Inhibition of Clostridium perfringens Spore Germination and Outgrowth by Buffered Vinegar and Lemon Juice Concentrate during Chilling of Ground Turkey Roast Containing Minimal Ingredients

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ABSTRACT

Inhibition of Clostridium perfringens spore germination and outgrowth in ground turkey roast containing minimal ingredients (salt and sugar), by buffered vinegar (MOstatin V) and a blend (buffered) of lemon juice concentrate and vinegar (MOstatin LV) was evaluated. Ground turkey roast was formulated to contain sea salt (1.5%), turbinado sugar (0.5%), and various concentrations of MOstatin V (0.75, 1.25, or 2.5%) or MOstatin LV (1.5, 2.5, or 3.5%), along with a control (without MOstains). The product was inoculated with a three-strain spore cocktail of C. perfringens to obtain initial spore levels of ca. 2.0 to 0.5 log CFU/g. Inoculated products were vacuum packaged, heat shocked for 20 min at 75°C, and cooled exponentially from 54.4 to 4.0°C in 6.5, 9, 12, 15, 18, or 21 h. In control samples without MOstatin V or MOstatin LV, C. perfringens populations reached 2.98, 4.50, 5.78, 7.05, 7.88, and 8.19 log CFU/g (corresponding increases of 0.51, 2.29, 3.51, 4.79, 5.55, and 5.93 log CFU/g) in 6.5, 9, 12, 15, 18, and 21 h of chilling, respectively. MOstatin V (2.5%) and MOstatin LV (3.5%) were effective in inhibiting C. perfringens spore germination and outgrowth in ground turkey roast to <1.0 log CFU/g during abusive chilling of the product within 21 h. Buffered vinegar and a blend (buffered) of lemon juice concentrate and vinegar were effective in controlling germination and outgrowth of C. perfringens spores in turkey roast containing minimal ingredients.

Clostridium perfringens is a gram-positive, anaerobic, spore-forming, rod-shaped bacterium. Although an anaerobe, the organism can tolerate brief exposure to air (16). It is a natural inhabitant of human and animal intestinal tracts, and has been isolated from a variety of foods of animal origin (9, 17). C. perfringens food poisoning is caused by a small group (<<5%) of C. perfringens type A isolates that produce enterotoxin (18). The Centers for Disease Control and Prevention estimated that more than 248,000 cases of foodborne illness occur annually in the United States due to C. perfringens (19), and ranks it as the third most commonly reported foodborne illness in the United States (18, 21).

Meat and poultry products are generally implicated in the majority of the outbreaks, with beef products responsible for about 40% of C. perfringens foodborne outbreaks (3). The ability of C. perfringens spores to survive traditional thermal processing procedures along with an unusually short generation time (<10 min in meat) between temperatures of 43 and 46°C contribute to its role in outbreaks of foodborne illness. Although C. perfringens vegetative cells do not survive the normal cooking schedules used in the meat industry, the spores of the organism can survive those heat treatments (12). Heat-activated spores can germinate and grow rapidly in products that are improperly chilled or temperature abused during subsequent handling, potentially resulting in a foodborne illness.

The U.S. Department of Agriculture, Food Safety and Inspection Service (FSIS) published compliance guidelines to minimize the risk of C. perfringens outgrowth during chilling of thermally processed meat and poultry products (29, 30). The guidelines recommend that meat and poultry processors cool the cooked, ready-to-eat meat and poultry products from 54.4 to 26.7°C within 1.5 h, and further to 4.4°C within an additional 5 h. Juneja et al. (13) reported that abusive cooling of autoclaved ground beef beyond 15 h (exponential cooling) resulted in C. perfringens population increases to a potentially hazardous level (>6 log CFU/g). The organic acid salts such as sodium; potassium; or calcium salts of acetic, citric, or lactic acids were shown to inhibit C. perfringens spore germination and outgrowth during extended cooling periods in a variety of meat systems (14, 23, 28, 31).

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The rapid growth in the natural and organic foods market has resulted in meat and poultry processors producing products for this market that comply with regulatory rules and guidelines. The growth of this market segment has been precipitated by consumer concerns about the use of antibiotics, pesticides, and/or hormones in conventionally produced foods and genetic modification of plants and animals, and the use of chemical additives (25). While organic products and their production have been well defined in the United States, the definition for products that constitute “natural” meat products has been less than transparent.

Processed meat and poultry products labeled “natural” must comply with the definition “...does not contain any artificial flavor or flavoring, coloring ingredient, or chemical preservative or any other artificial or synthetic ingredient; and the product and its ingredients are not more than minimally processed” (5). While earlier interpretations permitted the use of preservatives of natural origin (lactates prepared from natural sources such as corn syrup and salts of citrates), current interpretations preclude their use in natural meat products.

Due to the challenges in developing meat and poultry products that meet the definition of natural meat products, processors are increasingly developing products that have “clean labels,” i.e., products that do not contain ingredients that can be classified as chemicals (lactate, citrate, etc.). In addition, processors that aspire to produce clean label meat and poultry products also refrain from using traditional meat processing ingredients such as nitrates and/or nitrites and phosphate. These traditional ingredients provide functionality (color stability, water binding, etc.) to the products as well as antimicrobial activity, improving the safety of such products.

Thus, there is a need to search for ingredients that fit the definition of clean label, while providing the antimicrobial effect to these products and assure their safety. The ready-to-eat meat and poultry processing industry has used multifunctional ingredients such as fruit juice concentrates (e.g., lime and lemon juices) to contribute to the acidity of the products. However, their ability to modulate the product pH can contribute to the antimicrobial efficacy as well.

The objective of our research was to evaluate the efficacy of buffered vinegar (natural source of acetic acid) and a blend (buffered) of lemon juice concentrate (natural form of citric acid) and vinegar to inhibit germination and outgrowth of C. perfringens spores in ground turkey containing minimal ingredients during abusive cooling.

**MATERIALS AND METHODS**

C. perfringens cultures. Three different enterotoxin-producing strains of C. perfringens, NCTC 8238 (Hobbs serotype 2), NCTC 8239 (Hobbs serotype 3), and NCTC 10240 (Hobbs serotype 13), were used in the study. The source of these cultures and their maintenance has been described elsewhere (14).

Preparation of C. perfringens spore cocktail. The spore crop from each strain of C. perfringens was prepared separately, following the protocol outlined by Juneja et al. (11). An aliquot (0.1 ml) from the stock culture was inoculated into 10 ml of freshly prepared fluid thioglycollate medium (Difco, Becton Dickinson, Sparks, MD). Inoculated tubes were heat shocked for 20 min at 75°C in a submerged-coil water bath (Isotemp 3013H, Fisher Scientific, Fair Lawn, Nj), cooled in chilled water, and incubated for 18 h at 37°C. A 1.0-ml portion of this culture was transferred to 10 ml of freshly steamed fluid thioglycollate medium and then incubated for 4 h at 37°C. The fresh culture (1%) was then transferred to modified Duncan-Strong medium and incubated aerobically for 24 h at 37°C. The original Duncan-Strong formulation was modified by replacing starch with 0.4% raffinose (Sigma Chemical Co., St. Louis, MO) and supplemented with 100 mg/ml caffeine (Sigma) to enhance sporulation. The cultures of each strain were then harvested by centrifugation at 7,012 × g for 20 min at 4°C (GS-15R, Beckman, Palo Alto, CA) and washed twice with 50 ml of sterile distilled water. The spore crop of each strain was stored separately at 4°C until use. A spor cocktail containing all three strains of C. perfringens was prepared immediately before experiments by mixing equivalent numbers of spores from each suspension. The spore population was enumerated by subjecting the suspension to a heat treatment of 15 min at 75°C and subsequent plating as described in the “Enumeration of C. perfringens” section, below.

**Meat preparation.** Fresh turkey breasts were obtained from a local supplier and stored under refrigeration for a maximum of 2 days and then used. The raw turkey breasts were ground through a 3.175-mm grinder plate (model 4732, Hobart, Troy, OH). Other ingredients, salt (1.5% [wt/wt]) and turbidino sugar (0.5% [wt/wt]), were mixed in water (15% [wt/wt]) of the meat block), added to the ground turkey, and mixed for 4 min in a Leland mixer (model 100 DA, Leland, Detroit, MI). The prepared turkey roast was then mixed with various concentrations of MOstatin V and MOstatin LV (WTI Inc., Jefferson, GA) separately in a mixer (model KSSSWH, Kitchen Aid, Troy, OH) for 2 min to obtain various treatments. Five-gram portions of each treated sample were weighed into small vacuum pouches (nylon; 3-mil standard barrier; water vapor transmission rate was 10 g/liter/m²/24 h at 37.8°C and 100% relative humidity; oxygen transmission rate was 3,000 cm³/liter/m²/24 h at 23°C and 1 atm [101.29 kPa]), measuring 2.5 by 5 in. (Prime Source, Kansas City, MO), vacuum sealed at 12 mbar (1.2 KPa) in a vacuum-packaging machine (A300/H, Multivac, Wolfertschwenden, Germany), and frozen at –20°C until use.

**Treatments.** Seven treatments were evaluated: control turkey formulation (no MOstatin V or MOstatin LV); turkey formulation containing MOstatin V at 0.75, 1.25, or 2.5%; and turkey product containing MOstatin LV at 1.5, 2.5, or 3.5%. These concentrations were suggested by the supplier as appropriate for turkey products, based on their preliminary sensory analysis data (not presented).

**Spore inoculation, heat shock, and cooling procedure.** Before the experiment, meat samples were thawed overnight at 5°C in a refrigerator and aseptically inoculated with 100 μl of the three-strain spore cocktail of C. perfringens to attain final spore populations of ca. 2.0 to 2.5 log CFU/g of meat. The inoculated samples were vacuum sealed as described, massaged manually for 30 s to evenly distribute spores, and flattened to a uniform thickness of ca. 2 mm prior to heat shock. A pair of pouches containing the inoculated meat for each treatment was submerged in a water bath (Isotemp 3013H, Fisher Scientific) set at 75°C for 20 min to activate the C. perfringens spores. After heat shock, one of the two pouches was chilled immediately in an ice water bath, and the C. perfringens population was determined as described in the “Enumeration of C. perfringens” section, below. The second pouch was transferred to a refrigerated bath (submerged coil apparatus) with water circulation capabilities (RTE 740, Thermo...
TABLE 1. Mean pH and water activity values of ground turkey roast containing 0.75 to 3.5% MOstatin V and MOstatin LV<sup>a</sup>

<table>
<thead>
<tr>
<th>Treatment (%)</th>
<th>pH</th>
<th>Water activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.70 ± 0.06</td>
<td>0.979 ± 0.012</td>
</tr>
<tr>
<td>MOstatin V</td>
<td>0.75</td>
<td>5.61 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>5.61 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>2.50</td>
<td>5.57 ± 0.04</td>
</tr>
<tr>
<td>MOstatin LV</td>
<td>1.50</td>
<td>5.64 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>2.50</td>
<td>5.61 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>3.50</td>
<td>5.62 ± 0.03</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are averages of three independent replicates ± standard deviations; values for each sample were measured in triplicate.

Neslab, Portsmouth, NH) set at 54.4°C; allowed to equilibrate to this temperature for 10 min; and chilled from 54.4 to 4.0°C within 6.5, 9, 12, 15, 18, and 21 h. Exponential chilling rates (temperature decline) followed for all the chilling times replicated the cooling of meat products in the meat industry.

**Enumeration of C. perfringens.** After cooling, the contents of each package were aseptically transferred into a filter stomacher bag (BagFilter, Spiral Biotech, Norwood, MA). Twenty milliliters of 0.1% sterile peptone water was added, and the contents were stomached for 2 min in a laboratory blender (Stomacher 400, Seward Medical, London, UK). Ten-fold serial dilutions were made in 0.1% peptone water and appropriate dilutions were poured plated or spiral plated on tryptose-sulfite-cycloserine agar (Oxoid, Ltd., Basingstoke, UK) without egg yolk. After solidification of the agar, plates were overlaid with an additional 8 to 10 ml of tryptose-sulfite-cycloserine agar and incubated at 35°C for 18 h in an anaerobic jar (AnaeroPack System, Mitsubishi Gas Chemical Co., Inc., New York, NY). Typical *C. perfringens* colonies were enumerated, and the counts were expressed as log CFU per gram of meat.

**Measurement of pH and water activity.** Five-gram portions of the sample were homogenized with 20 ml of deionized water for 2 min in the stomacher blender, and the pH of each sample was measured by immersing the electrode of the pH meter (model Accumet-Classic AB15, Fisher Scientific) in the sample homogenate. The water activity of the samples was measured with an AquaLab 3TE water activity meter (Decagon Devices, Inc., Pullman, WA), following the manufacturer’s instructions.

**Statistical analyses.** Three independent replications were performed for each of the six exponential chilling rates. Data (log CFU per gram) were analyzed, and the results of each treatment were compared with analysis of variance of the General Linear Model procedure of the Statistical Analysis System (SAS Institute, Inc., Cary, NC). Fisher’s least significant difference (LSD; α = 0.05) was used to separate means of the residual *C. perfringens* populations (log CFU per gram) of the samples.

**RESULTS AND DISCUSSION**

The pH and water activity of ground turkey roast are shown in Table 1. The addition of MOstatin V (pH 5.31) and MOstatin LV (pH 5.55) reduced (P ≤ 0.05) the pH of ground turkey roast, except for MOstatin LV added at 1.5%. Considering the *C. perfringens* growth range of pH between 5.0 and 9.0, with an optimum between 6.0 and 7.0, the decreases in pH values due to the addition MOstatin V and MOstatin LV should have limited influence on *C. perfringens* spore germination and outgrowth in the current study. The incorporation of MOstatin V and LV did not affect (P > 0.05) the water activity of ground turkey roast.

The germination and outgrowth of *C. perfringens* spores in ground turkey roast is shown in Figures 1 through 6. Minimal *C. perfringens* population increases (<1.0 log CFU/g) were observed in the ground turkey roast during cooling from 54.4 to 4.0°C in 6.5 h for all treatments. A *C. perfringens* population increase of 0.51 log CFU/g (Fig. 1) was observed in the control sample (without MOstatin V and MOstatin LV), which is within the limits of the FSIS performance standard of <1.00 log CFU/g of growth. These results closely agree with the findings of Kalinowski et al. (15), who reported an increase of 0.83 log CFU/g of the *C. perfringens* population in nuncured turkey during 6.5 h of chilling time. Velugoti et al. (31) also reported a minimal increase of 0.41 log CFU/g of the *C. perfringens* population in injected pork during exponential chilling profile of 6.5 h. These findings clearly indicate that proper cooling of cooked meat products from 54.4 to 4.0°C within 6.5 h would be adequate to prevent the germination and outgrowth of *C. perfringens* spores to <1.00 log CFU/g in nuncured meat and poultry products even in the absence of antimicrobials. Incorporation of MOstatin V and MOstatin LV at different levels either resulted in minimal increases or slight reductions in *C. perfringens* populations in ground turkey roasts containing MOstatin V and MOstatin LV in a concentration-dependent manner.

Incorporation of MOstatin V at 0.75% in the ground turkey roast formulation resulted in >1.00 log CFU/g growth of *C. perfringens* population when the product was cooled after a 9-h chilling time. During the 9-h abusive chilling time, incorporation of MOstatin V (1.25 and 2.5%) and MOstatin LV (1.5, 2.5, and 3.5%) inhibited *C. perfringens* growth in ground turkey roast to <1.00 log CFU/g (Fig. 2).

Deviations from the FSIS stabilization guidelines do occur occasionally in meat and poultry processing estab-
lishments, due to inadequate chilling capacity, increase in production volume, breakdown in refrigeration system, or power failures. In such cases, the presence of an antimicrobial agent at a concentration sufficient to prevent germination and outgrowth of *C. perfringens* spores will provide an additional safety measure.

There is a need for identifying and incorporating functional ingredients that possess antimicrobial activity, especially in minimizing *C. perfringens* spore germination and outgrowth in meat and poultry products within the organic, natural, and/or clean label categories as a margin of safety. Traditional antimicrobials such as nitrates and/or nitrites, phosphates, and organic acid salts as currently used in the meat processing industry are not allowed in the manufacture of organic and natural meat products. While the definitions and the degree of leniency in allowing antimicrobial agents, whether as functional or innocuous ingredients differ within these categories of products, the need for such ingredients to assure safety is magnified in organic or natural meat and poultry products when compared with their traditional counterparts.

Ingredients that have been used traditionally in food processing, such as vinegar and fruit juices (lemon and lime juices) do provide functionality (flavor and tangy taste) when they are added to meat and poultry products. In addition, they can contribute to the antimicrobial activity and help assure product safety.

Increasing the concentration of MOstatin V to 1.25% resulted in minimal increase in the *C. perfringens* population by 0.70 log CFU/g during 9 h of chilling. However, *C. perfringens* populations increased by 1.29, 1.93, 2.87, and 3.43 log CFU/g during the extended cooling times of 12, 15, 18, and 21 h, respectively. Further increasing the concentration of MOstatin V to 2.5% resulted in minimal increases in *C. perfringens* population (<1.00 log CFU/g) for all chilling times.

Incorporation of MOstatin LV at 1.5% concentration in the turkey roast resulted in a 0.87-log CFU/g increase during a 9-h chill time (Fig. 2), but chilling times beyond 9 h allowed >1-log CFU/g growth of *C. perfringens* in ground turkey roast. The mean *C. perfringens* populations increased by 1.46, 2.61, 2.61, and 3.82 for 12-, 15-, 18-, and 21-h exponential chilling profiles, respectively (Figs. 3 through 6). The incorporation of MOstatin LV at a concentration of 2.5% was effective in preventing the germination and outgrowth of *C. perfringens* spores to <1 log CFU/g during 9-, 12-, and 15-h chilling times, and the corresponding increases were 0.14, 0.23, and 0.91 log CFU/g, respectively. However, increasing chilling time to 18 or 21 h resulted in increases of >1 log CFU/g. Further increase in the concentration of MOstatin LV to 3.5% was able to minimize *C. perfringens* growth to <1.00 log CFU/g during the extended chilling times of 18 and 21 h. In ground turkey roast containing 3.5% MOstatin LV, the population of *C. perfringens* increased by 0, 0.08, 0.58, 0.38, and 0.80 log CFU/g for 9-, 12-, 15-, 18-, and 21-h chilling times, respectively.

Literature on the use of vinegar and mixtures of lemon juice concentrate and vinegar for controlling the germination and outgrowth of *C. perfringens* spores in meat products is lacking. The major and active components of vinegar and lemon juice are acetic acid and citric acids, respectively. While the precise composition of MOstatin V and MOstatin LV is not known, MOstatin V was more inhibitory to *C. perfringens*, compared with MOstatin LV in ground turkey roast. Incorporation of MOstatin V (2.5%) resulted in an increase in the *C. perfringens* population by 0.75 log CFU/g of turkey roast after a 21-h chilling profile. In order to achieve a similar inhibition in *C. perfringens* germination and outgrowth, higher MOstatin LV concentration (3.5%) was required. The observed differences could be explained by the mode of action of organic acids.

The organic acids added to foods exist in a pH-dependent equilibrium between the undissociated and dissociated state (4). The antimicrobial activity of organic acids primarily results from the uncharged, undissociated state of the molecule, a major species at low pH. The undissociated acid molecules freely cross the plasma membrane to enter the microbial cell. Once inside the cell, where the pH is maintained close to neutral (pH of 7.0) due
to active homeostasis by the microbial cell, the acid molecule dissociates, resulting in the release of the charged anions and protons in the cytoplasm, which could not have crossed the cell membrane on their own (4). These accumulating anions in the cytoplasm inhibit metabolic reactions (6) and the protons stress the intracellular pH homeostasis mechanisms (22). The $pK_a$ of an organic acid determines its effectiveness in inhibiting microbial growth. Organic acids with higher $pK_a$ values are more effective preservatives, and their antimicrobial efficacy generally improves with increasing chain length and degree of unsaturation (8).

The $pK_a$ values of acetic acid and citric acid are 4.75 and 3.13, respectively (8). Thus, at the pH (5.6) of ground turkey roast, a greater proportion of the acetic acid will be in the undissociated form, compared with the citric acid and hence, greater inhibition of $C. \text{perfringens}$ by vinegar, compared with lemon juice concentrate and vinegar blend. Miller et al. (20) reported that acetic acid salts were more effective in delaying the toxin production by $C. \text{botulinum}$ than were citric acid salts. The authors suggested that the

antibotulinal activity of acetic acid (monocarboxylic acid) was related to its $pK_a$, while citric acid (tricarboxylic acid) probably exhibited its action by the chelation mechanism. The superior inhibitory activity of acetic acid, compared with citric acid against $Escherichia \text{coli}$ O157:H7 (1, 7) and $Listeria \text{monocytogenes}$ (10, 27) has been reported. Acetic acid has also been reported to inhibit spore germination and vegetative cell growth of $Bacillus \text{cereus}$ (32).

Although literature on the use of acetic and citric acids per se in meat products is lacking, sodium salts of citric and acetic acids have been reported to be effective in preventing germination and outgrowth of $C. \text{perfringens}$ in a variety of meat systems (14, 22, 23, 28). In contrast to our findings, Sabah et al. (23) reported that sodium citrate was more effective in inhibiting the germination and outgrowth of $C. \text{perfringens}$, compared with sodium acetate and sodium diacetate in restructured roast beef. Incorporation of sodium acetate and sodium diacetate in restructured roast beef (0.25%) resulted in an increase of $C. \text{perfringens}$ populations by 1.21 and 0.68 log CFU/g, while buffered sodium citrate (2.0 and 4.8%) mostly reduced the $C. \text{perfringens}$ populations during the 18-h exponential chilling time. It was suggested that the citrate might have exerted its action by chelation rather than by the traditional weak acid mechanism of the undissociated molecule. The observed differences in the inhibitory activity of sodium citrate, sodium acetate, and sodium diacetate could have also resulted from the effective concentrations of active components, i.e., citric acid and acetic acid available from the added salts for the $C. \text{botulinum}$ growth inhibition mechanism of the undissociated molecule. The observed differences in the inhibitory activity of sodium citrate, sodium acetate, and sodium diacetate could have also resulted from the effective concentrations of active components, i.e., citric acid and acetic acid available from the added salts for the inhibited growth of $C. \text{botulinum}$ in restructured roast beef. Sabah et al. (23) also noted that sodium diacetate (60:40 [wt/wt] mixture of sodium acetate and acetic acid) was more effective than was sodium acetate in inhibiting $C. \text{perfringens}$ spore germination and outgrowth (0.68 to 1.21 log CFU/g of sodium
diacate and sodium acetate, respectively). This improved antimicrobial activity may be due to the pH lowering effect of the acetic acid in combination with the ability of the salt (sodium acetate) to enter the cytoplasm and reduce the internal pH of the cell, effectively imposing a greater strain on the cell pH homeostasis due to lower external and internal pH values. Sabah et al. (22) reported that sodium citrate (2%) was more effective, compared with sodium lactate (2%) in inhibiting _C. perfringens_ germination and outgrowth during cooling of cooked ground beef. The authors stated that effective concentrations of citrate and lactate available from the added salts (from the ingredients used) could have been one of the factors for the observed difference in the inhibitory activity of these two salts.

Juneja and Thippareddi (14) reported that buffered sodium citrate (IONAL, 1% [wt/wt]) resulted in reduction of _C. perfringens_ germination and outgrowth population (total spore and vegetative cells), while the use of buffered sodium citrate supplemented with 8% sodium diacetate (IONAL Plus) and sodium acetate (1% [wt/wt]) resulted in minimal increase (<0.32 log CFU/g) of _C. perfringens_ in the ground turkey meat after a 15-h chilling period. Further increasing the chilling time to 21 h resulted in _C. perfringens_ population increases of 0.74, 1.14, and 1.33 log CFU/g for IONAL, IONAL Plus, and sodium acetate (all antimicrobials used at 1% [wt/wt]), respectively. Increasing sodium acetate concentration to 2% (wt/wt) resulted in a 0.22-log CFU/g decrease in the _C. perfringens_ population in turkey breast meat. Thus, the increase or decrease of the _C. perfringens_ population in meat products in the presence of organic acid salts such as sodium citrate and sodium acetate depended on the actual amount of active antibacterial principle, i.e., citric acid and acetic acid. Similarly, Thippareddi et al. (28) reported that IONAL was more effective than IONAL Plus was, possibly due to lack of synergism between citrate and acetate or decrease in the total effective acetate ion concentration available for inhibition of germination and outgrowth. In all the above studies involving the use of citrates and acetates to inhibit _C. perfringens_ germination and outgrowth, the product formulations contained phosphates as one of the ingredients, and was not used in the present study. Phosphates have been reported to exhibit antimicrobial properties and act synergistically with nitrates and sodium chloride (10, 11). The phosphates act as chelators of cations essential for microbial growth (2, 25), a similar mechanism suggested for citric acid in the observed inhibition of _C. perfringens_ germination and outgrowth in meat systems (14, 23). Research on the synergism between phosphates and citrates in inhibiting the _C. perfringens_ germination and outgrowth in different meat systems is in progress in our laboratory.

Vinegar and lemon juice concentrate, either alone or as a mixture, were effective in controlling germination and outgrowth of _C. perfringens_ spores in ground turkey roast without other conventional meat processing ingredients like nitrite and/or nitrate and phosphates during extended chilling times. The use of these multifunctional ingredients offer the ready-to-eat meat and poultry manufacturers a means to claim a clean label, as consumers perceive these added ingredients as natural products. The sensory acceptability and shelf stability of these products containing minimal quantities of all natural ingredients like vinegar, lemon juice, sea salt, and turbinado sugar but free from traditionally used ingredients like nitrite–nitrate, phosphates should be investigated.

Caution should be exercised by meat and poultry processors when developing cooked meat and poultry products containing minimal ingredients as such products may present greater microbial risks, as evidenced in the current study. Properly designed microbial challenge studies should be conducted to evaluate the production practices of products that are termed “organic,” “natural,” or “clean label” to assure their microbial safety.

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