DETERMINATION OF THE MYOGLOBIN STATES IN BEEF USING REFLECTANCE SPECTRA AND MULTIVARIATE REGRESSION

T. Isaksson[1], M. Khatri[1], M. Bjelanovic[1], O. Sørheim[2], E. Slinde[1,3] and B. Egelandsdal[1]

[1]Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Ås, Norway; [2]Nofima, P.O. Box 210 , N-1431 Ås, Norway; [3]Institute of Marine Research P.O. Box 1870, Nordnes, N-5817 Bergen, Norway.

Abstract – A new, fast reflectance spectroscopic method for determination of myoglobin states is presented. Beef steak and ground beef samples were chemically treated to achieve one of three desired myoglobin states; deoxymyoglobin (DMb), oxymyoglobin (OMb) and metmyoglobin (MMb). Reflectance spectra were measured and multivariate regressions were performed. Validation of the models gave after correction and normalization prediction errors of about 4 % for whole steaks and 5 % for ground beef. The protocol of the American Meat Science Association from 1991 resulted in prediction errors of myoglobin states of about 8 – 18 % for whole steaks. It is concluded that the present new method performed well for determinations of OMb, DMb and MMb states, both in whole steaks and in ground beef samples.

Keywords – Myoglobin states, oxymyoglobin, deoxymyoglobin, metmyoglobin, spectroscopy, multivariate, partial least square regression

I. INTRODUCTION

It is important to have fast and accurate methods to determine color and the myoglobin states deoxymyoglobin (DMb), oxymyoglobin (OMb) and metmyoglobin (MMb) in beef and products of beef. Several spectroscopic methods are suggested and discussed in the literature. The benchmark method was published in 1991 "American Meat Science Association (AMSA) guidelines for meat color evaluation"[1]. The present paper suggests a new spectroscopic method and an alternative treatment of the samples. We also introduce the use of the multivariate regression method: partial least square regression (PLSR) [2].

II. MATERIALS AND METHODS

Samples: Two (*M. semimembranosus*) sets of calibration samples were studied. One set of 8 fresh, 3 days *post mortem*, whole beef muscles was analyzed [3]. Each muscle was cut parallel to the fiber direction in 15 mm slices into four steaks providing in total 32 steak samples. The second sample set consisted of ground beef from four different animals [4]. These samples were added about 10 w/w-% of water to a mince of beef and pork adipose tissue (giving 14% w/w of fat). This ground mixed meat sample set gave in total 78 different samples. In addition, 927 spectra from ground beef with unknown myoglobin state were predicted, here called additional ground beef samples. To ensure biological variation and variation in the myoglobin contents, animals with different age (1.5 – 5 years old) were used.

Preparation of myoglobin states: Both whole beef samples and ground beef samples were forced by chemical treatments to be in the specific OMb, DMb and MMb states. OMb: Each sample was placed in trays with 75 % O_2 and 25 % CO_2, at 4 ^{0}C, for minimum 24 h prior to spectroscopic measurements. DMb: Each sample was vacuum packed and sealed with no access to O_2 at 4 ^{0}C for minimum 48 h. MMb: Each steak sample was flushed with about 60 %CO_2/ 40 % N_2 added and adjusted to 1.5 % O_2 for minimum 7 days. Each ground beef sample was impregnated with 1.0 % $K_3[Fe(CN)_6]$ solution, at 4 ^{0}C, for 1 minute, and swabbed for excess solution at 4 °C for 12 hours prior to measurements.

Spectroscopy: A grating instrument (Foss NIRSystems, Model 6500, Hillerød, Denmark) was equipped with an interactance fiber optic probe (NR-6770-A, Foss NIRSystems) and the instrument software (Vision 2001, NIRSystems). The probe head consists of seven about 1.0x20.0 [mm^2] rectangular parallel glass windows, about 2.0 [mm] apart, mounted in the middle of a 40.0x40.0 [mm^2] metal block (Figure 1). Every second (nr. 2, 4 and 6) window emits

monochrome light into the sample and every other second (nr. 1, 3, 5 and 7) window collects the reflected light from the sample to the detector. This construction forces the emitted light to penetrate several [mm] into a meat sample. The instrument have an average spectral bandwidth of about 8.5 [nm = 10^{-9} m] from the monochromator. The instrument with the probe was tested (performance test and wavelengths linearization) as recommended by the manufacturer and passed all these tests. It was scanned at every second wavelengths in the range 400 – 1098 [nm], giving 350 spectral variables. Thirty two scans for each single measurement were averaged. For whole steaks, three such single measurements were performed on randomly different locations on each sample, resulting in 96 spectra for whole steaks and 72 spectra for the ground beef. The total measurement time was about 120 seconds. The samples at room temperature was placed in a high density polyethylene cup (Dyno 516, SWF Companies, Redley, USA) and covered with one sheet of low density polyethylene film (Toppits-glad, Melitta Group, Minden, Germany). The probe was placed by its own gravity on the covered sample for the whole steak samples. For the ground beef samples the samples were turned upside down (to transparent film side) and placed on the probe. A reference spectrum using a ceramic white tile (L* = 101.01, a* = 1.74 and b* = 5.3) was measured every day before the measurements. The data collection software delivered the spectroscopy data in absorbance (*A*) units *vs*. wavelengths [nm]. Extended Multiplicative Signal Correction (EMSC) [5], using second order polynomial of the wavelengths and no other external reference data was done on all spectra. The samples were measured in replicates such that it resulted in 96 spectra for the whole steak sample set and 72 spectra for the ground beef sample set.

Multivariate regression: Partial least square regression (PLSR) is probably the most used method for multivariate calibration in near infrared applications. This type of multivariate regression can be popularly described in four steps; 1) Spectra (with *K* variables) and a reference method, here making samples with specific myoglobin states (OMb, DMb and MMb) by the method described above, are measured for a number of samples (*I*) and are represented as two matrices, $\mathbf{X}_{IxK}$ (spectra) and $\mathbf{y}_{Ix1}$(states), respectively. This can be referred to as the calibration set of samples. The reference values were set to 0 for samples not in the focused myoglobin state and 1 for the myoglobin state that was prepared. 2) A calibration model is estimated. For PLSR this is done by decomposing the centered **X** matrix into a score matrix ($\mathbf{T}_{IxA}$, were *A* is the number of factors), a loading weight matrix ($\mathbf{W}_{KxA}$) and a residual matrix ($\mathbf{E}_{IxK}$), formulated as $\mathbf{X}_c = \mathbf{TW}^t + \mathbf{E}$. Followed by estimation of the regression coefficient vector $\mathbf{q}_{Ax1}$ for **T** as $\mathbf{q} = (\mathbf{T}^t\mathbf{T})^{-1}\mathbf{T}^t\mathbf{y}$ and the regression coefficient vector $\mathbf{b}_{Kx1}$ for **X** as $\mathbf{b} = \mathbf{W}(\mathbf{P}^t\mathbf{W})^{-1}\mathbf{q}$, for the regression model $\mathbf{y} = \mathbf{Tq} + \mathbf{e}$ and $\mathbf{y} = \mathbf{Xb} + \mathbf{e}$, where $\mathbf{e}_{Ix1}$ is the residual vector, respectively. Each myoglobin state is modeled separately, and gives consequently here three models, one for each myoglobin state. 3) These models were validated, by leave-one-out cross validation, giving the quality of the models, the accuracy of the models, here expressed as root mean square error of cross validation, or validation prediction error, formulated as $RMSECV = \left(I^{-1}\sum_{i=1}^{I}(\hat{y}_i - y_i)^2 \right)^{1/2}$, where $\hat{y}_i$ is the predicted myoglobin states and y_i is the reference values for each myoglobin state; i.e. the pure states (1) that were prepared. The lower the *RMSECV* value is, the better is the quality of the model. The *RMSECV* values can be used to define a prediction confidence interval. Assuming, normal distribution and no bias, if one predict a sample to $\hat{y}_n$, one can expect with about 95% confidence that y_n will be within the interval [$\hat{y}_n \pm 2RMSECV$]**.** The linear correlation coefficient (*R*), between the predicted myoglobin states and the reference values for each myoglobin state is also calculated. The validation step also identifies the number of PLSR factors (*A*). 4) After the best model is chosen and validated, any number of new samples ($\mathbf{x}_{i(1xK)}$) can be predicted as $\hat{y}_i = \mathbf{xb}$, for each of the myoglobin states. Both in step 3) and 4) the predictions were first corrected ($\hat{y}_i >$ 1.00 were set to 1.00 and $\hat{y}_i <$ 0.00 were set to

0.00) and then normalized ($\hat{y}_{i,OMb} + \hat{y}_{i,DMb} + \hat{y}_{i,MMb} = 1.00$).

The regressions were done using the Unscrambler (Ver. 9.2, Camo ASA, Oslo, Norway) and the corrections were done using Excel (Microsoft Office 2007, Seattle, WA, USA).

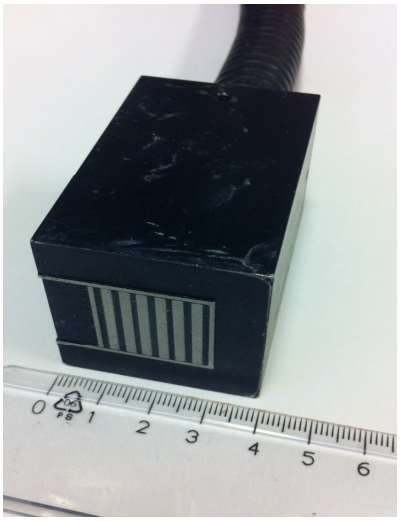

Figure 1. A photography of the fiber optical probe connected to the grating instrument, used in this study.

III. RESULTS AND DISCUSSION

Table 1. The prediction performances of the cross validated corrected and normalized predictions of DMb, OMb and MMb states using EMSC(*A*) and PLSR for whole steak and ground beef sample set.

Sample set	Myoglo-bin state	RMSECV [fraction (# PLSR factors)]	R
Whole steaks	DMb	0.042 (2)	0.997
	OMb	0.041 (3)	0.997
	MMb	0.039 (3)	0.997
Ground beef	DMb	0.051 (2)	0.996
	OMb	0.055 (3)	0.995
	MMb	0.045 (5)	0.997

For the ground beef 8 spectra from the longer stored minces were taken out as outliers, resulting in 64 spectra. The prediction performances from the cross validations of the two datasets are presented in Table 1. The prediction errors were about 4 % for whole steaks and about 5 % for the ground beef, respectively. The correlation coefficients between the predicted and reference values were high, $R > 0.997$ for whole steaks and $R > 0.995$ for ground beef, respectively.

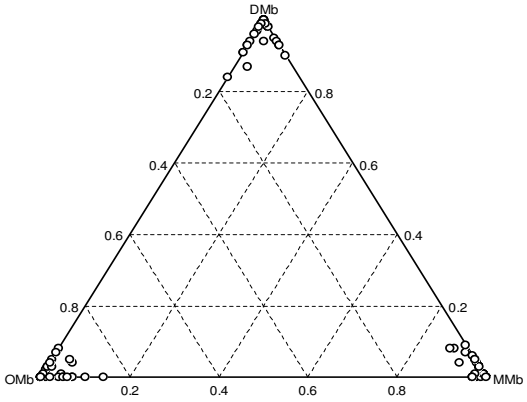

Figure 2. The cross validated corrected and normalized predictions of DMb, OMb and MMb states using EMSC(*A*) and PLSR for the whole steak sample set ($I = 96$). See Table 1 for statistical details.

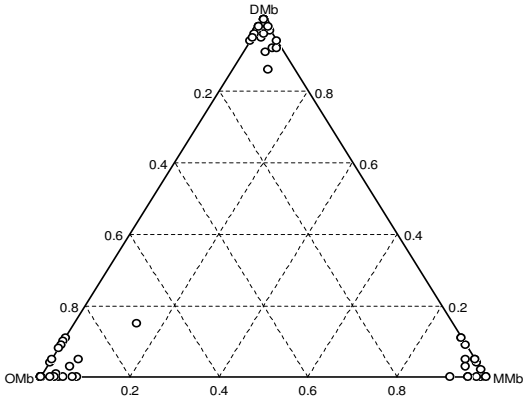

Figure 3. The cross validated corrected and normalized predictions of DMb, OMb and MMb states using EMSC(*A*) and PLSR for the ground beef sample set ($I = 64$). See Table 1 for statistical details.

The prediction performances are also illustrated in Figure 2 and 3 for whole steak and ground beef, respectively. If the predictions had been without any errors, meaning that the prepared myoglobin states were 100 % pure, and the

instrument and the modeling were perfect, one would expect all samples to be perfect in the corners of the triangles. Real world is never without errors, so the predicted samples clustered close to the corners.

In a previous study [3] we compared the present method with the AMSA method [1]. The AMSA method then resulted in prediction errors of *RMSECV* = 0.18 (*R* = 0.960) for DMb, *RMSECV* = 0.16 (*R* = 0.947) for OMb and *RMSECV* = 0.079 (*R* = 0.993) for MMb, respectively. To compare, the results are illustrated in Figure 4.

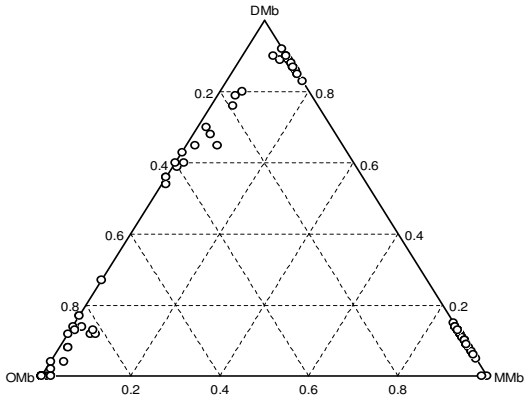

Figure 4. The corrected and normalized calculations of DMb, OMb and MMb states using the AMSA method for the whole steak sample set (*I* = 96).

The present, new method outperformed the results from the AMSA method (compare Figure 2 and 4).

In our study we also had 927 spectra from 156 ground beef samples, with unknown myoglobin states. Without knowing the true values for the myoglobin states, it is of course not possible to calculate any prediction errors or correlation coefficients of the prediction performance. However, the prediction results can be illustrated as in Figure 5.

IV. CONCLUSION

The present method gave good prediction performances, from cross validations for all three myoglobin states. Prediction of myoglobin states of whole steaks gave slightly better results compared to measurements on ground beef. In a previous comparison the present new method gave better prediction performances compared to the AMSA method from 1991.

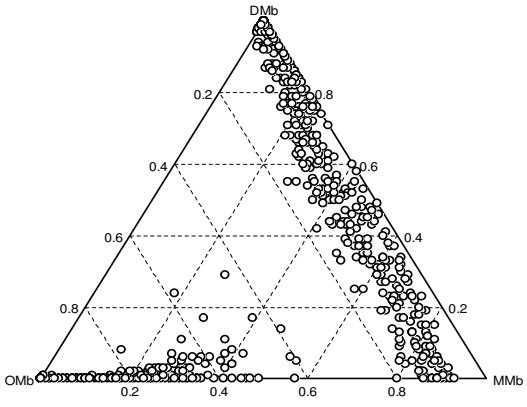

Figure 5. Corrected and normalized predictions of DMb, OMb and MMb states using EMSC(*A*) and PLSR for the additional ground beef samples set (*I* = 927).

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