Carvacrol, Cinnamaldehyde, Oregano Oil, and Thymol Inhibit Clostridium perfringens Spore Germination and Outgrowth in Ground Turkey during Chilling¹

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ABSTRACT

Inhibition of Clostridium perfringens by plant-derived carvacrol, cinnamaldehyde, thymol, and oregano oil was evaluated during abusive chilling of cooked ground turkey. Test substances were mixed into thawed turkey product at concentrations of 0.1, 0.5, 1.0, or 2.0% (wt/wt) along with a heat-activated three-strain C. perfringens spore cocktail to obtain final spore concentrations of 2.2 to 2.8 log CFU spores per g of turkey meat. Aliquots (5 g) of the ground turkey mixtures were vacuum packed and then cooked in a water bath, where the temperature was raised to 60°C in 1 h. The products were cooled from 54.4 to 7.2°C in 12, 15, 18, or 21 h, resulting in 2.9-, 5.5-, 4.9-, and 4.2-log CFU/g increases, respectively, in C. perfringens populations in samples without antimicrobials. Incorporation of test compounds (0.1 to 0.5%) into the turkey completely inhibited C. perfringens spore germination and outgrowth (P ≤ 0.05) during exponential cooling in 12 h. Longer chilling times (15, 18, and 21 h) required greater concentrations (0.5 to 2.0%) to inhibit spore germination and outgrowth. Cinnamaldehyde was significantly (P < 0.05) more effective (<1.0-log CFU/g growth) than the other compounds at a lower concentration (0.5%) at the most abusive chilling rate evaluated (21 h). These findings establish the value of the plant-derived antimicrobials for inhibiting C. perfringens in commercial ground turkey products.

Contamination of turkey meat with spoilage and pathogenic microorganisms, as well as by yeast, may adversely affect the sensory and nutritional quality of the meat and occasionally cause human illness. The following studies are relevant to the theme of the present study. (i) Anaerobic spore-forming clostridia caused spoilage of cooked roast beef and turkey breast stored at 4°C or below (12, 13). (ii) Growth of the isolates in broth media was inhibited by 3.0% NaCl, 100-ppm nitrite, 2% sodium lactate, or 0.2% sodium diacetate (12, 13). (iii) Sodium diacetate and sodium lactate also inhibited a spore-forming Clostridium isolate recovered from a pink cook-in-bag turkey breast with an off-odor (16). (iv) Clostridium botulinum type B spores produced a toxin in cook-in-bag turkey breast, even when stored under strict refrigeration conditions (14). (v) Sodium diacetate with nitrite, lactate, or pediocin inhibited the growth of Listeria monocytogenes in turkey slurries (20). (vi) Coating turkey frankfurters with zein coatings containing nisin, sodium lactate, and sodium diacetate inhibited growth and recontamination by L. monocytogenes (15).

Lactobacillus sakei and Leuconostoc mesenteroides are the predominant spoilage bacteria that contaminated turkey breast during slicing and vacuum-packaging operations (19). Yarrowia lipolytica and Candida zeylanoides are the predominant yeast species that grew in poultry meats at refrigerated temperatures. Spoilage of poultry products induced by these yeast species results from lipolytic and proteolytic activities of yeast enzymes (3, 8). Collectively, these studies establish the need for new ways to inhibit both pathogenic and spoilage bacteria and yeast in turkey products.

In a previous study, we found that adding plant-derived carvacrol, cinnamaldehyde, thymol, and oregano oil to ground beef completely inhibited C. perfringens spore germination and outgrowth during exponential cooling (11). The main objective of this study was to find out whether the addition of these same natural antimicrobials would induce similar inhibition of the spores in retail ground turkey.

MATERIALS AND METHODS

Test compounds. Carvacrol (>98% pure) was a gift from Millenium Chemical Co. (Boca Raton, Fla.), cinnamaldehyde (>99.5% pure) and thymol (>98% pure) were obtained from Sigma (St. Louis, Mo.), and oregano oil was purchased from Lhasa Kamak Herb Co. (Berkeley, Calif.).

Test organisms and spore production. C. perfringens strains NCTC 8238, NCTC 8239, and ATCC 10288 from the Microbial Food Safety Research Unit, Eastern Regional Research Unit (Wyndmoor, Pa.), culture collection were maintained as spore-forming stock cultures in a cooked meat medium (Difco Laboratories, Detroit, Mich.). An active culture was prepared in freshly

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prepared fluid thioglycollate medium, and sporulation was stimulated in Duncan and Strong sporulation medium as previously described (10). After the spore crop of each strain had been washed twice and resuspended in sterile distilled water, the spore suspensions were stored at 4°C. A spore cocktail containing the three strains of C. perfringens was prepared immediately prior to use by mixing of approximately the same number of spores from each suspension. This composite of spore strains was heat shocked for 20 min at 75°C just prior to use.

Preparation and inoculation of ground turkey. Cooked ground turkey was obtained from a local retail market and stored frozen (−5°C) until used (approximately 40 days). Carvacrol, cinnamaldehyde, oregano oil, or thymol was separately mixed into ground turkey samples with a Hobart mixer to a final concentration of 0.1 to 2.0% (wt/wt). Duplicate 5-g samples were aseptically weighed into filter stomacher 400 polyethylene bags containing noninoculated turkey. The bags were then evacuated to a negative pressure of 1,000 millibars and were heat sealed by using a Multivac gas-packaging machine (model A300/16, Multivac Inc., Kansas City, Mo.).

Cooking and cooling procedures. The Spiral Biotech bags containing inoculated products were sandwiched between stainless steel wire racks as described previously (22). The product was then submerged completely in a circulating water bath (Exacal, model RTE-221, NESLAB Instruments, Inc., Newington, N.H.). The temperature of the water bath was increased linearly to 60°C in a period of 1 h to mimic cooking in retail food service establishments and the food industry. The bags were then removed, chilled immediately in an ice water bath, and plated as described below. A second set of racks containing the product for each treatment was cooked (60°C/1 h) and then transferred to a programmable water bath set at 54.5°C. The bath was allowed to equilibrate at this temperature for 10 min and then chilled at an exponential rate from 54.5 to 7.2°C according to the target chilling times shown in Table 1.

Enumeration of bacteria. Immediately after cooking and/or chilling, samples were removed and their total germinated C. perfringens populations were enumerated by spiral plating (Spiral Systems Model D Plating Instruments, Cincinnati, Ohio) on tryptose-sulfite-cycloserine agar (Difco) as described previously (9). The total C. perfringens population was determined after 48 h of incubation at 37°C in a Bactron anaerobic chamber (Bactron IV, Sheldon Laboratories, Cornelius, Ore.). The lower limit of detection by this procedure is 21 CFU/ml. Uninoculated ground turkey and cooked turkey (25 g) were used to verify the absence of

### TABLE 1. Populations of C. perfringens in cooked ground turkey containing 0.1 to 2.0% thymol, cinnamaldehyde, oregano oil, or carvacrol immediately after heat treatment (cooked to 60°C in 1 h) and following cooling (chill) from 54.4 to 7.2°C exponentially in 21, 18, 15, or 12 h

<table>
<thead>
<tr>
<th>Treatment</th>
<th>21 h Cook (60°C/h)</th>
<th>21 h Chill</th>
<th>18 h Cook (60°C/h)</th>
<th>18 h Chill</th>
<th>15 h Cook (60°C/h)</th>
<th>15 h Chill</th>
<th>12 h Cook (60°C/h)</th>
<th>12 h Chill</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.84 (0.17)</td>
<td>7.01 (0.25)</td>
<td>2.63 (0.44)</td>
<td>7.18 (0.43)</td>
<td>2.19 (0.49)</td>
<td>7.69 (0.14)</td>
<td>2.5 (0.38)</td>
<td>5.35 (0.41)</td>
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<tr>
<td>Thymol (%)</td>
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</tr>
<tr>
<td>0.1</td>
<td>2.86 (0.13)</td>
<td>7.07 (0.12)</td>
<td>2.86 (0.13)</td>
<td>7.59 (0.1)</td>
<td>2.86 (0.13)</td>
<td>6.06 (0.01)*</td>
<td>2.86 (0.13)</td>
<td>3.59 (0.27)*</td>
</tr>
<tr>
<td>0.5</td>
<td>2.75 (0.08)</td>
<td>7.32 (0.13)</td>
<td>2.75 (0.08)</td>
<td>7.36 (0.01)</td>
<td>2.75 (0.08)</td>
<td>6.01 (0.01)*</td>
<td>2.75 (0.08)</td>
<td>3.24 (0.01)*</td>
</tr>
<tr>
<td>1.0</td>
<td>3.26 (0.3)</td>
<td>4.54 (0.46)*</td>
<td>2.44 (0.26)</td>
<td>3.38 (0.5)*</td>
<td>2.46 (0.57)</td>
<td>2.58 (0.25)*</td>
<td>2.58 (0.13)</td>
<td>2.83 (0.04)*</td>
</tr>
<tr>
<td>2.0</td>
<td>3.21 (0.39)</td>
<td>2.56 (0.2)*</td>
<td>2.44 (0.57)</td>
<td>2.36 (0.5)*</td>
<td>2.36 (0.35)</td>
<td>2.46 (0.21)*</td>
<td>2.38 (0.13)</td>
<td>2.46 (0.35)*</td>
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<tr>
<td>Cinnamaldehyde (%)</td>
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<tr>
<td>0.1</td>
<td>2.71 (0.05)</td>
<td>6.82 (0.03)</td>
<td>2.71 (0.05)</td>
<td>7.58 (0.01)</td>
<td>2.71 (0.05)</td>
<td>5.88 (0.04)*</td>
<td>2.71 (0.05)</td>
<td>3.38 (0)*</td>
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<tr>
<td>0.5</td>
<td>2.61 (0.29)</td>
<td>4.04 (0.23)*</td>
<td>2.61 (0.29)</td>
<td>4.04 (0.28)*</td>
<td>2.61 (0.29)</td>
<td>4.06 (0.82)*</td>
<td>2.61 (0.29)</td>
<td>3.16 (0.02)*</td>
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<tr>
<td>1.0</td>
<td>3.41 (0.17)</td>
<td>2.81 (0.08)*</td>
<td>2.86 (0.23)</td>
<td>2.66 (0.19)*</td>
<td>2.98 (0.15)</td>
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<td>3.15 (0.22)*</td>
<td>3.03 (0.06)</td>
<td>2.81 (0.39)*</td>
<td>2.95 (0.17)</td>
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<td>2.49 (0.02)</td>
<td>2.95 (0.01)*</td>
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<tr>
<td>Oregano oil (%)</td>
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<tr>
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<td>2.69 (0.18)</td>
<td>7.29 (0.08)</td>
<td>2.69 (0.18)</td>
<td>7.7 (0.21)</td>
<td>2.69 (0.18)</td>
<td>7.37 (0.09)</td>
<td>2.69 (0.18)</td>
<td>3.84 (0.04)*</td>
</tr>
<tr>
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<td>7.43 (0.1)</td>
<td>2.81 (0.18)</td>
<td>7.41 (0.08)</td>
<td>2.81 (0.18)</td>
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<td>2.81 (0.18)</td>
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</tr>
<tr>
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<td>4.87 (0.31)</td>
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<td>4.65 (0.4)*</td>
<td>2.72 (0.6)</td>
<td>3.70 (0.49)*</td>
<td>2.39 (0.03)</td>
<td>2.72 (0.06)*</td>
</tr>
<tr>
<td>2.0</td>
<td>3.03 (0.16)</td>
<td>2.59 (0.14)</td>
<td>2.31 (0.38)</td>
<td>2.6 (0.81)*</td>
<td>2.41 (0.24)</td>
<td>2.19 (0.23)*</td>
<td>2.26 (0.06)</td>
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<tr>
<td>Carvacrol (%)</td>
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<td></td>
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<td>2.67 (0.04)</td>
<td>7.19 (0.2)</td>
<td>2.67 (0.04)</td>
<td>7.59 (0.17)</td>
<td>2.67 (0.04)</td>
<td>6.09 (0.04)*</td>
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<td>3.31 (0.01)*</td>
</tr>
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<td>0.5</td>
<td>2.53 (0.06)</td>
<td>6.98 (0.2)</td>
<td>2.53 (0.06)</td>
<td>7.47 (0.2)</td>
<td>2.53 (0.06)</td>
<td>6.02 (0.04)*</td>
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<td>3.16 (0.02)*</td>
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<td>1.0</td>
<td>3.06 (0.25)</td>
<td>2.84 (0.53)*</td>
<td>2.08 (0.45)</td>
<td>2.44 (0.2)*</td>
<td>2.43 (0.43)</td>
<td>2.16 (0.47)*</td>
<td>2.36 (0.03)</td>
<td>2.33 (0.13)*</td>
</tr>
<tr>
<td>2.0</td>
<td>2.84 (0.21)</td>
<td>2.37 (0.25)*</td>
<td>2.13 (0.56)</td>
<td>2.19 (0.49)*</td>
<td>2.48 (0.33)</td>
<td>2.27 (0.31)*</td>
<td>2.18 (0.03)</td>
<td>2.36 (0.03)*</td>
</tr>
</tbody>
</table>

*a Values are averages of two separate determinations each in duplicate with standard deviations shown in parentheses. *, significantly less than the corresponding control (P < 0.05) by Dunnett’s test.
naturally occurring \textit{C. perfringens}. This verification involved plat-
ing lactose-gelatin and nitrate-motility medium (21).

\textbf{Statistical analyses.} Two independent trials, in duplicate, as
defined by a new batch of turkey meat, were performed for each
of the exponential chilling rates (12, 15, 18, and 21 h). Data were
analyzed by analysis of variance with the General Linear Model
procedure of the Statistical Analysis System (release 9.1.3 [2000],
SAS Institute, Inc., Cary, N.C.). Dunnett’s test was used to test
for a decrease from the control for \textit{C. perfringens} populations (in
numbers of log CFU per gram) (17).

\textbf{RESULTS AND DISCUSSION}

The programmed time-temperature profiles of the products
for the 12-, 15-, 18-, and 21-h exponential chill rates are
described elsewhere (11). These profiles represent ex-
tended chilling rates based on the U.S. Department of Ag-
ticulture/Food Safety and Inspection Service (USDA/FSIS)
stabilization requirements for chilling of uncurled, cooked
meat and poultry products (23). Table 1 shows that chilling
of ground turkey from 54.4 to 7.2°C resulted in germina-
tion and growth of \textit{C. perfringens} spores from initial popula-
tions after cooking of about 2.2 to 2.8 log CFU/g of ground
turkey by 2.9, 5.5, 4.9, and 4.2 log CFU/g following 12-
15-, 18-, and 21-h exponential chill rates, respectively. Ta-
ble 1 also shows that adding 0.1 to 2% carvacrol, cin-
namaldehyde, oregano oil, or thymol to the poultry meat gen-
-erally inhibited germination and outgrowth of \textit{C. perfrin-
gens} spores at all four exponential chill rates. The inhibition
was concentration dependent.

To facilitate the use of the data as a guide for large-
scale commercial evaluation of the antimicrobials, we offer
the following overview of the observed effects for each
antimicrobial at each concentration for the four chill rates
compared to respective controls:

At the 21-h chill rate, thymol had no effect at the 0.1
and 0.5% levels but caused a decrease from the control of
2.5 log CFU/g at the 1% level and a decrease of 4.5 log
CFU/g at the 2% level. Thymol completely restricted
growth from spores at the 2% level (levels were the same
as controls). At the 18-h chill rate, there was no effect at
the 0.1 and 0.5% levels, a decrease of 3.8 log CFU/g at the
1% level, and a decrease of 4.8 log CFU/g at the 2% level.
The spores were completely inhibited at the 2% level.
At the 15-h chill rate, there was a decrease of \sim 16 log CFU/
g at both the 0.1 and 0.5% levels and of \sim 5.2 log CFU/g at
both the 1 and 2% levels. The spores were completely
inhibited at the 1 or 2% level. At the 12-h chill rate, the
decrease at the 0.1% level was 1.8 log CFU/g and, at the
0.5 to 2% levels, 2.1 to 2.9 log CFU/g. The spores were
completely inhibited at the 0.5, 1, or 2% level of thymol.

With cinnamaldehyde at the 21-h chill rate, there was
no effect at the 0.1% level and a decrease from the control
of 3.0 log CFU/g at the 0.5% level and of \sim 3.9 to 4.2 log
CFU/g at the 1 and 2% levels. A cinnamaldehyde level of
1 or 2% completely inhibited the spores. At the 18-h chill
rate, there was no effect at the 0.1% level and a decrease
of 3.1 log CFU/g at the 0.5% level and of 4.5 to 4.8 log
CFU/g at the 2% level. A cinnamaldehyde level of 1 or 2% completely inhibited the spores. At the 15-h chill rate, there
was a decrease of 1.8 log CFU/g at the 0.1% level, of 3.6
log CFU/g at the 0.5% level, and of 4.5 to 4.6 log CFU/g
at the 1 and 2% levels. The spores were completely inhibited
at a concentration of 1 or 2%. At the 12-h chill rate, the
decrease at the 0.1 to 2% levels was \sim 2.0 to 2.5 log
CFU/g. All four concentrations completely inhibited the
spores.

With oregano oil at the 21-h chill rate, there was no effect
at the 0.1 and 0.5% levels and a decrease from the control
of 2.1 log CFU/g at the 1% level and of 4.4 log
CFU/g at the 2% level. Concentrations of oregano oils of
\sim 2% completely inhibited the spores. At the 18-h chill rate,
there was no effect at the 0.1 and 0.5% levels and a decrease
of 2.5 log CFU/g at the 1% level and of 4.6 at the
2% level. Concentrations of \sim 2% completely inhibited the
spores. At the 15-h chill rate, there was no effect at the 0.1
and 0.5% levels and a decrease from the control of 4.0 log
CFU/g at the 1% level and of 5.5 log CFU/g at the 2% level.
The spores were completely inhibited at 2%. At the
12-h chill rate, the decrease from the control at the 0.1 to
2% levels was 1.5 to 2.9 log CFU/g. The spores were com-
pletely inhibited at the 0.5 to 2% levels.

With carvacrol at the 21-h chill rate, there was no effect
at the 0.1 and 0.5% levels and a decrease from the control
of 4.2 to 4.6 log CFU/g at the 1 and 2% levels. Concentrations
of 1 or 2% of carvacrol completely inhibited the
spores. At the 18-h chill rate, there was no effect at the 0.1
and 0.5% levels and a decrease from the control of
4.7 to 5.0 log CFU/g at the 1 and 2% levels. A concen-
tration of 1 or 2% completely inhibited the spores. At the
15-h chill rate, there was a decrease from the control of 1.6
log CFU/g at both the 0.1 and 0.5% levels and of \sim 5.0 at
both the 1 and 2% levels. The spores were completely
inhibited at a level of 1 or 2%. At the 12-h chill rate, the
decrease from the control at the 0.1 to 1% levels was \sim 2.0
log CFU/g and, at the 2% level, 3.0 log CFU/g. The spores
were completely inhibited at all four levels.

The presence of \textit{C. perfringens} spores in foods is a
potential health hazard (18). \textit{C. perfringens} is frequently
found in meats, generally through fecal contamination of
carcasses, contamination from other ingredients, and/or
from postprocessing contamination. Abusive conditions
during distribution of the product in the food chain could
favor germination and outgrowth of the spores.

The USDA/FSIS draft compliance guidelines for
ready-to-eat meat and poultry products state that such products
should be cooled at a rate sufficient to allow no more than
a 1-log total increase of \textit{C. perfringens} cells per g of
meat (23). These federal guidelines also state that cooling
from 54.4 to 26.6°C should take no longer than 1.5 h and
that cooling from 26.6 to 4.4°C should take no longer than
5 h (23). Additional guidelines allow for the cooling of
certain cured and cooked meats from 54.4 to 26.7°C in 5
and from 26.7 to 7.2°C in 10 h. Processors can use either
customized or alternate chilling regimens as long as a <1-
log increase of \textit{C. perfringens} levels in the finished product
 can be documented.

Numerous studies have been published on the antimi-
crobial activities of plant essential oils and their constitu-
ents against foodborne pathogens as reviewed by Burt (2) and by Friedman (4). However, there are no published data on the efficacy of plant-derived compounds against *C. perfringens* during chilling of cooked ground turkey. Because carvacrol, cinnamaldehyde, oregano oil, and thymol were previously found to exhibit strong antimicrobial effects against foodborne pathogens (2), the main objective of this study was to evaluate the ability of these plant-derived substances to control or inhibit germination and outgrowth of *C. perfringens* from spores in ground turkey meat sold at retail during extended chill processing. The results herein showed that (i) all of the test substances inhibited germination at dose-dependent rates; (ii) the extent of inhibition by the antimicrobials was approximately in the following order: cinnamaldehyde > thymol > carvacrol > oregano (*P* ≤ 0.05); and (iii) the extent of inhibition of the spores in ground turkey was similar to that previously described for ground beef (11).

The results of the present study with ground turkey and the previous study with ground beef indicate that thymol (1 to 2%), cinnamaldehyde (0.5 to 2%), oregano oil (2%), and carvacrol (1 to 2%) can be used as ingredients at the indicated levels in processed turkey and meat products to protect against a *C. perfringens* hazard during chilling. The described inhibition may reduce the risk of illness in case of product temperature abuse. Further research should investigate the antimicrobial effects of the essential oils and oil compounds in combination with other ingredients typically used in the meat industry (nitrite, nisin, sodium chloride, sodium diacetate, and sodium lactate) in order to optimize their use at the lowest possible levels to afford microbiological safety.

Cinnamaldehyde, which had the highest activity against *C. perfringens* in turkey meat, is present in numerous commercial foods (7) and is listed as generally accepted as safe (1). Levels of carvacrol, thymol, and oregano oil tested in this study may also be safe (2). Unresolved are the questions as to whether the products also exhibit acceptable sensory properties and how the turkey matrix and storage temperatures and times may influence antibacterial effects.

Because the plant compounds evaluated in this study were also previously found to inhibit growth of *Campylobacter jejuni*, *Escherichia coli* O157:H7, *L. monocytogenes*, and *Salmonella enterica* (4, 6), as well as antibiotic-resistant bacteria (5), these and other plant essential oils and their compounds may be able to control growth of *C. perfringens* as well as one or more of the other nonresistant as well as antibiotic-resistant pathogens during storage and distribution of poultry products. This possibility merits further study.

In conclusion, the present study suggests that it may be possible to prevent the germination and outgrowth of *C. perfringens* spores in turkey products by incorporating natural antimicrobials, thus further minimizing risk to the consumer.

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