Control of *Clostridium perfringens* in Cooked Ground Beef by Carvacrol, Cinnamaldehyde, Thymol, or Oregano Oil during Chilling†

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**ABSTRACT**

Inhibition of *Clostridium perfringens* spore germination and outgrowth by carvacrol, cinnamaldehyde, thymol, and oregano oil was evaluated during abusive chilling of cooked ground beef (75% lean) obtained from a local grocery store. Test substances were mixed into thawed ground beef 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 ca. 2.8 log spores per g. Aliquots (5 g) of the ground beef mixtures were vacuum-packed and then cooked in a water bath, the temperature of which 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 3.18, 4.64, 4.76, and 5.04 log CFU/g increases, respectively, in *C. perfringens* populations. Incorporation of test compounds (≥0.1%) into the beef completely inhibited *C. perfringens* spore germination and outgrowth (*P* ≤ 0.05) during exponential cooling of the cooked beef in 12 h. Longer chilling times (15, 18, and 21 h) required greater concentrations to inhibit spore germination and outgrowth. Cinnamaldehyde was significantly (*P* < 0.05) more effective (<1.0 log CFU/g growth) at a lower concentration (0.5%) at the most abusive chilling rate evaluated (21 h) than the other compounds. Incorporation of lower levels of these test compounds with other antimicrobials used in meat product formulations may reduce the potential risk of *C. perfringens* germination and outgrowth during abusive cooling regimes.

The presence of *C. perfringens* spores in foods is a potential health hazard. This organism is commonly found in soil and dust, in the intestinal tract of humans and animals, in spices, and on the surfaces of vegetable products, as well as in other raw and processed foods. *C. perfringens* is also frequently found in meats, generally through fecal contamination of carcasses, contamination from other ingredients and/or postprocessing contamination. Thus, the organism was detected in 36, 80, and 2% of fecal samples from cattle, poultry, and swine, respectively (37). *C. perfringens* was isolated from 43.1% of processed and unprocessed beef, veal, lamb, pork, and chicken products (16); 47.4% of raw ground beef samples (24); 38.9% of commercial pork sausage samples (2); and was found on raw and cooked beef, and on equipment of food service establishments lacking safety standards (5). A mean level of 45 *C. perfringens* CFU/cm² was detected on raw beef carcass surface samples (31).

*C. perfringens* outbreaks linked to meat usually result from improper handling and mistreatment in food service environments (3, 4, 34), and rarely involve commercial meat processors. Fifty-seven outbreaks and 2,772 cases of *C. perfringens* food poisoning were reported to the Centers for Disease Control and Prevention (CDC) between 1993 and 1997 (8). Because most cases of *C. perfringens* food poisoning are mild and not reported, the actual number of cases in the United States is estimated to be as high as 248,520 per year (25), resulting in an estimated cost of $200 per case (36). Meat and poultry products were associated with the vast majority of these outbreaks in the United States (4, 34), possibly because the organism requires more than a dozen amino acids and several vitamins for growth that are typically present in meat (6, 23). *C. perfringens* grows rapidly in meat systems. The growth temperature ranges from 6 to 50°C, with a doubling time as short as 7.1 to 10 min (17). Due to its rapid growth and wide distribution, growth of *C. perfringens* is used as a standard to assess the safety of cooling processes for meat and poultry products. Although the potential risk of outgrowth in these products may be low, abusive conditions during distribution of the product in the food chain could favor spore germination and outgrowth.

The U.S. Department of Agriculture/Food Safety Inspection Service (USDA/FSIS) draft compliance guidelines for ready-to-eat (RTE) meat and poultry products state that such products should be cooled at a rate sufficient to prevent more than a 1-log increase of *C. perfringens* cells (40). 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 (40). Additional guidelines allow for the cooling of certain cured cooked meats from 54.4 to 26.7°C in 5 h 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 C. perfringens levels in the finished products can be documented. These guidelines do not consider the antimicrobial efficacies of added antimicrobial compounds that are widely used in the food industry as flavoring agents.

A recent study (21) reported that C. perfringens levels in cooked RTE meat and poultry products that do not meet the USDA/FSIS stabilization requirements were <10 CFU/g (79% samples). However, 16% of the samples had total C. perfringens populations >10 CFU/g and 5% had >100 CFU/g, indicating that the meat formulations/batters contained spores of this pathogen. Highest levels for this pathogen (>100 CFU/g) were observed in a barbecued product.

Numerous studies have been published on the antimicrobial activities of plant essential oils and their constituents against foodborne pathogens (7). Essential oil components such as carvacrol, cinnamaldehyde, and thymol can inhibit growth of foodborne pathogens in alfalfa seeds and sprouts (43), apple juice (12), carrot broth (42), fresh cut melon (27), meat (9, 33), poultry (15), rice (38), seafood (22), and pathogenic oral human bacteria (10). However, there are no published data on the efficacy of plant-derived compounds against C. perfringens during chilling of cooked ground beef. Consequently, the main objective of this study was to evaluate the ability of the plant-derived substances carvacrol, cinnamaldehyde, oregano oil, and thymol to control or inhibit germination and outgrowth of C. perfringens from spores in ground beef during extended chill processing.

MATERIALS AND METHODS

Test compounds. Carvacrol was a gift from Millenium Chemical Co. (Boca Raton, Fla.), cinnamaldehyde and thymol were obtained from Sigma (St. Louis, Mo.), and oregano oil came from Lhasa Karnark Herb Co. (Berkeley, Calif.).

Test organisms and spore production. Clostridium 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 sporulated stock cultures in a cooked meat medium (Difco, Becton Dickinson, Sparks, Md.). Active cultures were prepared in fresh fluid thioglycollate medium. Sporulation was stimulated in Duncan and Strong sporulation medium as previously described (18). Spore numbers were determined by spiral plating (Spiral Systems Model D Plating Instruments, Cincinnati, Ohio) on tryptose-sulfite-cycloserine (TSC) agar (Difco) as described previously (19). After the spore crop of each strain was 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 before use by combining an equal number of spores from each suspension. This composite of spore strains was then heat shocked for 20 min at 75°C prior to use.

Preparation and inoculation of meat. Ground beef (75% lean) was obtained from a local retail market and stored frozen (−5°C) until used (approximately 40 days). Thymol, cinnamaldehyde, oregano oil, or carvacrol were each mixed separately into ground beef samples with a Hobart mixer to final concentrations of 0.1, 0.5, 1.0, or 2.0% (wt/wt). All treatments were duplicated. Duplicate 5-g samples were aseptically weighed into filter Stomacher 400 polyethylene bags (SFB-0410, Spiral Biotech, Bethesda, Md.) and inoculated with 1 ml of the heat-shocked C. perfringens spore cocktail to a final spore concentration of ca. 2.8 log CFU/g. The contents of the bags were thoroughly mixed to ensure even distribution of the spores in the meat sample. Negative controls consisted of bags containing uninoculated beef. The bags were then evacuated to a negative pressure of 1,000 millibars and heat sealed using a Multivac gas-packaging machine (model A300/16, Multivac Inc., Kansas City, Mo.).

Heat shock and cooling procedures. The bags containing inoculated products were sandwiched between stainless steel wire racks as described elsewhere (35). They were then submerged completely in a circulating water bath (Exacal, model RTE-221, NESLAB Instruments, Inc., Newington, N.H.) and then transferred to a programmed water bath set at 54.5°C. The bath was allowed to equilibrate at this temperature for 10 min and then cooled 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 enumerated for total germinated C. perfringens population by spiral plating. The total C. perfringens population was determined after 48 h incubation at 37°C in a Bactron anaerobic chamber (Bactron IV, Sheldon Laboratories, Cornelius, Oreg.). Both uninoculated raw beef and cooked beef (25 g) were used to verify the absence of naturally

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occuring C. perfringens. This verification involved the use of lactose-gelatin and nitrate-motility medium (30).

**Statistical analyses.** Two independent trials, in duplicate, as defined by a new batch of meat, were performed for each of the exponential chilling rates (12, 15, 18, and 21 h). Data were analyzed by variance using the General Linear Model procedure of the Statistical Analysis System (SAS Institute Inc., Cary, N.C., 2000; Release 8.01). The Bonferroni LSD method was used to separate means of the C. perfringens populations (log CFU per gram) (26).

### RESULTS AND DISCUSSION

Table 1 shows the programmed time-temperature profiles of the products for the 12-, 15-, 18-, and 21-h exponential chill rates. These profiles represent extended chilling rates based on the USDA/FSIS stabilization requirements for chilling of un cured, cooked meat, and poultry products (40). In the present study, we have demonstrated/validated the use of vacuum bags and small (5 g) portions of meat for conducting challenge studies using C. perfringens spore-inoculated product. This method was easy to perform and addressed the small amount of product at the center of a large beef roast, considered to be the cold spot (worst-case scenario). The water bath generated highly reproducible product temperature profiles and can be used in future validation studies or for evaluation of microbiological safety of much larger quantities of meat.

Table 2 and Figures 1 and 2 show that the chilling of ground beef from 54.4 to 7.2°C resulted in germination and outgrowth of C. perfringens spores from initial populations of about 2.8 log CFU/g to 3.2, 4.6, 4.8, and 5.0 log CFU/g following 12, 15, 18, and 21 h of exponentially chilling, respectively. The extent of growth observed in the present study is higher than that observed previously in our laboratory (20, 35) when ingredients such as salt (NaCl) and phosphates were evaluated.

Table 2 and Figures 1 and 2 also show that adding 0.1 to 2% carvacrol, cinnamaldehyde, oregano oil, or thymol to the meat inhibited germination and outgrowth of C. perfringes in cooked ground beef containing thymol, cinnamaldehyde, oregano oil, or carvacrol immediately after heat treatment (HS, 60°C in 1 h) and following cooling (CHILL) from 54.4 to 7.2°C exponentially in 18 h.
fringens spores at 12 h exponential chill rates. When the rate and extent of cooling from 54.4 to 7.2°C was increased to 15 h, 1% carvacrol, cinnamaldehyde, or thymol adequately restricted growth from spores. However, the rates for germination and multiplication were slower using 1% oregano oil. At a concentration of 2%, oregano oil completely controlled C. perfringens growth during 15 h cooling. At concentrations of 0.1, 0.5, or 1%, these antimicrobials inhibited germination and outgrowth of C. perfringens at a 15-h exponential chill rate in the following order: thymol > cinnamaldehyde > carvacrol > oregano ($P \leq 0.05$). Supplementation of beef with 0.1 or 0.5% oregano oil resulted in greater germination and outgrowth of C. perfringens spores than with thymol, cinnamaldehyde or carvacrol, with a maximum population of 7.4 log CFU/g after exponential chilling in 15 h compared to an initial spore population of 2.75 log CFU/g.

Addition of 0.1 or 0.5% carvacrol, oregano oil, or thymol to the meat did not control germination and outgrowth of C. perfringens spores following 18 or 21 h of cooling. Starting with an initial population of about 2.5 log CFU/g, final C. perfringens populations in all samples were about 7 log CFU/g after cooling. The only exception was 0.5% cinnamaldehyde under which conditions the final C. perfringens levels after cooling were <4 log CFU/g. At higher concentrations (1.0 or 2.0%), all compounds used in the study effectively controlled germination and outgrowth of C. perfringens, with cinnamaldehyde and carvacrol being significantly more effective ($P \leq 0.05$) than oregano oil. At a level of 2%, oregano oil was also highly effective in restricting the growth of C. perfringens. These results indicate that the antimicrobial effects are concentration-dependent.

We did not observe decreased populations of C. perfringens after exponential 18 and 21 h chilling as compared to the populations after cooking and/or before the start of chilling. This is in contrast to previous findings in which total C. perfringens populations in inoculated ground roast beef supplemented with Ional or Ional Plus (1.3%) and with Purasal or Optiform (3.0 and 4.8%) decreased during 18 and 24 h of exponential chilling (35).

Previously, we also reported C. perfringens population increases of 1.5 and 5.3 log CFU/g in roast beef following 18-h and 21-h chill rates and increases of 4.1 and 4.4 log CFU/g in roast beef following 18 and 21 h chill rates, respectively (20, 35). In these previous studies, we assessed the fate of C. perfringens in ground beef that was obtained by grinding beef top rounds injected with minimal levels of salt (NaCl, 0.85%), potato starch (0.25%), and potassium tetra pyrophosphate (0.2%) at a 12% pump rate. By contrast, in the present study, we inoculated ground beef without these ingredients to obtain conservative estimates of the growth of C. perfringens from spores. The different results may be attributed to the presence of additives in the previous studies, resulting in lower growth of C. perfringens during 18 or 21 h cooling. Nevertheless, these germination and outgrowth rates exceed the USDA/FSIS stabilization performance standards for control of C. perfringens.

Previously, we also heat treated the product at 75°C for 20 min to serve the dual purpose of heat activating the spores and simulating the cooking process (35). In the present study, we cooked the product in a water bath in which the temperature was raised to 60°C during 1 h to mimic heat processing in the meat industry. Both the earlier and present studies simulated a worse case scenario that would result in heat activation of spores and subsequent growth during abusive cooling from 54.4 to 7.2°C.

In a related study, Shigehisa et al. (32) reported on the germination and growth of C. perfringens spores inoculated into ground beef heated at 60°C and then cooled to 15°C at a linear cooling rate of 5 to 25°C/h. Under these conditions, the organism did not grow during exposure to falling temperature rates of 25 to 15°C/h. However, growth was observed at cooling rates less than 15°C/h. This study is not totally applicable to typical retail food operations because cooling is not linear; it is exponential, as employed in the present study.

Other studies have demonstrated the efficacy of various antimicrobial agents against the growth of C. perfringens during cooling of meat products. For example, Sabah et al. (28, 29) found that 0.5 to 4.8% sodium citrate inhibited growth of C. perfringens in cooked vacuum-packaged restructured beef cooled from 54.4 to 7.2°C within 18 h and that oregano oil in combination with organic acids inhibited growth of the organism during cooling of sous-vide cooked ground beef products. Juneja and Thippareddi (20) observed that organic acid salts such as 1% sodium lactate, 1% sodium acetate, or 1% buffered sodium citrate (with or without sodium diacetate) inhibited germination and outgrowth of C. perfringens spores during the chilling of marinated ground turkey breast. Some of the meat products sampled after process deviations (stabilization) contained populations of >10 CFU/g (up to a maximum of 710 CFU/g) (21). Such levels of C. perfringens could be due to germination and outgrowth during deviations in the cooling process and/or to the survival of the spores during cooking.

The time/temperature guidelines for cooling cooked products specify that the maximum internal temperature should not remain between 54.4 and 26.7°C for more than 1.5 h or between 26.7 and 4.4°C for more than 5 h (40). The U.S. Food and Drug Administration (FDA) Division...
of Retail Food Protection recognized that inadequate cooling poses a major food safety problem and, thus, established a recommendation that all food should be cooled from 60 to 21°C (140 to 70°F) in 2 h and from 21 to 5°C (70 to 41°F) in 4 h (41). Moreover, cooling process deviations do occur and processors have to evaluate microbiological safety of the products in such process deviations. While the guidelines suggest use of microbiological predictive models to evaluate potential \( C. \) *perfringens* spore germination and outgrowth, these predictive models do not consider the antimicrobial effects of some ingredients, including phosphates, nitrates and organic acid salts, that are used in the processed meat industry.

Sodium and potassium salts of propionic, lactic, pyruvic, acetic, and citric acids are extensively used in meat and poultry products as either flavor enhancers or to extend microbiological shelf stability. Sodium citrate and citric acid are Generally Recognized as Safe (GRAS) ingredients and have been shown to inhibit growth of pathogens in meat products. However, the apparent reluctance of meat processors to use organic acid salts at concentrations sufficient to inhibit foodborne pathogens is due to the accompanying undesirable organoleptic quality changes.

Incorporation of natural antimicrobials that can inhibit germination and outgrowth of *C. perfringens* may reduce the risk of illness if the product is temperature abused. Based on results of the present study, plant-derived antimicrobials such as thymol (1 to 2%), cinnamaldehyde (0.5 to 2%), oregano oil (2%), and carvacrol (1 to 2%) can be used as ingredients in processed meat products to provide an additional measure of safety to address the *C. perfringens* hazard during chilling of meat products. Because the present study demonstrated that essential oils and oil compounds can control *C. perfringens* spore germination and outgrowth in a meat system, it is likely that combining low concentrations of the antimicrobial salts mentioned earlier with low levels of the antimicrobials evaluated in this study may act additively or synergistically, thus providing both safety and efficacy in controlling *C. perfringens* spore germination and outgrowth during extended cooling.

We have shown that oregano oil and the plant essential oil compounds carvacrol, cinnamaldehyde, and thymol can control *C. perfringens* spore germination and outgrowth during extended chill rates in a meat system. Further research should investigate the antimicrobial effects of these essential oils/oil compounds in combination with other ingredients typically used in the meat industry in order to optimize their use at the lowest possible levels to enhance the microbiological safety of meat products.

It should also be noted that cinnamaldehyde which is present in numerous commercial foods (14) is listed as GRAS by the Flavor and Extract Manufacturers Association (1). The literature also suggests that low levels of carvacrol, thymol, and oregano oil may be safe to consumers (7). Unresolved are the questions as to whether the products also exhibit acceptable sensory properties and how the meat matrix and storage temperatures and times may influence antibacterial effects.

The USDA/FSIS also has approved the use of sodium or potassium salts of lactic acid and sodium diacetate as antimicrobial ingredients in meat products at concentrations up to 4.8% to control *Listeria monocytogenes* and other pathogens (39). Because the plant compounds evaluated in this study are also reportedly inhibitory to *Campylobacter jejuni*, *Escherichia coli O157:H7*, *L. monocytogenes*, and *Salmonella enterica* (11, 13), these spices may be able to simultaneously control growth of *C. perfringens* and other pathogens in meat products during storage and distribution. This aspect merits study.

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

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