The stability of carboxymyoglobin in presence of oxygen in meat

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Abstract – This paper establishes a method to differentiate between carboxymyoglobin (COMb) and oxymyoglobin (OMb) in meat, based on their reflectance in the visible spectral range. The different ratio of the two reflectance minima between 540 and 582 nm was found to be suitable to estimate relative proportions of COMb and OMb. In addition, results indicate that COMb is rapidly converted into OMb on the meat surface in presence of oxygen.

Key Words – carbon monoxide, meat, myoglobin, reflectance spectroscopy

I. INTRODUCTION

The effects of carbon monoxide (CO) on meat are subject of various studies, while in many cases meat is exposed to both, CO and oxygen [1, 2]. Recently, also a pretreatment of meat with CO is discussed as a technology which improves meat quality [3]. However, there is no method to measure the stability of COMb in presence of oxygen, since reflectance methodology to differentiate between COMb and OMb on the surface of meat is not available. Traditional equations used to estimate myoglobin redox forms [4, 5] do not account for existence of COMb. Published absorbance spectra for purified *bovine* COMb solutions [6] suggest, that the ratio of the two absorption maxima between 540 and 582 nm could be used to differentiate between COMb and OMb. However those ratios correspond to absorbance and not reflectance. Hence, this paper describes a method to determine the content of COMb as well as its stability in presence of oxygen, based on surface reflectance from 450 to 600 nm.

II. MATERIALS AND METHODS

Sample preparation

Four fresh (24 hours *post mortem*) heifer *M. semitendinosus* muscles (different days of slaughter) were sliced into 0.5 cm thick steaks and cut in the middle (to get enough samples). For the induction of the COMb form, the samples were prepared in a carbon dioxide flow (to start with deoxymyoglobin) and immediately packaged in a prepared gas mixture containing 10 % CO and 90 % nitrogen (Linde, Pullach, Germany). To convert all myoglobin into the carboxy form, the samples were stored at 3 °C for 48 hours. In order to create the 100 % OMb form, the samples were packaged in 100 % oxygen and stored at 0.5 °C for 24 hours before reflectance measurement. Each sample was individually packaged (1 steak = 1 package) in a polypropylene tray of size 22.7 cm x 17.8 cm x 5.0 cm (ES-Plastic, Hutthurm, Germany) and sealed with a 5/12 µm EVOH/PET layer (EK-Pack, Ermengerst-Wiggensbach, Germany). The bottom of the tray was covered with 1.0 cm thick 10 PPI foam (JBL, Neuhofen, Germany) to achieve a gas exposition on the entire meat surface. MAP (modified atmosphere packaging) was accomplished using a tray sealing packaging machine (TS300N, VC999, Herisau, Switzerland). For stability measurement of COMb, samples were repacked in HiOx-MA (high oxygen modified atmosphere) containing 80 % oxygen and 20 % carbon dioxide and stored at 0.5 °C for 10 minutes up to 24 hours, before scanning.

Reflectance measurement

After opening the package, the samples were cut out of the meat parts free from fat and connective tissue using a sharp cylinder and placed in a round sample holder (5.0 x 17.0 mm). Then samples were vacuum-packaged and immediately scanned. Reflectance was measured from 450 to 600 nm at 0.5 nm increments using a spectrophotometer (Specord 205, Analytik Jena, Jena, Germany) equipped with an integrating sphere. Calibration was carried out through the vacuum foil. All measurements were performed within 96 hours *post mortem* to ensure that myoglobin was in the reduced state (metmyoglobin cannot bind CO and oxygen). For each treatment, the ratio K/S min(I) / K/S min(II), with min(I) = reflectance minimum between 540 and 545 nm and min(II) = reflectance minimum between 576 and 582 nm was determined. The relative COMb content was calculated based on a linear 3 point calibration curve from 24 samples with 0, 50, 100 % COMb. For 0 % COMb the sample holder was filled with an OMb sample, for 50 % COMb one half of the sample holder was filled with an OMb sample and the other half was filled with a COMb sample.

III. RESULTS AND DISCUSSION

As shown in Table 1, for OMb reflectance magnitudes at minimum(I) were greater than at minimum(II), whereas in case of COMb the reverse was true. However, all maxima and minima for COMb were consistently located in a slight shift to shorter wavelengths compared to those for OMb. Therefore, no fixed wavelengths could be used for calculation and the two reflectance minima(I, II) had to be determined for each COMb proportion individually. The ratio K/S min(I) / K/S min(II) and % COMb indicated a high linear relationship with a coefficient of determination of $R^2 = 0.999$. Therefore it was concluded that the used calculation method would lead to proper results.

Table 1

Surface reflectance minima and maxima for carboxymyoglobin and oxymyoglobin in the spectral range from 450 nm to 600 nm in meat.

Pigment[a]	Max (nm)	Min (nm)	Reflectance (%)[b]	K/S value[c]	$K/S_{min(I)} / K/S_{min(II)}$
COMb	494.0		10.12	4.10	
	558.5		5.52	8.44	
		541.5 (I)	4.68 (I)	10.32 (I)	1.03 (0.027[e])
		577.5 (II)	4.79 (II)	10.03 (II)	
OMb	509.0		9.95	4.16	
	563.0		6.69	6.67	
		544.0 (I)	4.65 (I)	10.03 (I)	0.91 (0.015[e])
		580.5 (II)	4.25 (II)	11.08 (II)	
50 % COMb + 50 % OMb[d]	498.0		10.43	4.06	
	561.5		6.25	7.50	
		543.5 (I)	4.82 (I)	10.10 (I)	0.97 (0.009[e])
		579.0 (II)	4.75 (II)	10.32 (II)	

[a] OMb is oxymyoglobin, COMb is carboxymyoglobin.
[b] Mean values calculated from 24 scans (6 scans per muscle) for each form.
[c] Reflectance data converted to K/S values by the Kubelka-Munk equation ($K/S = (1 - R)^2 / 2R$), with reflectance as a decimal (R = % Reflectance / 100), mean values of the calculations for each scan (n = 24).
[d] Both forms are part of the integrating sphere, one half of the sample holder is filled with an OMb sample and the other half is filled with a COMb sample.
[e] Standard deviation.

Reflectance curves presented in Figure 1 (A) suggest that a major proportion of surface COMb was converted into the OMb form after a storage time of 24 hours in HiOx-MA, since spectral curves were almost identical to those of OMb. As shown in Figure 1 (B), already about 50 % of COMb was converted into OMb after four hours in HiOx-MA. It must be taken into account that meat is a three-dimensional model and replacement of CO by oxygen on the heme occurs more rapidly in the outer layers than in the inner layers of the meat surface. Therefore a logarithmic regression model indicated to be the most appropriate.

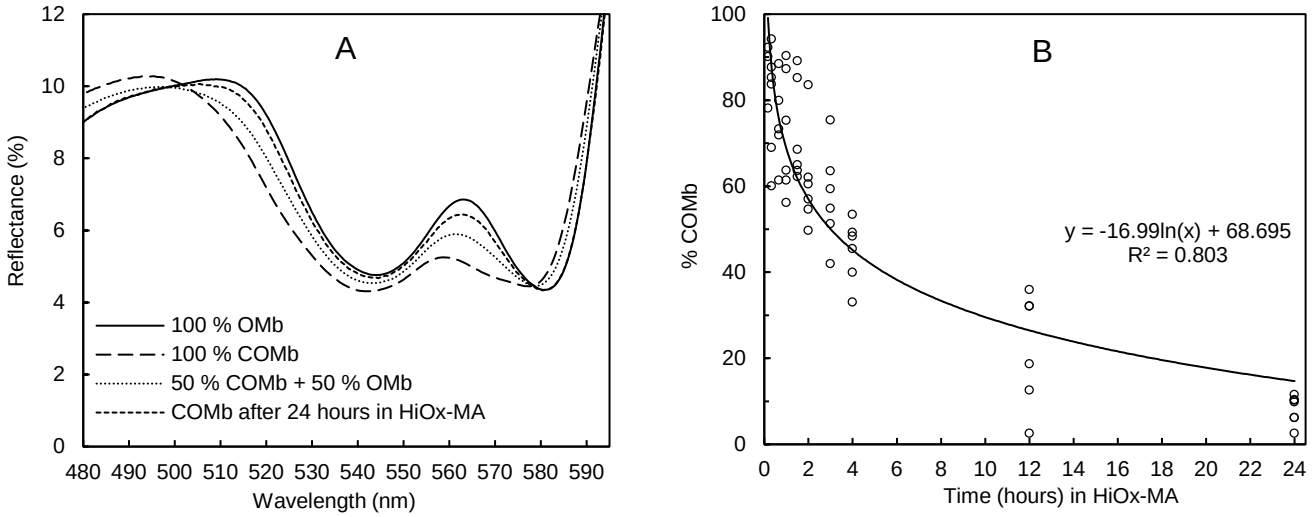

Figure 1. Surface reflectance spectra (A) for meat samples containing different proportions of COMb (carboxymyoglobin) and OMb (oxymyoglobin) and time dependent changes in the relative contend of COMb (B) during incubation of CO-treated samples in HiOx-MA (high oxygen modified atmosphere) containing 80 % oxygen and 20 % carbon dioxide. Spectral curves (A) are presented as mean values from 24 scans and changes in % COMb (B) are shown as scattered values (6 scans per incubation time) with logarithmic regression (R^2 is coefficient of determination).

IV. CONCLUSION

Although COMb and OMb have similar reflectance spectra, proportions of both forms can be determined due to the different ratio of the two reflectance minima between 540 and 582 nm in meat. In addition, the results strongly support the consideration of oxygen in investigations conducted with carbon monoxide treated meat, since COMb is rapidly converted into OMb in presence of oxygen.

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