Use of Natural Ingredients to Control Growth of *Clostridium perfringens* on Frankfurters and Ham

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Summary and Implications

Three out of seven naturally cured frankfurter and naturally cured ham treatments with natural or clean label inhibitors showed no significantly greater ($P<0.05$) growth by inoculated *Clostridium perfringens* than that of the control. These results will be used to prepare guidelines for manufacturing these products in a manner that will achieve a safety level that is equivalent to traditionally cured meat products without altering the uniqueness of this category of processed meats.

Introduction

"Natural" and "organic" foods have become extremely popular with consumers. These foods are generally more expensive than its traditional counterpart. However, because there is such a positive connation with the words "natural" and "organic", consumers are willing to pay more for this unique group of products. The meat industry has recognized this trend and has begun to manufacture products that simulate traditionally cured meat products, but without direct addition of nitrite. The main concern with these processed meats marketed as "natural" and "organic" is that they do not contain formulated sodium nitrite ($NaNO_2^-$) in concentrations known to be highly effective in inhibiting the growth of many foodborne pathogens. These products contain natural sources of nitrite/nitrate (e.g. celery powder, celery juice and sea salt). Sodium nitrite has a long-standing history of effectively inhibiting foodborne pathogens such as *Clostridium botulinum*. To date, there is no known replacement for this substance. The "natural" curing process has been shown to result in less nitrite than conventionally cured products. In addition, an earlier study of the potential for *C. perfringens* growth in commercially available natural/organic frankfurters illustrated that there is wide variation in the potential for pathogen growth among the commercially available natural/organic frankfurters, meaning that the bacterial safety of these products is not well understood or well controlled. These results were reported in the Iowa State University Animal Industry Report 2009. A similar study to evaluate commercially available bacon and ham is reported in the Iowa State University Animal Industry Report 2010.

Consequently, the development of supplemental treatments to increase the level and consistency of antimicrobial protection in these products is important to provide consumers with the degree of safety that they have come to expect from conventionally cured processed meats. Therefore, the objective of this study was to identify and test ingredients that might improve product safety properties without altering the unique natural/organic status of these products.

Materials and Methods

C. perfringens strains ATCC 10258, 3124 and 12917 were obtained from the Food Safety Research Laboratory at Iowa State University. The organism was cultured in fluid thioglycollate medium and sporulation was induced in Duncan-Strong sporulation medium. The spore crop was harvested by centrifugation (9,500 rpm x g, 10 min., 4ºC) and then re-suspended in physiological saline (0.85% wt/vol sodium chloride). The three strains were combined and vortexed just before inoculation took place.

Eight treatments of frankfurters were manufactured, processed and packaged at the Iowa State University (ISU) Meat Laboratory. Following processing, the frankfurters were placed in a cooler at the ISU Meat Laboratory. The next day, the frankfurters were vacuum packaged. Frankfurters were taken to the Food Safety Research Laboratory at Iowa State University the day after packaging to begin Day 0 of the study. All frankfurter treatments contained the base ingredients of 80/20 beef trim, 50/50 pork trim, frankfurter spice blend, water/ice, dextrose and salt. In addition, Treatment A served as the positive control and contained sodium erythorbate, sodium nitrite and lactate/diacetate blend. Treatment B served as the negative control and only contained the base ingredients of 80/20 beef trim, 50/50 pork trim, frankfurter spice blend, water/ice, dextrose and salt. Treatment C contained a natural nitrate source and a nitrate reducing starter culture (*Staphlococcus carnosus*). Treatment D contained a natural nitrate source, a nitrate reducing starter culture (*Staphlococcus carnosus*) and natural antimicrobial A (vinegar, lemon powder and cherry powder blend). Treatment E contained a natural nitrate source, a nitrate reducing starter culture (*Staphlococcus carnosus*) and clean label antimicrobial B (cultured corn sugar and vinegar blend). Treatment F contained a natural source of nitrite without additional antimicrobials. Treatment G contained a natural nitrite source and natural antimicrobial A (vinegar, lemon powder and cherry powder blend). Treatment H contained a natural nitrite source and clean label antimicrobial B (cultured corn sugar and vinegar blend).

Eight treatments of ham were manufactured, processed, sliced and packaged at the ISU Meat Laboratory. Each treatment was placed in a bag and left unsealed. The bag

was immediately delivered to the Food Safety Research Laboratory to begin Day 0 of the study. The ham treatments contained the base ingredients of ground ham, salt, sugar and water. Treatment A served as the negative control and only contained the base ingredients of ground ham, salt, sugar and water. Treatment B served as the positive control and contained sodium erythorbate, sodium nitrite and lactate/diacetate blend. Treatments C through H were the same as for the frankfurters.

While in the Food Safety Research Laboratory, 25-gram samples of each treatment of frankfurters and ham were placed in 5 X 16 vacuum package bags (Cryovac Packaging, Duncan, SC) and inoculated with 0.1 ml of the 3-strain cocktail of *C. perfringens* to give a final spore concentration of ~5 log colony forming units (CFU) per sample. Frankfurters were interiorly inoculated using a 1 cc needle (Difco, Becton Dickinson, Sparks, MD). Ham was surface inoculated. After packages were sealed under vacuum, all samples were heat shocked in a water bath (NESLAB Instruments, Inc., Newington, N.H. RTE-211) to an internal temperature of 75^{o} C to ensure that all vegetative cells were inactivated and only spores remained. A thermometer was used in non-inoculated samples to monitor temperature during the heat shocking process. Following the heat shocking process, all product was chilled according to the USDA guidelines for *C. perfringens* control in cured meats (54.4^{o}C to 26.6^{o}C within 5 hours, and 26.6^{o}C to 7.2^{o}C within the next 10 hours). After the product reached an internal temperature of 7.2^{o}C, the product was stored in storage containers at room temperature (~20^{o}C). Sampling was conducted on day 0, 1, 2, 4, 6, 8, 10 for frankfurters and day 0, 2, 4, 6, 8 for ham. These sampling days were determined by results from preliminary studies.

Microbiological analysis

On the appropriate day, a package was collected for each treatment and opened aseptically. Sampling was achieved by blending each 25-gram sample with 225 ml of 0.1% peptone water in a sterile Whirl-Pak stomacher bag (Nasco, Ft. Atkinson). Each sample was stomached for 30 seconds in the laboratory blender (Stomacher 400, Seward Medical, London, UK). All blended samples were maintained on an ice slurry. Appropriate dilutions were plated with a glass rod in duplicate on perfringens agar with Tryptose Sulphite Cycloserine and egg yolk emulsion (Oxoid, Basingstroke, UK). Agar plates were incubated at 35°C in anaerobic jars with Gas Pak palladium catalyst envelopes (Oxoid Ltd., Basingstoke, UK) for 24 hours. In an effort to ensure the anaerobic jars were functioning properly, anaerobic indicators were included in each jar.

Data Analysis

Two independent replicate experiments were performed for the frankfurters and three independent replicate experiments were performed for ham manufactured at ISU Meat Laboratory. Viable *C. perfringens* populations were determined by calculating the log value of bacterial counts on duplicate plates for each sample that was analyzed. A F-test was performed to confirm that there was a difference among treatments. In the pairwise comparisons of the means, Tukey's Honestly Significant Difference (HSD) procedure was used to adjust for the multiple comparisons when testing for a significant difference between means of treatments within a particular product (e.g. frankfurters and ham). Significant levels were determined at $P<0.05$. Data were analyzed using PROC GLM (general linear models) procedure of the Statistical Analysis System software program (SAS Institute Inc., Cary, N.C.).

Results and Discussion

Figure 1 illustrates the effect of treatment on growth of *C. perfringens* over time from spore inocula in frankfurters manufactured with natural ingredients. Growth of *C. perfringens* was significantly faster ($P<0.05$) than the control (A) in the naturally cured treatments (B, F, C) and in the Treatment E. Figure 2 illustrates the effect of treatment on growth of *C. perfringens* over time from spore inocula in ham manufactured with natural ingredients. Growth was again faster in the naturally cured products without antimicrobials (A, F).

Addition of the antimicrobials appears to improve control of *C. perfringens*, but these products demonstrated a considerable variation of inhibitory activity. The results from this project will be used to prepare guidelines for manufacturing these products that will be communicated to the meat industry and consumers.

Acknowledgments

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Figure 1. Effect of treatment on growth of *C. perfringens* from spore inocula in frankfurters manufactured with natural ingredients.

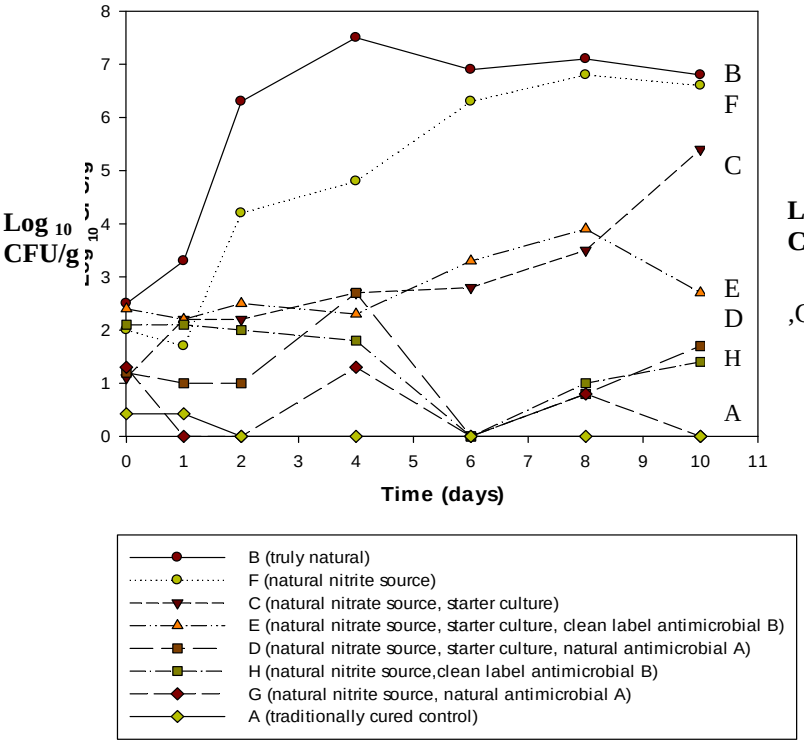

Figure 2. Effect of treatment on growth of *C. perfringens* from spore inocula in ham manufactured with natural ingredients.