



Review

Current research in meat color

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This review surveyed recent literature focused on factors that affect myoglobin chemistry, meat color, pigment redox stability, and methodology used to evaluate these properties. The appearance of meat and meat products is a complex topic involving animal genetics, ante- and postmortem conditions, fundamental muscle chemistry, and many factors related to meat processing, packaging, distribution, storage, display, and final preparation for consumption. These factors vary globally, but the variables that affect basic pigment chemistry are reasonably consistent between countries. Essential for maximizing meat color life is an understanding of the combined effects of two fundamental muscle traits, oxygen consumption and metmyoglobin reduction. In the antemortem sector of research, meat color is being related to genomic quantitative loci, numerous pre-harvest nutritional regimens, and housing and harvest environment. Our knowledge of postmortem chilling and pH effects, atmospheres used for packaging, antimicrobial interventions, and quality and safety of cooked color are now more clearly defined. The etiology of bone discoloration is now available. New color measurement methodology, especially digital imaging techniques, and improved modifications to existing methodology are now available. Nevertheless, unanswered questions regarding meat color remain. Meat scientists should continue to develop novel ways of improving muscle color and color stability while also focusing on the basic principles of myoglobin chemistry.

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Keywords: Color; Color stability; Myoglobin; Hemoglobin; MAP; Bone marrow; Antimicrobials; Quantitative loci

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1. Introduction

Meat purchasing decisions are influenced by color more than any other quality factor because consumers use discoloration as an indicator of freshness and wholesomeness. As a result, nearly 15% of retail beef is discounted in price due to surface discoloration, which corresponds to annual revenue losses of \$1 billion (Smith, Belk, Sofos, Tatum, & Williams, 2000). Economic improvements associated with product that achieves its color life potential depends on our knowledge of pre- and postmortem myoglobin chemistry.

Our objectives were to review published (primarily *Meat Science*, *Journal of Muscle Foods*, *Journal of Animal Science*, *Journal of Food Science*) fresh meat color research conducted within the last five years (1999 through 2004). The goal was not to specifically indicate what is new or novel but rather to discuss the current focus of fresh meat color research. As such, our survey of recent peer-reviewed literature suggests that the following were the major topics/areas of interest to researchers.

- Myoglobin chemistry
- Color measurement
- Pre-harvest factors affecting beef color
- Pre-harvest factors affecting pork color
- Antimicrobials
- Modified atmosphere packaging (MAP)
- Bone marrow discoloration
- Cooked color

1.1. Myoglobin redox-form dynamics

Myoglobin is the principle protein responsible for meat color, although other heme proteins such as hemoglobin and cytochrome C may also play a role in beef, lamb, pork, and poultry color. Myoglobin is a water-soluble protein containing 8 α -helices (A–H) linked by short nonhelical sections. Of the numerous residues in

myoglobin, histidine has received the most attention because of its key role in myoglobin structure and function. Myoglobin also contains a prosthetic group located within the protein's hydrophobic pocket. The heme ring has a centrally located iron atom that can form six bonds. Four of these bonds are with pyrrole nitrogens while the 5th coordinates with the proximal histidine-93. A 6th site is available to reversibly bind ligands. A distal histidine-64 also influences color dynamics by affecting space relations within the hydrophobic heme pocket. The ligand present and the valence of iron dictate muscle color (Fig. 1). Therefore, four major chemical forms of myoglobin are primarily responsible for meat color.

1.1.1. Reaction 1: Oxygenation

Deoxymyoglobin occurs when no ligand is present at the 6th coordination site and the heme iron is ferrous (Fe^{2+}). This results in the purplish-red or purplish-pink color typically associated with vacuum packaged product and muscle immediately after cutting. Very low oxygen tension (<1.4 mm Hg; Brooks, 1935) is required to maintain myoglobin in a deoxygenated state. Oxygenation occurs when myoglobin is exposed to oxygen and is characterized by the development of a bright cherry-red color. No change in iron's valence occurs during oxygenation although the 6th coordination site is now occupied by diatomic oxygen. In addition, the distal histidine interacts with bound oxygen, altering myoglobin's structure and stability. As exposure to oxygen increases, the oxymyoglobin penetrates deeper beneath the meat's surface. Depth of oxygen penetration and thickness of the oxymyoglobin layer depend on the meat's temperature, oxygen partial pressure, pH, and competition for oxygen by other respiratory processes.

1.1.2. Reaction 2: Oxidation

Discoloration results from oxidation of both ferrous myoglobin derivatives to ferric iron (Livingston & Brown, 1982; Wallace, Houtchens, Maxwell, &

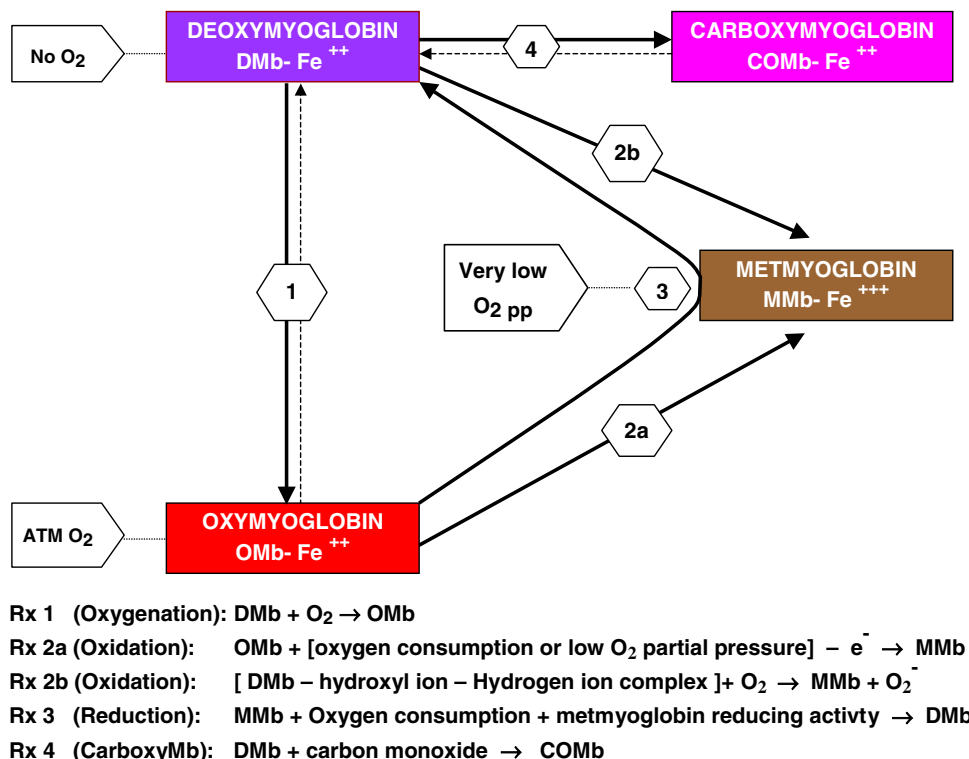


Fig. 1. Visible myoglobin redox interconversions on the surface of meat. Based in the part on: Brooks (1935), Livingston and Brown (1981) and Wallace et al. (1982).

Caughey, 1982). Although discoloration is often referred to as the amount of surface area covered by metmyoglobin, subsurface myoglobin forms also play a role in product appearance. This is because metmyoglobin beneath the surface (located between superficial oxymyoglobin and interior deoxymyoglobin) gradually thickens and moves towards the surface. Metmyoglobin formation depends on numerous factors including oxygen partial pressure, temperature, pH, meat's reducing activity, and in some cases, microbial growth.

1.1.3. Reaction 3: Oxidation plus reduction

Reduction of metmyoglobin is crucial to meat color life and greatly depends on muscle's oxygen scavenging enzymes, reducing enzyme systems, and the NADH pool, which is limited in postmortem muscle. Unfortunately, both enzyme activity and the NADH pool are continually depleted as time postmortem progresses. Although vital for meat color stability, postmortem replenishment of the NADH pool has received little attention.

Note in the color diagram, that reduction of oxymyoglobin on the surface of fresh meat is a two-step reaction. As a result, oxymyoglobin is not converted directly to deoxymyoglobin, but first proceeds through the ferric redox state at low-oxygen partial pressures. Endogenous removal of oxygen to achieve low-oxygen partial pres-

ures occurs via oxygen consumption, which likely results in oxidation of oxy- to metmyoglobin. From a practical standpoint, this is often troublesome because subsequent deoxymyoglobin formation then depends on the muscle's reducing capacity plus further reduction in oxygen tension. For example, chemical reduction of oxymyoglobin poses a problem when packaging bloomed product in vacuum or ultra-low-oxygen atmospheres because the meat chemistry may not be capable of further oxygen consumption coupled with reduction of ferric to ferrous iron (i.e. reaction three cannot be completed).

1.1.4. Reaction 4: Carboxymyoglobin formation

Carboxymyoglobin is a relevant chemical state of myoglobin because of the current increased interest in packaging with low levels of carbon monoxide (Luno, Roncales, Djenane, & Beltran, 2000; FDA, 2002, 2004; Hunt et al., 2004; Sørheim, Nissen, Aune, & Nesbakken, 2001). Exactly which myoglobin derivatives can form carboxymyoglobin is unclear. Obviously, carbon monoxide can bind to the vacant 6th position of deoxymyoglobin and form a very bright-red color that is relatively stable. However, can carbon monoxide displace oxygen coordinated at the 6th position? Similarly, while metmyoglobin is physiologically inert, can carbon monoxide promote reduction and form a bright-red color from oxidized meat? Several fundamental concepts of car-

boxymyoglobin chemistry remain unanswered, regardless of their importance to meat color life. It appears that deoxymyoglobin is more readily converted to carboxymyoglobin than is oxy- or metmyoglobin. Nevertheless, carbon monoxide will slowly dissociate from myoglobin after carboxymyoglobin is exposed to atmospheres free of carbon monoxide.

2. Myoglobin chemistry

Recent work on myoglobin chemistry has examined the regeneration of postmortem reducing equivalents, the relationship between pigment and lipid oxidation, and factors involved in myoglobin stability (Table 1). A key ingredient in meat color life is metmyoglobin reduction, a process that requires NADH. This requirement has, to some extent, questioned the importance of postmortem metmyoglobin reduction because NADH is available in such small amounts and mechanisms involved in its regeneration are not straightforward. Bekhit, Geesink, Ilian, Morton, and Bickerstaffe (2003) suggested that the amount of NADH present plays a greater role in beef color stability than does metmyoglobin reducing activity. Other work has concluded that there is little dependence of ovine muscle color stability on metmyoglobin reducing ability (Bekhit, Geesink, Morton, & Bickerstaffe, 2000). However, when evaluating the relation between color stability and muscle reducing capacity, location of metmyoglobin reducing

activity within extracts (sarcolemmal versus particulate fractions) was critical and therefore, may influence results (Bekhit et al., 2004).

In a system that used enhanced beef as a model, lactate dehydrogenase was involved in both regeneration of postmortem NADH and metmyoglobin reduction (Mancini, Hunt, Kim, & Lawrence, 2004b). This mechanism was responsible for the color stability associated with lactate-enhanced beef (Fig. 2). The authors proposed that lactate dehydrogenase converted postmortem-injected lactate to pyruvate and NADH, which replenished the reducing equivalent pool of postmortem muscle and chemically reduced metmyoglobin due to greater metmyoglobin reducing activity. From a meat quality standpoint, lactate dehydrogenase may be an overlooked endogenous enzyme that has potential to influence color life.

Sammel et al. (2002a, 2002b) described conditions that were damaging to myoglobin reducing chemistry during carcass chilling. The more rapid chilling resulting from the hot boning of the large beef *semimembranosus* muscle resulted in less protein denaturation and more reducing chemistry and pigment redox stability. They also compared methods for measuring reducing capacity of muscle and found the aerobic reduction and nitric oxide reduction methodology to be most correlated with visual and instrumental color stability.

Alderton, Faustman, Liebler, and Hill (2003) reported that 4-hydroxy-2-nonenal, a byproduct of lipid oxidation, induces redox instability of bovine skeletal

Table 1
Summary of research evaluating factors affecting myoglobin chemistry

Publication	Results/conclusions
Faustman et al. (1999)	Secondary lipid oxidation products such as α - and β -aldehydes decrease oxymyoglobin redox stability, possibly by covalent modification of myoglobin
Bekhit et al. (2000)	There is little dependence of ovine muscle color stability on metmyoglobin reducing ability. Electrical stimulation and preslaughter stress had no effect on metmyoglobin reduction
Lynch and Faustman (2000)	Aldehyde lipid oxidation products can decrease both oxymyoglobin stability and the likelihood of metmyoglobin reduction via enzymatic processes
Connolly et al. (2002)	Peroxynitrite can induce rapid and extensive oxymyoglobin instability at concentrations lower than those needed to catalyze lipid oxidation. Oxidation of iron via peroxynitrite is the most likely mode of action
Richards et al. (2002)	Deoxyhemoglobin is a potent catalyst of lipid oxidation in fish muscle. Deoxyhemoglobin's high-spin ferrous iron and less compact structure can contribute to its catalytic nature
Sammel et al. (2002a)	The inner, deep part of the beef <i>semimembranosus</i> muscle is less color stable, lower in NAD, MRA and OCR than the outer portion of the muscle. Two best MRA methods are aerobic reduction and nitric oxide reduction
Sammel et al. (2002b)	Traditional chilling of large, thick beef muscles is damaging to color stability as the rapid pH decline with higher meat temperatures in the deep muscle causes more protein denaturation, less ARA, OCR, WHC and color stability
Alderton et al. (2003)	4-Hydroxy-2-nonenal induces redox instability in bovine skeletal muscle oxymyoglobin via adduction to histidine. This mechanism likely is involved in the interrelationship between lipid and pigment oxidation
Bekhit et al. (2003)	Location of metmyoglobin reducing activity within extracts (sarcolemmal versus particulate fractions) should be considered when researchers are evaluating muscle reducing capacity and its relation to color stability
Lee et al. (2003)	4-Hydroxy-2-nonenal increases the redox instability of porcine oxymyoglobin via covalent modification of myoglobin histidine residues. Species can influence the factors affecting lipid and pigment oxidation
Tang et al. (2003)	Oxymyoglobin redox stability increases with the addition of glutathione to bovine skeletal muscle cytosol. High-molecular weight components within the cytosol influence glutathione effectiveness
Mancini et al. (2004b)	Lactate dehydrogenase may help replenish the pool of postmortem NADH. Production of reducing equivalents via lactate dehydrogenase seemed to be used for metmyoglobin reduction

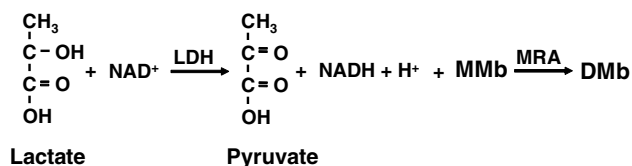


Fig. 2. Proposed mechanism for lactate stabilization of meat color.

muscle oxymyoglobin via adduction to histidine residues. The authors suggested that this covalent modification of myoglobin, particularly at proximal and distal histidines, likely plays a role in the interrelationship between pigment and lipid oxidation. Others have indicated that aldehydes decrease myoglobin redox stability and limit the likelihood of metmyoglobin reduction via enzymatic processes (Faustman, Liebler, McClure, & Sun, 1999; Lee, Phillips, Liebler, & Faustman, 2003; Lynch & Faustman, 2000).

Hemoglobin is more involved in fish pigment and lipid oxidation than beef and pork color due, in part, to increased muscle hemoglobin content post-harvest. In particular, Richards, Modra, and Li (2002) reported that deoxyhemoglobin is a potent catalyst of lipid oxidation in fish muscle. These researchers proposed the following mechanisms to explain the role of deoxyhemoglobin in lipid oxidation. Compared with oxyhemoglobin, deoxyhemoglobin is more unstable and more likely to form methemoglobin, which can further propagate lipid oxidation. This is because deoxyhemoglobin has a high-spin, 5-bond iron complex, consisting of four bonds between iron and the porphyrin ring and one bond between iron and histidine. On the other hand, oxyhemoglobin results from interaction between iron and diatomic oxygen. This forms a 6-bond coordination complex that has a low-spin state and is more stable against oxidation than its high-spin deoxygenated counterpart.

Deoxyhemoglobin's structure also makes it more likely to promote lipid oxidation. When oxyhemoglobin forms, interaction between histidine and diatomic oxygen pulls iron further into the plane of the heme ring, unlike deoxyhemoglobin, where the lack of an oxygen-histidine interaction causes iron to be displaced from the plane (Richards et al., 2002). This allosteric mechanism is transmitted through the rest of the protein, resulting in a change in tertiary structure from a more open deoxyhemoglobin to a more compact oxyhemoglobin. Without the presence of a ligand in deoxyhemoglobin, increased heme cavity flexibility can result, which promotes cavity access to oxidants that normally would be excluded from oxyhemoglobin's iron. Less oxygenated hemoglobins also may be more likely to release heme because these molecules have fewer protein-heme interactions.

Connolly, Brannan, and Decker (2002) reported that peroxynitrite induced rapid and extensive oxymyoglo-

bin instability. Oxidation increased as peroxynitrite concentration increased, with lower levels required to catalyze pigment oxidation than those needed to catalyze lipid oxidation. The mechanism of peroxynitrite-induced myoglobin instability was attributed to direct oxidation of iron rather than altered protein structure. However, small changes in myoglobin's structure resulting from peroxynitrite may limit pigment reduction. Tang, Faustman, Lee, and Hoagland (2003) reported that adding glutathione to bovine muscle cytosol improved oxymyoglobin redox stability. However, glutathione's positive effect on myoglobin redox stability depended on heat sensitive high-molecular weight components within the cytosol of bovine skeletal muscle.

3. Color measurement

3.1. Computer vision

Computer vision based on analysis of digital camera images has distinct advantages over traditional color evaluation (Table 2). For example, O'Sullivan et al. (2003b) noted several benefits associated with digital camera derived j.peg images, including (1) compared with a colorimeter, only a single digital observation is needed for a representative assessment of color, (2) digital images can account for surface variation in myoglobin redox state, and (3) digital image data can be converted to numerous color measurement systems (Hunter, CIE, XYZ, etc.).

Lu, Tan, Shatadal, and Gerrard (2000) acquired digital images using a color camera. The image was then processed to remove background, fat, and bone. RGB and $L^*a^*b^*$ values can be measured on a given area of j.peg images (O'Sullivan et al., 2003b). Sensory color scores can be predicted with statistical and/or neural network models (Lu et al., 2000).

Overall, computer vision is a promising method for predicting visual color. Lu et al. (2000) reported that computer vision, particularly digital image analysis combined with either a neural network or statistical modeling, predicted pork loin visual color. These researchers found moderate correlations between original trained panelist sensory scores and color scores predicted using a multi-layer neural network ($r = 0.75$) and a partial least squares method ($r = 0.52$; Lu et al., 2000). In addition, the neural network resulted in negligible prediction errors for 93% of the samples; 84% of the samples had negligible errors when statistical models were used. O'Sullivan et al. (2003b) reported that instrumental color measures derived from digital camera images can predict red and brown sensory terms. Because the camera measured the entire sample surface, it was more representative of sensory descriptors than

Table 2
Summary of research evaluating meat color measurement methodology

Publication	Results/conclusions
Lu et al. (2000)	Computer vision and digital image analyses combined with either a neural network or statistical modeling, can predict pork loin visual color
Abril et al. (2001)	Cluster analysis segregates beef CIE $L^*a^*b^*C^*h^*$ values into two beef pH groups (<6.1 and ≥ 6.1). Using stepwise discriminant analysis, b^* is best for discriminating between the two pH groups
Alcalde and Noguera (2001)	Discriminant analysis improves when the number of measurement sites increases compared with increasing the number of variables measured at a given site
Barbut (2001)	Incandescent light results in more preferable beef color than fluorescent lighting. Incandescent light has luminance in the red region of the spectrum, whereas fluorescent light produces virtually no red
Brewer et al. (2001)	L^* is the best indicator of PSE and/or DFD pork and Illuminant and instrument type influence instrumental color measures
Carpenter et al. (2001)	Packaging type influences red color perception mainly due to packaging film that comes into contact with the meat (PVC or vacuum), which contrasted with packaging that requires headspace (MAP)
Hulsegge et al. (2001)	Use of large apertures can result in “edge-loss” if the aperture is larger than the measured surface. As a result, light emitted from the instrument is lost and thus, considered absorbed by the sample
Lindahl et al. (2001)	Pigment content and redox state accounts for a large amount of variation in a^* values while only redox state influences b^* . Haem pigment and metmyoglobin contents are slightly correlated with L^*
Ringkob (2001, 2002, 2003)	Image analysis is useful for monitoring both muscle and fat color. Computer programs can be designed to calculate percent metmyoglobin surface coverage
Garcia-Esteban et al. (2003)	Hunter L and CIE L^* produce more reproducible lightness data than $L^*u^*v^*$ and XYZ systems. Angle of observer and illuminant has no effect on measures of lightness from these systems
Mancini et al. (2003)	K/S 610 + K/S 525 is accurate and repeatable and can be used to quantify surface oxymyoglobin concentration and discoloration of ground beef
O’Sullivan et al. (2003a)	Assessment of pork colors associated with b^* (blue to yellow) is more difficult for panelists. Trained panelists were objective and discriminated between red and brown colors. When product color is familiar to panelists and less discriminative evaluation is required, evaluation can be done without training. Nevertheless, trained panelists are more repeatable
O’Sullivan et al. (2003b)	Color measures derived from a digital camera images can predict red and brown sensory terms. Because the camera measures the entire sample, it is more representative than the colorimeter
Mancini et al. (2004a, 2004b)	a^* , chroma, and reflectance spectrums are useful for tracking bone marrow color changes. L^* is less reliable for assessing vertebrae color changes early in display
O’Neill et al. (2003)	Cranial, medial, and caudal locations within the beef <i>longissimus</i> have no effect on instrumental color. Most color change during bloom is completed within the first h of pigment oxygenation

the colorimeter, which was based on point-to-point measurements.

Ringkob (2001) also has evaluated image analysis for measuring meat color. Using special software, the discolored areas on the surface of steaks were mapped and the percentage of the surface covered by metmyoglobin was estimated. Ringkob (2002) and Ringkob (2003) found that image analysis was useful for assessing pork color, particularly detecting differences between yellow and white fat.

3.2. Instrumental color

Currently, many options are available for instrumental color analysis. For example, several types of instruments (colorimeters and spectrophotometers) are available. To further complicate the issue, each instrument offers a variety of options that allow researchers to choose from several (1) color systems (Hunter, CIE, and tristimulus); (2) Illuminants (A, C, D65, and Ultralume); (3) observers (2° and 10°); and (4) aperture sizes (0.64–3.2 cm). Brewer, Zhu, Bidner, Meisinger, and McKeith (2001) reported that illuminant and instrument type influenced color measures

of pork chops, thus suggesting that careful consideration should be given to selecting instrumental color measures if data are compared between carcasses, plants, and experiments. Other work has reported no effects of illuminant and angle of observer on lightness measures from the CIE $L^*a^*b^*$ and Hunter Lab systems (Garcia-Esteban, Ansorena, Gimeno, & Astiasaran, 2003). Hulsegge, Engel, Buist, Merkus, and Klont (2001) speculated that the use of large apertures may promote “edge-loss” if the aperture is larger than the measured surface. As a result, light emitted from the instrument is lost and, thus, considered absorbed by the instrument’s detector.

Selecting the most appropriate color variable is project specific and dependent on the objectives of each experiment. For example, although CIE is commonly used by researchers to measure color, Alcalde and Noguera (2001) reported that tristimulus coordinates (XYZ) also were useful for measuring lamb carcass color. Researchers often report several variables because they are easy to obtain and readily available with most instrumentation. However, depending on the experiment’s objective, increasing the number of variables measured may provide little additional information.

Thus, discriminant analysis may be improved when the number of measurement sites is increased rather than increasing the number of variables measured at a given site (Alcalde & Negueruela, 2001). In addition, location within a muscle should also be considered when making instrumental color measurements (O'Neill, Lynch, Troy, Buckley, & Kerry, 2003; Norman, Berg, Ellersieck, & Lorenzen, 2004; Sammel et al., 2002a, 2002b).

One variable that has received little attention but may be useful is ΔE , which measures total color change by accounting for combined changes in L^* , a^* , and b^* . Abril et al. (2001) reported that total color differences for $\Delta E \geq 0.9$ were visually detectible and useful for differentiating meat of pH < 6.1 vs. >6.1. Future work might be designed to test the utility of ΔE . Mancini, Hunt, Hachmeister, Kropf, and Johnson (2004a) used ΔC^* (Δ Chroma) to measure bone marrow discoloration.

In measuring bloom on the surface of pork muscles, Brewer et al. (2001) reported that L^* was not affected by bloom time whereas a^* and b^* increased within the first 10 min but not thereafter. Hue was less responsive to color changes during bloom (5 min) whereas chroma continued to increase for 20 min. Thus, the authors suggested that each instrumental color measure responds differently to the changes on the surface of fresh cut pork. As a result, they concluded that muscle, instrumental variable, and bloom time must be specified before starting a project if the researchers intend to make comparisons.

If research objectives include characterizing pork color, Brewer et al. (2001) reported that L^* was most correlated to visual determinations of chop pinkness ($r = -0.67$ to -0.80). Using L^* in conjunction with a^* explained 69% of the variability in visual pink color. Thus, the authors suggested that L^* may be the best indicator of PSE and/or DFD pork. Other work has shown that haem pigment and metmyoglobin contents are only slightly correlated with pork L^* values ($r = 0.35$ – 0.45) (Lindahl, Lundstrom, & Tornberg, 2001). Haem pigment and metmyoglobin contents were less correlated with b^* than a^* ($r = 0.40$, 0.50 , respectively), whereas fiber optic probe measurements were more correlated ($r = 0.20$) to b^* . Pigment content and redox state accounted for much of the variation in a^* values while only redox state influenced b^* .

In evaluating veal color, Hulsegge et al. (2001) reported that visual color scores of veal carcasses (*rectus abdominis*) were moderately correlated with L^* ($r = -0.68$) and a^* ($r = 0.69$). In addition, L^* and a^* values correctly assigned half of the carcasses to the same classification as visual assessment. Garcia-Esteben et al. (2003) reported that Hunter measurements for L , a , and b (Illuminant A) were best suited for measuring the color of dry-cured ham. Hunter Lab and CIE $L^*a^*b^*$ provided more reproducible lightness data than $L^*u^*v^*$ and XYZ systems.

Instrumental measures of L^* and a^* are straightforward and can easily be applied to muscle color. On the other hand, the colors represented by b^* (blue and yellow) are not typical or intuitively related to meat. For example, b^* was more correlated to brown as described by sensory panelists than to sensory evaluation of blue and yellow descriptors (O'Sullivan et al., 2003b). They suggested that assessment of b^* (blue to yellow) was difficult for panelists. More training is required for b^* than a^* or L^* because it is difficult for panelists to completely understand the b^* descriptor.

Recent adoption of MAP by the industry has presented several problems in evaluating color. For example, whereas repeated measures during display were easily done when product was overwrapped in oxygen permeable film, the use of packaging with headspace is not conducive to instrumental color measurement without compromising package integrity and atmosphere. To maintain package gas composition, some researchers invert or flip packages over when they have headspace so that the meat is in contact with the film; researchers can then obtain instrumental color measures. However, this process affects film transparency as purge and fat collect on the film. Other researchers open packages before instrumental color measurement, allowing direct contact between the meat surface and the instrument's aperture. Although this is a preferred methodology, it unfortunately requires more packages than traditional oxygen-permeable overwrap or vacuum-skin packaging methods. With this approach, color measurements must be done immediately after the package is opened in order to maintain the effects of the specific gas compositions.

Current literature makes more use of color coordinates (Lab, $L^*a^*b^*$, or XYZ) than spectral data, possibly because these coordinates provide a simple, albeit less direct estimate of discoloration. Although using myoglobin's redox state to quantify discoloration is more time consuming and more difficult than estimating pigment oxidation using a loss of red (decrease in a^*), reflectance spectra has greater diagnostic benefits for cause and effect. Myoglobin redox profiles are assessed traditionally using an accumulation of metmyoglobin. However, Mancini, Hunt, and Kropf (2003) concluded that $K/S\ 610 \div K/S\ 525$ [K is an absorption coefficient and S is a scattering coefficient where $K/S = (1 - \text{reflectance as a decimal})^2 \div (2 \times \text{reflectance})$] was an accurate and repeatable method for quantifying surface oxymyoglobin concentration and discoloration of ground beef. These researchers also suggested that obtaining unrealistic myoglobin estimates (values <0 or >100%) would likely increase significantly if standard values from other work (values published in literature) were used. Therefore, reference samples that provide 100% and 0% of each

pigment form and meet the specific conditions of each experiment (rather than using published standards) will reduce outliers.

Using near infrared reflectance (NIR), Liu et al. (2003) identified bands at 440, 475, 535, and 635 nm for deoxymyoglobin, metmyoglobin, oxymyoglobin, and sulfmyoglobin, respectively. Prediction of Hunter Lab variables with visible/NIR spectroscopy was moderately strong, accounting for 55–90% of the variability. Leroy et al. (2003) reported higher correlations between NIR and L^* (R^2 between predicted and measured values = 0.85) and b^* ($R^2 = 0.75$) than for NIR and a^* ($R^2 = 0.49$). This was attributed to a lack of red color wavelengths (i.e., 580–630 nm) in the NIR spectra (833–2035 nm). Near-infrared spectra between 833 and 2035 nm may be used to predict beef *longissimus* L^* and b^* and to predict beef steak color during aging.

The inseparable relationship between product color and pH is widely accepted. Brewer et al. (2001) suggested that L^* was a reliable indicator of PSE and/or DFD pork and, therefore, can be used as a benchmark for pork quality. For beef samples, cluster analysis segregated CIE $L^*a^*b^*$ values into two groups: samples with ultimate pH less than 6.1 and samples with ultimate pH of 6.1 or more (Abril et al., 2001). Using stepwise discriminant analysis, b^* was selected as the best variable for discriminating between the two pH groups, correctly segregating samples 86–95% of the time and accounting for nearly 90% of the variability in pH. Hue angle also was highly discriminant. When muscle pH was less than 6.1, color change was visible to the naked eye whereas color change was minimal when muscle pH was more than 6.1.

Since consumer purchasing decisions depend on product color, it is interesting that few researchers have evaluated fat and bone color, even though these two contribute to overall product appearance. Ringkob (2003) noted the importance of pork fat color, particularly to the international market, and suggested that image analysis might be useful for measuring fat color.

Mancini et al. (2004a) and Mancini, Hunt, Hachmeister, Kropf, and Johnson (2005a) used instrumental and trained color panelists to assess marrow discoloration. These researchers suggested that a^* was useful for evaluating marrow color, whereas L^* was a less reliable indicator of marrow discoloration. Although bone discoloration is often referred to as “bone darkening” or “bone blackening,” these are misnomers; in fact, “bone graying” was the most common form of discoloration seen shortly after packaging. As a result, L^* increased early in display, not decreasing as the name “black bone” would suggest. Thus, chroma is a more reliable indicator of marrow discoloration because it quantifies graying as an accumulation of white within a pure red color.

3.3. Myoglobin redox forms

For some studies, having a method to quantitatively determine specific myoglobin redox forms is critical for diagnostics and trouble-shooting purposes. Historically, these methods have involved ratios of isobestic wavelengths specific to either 0% or 100% of the redox states. However, some data may produce myoglobin values that are either negative or more than 100%, which causes problems with accuracy of the specific forms. To address these problems, Tang, Faustman, and Hoagland (2004) critically reevaluated the widely used equations of Krzywicki and suggested that new wavelengths be used for determining metmyoglobin (545–503 nm), deoxymyoglobin (565–557 nm), and oxymyoglobin (572–582 nm).

Spectral curves for reflectance also helped in assessing the redox state of hemoglobin on the surface of cut vertebrae and ribs (Mancini et al., 2005a). Similarly, reflectance minima for subcutaneous fat resulted from the redox state of residual hemoglobin (capillaries and/or hemorrhage; Irie, 2001). Darker fat color was attributed to the presence of either deoxyhemoglobin or methemoglobin. Marrow discoloration was attributed to methemoglobin.

The effects of time on intramuscular fat and marrow color were a direct result of hemoglobin derivative inter-conversions (Irie, 2001; Mancini et al., 2005a). For fat color, deoxyhemoglobin predominated immediately after cutting, oxyhemoglobin was present after 1 h, and methemoglobin formed after 3 days (Irie, 2001). Thus, time post-fabrication should be specified when evaluating fat color. Fat color and hemoglobin redox state was affected by texture (soft versus hard fat), which determines oxygen permeability. Although marrow pigment oxygenation (bloom) also was due to a conversion of deoxy- to oxyhemoglobin, pigment oxidation occurred rapidly in vertebrae marrow, and methemoglobin formed within 6 h after packaging in high-oxygen MAP. Mancini et al. (2005a) suggested that marrow discoloration depended on localized oxygen concentration and oxygen diffusion into cut bone.

3.4. Visual color

Carpenter, Cornforth, and Whittier (2001) noted a strong association between color preference and purchasing intent with consumers discriminating against beef that is not red (i.e., beef that is purple or brown). Therefore, visual determinations are the gold standard for assessing treatment effects and estimating consumer perception.

Package type can influence red color perception. Meat packaged with film contact (PVC overwrap or vacuum) was perceived as more red than meat packaged with headspace (Carpenter et al., 2001). These authors

also noted that panelist descriptions of color may depend on individual cognition when references are not used. This point re-emphasizes the need for visual panels to be trained, screened, and selected based on their abilities to consistently evaluate desired color traits. Carpenter et al. (2001) concluded that consumer preference for bright-red colored beef overwrapped in PVC might slow industry's move toward central packaging (MAP and vacuum-skin packaging).

Lighting used for visual color evaluation is often overlooked; however, it can dramatically influence visual color perception. Lighting type and intensity must be standardized from sample to sample, panelist to panelist, and replication to replication. To maximize appearance yet minimize photo-oxidation, recommended lighting is 1614 lux (150 foot candles) of fluorescent lighting, which should have a color temperature of 3000–3500 K (lamps such as Deluxe Warm White, Natural, Deluxe Cool White, SP 3000, SP 3500). Cool White or lamps giving unreal pink, blue, or green tints should be avoided. Barbut (2001) reported that incandescent light increased beef and pork color desirability likely because its spectrum included more red wavelengths. Fluorescent lighting used in this study had virtually no luminance in the red region and, thus, the beef was considered less desirable than beef displayed under incandescent lighting.

O'Sullivan, Byrne, and Martens (2003a) and O'Sullivan et al. (2003b) reported that although trained color panelists can be objective and discriminating, researchers often ignore the relevance of visual panels and, as a result, are more willing to accept instrumental data, likely because obtaining instrumental measurements seems simpler than conducting visual analysis. They suggested that when product color was familiar to the

panelist and a lesser degree of discriminative evaluation was required, visual color evaluation may be done without training. This approach may be more comparable to consumer perceptions of color differences than trained panel observations. Nevertheless, trained panelists produced more repeatable data with a more normal distribution than untrained panelists. Trained panelists also used the red and brown scales more easily than untrained panelists. Thus, training was beneficial, and the use of reference samples resulted in a cognitively clearer use of red and green descriptors by the panelists and improved the degree of sensitivity for detection of muscle discoloration due to metmyoglobin. O'Sullivan et al. (2003a) suggested that future research might be designed to evaluate the level of panelist training, the use of color references, and the repeatability of human color memory.

4. Pre-harvest factors affecting beef color

Recent work has attributed the effects of diet on muscle color to either altered glycogen storage, chilling rate, or antioxidant accumulation, all of which can ultimately relate to muscle's fundamental intrinsic color traits, pH, oxygen consumption, and metmyoglobin reducing activity (Table 3). Supplementing forage-fed cattle with soyhulls improved muscle color without affecting fat color (Baublits et al., 2004). Changes in muscle lightness and yellowness were attributed to dietary effects on pre-harvest glycogen and marbling levels. This agrees with the ability of b^* to detect pH differences (Abril et al., 2001). Vestergaard, Oksberg, and Henckel (2000) reported that forage-based diets fed in restricted amounts might promote oxidative metabolism, rather than

Table 3
Summary of research evaluating beef nutritional factors affecting meat color

Publication	Results/conclusions
French et al. (2000)	Grazing increases the yellowness of subcutaneous fat due to a greater amount of β -carotene in pasture than in concentrates. No dietary effects on <i>longissimus</i> color were reported
French et al. (2001)	When comparing finishing cattle on grass and concentrate, no diet effects on <i>longissimus</i> color will occur. Significant correlations between b^* and carcass fat score (0.29) were noted
Vestergaard et al. (2000)	Forage-based, restricted diets promote oxidative, rather than anaerobic muscle metabolism. This would limit glycogen storage and result in a darker color compared with ad libitum concentrate diets
Lynch et al. (2002)	α -tocopherol levels in adipose tissue are higher in over-wintered than pastured heifers. This increases lipid stability, which could improve <i>longissimus</i> color life
Muramoto et al. (2003)	The yellowness of <i>semimembranosus</i> and <i>longissimus</i> muscles from Japanese black cattle are not affected by dietary β -carotene supplementation prior to slaughter (7500 mg/day for 28 days)
O'Sullivan et al. (2003c)	Although diet (high herbage versus ad libitum concentrate) has no effect on the color of over-wrapped <i>longissimus</i> steaks, herbage diets improve the color stability of steaks packaged in high-ox MAP
Baublits et al. (2004)	Supplementing forage-fed cattle with soyhulls improves muscle color without affecting fat color. Frame size (small, medium, and large) will have little influence on muscle or fat color
Bruce et al. (2004)	Compared with pastured steers, muscle from grain-finished steers is less dark and more red, which can be attributed to subcutaneous fat and slower postmortem chilling
Realini et al. (2004)	During a 21-day display, <i>longissimus</i> $L^*a^*b^*$ was greater for cattle finished on pasture versus finishing on concentrate. Adding vitamin C to ground beef improves color stability during display

anaerobic muscle metabolism and glycogen storage. As a result, bulls fed forage-based, restricted diets had less glycogen, higher muscle pH, and darker muscle color than bulls fed ad libitum concentrates. Similarly, Immonen, Ruusunen, and Puolanne (2000) reported that increasing residual glycogen concentration within the *longissimus* (<25 mM/kg, 25.1–49.9 mM/kg, >50 mM/kg) decreased redness (19.9 versus 18.5 versus 17.3) and increased yellowness (10.0 versus 8.5 versus 7.8).

Muscle from pastured steers was darker than grain-finished steers due to the dietary effects of more subcutaneous fat and slower postmortem chilling, which when combined with lower muscle pH should increase protein denaturation in grain finished animals relative to pasture finished animals (Bruce, Stark, & Beilken, 2004). To support this, the authors reported significant correlations between subcutaneous fat depth and instrumental color measures taken 1 day postmortem (L^* , $r = 0.63$; a^* , $r = 0.60$; b^* , $r = 0.67$). Subcutaneous fat depth also was strongly correlated with pH ($r = -0.82$).

Lynch et al. (2002) reported that breed, feeding regime, and housing influenced display color variability of beef from heifers. In general, *longissimus a** and color stability were greater in over-wintered cattle (finished indoors on silage and concentrate) than in cattle finished on grass. Feeding effects on color were attributed to the relationship between lipid and pigment oxidation, particularly the instability of polyunsaturated fatty acids. For example, pastured cattle had more 18:3 fatty acid. In addition, α -tocopherol levels in adipose tissue were greater in over-wintered than pastured heifers. They suggested that this would increase lipid stability, which in turn would improve *longissimus* color life. O'Sullivan et al. (2003c) reported that dietary treatment (high herbage versus ad libitum concentrate) had no significant effect on the color of overwrapped *longissimus* steaks, whereas *longissimus* color stability of steaks from high-herbage diets packaged in high-oxygen MAP was improved compared to *longissimus* from cattle fed ad libitum concentrate. This was attributed to dietary effects on accumulation of lipid-soluble antioxidants and reduced intramuscular fat.

Muramoto, Nakanishi, Shibata, and Aikawa (2003) reported that dietary β -carotene supplementation (7500 mg/day for 28 days prior to harvest) lengthened color life (time to 20% MMB) by 1.5 and 3 days for the *semimembranosus* and *longissimus*, respectively. However, supplementation had no effect on *semimembranosus* and *longissimus b** values of Japanese Black cattle.

Fat color has received less attention in the literature; however, dietary regime can affect carotenoid content, which can influence fat color. French et al. (2000) reported that yellowness of subcutaneous fat was inversely and linearly related to the amount of dietary concentrate ($r = -0.52$). This was attributed to a lower amount of β -

carotene in concentrates than in pasture, resulting in less carotene accumulation in the fat of cattle fed concentrates.

Housing system may affect beef color through changes in physical activity, which could influence muscle fiber type and metabolism. Vestergaard et al. (2000) reported both a darker muscle color and increased pigmentation for loose-housed bulls fed a roughage-based diet compared with bulls fed ad libitum concentrates in a tie stall. Interestingly, the authors reported little difference in muscle pH (0.07 units higher for loose housing). Loose-housing combined with a roughage-based diet also decreased a^* and chroma. Thus, increased pigmentation resulting from loose-housing resulted in darker, but not more red muscle color. Color and pigmentation differences were attributed to physical activity, rather than feeding level and diet composition. Compared with tie-stall housing and ad libitum concentrates, loose housing and roughage-based diets increased the amount of slow-contracting fibers, vascularization, and muscle oxidative metabolic potential. The authors suggested that increased oxidative muscle potential could decrease lactate production while increasing pyruvate oxidation within mitochondria, β -oxidation, and time to muscle exhaustion. Enzyme changes were likely the result of alterations in muscle fiber type populations. In support of this interrelationship between fiber type characteristics and color, Ozawa et al. (2000) reported significant correlations between α -red fiber diameter and a^* ($r = 0.32$) and β -red fiber diameter and a^* ($r = 0.30$). However, other than positive correlations noted between red fiber diameter and a^* values, fiber type diameter tended to have little relation to measures of instrumental color.

5. Pre-harvest factors affecting pork color

5.1. Genetics

Brewer et al. (2004) evaluated several sire lines (Duroc, Synthetic, Duroc/Landrace, Pietrain, Duroc/Hampshire, and large white) and reported that genetic line affected loin chop two-toning, lightness, pinkness, and a^* (Table 4). Similarly, Edwards, Bates, and Osburn (2003) suggested that Duroc progeny had more favorable visual color, higher pH, and increased redness than Pietrain sired pigs. Although enhancement (10% injection of water plus 0.25% salt and 0.4% tripolyphosphate) tended to minimize the effects of genetic background on pork loin color, variability in the effects of enhancement on pork loin color were attributed to genetic background (Brewer et al., 2004).

Recent work also has focused on the effects of halothane, ryanodine, and napole genotypes on pork color. In summary, fresh pork color is detrimentally affected

Table 4
Summary of research evaluation pork genetics affecting meat color

Publication	Results/conclusions
Bertram et al. (2000)	Both L^* and a^* are normally distributed for the RN– pigs. Loins from normal pigs were less red than loins from pigs with the RN– gene. This is attributed to lower pigment content
Channon et al. (2000)	Pork <i>longissimus</i> color is detrimentally affected by the presence of the n allele. The effects of stunning method and pre-slaughter handling on pork color can be dependent on halothane genetics
Fisher et al. (2000)	Halothane negative pigs (NN) has higher initial and 24-h pH and a lower incidence of PSE (8%) compared with nn genotypes (100% PSE)
Kuchenmeister et al. (2000)	<i>Longissimus</i> of pigs from a malignant hyperthermia resistant line (no mutation in the RYR-1 gene) have lower L^* values than homozygous malignant hyperthermia susceptible pigs (mutant allele in RYR-1)
Piedrafita et al. (2001)	The stress gene (nn) detrimentally affects color (increased paleness), protein denaturation (increased transmittance) and 45-min pH (more rapid decline) but not 24-h pH measurements
Van Oeckel et al. (2001)	<i>Longissimus</i> from homozygous halothane negative pigs (NN) had increased pH and darkness. Removal of the halothane gene may not improve the eating quality of pork
Velarde et al. (2001)	Loins from pigs with one copy of the halothane gene (Nn) are lighter and more yellow than NN pigs. Variability within responses to stunning types is attributed to halothane genotypes
Brewer et al. (2002)	Hampshires with the RN– gene have loins that were less pink than rn+ Hampshires. Breed effects on pinkness and a^* also are noted for Duroc, Pietrain-NN, Hampshire, and Berkshire pigs
Eggert et al. (2002)	Loins from Nn pigs had lower 45-min pH and are paler than loins from NN pigs. An increased amount of Type I or IIA fibers may decrease muscle yellowness
Fabrega et al. (2002)	Loins from pigs containing the halothane gene (Nn) are paler but more red than homozygous NN pigs. Increased a^* is attributed to genotype effects on increased drip loss and oxymyoglobin concentration
Fernandez et al. (2002)	Halothane genotype influences <i>longissimus</i> 40-min pH (nn < Nn < NN) and $L^*a^*b^*$ (nn > Nn=NN) but not 24-h pH. Heterozygous genotypes are intermediate to the homozygous genotypes
Moelich et al. (2003)	Halothane genotype did not affect cooked pork loin roast instrumental color but influences fresh loin color (L^* : nn > Nn > NN)
Brewer et al. (2004)	Genetic Sire lines affect loin chop two-toning, lightness, pinkness, and a^* . Sire lines tested include Duroc, Synthetic, Duroc/Landrace, Pietrain, Duroc/Hampshire, and large white
Lindahl et al. (2004)	Redness differences between alleles at the PRKAG3 (RN) locus are due to myoglobin redox state, oxymyoglobin stability, and metmyoglobin reductase activity

by the presence of the halothane allele (Nn or nn; Channon, Payne, & Warner, 2000; Eggert, Depreux, Schinckel, Grant, & Gerrard, 2002; Fabrega et al., 2002; Fernandez, Neyraud, Astruc, & Sante, 2002; Fisher, Mellett, & Hoffman, 2000; Moelich, Hoffman, & Conradie, 2003; Van Oeckel, Warrants, Boucque, Delputte, & Depuydt, 2001; Velarde et al., 2001). Conversely, halothane effects were not detected for cooked color (Moelich et al., 2003). Mutation on the allele for RYR-1 (homozygous susceptibility for malignant hyperthermia) also increased loin paleness (Kuchenmeister, Kuhn, & Ender, 2000). Other research has suggested that the rn+/rn+ genotype (noncarriers for RN) resulted in darker loins than RN–/rn+ (Moeller, Baas, Leeds, Emnett, & Irvin, 2003). Although the RN– genotype detrimentally affected lightness, the gene improved *longissimus* redness possibly via increased pigment content (Bertram, Petersen, & Andersen, 2000). Lindahl et al. (2004) reported that redness and yellowness differences

between alleles at the PRKAG3 (RN) locus were primarily attributed to myoglobin redox state. Genotypes with less discoloration resulted from the positive effects of increased pH on oxygen consumption, oxymyoglobin stability, and metmyoglobin reductase activity (Lindahl et al., 2004). Similar results were provided by Fabrega et al. (2002), who reported that pigs containing the halothane gene (Nn) had loins that were more red than homozygous NN pigs because of increased oxymyoglobin concentration and drip loss.

5.2. Quantitative trait loci

Ovilo et al. (2002) reported significant quantitative trait loci positions for L^* (109 cM), a^* (89 cM), and pigment content (109 cM) on porcine autosome SSC4 (Table 5). Similarly, quantitative trait loci on SSC7 were found to significantly affect pigment content (position 87 cM), L^* (position 77 cM), and a^* (positions 50 cM).

Table 5
Summary of research evaluating pork genomic factors affecting meat color

Publication	Results/conclusions
de Koning et al. (2001)	Markers and positions for instrumental color variables are found on four separate chromosomes. Quantitative trait loci affecting pork color are detected
Ovilo et al. (2002)	Quantitative trait loci positions are found for L^* , a^* , and pigment content but not b^* , hue angle, and chroma. Therefore, genomic regions account for phenotypic variation in pork <i>longissimus</i> color

Another quantitative trait loci for a^* was located at 81 cM of SSC8. Of the 18 autosomes assessed, no quantitative trait loci were detected for b^* , hue angle, or chroma. Similarly, de Koning et al. (2001) reported markers and positions for instrumental color variables on four separate chromosomes. Thus, quantitative trait loci and genomic regions that account for phenotypic variation in pork *longissimus* color have been detected (de Koning et al., 2001; Ovilo et al., 2002).

5.3. Diet

Dietary supplementation has been examined as a means of improving pork color (Table 6). Supplementing the diets of growing-finishing pigs with a manganese amino acid complex improved pork color (Apple et al., 2004). Similarly, adding magnesium mica to growing-finishing diets improved pork color (a^* , chroma, and visual assessment; Apple, Maxwell, deRodas, Watson, & Johnson, 2000). Feeding magnesium sulfate or proteinate at 1.6 g day⁻¹ for only 1 day darkened pork *longissimus* color and reduced drip losses more than did feeding 3.2 g day⁻¹ (Hamilton, Ellis, McKeith, & Eggert, 2003a). Other work has reported that fortifying diets with magnesium did not influence *longissimus* a^* and b^* , but 2-day supplementation darkened loin color (Hamilton et al., 2002). Magnesium can minimize stress

before harvest, influence intracellular calcium gradients, and promote high-energy phosphates involved in glycolysis (Frederick, van Heugten, & See, 2004).

Wilborn, Kerth, Owsley, Jones, and Frobish (2004) speculated that feeding vitamin D3 might elevate intracellular calcium levels, promote slow-twitch fiber expression (activate calcineurin), and increase oxidative muscle metabolism. Although the mechanism remains unclear, vitamin D3 improved pork color and may benefit pork intended for export (Wiegand et al., 2002; Wilborn et al., 2004).

Matthews, Southern, Higbie, Persica, and Bidner (2001b) speculated that betaine (trimethylamine glycine, known to reduce carcass fat content) accumulation in pig muscle may serve as an osmo-protectant by altering intracellular osmotic regulation. However, the effects of feeding betaine on pork color were minimal (Matthews, Southern, Bidner, & Persica, 2001a; Matthews et al., 2001b).

5.4. Glycolytic potential

Glycolytic potential indicates the capacity for anaerobic metabolism by accounting for the various substrates found in muscle that can be converted to lactic acid (Table 7). Hamilton, Miller, Ellis, McKeith, and Wilson (2003b) reported that glycolytic potential was

Table 6
Summary of research evaluating pork nutritional factors affecting meat color

Publication	Results/conclusions
Apple et al. (2000)	Supplementing pigs with magnesium mica improves <i>longissimus</i> color by increasing a^* and chroma (hue decreased) with no effect on L^* or b^*
Matthews et al. (2001a)	Loins from barrows finished with inadequate pen space are darker than loins from pigs receiving adequate pen space due to a reduced response to stress in barrows finished in inadequate space
Matthews et al. (2001b)	Betaine supplementation has no effect on loin color but at 0.250% darkens <i>biceps femoris</i> color
Rosenvold et al. (2001a)	Strategic finishing of pigs in order to reduce muscle glycogen stores increased 45 min muscle pH and tended to darken loins; therefore, this feeding regime has potential to improve pork quality
Rosenvold et al. (2001b)	Strategic finishing of pigs with diets low in digestible carbohydrates alters glycogen stores within muscle and results in a darker <i>longissimus</i> color without affecting pH
Gentry et al. (2002)	Visual and instrumental color measures are not influenced by housing system: Indoor slatted floor, indoor deep bedding, outdoor housing on dirt, and outdoor housing on alfalfa
Hamilton et al. (2002)	Fortifying swine diets with magnesium did not influence <i>longissimus</i> a^* or b^* , but tended to darken loin color (2-day supplementation). Glycolytic potential affected ultimate pH but not loin color
Rosenvold et al. (2002)	Preslaughter exercise decreased b^* values compared to nonexercised pigs
Wiegand et al. (2002)	Short term feeding of vitamin D3 may be helpful for pork intended for export (lower L^* and higher a^* are noted after 14 days of storage)
Hamilton et al. (2003a)	Feeding magnesium sulfate or proteinate at 1.6 g/day for 1 day will darken pork <i>longissimus</i> color and increase water-holding capacity more than does magnesium at 3.2 g/day. Controls were lightest and with more drip
Rosenvold and Andersen (2003)	Stress-related changes in postmortem muscle temperature may inactivate metmyoglobin reductase and proteins involved in oxygen consumption. Therefore, muscle temperature can significantly affect color and color stability
Apple et al. (2004)	Adding manganese amino acid complex to growing-finishing diets improves pork color (a^* , chroma, and visual assessment)
Frederick et al. (2004)	Supplementing the drinking water of hogs with magnesium improves the color of vacuum packaged loins by reducing muscle paleness (redness and yellowness were not affected by magnesium)
Gentry et al. (2004)	Pigs born and reared outdoors have redder loins than pigs born and reared indoors. Pork color may be improved by altering available space during birth and finishing phases
Wilborn et al. (2004)	Supplementing finishing pigs with 80 IU/kg of vitamin D3 resulted in <i>longissimus</i> with a darker, less gray color. Supranutritional vitamin D3 may improve <i>longissimus</i> color

Table 7
Summary of research evaluating pork glycolytic potential and meat color

Publication	Results/conclusions
Meadus and MacInnis (2000)	Glycolytic potential is correlated to L^* ($r = 0.40$) and b^* ($r = 0.43$), which suggests that increases in glycolytic potential promote acidity, paleness, and yellowness
Allison et al. (2003)	Variation in <i>longissimus</i> color of HAL-1843 free pigs is not related to rate limiting glycolytic enzymes such as pyruvate kinase and phosphofructokinase
Hamilton et al. (2003b)	<i>Longissimus</i> quality is inversely related to glycolytic potential and free glucose. L^* values increase 0.99 and 1.32 units for every one standard deviation in ante- and postmortem glycolytic potential
Moeller et al. (2003)	Loin glycolytic potential is correlated to L^* ($r = 0.33$)

moderately correlated to *longissimus* L^* values ($r = 0.23$ antemortem and 0.31 postmortem). In addition, free glucose content was related to muscle darkening ($r = 0.52$). The authors suggested that L^* values increase 0.99 and 1.32 units for every one standard deviation in ante- and postmortem glycolytic potential, respectively. As a result, *longissimus* quality was inversely related to glycolytic potential and free glucose. Moeller et al. (2003) also reported positive correlations between loin glycolytic potential and L^* ($r = 0.33$). Glycolytic potential was correlated to L^* ($r = 0.40$) and b^* ($r = 0.43$), which suggests that increases in glycolytic potential promote acidity, paleness (lower L^*), and yellowness (greater b^* ; Meadus & MacInnis, 2000). However, Hamilton et al. (2002) reported that glycolytic potential affected ultimate pH but not loin color. Nevertheless, strategic finishing of pigs with diets low in digestible carbohydrates will reduce glycogen stores within muscle and result in darker *longissimus* color without affecting pH (Rosenfold et al., 2001b). Other work has concluded that strategic finishing can be used to reduce muscle glycogen and improve pork color (Rosenfold et al., 2001a). Variation in *longissimus* color of HAL-1843 free pigs was not related to rate-limiting glycolytic enzymes such as pyruvate kinase and phosphofructokinase (Allison, Bates, Booren, Johnson, & Doumit, 2003). Ultimate lac-

tate level and L^* were linearly related, with increased paleness during storage resulting from higher lactate levels (Juncher et al., 2001). Most data indicate that reducing glycolytic potential and free glucose may improve pork muscle color.

The effects of pre-harvest environment, particularly housing and pen space, on pork color have received some recent attention (Table 8). Although neither birth nor rearing environment influenced loin darkness or yellowness, pigs born and reared outdoors had redder loins than pigs born and reared indoors (Gentry, McGlone, Miller, & Blanton, 2004). The mechanism by which rearing environment influenced muscle color was unknown but was attributed to environmental components such as space, soil, and vegetation. Because loins from pigs born and finished outdoors were higher in Type I and IIA fibers, these authors also suggested that rearing pigs in an outdoor production system may increase muscle redness by delaying the conversion of IIA to IIB muscle fibers ($r = 0.44$ between a^* and percent Type I fibers). Matthews et al. (2001a) concluded that loins from barrows finished with inadequate pen space were darker than loins from pigs in adequate pen space. This difference was attributed to a reduced response to stress at harvest in barrows finished in inadequate pen space.

Table 8
Summary of research evaluating pork pre-harvest factors affecting meat color

Publication	Results/conclusions
Channon et al. (2000)	Pre-slaughter handling and stunning method influence pork color. These effects can be dependent on halothane genotype
Kuchenmeister et al. (2000)	Seasonal effects on L^* values (more pale muscle in the summer) are attributed to changes in the calcium release channels
Matthews et al. (2001)	Loins from barrows finished with inadequate pen space are darker than loins from pigs receiving adequate pen space due to a reduced response to stress in barrows finished in inadequate space
Velarde et al. (2001)	Pigs stunned with carbon dioxide have loins that are darker and less yellow than those from pigs electrically stunned. Variability within stunning type is attributed to halothane genotypes
Gentry et al. (2002)	Visual and instrumental color measures are not influenced by housing system: Indoor slatted floor, indoor deep bedding, outdoor housing on dirt, and outdoor housing on alfalfa
O'Neill et al. (2003)	Time of year influences loin pH and instrumental color. In addition to weather, seasonal increases in demand, production, and slaughter rate may detrimentally affect pork color
Rosenfold and Andersen (2003)	In pigs lacking the genetics for rapid pH decline, postmortem muscle temperature rather than pH has a greater influence on color stability, possibly via denaturation of proteins involved in oxygen consumption
Gentry et al. (2004)	Pigs born and reared outdoors had redder loins than pigs born and reared indoors. Therefore, pork color may improve by altering available space during birth and finishing phases

Rosenvold and Andersen (2003) reported that pre-harvest stress did not affect 24-h color and pH but did increase degree of bloom. A stress-induced increase in early postmortem *longissimus* temperature may have promoted denaturation of proteins involved in oxygen consumption, resulting in greater surface oxygenation due to less competition for oxygen by enzymes. Conversely, in the *biceps femoris* and *semitendinosus*, this stress-related elevation of early postmortem muscle temperature may have inactivated metmyoglobin reductase, which subsequently negated the benefits of limited oxygen consumption and resulted in decreased color stability during display. Thus, in pigs lacking the genetics conducive to rapid postmortem pH decline, muscle temperature influences color and color stability more than pH. Other recent reports on the effects of pre-harvest conditions on pork color were provided by Channon et al. (2000), Owen, Montgomery, Ramsey, and Miller (2000), Aaslyng and Barton Gade (2001), Juncher et al. (2001), Rosenvold et al. (2001a), Stoier, Aaslyng, Olsen, and Henckel (2001), and Rosenvold et al. (2002).

Finally, pre-harvest handling and stunning method also influenced pork color (Channon et al., 2000). Pigs stunned with carbon dioxide had loins that were darker and less yellow than those from pigs electrically stunned (Velarde et al., 2001). Variability within stunning type was attributed to halothane genotypes. Seasonal effects on L^* values (more pale muscle in the summer) were attributed to changes in the calcium release channels (Kuchenmeister et al., 2000). Others have reported that in addition to weather, seasonal increases in demand, production, and harvest rate may detrimentally affect pork color (O'Neill et al., 2003).

6. Antimicrobials

Although antimicrobials have been investigated as intervention treatments to extend shelf life and control pathogens, researchers often evaluate only microbial growth and pay less attention to the effects of antimicrobials on color (Table 9). Ideally, antimicrobial technologies should minimize microbial growth while either not affecting or improving product color.

Pohlman, Stivarius, McElyea, Johnson, and Johnson (2002a) reported that 1% ozonated water followed by 5% acetic acid decreased redness, oxymyoglobin content, and color stability during display, likely because the acetic acid promoted pigment oxidation by reducing muscle pH. Similarly, applying either 1% ozonated water followed by 0.5% cetylpyridinium chloride or 1% ozonated water followed by 5% acetic acid to beef trimmings lightened ground beef color, whereas 200 ppm chlorine dioxide followed by 10% trisodium phosphate darkened color. Differences in lightness were attributed to ground beef pH, which was decreased by treatments containing acetic acid (pH = 4.6) and cetylpyridinium (pH = 5.4) and increased by the chlorine dioxide treatment (pH = 7.0). Thus, the authors concluded that 200 ppm chlorine dioxide followed by 10% trisodium phosphate reduced microbial growth while also improving color stability during display. Stivarius, Pohlman, McElyea, and Apple (2002a) also reported that applying acetic acid to beef trimmings tended to negatively influence ground beef color (decreased redness and oxymyoglobin). This was attributed to a lower ground beef pH as a result of adding acetic acid (pH = 4.4). Similarly, Stivarius, Pohlman, McElyea, and Waldroup (2002b) reported that treatment of

Table 9
Summary of research evaluating antimicrobial interventions affecting meat color

Publication	Results/conclusions
Stivarius et al. (2002a)	Applying acetic acid to beef trimmings tends to negatively influence ground beef color. Trisodium phosphate at 5% does not influence ground beef color during display
Stivarius et al. (2002b)	Lactic acid (5%) applied to beef trimmings results in lighter colored ground beef with less surface oxymyoglobin and less redness
Pohlman et al. (2002a)	Ground beef made from trimmings treated with chlorine dioxide (200 ppm) followed by 10% trisodium phosphate reduces microbial growth and improves color stability during display
Pohlman et al. (2002b)	Ground beef made from trimmings treated with 0.5% cetylpyridinium followed by 10% trisodium phosphate has reduced microbial growth and improves color stability during display
Pohlman et al. (2002c)	Treating beef trimmings with either 10% trisodium phosphate or 0.5% cetylpyridinium chloride limits microbial growth and improved ground beef color stability during display
Jimenez-Villarreal et al. (2003a)	Treating beef trimmings with cetylpyridinium followed by trisodium phosphate or chlorine dioxide followed by trisodium phosphate improves ground beef oxymyoglobin stability during display
Jimenez-Villarreal et al. (2003b)	Treating beef trimmings with combinations of either cetylpyridinium chloride and trisodium phosphate or chlorine dioxide and trisodium phosphate improves ground beef color stability
Jimenez-Villarreal et al. (2003c)	Cetylpyridinium and trisodium phosphate affects microorganism growth and ground beef pH, which results in a redder and more stable color during display
Vasavada et al. (2003)	Sodium levulinate was an antimicrobial in fresh pork and fresh turkey sausage, with no undesirable effects on color. Raw meat redness values decreased with storage time in control and levulinate-treated samples."

trimmings with hot water and lactic acid resulted in a lighter, less-red ground beef.

Pohlman, Stivarius, McElyea, and Waldroup (2002c) reported that treating beef trimmings with either 10% trisodium phosphate or 0.5% cetylpyridinium chloride limited microbial growth and improved ground beef color stability during display (increased redness and oxymyoglobin content). Jimenez-Villarreal, Pohlman, Johnson, Brown, and Baublits (2003c) concluded that trisodium phosphate, cetylpyridinium chloride, chlorine dioxide, and lactic acid had little to no effect on ground beef color, and thus, were effective antimicrobial interventions that will not detrimentally influence color. Additional work (Jimenez-Villarreal, Pohlman, Johnson, & Brown, 2003b) indicated that treating beef trimmings with combinations of either cetylpyridinium chloride and trisodium phosphate or chlorine dioxide and trisodium phosphate improved ground beef color stability. Increases in oxymyoglobin content were attributed to antimicrobial effects of slightly elevated pH and reduced microbial growth (Jimenez-Villarreal, Pohlman, Johnson, & Brown, 2003a). A similar reports was provided by Pohlman, Stivarius, McElyea, Johnson, and Johnson (2002b), who concluded that 0.5% cetylpyridinium followed by 10% trisodium phosphate reduced microbial growth while also improving ground beef color stability during display. An antimicrobial-induced elevation of pH was suggested as the mechanism by which trisodium phosphate improved color. Vasavada, Carpenter, Cornforth, and Ghorpade (2003) suggested that sodium levulinate was an antimicrobial in both fresh pork and fresh turkey sausage, with no undesirable effects on color.

7. Modified atmosphere packaging

Because consumers use meat color as an indicator of wholesomeness, recent advances in MAP have focused

on finding the correct blend of gases that maximizes initial color, color stability, and shelf life while also minimizing microbial growth, lipid oxidation, and gaseous headspace (Table 10). High-oxygen atmospheres (80% O₂) promote pigment oxygenation, and therefore, prolong the time before metmyoglobin is visible on the muscle surface. The drawback to high-oxygen MAP is, although it maintains redness during storage, rancidity often develops while color is still desirable (Jayasingh, Cornforth, Brennand, Carpenter, & Whittier, 2002). Jakobsen and Bertelsen (2000) reported that while oxygen levels higher than 20% were necessary to promote meat color, package oxygen contents higher than 55% did not result in additional color stabilizing benefits. Ultra-low-oxygen atmospheres minimize lipid oxidation and aerobic microorganism growth; however, muscle reducing capacity coupled with poor blooming (deoxymyoglobin oxygenation) after long storage can be major drawbacks to this system if ultra-low levels of residual oxygen are not maintained. The levels of O₂ historically quoted (1–2%) are too high; oxygen needs to be less than 1% for pork and less than 0.05% for beef. If the meat lacks sufficient intrinsic oxygen consumption, scavengers may be needed.

To eliminate the disadvantages of commercial ultra-low-oxygen MAP, carbon monoxide has been added to packages because of its high affinity for myoglobin and its ability to form a bright-cherry red color on the surface of beef (Hunt et al., 2004; Jayasingh, Cornforth, Carpenter, & Whittier, 2001; Luno et al., 2000; Sørheim, Nissen, & Nesbakken, 1999). Carbon monoxide (0.5%) also improves the color stability of both injected and noninjected pork chops (Krause, Sebranek, Rust, & Honeyman, 2003).

Hunt et al. (2004) concluded that the use of 0.4% carbon monoxide during storage in MAP improved beef color without masking spoilage. Upon removal of product from carbon monoxide packaging, meat color (likely

Table 10
Summary of research evaluating modified atmosphere packaging (MAP) effects on meat color

Publication	Results/conclusions
Jakobsen and Bertelsen (2000)	Package oxygen content higher than 20% is beneficial for color stability. However, increasing package oxygen content to levels higher than 55% may not provide additional benefits. Color and TBARS were modeled
Luno et al. (2000)	Combining 0.5, 0.75, or 1.0% carbon monoxide with 24% oxygen can stabilize beef color. Lower levels of carbon monoxide are less effective in the presence of 24% oxygen
Jayasingh et al. (2001)	Pretreating steaks with 0.5% carbon monoxide can improve color stability during subsequent vacuum packaging. Using a 0.5% carbon monoxide MAP system also improves steak color stability
Jayasingh et al. (2002)	High-oxygen MAP can benefit color stability but reduce odor and flavor stability. Off-odors and flavors may develop before color has deteriorated
Kusmider et al. (2002)	The detrimental effects of irradiation (2.0 and 4.5 kGy) on color were minimized by using packaging with 0.5% carbon monoxide
Krause et al. (2003)	0.5% carbon monoxide can improve the color stability of injected and noninjected pork chops. The depth of carbon monoxide penetration from the surface increases as exposure time increases
Hunt et al. (2004)	Combining 0.4% carbon monoxide with ultra-low oxygen packaging will improve beef color stability without masking spoilage. Muscle biochemical profile may influence its response to carbon monoxide
John et al. (2004)	High-oxygen MAP increases, whereas low oxygen and 0.4% CO prevent premature browning in ground beef

a combination of COMb and OMb) deteriorated during display in a manner not different from product exposed only to air. Jayasingh et al. (2001) reported that packaging steaks and ground beef in 0.5% carbon monoxide improved color stability. Whereas Hunt et al. (2004) and Jayasingh et al. (2001) combined oxygen exclusion and low levels of carbon monoxide, Luno et al. (2000) tested a carbon monoxide packaging system that also contained 24% oxygen. In this system, carbon monoxide at 0.1% and 0.25% was comparable to high-oxygen whereas 0.5%, 0.75%, and 1.0% carbon monoxide improved beef color stability beyond that of high-oxygen. Thus, Luno et al. (2000) concluded that a minimum of 0.5% carbon monoxide is required to stabilize beef color even in the presence of 24% oxygen. Higher levels of carbon monoxide may increase the risk of masking spoilage with desirable color; however, the off-odors of spoilage should be present.

Another packaging system involves pretreating beef with carbon monoxide prior to vacuum packaging in order to improve product color stability (Jayasingh et al., 2001). Increasing carbon monoxide levels during pretreatment decreased the exposure time necessary to promote depth of penetration. Jayasingh et al. (2001) reported that 5% carbon monoxide resulted in 2.2 times more carbon monoxide penetration within 24 h after packaging than 0.5%. Krause et al. (2003) reported that the depth of carbon monoxide penetration from the surface of pork chops (thickness of the carboxymyoglobin layer) increased as exposure time increased. Depth of penetration was 5 mm within 1 day after packaging and 10 mm after 14 days. Within 36 days, carbon monoxide had completely penetrated the chops, and the myoglobin in the interior appeared to be converted to carboxymyoglobin.

Kusmider, Sebranek, Lonergan, and Honeyman (2002) suggested that packaging beef in 0.5% carbon monoxide could counteract the detrimental effects of irradiation on color. Combining carbon monoxide packaging technology with irradiation (2.0 or 4.5 kGy) produced attractive and safe beef patties.

The traditional “meat color triangle” is well established in the literature, and the interconversions between deoxy-, oxy-, and metmyoglobin have been extensively studied (Fig. 1). However, rarely is carboxymyoglobin considered in this triangle. From a meat-science standpoint, little work has assessed the role of carbon monoxide in basic myoglobin chemistry. Most meat researchers refer to the formation of carboxymyoglobin simply by implicating deoxymyoglobin’s strong affinity for carbon monoxide; but they never consider carboxymyoglobin stability and dynamics other than to state that the carboxy-pigment is “stable.” It is likely that carboxymyoglobin is stable in an atmosphere containing carbon monoxide, but whether it is stable in oxygen-containing atmospheres is unclear. This stability is critical because

one of the systems approved for use in the US requires removing packages from carbon monoxide-containing atmospheres prior to display and sale. The most recently approved system in the US (FDA, 2004), however, allows the meat to be sold with carbon monoxide in the retail package atmosphere.

The stability of carboxymyoglobin once the molecule is challenged with an oxygen-enriched atmosphere is not straightforward. The idea of carbon monoxide dissociation (myoglobin’s ability to hold onto a carbon monoxide ligand) and replacement with oxygen once carboxymyoglobin is exposed to the atmosphere is a fundamental mechanism that should be addressed. In addition, the likelihood of replacing an oxygen ligand with carbon monoxide (conversion of oxy- to carboxymyoglobin) should receive some future attention. Likely, it is a matter of molecular affinity and the relative partial pressures of oxygen and carbon monoxide. This concept does not contradict myoglobin’s binding affinity for carbon monoxide but rather suggests that myoglobin may prefer to hold onto oxygen rather than carbon monoxide. This is particularly important when packaging previously bloomed steaks in carbon monoxide.

When beef was removed from a carbon monoxide atmosphere and vacuum packaged, depth of carbon monoxide penetration gradually decreased (Jayasingh et al., 2001). Conversely, when product remained in carbon monoxide with no oxygen, depth of penetration increased steadily during storage. Other work has noted that carboxymyoglobin remained stable even after chops were removed from a 0.5% carbon monoxide atmosphere and vacuum packaged (Krause et al., 2003).

8. Bone marrow discoloration

With the increased use of high-oxygen MAP, the US meat industry has reported that bone marrow discoloration can decrease beef and pork shelf life. Mancini et al. (2004a, 2005a) reported that the aqueous phase of bone marrow was primarily responsible for discoloration, whereas the lipid portion had no significant role in marrow color stability. Within the water-soluble phase, hemoglobin’s redox state was the principal determinant of marrow color. Promoting the ferrous state of hemoglobin with topical application of water-soluble reducing agents or by excluding oxygen from the packaging atmosphere were most effective for maximizing marrow color stability during storage and display. Increasing lipid stability or metal chelation had no positive effect on hemoglobin oxidative stability and marrow color.

No-oxygen, ultra-low-oxygen, and 0.4% carbon monoxide packaging maintained hemoglobin in its ferrous form and improved marrow color stability (Mancini

et al., 2005a). As reported by industry, high-oxygen MAP decreased the redox stability of hemoglobin within marrow. Applying ascorbic acid or sodium erythorbate to the surface of ribs and vertebrae inhibited discoloration. However, increasing ascorbic acid's lipophilicity (ascorbate-6-palmitate) diminished the reducing agent's ability to stabilize marrow color. Similarly, lipid soluble antioxidants had no positive effects on vertebrae marrow color. To improve the color stability of marrow of ribs and vertebrae, the beef industry should use technologies that minimize hemoglobin oxidation.

9. Cooked color

The increased use of MAP has brought about several changes in cooked meat color (Table 11) due to the effect of packaging atmosphere on myoglobin redox chemistry, which is the primary determinant of cooked color (Hunt, Sørheim, & Slinde, 1999). Tørngren (2003) and Seyfert, Hunt, Mancini, Kropf, and Stroda (2004a) reported that packaging beef steaks in high-oxygen MAP increased the incidence of premature browning (cooked appearance at temperatures too low to kill pathogens). Specifically, injection-enhanced beef round steaks packaged 7 d in high-oxygen MAP were prematurely brown when cooked to 71.1 °C (medium degree of doneness), a temperature that should have some pink

internal color and be safe to consume. The high levels of oxygen in this packaging system maximized oxymyoglobin formation on the surface and in some cases, deep within the product interior (Seyfert et al., 2004a). While high-oxygen MAP was a benefit for fresh meat color, it predisposed cooked product to premature browning at temperatures less than that required for food safety because oxymyoglobin and metmyoglobin were more heat labile (less stable) than deoxymyoglobin (Hunt et al., 1999) and carboxymyoglobin (Ballard, 2004; John et al., 2004). Thus, these authors concluded that internal cooked color should not be used as an indicator of doneness because of its strong dependence on myoglobin's redox state prior to cooking. Lyon, Berry, Soderberg, and Clinch (2000) also suggested that juice color and texture are not reliable indicators of product doneness.

John et al. (2004) and Seyfert, Mancini, and Hunt (2004b) concluded that high-oxygen MAP increased premature browning in ground beef patties. In these studies vacuum-packaged product had predominately deoxymyoglobin in the patty interior prior to cooking and therefore, maintained a slightly pink color to endpoint temperatures of 71.1 °C. In addition, Seyfert et al. (2004b) noted that the internal color of cooked patties exhibited an increase in redness (bloom) upon exposure to the atmosphere. This reemphasizes the thermal stability of the deoxygenated pigment and its role in cooked color. Conversely, ground beef packaged in

Table 11
Summary of research evaluating factors affecting cooked meat color

Publication	Results/conclusions
Hunt et al. (1999)	Myoglobin's redox state influences cooked color because each derivative differs in its thermal stability. Deoxymyoglobin is more stable than oxy- and metmyoglobin
Berry and Bigner-George (2000)	Increasing fat content, endpoint temperature, and time post-cooking before evaluation will decrease the redness of cooked ground beef patties. Frozen storage also affects cooked color
Lyon et al. (2000)	Cooked meat color, juice color, and product texture should not be used to predict degree of doneness
Killinger et al. (2000)	Ground beef patties that had predominately oxy- and metmyoglobin show more premature browning than patties made from ground beef containing high levels of deoxymyoglobin
Berry and Bigner-George (2001)	The thickness of ground beef patties and post-cooking temperature rise will affect cooked color
Phillips et al. (2001)	Erythorbic acid can be helpful in minimizing the incidence of premature browning in ground beef patties
Lien et al. (2002a)	The redox state of porcine myoglobin in addition to muscle pH will dictate cooked chop color
Osborn et al. (2003)	A NADH-metmyoglobin reducing system is present in heated beef muscle extracts. This reducing system can produce an oxymyoglobin-like color from metmyoglobin formed during heating (50–70 °C)
Tørngren (2003)	Use of high-oxygen MAP could increase the incidence of premature browning in steaks
Ballard (2004)	Carboxymyoglobin is more heat stable than deoxymyoglobin and will appear about 1/2–2/3 of a degree of doneness less well done than deoxymyoglobin. Carbon monoxide in packages may increase the incidence of persistent pinking
John et al. (2004)	0.4% CO in low-oxygen MAP increases the endpoint temperature needed to denature carboxymyoglobin and decreases the incidence of premature browning
Seyfert et al. (2004a)	Injection-enhanced beef round steaks packaged in high-oxygen MAP are brown when cooked to 71.1 °C because the high-oxygen MAP promotes oxymyoglobin formation in the product interior
Seyfert et al. (2004b)	High-oxygen MAP increases premature browning in ground beef patties. Premature browning increases as the amount of oxy- and metmyoglobin increase
Mancini et al. (2005b)	Cooked pork loins may develop a pink color during storage. This color reversion is influenced by endpoint temperature and muscle pH
Suman et al. (2004)	Muscle source, location, and inherent biochemical profile can influence ground beef cooked color
Suman et al. (2005)	Adding erythorbate to ground beef increases reducing activity prior to cooking and reduces the incidence of premature browning

high-oxygen MAP had more oxy- and metmyoglobin prior to cooking and, as a result, browned prematurely. Location within a package also had an effect on cooked color with ground beef on the surface (exposed to light during display) being more likely to brown prematurely than ground beef at the bottom of the package (less metmyoglobin than surface beef). Deeper penetration of oxymyoglobin in ground beef in high-oxygen MAP also promotes premature browning. Thus, the ability of high-oxygen ground beef to prematurely brown is strongly related to the amount of oxy- and/or metmyoglobin. Ground beef in high-oxygen MAP could pose a food safety concern if consumers do not measure internal product temperature (Seyfert et al., 2004b). Killinger, Hunt, Campbell, and Kropf (2000) also reported location effects within a package for store-purchased ground beef (overwrapped with polyvinyl chloride). Patties made from ground beef from the upper surface of packages had much more oxy- and metmyoglobin and as a result, had the highest incidence (62.5%) of premature browning. Conversely, patties made from ground beef in the bottom portion of packages had more deoxymyoglobin and the lowest incidence (25%) of premature browning.

Given the role of myoglobin's redox state in cooked color, John et al. (2004) clearly demonstrated that carbon monoxide in modified atmospheres would prevent premature browning of ground beef patties. In addition, Phillips, Mancini, Sun, Lynch, and Faustman (2001) and Suman et al. (2005) suggested that erythorbate may be used to minimize premature browning. These researchers reported greater total reducing activity prior to cooking for ground beef containing erythorbate, which indicates the importance of reduced pigments in developing cooked color. The effects of myoglobin form on cooked color also have been demonstrated in ground and whole muscle pork (Lien et al., 2002a, 2002b). Other factors affecting cooked color include muscle source; anatomical location and inherent biochemical profile; fat content; refrigerated and frozen storage; endpoint temperature; and post-cooking temperature rise (Berry & Bigner-George, 2000; Berry & Bigner-George, 2001; Killinger et al., 2000; Suman et al., 2004).

The use of carbon monoxide in MAP has caused researchers to investigate cooked color of meat containing carboxymyoglobin. John et al. (2004) reported that carboxymyoglobin was more heat stable compared to oxymyoglobin, and Ballard (2004) reported greater denaturation for deoxymyoglobin (58%) at 71.1 °C compared to 49% for carboxymyoglobin. Thus, adding carbon monoxide to low-oxygen packages may increase the incidence of persistent pinking, which John et al. (2004) reports may be due to a heat-denatured CO-hemochrome rather than undenatured carboxymyoglobin. Ballard, 2004 compared *triceps brachii* steaks packaged in vacuum to steaks packaged in carbon monoxide

(0.4% and 2.0%) and found a redder internal cooked color for those in carbon monoxide. Further work is needed to confirm industry observations that carbon monoxide (at 0.4%) decreases cooked color by 0.5–0.7 of a degree of doneness.

Higher pH protects myoglobin from heat denaturation, allowing the maintenance of red or pink color during and after cooking (Hunt et al., 1999; Lien et al., 2002a). In addition to persistent pink color, cooked pork products may develop a pink color during storage (Mancini, Kropf, Hunt, & Johnson, 2005b). As a result, a product that is thoroughly cooked and otherwise appears "done" may gradually undergo color reversion, becoming more pink or red over time. The mechanism of pink color development (return of redness) during storage is not fully understood. In heated beef muscle extracts, metmyoglobin can be chemically reduced, producing an oxymyoglobin-like color (Osborn, Brown, Adams, & Ledward, 2003). Thus, heat-induced (50–70 °C) metmyoglobin formation can be reversed by a NADH-metmyoglobin reducing system (Osborn et al., 2003). Other work has reported that porcine metmyoglobin denatured with urea was reduced by Na₂S₂O₄, although to a lesser extent than native metmyoglobin (Zhu & Brewer, 2003). While pigment reduction is possible, heat denaturation of porcine metmyoglobin likely is not reversible (Zhu & Brewer, 2002).

10. Conclusions

Considerable fresh meat color research has been conducted in the last five years, but several fundamental concepts are still unanswered. In particular, carboxymyoglobin chemistry could receive attention. The role of genetics and quantitative trait loci in meat color merit further evaluation. Additional evaluation of the fundamental relationships between metmyoglobin reduction and oxygen consumption could help solve numerous practical meat color problems. Methodology for color measurement, especially those utilizing scanning and digital technologies need to be refined or developed into practical systems. Although no meat color research was found utilizing nanotechnology, this area should have interesting potential applications. Future research might utilize biomedical techniques and applications to make significant advances in our knowledge of meat color chemistry compared to our current, more applied product research.

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