Survival and Growth of *Clostridium perfringens* on Commercial No-Nitrate-or-Nitrite Added (Natural and Organic) Bacon and Ham

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Armitra Jackson, graduate research assistant; Gary Sullivan, graduate research assistant; Joseph Sebranek, distinguished professor; James Dickson, professor

Summary and Implications

Four out of nine commercial brands of natural/organic bacon showed significantly greater ($P<0.05$) growth by inoculated *Clostridium perfringens* than that observed for the conventionally cured control (n=1). All natural/organic commercial ham analyzed in the study (n=7) showed significantly greater ($P<0.05$) growth by inoculated *Clostridium perfringens* than that observed for the conventionally cured control hams (n=4). These products also demonstrated a wide variation in growth response. This means that natural and organic processed meats may require additional protective measures in order to consistently provide the same level of safety from bacterial pathogens that is achieved by conventionally cured meat products.

Introduction

In the last decade, consumers have become infatuated with the idea of consuming foods they deem to be "healthier". This makes processed meats marketed as "natural" and "organic" extremely attractive. To that end, consumers are willing to pay premium prices for these products with the belief they are consuming a product that is safer than its conventional counterpart. Due to the increased consumer demand for "natural" and "preservative-free" food products, a significant number of meat processors have begun to process meats without formulated nitrite (e.g. sodium nitrite). Because nitrite is a preservative, it is not permitted in natural and organic meat products. In an effort to replace sodium nitrite, there is currently a new trend to process meats with natural sources of nitrite/nitrate. These products are manufactured to simulate traditionally cured meat products, but without direct addition of nitrite. Natural sources of nitrate/nitrite include celery juice, celery powder and sea salt. However, the "natural" curing process has been shown to result in less nitrite than conventionally cured products. The major concern with these products is that they do not contain formulated nitrite in concentrations known to be highly effective in inhibiting the growth of *C. perfringens* and many other foodborne pathogens. As a result, these products are more susceptible to foodborne pathogens.

The objective of this study was to quantify the potential for *C. perfringens* growth in commercially available processed meats manufactured to simulate traditionally cured products, but without the direct addition of nitrite or nitrate and in products to which no nitrite/nitrate source was used. Results for 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. In this report, commercially available natural/organic bacon and ham were also evaluated.

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.

For each of the three replications, commercial brands of bacon and ham from the same sell by date were purchased at grocery stores located in the Midwest region of the United States and online stores. Product purchased from online distributors was shipped overnight to Iowa State University Meat Laboratory under refrigeration and was received during normal business hours. As soon as the product arrived, the temperature was taken to ensure temperature abuse did not occur. Product was stored in the cooler at the Food Safety Research Laboratory at Iowa State University and was analyzed within one week of purchase. Each product was assigned a letter code. Bacon brands A, B, C, D, E, F, G, H, I were marketed as "No Nitrite or Nitrate Added" and were naturally cured using sea salt or celery juice to simulate the typical curing process. Bacon brand J was conventionally cured, thus serving as the control. Ham brands A, B, C, D, E, F, G were marketed as "No Nitrite or Nitrate Added" and were naturally cured using sea salt or celery juice to simulate the typical curing process. Ham Brands H, I, J, K were conventionally cured and served as controls.

For the bacon and ham, 25-gram samples from each brand of product (e.g. bacon and ham) were placed in 5 X 16 vacuum package bags (Cryovac Packaging, Duncan, SC). and surface 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. 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° 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°C to 26.6°C within 5 hours, and 26.6°C to 7.2°C within the next 10 hours). After the product reached an internal temperature of 7.2°C, the product was stored in containers at room temperature (~20°C). Sampling was conducted on 0, 1, 2, 6, 8, 10 days for bacon and ham. These sampling days were determined by results from preliminary studies. One control was used for the bacon while four controls were used for the ham.

Microbiological analysis

On the appropriate day, one 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

Three independent replicate experiments were performed for the bacon and ham. 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 brands. 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 brands within a particular product. 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 growth of *C. perfringens* over time with different brands of commercially available bacon. Products are labeled in the manner in which they are marketed (e.g. natural, organic, traditionally cured, etc). Differences in overall (e.g. without adjusting for day effect) inhibitory response ($P<0.05$) were observed in Brands D, B, C and G when compared to Control J. Inhibitory response by Brands I, H and E were the same as that of Control J. Brand E contained sodium lactate, a significant antimicrobial, listed in the ingredients statement on the package. It is possible that Brands H and I may have contained other ingredients not listed on the label that may have aided in the inhibition of *C. perfringens*. Figure 2 illustrates the growth of *C. perfringens* over time on commercially available ham. Products are labeled in the manner in which they are marketed (e.g. natural, organic, salt cured, etc). Without the consideration of the day effect, differences in inhibitory response ($P<0.05$) were observed in Brands C, E, B, F and A when compared to the Control H. Differences in inhibitory response ($P<0.05$) were observed in Brands C, E, B, F, A, and G when compared to the Controls I and K. Differences in overall (e.g. without adjusting for day effect) inhibitory response ($P<0.05$) were observed in Brands C, E, B, F, A, G and D when compared to the Control J. These results are most likely due to the fact that the four traditionally cured controls are cured with conventional concentrations of sodium nitrite. The other brands used natural sources of nitrate/nitrate.

These results indicate that commercial natural and organic cured meats have more potential for pathogen growth than conventionally cured products. It is also evident from Figures 1 and 2 that there is wide variation in the potential for pathogen growth among the commercially available natural/organic bacon and ham meaning that the bacterial safety of these products is not well understood or well controlled. Consequently, 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.

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Figure 1. Effect of treatment on growth of *C. perfringens* from spore inocula in commercially available bacon.

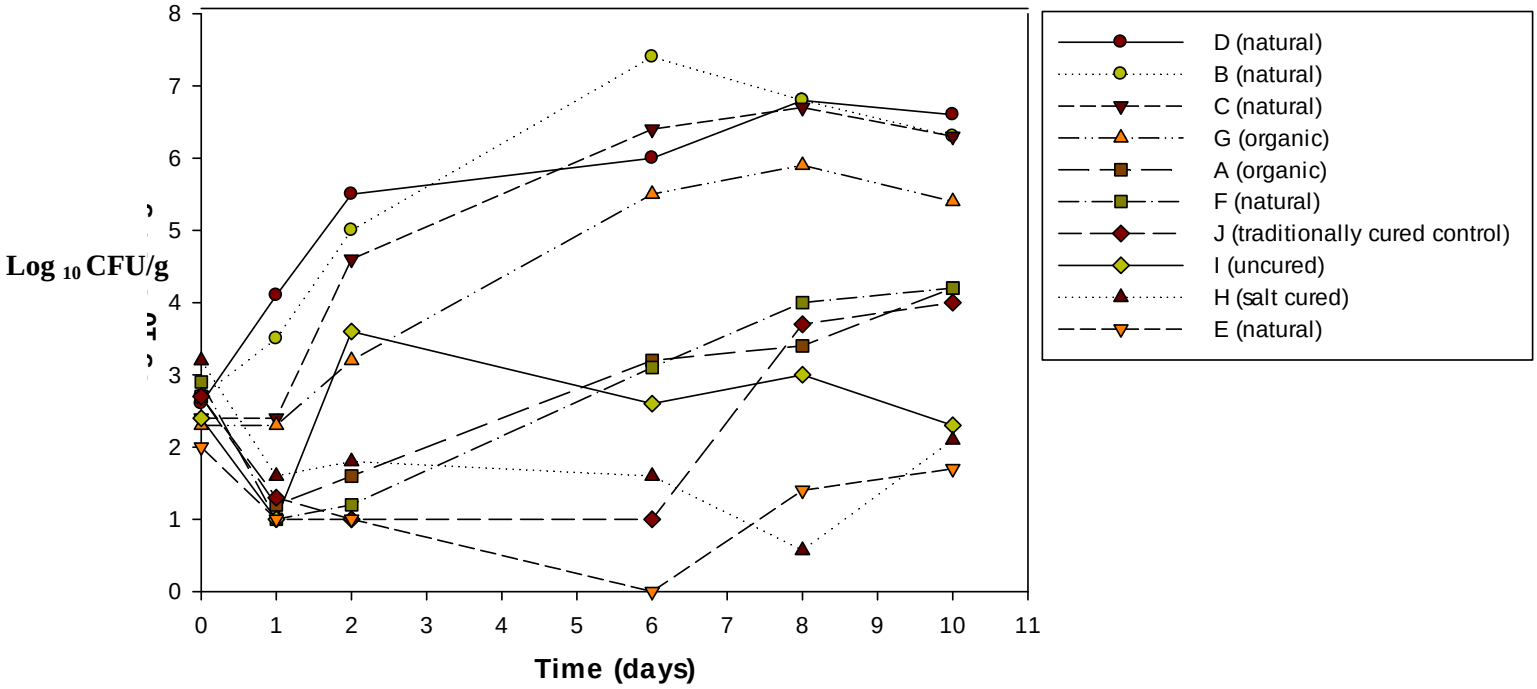

Figure 2. Effect of treatment on growth of *C. perfringens* from spora inocula in commercially available ham.