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Effects of Enhancement Solution pH on Fresh and Cooked Color of Dark-Cutting Beef

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Effects of Enhancement Solution pH on Fresh and Cooked Color of Dark-Cutting Beef

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ABSTRACT

In two experiments, dark-cutting (**DC**) beef strip loins were used to test the effects of citric acid-enhancement pH on visual and instrumental color of fresh and cooked steaks. Dark cutting and normal pH strip loin were injected to 111% of raw product weight with pH 3.5, 4.0, 4.5, or 5.0 solutions by mixing citric acid in either 0.05% phosphate solution or tap water (Exp 1), or with pH 2.0, 2.5, 3.0, and 3.5 solutions made by mixing citric acid in either a 0.5% orthophosphate solution (**PO_4**) or a 0.5% tripolyphosphate solution (**STP**) (Exp2). Loin sections were cut into 2.5-cm-thick steaks and assigned to either simulated retail display for five days or cooked. Post-enhancement pH of enhanced DC steaks did not ($P \geq 0.180$) differ from that of non-enhanced DC steaks, regardless of solution pH (Exp 1); however, decreased linearly ($P < 0.001$) as solution pH decreased from 3.5 to 2.0, and the proportions of free and bound moisture of DC steaks enhanced with pH 2.5 solution were comparable ($P \geq 0.141$) to that of CH (Exp 2). On d 1 and 3 of display, fresh color scores of enhanced DC steaks were greater ($P < 0.001$) than untreated DC (Exp 1), but color scores of CH steaks were greater ($P < 0.001$) than enhanced DC steaks (Exp 1 and 2). Conversely, in experiment 1, degree of doneness scores increased linearly (indicating greater doneness; $P = 0.032$) as solution pH increased from 3.5 to 5.0, and steaks enhanced with pH 4.0 and 4.5 solutions received lower (more red; quadratic, $P = 0.012$) cooked color scores than non-enhanced DC steaks, but neither score was comparable ($P < 0.001$) to those for CH steaks. While in experiment 2 enhancing DC sections with pH 2.5 solutions produced cooked color and degree of doneness scores that were similar ($P \geq 0.113$) to non-enhanced CH steaks. Thus, enhancement with pH 2.5 citric acid solutions can effectively eliminate the persistent red cooked color typically associated with DC beef; however, citric acid enhancement failed to improve the fresh color of DC beef comparable to that of CH beef.

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Chapter I

LITERATURE REVIEW

INTRODCUTION AND REVIEW OF LITERATURE

Dark, Firm, and Dry

Dark cutting beef (DC) is a well known beef quality defect that has been extensively researched since the mid 1900's. Also know as Dark, Firm, and Dry (DFD), this quality defect exhibits a dark red to blackish lean color, a sticky surface texture, increased water-holding capacity, and ultimate muscle pH value in excess of 6.0. These characteristics cause DC carcasses to cost the beef industry millions of dollars a year.

Early research produced conflicting theories of what could cause DC beef. Guilbert (1937) dismissed the factors of age, exercise, feed, delayed bleeding, and pigmentation as possible causes. Mackintosh and Hall (1935) concluded that what they called "black beef" was the result of some undetermined factor affecting the condition of the muscle hemoglobin rather than the quantity of hemoglobin present. Hall, Latschar and Mackintosh (1944) reported a positive relationship between high muscle pH and the incidence of DC beef, which then was confirmed by Lawrie (1958) and Hedrick et al. (1959) who also linked the occurrence of DC beef with stress and the release of epinephrine. Using subcutaneous injections of epinephrine administered for 24 to 48 hr prior to slaughter, Ashmore et al. (1971) established that in DC beef, epinephrine acts on muscle tissue to activate one of the rate limiting enzymes glycogen phosphorolase, thereby accelerating glycogen metabolism. A depletion of muscle glycogen at the time of slaughter is understood to cause the carcass to have higher ultimate pH, as anaerobic metabolism and the use of glycogen postmortem is responsible for lowering postmortem pH (Ashmore et al. 1973)

Weather, growth promotants, genetics, disposition, and handling practices prior to animal slaughter can contribute to decrease in muscle glycogen reserves (Hedrick et al 1959; Smith et al

1993; Voisinet et al. 1997; Scanga et al. 1998). The lack of glycogen in the muscle at the time of slaughter limits the extent of glycolysis resulting in fewer cycles through glycolysis; therefore, less lactic acid is produced. With the reduced levels of lactic acid, the pH of the muscle is unable to drop to normal ultimate pH levels of ~5.5. The muscle proteins are therefore not denatured to the extent of that in a normal muscle and the muscle proteins hold in more water giving it the dark color, firm texture, and dry appearance. Although the name DFD may imply that there is little moisture this is not the case. When the pH is further away from isoelectric point the muscle holds the water intercellular, which gives the meat surface a dry appearance and dry feel (Price and Schweigert, 1987). The relationship between the ultimate muscle pH and muscle glycogen content at slaughter is given by the equation $pH_u = 7.1 – 0.028g$; where g is the glycogen concentration of beef *longissimus* muscle (LM) expressed in μmoles of glucose equivalents per gram of wet tissue (McVeigh, 1980). This relationship is only valid for glycogen concentrations below a limiting concentration of approximately 60 μmoles/g. Thus, to achieve a meat pH of 5.5, the LM glycogen content in beef at slaughter must be at least 57 μmoles/g. Therefore, any factor that depletes the glycogen level below this concentration will increase the ultimate pH value.

Only 1.9 to 2.3 % of the annual cattle slaughter in the U.S. produce DC beef (McKenna et al., 2002; Garcia et al., 2008); however, DC carcasses are penalized from 1/3 to a full USDA quality grade and most beef packers will assess additional carcass value discounts to DC carcasses (Kreikemeir & Unruh, 1998). It has been estimate that this beef quality defect can cost the beef industry anywhere from $175 to $500 million each year.

Meat purchasing decisions are influenced by color more than any other quality factor because consumers use discoloration as an indicator of freshness and wholesomeness (Mancini

& Hunt, 2005). DC beef is not sold to high-end foodservice because when cooked DC exhibit a persistent internal red, undercooked appearance at internal temperatures adequate for browning of normal pH beef cuts (Hague et al., 1994; Cornforth et al., 1991; Mendenhall, 1989; Trout, 1989).

Fresh Color

Hemoglobin and myoglobin are the two primary muscle pigments responsible for meat color. Hemoglobin is located in red blood cells and transports oxygen in the blood stream and delivers it to all the body cells in a living animal, while myoglobin is located in cardiac and skeletal muscle, and accepts oxygen from hemoglobin and facilitates oxygen transport to the mitochondria. However, myoglobin is used in most studies to determine fresh meat color, as both pigments have similar absorption characteristics. Myoglobin measurements would also include the contribution of hemoglobin that was not removed during exsanguination (Govindarajan, 1973).

Myoglobin is a single-chain globular protein of 153 amino acids, containg a heme prosthetic group in the center, with a molecular weight of ~17,800 daltons (Giddings, 1977). The heme prosthetic group of myoglobin is composed of an iron atom which bound within protoporphyrin ring by four of the iron atom's six coordination sites (Lehninger, 1982). The heme group is attached to the apoprotein at the fifth coordination site by a bond between the iron atom and a histidine (proximal) residue. The sixth site is available for binding a variety of ligands (Faustman and Cassens, 1990).

The heme iron may exist in a reduced ferrous (+2), or oxidized ferric (+3) form. Ferrous heme iron which lacks a sixth ligand is called deoxymyoglobin. In the literature, the term "myoglobin" is often used to denote deoxymyoglobin and can result in confusion. A piece of

meat in which deoxymyoglobin is the predominant pigment form will appear purplish-red in color. When oxygen occupies the sixth binding site of ferrous heme iron, oxymyoglobin results and is responsible for the desirable cherry-red appearance of fresh meat. These two reduced forms of myoglobin readily oxidize to the undesirable brownish-red metmyoglbin in which the heme iron is converted to the ferric state and water occupies the sixth coordination position. Metmyoglobin is incapable of binding oxygen and is thus physiologically inactive (Faustman and Cassens, 1990)

The oxidation of myoglobin, or lack thereof, can explain why DC beef display a dark color. It is reported that the overall darker color of DC beef is directly related to a higher mitochondrial respiration rate which keeps oxymyoglobin concentration low. In normal pH muscle, the reduction of postmortem muscle pH through glycolysis impairs the overall level of oxygen that mitochondria can consume (Ashmore et al., 1973). Whereas, Lawrie (1958) found that mitochondrial cytochrome oxidase was more active at higher pH values (>6.0), and concluded that the increases in oxygen consumption of DC meat could increase the concentration of DMb; thus, producing the characteristic dark color.

Cooked Color

The effect pH has on myoglobin to increase the temperature required to denature to the same degree as normal pH cuts at lower temperature is unclear. However, it is well published that increased pH does cause a lack of denaturation of myoglobin at temperatures that under normal pH levels would be fully denatured and present a higher degree of doneness. Most of the previous research focused on prescient pink color due to pH in ground meat products. Mendenhall (1989) found when a range of beef cuts with varying pH values were used to make ground beef patties and cooked to internal temperature of 71° C; patties with a pH near 5.7 had a

red and pink internal color. van Laack, Berry, and Solmon (1996) reported ground beef patties with a pH below 5.9 were associated with a brown cooked color, and the red color occurred mostly in patties with pH Values greater than 5.95. Similar results were reported by multiple other researches that myoglobin denaturation decreases with increasing pH (Trout, 1989; King & White, 2006; Conforth et al., 1986). Interestingly, Hunt et al. (1999) concluded that myoglobin's redox stat influences cooked color because each derivative differs in its thermal stability, and the Deoxymyoglobin is more stable than oxy- and metmyoglobin. However, the precistant pink color is not only limited to beef, Lien et al. (2002) observed similar results in pork and Schmidt and Trout (1984) showed that, even when cooked to the same internal temperature, high-pH beef, pork and turkey muscle was redder than low-pH muscle and appeared undercooked.

However, more recently Sawyer et al. (2008, 2009) has looked at cooked color of dark-cutting beef steaks, and discovered similar results as ground meat products, where increased pH values were related to decreased myoglobin denaturation and the steaks showed a presicestent pink color.

Marination

The word marinade was originally related to fish, and was used as a pickling method in the fish industry; however, the current usage of marinade encompasses all facets of red meats and poultry. Presently marinades are designed to contain such ingredients as a type of lactate, salt phosphates, natural flavorings, and water (Miller, 1998) and are used as a means to decrease the of variation between beef cuts of lower quality grades. Marinade can also help ensure a more flavorful, juicy, and tender product in the event that products are overcooked. Previous research has mainly focused on using marination to influence the tenderness of meat products. Numerous

studies listed in the following paragraphs have identified acid concentration as the operational component that ultimately determines the marinade pH and muscle tenderness.

Griswold (1955) and Howat, Sievert, Myers, Koonce, and Binder (1983) reported that acid marination did not improve meat tenderness. However, Lind, Griswold and Bramblett (1971) reported that meat marinated with 55% (v/v) acetic acid for 48 h (pH 2.85) increased tenderness. More specifically, Wenham and Locker (1976) and Gault (1985,1991) reported that tenderness of meat marinated with acid solutions (pH 2.58 and 3.17) increased, whereas Seuss and Martin (1993) indicated that meat became somewhat more tender as acid concentration increased at pH values between 1.8 and 3.0. The mechanism of increased tenderness observed in meat with pH values below 5.0 is believed to be caused by the intrinsic factors of the acid on the water-holding capacity of muscle proteins. It is believed that marinades act by altering the ultimate muscle pH, which, in turn, alters the physical and/or chemical properties of meat (Oreskovich, Bachtel, McKeith, Novakofski, & Basgall, 1992)

Harrell et al., reported that muscles that contain high pH values (6.38) were more tender than muscles with low pH (5.05) values when exposed to marinade solutions containing citric acid or disodium phosphate (pH 4.6 and 6.65, respectively); however, multiple researchers (Khan & lentz, 1973; Freedeen, Martin, &Weiss, 1974; Dransfield, 1977; Yu & Lee, 1986) have shown that meat with ultimately high postmortem pH (> 6.0; i.e. dark0cutters) are more tender than meat with normal postmortem pH (5.3 to 5.8) values.

The process of acidic marination has been defined as the process of marination meat through the immersion of meat in an acid solution of vinear, wine, or fruit juice (Lewis & Purslow, 1991; Stanton & Light, 1990). Acidic marinades are believed to involve several factors that impact the weakening of muscle structures due to swelling, increased proteolysis by

cathepsins, and an increase in the conversion of collagen to gelatin at low pH during the cooking process (Bege, Ertbjerg, Larsen, Astruc, Vignon, & Moller, 2001; Offer & Kinight, 1988); however, the exact mechanism by which acid martination impacts postmortem muscle is still not thoroughly understood.

The use of organic acid marination to improve the fresh and cooked color of DC beef is limited; however, recent research from Sawyer (2008, 2009) has shown that enhancing longissimus muscle sections from DC carcass with 0 to 1% lactic acid (LA) altered visual and instrumental measurements of internal color of DC beef when cooked to a medium degree of doneness and fresh beef color, equivalent to that with normal ultimate pH values.

Literature Cited

Aktas, N., & Kaya, M. (2001). The influence of marinating with weak organic acids and salts on the intramuscular connective tissue and sensory properties of beef. *European Food Research and Technology, 213,* 88-94.

Ashmore, C. R., Doerr, L., Foster, G., & Carroll, F. (1971). Respiration of mitochondria isolated from dark-cutting beef. *Journal of Animal Science, 33,* 574-577.

Ashmore, C. R., Carroll, F., Doerr, L., Tompkins, G., Stokes, H., & Parker, W. (1973). Experimental prevention of dark-cutting meat. *Journal of Animal Science, 36,* 33-36.

Berge, P., Ertbjerg, P., Larsen, L. M., Astruc, T., Vignon, X., & IVMler, A. J. (2001). Tenderization of beef by lactic acid injected at different times post mortem. *Meat Science, 57,* 347-357.

Cornforth, D. P., Vanhabzadeh, F., Carpenter, C. E., artholomew, D. T. (1986). Role of Reduced Hemochromes in Pink Color Defect of Cooked Turkey Rolls. Journal of Food Science, 51, 1132-1135.

Cornforth, D., Calkins, C. R, & Faustman, C. (1991). Methods for identification and prevention of pink color in cooked meat. *Reciprocal Meat Conf. Proc. 44,* 53-58.

Dransfield, E. (1977). Intramuscular composition and texture of beef muscles. *Journal of the Science of Food and Agriculture, 28,* 833-842.

Faustman C., Cassens, R.G., (1990). The biochemical basis for discoloration in fresh meat: A review. *Journal of Muscle foods,* 1, 217-243

Freeden, H. T., Martin, A. H., & Weiss, G. M. (1974). Changes in tenderness of beef longissimus dorsi as related to muscle color and pH. *Journal of Food Science, 39,* 532-536.

Garcia, L.G., Nicholson, K.L., Hoffman, T.W., Lawrence, T.E., Hale, D.S., Griffin, D.B., Savell, J.W., VanOverbeke, D.L., Morgan, J.B., Belk, K.E., Field, T.G., Scanga, J.A., Tatum, J.D., Smith, G.C., (2008). National Beef Quality Audit-2005: Survey of targeted cattle and carcass characteristics related to quality, quantity, and value of fed steers and heifers. *Journal of Animal Science,* 86, 3533-3543

Gault, N. F. (1985). The relationship between water-holding capacity and cooked meat tenderness in some beef muscles as influenced by acidic conditions below the ultimate pH. *Meat Science, 15,* 15-30.

Gault, N. F. (1991). Marinaded meat. In R. Lawrie (Ed.), *Developments in meat science* - 5 (pp. 191-245). London: Elsevier Science.

Griddings, G.G., (1977). The basis of color in muscle foods. CRC Crit. Review Food Technolgy. 5, 143.

Govindarajan, S., (1973) Fresh meat color. CRC Crit. Review Food Technolgy. 4, 117.

Griswold, R. (1955). The effect of different methods of cooking beef round of commercial and prime grades. 1. Palatability and shear values. *Food Research, 20,* 160-168.

Guilbert, H. R. (1937). What causes black-cutter beef? *Journal of Heredity, 28,* 213-215.
Hall, J. L., Latschar, C. E., & Mackintosh, D. L. (1944). Characteristics of dark-cuttingbeef. Survey and preliminary investigations. *Kansas Agricultural Experiment Station Technical Bulletin, 58,* Manhattan, KS.

Hall, J.L., Latschar, C.E., and Mackintaosh, D.L., (1944). Characteristics of dark-cutting beef. Survery and preliminary investigations. Kansas Agriculture Experment Station Technical Bulletin, 58

Hague, M. A., Warren, K. E., Hunt, M. C, Kropf, D. H., Kastner, C. L., Stroda, S. L., & Johnson, D. E. (1994). Endpoint temperature, internal cooked color, and expressible juice color relationships in ground beef patties. *Journal of Food Science, 59,* 465-470.

Hedrick, H. B., Boillot, J. B., Brady, D. E., & Naumann, H. D. (1959). Etiology of darkcutting beef. *Missouri Agriculture Experiment Station Research Bulletin, Columbia, MO. 111.*

Howart, P. M., Sievert, L. M., Myers, P. J., Koonce, K. L., & Bidner, T. D. (1983). Effect of marination upon mineral content and tenderness of beef. *Journal of Food Science, 48,* 662-663.

Hunt, M. C, S0rheim, 0. & Slinde, E. (1999). Color and heat denaturation of myoglobin forms in ground beef. *Journal of Food Science, 64,* 847-851.

Khan, A. W., & Lentz, C. P. (1973). Influence of ante-mortem glycolysis and dephosphorylation of high energy phosphates on beef aging and tenderness. *Journal of Food Science, 38,* 56-58.

King, N. J., & White, R. (2006). Does it looked cooked? A review of factors that influence cooked meat color. *Journal of Food Science, 71,* 31-40.

Kreikemeir, K.K., Unruh, J.A., & Eck, T.P., (1998). Factors affecting the occurrence of dark-cutting beef and selected carcass traits in finished beef cattle. *Journal of Animal Science,* 76, 388-395.

Lawrie, R. A. (1958). Physiological stress in relation to dark-cutting beef. *Journal of the Science of Food and Agriculture, 11,* 721-725.

Lehninger, A.H., (1982). Principles of Biochemistry, Worth Publishers, New York.

Lewis, G. J., & Purslow, P. P. (1991). The effect of marination and cooking on mechanical properties of intramuscular connective tissue. *Journal of Muscle Foods, 2*, 177-195.

Lien, R., Hunt, M.C., Anderson, S., Kropf, D.H., Loughin, T.M., Dikeman, M.E.,& Velazco, J., (2002). Effects of endpoint temperature on the internal color of pork loin chops of different quality. *Journal of Food Chemistry and Toxicology*, 67, 1007-1010.

Lind, J. M., Griswold, R. M., & Bramblett, V. D. (1971). Tenderizing effect of wine vinegar marinade on beef round. *Journal of American Dietetic Association, 58,* 133-139.

Mackintosh, D. L., & Hall, J. L. (1935). Some factors related to color of meat. *American Society of Animal Production, 28,* 281-286.

Mancini, R.A, Hunt, M.C. (2005). Current research in meat color. *Meat Science*, 71, 100-121

McKenna, D. R., Roeber, D. L., Bates, P. K., Schmidt, T. B., Hale, D. S., Griffin, D. B., Savell, J. W., Brooks, J. C, Morgan, J. B., Montgomery, T. H., Belk, K. E., & Smith, G. C. (2002). National beef quality audit-2000: survey of targeted cattle and carcass characteristics related to quality, quantity, and value of fed steers and heifers. *Journal ofAnimal Science, 80,* 1212-1222.

McVeigh, J. M. (1980). *In vivo* glycogen metabolism in bovine skeletal muscle in relation to dark-cutting in beef. Ph. D. *thesis.* National University of Ireland.

Mendenhall, V. T. (1989). Effect of pH and total pigment concentration on the internal color of cooked ground beef patties. *Journal of Food Science, 54,* 1-2. 21

Miller, R. K. (1998). Functionality of non-meat ingredients used in enhanced pork. *Pork quality facts.* Des Moines, IA: National Pork Board.

Offer, G., & Knight, P. (1988). The structural basis of water-holding in meat - Part 1. General principles and water uptake in meat processing. In R. Lawrie, *Developments in meat science-4* (pp. 63-171). Barking, UK: Elsevier Science.

Oreskovich, D. C, Bechtel, P. J., McKeith, F. K., Novakofski, J., & Basgall, E. J. (1992). Marinade pH affects textural properties of beef. *Journal of Food Science, 57,* 305-311.

Price, J. F., & Schweigert, B. S. (1987). The science of meat and meat products (3rd Ed.). J. Wiley and Sons, New York.

Sawyer, J.T., Apple, J.K., Johnson, Z.B., (2008). The impact of lactic acid concentration and sodium chloride on pH, water-holding capacity, and cooked color of injection-enhanced dark-cutting beef.. *Meat science.* 79, 317-325

Sawyer, J.T., Apple, J.K., Johnson, Z.B., Baublits, R.T., Yancey, J.W.S. (2009). Fresh and cooked color of dark-cutting beef can be altered by post-rigor enhancement with lactic acid. *Meat science.* 83, 263-270

Scanga, J. A., Belk, K. E., Tatum, J. D., Grandin, T., & Smith, G. C. (1998). Factors contributing to the incidence of dark-cutting beef. *Journal of Animal Science, 76,* 2040-2047.

Schmidt, G., & Trout, G., (1984) Ph and Color. Meat Ind. Aug:30

Seuss, L, & Martin, M. (1993). The influence of marinating with food acids on the composition and sensory properties of beef. *Fleischwirtschaft, 73,* 292-295.

Smith, G. C, Tatum, J. D., & Morgan, J. B. (1993). Dark cutting beef: physiology, biochemistry and occurrences. Colorado State University, Fort Collins.

Stanton, C, & Light, N. (1990). The effects of conditioning on meat collage: Part 4 - the use of pre-rigor lactic acid injection to accelerate conditioning in bovine meat. *Meat Science, 27,* 141-159.

Trout, G. (1989). Variation in myoglobin denaturation and color of cooked beef, pork, and turkey meat as influence by pH, sodium chloride, sodium tripolyphosphate, and cooking temperature. *Journal of Food Science, 54,* 536-544.

Van Laack, R., Berry, B. & Solomon, m. (1996). Effect of precooking conditions on color of cooked beef patties. *Journal of Food Protection, 59,* 976-983.

Voisinet, B. D., Grandin, T., O'Connor, S. F., Tatum, J. D., & Deesing, M. J. (1997). *Bos indicus-cross* feedlot cattle with excitable temperaments have tougher meat and a higher incidence of borderline dark cutters. *Meat Science, 46,* 367-377.

Wenham, L. M., & Locker, R. H. (1976). The effect of marinading on beef. *Journal of the Science of Food and Agriculture, 27,* 1079-1084.

Yu, L. P., & Lee, Y. B. (1986). Effects of postmortem pH and temperature on bovine muscle structure and meat tenderness. *Journal of Food Science, 51,* 774-780.

Chapter II

CITRIC ACID ENHANCEMENT AT SOLUTION PH VALUES BETWEEN 3.5 AND 5.0 DOES NOT ALTER THE FRESH AND COOKED COLOR OF DARK CUTTING-BEEF

Abstract

Dark-cutting (**DC**) beef strip loins were used to test the effects of citric acid-enhancement pH on visual and instrumental color of fresh and cooked steaks. Each DC (mean pH = 6.65) and normal pH, USDA Choice (**CH**; mean pH = 5.48) strip loin was cut into 2 equal-length sections, and DC sections were injected to 111% of raw product weight with pH 3.5, 4.0, 4.5, or 5.0 solutions by mixing citric acid in either 0.05% phosphate solution or tap water. After injection and vacuum-tumbling, sections were cut into 2.5-cm-thick steaks, which were assigned to either simulated retail display at 4°C under 1,600 lux of warm-white lighting for five days or cooked to an internal temperature of 71°C on a gas-fired, open-hearth grill. Post-enhancement pH of enhanced DC steaks did not ($P \geq 0.180$) differ from that of non-enhanced DC steaks, regardless of solution pH. On d 1 and 3 of display, fresh color scores of enhanced DC steaks were greater ($P < 0.001$) than untreated DC, but color scores of CH steaks were greater ($P < 0.001$) than enhanced DC steaks. Conversely, degree of doneness scores increased linearly (indicating greater doneness; $P = 0.032$) as solution pH increased from 3.5 to 5.0, and steaks enhanced with pH 4.0 and 4.5 solutions received lower (more red; quadratic, $P = 0.012$) cooked color scores than non-enhanced DC steaks, but neither score was comparable ($P < 0.001$) to those for CH steaks. Results indicated that pH values (3.5 to 5.0) of citric acid enhancement solutions, regardless of base solution, were insufficient to improve fresh or cooked color of DC beef comparable to that of normal pH beef.

Keywords: *Citric acid enhancement; Cooked color; Dark-cutting beef; Fresh Color; Water-holding Capacity; pH*

1. Introduction

Dark-cutting (**DC**) beef is characterized by abnormally elevated muscle pH, increased water-holding capacity, a sticky texture, and a dark, undesirable lean color, which has been attributed to a number of antemortem factors that decrease muscle glycogen reserves prior to slaughter (Hedrick, Boillot, Brady, & Naumann, 1959; Smith, Tatum, & Morgan, 1993; Voisinet, Grandin, O'Connor, Tatum, & Deesing, 1997; Scanga, Belk, Tatum, Grandin, & Smith, 1998). Dark-cutting beef is not typically sold as retail cuts because consumers discriminate against the dark red to almost black fresh lean color (Viljoen, de Kock, & Webb, 2002), and DC beef isn't routinely marketed through foodservice because, when cooked, DC beef will exhibit a persistent red, undercooked appearance at internal temperatures adequate for browning of normal pH beef cuts (Mendenhall, 1989; Trout, 1989; Cornforth, Calkins, & Faustman, 1991).

Organic acids have been used successfully as an antimicrobial intervention for carcasses, primal cuts, and retail cuts for several years (Acuff, Vanderzant, Savell, Jones, Griffin, & Ehlers, 1987; Abugroun, Cousin, & Judge, 1993; Cutter & Siragusa, 1994; Yoon, Mukherjee, Belk, Scanga, Smith, & Sofos, 2009; Elgadir, Mariod, Abdelwahab, Jamilah, Rahman, & Che Man, 2011). In addition, a number of studies have shown that marinating meat from mature animals (Eilers et al., 1994; Morris, Theis, Miller, Acuff, & Savell, 1997; Ertbjerg, Larsen, & Møller, 1999a; Ertbjerg, Mielche, Larsen, & Møller, 1999b; Aktaş, Aksu, & Kaya, 2003) or from muscles with high amounts of connective tissue (Arganosa & Marriott, 1989; Burke & Monahan, 2003; Chang, Wang, Zhou, Xu, & Li, 2010) in organic acids improves cooked meat tenderness. And, more recently, enhancing post-rigor DC beef with lactic acid solutions has been shown to improve both fresh (Apple, Sawyer, Meullenet, Yancey, & Wharton, 2011) and cooked beef color (Sawyer, Apple, & Johnson, 2008; Sawyer, Apple, Johnson, Baublits, & Yancey, 2009;

Apple et al., 2011); however, enhancing DC beef with solutions containing 0.50, 0.75, and 1.00% lactic acid reduced post-enhancement muscle pH to between 4.1 and 4.6, resulting in decreased water-holding capacity and development of some abnormal fresh and cooked color variations (Sawyer et al., 2009). On the other hand, Sammel and Claus (2003, 2006) reported that marinating of turkey breast in solutions containing citric acid effectively reduced the persistent pink color of cooked ground and whole breasts treated with pinking agents. Because citric acid has three pKa values, it is plausible that enhancement solutions formulated with citric acid may not reduce post-enhancement pH as severely as lactic acid. Therefore, the objective of this study was to test the effects of the pH of citric acid enhancement solutions on fresh and cooked color of DC beef.

2. Materials and methods

2.1 Muscles

Normal pH, low U. S. Choice (**CH**; n = 5) and dark-cutting (**DC**; n = 41) beef strip loins (IMPS #180) were selected based on 24-hour postmortem pH and purchased from a large commercial slaughter facility. Upon arrival to the University of Arkansas Red Meat Abattoir, strip loins were removed from the vacuum package and trimmed free of fat and adjacent muscles. Then, pH was measured for each strip loin with a ceramic-tipped, hand-held pH probe (Testo 205 pH, Sparta, NJ, USA) before being cut into two equal-length sections (mean pH for DC and CH beef strip loins was 6.74 and 5.48, respectively).

2.2 *Enhancement Treatments*

The DC muscle sections (n = 9/treatment) were assigned randomly to 1 of 9 enhancement treatments of pH 3.5, 4.0, 4.5, or 5.0 solutions, made by mixing citric acid (**CA**; Cargill, Inc., Eddyville, IA, USA) in either a 0.5% orthophosphate solution (**PO_4**; B. K. Giulini Corp., Simi

Valley, CA, USA) or tap water (**H_2O**), and a non-injected negative control, whereas the CH sections served as a non-injected positive control. Enhancement solutions were prepared in 4°C tap water, and, under continuous agitation with a Rotosolver high-shear mixer (Admix Inc., Manchester, NH, USA), citric acid was titrated into either the H2O or PO4 base solutions to achieve solution pH values of 3.5, 4.0, 4.5, and 5.0. The weight and pH of each DC section were recorded before being injected to a targeted 111% of raw weight with their respective enhancement solution using a Fomaco 20/40 injector (Reiser Inc., Canton, MA, USA). Post-injection weight of each section was recorded, and injected DC sections were tumbled at 42 rpm under 12.4 N/cm^2 of vacuum for 5 min. After tumbling, the enhanced sections were weighed, placed on shelves, allowed to equilibrate for 30 minutes before recording post-enhancement weights and pH values. Both pH and weight of non-enhanced DC and CH sections were recorded at comparable times, and yields during the enhancement process were calculated based on the fresh, pre-enhanced section weight.

2.3 Sample preparation

Sections were cut into three 2.54-cm-thick steaks and one 1.27-cm-thick steak. One 2.54-cm-thick steak was vacuum-packaged and stored at -20°C for evaluation of cooked color, whereas the remaining 2.54-cm-thick steaks were individually placed onto foam trays with absorbent pads, over-wrapped with a polyvinyl chloride film (O_2 transmission rate = 14,000 cc $O_2/m^2/24h/atm$; Koch Supplies Inc., Kansas City, MO, USA), and stored at 2° C. Steaks designated for instrumental (n = 90) and visual color (n = 90) analyses were stored under simulated retail display conditions (2° C and 1,600 lux deluxe warm white fluorescent lighting; Philips Inc., Somerset, NJ, USA) for 6 d. The 1.27-cm-thick steak was used to measure water-holding capacity (WHC) and moisture content.

2.4 Simulated retail display

Instrumental color of steaks under simulated retail display conditions was measured on days 0, 1, 2, 3, 4, and 5 using a Hunter MiniScan XE (Model 45/0-L, Hunter Associates Laboratory Inc., Reston, VA, USA) calibrated daily against black and white tiles. The L*, a*, and b* values, were determined from the mean of three readings on the surface of each steak using Illuminant A, a 10° standard observer, and a 2.54-cm aperture. Additionally, hue angle was calculated as: $\tan^{-1}(b^*/a^*)$, whereas chroma (C*) was calculated as: $\sqrt{}$ (AMSA, 2012).

A five-member, trained sensory panel was used to evaluate sensory color of steaks during retail display. Panelists were selected and trained according to AMSA (1991) guidelines. Sensory panelists evaluated each steak under display for fresh beef color (8 = extremely bright cherry-red; 7 = bright cherry-red; 6 = moderately bright cherry-red; 5 = slightly bright-cherry red; 4 = slightly dark cherry-red; 3 = moderately dark red; 2 = dark red; and 1 = extremely dark red; AMSA, 2012), percent discoloration (8 = no [0 to 5%] discoloration to 1 = total [≥ 96%] discoloration), and overall acceptability (7 = extremely desirable; 6 = desirable; 5 = slightly desirable; 4 = acceptable; 3 = slightly undesirable; 2 = undesirable; and 1 = extremely undesirable; AMSA, 2012) on each day of simulated retail display.

2.5 *Cooked color*

Steaks (2.54-cm-thick) were thawed for approximately 16 h at 1° C before being cooked on a gas-fired, open-hearth grill (Star Manufacturing, Inc., Smithville, TN, USA). Steaks were turned every three minutes until the internal temperature of the steak reached 71° C (AMSA, 1995), and rested 5 minutes at room temperature before slicing for visual and instrumental color analysis. Internal steak temperature was monitored using Omega Hypodermic probe (Omega Technologies, Stamford, CT, USA) attached to a K28 Foodcheck Thermometer (Comark

Instruments, Beaverton, OR, USA). Steaks were cut just off the center (perpendicular to the steak surface), and, within 20 seconds of cutting, a ten-member, trained sensory panel (selected and trained according to AMSA [1991] guidelines) evaluated each cut surface (to the nearest 0.5) for internal cooked color (7 = brown, 6 = gray brown, 5 = pinkish gray, 4 = slightly pink, 3 = pink, 2 = medium red, and 1 = very red; AMSA, 2012) and internal doneness (7 = very well, 6 = well done, 5 = medium well; 4 = medium, 3 = medium rare, 2 = rare, and 1 = very rare; AMSA, 2012). Sensory evaluation was conducted with two sensory color sessions a day (n = 9 steaks/session) over a five-day period. Instrumental cooked color readings of steaks were measured concurrently with visual analysis on half of the steak wrapped immediately after cutting with a polyvinyl chloride film (O_2 transmission rate = 14,000 cc $O_2/m^2/24h/atm$; Koch Supplies Inc., Kansas City, MO, USA) to minimize post-cookery blooming. Values were measured using a Hunter MiniScan XE (Model 45/0-L, Hunter Associates Laboratory Inc., Reston, VA, USA) calibrated before every session against black and white tiles. The L*, a*, and b* values were determined from the mean of three readings on the cut surface of each steak using Illuminant A, a 10° standard observer, and a 1.27-cm aperture. In addition to calculating hue angle and C* as described previously, the reflectance at 630 nm was divided by the reflectance at 580 nm to calculate the red-to-brown ratio (AMSA, 2012).

2.6 Water-holding Capacity

Water-holding capacity of muscle samples was measured approximately 30 min after the fabrication of the sections using the methodology of Wierbicki and Deatherage (1958). Briefly, 500 mg of fresh never frozen muscle tissue was weighted onto a piece of Whatman No. 1 filter paper, which had been stored in a desiccator over saturated potassium chloride. The sample was then pressed at 345 N/cm^2 for 1 min in a Carver Press (Fred S. Carver Inc., Summit, NJ). Areas

of the meat and moisture were traced and subsequently measured using a compensating planimeter (Planix 8, Sokkia Corp., Overland Park, KS, USA). All samples were run in duplicate, and the percentage of free water was calculated by the fallowing equation: free water (%) = (((moisture surface area – meat surface area) 61.1) / total moisture) 100, whereas the percentage of bound water was calculated by subtracting the free water from 100.

2.7 Percentage of Moisture

Percent moisture analysis was conducted using five-gram samples of *Longissimus* muscle (LM) according to the freeze-drying method of Apple, Davis, Rakes, Maxwell, Stivarius, and Pohlman (2001). Samples were freeze-dried for 96 h in a Labconoco freeze dryer (Labconoco Corp., Kansas City, MO, USA) with settings for vacuum pressure of <10 μm Hg and a temperature of -50° C. After drying, moisture percentage was calculated as the difference between the wet and dry sample weights divided by the wet weight.

2.8 Statistical analysis

Data were analyzed as a completely randomized design, with treatments in a 2 × 4 factorial arrangement, plus positive (CH) and negative (DC) controls, and loin sections as the experimental unit. The ANOVA was generated using the mixed model procedure of SAS (SAS Inst., Inc., Cary, NC), and the statistical model for pH and water-holding capacity, as well as all cooked color characteristics, included enhancement treatment as the lone fixed effect, whereas the fresh steak color data collected for five days of simulated retail display was analyzed as a repeated measure, with treatment, display day, and the treatment × day interaction as the fixed effects in the model. In the analysis of visual appraisal of fresh and cooked color, panelist was included in the model as a random effect. Least squares means were calculated for all treatments, and, because of the unique treatment structure, preplanned contrasts were used to test: 1) based

solution differences (PO_4 vs. H_2O); 2) linear and quadratic responses to decreasing enhancement solution pH; 3) differences between each enhancement solution pH and untreated, low Choice; and 4) differences between each enhancement solution pH and non-enhanced, DC control.

3. Results and Discussion

3.1 pH and moisture retention

As expected, pre-enhancement pH of DC sections was substantially greater ($P < 0.001$) than CH sections, and, regardless of solution pH, post-enhancement pH of enhanced DC sections remained considerably greater ($P < 0.001$) than CH sections (Table 1). Although post-enhancement pH values were similar ($P \geq 0.180$) among enhanced and non-enhanced DC sections, sections enhanced with PO_4-based solutions had lower ($P < 0.001$) pH values than those enhanced with H_2O-based solutions. Contrary to the present results, a number of studies have clearly demonstrated that subjecting beef to citric acid marination reduced muscle pH (Arganosa & Marriott, 1989; Ke, Huang, Decker, & Hultin, 2009; Elgadir et al., 2011). A possible reason for the discrepancy between results of the present study and published results could be the solution pH. For example, Howat, Sievert, Myers, Koonce, and Bidner (1983) reduced the pH of *semimembranosus* steaks from 5.7 to 4.7 in a lime juice marinade with a solution pH of 2.56. Moreover, when LM steaks were marinated in solutions formulated with 0.5, 1.0, and 1.5% citric acid, Aktaş et al. (2003) observed a reduction in pH from 5.72 to between 4.28 and 3.70, but the marinade pH values were 2.73, 2.50, and 2.40, respectively. Both Sawyer et al. (2009) and Apple et al. (2011) reported that enhancing DC beef with solutions containing 0.15 to 0.35% lactic acid failed to reduce muscle pH below 6.0, but the pH of the LM from DC carcasses was reduced to values below 5.0 by lactic acid enhancement at concentrations between 0.50 and 2.0% (Sawyer et al., 2008, 2009). Because citric acid has three pKa values

(3.06, 4.74, and 5.40), Aktaş et al. (2003) explained that citric acid cannot induce dramatic reductions in post-rigor muscle pH because it can act as a buffer when it binds to muscle, whereas lactic acid can dramatically reduce muscle pH because it has only one pKa at 3.86; therefore, it would take greater quantities of citric acid, resulting in a much lower enhancement solution pH, to reduce the muscle pH of DC beef.

Total moisture content was greater ($P = 0.053$) in non-enhanced DC steaks than normal-pH, CH steaks, and the moisture content of steaks from enhanced DC sections was greater ($P < 0.001$) than both untreated DC and CH steaks, regardless of base solution (Table 1). When comparing non-enhanced controls, DC steaks had less ($P < 0.001$) free moisture and more ($P < 0.001$) bound moisture than CH steaks. Regardless of solution pH, the free moisture content of enhanced DC sections was less ($P < 0.001$) than CH and greater ($P \leq 0.021$) than DC, whereas the bound moisture content of enhanced DC sections was greater ($P < 0.001$) than CH and less ($P \leq 0.021$) than DC. In addition, DC sections enhanced with PO_4-base solutions had less ($P < 0.001$) free, and more ($P < 0.001$) bound, moisture than DC sections enhanced with H_2O-base solutions. Not surprising, steaks from non-enhanced DC sections had lower ($P = 0.006$) display losses than CH steaks during the five days of simulated retail display. Moreover, steaks from enhanced DC sections had greater ($P \leq 0.001$) display losses than steaks from either untreated DC or untreated CH sections, and display losses increased (linear, $P = 0.004$) as solution pH increased from 3.5 to 5.0, even though losses were similar ($P = 0.165$) between PO_4- and H_2O-based solutions.

One of the well-established characteristics of DC meat is its exceptional high water-holding capacity (Sawyer et al., 2008, 2009; Apple et al., 2011), because the ultimate pH exceeds the isoelectric point of 5.0 to 5.3 of myofibrillar proteins (Wismer Pedersen, 1971; Hamm,

1986). Oreskovich, Bechtel, McKeith, Novakofski, and Basgall (1992) demonstrated that the total and bound moisture contents of beef increased when pH was either decreased to 4.25, or less, or increased to 7.0, or greater, because there are a greater number of reactive groups on the myofibrillar proteins to bind water at pH values in excess of 6.0 and below 4.0 (Gault, 1985). When LM pH was decreased to less than 4.3 by citric acid marination, moisture content of beef LM (Aktaş et al. 2003) and *semitendinosus* (Ke et al., 2009) was increased between 11 and 15%. Conversely, the WHC of either beef or pork was not affected when pH was deceased only 0.04 to 0.15 pH units by marination with organic acids (Mendonca, Molins, Kraft, & Walker, 1989; Huang, Ho, & McMillan, 2005). When the pH of DC beef strip loins was reduced from 6.90 to 5.79 by post-rigor lactic acid enhancement (0.5% lactic acid), Apple et al. (2011) reported total, free, and bound moisture contents similar to the normal-pH (5.58) strip loins, whereas, when pH of DC beef was reduced to between 4.13 and 4.64 by enhancement with 1.0 to 2.0% lactic acid, LM moisture (total, free, and bound) contents were similar to that of non-enhanced DC strip loins (Sawyer et al., 2009). In addition, when enhancing DC strip loins with solutions containing less than 0.5% lactic acid, both Sawyer et al (2008) and Apple et al. (2011) noted no change in post-enhancement pH from untreated DC strip loins; yet, lactic acid enhancement at these low concentrations resulted in increases in total and bound moisture and decreases in free moisture similar to what was observed in the present experiment. They concluded that the increases in total and bound moisture were simply the greater number of reactive protein side-groups binding more water from the enhancement solution.

3.2 Fresh beef color

3.2.1 *Visual fresh beef color during simulated retail display*

Even though fresh color scores of steaks from enhanced DC sections were greater ($P < 0.001$) than those from non-enhanced DC sections during the first three days of display, the fresh color scores of untreated normal-pH steaks were superior ($P < 0.001$) to steaks from either non-enhanced or enhanced DC sections throughout simulated retail display (Table 2). Base solution had no ($P \geq 0.064$) effect on fresh color scores during the first three days of display; however, steaks enhanced with PO_4-solutions received greater ($P = 0.011$) scores than those enhanced with H_2O-base solutions on day 4 of display, whereas steaks enhanced with H_2O-solutions received greater ($P = 0.014$) fresh color scores than steaks enhanced with PO_4-solutions on day 5.

After one day of display, CH steaks were less discolored (greater discoloration scores, $P < 0.001$) than steaks from enhanced and non-enhanced DC sections, but discoloration scores did not differ between enhanced DC and CH steaks ($P \geq 0.101$), enhanced DC and non-enhanced DC steaks ($P \geq 0.086$), or untreated DC and CH steaks ($P \geq 0.202$) on days 2 and 3 of simulated retail display (Table 2). Conversely, panelist rated DC and enhanced DC steaks less discolored (greater discoloration scores; $P < 0.001$) than CH steaks over the last two days of display. Moreover, steaks from DC sections enhanced with PO_4-base solutions were more discolored (lower discoloration scores; $P \leq 0.028$) than those enhanced with H_2O-base solutions on day 1 and 2 of display, but discoloration scores were not ($P \geq 0.241$) different between base solutions thereafter.

Panelist rated CH steaks more acceptable (greater overall acceptability scores; $P < 0.001$) than steaks from either non-enhanced or enhanced DC sections over the first two days of display; however, untreated DC steaks received greater ($P \leq 0.024$) acceptability scores than CH steaks over the last three days of display (Table 2). On day 3 of display, steaks from DC sections enhanced with pH 3.5, 4.5, and 5.0 solutions were also deemed less ($P \leq 0.047$) acceptable than

untreated DC steaks, whereas steaks enhanced with pH 3.5 and 4.5 solutions received acceptability scores intermediate to non-enhanced DC ($P \leq 0.007$) and CH ($P \leq 0.048$) steaks on day 4 of display. By day 5, enhanced DC steaks received greater ($P \leq 0.001$) acceptability scores than CH steaks, regardless of solution pH. In addition, steaks from DC sections enhanced with H_2O-base solutions received higher overall acceptability ratings than those from DC sections enhanced with PO_4-base solutions on days 0 ($P = 0.031$), 2 ($P = 0.051$), and 5 ($P = 0.020$) of display, but the opposite was observed on day 4 ($P = 0.050$) of simulated retail display.

Acuff et al. (1987) reported that steaks from normal pH beef strip loins treated with 1% lactic acid were more desirable in appearance to those from untreated strip loins, whereas Kotula and Thelappurate (1994) found that neither lactic acid nor acetic acid affected visual color scores of fresh beef steaks. Conversely, Aktaş and Mükerrem (2001) reported that citric acid marination produced a brown color more quickly than untreated steaks, and Naveena, Muthukumar, Sen, Babji, and Murthy (2006) reported that buffalo steaks marinated in 2% lactic acid were more discolored during the first three days of display when compared to untreated buffalo steaks. Enhancing DC beef strip loins with lactic improved fresh beef colors scores over the non-enhanced DC control, but visual color scores never approached those of untreated CH strip steaks (Apple et al., 2011; Sawyer et al., 2009). In addition, discoloration scores of untreated DC steaks were virtually unchanged across the five-day display period, but CH steaks were noticeably discolored after three days of display (Apple et al., 2011). In the present study, discoloration increased with increasing days in simulated retail display, with steaks receiving similar discoloration scores on days 2 and 3, regardless of treatment; however, over the last two days of display, enhanced and untreated DC steaks were rated less discolored than untreated CH steaks. Additionally, panelists noted greater discoloration of citric acid-enhanced DC steaks on

day 0 than CH steaks, which was similar to the results of Sawyer et al. (2009) and Apple et al. (2011), where panelists observed greater discoloration in DC steaks enhanced with 0.5 to 1.0% lactic acid compared to untreated CH steaks on the first day of simulated display.

3.2.2 *Instrumental fresh beef color during simulated retail display*

Fresh CH strip loin steaks were lighter (greater L* values; $P < 0.001$) than fresh steaks from DC strip loins, and the L* values of raw steaks from enhanced DC strip loin sections were intermediate ($P < 0.001$) to either non-enhanced control (Figure 1), regardless of base solution (PO_4 vs. H_2O, $P = 0.999$) or solution pH ($P \geq 0.201$). Citric acid enhancement did not alter L* values of fresh beef steaks (Aktaş & Mükerrem, 2001; Hinkle, Calkins, de Mellow, Jr., Senaratne, & Pokharel, 2010) or pork LM chops (Huang, Ho, & McMillin, 2005); however, other research has demonstrated that citric acid marination increased L* values of raw beef (Arganosa & Marriott, 1989; Önenç, Serdaroğlu, & Abdraimov, 2004; Elgadir et al., 2011) and buffalo steaks (Naveena et al., 2006). When DC beef was enhanced with lactic acid, Sawyer et al. (2009) reported that non-enhanced CH steaks were lighter than non-enhanced DC steaks, but steaks from DC sections enhanced with 0.25 to 0.75% lactic acid had similar L* values to the CH steaks. Yet, similar to the results of the present study, Apple et al. (2011) observed that untreated DC steaks were darker (lower L* values) than CH steaks, with steaks from DC strip loins enhanced with 0.35 and 0.50% lactic acid having L* values intermediate to either non-enhanced DC or CH steaks.

Initially (day 0), CH steaks were redder (greater a* values, $P < 0.001$) than non-enhanced and enhanced DC steaks, regardless of pH or base-solution (Table 3). Redness (a*) values of enhanced DC steaks were intermediate to those of non-enhanced DC and CH steaks after 1 ($P \leq 0.002$) and 2 ($P \leq 0.008$) days of simulated display, with a* values increasing (linear, $P = 0.05$)

with increasing solution pH on day 1 of display. Interestingly, a* values did not differ (P = 0.181) between DC and CH steaks on day 3 of display, whereas steaks from DC sections enhanced with pH 4.0 (P = 0.015) and 4.5 (P = 0.003) solutions were redder than untreated DC steaks. Conversely, DC steaks had greater ($P \leq 0.029$) a* values than CH over the last two days of display, whereas steaks from DC sections enhanced with pH 3.5, 4.0, and 4.5 were redder ($P \leq$ 0.004) than CH steaks on day 4, and all enhanced DC steaks were redder ($P < 0.001$) than CH steaks on day 5, of simulated retail display.

Steaks from untreated DC sections were closer to the true red axis (lesser hue angles, $P <$ 0.001) than CH steaks on each day of simulated retail display (Table 3). Because of lower a* and b* values, it was not surprising that steaks from enhanced DC sections also had lower ($P <$ 0.001) hue angles than CH steaks across the five-day display period. In addition, steaks from DC section enhanced with pH 4.5 and 5.0 solutions had greater hue angles than untreated DC steaks after day 1 ($P \leq 0.005$) and 2 ($P \leq 0.023$) of display, whereas hue angles of pH 4.5- and pH 5.0-enhanced DC steaks were greater than non-enhanced DC steaks on days 3 (P = 0.002) and 5 (P = 0.010) of simulated retail display, respectively.

A number of studies have shown that citric acid marination reduces the redness (reduced a* and greater hue angle values) of fresh steaks from beef (Arganosa & Marriott, 1989; Aktaş & Mükerrem, 2001; Önenç et al., 2004; Elgadir et al., 2011), pork (Huang et al., 2005), and buffalo (Naveena et al., 2006). Sawyer et al. (2009) reported that fresh CH steaks had greater a* values and hue angles than untreated DC steaks, but steaks from DC strip loins enhanced with 0.5 to 1.0% lactic acid were considerable less red (lower a* values and greater hue angles) than untreated CH steaks. Additionally, Apple et al. (2011) observed that a* values and hue angles indicated that untreated CH steaks were redder than untreated DC steaks over the first three days

of simulated retail display; yet, a* values were similar between steaks from untreated CH and DC strip loins enhanced with 0.25% lactic acid on the fifth day of display, which is consistent with the results of the present study. The citric acid solutions used in the present study failed to reduce the post-enhancement pH of the DC strip loin sections used in the present experiment; thus, it is not surprising that the redness of fresh steaks was not really altered among non-enhanced and enhanced DC steaks.

Across all days of simulated retail display, yellowness (b*) values were greater ($P \leq 0.015$) for CH than non-enhanced DC steaks, whereas steaks from DC sections enhanced with H_2O-base solutions were more yellow (greater b* values) than DC sections enhanced with PO_4-base solutions on days 1 ($P = 0.042$), 4 ($P = 0.003$), and 5 ($P = 0.018$) of display (Table 3). Initial (day 0) b* values of steaks from enhanced DC sections were only greater ($P < 0.001$) than untreated DC steaks, whereas b* values of enhanced DC steaks were greater ($P \leq 0.014$) than DC but less ($P < 0.001$) than CH after one and two days of simulated retail display. On day 3 of display, steaks from DC sections enhanced with pH 3.5 and 4.0 solutions had b* values intermediate to CH ($P \leq 0.007$) and DC ($P \leq 0.051$), whereas steaks from pH 4.5-enhanced DC sections were more ($P < 0.001$) yellow than untreated DC and steaks from pH 5.0-enhanced DC sections were less ($P = 0.001$) yellow than CH. Although steaks from DC sections enhanced with pH 4.5 solutions had b* values similar to CH ($P =0.279$) and DC ($P = 0.076$) on day 4 of display, CH steaks were still more ($P \leq 0.027$) yellow on day 4 than those from DC section enhanced with pH 3.5, 4.0, and 5.0 solutions. On the last day of simulated display, steaks from DC sections enhanced with pH 5.0 solutions had lower b* values than both untreated DC ($P = 0.005$) and CH ($P < 0.001$) steaks, and CH steaks were more ($P < 0.001$) yellow than steaks from DC sections enhanced with pH 3.5, 4.0, and 4.5 solutions.

Neither Aktaş and Mükerrem (2001) nor Hinkle et al. (2010) found an effect of citric acid marination on b* values of raw beef steaks, whereas Arganosa and Marriott (1989) and Elgadir et al. (2011) reported that enhancement with citric acid solutions increased the yellowness of fresh beef steaks. Sawyer et al. (2009) reported that CH steaks were more yellow than DC steaks, and enhancing DC strip loins with lactic acid actually reduced b* values compared to untreated DC steaks. However, similar to results of the present study, Apple et al. (2011) observed that lactic acid enhancement increased b* values of DC steaks, but b* values were never comparable to those of non-enhanced CH steaks.

Initial (day 0) C* values were greater ($P < 0.001$) for CH than either non-enhanced or enhanced DC steaks (Table 3). Again on days 1, 2, and 3 of simulated retail display, CH steaks had a more vivid (greater C* values, $P \leq 0.005$) color than untreated DC steaks, whereas steaks from DC sections enhanced with citric acid, regardless of solution pH, had C* values less ($P < 0.001$) than CH but greater ($P \leq 0.007$) than DC after the first two days of display. Even though steaks from DC section enhanced with pH 3.5, 4.0, and 4.5 solutions had a more vivid color (greater C* values, $P \leq 0.052$) than untreated DC steaks on day 3 of display, C* values were similar among enhanced DC steaks and CH on the third ($P \geq 0.144$) and fourth ($P \geq 0.101$) days of display. On day 5 of display, untreated and pH 4.5-enhanced DC steaks had greater ($P \leq 0.016$) C* values than CH, and pH 5.0-enhanced DC steaks had lower ($P = 0.048$) C* values than untreated DC steaks. Similarly, Sawyer et al. (2009) observed that fresh C* values were greater in non-enhanced CH steaks compared to steaks from untreated DC and from DC strip loins enhanced with 0.25 and 0.50% lactic acid, but steaks from DC strips enhanced with 0.75 or 1.00% lactic acid were considerably less vivid than non-enhanced CH and DC steaks. And, Apple et al. (2011) also demonstrated that lactic acid enhancement increased C* values of DC

steaks across five days of simulated retail display; however, in agreement with the present results, C* values were still less than those from non-enhanced CH LM steaks.

3.3 Cooked beef color

Non-enhanced and enhanced DC steaks received lower ($P < 0.001$) cooked color scores than CH steaks (pink vs. slightly pink internal cooked color), and steaks from DC sections enhanced with pH 4.0 and 4.5 received lower ($P \leq 0.003$) cooked color scores than untreated DC steaks (Table 4). In addition, degree of doneness scores were greater ($P < 0.001$) for CH than non-enhanced and enhanced DC steaks ("medium" vs. "medium rare" at the same internal endpoint temperature of 71°C).

A problematic characteristic of dark-cutting meat is the persistent red appearance when cooked to endpoint temperatures of 71°C, or greater, and previous research from this laboratory has demonstrated that enhancing DC LM steaks with 0.5% lactic acid could produce cooked color scores and degree of doneness scores that were not different from non-enhanced CH steaks; however, enhancement with solutions containing 0.75 to 2.00% lactic acid actually produced an overcooked appearance (Sawyer et al., 2008, 2009; Apple et al., 2011). Moreover, Sawyer et al. (2008, 2009) attributed the improvements in internal cooked color to increased myoglobin denaturation in steaks from enhanced DC strip loins (53.7 to 71.2%), which was greater than non-enhanced DC steaks (35.8 to 37.1%) but similar to non-enhanced CH steaks (60.1 and 57.8%, respectively). Interesting, Aktaş and Mükerrem (2001) also noted that the internal cooked color of steaks marinated in 0.5 to 1.5% citric acid had a white, overcooked appearance.

There were no differences in internal cooked L*, a*, b*, and C* values between CH and DC steaks ($P \geq 0.096$), among enhanced and non-enhanced DC steaks ($P \geq 0.090$), or among enhanced DC steaks and CH steaks ($P \geq 0.065$; Table 4). Yet, hue angles were lower ($P = 0.024$)

for non-enhanced and enhanced DC steaks compared with CH steaks, whereas the reflectance ratio of 630 nm:580 nm was greater ($P \leq 0.053$) in cooked steaks from untreated and pH 5.0-enhanced DC steaks than CH steaks.

Similar to the current results, L*, b*, and C* values were not affected by enhancing DC strip loin sections with lactic acid (Sawyer et al., 2008; Apple et al., 2011), but the internal redness of cooked DC steaks was decreased by enhancing DC strip loins with lactic acid, with cooked a* values and red-to-brown ratios of steaks from DC sections enhanced with 0.50% lactic acid comparable to those of untreated CH steaks. Conversely, enhancing DC beef with 0.75 to 2.00% lactic acid produced the appearance of overcooked ("well done" to "very well done") beef even when cooked to the same internal endpoint temperature of 71°C (Sawyer et al., 2008, 2009).

Because citric acid has three pKa values, it was hypothesized that enhancement solutions formulated with citric acid would not reduce post-enhancement pH as severely as lactic acid. Moreover, Sammel & Claus (2003) postulated that citric acid could reduce, or eliminate, persistent pink color in two ways: 1) as a metal chelator, citric acid could potentially bind iron in the porphyrin ring of myoglobin; and/or 2) promote protein denaturation. In a series of experiments, sodium nitrite and sodium nicotinamide were used to promote pink color formation in ground and whole turkey breast meat and subsequently treated with varying levels of citric acid. Kieffer, Claus, and Wang (2000), as well as Sammel and Claus (2003), reported that citric acid decreased the redness (a*) values of cooked ground turkey, whereas Sammel and Claus (2006) observed a reduction in a* values when irradiated turkey rolls were treated with 0.3% citric acid. However, Sammel and Claus (2003) noted that pink color development was not curtailed in cooked, intact turkey breasts by citric acid enhancement.

4. Conclusion

Results obtained from this study indicate that enhancing dark-cutting (DC) beef with citric acid solutions formulated to pH values of 3.5 to 5.0 cannot alter the postmortem muscle pH, fresh color, or cooked color equivalent to that of beef from CH sections with normal ultimate pH values. Even though the pH range of the citric acid enhancement solutions was ineffective in this study, the minor changes in some of the quality characteristics with citric acid enhancement would suggest that reducing the solution pH to below 3.5 may be required to produce the desired improvements in fresh and cooked color of DC beef.

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Literature Cited

Abugroun, H. A., Cousin, M. A., & Judge, M. D. (1993). Extended shelf-life of unrefrigerated prerigor cooked meat. *Meat Science, **33**,* 207-229.

Acuff, G. R., Vanderzant, C., Savell, J. W., Jones, D. K., Griffin, D. B., & Ehlers, J. G. (1987). Effect of acid decontamination of beef subprimal cuts on the microbiological and sensory characteristics of steaks. *Meat Science, **19**,* 217-226.

Aktaş, N., & Mükerrem, K. (2001). The influence of marinating with weak organic acids and salts on the intramuscular connective tissue and sensory properties of beef. *European Journal of Food Research and Technology, **213**,* 88-94.

Aktaş, N., Aksu, M. İ., & Kaya, M. (2003). The effect of organic acid marination on tenderness, cooking loss and bound water content of beef. *Journal of Muscle Foods, **14**,* 181-194.

AMSA (1991). *Guidelines for meat color evaluation*. Chicago, IL: American Meat Science Association and National Live Stock and Meat Board.

AMSA (1995). *Guidelines for cookery, sensory evaluation and instrumental tenderness measurements of fresh meat*. Chicago, IL: American Meat Science Association and National Live Stock and Meat Board.

AMSA (2012). *Meat color measurement guidelines*. Champaign-Urbana, IL: American Meat Science Association.

Apple, J. K., Davis, J. R., Rakes, L. K., Maxwell, C. V., Stivarius, M. R., & Pohlman, F. W. (2001). Effects of dietary magnesium and duration of refrigerated storage on the quality of vacuum-packaged, boneless pork loins. *Meat Science, **57**,* 43-53.

Apple, J. K., Sawyer, J. T., Meullenet, J. –F., Yancey, J. W. S., & Wharton, M. D. (2011). Lactic acid enhancement can improve the fresh and cooked color of dark-cutting beef. *Journal of Animal Science, **89**,* 4207-4220.

Arganosa, G. C., & Marriott, N. G. (1989). Organic acids as tenderizers of collagen in restricted beef. *Journal of Food Science, **54**,* 1173-1176.

Burke, R. M., & Monahan, F. J. (2003). The tenderization of shin beef using a citrus juice marinade. *Meat Science, **63**,* 161-168.

Chang, H. –J., Wang, Q., Zhou, G. –H., Xu, X. –L., & Li, C. –B. (2010). Influence of weak organic acids and sodium chloride marination on characteristics of connective tissue collagen and textural properties of beef semimembranosus muscle. *Journal of Texture Studies, **41**,* 279-301.

Cornforth, D., Calkins, C. R., & Faustman, C. (1991). Methods for identification and prevention of pink color in cooked meat. *Proceedings of the 44th Annual Reciprocal Meat Conference, **44**,* 53-58.

Cutter, C. N., & Siragusa, G. R. (1994). Efficacy of organic acids against *Escherichia coli* O157:H7 attached to beef carcass tissue using a pilot scale model carcass washer. *Journal of Food Protection, **57**,* 97-103.

Eilers, J. D., Morgan, J. B., Martin, A. M., Miller, R. K., Hale, D. S., Acuff, G. R., & Savell, J. W. (1994). Evaluation of calcium chloride and lactic acid injection on chemical, microbiological and descriptive attributes of mature cow beef. *Meat Science, **38**,* 443-451.

Elgadir, M. A., Mariod, A. A., Abdelwahab, S. I., Jamilah, B., Rahman, R. A., & Che Man, Y. B. (2011). Physiochemical and microbial attributes of organic infused beef cuts (longissimus dorsi). *Journal of Food Safety, **31**,* 326-333.

Ertbjerg, P., Larsen, L. M., & Møller, A. J. (1999a). Effect of prerigor lactic acid treatment on lysosomal enzyme release in bovine muscle. *Journal of the Science of Food and Agriculture, **79**,* 95-100.

Ertbjerg, P., Mielche, M. M., Larsen, L. M., & Møller, A. J. (1999b). Relationship between proteolytic changes and tenderness in prerigor lactic acid marinated beef. *Journal of the Science of Food and Agriculture, **79**,* 970-978.

Gault, N. F. S. (1985). The relationship between water-holding capacity and cooked meat tenderness in some beef muscles as influenced by acidic conditions below the ultimate pH. *Meat Science, **15**,* 15-30.

Hamm, R. (1986). Functional properties of the myofibrillar system and their measurements. In P. J. Bechtel (Ed.), *Muscle as Food* (pp 135 – 199). Orlando, FL: Academic Press, Inc.

Hedrick, H. B., Boillot, J. B., Brady, D. E., & Naumann, H. D. (1959). *Etiology of darkcutting beef*. Missouri Agriculture Experiment Station Research Bulletin, Columbia, MO.

Hinkle, J. B., Calkins, C. R., de Mellow, Jr., A. S., Senaratne, L. S., & Pokharel, S. (2010). Acid marination for tenderness enhancement of beef bottom round. M. *S. Thesis, University of Nebraska – Lincoln* (http://digitalcommons.unl.edu/animalscidiss/12).

Howat, P. M., Sievert, L. M., Myers, P. J., Koonce, K. L., & Bidner, T. D. (1983). Effect of marination upon mineral content and tenderness of beef. *Journal of Food Science, **48**,* 662-663.

Huang, N. –Y., Ho, C. –P., & McMillin, K. W. (2005). Retail shelf-life of pork dipped in organic acid before modified atmosphere or vacuum packaging. *Journal of Food Science, **70**,* M382-M387.

Ke, S., Huang, Y., Decker, E. A., & Hultin, H. O. (2009). Impact of citric acid on the tenderness, microstructure and oxidative stability of beef muscle. *Meat Science*, ***82***, 113-118.

Kieffer, K. J., Claus, J. R., & Wang, H. (2000). Inhibition of pink color development in cooked, uncured ground turkey by addition of citric acid. *Journal of Muscle Foods*, ***11***, 235-243.

Kotula, K. L., & Thelappurate, R. (1994). Microbiological and sensory attributes of retail cuts of beef treated with acetic and lactic acid solutions. *Journal of Food Protection*, ***57***, 665-670.

Mendenhall, V. T. (1989). Effect of pH and total pigment concentration on the internal color of cooked ground beef patties. *Journal of Food Science*, ***54***, 1-2.

Mendonca, A. F., Molins, R. A., Kraft, A. A., & Walker, H. W. (1989). Microbiological, chemical, and physical changes in fresh, vacuum-packaged pork treated with organic acids and salts. *Journal of Food Science*, ***54***, 18-21.

Morris, C. A., Theis, R. L., Miller, R. K., Acuff, G. R., & Savell, J. W. (1997). Improving the flavor of calcium chloride and lactic acid injected mature beef top round steaks. *Meat Science*, ***45***, 531-537.

Naveena, B. M., Muthukumar, M., Sen, A. R., Babji, Y., & Murthy, T. R. K. (2006). Improvement of shelf-life of buffalo meat using lactic acid, clove oil and vitamin C during retail display. *Meat Science*, ***74***, 409-415.

Önenç, A., Serdaroğlu, M., & Abdraimov, K. (2004). Effect of various additives to marinating baths on some properties of cattle meat. *European Food Research and Technology*, ***218***, 114-117.

Oreskovich, D. C., Bechtel, P. J., McKeith, F. K., Novakofski, J., & Basgall, E. J. (1992). Marinade pH affects textural properties of beef. *Journal of Food Science*, ***57***, 305-311.

Sammel, L. M., & Claus, J. R. (2003). Citric acid and sodium citrate effects on reducing pink cooked defect of cooked intact turkey breasts and ground turkey rolls. *Journal of Food Science*, ***68***, 874-878.

Sammel, L. M., & Claus, J. R. (2006). Citric acid and sodium citrate effects on pink color development of cooked ground turkey irradiated pre- and post-cooking. *Meat Science*, ***72***, 567-573.

Sawyer, J. T., Apple, J. K., & Johnson, Z. B. (2008). The impact of lactic acid concentration and sodium chloride on pH, water-holding capacity, and cooked color of injection-enhanced dark-cutting beef. *Meat Science*, ***79***, 317-325.

Sawyer, J.T., Apple, J.K., Johnson, Z.B., Baublits, R.T., & Yancey, J.W.S. (2009). Fresh and cooked color of dark-cutting beef can be altered by post-rigor enhancement with lactic acid. *Meat Science. **83***, 263-270.

Scanga, J. A., Belk, K. E., Tatum, J. D., Grandin, T., & Smith, G. C. (1998). Factors contributing to the incidence of dark-cutting beef. *Journal of Animal Science, **76**,* 2040-2047.

Smith, G. C, Tatum, J. D., & Morgan, J. B. (1993). *Dark cutting beef: physiology, biochemistry and occurrences*. Colorado State University, Fort Collins, CO.

Trout, G. R. (1989). Variation in myoglobin denaturation and color of cooked beef, pork, and turkey meat as influenced by pH, sodium chloride, sodium tripolyphosphate. *Meat Science, **28**,* 203-210.

Viljoen, H. F., de Kock, H. L., & Webb, E. C. (2002). Consumer acceptability of dark, firm and dry (DFD) and normal pH beef steaks. *Meat Science, **61***, 181-185.

Voisinet, B. D., Grandin, T., O'Connor, S. F., Tatum, J. D., & Deesing, M. J. (1997). *Bos indicus*-cross feedlot cattle with excitable temperaments have tougher meat and a higher incidence of borderline dark cutters. *Meat Science, **46**,* 367-377.

Wierbicki, E., & Deatherage, F. E. (1958). Determination of water-holding capacity of fresh meats. *Journal of Agricultural and Food Chemistry, **6**,* 387-392.

Wismer Pedersen, J. (1971). Water. In J. R. Price & B. S. Schweigirt (Eds.), *The Science of Meat and Meat Products* (pp. 177 – 191). San Francisco, CA: W. H. Freeman and Co.

Yoon, Y., Mukherjee, A., Belk, K. E., Scanga, J. A., Smith, G. C., & Sofos, J. N. (2009). Effect of tenderizers combined with organic acids on *Escherichia coli* O157:H7 thermal resistance in non-intact beef. *International Journal of Food Microbiology, **133**,* 78-85.

Appendix

Figure 2.1.

Effects of enhancement solution pH on lightness (L*) values of fresh dark-cutting beef steaks across five days of simulated retail display. Bars with an asterisk (*) differ ($P < 0.05$) from normal pH (**CH**) control, whereas bars with a single-cross (†) differ ($P < 0.05$) from dark-cutting (**DC**) control.

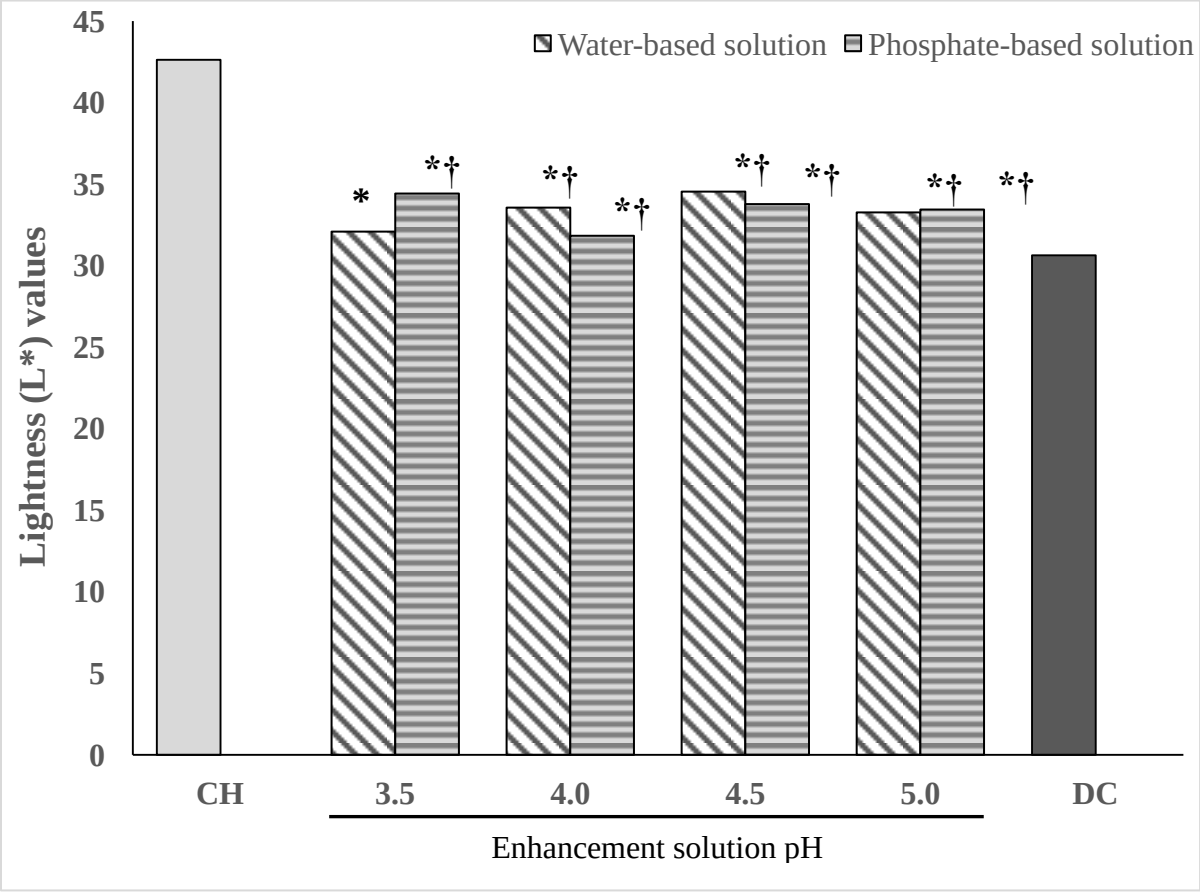

Table 2.1 Effects of enhancement solution pH on pH and water-holding capacity of dark-cutting beef steaks

Treatments	Pre-enhancement pH[1]	Post-enhancement pH[2]	Pump (%)[3]	Total moisture (%)[4]	Free moisture (%)[5]	Bound moisture (%)[6]	Display loss (%)[7]
Non-enhanced, normal pH (CH)	5.48	5.48	---	71.2	64.56	35.44	5.07
Non-enhanced dark-cutter (DC)	6.65	6.65	---	73.3	41.07	58.93	3.91
Water-based (H_2O) solution, pH 3.5	6.76*	6.73*	9.52	75.9*†	51.30*†	48.70*†	6.47*†
Water-based (H_2O) solution , pH 4.0	6.75*	6.72*	11.46	76.0*†	56.62*†	43.38*†	6.63*†
Water-based (H_2O) solution, pH 4.5	6.62*	6.60*	12.41	77.7*†	51.62*†	48.38*†	7.16*†
Water-based (H_2O) solution, pH 5.0	6.79*	6.75*	12.27	77.0*†	52.39*†	47.61*†	6.64*†
Phosphate-based (PO_4) solution, pH 3.5	6.82*	6.32*	9.74	77.2*†	44.28*†	55.72*†	6.01*†
Phosphate-based (PO_4) solution, pH 4.0	6.80*	6.64*	10.05	77.0*†	42.68*†	57.32*†	6.02*†
Phosphate-based (PO_4) solution, pH 4.5	6.75*	6.55*	10.93	76.6*†	45.78*†	54.22*†	6.34*†
Phosphate-based (PO_4) solution, pH 5.0	6.68*	6.47*	11.39	76.9*†	49.78*†	50.22*†	7.39*†
SEM	0.066	0.079	0.792	0.76	2.340	2.340	0.290
Contrasts[8]	C	C, P		C	C, P	C, P	C, L

Within a column, least squares means with an asterisk (*) differ ($P < 0.05$) from CH and least squares means with a single-cross (†) differ ($P < 0.05$) from DC.

[1]pH measured immediately before enhancement.

[2]pH measured after 30-min equilibration.

[3]Product yield after 30-min equilibration = (post-enhancement section weight ÷ pre-enhancement section weight) × 100.

[4]Total moisture = ((post-enhancement sample weight – freeze-dried sample weight) ÷ post-enhancement sample weight) × 100.

[5]Free moisture = (((moisture surface area – meat surface area) × 61.1) ÷ total moisture) × 100 (Wierbicki and Deatherage, 1958).

[6]Bound moisture = 100% - free moisture (Wierbicki and Deatherage, 1958).

[7]Display loss = ((0-d strip steak weight = 5-d strip steak weight) ÷ 0-d strip steak weight) × 100.

[8]Contrast abbreviations: **C** = CH vs. DC; **P** = H_2O vs. PO_4; **L** = linear solution pH effect; and **Q** = quadratic solution pH effect.

Table 2.2 Effects of enhancement solution pH on the visual color characteristics of dark-cutting beef steaks during simulated retail display

Treatments	Simulated retail display (day)					
	0	1	2	3	4	5
	---------- Fresh color score[2] (treatment × day, $P < 0.001$) ----------					
Non-enhanced, normal pH (CH)	6.5	6.2	5.4	4.8	4.7	3.9
Non-enhanced dark-cutter (DC)	2.1	2.2	2.3	2.4	2.7	2.5
Water-based (H_2O) solution, pH 3.5	2.8*†	3.0*†	3.2*†	3.4*†	2.7*	2.2*
Water-based (H_2O) solution , pH 4.0	3.1*†	3.3*†	3.3*†	3.4*†	2.8*	2.9*
Water-based (H_2O) solution, pH 4.5	3.1*†	3.4*†	3.8*†	3.7*†	3.0*	3.5*†
Water-based (H_2O) solution, pH 5.0	2.9*†	3.1*†	3.3*†	3.1*†	2.7*	2.9*†
Phosphate-based (PO_4) solution, pH 3.5	3.1*†	3.2*†	3.7*†	3.7*†	3.2*	2.4*
Phosphate-based (PO_4) solution, pH 4.0	2.8*†	2.8*†	2.9*†	3.3*†	3.0*	2.3*
Phosphate-based (PO_4) solution, pH 4.5	2.9*†	3.4*†	3.7*†	3.6*†	3.1*	2.9*†
Phosphate-based (PO_4) solution, pH 5.0	3.1*†	3.2*†	3.4*†	3.9*†	3.1*	2.9*†
SEM	0.23	0.42	0.34	0.48	0.38	0.55
Contrasts[1]	C	C	C	C	C, P	C, P, Q
	---------- Discoloration score[3] (treatment × day, $P < 0.001$) ----------					
Non-enhanced, normal pH (CH)	7.5	6.9	5.5	4.4	2.8	2.1
Non-enhanced dark-cutter (DC)	6.0	5.5	5.4	4.8	4.4	3.6
Water-based (H_2O) solution, pH 3.5	5.9*	5.6*	5.4	4.4	4.3*	3.4*†
Water-based (H_2O) solution , pH 4.0	6.1*	5.7*	5.4	4.9	3.8*	3.3*
Water-based (H_2O) solution, pH 4.5	5.8*	5.4*	5.3	4.9	3.6*†	3.3*
Water-based (H_2O) solution, pH 5.0	5.8*	5.5*	5.2	4.3	4.0*	3.2*
Phosphate-based (PO_4) solution, pH 3.5	5.5*	5.0*	5.1	4.3	3.9*	3.0*†
Phosphate-based (PO_4) solution, pH 4.0	5.9*	5.3*	4.9	4.5	4.5*	3.3*
Phosphate-based (PO_4) solution, pH 4.5	5.8*	5.2*	4.9	4.5	3.7*†	3.3*
Phosphate-based (PO_4) solution, pH 5.0	6.1*	5.3*	5.1	4.7	4.0*	3.4*
SEM	0.95	0.94	0.82	0.97	0.66	0.69
Contrasts[1]	C, U	C, P	P		C	C

Table 2.2 (Cont.) Effects of enhancement solution pH on the visual color characteristics of dark-cutting beef steaks during simulated retail display

Treatments	Simulated retail display (day)					
	0	1	2	3	4	5
	---------- Overall acceptability[4] (treatment × day, $P < 0.001$) ----------					
Non-enhanced, normal pH (CH)	5.4	5.0	4.1	2.8	2.0	1.3
Non-enhanced dark-cutter (DC)	3.3	3.4	3.4	3.4	2.8	1.9
Water-based (H_2O) solution, pH 3.5	3.4*	3.3*	3.5*	2.8†	2.5*†	1.8*†
Water-based (H_2O) solution , pH 4.0	3.7*	3.5*	3.5*	3.2	2.3*	1.9*
Water-based (H_2O) solution, pH 4.5	3.6*	3.3*	3.3*	2.8†	2.2*†	2.1*
Water-based (H_2O) solution, pH 5.0	3.5*	3.2*	3.4*	2.6†	2.1†	1.9*
Phosphate-based (PO_4) solution, pH 3.5	3.1*	3.0*	3.2*	2.8†	2.2*†	1.6*†
Phosphate-based (PO_4) solution, pH 4.0	3.3*	3.2*	3.2*	3.2	2.8*	1.7*
Phosphate-based (PO_4) solution, pH 4.5	3.3*	3.4*	3.2*	2.9†	2.4*†	1.8*
Phosphate-based (PO_4) solution, pH 5.0	3.4*	3.2*	3.3*	3.3†	2.4†	1.9*
SEM	0.50	0.52	0.47	0.33	0.26	0.14
Contrasts[1]	C, P	C, Q	C, P	C	C, P	C, P, L

Within a column, least squares means with an asterisk (*) differ ($P < 0.05$) from CH and least squares means with a single-cross (†) differ ($P < 0.05$) from DC.

[1]Contrast abbreviations: C = CH vs. DC; P = H_2O vs. PO_4; L = linear solution pH effect; and Q = quadratic solution pH effect.

[2]Fresh color score: 1 = extremely dark red to 8 = extremely bright cherry red (AMSA, 2012).

[3]Discoloration score: 1 = total (≥ 96%) discoloration; 2 = 81 to 95% discoloration; 3 = 66 to 80% discoloration; 4 = 51 to 65% discoloration; 5 = 36 to 50% discoloration; 6 = 21 to 35% discoloration; 7 = 6 to 20% discoloration; and 8 = no (0 to 5%) discoloration.

[4]Overall acceptability: 1 = extremely undesirable to 7 = extremely desirable (AMSA, 2012).

Table 2.3 Effects of enhancement solution pH on the instrumental color characteristics of dark-cutting beef steaks during simulated retail display

Treatments	Simulated retail display (day)					
	0	1	2	3	4	5
	---------- Redness (a*) values[2] (treatment × day, $P < 0.001$) ----------					
Non-enhanced, normal pH (CH)	32.34	29.67	27.08	24.95	20.57	15.77
Non-enhanced dark-cutter (DC)	20.54	21.50	23.16	23.91	22.74	21.73
Water-based (H_2O) solution, pH 3.5	20.61*	22.77*†	24.49*†	25.17	21.22*	19.79*
Water-based (H_2O) solution , pH 4.0	21.57*	22.69*†	25.11*†	25.21†	23.04*	21.15*
Water-based (H_2O) solution, pH 4.5	21.03*	23.88*†	24.96*†	25.81†	22.88*	21.59*
Water-based (H_2O) solution, pH 5.0	21.16*	23.95*†	25.09*†	24.80	21.83	21.04*
Phosphate-based (PO_4) solution, pH 3.5	21.00*	23.93*†	24.94*†	25.07	24.93*	22.17*
Phosphate-based (PO_4) solution, pH 4.0	20.81*	23.76*†	24.53*†	25.91†	23.10*	21.15*
Phosphate-based (PO_4) solution, pH 4.5	21.76*	24.11*†	25.59*†	26.03†	24.94*	22.13*
Phosphate-based (PO_4) solution, pH 5.0	21.56*	24.11*†	24.88*†	25.21	21.13	20.27*
SEM	0.570	0.570	0.570	0.570	0.570	0.570
Contrasts[1]	C	C, P, L	C		C, P	C
	---------- Yellowness (b*) values[2] (treatment × day, $P < 0.001$) ----------					
Non-enhanced, normal pH (CH)	24.58	22.83	21.02	20.18	18.04	16.56
Non-enhanced dark-cutter (DC)	12.59	14.12	15.71	16.56	15.23	13.72
Water-based (H_2O) solution, pH 3.5	12.42*	14.95*†	16.65*†	17.59*†	13.32*	10.96*
Water-based (H_2O) solution , pH 4.0	13.48*	15.35*†	17.59*†	18.03*†	15.50*	12.65*
Water-based (H_2O) solution, pH 4.5	13.56*	16.88*†	18.09*†	19.28†	15.72	12.09*
Water-based (H_2O) solution, pH 5.0	13.53*	16.68*†	17.81*†	17.56*	13.76*	11.65*†
Phosphate-based (PO_4) solution, pH 3.5	13.01*	16.89*†	17.88*†	18.28*†	18.37*	15.26*
Phosphate-based (PO_4) solution, pH 4.0	12.35*	16.06*†	16.93*†	18.48*†	15.18*	11.66*
Phosphate-based (PO_4) solution, pH 4.5	13.79*	16.93*†	18.41*†	19.13†	18.23	14.46*
Phosphate-based (PO_4) solution, pH 5.0	13.60*	16.85*†	17.76*†	18.20*	13.30*	10.78*†
SEM	0.612	0.612	0.612	0.612	0.612	0.612
Contrasts[1]	C	C, P, L	C	C, Q	C, P	C, P
	---------- Hue angles (°)[3] (treatment × day, $P < 0.001$) ----------					
Non-enhanced, normal pH (CH)	37.25	37.56	37.81	38.98	41.35	46.91
Non-enhanced dark-cutter (DC)	31.41	33.24	34.13	34.69	33.72	32.12
Water-based (H_2O) solution, pH 3.5	30.94*	33.15*	34.11*	34.85*	31.72*	28.68*
Water-based (H_2O) solution , pH 4.0	31.93*	34.00*	34.98*	35.54*	33.76*	30.76*

Table 2.3 (Cont.) Effects of enhancement solution pH on the instrumental color characteristics of dark-cutting beef steaks during simulated retail display

Treatments	Simulated retail display (day)					
	0	1	2	3	4	5
Water-based (H_2O) solution, pH 4.5	32.46*	35.24*†	35.84*†	36.74*†	34.23*	29.18*
Water-based (H_2O) solution, pH 5.0	32.48*	34.85*†	35.35*†	35.22*	32.05*	28.97*†
Phosphate-based (PO_4) solution, pH 3.5	31.59*	35.15*	35.60*	36.08*	36.34*	34.34*
Phosphate-based (PO_4) solution, pH 4.0	30.56*	33.97*	34.58*	35.41*	33.03*	28.67*
Phosphate-based (PO_4) solution, pH 4.5	32.22*	35.07*†	35.71*†	36.29*†	36.15*	32.99*
Phosphate-based (PO_4) solution, pH 5.0	32.19*	34.95*†	35.49*†	35.76*	32.01*	28.03*†
SEM	0.711	0.711	0.711	0.711	0.711	0.711
Contrasts[1]	C	C, L	C	C	C	C
	---------- Chroma (C*) values[4] (treatment × day, $P < 0.001$) ----------					
Non-enhanced, normal pH (CH)	40.62	37.44	34.29	32.09	27.37	22.96
Non-enhanced dark-cutter (DC)	24.11	25.74	27.99	29.09	27.38	25.72
Water-based (H_2O) solution, pH 3.5	24.08*	27.25*†	29.63*†	30.72†	25.10	22.67
Water-based (H_2O) solution , pH 4.0	25.44*	27.40*†	30.66*†	31.00†	27.79	24.68
Water-based (H_2O) solution, pH 4.5	24.98*	29.25*†	30.84*†	32.22†	27.79	24.77*
Water-based (H_2O) solution, pH 5.0	25.13*	29.20*†	30.79*†	30.40	25.86	24.11†
Phosphate-based (PO_4) solution, pH 3.5	24.72*	29.30*†	30.70*†	31.04†	30.99	26.96
Phosphate-based (PO_4) solution, pH 4.0	24.20*	28.69*†	29.81*†	31.83†	27.68	24.18
Phosphate-based (PO_4) solution, pH 4.5	25.78*	29.47*†	31.53*†	32.31†	30.90	26.46*
Phosphate-based (PO_4) solution, pH 5.0	25.50*	29.42*†	30.57*†	31.10	24.99	22.97†
SEM	0.771	0.771	0.771	0.771	0.771	0.771
Contrasts[1]	C	C, P, L	C	C	P	C

Within a column, least squares means with an asterisk (*) differ ($P < 0.05$) from CH and least squares means with a single-cross (†) differ ($P < 0.05$) from DC.

[1]Contrast abbreviations: C = CH vs. DC; P = H_2O vs. PO_4; L = linear solution pH effect; and Q = quadratic solution pH effect.

[2]L* values are a measure of darkness to lightness (greater value indicates a lighter color); a* values are a measure of redness (greater value indicates a redder color); and b* values are a measure of yellowness (greater value indicates a more yellow color).

[3]Hue angle represents the change from the true red axis (greater angle indicates a greater shift from the true red axis).

[4]Chroma, or saturation index, is a measure of the total color (a greater value indicates a more vivid color).

Table 2.4 Effects of enhancement solution pH on the visual and instrumental color characteristics of dark-cutting beef steaks cooked to an internal endpoint temperature of 71°C

Treatments	Cooked color score[1]	Degree of doneness score[2]	Lightness (L*) value[3]	Redness (a*) value[3]	Yellowness (b*) value[3]	Hue angle[4]	Chroma (C*) value[5]	Red-to-brown ratio[6]
Non-enhanced, normal pH (CH)	4.7	4.3	49.64	18.68	19.79	45.58	27.09	2.7
Non-enhanced dark-cutter (DC)	3.8	3.5	52.20	21.67	19.73	41.58	29.21	3.3
ater-based (H_2O) solution, pH 3.5	3.8*	3.4*	50.98	18.74	16.81	41.76*	25.05	3.1
Water-based (H_2O) solution , pH 4.0	3.0*†	2.9*	50.42	21.74	19.10	40.68*	28.78	3.3
Water-based (H_2O) solution, pH 4.5	3.1*†	3.1*	51.45	21.08	18.65	40.87*	27.99	3.1
Water-based (H_2O) solution, pH 5.0	3.8*	3.7*	53.67*	20.97	18.66	41.07*	27.91	3.1*
Phosphate-based (PO_4) solution, pH 3.5	3.4*	3.0*	52.16	20.71	18.90	42.08*	27.94	3.0
Phosphate-based (PO_4) solution, pH 4.0	3.6*†	3.3*	53.00	21.91	18.86	40.40*	28.81	3.2
Phosphate-based (PO_4) solution, pH 4.5	3.6*†	3.4*	51.05	19.47	17.59	41.76*	26.05	3.0
Phosphate-based (PO_4) solution, pH 5.0	3.3*	3.1*	52.26*	22.41	18.83	39.25*	29.01	3.5*
SEM	0.14	0.13	1.272	1.370	0.883	1.399	1.298	0.25
Contrasts[7]	C, Q	C, L				C		C

Within a column, least squares means with an asterisk (*) differ ($P < 0.05$) from CH and least squares means with a single-cross (†) differ ($P < 0.05$) from DC.

[1]Cooked color: 1 = very red to 7 = brown (AMSA, 2012).

[2]Degree of doneness: 1 = very rare to 7 = very well (AMSA, 2012).

[3]L* values are a measure of darkness to lightness (greater value indicates a lighter color); a* values are a measure of redness (greater value indicates a redder color); and b* values are a measure of yellowness (greater value indicates a more yellow color).

[4]Hue angle represents the change from the true red axis (greater angle indicates a greater shift from the true red axis).

[5]Chroma, or saturation index, is a measure of the total color (a greater value indicates a more vivid color).

[6]Spectral reflectance ratio of 630 nm/580 nm is an estimate of cooked color change from red to brown (greater value indicates a redder color; AMSA, 1991).

[7]Contrast abbreviations: **C** = CH vs. DC; **P** = H_2O vs. PO_4; **L** = linear solution pH effect; and **Q** = quadratic solution pH effect.

Chapter III

CITRIC ACID ENHANCEMENT AT PH VALUES LESS THAN 3.5 CAN IMPROVE THE COOKED COLOR, BUT NOT FRESH COLOR, OF DARK-CUTTING BEEF

Abstract

Beef strip loins were used to test the effects of citric acid-marination pH on visual and instrumental color of fresh and cooked beef steaks. Dark-cutting (**DC**; mean pH = 6.61) and normal pH, USDA Choice (**CH**; mean pH = 5.38) strip loins were cut into 2 equal-length portions, and DC sections were injected to a target of 111% of raw product weight with pH 2.0, 2.5, 3.0, and 3.5 solutions made by mixing citric acid in either a 0.5% orthophosphate solution (**PO_4**) or a 0.5% tripolyphosphate solution (**STP**). After injection and vacuum-tumbling, sections were cut into 2.5-cm-thick steaks destined for either simulated retail display or cooked to an endpoint temperature of 71°C. Post-enhancement pH decreased linearly ($P < 0.001$) as solution pH decreased from 3.5 to 2.0, and the proportions of free and bound moisture of DC steaks enhanced with pH 2.5 solution were comparable ($P \geq 0.141$) to that of CH. Even though fresh color scores were improved ($P < 0.001$) by citric acid-marination over untreated DC during the first three days of display, fresh steak color never ($P < 0.001$) approached that of CH steaks. Conversely, enhancing DC sections with pH 2.5 solutions produced cooked color and degree of doneness scores that were similar ($P \geq 0.113$) to non-enhanced CH steaks. Thus, enhancement with pH 2.5 citric acid solutions can effectively eliminate the persistent red cooked color typically associated with DC beef; however, citric acid enhancement failed to improve the fresh color of DC beef comparable to that of CH beef.

Keywords: *Citric acid enhancement; Cooked color; Dark-cutting beef; Fresh Color; Water-holding Capacity; pH*

1. Introduction

Dark-cutting (**DC**) beef is characterized by muscle pH values in excess of 6.0, as well as elevated water-holding capacity, and a dark red to almost black, undesirable lean color. Dark-cutting beef is rarely marketed in the retail sector because consumers associate the dark-red color with deteriorated wholesomeness (Viljoen, de Kock, & Webb, 2002), and it isn't usually sold to foodservice because, when cooked to internal temperatures adequate for browning of normal pH beef, DC beef will exhibit a persistent red, undercooked internal temperature (Mendenhall, 1989; Trout, 1989; Cornforth, Calkins, & Faustman, 1991).

Organic acids have been used as antimicrobial interventions for some time (Acuff, Vanderzant, Savell, Jones, Griffin, & Ehlers, 1987; Abugroun, Cousin, & Judge, 1993; Cutter & Siragusa, 1994; Yoon, Mukherjee, Belk, Scanga, Smith, & Sofos, 2009; Elgadir, Mariod, Abdelwahab, Jamilah, Rahman, & Che Man, 2011), and research has shown that marinating meat from mature animals (Eilers et al., 1994; Morris, Theis, Miller, Acuff, & Savell, 1997; Ertbjerg, Larsen, & Møller, 1999a; Ertbjerg, Mielche, Larsen, & Møller, 1999b; Aktaş, Aksu, & Kaya, 2003) or from muscles with high amounts of connective tissue (Arganosa & Marriott, 1989; Burke & Monahan, 2003; Chang, Wang, Zhou, Xu, & Li, 2010) in organic acids can improve cooked meat tenderness. More recently, research from this laboratory has demonstrated that enhancing post-rigor DC beef with lactic acid solutions can improve fresh (Apple, Sawyer, Meullenet, Yancey, & Wharton, 2011) and cooked beef color (Sawyer, Apple, & Johnson, 2008; Sawyer, Apple, Johnson, Baublits, & Yancey, 2009; Apple et al., 2011). Conversely, marinating DC beef with solutions containing greater than 0.50% lactic acid decreased post-enhancement muscle pH to 4.6, or less, actually reduced water-holding capacity, and produced some atypical fresh and cooked color variations (Sawyer et al., 2009). Sammel and Claus (2003, 2006) reported

that marinating turkey breast in solutions containing citric acid effectively reduced the persistent pink color of cooked ground and whole breasts treated with pinking agents. Yet, there were no positive effects on either fresh or cooked color of DC beef when enhanced with pH 3.5 to 5.0 citric acid solutions (Stackhouse, Apple, Yancey, Keys, Johnson, & Mehall, 2015). These authors noted that post-enhancement pH was not reduced below 6.0 in citric acid-enhanced DC sections; therefore, the objective of this experiment was to test the effects of lower pH (2.0 to 3.5), citric acid enhancement solutions on fresh and cooked color of DC beef.

2. Materials and methods

2.1 Muscles

Normal pH, low U. S. Choice (**CH**; n = 5) and dark-cutting (**DC**; n = 41) beef strip loins (IMPS #180) were selected based on 24-hour postmortem pH and purchased from a large commercial slaughter facility. Upon arrival at the University of Arkansas Red Meat Abattoir, strip loins were removed from the vacuum package and trimmed free of fat and adjacent muscles. Then, pH was measured for each strip loin with a ceramic-tipped, hand-held pH probe (Testo 205 pH, Sparta, NJ, USA) before being cut into two equal-length sections (mean pH for DC and CH beef strip loins was 6.74 and 5.39, respectively).

2.2 Enhancement Treatments

The DC muscle sections were randomly assigned to 1 of 9 enhancement treatments (n = 9/treatment) of pH 2.0, 2.5, 3.0, or 3.5 solutions, made by mixing citric acid (Cargill, Inc., Eddyville, IA, USA) in either a 0.5% orthophosphate solution (**PO_4**; B. K. Giulini Corp., Simi Valley, CA, USA) or 0.5% sodium tripolyphosphate (**STP**; B. K. Giulini Corp., Simi Valley, CA, USA), and a non-injected negative control, whereas the CH sections served as a non-

injected positive control. Enhancement solutions were prepared in 4°C tap water, and, under continuous agitation with a Rotosolver high-shear mixer (Admix Inc., Manchester, NH, USA), citric acid was titrated into either the PO_4- or STP-base solutions to achieve solution pH values of 2.0, 2.5, 3.0, and 3.5. Weight and pH of each DC section were recorded before being injected to a targeted 111% of raw weight with their respective enhancement solution using a Fomaco 20/40 injector (Reiser Inc., Canton, MA, USA). Injected DC sections were tumbled at 42 rpm under 12.4 N/cm^2 of vacuum for 5 min. After tumbling, the enhanced sections were placed on shelves and allowed to equilibrate for 30 minutes before recording post-enhancement weights and pH values. The pH of the *longissimus lumborum* (**LM**) from non-enhanced DC and CH sections was recorded at the same time to compare against that of enhanced DC sections.

2.3 Sample preparation

Sections were cut into three 2.54-cm-thick steaks and one 1.27-cm-thick steak. One 2.54-cm-thick steak was vacuum-packaged and stored at -20°C for evaluation of cooked color, whereas the remaining 2.54-cm-thick steaks were individually placed onto foam trays with absorbent pads, over-wrapped with a polyvinyl chloride film (O_2 transmission rate = 14,000 cc $O_2/m^2/24h/atm$; Koch Supplies Inc., Kansas City, MO, USA), and stored at 2°C. Steaks designated for instrumental (n = 90) and visual color (n = 90) analyses were stored under simulated retail display conditions (2°C and 1,600 lux deluxe warm white fluorescent lighting; Philips Inc., Somerset, NJ, USA) for five days. The 1.27-cm-thick steak was used to measure water-holding capacity (**WHC**) and moisture content.

2.4 Simulated retail display

Instrumental color of steaks under simulated retail display conditions was measured on days 0 (immediately after packaging and placement in display), 1, 2, 3, 4, and 5 using a Hunter

MiniScan XE (Model 45/0-L, Hunter Associates Laboratory Inc., Reston, VA, USA) calibrated daily against black and white tiles. The L*, a*, and b* values were determined from the mean of three readings on the surface of each steak using Illuminant A, a 10° standard observer, and a 2.54-cm aperture. Additionally, hue angle was calculated as: $\tan^{-1}(b^*/a^*)$, whereas chroma (C*) was calculated as: $\sqrt{}$ (AMSA, 2012).

A five-member, trained panel was used to evaluate visual color of steaks during retail display. Panelists were selected and trained according to AMSA (1991) guidelines. Panelists evaluated each steak under display conditions for fresh beef color (8 = extremely bright cherry-red; 7 = bright cherry-red; 6 = moderately bright cherry-red; 5 = slightly bright-cherry red; 4 = slightly dark cherry-red; 3 = moderately dark red; 2 = dark red; and 1 = extremely dark red; AMSA, 2012), percent discoloration (8 = no [0 to 5%] discoloration to 1 = total [≥ 96%] discoloration), and overall acceptability (7 = extremely desirable; 6 = desirable; 5 = slightly desirable; 4 = acceptable; 3 = slightly undesirable; 2 = undesirable; and 1 = extremely undesirable; AMSA, 2012) on each day of simulated retail display.

2.5 Cooked color

Steaks (2.54-cm-thick) were thawed for approximately 16 h at 1° C before being cooked on a gas-fired, open-hearth grill (Star Manufacturing, Inc., Smithville, TN, USA). Steaks were turned every three minutes until the internal temperature of the steak reached 71°C (AMSA, 1995), and rested five minutes at room temperature before slicing for visual and instrumental color analysis. Internal steak temperature was monitored using an Omega Hypodermic probe (Omega Technologies, Stamford, CT, USA) attached to a K28 Foodcheck Thermometer (Comark Instruments, Beaverton, OR, USA). Steaks were cut just off the center (perpendicular to the steak surface), and, within 20 seconds of cutting, a ten-member, trained panelists (selected

and trained according to AMSA [1991] guidelines) evaluated each cut surface (to the nearest 0.5) for internal cooked color (7 = brown, 6 = gray brown, 5 = pinkish gray, 4 = slightly pink, 3 = pink, 2 = medium red, and 1 = very red; AMSA, 2012) and internal doneness (7 = very well, 6 = well done, 5 = medium well; 4 = medium, 3 = medium rare, 2 = rare, and 1 = very rare; AMSA, 2012). Sensory evaluation was conducted with two sensory color sessions a day (n = 9 steaks/session) over a five-day period. Instrumental cooked color readings of steaks were measured concurrently with visual analysis on half of the steak wrapped immediately after cutting with a polyvinyl chloride film (O_2 transmission rate = 14,000 cc $O_2/m^2/24h/atm$; Koch Supplies Inc., Kansas City, MO, USA) to minimize post-cookery blooming. Values were measured using a Hunter MiniScan XE (Model 45/0-L, Hunter Associates Laboratory Inc., Reston, VA, USA) calibrated before every session against black and white tiles. The L*, a*, and b* values were determined from the mean of three readings on the cut surface of each steak using Illuminant A, a 10° standard observer, and a 1.27-cm aperture. In addition to calculating hue angle and C* as described previously, the reflectance at 630 nm was divided by the reflectance at 580 nm to calculate the red-to-brown ratio (AMSA, 2012).

2.6 Water-holding Capacity

Water-holding capacity of muscle samples was measured approximately 30 min after the fabrication of the sections using the methodology of Wierbicki and Deatherage (1958). Briefly, 500 mg of fresh LM was weighted onto a piece of Whatman No. 1 filter paper, which had been stored in a desiccator over saturated potassium chloride. The sample was then pressed at 345 N/cm^2 for one minute in a Carver Press (Fred S. Carver Inc., Summit, NJ). Areas of the meat and moisture were traced and subsequently measured using a compensating planimeter (Planix 8, Sokkia Corp., Overland Park, KS, USA). All samples were run in duplicate, and the percentage

of free water was calculated by the equation: free water (%) = (((moisture surface area – meat surface area) 61.1) / total moisture) 100, whereas the percentage of bound water was calculated by subtracting the free water from 100. Percent moisture analysis was conducted using five-gram samples of LM according to the freeze-drying method of Apple, Davis, Rakes, Maxwell, Stivarius, and Pohlman (2001).

2.7 Statistical analysis

Data were analyzed as a completely randomized design, with treatments in a 2 × 4 factorial arrangement, with positive (CH) and negative (DC) controls, and loin sections as the experimental unit. The ANOVA was generated using the mixed model procedure of SAS (SAS Inst., Inc., Cary, NC, USA), and the statistical model for pH and water-holding capacity, as well as all cooked color characteristics, included enhancement treatment as the lone fixed effect, whereas the fresh steak color data collected for five days of simulated retail display was analyzed as a repeated measure, with treatment, display day, and the treatment × day interaction as the fixed effects in the model. In the analysis of visual appraisal of fresh and cooked color, panelist was included in the model as a random effect. Least squares means were calculated for all treatments, and, because of the unique treatment structure, preplanned contrasts were used to test: 1) base solution differences (PO_4 vs.STP); 2) linear and quadratic responses to decreasing enhancement solution pH; 3) differences between each enhancement solution pH and the untreated CH control; and 4) differences between each enhancement solution pH and the non-enhanced DC control.

3. Results and Discussion

3.1 pH and moisture retention

Prior to enhancement, DC sections had substantially greater ($P < 0.001$) LM pH values than CH sections, and, after enhancement, LM pH decreased ($P < 0.001$) linearly with decreasing solution pH (Table 1). Interestingly, the pH of DC sections enhanced with pH 3.5 solutions was similar ($P = 0.229$) to DC, whereas enhancement with pH 2.0 solutions reduced ($P \leq 0.038$) LM pH almost 2.6 and 1.4 units below non-enhanced DC and CH sections, respectively. The post-enhancement pH of DC sections marinated in pH 2.0, 2.5 and 3.0 solutions was less ($P \leq 0.002$) than DC, but only LM pH values of pH 2.5-enhanced DC sections were similar ($P = 0.141$) to CH.

Previous studies have demonstrated that marinating beef in citric acid lowered muscle pH (Arganosa & Marriott, 1989; Ke, Huang, Decker, & Hultin, 2009; Elgadir et al., 2011). However, enhancing DC beef with citric acid solutions with pH values ranging from 3.5 to 5.0 did not alter muscle pH (Stackhouse et al., 2015). Current results were similar in that citric acid solutions with a pH of 3.5 did not alter muscle pH in DC beef. Aktaş et al. (2003) stated the citric acid has three carboxyl groups and, thus, three pKa values (3.06, 4.74, and 5.40). The three extra carboxyl groups act to buffer the muscle; therefore, very low pH enhancement solutions would be required to lower the pH of DC muscle.

The pH 2.0 citric acid treatment dramatically reduced muscle pH in the present study (below 5.0). Sawyer et al. (2009) also reported dramatically reduced muscle pH when DC strips were injected with lactic acid solutions greater than 0.5% (solution pH = 2.28), but not at lactic acid concentrations of 0.25 (Sawyer et al., 2009), 0.15 or 0.35% (Apple et al., 2011).

The moisture content of the LM did not differ between non-enhanced DC and CH (P = 0.383) or between steaks from DC sections enhanced with pH 2.0 solutions and untreated DC (P = 0.443) or CH (P = 0.809); however, total moisture content increased (linear, $P < 0.001$) with increasing solution pH, with the LM from DC sections enhanced with pH 2.5, 3.0, and 3.5 solutions having greater ($P \leq 0.001$) percentages of moisture than either DC or CH steaks (Table 1). In DC beef, the ultimate pH greatly exceeds the isoelectric point of the myofibrillar proteins (Wismer Pedersen, 1971; Hamm, 1986), resulting in its characteristically high water-holding capacity (Sawyer et al., 2008, 2009; Apple et al., 2011). For the pH 2.5, 3.0, and 3.5 solutions, the increased total moisture likely resulted from the increased muscle pH of the DC strips in combination with the additional water added in the solution. Previous research injecting DC strips with citric acid solutions with pH values between 3.5 and 5.0 also elevated total moisture content compared to non-injected DC and CH controls (Stackhouse et al., 2015). When DC strips were injected with lactic acid and final muscle pH was greater than 6.0, total moisture was greater than non-injected CH but not non-injected DC strips (Sawyer et al., 2008, 2009).

The decreased total moisture in DC strips injected with citric acid solutions of 2.0 compared to other injected DC strips is in contrast to previous research (Aktaş et al. 2003; Ke et al., 2009), which showed that total moisture increased with pH values decreasing away from the isoelectric point of the muscle proteins. In the pH 2.0-injected DC strips, free moisture and display loss were greater than CH; thus, it is likely that the moisture injected with the solution was lost free moisture. The decreased muscle pH of the DC strips injected with citric acid solution with a pH of 2.0 may have denatured muscle proteins, but reduced total moisture was not reported when lactic acid solutions were used to lower the pH of DC strips to very low pH (Sawyer et al., 2008, 2009).

The LM from non-enhanced DC sections had less ($P < 0.001$) free moisture, and more ($P < 0.001$) bound moisture, than the LM from CH sections, and the proportions of free moisture increased (linear, $P < 0.001$), whereas the percentages of bound moisture decreased (linear, $P < 0.001$), as solution pH decreased from 3.5 to 2.0 (Table 1). More specifically, enhancement with pH 2.0 solutions produced steaks with greater ($P \leq 0.003$) proportions of free moisture and lesser ($P \leq 0.003$) proportions of bound moisture than untreated DC and CH steaks, whereas enhancement with pH 3.5 solutions produced free and bound moisture percentages intermediate to either DC ($P < 0.001$) or CH ($P = 0.014$) steaks. Moreover, the LM from DC sections enhanced with pH 2.5 had free and bound moisture percentages similar ($P = 0.230$) to untreated CH steaks, whereas the proportions of free and bound moisture of the LM from DC sections enhanced with pH 3.0 solutions did not differ ($P = 0.079$) from untreated DC steaks. It is important to note that enhancement with PO_4-base solutions increased ($P = 0.014$) the free moisture content while decreasing ($P = 0.014$) bound moisture content compared to enhancement with STP-based solutions. Strips enhanced using solutions with pH of 2.5 had final muscle pH values similar to non-injected CH strips and, therefore, had percentages of free and bound moisture similar to CH strips. Sawyer et al. (2009) also found that when the pH of DC strips was lowered to pH values similar to CH with lactic acid solutions, the percentages of free and bound water were also similar to CH. In the current study, DC strips enhanced with citric acid solutions of pH 3.5 had percentages of free moisture that were greater than CH but less than DC. Pervious research injecting DC strips with citric acid solutions at pH 3.5 to 5.0 also altered the percentages of free and bound moisture but not to the extent of CH. It should be noted that that this lack of similarity with CH could be advantageous in industry applications because treatments with greater bound water also had greater cooking yields (Apple et al., 2011).

Display loss percentage was similar ($P = 0.482$) between non-enhanced DC and CH steaks; yet, display losses increased (linear, $P < 0.001$) as solution pH decreased from 3.5 to 2.0, and steaks from enhanced DC sections had greater display losses than both untreated DC ($P < 0.001$) and CH ($P \leq 0.004$) steaks. In addition, steaks from sections enhanced with PO_4-base solutions had greater ($P = 0.014$) display losses than those enhanced with STP-base solutions. Injecting DC strips with citric acid solutions with greater pH values than used in the present study also increased display loss compared to CH and DC (Stackhouse et al., 2015). Display loss was also increased over non-enhanced, normal pH treatments when DC strips were enhanced with 0.50% lactic acid, but not at 0.25, 0.75 or 1.0% solutions (Sawyer et al., 2009).

3.2 Fresh beef color

3.2.1 *Visual fresh beef color during simulated retail display*

Non-enhanced CH steaks received substantially greater ($P < 0.001$) fresh color scores than untreated and enhanced DC steaks on days 0, 1, 2, 3, and 4, and, even though steaks from enhanced DC sections received greater ($P \leq 0.003$) color scores than untreated DC steaks over the first three days of display, fresh color scores of enhanced DC steaks never approached those of untreated CH steaks (Table 2). At the end of the fourth day of display, CH steaks still received greater ($P < 0.001$) color scores than enhanced DC steaks, and steaks from DC sections enhanced with pH 2.5, 3.0, and 3.5 solutions received greater ($P \leq 0.018$) color scores than untreated DC steaks, but steaks enhanced with pH 2.0 solutions and non-enhanced DC steaks received similar ($P = 0.398$) fresh color scores. At the end of simulated display (day 5), CH steaks received greater ($P < 0.001$) color scores than non-enhanced and enhanced DC steaks, and the fresh color of steaks from DC sections enhanced with pH 3.0 and 3.5 was rated higher ($P \leq 0.011$) than untreated DC steaks, but color scores did not ($P \geq 0.108$) differ between DC steaks and those

from DC sections enhanced with pH 2.0 and 2.5 solutions. In addition, DC steaks enhanced with PO_4-base solutions received greater ($P = 0.034$) fresh color scores than steaks enhanced with STP-base solutions on day 1 of display; otherwise, base solution did not ($P \geq 0.066$) affect fresh color scores on days 0, 2, 3, 4, or 5.

There is very little, if any, available information on the effects of citric acid enhancement of DC strips on fresh beef color scores, especially steaks in simulated retail display. Research from this laboratory produced similar results, in that fresh color scores of DC strips injected with citric acid solutions (pH $\geq$ 3.5) were greater than non-injected DC, but not improved to the point of CH in the first four days of display (Stackhouse et al., 2015). When lactic acid was injected into DC strips, fresh color scores were also improved over non-injected DC strips (Sawyer et al., 2009). Acuff et al. (1987) showed improvements in fresh appearance when normal pH strips were treated with lactic acid, acetic acid, or a mixture of organic acids including acetic, lactic, citric, and ascorbic.

When placed into simulated retail display (day 0), CH steaks were less discolored (greater discoloration scores, $P < 0.001$) than DC steaks, and steak discoloration increased linearly ($P < 0.001$) as solution pH decreased from 3.5 to 2.0, with steaks from DC sections enhanced with pH 2.5 and 2.0 solutions being more than 50% discolored upon placement in display (Table 2). Even though untreated DC steaks received lesser ($P \leq 0.010$) discoloration scores than CH steaks on days 1, 3, and 4 of display, mean scores were never more than 1.1 points different, and discoloration scores were similar between non-enhanced DC and CH steaks on days 2 ($P = 0.915$) and 5 ($P = 0.128$) of display. Conversely, steaks from enhanced DC sections were more discolored than non-enhanced DC ($P \leq 0.034$) and CH steaks ($P \leq 0.008$) throughout the five days of simulated display, with pH 2.0-enhanced DC steaks receiving

discoloration scores indicative of "total discoloration" from the end of day 1 to completion of the display period. At the end of day 5, DC steaks enhanced with STP-base solutions were deemed less ($P = 0.009$) discolored than those enhanced with PO_4-base solutions, but discoloration scores did not ($P \geq 0.061$) differ between phosphate solutions during the first four days of display.

Similar to our results, DC strips injected with citric acid solutions with pH 3.5 and greater had similar discoloration scores to non-injected DC in the first two days of display but, on days 2 and 3 of display, all treatments were similarly discolored (Stackhouse et al., 2015). Furthermore, citric acid injected DC strips were inferior to both non-enhanced DC and CH strips on days 4 and 5 of display (Stackhouse et al., 2015). Sawyer et al. (2009) reported DC strips injected with 0.25% lactic acid had similar discoloration scores on days 0 through 4 of display, but those injected with 0.5, 0.75, or 1.0% lactic acid were inferior to DC and CH throughout display.

Normal pH steaks received greater ($P < 0.001$) overall acceptability scores than non-enhanced DC steaks, with CH steaks receiving scores indicative of "acceptable" to "desirable" across the first four days of simulated retail display (Table 2). In addition, desirability scores declined (linear, $P < 0.001$) as the solution pH decreased from 3.5 to 2.0, with pH 2.0-enhanced DC steaks deemed "extremely undesirable" immediately after packaging (day 0) and throughout simulated display. Steaks from DC sections enhanced with pH 3.5 solutions were similar ($P \geq 0.135$) to untreated DC steaks on days 0, 2, and 3, more ($P = 0.005$) acceptable than DC steaks on d 1, and less ($P \leq 0.008$) desirable on days 4 and 5 of display; however, panelists rated steaks enhanced with pH 2.5, 3.0, and 3.5 less ($P < 0.001$) desirable than CH steaks throughout the five-day display period. Base solution did not affect overall acceptability scores on days 0 ($P = 0.407$), 1 ($P = 0.116$), 3 ($P = 0.355$), or 5 ($P = 0.069$) of display, but steaks from DC sections

enhanced with STP-base solutions received greater ($P \leq 0.040$) acceptability scores on days 2 and 4 of simulated display than steaks from DC section enhanced with PO_4-base solutions.

Panelists ranked citric acid injected steaks as inferior in acceptability to non-injected CH steaks regardless of base PO_4 solution or solution pH throughout display. Stackhouse et al. (2015) also found inferior acceptability scores for citric acid-injected DC strips at days 0, 1, and 2 of display, but, on day 3, several treatments were similar to CH. Nevertheless, by that time, all treatments were rated as undesirable by the panelists (score of 3.5 of a 7-point scale). It is clear that the citric acid solutions used to enhance these DC strips improved some aspects of visual color over non-injected DC strips, but the final appearance is visually not on par with that of normal pH steaks.

3.2.2 *Instrumental fresh beef color during simulated retail display*

Initial (day 0) L* values were greater ($P < 0.001$) for CH than untreated DC steaks, and CH steaks remained lighter (greater L* values; $P < 0.001$) than DC steaks throughout the five-day display period (Table 3). Furthermore, across the simulated retail display period, DC steaks became lighter (linear, $P < 0.001$) as the solution pH decreased from 3.5 to 2.0, with steaks from DC sections enhanced with pH 2.0 solutions being lighter than CH ($P \leq 0.002$) and DC steaks ($P < 0.001$) from day 1 to 5 of display. In addition, 2.5 pH-enhanced DC steaks had similar ($P \geq 0.142$) fresh L* values to CH steaks, whereas L* values of steaks from DC sections enhanced with pH 3.0 and 3.5 solutions were intermediate to the CH ($P < 0.017$) and DC controls ($P \leq 0.045$) on days 1, 2, 3, and 5 of display. Interestingly, L* values were not ($P \geq 0.087$) affected by the base solution on days 0, 1, or 2, but, on days 3 through 5 of display, steaks enhanced with PO_4-base solutions had greater ($P \leq 0.035$) L* values than those enhanced with the STP-base solutions. Previous research has shown citric acid to either increase (Arganosa & Marriott, 1989;

Önenç, Serdaroğlu, & Abdraimov, 2004; Elgadir et al., 2011) or have no effect (Aktaş & Mükerrem, 2001; Hinkle, Calkins, de Mellow, Jr., Senaratne, & Pokharel, 2010) on L* values of fresh, normal pH beef steaks. In the current study, the DC strips injected with citric acid solutions with a pH of 2.5 had a final muscle pH similar to non-injected CH. Research from this laboratory demonstrated that citric acid enhancement of DC strips increased L* over non-injected DC strips, but was still darker than non-injected CH (Stackhouse et al., 2015). In that study, final pH values of the injected DC strips remained greater than the non-injected CH. When DC strips were injected with 0.25, 0.50, and 0.75% lactic acid, the L* values were similar to non-injected CH (Sawyer et al., 2009). Muscle pH values in that study were both above and below that of CH steaks. Apple et al. (2011) created pH values similar to CH by injecting lactic acid into DC strips, but injected DC steaks did not have similar fresh L* values to CH.

Initial (day 0) a* values were greater ($P < 0.001$) in untreated CH than DC steaks, and redness increased (quadratic, $P < 0.001$) with decreasing solution pH, with pH 2.5-enhanced DC steaks having a* values intermediate to either CH ($P < 0.001$) or DC ($P = 0.001$) steaks (Table 3). It should be noted that steaks from DC sections enhanced with pH 2.0 solutions were the least red, having lesser ($P < 0.001$) a* values than the CH and DC controls throughout display (days 0 to 5), whereas pH 2.5-enhanced steaks had intermediate redness values to both untreated CH (days 1 to 5; $P < 0.001$) and DC (days 3 to 5; $P \leq 0.006$). In addition, a* values were similar ($P \geq 0.03$) among untreated DC steaks and steaks from DC sections enhanced with pH 3.0 and 3.5 solutions across the display period. With the exception that DC steaks enhanced with STP-base solutions were redder ($P = 0.009$) than those enhanced with PO_4-base solutions on day 5 of display, a* values did not ($P \geq 0.061$) differ between base phosphate solutions.

Calculated hue angles indicated that DC ($P \leq 0.024$) and 3.5 pH-enhanced DC steaks ($P \leq 0.048$) were closer to the true red axis than CH steaks throughout simulated retail display, and that hue angles increased linearly ($P < 0.001$) as solution pH decreased from 3.5 to 2.0 (Table 3). In accordance with a* results, pH 2.0-enhanced DC steaks had the greatest hue angles, indicating the fresh color of these steaks was closer ($P < 0.001$) to the true yellow axis on each day of display than either untreated control. Moreover, hue angles were similar ($P \geq 0.094$) for CH and pH 2.5-enhanced DC steaks between days 1 and 5 of display, and hue angles were not ($P \geq 0.169$) affected by base phosphate solution during the entire display period.

Normal pH (CH) steaks were more yellow (greater b* values; $P < 0.001$) throughout simulated retail display than either non-enhanced or pH 3.5-enhanced DC steaks, and b* values increased (quadratic, $P < 0.001$) as solution pH increased from 2.0 to 3.5, with pH 2.0-enhanced DC steaks having lesser b* values than untreated DC ($P \leq 0.042$) and CH steaks ($P < 0.001$) between days 1 and 5 of display (Table 3). Day 0 and 1 b* values for steaks from sections enhanced with pH 2.5 and 3.0 solutions were intermediate to CH ($P < 0.001$) and DC ($P \leq 0.016$) steaks, but b* values were similar ($P \geq 0.071$) among non-enhanced, pH 2.5-enhanced, and pH 3.0-enhanced DC steaks on days 2, 3, 4, and 5 of display. And, even though steaks enhanced with STP-base solutions were more ($P = 0.028$) yellow than steaks enhanced with PO_4-base solutions on day 3 of display, base phosphate solution did not ($P \geq 0.070$) affect b* values of DC steaks.

Following the relatively same pattern as b* values, CH steaks had a more vivid (greater C* values; $P \leq 0.026$) fresh color than DC steaks, and C* values increase quadratically ($P < 0.001$) as solution pH increased from 2.0 to 3.5 across the five days of simulated retail display (Table 3). Fresh color of pH 2.0-enhanced DC steaks was the least ($P \leq 0.004$) vivid compared to

either the CH or DC controls, and, with the exception of initial (day 0) C* values, steaks from DC sections enhanced with pH 2.5, 3.0, and 3.5 solutions were less vivid (lower C* values; $P \leq 0.001$) than CH steaks each day of display. Again, C* values were similar ($P \geq 0.066$) between DC steaks enhanced with PO_4- and STP-base solutions on days 0, 1, 2, 4, and 5 of display, but STP-enhanced DC steaks had greater ($P = 0.018$) C* values than PO_4-enhanced DC steaks on day 3 of display.

In normal pH beef, citric acid marination has been shown to reduce redness (Arganosa & Marriott, 1989; Aktaş & Mükerrem, 2001; Önenç et al., 2004; Elgadir et al., 2011). Previous research in this laboratory reported that DC strips injected with citric acid solutions (pH 3.5 to 5.0) were less red than non-injected CH and similar to non-injected DC controls on day 0 of display, but were redder than DC controls on days 1 and 2 (Stackhouse et al., 2015). Although the color of CH steaks deteriorated with days of display in the present study, fresh color of CH steaks remained redder than injected DC steaks. Stackhouse et al. (2015) reported that citric acid injected DC strips were similar to CH controls after five days of display. When DC strips were injected with lactic acid, redness in fresh steaks was similar, or inferior, to non-injected DC steaks (Sawyer et al., 2009; Apple et al., 2011).

Fresh beef color is largely driven by the proportion of myoglobin in the red, oxygenated state (Seideman, Cohen, & Schollmeyer, 1984). The typical bright red color of fresh beef is replaced by a darker, purple-red coloration in DC beef due to the high functionality of mitochondria preserved by the high muscle pH (Ashmore, Doerr, & Parker, 1972) and the high oxygen-consumption rate (Zhu & Brewer, 1998). Previous research injecting normal pH beef with lactate (Knock et al., 2006; Kim et al., 2006; Mancini & Ramanathan, 2008) and DC beef with lactic acid (Apple et al., 2011) credited increased lactate dehydrogenase activity and

decreased oxygen consumption rate through NADH production with improvements in fresh color. In energy metabolism, citrate enters the Krebs cycle, so an introduction of citrate into the postmortem muscle was hypothesized to increase NDAH and increase color stability through decreased oxygen consumption. Nevertheless, the introduction of citric acid to DC strips did not improve fresh beef color over that of non-injected DC controls.

3.3 Cooked beef color

Cooked color and degree of doneness scores were similar ($P \geq 0.113$) between CH steaks and steaks from DC sections enhanced with pH 2.5 solutions, and both received greater ($P < 0.001$) cooked color and degree of doneness scores than untreated DC steaks (Table 4). Enhancement with pH 3.5 solutions did not ($P \geq 0.096$) change the internal cooked color or degree of doneness of DC steaks, whereas visual scores of pH 3.0-enhanced DC steaks were intermediate to untreated DC ($P \leq 0.053$) and CH steaks ($P < 0.001$). In agreement with the fresh color results, enhancement with pH 2.0 solutions caused cooked color ("gray brown") and degree of doneness ("medium well" to "well done") scores of DC steaks to be elevated ($P < 0.001$) over untreated CH and DC steaks, even though all steaks were cooked to an internal endpoint temperature of 71°C. Base phosphate solution did not ($P \geq 0.433$) affect either visual internal color score.

In addition to the dark, unappealing fresh appearance of DC beef, the high pH of DC protects myoglobin from denaturation during cooking, resulting in a red, underdone cooked color when steaks are cooked to endpoint temperatures of 71°C or higher (Trout, 1989). In the present study, treatments with final muscle pH values similar to CH also had cooked color and degree of doneness scores similar to CH. When DC strips were injected with citric acid solutions at higher

pH values (3.5 to 5.0) than the present study, cooked color and degree of doneness scores were more pink and more rare appearing than CH (Stackhouse et al., 2015).

In the present study, solutions of citric acid with very low pH resulted in cooked color and degree of doneness scores much more brown and well-done than all the other treatments. Instrumental readings confirmed that those samples were darker, less red and more brown than other treatments (Table 4). Sawyer et al. (2008, 2009) injected DC strips with lactic acid, resulting in very low final muscle pH values (below 5.0) and cooked color and degree of doneness scores indicative of brown and well done, respectively. In that study, the undenatured myoglobin found in raw and cooked samples was greatly decreased in samples with low pH values (< 5.0). The very acidic environment was responsible for denaturing myoglobin in enhanced samples even before cooking (Sawyer et al., 2009).

Base phosphate solution had no ($P \geq 0.247$) effect on any instrumental color measure of internal cooked color (Table 4). Moreover, neither cooked L* nor cooked a* values differed ($P \geq 0.671$) between non-enhanced DC and CH steaks, and both cooked L* ($P \geq 0.181$) and a* values ($P \geq 0.158$) were similar among CH steaks, untreated DC steaks, and DC steaks enhanced with pH 2.5, 3.0, and 3.5 solutions. However, enhancement with pH 2.0 solutions markedly reduced ($P < 0.001$) the internal lightness (L*) and redness (a*) values compared to untreated CH and DC steaks. Internal yellowness (b* values) was greater in CH steaks ($P < 0.024$) and steaks from DC sections enhanced with pH 2.5, 3.0, and 3.5 solutions ($P \leq 0.017$) than non-enhanced DC steaks, whereas pH 2.0-enhanced steaks were considerably less ($P \leq 0.001$) yellow than CH and DC steaks. Hue angles of CH and pH 2.5-enhanced DC steaks were greater ($P \leq 0.039$) than non-enhanced DC steaks, but hue angles were similar ($P \geq 0.085$) among CH steaks, DC steaks, and steaks enhanced with pH 3.0 and 3.5 solutions. Although C* values did not ($P \geq 0.092$) differ

among CH, DC, pH 2.5-, and pH 3.0-enhanced steaks, internal cooked color of steaks from DC sections enhanced with pH 3.5 solutions was more vivid (greater C* value; $P = 0.041$) than that of untreated DC steaks. Again, enhancement with pH 2.0 solutions detrimentally impacted hue angles ($P \leq 0.001$) and C* values ($P < 0.001$) of cooked steaks compared with untreated CH and DC steaks. In addition, the red-to-brown ratio of steaks enhanced with pH 2.0 solutions was substantially lower ($P \leq 0.001$) than DC and CH steaks, indicative of an overcooked appearance.

Similar to the present study, previous work from this laboratory found that injecting DC strips with citric acid solutions of pH 3.5 to 5.0 did not alter instrumental cooked color measurements compared to CH or DC controls (Stackhouse et al., 2015). When DC strips were injected with lactic acid, L*, b* and C* values of cooked steaks were not affected (Sawyer et al., 2008; Apple et al 2011), but redness was decreased in DC steaks enhanced with 0.5% lactic acid as measured by a* values and red-to-brown ratios. Other than the DC strips enhanced with solutions with pH 2.0, the range in instrumental color values of the cooked steaks was rather small, making it difficult to detect differences between treatments. Visual evaluations indicated that when muscle pH values were similar to CH, the cooked color scores were also similar to that of CH steaks.

4. Conclusion

Enhancement of DC beef strip loins with pH 2.5 citric acid solutions effectively changed the raw LM pH and internal cooked LM color to that similar to non-enhanced, normal pH (CH) steaks, but failed to positively impact fresh visual color. Conversely, enhancing DC beef with pH 3.0 or 3.5 solutions produced fresh and cooked steak colors virtually unchanged from the non-enhanced DC controls, whereas enhancement with pH 2.0 solutions caused LM pH to decline to values less than 4.1, resulting in considerable discoloration of fresh steaks even on the first day

of simulated retail display and an overcooked internal appearance. Furthermore, base phosphate solution had no appreciable effects on either fresh or cooked color of enhanced steaks. Thus, it is plausible that steaks from post-rigor DC beef enhanced with pH 2.5 citric acid solutions could be marketed to foodservice outlets because it can eliminate the persistent red, undercooked appearance of DC beef.

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Literature Cited

Abugroun, H. A., Cousin, M. A., & Judge, M. D. (1993). Extended shelf-life of unrefrigerated prerigor cooked meat. *Meat Science*, ***33***, 207-229.

Acuff, G. R., Vanderzant, C., Savell, J. W., Jones, D. K., Griffin, D. B., & Ehlers, J. G. (1987). Effect of acid decontamination of beef subprimal cuts on the microbiological and sensory characteristics of steaks. *Meat Science*, ***19***, 217-226.

Aktaş, N., & Mükerrem, K. (2001). The influence of marinating with weak organic acids and salts on the intramuscular connective tissue and sensory properties of beef. *European Journal of Food Research and Technology*, ***213***, 88-94.

Aktaş, N., Aksu, M. İ., & Kaya, M. (2003). The effect of organic acid marination on tenderness, cooking loss and bound water content of beef. *Journal of Muscle Foods*, ***14***, 181-194.

AMSA (1991). *Guidelines for meat color evaluation*. Chicago, IL: American Meat Science Association and National Live Stock and Meat Board.

AMSA (1995). *Guidelines for cookery, sensory evaluation and instrumental tenderness measurements of fresh meat*. Chicago, IL: American Meat Science Association and National Live Stock and Meat Board.

AMSA (2012). *Meat color measurement guidelines*. Champaign-Urbana, IL: American Meat Science Association.

Apple, J. K., Davis, J. R., Rakes, L. K., Maxwell, C. V., Stivarius, M. R., & Pohlman, F. W. (2001). Effects of dietary magnesium and duration of refrigerated storage on the quality of vacuum-packaged, boneless pork loins. *Meat Science*, ***57***, 43-53.

Apple, J. K., Sawyer, J. T., Meullenet, J. –F., Yancey, J. W. S., & Wharton, M. D. (2011). Lactic acid enhancement can improve the fresh and cooked color of dark-cutting beef. *Journal of Animal Science*, ***89***, 4207-4220.

Arganosa, G. C., & Marriott, N. G. (1989). Organic acids as tenderizers of collagen in restricted beef. *Journal of Food Science*, ***54***, 1173-1176.

Ashmore, C. R., Doerr, L., & Parker, W. (1972). Respiration of mitochondria isolated from dark-cutting beef: postmortem changes. *Journal of Animal Science*, ***34***, 46-48.

Burke, R. M., & Monahan, F. J. (2003). The tenderization of shin beef using a citrus juice marinade. *Meat Science*, ***63***, 161-168.

Chang, H. –J., Wang, Q., Zhou, G. –H., Xu, X. –L., & Li, C. –B. (2010). Influence of weak organic acids and sodium chloride marination on characteristics of connective tissue collagen and textural properties of beef semimembranosus muscle. *Journal of Texture Studies*, ***41***, 279-301.

Cornforth, D., Calkins, C. R., & Faustman, C. (1991). Methods for identification and prevention of pink color in cooked meat. *Proceedings of the 44th Annual Reciprocal Meat Conference, **44***, 53-58.

Cutter, C. N., & Siragusa, G. R. (1994). Efficacy of organic acids against *Escherichia coli* O157:H7 attached to beef carcass tissue using a pilot scale model carcass washer. *Journal of Food Protection, **57***, 97-103.

Eilers, J. D., Morgan, J. B., Martin, A. M., Miller, R. K., Hale, D. S., Acuff, G. R., & Savell, J. W. (1994). Evaluation of calcium chloride and lactic acid injection on chemical, microbiological and descriptive attributes of mature cow beef. *Meat Science, **38***, 443-451.

Elgadir, M. A., Mariod, A. A., Abdelwahab, S. I., Jamilah, B., Rahman, R. A., & Che Man, Y. B. (2011). Physiochemical and microbial attributes of organic infused beef cuts (longissimus dorsi). *Journal of Food Safety, **31***, 326-333.

Ertbjerg, P., Larsen, L. M., & Møller, A. J. (1999a). Effect of prerigor lactic acid treatment on lysosomal enzyme release in bovine muscle. *Journal of the Science of Food and Agriculture, **79***, 95-100.

Ertbjerg, P., Mielche, M. M., Larsen, L. M., & Møller, A. J. (1999b). Relationship between proteolytic changes and tenderness in prerigor lactic acid marinated beef. *Journal of the Science of Food and Agriculture, **79***, 970-978.

Hamm, R. (1986). Functional properties of the myofibrillar system and their measurements. In P. J. Bechtel (Ed.), *Muscle as Food* (pp 135 – 199). Orlando, FL: Academic Press, Inc.

Hinkle, J. B., Calkins, C. R., de Mellow, Jr., A. S., Senaratne, L. S., & Pokharel, S. (2010). Acid marination for tenderness enhancement of beef bottom round. M. *S. Thesis, University of Nebraska – Lincoln* (http://digitalcommons.unl.edu/animalscidiss/12).

Ke, S., Huang, Y., Decker, E. A., & Hultin, H. O. (2009). Impact of citric acid on the tenderness, microstructure and oxidative stability of beef muscle. *Meat Science, **82***, 113-118.

Kim, Y. H., Hunt, M. C., Mancini, R. A., Seyfert, M., Loughin, T. M., Kropf, D. H., & Smith, S. (2006). Mechanism for lactate-color stabilization in injection-enhanced beef. *Journal of Agricultural and Food Chemistry, **54***, 7856-7862.

Knock, R. C., Seyfert, M., Hunt, M. C., Dikeman, M. E., Mancini, R. A., Unruh, J. A., Higgins, J. J., & Monderen, R. A. (2006). Effects of potassium lactate, sodium chloride, sodium tripolyphosphate, and sodium acetate on colour, colour stability, and oxidative properties of injection-enhanced beef rib steaks. *Meat Science, **74***, 312-318.

Mancini, R. A., & Ramanathan, R. (2008). Sodium lactate influences myoglobin redox stability in vitro. *Meat Science, **78***, 529-532.

Mendenhall, V. T. (1989). Effect of pH and total pigment concentration on the internal color of cooked ground beef patties. *Journal of Food Science*, ***54***, 1-2.

Morris, C. A., Theis, R. L., Miller, R. K., Acuff, G. R., & Savell, J. W. (1997). Improving the flavor of calcium chloride and lactic acid injected mature beef top round steaks. *Meat Science*, ***45***, 531-537.

Önenç, A., Serdaroğlu, M., & Abdraimov, K. (2004). Effect of various additives to marinating baths on some properties of cattle meat. *European Food Research and Technology*, ***218***, 114-117.

Sammel, L. M., & Claus, J. R. (2003). Citric acid and sodium citrate effects on reducing pink cooked defect of cooked intact turkey breasts and ground turkey rolls. *Journal of Food Science*, ***68***, 874-878.

Sammel, L. M., & Claus, J. R. (2006). Citric acid and sodium citrate effects on pink color development of cooked ground turkey irradiated pre- and post-cooking. *Meat Science*, ***72***, 567-573.

Sawyer, J. T., Apple, J. K., & Johnson, Z. B. (2008). The impact of lactic acid concentration and sodium chloride on pH, water-holding capacity, and cooked color of injection-enhanced dark-cutting beef. *Meat Science*, ***79***, 317-325.

Sawyer, J.T., Apple, J.K., Johnson, Z.B., Baublits, R.T., & Yancey, J.W.S. (2009). Fresh and cooked color of dark-cutting beef can be altered by post-rigor enhancement with lactic acid. *Meat Science.* ***83***, 263-270.

Seideman, S. C., Cohen, S. H., & Schollmeyer, J. V. (1984). Some factors influencing ante-mortem changes in muscle: a brief review. *Food Microstructure*, ***3***, 127-132.

Stackhouse, R. J., Apple, J. K., Yancey, J. W. S., Keys, C. A., Johnson, T. M., & Mehall, L. N. (2015). Citric acid enhancement at solution pH values between 3.5 and 5.0 does not alter the fresh and cooked color of dark-cutting beef. *Meat Science* (*in review*).

Trout, G. R. (1989). Variation in myoglobin denaturation and color of cooked beef, pork, and turkey meat as influenced by pH, sodium chloride, sodium tripolyphosphate. *Meat Science*, ***28***, 203-210.

Viljoen, H. F., de Kock, H. L., & Webb, E. C. (2002). Consumer acceptability of dark, firm and dry (DFD) and normal pH beef steaks. *Meat Science*, ***61***, 181-185.

Wierbicki, E., & Deatherage, F. E. (1958). Determination of water-holding capacity of fresh meats. *Journal of Agricultural and Food Chemistry*, ***6***, 387-392.

Wismer Pedersen, J. (1971). Water. In J. R. Price & B. S. Schweigirt (Eds.), *The Science of Meat and Meat Products* (pp. 177 – 191). San Francisco, CA: W. H. Freeman and Co.

Yoon, Y., Mukherjee, A., Belk, K. E., Scanga, J. A., Smith, G. C., & Sofos, J. N. (2009). Effect of tenderizers combined with organic acids on *Escherichia coli* O157:H7 thermal resistance in non-intact beef. *International Journal of Food Microbiology*, ***133***, 78-85.

Zhu, L. G., & Brewer, M. S. (1998). Metmyoglobin reducing capacity of fresh normal, PSE, and DFD pork during retail display. *Journal of Food Science*, ***63***, 390-393.

Table 3.1Effects of citric acid enhancement solution pH on pH and water-holding capacity of dark-cutting beef steaks

Treatments	Pre-enhancement pH[1]	Post-enhancement pH[2]	Pump (%)[3]	Total moisture (%)[4]	Free moisture (%)[5]	Bound moisture (%)[6]	Display loss (%)[7]
Non-enhanced, normal pH (CH)	5.39	5.43	---	70.7	54.08	45.92	4.51
Non-enhanced dark-cutter (DC)	6.62	6.67	---	71.6	37.89	62.11	3.88
Orthrophosphate-based (PO_4) solutions							
pH 2.0	6.68*	4.06*†	10.66	71.3	66.05*†	33.95*†	17.33*†
pH 2.5	6.74*	5.69†	6.99	74.6*†	50.46†	49.54†	13.03*†
pH 3.0	6.71*	6.14*†	8.94	74.7*†	43.47*	56.53*	6.60*†
pH 3.5	6.53*	6.35*	9.22	74.5*†	52.68*†	47.32*†	6.81*†
Tripolyphosphate-based (STP) solutions							
pH 2.0	6.81*	4.62*†	9.57	70.6	58.34*†	41.66*†	16.34*†
pH 2.5	6.89*	5.70†	6.33	74.0*†	51.22†	48.78†	9.22*†
pH 3.0	6.81*	6.07*†	9.25	74.8*†	41.84*	58.16*	6.93*†
pH 3.5	6.85*	6.54*	11.45	74.7*†	42.04*†	57.96*†	6.82*†
SEM	0.049	0.147	0.707	0.68	2.183	2.183	0.627
Contrasts[8]	C	C, L		L	C, P, L	C, P, L	P, L

Within a column, least squares means with an asterisk (*) differ ($P < 0.05$) from CH and least squares means with a single-cross (†) differ ($P < 0.05$) from DC.

[1]pH measured immediately before enhancement.

[2]pH measured after 30-min equilibration.

[3]Product yield after 30-min equilibration = (post-enhancement section weight ÷ pre-enhancement section weight) × 100.

[4]Total moisture = ((post-enhancement sample weight – freeze-dried sample weight) ÷ post-enhancement sample weight) × 100.

[5]Free moisture = (((moisture surface area – meat surface area) × 61.1) ÷ total moisture) × 100 (Wierbicki & Deatherage, 1958).

[6]Bound moisture = 100% - free moisture (Wierbicki & Deatherage, 1958).

[7]Display loss = ((0-d strip steak weight = 5-d strip steak weight) ÷ 0-d strip steak weight) × 100.

[8]Contrast abbreviations: **C** = CH vs. DC; **P** = PO_4 vs. STP; **L** = linear solution pH effect; and **Q** = quadratic solution pH effect.

Table 3.2 Effects of citric acid enhancement solution pH on the visual fresh color characteristics of dark-cutting beef steaks during simulated retail display

Treatments	Simulated retail display (day) 0	1	2	3	4	5
	---------- Fresh color score[2] (treatment × day, $P < 0.001$) ----------					
Non-enhanced, normal pH (CH)	7.0	6.1	5.2	4.6	5.0	4.8
Non-enhanced dark-cutter (DC)	1.9	2.0	2.5	2.2	1.8	3.2
Orthrophosphate-based (PO_4) solutions						
pH 2.0	2.6*†	3.0*†	3.7*†	3.4*†	2.0*	2.8*
pH 2.5	3.4*†	3.1*†	3.8*†	3.4*†	3.0*†	3.5*
pH 3.0	2.7*†	2.8*†	3.7*†	3.1*†	2.5*†	2.6*†
pH 3.5	3.0*†	3.5*†	3.6*†	2.9*†	1.9*†	2.4*†
Tripolyphosphate-based (STP) solutions						
pH 2.0	2.4*†	2.8*†	3.5*†	3.4*†	2.1*	2.9*
pH 2.5	3.4*†	3.0*†	3.9*†	3.6*†	2.9*†	3.0*
pH 3.0	2.9*†	2.8*†	3.3*†	2.9*†	2.7*†	2.6*†
pH 3.5	2.6*†	3.0*†	3.3*†	2.7*†	3.0*†	2.8*†
SEM	0.20	0.25	0.41	0.39	0.44	0.41
Contrasts[1]	C	C, P, L	C	C, L	C	C, L
	---------- Discoloration score[3] (treatment × day, $P < 0.001$) ----------					
Non-enhanced, normal pH (CH)	8.0	7.7	7.6	7.2	6.6	6.7
Non-enhanced dark-cutter (DC)	6.8	6.7	7.6	6.5	5.5	6.3
Orthrophosphate-based (PO_4) solutions						
pH 2.0	2.3*†	1.7*†	1.9*†	1.1*†	1.3*†	1.0*†
pH 2.5	4.6*†	4.0*†	4.6*†	4.2*†	3.4*†	3.6*†
pH 3.0	6.2*†	5.3*†	6.8*†	6.1*†	4.2*†	5.1*†
pH 3.5	6.8*	6.4*	7.1*†	6.2*	3.9*†	5.1*†
Tripolyphosphate-based (STP) solutions						
pH 2.0	2.6*†	1.9*†	1.7*†	1.2*†	1.1*†	1.0*†
pH 2.5	4.7*†	4.3*†	5.2*†	4.8*†	3.4*†	3.9*†
pH 3.0	6.4*†	5.0*†	6.7*†	6.1*†	4.2*†	5.4*†
pH 3.5	6.7*	5.8*	7.2*†	6.5*	5.1*†	5.8*†
SEM	0.49	0.63	0.40	0.54	0.79	0.58
Contrasts[8]	C, L	C, L	L	C, L	C, L	P, L

Table 3.2 (Cont.) Effects of citric acid enhancement solution pH on the visual fresh color characteristics of dark-cutting beef steaks during simulated retail display

Treatments	Simulated retail display (day)					
	0	1	2	3	4	5
	---------- Overall acceptability[4] (treatment × day, $P < 0.001$) ----------					
Non-enhanced, normal pH (CH)	6.0	5.4	5.3	4.7	4.5	3.8
Non-enhanced dark-cutter (DC)	3.7	3.4	4.2	3.6	2.8	3.4
Orthrophosphate-based (PO_4) solutions						
pH 2.0	1.3*†	1.0*†	1.0*†	1.0*†	1.0*†	1.0*†
pH 2.5	2.4*†	2.1*†	2.1*†	1.9*†	1.8*†	1.8*†
pH 3.0	3.4*†	3.2*†	3.6*†	3.3*†	2.0*†	2.0*†
pH 3.5	4.0*	4.1*†	4.0*	3.5*	1.7*†	2.1*†
Tripolyphosphate-based (STP) solutions						
pH 2.0	1.3*†	1.1*†	1.0*†	1.0*†	1.0*†	1.0*†
pH 2.5	2.3*†	2.1*†	2.8*†	2.1*†	1.8*†	1.8*†
pH 3.0	3.4*†	2.9*†	3.7*†	3.2*†	2.3*†	2.2*†
pH 3.5	3.8*	3.7*†	4.0*	3.7*	2.7*†	2.6*†
SEM	0.30	0.34	0.38	0.34	0.22	0.16
Contrasts[8]	C, L	C, L	C, P, L	C, L	C, P, L	C, L

Within a column, least squares means with an asterisk (*) differ ($P < 0.05$) from CH and least squares means with a single-cross (†) differ ($P < 0.05$) from DC.

[1]Contrast abbreviations: **C** = CH vs. DC; **P** = PO_4 vs. STP; **L** = linear solution pH effect; and **Q** = quadratic solution pH effect.

[2]Fresh color score: 1 = extremely dark red to 8 = extremely bright cherry red (AMSA, 2012).

[3]Discoloration score: 1 = total (≥ 96%) discoloration; 2 = 81 to 95% discoloration; 3 = 66 to 80% discoloration; 4 = 51 to 65% discoloration; 5 = 36 to 50% discoloration; 6 = 21 to 35% discoloration; 7 = 6 to 20% discoloration; and 8 = no (0 to 5%) discoloration.

[4]Overall acceptability: 1 = extremely undesirable to 7 = extremely desirable (AMSA, 2012).

Table 3.3 Effects of citric acid enhancement solution pH on the instrumental fresh color characteristics of dark-cutting beef steaks during simulated retail display

Treatments	Simulated retail display (day)					
	0	1	2	3	4	5
	---------- Lightness (L*) values[2] (treatment × day, $P = 0.013$) ----------					
Non-enhanced, normal pH (CH)	42.84	40.72	40.71	39.22	38.79	39.89
Non-enhanced dark-cutter (DC)	31.05	31.65	32.56	31.30	32.47	32.10
Orthrophosphate-based (PO_4) solutions						
pH 2.0	44.56†	45.33*†	46.65*†	46.02*†	45.78*†	45.90*†
pH 2.5	40.02*†	39.85†	41.52†	40.20†	38.71†	40.16†
pH 3.0	36.09*†	34.95*†	36.98*†	36.21*†	34.66*	35.06*†
pH 3.5	35.17*†	36.13*†	36.96*†	36.73*†	35.24*	36.31*†
Tripolyphosphate-based (STP) solutions						
pH 2.0	43.18†	43.45*†	44.59*†	44.16*†	44.00*†	44.67*†
pH 2.5	37.45*†	38.17†	39.41†	38.62†	38.02†	36.73†
pH 3.0	36.35*†	35.67*†	37.98*†	35.00*†	34.48*	35.09*†
pH 3.5	32.95*†	34.50*†	34.85*†	34.20*†	32.71*	33.57*†
SEM	1.259	0.941	1.076	0.921	0.900	1.138
Contrasts[1]	C, L	C, L	C, L	C, P, L	C, P, L	C, P, L
	---------- Redness (a*) values[2] (treatment × day, $P < 0.001$) ----------					
Non-enhanced, normal pH (CH)	34.66	30.80	29.65	28.55	27.31	24.77
Non-enhanced dark-cutter (DC)	21.82	23.53	25.52	25.85	25.16	23.60
Orthrophosphate-based (PO_4) solutions						
pH 2.0	14.49*†	12.88*†	10.14*†	10.41*†	9.26*†	9.16*†
pH 2.5	24.20*†	24.32*	23.55*	22.30*†	22.29*†	20.35*†
pH 3.0	23.13*	24.43*	24.80*	24.70*	24.63*	21.42*
pH 3.5	22.80*	24.35*	25.28*	24.75*	22.52*	19.25*†
Tripolyphosphate-based (STP) solutions						
pH 2.0	15.41*†	13.33*†	11.36*†	10.81*†	9.96*†	9.14*†

Table 3.3 (Cont.) Effects of citric acid enhancement solution pH on the instrumental fresh color characteristics of dark-cutting beef steaks during simulated retail display

	Simulated retail display (day)					
Treatments	0	1	2	3	4	5
pH 2.5	24.85*†	24.21*	24.13*	23.91*†	22.86*†	20.77*†
pH 3.0	22.35*	25.06*	25.16*	26.08*	24.66*	22.09*
pH 3.5	21.82*	24.83*	25.80*	26.05*	24.94*	22.53*†
SEM	0.628	0.709	0.732	0.741	0.767	0.907
Contrasts[8]	C, Q	C, Q	C, L	C, P, L	C, Q	Q
	---------- Yellowness (b*) values[2] (treatment × day, $P < 0.001$) ----------					
Non-enhanced, normal pH (CH)	26.84	23.68	22.99	22.50	21.57	20.38
Non-enhanced dark-cutter (DC)	13.65	15.82	18.14	18.60	18.19	16.30
Othrophosphate-based (PO_4) solutions						
pH 2.0	13.86*	13.93*†	13.05*†	13.15*†	12.91*†	12.91*†
pH 2.5	18.11*†	19.12*†	19.61*	18.69*	18.65*	18.04*
pH 3.0	15.49*†	17.32*†	18.62*	18.34*	18.32*	15.31*
pH 3.5	14.78*	17.22*	18.57*	18.16*	15.79*	11.79*†
Tripolyphosphate-based (STP) solutions						
pH 2.0	14.73*	14.40*†	14.02*†	13.82*†	13.52*†	13.39*†
pH 2.5	17.86*†	17.96*†	19.38*	19.10*	18.42*	16.74*
pH 3.0	14.99*†	18.55*†	19.48*	19.90*	18.79*	16.70*
pH 3.5	13.31*	17.51*	18.63*	19.03*	17.81*	14.92*†
SEM	0.523	0.597	0.604	0.565	0.669	0.713
Contrasts[8]	C, Q	C, Q	C, Q	C, P, Q	C, Q	C, Q
	---------- Hue angles (°)[3] (treatment × day, $P < 0.001$) ----------					
Non-enhanced, normal pH (CH)	37.75	37.55	37.79	38.72	38.36	39.73
Non-enhanced dark-cutter (DC)	31.91	33.83	35.34	35.67	35.77	34.56
Orthrophosphate-based (PO_4) solutions						
pH 2.0	43.87*†	47.62*†	52.74*†	51.97*†	54.44*†	54.95*†
pH 2.5	36.73*†	38.17†	39.85†	40.11†	40.06†	41.98†
pH 3.0	33.66*†	35.23†	36.88†	36.55	36.58	35.43*
pH 3.5	32.93*	35.17	36.26*	36.21*	34.79*	31.08*†

Table 3.3 (Cont.) Effects of citric acid enhancement solution pH on the instrumental fresh color characteristics of dark-cutting beef steaks during simulated retail display

	Simulated retail display (day)					
Treatments	0	1	2	3	4	5
Tripolyphosphate-based (STP) solutions						
pH 2.0	44.04*†	48.16*†	51.66*†	52.65*†	54.05*†	55.90*†
pH 2.5	35.66*†	36.51†	38.76†	38.70†	38.85†	38.89†
pH 3.0	33.86*†	36.47†	37.66†	37.38	37.25	37.13*
pH 3.5	31.36*	35.08	35.73*	36.05*	35.46*	33.33*†
SEM	0.579	0.767	0.743	0.809	0.688	0.932
Contrasts[8]	C, L	C, L	C, L	C, L	C, L	C, L
	---------- Chroma (C*) values[4] (treatment × day, $P < 0.001$) ----------					
Non-enhanced, normal pH (CH)	43.83	38.85	37.52	36.34	34.80	32.09
Non-enhanced dark-cutter (DC)	25.75	28.36	31.32	31.85	31.05	26.69
Orthrophosphate-based (PO_4) solutions						
pH 2.0	20.06*†	19.00*†	16.58*†	16.81*†	15.90*†	15.86*†
pH 2.5	30.24*†	30.94*	30.66*	29.13*	29.08*	27.24*
pH 3.0	27.85*	29.96*	31.02*	30.78*	30.71*	26.35*
pH 3.5	27.18*	29.84*	31.37*	30.70*	27.53*	22.63*†
Tripolyphosphate-based (STP) solutions						
pH 2.0	21.34*†	19.69*†	18.08*†	17.59*†	16.82*†	16.23*†
pH 2.5	30.61*†	30.15*	30.96*	30.62*	29.37*	26.70*
pH 3.0	26.93*	31.18*	31.83*	32.83*	31.01*	27.70*
pH 3.5	25.56*	30.39*	31.83*	32.26*	30.65*	27.05*†
SEM	0.777	0.874	0.906	0.874	0.975	1.085
Contrasts[8]	C, Q	C, Q	C, Q	C, P, Q	C, Q	C, Q

Within a column, least squares means with an asterisk (*) differ ($P < 0.05$) from CH and least squares means with a single-cross (†) differ ($P < 0.05$) from DC.

[1]Contrast abbreviations: **C** = CH vs. DC; **P** = PO_4 vs. STP; **L** = linear solution pH effect; and **Q** = quadratic solution pH effect.

[2]L* values are a measure of darkness to lightness (greater value indicates a lighter color); a* values are a measure of redness (greater value indicates a redder color); and b* values are a measure of yellowness (greater value indicates a more yellow color).

[3]Hue angle represents the change from the true red axis (greater angle indicates a greater shift from the true red axis).

[4]Chroma, or saturation index, is a measure of the total color (a greater value indicates a more vivid color).

Table 3.4 Effects of enhancement solution pH on the visual and instrumental color characteristics of dark-cutting beef steaks cooked to an internal endpoint temperature of 71°C

Treatments	Cooked color score[1]	Degree of doneness score[2]	Lightness (L*) value[3]	Redness (a*) value[3]	Yellowness (b*) value[3]	Hue angle[4]	Chroma (C*) value[5]	Red-to-brown ratio[6]
Non-enhanced, normal pH (CH)	4.0	3.9	51.45	18.92	19.96	46.77	27.55	2.4
Non-enhanced dark-cutter (DC)	3.3	3.2	51.79	18.16	17.02	42.83	24.93	2.5
Orthrophosphate-based (PO_4) solutions								
pH 2.0	6.7*†	5.9*†	45.67*†	8.69*†	11.97*†	54.04*†	14.80*†	1.3*†
pH 2.5	4.4†	4.0†	51.63	20.01	20.36†	45.79†	28.58	2.7
pH 3.0	3.5*†	3.4*†	52.92	19.29	19.44†	45.38	27.41	2.6
pH 3.5	3.3*	3.1*	53.22	20.57	19.55†	43.73	28.43†	2.8
Tripolyphosphate-based (STP) solutions								
pH 2.0	6.2*†	5.5*†	45.07*†	10.96*†	13.80*†	51.89*†	17.66*†	1.5*†
pH 2.5	4.1†	3.9†	52.15	18.78	19.79†	46.95†	27.36	2.5
pH 3.0	3.6*†	3.5*†	50.33	20.06	20.04†	45.85	28.45	2.8
pH 3.5	3.8*	3.5*	53.48	20.25	20.59†	45.83	28.90†	2.8
SEM	0.12	0.10	1.124	1.259	0.860	1.210	1.411	0.21
Contrasts[7]	C, L	C, L	L	L	C, L	C, L	L	L

Within a column, least squares means with an asterisk (*) differ ($P < 0.05$) from CH and least squares means with a single-cross (†) differ ($P < 0.05$) from DC.

[1]Cooked color: 1 = very red to 7 = brown (AMSA, 2012).

[2]Degree of doneness: 1 = very rare to 7 = very well (AMSA, 2012).

[3]L* values are a measure of darkness to lightness (greater value indicates a lighter color); a* values are a measure of redness (greater value indicates a redder color); and b* values are a measure of yellowness (greater value indicates a more yellow color).

[4]Hue angle represents the change from the true red axis (greater angle indicates a greater shift from the true red axis).

[5]Chroma, or saturation index, is a measure of the total color (a greater value indicates a more vivid color).

[6]Spectral reflectance ratio of 630 nm/580 nm is an estimate of cooked color change from red to brown (greater value indicates a redder color; AMSA, 1991).

[7]Contrast abbreviations: **C** = CH vs. DC; **P** = PO_4 vs. STP; **L** = linear solution pH effect; and **Q** = quadratic solution pH effect.

Chapter IV

CONCLUSION

Enhancement of DC beef strip loins with pH 2.5 citric acid solutions effectively changed the raw LM pH and internal cooked LM color to that similar to non-enhanced, normal pH (CH) steaks, but failed to positively impact fresh visual color. Conversely, enhancing DC beef with pH 3.0 or 3.5 solutions produced fresh and cooked steak colors virtually unchanged from the non-enhanced DC controls, whereas enhancement with pH 2.0 solutions caused LM pH to decline to values less than 4.1, resulting in considerable discoloration of fresh steaks even on the first day of simulated retail display and an overcooked internal appearance. Furthermore, base phosphate solution had no appreciable effects on either fresh or cooked color of enhanced steaks. Thus, it is plausible that steaks from post-rigor DC beef enhanced with pH 2.5 citric acid solutions could be marketed to foodservice outlets because it can eliminate the persistent red, undercooked appearance of DC beef.