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ACID MARINATION FOR TENDERNESS ENHANCEMENT OF BEEF BOTTOM ROUND

By

Jeremey Blake Hinkle

A THESIS

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ACID MARINATION FOR TENDERNESS ENHANCEMENT OF BEEF ROUND

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University of Nebraska, 2010

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Acid marination is known to improve meat tenderness. The objective of this study was to document the tenderness and color effects of marinating m. *biceps femoris* and to determine optimal acid levels. Acid solutions were prepared to 0.1 M (low) and 0.5 M (high) and pumped to 7% of initial wt. in experiment 1; 0.75 M (low) and 1.5 M (high) and pumped to 10 % of initial wt. in experiment 2. Lactic acid, acetic acid and sodium citrate dihydrate (food grade citric acid) at low and high concentrations gave 6 treatments per experiment. Objective color measurements were taken at 0, 1, and 8 h after marination for experiments one and two. Cooking loss and tenderness were analyzed from steaks cut at 0, 1, and 8 h and at 1, 3, 5, 7, 14, 21 and 28 d after injection and from steaks cut at 0, 1, and 8 h and at 1, 3, 5, 7, 14 d for experiments 1 and 2, respectively. Muscles were cut into steaks at 8 h and frozen at the times indicated after injection. Color was measured after given time to bloom at 0, 1 and 8 h post-injection for both experiments 1 and 2. Shear force measurements were obtained from an Instron with a Warner-Bratzler shear force attachment. No effects on tenderness (P = 0.11) were observed from experiment 1. A significant (P = 0.02) increase in tenderness was observed from 1 to 14 days during experiment 2. Sodium citrate dihydrate has little to no effect on tenderness; acetic and lactic acids at 0.75 M to 1.5 M had a positive effect on

tenderness. In experiment 1, muscles marinated with 0.5 M sodium citrate dihydrate decreased in lightness (L*), whereas samples with 0.1 M lactic acid increased in L* at 1 h ($P \leq 0.05$). All muscles marinated with 0.1 M sodium citrate, acetic and lactic acid increased significantly in lightness at 8 h ($P \leq 0.05$). Both redness (a*) and yellowness (b*) decreased for all six treatment groups from 0 to 8 h ($P \leq 0.05$) of experiment 1. There was a significant effect of treatment x time ($P = 0.04$) on L* values. When using either acetic or lactic acids at higher concentrations, at the injection site, the color of the meat turned from red to a dark gray. Results of this work indicate that acid marination with 0.1 to 0.5 M of the three analyzed acids had no effect on tenderness, but at higher concentrations of acid (0.75 to 1.5 M) significantly ($P = 0.02$) improved tenderness from 1 to 14 d.

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INTRODUCTION

The primary attribute consumer's associate with the palatability of a good steak is tenderness. When asked what they disliked about beef products, U.S.D.A. Select grade steaks were identified as being tough (Savell et al., 1989). Miller et al. (2001) found 15-20% of the steaks sold to consumers were described as tough. Muscle tenderness was found to be variable in a carcass depending on its muscle function (Von Seggern, et al., 2005). Variability in muscle tenderness was also found within each muscle (Senaratne, et al., 2009). Since tenderness is an important attribute for consumers, the meat industry should strive for a more consistently tender product. One possible method to enhance tenderness is acid marination.

Wenham and Locker (1976) evaluated the tenderness of meat when marinated in 1.5 % acetic acid. They found a 39% improvement in tenderness within the first 8 hours and an additional 21% improvement 43 hours post-marination. Some researchers studied the effects of acid concentration on meat and disregarded the effects of time (Wenham and Locker, 1976, Aktas et al., 2003). Saunders (1994) also focused on the effects acid concentration at a specific time point finding that it decreased the band intensity of all major myofibrillar proteins including the myosin heavy chains, and new smaller bands appeared as a result of degradation.

Acids have been shown to enhance tenderness, but little work has been documented on the recommendation of acid strength to optimize tenderness and the length of time which the muscle would stay acceptable to consumers. The objectives of this study were to evaluate the tenderness and color effects of marinating m. *biceps*

femoris (72 bottom rounds) with lactic, acetic and sodium citrate dihydrate (food grade citric acid) over time and to determine optimal acid levels.

REVIEW OF LITERATURE

Consumer perceptions on steak tenderness

The primary attribute consumer's associate with the palatability of a good steak is tenderness. When asked what they disliked about beef products, U.S.D.A. Select grade steaks were identified as being tough (Savell et al., 1989). Smith et al. (1987) also reported that U.S.D.A. Prime steaks are more palatable than steaks from a lower quality grade. Miller et al. (2001) found 15-20% of the steaks sold to consumers were described as tough. A tough steak is commonly characterized as being hard to chew or "stringy". Researches often quantify overall tenderness as the amount of connective tissue and the muscle fiber tenderness of a steak (McKeith et al., 1985, Smith et al., 1987). Tenderness is commonly evaluated using, trained and consumer taste panels and Warner-Bratzler shear force measurements. Warner-Bratzler shear force (WBSF) is commonly reported in kg or lb/force. Using the WBSF, muscles in a beef carcass were shown to be variable in tenderness (Von Seggern, et al., 2005). Later, tenderness was shown to be variable in tenderness within a single muscle from one end of the muscle to the other end (Senaratne, et al., 2009). Since tenderness is an important attribute for consumers, the meat industry should strive for a more consistently tender product. One method to accomplish this is through acid marination.

Acid Types for Marinating Solution

In general, marinades are widely used for enhancing the flavor and tenderness of meat. Marinades can be found in many regions of the world, each offering different flavors. Many tropical regions utilize the abundant fruits for marinating meat, such as tropical fruits. Papaya, kiwi and pineapple can also be used for marinating to enhance tenderness through enzymatic action. Citrus fruits also provide organic acids that can be used for acid marination. Other types of marinades with strong flavorings are used to mask the "gamey" flavor of meats like venison, wild boar and small game (Manteuffel-Gross and Ternes, 2009).

Organic acids are commonly used in marinating meat. Laboratory grade acids cannot be used in food production so consequently food grade organic acids are often studied. Common house-hold marinating ingredients are vinegar and fresh citric juices such as grapefruit, lemon, lime, and orange. Other marinades commonly used on game meats are teriyaki sauce, soy sauce, and red wines, alone or in combination (Manteuffel-Gross and Ternes, 2009). Many tropical regions utilize lemons and limes to marinate fish and poultry, whereas, in Midwest of the United States soy sauce and wine-based marinades are more common (Aktas et al., 2003, Serdaroglu et al., 2007, Manteuffel-Gross and Ternes, 2009).

The two most studied acids for meat enhancement or tenderness enhancement are citric and lactic acids. Lactic acid is often used in the meat industry as an antimicrobial during carcass slaughter and fabrication to help control the spread of E. coli O157: H7 and other pathogens. Lactic acid is also generally perceived as a natural acid since it is a by-product of fermentation during food production. Citric acid is widely perceived as a

safe organic acid and used in a variety of foods such as soft drinks, candies, wines and in canning of vegetables. Acetic acid is often used as a flavoring in spinach, ketchup, mustard, and hot sauces.

Soy sauce is a very common base for marinades as it offers acidity, salt flavor and color. Soy and teriyaki sauces contain a multitude of acids so identifying the acid that improves tenderness is difficult when using soy sauce as the main acidulant. The main acids in soy sauce are lactic acid and pyroglutamic acid, with lactic being the most prevalent acid. Pyroglutamic acid was found to be more prevalent than lactic acid in certain types of sauces due to the extent of fermentation. Other minor organic acids found in soy sauce are, in order of prevalence, acetic, formic, citric and succinic acids (Neidleman and Laskin, 1997). Teriyaki sauce's main ingredient is soy sauce, also making this flavoring/ acidulant more difficult to study.

There are two main types of soy sauce production: traditional fermentation using wheat and soybean or chemical production by hydrolysis of soybeans. In the United States, the hydrolysis method of production is preferred over the fermentation method because of the consistent flavor profile and large batches that can be produced. Gourmet or high quality soy sauces are also made by the fermentation process, but are more expensive to produce (Kikkoman, 2009).

Muscle Proteins

There are 3 basic muscle protein categories that comprise skeletal muscle: stromal sarcoplasmic, and myofibrillar proteins. Stromal proteins are insoluble in both water and salt. These proteins form the harness that holds together the muscle cells. More specifically, the stromal proteins (collagen and elastin) are found in connective tissues.

Sarcoplasmic proteins are water soluble and globular in shape. Myoglobin, a common sarcoplasmic protein that is important in meat color.

Myofibrillar proteins are salt soluble, and consist of many individual proteins that perform different functions. Two basic functional categories of myofibrillar proteins are structural and contractile proteins. Some common structural proteins are titin (connectin), desmin (skeletin), and Z-protein. The important contractile proteins are actin, myosin, actomyosin and troponin. Actin (thin filament) and myosin (thick filaments) are the main components involved in contraction (Price and Schweigert, 1987).

Effects of acid on myofibrillar proteins

When the pH of a muscle decreases, reaching the isoelectric point of approximate pH 5.3, the protein repulsion would be at the lowest point indicating the equal amounts of positive and negative charges. As the pH becomes more acidic the balance of charges is disrupted by an increase in positive charges, causing repulsion. The same process occurs with negative charges when the pH becomes more basic.

Ke et al. (2008) suggest that tenderness is related to the pH of the muscle. They reported that Warner-Bratzler shear force decreased as muscle pH was lowered to 3.52; then shear force significantly increased as the pH was buffered back to pH 5.26. The researchers reported seeing a disappearance of the microstructure of the muscle at low pH, except they could still see the Z-discs and faint traces of the M-line. The microstructure returned to a surprisingly normal structure as it was buffered back to a normal postmortem pH. The disappearance of the microstructure was believed to be caused by the accumulation of the net positive charges on the myofibrillar/cytoskeletal protein resulting in greater repulsive forces in the myofibrils pushing the myofibrils apart.

As the muscle is buffered back to the normal postmortem pH, less repulsion of the myofibril would occur allowing the microstructure to return to its natural state.

Similarly, Oreskovich et al. (1992) found that the Z-line structure remained intact at pH 1.94 using a 0.4 M phosphoric acid except the M-line structure and the thick and thin filaments were lost. Transmission electron microscopy was used to study the Z-line, M-line and thick and thin filaments. They tended to degrade as the pH is lowered from 5.3 to approximately 3.5 (Rao et al., 1989a, Oreskovich et al., 1992, Saunders, 1994, and Ke et al., 2008). Saunders (1994) using SDS-Page, reported that myofibrillar proteins specifically the myosin heavy chains do not start to break down until a pH 4.5 is reached, where the band widths become smaller and new bands start to appear.

Several studies have suggested tenderness is directly influenced by water holding capacity. Water holding capacity is the amount of water that can be held within a muscle during some form of mechanical forces such as cutting, tumbling, etc. As the enhancement/acid solution is added the myofibrillar protein swell, allowing water into the muscle. Others have noted this swelling effect in enhancement solutions containing salt and water, perhaps through myofibrillar proteins extraction from muscle microstructure (Offer and Trinick, 1983). Cooking loss was also noted to be less in enhancement products versus non-enhanced meats (Gault, 1985).

Some data have shown that the tenderness benefits of acid marination occur within the first few days of marinating. A slight decrease in shear force can be achieved after an extended period of time of either 14 to 21 days after the initial increase in tenderness, indicating that there should not be a concern or over-tenderizing (Wenham et al., 1976, Ertbjerg et al., 1999, and Berge et al., 2001). Ertjerg et al. (1999) suggests that

the continual increase in tenderness after the initial benefits of acid marination is attributed to an enhanced release of cathepsin enzymes. This allows the enzymes to increase degradation of the myofibrillar proteins.

Berge et al. (2000) looked at steaks marinated at extended periods of time for 14 to 21 days. Others have only looked at only short time periods with 1 or 2 points of data collection of 24 to 48 hours after marination (Aktas et al., 2003, Serdaroglu et al., 2006). Wenham and Locker (1976) took data at 8 hours and again at 2 days. Similarly, Berge et al. (2001) injected lactic acid 1 hour postmortem (pre-rigor), and again 24 hours postmortem (post rigor) and allowed the meat to marinate for 2 and 14 days. There is a lack of published information on the effects of acid marination on meat tenderness, color and other attributes over time. This information would be valuable for transportation and product development.

The majority of the research in acid marination involves low concentrations of acids ranging from 0 to 0.5 M and/or 0 to 1.5% (Wenham and Locker, 1976, Berge et al. 2001, Aktas and Kaya, 2001, Burke et al., 2002 and Ke et al., 2008). Higher concentrations of acid for marination have seldom been studied.

Collagen

There are eleven types of collagen found in the body. Type I is found in skin, tendons, vascular system, organs and bones. Types II, IX, X, and XI collagen are mainly found in cartilage. Type III and VII collagen is found in skin, vascular system and intestine. Type IV collagens are usually found in basement membranes. Type VIII is found in the Aortic endothelium (Pearson et al., 1987).

Marsh (1977) stated that "The connective tissue serves many vital purposes in life: it supports the soft muscle substance within its boundaries, provides a bed for blood vessels and nerves, and protects the contractile structure from damage by over-extension." Although connective tissue is important in live animal tissue, it also plays a large role in muscle tenderness.

Epimysial cores were taken from the *Longissium*, *Triceps Brachii* and *Biceps Femoris* muscles of 20 mo old bulls and found to have significantly higher Warner-Bratzler shear force value than younger steers (Field et al., 1969). This finding is consistent with the well known fact that older animals have a greater abundance of cross-linked collagen. A compilation of studies and reviews from Bailey and co-workers Pearson et al. (1987) showed that collagen contains four types of cross-links. Two of those cross-links of interest are: dilute acid- and heat-liable aldimines and dilute acid- and heat-stable oxo-imines (Pearson et al., 1987, Shimokomaki et al., 1972). Hill (1966) showed that collagen solubility in muscle decreases with physiological age, which in turn increases the amount of insoluble cross-liked collagen.

Effects of Acid on Collagen

A weakening of the perimysial connective tissue was noted when marinated in an acidic solution, but the mechanism by which the weakening had occurred is unclear (Lewis et al., 1991). Some have suggested that a weakening of connective tissue and collagen by acid marination will cause a reduction in thermal stability by shifting the denaturation temperature downward by 5-10°C (Lewis et al., 1991, Miles et al., 1995., and Berge et al., 2001). Lewis et al. (1991) observed that the effect of acid marination on connective tissue is much less than the effect on myofibrillar proteins. The denaturation

temperature is significantly less if marinated in acid as compared to being marinated in water or salt (Miles et al., 1995, Aktas and Kaya, 2001). Another study revealed that beef cores marinated in acetic acid (0.7 M at 2.5 pH for 72 hours) had an eighty percent reduction in collagen, while marinated beef cores marinated in phosphoric acid (0.4 M at 1.5 pH for 72 hours) reduced in collagen content by forty percent through acid hydrolysis as compared to the control (Oreskovich et al., 1993).

When bovine collagen is exposed to pH either above or below 5.3, will swell and become translucent. At postmortem pH the collagen will stay opaque (white in color) and does not swell (Pearson et al., 1985). Swelling of collagen was also noted in unrestrained tendon that was exposed to 0.05 M acetic acid (Miles et al., 1995).

The true mechanism by which collagen becomes soluble is unclear. Offer and Knight (1988) suggest that collagen may be soluble through the breakdown of cross-links. Some of these bonds are Schiff base aldimine bonds being ruptured easily by pH, heat changes, and denaturing agents. Burke (2003) acknowledged some other theories that are not widely accepted: (1) peptide bond hydrolysis and (2) slow breakage of covalent cross-links.

Enzyme Activity

The three proteolytic systems are the calpains which are calcium activated at neutral pH, the lysosomal enzymes which function in acidic conditions (Koohmaraie, 1992), and the multicatalytic proteinase complex at optimium pH 7.5 to 8.5 in temperatures of 50 to 60°C (Arbona and Koohmaraie, 1993). Certain myofibrillar structures such as tropmyosin, troponin T, troponin I and α-actinin, and the Z-line have

been shown to be susceptible to cathepsin activity (Penny and Ferguson-Price, 1979, Hutton et al., 1981).

Hutton (1981) revealed that lysosomal enzyme activity decreases when heated with either a microwave or conventional heat from 40 to 70°C. In agreement, Moeller (1977) found that cathepsin C activity is greater in the soluble and insoluble fraction at 2°C as compared to 37°C. Contradicting results from Lutalo-Bosa and Mackrae (1969) concluded that cathepsin enzymes exhibited maximal activity around 40°C and then decreased with higher temperatures. It is clear that temperature of the meat plays an important role on enzyme activity.

Acid injection pre-rigor could have beneficial effects on meat tenderness by releasing lysosomal enzymes earlier than seen in non-injected meat (Ertbjerg et al., 1999, Berge et al., 2001). Lysosomal enzyme increased in activity within the soluble fraction over storage time and concentration. This shows a release of enzymes from the insoluble fraction (membrane and myofibrillar) into the soluble fraction, increasing lysosomal activity while increasing concentration of the acid (Ertbjerg et al., 1999). Saunders (1993) claimed the degradation of isolated myofibrils were a result of cathepsin activity, not acidic conditions directly. Calpains are not considered a contributor to meat tenderness in acid marination because of their low tolerance to acidic conditions (Geesink and Koohmaraie, 1999).

Color

The color of meat is primarily developed from two pigments hemoglobin and myoglobin. Color can change when the chemical state of the myoglobin changes within the metmyglobin reducing activity cycle. The pigment can either be oxidized to the

metmyoglobin state or the meat could have enough reducing equivalents to allow it to be reduced to one of the two reduced states, myoglobin or oxymyoglobin.

The ferrous ($Fe2^{+}$) state of myoglobin can bind oxygen to the heme group, changing the chemical state to oxymyoglobin. Oxymyoglobin can often be characterized by the distinctive bright cherry red color after the meat has been given a chance to bloom. Metmyoglobin also has a distinctive brown color. When oxidized, myoglobin will revert back to the ferric ($Fe3^{+}$) state, metmyoglobin.

Myoglobin, a common sarcoplasmic protein within muscle cells has eight α-helical segments enclosing a heme-ring. Hemoglobin is a blood protein made up of 4 alternating subunits, 2-alpha and 2-beta, with each subunit enclosing a heme ring. The hemoglobin subunits are structurally similar to myoglobin. Myoglobin has a high affinity for binding oxygen (Lehninger, 2005).

Holomyoglobin is the native myoglobin with the heme group intact. If the heme group is removed, the myoglobin is referred to as apomyoglobin (N) or native apomyoglobin. The conformational state is similar to myoglobin except the heme group is removed. After the heme is removed, the conformational states change from N to I*complementary sub-domain to I (intermediate) and U is apomyoglobin completely unfolded. At physiological pH 7, apomyoglobin is in the N-state. As the pH drops the protein starts to unfold at pH 6 (N-I) and the conformation shifts from the I-state to the U-state around pH 3, giving a completely unfolded apomyoglobin. (Yang and Honig, 1994). Holomyoglobin, N, I*, I, and U are in order from folded to unfolded, respectively, as N, I*, and I are partially unfolded, while U is in a state of an unfolded random coil.

In general, as pH increases the negatively charged ions begin to increase in concentration and then cause repulsion of muscle proteins. As the repulsion occurs the proteins allow light to be absorbed giving the appearance of dark-colored meat. At the isoelectric point this is not the case, as the negatively and positively charged ions are at a state of equilibrium. The proteins are not being repulsed, thus, the light is reflected and the appearance of the meat is pale.

Knowing that pH is indicative of apomyoglobin's folding state we can infer what will happen to the color when an acid is applied to fresh meat. As the pH drops the myoglobin begins to unfold. Simultaneously, the myofibrillar proteins are accumulating like charges causing repulsion in the myofibrillar proteins. The build-up of positive charges causing repulsion in myofibrillar proteins coupled with the folding state of the myoglobin at low pH will allow light to be absorbed easily giving the appearance of dark color meat (Aktas et al., 2003, Serdaroglu et al., 2006, Saunders, 1993, Offer and Trinick, 1993, Hamm, 1960).

Negative color attributes have been observed using lactic acid in marinades, turning the meat a dark gray or gray-brown color (Burke, 2003; Sawyer, 2008, 2009). Burke et al. (2003) performed initial studies on beef, noting that swelling, darkening, and gelatinization would occur when using greater than 0.3 M organic acids (lactic, acetic and citric). Similarly, Sawyer (2008, 2009) reported that injecting 0.75 % to 2.00% lactic acid negatively impacted fresh beef color, which panelists described as shades of gray to black and suggested it was a well done cooked appearance.

Diffusion rates

Tumbling can positively impact attributes by tenderizing, solublizing proteins, and dispersing cure solutions (Krause et al., 1978a, 1978b, Lachowicz et al., 2003) Another positive attribute of tumbling is that cooking yields will increase by allowing added moisture to be drawn back into the meat (Lachowicz et al., 2003, Pietrasik, 2004, 2005). Krause et al. (1978a) demonstrated this by injecting cubed porcine muscle (15 cm long, 10 cm wide, and 7.5 cm deep) in the geometric center to 3% by weight. After tumbling, an increase in the migration of salt was seen. Also, the overall residual sodium nitrite levels remained higher in the tumbled samples than the samples that did not receive tumbling.

Graiver et al. (2009) modeled dispersion rates of sodium chloride, sodium nitrite, and potassium nitrate at 4°C. The model took into account the diffusion coefficients of the solutes as functions of sodium chloride concentration, convective contribution and mass transfer coefficient in the immersion brine. The validation experiment was conducted on pork *L. dorsi* muscle, free from visible fat, fabricated to the shape of a cylinder. To find the rate at which the salts would transfer, the Stokes-Einstein equation was used. The average velocity for the mass transfer coefficient (K_c) of sodium chloride, sodium nitrite, and potassium nitrate was found to be 1.3×10^{-7} m^2/s. To achieve a higher concentration of salt in the cylinder of pork a longer immersion time in the brine solution was required. The data presented by Vestergaard et al. (2005) are consistent with Graiver et al. (2009), except the experiment was conducted using dry salt instead of brine. The dry cured ham method required 6 days for a salt gradient to penetrate 2 cm into the ham.

The underlying conclusion from the two experiments shows that water and salt is required to act as a transport medium.

Offer and Trinick (1983) demonstrated that 1.0 M salt causes the myofibrils to swell. As a consequence, the swelling allows more water to enter into the muscle. Pinotti et al. (2000) conducted an experiment similar to that of Gravier et al. (2009) using a cylinder of pork *L. dorsi* muscle. Pinotti et al. (2000) concluded that both NaCl and temperature alter diffusion coefficients. As the temperature increases from $4^{o}C$ and $20^{o}C$, the sodium nitrite dispersion increased through the pork muscle from 3.8×10^{-10} m^2/s and 4.1×10^{-10} m^2/s, respectively. Pinotti et al. (2000) further reported that the diffusion rates of $NaNO_2$ increases with increasing amounts of NaCl.

Gerla and Rubiolo (2003) performed an experiment modeling diffusion coefficients in cheese. They found that the presence of NaCl increased the diffusion rate of lactic acid in cheese. Even though cheese protein (casein) is not a myofibrillar protein, the effect of salt followed a similar trend as shown in meat (Pinotti et al., 2000, Gravier et al., 2009). Further research needs to be conducted to discover diffusion rates using organic acids and NaCl.

The main factors that affect diffusion of solutes through muscle protein are time, temperature, species type and concentration gradients. Muscle fiber direction was once thought to have a significant impact on diffusion of the solutes, but Wood (1966) and Djelveh and Gros (1988) concluded there were no significant relationships between fiber direction and solute dispersion rates. It is commonly thought that the colder the curing solution during ham production the better it will disperse in the meat. This common perception is contrary to the results found by Djelveh and Gros (1988) and Pinotti et al.

(2000) who concluded that as the temperature increases the diffusion rate also increases. Another factor that influences the rate of diffusion is the species type. Djelveh and Gros (1988) showed that bovine muscle has a slower rate of diffusion than porcine muscle. Similarly, Wood (1966), Djelveh and Gros (1988), and Gravier et al. (2009) have all shown the diffusion rates of sodium chloride in porcine muscle to be around 1.3×10^{-7} m^2/s, whereas, Djelveh and Gros (1988) showed slower diffusion rates in beef ranging from 4.06 to 9.4×10^{-10} m^2/s. There is a lack of understanding as to why the rates between species are different.

Antimicrobial effects

Often the food industry uses organic acids to control microbial growth. Organic acids can be found in many foods, either from direct addition (acidulates) or naturally occurring from fermentation (lactic acid bacteria). Some common acids can be purchased in the form of either fresh citrus fruits or vinegar. Lactic acid cannot be purchased in pure form but can be found in yogurts and fermented meats. Lactic acid is produced as a by-product of fermentation. Allowing lactic acid bacteria to thrive in optimal conditions (moisture, nutrients and temperature) allows the fermentation process to begin. As the bacteria grow in population they produce lactic acid which lowers the pH and reduces the population of pathogenic microorganisms from contamination.

In the beef industry, packers use USDA approved acids such as lactic acid and chlorine rinses for E. coli O157:H7 and other pathogens. This procedure is effective if the antimicrobial solution reaches the appropriate pH. It has been shown that E. coli O157:H7 and L. monocytogenes can become acid resistant and develop acid tolerance. Acid adaptation of E. coli O157:H7 may occur after exposure to weak organic acids such

as benzoic or phenylacetic acid which is close to the conditions that are present in the rumen or in the feces (Leyer et al., 1995). Similarly, Cutter and Siragusa (1994) found when using 1, 3, 5% acetic, lactic or citric acids applied on the carcass at 24°C then incubated for 24 hrs at 4°C the organic acids reduced the bacterial load, but did not completely inactivate the organism.

Listeria monocytogenes can grow at refrigerator temperatures and is less responsive to lactic acid as an antimicrobial. Acetic acid is the most effective for controlling growth of Listeria by lowering the intracellular pH and inhibiting intracellular processes instead of degrading or solublizing the cell wall (Farber et al. 1989, Ita et al. 1990). Sorrells et al. (1989) suggest that bacterial inhibition should be studied by a variety of factors, not just one factor. The extent of bacterial count reduction was dependent on acid type, temperature, and time.

Summary: Effects of Acid on Muscle

The pH of fresh meat is normally around 5.3 to 5.8. Upon injection of organic acids the pH of the muscle will begin to drop. When the pH falls below 4.5 protein degradation starts to occur (Saunders, 1993). Simultaneously, collagen becomes translucent and swells as the pH drops (Miles et al., 1995). The Myofibrillar structures also begin to swell and take on water as the muscle deviates from the isoelectric point repelling the like positive charges. As the swelling occurs the muscle continues to degrade, optimally at pH 3.0 by cathepsin activity (Saunders, 1993). As concentration of the organic acids and time increase the membrane fraction of the muscle will begin to rupture, releasing lysosomal enzymes allowing them to degrade myofibrillar proteins. Collagen also begins to solublize and migrate out of the muscle slightly, leaving less

connective tissue (Oreskovich et al., 1993). A decrease in cooking loss occurred, when samples were injected with 1.0 M lactic acid versus 0.5 M lactic acid, implying that the acid-treated sample retained more liquid during cooking due to the swelling and retention of water (Gault 1985; Ertbjerb et al., 1999). A color change inherently occurs as the myoglobin proteins begin to unfold and the myofibrillar structure widens as the proteins repel from the like charges accumulating under acid conditions (Gault, 1985; Yang and Honig, 1994). The swelling of the meat will cause the light to be absorbed instead of reflected giving the appearance that the muscle is darker in color. If the meat is injected with acid pre-rigor the pH is lowered more quickly than normal and the lysosomal enzymes are activated earlier (Ertjberg et al., 1999). Acidic conditions tend to inactivate calpains and activated the lysosomal enzymes (Ertbjerg et al., 1999). Lysosomal enzymes will cause degradation of the myofibrillar proteins.

Summary

Meat tenderness is important for beef consumers. Acid marination can improve tenderness but is not widely used in industry. Organic acids such as vinegar (acetic acid) and fruit juices (citric acid) are readily available. Organic acids will not only degrade myofibrillar proteins but will soften stromal proteins as well. The benefits from acid marination are flavor and enhanced tenderness, although meat color may become compromised and is not desirable.

MATERIALS AND METHODS

Experiment 1

Experimental Design

Choice grade beef bottom round, *M. biceps femoris* (IMPS #171B; NAMP, 2007), were obtained from Greater Omaha Packing Company Inc. in Omaha, Nebraska, USA. The *M. biceps femoris* was chosen based on high connective tissue content (Von Seggern et al., 2005). Sodium citrate dihydrate (≥ 99% FG, food grade) and acetic acid (≥ 99.5% FG) were obtained from Sigma-Aldrich in St. Louis, Missouri, USA. Lactic acid (88% FG) was purchased from Birko Co. Henderson, Colorado, USA. Double distilled water was used in preparing all solutions. Marination times were allocated randomly to each muscle by steak position. See appendix 1 for steak positions and appendix 3 for process flow diagram. The data were analyzed as a split plot design where acid treatment was the whole plot, and day the split plot. Muscle *Biceps femoris* within acid treatment was considered the whole plot error and day by acid treatment the split plot error.

Acid solution preparation

Solutions were diluted to low (0.1 M) and high (0.5 M) concentrations for lactic acid, acetic acid and sodium citrate dihydrate, resulting in six treatments. All solutions were prepared with double distilled water. Samples of each solution were collected, and then titrated for accuracy using 3-4 drops of phenolphthalein as an indicator solution and a dilute sodium hydroxide. Sodium hydroxide was titrated in the solution until the color changed from clear to pink indicating the solution was titrated

Muscle preparation

The *M. biceps femoris* was trimmed of excess fat. The medial portion of the muscle contains an ischiatic head that was removed closely to the seam of connective tissue, leaving one continuous muscle from which to fabricate 2.54 cm thick steaks. Remaining trim was discarded. A control steak was removed from the fabricated muscle prior to injection. The locations of steaks, removed as a control, or after 1 and 8 hr, 1, 3, 7, 14, 21, 28 d, were randomized. No treatments were performed on the control steaks. Each muscle was injected with an acid solution to 107% of the fresh weight. Ten muscles were injected for each of the 6 treatments of either low or high concentration. The enhanced muscles were bagged and clipped individually, and then gently tumbled for 30 minutes, 18 revolutions/min with approximately 50 lbs per batch, with no vacuum using a Roschermatic Type TU-120 (Roscherwerke GMBH, Postiacn 3566 D-4500 Osnabruck/ W-Germany) to disperse the acid solution throughout the meat. A steak was cut 1 h post-injection, and then the remaining muscles were cut into steaks at 8 hours. All steaks were individually vacuum packaged and frozen at the times indicated.

Color

All color readings were taken using a Hunter Lab ® MiniScan XE Plus (Model 45/0-L, Hunter Laboratory Associates, Inc., Reston, VA) portable colorimeter equipped with a 2.54 cm orifice and using illuminant D65 at 10° standard observer to determine CIE (1976) L* (measure of darkness to lightness), a* (measure of redness), and b* (measure of yellowness) values. The colorimeter was standardized using a black tile and white tile (X=78.5, Y=83.2, and Z=88.7) prior to each session. All steaks were allowed

to bloom at least 1 hour before taking measurements. Color measurements were taken on the control, 1 and 8 hr post-injection steaks. The mean of three random readings from each steak were used for statistical analyses.

Cooking loss

Cooking loss was calculated by the equation "cook loss % = ((fresh weight-cooked weight)/fresh weight) x 100". All steaks were cooked to a minimum internal temperature of 71°C, using an Omega thermocouple set by Omega Engineering, Inc. Stanford, Connecticut, USA.

Tenderness evaluation

Steaks were grilled on a Hamilton Beach Indoor-Outdoor Grill (Model 31605A, Proctor-Silex Inc., Washington, NC) turned over once at 35°C, until they reached an internal temperature of 71°C. Grilled steaks were cooled at 4°C for 24 h. Cores were taken parallel with the muscle fiber orientation, using a 1.27 cm diameter coring bit. All the cores were sheared on an Instron Universal Testing Machine Model 55R1123 (Canton, MA) using a Warner-Bratzler shear force (WBSF) attachment, 500 kg load cell at 250 mm/min crosshead speed. A mean of 6 measurements per steak were taken for statistical analysis.

Solution and Muscle pH

The solution and meat pH was measured using an Orion 4 STAR pH ISE Bench-top meter (Thermo Electron Corporation, Waltham, MA). Five M. biceps femoris steaks were used in the pH analysis; but, within each steak the samples were divided into light and dark portions giving 10 samples to compare for statistical analysis. A delay of a year

occurred prior to the pH analysis of the muscles for experiment one and solutions for experiment two. No pH was measure on muscles from experiment two. The pH meter was calibrated using standard buffer solutions of pH 7.0 and 4.0 before each session. Injection solution pH was directly measured using the calibrated pH meter. If no discoloration was present in the steak, the steaks were diced into small pieces, and then re-frozen using liquid nitrogen (approximately -210 °C). Where discoloration was present in the steaks, the dark portions were separated from the light portions and then frozen. The pH analysis was performed on the segregated samples. A Waring blender model 51BL32 (Waring Commerical, Torrington, CT) was used to pulverize the meat cubes into pieces. Raw material was weighed out into beakers at 10 g of sample per beaker. Ninety mL of distilled, deionized water was added to each beaker and then homogenized for 30 seconds at 10, 800 rpm. A stir bar was placed in each beaker, stirring the homogenized solution while the pH was being measured. The electrode and beakers were cleaned between samples.

Statistical Analysis

Data of this experiment were analyzed as a split plot design where acid treatment was the whole plot, and day the split plot. Muscle *Biceps femoris* within acid treatment was considered the whole plot errors and day by acid treatment the split plot error. Data were analyzed using the GLIMMIX procedure of SAS (Version 9.1, Cary, N.C., 2002). When significance ($P \leq 0.05$) was indicated by ANOVA, mean separations were performed using the LSMEANS and DIFF functions of SAS.

Experiment 2

Experimental Design

Choice grade beef bottom round, *M. biceps femoris* (IMPS #171B; NAMP, 2007), were obtained from Greater Omaha Packing Company Inc. in Omaha, Nebraska, USA. The *M. biceps femoris* was selected for high connective tissue content (Von Seggern et al., 2005). Sodium citrate dihydrate (≥ 99% FG, food grade) and acetic acid (≥ 99.5% FG) were obtained from Sigma-Aldrich in St. Louis, Missouri, USA. Lactic acid (88% FG) was purchased from Birko Co. Henderson, Colorado, USA. Double distilled water was used in preparing all solutions. Marination times were allocated randomly to each muscle by steak position. See appendix 2 for steak positions and appendix 3 for the process flow diagram. The data were analyzed as a split plot design where acid treatment was the whole plot and day the split plot. Muscle *Biceps femoris* within acid treatment was considered the whole plot error and day by acid treatment the split plot error.

Acid solution preparation

Solutions were diluted to low (0.75 M) and high (1.5 M) concentrations for lactic, acetic acid and sodium citrate dihydrate, resulting in six treatments. Samples of each solution were collected prior to injection, and then titrated for accuracy using an indicator solution and sodium hydroxide as described above in experiment #1. All solutions were prepared with double distilled water.

Muscle preparation

Fabrication, tumbling and color measurements were performed as described in experiment 1. Each muscle was injected with an acid solution to 110% of the fresh

weight. A control steak was removed from the fabricated muscle prior to injection. The location of steaks, removed as a control, or after 1 and 8 hr, 1, 3, 7, 14 d were randomized. No treatments were performed on the control steaks. Three muscles were injected with each acid type of the low concentration and, 4 muscles were injected with each acid type of the high concentration. The enhanced muscles were bagged and clipped individually, and then gently tumbled for 30 minutes to disperse the acid solution throughout the meat. A steak was removed at 1 hr post-injection while all other steaks were cut at 8 hr post-injection. All steaks were individually vacuum packaged and frozen at the indicated times.

Statistical Analysis

Data of this experiment were analyzed as a split plot design where acid treatment was the whole plot, and day the split plot. Muscle *Biceps femoris* within acid treatment was considered the whole plot and day by acid treatment the split plot errors. Data were analyzed using the GLIMMIX procedure of SAS (Version 9.1, Cary, N.C., 2002). When significance ($P \leq 0.05$) was indicated by ANOVA, means separations were performed using the LSMEANS and DIFF functions of SAS.

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Acid Marination for Tenderness Enhancement of the Beef Bottom Round

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Acid Marination for Tenderness …

Sensory and Food Quality

ABSTRACT:

The objective of this study was to evaluate the tenderness and color effects of marinating M. *biceps femoris* (IMPS #171B; NAMP, 2007) (n = 72 bottom rounds) and determine optimal acid levels. During experiment one, solutions of lactic acid, acetic acid and sodium citrate dihydrate were prepared at 0.1 M and 0.5 M and samples injected to 7% pump. For the second experiment, solutions of lactic acid, acetic acid and sodium citrate dihydrate were prepared at 0.75 M and 1.5 M and pumped to 10% pump. Objective color measurements were taken on steaks at 0, 1, and 8 h after marination for experiments one and two. Color was measured after the meat was allowed to bloom at for 30 minutes at 0 h (no injection), and after injection and marination of 1 h and 8 h for both experiments. Cooking loss and tenderness were analyzed from steaks cut at 0, 1, and 8 h and at 1, 3, 5, 7, 14, 21 and 28 d after injection for experiment one whereas, experiment two stopped collecting samples on 14 d. No effects on tenderness (P = 0.11) were observed from experiment one. A significant (P = 0.02) increase in tenderness was observed from 1 to 14 d during experiment two. At the injection site, both acetic and lactic acids altered the color of meat from red to a dark gray. Results of this work demonstrated that acid marinating at low concentration has no effects on tenderness. Although, when concentrations of acid solutions are increased to 0.75 M and use a 10% pump tenderness (P = 0.02) can be significantly enhanced.

KEYWORDS: Beef, Acid Marination, Tenderness, Color.

INTRODUCTION

The primary attribute consumer's associate with the palatability of a good steak is tenderness. When asked what they disliked about beef products, U.S.D.A. Select grade steaks were identified as being tough (Savell et al., 1989). Miller et al. (2001) found 15-20% of the steaks sold to consumers were described as tough. Muscle tenderness was found to be variable in a carcass depending on its muscle function (Von Seggern, et al., 2005). Variability in muscle tenderness was also found within each muscle (Senaratne, et al., 2009). Since muscle tenderness is a major attribute to consumer acceptability of meat, and large variability in tenderness exists, industry should strive to minimize the large variability in tenderness. One possible method to enhance tenderness is acid marination.

Wenham and Locker (1976) evaluated 1.5% acetic acid. They found a 39% improvement in tenderness within the first 8 h and an additional 21% improvement 43 h post-marination. Ertbjerg et al. (1999) found a slight decrease in shear force over a 21 d period post-marination with lactic acid suggesting that there would be no concern for over-tenderization. Berge et al. (2001) found little difference in beef samples between 2 and 14 d post-marination with 0.5 M lactic acid. Thus, the benefits of acid marination occurred within the first two days in their study.

Acids have been shown to enhance tenderness, but little work has reported acid strength to optimize tenderness and the length of time which the muscle would stay acceptable to consumers. The objective of this study was to evaluate the effect of acid marinate on tenderness and color of M. *biceps femoris* (n = 72 bottom rounds) and to determine optimal acid levels.

MATERIALS AND METHODS

Experiment 1

Materials

Beef bottom round, *M. biceps femoris* (IMPS #170;NAMP, 2007), (n = 30) were obtained from Greater Omaha Packing Company Inc. in Omaha, Nebraska, USA. The *M. biceps femoris* was chosen based on high amount of connective tissue as compared to other muscles (Von Seggern et al., 2005). Stock solutions of sodium citrate dihydrate (≥ 99% FG, food grade) and acetic acid (≥ 99.5% FG) were obtained from Sigma-Aldrich in St. Louis, Missouri, USA. Lactic acid (88% FG) was purchased from Birko Co. Henderson, Colorado, USA. Double distilled water was used in preparing all solutions.

Design

Marination times were allocated randomly to each muscle by steak position. See appendix 1 for steak positions. The data were analyzed as a split plot design where acid treatment was the whole plot, and day the split plot. Muscle *biceps femoris* within acid treatment was considered the whole plot error and day by acid treatment the split plot error.

Acid solution preparation

Acid solutions were prepared to 0.1 M (low) and 0.5 M (high) concentrations for lactic acid, acetic acid and sodium citrate dihydrate, resulting in six treatments. All solutions were prepared with double distilled water. Samples of each solution were collected, and then titrated for accuracy of the injection solution using 3-4 drops of phenolphthalein as an indicator solution and a dilute sodium hydroxide. Sodium

hydroxide was titrated in the solution until the color changed from clear to pink indicating the solution was titrated.

Muscle preparation

The beef bottom round, *M. bicep femoris* (IMPS #170;NAMP, 2007), were trimmed of excess fat. The medial portion of the muscle contains an ischiatic head that was removed closely to the seam of connective tissue, leaving one continuous muscle from which to fabricate 2.54 cm thick steaks. Remaining trim was discarded. A control steak was removed from the fabricated muscle prior to injection. A steak was cut 1 hr after injection and tumbling then remaining muscles were cut into steaks at 8 h. All steaks were individually vacuum packaged and frozen at the times indicated. For complete detail of the times and treatments see appendix 1. The location of steaks, removed as a control, or after 1 and 8 h, 1, 3, 7, 14, 21, 28 d were randomized. No treatments were performed on the control steaks. Each muscle was injected with an acid solution to 107% of the fresh weight. Five muscles per treatment were injected for each of the 6 treatments of either low or high concentration. The enhanced muscles were bagged and clipped individually, and then gently tumbled for 30 minutes, 18 revolutions/min with approximately 50 lbs per batch , with no vacuum, using a Roschermatic Type TU-120 (Roscherwerke GMBH, Postiacn 3566 D-4500 Osnabruck/ W-Germany) to disperse the acid solution throughout the meat.

Color Measurement

All color readings were taken using Hunter Lab ® MiniScan XE Plus (Model 45/0-L, Hunter Laboratory Associates, Inc., Reston, VA) portable colorimeter equipped with a 2.54 cm orifice and using illuminant D65 at 10° standard observer to determine

CIE (1976) L* (measure of darkness to lightness), a* (measure of redness), and b* (measure of yellowness) values. The colorimeter was standardized using a black tile and white tile (X=78.5, Y=83.2, and Z=88.7) prior to each session. All steaks were allowed to bloom at least 1 h before taking measurements. Color measurements were taken on the control (no injection). Steaks were cut at 1 and 8 h after injection of the whole muscle, and then color measurements were taken after bloom. The mean of three random readings from each steak were used for statistical analyses.

Cooking and Cooking loss

Steaks were grilled on a Hamilton Beach Indoor-Outdoor Grill (Model 31605A, Proctor-Silex Inc., Washington, NC) turned over once at 35°C, until they reached an internal temperature of 71°C. All steaks were cooked to a minimum internal temperature of 71°C, using an Omega thermocouple set by Omega Engineering, Inc., Stamford, Connecticut, USA. Cooking loss was calculated by the equation "cook loss % = ((fresh weight- cooked weight)/fresh weight) x 100".

Tenderness evaluation

Grilled steaks were cooled at 4°C for 24 h. Cores were taken parallel with the muscle fiber orientation, using a 1.27 cm diameter coring bit. All the cores were sheared on an Instron Universal Testing Machine Model 55R1123 (Canton, MA) using a Warner-Bratzler shear force (WBSF) attachment, 500 kg load cell at 250 mm/min crosshead speed. A mean of 6 measurements per steak were taken for statistical analysis.

Solution and Muscle pH

The solution and meat pH was measured using an Orion 4 STAR pH, ISE Bench-top meter (Thermo Electron Corporation, Waltham, MA). Before each session the pH meter was calibrated using standard buffer solutions of pH 7.0 and 4.0. Injection solution pH was directly measured using the calibrated pH meter. If no discoloration was present in the steak, the steaks were diced into small pieces, and then re-frozen using liquid nitrogen (approximately -210 °C). Where discoloration was present in the steaks, the dark were separated from the light and then frozen and pH analysis was performed on the segregated samples. A Waring blender model 51BL32 (Waring Commerical, Torrington, CT) was used to pulverize the meat cubes into pieces. Raw material was weighed out into beakers at 10g of sample per beaker. Ninety mL of distilled, deionized water was added to each beaker and then homogenized for 30 seconds at 10, 800 rpm. A stir bar was placed in each beaker, stirring the homogenized solution while the pH was being measured. The electrode and beakers were cleaned between samples.

Statistical Analysis

Data of this experiment were analyzed as a split plot design where acid treatment was the whole plot and day the split plot. Muscle *Biceps femoris* within acid treatment was considered the whole plot error and day by acid treatment the split plot error. Data were analyzed using the GLIMMIX procedure of SAS (Version 9.1, Cary, N.C., 2002). When significance ($P \leq 0.05$) was indicated by ANOVA, means separations were performed using the LSMEANS and DIFF functions of SAS.

Experiment 2

Beef bottom round, *M. bicep femoris* (IMPS #170;NAMP, 2007), (n = 42) were obtained from Greater Omaha Packing Company Inc. in Omaha, Nebraska, USA. The *M. bicep femoris* was chosen based on high connective tissue content (Von Seggern et al., 2005). Sodium citrate dihydrate ($\geq$ 99% FG, food grade) and acetic acid ($\geq$ 99.5% FG) were obtained from Sigma-Aldrich in St. Louis, Missouri, USA. Lactic acid (88% FG) was purchased from Birko Co. Henderson, Colorado, USA. Double distilled water was used in preparing all solutions. Marination times were allocated randomly to each muscle by steak position. See appendix 2 for steak positions. The data were analyzed as a split plot design where acid treatment was the whole plot, and day the split plot. Muscle *Biceps femoris* within acid treatment was considered the whole plot error and day by acid treatment the split plot error.

Muscle preparation

Fabrication, tumbling and color measurements were performed as mentioned in experiment one. Each muscle was injected with an acid solution to 110% of the fresh weight. A control steak was removed from the fabricated muscle prior to injection. The location of steaks, removed as a control, or after 1 and 8 h, 1, 3, 7, 14 d were randomized. No treatments were performed on the control steaks. 3 muscles were injected with each acid type of the low concentration and, 4 muscles were injected with each acid type of the high concentration. The enhanced muscles were bagged and clipped individually, and then gently tumbled for 30 minutes to disperse the acid solution throughout the meat as described in experiment one. A steak was removed at 1 h post injection while all other

steaks were cut at 8 h post-injection. All steaks were individually vacuum packaged and frozen at the indicated times.

Acid solution preparation

Solutions were diluted to 0.75 M (low) and 1.5 M (high) concentrations for lactic, acetic acid and sodium citrate dihydrate, resulting in six treatments. Samples of each solution was collected prior to injection, and then measured for pH. All solutions were prepared with double distilled water.

Color Measurement

All color readings were taken using Hunter Lab ® MiniScan XE Plus (Model 45/0-L, Hunter Laboratory Associates, Inc., Reston, VA) portable colorimeter equipped with a 2.54 cm orifice and using illuminant D65 at 10° standard observer to determine CIE (1976) L* (measure of darkness to lightness), a* (measure of redness), and b* (measure of yellowness) values. The colorimeter was standardized using a black tile and white tile (X=78.5, Y=83.2, and Z=88.7) prior to each session. All steaks were allowed to bloom at least 1 h before taking measurements. Color measurements were taken on the control, 1 and 8 hr post injection steaks. The mean of three random readings from each steak was used for statistical analysis.

Cooking loss

Steaks were grilled on a Hamilton Beach Indoor-Outdoor Grill (Model 31605A, Proctor-Silex Inc., Washington, NC) turned over once at 35°C, until they reached an internal temperature of 71°C. All steaks were cooked to an internal temperature of 71°C, using an Omega thermocouple set by Omega Engineering, Inc. Stamford, Connecticut,

USA. Cooking loss was calculated by the equation "cook loss % = ((fresh weight- cooked weight)/fresh weight) x 100".

Tenderness evaluation

Grilled steaks were cooled at 4°C for 24 h. Cores were taken parallel with the muscle fiber orientation, using a 1.27 cm diameter coring bit. All the cores were sheared on an Instron Universal Testing Machine Model 55R1123 (Canton, MA) using a Warner-Bratzler shear force (WBSF) attachment, 500 kg load cell at 250 mm/min crosshead speed. A mean of 6 measurements per steak was taken for statistical analysis.

Statistical Analysis

Data of this experiment were analyzed as a split plot design where acid treatment was the whole plot and day the split plot. Muscle *Biceps femoris* within acid treatment was considered the whole plot error and time after injection by acid treatment the split plot error. Data were analyzed using the GLIMMIX procedure of SAS (Version 9.1, Cary, N.C., 2002). When significance ($P \leq 0.05$) was indicated by ANOVA, means separations were performed using the LSMEANS and DIFF functions of SAS.

RESULTS AND DISCUSSION

Experiment 1

Color Measurements

Treatments of acetic acid in high concentration (0.5 M) and lactic acid in low concentration (0.1 M) were significantly ($P < 0.05$) lighter than citric acid at low and high concentration at 1 h after injection (Table 1). There were no significant differences in lightness among any of the treatments after 8 h of marination. All treatments significantly

became lighter from 0 to 8 h. Muscles marinated with 0.5 M sodium citrate dihydrate decreased in lightness (L*) from 1 to 8 h. Whereas, other samples treated with 0.1 M lactic acid increased in L* at 1 h ($P \leq 0.05$). All treatments, from 1 to 8 h were significantly ($p < 0.0001$) less red (Table 2). Meat treated with acetic or lactic acid had very slight discoloration at the injection sites. Wenham et al. (1976) reported a similar finding of meat discoloration (dull gray color) when acid is applied. All discoloration remained unaltered during aging. Acetic acid with high concentration (0.05 M) had the greatest effect in decreasing redness over 8 hours. At 1 h after injection, there were no significant differences among the treatments in L*. Lactic acid with high concentration and acetic acid in high concentration (0.05 M) were significantly less red as compared to other treatments at 8 h. Both redness (a*) and yellowness (b*) decreased for all six treatment groups from 0 to 8 h ($P \leq 0.05$). Muscles marinated with 0.1 M lactic acid had the highest a* and b* values, whereas 0.5 M acetic acid had the lowest a* at 8 h. Both acetic and lactic acids had a significant time x treatment interaction from 0 to 8 h; by decreasing b* (Table 3).

Cooking loss

Lactic acid with high concentration had consistently lower cooking losses as compared to the other treatments over time, but there were no significant effects of treatment x time ($P = 0.17$). At 28 d, lactic acid with high concentration (0.05 M) had a cooking loss of 17.31 percent (Table 4). Citric acid with high concentration had the greatest cooking loss of 32.04 percent at 28 d. At 1 h acetic acid both low and high concentrations possessed the highest cooking loss of 35 percent among the treatments (Table 4). This cooking loss is probably due to a pH affect as the pH of the two solutions was close to pH 2.6.

Tenderness

No significant differences were found in tenderness values when comparing acid treatments (Figure 1). Apparently, the low concentrations of acids used for marinating were not sufficient to degrade the connective tissue.

Solution and Muscle pH

Solution pH was highly acidic at approximately pH 2.0 for both lactic and acetic acids. Sodium citrate dihydrate is considered a salt, so as expected the pH was basic, around 8.1 (Appendix 4). The discolorations within the meat brought concerns about dispersion (Appendix 5). That is, when the acid solutions were injected, the solutions were not able to disperse through the meat. After segregating the dark and light portions and performing a pH analysis, we found that there were no significant (P = 0.19) differences between the samples, indicating that the solution did eventually disperse within the meat.

Experiment 2

Color Measurement

Acetic acid with high concentration has a significant effect on lightness causing the meat to become darker from 0 to 1 h and then significantly darker from 1 to 8 h. Citric acid with high concentration and lactic acid with high concentration both became significantly darker from 0 to 1 h, but did not become darker from 1 to 8 h (Table 5). There were no significant differences in lightness from 0 to 8 h for acetic acid at low concentration, citric acid at low concentration or lactic acid at low concentration. There was a significant treatment x time effect (P = 0.04) on L* values. At the injection site, using either acetic or lactic acids the color of the meat was altered from red to a dark

gray. Sawyer et al. (2009) reported on the unusual color that dark cutting steaks acquired after treatment from 0.5 to 1.0% lactic acid solutions. Sawyer (2008) also reported on a dark cutting steak enhanced with 1.00% lactic acid solution were cooked to “medium rare”. A sensory panel results were indicative of gray-brown to brown scores, “well” to “very well done” internal color. All discolorations were unaltered during aging. The acetic acid with high concentration and acetic acid with low concentration both became significantly less red from 0 to 1 h and then again significantly less red from 1 to 8 h. Lactic acid with high concentration and lactic acid with low concentration both became significantly less red from 0 to 1 h, but there were no significant differences from 1 to 8 h (Table 6). There were no significant differences in redness from 0 to 8 h for treatments citric acid with high concentration and citric acid with low concentration. Acetic acid with high concentration, acetic acid with low concentration, citric acid with high concentration and lactic acid with low concentration had no significant differences in yellowness from 0 to 1 h, but had a significant difference from 1 to 8 h (Table 7). There was a significant effect on treatment x time (P = 0.003) on b* values.

Solution dispersion within the muscle would have been increased if NaCl were present. Offer and Trinick (1983) demonstrated that 1.0 M salt will cause the myofibrils to swell. Pinotti et al. (2000) conducted an experiment similar to that of Gravier et al. (2009) using a cylinder of pork *L. dorsi* muscle. Pinotti et al. (2000) concluded that both NaCl and temperature alter diffusion coefficients. As the temperature increases from 4°C and 20°C, the sodium nitrite dispersion increased through the pork muscle from 3.8 x 10^{-10} m^2/s and 4.1 x 10^{-10} m^2/s, respectively. Pinotti et al. (2000) further reported that the diffusion rates of $NaNO_2$ increases with increasing amounts of NaCl.

Cooking loss

There was a significant treatment x time effect ($P < 0.0001$) on cooking loss. Only acetic acid with low concentration and acetic acid with high concentration showed significant cooking losses from the control to 1 h. Acetic acid with low concentration and acetic acid with high concentration had shown an increase in cooking losses from control to 1 h. Lactic acid with high concentration and citric acid with low concentration had no significant differences in cooking loss from control to 14 d, but citric acid with high concentration had a decrease in cooking loss from control to 14 d (Table 8).

Tenderness

Lactic and acetic acids at low and high concentrations significantly increased tenderness; whereas, sodium citrate dihydrate affected tenderness slightly at a high concentration (Figure 2). Overall, all the treatments initially increased tenderness significantly from control to 1 h (Figure 3). A significant ($P = 0.04$) decrease in tenderness was observed from 1 h to 1 d and then a significant ($P = 0.02$) increase in tenderness was observed from 1 to 14 d. Berge (2001) showed similar results of a significant decrease in shear force values then little change in tenderness over time using lactic acid. This tenderness could be attributed to the results of Ertbjerg et al. (1999), who suggested the lysosomal enzymes increased in activity with storage time and acid concentration of the soluble fraction. When activity in the soluble fraction increases, it allows more degradation to take places in other regions of the muscle. Sodium citrate dihydrate has little to no effect on tenderness; although, acetic and lactic acids at 0.75 M to 1.5 M had a significant positive effect on tenderness.

CONCLUSIONS

Acid marination could be used to marinate meat to increase beef tenderness during distribution. Meat marinated in lactic or acetic acid at either 0.75 M or 1.5 M for 14 d was not over tenderized, indicating both acids and concentrations are good for marination. Tenderness enhancement can be achieved using either acetic or lactic acid at 0.75 M. The discolorations in the meat caused by the acid treatments would not be accepted by consumers. Further research need to be conducted to minimize the discolorations.

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Table 1. Experiment 1. Lightness values (L*) of flat round steaks (m. *Biceps femoris*) acid marinated with high and low concentrations of acetic, citric, or lactic acids. [Significant effect = trt*time (P = 0.04)]

Time	Treatments						Contrasts					
	AH	AL	CH	CL	LH	LL	AH vs AL	CH vs CL	LH vs LL	A vs C	A vs L	C vs L
0	40.20	38.17[B]	40.57[A]	36.54[B]	38.52	38.37[B]	0.30	0.04	0.94	0.64	0.59	0.94
1	41.33	38.99[B]	35.79[B]	37.53[B]	39.66	42.13[A]	0.23	0.37	0.21	0.02	0.59	0.005
8	40.41	42.20[A]	40.28[A]	42.18[A]	41.51	42.44[A]	0.18	0.16	0.49	0.94	0.47	0.43

[ABC] Means in the same column having different superscripts are significant.

AH = acetic acid high, AL = acetic acid low; CH = citric acid high, CL = citric acid low; LH = lactic acid high, and LL = lactic acid low.

A = acetic acid, C = citric acid, L = Lactic acid

Table 2. Experiment1. Redness values (a*) of flat round steaks (m. *Biceps femoris*) acid marinated with high and low concentrations of acetic, citric, or lactic acids. [Significant effects= trt (P= 0.0002) and time (P<0.0001)]

	Treatments							Contrasts					
Time	AH	AL	CH	CL	LH	LL	Means	AH vs AL	CH vs CL	LH vs LL	A vs C	A vs L	C vs L
0	24.47	24.03	24.13	25.09	25.30	26.47	24.92^{a}	0.72	0.43	0.34	0.67	0.06	0.14
1	23.31	23.01	23.97	25.56	23.98	26.21	24.34^{a}	0.83	0.28	0.13	0.13	0.07	0.75
8	16.36	19.68	19.76	20.35	17.20	22.08	19.24^{b}	0.02	0.66	0.0012	0.04	0.1	0.66
Means	21.38^{C}	22.24^{BC}	22.62^{BC}	23.67^{BA}	22.16^{BC}	24.92^{A}							

^{A,B,C} Means in the same row having different superscripts are significant.

^{a,b} Means in the same column having different superscripts are significant.

AH = acetic acid high, AL = acetic acid low; CH = citric acid high, CL = citric acid low; LH = lactic acid high, and LL = lactic acid low.

A = acetic acid, C = citric acid, L = Lactic acid

Table 3. Experiment 1. Yellowness values (b*) of flat round steaks (m. *Biceps femoris*) acid marinated with high and low concentrations of acetic, citric, or lactic acids. [Significant effect = trt*time (P = 0.003)]

Time	Treatments						Contrasts					
	AH	AL	CH	CL	LH	LL	AH vsAL	CH vs CL	LH vs LL	A vs C	A vs L	C vs L
0	23.16^{A}	23.45^{A}	20.89^{A}	25.15^{B}	25.94^{A}	26.53^{A}	0.83	0.004	0.66	0.76	0.004	0.002
1	24.40^{A}	21.30^{A}	22.04^{A}	27.42^{A}	22.46^{B}	27.63^{A}	0.02	0.0002	0.0002	0.04	0.02	0.71
8	13.83^{B}	15.09^{B}	13.67^{B}	15.39^{C}	13.63^{C}	18.12^{B}	0.18	0.07	<0.0001	0.92	0.04	0.05

^{ABC} Means in the same column having different superscripts are significant.

AH = acetic acid high, AL = acetic acid low; CH = citric acid high, CL = citric acid low; LH = lactic acid high, and LL = lactic acid low.

A = acetic acid, C = citric acid, L = Lactic acid

Table 4. Experiment 1.Treatment & Time effects on cooking loss percentage of flat round steaks (m. *Biceps femoris*) acid marinated with high and low concentrations of acetic, citric, or lactic acids. (*P* values = trt < 0.0001, time = 0.0073, trt*time = 0.17)

Treatment	Time								
	Control	1h	8h	1d	3d	7d	14d	21d	28d
LL	20.23^{b}	26.32^{b}	24.43^{b}	25.85^{ab}	21.73^{b}	19.76^{b}	24.63^{bc}	21.37^{bc}	24.42^{b}
LH	23.2^{ab}	24.88^{b}	21.75^{b}	21.11^{b}	23.59^{ab}	19.73^{b}	19.12^{c}	18.3^{c}	17.31^{c}
AL	24.58^{ab}	35.49^{a}	29.08^{ab}	31.34^{a}	35.27^{a}	29.82^{a}	25.61^{bc}	26.25^{ba}	32.22^{a}
AH	26.19^{aB}	34.47^{aA}	33.46^{aA}	32.49^{aA}	34.98^{aA}	31.91^{aA}	33.77^{aA}	29.89^{aAB}	30.21^{abAB}
CL	24.58^{aB}	29.82^{abAB}	33.20^{aA}	30.70^{aAB}	27.29^{abB}	30.64^{aAB}	28.05^{abAB}	27.67^{abAB}	27.75^{abAB}
CH	26.3^{abABCD}	26.95^{bABC}	23.82^{bBCD}	21.07^{bD}	23.10^{abBCD}	21.19^{bCD}	$26.11^{abcABCD}$	27.52^{baAB}	32.04^{aA}

[A,B,C]Means in the same row having different superscripts are significant.

[a,b] Means in the same column having different superscripts are significant.

AH = acetic acid high, AL = acetic acid low; CH = citric acid high, CL = citric acid low; LH = lactic acid high, and LL = lactic acid low.

Table 5. Experiment 2. Lightness values (L*) of flat round steaks (m. *Biceps femoris*) acid marinated with high and low concentrations of acetic, citric, or lactic acids . No significant trt *time effect (p = 0.62).

Time	Treatments							Contrasts					
	AH	AL	CH	CL	LH	LL	Means	AH vs AL	CH vs CL	LH vs LL	A vs C	A vs L	C vs L
0	43.88	43.21	45.46	41.11	42.53	40.39	36.65[a]	0.76	0.06	0.34	0.87	0.19	0.25
1	39.20	40.78	40.39	37.95	38.99	39.80	34.02[b]	0.47	0.17	0.71	0.72	0.70	0.98
8	35.11	39.62	39.86	38.58	36.20	38.33	33.67[b]	0.05	0.57	0.34	0.24	0.95	0.22
Means	39.40[B]	41.20[AB]	42.09[A]	39.22[B]	39.24[B]	39.51[B]							

[A,B,C] Means in the same row having different superscripts are significant.

[a,b] Means in the same column having different superscripts are significant.

AH = acetic acid high, AL = acetic acid low; CH = citric acid high, CL = citric acid low; LH = lactic acid high, and LL = lactic acid low.

A = acetic acid, C = citric acid, L = Lactic acid

Table 6. Experiment 2. Redness values (a*) of flat round steaks (m. *Biceps femoris*) acid marinated with high and low concentrations of acetic, citric, or lactic acids. Significant trt *time interaction ($P < 0.0001$).

Time	Treatments						Contrasts					
	AH	AL	CH	CL	LH	LL	AH vs AL	CH vs CL	LH vs LL	A vs C	A vs L	C vs L
0	31.84^{A}	32.48^{A}	33.12	34.02	32.48^{A}	32.59^{A}	.51	.35	.98	.04	.52	.16
1	25.17^{B}	27.87^{B}	32.23	33.86	20.27^{B}	25.58^{B}	.20	.44	.01	<0.0001	.02	<0.0001
8	17.12^{C}	21.32^{C}	31.64	32.32	20.03^{B}	23.38^{B}	.10	.79	.19	<0.0001	.17	<0.0001

^{ABC} Means in the same column having different superscripts are significant.

AH = acetic acid high, AL = acetic acid low; CH = citric acid high, CL = citric acid low; LH = lactic acid high, and LL = lactic acid low.

A = acetic acid, C = citric acid, L = Lactic acid

Table 7. Experiment 2. Yellowness values (b*) of flat round steaks (m. *Biceps femoris*) acid marinated with high and low concentrations of acetic, citric, or lactic acids. No significant trt *time effect (P = 0.62).

Time	Treatments						Contrasts					
	AH	AL	CH	CL	LH	LL	AH vs AL	CH vs CL	LH vs LL	A vs C	A vs L	C vs L
0	24.56[A]	26.77[A]	26.44	29.29[A]	24.74[A]	26.61[A]	.28	.17	.36	.13	.99	.13
1	20.34[B]	24.98[A]	26.74	28.49[A]	15.20[B]	22.55[B]	.007	.29	<0.0001	<0.0001	.0024	<0.0001
8	14.98[C]	16.58[B]	24.61	23.75[B]	18.04[B]	21.89[B]	.49	.71	.11	<0.0001	.01	.01

[ABC] Means in the same column having different superscripts are significant.

AH = acetic acid high, AL = acetic acid low; CH = citric acid high, CL = citric acid low; LH = lactic acid high, and LL = lactic acid low.

A = acetic acid, C = citric acid, L = Lactic acid

Table 8. Experiment 2.Treatment x Time effects on cooking loss percentage of flat round steaks (m. *Biceps femoris*) acid marinated with high and low concentrations of acetic, citric, or lactic acids. [Significant effect = treatment*time ($P < 0.0001$)]

Treatment	Time						
	Control	1h	8h	1d	3d	7d	14d
LL	28.41^{abB}	29.59^{bAB}	31.47^{abAB}	32.72^{aAB}	32.86^{aAB}	32.03^{aAB}	33.94^{aA}
LH	30.71^{a}	30.2^{b}	32.24^{ab}	32.69^{a}	31.08^{a}	30.53^{ab}	32.64^{a}
AL	25.81^{bB}	34.57^{aA}	35.18^{aA}	33.17^{aA}	33.56^{aA}	35.1^{aA}	35.57^{aA}
AH	28.11^{abB}	33.69^{aA}	31.18^{abAB}	33.93^{aA}	33.33^{aA}	33.73^{aA}	32.37^{aA}
CL	26.78^{abAB}	29.43^{bA}	28.12^{bcAB}	28.3^{bA}	23.72^{bB}	26.65^{bAB}	25.69^{bAB}
CH	27.99^{abA}	25.78^{cAB}	25.5^{cAB}	22.1^{cBC}	22.3^{bBC}	20.78^{cAB}	22.77^{bBC}

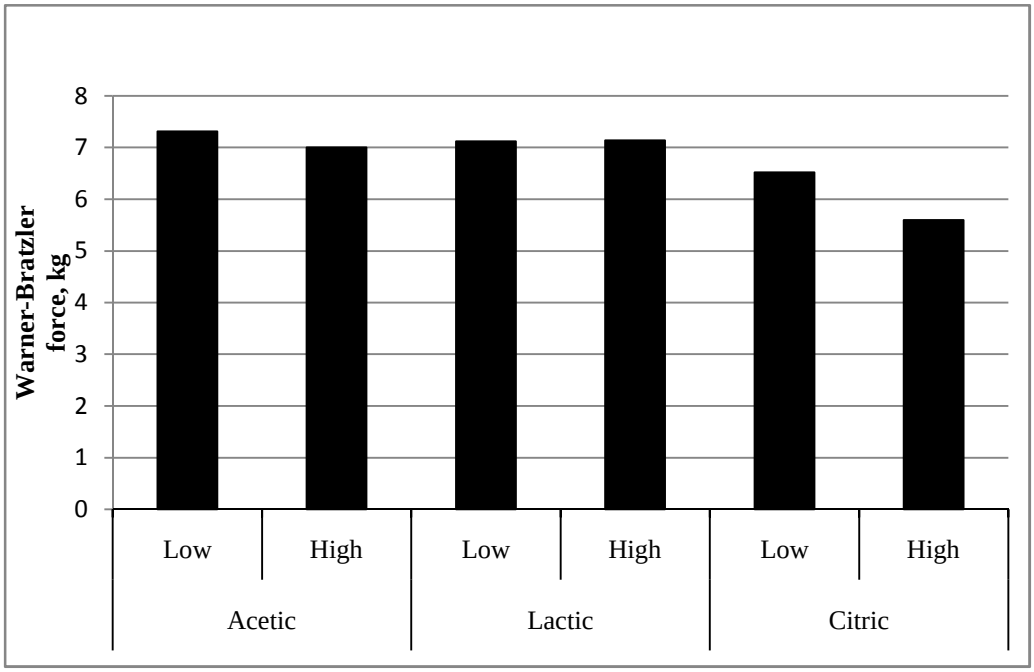
[A,B,C]Means in the same row having different superscripts are significant.

[a,b] Means in the same column having different superscripts are significant.

AH = acetic acid high, AL = acetic acid low; CH = citric acid high, CL = citric acid low; LH = lactic acid high, and LL = lactic acid low.

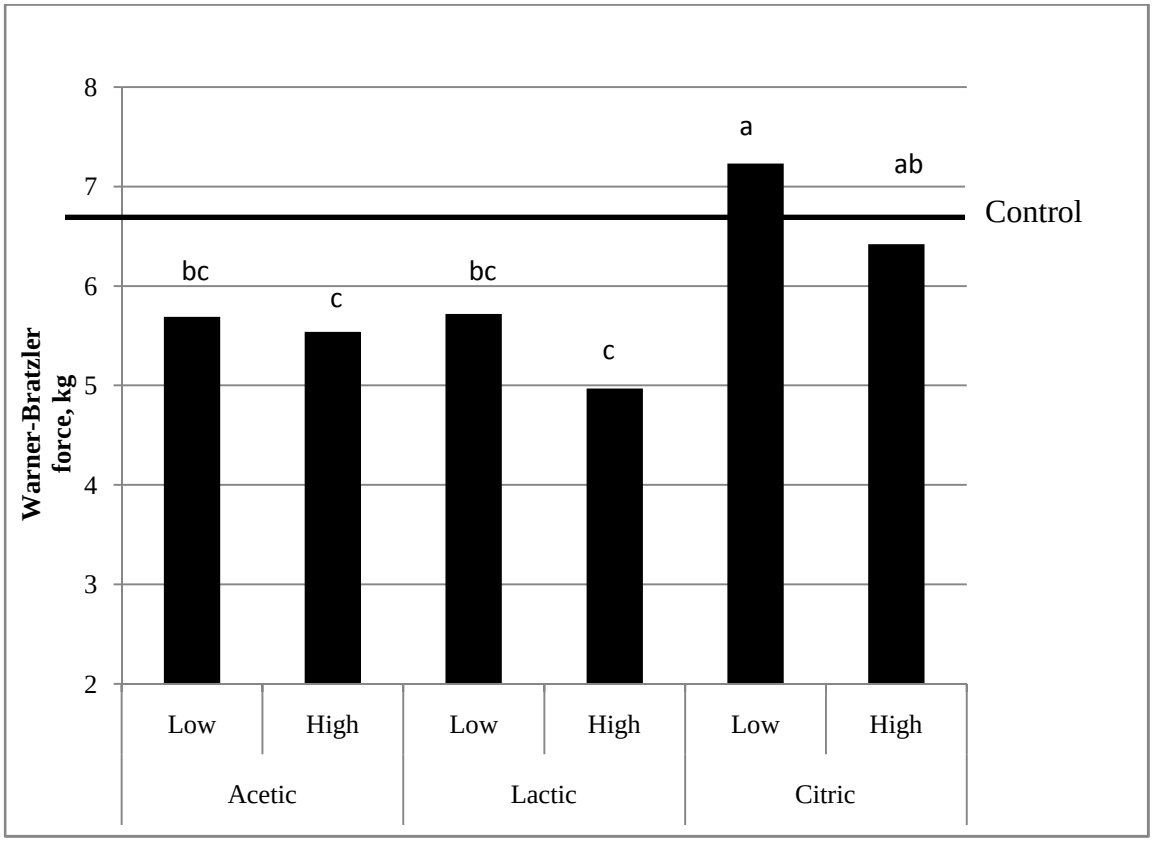
FIGURES

Figure 1. Experiment 1. Acid treatment vs. Warner-Bratzler shear force



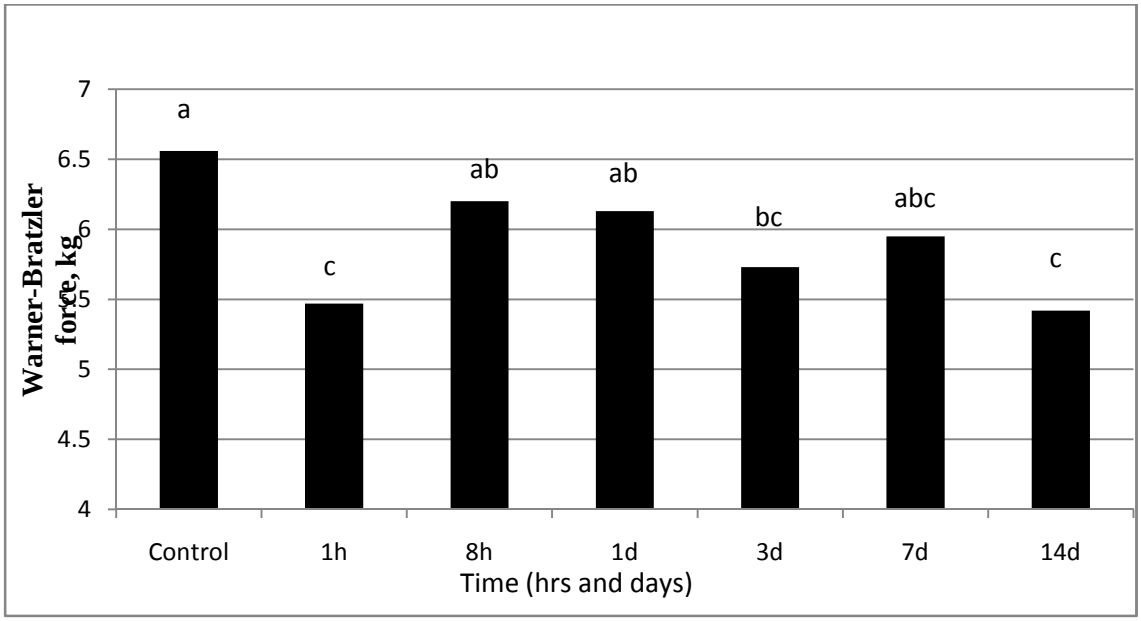
[a,b,c] Means having different superscripts are significant ($P < 0.05$). Low = 0.1 M, High = 0.5 M.

Figure 2. Experiment 2. Acid treatments vs Warner-Bratzler shear force



[a,b,c] Means in the columns having different superscripts are significant ($P < 0.05$).
Low = 0.1 M, High = 0.5 M

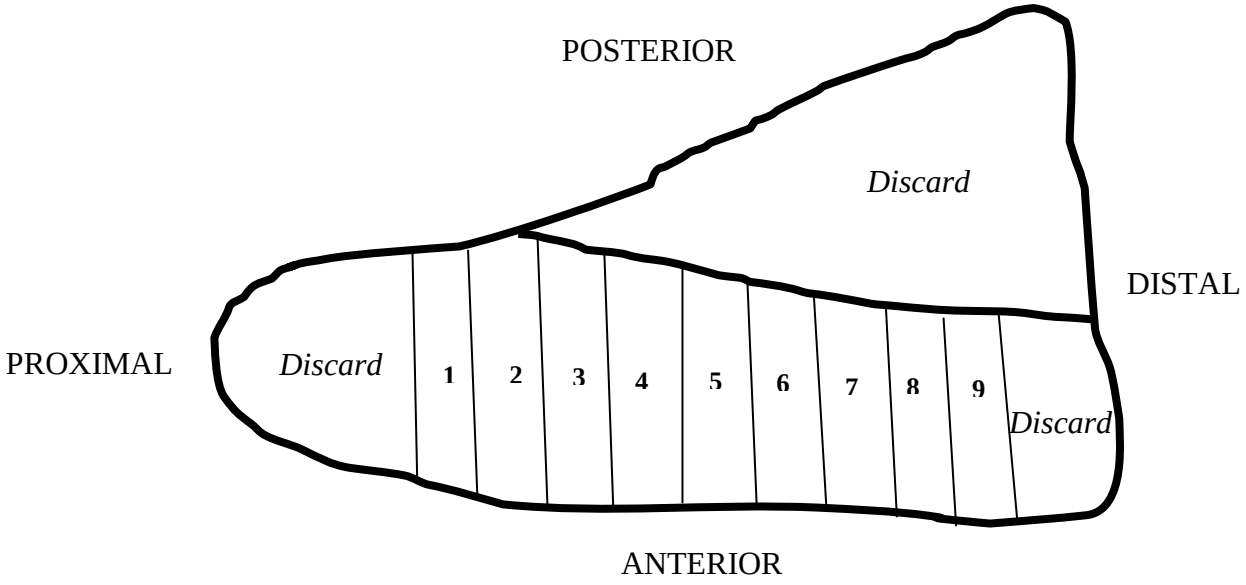
Figure 3. Experiment 2. Marination time vs. Warner-Bratzler shear force



[a,b,c] Means in the columns having different superscripts are significant ($P < 0.05$). Control (0 hr), 1 h, 8 h, 1 d, 3 d, 7 d and 14 d = marination time after injection.

APPENDICES

Appendix 1. Randomized treatment allocation by location, marination time and concentration of acids-Experiment 1.



		Lactic Acid – Repetition 1								
	Muscle	Steak Position								
Conc.	#	1	2	3	4	5	6	7	8	9
0.1 M	1	28d	1d	c	3d	7d	8h	14d	21d	1h
	2	1d	28d	21d	14d	3d	7d	8h	1h	c
	3	7d	8h	1d	14d	c	28d	3d	1h	21d
	4	8h	28d	1d	21d	14d	c	3d	1h	7d
	5	c	1h	8h	1d	28d	21d	14d	7d	3d
0.5 M	6	c	21d	7d	1d	1h	8h	3d	14d	28d
	7	28d	c	14d	8h	7d	1d	1h	21d	3d
	8	8h	14d	21d	1h	1d	28d	3d	7d	c
	9	3d	1h	1d	c	8h	14d	21d	7d	28d
	10	7d	c	14d	1d	28d	1h	8h	3d	21d
	Proximal		→							Distal

C = 0 hr (control), h = hr, d = day

		Aectic Acid – Repetition 1								
	Muscle	Steak Position								
Conc.	#	1	2	3	4	5	6	7	8	9
0.1 M	11	14d	7d	28d	21d	1h	1d	8h	c	3d
	12	28d	1d	c	8h	21d	7d	1h	14d	3d
	13	1d	c	3d	1h	21d	8h	7d	28d	14d
	14	1h	c	1d	8h	14d	28d	21d	3d	7d
	15	21d	14d	1h	3d	7d	28d	c	8h	1d
0.5 M	16	1h	14d	7d	1d	28d	3d	c	21d	8h
	17	21d	1h	28d	1d	14d	3d	8h	c	7d
	18	14d	1d	c	1h	21d	3d	28d	8h	7d
	19	c	14d	8h	21d	1h	1d	28d	3d	7d
	20	28d	1h	14d	8h	c	1d	7d	3d	21d
	Proximal	→								Distal

C = 0 hr (control), h = hr, d = day

		Citric Acid – Repetition 1								
	Muscle	Steak Position								
Conc.	#	1	2	3	4	5	6	7	8	9
0.1 M	21	8h	14d	c	28d	7d	21d	3d	1d	1h
	22	21d	8h	c	28d	7d	1d	1h	14d	3d
	23	28d	3d	21d	1h	14d	7d	8h	1d	c
	24	c	1d	21d	14d	8h	7d	3d	1h	28d
	25	1d	14d	7d	1h	c	8h	3d	28d	21d
0.5 M	26	8h	1d	14d	1h	21d	c	28d	3d	7d
	27	28d	1h	c	1d	14d	7d	8h	3d	21d
	28	28d	1d	14d	c	21d	1h	7d	3d	8h
	29	21d	3d	1d	28d	14d	c	1h	7d	8h
	30	c	8h	1h	14d	28d	3d	1d	21d	7d
	Proximal	→								Distal

C = 0 hr (control), h = hr, d = day

		Lactic Acid – Repetition 2								
	Muscle #	Steak Position								
Conc.		1	2	3	4	5	6	7	8	9
0.1 M	31	28d	1d	c	3d	7d	8h	14d	21d	1h
	32	1d	28d	21d	14d	3d	7d	8h	1h	c
	33	7d	8h	1d	14d	c	28d	3d	1h	21d
	34	8h	28d	1d	21d	14d	c	3d	1h	7d
	35	c	1h	8h	1d	28d	21d	14d	7d	3d
0.5 M	36	c	21d	7d	1d	1h	8h	3d	14d	28d
	37	28d	c	14d	8h	7d	1d	1h	21d	3d
	38	8h	14d	21d	1h	1d	28d	3d	7d	c
	39	3d	1h	1d	c	8h	14d	21d	7d	28d
	40	7d	c	14d	1d	28d	1h	8h	3d	21d
	Proximal	→								Distal

C = 0 hr (control), h = hr, d = day

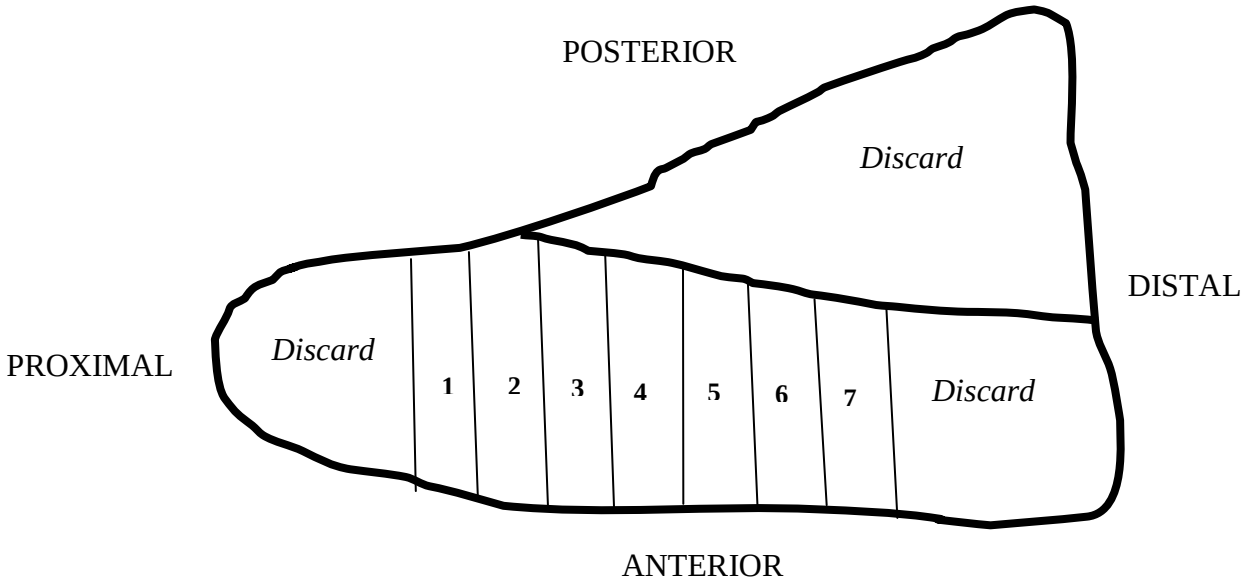
		Acetic Acid – Repetition 2								
	Muscle #	Steak #								
Conc.		1	2	3	4	5	6	7	8	9
0.1 M	41	1h	7d	14d	3d	1d	28d	c	21d	8h
	42	7d	1h	3d	21d	28d	8h	1d	14d	c
	43	1d	14d	7d	1h	8h	c	28d	3d	21d
	44	1d	7d	28d	21d	14d	c	8h	1h	3d
	45	c	21d	1h	3d	14d	8h	1d	7d	28d
0.5 M	46	21d	8h	7d	3d	c	1d	1h	14d	28d
	47	8h	1d	c	1h	28d	21d	7d	3d	14d
	48	1d	c	21d	28d	3d	1h	14d	8h	7d
	49	c	7d	28d	21d	8h	1h	1d	3d	14d
	50	3d	8h	21d	7d	1h	28d	c	14d	1d
	Proximal	→								Distal

C = 0 hr (control), h = hr, d = day

		Citric Acid – Repetition 2								
	Muscle #	Steak #								
Conc.		1	2	3	4	5	6	7	8	9
0.1 M	51	8h	7d	c	1h	14d	3d	1d	28d	21d
	52	28d	c	1d	3d	7d	14d	8h	1h	21d
	53	1d	c	8h	7d	14d	1h	28d	21d	3d
	54	7d	21d	c	8h	3d	1d	1h	14d	28d
	55	21d	8h	1h	1d	7d	c	3d	14d	28d
0.5 M	56	7d	c	8h	1h	21d	3d	14d	28d	1d
	57	1h	8h	3d	28d	14d	21d	1d	c	7d
	58	3d	21d	1d	1h	8h	14d	28d	c	7d
	59	7d	c	3d	8h	1h	14d	1d	28d	21d
	60	14d	28d	8h	1d	7d	1h	3d	21d	c
	Proximal		→							Distal

C = 0 hr (control), h = hr, d = day

Appendix 2. Randomized treatment allocation by location, marination time and concentration of acids-Experiment 2



		Lactic Acid - Repetition 1						
	Muscle	Steak Position						
Conc.	#	1	2	3	4	5	6	7
0.75 M	1	8h	7d	3d	14d	1h	c	1d
	2	14d	7d	3d	8h	1h	c	1d
	3	c	1h	14d	7d	3d	1d	8h
1.5 M	4	7d	1h	1d	c	8h	3d	14d
	5	14d	1h	8h	7d	1d	3d	c
	6	14d	c	7d	3d	1d	1h	8h
	7	1h	8h	c	14d	1d	3d	7d
	Proximal	→						Distal

C = 0 hr (control), h = hr, d = day

		Acetic Acid - Repetition 1						
	Muscle	Steak Position						
Conc.	#	1	2	3	4	5	6	7
0.75 M	8	1h	14d	3d	8h	7d	1d	c
	9	1d	14d	c	8h	7d	1h	3d
	10	3d	c	14d	7d	1h	1d	8h
1.5 M	11	c	3d	14d	1d	1h	7d	c
	12	7d	3d	c	1d	14d	1h	8h
	13	7d	3d	8h	1d	c	1h	14d
	14	c	1h	14d	7d	1d	8h	3d
	Proximal	→						Distal

C = 0 hr (control), h = hr, d = day

		Citric Acid - Repetition 1						
	Muscle	Steak Position						
Conc.	#	1	2	3	4	5	6	7
0.75 M	15	14d	7d	8h	1d	c	3d	1h
	16	7d	1h	1d	14d	c	8h	3d
	17	7d	8h	1d	3d	1h	14d	c
1.5 M	18	1h	c	1d	14d	3d	8h	7d
	19	c	8h	1h	3d	1d	14d	7d
	20	7d	1h	3d	1d	c	8h	14d
	21	8h	7d	3d	c	14d	1d	1h
	Proximal	→						Distal

C = 0 hr (control), h = hr, d = day

		Lactic Acid - Repetition 2						
	Muscle #	Steak Position						
Conc.		1	2	3	4	5	6	7
0.75 M	22	3d	1d	8h	14d	c	7d	1h
	23	c	1h	14d	7d	1d	8h	3d
	24	3d	1h	7d	c	14d	1d	8h
1.5 M	25	14d	8h	3d	1h	1d	7d	c
	26	8h	3d	1h	1d	c	14d	7d
	27	14d	c	3d	1h	8h	1d	7d
	28	14d	c	7d	1d	1h	8h	3d
	Proximal	→						Distal

C = 0 hr (control), h = hr, d = day

		Acetic Acid - Repetition 2						
	Muscle #	Steak Position						
Conc.		1	2	3	4	5	6	7
0.75 M	29	14d	3d	8h	1d	c	7d	1h
	30	3d	c	1d	1h	8h	7d	14d
	31	c	14d	1d	7d	3d	1h	8h
1.5 M	32	8h	c	3d	14d	1d	7d	1h
	33	1h	7d	14d	1d	c	8h	3d
	34	14d	3d	7d	c	1d	1h	8h
	35	1h	8h	7d	3d	c	1d	14d
	Proximal	→						Distal

C = 0 hr (control), h = hr, d = day

		Citric Acid - Repetition 2						
	Muscle #	Steak Position						
Conc.		1	2	3	4	5	6	7
0.75 M	36	1h	3d	7d	8h	c	1d	14d
	37	3d	c	7d	8h	1d	14d	1h
	38	1d	7d	3d	8h	1h	14d	c
1.5 M	39	1h	8h	7d	c	1d	3d	14d
	40	1d	1h	7d	14d	c	3d	8h
	41	c	3d	1h	7d	14d	1d	8h
	42	3d	1h	8h	c	14d	7d	1d
	Proximal	→						Distal

C = 0 hr (control), h = hr, d = day

Appendix 3. Process flow diagram for acid marination of the beef bottom round for experiments 1 and 2.

<u>Processing</u>

Trim excess fat and remove "wing" of *m. biceps femoris*

⇩

Locate and cut control steaks (See appendix 1 and 2 for treatment allocations).

⇩

Inject with multi-needle injector with acid solution to 7 or 10 percent pump

⇩

Tumble for 30 minutes for dispersion of acid solution

⇩

Cut steaks at 1 h for color measurements and shear force (See appendix 1 and 2 for treatment allocations)

⇩

Cut remaining steaks at 8 h for color measurements and shear force (See appendix 1 and 2 for treatment allocations)

⇩

Vacuum packaging

⇩

Place all steaks in the freezer according to the time allocated to each steak post-injection (See appendix 1 and 2 for treatment allocations)

Appendix 4. Solution and Muscle pH for acid marination of the beef bottom round for experiments 1 and 2.

Experiment 1	Rep 1	Rep 2
Solution pH	pH	pH
Acetic High (0.5 M)	2.22	2.57
Acetic Low (0.1 M)	2.92	2.97
Lactic High (0.5 M)	1.98	1.98
Lactic Low (0.1 M)	2.33	2.44
Citric High (0.5 M)	8.31	8.35
Citric Low (0.1 M)	8.35	8.29

Experiment 2	Rep 1	Rep 2
Solution pH	pH	pH
Acetic High (1.5 M)	2.3	2.25
Acetic Low (0.75 M)	2.58	2.54
Lactic High (1.5 M)	1.84	1.86
Lactic Low (0.75 M)	1.89	2.05
Citric High (1.5 M)	8.16	8.25
Citric Low (0.75 M)	8.58	8.3

Experiment 1. Muscle pH

	Sample #	Dark	Light
Lactic Acid 0.5 M	36	4.6	5.11
	38	4.65	5.11
	40	4.72	4.8
Acetic Acid 0.5 M			
	46	5.23	5.24
	50	4.96	4.9

Appendix 5. Pictures of beef bottom round when marinated with acetic and lactic acids at 0.5 M concentration.

FUTURE RESEARCH RECOMMENDATIONS

Acid Marination

Relatively little research has been done on acid marination for beef tenderness. Almost every country they has their own form of marination from fermentation (production of lactic acid) to direct acidification with acids from fruits or vinegar. This current research shows that acid marination does improve tenderness. Acid marination has some negative impacts on meat, such as color and flavor changes.

I propose that the color changes from red to dark gray be looked at more in-depth to understand the rapid color change. I recommend a study using differential scanning calorimetry to see if the acids are denaturing the proteins or degrading them. Differential scanning calorimetry provides a potential method for examining changes in connective tissues and myofibrillar proteins. Objectives of this study should identify the cause of the discoloration. A second objective should be to identify a method that would limit the discolorations.

Another future research project should be to see the extent in which organic acids will disperse through meat with NaCl, facilitated by tumbling, injection or immersion, or a combination of the methods. In this current research tumbling was applied, but the treatments left distinct lines of separation between the gray discoloration and the red pigments. Objectives of this study should be to determine if the distribution of the acid is being inhibited, and if so what is causing the inhibition. Secondly, the dispersion rates should be determined using the methods of Gravier et al. (2009).