REFLECTANCE AT 610 NANOMETERS FOR ESTIMATING OXYMYOGLOBIN OF GROUND BEEF

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Background

Using reflectance to quantify myoglobin derivatives on the surface of meat is one of many techniques employed to evaluate meat discoloration. Estimating the concentration of myoglobin forms is based on formulas that utilize isobestic wavelengths for each pigment derivative (AMSA, 1991). However, before applying reflectance data to formulas, one must determine reference values for 0 and 100% of deoxymyoglobin (DMb), oxymyoglobin (OMb), and metmyoglobin (MMb). In addition, reflectance data must be transformed into K/S values by the Kubelka-Munk equation $[(1 - R)^2 \div 2R]$, which accounts for the light absorbance and scattering properties of samples.

Historically, researchers have evaluated discoloration using an accumulation of MMb, which is determined by the ratio of K/S 572 and K/S 525 (Stewart et al., 1965). Seldom has discoloration been evaluated using OMb. When OMb has been used, it was determined indirectly using the difference between 100% and the sum of MMb and DMb (K/S 474 ÷ K/S 525). Thus, OMb by difference is only as accurate as the MMb and DMb determinations. Previous work by Ledward (1970) suggested that % MMb was accurate to only 6 to 7%, therefore indirect determination of OMb would be equally limited. Since 610 nm is isobestic for DMb and MMb, OMb may be determined directly using the ratio of K/S 610 ÷ K/S 525. Although previous research has evaluated the relationship between visual color and %MMb, little work has assessed the utility of K/S 610 ÷ K/S 525 for estimating OMb.

Quantifying myoglobin with reflectance may result in unrealistic concentrations of the three forms that are greater than 100% or less than 0%. Since these values are impractical, it may be useful to adjust unrealistic outlier data to 0% or 100%, but no research has demonstrated the effects of data adjustment. Outliers may result when researchers use published reference values for 0 or 100% of the myoglobin derivatives. Thus, it is generally recommended that standards for each myoglobin form be determined for each individual project to help minimize outliers (Hunt, 1980).

Objectives

This research assessed the utility of the K/S 610 ÷ K/S 525 method as a measure of OMb and discoloration of ground beef. In addition, comparisons were made between direct and indirect methods of OMb determination and using data adjusted for outliers.

Methods

Coarse ground (1.27 cm plate) beef chubs (4.55 kg) containing 81% lean were stored for 6 days at 0°C before each was assigned randomly to one of twelve combinations of three storage temperatures (0°, 4.5°, and 8.9°C) and four storage times (0, 4, 8, and 12 days). Three replications were conducted.

After storage, each chub was mixed by hand and ground once through a 0.32 cm plate. Approximately 454 g of ground beef was placed on each 2S Styrofoam® tray containing an absorbent pad and overwrapped in polyvinyl chloride film (oxygen transmission rate of 23,250 $cc/m^3/24h$ @ 23°C and 0% RH). Ground beef was displayed continuously at 0°, 4.5°, or 8.9°C in three 2.44 m open-top display cases under 1614 lux of Ultra-Lume fluorescent light (3000K). Combinations of these storage and display treatments provided variation in surface discoloration and myoglobin form concentrations, which were necessary to test the objectives of this experiment.

Ground beef surface color was analyzed visually and instrumentally at 0 (30 minutes after grinding), 24, and 48 hours of display. Visual color was appraised by seven trained panelists (AMSA, 1991), all of whom passed the Farnsworth-Munsell 100-Hue Test. A five-point color scale of 1=very bright cherry red, 2=bright cherry red, 3=slightly dark red to tannish red, 4=moderately grayish/tan to brown, and 5=tan to brown was used in increments of 0.5. Instrumental color included spectrophotometric measurements (400-700 nm at 10 nm increments) using a HunterLab MiniScan™ with a 3.18 cm diameter aperture and a 10° observer. Percent DMb (K/S 474 ÷ K/S 525) and %MMb (K/S 572 ÷ K/S 525) content were determined following the procedure of AMSA (1991). Oxymyoglobin content was calculated directly using the equation: K/S 610 ÷ K/S 525 (AMSA, 1991). Oxymyoglobin also was calculated by difference according to the equation: Indirect OMb = 100% - (%DMb + %MMb). Data adjustment included transforming values that were less than 0% to 0% and values greater than 100% to 100%.

Spectral data for reference values for 0 and 100% of DMb, OMb, and MMb were determined as follows. Ground beef was vacuum packaged and allowed to deoxygenate for 24 hours at 4°C to obtain 100% DMb. Values for 100% OMb were obtained by exposing ground beef to 100% oxygen. Values for 100% MMb were obtained from fully oxidized and discolored ground beef.

Data analyses were performed using SAS (2000). Pearson correlation coefficients for myoglobin forms (both adjusted and unadjusted for outliers) and visual scores were used to assess the utility of the K/S 610 ÷ K/S 525 method and the effects of data adjustment. The utility of the K/S 610 ÷ K/S 525 method also was evaluated using the following hypotheses at $\alpha = 0.05$: H_0: $\mu_d = 0$ and H_a: $\mu_d \neq 0$, where μ_d is the mean difference between direct and indirect measurements.

Results and discussion

Direct quantification of OMb with the K/S 610 ÷ K/S 525 method had a strong linear relationship (r = -0.93, Table 1A) with visual color, whereas MMb (572 ÷ K/S 525) had a correlation of 0.90 with visual color. Directly determined oxymyoglobin also had a strong inverse relationship (r = -0.98) with MMb, which indicates that K/S 610 ÷ K/S 525 accounted for non-brown pigments (i.e. OMb and DMb). The associations between K/S 610 ÷ K/S 525, visual color, and MMb, suggest that K/S 610 ÷ K/S 525 will accurately represent a decline in OMb during display. Thus, ground beef visual color stability during display may be evaluated using K/S 610 ÷ K/S 525 rather than 572 ÷ K/S 525 (Table 1).

The degree of association between direct measurement of OMb (K/S 610 ÷ K/S 525) and visual color was essentially the same as that between the traditional method of OMb quantification by subtraction and visual color (Table 1A, r = -0.93 versus -0.92). In addition, the mean difference between direct OMb and indirect OMb values was not statistically different than 0 (actual mean was 0.03% OMb, $P = 0.919$), demonstrating that direct and indirect methods estimated equivalent amounts of OMb. The 95% confidence interval about the mean difference of the two methods was –0.53% to 0.59% OMb. The sum of the mean values (not adjusted for outliers) for directly estimated OMb, MMb, and DMb accounted for 99.9% of the total pigment on the surface of ground beef. Oxymyoglobin determined by difference plus percentages of MMb and DMb also totaled 99.9%; however, this was due the nature of the basic equation for indirect calculation. Thus,

direct determination of OMb when combined with independently calculated percentages of MMb and DMb accounted for as much total pigment as indirect OMb per se.

Quantifying myoglobin with reflectance commonly produces unrealistic amounts (<0 and >100% of the pigment forms), which result from reflectance values exceeding the reference values that represent 100% for each pigment form. In this investigation, the maximum value obtained from direct calculation was 103% OMb, whereas indirect calculation of OMb went up to 107%. Direct calculation resulted in approximately 12% fewer unrealistic OMb values than indirect calculation (3.7% for direct vs. 16.4% for indirect). Approximately 28.6% of DMb and 18.2% of MMb values were less than 0%, which might account for more inflated OMb values (unrealistic values > 100) calculated by the indirect method. Since OMb by difference was based primarily on the MMb content of oxygenated meat, the strong relationship of the two (r = -0.99) confirms the likelihood that unrealistic MMb estimates will affect indirect determinations of OMb. Direct determination of OMb is independent of these erroneous outliers for MMb and DMb. Determining OMb using K/S 610 ÷ K/S 525 should more accurately represent OMb concentration on the surface of ground beef early in display when MMb content is minimal and could be measured as less than 0%. However, direct and indirect calculations resulted in a similar number of outliers less than 0% OMb (1 and 2 %, respectively). For data analysis, these unrealistic estimates could have been used "as is" or transformed into more practical values, limiting the distribution of data to between 0 and 100%. Correlation coefficients for adjusted and unadjusted data were similar for each quantification method (Table 1A vs. 1B). Since both visual color scores and adjusted reflectance values responded similarly to visual color changes during display, adjusted data should be sufficient to evaluate discoloration. However, data adjustment is a valuable practice because greater than 100% (or less than 0%) of any one pigment form is theoretically impossible and extreme outlier values may bias results. Thus, percentage myoglobin forms from adjusted values were considered more representative of actual pigment proportions.

Although reference values specific for this project were determined, each method of myoglobin quantification still produced some degree of unrealistic (< 0 or > 100%) estimates. However, the probability of obtaining outliers likely would have increased if standard values from published literature were used. Therefore, it is crucial that reference samples providing 100% of each pigment form are created under the specific conditions of each experiment and use of pre-existing standards is not recommended if research objectives include exacting estimates of myoglobin forms.

Conclusions

The K/S 610 ÷K/S 525 reflectance method will estimate the proportion of OMb on the surface of ground beef and can be used assess ground beef discoloration. Direct calculation of OMb was highly representative of visual color and closely, but inversely related to MMb. Data with and without adjustment for outliers responded similarly to changes in color during display. However, since adjusting for outliers was a simple task, data adjustment is recommended so that percentages of the myoglobin forms will more accurately represent the actual amounts of pigment forms on the surface of ground beef.

Pertinent literature

AMSA. (1991). Guidelines for meat color evaluation. In *Proceedings 44th Annual Reciprocal Meat Conference*, (pp. 1-17), 9-12 June 1991, Kansas, USA.

Hunt, M. C. (1980). Meat color measurements. In *Proceedings 33rd Annual Reciprocal Meat Conference*, (pp. 41-46), 22-25 June 1980, Indiana, USA.

Ledward, D. A. (1970). Metmyoglobin formation in beef stored in carbon dioxide enriched and oxygen depleted atmospheres. *Journal of Food Science,* 35(1), 33-37.

SAS (2000). *SAS User's Guide* (8th ed.). Cary, NC: SAS Institute Inc.

Stewart, M. R., Zipser, M. W., & Watts, B. M. (1965). The use of reflectance spectrometry for the assay of raw meat pigments. *Journal of Food Science*, 30(3), 464-469

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Table 1
Relationships[ab] between myoglobin quantification methods and ground beef visual color scores.

Table 1A Unadjusted data	Visual	OMb, direct	OMb, indirect	MMb
OMb, direct	-0.93			
OMb, indirect	-0.92	0.98		
MMb	0.90	-0.98	-0.99	
DMb	0.17	-0.07[b]	-0.09[b]	-0.05[b]

Table 1B Adjusted data	Visual	OMb, direct	OMb, indirect	MMb
OMb, Direct	-0.93			
OMb, Indirect	-0.92	0.98		
MMb	0.91	-0.98	-0.99	
DMb	0.19	-0.11	-0.12	0.01[b]

[a] Assessed by Pearson correlation coefficients, n=324.
[b] Correlations less than 0.1 were not significant ($P > 0.05$)