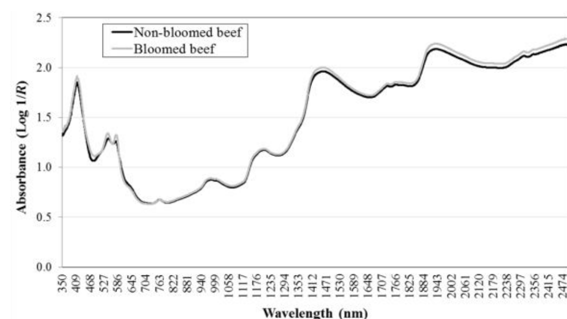

Fig. 1. Average Vis-NIR spectra (n = 120) of non-bloomed and bloomed beef samples

C. Statistical analysis

Principal component analysis (PCA) was performed to decompose and compress the data matrix. Partial least squares discriminant analysis (PLS2-DA, [12]) was applied to classify samples into each of the carcass grades studied (normal and dark cutter). 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 (normal = 1, dark cutter = 2). According to this equation, a sample was classified as meat belonging to a specific category (normal or dark cutter) 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).

III. 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 normal and dark cutter 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 normal and dark cutter 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 Discrimination results based on spectra collected on non-bloomed and bloomed beef samples

			Classified (%)			
			Calibration		Cross-Validation	
Analysis mode	PLS terms	Carcass grade	Normal	Dark cutter	Normal	Dark cutter
Non-bloomed	4	Normal	**94.9**	5.1	**94.9**	5.1
		Dark cutter	5.1	**94.9**	5.1	**94.9**
Bloomed	3	Normal	**88.1**	11.9	**88.1**	11.9
		Dark cutter	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 [13]. 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 normal and dark cutters were observed due to the redox states of myoglobin (548, 580 and 762 nm; [13]) in the Vis region, and the absorption of O-H bonds of water (890, 970, 1450 and 1940 nm) and N-H bonds of protein (2180, 2300, 2352 and 2470 nm) [14] in the NIR region.

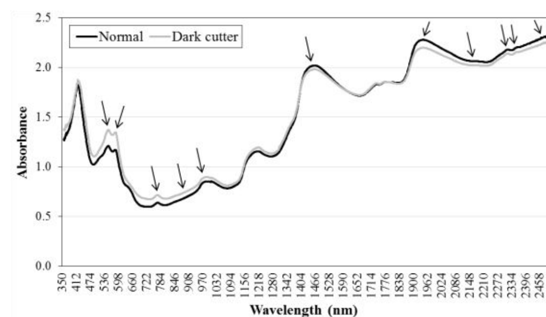

Fig. 2. Average Vis-NIR spectra of non-bloomed normal (n = 60) and dark cutter (n = 60) samples

Additionally, dark cutters are assumed to have a glycolytic potential of less than 100 μmol of glycogen/g of muscle [3]. 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.

IV. CONCLUSION

Vis-NIRS technology has the potential to objectively assist in segregating dark cutters from normal beef. Partial least squares discriminant analysis based on Vis-NIR spectra correctly classified 95% of the non-bloomed beef samples from both normal and dark cutter 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.

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