Meat and Muscle Biology™

Effect of Instrument Settings and Measurement Environment on Pork Color Measurements and Variability

K. E. Barkley[1], H. Rode[2], D. R. McKenna[2], D. D. Boler[1], B. N. Harsh[1], and A. C. Dilger[1]*

[1]Department of Animal Sciences, University of Illinois Urbana-Champaign, Urbana, IL 61801, USA
[2]Tyson Fresh Meats Inc., Dakota Dunes, SD 57049, USA
*Corresponding author. Email: adilger2@illinois.edu (A. C. Dilger)

Abstract: The study objectives were to determine how different instrumental settings and measurement environments affect the means and variability of instrumental muscle color in pork loins and Boston butts (*serratus ventralis*). Three studies were conducted testing different variables; study 1 tested aperture type (closed vs. open), study 2 tested illuminant (D65 vs. C), and study 3 tested measurement environment (commercial facility vs. university; loins only). Within each set of loins and Boston butts, the 100 greatest and lowest lightness (L^*), redness (a^*), and yellowness (b^*) measurements were determined for each machine/setting combination. Color data within a set were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Variances and coefficient of variation were calculated using the MEANS procedure. Coefficients of determination between machines within a set were calculated using the REG procedure. L^* and a^* measurements in loins and Boston butts were more variable when using an open aperture than a closed aperture ($P \leq 0.02$). Illuminant did not affect L^* or a^* variability in either muscle ($P \geq 0.16$). In loins and Boston butts, measurements from machine 1 explained 11%–54% ($P < 0.0001$) of variation in machine 2 measurements when settings differed, and there was 17%–65% agreement between machines for extreme values. In loins, machine 1 measurements explained 41%–49% ($P < 0.0001$) of variation in machine 2 measurements under commercial conditions and 86%–92% ($P < 0.0001$) under controlled conditions. With identical settings, there was 49%–73% agreement between machines for the greatest and lowest 100 values under commercial conditions and 84%–90% agreement under university conditions. Overall, using a closed aperture decreased overall color variability compared with using an open aperture. However, it is difficult to compare studies measuring instrumental color when operational settings differ or the environment is not well controlled.

Key words: color, instrumental color, pork, variation, variability
Meat and Muscle Biology 5(1): 27, 1–15 (2021) **doi:10.22175/mmb.12245**
Submitted 21 January 2021 Accepted 13 April 2021

Introduction

Color is one of the most important traits when evaluating fresh pork quality (Mancini and Hunt, 2005). When conducting meat quality research, investigators will often use a color-detecting instrument to obtain objective color measurements. When measuring instrumental color, a wide variety of operational settings exist that make replicability of measurements between groups difficult. Barkley et al. (2018) reported that when settings between machines of the same model were kept constant and measurements were obtained in the same environment, differences in color traits were not large enough to be of practical significance. However, it may not always be practical for researchers or technicians to use identical settings, because not all research groups collect color data using the same equipment. Furthermore, even if 2 machines use identical settings, observations may be collected in different environments (e.g., university lab vs. processing plant), contributing further variation to instrumental readings.

Variability in instrumental fresh pork color has primarily been addressed using the *longissimus* (Overholt et al., 2016; Arkfeld et al., 2017), and limited data are available for other muscles. Arkfeld et al. (2016) reported that instrumental lightness and redness variability differed among the *longissimus* (L^* coefficient of variation [CV] = 4.73, a^* CV = 15.55), *gluteus profundus* (L^* CV = 8.89, a^* CV = 13.79), *gluteus medius* (L^* CV = 7.38, a^* CV = 20.19), and *semimembranosus* (L^* CV = 6.73, a^* CV = 19.53). This suggested that differences in measurement conditions may have more pronounced effects on instrumental variability in some muscles than others.

In order to maximize replicability of instrumental color data, it is important to understand how differences in observation conditions affect color readings. Although it is understood that using different settings will result in different mean values (Brewer et al., 2001), it is not known whether those differences are relative or also have different associated variabilities. Therefore, the objective of this study was to determine how purposefully altering measurement settings affects means and variability of color measurements in loins and Boston butts. It was hypothesized that measuring color under different conditions would result in mean differences that also had different amounts of variability and that the amount of variability contributed would differ between muscles.

Materials and Methods

All samples used in this study were collected from pigs harvested under FSIS supervision at a commercial abattoir; therefore, Institutional Animal Care and Use Committee approval was not necessary. No information was made available to the research team regarding the identity of the animals.

To determine the effect of instrumental settings differences on measurement means and variability, 3 studies were conducted. For each study, instrumental Commission Internationale de l´Eclairage (CIE; "International Commission on Illumination") lightness (L^*), redness (a^*), and yellowness (b^*) (CIE, 1978) were measured on all loins using 2 Minolta CR-400 Chroma meters (Minolta Camera Company, Osaka, Japan) equipped with a 2° observer and an 8 mm aperture. The devices were calibrated one time with a white tile specific to that machine at the beginning of each study. Study 1 investigated differences in aperture type (closed vs. open) using the same illuminant (D65). The device with a closed aperture had a glass cover over the opening where color was observed, while the device with an open aperture had no cover over the opening. Study 2 investigated differences in illuminant (D65 vs. C) using the same aperture type (closed). Study 3 investigated differences in measurement environment (commercial plant setting vs. university setting; loins only) with identical instrumental settings. Two separate sets of loins were used in study 3; the loins measured in a commercial plant were different from those measured in a university setting.

Studies 1 and 2: Instrumental settings

Pigs used in studies 1 and 2 were immobilized by carbon dioxide stunning and terminated via exsanguination. Carcasses were blast-chilled and transferred into a temperature equilibration cooler. At approximately 22 h postmortem, carcasses were fabricated into primal pieces. Bone-in loins were fabricated into boneless center-cut pork loins (Canadian back loin; NAMP #414; NAMP, 2007), and shoulders were fabricated into bone-in Boston butts (NAMP #406) (NAMP, 2007). Instrumental color measurements were collected in loins at approximately 23 h postmortem and in Boston butts at approximately 25 h postmortem. Loins and Boston butts were removed from respective boning and trimming lines at the time of cutting to be evaluated by trained technicians. Because samples were removed from the boning and trimming line for analysis, they were not given time to oxygenate. All loin color measurements were made on the ventral surface of the loin after back rib removal at the approximate location of the 10th rib. Measurements were assessed on the top of the loin as presented to the technician, with the technician holding the instrument vertically. Color measurements in Boston butts were assessed on the *serratus ventralis* on the face where the Boston butt was removed from the loin. The *serratus ventralis* was chosen to represent the Boston butt because of its large size, ease of access, and value relative to other muscles in the shoulder, making it suitable for quick observations in a processing plant or research setting. Measurements were assessed on the side face of the Boston butt while on the boning and trimming line, with the technician holding the instrument horizontally. For study 1, machine 1 had a closed aperture and machine 2 had an open aperture. For study 2, machine 1 used a D65 illuminant while machine 2 used a C illuminant.

Study 3: Environment

Commercial loins used in study 3 ($n = 600$) were collected and measured in the same manner as loins

from studies 1 and 2. An additional, separate set of loins ($n = 250$) was evaluated at the University of Illinois Meat Science Laboratory (Urbana, IL) under more controlled conditions than in the commercial facility. These loins were placed on tables and allowed to oxygenate for at least 20 min prior to evaluation. Loins were measured by a single technician using 2 Minolta CR-400 Chroma meter devices on the ventral face at the approximate location of the 10th rib. For each loin, 3 consecutive measurements were observed on ventral surface using the first Minolta, and then an additional 3 measurements were observed in the exact same location with the second Minolta. The 2 Minolta devices used for this set of loins were different from the devices used for measurements at the commercial facility.

Statistical analyses

Color data within a study set were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) as a 1-way ANOVA with 2 treatments: machine 1 and machine 2. Variances were calculated using the MEANS procedure and tested for homogeneity using the Levene test of the GLM procedure. CV were calculated in addition to variances as a means of comparing variability between loins and Boston butts. However, because of the occurrence of several negative b^* values (indicating that samples were more blue than yellow) in the data, yellowness CV may not be suitable for comparisons between muscles (Livers, 1942). Means and variances were considered different at $P \leq 0.05$.

Within each set, the 100 darkest, the 100 least red, and the 100 least yellow measurements assessed using each Minolta were determined and analyzed in the same manner as the full data sets. This was also repeated with the 100 lightest, 100 reddest, and 100 most yellow measurements. These measurements were then used to determine which loins had the 100 greatest or 100 lowest lightness, redness, or yellowness values when measured using both machines for each set, e.g., if the loins that had the 100 lightest measurements when observed using the first machine also had the 100 lightest measurements when observed using the second machine (percentage machine agreement). For each set (lightest/darkest, least/most red, and least/most yellow), the frequency of loins or Boston butts that appeared in both groups regardless of instrument type was calculated. This analysis was used to determine whether samples selected to meet a certain criterion, i.e., light loins for a certain consumer base, were selected consistently regardless of the machine type used. Ideally, the 100 samples with the highest lightness values measured using one machine would be the same 100 samples determined to be lightest when using the second machine.

Coefficients of determination (R^2) were calculated using the REG procedure of SAS and used to determine the ability for the measurements observed using the first Minolta to predict measurements observed using the second Minolta for each study. Excessive influence of individual observations on estimated prediction equations were determined using the Difference of Fit (DFITTS) statistic. Observations were determined to have excessive influence when DFITTS > 2 $[(p/n)^{1/2}]$, in which p = the number of parameters and n = the number of observations. Observations that met this criterion were removed from the dataset for regression analyses.

Results

Study 1: Aperture

Loins. Open aperture measurements ($n = 538$) were 1.93 L^* units lighter ($P < 0.0001$), 2.08 a^* units less red ($P < 0.0001$), and 4.89 b^* units more yellow ($P < 0.0001$) than measurements evaluated using a closed aperture ($n = 538$; Table 1). Lightness and redness measurements had greater variances ($P < 0.01$) and CV when measured using an open aperture than when using a closed aperture. Variance in yellowness did not differ between instruments ($P = 0.34$), while closed aperture measurements had greater CV than open aperture measurements. Lightness values assessed on loins using a closed aperture explained 54% of variation in lightness values measured using an open aperture ($R^2 = 0.54$, $P < 0.0001$; Table 1), redness values observed using a closed aperture explained 48% of variation in open aperture redness ($R^2 = 0.48$, $P < 0.0001$), and yellowness values observed using closed aperture explained 41% of variation in open aperture yellowness ($R^2 = 0.41$, $P < 0.0001$).

Differences in means and variability of the 100 lowest (darkest, least red, and least yellow) and 100 greatest (lightest, reddest, and most yellow) color values between machines with different aperture settings are shown in Figure 1. Measurements assessed using an open aperture were lighter, less red, and more yellow for low and high measurements ($P < 0.0001$). No color value variances differed between machines ($P \geq 0.08$) with the exception of the most red measurements, which were more variable when using an open

Table 1. Instrumental color least squares means, variability, and prediction ability for loins and Boston butts when using different aperture types.

	Loins[1]			Boston Butts[1]		
	Closed[2]	Open[2]	*P*-value	Closed[2]	Open[2]	*P*-value
Lightness (L)*[3]						
Samples, n	538	538		504	499	
Mean	47.8	49.73	<0.0001	41.34	44.51	<0.0001
Variance	7.47	9.61	<0.01	5.11	6.9	<0.01
CV (%)	5.72	6.23		5.47	5.90	
Slope[4]	0.83			0.39		
R^2	0.54		<0.0001	0.11		<0.0001
Redness (a)*[3]						
Samples, n	538	538		504	499	
Mean	7.71	5.63	<0.0001	15.87	15.89	0.84
Variance	0.98	1.31	<0.01	2.69	3.49	0.01
CV (%)	12.84	20.33		10.33	11.76	
Slope[4]	0.81			0.52		
R^2	0.48		<0.0001	0.21		<0.0001
Yellowness (b)*[3]						
Samples, n	538	538		504	499	
Mean	-1.23	3.66	<0.0001	-0.28	6.39	<0.0001
Variance	0.79	0.85	0.34	1.03	1.33	0.02
CV (%)	-72.26	25.19		-362.46	18.05	
Slope[4]	0.67			0.53		
R^2	0.41		<0.0001	0.21		<0.0001

[1]Samples were measured using a Minolta CR-400 Chromameter colorimeter equipped with a 2° observer, 8 mm aperture, and calibrated with a white tile specific to that machine. Devices used in this study also had a D65 illuminant.

[2]Aperture type that was used for each machine. "Closed" refers to an aperture that was covered with a piece of glass, while "Open" refers to an aperture that had no covering.

[3]L* measures darkness to lightness (greater L* indicates a lighter color), a* measures redness (greater a* indicates a redder color), b* measures yellowness (greater b* indicates a more yellow color).

[4]Slope and R2 statistics were determined using closed aperture measurements as the predictive variable and open aperture measurements as the dependent variable.

aperture ($P = 0.02$). The darkest and most yellow measurements had greater CV when measured using a closed aperture, whereas the lightest, least red, reddest, and least yellow measurements had greater CV when measured using an open aperture. There was 65% agreement between machines for the darkest observations and 52% agreement between machines for the lightest observations. The least red observations had 58% agreement between machines, and the reddest measurements had 53% agreement between machines. There was 58% agreement between machines for the least yellow measurements and 47% agreement between machines for the most yellow measurements.

Boston butts. Measurements on Boston butts evaluated using an open aperture ($n = 499$) were 3.17 *L** units lighter ($P < 0.0001$) and 6.67 *b** units more yellow ($P < 0.0001$) than measurements evaluated using a closed aperture ($n = 504$) but did not differ in redness between instruments ($P = 0.84$; Table 1). Lightness, redness, and yellowness were all more variable when measured using an open aperture compared with a closed aperture ($P \leq 0.02$). Lightness values assessed on Boston butts using a closed aperture explained 11% of variation in lightness values measured using an open aperture ($R^2 = 0.11$, $P < 0.0001$), while redness values observed using a closed aperture explained 21% of variation in open aperture redness ($R^2 = 0.21$, $P < 0.0001$), and yellowness values observed using a closed aperture explained 21% of variation in open aperture yellowness ($R^2 = 0.21$, $P < 0.0001$).

Differences in means and variability for the 100 lowest (darkest, least red, and least yellow) and 100 greatest (lightest, reddest, and most yellow) Boston butt color values between machines with different aperture settings are shown in Figure 2. Measurements evaluated using an open aperture were lighter, less red, and more yellow for low and high values ($P \leq 0.04$). Variances of the lightest, least red, reddest, and most yellow

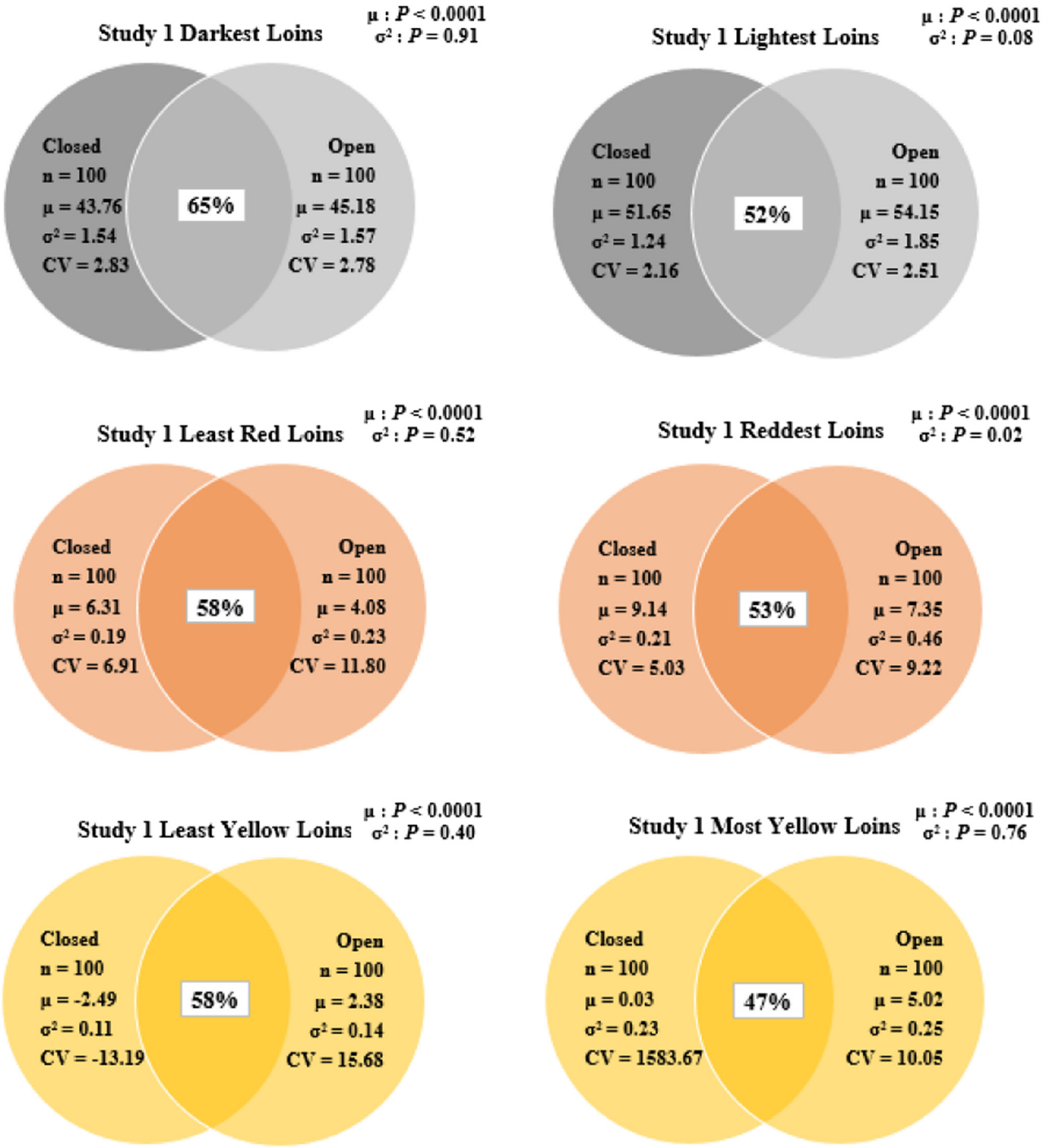

Figure 1. Proportion of loin color measurements observed with each machine that were among the greatest or lowest measurements when measured using machines with different apertures.

measurements did not differ between machines ($P \geq 0.18$), while the darkest and least yellow measurements had greater variances when measured using an open aperture ($P \leq 0.01$). The darkest, least red, and least yellow measurements had greater CV when measured using an open aperture, whereas the lightest, reddest, and most yellow measurements had greater CV when measured using a closed aperture. There was 47% agreement between machines for the darkest measurements and 30% agreement for the lightest measurements. Both the least red and reddest measurements had 24% agreement between machines. There was 39% agreement between machines for the least yellow Boston butts and 38% agreement between machines for the most yellow Boston butts.

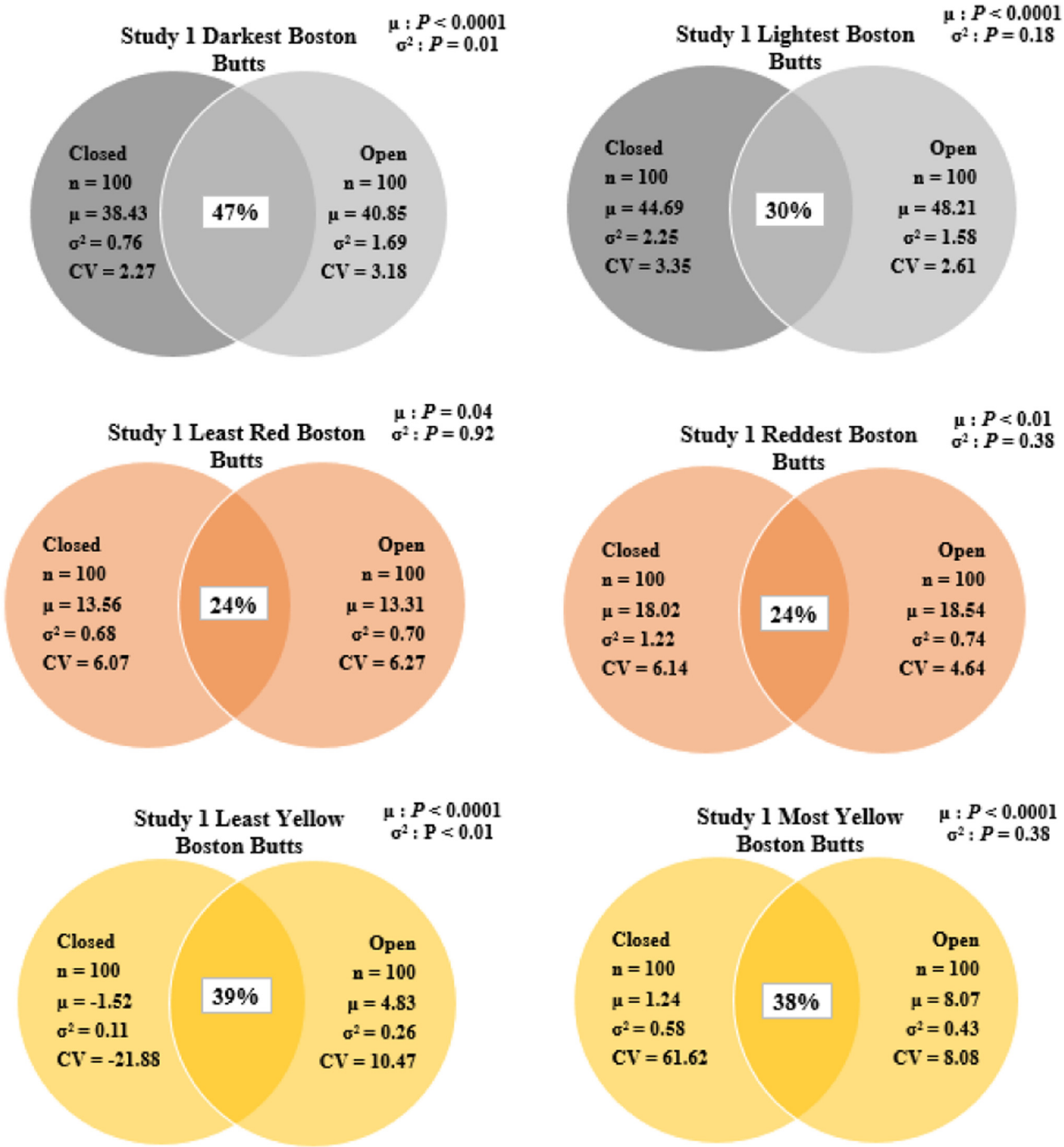

Figure 2. Proportion of Boston butt color measurements observed with each machine that were among the greatest or lowest measurements when measured using machines with different apertures.

Study 2: Illuminant

Loins. C illuminant measurements ($n = 589$) were 1.16 L^* units darker ($P < 0.0001$) and 0.61 b^* units more yellow ($P < 0.0001$) than measurements evaluated using a D65 illuminant ($n = 598$), but redness measurements did not differ between machines ($P = 0.16$). No color traits variances differed between instruments ($P \geq 0.21$), while C illuminant CV were lower than D65 illuminant values for lightness and greater for redness and yellowness. Lightness values assessed on loins using a D65 illuminant explained 48% of variation in lightness values measured using a C illuminant ($R^2 = 0.48$, $P < 0.0001$), whereas redness values observed using a D65 illuminant explained 40% of variation in

C illuminant redness ($R^2 = 0.40$, $P < 0.0001$), and yellowness values observed using a D65 illuminant explained 43% of variation in C illuminant yellowness ($R^2 = 0.43$, $P < 0.0001$).

Differences in means and variability of the 100 lowest and 100 greatest loin color values between machines with different illuminant settings are shown in Figure 3. The highest and lowest average L^* and b^* values were greater when using D65 illuminant ($P < 0.0001$), whereas redness values did not differ ($P \geq 0.16$). No color value variances differed between machines ($P \geq 0.29$). The darkest, least red, least

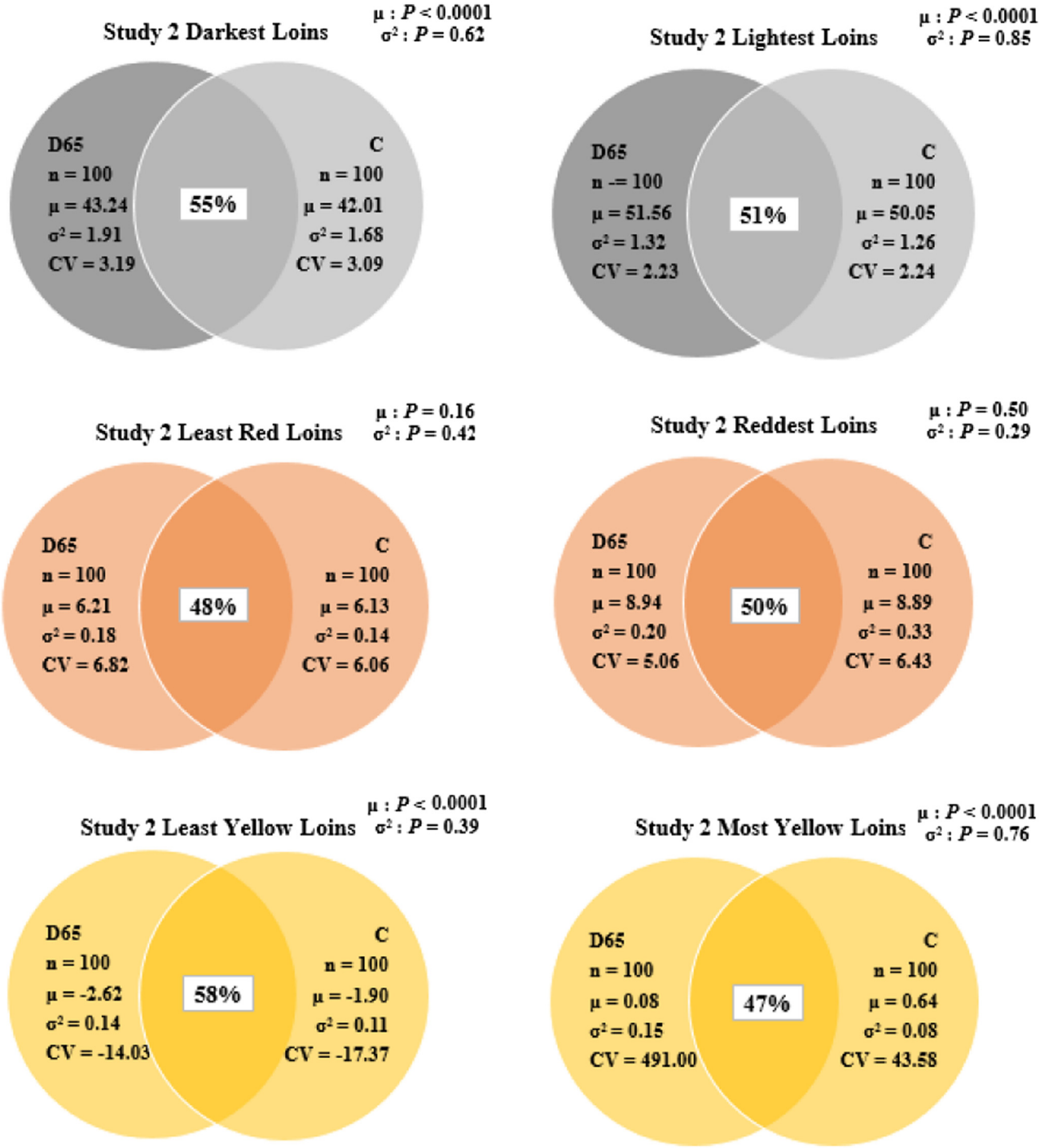

Figure 3. Proportion of loin color measurements observed with each machine that were among the greatest or lowest measurements when measured using machines with different illuminants.

yellow, and most yellow measurements had greater CV when measured using a D65 illuminant, whereas the lightest and reddest measurements had greater CV when measured using a C illuminant. There was 55% agreement between machines for the darkest measurements and 51% agreement between machines for the lightest measurements. The least red measurements had 48% agreement between machines, and the reddest measurements had 50% agreement between machines. There was 58% agreement between machines for the least yellow measurements and 47% agreement between machines for the most yellow measurements.

Boston butts. Measurements on Boston butts evaluated using a C illuminant ($n = 523$) were 2.37 L^* units darker ($P < 0.0001$), 0.78 a^* units less red ($P < 0.0001$), and 1.49 b^* units more yellow ($P < 0.0001$) than measurements made using a D65 illuminant ($n = 521$; Table 2). Yellowness was more variable when measured using a D65 illuminant than a C illuminant ($P = 0.02$), but lightness and redness variance did not differ between instruments ($P \geq 0.16$). Lightness values assessed using a D65 illuminant explained 26% of variation in lightness values measured using a C illuminant ($R^2 = 0.26$, $P < 0.0001$), whereas redness values observed using a D65 illuminant explained 15% of variation in C illuminant redness ($R^2 = 0.15$, $P < 0.0001$), and yellowness values observed using a D65 illuminant explained 28% of variation in closed C yellowness ($R^2 = 0.28$, $P < 0.0001$).

Differences in means and variability for the 100 lowest and 100 greatest Boston butt color values between machines with different illuminant settings are shown in Figure 4. Measurements evaluated using a D65 illuminant were lighter, redder, and less yellow for low and high values ($P < 0.0001$). No color values except the least yellow measurements differed in variance between machines ($P \geq 0.08$), whereas the least yellow values had a greater variance when measured using a D65 illuminant ($P = 0.02$). The lightest, least red, least yellow, and most yellow values had greater

Table 2. Instrumental color least squares means, variability, and prediction ability for loins and Boston butts when using different illuminants.

	Loins[1]			Boston Butts[1]		
	D65[2]	C[2]	*P*-value	D65[2]	C[2]	*P*-value
Lightness (L)*[3]						
Samples, n	598	589		521	523	
Mean	47.32	46.16	<0.0001	40.19	37.82	<0.0001
Variance	7.23	7.29	0.91	6.64	5.39	0.17
CV (%)	5.68	5.85		6.41	6.14	
Slope[4]	0.7			0.52		
R^2	0.48		<0.0001	0.18		<0.0001
Redness (a)*[3]						
Samples, n	598	589		521	523	
Mean	7.51	7.43	0.13	16.07	15.29	<0.0001
Variance	0.85	0.86	0.83	2.28	1.92	0.16
CV (%)	12.28	12.48		9.40	9.06	
Slope[4]	0.64			0.35		
R^2	0.40		<0.0001	0.15		<0.0001
Yellowness (b)*[3]						
Samples, n	598	589		521	523	
Mean	-1.29	-0.68	<0.0001	-0.49	1.00	<0.0001
Variance	0.8	0.72	0.21	1.11	0.90	0.02
CV (%)	-69.34	-124.78		-215.01	94.87	
Slope[4]	0.62			0.48		
R^2	0.43		<0.0001	0.28		<0.0001

[1]Samples were measured using a Minolta CR-400 Chromameter colorimeter equipped with a 2° observer, 8 mm aperture, and calibrated with a white tile specific to that machine. Devices used in this study also had a closed (covered) aperture.

[2]Illuminant type that was used for each machine.

[3]L* measures darkness to lightness (greater L* indicates a lighter color), a* measures redness (greater a* indicates a redder color), b* measures yellowness (greater b* indicates a more yellow color).

[4]Slope and R2 statistics were determined D65 illuminant measurements as the predictive variable and C illuminant measurements as the dependent variable.

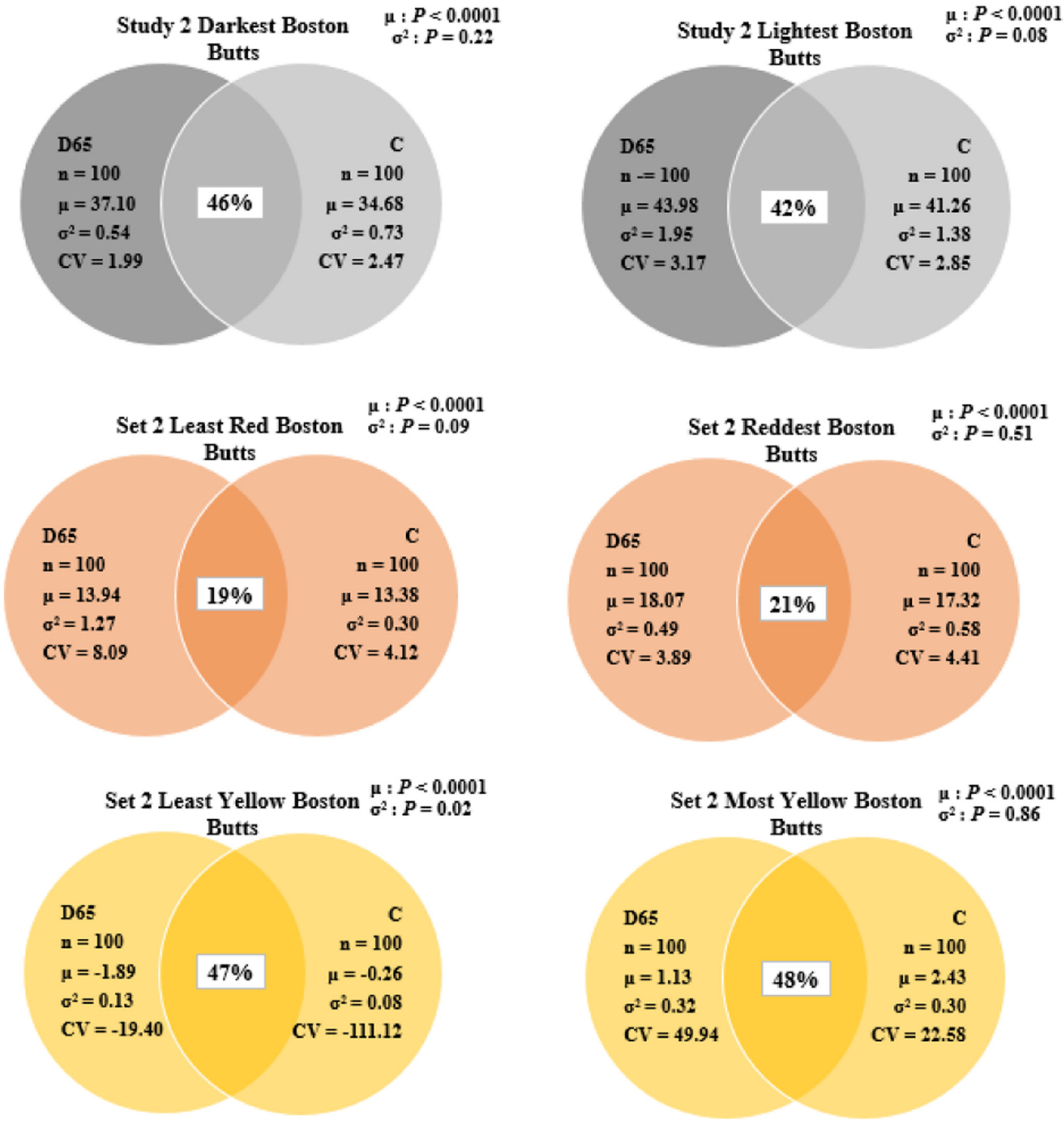

Figure 4. Proportion of Boston butt color measurements observed with each machine that were among the greatest or lowest measurements when measured using machines with different illuminants.

CV when measured using a D65 illuminant, whereas the darkest and reddest values had greater CV when measured using a C illuminant. There was 46% agreement between machines for the darkest Boston butt measurements and 42% agreement between machines for the lightest Boston butt measurements. The least red measurements had 19% agreement between machines, and the reddest measurements had 21% agreement between machines. There was 47% agreement between machines for the least yellow measurements and 48% agreement between machines for the most yellow measurements.

Study 3: Environment

Commercial plant setting. Loin measurements evaluated in a commercial plant using the first machine ($n = 599$) were 1.66 L^* units darker ($P < 0.0001$), 2.66

*a** units less red ($P < 0.0001$), and 3.45 *b** units more yellow ($P < 0.0001$) than measurements evaluated using the second machine ($n = 600$; Table 3). No color traits variances differed between machines ($P \geq 0.11$). Lightness, redness, and yellowness CV were greater when using machine 2 than machine 1. Lightness values assessed on loins using machine 1 explained 49% of variation in lightness values measured by machine 2 ($R^2 = 0.49$, $P < 0.0001$), whereas redness values observed using machine 1 explained 42% of variation in machine 2 redness ($R^2 = 0.42$, $P < 0.0001$), and yellowness values observed using machine 1 explained 41% of variation in machine 2 yellowness ($R^2 = 0.41$, $P < 0.0001$).

Differences in means and variability for the 100 lowest and 100 greatest color values between machines used in a commercial plant setting are shown in Figure 5. Machine 1 measurements were lighter, redder, and less yellow than machine 2 measurements for high and low values ($P < 0.0001$). No color values differed in variance between machines ($P \geq 0.12$). All color values had greater CV when measured using machine 1. There was 73% agreement between machines for the darkest measurements and 67% agreement between machines for the lightest measurements. The least red measurements had 60% agreement between machines, and the reddest measurements had 49% agreement between machines. There was 55% agreement between machines for the least yellow measurements and 54% agreement between machines for the most yellow measurements.

University setting. Measurements on loins evaluated in a university setting using machine 1 ($n = 250$) were 0.74 *L** units lighter ($P < 0.0001$) and 1.04 *b** units less yellow ($P < 0.0001$) than measurements evaluated using machine 2 ($n = 250$) but did not differ in redness ($P = 0.89$; Table 3). No color measurement variances differed between machines ($P \geq 0.29$). Lightness and yellowness measurements had greater CV when measured using machine 1, whereas redness CV were greater when measured using machine 2. Lightness values assessed on loins using machine 1 explained 92% of variation in lightness values measured

Table 3. Instrumental color least squares means, variability, and prediction ability for loins when measured in a commercial processing facility or a university laboratory.

	Commercial Plant[1]			University[1]		
	Machine 1	Machine 2	*P*-value	Machine 1	Machine 2	*P*-value
Lightness (L)*[2]						
Samples, n	599	600		250	250	
Mean	48.18	46.52	<0.0001	48.01	47.27	< 0.01
Variance	6.74	7.63	0.11	10.71	9.40	0.29
CV (%)	5.39	5.94		6.82	6.49	
Slope[3]	0.66			0.9		
R^2	0.49		<0.0001	0.92		<0.0001
Redness (a)*[2]						
Samples, n	599	600		250	250	
Mean	7.52	4.86	<0.0001	9.30	9.32	0.89
Variance	0.95	0.92	0.73	1.37	1.57	0.30
CV (%)	12.93	19.71		12.57	13.44	
Slope[3]	0.66			0.99		
R^2	0.42		<0.0001	0.86		<0.0001
Yellowness (b)*[2]						
Samples, n	599	600		250	250	
Mean	-1.42	2.03	<0.0001	4.00	5.04	< 0.0001
Variance	0.68	0.74	0.34	1.56	1.67	0.60
CV (%)	-58.15	42.37		31.18	25.67	
Slope[3]	0.62			0.96		
R^2	0.41		<0.0001	0.85		<0.0001

[1]Samples were measured using a Minolta CR-400 Chromameter colorimeter equipped with a 2° observer, 8 mm aperture, and calibrated with a white tile specific to that machine.

[2]L* measures darkness to lightness (greater L* indicates a lighter color), a* measures redness (greater a* indicates a redder color), b* measures yellowness (greater b* indicates a more yellow color).

[3]Slope and R2 statistics were determined D65 illuminant measurements as the predictive variable and C illuminant measurements as the dependent variable.

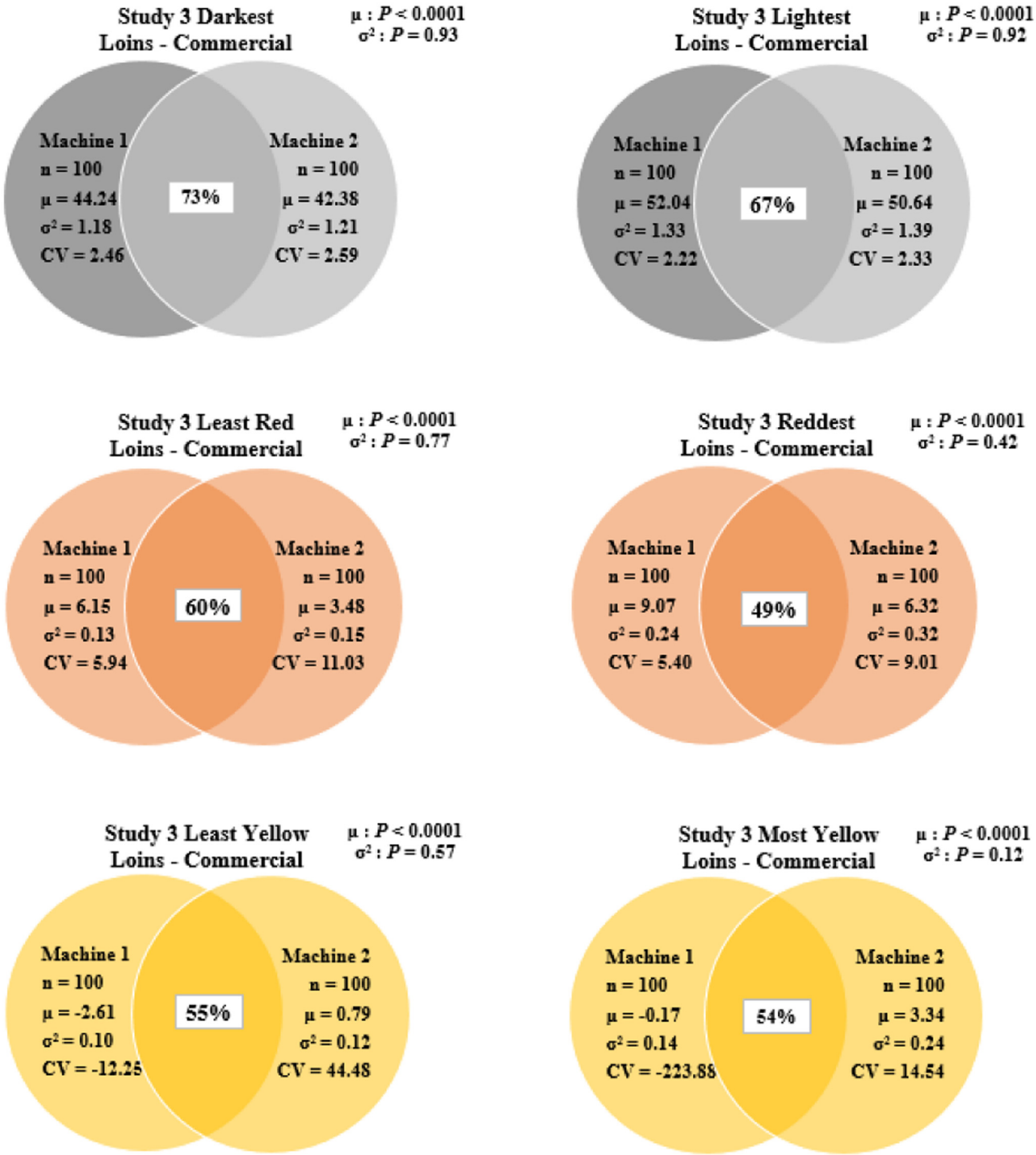

Figure 5. Proportion of loin color measurements observed with each machine that were among the greatest or lowest measurements when measured using machines with identical settings in a commercial plant setting.

by machine 2 ($R^2 = 0.92$, $P < 0.0001$), whereas redness values observed using machine 1 explained 89% of variation in machine 2 redness ($R^2 = 0.89$, $P < 0.0001$), and yellowness values observed using machine 1 explained 88% of variation in machine 2 yellowness ($R^2 = 0.88$, $P < 0.0001$).

Differences in means and variability for the 100 lowest and 100 greatest loin color values measured in a university setting are shown in Figure 6. For this set, the average L^* and b^* values of the high and low groups were different ($P < 0.05$), whereas average a^* values did not differ ($P \geq 0.21$). No color values

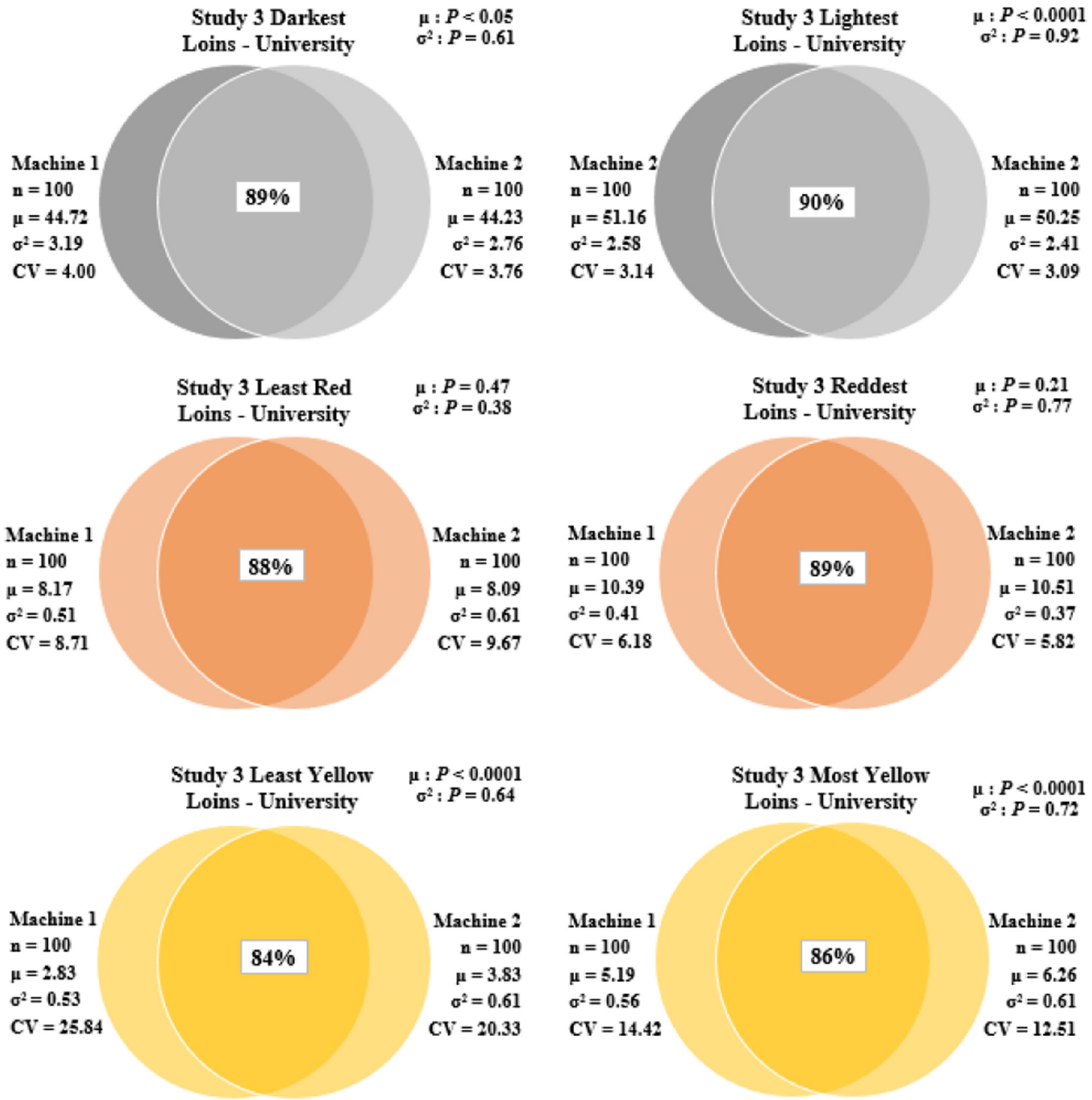

Figure 6. Proportion of loin color measurements observed with each machine that were among the greatest or lowest measurements when measured using machines with identical settings in a university plant setting.

differed in variance between machines ($P \geq 0.38$). All color values except for the least red measurements had greater CV when measured using machine 1; by contrast, the least red measurements had a greater CV when measured using machine 2. There was 89% agreement between machines for the darkest measurements and 90% agreement between machines for the lightest measurements. The least red measurements had 88% agreement between machines, and the reddest measurements had 89% agreement between machines. There was 84% agreement between machines for the least yellow measurements and 86% agreement between machines for the most yellow measurements.

Discussion

When conducting meat color analyses with an instrument, a wide variety of operational settings exist that make replicability of measurements between groups

difficult. Even when instrumental settings are held constant, the environment in which observations are collected may impact measurement variability. To maximize replicability of instrumental color measurements, it is important to have an understanding of which variables may impact instrumental variability. Barkley et al. (2018) conducted a study analyzing the effect of the machine itself on instrumental color measurements when all settings were kept constant and when loins were measured in a controlled environment. While statistical differences in color traits between machines were present, the study concluded that said differences were too small to be of practical importance and that those machines could be used interchangeably as long as each machine used identical settings. In order to build on the findings of Barkley et al. (2018), the current study was conducted to determine how purposely altering operating conditions, including machine settings and measurement environment, would affect the replicability of instrumental color readings.

Loins and Boston butts measured using an open aperture were lighter and more yellow than when measured using a closed aperture, whereas loins were more red when measured with a closed aperture, and redness did not differ in Boston butts. These findings were expected, because color values were different between machines when different operational settings were used in a previous study (Brewer et al., 2001). Additionally, Barkley et al. (2018) determined that means differ between machines even when operational settings remained constant. However, both of the previous studies only investigated differences in mean values, and not variability of those readings. All loin and Boston butt color measurements, except loin yellowness, were more variable when evaluated using an open aperture than when using a closed aperture. Variability differences within a muscle type were most prevalent in loin redness CV, as the CV for redness measured with an open aperture was nearly twice as large as when using a closed aperture. However, CV are known to be sensitive to means closer to 0, so the CV observed for loin redness using an open aperture may be inflated by the small mean (Liver, 1942). Overall, variability between loins and Boston butts was similar, and using an open aperture had a similar effect on instrumental color variance and CV for both muscle types.

When using an open aperture, if too much pressure is applied, a "pillowing" effect may occur, causing the sample to form a curved surface inside the aperture as the measurement is taken (American Meat Science Association, 2012). Pillowing changes the surface—and therefore the reflectance—of the sample, which may increase color measurement variability compared with using a closed aperture. When using a closed aperture, protein or fat may smudge the glass cover and contribute additional error as well. Nevertheless, results from this study suggest that using an open aperture had a more pronounced effect on overall color variability of loins and Boston butts than using a closed aperture. Therefore, using a closed aperture may decrease variability in instrumental color readings, and thus increase replicability compared with using an open aperture.

Color traits evaluated using an open aperture were able to predict some variation in all corresponding color traits measured using a closed aperture in loins and Boston butts. However, no color trait assessed with an open aperture was able to explain more than 54% of variation in the corresponding trait in loins, or 21% of variation in Boston butts. Because one machine was only able to explain slightly greater than half of the variation for any given trait, it would be difficult for technicians to compare or replicate measurements from studies using differing aperture types, especially when evaluating Boston butt measurements. This lack of replicability between machines was also reflected by the proportion of loins or Boston butts that had extreme values when measured using both machines. Of the 100 loins and Boston butts considered to have the highest or lowest lightness values when using an open aperture, only 65 loins and 49 Boston butts, at maximum, were also among the 100 samples with the highest values measured using a closed aperture. These data show that when using multiple machines to evaluate color, a maximum of 65 samples would properly be identified as "light" using either machine, but the remaining 35 could be dark or intermediate and would be improperly selected. Because consumers value consistency in color between products, it would be problematic to improperly select samples because of a lack of consistency between machines. These results suggested that consistency between values was greater in the loin than in the Boston butt. This may have been caused by measurement differences between muscles. Loin measurements were observed on the top of the muscle with the machine held vertically, whereas Boston butt measurements were observed on the side of the muscle, requiring the device to be held horizontally. Holding the instrument horizontally is more difficult and may have produced more variation, especially if measurements were made at an angle. Overall, it is difficult to compare results from color studies when aperture type is not consistent due to variability differences between machines.

Using a C illuminant decreased lightness and increased yellowness measurements in loins and Boston

butts compared with using a D65 illuminant. C illuminant redness measurements were less red in Boston butts but did not differ from D65 measurements in loins. Brewer et al. (2001) also reported an increase in *longissimus* yellowness measurements when using a C illuminant compared with a D65 illuminant. However, this same study also reported that using a C illuminant increased lightness measures compared with a D65 illuminant in the *longissimus*, the opposite of what was observed in the current study. The machines used in that study were different from the machines used in the current study, and inherent mechanical differences may be contributing to discrepancies between the 2 studies. Minimal differences in variability were present between C and D65 illuminants. Aside from Boston butt yellowness, no statistical differences were present between loin or Boston butt color variances when using different illuminants, and only slight differences in CV were observed. The 2 chosen illuminants use similar light sources (6,500 Kelvin [K] for C, 6,774 K for D65), which may account for the lack of variability differences (American Meat Science Association, 2012). Therefore, C and D65 illuminant may have a similar impact on overall variability of instrumental color measurements in muscles that have not oxygenated.

Although overall variability was similar between machines using different illuminants, D65 measurements were unable to predict more than 48% of variability in C illuminant measurements for loins, or 28% of variability in Boston butts. Furthermore, no more than 59% of loins and 48% of Boston butts were considered to have the same "high" or "low" categorization when using both illuminants, indicating low replicability between machines. The American Meat Science Association color guidelines (2012) specify that color data can be converted from one illuminant to another as long as data are collected in spectral form. Because the Minolta device used in this study was a colorimeter instead of a spectrophotometer, data were collected in tristimulus form rather than spectral form, which may have resulted in a lower predictive ability. Furthermore, variability between machines differed enough in individual loins that values from one machine were not able to consistently predict values from the second machine, causing a decrease in R^2 values and machine agreement even though overall variability was similar. Overall, it is difficult to compare results from studies using different illuminants because of variability differences and the lack of replicability between machines.

When loins were measured using machines with identical settings, mean lightness and yellowness differences were present when measuring both under commercial conditions and under university conditions. Redness values differed between machines under commercial conditions, but not under controlled university conditions. Barkley et al. (2018) concluded that differences between machines with identical settings were likely not large enough to warrant concern. However, lightness and yellowness differences in commercial loins were more than twice as large as differences from university loins (1.66 L^* units vs. 0.71 L^* units and 3.45 b^* units vs. 0.96 b^* units) and may be large enough to affect conclusions. However, there were no differences in variance between machines for any color trait in either set of loins. Despite the lack of variance differences between machines under commercial conditions, color measurements from the first machine were only able to predict 41%–49% of variation in measurements from the second machine, and only 49%–73% of high or low color measurements were among the greatest or lowest measurements when assessed using both machines. Contrarily, when color measurements were observed using machines in a controlled university setting, measurements from one machine were able to predict 85%–92% of variation in measurements observed using the second machine. Additionally, of the 100 greatest or lowest measurements observed using the first machine, 84%–90% of measurements were also among 100 greatest or lowest color measurements using the second machine.

These data indicate that replicability can decrease by nearly 50% when taking measurements under less controlled conditions, even when the machine type and instrumental settings are identical. There are multiple factors that could be contributing to variability discrepancies between the 2 data sets. Even though both sets of loins were measured using the machines of the same type with identical settings, the loins came from different populations. The loins measured in the university facility were inherently more variable, which could have improved the predictive ability overall. Furthermore, the 2 machines used under commercial conditions and the 2 machines used under university conditions were not the same, and each machine may have contributed different amounts of variability. Perhaps more importantly, the location of measurements differed slightly between the 2 sets. Loins measured under controlled conditions were laid out on tables, and technicians were able to ensure that color measurements assessed using the second machine were observed in the exact same location as measurements made using the first machine. Because color measurements from the other set were evaluated at line speed, it was more difficult for technicians to ensure that loins were

measured in the exact same area using both machines, and although measurements from each machine were made on the same loin, they may not have been evaluated in the exact same location. Redifer et al. (2020) reported that even small distances between loin locations can cause differences in color and marbling. Additionally, 3 measurements per machine were observed on each loin and averaged in the university study, decreasing variability contributed by measurement position. Alternatively, loins measured at line speed were only measured once with each machine, and therefore measurement location may have contributed more to overall variability.

Practically speaking, these data suggest that comparisons can reasonably be made between studies completed at different times or at different controlled locations. However, when conducting research under commercial conditions, the inherent variability of the environment may affect the ability of researchers to draw conclusions, especially if samples sizes are limited. Additionally, researchers comparing data collected in commercial facilities at different times or locations should proceed with caution. Given the data presented herein, 2 researchers aiming to collect the 100 lightest and 100 darkest loins from the same set of 600 loins would only agree on 67%–73% of those selections because they used 2 different machines. For some applications, this amount of agreement is acceptable. However, knowing the inherent variability of measurements is important when drawing overall conclusions, as some research and commercial trials may require a smaller level of variability when comparing data.

In conclusion, when conducting studies measuring instrumental meat color, using a closed aperture will decrease overall color variability compared with using an open aperture, whereas C and D65 illuminants have a similar effect on variability. As expected, differences in mean values are present when using different instrumental settings; however, results from this study would indicate that these differences are not relative and that the ranges of mean values are more important. When comparing or replicating studies measuring instrumental color, it is difficult to make comparisons when machine settings are not the same or if measurements are observed under conditions that are not well controlled. Utilizing this information will help to improve the replicability of future color assessments and make meat color research more accurate as a whole.

Literature Cited

American Meat Science Association. 2012. Meat color measurement guidelines. American Meat Science Association. Champaign, IL. Revised December 2012. http://www.meatscience.org/docs/default-source/publications-resources/Hot-Topics/2012_12_meat_clr_guide.pdf?sfvrsn=0. (Accessed February 16, 2018).

Arkfeld, E. K., K. B. Wilson, M. F. Overholt, B. N. Harsh, J. E. Lowell, E. K. Hogan, B. J. Klehm, B. M. Bohrer, D. A. Mohrhauser, D. A. King, T. L. Wheeler, A. C. Dilger, S. D. Shackelford, and D. D. Boler. 2016. Pork loin quality is not indicative of fresh belly or fresh and cured ham quality. J. Anim. Sci. 94:5155–5167. https://doi.org/10.2527/jas.2016-0886.

Arkfeld, E. K., D. A. Mohrhauser, D. A. King, T. L. Wheeler, A. C. Dilger, S. D. Shackelford, and D. D. Boler. 2017. Characterization of variability in pork carcass composition and primal quality. J. Anim. Sci. 95:697–708. https://doi.org/10.2527/jas2016.1097.

Barkley, K. E., B. Fields, A. C. Dilger, and D. D. Boler. 2018. Rapid communication: Effect of machine, anatomical location, and replication on instrumental color of boneless pork loins. J. Anim. Sci. 96:2747–2752. https://doi.org/10.1093/jas/sky223.

Brewer, M. S., L. G. Zhu, B. Bidner, D. J. Meisinger and F. K. McKeith. 2001. Measuring pork color: effects of bloom time, muscle, pH and relationship to instrumental parameters. Meat Sci. 57:169–176. https://doi.org/10.1016/S0309-1740(00)00089-9.

CIE. 1978. CIE recommendations on uniform color spaces, color-difference equations, and metric color terms. Psychometric Color Terms. Paris: Commission Internationale de l'Eclairage. Supplement 2 of CID Publication 15 (E-1.3.1) 1971. p. 8–12. https://doi.org/10.1002/j.1520-6378.1977.tb00102.x.

Livers, J. T. 1942. Some limitations to use of coefficient of variation. J. Farm Econ. 24:892–895. https://doi.org/10.2307/1232009.

Mancini, R. A., and M. C. Hunt. 2005. Current research in meat color. Meat Sci. 71:100–121. https://doi.org/10.1016/j.meatsci.2005.03.003.

NAMP. 2007. Meat Buyer's Guide. North Am. Meat Proc. Assoc., Reston, VA.

Overholt, M. F., E. K. Arkfeld, D. A. Mohrhauser, D. A. King, T. L. Wheeler, A. C. Dilger, S. D. Shackelford, and D. D. Boler. 2016. Comparison of variability in pork carcass composition and quality between barrows and gilts. J. Anim. Sci. 94:4415–4426. https://doi.org/10.2527/jas.2016-0702.

Redifer, J. D., J. E. Beever, C. A. Stahl, D. D. Boler, and A. C. Dilger. 2020. Characterizing the amount and variability of intramuscular fat deposition throughout pork loins using barrows and gilts from two sire lines. J. Anim. Sci. 98:1–10. https://doi.org/10.1093/jas/skaa275.