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Prediction of myoglobin states using filter based reflectance measurements. (#383)

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Introduction

Minolta instruments have become very popular as a simple measurements tool for color. Typically the variables L*, a*, b* are given both in research and more applied reports. However, the variables L*, a*, b* are difficult to interpret in terms of oxymyoglobin (oxyMb), deoxymyoglobin (deoxyMb) and metmyoglobin (metMb) changes that are more informative. The AMSA guidelines (1) suggest four wavelengths as adequate for Mb state determination, namely 474, 525, 572 and 610 nm. Minolta CM-700d measures 40 wavelengths, at 10 nm intervals. We have used reflectance spectra to predict Mb states using the method published by Bjelanovic et al (2). This method does not use isobestic points but simply make use of all spectral readings available. Our work was carried out to determine how accurate Minolta CM-700d would predict Mb states.

Methods

Pure states for predictive model generation: Three *M. Semimembranosus* from 3 animals were sliced (2.5 cm thickness) and prepared into oxy-, deoxy- and metMb using high oxygen, vacuum packaging and chemical transformation (immersion in 1% potassium ferric cyanide) according to AMSA guidelines (1). Minolta CM-700d spectrophotometer (400 - 700 nm) was calibrated according to the manual. The spectra, 128 per Mb state, were collected at 16°C with meat covered with a transparent EVOH film.

*Application of predictive model for monitoring the effect of additives:*All meat samples were made and packed at a commercial company Norfersk AS (Hærland, Norway). Color stabilizing solutions were added to minced meat (Table 1). The meat samples were 300 g, shaped into 2 hamburger "patties", and packed in black plastic trays sealed with transparent film (OTR 24cm^3/m^2 per 24 h, 0% R.H., 1 bar, LID1050/LID 2050 Lidstock, modified atmosphere). The effect of the additives was monitored over 16 days. Each sample was prepared 3 times and measured 3 times at 3 different places. The total number of spectra acquired was 1944.

Statistics: The spectra were subjected to multiplicative scatter correction (MSC, Unscrambler X10.3). MSC is a pre-processing method commonly used in order to decrease the challenges with scaling and offset (baseline) effects due to light scattering, and thereby corrects the signal to noise due to differences in the light path. Following MSC, predictive models for myoglobin states were calculated using partial least square regression with full cross validation. These models were used to predict the influence of the additives (Table 1) on Mb states. Differenences between additives were assessed with ANOVA and Tukey as post hoc test.

Results

Generation of predictive models: Table 2 shows that the error dropped to 0.06-0.08 for the Mb states when MSC was applied. Figure 1 shows that despite the relatively large prediction error for Mb states (Table 2), the curves in Figure 1 were relatively smooth indicating a robust prediction of changes in Mb states. In addition, the sum of states for the samples with 0.5 % NaCl, 0.5% NaCl with Purasal S (lactate) and 0.5 % NaCl with Addcon (succinate/glutamate) were 0.975±0.078 (mean± std. dev.), 0.960±0.095, 0.980±0.029, respectively. The sum would be 1.0 without any analytical errors. OxyMb was the only state that sometimes was predicted as negative and then the minimum value was -0.055. Negative predictions are possible with our method since all three predictions are done with independent models. We did not remove any outliers (See Table 2, left figure). However, we know that the prediction error can drop to about 0.05 or below (Bjelanovic et al., 2013) using similar type of samples for calibration but ithen with instrumentation with higher resolution. In that context the data in Figure 1 are rather impressive.

The predictive model used to differentiate between possible color stabilizers: Systems (meat from a cattle 5 day *post mortem*) with 0.5 % NaCl added were maximally reduced (91.2 % deoxyMb after 3 days) and then the amount of deoxyMb started to decline. The same optimum was observed for Purasal S, but the deoxyMb fraction declined less rapidly. Addcon reached a deoxyMb fraction of 0.930 already after 1 day. However, its fraction of deoxyMb declined at a rate intermediate between only NaCl addition and Purasal S plus 0.5 % NaCl. The reason for these differences is that Addcon substrates act directly on the electron transport chain, while Purasal S. using sodium lactate as active ingredient produced reducing equivalents more slowly. Addcon would require lower oxygen diffusion films than used in our experiment. The difference in absolute amount of additived between Purasal S and Addcon may also matter.

Conclusion

Minolta CM-700 d gave useful prediction models of Mb states with error 0.06-0.08. The models were relevant for elucidating differences between color stabilizing agents

References

1.American Meat Science Association. 2012. AMSA Meat Color Measurement Guidelines. At: http://www.meatscience.org

Notes

2.Bjelanovic, M., Egelandsdal, B., Sørheim, O., Slinde, E., Puolanne E. & Isaksson, T. (2013). Determination of the myoglobin states in ground beef using non-invasive reflectance spectrometry and multivariate regression analysis. *Meat Science*, 95, 451-457.

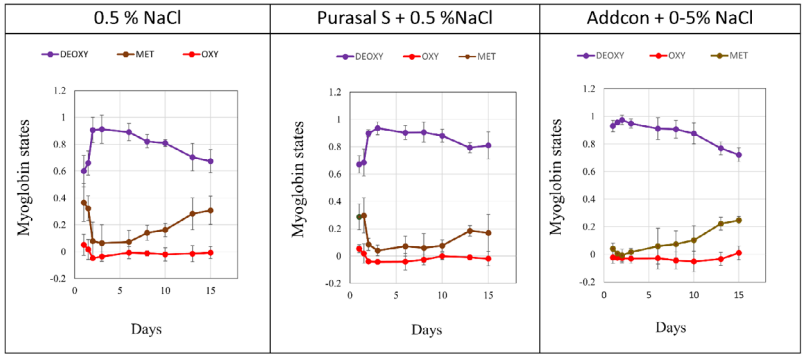

Fig 1-Effect of different color stabilizing additives on myoglobin states present in modified atmosphere packaged minces.

Table 1-2 and Figure 1
Three units to accompany abstracts.

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