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Antimicrobial Effect of Herb Extracts against *Escherichia coli* O157:H7, *Listeria monocytogenes,* and *Salmonella* Typhimurium Associated with Beef†

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ABSTRACT

The effects of plant extracts against pathogenic bacteria in vitro are well known, yet few studies have addressed the effects of these compounds against pathogens associated with muscle foods. A series of experiments was conducted to determine the effectiveness of a commercially available, generally recognized as safe, herb extract dispersed in sodium citrate (Protecta One) or sodium chloride (Protecta Two) against *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* associated with beef. In the first experiment, *E. coli* O157:H7, *Salmonella* Typhimurium, and *L. monocytogenes* inoculated onto beef and subjected to surface spray treatments with 2.5% solutions of Protecta One or Protecta Two were not affected by immediate application (day 0) of the herbal extracts. However, after 7 days of storage at 4°C, *E. coli* O157:H7 was reduced by >1.3 $\log_{10}$ CFU/cm^2 by Protecta Two; *L. monocytogenes* was reduced by 1.8 and 1.9 $\log_{10}$ CFU/cm^2 by Protecta One and Protecta Two, respectively; *Salmonella* Typhimurium was not reduced >0.3 $\log_{10}$ CFU/cm^2 by either extract by day 7. In the second experiment, 2.5% Protecta Two (wt/vol or wt/wt) added to inoculated lean and adipose beef trim, processed, and packaged as ground beef chubs (80% lean, 20% adipose), did not reduce pathogen populations >0.5 $\log_{10}$ CFU/g up to 14 days at 4°C. In the third experiment, surface spray treatments of beef with 2.5% lactic acid or 2.5% solutions of Protecta One or Protecta Two, vacuum packaged, and stored up to 35 days at 4°C did reduce *E. coli* O157:H7, *L. monocytogenes,* and *Salmonella* Typhimurium slightly. These studies suggest that the use of herb extracts may afford some reductions of pathogens on beef surfaces; however, the antimicrobial activity may be diminished in ground beef by adipose components.

Organic acids, chlorine dioxide, trisodium phosphate, heat, steam, or hot water are generally recognized as safe interventions and are used extensively by the meat and poultry industries to reduce visible and bacterial contamination on meat surfaces *(11, 24).* These interventions have been found to be effective for immediately reducing foodborne pathogens such as *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes.* Despite the widespread use and effectiveness of these interventions, researchers are continually investigating the use of other novel antimicrobial agents or preservatives to reduce or inhibit pathogens or spoilage organisms in fresh or processed foods. Examples of such compounds include a variety of natural plant antimicrobials with the potential to reduce foodborne pathogens.

Antimicrobial compounds of plant origin may be found in plant stems, roots, leaves, bark, flowers, or fruits *(2, 6).* According to Mitscher *(18),* plants may be a poorly exploited source of antimicrobial agents because the structures and modes of action are not known *(20).* Additionally, there are over 1,300 plants exhibiting a potential source of antimicrobial agents *(15, 20, 28).* Antimicrobial agents derived from plants may include phytoalexins, isothiocyanates, alliin or allicins, plant pigments, and phenolic compounds from herbs or spices.

Phytoalexins are considered low molecular weight, broad-spectrum, antimicrobial compounds that are synthesized by higher plants in response to injury, infection, or treatment and have not been identified in healthy plant tissues *(2, 3, 6, 20).* Phytoalexins, produced by beans, potatoes, peppers, eggplants, grapes, and carrots, are broad-spectrum antimicrobials that exhibit both antibacterial and antifungal properties *(6).* The mode of action of these compounds against bacteria occurs at the cell membrane where they appear to disturb membrane-associated functions *(3).*

Isothiocyanates are compounds stored in the cell vacuoles of plants and are released when plant tissues are injured or the integrity is disrupted *(6).* These compounds are found in horseradish, mustard, turnip, cabbage, Brussels sprouts, kale, collards, cauliflower, radish, charlock, rutabaga, spinach, and watercress and include phenolic compounds, diphenol oxidase enzymes, and quinone inhibitors *(2, 6).* The mode of action of isothiocyanates against bacteria appears to be the oxidative cleavage of disulfide bonds leading to inactivation of intracellular enzymes *(8).*

Alliin and allicins are produced by garlic or onion

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† Names are necessary to report factually on available data; however, the U.S. Department of Agriculture neither guarantees nor warrants the standard of the product, and the use of the name by U.S. Department of Agriculture implies no approval of the product to the exclusion of others that may also be suitable.

plants. Alliin, the precursor to allicin, is produced when the plant or bulb tissue is disrupted *(6)*. Alliin is hydrolyzed to yield allicin, pyruvate, and ammonia, and as a broad-spectrum antimicrobial, allicin appears to inhibit sulfhydryl enzymes in a wide variety of bacteria.

Plant pigments such as anthocyanins can be found in flowers and fruits. It is thought that the antimicrobial activity of these compounds is due to the chelation of metal ions resulting in inhibition of certain bacterial enzymes and, ultimately, inhibition of bacterial growth *(6, 26)*.

Essential oils are considered a group of compounds that are responsible for the odor, aroma, and flavor of spices or herbs and are generally soluble in ethanol *(20)*, exhibiting activity against both gram-positive and gram-negative bacteria, with gram-positive bacteria being more sensitive *(2, 20)*. Shelef *(23)* noted that phenolic compounds found in the essential oils might be responsible for the antimicrobial activity of these compounds. The antimicrobial action of phenolic compounds, such as oleuropein from olives, hydroxycinnamic acids, cinnamic acids, tannins, and tannic acid *(6, 7)* has been studied extensively *(6, 23)*. Conner and Beuchat *(5)* suggested that the potential mode of action of these compounds against yeast might be due to the impairment of enzymatic processes involved in energy production and structural component synthesis. Nychas *(20)* theorized that phenols affect bacteria by weakening or destroying the permeability barrier of the cell membrane, resulting in the release of cellular constituents, or by altering the physiological status of the cells by changing the fatty acid composition and phospholipid content of bacteria, interfering with energy metabolism, disrupting electron transport or nutrient uptake, and affecting nucleic acid synthesis. Based on these observations, it appears that phenolic compounds do not have a single target associated with antimicrobial activity or a common mechanism of action *(20)*.

While numerous in vitro studies have demonstrated the effectiveness of phytoalexins, pigments, herbs, spices, or plant extracts and their active ingredients against pathogens, few studies have addressed the use of plant-derived antimicrobials to inhibit pathogenic or spoilage organisms associated with meat. Farbood et al. *(13)* demonstrated that a rosemary spice extract was effective against *Staphylococcus aureus* but not against total plate counts in mechanically deboned poultry meat, turkey breast, and beef. Stecchini et al. *(27)* demonstrated that the essential oils of coriander or clove could significantly reduce *Aeromonas* spp. in vacuum-packaged, refrigerated, noncured cooked pork. Treatments of ready-to-eat pork liver sausage with rosemary delayed the growth of *L. monocytogenes (21)*. *Aeromonas hydrophila* and *L. monocytogenes* were inhibited on cooked chicken breast meat by eugenol and pimento extracts *(16)*. Other researchers have demonstrated that the direct addition of cloves or cinnamon significantly reduced *E. coli* O157:H7 in ground beef *(1)*. In fermented sausage, *E. coli* O157:H7 also was reduced when treated with garlic, cloves, or cinnamon *(1)*. The following study was conducted to determine if a commercially available, generally regarded as safe, herb extract could inhibit or reduce pathogen populations on the surfaces of beef or when inoculated beef trim was processed into ground beef.

MATERIALS AND METHODS

Bacterial cultures. *E. coli* O157:H7 ATCC 43895, *L. monocytogenes* Scott A, and *Salmonella* Typhimurium ATCC 14028 were obtained from the Roman L. Hruska U.S. Meat Animal Research Center (MARC) culture collection and maintained in 75% glycerol at −20°C. *E. coli* O157:H7, *L. monocytogenes,* and *Salmonella* Typhimurium were propagated for 18 h in trypticase soy broth (Difco, Detroit, Mich.) at 37°C for 18 h. Overnight cultures were diluted 1:100 in 2% buffered peptone water (BPW) to obtain a viable cell population of approximately 6 $\log_{10}$ CFU/ml. A pathogen cocktail consisting of equal amounts of *E. coli* O157:H7, *L. monocytogenes,* and *Salmonella* Typhimurium was made and used to inoculate tissues in the subsequent meat experiments.

Meat experiment 1. Prerigor lean tissues were obtained from the cutaneous trunci within 15-min postexsanguination from beef carcass sides processed at MARC. Tissues were vacuum packaged (Hollymatic model LV10G, Hollymatic, Inc., Countryside, Ill.) in a standard vacuum-packaging bag (3.2 mil nylon/copolymer bag with oxygen transmission rate at 23°C of 52 cc/m^2; Hollymatic, Inc.) and held for 18 h at 4°C to undergo rigor. Tissues were frozen (−20°C) until the day before the experiment and then thawed at 4°C overnight. On the day of the experiment, thawed tissues were cut aseptically to 12 cm by 12 cm and placed on sterile trays. The pathogen cocktail was paintbrush-inoculated onto the fascia surface of the lean or adipose surfaces and allowed to remain undisturbed for 15 min. Bacterial levels of approximately 5 $\log_{10}$ CFU/cm^2 were obtained using this methodology.

The commercially available, GRAS, blended herb extracts used in the following experiments were dispersed in sodium citrate (Protecta One) or sodium chloride (Protecta Two; Bavaria Corp., Apopka, Fla.). According to the manufacturer, the concentrations of sodium citrate and sodium chloride used as carriers for the herbal extracts do not contribute significantly to the antimicrobial activity of the compounds. Other properties of these compounds are depicted in Table 1. Protecta One or Protecta Two were added to sterile distilled water to make a 2.5% concentration (wt/vol), transferred to a sterile spray bottle, and chilled to 4°C (manufacturer's information).

Inoculated, postrigor lean tissues were treated by spraying Protecta One, Protecta Two, or sterile distilled water evenly over the tissues for 15 s with a handheld spray bottle (Ace Hardware; approximate volume, 20 ml). Tissues were allowed to drip for approximately 1 min. Day 0 samples were excised from the tissues for bacterial enumeration (see below). The remaining tissue was stored in an aseptic container for 7 days at 4°C and then analyzed.

Meat experiment 2. Approximately 10,000 g of postrigor lean beef trim and 2,400 g of postrigor beef adipose were obtained from the MARC abattoir or a local slaughter facility. The lean or adipose tissues were cut aseptically into approximately 25-g cubes. For each replication and treatment conducted, 800 g of the cubed lean and 200 g of the cubed adipose tissues were added to a sterile bag, inoculated with 300 ml of a 1:100 dilution (BPW) of the pathogen cocktail allowed to remain undisturbed for 15 min, then remaining liquid poured off.

Immediately after inoculation, the lean and adipose cubes were subjected to one of the following four treatments: (i) inoculated, untreated cubes were coarse ground twice (4.5 mm head on a model MG12, 0.25 hp grinder; Daypol Enterprises, Inc., New York); (ii) 300 ml of 2.5% liquid Protecta Two (room temperature;

TABLE 1. *Description of herb extracts*

Description/composition[a]	Protecta One[b]	Protecta Two[c]
Activity	Yeast, molds, bacteria	Bacteria
Color	Yellowish	Yellowish
Texture	Powder	Powder
Flavor	Neutral	Typical (salty)
Recommended concentration	2–2.5% in water, 4°C	2% in water, 4°C
pH of 2.5% solution[d]	6.19	5.06

[a] Obtained from the literature provided by manufacturer.
[b] Blend of natural herb extracts dispersed in sodium citrate.
[c] Combination of natural herb extracts dispersed in a sodium chloride carrier.
[d] Obtained from this study.

wt/vol) was added to inoculated cubes, remained undisturbed for 15 min, then coarse ground twice; (iii) inoculated cubes were coarse ground, 300 ml of 2.5% liquid Protecta Two (room temperature; wt/vol) was added, and mixture coarse ground again; and (iv) 2.5% crystalline Protecta Two (wt/wt) was added directly to the inoculated cubes and coarse ground twice. Any remaining solution was processed with the meat and not decanted. Untreated and Protecta-treated ground beef samples (approximately 23% adipose as determined by proximate analyses) were transferred to 3.2 mil nylon/copolymer bags with an oxygen transmission rate at 23°C of 52 cc/m^2 (Hollymatic, Inc.), heat sealed with a Hollymatic model LV10G, and held at 4°C until sampled at days 0, 1, 2, 7, and 14.

Meat experiment 3. Frozen, beef strip loins processed at MARC were sawed in half to expose both lean and adipose surfaces. Frozen halves were vacuum packaged and stored at −20°C until needed. Three days prior to the experiment, frozen halves were thawed at 4°C. On the day of the experiment, thawed halves were placed on sterile trays with lean or adipose surfaces exposed. A 1:100 dilution of the pathogen cocktail was paintbrush-inoculated onto the lean or adipose surfaces and allowed to remain undisturbed for 15 min. Pathogen levels of approximately 4 to 5 $\log_{10}$ CFU/cm^2 were obtained using this methodology.

Solutions of 2.5% Protecta One (wt/vol), Protecta Two (wt/vol), or lactic acid (vol/vol; Sigma, St. Louis, Mo.) were made in sterile distilled water, transferred to a sterile spray bottle, and chilled to 4°C. Inoculated lean or adipose tissues were treated by spraying 2.5% Protecta One, Protecta Two, or lactic acid water evenly over the tissues for 15 s with a handheld spray bottle (approximate volume, 20 ml). Tissues were allowed to drip for approximately 1 min. Six, 25-cm^2 samples were excised from the tissues; one sample was saved for bacterial enumeration on day 0 (see below). The five remaining pieces were vacuum packaged and stored at 4°C until sampled on days 2, 7, 21, and 35.

Bacterial enumeration. Following excision of 25-cm^2 beef surface tissues or removal of 25-g samples on the specified days, samples were pummeled for 2 min (Stomacher 400, Tekmar, Inc., Cincinnati, Ohio) in a Sterefil Stomacher bag (Spiral Biotech, Bethesda, Md.) with 25 ml of buffered peptone water (pH 7.0; BBL, Cockeysville, Md.) containing 0.1% Tween 20 (Fisher, St. Louis, Mo.). Each stomachate was serially diluted in buffered peptone water, and either spiral plated (model D Spiral Plater; Spiral Biotech) in duplicate. For the detection of *E. coli* O157:H7, *L. monocytogenes,* or *Salmonella* Typhimurium, stomachates were spiral plated onto sorbitol McConkey agar (Difco), Listeria selective agar (Oxoid Division, Unipath Co., Ogdensburg, N.Y.), or Rambach agar (Merck, Darmstadt, Germany), respectively. Trypticase soy agar (Difco) was used for enumeration of mesophilic aerobic plate counts (APC). All plates were enumerated manually or with the CASBA IV image analyzer (Spiral Biotech) after incubation for 48 h at 37°C. The lowest level of detection of organisms was 1.30 $\log_{10}$ CFU/cm^2 or CFU/g using spiral plating procedures.

Plate overlay assays. The antimicrobial activity of Protecta One or Protecta Two was determined in a series of plate overlay assays. Protecta One or Protecta Two was added to sterile distilled water to a final concentration of 2.5%, and 20 μl was spotted onto lawns of *E. coli* O157:H7, *L. monocytogenes,* or *Salmonella* Typhimurium made as follows. Briefly, trypticase soy agar plates were overlaid with 8 ml of semisoft trypticase soy agar (0.5% wt/vol agar) seeded with 80 μl of an overnight broth culture of *E. coli* O157:H7, *L. monocytogenes,* or *Salmonella* Typhimurium. The seed density was approximately 6 $\log_{10}$ CFU/ml of overlay. Duplicate plates were scored (±) for zones of inhibition after 24 h of incubation at respective temperature and recorded *(25).*

Calculations and statistical analyses of population data. After enumeration, bacterial populations from duplicate plates were averaged and converted to $\log_{10}$ CFU/cm^2 or CFU/g. Least-squared means of bacterial populations ($\log_{10}$ CFU/cm^2 or CFU/g) from each treatment were calculated from three replications for all meat experiments. Statistical analyses were conducted using analysis of variance and the general linear models procedure of SAS (SAS for Windows, release ver. 6.12, SAS Institute, Inc., Cary, N.C.). Statistical significance was defined as $P \leq 0.05$, unless otherwise noted.

RESULTS

Meat experiment 1. Initial plate overlay assays indicated that 1, 2, and 2.5% concentrations of Protecta One or Protecta Two exhibited antimicrobial activity, as demonstrated by clear zones of inhibition, against *E. coli* O157:H7, *Salmonella* Typhimurium, and *L. monocytogenes.* Based on these findings, meat experiments were conducted. The effects of spray treatments of Protecta One and Protecta Two on APC and pathogen populations associated with postrigor beef surfaces are depicted in Table 2. Immediate application of the herbal extracts resulted in slight reductions of *E. coli* O157:H7 of approximately 0.30 $\log_{10}$ CFU/cm^2. By day 7 of aerobic, refrigerated storage, populations were reduced 1.3 $\log_{10}$ CFU/cm^2 by treatments with Protecta Two. Water or herbal extracts did not significantly reduce populations of *L. monocytogenes* after application on day 0. However, by day 7 of refrigerated storage, treat-

TABLE 2. *Meat experiment 1*[a]

Treatment	*E. coli* O157:H7		*L. monocytogenes*		*Salmonella* Typhimurium		Aerobic plate counts	
	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7
Untreated	5.6 A	5.4 A	5.3 A	7.5 A	5.0 A	4.8 A	5.4 A	7.5 A
Water	5.4 B	5.1 A	4.9 A	7.3 A	4.7 B	4.2 B	5.2 A	6.6 B
Protecta One	5.3 B	5.2 A	5.1 A	5.5 B	4.6 B	4.6 AB	5.0 A	5.7 C
Protecta Two	5.3 B	4.1 B	4.9 A	5.6 B	4.5 B	4.4 B	5.0 A	5.7 C

[a] Effect of 2.5% Protecta One or 2.5% Protecta Two on populations ($\log_{10}$ CFU/cm^2) of *E. coli* O157:H7, *L. monocytogenes, Salmonella* Typhimurium, and aerobic plate counts on postrigor lean beef carcass tissue immediately after treatment and after 7 days of storage at 4°C. Means within a column sharing the same letter are not different.

ments with Protecta One and Protecta Two significantly reduced (>1.9 and 2 $\log_{10}$ CFU/cm^2, respectively) *L. monocytogenes.* Populations of *Salmonella* Typhimurium were reduced slightly by immediate treatments with water or herbal extracts, with a 0.4-$\log_{10}$ CFU/cm^2 reduction observed with Protecta Two after 7 days of refrigerated storage. As was observed with *L. monocytogenes* and *E. coli* O157:H7, APC were not reduced immediately by treatments with the herbal extracts. By day 7, Protecta One and Protecta Two affected a reduction of 1.8 $\log_{10}$ CFU/cm^2 against APC. These results suggest that Protecta One or Protecta Two does not elicit immediate activity against bacterial populations. Rather, activity occurs some time after application and during the 7 days of refrigerated storage.

Meat experiment 2. Three treatments with a 2.5% concentration of Protecta One were applied to experimentally inoculated beef lean and adipose trim or ground beef in an attempt to reduce pathogens and APC. The addition of a liquid 2.5% solution of Protecta One to inoculated beef trim or ground beef and subsequent grinding afforded some immediate and sustained reductions in bacterial populations of APC and *Salmonella* Typhimurium (Table 3). Populations of *L. monocytogenes* and *E. coli* O157:H7 were reduced to the greatest extent on days 0 and 1 by treating the trim with a 2.5% liquid solution of Protecta One (Table 3). However, by day 14, the antimicrobial effect was negated as populations of *L. monocytogenes* and *E. coli* O157:H7 remaining from all treatments were statistically similar (Table 3). When examined against all pathogen populations, application of 2.5% (wt/wt) Protecta One in a powder form to ground beef did not result in any reductions, as compared to untreated ground beef (Table 4). The addition of powdered Protecta One to ground beef resulted in increased APC on all days, as compared to untreated ground beef (Table 3). While neither Protecta One nor Protecta Two were sterile, it is possible that the addition of water activated or solubilized the antimicrobial compounds leading to the observed population differences. Additionally, treating beef trim or ground beef with a 2.5% liquid solution of Protecta One for 15 min and grinding did result in bac-

TABLE 3. *Meat experiment 2*[a]

Treatment	Organism	Day				
		0	1	2	7	14
Untreated	APC	6.5 B	6.5 B	6.9 A	6.6 B	6.3 B
Trim treated with Protecta One, 2.5% liquid	APC	6.1 C	6.4 B	6.6 B	6.3 C	6.2 B
Ground beef treated with Protecta One, 2.5% liquid	APC	6.1 C	6.5 B	6.1 C	6.1 C	6.0 B
Ground beef treated with Protecta One, 2.5% powder	APC	7.3 A	7.6 A	7.2 A	7.2 A	7.3 A
Untreated	*Salmonella* Typhimurium	5.4 A	5.4 AB	5.2 AB	5.1 B	4.5 B
Trim treated with Protecta One, 2.5% liquid	*Salmonella* Typhimurium	4.8 C	4.9 C	4.9 C	4.5 C	4.2 C
Ground beef treated with Protecta One, 2.5% liquid	*Salmonella* Typhimurium	5.1 B	5.2 B	5.1 BC	5.0 B	4.5 B
Ground beef treated with Protecta One, 2.5% powder	*Salmonella* Typhimurium	5.4 A	5.5 A	5.3 A	5.4 A	5.0 A
Untreated	*L. monocytogenes*	5.4 A	5.4 A	5.7 A	5.5 A	5.2 A
Trim treated with Protecta One, 2.5% liquid	*L. monocytogenes*	5.1 B	4.9 B	5.3 B	5.2 AB	5.1 A
Ground beef treated with Protecta One, 2.5% liquid	*L. monocytogenes*	5.4 A	5.2 A	5.5 AB	5.1 B	5.3 A
Ground beef treated with Protecta One, 2.5% powder	*L. monocytogenes*	5.5 A	5.3 A	5.5 A	5.5 A	5.3 A
Untreated	*E. coli* O157:H7	5.7 AB	5.7 A	5.4 A	5.58 A	5.2 A
Trim treated with Protecta One, 2.5% liquid	*E. coli* O157:H7	5.4 C	5.4 B	5.3 A	5.3 B	5.2 A
Ground beef treated with Protecta One, 2.5% liquid	*E. coli* O157:H7	5.5 BC	5.7 A	5.3 A	5.5 AB	5.3 A
Ground beef treated with Protecta One, 2.5% powder	*E. coli* O157:H7	5.8 A	5.8 A	5.4 A	5.6 A	5.3 A

[a] Effect of Protecta One on populations ($\log_{10}$ CFU/g) of aerobic, mesophilic plate counts (APC), *Salmonella* Typhimurium, *L. monocytogenes, E. coli* O157:H7 in ground beef immediately after treatment and after 1, 2, 7, and 14 days of storage at 4°C. Means within a column for a given organism sharing the same letter are not different.

TABLE 4. *Meat experiment 3*[a]

Treatment	Organism	Day 0		Day 2		Day 7		Day 21		Day 35	
		Lean	Adipose	Lean	Adipose	Lean	Adipose	Lean	Adipose	Lean	Adipose
Untreated	APC	5.6 A	6.0 A	5.7 A	7.0 A	6.0 A	7.1 A	6.0 A	6.6 A	6.0 A	6.0 A
Protecta One	APC	5.0 B	5.8 A	5.3 A	6.2 A	5.6 A	6.4 A	5.7 A	6.8 A	6.0 A	5.9 A
Protecta Two	APC	5.3 AB	5.5 A	5.7 A	5.9 AB	6.1 A	6.5 A	5.8 A	5.5 B	5.6 AB	5.4 AB
Lactic acid	APC	5.1 AB	5.6 A	5.5 A	5.5 B	5.7 A	6.2 A	5.6 A	5.1 B	5.4 B	4.6 B
Untreated	ST	5.3 A	4.1 A	4.8 A	4.1 A	4.9 A	3.7 AB	3.9 A	3.1 A	4.2 A	2.8 A
Protecta One	ST	4.9 A	3.8 A	4.7 A	3.7 A	4.0 B	4.0 A	3.7 A	2.4 AB	3.7 A	2.0 AB
Protecta Two	ST	5.1 A	4.4 A	4.9 A	3.9 A	5.1 A	3.6 AB	4.3 A	2.0 B	3.7 A	1.3 B
Lactic acid	ST	4.9 A	3.4 A	5.1 A	2.6 B	4.8 A	2.9 B	3.6 A	1.4 B	2.5 B	1.3 B
Untreated	LM	5.6 A	4.9 A	5.0 A	3.9 A	5.0 A	4.3 A	4.7 A	2.2 A	4.2 A	1.9 A
Protecta One	LM	4.9 A	4.4 AB	4.7 A	3.5 A	4.6 AB	3.9 A	4.1 AB	2.5 A	3.1 B	2.1 A
Protecta Two	LM	5.4 A	4.5 AB	5.1 A	3.8 A	4.9 A	3.3 A	4.2 A	1.9 AB	3.2 B	2.2 A
Lactic acid	LM	5.0 A	3.8 B	4.8 A	2.3 B	4.2 B	1.9 B	3.4 B	1.3 B	1.8 C	1.3 B
Untreated	EC	6.1 A	4.9 A	4.6 A	4.5 A	5.1 A	4.6 A	4.9 A	3.7 A	4.3 A	2.9 A
Protecta One	EC	5.4 B	4.4 A	4.7 A	3.8 A	4.4 B	4.1 A	4.5 A	3.0 AB	3.9 A	2.1 AB
Protecta Two	EC	6.1 A	4.2 A	4.8 A	4.2 A	5.0 A	3.7 AB	4.8 A	2.5 B	4.0 A	1.3 B
Lactic acid	EC	5.5 AB	4.0 A	4.9 A	3.7 A	5.0 A	3.5 B	4.6 A	2.5 B	3.9 A	1.8 B

[a] Effect of 2.5% Protecta One, 2.5% Protecta Two, or 2.5% lactic acid on populations ($\log_{10}$ CFU/cm^2) of aerobic, mesophilic plate counts (APC), *Salmonella* Typhimurium (ST), *L. monocytogenes* (LM), and *E. coli* O157:H7 (EC), on vacuum-packaged lean or adipose beef immediately after treatment and after 2, 7, 21, and 35 days of storage at 4°C. Means within a column for a given organism and tissue type sharing the same letter are not different.

terial reductions; however, reductions were approximately 1 $\log_{10}$ CFU/cm^2. It is possible that processing of the intact tissues into ground beef interfered with the antimicrobial activity of the extracts presumably by binding to the lipid fraction *(13)*.

Meat experiment 3. To determine if surface application of Protecta One or Protecta Two followed by refrigerated, vacuum-packaged storage for up to 35 days could result in reductions of pathogens and APC, another meat experiment was conducted. This experiment also compared the effectiveness of lactic acid and herbal extracts against foodborne pathogens.

At day 0, remaining APC were reduced slightly on lean beef surfaces treated with both herbal extracts as well as lactic acid, as compared to untreated surfaces (Table 4). However, no significant differences were observed for APC on adipose surfaces following any of the treatments on day 0. After 2 days of refrigerated, vacuum-packaged storage, APC were not dramatically reduced on lean surfaces; APC reductions of >1.1 $\log_{10}$ CFU/cm^2 were observed on adipose surfaces following treatments with Protecta Two or lactic acid. At day 7, no significant differences in populations were observed for APC on either lean or adipose surfaces left untreated or treated with the herbal extracts or lactic acid. By day 21, no significant differences in populations were observed for APC on lean surfaces; yet Protecta Two and lactic acid exerted reductions >1 $\log_{10}$ CFU/cm^2 against APC on adipose surfaces. Slight reductions in APC were observed on day 35 when lean and adipose surfaces were treated with Protecta Two and lactic acid.

While Protecta One and lactic acid treatments resulted in immediate reductions in *E. coli* O157:H7 on lean surfaces, no significant differences in populations were observed against the pathogen on adipose surfaces (Table 4). By day 2, no differences in populations were observed for any of the treatments on either tissue type. Treatments with Protecta One resulted in a slight reduction of *E. coli* O157:H7 on lean surfaces at day 7. Reductions were observed against the pathogen when treated with Protecta Two and lactic acid, respectively, on day 7. By day 35, pathogen populations were significantly different on adipose surfaces treated with either herbal extract or lactic acid, but no differences in populations were observed on lean surfaces following any of the treatments.

Protecta One, Protecta Two, or lactic acid exerted little effect on populations of *Salmonella* Typhimurium on either lean or adipose tissues at day 0 (Table 4). By day 2, lactic acid treatments affected a reduction of >1 $\log_{10}$ CFU/cm^2 of the pathogen on adipose, but no significant differences in pathogen populations were observed on lean surfaces with any of the treatments. Slight differences in populations of *Salmonella* Typhimurium were observed at day 7 for lean surfaces treated with Protecta One and adipose surfaces treated with lactic acid. Day 21 data indicated that *Salmonella* Typhimurium on lean surfaces were unaffected by any of the treatments; but Protecta Two and lactic acid affected the pathogen significantly (reductions of >1 $\log_{10}$ CFU/cm^2) on adipose surfaces. Lactic acid treatments afforded the greatest reduction of *Salmonella* Typhimurium on both lean and adipose surfaces by day 35 of refrigerated, vacuum-packaged storage.

As was observed with *E. coli* O157:H7 and *Salmonella* Typhimurium, populations of *L. monocytogenes* were not immediately reduced by Protecta One, Protecta Two, or lac-

tic acid on lean surfaces (Table 4). Slight population differences were observed on adipose surfaces treated with all three compounds at day 0. However, by day 2, only treatments with lactic acid resulted in differences in populations of *L. monocytogenes*. At days 7, 21, and 35 of refrigerated, vacuum-packaged storage, only treatment with lactic acid appeared to reduce *L. monocytogenes* significantly on lean or adipose surfaces.

DISCUSSION

Plant-derived antimicrobial compounds have been recognized for hundreds of years as a means of inhibiting undesirable bacteria. Numerous reviews and research papers have described the antimicrobial properties of plant essential oils *(2, 7, 17, 19, 21)*, isothiocyanates *(8)*, allicin *(12)*, phytoalexins *(2, 20)*, and pigments *(6)*. While these reports have provided extensive in vitro information on the spectrum of activity, mode of action, etc., reports pertaining to the effectiveness of these types of compounds against microbes in food systems appear to be limited. Specifically, the use of plant-derived antimicrobials in foods may be hampered by effective dosages, interference by food constituents or other food-grade compounds, unsuitable water activity, incompatible pH, or processing regimens *(14)*.

In the present study, Protecta One and Protecta Two, at a concentration of 2.5%, exhibited pH values of 6.19 and 5.06, respectively. The pH difference of the two compounds may be attributed to the presence of citrate. Previously, researchers demonstrated the antimicrobial effectiveness of sodium citrate against *E. coli* O157:H7. *L. monocytogenes*, and lactic acid bacteria *(4)*. Based on meat experiments 1 and 3 conducted in this study, it appears that Protecta Two was more effective than Protecta One. While the manufacturer claims that sodium citrate or sodium chloride carriers do not contribute to antimicrobial activity, a study suggests that sodium chloride actually may enhance activity. Dickson *(10)* demonstrated that addition of sodium chloride reduced water activity and induced osmotic stress that improved the activity of organic acids against *S.* Typhimurium. It is possible that the sodium chloride or sodium citrate associated with Protecta One or Protecta Two may reduce water activity and/or induce osmotic stress. Without separating and testing the individual components associated with these compounds, this observation cannot be confirmed. Given the chemical makeup of the herb extract in these compounds is not known, the mechanism of action of active compounds against microbes cannot be elucidated. However, like many other plant-derived antimicrobials, this herb extract may contain phenolic compounds *(2, 13, 23)*. As described previously, phenolic compounds may interfere with energy metabolism, disrupt electron transport or nutrient uptake, or affect nucleic acid synthesis, ultimately resulting in cellular death *(20)*.

The application of Protecta One or Protecta Two to inhibit foodborne pathogens on beef lean and adipose surfaces or in ground beef has not been reported previously in the literature. Preliminary research conducted by Dickens et al. *(9)* demonstrated that poultry carcasses treated with 2% Protecta Two had lower counts of *Campylobacter* spp., coliforms, *E. coli*, and total plate counts, as compared to water-treated carcasses. In the present study, spray treatments of meat surfaces with the herbal extracts appeared to be a suitable method of delivery of the compounds to the meat surfaces, as indicated by the small reductions of APC, *E. coli* O157:H7, *Salmonella* Typhimurium, and *L. monocytogenes* (meat experiment 1). Protecta One and Protecta Two appeared to be equally effective against the different bacterial populations. However, greater reductions were observed after 7 days of refrigerated storage, rather than immediately after application. Other researchers have noted that lower temperatures and prolonged storage may enhance the inhibitory activity of plant extracts *(2, 16)*.

According to the manufacturer, Protecta One has been "... successful in eliminating heavy loads of bacteria on the trimmings of beef" (Bavaria Corp.). Based on the findings of meat experiment 2, application of Protecta One or Protecta Two to trim subsequently processed into ground beef did not reduce any of the bacterial populations >0.5 $\log_{10}$ CFU/g. However, application of 2.5% Protecta One (wt/vol) to experimentally inoculated beef trim appeared to be more effective than treating the ground beef or adding the dry powder (2.5%, wt/wt) directly to the ground beef. Meat experiment 2 also demonstrates that activity of the herb extract, applied either as a solution or powder, is reduced in ground beef. Farbood et al. *(13)* reported that a higher lipid content of meat (as may be seen in ground beef) might result in a drastic reduction in the concentration of rosemary spice extract. This phenomenon may be due to the solubilization of the antimicrobial agents into the lipid fraction of the food, thereby reducing their availability for antimicrobial activity *(4, 22, 27)*.

Application of Protecta Two in combination with vacuum-packaged, refrigerated storage reduced populations ($\sim$1 $\log_{10}$ CFU/cm^2) of *E. coli* O157:H7 and *Salmonella* Typhimurium on lean and adipose beef surfaces after 35 days (meat experiment 3). However, treatments of vacuum-packaged lean or adipose surfaces with Protecta Two did not reduce *L. monocytogenes* or APC significantly after 35 days at 4°C. Stecchini et al. *(27)* reported that the lethal effect of essential oils was enhanced against *A. hydrophila* by vacuum packaging. It was theorized that the lower oxygen tension might increase the sensitivity of susceptible gram-negative bacterial cells to the antimicrobial activity of the herb extract *(27)*. It should be noted that pathogens used in meat experiment 3 exhibited a gradual decline in populations, regardless of treatment, during the 35 days of storage. This observation could be attributed to a combination of antimicrobial compounds produced by APC (which did not decline over time), prescribed treatments, as well as vacuum-packaged, refrigerated storage.

Given the low numbers of pathogens generally found in beef products, the use of herb extracts may afford minimal protection (approximately 1 $\log_{10}$ reduction) on intact beef lean or adipose surfaces stored under refrigerated or vacuum-packaged conditions. However, any antimicrobial activity associated with herbal extracts may be diminished by the presence of adipose components in ground beef.

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