

SIMPLIFIED METHODOLOGY FOR MEASURING MEAT COLOR

INTRODUCTION

THE COLOR of fresh meat is determined mainly by the relative proportions of three meat pigments: purple reduced myoglobin (Mb), red oxymyoglobin (MbO₂) and brown metmyoglobin (MetMb). Under normal marketing conditions reduced myoglobin in the presence of O₂ is completely changed to MbO₂ within a few hours (Van den Oord and Wesdorp, 1971a). The bright red of the MbO₂ is the preferred color of fresh beef packaged for retail sales. The change of MbO₂ to MetMb can be effected by bacterial growth or oxygen tension levels, resulting in slow oxidation of the heme iron to its ferric state. As this change occurs the meat becomes less acceptable to consumers. The relative proportions of MbO₂ and MetMb are a prime factor in the acceptability of fresh beef color to consumers. At approximately 50% conversion to MetMb the meat is unacceptable to most consumers and therefore unsuitable for retail sale (Van den Oord and Wesdorp, 1971a).

The preferred method for measuring consumer acceptability is color evaluation by a panel of trained observers. This method has several serious disadvantages for continuous evaluation of meat color changes. Panel measurements are time consuming, prone to subjective errors and limited in the number of evaluations which can be made at one time.

Reflectance measurement is the instrumental technique of choice because it measures the color on the surface of the meat as observed by the consumer and it is nondestructive. Two types of instruments can be used in reflectance studies: colorimeters and spectrophotometers with reflectance attachments. Previous investigators have employed a variety of techniques for meat studies. Haas and Bratzler (1965) used Munsell disk colorimetry and a Gardner color difference meter to follow oxygenation rates in beef, pork and lamb. Snyder (1965) used the Gardner R_d, a, b, and a/b values to indicate changes taking place in intact beef samples. The "R_d" value is a measure of the total light reflected while "+a" is a measure of redness, "-a" is a measure of greenness, "+b" is a measure of yellowness and "-b" is a measure of blueness in the reflected light. He found that a high a/b value indicated a high concentration on the surface of the meat of either Mb or MbO₂, while a low a/b value indicated a high concentration of MetMb. Romans et al. (1965) attempted correlation of Munsell disk colorimetry values with myoglobin concentration but found low correlation coefficients ($r = -0.5$). Jeremiah et al. (1972) related color difference values to consumer acceptability of beef color. Their study was designed to measure the preferred intensity of color from MbO₂. They correlated the values from the Macbeth-Munsell disk colorimeter, the Gardner color difference meter, and the Photovolt-610 reflectance meter. Dean and Ball (1960) used the Gardner a_L value as a measure of redness or bloom in beef.

Other reflectance methods which could be used for estimating consumer acceptability of beef color measure the concentration of the myoglobin pigments with a reflectance attachment to a spectrophotometer. Dean and Ball (1960) calculated % Mb, % MbO₂ and % MetMb by using ratios of K/S values, the ratio of absorption coefficient over the scattering coefficient (Judd, 1952) of % reflectances at 507 and 573 nm and at 473 and 597 nm. The wavelengths of 507 and 573 nm were chosen, since Broumand et al. (1958) found these to be absorp-

tion isobestic points for MbO₂ and MetMb. Snyder (1965) used R_a values (reflectance value on a log scale corresponding to absorbance, used in transmittance data) from the reflectance spectra after R_a had been adjusted to 1.0 at 525 nm (an isobestic point for the three forms of myoglobin). He measured concentration of the pigments by plotting R_a at 473 nm for MetMb and MbO₂ and at 571 nm for Mb and MbO₂ and from these plots he calculated the concentration of the individual pigments. Standard curves for Mb, MbO₂ and MetMb were made from suspensions of the myoglobin derivatives in nonfat milk. Snyder and Armstrong (1967) found that with suspensions of MetMb or MbO₂ in nonfat milk, the K/S ratio at a single wavelength (571 nm) was sufficient for accurate measurement of MetMb. However, the authors stressed that for intact meat, ratios of K/S values such as proposed by Snyder (1965) were preferable. Stewart et al. (1965) used the K/S ratio at 525 nm to give total pigment concentration. Franke and Solberg (1971) found that % MetMb could be measured using: K/S 572 nm/K/S 525 nm, R_a 572 nm/R_a 525 nm, or a value called ΔR_a 632 nm. The ΔR_a 632 nm was found by setting the instrument at a constant value for 750 nm and subtracting the R_a 632 nm from the constant R_a 750 nm. Van den Oord and Wesdorp (1971b) stated that the myoglobin concentration and the relative proportions of oxy- and metmyoglobin are determined by using the difference R_a 630 nm - R_a 580 nm. These two wavelengths correspond to the minimums of met- and oxymyoglobin respectively. The above papers have established a linear relationship between reflectance measurements and concentration of the various myoglobin pigments. Van den Oord and Wesdorp (1971a) established a relationship between consumer acceptability of color, as related to aging of beef, and R_a 630 nm - R_a 580 nm.

We have investigated the relationships between beef color quality as judged by a trained panel and various types of reflectance measurements. The instruments used to make the measurements were color difference meters and reflectance spectrophotometers. Linear correlation coefficients were obtained for the various parameters measured versus the panel ratings (hedonic scale) as well as the correlation coefficients between the different parameters. The results from this study enable one to select a quick and accurate method for replacing a hedonic evaluation of color in storage studies on beef.

EXPERIMENTAL

Procedure

Eye-round roasts (USDA Choice semitendinosus muscles) obtained from commercial sources were used in the experiments. All equipment was scrubbed with a 3% hexachlorophene solution and rinsed with 70% ethanol to minimize bacterially produced discoloration of the meat samples. The eye-rounds were carefully trimmed to remove all fat and to expose fresh surfaces. Samples, approximately 1 × 1 × 2 in., were sliced across the grain from the trimmed roasts. One sample from each run (oxidized control) was sprayed with 1 ml of 1/2% K₃Fe(CN)₆ solution. All samples were wrapped in an oxygen permeable commercial wrap (PVC stretch film MC-FMC Corp.). The samples were packaged to approximate commercial supermarket methods, i.e., close contact with the meat surface. The samples were allowed to bloom at 4°C in the dark for 3 hr before initial hedonic evaluation. The samples were displayed on a white background over an ice bed. Lighting for hedonic appraisal was G.E. Cool White and illumination was an average of 80

ft-c at the meat surface. The color difference meter readings and the reflectance spectrophotometer readings were made immediately after the panel evaluations. A 10-member panel was selected to judge these samples on the basis of color quality. A scale of 0 to 50 was used with 50 being extremely acceptable, 30 being marginally acceptable and 0 being totally unacceptable. Each sample was rated by the panel and these ratings (H values) were compared with values obtained with a color difference meter and a reflectance spectrophotometer. Such procedures were carried out each day for a period of from 5 to 8 days. After this length of time most samples had unacceptable color.

Apparatus

A Beckman D.U. Model 2400 spectrophotometer with an integrating sphere reflectance attachment was used to make reflectance measurements at 520, 540, 560, 580, 600, 620, 630, 640, 650 and 700 nm. A magnesium carbonate block wrapped with the same packaging material as the samples was the reference standard. Color difference measurements were made by a Gardner color difference meter which was balanced using a standard tomato red ceramic plate: R_d 6.1, "a" 32.4, "b" 14.8. A clear glass optical flat was placed between the wrapped meat sample and the source (over the sample port) to provide a flat meat surface for the measurements.

Calculations

Method 1. The linear correlation coefficient for the Gardner "a" value versus the panel or hedonic (H) evaluation was calculated. Since linear correlation coefficients are not normalized functions, their confidence limits cannot be calculated in the normal manner. Confidence limits on the linear correlation coefficient were established by the following type of calculation (Steel and Torrie, 1960):

r = linear correlation coefficient, an estimate of ρ the population correlation coefficient;

Z = transformed r , Z is approximately normal with mean $0.5 \ln [(1+r)/(1-r)]$ and standard deviation $1/\sqrt{N-3}$;

N = number of data points; and

$t_{.05}$ = 1.96 for a normally distributed function:

$$Z = 1/2 \ln [(1+r)/(1-r)]$$

$$Z^- = Z - \frac{1.96}{\sqrt{N-3}}$$

$$Z^+ = Z + \frac{1.96}{\sqrt{N-3}}$$

The 95% confidence limits for ρ are:

$$\frac{e^{2Z^-} - 1}{e^{2Z^-} + 1} \leq \rho \leq \frac{e^{2Z^+} - 1}{e^{2Z^+} + 1}$$

Linear combinations of "a," "R_d" and "b" versus H were also calculated but no significant improvement in fit was noted over the fit with "a" alone. Higher order polynomial fits of "a" versus H were calculated but the advantage of the slightly better fit obtained with a fifth degree polynomial in "a" over a simple correlation was outweighed by the complexity of the calculation necessary.

Method 2. Linear correlation coefficients for %R values versus H or "a" at selected wavelengths and the linear correlation coefficients' 95% confidence limits were calculated as described in Method 1. The wavelengths selected were 580 nm, which is a maximum for MbO₂; 630 nm, which is a maximum for MetMb; and 700 nm, which is independent of H. Linear correlation coefficients and their 95% confidence limits were also calculated for combinations (differences) of the selected wavelengths. The difference in two population values of r was tested by the following variation of a "large sample normal test" (Steel and Torrie, 1960). The example used to illustrate this test is r_1 for "a" versus H and r_2 for H versus %R 630 - %R 580.

N = number of data points;

r = linear correlation coefficient;

Z = transformed r :

	N	r	Z	$\frac{1}{N-3}$
H vs. "a"	629	.910	1.53	.0016
H vs. %R 630 - %R 580	277	.862	130	.0036

$$z = \frac{|Z_1 - Z_2|}{\sqrt{\frac{1}{N_1-3} + \frac{1}{N_2-3}}}$$

where z is normally distributed with a mean of zero and a standard deviation of 1. P , the probability that $\rho_1 = \rho_2$, is calculated by

$$P = 2 \int_z^{\infty} \frac{1}{\sqrt{2\pi}} e^{-X^2/2} dX$$

For this example $P = 0.001$, and it is safe to assume $\rho_1 > \rho_2$.

Method 3. Linear correlation coefficients for the logarithmic transformations of the %R at the selected wavelengths versus H or "a" and their 95% confidence limits were calculated as described in Method 1. Combinations (differences) of the selected wavelengths are expressed as logarithms of the ratios of the wavelengths. Comparisons were made among the linear correlation coefficients found using the different methods described in the manner shown in Method 2.

RESULTS & DISCUSSION

FIGURE 1 represents the reflectance spectra in terms of %R of three forms of myoglobin. Myoglobin itself is unstable in air and readily oxygenated to the oxymyoglobin form. The reflectance spectrum of oxymyoglobin has minimums between 540 nm and 580 nm and high reflectance in the 600-700 nm region, while the reflectance spectrum of metmyoglobin has increased reflectance in the 540-580 nm or yellow region and decreased reflectance in the red region, particularly at 630 nm. Meat surfaces which contain a high percentage of the oxymyoglobin form of the pigment are bright red in color while those surfaces which contain most of their pigment in the metmyoglobin form are brown or dark yellow in color.

Since the proportion of red color present on the surface of the meat was the criterion used for evaluating the acceptability of the meat color to consumers, the Gardner color difference

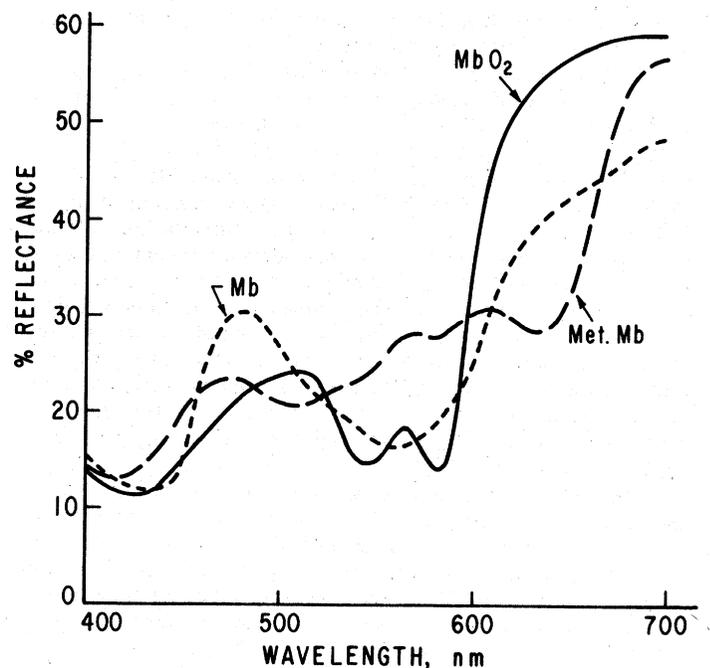


Fig. 1—Reflectance spectra of Mb, MbO₂, and MetMb in terms of % reflectance.

Table 1—Correlation of H^a with reflectance at selected wavelengths

Source of reflectance values	Linear correlation coefficient	95% Confidence limits	
%R 580 nm	-0.66	-0.59,	-0.72
%R 630 nm	0.78	0.72,	0.82
%R 700 nm	0.21 ^b	0.10,	0.32
%R 700 nm - %R 580 nm	0.57	0.48,	0.64
%R 700 nm - %R 630 nm	-0.64	-0.57	-0.71
%R 630 nm - %R 580 nm	0.86	0.83,	0.89
2-1n %R 580 nm	0.70	0.63,	0.75
2-1n %R 630 nm	-0.76	-0.71	-0.81
2-1n %R 700 nm	-0.14 ^b	-0.02,	-0.25
1n (%R 580 nm/%R 700 nm)	-0.69	-0.62,	-0.74
1n (%R 630 nm/%R 700 nm)	0.64	0.57,	0.71
1n (%R 580 nm/%R 630 nm)	-0.86	-0.83,	-0.89

^a Consumer acceptability of color

^b Not significant at 99% confidence level. N for all samples is 277.

meter value "a," which is a mathematical representation involving a weighted integration of the reflectance primarily from the red region of the visible spectrum, was correlated with the hedonic (H) or panel score. The linear correlation coefficient (r) found for "a" versus H (hedonic score) is 0.91 with 95% confidence limits of (0.90, 0.92).

The reflectance spectra were determined using an integrating sphere reflectance instrument. The reflectance at three wavelengths 580 nm (a minimum for MbO₂), 630 nm (a minimum for MetMb) and 700 nm (highest reflectance found for the meat samples in the visible region of the spectrum) were selected for correlation with H.

Table 1 is a compilation of the linear correlation coefficients and their 95% confidence limits for the selected wavelengths versus the hedonic (H). Linear correlation coefficients were evaluated for both %R and 2-1n %R at the selected wavelengths versus H. The 2-1n %R values were included in the analysis of data because instrumentation in spectrophotometers often is designed to permit greater precision in reading absorbance or 2 - log %R values. Logarithmic trans-

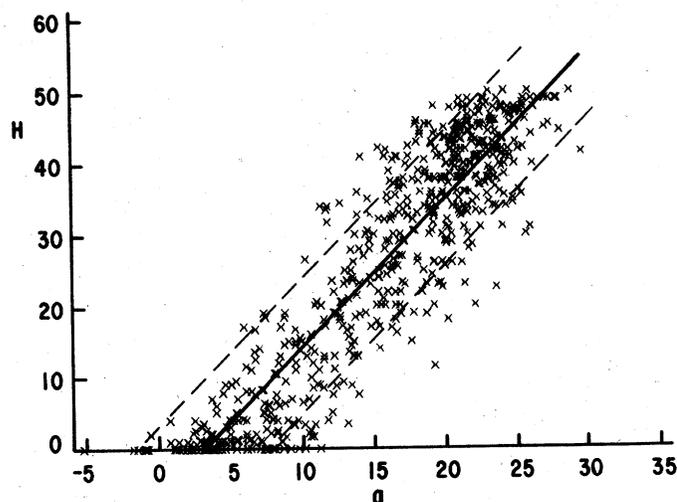


Fig. 2—Gardner Color Difference meter "a" vs. hedonic H.

Table 2—Correlation of "a"^a with reflectance at selected wavelengths

Source of reflectance values	Linear correlation coefficient	95% Confidence limits	
%R 580 nm	-0.71	-0.65,	-0.76
%R 630 nm	0.78	0.73,	0.83
%R 700 nm	0.24 ^b	0.12,	0.34
%R 700 nm - %R 630 nm	-0.64	-0.56,	-0.70
%R 630 nm - %R 580 nm	0.89	0.86,	0.91
2-1n %R 580 nm	0.73	0.67,	0.78
2-1n %R 630 nm	-0.78	-0.73,	-0.82
2-1n %R 700 nm	-0.14 ^b	-0.02,	-0.26
1n (%R 580 nm/%R 700 nm)	-0.72	-0.66,	-0.77
1n (%R 630 nm/%R 700 nm)	0.66	0.59,	0.72
1n (%R 580 nm/%R 630 nm)	-0.90	-0.88,	-0.92

^a From the Gardner Color Difference Meter

^b Not significant at the 99% level. N for all samples is 277.

formations permit small variations in straight line fits to be eliminated. Although the use of natural instead of common logarithms, as employed here, will change the slope of the line, the linear correlation coefficients are not affected.

The extremely low linear correlation coefficients for H versus reflectance at 700 nm indicates that the reflectance at 700 nm is independent of both MbO₂ and MetMb concentrations. With a plot of H versus reflectance at 580 nm, the decrease in MbO₂ concentration is apparent. Similarly a plot of H versus reflectance at 630 nm shows an increase in MetMb concentration. However, linear correlation coefficients for both %R and for 2-1n %R at both 580 and 630 nm versus the hedonic are significantly lower than the linear correlation coefficient found for "a" versus H. The low values for these correlation coefficients indicate that a combination of wavelengths or other correction is needed.

Comparison of the linear correlation coefficients for the reflectance, either % or 1n%, at 630 and 580 nm versus H with the linear correlation coefficients for the differences in reflectance for either the %R or 1n %R, of 700 nm - 580 nm and

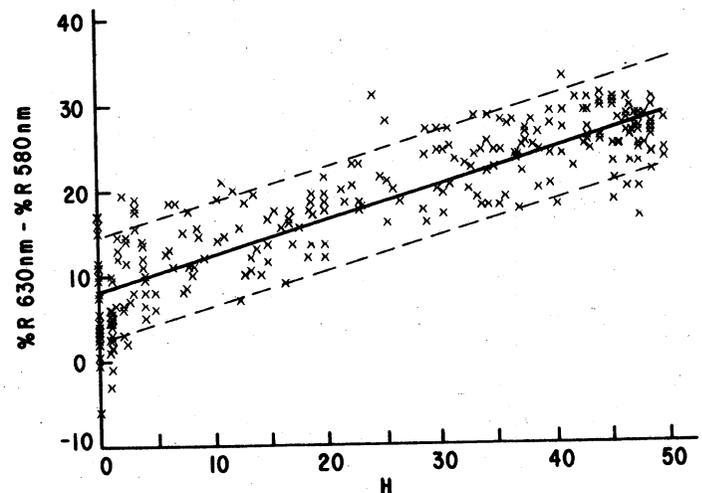


Fig. 3—Hedonic H vs. %R 630 nm - %R 580 nm.

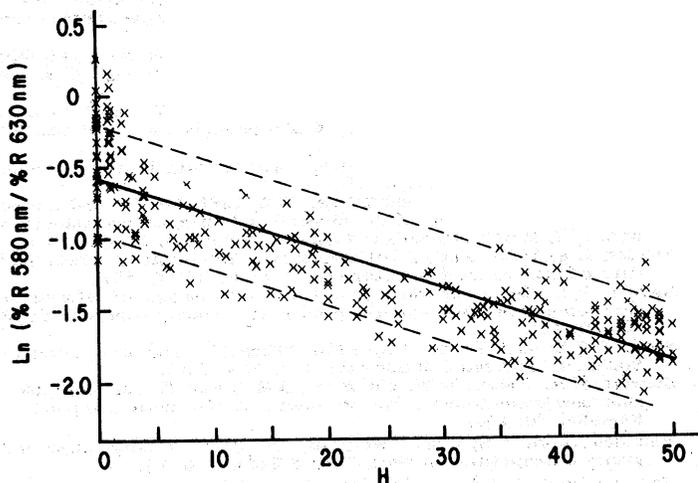


Fig. 4—Hedonic H vs. $\ln (\%R 580 \text{ nm} / \%R 630 \text{ nm})$.

700 nm - 630 nm, shows no improvement in a straight line fit with H. However, the linear correlation coefficients for the differences involving the wavelengths 630 nm and 580 nm show improvement in straight line fits with the hedonic score. This type of combination takes into account both the appearance of MetMb and the disappearance of MbO₂.

Figures 2, 3 and 4, respectively, show the graphs of "a" versus H, H versus %R 630 nm - %R 580 nm, and H versus $\ln (\%R 580 \text{ nm} / \%R 630 \text{ nm})$. Visual analysis of the graph of "a" versus H shows clustering of points about the values of 0 and 50 on the hedonic scale. This clustering is caused by boundary effects. The H value is not as elastic as the "a" value in describing either extreme in meat quality. This effect can be noted in all the graphs where the H value is one of the coordinates. A 5th degree polynomial fit of "a" with H was found. The curve given by this polynomial was sigmoid in shape with flattening about 0 and 50 of H. Since we have assumed that this flattening is caused by boundary effects in H, a straight line fit should give a more accurate measure of the color of the meat.

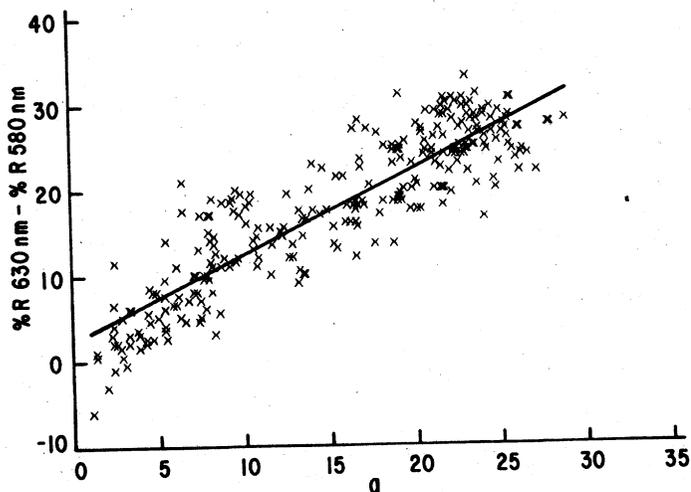


Fig. 5—Gardner Color Difference meter "a" vs. %R 630 nm - %R 580 nm).

To check this hypothesis, comparisons of the two instrumental methods were made. The linear correlation coefficients found are listed in Table 2. The P value for the comparison of H versus %R 630 nm - %R 580 nm with "a" versus %R 630 nm - %R 580 nm is 0.156 and the P value for the comparison H versus $\ln (\%R 580 \text{ nm} / \%R 630 \text{ nm})$ with "a" versus $\ln (\%R 580 \text{ nm} / \%R 630 \text{ nm})$ is .036. These P values demonstrate the improvement in straight line fits, as shown by higher (in absolute value) linear correlation coefficients caused by eliminating the H value and substituting the "a" value from the Gardner color difference meter. The higher linear correlation coefficients are probably due to the objectivity of the instruments versus the subjectivity of the panel and support the boundary effect hypothesis. Visual inspection of the graphs for "a" versus %R 630 nm - %R 580 nm (Fig. 5) and for "a" versus $\ln (\%R 580 \text{ nm} / \%R 630 \text{ nm})$ (Fig. 6) shows a decrease in the clustering of points at both ends of the lines.

All data reported here were obtained on two instruments, a Gardner color difference meter and a Beckman D.U. spectrophotometer with an integrating sphere reflectance attachment. Similar instruments were evaluated for comparableness. A Neotec color difference meter and a Color Master color difference meter were checked. The absolute value of "a" or redness for these instruments differed from the Gardner "a" value but analysis of the data obtained from these instruments indicated that either instrument could be used to replace a hedonic evaluation of meat samples if a standard line of "a" or red value versus H was first obtained. Data from two spectrophotometers, a Beckman DBG and a B&L Spectronic 20, both equipped with integrating sphere reflectance attachments, gave extremely close agreement with the data obtained using the Beckman D.U.

From the linear correlation coefficients the three most effective methods for replacing the H are the Gardner "a" value, %R 630 nm - %R 580 nm and $\ln (\%R 580 \text{ nm} / \%R 630 \text{ nm})$. The P value for the comparison of "a" versus H with H versus %R 630 nm - %R 580 nm is .001 and the P value for the comparison of "a" versus H with H versus $\ln (\%R 580 \text{ nm} / \%R 630 \text{ nm})$ is .002. These P values indicate that the "a" value from the Gardner color difference meter is the most accurate replacement for the hedonic. The colorimeter requires less than 30 sec per measurement and can therefore be employed for large numbers of samples. Its main disadvantage is that a standard curve must be found for each different type of color difference meter used.

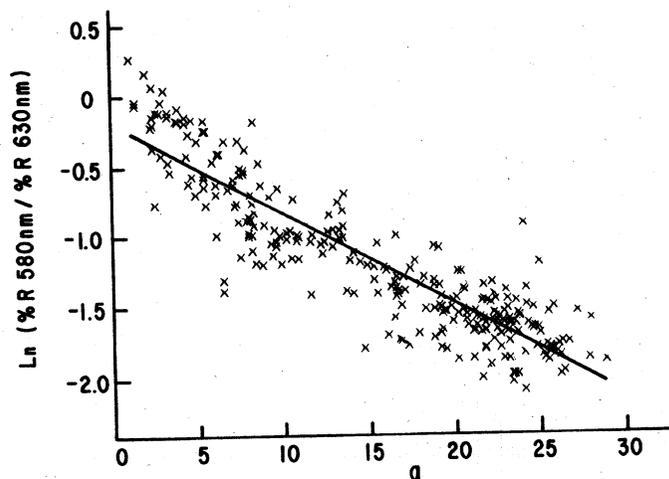


Fig. 6—Gardner Color Difference meter "a" vs. $\ln (\%R 580 \text{ nm} / \%R 630 \text{ nm})$.

The spectrophotometric methods outlined are slower, do not give quite as good agreement with the panel evaluation and entail expensive equipment. The main advantages of a reflectance spectrophotometric method are that the results from one instrument are comparable to results from another instrument, provided that the same type reflectance attachment is used, and that spectrophotometers are commonly available in laboratories. Analysis of the linear correlation coefficients of H versus %R 630 nm - %R 580 nm and H versus \ln (%R 580 nm/%R 630 nm) gives a P value of .90. This P value indicates that there is no reason to prefer one method of calculation over the other.

The use of either colorimeters or reflectance spectrophotometers provides a quick, accurate replacement for panel or hedonic evaluations of meat color. Both methods require little training on the part of the operator for either procedure or interpretation.

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