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Interrelationships Between Visual and Instrumental Measures of Ground Beef Color

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Abstract: Two experiments were conducted to understand the interrelationship among visual color score, Farnsworth-Munsell values, instrumental meat color measurements, and their impact on beef color evaluation. In experiment 1, *L**, *a**, and *b** values and various reflectance traits of the Farnsworth-Munsell 100-Hue Test tiles were measured. Incremental changes of the tiles were used to predict changes in *a** values and other color variables that can be seen by a trained visual panel. Regression analysis suggests that trained panelists that pass the Farnsworth-Munsell Hue Test can discriminate a change of 0.95 in *a**, 0.9 in *b**, and 2.54 in hue angle values when Illuminant A is used. In experiment 2, ground beef was assigned randomly to one of 36 combinations of 3 storage temperatures, 4 storage times, and 3 display temperatures to create a variety of surface colors. A 10% change in ground beef metmyoglobin content corresponded to 3.2 units of *a** ($R^2 = 0.95$). Of all the instrumental measurements, *a** ($r = -0.97$) and chroma ($r = -0.97$) best represented the red color intensity that panelists saw on the surface of ground beef. Significant surface discoloration occurred at 37.1% metmyoglobin and an *a** value of 25.4 (with Illuminant A). Using a 5-point visual lean color scale, the change required in *a** value for a unit change visual color score was 4.6. In conclusion, *a** and chroma are highly related to visual color scores and changes in metmyoglobin, and a change in *a** of 0.95 can be observed by visual panelists that have passed the Farnsworth-Munsell test.

Keywords: meat color, reflectance measurements, myoglobin quantification, visual color, Farnsworth-Munsell color tiles
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Introduction

Visual color scores best represent what consumers see and are the standard for determining consumer preference. However, disadvantages associated with conducting trained visual panels, such as availability, fatigue, and consistency (King et al. In Press), have caused researchers to rely more on rapid, non-invasive measures of color, such as reflectance using a colorimeter, to assess treatment differences (Kropf, 1984). As a result, instrumental measurements using HunterLab MiniScan spectrophotometers or Minolta chroma meters often are the only color evaluations used in meat color studies.

Both the CIELAB color space and measurements calculated from these color space values, such as chroma, hue angle, and *a**/*b**, are frequently used to measure initial color, discoloration, and differences in color stability (Kim et al., 2006; Mancini et al., 2010; Mohan et al., 2010; Khatri et al., 2012; English et al., 2016; Tomasevic et al., 2019; Ramanathan et al., 2020). In addition, several different reflectance measurements also are available to characterize myoglobin redox changes during storage and display. More specifically, isobestic wavelengths can be used to estimate the amount of each myoglobin derivative with formulas that require 0% and 100% myoglobin reference values (King et al. In Press). Direct quantification of deoxymyoglobin (DMb) using absorbance coefficient (K) and scattering coefficient (S) ratios (K/S) 474 ÷ K/S 525 (Snyder, 1965), oxymyoglobin (OMb) using K/S 610 ÷ K/S 525 (Mancini et al., 2003), and metmyoglobin (MMb) using K/S 572 ÷ K/S 525 (Franke and Solberg, 1971) are established methods for characterization of the myoglobin redox forms on the surface of meat. However, because estimating myoglobin forms is time-consuming, other measures of color such as a^*, b^*, and calculations of total color change (delta E) are often used when quantification of myoglobin derivatives is not necessary.

Previous research has utilized both instrumental and visual color to characterize color changes, and some studies determined the relationship between instrumental color and visual panel/web-based color evaluation (Eagerman et al., 1977, 1978; Harrison et al., 1980; Holman et al., 2016; Wills et al., 2017; Cassens et al., 2021; Yoder et al., 2021). Although instrument settings such as illuminant and aperture size can influence color measurements, Tapp et al. (2011) reported that a significant amount of published research does not report several instrumental parameters (such as illuminant or aperture size) as suggested by the AMSA Color Guidelines (2012).

Given the abundant use of instrumental color measurements, meat color researchers often ask how changes in reflectance values relate to visual color. More specifically, it would be interesting to know "what is a typical change in a^* that can be seen by a visual panel trained to evaluate meat color?" Researchers also speculate about the average change in a^* per 1-unit change in the visual color score as well as the color change in myoglobin associated with a 1-unit change in a^*. Limited research is available to better understand the practical applications of reflectance values and how instrumental meat color measurements are interpreted. Therefore, the objectives of this study were to (1) compare instrumental color with incremental changes in Farnsworth-Munsell color tiles to predict changes in a^* values and other color variables that can be seen by a trained visual panel and (2) determine instrumental color values that correspond to 1-unit changes in trained visual color scores of ground beef.

Materials and Methods

Two separate experiments were conducted to determine the relationship between visual and instrumental color measurements.

Experiment 1: Use of Farnsworth-Munsell 100-Hue Test to determine a threshold of instrumental color discrimination between adjacent tiles

The Farnsworth-Munsell 100-Hue Test is commonly used to evaluate the ability of humans to discriminate small differences in color (Kinnear and Sahraie, 2002). Trained panelists used to evaluate meat color should be screened with the Farnsworth-Munsell Hue Test to determine their eligibility to serve on trained color panels (King et al. In Press).

A HunterLab MiniScan XE Plus spectrophotometer (Model 45/0 LAV, 2.5-cm diameter aperture, 10° observer; HunterLab, Reston, VA) was used to measure L^*, a^*, b^* values, chroma $[(a^2 + b^2)^{½}]$, and hue angle ($\tan^{-1} b^*/a^*$) of the 100-hue tiles (King et al. In Press). Three readings of each tile were recorded using Illuminants A, C, and D65 and averaged for statistical analysis. Only tiles that corresponded to positive a^* and b^* values were used in the data analysis because these tiles are more closely related to beef surface color.

Experiment 2: Predicting instrumental color values associated with changes in trained visual color scores

To create a variety of ground beef colors, chubs of ground beef containing 19% fat were obtained from a local purveyor, shipped to the Kansas State University Meat Laboratory, and stored at 0°C for 6 d before each chub was assigned randomly to 1 of 12 storage temperature (0°C, 4.5°C, and 8.9°C) and storage time (0, 4, 8, and 12 d) combinations. After storage, each chub was mixed by hand and ground through a 0.32 cm plate (Model 4732, Hobart Mfg. Co., Troy, OH), and 454 g was placed on 2S Styrofoam® trays (Tenneco, Lake Forest, IL) with a Dry-Loc pad (AC-50, Cryovac, Duncan, SC). Trays were overwrapped with polyvinyl chloride film (23,250 cc/m^2/24 h at 23°C and 0% relative humidity, Borden Packaging and Industrial Products, North Andover, MA). After packaging, 1 package per chub was displayed continuously for 48 h at 0°C, 4.5°C, or 8.9°C in three 2.44-m-long open-top display cases (Model DMF8, Tyler Refrigeration Corporation, Niles, MI) under 1,614 lux of Ultra-Lume fluorescent light (3,000 K, Philips Lighting, Salina, KS).

Tissue pH was determined by homogenizing 10 g of ground beef with 100 mL of deionized-distilled water for 30 s with a Stomacher Lab Blender

(Seward Stomacher 400 Lab Blender, Seward Medical, UK). Following homogenization, a combination electrode attached to an Accumet pH meter (Accumet standard, Fischer Scientific, Pittsburgh, PA) was used to measure pH.

Ground beef surface color was measured instrumentally every 6 h for 48 h of display. Visual color was appraised at 0, 24, and 48 h by 7 panelists trained following AMSA Color Guidelines (2012). All panelists had scores < 50 on the Farnsworth-Munsell 100-Hue Test. Training sessions included ground beef samples with a variety of colors (multiple samples that aligned with each whole-point visual color score as well as several 0.5 interval scores). A discussion outlining visual color evaluation of these samples was used (e.g., assessing overall color vs. worst point color). In addition, color evaluation of these samples independent of other panelist input was followed by group discussion to better understand the intricacies of color measurement and the scale used. Initial color (0 h) was evaluated 30 min after the meat was packaged using a 5-point scale (with increments of 0.5), in which 1 = very bright cherry red, 2 = moderately bright red, 3 = slightly dark red to tannish red, 4 = dark red to tannish red, and 5 = tan to brown (Mancini et al., 2003).

Reflectance measurements included CIE L^*, a^*, and b^* values (CIE, 1976), and spectral readings from 400 to 700 nm were recorded using a HunterLab Miniscan (for illuminant A, 3.18 cm diameter aperture, and 10° Observer; Hunter Associates Laboratory Inc., Reston, VA). Additional instrumental color measurements calculated were chroma, hue angle, a^*/b^*, percentage R630 − percentage R580, and percentage R630 ÷ percentage R580 (R = reflectance at a specific wavelength). Percentage myoglobin redox forms were estimated using isobestic formulas for %OMb, %DMb, and %MMb (King et al. In Press).

Statistical analysis

A total of 36 treatment combinations were created by crossing 3 storage temperatures (0°C, 4.5°C, and 8.9°C; without light), 4 storage times (0, 4, 8, and 12 d; without light), and 3 display temperatures (0°C, 4.5°C, and 8.9°C; with light). The storage temperature and storage time represent dark storage prior to retail display. The overall experiment was replicated 3 times using a total of $n = 36$ course-ground beef chubs. These numerous storage and display treatments produced a wide range of discoloration to enhance correlation and regression analysis (Figure 1). Overall, as chub storage times × temperatures and display times × temperatures increased, surface discoloration also increased.

Correlation coefficients of instrumental measurements and visual scores were calculated using the Proc Corr procedure of SAS (SAS Institute Inc., Cary, NC). The Reg and Rsquare procedures of SAS were used to (1) determine the amount of variability in ground beef visual color scores accounted for by independent variables (L^*, a^*, b^*, chroma, hue angle, saturation index, a^*/b^*, percentage R630 − percentage R580, percentage R630 ÷ percentage R580, %OMb, %MMB, and %DMb) and (2) predict visual color scores from independent variables. Best-fit regression models, which were based on adjusted R^2, root mean square error, and the parsimony concept, were used to estimate instrumental values. The Proc Reg option was used to determine how changes in visual color score affected instrumental reflectance parameters.

Results

Experiment 1: Relationships between spectrophotometric color measurements of Farnsworth-Munsell tiles and trained panelist visual acuity

The Farnsworth-Munsell 100-Hue Test tiles in the quadrant most related to visual beef visual surface color had a strong positive correlation with a^*, b^*, chroma, and hue angle values regardless of the illuminant used (Table 1). Although a slight increase in R^2 was observed for b^* and chroma when D65 and C were used, the illuminant type had no influence on the relationship between a^* and the Farnsworth-Munsell tiles. For all 3 illuminants tested, regression analysis indicated that trained panelists that had passed the Farnsworth-Munsell 100-Hue Test could discriminate a change of approximately 1-unit for a^*. Compared with Illuminant A, there was a slight increase in the hue angle value that corresponded to Farnsworth-Munsell tile changes when Illuminants C and D65 were used.

Experiment 2: Relationships between ground beef visual color scores and instrumental color measurements

All instrumental reflectance measures except % DMb were highly correlated to ground beef visual color scores (Table 2). This was expected since % DMb was not a major redox form on the surface of oxygenated ground beef used in this experiment. Of all the

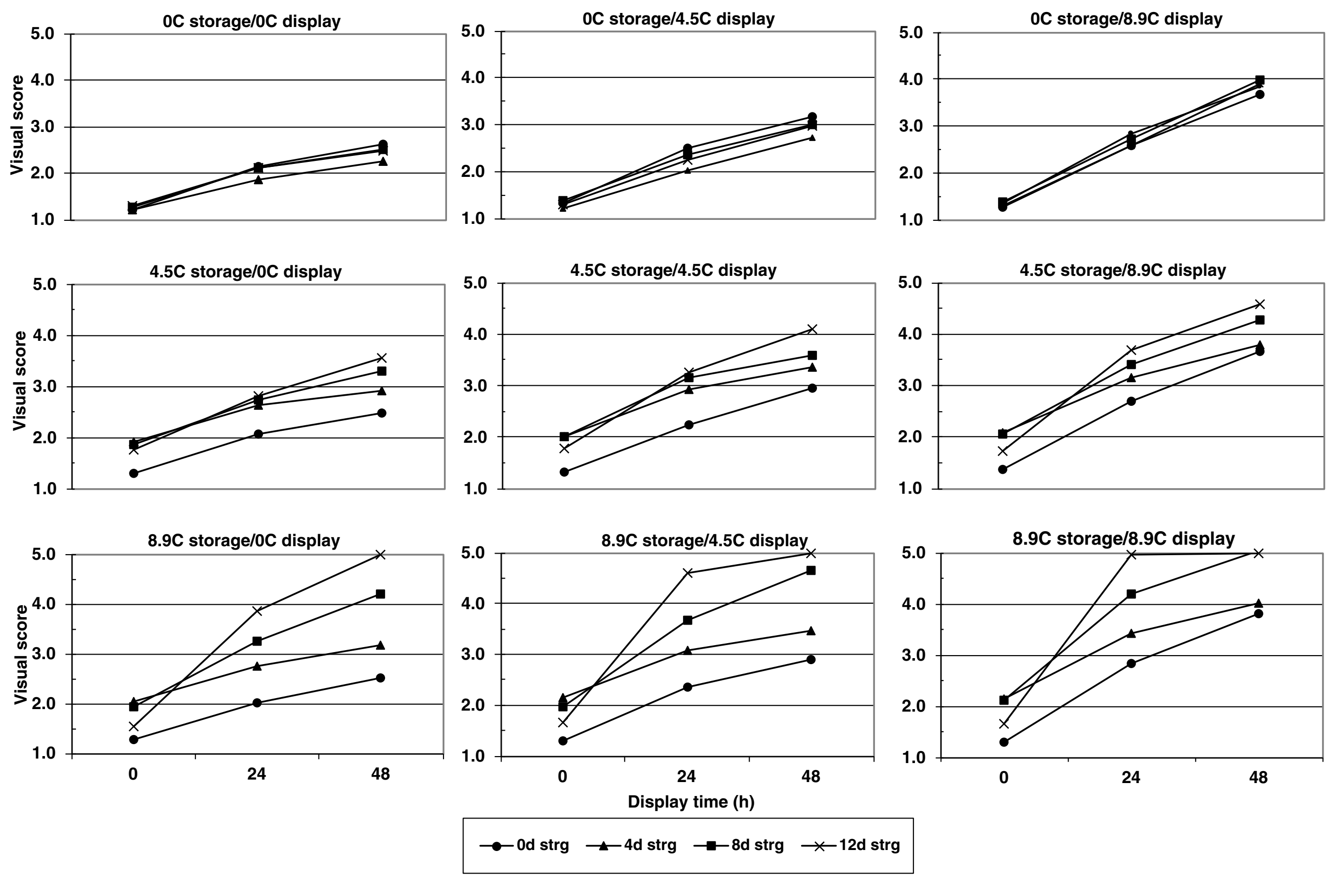

Figure 1. Effects of storage temperature, storage time display temperature, and time on visual color of ground beef patties. To create a variety of ground beef colors, ground beef chubs were stored at 0°C for 6 d before each chub was assigned randomly to one of 12 storage temperature (0°C, 4.5°C, and 8.9°C) and storage time (0, 4, 8, and 12 d) combinations. After storage, each chub was mixed by hand and ground, and 454 g was placed on 2S Styrofoam™ trays with a Dry-Loc pad. Trays were overwrapped with polyvinyl chloride film. After packaging, one package per chub was displayed continuously for 48 h at 0°C, 4.5°C, or 8.9°C in three 2.44-m-long open-top display cases. Surface color was evaluated using a 5-point scale of 1 = very bright cherry red, 2 = moderately bright red, 3 = slightly dark red to tannish red, 4 = dark red to tannish red, and 5 = tan to brown.

Table 1. Instrumental color change for incremental changes in Farnsworth-Munsell tile[1]

Illuminant type	Parameter	Instrumental color change for incremental changes in Farnsworth-Munsell tile	R^2
A10	a^*	0.95	0.94
D65	a^*	0.98	0.94
C10	a^*	0.96	0.94
A10	b^*	0.99	0.95
D65	b^*	1.08	0.98
C10	b^*	1.08	0.98
A10	Chroma	0.50	0.91
D65	Chroma	0.46	0.94
C10	Chroma	0.49	0.95
A10	Hue angle	2.54	0.99
D65	Hue angle	3.18	0.99
C10	Hue angle	3.17	0.99

[1]Only tiles that corresponded to positive a^* and b^* values were used in the data analysis because these tiles more closely related to beef surface color. R^2 = regression coefficient.

instrumental measurements evaluated, a^* ($r = -0.97$) and chroma ($r = -0.97$) best represented the red color intensity that panelists saw on the surface of ground beef. In addition, a^* had a strong correlation to both %OMb ($r = 0.94$) and %MMb ($r = -0.94$). As a result, a^* decreased as MMb on the ground beef surface increased (Figure 2). A 10% increase in surface %MMb resulted in a 3.2-unit decrease in a^* with Illuminant A as the light source.

Color variables based on reflectance at 580 nm and 630 nm were highly correlated with %MMb and %OMb as well as visual color (Table 2). Although chroma had a strong correlation with visual color, %OMb, and %MMb, other mathematical manipulations of individual coordinates such as a^*/b^* and hue angle were less correlated with visual color measurements and %MMb, though they were still highly correlated ($r > 0.85$).

Spearman correlation coefficients (data not shown) demonstrated that a^* and chroma had a high monotonic association ($r = -0.96$) with visual ground beef color.

Table 2. Pearson correlation coefficients for the relationship between visual color scores and reflectance measurements (Illuminant A) of ground beef

Variable	Visual	*a**	*b**	Chroma	*a**/*b**	Hue	R630 −R580	R630 ÷ R580	%OMb	%MMb
*L**	−0.80									
*a**	−0.97									
*b**	−0.94	0.97								
Chroma	−0.97	1.00	0.99							
*a**/*b**	−0.89	0.91	0.77	0.86						
Hue angle	0.85	−0.87	−0.72	−0.82	−1.00					
R630 −R580	−0.93	0.92	0.93	0.94	0.79	−0.75				
R630 ÷ R580	−0.89	0.90	0.87	0.90	0.78	−0.74	0.90			
%OMb	−0.93	0.94	0.88	0.92	0.92	−0.90	0.95	0.92		
%MMb	0.90	−0.94	−0.85	−0.90	−0.92	0.90	−0.94	−0.94	−0.97	
%DMb	0.15	−0.08*	−0.23	−0.14	0.17	−0.21	−0.22	−0.12	−0.06§	−0.05§

Surface color was evaluated using a 5-point scale of 1 = very bright cherry red, 2 = moderately bright red, 3 = slightly dark red to tannish red, 4 = dark red to tannish red, and 5 = tan to brown.

§Indicates correlation coefficient is not significant. Value without "§" indicates correlation coefficient is significant ($P < 0.05$).

%MMb = percentage metmyoglobin; %OMb = percentage oxymyoglobin; R580 = reflectance at 580 nm; R630 = reflectance at 630 nm.

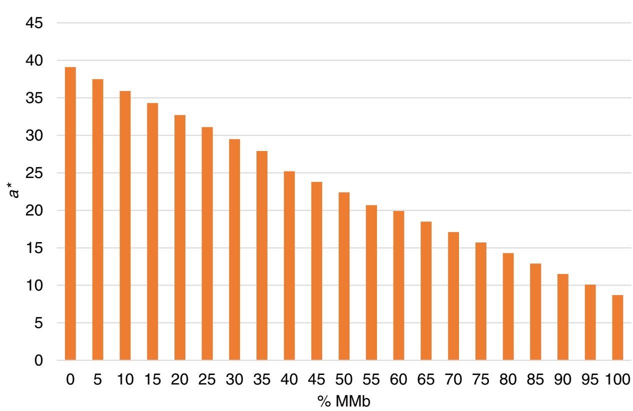

Figure 2. Relationship between percentage metmyoglobin (% MMb) and *a values**. The relationship between %MMb and *a** values (Illuminant A) on the ground beef surface color was derived using a regression equation ($R^2 = 0.95$; $y = [-1.6x + 40.7]$).

Thus, the inverse relationship of these variables indicated that larger *a** values characterized bright-red color and a numerical decrease represented a change from red color to brown. Percentages of OMb ($r = -0.93$) and MMb ($r = 0.90$) also had a high monotonic correlation with ground beef visual color.

Regression analysis suggests that *a** alone can predict 95% of the variability in visual color scores ($R^2 = 0.95$; Table 3). Adding variables such as chroma, hue angle, OMb, MMb, and 630–580 nm resulted in little or no change in R^2.

Using regression analysis, ground beef described by the trained visual panel as very bright cherry red (color score of 1) had an *a** value of 39.1 for Illuminant A (Table 4). A visual color score of 3, which characterized the ground beef surface color as slightly dark red to tannish red was associated with an *a** value of 26.1, a chroma value of 28.4, 63.1% OMb, and 37.1% MMb. Tan to brown ground beef surface color (score of 5) had an *a** value of 10.5.

Table 3. Parameter estimates and confidence levels for predicting visual color scores from ground beef instrumental color (Illuminant A) measures

*a**	DMb	630 – 580 nm	OMb	Chroma	Intercept	R^2	$MSE^{1/2}$
−0.14					6.52	0.95	0.25
−0.14	0.023				6.44	0.95	0.23
−0.12		−0.01			6.26	0.95	0.24
−0.12			−0.005		6.41	0.95	0.25
				−0.13	7.43	0.94	0.27

Surface color was evaluated using a 5-point scale of 1 = very bright cherry red, 2 = moderately bright red, 3 = slightly dark red to tannish red, 4 = dark red to tannish red, and 5 = tan to brown.

DMb, deoxymyoglobin; $MSE^{1/2}$, root mean square error; OMb, oxymyoglobin.

Using a 5-point scale to assess display color, the average change required in *a** and *b** values per unit change in visual color score was 4.6 and 2.5, respectively (Table 5). Changes required in chroma and hue angle for a unit change in display color score were 5.1 and 2.1, respectively. Both 630 nm–580 nm and 630 nm ÷ 580 nm had greater coefficients of variation than other color parameters.

Discussion

The color of beef is a critically important variable at nearly every segment of the beef chain. Qualitative

Table 4. Relationship between visual color score and instrumental red color (Illuminant A) characteristics[1]

Surface color score	a^*	Hue	Chroma	R630 nm −R580nm	R630 nm ÷ R580 nm
1 = very bright cherry red	39.1	35.5	43.6	32.7	6.1
2 = moderately bright red	32.6	37.6	38.6	26.6	5.1
3 = slightly dark red to tannish red	26.1	39.6	33.5	20.5	4.0
4 = dark red to tannish red	19.6	41.7	28.4	14.4	2.9
5 = tan to brown	13.1	43.8	23.3	8.3	1.8
R^2	0.91	0.60	0.90	0.92	0.91

[1]The values were derived from regression equation between visual color and instrumental red color during retail display. R^2 values are indicated.

R580, reflectance at 580 nm; R630, reflectance at 630 nm.

Table 5. Changes required in instrumental color (Illuminant A) for unit change in ground beef display color[1]

Instrumental parameter	R^2	Change in instrumental color per unit change in visual display color[2]	Root mean square error	Coefficient of variation
L^*	0.72	2.10	1.69	3.79
a^*	0.91	4.56	1.85	7.20
b^*	0.86	2.52	1.30	6.20
a^*/b^*	0.62	0.08	0.08	6.69
Chroma	0.90	5.08	2.10	6.32
Hue	0.60	2.06	2.19	5.51
R630 nm −R580 nm	0.92	6.11	2.28	11.2
R630 nm ÷ R580 nm	0.91	1.08	0.43	11.2

[1]Display color was measured on a 5-point scale (1 = very bright cherry red, 2 = moderately bright red, 3 = slightly dark red to tannish red, 4 = dark red to tannish red and 5 = tan to brown).

[2]The values were derived from regression equation between visual color and instrumental red color during retail display. R^2 = regression coefficient.

R580, reflectance at 580 nm; R630, reflectance at 630 nm.

and quantitative assessment of beef color may be done visually or instrumentally with varying degrees of precision and accuracy. The desired outcomes vary globally; however, the interpretation of the color data may not be well understood. This study attempts to clarify the working relationships and interrelationships among ground beef color measurements.

Numerous factors affect meat color, and pH is one of the most important because it is impacted by genetics, production systems, animal age, antemortem stress, and interactions of time and chilling dynamics of temperature on the conversion of muscle to meat. Raw material pH can greatly affect color and color stability; thus, we minimized pH effects by using ground beef that had a normal pH of 5.7.

What many researchers would like to know are the subtle relationships between visual and instrumental color measurements. The Farnsworth-Munsell Hue Test evaluates an individual's ability to arrange hue tiles in a particular order. More specifically, this test indicates color discrimination of closely related hues and is used to separate persons with normal color vision into classes of superior, average, or low (Farnsworth, 1957).

No research has utilized the CIE $L^*a^*b^*$ values of Farnsworth-Munsell tiles to discriminate corresponding redness and meat color. This technique was very useful in this study as the Farnsworth chips provided a "standard" that could be used to relate human visual acuity and instrumental appraisals of meat color traits. For example, the Farnsworth-Munsell Hue Test indicated that a trained visual color panelist can detect CIE a^* values differences of 0.95 tile units (using Illuminant A) and that Illuminant C and D65 had more influence on b^* than a^*.

Declining values of a^* and chroma (loss of saturation) on beef cuts negatively impact purchasing decisions. Previous studies noted both visual and instrumental color measurements were related with different levels of correlation (Zhu and Brewer, 1999; Goni et al., 2008). High correlations between a^* and visual color were noted in the current research. As a result, the current research suggests that a^* can be used to predict red to brown visual color changes, and additional variables such as chroma and hue angle may not be necessary. In addition, a^* can be used to assess changes in ground beef discoloration associated with MMb accumulation.

Differences in instrumental settings, lighting conditions, fat level, and other factors make it difficult to create a universal cut-off value for both subjective and objective measurements of meat color that represent discoloration. A survey using digital images noted that a^* values of 14.5 of beef steaks were acceptable for consumers (Illuminant D65; Holman et al., 2017).

Van den Oord and Wesdorp (1971) reported that 50% MMb results in rejection by consumers. In the current research using Illuminant A and a 10° Observer, an a^* value of 23.1 was associated with 50% MMb. In addition, trained panelists described ground beef as slightly dark red to tannish at a surface MMb content as low as 37%.

The current study suggests that both a^* and chroma can accurately characterize ground beef surface color when a visual panel is not necessary or available. In many situations, a^* alone may be the only variable necessary to assess changes in discoloration associated with a decrease in redness or an increase in MMb. The current study suggests that a decrease in a^* of 3.2 is associated with a 10% increase in %MMb.

Although data using Illuminant A indicate that a 1-unit change in visual color score corresponded to a 4.6-unit change in a^*, the actual change in a^* value that a trained visual color panelist can identify is likely lower. For example, trained panelists are often asked to score visual color to the nearest 0.5. More specifically, a change in a^* of 0.95 units can be identified by trained panelists that pass the Farnsworth-Munsell Hue Test. Researchers must consider the inherent differences due to factors such as type of spectrophotometer, light source, sensory color analysis scales, etc., when interpreting results from different studies (King et al. In Press).

Conclusions

Data from two experiments indicate the following relationships between visual and instrumental measurements of ground beef color:

- Regardless of the illuminant type, a^* is strongly correlated to the Farnsworth-Munsell 100-Hue Test tiles associated with ground beef visual surface color.
- Both visual and instrumental color are highly correlated.
- In the absence of a visual panel, researchers can use a^* values to determine changes in red color and %MMb.
- When predicting visual color, adding other instrumental variables to a^* may not improve predicting power.
- A 10% change in MMb content corresponds to 3.2 units of a^*.
- Trained panelists that pass the Farnsworth-Munsell Hue Test can identify changes in a^* of 0.95 units and 0.99 b^* units using Illuminant A.

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