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Electrostatic Atomization Of Antimicrobial Treatments On Ground Beef Processing, Instrumental Color, Sensory Color, Taste and Aroma Characteristics

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Electrostatic Atomization Of Antimicrobial Treatments On Ground Beef Processing,
Instrumental Color, Sensory Color, Taste and Aroma Characteristics

Electrostatic Atomization Of Antimicrobial Treatments On Ground Beef Processing,
Instrumental Color, Sensory Color, Taste and Aroma Characteristics

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Animal Science

by

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Instituto Tecnológico y de Estudios Superiores de Monterrey
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ABSTRACT

The general objectives of this research were to evaluate the interaction effects of sixteen different antimicrobial treatments throughout seven days of display on physical, chemical and sensory characteristics of ground beef patties, when compared to an un-treated control.

Antimicrobial treatments included: fumaric acid, malic acid, octanoic acid, decanoic acid, sodium propionate, propionic acid solution, potassium lactate/diacetate blend, sodium benzoate, hexanoic acid, pyruvic acid, levulinic acid, lactic acid/ citric acid blend, sodium diacetate, lemon juice and acetic acid. Prior to grinding, beef trimmings (80/20) were electrostatically sprayed with antimicrobial treatment solutions. Ground beef was processed into meat patties and sampled for 7 days. The packages were displayed under simulated retail conditions. Trained panelists evaluated palatability and meat sensory color, odor and processing abilities on days: 0, 1, 2, 3 and 7 of display. The use of the described antimicrobial agents maintained, improved or decreased the quality attributes of ground beef.

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Chapter I
Review Of Literature

A. Ground Beef Consumption

Ground beef is a staple commodity that is sold fresh for home consumption.

On average, 80 % of people in the United States consume ground beef at home every two weeks (Redson, 2010). Ground beef is eaten an average of 1.7 times per week by those reporting eating ground beef (Redson, 2010). A FoodNet Population Survey in 2006 showed that 80.1 % of the United States male population consumes ground beef. Of the 8,543 randomly selected respondents, 75.3 % reported consuming some type of ground beef in the previous 7 days (Taylor et al., 2011).

Hamburger patties and ground beef are similar products; burger patties can be defined as fresh or frozen chopped beef trimmings with less than 30% fat, excluding water and additives (Huffman et al., 1992). A high percentage of the American population consumes ground beef and the safety of this product is an important issue for beef producers and retailers involved in the process.

B. Ground Beef Safety

A past study by the Food Marketing Institute (FMI) reported that 69% of consumers considered product safety as a very important factor when it comes to food selection (FMI, 1994). Years later, another study by Yeung and Morris (2001) found that consumer's concern about food and public health has been increasing. It is clear that beef safety is becoming an issue of importance for costumers and can have an effect on future beef consumption levels. In 2010, a study by Radam et al. determined the perceptions and attitudes of Malay population towards safety of beef. The findings in this study indicate that most of the consumers may have misconceptions and negative attitudes towards beef.

Concerns about public health have increased among consumers but some still eat undercooked meat products, increasing the risk of foodborne illnesses.

From 1995 to 1996, 9% of Kansas's residents reported eating undercooked hamburger patties (Altekruse et al., 1999). Additionally, a FoodNet Population Survey in 2006 reported that 20 and 15.5% of the male and female population respectively, consumes pink ground beef patties at home. Even though ground beef consumption patterns can be affected by several factors such as gender, age, race and education, 18% of ground beef consumers in the United States frequently eat undercooked or pink burger patties (Taylor et al., 2012). Consumption of raw or undercooked bovine origin products is a risk factor for infection with *Escherichia coli* O157:H7, *Salmonella* and other foodborne pathogens (Hussein, 2007). Food is a perfect vehicle by which many pathogens can colonize. Even though food production techniques improve, food-borne pathogens such as *Salmonella* (sm) and *Escherichia Coli* (Ec) able to evolve. About 76 million cases of foodborne illnesses are estimated to occur annually in the United States; 325,000 result in hospitalization and 5,000 in deaths (Mead et al., 1999). There are over 200 known microbial, chemical or physical agents that can cause some kind of illness when ingested (Acheson, 1999).

According to a study by Scallan et al. (2011), 31 major pathogens in the United States annually cause 37.2 million episodes of foodborne illness, of which 36.4 million are home acquired and 11% of these diseases are caused by *Salmonella* spp. Similarly, these 31 pathogens cause 228,744 hospitalizations, of which 55,961 are related to foodborne illnesses with *Salmonella* spp. as the leading cause of the cases (35%). Of the 2,612 deaths caused annually by these pathogens, 1351 are caused by contaminated food eaten in the

United States. Simonsen et al. (2010), reported that *Salmonella* spp. and *Campylobacter* were also leading causes of foodborne illnesses in England, Wales and Australia.

Escherichia coli:

According to Banatvala et al (2001), *E. coli* O157:H7 infections can lead to hemolytic uremic syndrome (destruction of red blood cells), characterized by renal injury and hemolytic anemia. Rangel et al (2005) reported that *E. coli* O157:H7 causes 73,000 illnesses annually in the United States. This microorganism was first recognized as a pathogen in 1982 during an outbreak investigation of hemorrhagic colitis (Riley et al., 1983); the first *E. coli* O157:H7 ground beef outbreak was also reported in this year. It was not until 1993, after a large outbreak involving a well-known fast food restaurant chain and undercooked meat patties that *E. coli* O157:H7 was broadly recognized as an important threat in the United States (Bell et al, 1994). After this incident, the U.S. Food and Drug Administration (FDA) implemented new temperature guidelines for ground beef cookery in restaurants (FDA, 1993).

From 1982 to 2002, a total of 350 outbreaks in the United States were reported from 49 states and *E. Coli* O157:H7 infection was responsible for 8,598 cases (Rangel et al., 2005). Mead et al. (1999) reported *E. coli* was responsible for 73,000 illnesses and 250 annual deaths in the United States.

Salmonella:

Salmonella spp. colonise a wide range of hosts including poultry, cattle and pigs, producing contaminated meat products (Newell et al., 2010). According to Gantois et al (2008),

Salmonella has adapted to colonise the avian reproductive track and survive in hen's eggs. Some other studies had shown that *Salmonella* spp. have now evolved to colonize vegetables (Barak et al., 2005). It seems that *Salmonella* spp. are microorganisms able to evolve and adapt to environmental changes. Some stereotypes of *Salmonella* (Typhimurium, Virchow, Derby and Newport) have shown an antimicrobial resistance. (European Food Safety Authority, 2006). Many foodborne illnesses are related to *Salmonella* and there have been remarkable outbreaks of this pathogen throughout the years. In California, multidrug-resistant (MDR) *Salmonella* infections have predominantly occurred among the Hispanic population (Cody et al., 1999). Ground beef has been identified as the source for MDR *Salmonella*, Newport and *Salmonella* Typhimurium infections (Varma et al., 2006).

C. Quality Characteristics of Ground Beef

Although antimicrobials have been evaluated as intervention treatments to reduce pathogens and extend shelf life, researchers pay less attention to the effects of antimicrobials on meat color (Mancini & Hunt, 2005).

Color:

Consumers relate good quality beef with a bright red color, which is an indicator of freshness (Renner & Labadie, 1993). According to Smith et al. (2000), meat-purchasing decisions are influenced by color because consumers use discoloration as a freshness measurement. Nearly 15 % of retail beef is reduced in price due to discoloration, corresponding to annual losses of \$1 billion. Color is an important quality in purchasing fresh meat. Factors affecting meat color include: temperature, pH, humidity, lights, gaseous

atmosphere and microorganisms (Solberg, 1968). Kropf (1980), reported color as the greatest appearance factor when purchasing meat cuts. Also, muscle color is one of the main factors used to determine USDA quality grades for beef carcasses (USDA, 1997).

Color in meat is directly affected by myoglobin content and its chemical state among other different factors. Myoglobin is by nature a water-soluble protein containing 8 alpha helices linked by short non-helical sections. This protein contains a heme ring, which has a centrally located iron atom that can form at least six different bonds. Four major chemical forms of this protein are primarily responsible for meat color (deoxymyoglobin, carboxymyoglobin, oxymyoglobin and metmyoglobin). When myoglobin is exposed to oxygen (oxymyoglobin), a bright cherry-red color is developed in meat due to oxygenation. Depth of oxygen penetration depends on the meat's pH, temperature, oxygen partial pressure and competition for oxygen by other respiratory processes. Deoxymyoglobin occurs when no ligand is present at the 6th coordination site and the heme iron is ferrous (Fe 2+), resulting in a dark purplish-red or purplish-pink color. Carboxymyoglobin formation occurs when carbon monoxide attaches to the vacant 6th position of deoxymyoglobin, producing a stable bright-red color. Metmyoglobin is the oxidized brown colored form of myoglobin and it contains ferric iron (Fe 3+). It can be easily formed at low concentrations of oxygen (AMSA, 2012).

Discoloration is often referred as the amount of meat surface area covered by metmyoglobin (brown color). Discoloration in meat results from oxidation of both ferrous myoglobin derivatives to ferric iron (Livingston & Brown, 1982). The predominant myoglobin form in raw ground beef directly affects the color and appearance of cooked meat. Warren et al. (1996) reported that ground beef with high proportion of oxymyoglobin

or metmyoglobin appears well done (gray-brown) at low internal temperatures. When the previous condition occurs, meat can turn brown at lower than normal cooking temperatures, where food poisoning pathogens such as *Salmonella* spp. and *E. coli* O157:H7 can survive. Myoglobin is the principle protein responsible for meat color, but hemoglobin and cytochrome C may also play a role in beef, lamb, pork and poultry color (Mancini & Hunt, 2005).

Another factor that plays an important role in meat color is pH. Stivarius et al. (2002) reported that acetic acid tended to negatively influence beef color (decreased redness and oxymyoglobin content) due to its low pH.

One of the main challenges in the meat industry is to increase red color stability in meat. The use of modified atmospheres (MAP) has become a primary packaging option in the United States in case ready meats. High oxygen atmospheres (80% oxygen, 20% carbon dioxide) can maintain a red color in this type of product for 7 to 14 d. A study by John et al. (2004) concluded that high-oxygen packaging of ground beef maintained acceptable red color for at least 7 d, compared with 3 d of acceptable appearance for ground beef wrapped in polyvinyl-chloride film.

Instrumental color:

Many options are available for instrumental color analysis (colorimeters & spectrophotometers). Each instrument offers a variety of color systems, illuminants and observers to determine meat color. Current literature makes more use of color coordinates L^* (lightness), a^* (redness) and b^* (yellowness) when describing meat color. These coordinates provide a simple estimate of color and discoloration (Mancini & Hunt, 2005).

The spectral data (630/580nm reflectance) plays an important role when determining the oxymyoglobin fraction of the myoglobin pigment (Hunt et al., 1991). Townsend & Bratzel (1958) reported that light of 580 nm was absorbed by myoglobin, causing a photochemical oxidation of oxymyoglobin to metmyoglobin. Ratios of a^*/b^* , hue angle and saturation index are used to determine the discoloration of the meat.

Sensory color evaluation:

Sensory color is a standard for estimating consumer perception towards meat. Carpenter, Cornforth and Whittier (2001) reported a strong relationship between color preference and meat purchasing with consumers discriminating against beef that is not red. Panelist descriptions of color depend on individual cognition. For this reason, it is important to train sensory panelists (AMSA, 1995). Hunt et al. (1991) stated that marbling, shape, size, and colors of surrounding objects affect the perception of color when evaluating meat. For this reason, the use of scales in a trained panel is important in order to keep the collected data reliable.

Sensory Taste Evaluation:

Taste, as with color, is a physical measurement that relies on a sensory perception. Attributes such as taste, aroma and tenderness/texture are important when evaluating meat palatability. Tenderness is so important that costumers are willing to pay more for products (Miller et al., 1998). Flavor and juiciness also play an important role on ground beef eating quality and for this reason, taste panels need to be conducted appropriately and following special considerations in order to obtain reliable results.

According to the American Meat Science Association (AMSA, 1995), ground beef patties should be cut into 2.5 cm pieces and maintained at the same temperature when offered to the panelists. This is one of the main challenges for sensory panels due to the spacing in time between each sample presentation and the fact that panelists evaluate samples at their own pace. The American Meat Science Association recommends keeping samples in aluminum foil, placing them in a baking dish inside a food warmer or heated oven (54°C). In order to cleanse the palate between samples, panelists should be served water (room temperature) and unsalted crackers between the tasting of each sample (AMSA, 1995). Individual booths and proper lightning are necessary to avoid distracting factors that can affect meat perception.

D. Electrostatic Spray Technology

Electrostatic spray is a technology created in the 1930's to improve spray deposition. The basic principle of this design is the attraction of opposite charges and repulsion of similar charges. As the chemical mix in the sprayer leaves the nozzle, it is exposed to a negative charge that is attracted by the positive charges in the sprayed surface. This results in a better distribution of particle deposition. Bayat et al. (1994) found that electrostatic sprayers had a higher spray deposition on the undersides of leaves treated with pesticides, reducing pesticide losses and increasing biological efficiency against flies.

The electrostatic atomization of liquids is used in diverse fields such as crop spraying, paint spraying and the propulsion of space vehicles (Bailey, 1974). The use of electrostatic coating systems in food has become an emerging technology in the food industry. Electrostatic spraying has been used in the application of smoke flavors,

impregnation of bread with vegetable oil and for the application of liquid coating agents to confectionary and chocolate products (Anonymous, 1978). According to Abu-Ali & Barringer (2004) the field of electrostatics is under considerable study in the food industry due to its potential for producing even coatings. In electrostatic atomization, small droplets are produced and dispersed across the target. Smaller droplets produce more reproducible coating. As the charge to mass increases, the drop size produced by electrostatic spraying reduces, resulting in a better deposition (Wilkerson & Gaultney, 1989). Electrostatic coating systems can improve quality of operation and cost reduction. The application of water-soluble antimicrobials on beef trimmings by this technique should be effective.

E. Antimicrobials' Background and Profile

Organic acids have been used as food additives and preservatives for a long time, extending shelf-life and retarding food deterioration. They have been applied in both pre-harvest and post-harvest food production and processing (Ricke, 2003). When applying organic acids as meat decontaminants, several factors such as type of bacteria present and harvest and processing technology should be considered in order to achieve optimum activity. However, when organic acids are used in meat as preservatives, they are considered to be ingredients of the product since they will remain in the finished product at detectable levels (Theron & Lues, 2007).

- A. *Fumaric acid*: Fumaric acid is a white crystalline compound with molecular formula ($C_4H_4O_4$) that has a fruit-like taste. This organic acid is synthesized by the catalytic isomerization of malic acid at low pH aqueous solutions and can also be naturally found in some types of moss and mushrooms. Fumaric acid is use in many fields of the food

industry as an additive, especially in processed foods. Limited research in ground beef has been done with this short chain organic acid but its antimicrobial properties against *E. coli* have been demonstrated in several studies (Chikthimmah et al., 2003). Additionally, Lu et al. (2011) concluded that supplementing or replacing acetic acid with fumaric acid may accelerate the pathogenic killing process in acidified foods.

B. Malic acid: Malic acid is an organic compound with the molecular formula ($C_4H_6O_5$) found in metabolic cycles of all living organisms. It plays an important role in sourness of fruits; is the principal acid contained in apples and exists in two isomeric forms (L and D enantiomers). Malic acid is used as a food additive in many fields of the food industry, including confectioneries, beverages and baking. It has been shown in previous studies that this acid has antimicrobial properties as well. Mohan et al. (2012) reported a reduction of *E. coli* and *Salmonella* on inoculated ground beef with minimal impact on meat color when treated with 2% malic acid then displayed for 7 days. Another study conducted by Eswarnandam et al. (2004) concluded that 2.6% malic acid-incorporated soy protein films decreased log number CFU/mL of *L. monocytogenes*, *S. gaminara* and *E. coli* O157:H7 from 8.3, 9.0 and 8.9 log to 5.5, 3.0 and 6.8 log number CFU/mL, respectively. Additionally, high concentrations of malic acid (2.0 & 2.5%) were shown to be efficient when reducing and inactivating by more than 5-log cycles *E. Coli* O157:H7, *L. monocytogenes* and *S. Enteritidis* (Raynaudi-Massilia et al., 2009).

C. Octanoic acid (caprylic acid): Octanoic acid is an eight carbon saturated fatty acid that can be naturally found as a minor constituent of coconut and palm kernel oils. This organic acid

is known for having anti-viral, anti-bacteria and anti-fungal properties. In the food industry, caprylic acid is used as an equipment sanitizer, not as a food additive. Although little research has been done about this antimicrobial in ground beef, Mohan et al. (2012) reported a reduction of EC counts on beef trimmings before grinding by 1-log ($P < 0.05$) when treated with 0.04% octanoic acid on day 1 of display. Additionally, this treatment also reduced SM count by 1.1 log ($P < 0.05$) on the same day of display.

D. Decanoic acid (capric acid): Decanoic acid is a ten carbon saturated fatty acid naturally found in coconut oil and palm kernel oil. In its refined form, it can be found as white crystals or transparent, colorless liquid. The Environmental Protection Agency (EPA) classifies this organic acid as generally recognized as safe (GRAS). Toxicity profile indicates no significant risks for humans, even at high dosages. Limited research in beef has been done with this antimicrobial.

E. Sodium Propionate: Sodium propionate is a common manufactured food additive that can also occur in nature. Is the salt form of propionic acid, which is an organic acid, produced during the degradation of sugar. Although little research has been done in the use of sodium propionate on ground beef, this salt is approved for use in meat and poultry products by the Food and Safety Inspection Services (FSIS) of the US Department of Agriculture and food products by the FDA.

F. Propionic acid based solution: Propionic acid is a form of carboxylic acid that occurs naturally. This colorless liquid has an unpleasant odor. This acid has the natural ability to

prevent the growth of mold in foods and according to the (EPA) (1991), is a fungicide and bactericide, registered to control fungi and bacteria in stored grains, hay, poultry litter, and drinking water for livestock and poultry. For the study presented in this thesis, a propionic acid-based antimicrobial solution (BACTOCEASE®) (Kemin Food Technologies Inc., Des Moines, IL, USA) was evaluated as a treatment solution. Glass et al. (2013) reported a limit growth of *L. monocytogenes* on cured turkey to <1-log increase for all samples through 9 weeks of storage when treated with 0.3, 0.4 and 0.5% of a propionic acid-based solution. However, limited research has been done on the impact of this antimicrobial in ground beef color.

G. *Potassium lactate/potassium diacetate blend*: Potassium lactate is the potassium salt of lactic acid, obtained by neutralization of the acid of natural origin with a high purity potassium source or produced by bacteria in fermented food. It is used as a preservative in the food industry, mainly against yeast and fungi. It is used in meat and poultry products in order to extend shelf-life, inhibiting spoilage and pathogenic bacteria. Potassium diacetate is a compound of acetic acid and potassium acetate. This food additive acts as a preservative and acidity regulator. Fik and Leszczynska-Fik (2007) reported a significant inhibitory effect ($P < 0.05$) on bacterial growth in minced beef when treated with 0.65 and 1.3% potassium lactate-sodium diacetate blend. Additionally, Quilo et al. (2009) reported a redder color ($P < 0.05$), less discoloration ($P < 0.05$) and no difference in beef flavor ($P > 0.05$) for ground beef patties from a 3% potassium lactate treatment and compared to an untreated control on days 0 and 1 of retail display. Limited research has been done in the use of potassium diacetate as an antimicrobial on meat. For the study presented in this thesis, a 1:1 blend of potassium

lactate/ potassium diacetate (Jungbunzlauer Inc. Newton Centre, Massachusetts, USA) was used as a treatment solution at a concentration of 3%.

- H. Sodium Benzoate:* Sodium benzoate is the sodium salt of benzoic acid, which is found naturally in some fruits. In the food industry, is used as a preservative, mainly in acidic foods. Concentration as a preservative is limited by the FDA to 0.1% by weight.
- I. Hexanoic acid (caproic acid):* Hexanoic acid is a colorless, oily liquid derived from hexane. The primary use of this acid is in the manufacture of its esters for artificial flavors in the food industry. Very limited research has been done in the use of hexanoic acid on ground beef, but it has been used as a carcass decontaminant and has a GRAS status of not more than 3%.
- J. Pyruvic acid:* Pyruvic acid is a natural organic acid which supplies energy to living cells through the Krebs cycle in the presence of oxygen. Mohan et al. (2011) reported a significant ($P < 0.05$) reduction of coliforms on beef trimmings prior to grinding when treated with 3% pyruvic acid.
- K. Levulinic acid:* Levulinic acid can be produced by high temperature acid hydrolysis of carbohydrates such as glucose, sucrose, fructose and galactose. This organic acid can also be produced from biometric materials such as wood and starch. Levulinic acid can be isolated by partial neutralization filtration or by solvent extraction. This highly versatile chemical has been used as a resin, plasticizer, animal feed and as an antifreeze. A study by Zhao, Zhao and Doyle (2009) reported a reduction of *Salmonella* Enteritidis by 3.7 log CFU/cm² on

inoculated chicken skin when treated with 0.05% levulinic acid. Additionally, the same study reported a reduction of *Salmonella* Enteritidis on chicken wings by 2.6 and 4.0 log CFU/g when treated with 2 or 3% levulinic acid, respectively.

L. Lactic acid/citric acid blend: Lactic acid is an organic compound that results after carbohydrate breakdown in living organisms. This acid is also formed by natural fermentation in many products such as cheese and yogurts. Lactic acid is used as a pH regulator or as a food preservative in many food systems including beverages, vegetables, meat, poultry and fish. Several authors have reported enhancements in beef shelf-life when treated with lactic acid as an antimicrobial. Stivarius et al. (2002) concluded that lactic acid was effective ($P < 0.05$) for reducing *E. coli*, coliforms and aerobic plate count in ground beef but reduced ($P < 0.05$) ground beef redness. However, Jimenez-Villarreal et al. (2003) reported that ground beef patties from beef trimmings treated with 2% lactic acid followed by 0.5% cetylpyridinium chloride were similar ($P > 0.05$) in redness to an untreated control. Another study showed a reduction of *E. coli* O157:H7 and *Salmonella* Typhimurium in ground beef up to 0.5 and 0.6 log respectively, when treated with 5% lactic acid (Harris et al., 2012).

For this study, a USDA-approved blend of lactic and citric acids, developed as an antimicrobial (BEEFXIDE ®) (Birko Corp., Henderson, CO, USA) was used as a treatment solution.

M. Sodium diacetate: Sodium diacetate is an acidic sodium salt used as a food preservative and pH buffer. It is a simple combination of acetic acid and sodium acetate. As a preservative is

effective against mold and bacteria. Recent studies suggest that this compound is a pH regulator and an effective antimicrobial in meat and poultry products, especially with strains such as *C. botulinum* and *L. monocytogenes*. Combinations of potassium lactate and sodium diacetate have shown to be effective for controlling pathogenic bacteria. Knight et al. (2007) reported a reduction of *L. monocytogenes* on vacuum-packaged frankfurters when treated with a 3% potassium lactate- sodium diacetate solution. Mirosław et al. (2007) concluded that this same solution could be used as a good stabilizer of color and texture in minced meat production. Additionally, Ponrajan et al. (2011) reported a significant ($P < 0.05$) *E. coli* reduction of 0.6 log CFU/g on top rounds and sirloins when treated with a 1% solution of 80% sodium citrate plus 20% sodium diacetate.

N. Vinegar based powder: Vinegar is mainly a solution of water and acetic acid, which is produced by the fermentation of ethanol. Acetic acid has shown antimicrobial properties and has been studied by several authors. Stivarius et al. (2002) reported a reduction ($P < 0.05$) of *E. coli*, *Salmonella* Typhimurium coliforms and aerobic plate count by 0.9, 1.47, 1.25 and 1.25 CFU/g, respectively on beef trimmings before grinding when treated with 5% acetic acid. However, ground beef color of these treated beef trimmings tended to be less red ($P < 0.05$) and contain less oxymyoglobin when compared to an untreated control. Another study conducted by Harris et al. (2006) reported a significant ($P < 0.05$) reduction of *E. coli* O157:H7 and *Salmonella* Typhimurium in ground beef when treated with 2 or 4% acetic acid, without affecting sensory taste characteristics.

For this study, a buffered, vinegar-based ingredient (BACTOCEASE NV®) (Kemin Food Technologies Inc., Des Moines, IL, USA) was used as a treatment solution at a concentration of 1%.

O. Lemon juice: Lemon juice has shown to be a natural antimicrobial due to the presence of citric acid. The juice of lemon contains around 5 to 6 % citric acid, responsible for the sour taste. Citric acid is a weak organic acid with antimicrobial that occurs naturally in citrus fruits. Pohlman et al. (2012) concluded that decontamination of *biceps femoris* steaks using a solution of 20% (v/v) hydrochloric / citric acid blend is effective. Additionally, Choi et al. (2013) reported a reduction of 5.7 and 5.3 log CFU/ml in the populations of *C. sakazakii* and *S. Typhimurium*, respectively, after treated for 30 minutes with caprylic and citric acids (80 mM). Citric acid improves the tenderness of meat (Burke and Monahan, 2003) and at the same time reduces beef pathogens such as *Escherichia coli* and *Salmonella*. Different concentrations of lemon juice (2 ml, 5 ml, 10 ml and 15 ml) have been reported to be effective in the reduction ($P < 0.05$) of *E. coli* and *Salmonella* Enteritidis on inoculated raw meatballs (Bingol et al., 2011).

Chapter II

Long Chain Organic Acid Effects on Ground Beef Processing Instrumental Color and Sensory Color, Taste and Aroma Characteristics.

ABSTRACT

Beef trimmings (80/20) were sprayed with fumaric acid (F), malic acid (M), octanoic acid (O) and decanoic acid (D) all at 3% (w/v) versus untreated control (CON). Beef trimmings were ground, processed into meat patties and sampled for 7 days. The packages were displayed under simulated retail conditions. Trained panelists evaluated meat sensory color, odor and processing abilities on days: 0, 1, 2, 3 and 7 of display. The treatments D, F and O treatments significantly increased sensory evaluated overall meat color redness ($P < 0.05$), reduced percentage of discoloration ($P < 0.05$) and showed higher a^* values ($P < 0.05$) compared to the control CON on days 0 and 1 of display. There was no significant difference ($P > 0.05$) in beef flavor between CON and the rest of the treatments. The results suggest that the use of 3% solutions containing fumaric, malic, octanoic and decanoic acid as antimicrobials on beef trimmings prior to grinding may improve or maintain sensory retail display properties such as meat color and odor without affecting beef flavor of ground beef patties.

Keywords: beef trimmings, antimicrobials, meat safety, organic acids, meat color

INTRODUCTION

A past study by the Food Marketing Institute (FMI) reported that 69% of consumers considered product safety as a very important factor when it comes to food selection (FMI, 1994). Concern about public health has increased, but some people still consume undercooked meat products, increasing the risk of foodborne illnesses. Even though food production techniques have improve, food-borne pathogens such as *Salmonella* (sm) and *Escherichia coli* have the potential to evolve and thrive.

Ground beef is a perfect vehicle for pathogens to colonize. It has been identified as the source for multiple drug resistant (MDR) *Salmonella* Newport and *Salmonella* Typhimurium infections (Varma et al., 2006). About 76 million cases of foodborne illnesses are estimated to occur annually in the United States, resulting in 325,000 hospitalizations and 5,000 deaths (Mead et al., 1999). Additionally, Rangel et al. (2005) reported that *E. coli* O157:H7 causes 73,000 annually illnesses in the United States.

Due to concerns related to food safety, organic acids have been utilized in the industry to improve safety and extend shelf life by retarding food deterioration. They have been applied in both pre-harvest and post-harvest food production and processing systems (Ricke, 2003). Several authors have studied the impact of different organic acids on ground beef. Stivarius et al. (2002) concluded that lactic acid was effective ($P < 0.05$) for reducing *E. coli*, coliforms and aerobic plate count in ground beef. Limited research on ground beef has been done with fumaric acid but its antimicrobial properties against *E. coli* have been demonstrated in apple cider (Chikthimmah et al., 2003) and acidified foods (Lu et al., 2011). Antimicrobial properties of malic acid have been studied in ground beef by Mohan et al. (2012) who reported a reduction of *E. coli* and

Salmonella in inoculated ground beef with minimal impact on meat color characteristics. Eswarnandam et al. (2004) concluded that 2.6% malic acid-incorporated soy protein films decreased log number CFU/mL of *L. monocytogenes*, *S. gaminara* and *E. coli* O157:H7 from 8.3, 9.0 and 8.9 log to 5.5, 3.0 and 6.8 log number CFU/mL, respectively. Additionally, 2.0 & 2.5% concentrations of malic acid were shown to be efficient for reducing by more than 5-log cycles *E. coli* O157:H7, *L. monocytogenes* and *S. Enteritidis* in apple, pear and melon juices (Raynaudi-Massilia et al., 2009). Octanoic or caprylic acid has been effective against *Salmonella enterica* serovar Typhimurium in reconstituted infant formula (Choi et al., 2013) and against *E. coli* in inoculated ground beef (Mohan et al., 2012). This last author reported a reduction of *E. coli* counts on beef trimmings treated before grinding by 1-log ($P < 0.05$) when treated with 0.04% octanoic acid on day 1 of display. Additionally, this treatment also reduced SM count by 1.1 log ($P < 0.05$) on the same day 1 of display with minimal impact on meat redness.

Although many antimicrobials have been evaluated as intervention treatments to reduce pathogens and extend shelf life safety, researchers pay less attention to the effects of antimicrobials on meat color (Mancini & Hunt, 2005). Therefore the purpose of this research was to determine the impact of fumaric, malic, octanoic and decanoic acid on ground beef patty color, odor and sensory characteristics. All the antimicrobials used in this study are approved for use in meat and poultry products by the Food Safety Inspection Services (FSIS) of the US Department of Agriculture and food products by the Food and Drug Administration.

MATERIALS AND METHODS

Antimicrobial treatment application

The antimicrobial treatments for this study were 3% (w/v) fumaric acid (F) (A.E. Staley Manufacturing Company, Decatur, Illinois, USA), 3% (w/v) malic acid (M) (Sigma-Aldrich, St. Louis, Missouri, USA) 3% (w/v) octanoic acid (O) (Sigma-Aldrich), 3% (w/v) decanoic acid (D) (Sigma-Aldrich) and an untreated control (CON). Distilled water was used for the preparation of the antimicrobial solutions. Beef trimmings (80/20) were electrostatically (ESS; Electrostatic Spraying Systems Inc., Watkinsville, GA, USA) sprayed with organic acid antimicrobial treatment solutions at a rate of (~0.1 ml/g) until meat surfaces were saturated. Each treatment was repeated 2 times. Next, similarly to Quilo et al. (2009), beef trimmings were ground twice using an American Eagle AE-G12N grinder (American Eagle Food Machinery Inc., Chicago, IL), with a 3.0 mm plate. After grinding, beef was processed into 150 g meat patties, placed on plastic foam trays with absorbent diapers and over wrapped with polyvinyl chloride film with an oxygen transmission rate of 14,000 cc/mm²/24h/1atm (Kotch Supplies, Inc., Kansas City, Missouri, USA). The packages were displayed under simulated retail conditions (4°C; warm white fluorescent lightning; 1630 lx; Phillips Inc., Somerset, New Jersey, USA) for 7 days and patties from each treatment were frozen for further sensory evaluation. The pH of ground beef was determined on day 0 of display by homogenizing 2.0 g of ground beef in 20 ml of distilled water (1:10 ratio), and evaluating with an Orion 3 Star pH meter (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Processing properties, sensory color and odor

A nine member trained sensory panel was used to evaluate processing abilities: smearing and patty forming ability, sensory color and sensory odor characteristics of ground beef patties on days 0, 1, 2, 3 and 7 of simulated retail display. For each treatment, panelists evaluated smearing (6= extreme smearing, 5= moderate smearing, 4= slight smearing, 3= slight cut-grind, 2= moderate cut-grind, 1= extreme cut-grind) and patty forming ability (6= extremely fragile, 5= moderately fragile, 4= slightly fragile, 3= slightly cohesive, 2= moderately cohesive, 1= extremely cohesive). The ground beef patties were also evaluated for worst point color, overall color and percentage of discoloration under simulated retail display. The panelists evaluated worst point color (1= brown, 2= moderately brownish red, 3= slightly brownish red, 4= dull red, 5= bright red), which defines a discolored area of at least 2 cm in diameter, overall color (1= brown, 2= moderately brownish red, 3= slightly brownish red, 4= dull red, 5= bright red) and percentage of discoloration [1= total discoloration (96-100%), 2= extensive discoloration (80-95%), 3= moderate discoloration (60-79%), 4= modest discoloration (40-59%), 5= small discoloration (20-39%), 6= slight discoloration (1-20%), 7= no discoloration (0%)] on days 0, 1, 2, 3 and 7 of display. Ground beef patty packages were then opened and evaluated for beef odor and off odor characteristics. Beef odor was evaluated using an eight point scale where 8= extremely beef like, 7= very beef like, 6= moderately beef like, 5= slightly beef like, 4= slightly non beef like, 3= moderately non-beef like, 2= very non-beef like, 1= extremely non-beef like and off odor attributes using a five point scale (5= no off odor, 4= slight off odor, 3= small off odor, 2= moderate off odor, 1= no off odor) on the display days previously described.

Instrumental color

Instrumental color of ground beef patties was measured using a Hunter Lab Mini Scan Illuminant A/10° observer (Hunter Associates Laboratory, Inc., Reston, West Virginia, USA) on

days: 0, 1, 2, 3 and 7 of display. Samples were evaluated for CIE; L*, a* and b* color values.

The proportion of oxymyoglobin to metmyoglobin was estimated in the visible spectrum using 580 and 630 nm reflectance measurements (Hunt et al., 1991). Saturation index, which describes the brightness of color was calculated $[(a^{*2} + b^{*2})^{0.5}]$, as was the hue angle $[\tan^{-1}(b^*/a^*)]$, which represents the shift from red to yellow of the patties. The colorimeter was standardized each day before sampling using a white tile and a black tile. Three measurements were taken on different areas for each sample and averaged for statistical analysis.

Sensory evaluation

Sensory evaluation of ground beef patties was conducted after thawing ground beef samples under refrigerated conditions. A nine-member panel was trained following the American Meat Science Association Guidelines (AMSA, 1995). Specifically, ground beef patties treatment and control groups were thawed, removed from the foam trays and cooked for evaluation in a Blodgett/Zephaire forced air convection oven (Blodgett Oven, Burlington, VT) at 163 °C until an internal temperature of 71°C was reached (AMSA, 1995; Quilo et al., 2009). Patties were sectioned into squares (2.54 cm x 2.54 cm), wrapped in foil and maintained at 49°C in an Alto-Shaam commercial food warmer (Alto-Shaam Inc., Menomonee Falls, WI) for approximately 15 min until served to panelists. Ten samples were randomly presented to the panelists using a complete block design. Trained panelists evaluated samples at their own pace, indicating whenever the next sample was required. Panelists evaluated bind (1= extremely fragile, 2= very fragile, 3= moderately fragile, 4= slightly fragile, 5= lightly bind, 6= moderately bind, 7= very strong bind, 8= extremely bind), overall tenderness (1= extremely tough, 2= very tough, 3= moderately tough, 4=slightly tough, 5= slightly tender, 6= moderately tender, 7= very tender, 8=

extremely tender), juiciness (1= extremely dry, 2= very dry, 3= moderately dry, 4= slightly dry, 5= slightly juicy, 6= moderately juicy, 7= very juicy, 8=extremely juicy), beef flavor (1= extremely non-beef like, 2= very non-beef like, 3= moderately non-beef like, 4= slightly non-beef like, 5= slightly beef like, 6= moderately beef like, 7= very beef like, 8= extremely beef like) and off flavor intensity on a five point scale (1= extremely off flavor 2= moderate off flavor, 3= small off flavor, 4= slight off flavor, 5= no off flavor).

Statistical Analysis

The experiment was arranged in a completely randomized 5x5 factorial design. Data were analyzed using the General Linear Model procedure of SAS for interaction and main effects. Least-squares means for significant interactions or main effects were separated using the Probability of Difference procedure (PDIFF) of SAS (SAS Inst. Inc., Cary, North Carolina, USA). Tukey's post-hoc analysis test procedure of SAS was conducted for means separation of sensory panel data.

RESULTS AND DISCUSSION

Processing properties

The impact of antimicrobial treatments on patty forming ability is shown in Table 1. Panelists found treatments D, O and M less ($P < 0.05$) fragile than CON for patty forming ability through all seven days of retail display, where M showed more ($P < 0.05$) cohesiveness than the rest of the treatments. Patty cohesiveness remained relatively stable through 7 days of display

with patties from day 1 of display having similar cohesiveness' as patties on day 7 of display ($P > 0.05$) (Table 2).

Day by treatment interaction effect on instrumental color

The day by treatment interaction effect on CIE a^* value is summarized in Table 3. On day 0 of display, F, M and O were redder ($P < 0.05$) than patties left untreated (CON) and D ground beef patties, which were similar ($P > 0.05$) to the control. However, on day 1 of display all treatments were redder ($P < 0.05$) than CON. On days 2 and 3 of retail display, CON was redder ($P < 0.05$) than the rest of the treatments. By day 7 of display, M was redder ($P < 0.05$) than the rest of the treatments. The CIE a^* values on day 7 of display are greater ($P < 0.05$) than those on day 3 display for each treatment. This increase in redness on day 7 is probably due to the accumulation of purge (water soluble myoglobin) on the surface of ground beef patties, increasing the redness of all treatments and the untreated control (CON). These results partially agree with those of Mohan et al. (2012), who found octanoic and malic acids in concentrations of 0.04 and 2 % respectively, similar in redness to the control among 7 days of display. The higher concentration of both organic acids in this project, enhanced meat redness on days 0 and 1 of display when compared to CON.

The day by treatment interaction effect on CIE b^* value is summarized in Table 3. On day 0 of retail display, D, F and O patties were not different ($P > 0.05$) from CON. However, M patties were more ($P < 0.05$) yellow than the rest of the treatments, except F. On day 1 of display, treatments F, M and O were similar ($P > 0.05$) to CON while D treated patties were less yellow ($P < 0.05$) than the rest of the treatments. On day 2 of display, M and O patties were similar ($P > 0.05$) to those left untreated (CON) while D and F were less ($P < 0.05$) yellow than

the rest of the treatments. However, on day 7 of display, CON and O were less yellow ($P < 0.05$) than M.

Table 4 shows the day of display by treatment interaction effect on CIE L* value. In general, all ground beef patties became darker in color across the 7 days of display. On day 0 of display, the untreated control (CON) and M did not differ ($P > 0.05$) from each other in lightness and at the same time were lighter ($P < 0.05$) than treatments D, F and O. On day 1 of display, all treatments and the untreated control (CON) were darker ($P < 0.05$) than M. However, F and CON were similar ($P > 0.05$) and at the same time lighter ($P < 0.05$) than D and O. On day 2 of display, CON was darker in color ($P < 0.05$) than F and M and was similar ($P > 0.05$) to both D and O patties. On day 3 of display, CON was similar ($P > 0.05$) to D, which was darker ($P < 0.05$) than F and M. On day 7 of retail display, treatments D and O were darker ($P < 0.05$) than CON, F and M, which were not different ($P > 0.05$) from each other.

Table 4 also shows the day of display by treatment interaction effect on hue angle. On days 0 and 1 of display, the hue angle of all treatments except M, were lower ($P < 0.05$) than CON, except for M on day 0, which was similar ($P > 0.05$) to CON. On days 2 and 3 of display, CON was redder (a^* ; $P < 0.05$) than the rest of the treatments and at the same time its hue angle value was lower ($P < 0.05$). However, on day 7 of display the hue angle value of M was similar ($P > 0.05$) to CON and at the same time lower ($P < 0.05$) than treatments D, F and O.

Saturation index refers to the intensity of the a^* and b^* values and is expressed as vividness or brightness. The day by treatment interaction effect on saturation index is summarized in Table 4. On days 0 and 1 of display, the F, M and O treatments were more ($P < 0.05$) vivid in color compared to CON and D, which showed no difference ($P > 0.05$) between each other. However, on days 2 and 3 of display, the untreated control presented more ($P < 0.05$)

vividness than the rest of the treatments, except for M, which was similar ($P > 0.05$) to CON on day 3 of display. This relates again with the redder ($P < 0.05$) color showed by CON on these days of display compared to the rest of the treatments. On day 7 of display, the M patties were more ($P < 0.05$) vivid than CON and the rest of the treatments.

The estimation of oxymyoglobin content (630/580 nm), summarized in Table 4, was higher ($P < 0.05$) for all the treatments on days 0 and 1 of display when compared to the untreated control (CON). However, on days 2 and 3 of display, CON had higher ($P < 0.05$) estimations of oxymyoglobin content than the rest of the treatments. On day 7 of display, M treated patties showed no difference ($P > 0.05$) in oxymyoglobin content when compared to untreated patties (CON). Similarly to CIE a^* value, oxymyoglobin ratio tended to increase from day 3 to day 7 of display. Our results are similar to those of Jimenez-Villarreal et al. (2003a; 2003b), who also observed an increase in oxymyoglobin proportions on day 7 of display, being similar ($P > 0.05$) to the first two days of display. Again, a possible justification for this is the accumulation of high levels of water-soluble myoglobin on the surface of the package, resulting in a redder color and higher oxymyoglobin proportions.

Day by treatment interaction effects on worst point color, overall color, percentage discoloration, beef odor, off odor and smearing

The day by treatment interaction effect on worst point color is summarized in Table 5. Panelist detected that all treatments were redder ($P < 0.05$) than CON on days 0 and 1 of display, except for D on day 0, which remained similar ($P > 0.05$) to CON. On day 2 of display, all treatments were similar ($P > 0.05$) in worst point color. However, on day 3 of display, CON was redder ($P < 0.05$) than D, F and M but at the same time similar ($P > 0.05$) to treatment O.

Likewise, on day 7 of display, CON was redder ($P < 0.05$) than treatments D, F and O but at the same time was not different ($P > 0.05$) from M treated patties. Interestingly, treatment M increased in redness of worst point color through the latter stages of display.

The discoloration behavior of the patties (Table 5) turned out to be similar to the overall color. On day 0 of display, all treatments had similar ($P > 0.05$) discoloration to CON patties, except for O, which had less ($P < 0.05$) discoloration than CON. On day 1 of display, all treatments showed less ($P < 0.05$) discoloration than CON. On days 2 and 3 of display, all treatments had a greater ($P < 0.05$) discoloration compared to CON, except for M on day 3, which was not different ($P > 0.05$) from CON. On day 7 of display, all treatments were similar ($P > 0.05$) in discoloration.

The overall color attribute is summarized in Table 5. On day 0 of display, panelists found a redder ($P < 0.05$) color for treatments F and O compared to CON, which was similar ($P > 0.05$) to M and D. Similarly, on day 1 of retail display, all treatments had a redder ($P < 0.05$) color than CON. Conversely, on days 2 and 3 of display, panelist found CON patties redder ($P < 0.05$) than the rest of the treatments, except for M on day 3, which was similar to CON. Similarly to instrumental *CIE a** value, on day 7 of display panelists found both untreated control (CON) and M treated patties redder ($P < 0.05$) than the rest of the treatments and again, M was shown to improve its values through the last days of display.

Treatment by day of display interaction effect on grinding ability is shown in Table 6. The F and O patties showed a greater particle definition ($P < 0.05$) compared to the rest of the treatments on days 0 and 1 of display. The D and M treatments were similar to CON ($P > 0.05$) on those days of display. On day 2 of display, CON, D and O patties showed the greatest particle

definition ($P < 0.05$). However, on days 3 and 7 of display, all treatments were similar ($P > 0.05$) in this particle definition attribute.

There were no significant differences in beef odor ($P > 0.05$) between CON and the rest of the treatments on day 0 of display except for M, showing a less intense ($P < 0.05$) beef odor (Table 6). On days 1, 2 and 3 of display, all treatments had a more intense ($P < 0.05$) beef odor than CON, except for treatment D on day 2 of display, which was similar ($P > 0.05$) in beef odor to CON. By day 7 of retail display, CON was similar ($P > 0.05$) in beef odor to all treatments, except for F, which had a more intense ($P < 0.05$) beef odor than CON but was not different ($P > 0.05$) from treatment O.

Table 6 also shows the day by treatment interaction effect for off odor. The D, F and O treated patties were similar ($P > 0.05$) to the untreated control (CON) on day 0 of display and at the same time had less ($P < 0.05$) off odor than M. However, on day 1 of display, panelists found all treatments to be similar ($P > 0.05$) in off odor to CON, except for F, which had less ($P < 0.05$) off odor than CON. Treatments F, M and O had similar ($P > 0.05$) off odor on day 2 of display and at the same time showed less ($P < 0.05$) off odor than CON and D. On day 3 of display, all treatments had less ($P < 0.05$) off odor than CON. All treatments were similar ($P > 0.05$) in off odor when compared to the untreated control on day 7 of display, except for F, which had less ($P < 0.05$) off odor than CON.

pH

Un-treated ground beef patties (CON) had the greatest ($P < 0.05$) pH on day 0 of retail display (Table 7). Past studies have shown a relationship between high pH and redness of color in meat (Pohlman et al., 2002; Lim et al., 2004). However, that relationship was not found in this study and a possible justification could be the proximity in pH values between treatments.

Effects of antimicrobial treatments on sensory taste characteristics

The effects of antimicrobial treatments on sensory taste characteristics are shown in Table 8. Trained panelists were unable to detect any differences ($P > 0.05$) in beef flavor, off flavor, bind and tenderness between CON and the rest of the treatments. The D and F treatments were similar ($P > 0.05$) in juiciness to CON and juicier ($P < 0.05$) than O and M. Therefore, the use of antimicrobials had little impact on sensory attributes of the resulting patties.

CONCLUSION

The results suggests that the use of solutions containing fumaric, malic, octanoic and decanoic acid as antimicrobials on beef trimmings prior to grinding may improve or maintain the same instrumental color and sensory retail display properties such as meat color and odor without affecting sensory taste of ground beef patties. However, the uses of octanoic acid tended to out perform the rest of the treatments in most of its quality effects when compared to CON. Treatment M was effective for some attributes such as redness (a^*), overall color, estimation of oxymyoglobin content and percentage discoloration, which were better demonstrated at the late stages of display (days 3 and 7). Therefore, the application of these antimicrobial treatments can be used to improve ground beef safety without affecting ground beef patty quality.

Table 1. Effect of antimicrobial treatments ¹ applied to beef trimmings on least-squares means (\pm SE) for processing abilities ² of ground beef

Attribute	Treatment					S.E
	CON	D	F	M	O	
<i>Processing abilities</i>						
Patty forming ability	3.25 ^a	2.63 ^{bc}	2.92 ^{ab}	2.07 ^d	2.49 ^c	0.13

¹ CON= control, D= 3% decanoic acid, F= 3% fumaric acid, M= 3% malic acid, O= 3% octanoic acid.

² Patty forming ability score: 6= extremely fragile; 1= extremely cohesive.

^{a-d} Least-squares means within an attribute bearing different superscripts differ ($P < 0.05$).

Table 2. Effect of duration of display on the least-squares means (\pmSE) for processing abilities ¹ of ground beef						
Attribute	Days of display					S.E
	0	1	2	3	7	
<i>Processing abilities</i>						
Patty forming ability	2.46 ^b	2.81 ^{ab}	2.93 ^a	2.70 ^{ab}	2.47 ^b	0.14

¹ Patty forming ability score: 6= extremely fragile; 1= extremely cohesive.
^{a-b} Least-squares means within an attribute bearing different superscripts differ ($P < 0.05$).

Table 3. Effect of days of display by antimicrobial treatment ¹ interaction effect on the least-squares means (\pm SE) for CIE L^* ², CIE a^* ³ and CIE b^* ⁴ of ground beef through simulated retail display

Attribute	Treatment	Days of display				
		0	1	2	3	7
<i>CIE L*</i>	CON	55.99 ^{ab}	52.36 ^{ef}	50.18 ^{ijk}	50.14 ^{jk}	49.65 ^{jkl}
	D	51.73 ^{e-h}	50.01 ^{jkl}	50.90 ^{g-j}	49.98 ^{jkl}	45.85 ⁿ
	F	52.76 ^{ef}	51.51 ^{fi}	54.21 ^{cd}	53.00 ^{de}	50.47 ^{h-k}
	M	56.11 ^a	54.71 ^{bc}	52.64 ^b	51.92 ^{efg}	49.18 ^{klm}
	O	50.95 ^{g-j}	48.13 ^m	49.79 ^{jkl}	48.67 ^{lm}	44.56 ⁿ
	S.E.	0.47				
<i>CIE a*</i>	CON	19.22 ^{cd}	10.91 ^j	17.45 ^e	19.60 ^{cd}	21.78 ^b
	D	20.09 ^c	12.74 ^h	10.13 ^j	10.40 ^j	18.63 ^{de}
	F	22.79 ^{ab}	12.92 ^h	10.80 ^j	10.14 ^j	16.66 ^{ef}
	M	22.13 ^{ab}	14.50 ^g	11.29 ^{ij}	15.73 ^{fg}	23.37 ^a
	O	21.66 ^b	15.40 ^g	12.30 ^{hi}	11.16 ^{ij}	19.80 ^{cd}
	S.E.	0.44				
<i>CIE b*</i>	CON	21.72 ^{bc}	19.00 ^{g-j}	19.49 ^{fgh}	18.85 ^{hij}	18.38 ^{ijk}
	D	21.03 ^{cd}	17.42 ^k	18.03 ^{jk}	19.29 ^{ghi}	19.23 ^{ghi}
	F	22.69 ^{ab}	19.53 ^{fgh}	18.36 ^{ijk}	19.31 ^{ghi}	19.23 ^{ghi}
	M	23.56 ^a	19.80 ^{fgh}	20.40 ^{def}	21.97 ^{bc}	19.74 ^{fgh}
	O	21.90 ^{bc}	19.94 ^{efg}	19.05 ^{g-j}	19.20 ^{ghi}	18.12 ^{jk}
	S.E.	0.37				

¹ CON= control, D= 3% decanoic acid, F= 3% fumaric acid, M= 3% malic acid, O= 3% octanoic acid.

² *CIE L**: 0= black and 100= white.

³ *CIE a**: -60= green and +60= red.

⁴ *CIE b**: -60= blue and +60= yellow.

^{a-k} Least-squares means within an attribute bearing different superscripts differ ($P < 0.05$).

Table 4. Effect of days of display by antimicrobial treatment ¹ interaction effect on the least-squares means (\pm SE) for hue angle ², saturation index ³ and oxymyoglobin content ⁴ of ground beef

Attribute	Treatment	Days of display				
		0	1	2	3	7
<i>Hue Angle</i>						
	CON	48.47 ^{gh}	60.06 ^{bc}	48.24 ^{ghi}	43.96 ^{kl}	40.16 ^m
	D	46.30 ^{ij}	53.79 ^{ef}	60.67 ^{abc}	61.67 ^{ab}	45.91 ^{jk}
	F	44.87 ^{jk}	56.54 ^d	59.53 ^c	62.29 ^a	49.26 ^g
	M	46.70 ^{hij}	53.81 ^{ef}	61.04 ^{abc}	54.43 ^e	40.19 ^m
	O	45.29 ^{jk}	52.33 ^f	57.15 ^d	59.84 ^{bc}	42.66 ^l
	S.E.	0.73				
<i>Saturation index</i>						
	CON	29.01 ^c	21.92 ^{klm}	26.22 ^{efg}	27.22 ^{de}	28.50 ^{cd}
	D	29.09 ^c	21.59 ^{klm}	20.68 ^m	21.91 ^{klm}	26.77 ^{ef}
	F	32.16 ^a	23.42 ^{ij}	21.31 ^{lm}	21.81 ^{klm}	25.48 ^{fgh}
	M	32.34 ^a	24.55 ^{hi}	23.31 ^{ij}	27.05 ^e	30.60 ^b
	O	30.81 ^b	25.20 ^{gh}	22.68 ^{jk}	22.20 ^{jkl}	26.90 ^e
	S.E.	0.47				
<i>630nm/580nm</i>						
	CON	2.18 ^e	1.04 ^l	1.91 ^f	2.33 ^{de}	2.76 ^{ab}
	D	2.43 ^{cd}	1.42 ^{hij}	1.03 ^l	1.00 ^l	2.14 ^e
	F	2.91 ^a	1.34 ^{ijk}	1.14 ^{kl}	1.01 ^l	1.81 ^{fg}
	M	2.52 ^{cd}	1.51 ^{hi}	1.01 ^l	1.44 ^{hi}	2.98 ^a
	O	2.63 ^{bc}	1.63 ^{gh}	1.20 ^{jkl}	1.02 ^l	2.47 ^{cd}
	S.E.	0.08				

¹ CON= control, D= 3% decanoic acid, F= 3% fumaric acid, M= 3% malic acid, O= 3% octanoic acid.

² Calculated as $\tan^{-1}(b^*/a^*)$.

³ Calculated as $(a^{*2} + b^{*2})^{0.5}$.

⁴ Calculated as the ratio 630nm/580nm reflectance.

^{a-1} Least-squares means within an attribute bearing different superscripts differ ($P < 0.05$).

Table 5. Effect of days of display by antimicrobial treatment ¹ interaction effect on the least-squares means (\pm SE) for overall color ², % discoloration ³ and worst point color ⁴ of ground beef through simulated retail display

Attribute	Treatment	Days of display				
		0	1	2	3	7
<i>Overall color</i>	CON	3.33 ^{d-g}	1.45 ^{klm}	3.44 ^{c-g}	3.33 ^{d-g}	3.81 ^{b-e}
	D	3.83 ^{bcd}	2.45 ^{hi}	1.38 ^{klm}	1.88 ^{ijk}	2.45 ^{hi}
	F	4.05 ^{ab}	3.02 ^{fgh}	1.00 ^m	1.38 ^{klm}	2.16 ^{ij}
	M	3.61 ^{b-f}	3.31 ^{d-g}	1.22 ^{lm}	3.00 ^{gh}	3.95 ^{abc}
	O	4.44 ^a	3.23 ^{efg}	1.66 ^{jkl}	1.61 ^{jkl}	2.95 ^{gh}
	S.E.	0.22				
<i>% Discoloration</i>	CON	5.76 ^{bcd}	1.91 ^{kl}	3.72 ^{hi}	4.66 ^{efg}	5.45 ^{cde}
	D	6.50 ^{ab}	3.17 ^{ij}	1.61 ^{kl}	2.38 ^{jk}	5.10 ^{def}
	F	6.50 ^{ab}	4.03 ^{ghi}	1.33 ^l	1.66 ^{kl}	4.67 ^{efg}
	M	6.05 ^{bc}	4.03 ^{ghi}	1.29 ^l	4.38 ^{fgh}	5.81 ^{bcd}
	O	6.88 ^a	4.03 ^{ghi}	1.94 ^{kl}	2.16 ^k	5.24 ^{cde}
	S.E.	0.32				
<i>Worst point color</i>	CON	3.05 ^d	1.30 ^{ijk}	1.44 ^{h-k}	2.44 ^{ef}	3.59 ^{bcd}
	D	3.61 ^{bcd}	2.37 ^{efg}	1.22 ^{jk}	1.82 ^{g-j}	1.94 ^{f-i}
	F	3.77 ^{bc}	3.02 ^{de}	1.11 ^k	1.08 ^k	1.87 ^{f-i}
	M	3.83 ^b	3.23 ^{bcd}	1.09 ^k	1.44 ^{h-k}	3.37 ^{bcd}
	O	4.61 ^a	3.16 ^{cd}	1.55 ^{h-k}	2.05 ^{fgh}	2.09 ^{fgh}
	S.E.	0.24				

¹ CON= control, D= 3% decanoic acid, F= 3% fumaric acid, M= 3% malic acid, O= 3% octanoic acid.

² Color score: 1=brown; 5= bright red.

³ Percentage discoloration: 1= total discoloration (96-100%) and 7= no discoloration (0%).

⁴ Color score: 1=brown; 5= bright red.

^{a-1} Least-squares means within an attribute bearing different superscripts differ ($P < 0.05$).

Table 6. Effect of days of display by antimicrobial treatment ¹ interaction effect on the least-squares means (\pm SE) for beef odor ², off-odor ³ and smearing ⁴ of ground beef through simulated retail display

Attribute	Treatment	Days of display				
		0	1	2	3	7
<i>Beef Odor</i>						
	CON	5.46 ^{ab}	3.97 ^{d-g}	3.50 ^{fg}	2.94 ^{gh}	1.90 ^{hi}
	D	6.08 ^a	5.64 ^{ab}	4.12 ^{c-f}	5.44 ^{ab}	1.62 ⁱ
	F	5.55 ^{ab}	5.26 ^{abc}	5.37 ^{ab}	5.72 ^{ab}	3.47 ^{fg}
	M	3.88 ^{efg}	5.12 ^{a-d}	4.87 ^{b-e}	4.11 ^{def}	1.13 ⁱ
	O	5.38 ^{ab}	5.76 ^{ab}	5.00 ^{bcd}	4.83 ^{b-e}	2.90 ^{gh}
	S.E.	0.43				
<i>Off odor</i>						
	CON	4.04 ^{ab}	3.44 ^{b-e}	2.50 ^{fgh}	2.16 ^{gh}	1.08 ^j
	D	4.00 ^{ab}	3.94 ^{ab}	2.81 ^{efg}	3.61 ^{abc}	1.22 ^{ij}
	F	4.11 ^{ab}	4.22 ^a	3.50 ^{bcd}	3.77 ^{ab}	1.94 ^{hi}
	M	3.11 ^{c-f}	3.94 ^{ab}	3.78 ^{ab}	2.94 ^{def}	1.08 ^j
	O	4.16 ^a	4.01 ^{ab}	3.70 ^{abc}	3.83 ^{ab}	1.36 ^{ij}
	S.E.	0.27				
<i>Smearing</i>						
	CON	3.77 ^{b-f}	4.06 ^{abc}	3.00 ^{d-h}	3.38 ^{b-g}	4.28 ^{ab}
	D	3.61 ^{b-f}	3.35 ^{b-g}	3.50 ^{b-g}	3.83 ^{a-e}	3.85 ^{a-e}
	F	2.88 ^{fgh}	2.64 ^{gh}	4.16 ^{ab}	3.88 ^{a-d}	3.42 ^{b-g}
	M	4.00 ^{abc}	4.06 ^{abc}	4.72 ^a	3.94 ^{abc}	4.06 ^{abc}
	O	2.11 ^h	2.92 ^{e-h}	3.16 ^{c-g}	3.61 ^{b-f}	3.49 ^{b-g}
	S.E.	0.36				

¹ CON= control, D= 3% decanoic acid, F= 3% fumaric acid, M= 3% malic acid, O= 3% octanoic acid.

² Beef odor score: 1= extremely non beef like and 8= extremely beef like.

³ Off-odor score: 1= extreme off odor and 5= no off odor

⁴ Grinding ability score: 6= extreme smearing; 1= extreme cut-grind.

^{a-j} Least-squares means within an attribute bearing different superscripts differ ($P < 0.05$).

Table 7. Effect of antimicrobial treatments ¹ applied to beef trimmings on pH of ground beef on day 0 of display.

Treatment	pH	S.E.
CON	5.56 ^a	0.04
D	5.14 ^c	
F	5.28 ^{bc}	
M	5.37 ^b	
O	4.88 ^d	

¹ CON= control, D= 3% decanoic acid, F= 3% fumaric acid, M= 3% malic acid, O= 3% octanoic acid.

^{a-d} Least-squares means within an attribute bearing different superscripts differ ($P < 0.05$).

Table 8. Effect of antimicrobial treatments ¹ applied to beef trimmings on least-squares means (\pm SE) bind ², tenderness ³, juiciness ⁴, beef flavor ⁵ and off flavor ⁶ characteristics of ground beef patties.

Attribute	Treatment					E.M.S.
	CON	D	F	M	O	
Bind	5.64 ^a	5.64 ^a	5.78 ^a	5.85 ^a	5.71 ^a	2.45
Tenderness	6.42 ^{ab}	6.85 ^a	6.35 ^{ab}	5.78 ^{ab}	5.21 ^b	1.52
Juiciness	5.92 ^a	5.71 ^a	5.07 ^{ab}	3.37 ^c	4.28 ^{bc}	1.45
Beef Flavor	6.35 ^a	5.57 ^a	5.35 ^a	6.36 ^a	6.35 ^a	1.14
Off flavor	3.92 ^{ab}	3.35 ^{ab}	2.78 ^b	4.43 ^a	4.14 ^a	1.37

¹ CON= control, D= 3% decanoic acid, F= 3% fumaric acid, M= 3% malic acid, O= 3% octanoic acid.

² Bind score: 1= extremely fragile and 8= extreme bind.

³ Tenderness score: 1= extremely tough and 8= extremely tender.

⁴ Juiciness score: 1= extremely dry and 8= extremely juicy.

⁵ Beef flavor score: 1= extremely non-beef like and 8= extremely beef like.

⁶ Off flavor score: 1= extreme off flavor and 5= no off flavor.

^{a-c} Least-squares means within an attribute bearing different superscripts differ ($P < 0.05$).

Chapter III

Antimicrobial Treatment Effects on Ground Beef Instrumental and Sensory Color, Sensory Aroma and Taste Characteristics.

ABSTRACT

Beef trimmings (80/20) were sprayed with sodium benzoate 0.1% w/v (BEN), sodium propionate 0.3% w/v (PROP), potassium lactate/potassium diacetate blend 3% w/v (POT) and a propionic acid-based antimicrobial solution 0.35% w/v (PAA) and compared to an untreated control (CON). Beef trimmings were ground, processed into meat patties and sampled for 7 days. The packages were displayed under simulated retail conditions and trained panelists evaluated meat sensory color, odor and processing abilities on days: 0, 1, 2, 3 and 7 of display. The treatments PAA and POT significantly improved the overall meat color redness ($P < 0.05$) and reduced the percentage of discoloration ($P < 0.05$) on days 0 and 1 of display. All of the treatments presented a similar beef odor ($P > 0.05$) compared to CON on days 0, 1, 2 and 3 of retail display. Treatments BEN, PAA and PROP were similar ($P > 0.05$) in beef flavor to CON. The results suggests that the use of propionic acid-based solutions and sodium propionate as antimicrobials on beef trimmings prior to grinding may improve sensory retail display properties such as meat color and odor without affecting beef flavor of ground beef patties.

Keywords: beef trimmings; antimicrobials; meat safety; meat color

INTRODUCTION

Food safety is an important issue for many consumers around the world. Consumer's concern about food and public health has been increasing over time. It is clear that beef safety is becoming an issue of importance to consumers and can have an impact on future consumption levels (Yeung Ruth and Morris, 2001). A study by Scallan et al (2011), reported that each year, 31 major pathogens acquired in the United States cause 37.2 million cases of foodborne illness, of which 36.4 million are home acquired. Approximately 11% of these illnesses are caused by *Salmonella*, a foodborne pathogen adaptable to many different environments. *Salmonella* colonise a wide range of hosts including poultry, cattle and pigs, producing contaminated meat products (Newell et al., 2010). All these contaminated food products cause hospitalizations and foodborne illnesses among consumers. According to Gantois et al. (2008), *Salmonella* pathogen is so resilient, it has adapted to colonise the avian reproductive track and survive in hen's eggs. Some other studies had shown that *Salmonella* spp. have now evolved to colonise even vegetables (Barak et al., 2005).

Ground beef is a top retail product, sold fresh for home consumption. On average, 80 % of persons in the United States consume ground beef at home every two weeks. Additionally, ground beef is eaten an average of 1.7 times per week by those reporting eating this product (Redson, 2010). Foodborne-illness pathogens find ground beef as a perfect vehicle to reproduce and colonise. Ground beef has been identified as the source for multiple drug resistant *Salmonella* Newport and *Salmonella* Typhimurium infections (Varma et al., 2006), and for this reason, consumers should take awareness and fully cook (71° C) ground beef products at home.

Organic acids and their salts have been used as food preservatives, extending shelf life and preventing food deterioration. Antimicrobial electrostatic spraying on beef trimmings before grinding could significantly reduce illnesses caused by ground beef pathogens.

The use of antimicrobials on ground beef can reduce pathogenic activity in meat but can directly affect the color, which may impact customer purchase and consumption. Stivarius et al. (2002) concluded that lactic acid was an effective treatment for reducing ($P < 0.05$) *E. coli*, coliforms and aerobic plate count on ground beef but at the same time reduced ($P < 0.05$) ground beef redness. However, Quilo et al. (2009b) reported a redder color ($P < 0.05$), less discoloration ($P < 0.05$) and no difference in beef flavor ($P > 0.05$) of ground beef patties treated with 3% potassium lactate (potassium salt of lactic acid) and compared to an untreated control on days 0 and 1 of retail display. Additionally, Fik and Leszczynska-Fik (2007) reported a significant inhibitory effect ($P < 0.05$) on bacterial growth in minced beef when treated with 0.65 and 1.3% potassium lactate-sodium diacetate blend. Propionic acid seems to be a promising antimicrobial in the food industry. Although little research has been done on the impact of this antimicrobial on meat sensory characteristics, Glass et al. (2013) reported a limit growth of *L. monocytogenes* on cured turkey to <1-log increase for all samples through 9 weeks of storage when treated with 0.3, 0.4 and 0.5% of the same propionic acid-based solution used for this study (propionic acid 1:1). However, limited research has been done on the antimicrobial impact of propionic acid, potassium diacetate and sodium propionate on ground beef color and sensory characteristics. Therefore, the purpose of this research was to determine the impact of sodium benzoate, sodium propionate, potassium lactate/potassium diacetate blend and a propionic acid-based antimicrobial solution (propionic acid 1:1) on ground beef patty color, odor and taste. All research was conducted on uninoculated beef. All the antimicrobials used in this study are approved for use in meat and poultry products by the

Food and Safety Inspection Services (FSIS) of the US Department of Agriculture and food products by the Food and Drug Administration (FDA).

MATERIALS AND METHODS

Antimicrobial treatment application.

The antimicrobial treatments for this study were sodium benzoate 0.1% w/v (BEN) (FBC Industries, Rochelle, Illinois, USA) sodium propionate 0.3% w/v (PROP) (Niacet Corporation, Niagara Falls, New York, USA) potassium lactate/potassium diacetate blend (14:1) 3% w/v (POT) (Jungbunzlauer Inc. Newton Centre, Massachusetts, USA), a propionic acid-based antimicrobial solution 0.35% w/v (PAA) (propionic acid 1:1) (Kemin Food Technologies Inc., Des Moines, Iowa, USA) and an untreated control (CON). Distilled water was used for the preparation of the antimicrobial solutions. Beef trimmings (80% lean / 20% fat) were electrostatically sprayed (ESS; Electrostatic Spraying Systems Inc., Watkinville, GA, USA) with organic acid antimicrobial treatment solutions at a rate of (~0.1 ml/g) until meat surfaces were saturated. Each treatment was repeated 2 times. Next, beef trimmings were ground twice using an American Eagle AE-G12N grinder (American Eagle Food Machinery Inc., Chicago, IL), with a 3.00 mm plate. After grinding, beef was manually processed into 150 g meat patties, placed on plastic foam trays with absorbent diapers and over wrapped with polyvinyl chloride film with an oxygen transmission rate of 14,000 cc/mm²/24h/1atm (Kotch Supplies, Inc., Kansas City, Missouri, USA). The packages were displayed under simulated retail conditions (4°C; warm white fluorescent lightning; 1630 1x; Phillips Inc., Somerset, New Jersey, USA) for 7 days. The pH of ground beef was determined on day 0 of display by homogenizing 2.0 g of

ground beef in 20 ml of distilled water 1:10 ratio, and evaluated with an Orion 3 Star pH meter (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Processing properties, sensory color and odor

A nine member trained sensory panel was used to evaluate sensory color and sensory odor characteristics of ground beef patties and processing abilities (smearing and patty forming ability), on days 0, 1, 2, 3 and 7 of simulated retail display. For each treatment, panelists evaluated processing abilities: patty forming ability (6= extremely fragile, 5= moderately fragile, 4= slightly fragile, 3= slightly cohesive, 2= moderately cohesive, 1= extremely cohesive) and smearing (6= extreme smearing, 5= moderate smearing, 4= slight smearing, 3= slight cut-grind, 2= moderate cut-grind, 1= extreme cut-grind). The ground beef patties were also evaluated for overall color, worst point color and percentage of discoloration under retail display. The panelists evaluated overall color (1= brown, 2= moderately brownish red, 3= slightly brownish red, 4= dull red, 5= bright red), worst point color (1= brown, 2=moderately brownish red, 3= slightly brownish red, 4= dull red, 5= bright red), which defines a discolored area of at least 2 cm in diameter and percentage of discoloration [1= total discoloration (96-100%), 2= extensive discoloration (80-95%), 3= moderate discoloration (60-79%), 4= modest discoloration (40-59%), 5= small discoloration (20-39%), 6= slight discoloration (1-20%), 7= no discoloration (0%)] on days 0, 1, 2, 3 and 7 of display. Patty packages were then opened and evaluated for beef odor and off odor. Beef odor was evaluated through an eight point scale (8= extremely beef like, 7= very beef like, 6= moderately beef like, 5= slightly beef like, 4= slightly non beef like, 3= moderately non beef like, 2= very non beef like, 1= extremely non beef like) and off odor attributes through

a five point scale (5= no off odor, 4= slight off odor, 3= small off odor, 2= moderate off odor, 1= extreme off odor) on the previously described display days.

Instrumental color

Instrumental color of ground beef patties was measured using a Hunter Lab Mini Scan Illuminant A/10° observer (Hunter Associates Laboratory, Inc., Reston, West Virginia, USA) on days: 0, 1, 2, 3 and 7 of display. Samples were evaluated for CIE L^* , a^* and b^* color values. The proportion of oxymyoglobin to metmyoglobin was estimated in the visible spectrum from 580 to 630 nm reflectance measurements (630 nm/ 580 nm) (Hunt et al., 1991). Saturation index, which describes the brightness of color, was calculated $[(a^{*2} + b^{*2})^{0.5}]$, as was the hue angle $[\tan^{-1}(b^*/a^*)]$, which represents the shift from red to yellow of the ground beef patties. The colorimeter was standardized every day before sampling using a white tile and a black tile. Three measurements were taken on different areas for each sample.

Sensory evaluation

Sensory evaluation of ground beef patties was conducted on previously frozen patties. Ground beef patties of the different treatments and control were thawed, removed from the foam trays and cooked for evaluation in a Blodgett/Zephaire forced air convection oven (Blodgett Oven., Burlington, VT) at 163 °C until an internal temperature of 71°C was reached (AMSA, 1995; Quilo et al., 2009b). Patties were cut into squares (2.54 cm x 2.54 cm), wrapped in foil and kept in a commercial food warmer operating at 49°C in an Alto-Shaam commercial food warmer (Alto-Shaam, Menomonee Falls, WI) for approximately 15 min until served. Ten samples were randomly presented to the panelists seating in individual booth under sodium color neutralizing

lights (POSC) using a complete block design. Trained panelists (following the American Meat Science Association Guidelines; AMSA, 1995) evaluated samples at their own pace, indicating whenever the next sample was required. Panelists evaluated bind (1= extremely fragile, 2= very fragile, 3= moderately fragile, 4= slightly fragile, 5= lightly bind, 6= moderately bind, 7= very strong bind, 8= extremely bind), overall tenderness (1= extremely tough, 2= very tough, 3= moderately tough, 4=slightly tough, 5= slightly tender, 6= moderately tender, 7= very tender, 8= extremely tender), juiciness (1= extremely dry, 2= very dry, 3= moderately dry, 4= slightly dry, 5= slightly juicy, 6= moderately juicy, 7= very juicy, 8=extremely juicy), beef flavor (1= extremely non-beef like, 2= very non-beef like, 3= moderately non-beef like, 4= slightly non-beef like, 5= slightly beef like, 6= moderately beef like, 7= very beef like, 8= extremely beef like) and off flavor intensity on a five point scale (1= extremely off flavor 2= moderate off flavor, 3= small off flavor, 4= slight off flavor, 5= no off flavor).

Statistical Analysis

The experiment was arranged in a completely randomized 5x5 factorial design. Data were analyzed using the General Linear Model procedure and least squares means were separated using the PDIFF procedure of SAS (SAS Inst. Inc., Cary, North Carolina, USA). For sensory panel data, Tukey's post-hoc analysis test procedure of SAS was conducted for means separation.

RESULTS AND DISCUSSION

Processing properties

Panelists found treatments PAA and POT similar ($P > 0.05$) in particle definition to CON, however PROP and BEN showed less ($P < 0.05$) particle definition than CON (Table 1). These results are similar to those found by Jimenez Villarreal et al. (2003b) and Quilo et al. (2009b), where some organic acid treated patties had slightly less ($P < 0.05$) particle definition than the control. However, the particle definition of all treated and untreated patties remained similar ($P > 0.05$) through the seven days of display (Table 2).

Day by treatment interaction effect on instrumental color

Table 3 shows the day of display by treatment interaction effect on CIE L^* value. On day 0 of display, treatments BEN and PROP were lighter ($P < 0.05$) than treatment CON, which was no different ($P > 0.05$) from POT and lighter ($P < 0.05$) than PAA. Similarly, on day 1 of display, treatments BEN and PROP were lighter ($P < 0.05$) than CON and the rest of the treatments. PAA treated patties were darker ($P < 0.05$) than CON and POT, which were similar ($P > 0.05$). Treatment PROP was lighter ($P < 0.05$) than the rest on day 2 of display. Treatments BEN and POT were lighter ($P < 0.05$) than CON on day 2 of display but remained similar to treatment PAA. On day 3 of display, BEN treated patties were lighter ($P < 0.05$) than the rest of the treatments and CON. Treatments POT and PROP were also lighter ($P < 0.05$) than CON and PAA, which again remained similar ($P > 0.05$). On day 7 of retail display, CON and PAA were similar ($P > 0.05$) and darker ($P < 0.05$) in color than the rest of the treatments. The BEN

treatment was lighter ($P < 0.05$) than both POT and PROP, which were not different ($P > 0.05$) from each other.

The day by treatment interaction effect on *CIE a** value is summarized in Table 3. On day 0 of display, PAA and POT were similar ($P > 0.05$) in redness to CON. All of these treatments were also redder ($P < 0.05$) than BEN and PROP. All treatments showed no difference ($P > 0.05$) in redness from CON from days 1 to 3 of display, except for PAA, which showed less ($P > 0.05$) redness than CON, BEN, POT and PROP on day 2 of retail display. By day 7 of display, all treatments were similar ($P > 0.05$) to each other and CON. These results agree with those of Quilo et al. (2009a; 2009b; 2010), who found a similarity ($P > 0.05$) in redness between potassium lactate treated ground beef patties and an untreated control through 7 days of retail display. However, limited research has been done on the impact of sodium propionate, sodium benzoate and propionic acid on meat color.

The day by treatment interaction effect on *CIE b** value is also summarized in Table 3. On day 0 of retail display, CON was similar ($P > 0.05$) in yellowness to PAA, POT, and PROP. However, BEN patties were less ($P < 0.05$) yellow than the rest of the treatments. On days 1 and 2 of display, treatments BEN and PROP showed similar ($P > 0.05$) yellowness when compared to each other and at the same time were more yellow ($P < 0.05$) than CON, PAA and POT, which remained similar ($P > 0.05$). On days 3 and 7 of display, all treatments were more ($P < 0.05$) yellow than the control (CON), except for PAA, which remained similar ($P > 0.05$) to CON on both days and POT, which remained similar ($P > 0.05$) to CON on day 7.

Table 4 shows the day of display by treatment interaction effect on hue angle, which represents the shift from red to yellow of the ground beef patties. On day 0 of display, the hue angle values of CON, PAA and POT were similar ($P > 0.05$) and lower ($P < 0.05$) than those of

BEN and PROP. However, treatment BEN had a higher ($P < 0.05$) hue angle than PROP and the rest of the treatments. On day 1 of display, PROP had a higher ($P < 0.05$) hue angle than CON and the rest of the treatments but was not different from BEN. However, the untreated control (CON) was similar ($P > 0.05$) in hue angle value to PAA and POT. On day 2 of display, CON was similar ($P > 0.05$) in hue angle to treatments POT and PROP, but lower ($P < 0.05$) than BEN and PAA, which were not different ($P > 0.05$) from each other. BEN and PROP were similar ($P > 0.05$) and had higher ($P < 0.05$) hue angle values than CON, PAA and POT on day 3 of retail display. However, CON, PAA and POT were similar ($P > 0.05$) on day 3 of display. By day 7 of display, treatments BEN, POT and PROP had higher ($P < 0.05$) hue angle values than CON and PAA, which were similar ($P > 0.05$).

Saturation index refers to the intensity of the a^* and b^* values and is expressed as vividness or brightness of color. The day by treatment interaction effect on saturation index is summarized in Table 4. On day 0 of display, CON was similar ($P > 0.05$) in saturation index to PAA and POT and was more ($P < 0.05$) vivid than BEN and PROP. However, on day 1 of display, there were no differences ($P > 0.05$) between CON and the rest of the treatments, except for BEN, which was more ($P < 0.05$) vivid. On day 2 of display, PROP was more ($P < 0.05$) vivid than CON and the rest of the treatments but not different ($P > 0.05$) from BEN. The CON treatment was similar ($P > 0.05$) to POT and more ($P < 0.05$) vivid than PAA on day 2 of display. On day 3 of display, CON was similar ($P > 0.05$) in vividness to PAA and POT but was less ($P < 0.05$) vivid than treatments BEN and PROP, which remained similar ($P > 0.05$) to each other. All treatments were similar ($P > 0.05$) on day 7 of display, except for BEN, which was more ($P > 0.05$) vivid in color than the rest of the treatments.

The estimation of oxymyoglobin content (630/580 nm) is summarized in Table 4. The PAA treatment had higher ($P < 0.05$) oxymyoglobin content than CON and the rest of the treatments on day 0 of display. Also, on day 0 of display, CON was similar ($P > 0.05$) to POT and higher ($P < 0.05$) than treatments BEN and PROP in oxymyoglobin content. On day 1 of display, CON patties were not different from the rest of the treatments. Similarly, on day 2 of display, all treatments were similar ($P > 0.05$) to CON in oxymyoglobin content, except for PAA, which had lower ($P < 0.05$) content than CON. On day 3 of display, CON was similar to PAA and POT and at the same time higher ($P < 0.05$) in oxymyoglobin content than treatments BEN and PROP. By day 7 of display, CON was similar ($P > 0.05$) to PAA and POT, however, it had higher ($P < 0.05$) oxymyoglobin content than BEN and PROP.

Day by treatment interaction effects for worst point color, overall color, percentage discoloration, patty forming ability, beef odor and off odor

The day by treatment interaction effect for worst point color is summarized in Table 5. On day 0 of display, panelists found a redder ($P < 0.05$) worst point color on treatments POT, PAA and PROP when compared to CON, which was redder ($P < 0.05$) than BEN. Similarly, on day 1 of display, PAA and POT were again redder ($P < 0.05$) than CON, which was similar ($P > 0.05$) to BEN and PROP. By day 2 of display, all treatments except PAA were redder ($P < 0.05$) in worst point color than the untreated control (CON) and similar ($P > 0.05$) between each other. However, PAA remained similar ($P > 0.05$) to CON on this day of display. There were no differences ($P > 0.05$) in worst point color between treatments and CON on days 3 and 7 of retail display, except for BEN, which was redder ($P < 0.05$) than CON on day 7 of display.

The discoloration behavior of the patties is shown in Table 5. On day 0 of display, CON was similar ($P > 0.05$) to all treatments except for BEN, which had more ($P < 0.05$) discoloration than CON. On day 1 of display, PAA treated patties showed the least ($P < 0.05$) discoloration, followed by POT patties, which had less ($P < 0.05$) discoloration than those of CON, BEN and PROP. On day 2 of display, treatments BEN and PROP had less ($P < 0.05$) discoloration than CON, which remained similar ($P > 0.05$) to POT. On days 3 and 7 of retail display, all treatments were similar ($P > 0.05$), except for PAA, which had greater ($P < 0.05$) discoloration than CON on these days of display.

The overall color attribute is summarized in Table 5. On day 0 of display, panelists found a redder ($P < 0.05$) color for treatments PAA, PROP and POT compared to CON and BEN. PAA treatment patties were the reddest ($P < 0.05$) on day 1 of retail display. The POT treatment was redder ($P < 0.05$) than CON, which remained similar ($P > 0.05$) in color to BEN and was redder ($P < 0.05$) than PROP on the same day of display. Treatments BEN and PROP were redder ($P < 0.05$) than PAA and CON, which was similar ($P > 0.05$) to POT on day 2 of retail display. All treatments except PAA were similar ($P > 0.05$) to CON in overall color on days 3 and 7 of display. PAA was less red ($P < 0.05$) than the rest of the treatments on these days.

Beef odor attribute is summarized on Table 6. From days 0 to 3 of display, CON was similar ($P > 0.05$) in beef odor to the rest of the treatments. However, by day 7 of retail display, BEN and PROP were similar ($P > 0.05$) and had a more intense ($P < 0.05$) beef odor than CON, which remained similar ($P > 0.05$) to PAA and POT. Table 6 also shows the day by treatment interaction effect on off odor. On day 0 of display, CON was similar ($P > 0.05$) in off odor to all of the treatments. On day 1 of display, CON was similar ($P > 0.05$) in off odor to all of the treatments, except for PAA, which had less ($P < 0.05$) off odor. No differences ($P > 0.05$) in off-

odor were found between CON and the rest of the treatments from days 2 to 7 of display, except for PROP, which had more intense ($P < 0.05$) off odor than CON on day 7 of display. These results agree with those of Quilo et al. (2009; 2010), who found minimal impact on beef odor and off-odor on potassium lactate treated patties when compared to an untreated control through 7 days of display.

Patty forming ability (Table 6) remained similar ($P > 0.05$) between CON and the rest of the treatments on day 0 of retail display. CON was similar ($P > 0.05$) to BEN and more fragile ($P < 0.05$) than PAA, POT and PROP on day 1 of display. On day 2 of display, PROP patties were found more fragile ($P < 0.05$) than those of CON, which were similar ($P > 0.05$) to those of BEN, PAA and POT. However, on days 3 and 7 of retail display, CON was no different ($P > 0.05$) from the rest of the treatments.

pH

Results show that all treatments were similar ($P > 0.05$) in pH to CON on day 0 of retail display (Table 7), except for POT, which was lower ($P < 0.05$) than CON. However, past studies have shown a relationship between high pH and high red color in meat (Pohlman et al., 2002; Lim et al., 2004).

Effects of antimicrobial treatments on sensory taste characteristics

Trained panelists found no difference ($P > 0.05$) in bind between CON, PAA, PROP and POT (Table 8). However, BEN treated patties were more fragile ($P < 0.05$) than those of CON. BEN and PROP were more tender ($P < 0.05$) than CON, which remained similar ($P > 0.05$) to PAA and POT. Only PROP was scored juicier ($P > 0.05$) than CON, which remained similar ($P > 0.05$) to BEN, PAA and POT. All treatments were found to be similar ($P > 0.05$) in beef

flavor, except for treatment POT, which had a less intense ($P < 0.05$) beef flavor than CON. However, panelists couldn't find any difference ($P > 0.05$) in off-flavor between treatments.

CONCLUSION

The results suggests that the use of propionic acid-based solutions and sodium propionate as antimicrobials on beef trimmings prior to grinding may improve sensory retail display properties such as meat color and odor characteristics without affecting beef flavor of ground beef patties.

Table 1. Effect of antimicrobial treatments ¹ applied to beef trimmings on least-squares means (\pm SE) for processing abilities ² of ground beef patties

Attribute	Treatment					S.E
	CON	BEN	PAA	POT	PROP	
<i>Processing abilities</i>						
Grinding ability	2.61 ^c	3.21 ^b	2.98 ^{bc}	3.00 ^{bc}	3.79 ^a	0.16

¹ CON= control, BEN=0.1% sodium benzoate, PAA= 0.35% propionic acid solution, POT= 3% potassium lactate/ diacetate solution, PROP= 0.3% sodium propionate.

² Grinding ability score: 6= extreme smearing; 1= extreme cut-grind.

^{a-c} Least-squares means within an attribute bearing different superscripts differ ($P < 0.05$).

Table 2. Effect of duration of display on the least-squares means (\pmSE) for processing abilities ¹ of ground beef						
Attribute	Days of display					S.E
	0	1	2	3	7	
<i>Processing abilities</i>						
Grinding ability	3.16	3.19	3.30	2.93	3.01	0.18

¹ Grinding ability score: 6= extreme smearing; 1= extreme cut-grind.
Least-squares means did not differ ($P > 0.05$).

Table 3. Effect of days of display by antimicrobial treatment ¹ interaction effect on the least-squares means (\pm SE) for CIE a^* ², CIE b^* ³ and CIE L^* ⁴ of ground beef through simulated retail display

Attribute	Treatment	Days of display				
		0	1	2	3	7
<i>CIE L*</i>	CON	45.20 ^{e-h}	43.04 ^{ghi}	39.15 ^{jk}	38.05 ^{klm}	37.55 ^{klm}
	BEN	50.96 ^{ab}	51.50 ^a	46.07 ^{def}	48.95 ^{abc}	48.48 ^{bcd}
	PAA	41.21 ^{ij}	38.37 ^{kl}	36.64 ^{klm}	35.88 ^{lm}	35.58 ^m
	POT	46.77 ^{cde}	45.75 ^{d-g}	43.05 ^{ghi}	42.52 ^{hi}	41.67 ^{ij}
	PROP	50.65 ^{ab}	49.36 ^{abc}	49.59 ^{ab}	43.78 ^{f-i}	43.66 ^{f-i}
	S.E.	1.08				
<i>CIE b*</i>	CON	21.81 ^a	17.42 ^{hij}	17.81 ^{f-i}	15.84 ^{kl}	13.91 ^{mn}
	BEN	18.78 ^{d-h}	19.99 ^{bcd}	21.23 ^{ab}	20.72 ^{abc}	18.36 ^{e-i}
	PAA	21.63 ^a	17.04 ^{ijk}	17.57 ^{f-j}	16.33 ^{jkl}	13.18 ⁿ
	POT	21.81 ^a	17.40 ^{hij}	19.04 ^{def}	17.61 ^{f-j}	15.05 ^{lm}
	PROP	20.84 ^{abc}	19.00 ^{d-g}	21.58 ^a	19.49 ^{cde}	15.83 ^{kl}
	S.E.	0.56				
<i>CIE a*</i>	CON	25.14 ^{a-d}	14.66 ^{jk}	21.43 ^{fgh}	24.10 ^{b-e}	22.84 ^{d-h}
	BEN	16.94 ^{ij}	15.13 ^{ij}	21.79 ^{fgh}	25.29 ^{abc}	23.71 ^{b-f}
	PAA	26.67 ^a	15.56 ^{ij}	17.19 ⁱ	23.64 ^{b-g}	22.57 ^{e-h}
	POT	25.57 ^{ab}	14.81 ^{jk}	21.01 ^h	24.72 ^{a-e}	23.15 ^{c-h}
	PROP	21.37 ^{gh}	12.72 ^k	23.37 ^{b-g}	24.58 ^{a-e}	22.97 ^{d-h}
	S.E.	0.81				

¹ CON= control, BEN=0.1% sodium benzoate, PAA= 0.35% propionic acid solution, POT= 3% potassium lactate/ diacetate solution, PROP= 0.3% sodium propionate.

² CIE a^* : -60= green and +60= red.

³ CIE b^* : -60= blue and +60= yellow.

⁴ CIE L^* : 0= black and 100= white.

^{a-n} Least-squares means within an attribute bearing different superscripts differ ($P < 0.05$).

Table 4. Effect of days of display by antimicrobial treatment ¹ interaction effect on the least-squares means (\pm SE) for hue angle ², saturation index ³ and oxymyoglobin content ⁴ of ground beef

Attribute	Treatment	Days of display				
		0	1	2	3	7
<i>Hue angle</i>						
	CON	40.97 ^{fg}	50.00 ^b	39.79 ^{fgh}	33.30 ^{jk}	31.34 ^{kl}
	BEN	48.18 ^{bc}	53.45 ^a	44.56 ^{de}	39.33 ^{gh}	37.73 ^{hi}
	PAA	39.02 ^{gh}	47.61 ^{bc}	45.79 ^{cd}	34.63 ^j	30.22 ^l
	POT	40.45 ^{fgh}	49.87 ^b	42.51 ^{ef}	35.33 ^{ij}	33.01 ^{jkl}
	PROP	44.32 ^{de}	56.24 ^a	42.83 ^{def}	38.40 ^{gh}	34.57 ^j
	S.E.	1.18				
<i>Saturation index</i>						
	CON	33.29 ^{ab}	22.79 ^m	27.92 ^{f-i}	28.85 ^{e-h}	26.75 ^{h-k}
	BEN	25.36 ^{jk}	25.14 ^{kl}	30.55 ^{cde}	32.70 ^{abc}	29.99 ^{def}
	PAA	34.35 ^a	23.08 ^{lm}	24.60 ^{klm}	28.74 ^{e-h}	26.15 ^{ijk}
	POT	33.61 ^{ab}	22.89 ^{lm}	28.42 ^{e-h}	30.37 ^{de}	27.61 ^{g-j}
	PROP	29.85 ^{d-g}	22.89 ^{lm}	31.83 ^{bcd}	31.43 ^{bcd}	27.90 ^{f-i}
	S.E.	0.88				
<i>630nm/580nm</i>						
	CON	3.91 ^{bcd}	1.54 ^{ij}	3.01 ^{fgh}	4.27 ^{ab}	4.13 ^{bc}
	BEN	2.01 ⁱ	1.52 ^{ij}	2.67 ^h	3.46 ^{def}	3.23 ^{efg}
	PAA	4.76 ^a	1.73 ^{ij}	1.85 ⁱ	3.95 ^{bcd}	4.24 ^{ab}
	POT	3.99 ^{bcd}	1.62 ^{ij}	2.64 ^h	3.88 ^{bcd}	3.75 ^{b-e}
	PROP	2.82 ^{gh}	1.21 ^j	2.86 ^{gh}	3.62 ^{cde}	3.51 ^{def}
	S.E.	0.21				

¹ CON= control, BEN=0.1% sodium benzoate, PAA= 0.35% propionic acid solution, POT= 3% potassium lactate/ diacetate solution, PROP= 0.3% sodium propionate.

² Calculated as $\tan^{-1}(b^*/a^*)$.

³ Calculated as $(a^{*2} + b^{*2})^{0.5}$.

⁴ Calculated as the ratio 630nm/580nm reflectance.

^{a-1} Least-squares means within an attribute bearing different superscripts differ ($P < 0.05$).

Table 5. Effect of days of display by antimicrobial treatment ¹ interaction effect on the least-squares means (\pm SE) for overall color ², percentage discoloration ³ and worst point color ⁴ of ground beef through simulated retail display

Attribute	Treatment	Days of display				
		0	1	2	3	7
<i>Worst point color</i>	CON	2.94 ^{f-i}	1.77 ^{klm}	1.91 ^{klm}	3.41 ^{c-g}	2.37 ^{ijk}
	BEN	2.16 ^{ijkl}	1.62 ^{lm}	4.03 ^{bc}	3.12 ^{e-i}	3.20 ^{d-h}
	PAA	4.38 ^{ab}	3.50 ^{c-f}	1.53 ^{lm}	2.78 ^{g-j}	2.20 ^{jkl}
	POT	4.83 ^a	2.66 ^{hij}	3.45 ^{c-g}	3.87 ^{b-e}	2.03 ^{jkl}
	PROP	3.88 ^{bcd}	1.33 ^m	4.28 ^{ab}	3.37 ^{c-g}	2.70 ^{g-j}
	S.E.	0.27				
	<i>% Discoloration</i>	CON	6.07 ^{abc}	3.33 ^h	4.96 ^{def}	5.79 ^{bcd}
BEN		3.83 ^{gh}	2.35 ^{ij}	6.21 ^{abc}	5.29 ^{c-f}	5.46 ^{cde}
PAA		6.94 ^a	6.06 ^{abc}	1.79 ^{jk}	4.29 ^{fg}	3.21 ^{hi}
POT		6.83 ^a	5.01 ^{def}	5.62 ^{bcd}	5.62 ^{bcd}	4.46 ^{efg}
PROP		6.44 ^{ab}	1.44 ^k	6.96 ^a	5.37 ^{cde}	5.46 ^{cde}
S.E.		0.37				
<i>Overall color</i>		CON	3.94 ^{c-f}	2.34 ^{kl}	3.49 ^{e-h}	3.91 ^{c-g}
	BEN	2.61 ^{jk}	1.96 ^l	4.24 ^{bcd}	3.74 ^{d-g}	3.74 ^{d-g}
	PAA	4.72 ^{ab}	4.44 ^{abc}	2.16 ^{kl}	3.07 ^{hij}	2.74 ^{ijk}
	POT	4.88 ^a	3.44 ^{fgh}	4.07 ^{cde}	4.12 ^{cde}	3.32 ^{ghi}
	PROP	4.00 ^b	1.34 ^m	4.24 ^{bcd}	3.99 ^{c-f}	3.49 ^{e-h}
	S.E.	0.23				

¹ CON= control, BEN=0.1% sodium benzoate, PAA= 0.35% propionic acid solution, POT= 3% potassium lactate/ diacetate solution, PROP= 0.3% sodium propionate.

² Color score: 1=brown; 5= bright red.

³ Percentage discoloration: 1= total discoloration (96-100%) and 7= no discoloration (0%).

⁴ Color score: 1=brown; 5= bright red.

^{a-m} Least-squares means within an attribute bearing different superscripts differ ($P < 0.05$).

Table 6. Effect of days of display by antimicrobial treatment ¹ interaction effect on the least-squares means (\pm SE) for beef odor ², off-odor ³ and patty forming ability ⁴ of ground beef through simulated retail display

Attribute	Treatment	Days of display				
		0	1	2	3	7
<i>Beef Odor</i>						
	CON	5.27 ^{ab}	4.66 ^{bcd}	3.53 ^{e-h}	2.72 ^{hi}	1.45 ^k
	BEN	5.38 ^{ab}	4.00 ^{def}	3.12 ^{fgh}	3.32 ^{e-h}	2.53 ^{hij}
	PAA	4.55 ^{bcd}	4.85 ^{bc}	3.78 ^{d-g}	2.82 ^{ghi}	2.03 ^{ijk}
	POT	5.27 ^{ab}	5.38 ^{ab}	4.03 ^{c-f}	2.82 ^{ghi}	1.53 ^{jk}
	PROP	6.11 ^a	3.88 ^{def}	4.20 ^{cde}	3.32 ^{e-h}	3.12 ^{fgh}
	S.E.	0.37				
<i>Off odor</i>						
	CON	4.77 ^a	3.72 ^{def}	2.88 ^{ghi}	1.85 ^{jkl}	1.13 ^m
	BEN	4.38 ^{abc}	3.93 ^{cde}	2.55 ^{hi}	2.35 ^{h-k}	1.72 ^{klm}
	PAA	4.55 ^{ab}	4.64 ^{ab}	3.38 ^{efg}	2.45 ^{hij}	1.80 ^{j-m}
	POT	4.66 ^{ab}	4.22 ^{bcd}	2.97 ^{gh}	1.85 ^{jkl}	1.30 ^{lm}
	PROP	4.77 ^a	3.27 ^{fg}	3.38 ^{efg}	2.25 ^{ijk}	2.30 ^{h-k}
	S.E.	0.24				
<i>Patty forming ability</i>						
	CON	2.33 ^{b-f}	3.38 ^a	1.90 ^{d-g}	2.23 ^{c-g}	1.84 ^{efg}
	BEN	1.88 ^{efg}	2.83 ^{ab}	1.98 ^{d-g}	1.90 ^{d-g}	1.56 ^g
	PAA	2.25 ^{c-f}	2.33 ^{b-f}	2.15 ^{c-g}	2.15 ^{c-g}	2.40 ^{b-f}
	POT	2.34 ^{b-f}	2.61 ^{bc}	2.01 ^{c-g}	1.81 ^{efg}	1.81 ^{efg}
	PROP	2.44 ^{b-e}	2.00 ^{d-g}	2.48 ^{b-e}	2.56 ^{bcd}	1.73 ^{fg}
	S.E.	0.25				

¹ CON= control, BEN=0.1% sodium benzoate, PAA= 0.35% propionic acid solution, POT= 3% potassium lactate/ diacetate solution, PROP= 0.3% sodium propionate.

² Beef odor score: 1= extremely non beef like and 8= extremely beef like.

³ Off-odor score: 1= extreme off odor and 5= no off odor.

⁴ Patty forming ability score: 6= extreme fragile; 1= extreme cohesive.

^{a-j} Least-squares means within an attribute bearing different superscripts differ ($P < 0.05$).

Table 7. Effect of antimicrobial treatments ¹ applied to beef trimmings on pH of ground beef on day 0 of display

Treatment	pH	S.E.
CON	5.68 ^{ab}	0.03
BEN	5.67 ^b	
PAA	5.68 ^{ab}	
POT	5.48 ^c	
PROP	5.81 ^a	

¹ CON= control, BEN=0.1% sodium benzoate, PAA= 0.35% propionic acid solution, POT= 3% potassium lactate/ diacetate solution, PROP= 0.3% sodium propionate.

^{a-d} Least-squares means within a column bearing different superscripts differ ($P < 0.05$).

Table 8. Effect of antimicrobial treatments ¹ applied to beef trimmings on least-squares means (\pm SE) bind ², tenderness ³, juiciness ⁴, beef flavor ⁵ and off flavor ⁶ characteristics of ground beef patties

Attribute	Treatment					E.M.S.
	CON	BEN	PAA	POT	PROP	
Bind	5.28 ^a	3.78 ^b	4.78 ^{ab}	5.14 ^{ab}	5.46 ^a	2.37
Tenderness	4.78 ^b	6.85 ^a	5.92 ^{ab}	6.00 ^{ab}	7.07 ^a	1.81
Juiciness	3.78 ^b	5.21 ^{ab}	4.35 ^b	5.07 ^{ab}	6.00 ^a	2.01
Beef Flavor	6.35 ^a	6.14 ^{ab}	5.00 ^{ab}	4.71 ^b	5.00 ^{ab}	2.24
Off flavor	4.78 ^a	4.42 ^a	4.07 ^a	3.78 ^a	3.64 ^a	1.26

¹ CON= control, BEN=0.1% sodium benzoate, PAA= 0.35% propionic acid solution, POT= 3% potassium lactate/ diacetate solution, PROP= 0.3% sodium propionate.

² Bind score: 1= extremely fragile and 8= extreme bind.

³ Tenderness score: 1= extremely tough and 8= extremely tender.

⁴ Juiciness score: 1= extremely dry and 8= extremely juicy.

⁵ Beef flavor score: 1= extremely non-beef like and 8= extremely beef like.

⁶ Off flavor score: 1= extreme off flavor and 5= no off flavor.

^{a-b} Least-squares means within an attribute bearing different superscripts differ ($P < 0.05$).

Chapter IV

Electrostatic Atomization of Levulinic, Pyruvic, Hexanoic and Lactic/Citric Acid on Beef Trimmings and Their Effects on Ground Beef Instrumental and Sensory Color, Sensory Aroma and Taste Characteristics.

ABSTRACT

Beef trimmings (80/20) were sprayed with levulinic acid 3% v/v (LEV), pyruvic acid 2.5% v/v (PYR), hexanoic acid 0.5% v/v (HEX), a lactic acid/citric acid commercial blend (LAC) (lactic acid/citric acid 3:2) 2.5% v/v and were compared to an untreated control (CON). Beef trimmings were ground, processed into meat patties and sampled for 7 days. The packages were displayed under simulated retail conditions and trained panelists evaluated meat sensory color, odor and processing abilities on days: 0, 1, 2, 3 and 7 of display. On day 1 of display, CON was similar ($P > 0.05$) in redness (a^*) to the rest of the treatments, except for HEX, which was less ($P < 0.05$) red. On this same day of display, LEV and CON were similar ($P > 0.05$) in sensory overall color. The untreated patties (CON) showed no difference ($P > 0.05$) in beef odor when compared to all treatments. There were no significant differences ($P > 0.05$) in tenderness, juiciness, beef flavor and off flavor among the untreated control (CON) and the rest of the treatments. The results of this project suggest that the use of levulinic and pyruvic organic acids as antimicrobials on beef trimmings prior to grinding may maintain sensory retail display properties such as color and odor in early stages of retail display without affecting sensory taste properties of ground beef patties.

Keywords: beef trimmings; antimicrobials; meat safety; meat color; organic acids

INTRODUCTION

The use of electrostatic coating systems in food has become an emerging technology in the food industry. Electrostatic spraying has been used in the application of smoke flavors, impregnation of bread with vegetable oil and for the application of liquid coating agents to confectionary and chocolate products (Anonymous, 1978). In electrostatic atomization, small droplets are produced and dispersed across the target. Smaller droplets produce more reproducible coating. As the chemical mix in the sprayer leaves the nozzle, it is exposed to a negative charge that is attracted by the positive charges on the sprayed surface. As the charge to mass increases, the droplet size produced by electrostatic spraying reduces, resulting in a better deposition (Wilkerson & Gaultney, 1989).

Organic acids have been used as food additives and preservatives for a long time, extending shelf life and preventing food deterioration. They have been applied in both pre-harvest and post-harvest food production and processing systems (Ricke, 2003). The effectiveness of these acids have been studied by several authors, including Mohan et al. (2011) who reported a significant ($P < 0.05$) reduction of coliforms on beef trimmings prior to grinding when treated with 3% of pyruvic acid. Stivarius et al. (2002) concluded that lactic acid was effective ($P < 0.05$) for reducing *E. coli*, coliforms and aerobic plate count in ground beef processed from beef trimmings: Additionally, Jimenez-Villarreal et al. (2003a) reported that ground beef patties treated with 2 % lactic acid followed by 0.5 % cetylpyridinium chloride were similar ($P > 0.05$) in redness to an untreated control. Another study by Harris et al., (2012) showed a reduction of *E. coli* O157:H7 and *Salmonella* Typhimurium in ground beef up to 0.5 and 0.6 log respectively, when treated with 5 % lactic acid.

Very limited research has been done on the use of hexanoic acid on ground beef, but it has been used as a carcass decontaminant and has a GRAS status of not more than 3 % usage. A study by Zhao, Zhao and Doyle (2009) reported a reduction of *Salmonella* Enteritidis by 3.7 log CFU/cm² on inoculated chicken skin when treated with 0.05 % levulinic acid. Additionally, the same study reported a reduction of *Salmonella* Enteritidis on chicken wings by 2.6 and 4.0 log CFU/g when treated with 2 or 3% levulinic acid, respectively.

Although these antimicrobials have been evaluated as intervention treatments to reduce pathogens and extend shelf life, researchers pay less attention to the effects of antimicrobials on meat color (Mancini & Hunt, 2005). Consumers relate beef quality with a bright red color, which is an indicator of freshness (Renerre & Labadie, 1993). Additionally, according to Smith et al. (2000), meat-purchasing decisions are influenced by color and nearly 15 % of retail beef is reduced in price due to discoloration, corresponding to annual losses of \$1 billion. Therefore, the purpose of this research is to determine the impact of levulinic, pyruvic, hexanoic and a lactic/citric acid commercial blend (lactic acid/citric acid 3:2) on ground beef patty color, odor and taste. All research was conducted on uninoculated beef and the antimicrobials used in this study are approved for use in meat products by the Food Safety and Inspection Service (FSIS) of the US Department of Agriculture and food products by the Food and Drug Administration (FDA).

MATERIALS AND METHODS

Antimicrobial treatment application.

The antimicrobial treatments for this study were levulinic acid 3% v/v (LEV), (Sigma-Aldrich, St. Louis, Missouri, USA) pyruvic acid 2.5% v/v (PYR) (Sigma-Aldrich, St. Louis, Missouri, USA) hexanoic acid 0.5% v/v (HEX) (Sigma-Aldrich, St. Louis, Missouri, USA), and a lactic acid/citric acid blend commercial solution 2.5% v/v (LAC) (lactic acid/citric acid 3:2) (Birko Corporation, Henderson, Colorado, USA) and an untreated control (CON). Distilled water was used for the preparation of the antimicrobial solutions. Beef trimmings (80% lean / 20% fat) were sprayed (ESS; Electrostatic Spraying Systems Inc., Watkinsville, GA, USA) with organic acid antimicrobial treatment solutions at a rate of (~0.1 ml/g) until meat surfaces were saturated. Each treatment was repeated 2 times. Next, beef trimmings were ground twice using an American Eagle AE-G12N grinder (American Eagle Food Machinery Inc., Chicago, IL), with a 3.2 mm plate. After grinding, beef was manually processed into 150 g meat patties, placed on plastic foam trays with absorbent pads and over wrapped with polyvinyl chloride film with an oxygen transmission rate of 14,000 cc/mm²/24h/1atm (Kotch Supplies, Inc., Kansas City, Missouri, USA). The packages were displayed under simulated retail conditions (4° C; warm white fluorescent lightning; 1630 1x; Phillips Inc., Somerset, New Jersey, USA) for 7 days. The pH of ground beef was determined on day 0 of display by homogenizing 2.0 g of ground beef in 20 ml of distilled water 1:10 ratio, and evaluated with an Orion 3 Star pH meter (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Processing properties, sensory color and odor

Eleven trained panelists evaluated sensory color and sensory odor characteristics of ground beef patties and processing abilities (smearing and patty forming ability), on days 0, 1, 2, 3 and 7 of simulated retail display. For each treatment, panelists evaluated patty forming ability (6= extremely fragile, 5= moderately fragile, 4= slightly fragile, 3= slightly cohesive, 2= moderately cohesive, 1= extremely cohesive) and smearing (6= extreme smearing, 5= moderate smearing, 4= slight smearing, 3= slight cut-grind, 2= moderate cut-grind, 1= extreme cut-grind). The ground beef patties were also evaluated for overall color, worst point color and percentage of discoloration under simulated retail display. The panelists evaluated overall color (1= brown, 2= moderately brownish red, 3= slightly brownish red, 4= dull red, 5= bright red), worst point color (1= brown, 2=moderately brownish red, 3= slightly brownish red, 4= dull red, 5= bright red), which defines a discolored area of at least 2 cm in diameter, and percentage of discoloration [1= total discoloration (96-100%), 2= extensive discoloration (80-95%), 3= moderate discoloration (60-79%), 4= modest discoloration (40-59%), 5= small discoloration (20-39%), 6= slight discoloration (1-20%), 7= no discoloration (0%)] on days 0, 1, 2, 3 and 7 of display. Patty packages were then opened and evaluated for beef odor and off odor characteristics. Beef odor was evaluated through an eight point scale as 8= extremely beef like, 7= very beef like, 6= moderately beef like, 5= slightly beef like, 4= slightly non beef like, 3= moderately non beef like, 2= very non beef like, 1= extremely non beef like, and off odor attributes through a five point scale as 5= no off odor, 4= slight off odor, 3= small off odor, 2= moderate off odor, 1= no off odor on the previously described display days.

Instrumental color

Instrumental color of ground beef patties was measured using a Hunter Lab Mini Scan Illuminant A/10° observer (Hunter Associates Laboratory, Inc., Reston, West Virginia, USA) on days: 0, 1, 2, 3 and 7 of display. Samples were evaluated for CIE L^* , a^* and b^* color values. The proportion of oxymyoglobin was estimated in the visible spectrum from 580 to 630 nm reflectance measurements (630 nm/ 580 nm). Saturation index, which describes the brightness of color was calculated $(a^{*2} + b^{*2})^{0.5}$, as was the hue angle $[\tan^{-1} (b^*/a^*)]$, which represents the shift from red to yellow of the ground beef patties.

The colorimeter was standardized each day before sampling using a white tile and a black tile. Three measurements were taken on different areas for each sample.

Sensory taste evaluation

Sensory taste evaluation of ground beef patties was conducted using previously frozen ground beef patties. Specifically, ground beef patties from the different treatments and control were thawed, removed from the foam trays and cooked for sensory panel evaluation in a Blodgett/Zephaire forced air convection oven (Blodgett Oven, Burlington, VT) operating at 163 °C until an internal temperature of 71°C was reached (AMSA, 1995). Patties were cut into squares (2.54 cm x 2.54 cm), wrapped in foil and kept at 49°C in an Alto-Shaam commercial food warmer (Alto-Shaam, Menomonee Falls, WI) for approximately 15 min until served. Ten samples were randomly presented to the panelists using a complete block design. Trained panelists (following the American Meat Science Association Guidelines) (AMSA, 1995) evaluated samples at their own pace, in individual booths under sodium color neutralizing light, one sample at a time. Panelists evaluated bind (1= extremely fragile, 2= very fragile, 3=

moderately fragile, 4= slightly fragile, 5= lightly bind, 6= moderately bind, 7= very strong bind, 8= extremely bind), overall tenderness (1= extremely tough, 2= very tough, 3= moderately tough, 4=slightly tough, 5= slightly tender, 6= moderately tender, 7= very tender, 8= extremely tender), juiciness (1= extremely dry, 2= very dry, 3= moderately dry, 4= slightly dry, 5= slightly juicy, 6= moderately juicy, 7= very juicy, 8=extremely juicy), beef flavor (1= extremely non-beef like, 2= very non-beef like, 3= moderately non-beef like, 4= slightly non-beef like, 5= slightly beef like, 6= moderately beef like, 7= very beef like, 8= extremely beef like) and off flavor intensity on a five point scale (1= extremely off flavor 2= moderate off flavor, 3= small off flavor, 4= slight off flavor, 5= no off flavor).

Statistical Analysis

The experiment was arranged in a completely randomized 5x5 factorial design. Data were analyzed using the General Linear Model procedure of SAS for the main effects of treatment and day and their interactions. Least squares means were generated for significant main effects and interactions and separated using the PDIFF procedure of SAS (SAS Inst. Inc., Cary, North Carolina, USA). For sensory panel data, Tukey's post-hoc analysis test procedure of SAS was conducted for mean separation.

RESULTS AND DISCUSSION

Processing properties and beef odor characteristics

Panelists found CON and LEV to have a greater ($P < 0.05$) particle definition than the rest of the treatments through all seven days of retail display, and found them similar ($P > 0.05$)

to each other (Table 1). These results are similar to those found by Jimenez Villarreal et al. (2003b) and Quilo et al. (2009), where antimicrobial treated patties such as lactic acid and trisodium phosphate had slightly less ($P < 0.05$) particle definition than the untreated control. The HEX treated patties were less ($P < 0.05$) fragile than CON and the rest of the treatments (Table 1). Smearing and patty forming ability values between all treated and untreated patties remained similar ($P > 0.05$) through 2 days of display (Table 2).

The untreated patties (CON) showed no difference ($P > 0.05$) in beef odor when compared to the rest of the treatments. As expected, beef odor of all patties was more intense ($P < 0.05$) on days 0 and 1 of display, and started to decrease from days 2 to 7 of display (Table 2).

Day by treatment interaction effect on instrumental color

Table 3 shows the day of display by treatment interaction effect on CIE L^* value. All treatments were lighter ($P < 0.05$) in color than CON across the seven days of retail display.

The day by treatment interaction effect on CIE a^* value is summarized in Table 3. On day 0 of display, CON was redder ($P < 0.05$) than the rest of the treatments. On day 1 of retail display, CON was similar ($P > 0.05$) in redness to all of the treatments, except for HEX, which was less ($P < 0.05$) red. On days 2 and 3 of display, CON was again redder ($P < 0.05$) than the rest of the treatments. By day 7 of display, all the treatments had similar ($P > 0.05$) redness compared to CON. It can be seen that on this last day of display, ground beef patties were redder ($P < 0.05$) than in early stages of display and the possible justification for this is the accumulation of high levels of water-soluble myoglobin on the surface of the package (purge), resulting in a redder color.

The day by treatment interaction effect on CIE b^* value is also summarized in Table 3. On day 0 of retail display, CON was more ($P < 0.05$) yellow than the rest of the treatments. However, on day 1 of display, treatments HEX and PYR were more ($P < 0.05$) yellow than the rest of the treatments and the untreated control (CON). The CON treatment was similar ($P > 0.05$) in yellowness to LAC and LEV on this same day of display. The HEX treated patties were again the most yellow ($P < 0.05$) on day 2 of display. The CON patties were similar ($P > 0.05$) to those of LAC and PYR but more ($P < 0.05$) yellow than LEV patties as well. On day 3 of display, all treatments were more ($P < 0.05$) yellow than the control (CON), except for LEV, which remained similar ($P > 0.05$) to CON. By day 7 of retail display, all treatments were more ($P < 0.05$) yellow than CON.

Table 4 shows the day of display by treatment interaction effect on hue angle. This attribute represents the shift from red to yellow of the ground beef patties. On day 0 of display, CON had lower ($P < 0.05$) hue angle than the rest of the treatments, but was similar ($P > 0.05$) to LEV and PYR. This explains the greater redness in color of CON patties over the rest of the treatments on day 0 of display. On day 1 of display, HEX had a higher ($P < 0.05$) hue angle than CON and the rest of the treatments. On days 2, 3 and 7 of retail display, CON had a lower ($P < 0.05$) hue angle than the rest of the treatments.

Saturation index refers to the intensity of the a^* and b^* values and is expressed as vividness or brightness. The day by treatment interaction effect on saturation index is summarized in Table 4. On day 0 of display, CON was more ($P < 0.05$) vivid in color than the rest of the treatments. However, on day 1 of display, there were no differences ($P > 0.05$) in saturation index between CON and the rest of the treatments. On days 2 and 3 of display, HEX and CON were similar

($P > 0.05$) to each other and more ($P < 0.05$) vivid than LAC, LEV and PYR. However, on day 7 of display, CON was less ($P < 0.05$) vivid than HEX and LAC but similar ($P > 0.05$) to LEV and PYR.

The estimation of oxymyoglobin content (630/580 nm) is summarized in Table 4. As expected after the $CIE a^*$ results, on day 0 of retail display CON had higher ($P < 0.05$) oxymyoglobin content than the rest of the treatments. However, on day 1 of display CON, LAC, PYR and LEV showed similar ($P > 0.05$) oxymyoglobin content between them and higher ($P < 0.05$) content than HEX. On days 2, 3 and 7 of retail display, CON patties were again higher ($P < 0.05$) in oxymyoglobin proportions than the rest of the treated patties. Our results are similar to those of Jimenez-Villarreal et al. (2003a; 2003b), who also observed an increase in oxymyoglobin proportions on day 7 of display.

Day by treatment interaction effects for worst point color, overall color, percentage discoloration and beef off odor

The day by treatment interaction effect for worst point color is summarized in Table 5. On day 0 of display, CON was similar ($P > 0.05$) in worst point color to LEV but was redder ($P < 0.05$) than LAC, HEX and PYR. Similarly, on day 1 of display, CON, PYR and LEV were similar ($P > 0.05$) and redder ($P < 0.05$) than LAC and HEX. On day 2 of display, all treatments were similar ($P > 0.05$). However, on day 3 of display, CON was redder ($P < 0.05$) than the rest of the treatments. By day 7 of retail display, HEX and PYR were redder ($P < 0.05$) than CON, which remained similar ($P > 0.05$) to LAC and LEV.

The discoloration behavior of the patties can be found in Table 5. On days 0 and 1 of retail display, CON, LEV and PYR remained similar ($P > 0.05$) in discoloration and had less (P

< 0.05) discoloration than LAC and HEX patties. However, on day 2 of display, all treatments remained similar ($P > 0.05$). On day 3 of retail display, CON had less ($P < 0.05$) discoloration than all the treatments. However, by day 7 of display, HEX treated patties had less ($P < 0.05$) discoloration than those of CON, LAC and PYR. On this same day of display, CON remained similar ($P > 0.05$) to all treatments, except for HEX.

The overall color attribute is summarized in Table 5. On day 0 of display, panelists found a redder ($P < 0.05$) color in CON compared to the rest of the treatments. However, on day 1 of display, LEV and CON were similar ($P > 0.05$) in color and redder ($P < 0.05$) than LAC, HEX and PYR. On day 2 of display, panelists found treatments HEX, LEV, and PYR similar ($P > 0.05$) in color and at the same time redder ($P < 0.05$) than CON. The untreated control patties (CON) were again redder ($P < 0.05$) than the rest of the treatments on day 3 of retail display. By day 7 of display, HEX treated patties were redder ($P < 0.05$) than those of CON and LAC and similar ($P > 0.05$) to LEV and PYR patties. These results agree with those of Jimenez-Villarreal et al. (2003b) who found patties from a 2% lactic acid treatment were less ($P < 0.05$) red on days 0 and 1 of display than the untreated control.

The off odor attribute is summarized on Table 5. On day 0 of retail display, CON patties were similar to those of LEV and had less ($P < 0.05$) off odor than those of LAC, HEX and PYR. Similarly, on day 1 of display, CON was similar ($P > 0.05$) to LEV and PYR, and had less ($P < 0.05$) off odor than LAC and HEX. On days 2 and 3 of display, CON was similar ($P < 0.05$) in off odor to all treatments, except for LEV and PYR, which had less ($P < 0.05$) off odor than CON. By day 7 of retail display, all treatments were similar ($P > 0.05$) in off odor.

pH

On day 0 of retail display, pH values of all treated patties remained similar ($P > 0.05$) to those of CON (Table 6). However, several studies have shown that higher pH results in a redder ground beef color (Pohlman et al., 2002; Jimenez-Villarreal et al., 2003a, 2003b; Lim et al., 2004).

Effects of antimicrobial treatments on sensory taste characteristics

Trained panelists found CON patties more ($P < 0.05$) cohesive than those of HEX, LEV and PYR (Table 7). However, CON was similar ($P > 0.05$) in bind to LAC. There were no significant differences ($P > 0.05$) in tenderness, juiciness, beef flavor and off flavor among the untreated control (CON) and the rest of the treatments. Sensory taste panel results show that all treated and non-treated patties tended to have similar sensory attributes.

CONCLUSION

The results of this project suggest that the use of levulinic and pyruvic organic acids as antimicrobials on beef trimmings prior to grinding may maintain sensory retail display properties such as color and odor in early stages of retail display with little effect on sensory taste characteristics of ground beef patties.

Table 1. Effect of antimicrobial treatments ¹ applied to beef trimmings on least-squares means (\pm SE) for grinding ability ², patty forming ability ³ and beef odor ⁴ of ground beef patties

Attribute	Treatment					S.E
	CON	LAC	HEX	LEV	PYR	
<i>Processing abilities</i>						
Grinding ability	2.87 ^c	4.11 ^b	4.52 ^a	2.97 ^c	3.77 ^b	0.13
Patty forming ability	2.76 ^a	2.54 ^a	1.90 ^b	2.47 ^a	2.64 ^a	0.11
<i>Sensory characteristics</i>						
Beef odor	3.30 ^{ab}	3.22 ^b	3.11 ^b	3.62 ^a	3.64 ^a	0.14

¹ CON= control, LAC =2.5% lactic acid/citric acid commercial blend, HEX= 0.5% hexanoic acid, LEV= 3% levulinic acid, PYR= 2.5% pyruvic acid.

² Grinding ability score: 6= extreme smearing; 1= extreme cut-grind.

³ Patty forming ability score: 6= extreme fragile; 1= extreme cohesive.

⁴ Beef odor score: 1= extremely non-beef like and 8= extremely beef like.

^{a-c} Least-squares means within an attribute bearing different superscripts differ ($P < 0.05$).

Table 2. Effect of duration of display on the least-squares means (\pmSE) for grinding ability ¹, patty forming ability ² and beef odor ³ of ground beef						
Attribute	Days of display					S.E
	0	1	2	3	7	
<i>Processing abilities</i>						
Grinding ability	3.52 ^b	3.58 ^{ab}	3.61 ^{ab}	3.59 ^{ab}	3.95 ^a	0.16
Patty forming ability	2.30 ^b	2.46 ^{ab}	2.53 ^{ab}	2.62 ^a	2.40 ^{ab}	0.14
<i>Sensory characteristics</i>						
Beef odor	4.78 ^a	4.73 ^a	3.17 ^b	2.38 ^c	1.82 ^d	0.17
¹ Grinding ability score: 6= extreme smearing; 1= extreme cut-grind. ² Patty forming ability score: 6= extreme fragile; 1= extreme cohesive. ³ Beef odor score: 1= extremely non-beef like and 8= extremely beef like. ^{a-c} Least-squares means within an attribute bearing different superscripts differ ($P < 0.05$).						

Table 3. Effect of days of display by antimicrobial treatment ¹ interaction effect on the least-squares means (\pm SE) for CIE L^* ², CIE b^* ³ and CIE a^* ⁴ of ground beef patties through simulated retail display

Attribute	Treatment	Days of display				
		0	1	2	3	7
<i>CIE L*</i>						
	CON	42.83 ^{gh}	41.52 ^h	39.21 ⁱ	38.06 ⁱ	37.42 ⁱ
	LAC	51.74 ^{ab}	51.60 ^{ab}	49.59 ^{cd}	49.38 ^{cd}	47.80 ^{def}
	HEX	51.94 ^{ab}	53.21 ^a	50.17 ^{bc}	48.73 ^{c-f}	47.45 ^{ef}
	LEV	47.46 ^{ef}	49.04 ^{cde}	47.33 ^{ef}	47.02 ^f	43.70 ^g
	PYR	50.49 ^{bc}	51.70 ^{ab}	49.61 ^{cd}	48.05 ^{def}	43.71 ^g
	S.E.	0.66				
<i>CIE b*</i>						
	CON	21.43 ^a	17.15 ^{lmn}	19.31 ^{gh}	17.79 ^{j-m}	16.69 ⁿ
	LAC	19.45 ^{e-h}	17.16 ^{lmn}	18.88 ^{hi}	19.63 ^{d-h}	21.16 ^{ab}
	HEX	20.41 ^{bcd}	18.34 ^{ij}	20.94 ^{abc}	21.71 ^a	21.45 ^a
	LEV	19.43 ^{e-h}	16.94 ^{mn}	17.33 ^{k-n}	18.02 ^{i-l}	19.40 ^{fgh}
	PYR	20.22 ^{c-f}	18.10 ^{ijk}	19.80 ^{d-g}	20.30 ^{b-e}	18.83 ^{hi}
	S.E.	0.31				
<i>CIE a*</i>						
	CON	24.93 ^{bc}	14.74 ^g	17.22 ^f	23.63 ^c	25.75 ^{ab}
	LAC	18.61 ^{ef}	13.49 ^{ghi}	12.08 ^{hij}	17.96 ^f	25.72 ^{ab}
	HEX	20.60 ^d	11.63 ^{ij}	14.70 ^g	20.08 ^{de}	27.64 ^a
	LEV	20.49 ^{de}	14.52 ^g	10.97 ^j	12.08 ^{hij}	25.06 ^{bc}
	PYR	21.44 ^d	13.89 ^{gh}	12.15 ^{hij}	17.36 ^f	25.52 ^{bc}
	S.E.	0.69				

1 CON= control, LAC=2.5% lactic acid/citric acid commercial blend, HEX= 0. 5% hexanoic acid, LEV= 3% levulinic acid, PYR= 2.5% pyruvic acid.

² CIE L^* : 0= black and 100= white.

³ CIE b^* : -60= blue and +60= yellow.

⁴ CIE a^* : -60= green and +60= red.

^{a-n} Least-squares means within an attribute bearing different superscripts differ ($P < 0.05$).

Table 4. Effect of days of display by antimicrobial treatment ¹ interaction effect on the least-squares means (\pm SE) for hue angle ², saturation index ³ and oxymyoglobin content ⁴ of ground beef patties

Attribute	Treatment	Days of display				
		0	1	2	3	7
<i>Hue Angle</i>						
	CON	40.65 ^{kl}	49.35 ^{efg}	48.53 ^{fg}	36.96 ^m	32.94 ⁿ
	LAC	46.50 ^{ghi}	52.16 ^{cde}	57.39 ^{ab}	47.70 ^{fgh}	39.42 ^{lm}
	HEX	44.80 ^{hij}	57.61 ^{ab}	55.02 ^{bc}	47.39 ^{fgh}	37.81 ^{lm}
	LEV	43.50 ^{ijk}	49.56 ^{def}	57.67 ^{ab}	56.31 ^{ab}	37.94 ^{lm}
	PYR	43.31 ^{jk}	52.53 ^{cd}	58.47 ^a	49.54 ^{def}	36.41 ^m
	S.E.	1.08				
<i>Saturation index</i>						
	CON	32.88 ^b	22.63 ^h	25.91 ^g	29.61 ^{de}	30.69 ^{cd}
	LAC	26.96 ^{fg}	21.92 ^{hi}	22.43 ^h	26.68 ^{fg}	33.32 ^{ab}
	HEX	29.01 ^e	21.74 ^{hi}	25.60 ^g	29.60 ^{de}	34.99 ^a
	LEV	28.24 ^{ef}	22.37 ^h	20.52 ⁱ	21.74 ^{hi}	31.72 ^{bc}
	PYR	29.48 ^{de}	22.82 ^h	23.23 ^h	26.73 ^{fg}	31.72 ^{bc}
	S.E.	0.34				
<i>630nm/580nm</i>						
	CON	3.84 ^b	1.59 ^{ghi}	1.76 ^{fgh}	3.64 ^b	4.70 ^a
	LAC	2.29 ^{de}	1.52 ^{g-j}	1.12 ^{kl}	1.91 ^{efg}	3.53 ^b
	HEX	2.52 ^{cd}	1.16 ^{jkl}	1.29 ^{i-l}	2.05 ^{ef}	3.93 ^b
	LEV	2.66 ^{cd}	1.66 ^{f-i}	1.03 ^l	1.14 ^{jkl}	3.75 ^b
	PYR	2.81 ^c	1.47 ^{h-k}	1.06 ^l	1.70 ^{fgh}	3.85 ^b
	S.E.	0.14				

¹ CON= control, LAC=2.5% lactic acid/citric acid commercial blend, HEX= 0. 5% hexanoic acid, LEV= 3% levulinic acid, PYR= 2.5% pyruvic acid.

² Calculated as $\tan^{-1}(b^*/a^*)$.

³ Calculated as $(a^{*2} + b^{*2})^{0.5}$.

⁴ Calculated as the ratio 630nm/580nm reflectance.

^{a-n} Least-squares means within an attribute bearing different superscripts differ ($P < 0.05$).

Table 5. Effect of days of display by antimicrobial treatment ¹ interaction effect on the least-squares means (\pm SE) for worst point color ², percentage discoloration ³, overall color ⁴ and off-odor ⁵ of ground beef patties through simulated retail display

Attribute	Treatment	Days of display				
		0	1	2	3	7
<i>Worst point color</i>	CON	4.72 ^a	3.07 ^c	1.49 ^{ij}	4.35 ^{ab}	2.35 ^{d-g}
	LAC	3.00 ^c	1.85 ^{g-j}	1.74 ^{g-j}	2.85 ^{cd}	2.60 ^{c-f}
	HEX	3.13 ^c	1.68 ^{hij}	2.05 ^{f-i}	3.07 ^c	3.10 ^c
	LEV	4.40 ^{ab}	2.79 ^{cde}	1.99 ^{f-i}	1.29 ^j	2.52 ^{c-f}
	PYR	4.09 ^b	2.57 ^{c-f}	1.82 ^{g-j}	2.23 ^{e-h}	3.18 ^c
	S.E.	0.22				
<i>% Discoloration</i>	CON	6.63 ^a	4.97 ^{de}	1.60 ^j	6.50 ^{ab}	4.53 ^{de}
	LAC	4.59 ^{de}	3.34 ^{fgh}	2.10 ^{ij}	4.60 ^{de}	5.19 ^{cde}
	HEX	5.18 ^{de}	2.99 ^{ghi}	2.23 ^{ij}	4.93 ^{de}	6.44 ^{ab}
	LEV	6.45 ^{ab}	5.01 ^{de}	2.42 ^{hij}	1.79 ^j	5.53 ^{bcd}
	PYR	6.09 ^{abc}	4.23 ^{ef}	2.42 ^{hij}	3.40 ^{fg}	4.94 ^{de}
	S.E.	0.41				
<i>Overall color</i>	CON	4.95 ^a	3.39 ^{ef}	1.37 ^k	4.50 ^{abc}	3.56 ^{def}
	LAC	3.31 ^{ef}	1.94 ^{ij}	1.81 ^{ijk}	3.44 ^{ef}	4.06 ^{cd}
	HEX	3.38 ^{ef}	1.66 ^{jk}	2.21 ^{hi}	3.66 ^{de}	4.73 ^{ab}
	LEV	4.45 ^{bc}	3.11 ^{fg}	1.95 ^{ij}	1.45 ^{jk}	4.23 ^{bc}
	PYR	4.31 ^{bc}	2.61 ^h	2.18 ^{hi}	2.66 ^{gh}	4.15 ^{bcd}
	S.E.	0.22				
<i>Off-odor</i>	CON	4.47 ^a	4.41 ^{ab}	2.91 ^{fg}	1.65 ^{jk}	1.32 ^k
	LAC	3.63 ^{de}	3.78 ^{cd}	3.07 ^{ef}	2.12 ^{hij}	1.52 ^{jk}
	HEX	3.81 ^{cd}	3.72 ^{cd}	2.42 ^{ghi}	2.15 ^{hij}	1.32 ^k
	LEV	4.13 ^{a-d}	4.22 ^{abc}	3.66 ^{cde}	2.72 ^{fgh}	1.92 ^{ijk}
	PYR	3.86 ^{bcd}	4.03 ^{a-d}	3.57 ^{de}	2.65 ^{fgh}	1.82 ^{ijk}
	S.E.	0.27				

¹ CON= control, LAC=2.5% lactic acid/citric acid commercial blend, HEX= 0. 5% hexanoic acid, LEV= 3% levulinic acid, PYR= 2.5% pyruvic acid.

² Color score: 1=brown; 5= bright red.

³ Percentage discoloration: 1= total discoloration (96-100%) and 7= no discoloration (0%).

⁴ Color score: 1=brown; 5= bright red.

⁵ Off-odor score: 1= extreme off odor and 5= no off odor

^{a-k} Least-squares means within an attribute bearing different superscripts differ ($P < 0.05$).

Table 6. Effect of antimicrobial treatments ¹ applied to beef trimmings on pH of ground beef on day 0 of display

Treatment	pH	S.E.
CON	5.56 ^{ab}	0.02
LAC	5.48 ^b	
HEX	5.64 ^a	
LEV	5.52 ^b	
PYR	5.53 ^b	

¹ CON= control, LAC= 2.5% lactic acid/citric acid commercial blend, HEX= 0.5% hexanoic acid, LEV= 3% levulinic acid, PYR= 2.5% pyruvic acid.

^{a-b} Least-squares means within a column bearing different superscripts differ ($P < 0.05$).

Table 7. Effect of antimicrobial treatments ¹ applied to beef trimmings on least-squares means (\pm SE) bind ², tenderness ³, juiciness ⁴, beef flavor ⁵ and off flavor ⁶ characteristics of ground beef patties

Attribute	Treatment					
	CON	LAC	HEX	LEV	PYR	E.M.S.
Bind	6.18 ^a	5.00 ^{ab}	4.37 ^b	4.37 ^b	3.75 ^b	2.94
Tenderness	4.63 ^a	5.12 ^a	5.62 ^a	5.50 ^a	5.93 ^a	3.04
Juiciness	3.96 ^a	4.06 ^a	4.68 ^a	4.62 ^a	4.93 ^a	2.1
Beef Flavor	6.13 ^a	5.75 ^a	6.00 ^a	5.56 ^a	5.37 ^a	2.29
Off flavor	4.71 ^a	4.64 ^a	4.21 ^a	4.21 ^a	3.92 ^a	0.64

¹ CON= control, LAC=2.5% lactic acid/citric acid commercial blend, HEX= 0.5% hexanoic acid, LEV= 3% levulinic acid, PYR= 2.5% pyruvic acid.

² Bind score: 1= extremely fragile and 8= extreme bind.

³ Tenderness score: 1= extremely tough and 8= extremely tender.

⁴ Juiciness score: 1= extremely dry and 8= extremely juicy.

⁵ Beef flavor score: 1= extremely non-beef like and 8= extremely beef like.

⁶ Off flavor score: 1= extreme off flavor and 5= no off flavor.

^{a-b} Least-squares means within an attribute bearing different superscripts differ ($P < 0.05$).

Chapter V

Electrostatic Antimicrobial Atomization of Sodium Propionate, Sodium Diacetate, Powdered Vinegar and Lemon Juice on Beef Trimmings and Their Effects on Ground Beef Instrumental and Sensory Color, Sensory Aroma and Taste Characteristics.

ABSTRACT

Beef trimmings (80/20) were sprayed with sodium propionate 0.5% w/v (PROP), sodium diacetate 0.25% w/v (DIAC), a commercial powder vinegar blend (NV) (> 90% acetic acid) 1% w/v and lemon juice 35% v/v (LMN). Treatments were compared to an untreated control (CON). Beef trimmings were ground, processed into meat patties and displayed for 7 days. The packages were displayed under simulated retail conditions and trained panelists evaluated meat sensory color, odor and processing abilities on days 0, 1, 2, 3 and 7 of display. On day 0 of display, LMN was redder (a^* ; $P < 0.05$) than CON and the rest of the treatments. CON was less ($P < 0.05$) red (a^*) than all treatments on this same day of display. Trained panelists found all treatments more ($P < 0.05$) red than CON on day 0 of display. CON was similar ($P > 0.05$) in beef odor to all treatments. There were no sensory differences ($P > 0.05$) in juiciness, tenderness, beef flavor and off flavor among the untreated control (CON) and the rest of the treatments. The results of this project suggest that the use of sodium propionate, sodium diacetate, powder vinegar and lemon juice as antimicrobials on beef trimmings prior to grinding may improve sensory properties such as color and odor in early stages of retail display without affecting sensory taste characteristics of ground beef patties.

Keywords: beef trimmings; antimicrobials; meat safety; meat color; lemon juice

INTRODUCTION

Consumption of raw or undercooked bovine origin products is a risk factor for infection with *Escherichia coli* O157:H7, *Salmonella* and other foodborne pathogens (Hussein, 2007). Food is a perfect vehicle by which many pathogens can colonize. Even though food production techniques have improved, food-borne pathogens such as *Salmonella* and *Escherichia coli* seem able to evolve and thrive. From 1982 to 2002, a total of 350 foodborne illness outbreaks were reported in the United States from 49 states. *E coli* O157 infection was responsible of 8,598 cases (Rangel et al., 2005). *Salmonella* spp. are microorganisms able to evolve and adapt to environmental changes. Some stereotypes of *Salmonella* (Typhimurium, Virchow, Derby and Newport) have shown an antimicrobial resistance (European Food Safety Authority, 2006). Many foodborne illnesses are related to *Salmonella* and there have been remarkable outbreaks of this pathogen throughout the years. Additionally, ground beef has been identified as the source for multiple drug resistant (MDR) *Salmonella* Newport and *Salmonella* Typhimurium infections (Varma et al, 2006).

Combinations of potassium lactate and sodium diacetate have shown to be effective for controlling pathogenic bacetria. Knight et al. (2007) reported a reduction of *L. monocytogenes* on vacuum-packaged frankfurters when treated with a 3% potassium lactate- sodium diacetate solution. Additionally, Fik et al. (2008) concluded that this same solution could be used as a good stabilizer of color and texture in minced meat production. Acetic acid has shown antimicrobial properties and has been studied by several authors. Stivarius et al. (2002) reported a reduction ($P < 0.05$) of *E.Coli*, *Salmonella* Typhimurium, coliforms and aerobic plate count by 0.9, 1.47, 1.25 and 1.25 CFU/g, respectively on beef trimmings before grinding when treated with 5% acetic acid. However, ground beef color of these treated beef trimmings tended to be

less red ($P < 0.05$) and contained less oxymyoglobin when compared to an untreated control. Another study conducted by Harris et al. (2006) reported a significant ($P < 0.05$) reduction of *E. coli* O157:H7 and *Salmonella* Typhimurium in ground beef when treated with 2 or 4% acetic acid, without affecting sensory taste characteristics. Citric acid is a weak organic acid with antimicrobial properties that occurs naturally in citrus fruits. Pohlman et al. (2012) concluded that decontamination of *biceps femoris* steaks using a solution of 20% (v/v) hydrochloric / citric acid blend is effective for reducing bacteria. Additionally, Choi et al. (2013) reported a reduction of 5.7 and 5.3 log CFU/ml in the populations of *C. sakazakii* and *S. Typhimurium*, respectively, after treated for 30 minutes with citric acid (80 mM). Citric acid improves the tenderness of meat (Burke and Monahan, 2003) and at the same time reduces beef pathogens such as *Escherichia coli* and *Salmonella*. Different doses of lemon juice (2 ml, 5 ml, 10 ml and 15 ml) have been reported to be effective for reducing ($P < 0.05$) *E. coli* and *Salmonella* Enteritidis on inoculated raw meatballs (Bingol et al., 2011).

Therefore, the purpose of this research is to determine the impact of sodium propionate, sodium diacetate, lemon juice and a commercial powder vinegar blend (> 90% acetic acid) as antimicrobials on ground beef patty color, odor and taste characteristics. All research was conducted on uninoculated beef and the antimicrobials used in this study are approved for use in meat products by the Food and Safety Inspection Services (FSIS) of the U.S. Department of Agriculture and food products by the Food and Drug Administration.

MATERIALS AND METHODS

Antimicrobial treatment application.

The antimicrobial treatments for this study were sodium propionate 0.5% w/v (PROP) (Niacet Corporation, Niagara Falls, NY, USA), sodium diacetate 0.25% w/v (DIAC) (PURAC America Inc., Lincolnshire, IL, USA), a commercial powder vinegar blend solution (> 90% acetic acid) 1% w/v (Kemin Food Technologies Inc., Des Moines, IL, USA), and lemon juice 35% v/v (LMN) (Wal-Mart Stores, Inc., Bentonville, AR, USA). Treatments were compared to an untreated control (CON). Distilled water was used for the preparation of the antimicrobial solutions. Beef trimmings 80 % lean and 20% fat (80/20) were sprayed (ESS; Electrostatic Spraying Systems Inc., Watkinsville, GA, USA) with organic acid antimicrobial treatment solutions at a rate of (~0.1 ml/g) until meat surfaces were saturated. Each treatment was repeated 2 times. Next, beef trimmings were ground twice using an American Eagle AE-G12N grinder (American Eagle Food Machinery Inc., Chicago, IL), with a 3.00 mm plate. After grinding, beef was processed into 150 g meat patties, placed on plastic foam trays with absorbent pads and over wrapped with polyvinyl chloride film with an oxygen transmission rate of 14,000 cc/mm²/24h/1atm (Kotch Supplies, Inc., Kansas City, Missouri, USA). The packages were displayed under simulated retail conditions (4°C; warm white fluorescent lighting; 1630 lx; Phillips Inc., Somerset, New Jersey, USA) for 7 days. The pH of ground beef was determined on days 0 and 7 of display by homogenizing 2.0 g of ground beef in 20 ml of distilled water in a 1:10 ratio, and evaluating with an Orion 3 Star pH meter (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Processing properties, sensory color and odor

Eight trained panelists evaluated sensory color and sensory odor characteristics of ground beef patties and processing abilities including smearing and patty forming ability, on days 0, 1, 2, 3 and 7 of simulated retail display. For each treatment, panelists evaluated patty forming ability (6= extremely fragile, 5= moderately fragile, 4= slightly fragile, 3= slightly cohesive, 2= moderately cohesive, 1= extremely cohesive) and smearing (6= extreme smearing, 5= moderate smearing, 4= slight smearing, 3= slight cut-grind, 2= moderate cut-grind, 1= extreme cut-grind). The ground beef patties were also evaluated for overall color, worst point color and percentage of discoloration under retail display. The panelists evaluated overall color (1= brown, 2= moderately brownish red, 3= slightly brownish red, 4= dull red, 5= bright red), worst point color (1= brown, 2=moderately brownish red, 3= slightly brownish red, 4= dull red, 5= bright red), which defines a discolored area of at least 2 cm in diameter, and percentage of discoloration [1= total discoloration (96-100%), 2= extensive discoloration (80-95%), 3= moderate discoloration (60-79%), 4= modest discoloration (40-59%), 5= small discoloration (20-39%), 6= slight discoloration (1-20%), 7= no discoloration (0%)] on days 0,1,2,3 and 7 of display. Patty packages were then opened and evaluated for beef odor and off odor. Beef odor was evaluated through an eight point scale (8= extremely beef like, 7= very beef like, 6= moderately beef like, 5= slightly beef like, 4= slightly non beef like, 3= moderately non beef like, 2= very non beef like, 1= extremely non beef like) and off odor attributes through a five point scale as (5= no off odor, 4= slight off odor, 3= small off odor, 2= moderate off odor, 1= no off odor) on the previous display days.

Instrumental color

Instrumental color of ground beef patties was measured using a Hunter Lab Mini Scan Illuminant A/10° observer (Hunter Associates Laboratory, Inc., Reston, West Virginia, USA) on days: 0, 1, 2, 3 and 7 of display. Samples were evaluated for *CIE L**, *a** and *b** color values. The proportion of oxymyoglobin was estimated in the visible spectrum from 580 to 630 nm reflectance measurements (630 nm/ 580 nm). Saturation index, which describes the brightness of color was calculated $(a^{*2} + b^{*2})^{0.5}$, as was the hue angle $[\tan^{-1} (b^*/a^*)]$, which represents the shift from red to yellow of the ground beef patties.

The colorimeter was standardized every day before sampling using a white tile and a black tile. Three measurements were taken on different areas for each sample.

Sensory taste evaluation

Sensory evaluation of ground beef patties was conducted on previously frozen patties. Specifically, ground beef patties of the different treatments and control were thawed, removed from the foam trays and cooked for the sensory panel in a Blodgett/Zephaire forced air convection oven (Blodgett Oven, Burlington, VT) operating at 163 °C until an internal temperature of 71°C was reached (AMSA, 1995). Patties were cut into squares (2.54 cm x 2.54 cm), wrapped in foil and kept at 49°C in an Alto-Shaam commercial food warmer (Alto-Shaam, Menomonee Falls, WI) for approximately 15 min until served. Ten samples were randomly presented to the panelists using a complete block design. Trained panelists (following the American Meat Science Association Guidelines) (AMSA, 1995) evaluated samples in individual sodium color neutralizing booths at their own pace, indicating whenever the next sample was

required. Panelists evaluated bind (1= extremely fragile, 2= very fragile, 3= moderately fragile, 4= slightly fragile, 5= lightly bind, 6= moderately bind, 7= very strong bind, 8= extremely bind), overall tenderness (1= extremely tough, 2= very tough, 3= moderately tough, 4=slightly tough, 5= slightly tender, 6= moderately tender, 7= very tender, 8= extremely tender), juiciness (1= extremely dry, 2= very dry, 3= moderately dry, 4= slightly dry, 5= slightly juicy, 6= moderately juicy, 7= very juicy, 8=extremely juicy), beef flavor (1= extremely non-beef like, 2= very non-beef like, 3= moderately non-beef like, 4= slightly non-beef like, 5= slightly beef like, 6= moderately beef like, 7= very beef like, 8= extremely beef like) and off flavor intensity on a five point scale (1= extremely off flavor 2= moderate off flavor, 3= small off flavor, 4= slight off flavor, 5= no off flavor).

Statistical Analysis

The experiment was arranged in a completely randomized 5x5 factorial design. Data were analyzed using the General Linear Model for day by treatment, day and treatment effects. Least squares means were generated for significant ($P < 0.05$) interaction and main effects and separated using the PDIFF procedure of SAS (SAS Inst. Inc., Cary, North Carolina, USA). For sensory panel data a panelist term was added to the model and Tukey's post-hoc analysis test procedure of SAS was conducted for means separation.

RESULTS AND DISCUSSION

Processing properties, beef odor, off odor and lightness

Panelists found CON and LMN to have a greater ($P < 0.05$) particle definition than the rest of the treatments through all seven days of retail display, and found them similar ($P > 0.05$) to each other as well (Table 1). These results agree with those of Jimenez-Villarreal et al. (2003) and Quilo et al. (2009), where some organic acid treated patties such as lactic acid, showed less ($P < 0.05$) particle definition than the untreated control. Overall, the greatest ($P < 0.05$) particle definition tended to occur on day 3 of retail display (Table 2). The untreated patties (CON) were less ($P < 0.05$) fragile than the rest of the treated patties (Table 1). Additionally, all patties turned out to be less ($P < 0.05$) fragile on day 0 of retail display.

The CON treatment was similar ($P > 0.05$) in beef odor to all treatments (Table 1). As expected, beef odor of all patties was more intense ($P < 0.05$) on day 0 of display, and started to decrease from days 1 to 3 of retail display (Table 2). Treatments LMN and PROP were similar ($P > 0.05$) to NV and had less ($P < 0.05$) off odor than CON and DIAC. However, the untreated patties (CON) were similar ($P > 0.05$) in this attribute to those of DIAC and NV (Table 1). All ground beef patties had less ($P < 0.05$) off odor on day 0 of retail display, and as expected, off odor increased with display time (Table 2).

Instrumental lightness ($CIE L^*$) of beef patties is shown on Tables 1 and 2. Treatment DIAC was lighter ($P < 0.05$) in color than the rest of the treatments and the untreated control (CON), which was ($P < 0.05$) darker in color than all of the treatments. Overall, all patties were

lighter ($P < 0.05$) on days 0 and 1 of display and darker ($P < 0.05$) by days 3 and 7 of retail display.

Day by treatment interaction effect on instrumental color

The day by treatment interaction effect on meat redness (a^* value) is summarized in Table 3. On day 0 of display, LMN was redder ($P < 0.05$) than CON and the rest of the treatments. The CON treatment was less ($P < 0.05$) red than all treatments on this same day of display. However, on days 1 and 2 of display, CON was redder ($P < 0.05$) than the rest of the treatments. On day 3 of display, all treatments were similar ($P > 0.05$) to CON. By day 7 of display, all treated patties were similar ($P > 0.05$) to those of CON, except for PROP, which was redder ($P < 0.05$).

The day by treatment interaction effect on CIE b^* value is also summarized in Table 3. On day 0 of retail display, LMN was more ($P < 0.05$) yellow than NV, PROP and CON, which was the least ($P < 0.05$) yellow. Similarly, on day 1 of display, all treatments were more ($P < 0.05$) yellow than CON and remained similar ($P > 0.05$) between them. All treated patties were again more yellow ($P < 0.05$) than CON on day 2 of display, except for those of LMN, which were similar ($P > 0.05$). On days 3 and 7 of retail display, all treatments were more ($P < 0.05$) yellow than CON.

The day of display by treatment interaction effect on hue angle is shown in Table 4. This attribute represents the shift from red to yellow of the ground beef patties. On day 0 of display, all treatments were similar ($P > 0.05$) to CON. From days 1 to 3 of display, CON had lower ($P < 0.05$) hue angle than the rest of the treatments. By day 7 of display, CON was similar ($P > 0.05$) to LMN and NV and had a lower ($P < 0.05$) hue angle than treatments PROP and DIAC.

The intensity of the *CIE* a^* and b^* values (saturation index) represents the vividness or brightness of patties. The day by treatment interaction effect on saturation index is also summarized in Table 4. On day 0 of display, all treatments were more ($P < 0.05$) vivid in color than CON, especially LMN, which was the most ($P < 0.05$) vivid in color. On days 1 and 2 of display, CON was more vivid ($P < 0.05$) than the rest of the treatments. On day 3 of display, CON was similar ($P > 0.05$) to all treatments, except for PROP, which was more ($P < 0.05$) vivid. By day 7 of retail display, all treated patties were more ($P < 0.05$) vivid than those of CON.

The estimation of oxymyoglobin content (630/580 nm) is summarized in Table 4. On day 0 of retail display all treatments were higher ($P < 0.05$) in oxymyoglobin content than the untreated control (CON). The LMN treated patties had the highest ($P < 0.05$) oxymyoglobin estimation content on this day of display. However, on days 1 to 3 of display CON was higher ($P < 0.05$) in oxymyoglobin proportions than NV, PROP, DIAC and LMN. By day 7 of retail display, CON patties were higher ($P < 0.05$) in oxymyoglobin proportions than those of DIAC, but similar ($P > 0.05$) to those of LMN, NV and PROP. These results are different from those of Stivarius et al. (2002) who found a less intense ($P < 0.05$) beef odor, less ($P < 0.05$) redness and less ($P < 0.05$) oxymyoglobin content with 5% acetic acid treated patties when compared to an untreated control among 7 days of display. In this study, 1% powder acetic acid treated patties had similar ($P > 0.05$) beef odor, more ($P < 0.05$) redness and higher ($P < 0.05$) oxymyoglobin content than those of the untreated control (CON). A possible explanation for this result is the difference in concentration of both acetic acid antimicrobial solutions.

Day by treatment interaction effects for worst point color, overall color and percentage discoloration

The discoloration behavior of the patties (Table 5) followed a similar pattern to that of the overall color. On day 0 of retail display, DIAC, LMN, NV and PROP remained similar ($P > 0.05$) and had less ($P < 0.05$) discoloration than CON. On days 1 and 2 of retail display, CON had less ($P < 0.05$) discoloration than all the treatments. However, on day 3 of display, CON remained similar ($P > 0.05$) to DIAC and PROP, and had less ($P < 0.05$) discoloration than LMN and NV. By day 7 of display, untreated CON patties were similar ($P > 0.05$) to all treated patties, except for those of PROP, which had less ($P < 0.05$) discoloration than CON.

The day by treatment interaction effect for worst point color is also summarized in Table 5. On day 0 of display, DIAC, LMN, NV and PROP treated patties were redder ($P < 0.05$) in worst point color than those of the untreated control (CON). From days 1 to 3 of display, CON was redder ($P < 0.05$) than the rest of the treatments. By day 7 of retail display, CON remained similar ($P > 0.05$) to DIAC, LMN, NV and PROP.

The overall color attribute is summarized in Table 5. On day 0 of display, panelists found treatments LMN, NV, DIAC and PROP similar ($P > 0.05$) and at the same time redder ($P < 0.05$) than CON. However, on days 1 to 3 of display, CON was (redder ($P < 0.05$) than DIAC, LMN, NV and PROP. On day 7 of display, panelists found all treatments and CON similar ($P > 0.05$) in overall color.

pH

On day 0 of retail display, CON had similar ($P > 0.05$) pH value to PROP and at the same time higher ($P < 0.05$) than those of LMN, NV and DIAC (Table 6). Overall, by day 7 of display, pH values of all treatments were higher ($P < 0.05$) than those of day 0. A justification for this is that soluble proteins in ground beef such as actin, myosin and the complex (actomyosin) act as buffers and neutralize pH of meat over time. However, on day 7 of retail display, NV had a higher ($P > 0.05$) pH value than the rest of the treatments. The pH value of CON was similar ($P > 0.05$) to PROP and higher ($P < 0.05$) than LMN and DIAC on this same day of display (7).

Effects of antimicrobial treatments on sensory taste characteristics

Trained panelists found CON patties to have more ($P < 0.05$) bind than those of DIAC, LMN, NV and PROP. However, there were no significant differences ($P > 0.05$) in tenderness, juiciness, beef flavor and off flavor among the untreated control (CON) and the rest of the treatments (Table 7).

CONCLUSION

The results of this project suggest that the use of sodium propionate, sodium diacetate, powder vinegar and lemon juice as antimicrobials on beef trimmings prior to grinding may improve sensory properties such as color and odor, and instrumental color on day 0 of retail display without affecting sensory taste characteristics of ground beef patties.

Table 1. Effect of antimicrobial treatments ¹ applied to beef trimmings on least-squares means (\pm SE) for grinding ability ², patty forming ability ³, beef odor ⁴, off odor ⁵ and CIE L* ⁶ value of ground beef patties

Attribute	Treatment					S.E
	CON	DIAC	LMN	NV	PROP	
Processing abilities						
<i>Grinding ability</i>	2.84 ^c	4.74 ^{ab}	3.21 ^c	4.77 ^a	4.34 ^b	0.16
<i>Patty forming ability</i>	1.64 ^c	3.04 ^a	2.37 ^b	3.04 ^a	2.50 ^b	0.14
Sensory characteristics						
<i>Beef odor</i>	4.07 ^a	3.83 ^a	4.13 ^a	4.05 ^a	3.92 ^a	0.20
<i>Off-odor</i>	2.72 ^b	3.20 ^b	3.24 ^a	2.97 ^{ab}	3.03 ^a	0.11
Instrumental color						
<i>CIE L*</i>	43.39 ^d	55.48 ^a	50.00 ^c	51.31 ^b	52.39 ^b	0.44

¹ CON= control, DIAC= 0.25% sodium diacetate, LMN= 35% lemon juice, NV= 1% powder vinegar blend, PROP= 0.5 % sodium propionate.

² Grinding ability score: 6= extreme smearing; 1= extreme cut-grind.

³ Patty forming ability score: 6= extreme fragile; 1= extreme cohesive.

⁴ Beef odor score: 1= extremely non-beef like and 8= extremely beef like.

⁵ Off-odor score: 1= extreme off odor and 5= no off odor.

⁶ CIE L*: 0= black and 100= white.

^{a-d} Least-squares means within an attribute bearing different superscripts differ ($P < 0.05$).

Table 2. Effect of duration of display on the least-squares means (\pm SE) for grinding ability ¹, patty forming ability ², beef odor ³, off odor ⁴ and *CIE L ⁵ value of ground beef patties**

Attribute	Days of display					S.E
	0	1	2	3	7	
Processing abilities						
<i>Grinding ability</i>	4.20 ^{ab}	3.80 ^{bc}	4.06 ^{ab}	3.57 ^c	4.29 ^a	0.17
<i>Patty forming ability</i>	2.17 ^b	2.77 ^a	2.77 ^a	2.46 ^{ab}	2.41 ^{ab}	0.14
Sensory characteristics						
<i>Beef odor</i>	4.96 ^a	4.21 ^{bc}	4.27 ^b	2.88 ^d	3.68 ^{bc}	0.22
<i>Off-odor</i>	4.89 ^a	3.56 ^b	3.19 ^c	1.90 ^d	1.62 ^d	0.12
Instrumental color						
<i>Lightness</i>	52.45 ^a	53.14 ^a	50.74 ^b	48.12 ^c	48.11 ^c	0.44

¹ Grinding ability score: 6= extreme smearing; 1= extreme cut-grind.

² Patty forming ability score: 6= extreme fragile; 1= extreme cohesive.

³ Beef odor score: 1= extremely non-beef like and 8= extremely beef like.

⁴ Off-odor score: 1= extreme off odor and 5= no off odor.

⁵ *CIE L**: 0= black and 100= white.

^{a-d} Least-squares means within an attribute bearing different superscripts differ ($P < 0.05$).

Table 3. Effect of days of display by antimicrobial treatment ¹ interaction effect on the least-squares means (\pm SE) for *CIE a ² and *CIE b** ³ values of ground beef through simulated retail display**

Attribute	Treatment	Days of display				
		0	1	2	3	7
<i>CIE a*</i>						
	CON	16.55 ^{jk}	22.46 ^{def}	24.79 ^{a-d}	23.85 ^{b-e}	22.55 ^{c-f}
	DIAC	22.99 ^{b-f}	11.67 ^l	15.65 ^k	23.79 ^{b-e}	24.26 ^{bcd}
	LMN	27.14 ^a	17.02 ^{ijk}	18.82 ^{hij}	20.50 ^{fgh}	24.16 ^{b-e}
	NV	22.82 ^{b-f}	11.98 ^l	17.80 ^{h-k}	23.34 ^{b-e}	24.23 ^{bcd}
	PROP	21.53 ^{efg}	11.85 ^l	19.29 ^{ghi}	25.25 ^{abc}	25.41 ^{ab}
	S.E.	0.96				
<i>CIE b*</i>						
	CON	15.69 ⁱ	16.81 ^{ghi}	16.51 ^{ghi}	16.07 ^{hi}	14.34 ^j
	DIAC	22.23 ^{ab}	19.07 ^{cd}	18.93 ^{cd}	19.62 ^{cd}	18.98 ^{cd}
	LMN	23.06 ^a	18.76 ^{c-f}	17.27 ^{gh}	17.56 ^{fg}	16.71 ^{ghi}
	NV	21.62 ^b	18.85 ^{cd}	18.57 ^{def}	18.73 ^{c-f}	17.63 ^{efg}
	PROP	21.11 ^b	18.78 ^{cde}	18.87 ^{cd}	19.86 ^c	19.01 ^{cd}
	S.E.	0.43				

¹ CON= control, DIAC= 0.25% sodium diacetate, LMN= 35% lemon juice, NV= 1% powder vinegar blend, PROP= 0.5 % sodium propionate.

² *CIE a**: -60= green and +60= red.

³ *CIE b**: -60= blue and +60= yellow.

^{a-1} Least-squares means within an attribute bearing different superscripts differ ($P < 0.05$).

Table 4. Effect of days of display by antimicrobial treatment ¹ interaction effect on the least-squares means (\pm SE) for hue angle ², saturation index ³ and oxymyoglobin content ⁴ of ground beef patties

Attribute	Treatment	Days of display				
		0	1	2	3	7
<i>Hue Angle</i>						
	CON	43.45 ^{def}	37.03 ^{h-k}	33.64 ^{kl}	34.16 ^{kl}	32.40 ^l
	DIAC	44.03 ^{c-f}	58.50 ^a	50.51 ^b	39.48 ^{ghi}	38.04 ^{g-j}
	LMN	40.37 ^{fgh}	47.79 ^{bc}	43.32 ^{def}	41.11 ^{efg}	34.64 ^{jkl}
	NV	43.48 ^{def}	57.53 ^a	46.98 ^{bcd}	38.75 ^{ghi}	36.01 ^{i-l}
	PROP	44.41 ^{cde}	57.75 ^a	45.44 ^{cd}	38.16 ^{g-j}	36.79 ^{h-k}
	S.E.	1.35				
<i>Saturation index</i>						
	CON	22.81 ^k	28.08 ^{e-h}	29.80 ^{b-e}	28.77 ^{d-g}	26.74 ^{g-j}
	DIAC	31.98 ^b	22.36 ^k	24.58 ^{jk}	30.85 ^{bcd}	30.80 ^{bcd}
	LMN	35.62 ^a	25.34 ^{ij}	25.75 ^{hij}	27.17 ^{f-i}	29.39 ^{c-f}
	NV	31.44 ^{bc}	22.34 ^k	25.93 ^{hij}	29.93 ^{b-e}	29.97 ^{b-e}
	PROP	30.15 ^{b-e}	22.21 ^k	27.16 ^{f-i}	32.13 ^b	31.74 ^{bc}
	S.E.	0.85				
<i>630nm/580nm</i>						
	CON	1.95 ^{jk}	3.20 ^{d-g}	3.95 ^{ab}	4.09 ^a	3.61 ^{a-d}
	DIAC	2.67 ^{hi}	1.08 ^l	1.51 ^{kl}	2.90 ^{fgh}	3.09 ^{e-h}
	LMN	3.73 ^{abc}	1.87 ^{jk}	2.21 ^{ij}	2.64 ^{hi}	3.42 ^{c-f}
	NV	2.88 ^{gh}	1.10 ^l	1.99 ^{jk}	3.02 ^{e-h}	3.35 ^{c-g}
	PROP	2.60 ^{hi}	1.09 ^l	2.19 ^{ij}	3.30 ^{c-g}	3.44 ^{b-e}
	S.E.	0.18				

¹ CON= control, DIAC= 0.25% sodium diacetate, LMN= 35% lemon juice, NV= 1% powder vinegar blend, PROP= 0.5 % sodium propionate.

² Calculated as $\tan^{-1}(b^*/a^*)$.

³ Calculated as $(a^{*2} + b^{*2})^{0.5}$.

⁴ Calculated as the ratio 630nm/580nm reflectance.

^{a-1} Least-squares means within an attribute bearing different superscripts differ ($P < 0.05$).

Table 5. Effect of days of display by antimicrobial treatment ¹ interaction effect on the least-squares means (\pm SE) for overall color ², percentage discoloration ³ and worst point color of ground beef patties through simulated retail display

Attribute	Treatment	Days of display				
		0	1	2	3	7
<i>% Discoloration</i>	CON	4.54 ^{fg}	6.27 ^{ab}	6.15 ^{abc}	6.33 ^{ab}	4.40 ^{fg}
	DIAC	6.83 ^a	1.37 ⁱ	3.74 ^{gh}	5.69 ^{bcd}	5.26 ^{e-f}
	LMN	6.83 ^a	4.33 ^{fg}	4.32 ^{fgh}	4.83 ^{def}	4.74 ^{d-g}
	NV	6.54 ^{ab}	1.61 ⁱ	3.32 ^h	4.26 ^{fgh}	4.80 ^{def}
	PROP	6.83 ^a	1.32 ⁱ	4.57 ^{efg}	5.61 ^{bcd}	5.57 ^{b-e}
	S.E.	0.38				
<i>Worst point color</i>	CON	2.85 ^d	4.16 ^{ab}	4.04 ^{ab}	3.64 ^{bc}	2.30 ^{d-g}
	DIAC	4.35 ^a	1.16 ⁱ	1.62 ^{ghi}	2.71 ^d	2.79 ^d
	LMN	4.71 ^a	2.66 ^d	2.70 ^d	2.50 ^{def}	1.79 ^{ghi}
	NV	4.07 ^{ab}	1.33 ^{hi}	1.87 ^{fgh}	2.57 ^{def}	1.95 ^{e-h}
	PROP	4.21 ^{ab}	1.38 ^{hi}	2.62 ^{de}	2.93 ^d	2.98 ^{cd}
	S.E.	0.26				
<i>Overall color</i>	CON	2.93 ^{bcd}	4.55 ^a	4.51 ^a	4.22 ^a	3.17 ^{bcd}
	DIAC	4.57 ^a	1.22 ^e	2.67 ^d	3.50 ^b	3.34 ^{bc}
	LMN	4.85 ^a	3.11 ^{bcd}	3.34 ^{bc}	3.36 ^{bc}	3.26 ^{bcd}
	NV	4.22 ^a	1.66 ^e	2.76 ^{cd}	3.43 ^b	3.17 ^{bcd}
	PROP	4.22 ^a	1.55 ^e	3.51 ^b	3.50 ^b	3.51 ^b
	S.E.	0.24				

¹ CON= control, DIAC= 0.25% sodium diacetate, LMN= 35% lemon juice, NV= 1% powder vinegar blend, PROP= 0.5 % sodium propionate.

² Percentage discoloration: 1= total discoloration (96-100%) and 7= no discoloration (0%).

³ Color score: 1=brown; 5= bright red.

⁴ Color score: 1=brown; 5= bright red.

^{a-i} Least-squares means within an attribute bearing different superscripts differ ($P < 0.05$).

Table 6. Effect of antimicrobial treatments ¹ applied to beef trimmings on pH of ground beef on days 0 and 7 of display

Treatment	Days of display	
	0	7
CON	5.57 ^d	6.89 ^b
DIAC	5.44 ^e	6.78 ^c
LMN	5.46 ^e	6.77 ^c
NV	5.44 ^e	7.04 ^a
PROP	5.49 ^{de}	6.89 ^b
S.E.	0.02	

¹ CON= control, DIAC= 0.25% sodium diacetate, LMN= 35% lemon juice, NV= 1% powder vinegar blend, PROP= 0.5 % sodium propionate.

^{a-e} Least- squares means within a column bearing different superscripts differ ($P < 0.05$).

Table 7. Effect of antimicrobial treatments ¹ applied to beef trimmings on least-squares means (\pm SE) bind ², tenderness ³, juiciness ⁴, beef flavor ⁵ and off flavor ⁶ characteristics of ground beef patties

Attribute	Treatment					
	CON	DIAC	LMN	NV	PROP	E.M.S.
<i>Bind</i>	6.70 ^a	3.80 ^b	2.90 ^b	2.70 ^b	2.70 ^b	2.73
<i>Tenderness</i>	5.30 ^a	6.40 ^a	7.00 ^a	5.60 ^a	6.80 ^a	1.83
<i>Juiciness</i>	4.80 ^a	5.20 ^a	5.30 ^a	4.10 ^a	5.50 ^a	2.19
<i>Beef Flavor</i>	5.60 ^a	5.80 ^a	6.20 ^a	5.60 ^a	5.50 ^a	1.78
<i>Off flavor</i>	4.50 ^a	4.80 ^a	4.70 ^a	4.40 ^a	4.50 ^a	0.42

¹ CON= control, DIAC= 0.25% sodium diacetate, LMN= 35% lemon juice, NV= 1% powder vinegar blend, PROP= 0.5 % sodium propionate.

² Bind score: 1= extremely fragile and 8= extreme bind.

³ Tenderness score: 1= extremely tough and 8= extremely tender.

⁴ Juiciness score: 1= extremely dry and 8= extremely juicy.

⁵ Beef flavor score: 1= extremely non-beef like and 8= extremely beef like.

⁶ Off flavor score: 1= extreme off flavor and 5= no off flavor.

^{a-b} Least-squares means within a row bearing different superscripts differ ($P < 0.05$).

Chapter VI

Conclusion

The overall results of this project suggest that electrostatic atomization of antimicrobials on beef trimmings, result in a better deposition and therefore, a more effective and uniform coating. However, some antimicrobial treatments showed better results than others. Of the 16 different antimicrobial treatments applied among four different projects, 3% fumaric, malic, octanoic, decanoic and levulinic acids, 0.35% propionic acid solution, 2.5% pyruvic acid and 35% lemon juice treatments applied on beef trimmings prior to grinding may improve sensory properties such as sensory color, odor, and instrumental color with minimal impact on sensory taste characteristics of ground beef patties.

Consumption of undercooked or pink beef patties has been associated with the risk of foodborne illnesses and the application of this spraying technology and antimicrobials on the meat industry can enhance meat and consumers safety.

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APPENDICES

APPENDIX I

Meat Sensory Panel Ballot Ground Beef Patties

Do not hesitate to request another sample if you feel the first one you tasted may not be representative

Date-----

Judge-----

Sample #	Bind	Overall Tenderness	Juiciness	Beef Flavor	Off Flavor
	Bind (1-8 Scale) 1= extremely fragile 2= very fragile 3= moderately fragile 4= slightly fragile 5= lightly bind 6= moderately bind 7= very strong bind 8= extremely bind	Tenderness (1-8 scale) 1= extremely tough 2= very tough 3= moderately tough 4= slightly tough 5= slightly tender 6= moderately tender 7= very tender 8=extremely tender	Juiciness (1-8 scale) 1= extremely dry 2= very dry 3= moderately dry 4= slightly dry 5= slightly juicy 6= moderately juicy 7= very juicy 8=extremely juicy	Beef flavor (1-8 scale) 1= extremely non-beef like 2= very non-beef like 3= moderately non-beef like 4= slightly non-beef like 5= slightly beef like 6= moderately beef like 7= very beef like 8= extremely beef like	Off flavor (1-5 scale) 1= extremely off flavor 2= moderate off flavor 3= small off flavor 4= slight off flavor 5= no off flavor

APPENDIX 3

Hunter Lab MiniScan operation

I. Pre-operation

1. Before taking any readings or measurements, allow MiniScan adapt to the working conditions (humidity, temperature) of the respective working location for at least 20 - 30 minutes.

II. Standarization

1. Check the tiles and sample port are clean, and if necessary, proceed to clean with isopropyl alcohol.
2. Screen will light up and display information. Follow standardization instructions until the words "READY TO READ BLACK TILE" appear.
3. Hold the center of the black glass and flat to the MiniScan sample port.
4. Press the READ key in the middle of the MiniScan, represented by a "lightening bulb".
5. A beep sound will follow the click after pressing the key, indicating the black tile reading. After this step, the words "READY TO READ WHITE TILE" will be displayed at the screen.
6. Place the white tile at sample port and press the READ key. MiniScan will measure the white tile and return to the last utilized screen.

III. Capturing data readings.

1. Place MiniScan in flat position with the sample.
2. Press the READ key (lightening bulb key). There will be a flash, a beep, and the previous log numbers disappear, showing the new reading values.
3. Continue to take color readings and store them all when finished.

APPENDIX 4



Office of Research Compliance
Institutional Review Board

April 21, 2014

MEMORANDUM

TO: Jorge Marcos
Palika Dias Morse
Fred Pohlman

FROM: Ro Windwalker
IRB Coordinator

RE: PROJECT CONTINUATION

IRB Protocol #: 13-04-695

Protocol Title: *Antimicrobial Treatment Effects on Ground Beef Instrumental and Sensory Color, Sensory Aroma and Taste Characteristics*

Review Type: EXEMPT EXPEDITED FULL IRB

Previous Approval Period: Start Date: 05/09/2013 Expiration Date: 05/08/2014

New Expiration Date: 05/08/2015

Your request to extend the referenced protocol has been approved by the IRB. If at the end of this period you wish to continue the project, you must submit a request using the form *Continuing Review for IRB Approved Projects*, prior to the expiration date. Failure to obtain approval for a continuation on or prior to this new expiration date will result in termination of the protocol and you will be required to submit a new protocol to the IRB before continuing the project. Data collected past the protocol expiration date may need to be eliminated from the dataset should you wish to publish. Only data collected under a currently approved protocol can be certified by the IRB for any purpose.

This protocol has been approved for 15 total participants. If you wish to make *any* modifications in the approved protocol, including enrolling more than this number, you must seek approval *prior to* implementing those changes. All modifications should be requested in writing (email is acceptable) and must provide sufficient detail to assess the impact of the change.

If you have questions or need any assistance from the IRB, please contact me at 210 Administration Building, 5-2208, or irb@uark.edu.

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Voice (479) 575-2208 • Fax (479) 575-3846 • Email irb@uark.edu

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UNIVERSITY OF ARKANSAS

Office of Research Compliance
Institutional Review Board

July 23, 2014

MEMORANDUM

TO: Jorge Marcos
Palika Dias Morse
Fred Pohlman

FROM: Ro Windwalker
IRB Coordinator

RE: PROJECT CONTINUATION

IRB Protocol #: 13-07-029

Protocol Title: *Impact of Sodium Benzoate, Sodium Propionate, Bromine and Propionic Acid on Ground Beef Instrumental and Sensory Color, Sensory Aroma and Taste Characteristics*

Review Type: EXEMPT EXPEDITED FULL IRB

Previous Approval Period: Start Date: 08/02/2013 Expiration Date: 08/01/2014

New Expiration Date: 08/01/2015

Your request to extend the referenced protocol has been approved by the IRB. If at the end of this period you wish to continue the project, you must submit a request using the form *Continuing Review for IRB Approved Projects*, prior to the expiration date. Failure to obtain approval for a continuation on or prior to this new expiration date will result in termination of the protocol and you will be required to submit a new protocol to the IRB before continuing the project. Data collected past the protocol expiration date may need to be eliminated from the dataset should you wish to publish. Only data collected under a currently approved protocol can be certified by the IRB for any purpose.

This protocol has been approved for 15 total participants. If you wish to make *any* modifications in the approved protocol, including enrolling more than this number, you must seek approval *prior to* implementing those changes. All modifications should be requested in writing (email is acceptable) and must provide sufficient detail to assess the impact of the change.

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UNIVERSITY OF ARKANSAS

Office of Research Compliance
Institutional Review Board

October 3, 2014

MEMORANDUM

TO: Jorge Marcos
Fred Pohlman

FROM: Ro Windwalker
IRB Coordinator

RE: New Protocol Approval

IRB Protocol #: 14-09-134

Protocol Title: *Antimicrobial Effects on Ground Beef Patties Instrumental and Sensory Colors, Sensory Aroma and Taste Characteristics*

Review Type: EXEMPT EXPEDITED FULL IRB

Approved Project Period: Start Date: 10/03/2014 Expiration Date: 10/02/2015

Your protocol has been approved by the IRB. Protocols are approved for a maximum period of one year. If you wish to continue the project past the approved project period (see above), you must submit a request, using the form *Continuing Review for IRB Approved Projects*, prior to the expiration date. This form is available from the IRB Coordinator or on the Research Compliance website (<http://vpred.uark.edu/210.php>). As a courtesy, you will be sent a reminder two months in advance of that date. However, failure to receive a reminder does not negate your obligation to make the request in sufficient time for review and approval. Federal regulations prohibit retroactive approval of continuation. Failure to receive approval to continue the project prior to the expiration date will result in Termination of the protocol approval. The IRB Coordinator can give you guidance on submission times.

This protocol has been approved for 15 participants. If you wish to make *any* modifications in the approved protocol, including enrolling more than this number, you must seek approval *prior to* implementing those changes. All modifications should be requested in writing (email is acceptable) and must provide sufficient detail to assess the impact of the change.

If you have questions or need any assistance from the IRB, please contact me at 210 Administration Building, 5-2208, or irb@uark.edu.

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