Use of Natural Ingredients to Control Growth of *Listeria monocytogenes* on Ham

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Summary and Implications

Five out of seven naturally cured ham products showed no significantly greater ($P<0.05$) growth by inoculated *Listeria monocytogenes* than that of the control. The manufacture of cured ham with natural nitrate in combination with starter culture had highest residual nitrite levels. The treatments produced similar results to those in traditionally cured ham. The pH was affected by the addition of different antimicrobials. 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

In the past ten years, there has been a steady interest among consumers in foods labeled as "natural" and "organic". According to the October 2009 issue of Meatingplace, sales of these products at natural stores have risen 5.8 percent while conventional stores experienced a 2.4 percent increase in sales. To meet consumer demands for this unique group of products, the meat industry has begun to manufacture products that simulate traditionally cured meat products, but without direct addition of nitrite. As another marketing tool, many of these products contain "clean labels", meaning they do not contain ingredients that would have the potential to cause a consumer to become overly concerned by a chemical name. This process of manufacturing this particular group of processed meats is being used despite the long proven track record of product safety due to sodium nitrite. These "natural" and "organic" foods have the potential of being a food safety hazard because they do not contain formulated sodium nitrite ($NaNO_2^-$) in concentrations known to be highly effective in inhibiting the growth of many foodborne pathogens such as *Listeria monocytogenes*. These products contain natural sources of nitrite/nitrate (e.g. celery powder, celery juice and sea salt).

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. Much variation was found among all analytical traits measured. Pathogenic growth was correlated to water activity (a_w), residual nitrite, salt, total pigment, percent cured pigment and protein for ham. In bacon, significant correlations to pathogen growth were found for a_w, salt and total pigment.

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

L. monocytogenes strains H7969, H7764, H7769, H7762 and Scott A were obtained from the Food Safety Research Laboratory at Iowa State University. A 250 ml bottle of Trypticase Soy Broth, supplemented with 0.6% Yeast Extract, was inoculated with 1 ml from each of the five *L. monocytogene*s strains. The inoculated broth was incubated at 35^0C for 24 hours. To disperse the cells, the bottle was shaken for 1 minute. A 10 ml aliquot was removed from the inoculated broth and dispensed into a 90 ml 0.1% peptone bottle to achieve a 1:10 dilution.

Eight ham treatments were manufactured, processed, sliced and packaged at the ISU Meat Laboratory. Each treatment was placed in bags and left unsealed. The bags were immediately delivered to the Food Safety Research Laboratory at Iowa State University to begin Day 0 of the study. All of the ham treatments contained the base ingredients of ground ham, salt, sugar and water. Treatment A served as the negative control and contained only the base ingredients of ham, salt, sugar and water. Treatment B served as the positive control and included sodium erythorbate, sodium nitrite and lactate/diacetate blend. Treatment C included a natural nitrate source and a nitrate reducing starter culture (*Staphlococcus carnosus*). Treatment D included a natural nitrate source, a nitrate reducing starter culture (*Staphlococcus carnosus*) and natural antimicrobial A (vinegar, lemon powder and cherry powder blend). Treatment E included a natural nitrate

source, a nitrate reducing starter culture (*Staphlococcus carnosus*) and clean label antimicrobial B (cultured corn sugar and vinegar blend). Treatment F included a natural source of nitrite without additional antimicrobials. Treatment G included a natural nitrite source and natural antimicrobial A (vinegar, lemon powder and cherry powder blend). Treatment H included a natural nitrite source and clean label antimicrobial B (cultured corn sugar and vinegar blend).

In the Food Safety Research Laboratory, the product was weighed to approximately 25 grams and placed into 5 X 16 vacuum package bags (Cryovac Packaging, Duncan, SC). A 0.1ml aliquot of the 10^{-1} dilution was then aseptically transferred onto the ham of each bag for the various treatments. The cell concentration at inoculation was approximately 10^4 cells per gram. The bags were then vacuum sealed and stored at 4^0C throughout the duration of the 35 day study. Sampling was conducted on day 0, 7, 14, 21, 28 and 35.

Microbiological analysis

On the appropriate day, one package was collected for each treatment and opened aseptically. Sampling was achieved by performing an initial 1:5 dilution using a diluter (Spiral System ASAP™ Diluter, Cincinnati, OH). Each sample was homogenized in a sterile Whirl-Pak stomacher bag (Nasco, Ft. Atkinson) for 1 minute in the laboratory blender (Stomacher 400, Seward Medical, London, UK). The product was further serially diluted, according to the sample date. Beginning sample days were diluted to the 10^{-3} while later sample dates were diluted to the 10^{-5} past the initial 1:5 dilution. An aliquot of 0.1ml of the appropriate dilution was dispensed onto Modified Oxford Medium Base (Difco, Becton Dickinson, Sparks, MD) supplemented with Modified Oxford Antimicrobic Supplement (Difco, Becton Dickinson, Sparks, MD). The plates were spread with a glass rod and incubated at 35^0C. After 24 – 48 hours, the plates were removed and colonies typical of *Listeria* were enumerated.

Data Analysis

Three independent replicate experiments were performed for ham manufactured at the ISU Meat Laboratory. Viable *L. monocytogenes* 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. Significant levels were determined at $P<0.05$. Data was analyzed using PROC GLM (general linear models) procedure of the Statistical Analysis System software program (SAS Institute Inc., Cary, N.C.).

Physiochemical traits were measured for ham samples to correspond to microbial sampling. On day 0, samples were evaluated for a_w, salt, fat, protein, moisture, nitrate, CIE L*, a*, b*, residual nitrite and pH. Residual nitrite and pH were also measured on days 8, 14, 21, 28 and 35 while nitrate content was measured on days 8, 21 and 35. Data were analyzed using Proc GLM procedure of SAS and means were separation was conducted using least significant difference procedure.

Results and Discussion

Figure 1 illustrates the effect of treatment on growth of *L. monocytogenes* in ham manufactured with natural ingredients. Treatments E, H, D, G and C showed no significantly greater ($P<0.05$) growth by inoculated *Listeria monocytogenes* than that of the control, thus suppressed growth quite effectively. This can be attributed to the antimicrobials that were included in the treatments (with the exception of C). Growth was faster in the naturally cured products manufactured without a nitrate/nitrite source (A) or without antimicrobials (F).

Addition of the antimicrobials appears to improve control of *L. monocytogenes*, but these products demonstrated a slight variation of inhibitory activity, suggesting other inhibitory factors are probably involved. 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.

All treatments with a natural nitrate source and starter culture (C, D and E) had the highest residual nitrite followed by traditionally cured samples (Table 1). Residual nitrite declined with time. Samples with the vinegar, lemon powder and cherry powder blend (antimicrobial A) had the highest pH followed by those with a natural nitrite source and no antimicrobials. Traditionally cured samples had the lowest pH. Ham with the direct addition of sodium nitrite (control) had the lightest color (highest L*) of cured samples and the treatments with the vinegar, lemon powder and cherry powder blend (antimicrobial A) were the darkest. All treatments with a natural nitrate source and starter culture (C, D and E) had the highest residual nitrite followed by traditionally cured samples. Traditionally cured samples had the most red color (highest a*) followed by those with a natural nitrate sources and starter culture. The lowest a* values were found from the treatments of natural nitrite. These likely related to the residual nitrite level found in the product. No differences were found for a_W, salt, protein, fat and moisture.

Acknowledgments

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Figure 1. Effect of treatment on growth of *L. monocytogenes* in ham manufactured with natural ingredients.

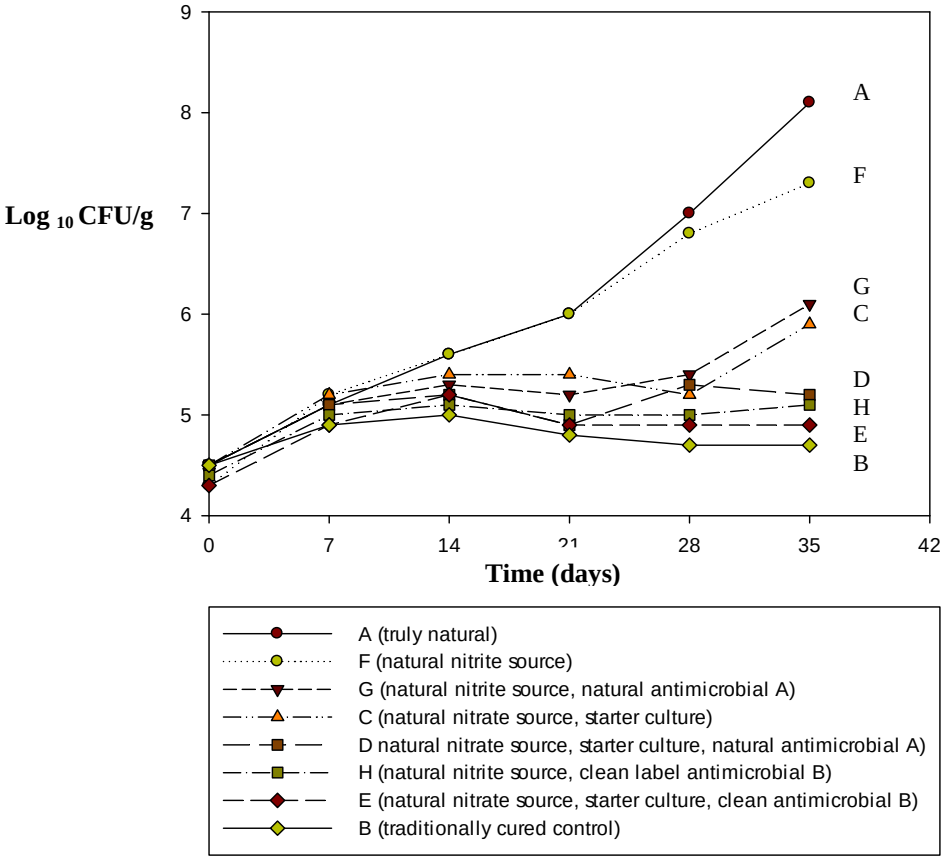

Table 1. Mean pH, residual nitrite, L* and a* for ham treatments.

	pH	Residual Nitrite (ppm)	L*	a*
A	6.13^{ef}	2.4^{f}	68.49^{a}	8.62^{e}
B	6.09^{f}	31.2^{d}	67.67^{b}	15.18^{a}
C	6.19^{c}	46.6^{a}	66.65^{cd}	14.41^{cd}
D	6.25^{b}	42.8^{b}	64.35^{e}	14.83^{b}
E	6.11^{f}	38.5^{c}	66.51^{cd}	14.76^{b}
F	6.21^{c}	23.7^{e}	66.87^{c}	14.11^{d}
G	6.32^{a}	22.6^{e}	64.44^{e}	14.69^{bc}
H	6.16^{de}	20.5^{e}	66.06^{d}	14.61^{bc}

Means with different superscripts within a given column differ by P>0.05.