EVALUATION OF VARIABILITY OF INSTRUMENTS USED IN PORK QUALITY ASSESSMENTS

BY

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THESIS

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ABSTRACT

In order to evaluate pork quality, various instruments are used to measure specific traits, including color and pH. Several instruments, each having several settings, are available for each instrument that impact the values observed, making it difficult for researchers to compare results when the settings are not exactly the same. Even when operational settings are kept the same; it is possible that differences between machines will persist. Additionally, it is not known how instrumental variability changes when using a different aperture type (open or closed) or illuminant (A, C, D65, etc.) when measuring color, or how instrumental variability changes among muscles. Therefore, the overall objectives of this work were to evaluate the variability of instruments used to measure color and ultimate pH, and to see how this variability changes when using different instrumental settings or when measuring different muscles (longissimus dorsi or serratus ventralis).

Instrumental color was measured 3 times on the anterior and 3 times on the posterior end of 250 pork loins with 2 different Minolta CR-400 Chroma meter devices. Each Minolta was programed to use a D65 illuminant, 2º observer with an 8 mm aperture, and calibrated with white tiles specific to each machine. Therefore, a total of 12 instrumental color measurements were collected on each loin. The VARCOMP procedure in SAS was used to estimate the proportion of variation contributed by each factor to CIE lightness (L*), redness (a*), and yellowness (b*), chroma and hue. Based on previous research, the average untrained consumer is able to distinguish approximately 3-L* units, 0.4-a* units, and 0.9-hue angle units. Loins evaluated with machine 1 were 0.71 L* units darker ($P < 0.01$), 1.09 b* units more yellow ($P < 0.01$), 0.47 chroma units more saturated ($P < 0.01$), and had a hue angle 5.12 units greater ($P < 0.01$) than when evaluated with machine 2 but did not differ ($P = 0.24$) in redness. The anterior portion of

the loin was lighter, less red, more yellow, more saturated and had a greater hue angle than the posterior end ($P < 0.01$). Loins became darker, less red, and less yellow ($P < 0.01$) as replication number increased. Inherent color differences among loins contributed the greatest proportion of variability for lightness (58%), redness (57%), yellowness (70%), saturation (70%) and hue angle (49%). Machine contributed 1% variability to lightness 3% to saturation, 23% to yellowness and 31% to hue angle (31%) but did not contribute to variability for redness. Anatomical location contributed 41% to lightness, 43% to redness, 7% to yellowness, 27% to saturation and 31% to hue angle. Replication did not contribute to total variation for any color traits, even though it did differ among measurements.

In a second experiment, three groups of loins and 3 groups of Boston butts were evaluated for instrumental color. In loins, the longissimus dorsi was measured at the approximate location of the 10^{th} rib, and in Boston Butts, the serratus ventralis was measured at the location where the shoulder was separated from the loin. Two Minolta CR-400 chroma meters (Minolta A and Minolta B) were used in this study that were equipped with an 8mm aperture, 2° observer, and calibrated with a white tile specific to that machine. All three groups of loins and Boston butts were measured using Minolta A equipped with a D65 illuminant and closed aperture. Each group of loins and Boston butts were also measured with Minolta B using for a different combination of illuminant (C or D65) and aperture (open or closed). Group 1 used an open aperture and D65 illuminant, group 2 used a closed aperture and C illuminant, and Group 3 used an open aperture and C illuminant. Three additional groups of loins and Boston butts were also evaluated for ultimate pH on three different days. All loins and butts were measured by using two pH meters (Meter 1 and Meter 2). Loins from sets 1 and 3 evaluated with Minolta B had greater variation in lightness ($P < 0.01$ for both sets) and redness ($P < 0.01$ for set 1, $P = 0.04$ for

set 3) than loins evaluated with Minolta A, but did not differ in yellowness. Loins from set 2 did not differ in variability for any color traits. Minolta B was able to predict 36 to 54% of variability in Minolta A lightness, 33 to 48% of variability in redness, and 33 to 43% of variability in yellowness. Boston butts from sets 1 and 3 evaluated with Minolta B had greater variation in lightness ($P < 0.01$ for set 1, $P = 0.03$ for set 3) than butts evaluated with Minolta A, but did not differ in yellowness. Boston butts from set 2 measured with Minolta A had greater variation in yellowness ($P = 0.02$) than Boston butts measured with Minolta B, but did not differ in variability of any other color traits. In Boston butts, Minolta B was able to predict 11 to 36% of variability in Minolta A lightness, 15 to 21% of variability in redness, and 21 to 27% of variability in yellowness. Meter B had greater variability than Meter A on all 3 days in loins, and on day 1 in butts; variability between machines did not differ on days 2 or 3 in butts. Meter A was able to predict 17 to 21% of Meter B variation in loins and 79 to 90% of Meter B variation in butts.

Overall, there were differences in instrumental color values between the two machines tested but those differences were likely less than the threshold for detection by a consumer. Even so, inherent color differences between loins were a greater contributor to total variability than the differences between the 2 machines. Therefore, it is more important to define the location of measurements than replication or machine when using a Minolta CR-400 when performing color evaluations, assuming the settings are the same. When using machines with different settings, variation in color traits was increased by using an open aperture but mostly unaffected by illuminant. One machine to measure instrumental color or ultimate pH cannot be used to predict measurements from a second machine when instrumental settings are not the same.

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CHAPTER 1: REVIEW OF THE LITERATURE

Introduction

People in the United States consumed an average of 29.2 kg of pork per person in the year 2017 (Pork Checkoff, 2018). With a population of approximately 326 million people in 2017, this adds up to nearly 10 billion kg of pork consumed in the United States alone (United States Census, 2019). To meet the demands of consumers, it is important for pork producers to create a consistently high quality product. Quality does not have a singular definition; however, for many consumers a high quality product is simply one that is consistent with other products available for sale. The majority of pigs in the U.S are marketed based on carcass weight and percent lean (Meyer, 2005); however, variation in pork quality can result in a loss in profit as well. Variation in pork quality traits (namely color, marbling, and flavor) may have contributed to a decrease in customer confidence in pork quality predictability, leading to pork products becoming less competitive for consumer spending (Arkfeld et. al., 2017). Some of this quality variation can be attributed to sources of variation from the animal itself, including sex, production focus, and season in which the pigs were raised. Arkfeld et al. (2017) reported that season and production focus contributed variation to loin lightness (9.1% and 17.2%, respectively), ultimate pH (6.2% and 2.4%, respectively), and slice shear force (23.4% and 11.2%, respectively). In this same study, sex contributed less than 4% of variation to all three of these quality traits.

Although quality traits differ depending on the application of the product (e.g. if sold fresh or used for further processing), certain traits are important to consumer regardless of the type of meat. Pork products undergo various analyses in both production and research settings to evaluate these quality traits. Two of the most important quality traits evaluated are fresh color

and ultimate pH. Color is the first thing a consumer sees when purchasing meat, and is quality trait consumers most commonly use when making meat-purchasing decisions (Mancini and Hunt, 2005). Brewer and McKeith (1999) reported that consumers preferred pork chops that appeared wetter and more intensely pink, while they discriminated against chops that were "very light pink." Ultimate pH is not something that is observed directly by the consumer, but has a direct impact on several quality traits, including visual color, water holding capacity, and sensory characteristics including tenderness, juiciness, and flavor (Huff-Longeran et. al., 2002; Boler et. al., 2010). Lonergan et. al. (2002) reported that 24 h ultimate pH was correlated with instrumental loin lightness (48 h, r = -0.32), percentage drip loss (r = -0.33), and sensory tenderness (r = 0.27), juiciness (r = 0.17), and flavor (r = 0.25). Boler et. al. (2010) also reported that 24 h pH was correlated with percentage purge loss (21 d, r = -0.43) and instrumental lightness (21 d, r = -0.53). Pork color can be evaluated using visual appraisal or instrumental analyses. Visual appraisal involves having a trained technician call a color score for a product based on a set of standards, such as the National Pork Producers Council (NPPC) color or Japanese color standards. Instrumental analysis requires using a machine to measure the surface of a product and output a set of values corresponding to the color of the item measured. Using a machine to measure color can provide objective measurements that are more precise and repeatable than subjective color measurement alone, while still being able to predict subjective color (Zhu and Brewer, 1999). Huff-Lonergan et. al. (2002) reported that loin lightness observed with a Hunter spectrophotometer was strongly correlated to subjective NPPC color (-0.69). Ultimate pH is measured using a meter and probe designed to measure the electric potential of meat. This electric potential depends on the number of free Hydrogen atoms present in meat; samples with more free Hydrogen will have a greater electric potential, and as a result a higher

"potential of Hydrogen," or pH. Many machines used to measure pH are not designed to operate in low temperatures typical for meat evaluations, which can make the measurement of meat pH more difficult if an inappropriate meter is used (Dutson, 1983). Fortunately, some newer models are designed to correct for deviations in temperature, and may be more suitable for meat pH measurement.

Several different instruments are available to measure color or ultimate pH, and within these instruments exist several operational settings that may change the resulting observations. Particularly in color measuring devices, several options are available for the instrument itself including illuminant, aperture size and type, degree of observer, and color space. Illuminant relates to the light source used to view an object. Samples measured with different illuminants can have different lightness, redness, and yellowness values (AMSA, 2012). Degree of observer and aperture size both affect how much of an object is viewed; a larger observer degree or aperture will cause a larger area to be observed (AMSA, 2012). The 10° observer is more commonly used for meat color measurement because it captures a larger portion of the sample to be analyzed. A decrease in aperture size been shown to decrease color values, particularly redness (Yancey and Kropf, 2008).

Although they do not possess the same number of customization options, there also exists a wide array of options for pH meters and probes. Glass tipped probes are often used in meat research, as they can pierce the meat surface more easily than some other types. Research has indicated that using different operational settings may result in different color values (Brewer et. al., 2001); however, research evaluating differences between pH meters is limited. With the amount of customization possible between machines, it is unlikely that researchers or processing plants will have the exact same instruments. This makes it difficult for researchers to replicate

results from literature, and for production companies to compare data across plants when using the same production practices. However, even if the equipment used between locations is the same, differences may still occur due to error inherent to the machine itself.

Current research has not indicated what differences are present between multiple machines of the same type when operational settings are kept constant. In order to satisfy the demands of consumers, producers must ensure that they are creating a consistent product by minimizing variability in quality traits, including color. For this to happen, it is important to understand how changes in instrumental operational settings affect not only the magnitude of observations, but also the variability attributed to these machines. Furthermore, it is important to understand how variability attributed to the machine relates to inherent variability of the muscle being measured. Therefore, the following review will discuss what variation is, and define what factors that affect variation in instrumental color and pH measurements when evaluating fresh pork.

Variability

Before discussing sources of variability in pork quality, it is important to have an understanding of variability itself. Variation is a statistical term describing how data is distributed in a data set. Several sources and types of variation exist. Two of the most important types of variation are variability and uncertainty (van Belle, 2008). Variability refers to variation in environmental quantities and uncertainty refers to the degree of precision with which these quantities are estimated. In the context of pork quality evaluation, an example of variability would be natural error within a pork loin while uncertainty would be error associated with the instrument used to measure color or pH itself. Due to the difficulties associated with separating variability from uncertainty, for this review variation and variability will be used

interchangeably. Kaps and Lamberson (2004) described four commonly used measures of variability: range, variance, standard deviation, and coefficient of variation (CV).

Range

Range is defined as the difference between the maximum and minimum values in a data set (Kaps and Lamberson, 2004). There are three major drawbacks associated with only using the range as a measure of variation: 1. only two values from the entire data set are used to calculate the range, 2. the interpretation of the range depends on the number of observations between the minimum and maximum values, and 3. the presence of outliers can greatly skew this value (Armitage et al., 2002). In order to circumvent these problems, it is more appropriate to use the range of a set of data as part of a box-plot. A box-plot, also known as a box-and-whisker plot, is a diagram with a box that contains the median, upper quartile, and lower quartile. The median is the middle number in a data set, or the 50^{th} percentile. The upper quartile, or 75^{th} percentile, represents the median of the half of the data above the overall median, whereas the lower quartile, or 25^{th} percentile, represents the median of the half of the data below the overall median and lines attached to the box called whiskers depicting the maximum and minimum values that are not considered outliers. The difference between the upper quartile and lower quartile is referred to as the interquartile range (IQR). In addition to the box, box-plots also contain 2 whiskers depicting the maximum and minimum values that are not outliers. Outliers are shown using upper and lower fences, which depict outliers both greater than the maximum and less than the minimum values, respectively. Both upper and lower fences consist of inner and outer fences as well; the upper inner fence is calculated as *Quartile* 3 + 1.5*(IQR)*, while the upper outer fence is calculated as *Quartile* 3 + 3*(IQR)*. Contrarily, the lower inner fence is calculated as *Quartile* 1 - 1.5*(IQR)*, while the upper outer fence is calculated as *Quartile* 1 - 3*(IQR)*. Values that fall

between the inner and outer fences are considered moderate outliers, while values that are greater than the upper outer fence or less than the lower outer fence are considered extreme outliers.

Variance and Standard Deviation

The sample variance of *n* observations is calculated as $s^2 = \frac{\sum(y_i - \bar{y})^2}{n-1}$, where y_i is an observation, $\bar{y}$ is the sample mean, *n* is the number of data points, and the units are the same as the original variable squared (Ott and Longnecker, 2001). By using $n - 1$ in the denominator instead of simply *n*, makes the variance an unbiased estimator of the population variance, σ^2 (Ott and Longnecker, 2001). In other words, if a large number of samples with the same sample size were pulled and the variance were calculated for each sample using $n - 1$, the average sample variance would equal the population variance. However, if dividing by *n* instead, the average sample variance would be less than the true population variance (Ott and Longnecker, 2001). Standard deviation is the square root of the variance and has the same units as the original variable. The empirical rule states that in a normal distribution $\bar{y} \pm s$ contains approximately 68% of the data in a population (where $\bar{y}$ = population mean and s = standard deviation), $\bar{x} \pm 2s$ contains approximately 95% of the data in a population, and $\bar{y} \pm 3s$ contains 99.7% of the data in a population (Ott and Longnecker, 2001).

Coefficient of Variation

The coefficient of variation (CV) is a relative measure of variability expressed as a percentage, and can be used to compare variation across variables that do not use the same units. (Kaps and Lamberson, 2004). This value is calculated as $\frac{s}{y} \times 100\%$ where s = the standard deviation of a sample and y = the sample mean.

Use of Variation Methods

Each method of qualifying variation is useful in different settings. While often not reliable on its own and certainly not useful when significant outliers are present, the range of a data set offers a quick and simple understanding of how the data may be distributed (Kaps and Lamberson, 2004). For more complex data sets, e.g. data with a large sample size or several outliers, a box-plot may be more useful to understand how the center of the data is distributed. Variance and standard deviation are more useful when a more sensitive measure of variability is desired (Ott and Longnecker, 2001) Standard deviation also provides a measure of variability with the same units as the original variable. When comparing variation across data sets, the coefficient of variation is appropriate as it converts variation into a percent that can be compared even when the original units of measurement are different. Overall, each of these measures of variation are useful tools when used appropriately. When performing a quick analysis, the range or a box-plot may be appropriate while variance and the coefficient of variation may be more suitable when performing more complex analyses or comparing values across data sets.

Variation in Fresh Pork Analyses

Muscle Characteristics

Variability in meat quality can be partially attributed to variation in characteristics of the muscle itself. Skeletal muscle consists of approximately 75.0% water, 18.5% protein, 3.0% lipid, and 1.0% non-protein nitrogenous substances, 1.0% carbohydrate, and 1.0% inorganic components (Aberle et. al., 2012). The exact composition of a muscle for an individual animal depends on the several factors including the physiological function of the muscle, genetics, and the sex of the animal (Rosenvold et al., 2003). The carbohydrate in muscle is generally glucose stored in the form of glycogen to be used for muscle contraction. Muscles undergo contraction

when an action potential (essentially an electric charge) is sent to the muscle, signaling a cascade of reactions involving the hydrolization of Adenosine triphosphate (ATP) to provide energy for contraction to occur. To generate ATP for this reaction, glycogen in the muscle is broken down through glycongenolysis to form glucose-1-phosphate (G1P), which is one glucose molecule bound to one phosphate molecule. This glucose product then undergoes glycolysis where one G1P molecule is converted into 2 pyruvate molecules. When oxygen is available, these pyruvate molecules are converted to Acetyl-CoA; these molecules pass through the tricarboxylic acid (TCA) cycle and eventually make their way to the electron transport chain, where 32 molecules of ATP are produced (Lawrie, 2006). When an animal is exsanguinated for slaughter, blood is removed and the system to carry oxygen throughout the body is no longer present. After the remaining oxygen in the body is utilized, muscle begins to go through a process called rigor mortis, a latin term for "the stiffening of death." In the absence of oxygen, pyruvate is instead converted to lactic acid to produce 2 ATP. This is why contraction is still able to occur postmortem, even though no blood is available to circulate oxygen. This glycolytic metabolism results in postmortem pH decline, as lactic acid accumulates in the muscle and decreases muscle pH from 7.2 to approximately 5.5 (Lawrie, 2006). The ultimate pH of meat will be discussed in further detail in a later section of this review.

One of the most important factors influencing meat quality is the muscle fiber profile of meat. In general, skeletal muscle fibers are classified into four categories based on their speed of contraction and metabolism; type I (slow, oxidative), type IIa (fast, oxidative and glycolytic), and types IIx and IIb (fast, glycolytic) (Lee et. al., 2010). These fiber types can also be categorized as red or white fibers depending on the amount of myoglobin present; types I and IIa are considered red fibers while types IIb and IIx are considered white fibers (Gerrard and Grant,

2006). Red muscle fibers contain more myoglobin; this is because more oxygen, which binds to myoglobin, circulates through these fibers to be used for oxidative metabolism (Choe et al., 2008). White muscle fibers have less of a need for oxygen since they primarily utilize glycolysis for energy metabolism, resulting in a decreased quantity of myoglobin. White fibers also possess greater amounts of glycogen; because red fibers primarily undergo aerobic metabolism, they do not need as much glucose to produce ATP as white fibers do.

Muscles contain different amounts of each muscle fiber depending on their physiological function (Gerrard and Grant, 2006). Muscles required to maintain tension over long times, such as those found in the shoulder or hindquarter, tend to contain more oxidative fibers. Alternatively, superficial muscles such as those found in the loin are primarily composed of faster-acting glycolytic fibers. These types of fibers provide quick and powerful movements for activities like running. However, even within a muscle the proportion of fibers may differ. Beecher et. al. (1988) reported that some portions of the semitendinosus and biceps femoris possessed a greater amount of red fibers similar to other muscles in the hindquarter, but that other portions of both muscles primarily consisted of white muscle fibers and had fiber profiles more similar to the longissimus dorsi. Van Oeckel and Warnants (2003) and Homm et. al. (2006) each reported differences in loin color depending on anatomical location where measurements were collected; this may have been because different portions of the loin had different types of muscle fibers present.

Genetics also plays a role in pork quality variability due to the heritability of some quality traits. Lo et. al. (1992) reported that loin color (0.11 ± 0.06), marbling (0.16 ± 0.07), firmness (0.29 ± 0.09), ultimate pH (0.14 ± 0.08), and sensory tenderness (0.45 ± 0.12) were all heritable when using reciprocal crosses of purebred Duroc and Landrace pigs. Another study

from van Wijk et. al. (2005) reported heritability of ultimate pH (0.11 ± 0.07), loin marbling (0.31 ± 0.12), and loin firmness (0.20 ± 0.08) when using sires from a synthetic Piétrain-Large White halothane-free boar line bred to sows from an unspecified single commercial line. Differences in quality traits also exist between sirelines. Lowell et. al. (2018) reported that early loin lightness was moderately correlated with aged loin lightness (r = 0.64) while weakly correlated in Pietrain-sired pigs (r = 0.35). Additionally, early loin subjective color was moderately correlated with aged ultimate pH (r = 0.44) and aged ventral L* (r = 0.57) in Duroc-sired pigs while weakly correlated ($r \leq 0.29$) in Pietrain-sired pigs.

Meat quality traits vary among sexes due to differences in circulating hormone levels (Aberle et. al, 2012). The presence of 5-androst-16-ene-3-one, a metabolite of testosterone, can give meat and onion-like or perspiratory odor known as "boar taint." Other quality traits have been shown to be affected by sex as well. Overholt et al. (2016) reported that loin subjective color was less variable ($P < 0.01$) in barrows than gilts, while subjective marbling was more variable ($P < 0.0001$). Lowell et. al. (2017) reported that subjective firmness scores were greater in barrows than in gilts ($P < 0.0001$). Additionally, early lightness was correlated with aged ventral pH (r = -0.56) and subjective color (r = -0.39) in barrows but not gilts.

Much of the research investigating pork variability has been performed using the loin. Arkfeld et. al. (2017) reported that the majority of variation (48.9% to 88.5%) in pork color traits (lightness, redness, and yellowness), ultimate pH, marbling score, and slice shear force was attributed to the pig and other factors (random error). The previous study also reported that sex contributed 0.2% to 12% of variation and production focus contributed 1.6% to 39.0% of variation to quality traits, while season contributed 6.2% to 23.4% of variability to each of these traits except for marbling (0% variation). As mentioned previously, Overholt et. al. (2016)

described differences in variability of subjective color and marbling between boars and gilts, while Lowell et. al. (2017) described differences in correlations between early and aged quality traits between sexes. Some research exists discussing differences in quality traits associated with muscles from the shoulder, but little information is available investigating differences in the variability of those quality traits. Future research should be conducted evaluating variation in these muscle groups as well.

Ultimate pH

The pH of a solution indicates how much free Hydrogen is available in a solution, and is expressed as a negative logarithm of the Hydrogen ion concentration ($-\log[H^+]$). This is measured on a scale of 0 (acidic) to 14 (basic), where a lower pH indicates a greater concentration of Hydrogen atoms and a pH of 7 indicates a neutral solution. As discussed previously, living muscle has a neutral pH of approximately 7.2 but declines to around 5.5 during the onset of rigor mortis (Lawrie, 2006). The ultimate pH of meat is the final pH after rigor mortis has concluded and muscle contraction no longer produces excess lactic acid. As the pH of muscle decreases during rigor, the muscles approach their isoelectric point (5.2 - 5.5), where the proteins in muscle possess a neutral charge. As the muscle becomes more neutral, it loses its ability to bind to more polar water molecules. This decreases the water holding capacity, and in turn decreases sensory juiciness (Huff-Lonergan and Lonergan, 2005; Moeller et. al, 2010). Because myoglobin is a water-soluble protein, a decrease in water holding capacity will also result in lower concentrations of myoglobin and the meat will appear paler in color (Choe et al., 2008). The ultimate pH of muscle may also influence meat tenderness. Moeller et al. (2010) and Lonergan et al. (2007) both reported greater sensory tenderness scores in pork loin chops with more alkaline pH values.

As discussed previously, meat ultimate pH is evaluated using a specific meter and probe. The purpose of the meter itself is simply to interpret the reading made by the electrode and display it in a readable form to the technician; electrodes present in the probe are making the actual pH measurement (Mirsky and Anson, 1929; American Chemical Society, 2004). Several types of probes are available to measure pH; for fresh meat, glass tipped probes are often utilized as they can easily penetrate the meat surface (Andersen et. al. 1999). In many cases, the glass tip is brightly colored so that it can be found easily if it were to break off during measurements. When measuring pH of any substance, two electrodes are utilized. The first electrode, called the sensitive electrode, has a silver-based wire suspended in a solution of potassium chloride, which is encased in a thin bulb made from a special glass containing metal salts. The second electrode, the reference electrode, contains a potassium chloride wire suspended in a potassium chloride solution. Meters designed to measure pH essentially work by measuring the voltage, or electric potential, of the sample of interest. The meter then compares this to the voltage of a known solution in the sensitive electrode, and uses the difference to determine the pH of the unknown solution; the reference electrode acts as a baseline.

One of the difficulties associated with pH variation is the simplicity of the measurement process; one merely needs to insert the probe into a meat sample and read the pH value that is displayed (Dutson, 1983). Because of this simplicity, it becomes easier for the technician to accept erroneous data, as nothing but the reading itself can indicate a problem. For this reason, technicians should be familiar with not only typical meat pH values, but also other factors affecting instrumental pH readings. One of the most important steps when preparing to measure pH is ensuring that the pH meter is properly calibrated. A pH meter must be calculated properly in order to obtain accurate results (Cheng and Zhu, 2005). This is performed by submerging the

electrodes in buffer solutions with known pH values. Typically, an electrode is submerged in two buffers: one of a neutral pH, and one of a higher or lower pH (usually either pH 4 or 10). The two buffers used depend on the pH of the substance to be measured; the pH of the sample should fall between the pH values of the two standards chosen. For example, because the average pH of meat is around 5.5, pH 4 and pH 7 buffers should be used as 5.5 falls between those values. When switching between buffers and when finished calibrating, care must be taken to clean the probe, as residue from one solution can dilute the other or affect the meat pH reading. Over time, the probe can degrade and become sluggish due to accumulated fat and protein clogging the electrode membranes; when this occurs, it takes a longer amount of time for the meter to equilibrate and read the actual pH of a sample (Dutson, 1983). If the technician does not wait a sufficient amount of time for the meter to reach the true pH, they may accept erroneous readings (Dutson, 1983). Additionally, if a technician is using a sluggish probe to measure meat pH in a plant while samples are moving on a line, the pH meter may not be able to keep up with line speed. The pH of samples could be read later, but not under the exact same conditions as the other readings. This can be prevented by soaking electrodes in a neutral buffer solution of pH 7.0 when not in use; however, if the probe has become too slow, it should be replaced as soon as possible.

Another problem associated with meat pH measurements relates to variation in pH throughout the muscle. As discussed previously, muscle fiber profiles can change throughout a muscle, resulting in different pH values as well. To obtain a representative pH for the entire muscle, multiple readings should be recorded at multiple anatomical locations (Dutson, 1983). If only interested in one specific area of the muscle, then this area should be specified when reporting results.

One problem associated with measuring the pH of meat as opposed to other solutions relates to the temperature that measurements are recorded in. Due to the microbiological concerns associated with meat, most readings are performed in a cold environment. However, many pH meters are not designed to operate at temperatures other than room temperature. This is because lower temperatures affect ionic adsorption and distribution in the electrode, which can alter the interpretation of differences in pH between the glass electrode and the solution of interest (Cheng and Zhu, 2005). Many more modern pH meters come equipped with built-in temperature correction systems; however, this may introduce more error and create more variability in the pH meter (Chemical Technicians' Ready Reference Handbook, 2011). One way to minimize temperature variation is to use two pH meters; this way one pH meter can always be kept at room temperature, and meters can be switched when the one in use becomes too cold (Dutson, 1983). However, the use of two pH meters can be problematic as well; each machine has a different uncertainty associated with it, which may contribute variation in a different form from temperature differences. Limited research is available examining differences in variability between pH meters of any type, and particularly how this variability affects meat pH evaluation.

Measuring meat pH is a process that appears simple, but requires significant preparation and understanding to ensure that variation in readings is minimized. Therefore, it is important to ensure that the technician has the appropriate skill and experience required to measure pH with a high level of accuracy and precision. However, there may be situations when more than one pH meter may be used, such as when working in cold temperatures. Furthermore, it is possible that researchers or plants will not share the same type of pH meter. For situations like this, future research should be conducted to determine if differences in variability between machines are

significant enough to warrant using only one pH meter, even when temperature issues are present.

Color

Color is the most important trait to consumers because they use it as an indicator of freshness and wholesomeness (Mancini and Hunt, 2005) Meat color revolves around myoglobin, the red-pigmented protein in muscle – muscles containing more myoglobin appear darker and more red to consumers. Observed meat color also depends on the state of myoglobin – whether it is reduced, oxygenated, or oxidized (AMSA, 2012). Myoglobin is protein that contains 8 alpha-helices and a prosthetic heme group containing centrally located iron atom. This iron atom contains 6 bonds; four connect iron to the heme ring, one attaches to proximal histidine-93, and the last site is available to reversibly bind oxygen. When there is no ligand attached, deoxymyoglobin is present, resulting in the dark-purple color characteristic of freshly cut or vacuum packaged meat. After binding to oxygen, oxymyoglobin is formed, giving meat its characteristic red color. Oxymyoglobin is the form most commonly presented to consumers, and is the form they expect to see when purchasing meat. The last form of myoglobin in fresh meat, metmyoglobin, occurs when the iron atom oxidized from a ferrous state (Fe^{2+}) to a ferric state (Fe^{3+}) and water is the bound ligand (AMSA, 2012). Metmyoglobin forms over time when exposed to small amounts of oxygen, and results in a brown color that indicates meat has gone bad to consumers. The color consumers see is affected by the reflection of light off the surface of meat. Humans are capable of seeing wavelengths ranging from 400 to 700 nm; this range is referred to as the visual color spectrum (AMSA, 2012). The human eye captures wavelengths that are reflected off the surface of an object and interprets these wavelengths as color. Meat

primarily reflects wavelengths in the 650-700 nm range, which is associated with the color red; this is why meat appears red in color.

As mentioned before, the evaluation of meat color can be performed in several ways, with two of the most common methods being visual appraisal and instrumental analysis. Visual evaluation is performed by a trained technician comparing a sample to a set of standards, and assigning a score accordingly. Some common sets of standards are the National Pork Producers Council (NPPC) color standards (NPPC, 1999), Japanese color standards, and Australian pork color standards. The set of standards used depends on the final destination of the final product; consumers from different locations may prefer a different colored product (Cho et al., 2007; Chen et al., 2010). The standards used often correspond to instrumental color values. For example, the NPPC color standards use a 1-point difference scale ranging from 1 to 6, where 1 is very light in color and 6 is very dark in color. Each point on the scale corresponds to an instrumental L* value, with a difference of 6 L* units between scores (instrumental color will be described in more detail later in this review).

Visual color evaluation is a useful tool when evaluating meat color, as it is evaluated in the same way that a consumer will evaluate color. However, visual color is subjective by nature; even when using trained technicians, because of differences present in the human eye, not all people will perceive color in the same way. This makes color visual color measurements difficult to reproduce among individuals (Zhu and Brewer, 1999). For this reason, instrumental color evaluation is often used either in addition to or in place of visual color assessment. Machines intended for color analysis are more objective by nature, making them more reproducible and precise than visual assessment alone (Zhu and Brewer, 1999).

Instrumental color is commonly measured using one of two devices: either a colorimeter or spectrophotometer. Both machines use their own light sources and illuminant conditions specific to that machine. Colorimeters measure tristimulus values; a set of three values representing the overall color of a sample. This is done by using a light source to illuminate the sample, then passing the reflected light through red, green, and blue filters – the three primary colors – and generating XYZ values. These lettered values are then converted into CIE L*a*b* values (CIE, 1978), as these values correspond better to perceived visual color values than the XYZ system (AMSA 2012). Instrumental L* represents lightness and darkness of a sample on a scale from 0 to 100, where an L* of 0 is perfectly black and an L* of 100 is perfectly white. The a* value measures redness and greenness on a scale of -60 (green) to +60 (red). The b* value also measures color on a scale of -60 to +60, but measures blueness (-60) to yellowness (+60) instead. CIE L*, a*, and b* values can also be used to calculate Chroma, hue angle, and ΔE values. Chroma represents how saturated a color is, and is calculated by $Chroma = \sqrt{(a^{*2} + b^{*2})}$. Hue angle represents the perceived color of a sample and is calculated by $h_{ab} = arctangent\left(\frac{b*}{a*}\right)$. The ΔE value is the change in overall color of a sample and is calculated by $\Delta E^*_{ab} = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a)^2 + (b^*_2 - b^*_1)^2}$. Spectrophotometers also measure tristimulus values, but in a different way. Colorimeters have a set wavelength and can only measure color at that wavelength; meanwhile, spectrophotometers measure the absorbance color over several wavelengths (generally in 1 – 10 nm intervals) then convert that to tristimulus values. This is particularly useful when performing tests like shelf life studies, where absorbances at multiple wavelengths are used to determine surface discoloration.

The light source, or illuminant, used by each machine can be modified depending on the samples evaluated. Several illuminants are available, but the three most commonly used in meat

evaluation are C (6774 K), D65 (6500 K), and A (2857 K). C and D65 illuminants both simulate daylight, where C represents average north sky daylight and D65 represents noon daylight. A illuminant represents incandescent light and places more emphasis on red wavelengths, making it more appropriate for studies where differences in redness are of interest (AMSA, 2012). While not used as commonly as the other three, F illuminant (fluorescent) may also be used when simulating retail display under fluorescent light sources. Use of different illuminants may cause differences in instrumental color values (Brewer et al, 2001). However, research is lacking examining the effect that illuminant has on overall color variability.

In meat research, the degree of observer used is most commonly 2° or 10°, depending on the instrument. The degree of observer refers to the viewing angle used to observe an object, where a larger degree results in a larger field of vision. In human eyes, this degree varies from person to person, making it more difficult for people to make consistent observations (AMSA, 2012). The CIE established 2° as the standard degree for colorimeters in 1931, as this was the value that most closely resembled human sight. In the 1960s further research was conducted determining that a 10° may be more representative of human sight. In current research, colorimeters generally have a set 2° observer and spectrophotometers generally have a set 10° observer. Because these values cannot be changed within a machine, limited research is available discussing how differences in observer alone affect color values or variability. Aperture size is often overlooked, but can significantly affect reported color values. As aperture size decreases, the percentage of light reflected also decreases, particularly at red wavelengths (Yancey and Kropf, 2008). If an aperture is too large, “edge-loss” may occur and the color of edges of a sample may be interpreted incorrectly (Hulsegge et al., 2001). The type of aperture (open or closed) may also affect instrumental color, particularly variability in readings. When using an

open aperture, applying too much pressure may cause “pillowing.” (AMSA, 2012). This results in the sample forming a concave surface in the opening of the aperture, causing light to reflect differently and resulting in erroneous readings. Multiple readings should also be taken when possible, especially when determining overall color of a meat sample.

Regardless of what instrumental settings are used, machines designed to measure color cannot provide accurate results if not calibrated properly. Calibration for color measurement differs by machine, but is generally simple. In general, calibration involves scanning a standardized black or white tile (AMSA, 2012). Instruments should always be calibrated before measuring samples, and the state of the calibration tile should match the sample being measured. For example, if measuring meat wrapped in polyvinyl chloride film, the calibration tile should also be covered in this film. In addition to reporting instrumental settings, the process for standardization should also be specified (AMSA, 2012).

It is clear that several factors affect instrumental color readings, and possibly the variation of the machine itself. In order to replicate a study, researchers need to ensure that they are using the exact same instrumental settings in order to minimize variation in results. However, one of the greatest difficulties associated with replicating previous color research lies simply in the lack of reporting details. Tapp III et al. (2011) reported that among 1,068 peer-reviewed journal articles investigating meat color, 48.69% of articles did not report illuminant, 73.6% did not report aperture size, and 52.4% did not report the number of readings per sample. Without appropriate knowledge of what settings are used, researchers cannot effectively replicate these studies. For this reason, a standardized set of parameters should be reported for all meat color evaluation (Tapp III et al., 2011).

Conclusions

Several studies have investigated sources of variation in meat itself, including muscle fiber typing, genetics, and sex. Some of these studies have also reported the effect these characteristics on quality traits of individual muscles. Variability within the loin has been well described; however, limited research is available variability of quality traits from muscles in the shoulder. While sufficient research has been conducted to evaluate and minimize variations in the animal itself, variability in the machinery used to measure these quality traits is often taken for granted. Research has been conducted evaluating the effects of machines used to measure these quality traits, particularly machines intended to measure color. However, no previous research has been conducted showing differences between machines when all instrumental settings are the same. Additionally, the majority of this research examines differences in magnitude of instrumental settings; limited research is available discussing how these settings affect color variability. Furthermore, differences in instrumental variability between muscle types are unknown as well.

Overall, two experiments were conducted investigating effects of instrumentation on variability. The first experiment involved measuring loins with two different colorimeters of the same model and equipped with the same operational settings. The objective of this study were to understand differences in machine when settings are controlled, and determine if the machine itself, the anatomical location of the measurement, or the number of replications had the greatest impact on variation in instrumental color. The second experiment expanded on the results observed by Brewer et al. (2001) when using different illuminants in the machine, but focused on differences in variation attributed to the illuminant and aperture type (open or closed). The objectives of the second experiment were to characterize variability in instrumental color and pH

for loins and Boston butts and to determine if color of pH measurements from one machine type can be used to predict measurements from a second machine type. Understanding the effects of instrumentation on variability could have a positive impact on minimizing overall variability in pork quality beyond what can be controlled in the animal itself.

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CHAPTER 2

EFFECT OF MACHINE, ANATOMICAL LOCATION, AND REPLICATION ON INSTRUMENTAL COLOR OF BONELESS PORK LOINS

Abstract

The objective was to determine the effect of machine, anatomical location and replication (multiple readings) on instrumental color and to characterize the amount of variation each factor contributed to overall color. Instrumental color was measured 3 times on the anterior and 3 times on the posterior end of 250 pork loins with 2 different Minolta CR-400 Chroma meter devices. Each Minolta was programed to use a D65 illuminant, 2º observer with an 8 mm aperture, and calibrated with white tiles specific to each machine. Therefore, a total of 12 instrumental color measurements were collected on each loin. The VARCOMP procedure in SAS was used to estimate the proportion of variation contributed by each factor to CIE L*, a*, b*, chroma and hue. Based on previous research, the average untrained consumer is able to distinguish between 3-L* units, 0.4-a* units, and 0.9-hue angle units. Loins evaluated with machine 1 were 0.71 L* units darker ($P < 0.01$), 1.09 b* units more yellow ($P < 0.01$), 0.47 chroma units more saturated ($P < 0.01$), and had a hue angle 5.12 units greater ($P < 0.01$) than when evaluated with machine 2 but did not differ ($P = 0.24$) in redness. The anterior portion of the loin was lighter, less red, more yellow, more saturated and had a greater hue angle than the posterior end ($P < 0.01$). All color trait values decreased ($P < 0.01$) as replication number increased. Inherent color differences among loins contributed the greatest proportion of variability for lightness (58%), redness (57%), yellowness (70%), saturation (70%) and hue angle (49%). Machine contributed 1% variability to lightness 3% to saturation, 23% to yellowness and 31% to hue angle (31%) but did not contribute to variability for redness. Anatomical location contributed 41% to lightness,

43% to redness, 7% to yellowness, 27% to saturation and 31% to hue angle. Replication did not contribute to total variation for any color traits, even though it did differ among measurements. Overall, there were differences in instrumental color values between the two machines tested but those differences were likely less than the threshold for detection by a consumer. Even so, inherent color differences between loins were a greater contributor to total variability than the differences between the 2 machines. Therefore, it is more important to define the location of measurements than replication or machine when using a Minolta CR-400 when performing color evaluations, assuming the settings are the same.

Introduction

Color has more influence on meat purchasing decisions by consumers than any other trait (Mancini and Hunt, 2005). To evaluate pork color, several visual measurement systems have been created where a trained technician assigns a score to pork based on perceived color. These grading systems include the National Pork Producers Council (NPPC, 1999), Japanese pork color standards, and Australian pork color standards. However, because of the subjective nature of visual appraisal, color interpretations are difficult to reproduce among individuals (Zhu and Brewer, 1999). For this reason, instrumental color analyses are often used instead of or in addition to visual appraisal. Colorimeters and spectrophotometers are able to simulate visual color while providing objective measurements that are more reproducible and precise (Zhu and Brewer, 1999). Multiple types of instruments with a wide variety of options for color space, illuminant, observer and aperture size are available (AMSA, 2012). Brewer et al. (2001) reported that illuminant and instrument type influenced color measurements of pork chops. With the amount of customization possible, it is likely that two researchers will not have the exact same instrument. These differences make it difficult to replicate or compare results from literature

regarding instrumental color (Tapp et al., 2011). This problem could potentially be remedied if researchers standardized equipment and used the same device with the same operational settings. However, even when these factors are controlled, differences may persist between machines. Additionally, it is not known if the loin itself, anatomical location of the loin, machine, or replication is the greatest contributor to variability in instrumental color in a population of loins. The expectation was that there would be differences in instrumental color readings when different machines were used. Therefore, the objective was to evaluate the effects of machine, anatomical location and replication number on instrumental color of boneless pork loins and to characterize the variation to color contributed by each of these factors.

Materials and Methods

Pigs were slaughtered under the supervision of the USDA Food Safety Inspection service at a federally inspected facility. Boneless loins were acquired from that facility and transported to the University of Illinois Meat Science Laboratory (Urbana, IL). Therefore, Institutional Animal Care and Use Committee approval was not obtained. Pigs were immobilized using electrical stunning and terminated via exsanguination. Carcasses were blast-chilled to rapidly cool the carcasses then held in an equilibration cooler until 1 d postmortem. At 1 d postmortem, carcasses were fabricated into primal cuts and loins were further cut into boneless Canadian back loins (NAMP #414). The posterior half (portion posterior to the spinalis dorsi) of the boneless loins were separated, vacuum-packaged, and transported on ice in coolers to the University of Illinois Meat Science Laboratory. Loins were collected from 2 groups of pigs (250 total) that were slaughtered 14 days apart. The first set contained 100 loins and the second set had 150 loins. Loins were aged until 14 d postmortem in the dark at 4°C. After aging, loins were removed from packaging and positioned on tables so that the ventral side was exposed to oxygen, then

allowed to oxygenate for at least 20 min. After oxygenation, instrumental CIE (CIE, 1978) L* (lightness), a*(redness), and b* (yellowness) readings were collected on each loin with two separate Minolta CR-400 Chroma meter devices (Minolta Camera Co., Ltd., Osaka, Japan). Both machines were programmed to use a D65 illuminant, a 2º observer with an 8 mm aperture, and calibrated with a white tile specific to the machine. For each loin, 3 consecutive measurements were taken on the anterior end of the ventral surface without moving the first Minolta, then an additional 3 measurements were taken in the exact same location with the second Minolta. Between the 3 readings, the head of the Minolta was not moved to ensure that each reading was taken in the exact same location. This process was repeated on the posterior end, for a total of 12 measurements per loin. From these measurements, chroma (measure of saturation;$\sqrt{a^{*2} + b^{*2}}$) and hue angle (description of color; Tan^{-1}(b*/a*)) were calculated. Hue angle is calculated by plotting a* and b* values on a 60-point scaled x-axis and y-axis respectively, then determining the angle made by the two values. This angle represents the color or hue of a sample.

Statistical Analyses

Color data were analyzed with the MIXED procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC) as a split-split plot design. Loin (250 total) served as the experimental unit and the blocking factor. The fixed effects in the model were machine (Minolta 1 vs Minolta 2), anatomical location (posterior vs anterior) and replication (multiple readings) and all possible interactions. The whole plot factor of machine was tested with the interaction between loin and machine. The split plot factor was location and was tested with the three-way interaction of loin, machine, and location. The split-split plot factor was replication and was tested by the four-way interaction of loin, machine, location, and replication. There were no statistically significant ($P < 0.05$) interactions among any fixed factors. Main effects means between machine, location, and

replication were considered significantly different from 0 at $P < 0.05$. Contribution to variability in instrumental color was determined for each independent variable (machine, anatomical location, and replication) using the VARCOMP procedure in SAS. Proportions of variance were calculated for each instrumental color reading (L*, a* and b*) as well as for calculated chroma and hue values. Variances from error that could not be attributed to an independent variable were attributed to inherent differences between loins as well as other factors that were not controlled in this study. Negative variances were treated as contributing zero variance to the population.

Results

Instrumental Color

Loins analyzed with Minolta 1 were 0.71 L* units darker ($P < 0.0001$), 1.09 b* units more yellow ($P < 0.0001$), 0.47 chroma units more saturated ($P < 0.0001$) and had a hue angle 5.12 units greater ($P < 0.0001$) than loins evaluated with Minolta 2 (Table 1). Instrumental redness (a*) did not differ between loins analyzed with the two machines ($P = 0.24$). From machine 1 to 2, L* increased by 1.57%, a* decreased by 0.78%, b* decreased by 29.78%, chroma decreased by 4.32%, and hue angle decreased by 25.93%. Anterior ends of the loins were instrumentally 4.06 L* units lighter ($P < 0.0001$), 1.78 a* units less red ($P < 0.0001$), 0.62 b* units less yellow ($P < 0.0001$), 0.95 chroma units more saturated ($P < 0.0001$), and had a hue angle 6.40 units greater ($P < 0.0001$) than the posterior ends. From the anterior end to the posterior end, L* decreased by 9.32%, a* increased by 19.12%, b* decreased by 15.9%, chroma decreased by 8.73%, and hue angle decreased by 33.50%. Instrumental L*, a*, b*, chroma and hue values decreased as the replication increased from the first reading to the third reading ($P < 0.0001$). From replication 1 to 3, L* decreased by 0.55%, a* decreased by 0.20%, b* decreased by 1.68%, chroma decreased by 0.45%, and hue angle decreased by 1.26%.

Proportions of Variance

Variation contributed by machine, anatomical location, and replication was estimated for each instrumental color parameter (L*, a* and b*), chroma and hue angle values of the loins (Fig. 1). The majority of variation for each color trait was contributed by random error within each loin that was not accounted for in this study (58% for L*, 57% for a*, 70% for b*, 70% for chroma and 49% for hue angle). Anatomical location contributed 41% of variation to L*, 43% to a*, 7% to b*, 27% to chroma, and 31% to hue angle. Machine contributed a small amount to the variation of L* (1%) and chroma (3%) and did not contribute at all to the variation of a*; however, it accounted for 23% of the total variation for b* and 20% for hue angle. Replication did not contribute significant variation to any of the color traits analyzed.

Discussion

While statistically significant, the differences between instrumental color values from two Minolta devices were likely not different enough to be visually distinguishable by consumers. Brewer et al. (2001) previously reported that using different instruments would lead to different reported color values; however, that particular study referred to devices with differing settings, such as color space or aperture size. One of the focuses of this experiment was to determine differences in instrumental color when using two devices produced by the same company and operated with the same settings (illuminant, aperture and observer). All of the color traits assessed were statistically different between the two machines. Even though the color values were statistically different between machines, the absolute difference between machines was small for L* and chroma. Additionally, differences in the magnitude of L* measurements represent less than 1 L* unit difference (much less than even a half NPPC color score (NPPC, 1999)). For L*, a* and chroma, no more than 3% of total variation was attributed by machine. In

sensory panels, b* tends to be more related to brownness than yellowness or blueness (O'Sullivan et al., 2003). This is because the colors represented by b*, yellow and blue, are not typically or intuitively related to meat (Mancini and Hunt, 2005). Therefore, differences in b* are more difficult to interpret than those in L* or a*. Hue angle had a greater difference between the two machines (5.12), and had 20% of its overall variation contributed by machine. However, hue angle is dependent on a* and b*, both of which had small values in the present study. When a* and b* have small values, such as in this experiment, small changes in magnitude can cause large changes in hue angle. Furthermore, machine did not contribute any variation to a*. Therefore, when measurements are taken with two different machines that are the same type, model and operating with the same settings, anatomical location must be standardized. Replications of measurements are not needed and can be disregarded. It remains to be determined whether 2 colorimeters of different types contribute an important amount of variation to color measurements.

Of the three factors evaluated (machine, location, replication), the greatest differences in instrumental color occurred between measurements made on the anterior and posterior ends of the loin. The anterior end of the loin was significantly lighter and less red than the posterior end, with lightness increasing by 9.3% and redness increasing by 22.8%. Additionally, anatomical location contributed the second most variation for all color traits except for b*, ranging from 27 through 43% (7% for b*). A number of studies have concluded that fresh pork color is not uniform throughout the length of the longissimus muscle (Ohene-Adjei et al., 2002; Van Oeckel and Warrants, 2003; Norman et al., 2004; Homm et al., 2006). Van Oeckel and Warrants (2003) reported that meat from the anterior portion of the loin was paler and less red than that from the posterior portion. Homm et al. (2006) also reported that loins were lighter on the anterior end,

but that the extreme posterior end was lighter than the rest of the loin and that the loin became less red from anterior to posterior. However, color measurements in that study were observed on the face of chops cut from the loin rather than the ventral portion of the loin. Lowell et al. (2017) reported that loin color is not necessarily indicative of chop color (barrows r =0.13; gilts r = 0.11). The results of this study are more consistent with the findings of Van Oeckel and Warrants (2003), as both that study and this study made color measurements on the ventral surface of the loin rather than chops. Therefore, when comparing data between studies, anatomical location of loin color measurements must be specified.

Overall, replication of measurement did not contribute to loin color variation, though it did differ among measurements. As the replication number increased, the color readings became lighter, less red, more yellow, less saturated, and had a smaller hue angle. Differences in replications were statistically significant; however, because of the small magnitude of difference and the large number of observations (1000 per replicate), it is possible that these differences can be attributed to type 1 statistical error. In statistics, a type I error occurs when the null hypothesis is rejected, even though no true difference is present (Kaps and Lamberson, 2004). Normally, statistical power of 0.75 to 0.80 is sufficient for means separation of practical differences. However, when a power test was conducted for experimental L* using a sample size of 1000 observations per replication, a minimal detectable difference of 3 units and a standard deviation of 4.01, the computed power was >.999, indicating that this portion of the experiment was more than sufficiently, and perhaps excessively, powered. Furthermore, although differences in replication were detected, replication did not contribute variation to any of the color traits analyzed. Therefore, much like the differences present between the machines, differences among replications were likely not great enough to be detectable by consumers.

One way to determine the importance of differences in color readings is to compare them to thresholds indicating how large of a difference in color a person is able to detect. Zhu and Brewer (1999) reported that when measured by a Hunter spectrocolorimeter under fluorescent light, the minimum instrumental differences required to be visually perceptual were 1.55-L* units, 0.4-a* units, and 0.9-hue angle units. The threshold for L* was calculated using a linear regression equation determined by Zhu and Brewer (1999) that was based on beef color under fluorescent lighting. In this study, the average differences for L*and a* were 0.71 and 0.08 units respectively, both of which are considerably less than the detectable thresholds reported by Brewer and Zhu (1999). However, because these thresholds were determined using a different instrument and color space from what was used in this study, those thresholds may not apply to these experimental conditions.

While differences between machines with identical settings were statistically significant, they were not great enough to be detectable by consumers. Loin and other factors that were not accounted for in this study contributed the greatest amount of variation to each of the measured color parameters, with anatomical location contributing the second most to L*, a*, chroma, and hue angle and machine contributing the second most to b*. Replication did not contribute variation to any of the color traits measured in this study. Using different machines of the same type with the same settings and taking multiple readings does not cause practical differences in the measurement of instrumental color of boneless pork loins. While differences in instrumental color readings may exist between machines, they were not great enough between these 2 machines to be of practical concern for research purposes, as inherent differences in the loin were a greater contributor to overall variation than machine. Therefore, it is more important to

define the anatomical location of color evaluation than the replication number when the model and settings are held constant.

Table

Table 2.1. Effects of colorimeter, anatomical location and replication on instrumental color of boneless pork loins

Colorimeter[1]	1	2	SEM	*P* - value	
Observations, n	250	250			
Lightness, L*[2]	45.25	45.96	0.20	< 0.0001	
Redness, a*[2]	10.24	10.16	0.08	0.24	
Yellowness, b*[2]	4.75	3.66	0.08	< 0.0001	
Saturation, chroma[3]	11.35	10.88	0.09	< 0.0001	
Hue angle, h_{ab}[4]	24.86	19.74	0.32	< 0.0001	
Location[1]	Anterior	Posterior	SEM	*P*-value	
Observations, n	500	500			
Lightness, L*[2]	47.64	43.58	0.20	< 0.0001	
Redness, a*[2]	9.31	11.09	0.08	< 0.0001	
Yellowness, b*[2]	4.51	3.89	0.08	< 0.0001	
Saturation, chroma[3]	11.83	10.88	0.09	< 0.0001	
Hue angle, h_{ab}[4]	25.50	19.10	0.32	< 0.0001	
Replication[1]	1	2	3	SEM	*P*-value
Observations, n	1,000	1,000	1,000		
Lightness, L*[2]	45.75[a]	45.58[b]	45.50[c]	0.18	<0.0001
Redness, a*[2]	10.21[a]	10.20[b]	10.19[c]	0.07	<0.0001
Yellowness, b*[2]	4.24[a]	4.20[b]	4.17[c]	0.07	<0.0001
Saturation, chroma[3]	11.14[a]	11.11[b]	11.09[c]	0.09	<0.0001
Hue angle, h_{ab}[4]	22.46[a]	22.28[b]	22.18[c]	0.32	<0.0001

[a, b, c] Means within a row for experimental treatments without a common superscript are different ($P \leq 0.05$)

[1]Observations were collected using 2 different Konica Minolta colorimeters. Each used D65 illuminant, 0° observer and an 8 mm aperture

[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]chroma is a measure of saturation (greater value indicates more saturated color) calculated using the equation $Chroma = \sqrt{a^{*2} + b^{*2}}$

[4]Hue angle is a description of color calculated using the arctangent (b*/a*).

Figure

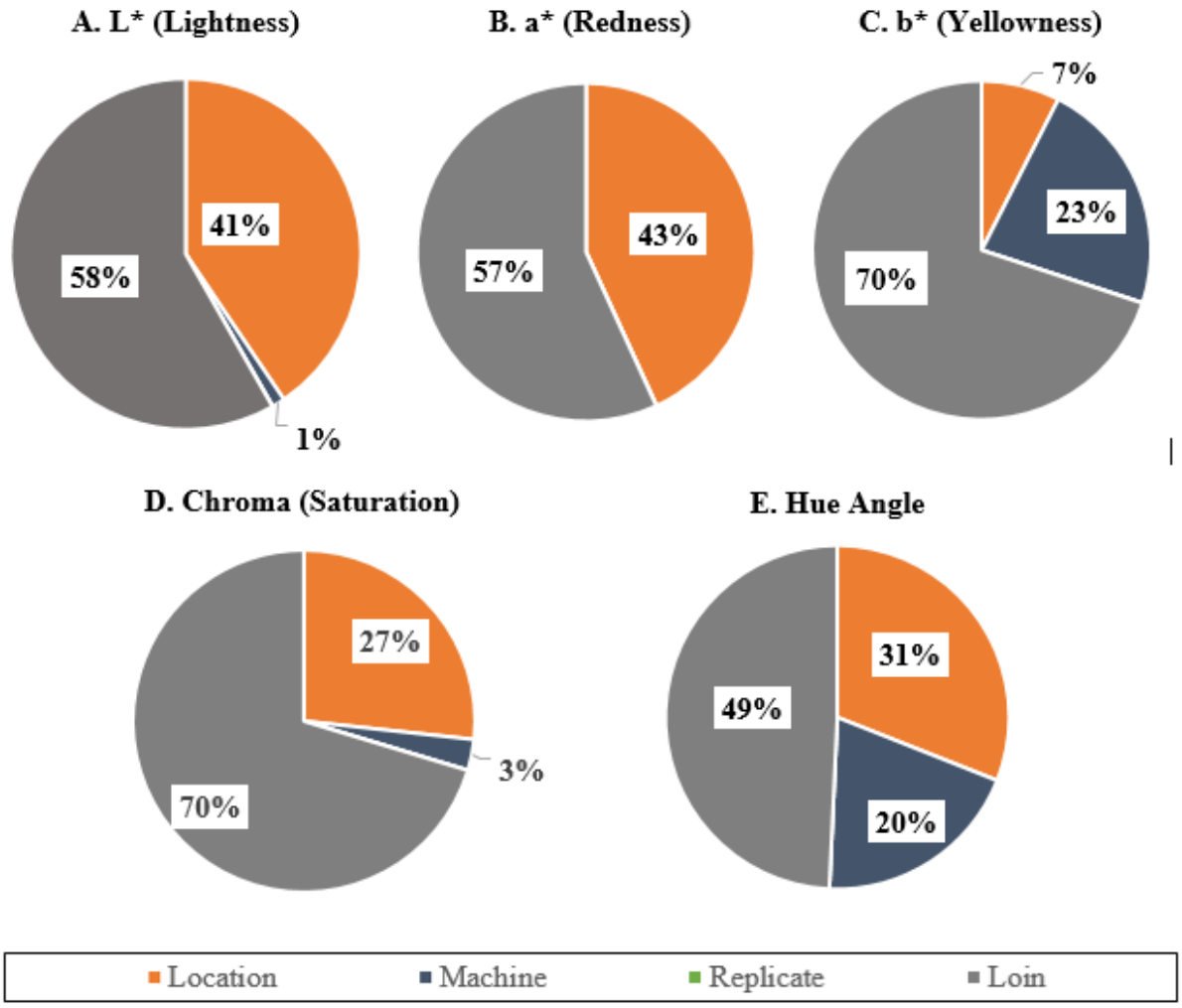

Figure 2.1. Percent of total variation that machine, anatomical location, replication and loin (random error) contributed to instrumental L*, a*, b*, chroma and hue angle

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CHAPTER 3

EVALUATION OF THE VARIABILITY OF INSTRUMENTS USED IN PORK QUALITY ASSESSMENTS

Abstract

The objectives were to characterize variability in instrumental color and pH for loins and Boston butts, and to determine if color or pH measurements from one machine type can be used to predict measurements from a second machine type. Three groups of loins and 3 groups of butts were evaluated for instrumental color; all 3 groups were measured using a Minolta CR-400 colorimeter equipped with a D65 illuminant, closed 8mm aperture, 2° observer, and calibrated with white tiles specific to that machine (Minolta A). Each group of loins and butts were also measured with a second Minolta CR-400 with identical settings except for illuminant and aperture combination (Minolta B). Group 1 had an open aperture and D65 illuminant, group 2 had a closed aperture and C illuminant, and Group 3 had an open aperture and C illuminant. Three additional groups of loins and butts were evaluated for ultimate pH on 3 different days. All loins and butts were measured using 2 pH meters (Meter A and Meter B). Loins from sets 1 and 3 evaluated with Minolta B had greater variation in lightness ($P < 0.01$ for both sets) and redness ($P < 0.01$ for set 1, $P = 0.04$ for set 3) than loins evaluated with Minolta A, but did not differ in yellowness. Loins from set 2 did not differ in variability for any color traits. Minolta B was able to predict 36 to 54% of variability in Minolta A lightness, 33 to 48% of variability in redness, and 33 to 43% of variability in yellowness. Butts from sets 1 and 3 evaluated with Minolta B had greater variation in lightness ($P < 0.01$ for set 1, $P = 0.03$ for set 3) than butts evaluated with Minolta A, but did not differ in yellowness. Butts from set 2 measured with Minolta A had greater variation in yellowness ($P = 0.02$) than butts measured with Minolta B,

but did not differ in variability of any other color traits. In butts, Minolta B was able to predict 11 to 36% of variability in Minolta A lightness, 15 to 21% of variability in redness, and 21 to 27% of variability in yellowness. Meter B had greater variability than Meter A on all 3 days in loins, and on day 1 in butts; variability between machines did not differ on days 2 or 3 in butts. Meter A was able to predict 17 to 21% of Meter B variation in loins and 79 to 90% of Meter B variation in butts. Overall, using an open aperture increased variation in instrumental color, and one machine cannot be used to predict measurements from a second machine when instrumental settings are varied between machines.

Introduction

Two of the most important quality traits when evaluating fresh pork are color and ultimate pH (Bendall and Swatland 1988; Mancini and Hunt, 2005). Fresh color is the most important trait for consumers when purchasing pork, as consumers use color as an indicator of freshness (Mancini and Hunt, 2005). Ultimate pH, the pH of muscle after the completion of rigor mortis, has an effect on several characteristics of pork, including color, water holding capacity, and tenderness (Bendall and Swatland, 1988; Watanabe et. al., 1996). In order to measure these quality traits effectively, researchers and producers need to be sure they are using appropriate instruments. Several instruments are available to measure both pH and color, and several settings within an instrument can influence instrumental color, making it difficult to know what type of instrument is the most appropriate (Brewer et. al. 2001). It has been historically proven that even within the same colorimeter illuminant type affects the magnitude of color measurements (Brewer et. al. 2001). However, it is not known whether different illuminants within a colorimeter or different pH meters possess different levels of variation, or if a colorimeter or pH meter can be used to predict the variability of a second machine. Furthermore,

it is not known if variability of machines differs between loins and Boston butts. The hypothesis was that because of inherent differences between machines and muscles, both colorimeters and pH meters would have different levels of variability, and that variability would differ further between muscles. Therefore, the objectives were to characterize variability in instrumental color and pH for loins and Boston butts, and to determine if color of pH measurements from one machine type can be used to predict measurements from a second machine type.

Materials and Methods

Evaluations were made on loins from pigs harvested under FSIS supervision at a commercial abattoir; therefore, Institutional Animal Care and Use Committee approval was not necessary. Pigs were immobilized by carbon dioxide stunning and terminated via exsanguination. Carcasses were blast-chilled and placed into a temperature equilibration cooler. At approximately 22 h postmortem, carcasses were fabricated into primal pieces.

Loins

Loins were removed from the boning and trimming line at the time of cutting to be evaluated for quality characteristics by trained technicians. Three different sets (n_1 = 538, n_2 = 600 and n_3 = 598 for each group, respectively) of loins were measured for instrumental color analysis. Color measurements were made on the ventral face at the approximate location of the 10^{th} rib. Instrumental CIE lightness (L*), redness (a*), and yellowness [b* (CIE, 1978)] were measured on all loins using a Minolta CR-400 Chroma meter equipped with a D65 illuminant, 2° observer, 8mm closed aperture, and calibrated with a white tile specific to the machine (Minolta A). Each set of loins were additionally measured using a second Minolta CR-400 Chroma meter with the same operational settings except for illuminant (C vs D65) and aperture type (open vs closed) (Minolta B). The first set of loins was measured with Minolta B using a D65 illuminant

with an open aperture, the second using a C illuminant with a closed aperture, and the third using a C illuminant with an open aperture. Ultimate pH was measured on three additional sets of loins (n_1 = 249, n_2 = 170, and n_3 = 285, respectively) using 2 separate pH meters in the same anatomical location that color measurements were made, with the 3 sets representing 3 different days of analyses (Meter A and Meter B). Both pH meters utilized glass electrode probes and were calibrated prior to analysis using pH 4 and pH 7 buffer calibration buffers. The loins used for pH analysis were separate from those used for color analysis.

Boston Butts

Shoulders were fabricated into bone-in Boston Butts (NAMP #406; NAMP, 2007). Quality measurements were observed on the serratus ventralis on the face where the Boston butt was removed from the loin. The serratus ventralis was chosen 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. Three different sets of butts (n_1 = 507, n_2 = 525, and n_3 = 524, respectively) were measured for instrumental color analysis in the same manner as for the loins. The combination of illuminant and aperture type for the first Minolta was the same for each set of butts as for the loins; for example, both set 1 of the loins and set 1 of the butts were measured using Minolta A equipped with a D65 illuminant and open aperture. Ultimate pH was measured on three additional sets of butts (n_1 = 522, n_2 = 524, n_3 = 525, respectively) in the same manner as for the loins, with the 3 sets of butts also representing 3 different days of analysis.

Statistical Analyses

Loin (1,736 total for color, 704 total for pH) or Boston Butt (1,556 total for color, 1,571 total for pH) served as the experimental unit in this study. Color and pH data were analyzed using the MIXED procedure of SAS as a 1-way ANOVA with two levels (Minolta A and Minolta B for color, Meter A and Meter B for pH). Variances for each treatment were calculated using the means procedure and tested for homogeneity using the Levene's test of the GLM procedure. Means and variances were considered different at $P \leq 0.05$. Coefficients of determination (R^2) were calculated using the REG procedure of SAS between Minolta readings and between pH meters. Influence of individual observations on estimated values were determined using the Difference of Fit (DFITTS) statistic. Observations were determined to have excessive influence when DFITTS > 2 $[(p/n)^{1/2}]$, where p = the number of parameters and n = the number of observations. Observations that met this criterion were removed from the data set for regression analyses.

Results

Loin Instrumental Color

Loins from set 1 evaluated using Minolta B (open aperture, D65 illuminant) 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 loins evaluated using Minolta A (Table 1). Lightness and redness were more variable ($P < 0.01$) when using Minolta B than Minolta A, but variation in yellowness did not differ between instruments ($P = 0.34$, Table 1). Loins from set 2 evaluated using Minolta B (closed aperture, C illuminant) were 1.16 L* units darker ($P < 0.0001$) and 0.61 b* units more yellow ($P < 0.0001$) than loins evaluated using Minolta A, but did not differ in redness between machines ($P = 0.16$). No color traits for loins from set 2 differed in variability between

instruments. Loins from set 3 evaluated using Minolta B (open aperture, C illuminant) were 1.59 L* units lighter ($P < 0.0001$), 0.39 a* units more red ($P < 0.0001$), and 1.92 b* units more yellow ($P < 0.0001$) than loins evaluated using Minolta A. Lightness ($P < 0.01$) and redness ($P = 0.04$) were more variable when using Minolta B than Minolta A, but variation in yellowness did not differ between instruments ($P = 0.98$).

Instrumental color of loins from set 1 measured by Minolta A explained 54% of variation in lightness ($R^2 = 0.54$, $P < 0.0001$), 48% of variation in redness ($R^2 = 0.48$, $P < 0.0001$), and 41% of variation in yellowness ($R^2 = 0.41$, $P < 0.0001$) measured by Minolta B (Figure 1). Instrumental color of loins from set 2 measured by Minolta A explained 48% of variation in lightness ($R^2 = 0.48$, $P <0.0001$), 40% of variation in redness ($R^2 = 0.40$, $P <0.0001$), and 43% of variation in yellowness ($R^2 = 0.43$, $P <0.0001$) measured by Minolta B (Figure 2). Instrumental color of loins from set 3 measured by Minolta A explained 36% of variation in lightness ($R^2 = 0.36$, $P <0.0001$), 33% of variation in redness ($R^2 = 0.33$, $P <0.0001$), and 33% of variation in yellowness ($R^2 = 0.33$, $P <0.0001$) measured by Minolta B (Figure 3).

Loin Ultimate pH

Loins evaluated using Meter A had an ultimate pH 0.11 units greater on day 1 ($P < 0.0001$), 0.39 units lower on day 2 ($P < 0.0001$), and 0.06 less on day 3 ($P < 0.0001$) than loins evaluated using Meter B (Table 2). Ultimate pH measured using Meter A was able to predict 21% of variation in ultimate pH measured by Meter B on day 1 ($R^2 = 0.15$, $P < 0.0001$, Figure 4), 17% of variation on day 2 ($R^2 = 0.17$ $P < 0.0001$, Figure 5), and 21% of variation on day 3 ($R^2 = 0.21$ $P < 0.0001$, Figure 6).

Boston Butt Instrumental Color

Boston butts from set 1 evaluated using Minolta B (open aperture, D65 illuminant) were 3.17 L* units lighter ($P < 0.0001$) and 6.67 b* units more yellow ($P < 0.0001$) than butts evaluated using Minolta A, but did not differ in redness between instruments (P = 0.84, Table 3). Lightness ($P < 0.01$), redness ($P = 0.01$), and yellowness ($P = 0.02$) were all more variable when measured by Minolta B than Minolta A (Table 3). Butts from set 2 evaluated using Minolta B (closed aperture, C illuminant) 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 butts measured using Minolta A. Yellowness was more variable when measured by Minolta A than Minolta B ($P = 0.02$), but variation in lightness and redness did not differ between instruments. Butts from set 3 evaluated using Minolta B (open aperture, C illuminant) were 2.13 L* units lighter ($P < 0.0001$), 2.55 a* units more red ($P < 0.0001$), and 4.06 b* units more yellow ($P < 0.0001$) than butts measured using Minolta A. Lightness ($P = 0.03$) and redness ($P < 0.0001$) were more variable when evaluated by Minolta B than Minolta A, but variation in yellowness did not differ between instruments ($P = 0.21$).

Instrumental color of butts from set 1 measured by Minolta A explained 11% of variation in lightness ($R^2 = 0.11$, $P < 0.0001$), 21% of variation in redness ($R^2 = 0.21$, $P < 0.0001$), and 21% of variation in yellowness ($R^2 = 0.21$, $P < 0.0001$) measured by Minolta B (Figure 7). Instrumental color of butts from set 2 measured by Minolta A explained 26% of variation in lightness ($R^2 = 0.26$, $P < 0.0001$), 15% of variation in redness ($R^2 = 0.15$, $P < 0.0001$), and 28% of variation in yellowness ($R^2 = 0.28$, $P < 0.0001$) measured by Minolta B (Figure 8). Instrumental color of butts from set 3 measured by Minolta A explained 36% of variation in

lightness ($R^2 = 0.36$, $P < 0.0001$), 17% of variation in redness ($R^2 = 0.17$, $P < 0.0001$), and 27% of variation in yellowness ($R^2 = 0.27$, $P < 0.0001$) measured by Minolta B (Figure 9).

Boston Butt Ultimate pH

Boston butts evaluated using Meter A had an ultimate pH 0.39 units greater on day 1 ($P < 0.0001$), 0.24 units lesser on day 2 ($P < 0.0001$), and 0.06 greater on day 3 ($P < 0.0001$) than loins evaluated using Meter B (Table 4). Ultimate pH measured using Meter A was able to predict 79% of variation in ultimate pH measured by Meter B on day 1 ($R^2 = 0.79$, $P < 0.0001$, Figure 10), 90% of variation on day 2 ($R^2 = 0.90$ $P < 0.0001$, Figure 11), and 88% of variation on day 3 ($R^2 = 0.88$ $P < 0.0001$, Figure 12).

Discussion

Barkley et. al. (2018) reported that when settings between machines of the same model are kept constant, differences in color traits are not large enough to be of practical significance. However, this study did not evaluate any differences in instrumental settings. In the present study, all loin and butt color traits, with the exception of butt redness, differed when using Minolta B equipped with an open aperture regardless of whether illuminant C or D65 was used. When using a closed aperture, all color traits also differed except for loin redness. Brewer et. al. (2001) also reported that even when using the same type of machine, the use of different instrumental settings may cause different color values. However, this study did not evaluate differences when using an open or closed aperture. Variability in loins did not differ between colorimeters for any color traits when using a Minolta with a C or D65 illuminant, despite differences being present in the magnitude of color traits. Variability in butt lightness and redness also did not differ, but variability in yellowness was greater when measured using a Minolta with a closed aperture and C illuminant. However, yellowness is not typically related to

fresh meat color, so it is of less concern than lightness or redness (O'Sullivan et. al., 2003). Variability in loin and butt lightness and redness were greater when using an open aperture regardless of the illuminant used. The difference is likely due to the potential for "pillowing". When using an open aperture, if too much pressure is applied, "pillowing" may occur, causing the sample to form a curved surface inside the aperture as the measurement is taken (AMSA, 2012). Due to the flat surface present with a closed aperture, pillowing is not possible and cannot contribute to increased variation. Pillowing changes the surface, and therefore the reflectance, of the sample, increasing variability in instrumental color compared to using a closed aperture. Therefore, when measuring pork color the state of the aperture must be taken into account if making comparisons between colorimeters.

Regardless of what settings were used, Minolta A was able to predict some variation in any color trait measured by Minolta B ($P < 0.05$). Minolta A was able to predict 33 to 54% of variation in all color traits in loins measured by Minolta B, and 11 to 36% of variation in all color traits in butts. However, no combination of settings for Minolta A, even when the same illuminant or aperture was used, was able to predict more than 54% of variation in any color trait. The AMSA color guidelines (AMSA, 2012) specify that color data can be converted from one illuminant to another, as long as spectral data is collected. As the Minolta device used is a colorimeter and not a spectrophotometer, the data was collected was in tristimulus form rather than spectral form, so it may not be possible to properly covert data between illuminants for this device. While not specifically addressed in the AMSA color guidelines, data from this study would also indicate that machines using different aperture types (open or closed) also cannot be interconverted. Additionally, Minolta B was able to predict more variation in color traits measured by Minolta A when measurements were made on loins than Boston butts. This

difference in prediction ability is likely because overall variation in butt color traits (11 to 36%) was less than that of loins (33 to 54%). The regression performed was to determine how well one colorimeter was able to predict variation in a second colorimeter; when there is less variation to predict, the dependent variable will be less able to predict overall variation (Kaps and Lamberson, 2004). This may indicate that there is less variation in color traits in Boston butts than loins. Limited research has been conducted evaluating color of Boston butts. However, it is known that muscles from the shoulder are primarily used for movement, and consist of more oxidative, red muscle fiber types than loins (Beecher et. al., 1965; Lefaucher et. al, 1986; Gerrard and Grant, 2006). Myoglobin is responsible for the color of meat, so muscles with a greater concentration of myoglobin will be darker (Klont et. al., 1998). Due to their more oxidative nature, muscles from the shoulder retain more myoglobin during rigor compared to muscles found in the loin, which are more glycolytic. (Klont et. al., 1998).

Ultimate pH differed between machines on all three days of analysis in both loins and butts. However, 1 meter did not consistently read higher on all days; in loins, Meter A read a greater pH on days 1 and 3 while Meter B was provided greater readings on day 2. In butts, Meter A read greater on day 1 and Meter B was greater on days 2 and 3. Korkeala et. al. (1986) also reported different ultimate pH values using identical meters equipped with different electrodes. This indicates that similar to colorimeters, different pH meters will not output the same measured values. In loins, Meter B had a greater variation on day 1 but variation did not differ on days 2 and 3, while variation in Meter B pH was greater on all 3 days in butts. Limited research is available discussing differences in pH variability in loins or Boston butts; however, these data would indicate that different machines also possess different levels of variability, and that this change in variability occurs with changes in muscle and days of use. Therefore, care

should be taken to ensure that the same pH meter is used for the duration of an experiment when serial data collection is part of the experimental objective.

Similar to what was observed between colorimeters, the ability of Meter A to predict variability in the ultimate pH measured by Meter B differed between muscles. In contrast to predictions made by colorimeters, however, Meter A was able to predict more variation from Meter B pH in butts (79 – 90% over 3 days) than in loins (17 – 21% over 3 days). Similar to what was observed with instrumental color, variation in butts was greater than in loins, which may be the reason overall predictability was greater in butts. However, the inference space in butts was also greater than that in butts; the ranges of pH data in butts were 1.12 to1.58 units for Meter A and 1.71 - 1.89 units for Meter B, while in loins ranges were only 0.56 to 0.80 for Meter A and 1.00 to 1.30 for Meter B. Because the data ranges in loins were less, a narrower inference space was available and the 2 machines may have had more difficulty detecting differences in variation in loins, decreasing the ability of one machine to predict variability of the other. Therefore, it may be necessary for a population of loins or butts to have a sufficient variability in order for one pH meter to be able to predict measurements made by a second pH meter.

Variability of Minolta output did not change between machines when using D65 or C illuminant, but variability in lightness and redness increased when using an open aperture, regardless of whether loins or butts were measured. Variability is also not constant between pH meters for loins or butts. Additionally, one colorimeter could not be used to predict values measured by a second colorimeter when the illuminant or aperture type is not constant between the 2 but predictions were stronger when measuring loins than butts. One pH meter was able to predict variability in a second pH meter, but only when the inference space was sufficiently

large. Overall, instruments used to measure color or pH cannot be used interchangeably when the operational settings are not constant between the two machines.

Tables

Table 3.1. Effect of instrument on instrumental color least squares means and variability of loins.

	Machine				Variance		
Item	Minolta A[1]	Minolta B[1]	SEM	*P*-value	Minolta A[1]	Minolta B[1]	Levene's P-value
Set 1							
Lightness (L*)[2]	47.80	49.73	0.18	<0.0001	7.47	9.61	<0.01
Redness (a*)[2]	7.71	5.63	0.07	<0.0001	0.98	1.31	<0.01
Yellowness (b*)[2]	-1.23	3.66	0.06	<0.0001	0.79	0.85	0.34
Set 2							
Lightness (L*)[2]	47.32	46.16	0.16	<0.0001	7.23	7.29	0.91
Redness (a*)[2]	7.51	7.43	0.05	0.13	0.85	0.86	0.83
Yellowness (b*)[2]	-1.29	-0.68	0.05	<0.0001	0.80	0.72	0.21
Set 3							
Lightness (L*)[2]	47.62	49.21	0.16	<0.0001	6.80	9.14	<0.01
Redness (a*)[2]	7.53	7.92	0.06	<0.0001	0.89	1.06	0.04
Yellowness (b*)[2]	-1.35	0.57	0.05	<0.0001	0.86	0.86	0.98

[1]Minolta A used a closed aperture and D65 illuminant for all three sets of loins, while Minolta B used a different combination of illuminant and aperture for each set (Set 1 = open aperture, D65 illuminant, Set 2 = closed aperture, C illuminant, Set 3 = open aperture, C illuminant)

[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).

Table 3.2. Prediction of instrumental loin lightness (L*), redness (a*), and yellowness (b*) values observed by Minolta B from color values observed by Minolta A.

Variable	Slope	R^2	P-value
Set 1[1]			
Lightness (L*)[2]	0.83	0.54	<0.0001
Redness (a*)[2]	0.81	0.48	<0.0001
Yellowness (b*)[2]	0.67	0.41	<0.0001
Set 2[1]			
Lightness (L*)[2]	0.70	0.48	<0.0001
Redness (a*)[2]	0.64	0.40	<0.0001
Yellowness (b*)[2]	0.62	0.43	<0.0001
Set 3[1]			
Lightness (L*)[2]	0.70	0.36	<0.0001
Redness (a*)[2]	0.63	0.33	<0.0001
Yellowness (b*)[2]	0.57	0.33	<0.0001

[1]Minolta A used a closed aperture and D65 illuminant for all three sets of loins, while Minolta B used a different combination of illuminant and aperture for each set (Set 1 = open aperture, D65 illuminant, Set 2 = closed aperture, C illuminant, Set 3 = open aperture, C illuminant)

[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).

Table 3.3. Effect of pH meter on ultimate pH least squares means and variability of loins.

		Machine				Variance		
Item	Day	Meter A	Meter B	SEM	*P*-value	Meter A	Meter B	Levene's P-value
pH	1	5.87	5.76	0.01	<0.0001	0.02	0.03	<0.0001
pH	2	5.81	6.20	0.03	<0.0001	0.03	0.12	<0.0001
pH	3	5.82	5.88	0.02	<0.0001	0.03	0.04	<0.01

Table 3.4. Prediction of ultimate loin pH observed by Meter B from ultimate pH observed by Meter A over 3 days.

Variable	Slope	R^2	P-value
Day 1	0.60	0.21	<0.0001
Day 2	1.15	0.17	<0.0001
Day 3	0.73	0.21	<0.0001

Table 3.5. Effect of instrument on instrumental color least squares means and variability of Boston butts.

	Machine				Variance		
Item	Minolta A[1]	Minolta B[1]	SEM	*P*-value	Minolta A[1]	Minolta B[1]	Levene's P-value
Set 1							
Lightness (L*)[2]	41.34	44.51	0.15	<0.0001	5.11	6.9	<0.01
Redness (a*)[2]	15.87	15.89	0.11	0.84	2.69	3.49	0.01
Yellowness (b*)[2]	-0.28	6.39	0.07	<0.0001	1.03	1.33	0.02
Set 2							
Lightness (L*)[2]	40.19	37.82	0.15	<0.0001	6.64	5.39	0.17
Redness (a*)[2]	16.07	15.29	0.09	<0.0001	2.28	1.92	0.16
Yellowness (b*)[2]	-0.49	1.00	0.06	<0.0001	1.11	0.90	0.02
Set 3							
Lightness (L*)[2]	41.44	43.57	0.16	<0.0001	5.58	6.97	0.03
Redness (a*)[2]	16.38	18.93	0.10	<0.0001	2.24	3.46	<0.0001
Yellowness (b*)[2]	-0.13	3.94	0.07	<0.0001	1.05	1.18	0.21

[1]Minolta A used a closed aperture and D65 illuminant for all three sets of loins, while Minolta B used a different combination of illuminant and aperture for each set (Set 1 = open aperture, D65 illuminant, Set 2 = closed aperture, C illuminant, Set 3 = open aperture, C illuminant)

[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).

Table 3.6. Prediction of instrumental Boston butt lightness, redness, and yellowness observed by Minolta B from color values observed by Minolta A.

Variable	Slope	R^2	P-value
Set 1[1]			
Lightness (L*)[2]	0.39	0.11	<0.0001
Redness (a*)[2]	0.52	0.21	<0.0001
Yellowness (b*)[2]	0.53	0.21	<0.0001
Set 2[1]			
Lightness (L*)[2]	0.52	0.18	<0.0001
Redness (a*)[2]	0.35	0.15	<0.0001
Yellowness (b*)[2]	0.48	0.28	<0.0001
Set 3[1]			
Lightness (L*)[2]	0.67	0.36	<0.0001
Redness (a*)[2]	0.52	0.17	<0.0001
Yellowness (b*)[2]	0.55	0.27	<0.0001

[1]Minolta A used a closed aperture and D65 illuminant for all three sets of Boston butts, while Minolta B used a different combination of illuminant and aperture for each set (Set 1 = open aperture, D65 illuminant, Set 2 = closed aperture, C illuminant, Set 3 = open aperture, C illuminant)

[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).

Table 3.7. Effect of pH meter on ultimate pH least squares means and variability of Boston butts.

		Machine				Variance		
Item	Day	Meter A	Meter B	SEM	*P*-value	Meter A	Meter B	Levene's P-value
pH	1	6.26	5.87	0.02	<0.0001	0.06	0.07	0.02
pH	2	6.30	6.54	0.02	<0.0001	0.10	0.12	0.42
pH	3	6.16	6.10	0.02	<0.0001	0.08	0.07	0.61

Table 3.8. Prediction of ultimate Boston butt pH observed by Meter B from ultimate pH observed by Meter A over 3 days.

Variable	Slope	R^2	P-value
Day 1	0.92	0.79	<0.0001
Day 2	1.04	0.90	<0.0001
Day 3	0.90	0.88	<0.0001

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