INHIBITION OF FOODBORNE PATHOGENS IN NO-NITRATE OR NITRITE-ADDED BACON BRINE FORMULATIONS

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Abstract – Consumers are concerned with the consumption of processed meats because of the nitrite content. Processed meats manufactured using natural curing ingredients may exhibit color, flavor and shelf-life similar to traditional products. However, few reports describe the effects of natural curing ingredients on survival and growth of foodborne pathogens in meat products. Therefore, the objective of this study was to evaluate the inactivation of *Clostridium perfringens* (CP)*, Listeria monocytogenes* (LM)*,* and *Salmonella* Typhimurium (ST) inoculated in bacon brine formulations made using natural nitrate with starter culture, natural nitrite with a natural cure accelerator, and conventional cure (sodium nitrite). Brine formulations were made, inoculated with a cocktail of the three foodborne pathogens, and stored at 35°C. Samples were taken at 0, 4, 8, 12, and 24 after inoculation. Natural nitrate inhibited the growth of LM to a greater degree (P < 0.05) than all other treatments. Natural nitrate, natural nitrite, and conventional cure control were not statistically different from each other throughout the 24 hours of sampling for CP and ST growth. Natural nitrite, natural nitrate with starter culture, and conventionally cured brine were effective at reducing viability below detectable limits for all three pathogens within 24 hours at 35ºC.

Key Words – bacon, foodborne pathogens, nitrite

I.   INTRODUCTION

As consumers become more health conscious, they often try natural or organic food alternatives. Organic food sales grew 7.7% from 2009 to 2010 [1]. Meat, poultry, and seafood products account for 2% of the total organic food market [2]. In 2005, meat, poultry, and seafood products were the fastest growing components of organic foods markets [3].

Traditionally cured meat products use sodium nitrite and other ingredients (i.e. sodium phosphates, sodium erythorbate) that are not permitted in natural, organic, or uncured meat products [4]. Nitrites in cured meat products help develop characteristic color, flavor, safety, shelf stability, antioxidant and antimicrobial properties. Natural curing ingredients such as vegetable juice powder often contain nitrates that can be converted to nitrite with the use of a starter culture. Current research indicates that products cured with vegetable juice powders can develop similar color and flavor as sodium nitrite-cured products [5, 6, 7]. Yet, there is limited information on the effects of natural cures to inhibit microbial growth.

There are several options available for manufacture of natural and organic processed meat products. Most products are manufactured using vegetable juice powder along with a starter culture capable of reducing nitrate to nitrite. A newer ingredient is a pre-reduced, vegetable juice powder in which the nitrate is already reduced to nitrite. These natural ingredients supply nitrite for meat curing reactions.

According to Sebranek & Bacus [8], nitrite reactivity is important to microbial inhibition. Ingoing and residual nitrite are important for antimicrobial effectiveness, which is dependent on several factors including pH [8] and the nitrite reaction sequence that generates nitric oxide and other reaction products [9]. The effects of nitrite and its inhibitory properties most likely differ with regard to bacterial species [9]. According to Tompkin [9], the residual nitrite present at the time of temperature abuse is critical to the antibotulinal effect. In addition, the depletion of residual nitrite during product storage will reach a point at which inhibitory effects also are depleted [9]. Therefore, nitrite is important to the shelf-life and stability of processed meat products. This stability is especially important in natural products

that use a natural nitrate source since the nitrite level is unknown at the time of manufacturing.

Nitrite retards microbial spoilage of cured meats and inhibits anaerobic and aerobic, spore-forming bacteria [10, 11]. Nitrite is inhibitory to anaerobic bacteria (i.e. *Clostridium botulinum*) [11] and important in the control of other pathogenic microorganisms (i.e. *Listeria monocytogenes*) [9, 12]. Nitrite is considered ineffective for control of Gram-negative enteric pathogens (i.e. *Escherichia coli* and *Salmonella*) [9]. However, *E. coli* survives longer and reached higher counts in salami without nitrite than with added nitrite [12]. *Staphylococcus* and *Salmonella* growth were slightly suppressed in frankfurters with nitrite compared to frankfurters that did not contain the compound [13]. The purpose of this study was to evaluate the effectiveness of natural curing systems to inhibit the growth of *L. monocytogenes, C. perfringens,* and *S.* Typhimurium in bacon brines.

II.   MATERIALS AND METHODS

The compounds used in this experiment were composed as follows. Treatment A (natural nitrate; Vegetable Juice Powder, Symrise, Teterboro, NJ with starter culture (CS-299Bactoferm, Chr Hansen, Inc., Milwaukee, WI) and Treatment B(natural nitrite; Celery Baste and Cherry Baste Aid, Newly Weds, Chicago, IL)were utilized at concentrations recommended by the manufacturer. Treatment C was composed of sodium nitrite and sodium erythorbate (conventionally cured control) and used at concentrations approved by the United States Department of Agriculture. Treatment D was composed of salt, sugar, and water. In all instances, salt and sugar were added to 20% and 5% of the formulation, respectively. Water was added and the brine was mixed for at least two minutes to insure all ingredients were dispersed. Treatment E consisted of distilled water.

L. monocytogenes (LM; ATCC Scott A; American Type Culture Collection, Manassas, VA), *C. perfringens*, (CP; ATCC 10543), and *S.* Typhimurium (ST; ATCC 14028) were obtained from the Muscle Foods Microbiology Lab of the Food Science Department at The Pennsylvania State University. LM and ST were cultured

separately in Tryptic Soy Broth (TSB, Becton Dickinson and Co., Sparks, MD) for 24 hours at 35°C. CP was cultured in Reinforced Clostridial Medium (RCM, Becton Dickinson and Co., Sparks, MD) for 36 hours at 35°C. Individual cultures were spun down and resuspended in 10 ml of buffered peptone water. The concentration of LM and ST was approximately 10^8 colony forming units per ml (CFU/ml), while CP was approximately 10^7 CFU/ml. Ten ml of each washed culture were mixed to make a cocktail and 4 ml of the cocktail was used to inoculate each brine formulation.

Sampling was conducted after incubation at 35ºC for 0, 4, 8, 12, and 24 h by removing 5 ml of the inoculated brine, serially diluting, and spread-plating on the appropriate selective agars, in duplicate, as follows: LM was isolated on Oxford medium base supplemented with Moxalactam (Remel, Lenexa, KS), CP was isolated on Perfringens agar base with egg yolk and perfringens selective supplement (Oxoid Ltd, Baskingstoke, UK), and ST was isolated on XLD agar (Becton Dickinson and Co. ,Sparks, MD). All inoculated agar plates were incubated at 35°C for 24-48 h, counted manually, and enumerated as log_{10} CFU per ml.

Three independent replications were conducted. The PROC MIXED procedure of Statistical Analysis System (SAS; version 9.3, SAS Institute Inc., Cary, NC) was used for statistical analysis. Pathogen growth was analyzed for treatment effects by hour. Means separation was conducted using LSMEANS function of SAS and Fisher's least significant difference (LSD) adjustment was performed for pathogen growth. Statistical significance was set at $P < 0.05$.

III.   RESULTS AND DISCUSSION

Natural nitrate with starter culture (B) decreased LM viability more (P<0.05) than all other treatments (A, C, D, and E) at 4 and 8 h (Figure 1). This observation may be attributed to an acidic pH (3.9) versus other treatments (A, C, D; pH > 5.0; data not presented). There were no significant differences (P>0.05) between treatments B (natural nitrate) and C (conventionally cured control), but A (natural nitrite), B (natural nitrate),

and E (water) were different from all other treatments at 4 and 8 h. All treatments containing nitrate or nitrite (A, B, C) were not statistically different (P>0.05) from each other, but were statistically different from uncured (D) and water (E) brines at 24 h. Treatment A demonstrated slower reduction of LM viability than treatments B and C. This observation may be due to pH differences of the brines. This finding corresponds to findings by Sullivan [14] who also found that natural nitrite was less effective at inhibiting LM.
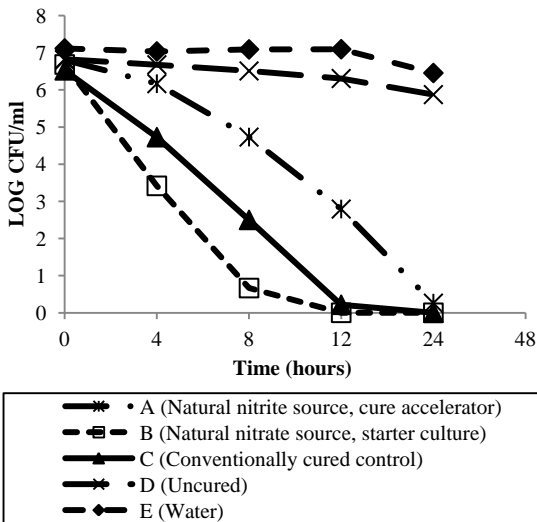

Figure 1. Effect of curing treatments on growth of *L. monocytogenes* in bacon brines during storage at 35°C. Natural Nitrite = pre-converted celery juice powder and cherry juice powder, Natural Nitrate = vegetable juice powder with starter culture.

At 0 h, treatments A, B, and C were not statistically different from each other, but were different from D and E (Figure 2). Treatment E (water) exhibited higher populations (P < 0.0001) than all other treatments (A, B, C, D) at all time points. Treatments A, B, and C were not different (P > 0.05) from each other at all time points. Uncured brine (D) had higher (P < 0.05) counts at 4, 8, and 12 h than treatment B (natural nitrate). Natural nitrite (A) and conventionally cured (C) had similar (P > 0.05) counts when compared to uncured (D) after 4 h. Natural nitrite (A) and uncured (D) exhibited similar (P > 0.05) counts after 4, 8, and 24 hours, but were significantly different (P < 0.05) at 12 h. Conventionally cured control (C) had lower (P < 0.05) counts at 8, 12, and 24 h than uncured (D). Jackson *et al.* [15] found that natural nitrite was less effective at

inhibiting CP growth than natural nitrate with a starter culture. This information differs from our finding, but could be attributed to the fact that we evaluated brines and not a meat system.
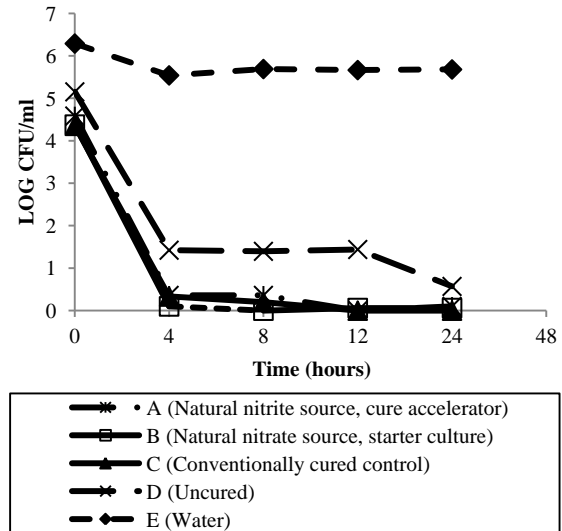

Figure 2. Effect of curing treatments on growth of *C. perfringens* in bacon brines during storage at 35°C. Natural Nitrite = pre-converted celery juice powder and cherry juice powder, Natural Nitrate = vegetable juice powder with starter culture.

Figure 3 illustrates the reduction in viability of ST following treatment with water (E), which was significantly different (P<0.05) from all other treatments (A, B, C, D) at all time points. Natural nitrite (A), natural nitrate with starter culture (B), and conventionally cured control (C) had significantly lower counts after 4, 8, and 12 h than uncured (D). There were no differences (P > 0.05) in counts between treatments A, B, C, and D at 24 h. Hinton [16] demonstrated that high salt (750 mM NaCl) concentrations can reduce the viability of ST. This may explain why there was not a difference at 24 h between treatments A, B, C, and D.
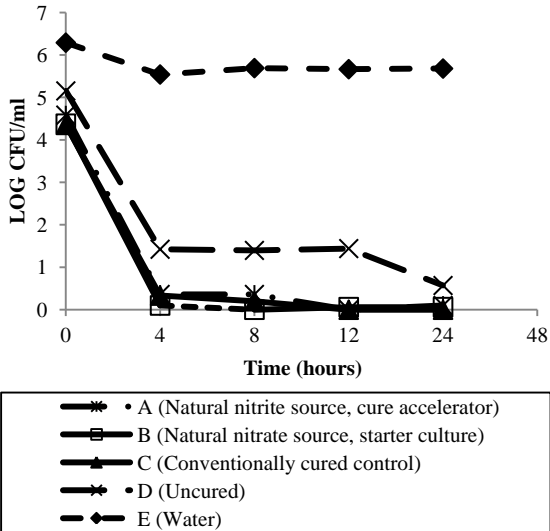
Figure 3. Effect of curing treatments on growth of *S.* Typhimurium in bacon brines during storage at 35°C. Natural Nitrite = pre-converted celery juice powder and cherry juice powder, Natural Nitrate = vegetable juice powder with starter culture.

IV.   CONCLUSIONS

Natural nitrite with a natural cure accelerator (A), natural nitrate with starter culture (B), and sodium nitrite (C) were all effectively reduced the viability of *L. monocytogenes, C. perfringens,* and *S.* Typhimurium in brine within 24 h of exposure. However, natural nitrate with starter culture (B) inhibited pathogen growth as effectively as sodium nitrite (C, conventionally cured).

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