Use of natural antimicrobials for *L. monocytogenes* control on ham

Gary A. Sullivan[1], Armitra L. Jackson[1], Steven E. Niebuhr[1], Kohl D. Schrader[1], Joseph G. Sebranek[1*], and James S. Dickson[1]

[1]Department of Animal Science, Iowa State University, Ames, Iowa, United States of America

[*]Corresponding author (phone: 515 294-1091; fax: 515 294 5066; e-mail: sebranek@iastate.edu)

***Abstract*—Consumers' demand for foods manufactured without the direct addition of sodium nitrite have resulted in a unique class of cured meat. This study evaluated *Listeria monocytogenes* growth on ham manufactured with natural curing methods with antimicrobials and assessed impacts on physio-chemical characteristics. Both of the natural antimicrobials evaluated inhibited growth similar to that of the traditionally cured control. Ham made with pre-fermented celery juice powder had lower residual nitrite concentrations and when no antimicrobial was added, *L. monocytogenes* growth was similar to that of the uncured control. Ham pH was influenced slightly by antimicrobials. Ham can be produced with natural curing methods and antimicrobials to inhibit *Listeria monocytogenes* growth with little changes to physio-chemical traits.**

***Index Terms*—No sodium nitrite added, *Listeria monocytogenes*, natural antimicrobials, ham**

I. INTRODUCTION

A recent survey by the Organic Trade Association (2009) reported that 73% of US households at least occasionally purchase organic foods. These respondents cited health benefits as the major reason for organic food purchases and 47% stated that they avoided artificial ingredients in food to improve health. In response to this demand, many meat processors have begun to manufacture processed meats with characteristics similar to traditionally cured products, but without the direct addition of sodium nitrite or sodium nitrate or other chemical antimicrobials. Five of eight brands of commercial frankfurters no-nitrite-or-nitrate frankfurters had greater *Listeria monocytogenes* growth than controls (Schrader, Sebranek, Cordray, Dickson & Mendonca, 2010). Both food-borne pathogens and spoilage bacteria grew at faster rates, resulting in increased likelihood of illness and decreased shelf life.

These products utilized ingredients such as vegetable powders, sea salts, and turbinado sugar that are high in nitrate and included nitrate reducing starter cultures to produce nitrite. Many factors during processing can impact microbial conversion of nitrate. To eliminate this problem, pre-fermented vegetable juice powders, containing nitrite, can standardize ingoing nitrite. These systems have significantly less ingoing nitrite than traditionally cured products. Antimicrobial properties of organic acids are well documented (Theron & Lues, 2007), and ingoing nitrite concentrations impacts effectiveness of organic acids (Qvist & Bernbom, 2000). Natural sources of organic acids have been identified for use as natural antimicrobials. The purpose of this study was to evaluate the effectiveness of natural curing systems and natural antimicrobials in inhibiting growth of *Listeria monocytogenes*.

II. MATERIALS AND METHODS

Bacterial strain and spore suspension *Listeria monocytogenes* strains H7969, H7764, H7769, H7762 and Scott A were obtained from the Food Safety Research Laboratory (FSRL) at Iowa State University (ISU). 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. 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.

Manufacture of hams Eight treatments (six experimental treatment combinations and two control treatments) were produced to evaluate the inhibition of *Listeria monocytogenes* growth by natural nitrite or nitrite sources and natural antimicrobials. Celery juice powder (natural nitrate) and pre-fermented celery juice powder (natural nitrite) were used as natural curing agents. Two commercially available natural antimicrobials were evaluated: a blend of cherry, lemon and vinegar powder (Antimicrobial A) and a cultured cane sugar and vinegar blend (Antimicrobial B). All commercial ingredients were utilized at concentrations recommended by the supplier.

Hams, 25% extended, were produced following formulations found in Table 1 at the Meat Laboratory at ISU with pork inside ham muscles obtained from a local supplier. The ham muscles were coarse-ground through a 6.35 mm plate. Ham and non-meat ingredients were added and mixed for two minutes using a double action mixer (Leland Southwest, Fort Worth, TX, USA). Mixed samples were reground using a 3.18 mm plate and stuffed into a 35 mm fibrous casing with a rotary vane vacuum-filling machine (Risco vacuum stuffer, Model RS 4003-165). Treatments with natural nitrate (D, E, G) were placed in a single truck smokehouse for fermentation at 42 °C for 2 hours. Conventionally cured control (H) and treatments with natural nitrite (B, C, G) were placed in the smoke house 90 minutes into fermentation to allow temperature to equilibrate. Treatment A (no nitrite in any form) was cooked in a separate smokehouse following the same cooking cycle. All products were cooked to an internal temperature of 73.9 °C. The hams were placed in a 0°C cooler overnight to stabilize. The next day, the hams were sliced to 1.5 mm thick

slices using a fully automatic slicing machine (Bizerba, Model A-500, Piscataway, NJ., USA) and vacuum packaged (Ulma Packaging, MINI Series, Ball Ground, GA, USA). Three replications were produced. Hams were then transferred to the FSRL or Analytical Laboratory at ISU to begin day 0 of the study.

Sample inoculation While in the FSRL, 25-gram samples of each treatment of ham were placed in 5 X 16 in vacuum bags (Cryovac Packaging, Duncan, SC., USA). 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 packaged and stored at 4°C 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 for each treatment was collected and opened aseptically. Sampling was achieved by performing an initial 1:5 dilution using a diluter (Spiral System ASAP^{TM} Diluter, Cincinnati, OH). Each sample was homogenized in a sterile Whirl-Pak stomacher bag (Nasco, Ft. Atkinson, WI, USA) for 1 min in the laboratory blender (Stomacher 400, Seward Medical, London, UK). The product was further serially diluted, according to the sample date. The early sample days were diluted to 10^{-3} while later sample dates were diluted to 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°C. After 24 – 48 hr, the plates were removed and colonies typical of *Listeria* were enumerated.

Physiochemical analysis Samples were analyzed for residual nitrite, pH, and CIE L*, a*, and b* on days 0, 8, 14, 21, 28 and 35. Residual nitrate was analyzed on days 0, 8, 21, and 35. Samples collected after mixing and fermentation were also evaluated for residual nitrite, nitrate and pH. Water activity (a_w) was analyzed on day 0. Packaged samples were held in a 4 °C dark walk-in cooler. Residual nitrite determination was conducted using the AOAC method (AOAC, 1990). The pH of ham samples was determined with a pH probe in a 9:1 water:sample slurry (Inlab Solids Pro probe, Metler Toledo MultiSeven meter). CIE L*, a* and b* were measured using Illuminate A, 10 ° standard observer and a 1.27 cm port. Residual nitrate was measured as described by Ahn & Maurer (1987) with modifications described by Sindelar, Cordray, Sebranek, Love & Ahn (2007). Water activity was determined using an Aqualab Series 3 water activity meter (Decagon, Pullman, WA).

Statistical analysis Data was analyzed using PROC GLM (general linear models) procedure of the Statistical Analysis System software program (version 9.2, SAS Institute Inc., Cary, NC). When significant ($P<0.05$) treatment effects were found, 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.

III. RESULTS AND DISCUSSION

Both antimicrobials were effective in inhibiting growth of *Listeria monocytogenes* over 35 days of storage at 4 °C (Figure 1). Treatments A (uncured control) and B (natural nitrite, no antimicrobial) showed similar growth and that was significantly greater ($P<0.05$) than all other treatments during storage. All remaining treatments had *L. moncytogenes* growth similar to that of the traditionally cured control. Although still similar to the traditional cured product, treatments C (natural nitrite, antimicrobial A) and D (natural nitrate, no antimicrobial) began to increase by day 35. These antimicrobials showed similar inhibitory affects for *L. monocytogenes* on frankfurters (Schrader, Cordray, Sebranek, Dickson, & Mendonca 2010). Curing method played a greater role in inhibition than antimicrobial for *Clostridium perfringens* inhibition on ham and frankfurters (Jackson, Sullivan, Sebranek, & Dickson, 2010).

Physio-chemical traits can be found in Table 2. Samples collected after mixing provide a reference point for ingoing nitrite and nitrate levels. Natural nitrite source treatments had nitrite levels ranging from 20.2-23.5 ppm residual nitrite and 11.0-13.7 ppm residual nitrate following mixing. Natural nitrate sources had ingoing nitrate concentrations of 94.7-99.0 ppm and 0.7-0.8 ppm nitrite. Following fermentation, nitrate concentrations were reduced to 14.3-16.5 ppm and residual nitrite concentrations ranged from 40.4-51.2 ppm. No differences ($P>0.05$) were found in nitrite or nitrate concentrations among natural nitrate treatments suggesting that the antimicrobials did not have an impact on the starter culture activity. Natural nitrate samples had the greatest amounts of residual nitrite over storage ($P<0.05$). Samples containing antimicrobial A had higher pH ($P<0.05$) than antimicrobial B when compared to the corresponding nitrite source. Samples with antimicrobial A had the lowest L* which could be related to the higher product pH. Although means separation occurred among cured samples for a*, values ranged from 14.1-15.2. Therefore, it is unlikely this would impact visual appearance. Naturally cured samples with no antimicrobials had the lowest ($P<0.05$) b* values but again little numerical difference existed. No differences were found in water activity.

IV. CONCLUSION

Through the use of natural curing ingredients and antimicrobials, ham can be produced that possess similar *Listeria monocytogenes* inhibition when compared to traditionally cured controls during 35 day of storages. Method of natural curing used impacted the amount of nitrite and nitrate in the system. The natural antimicrobials may have small

impacts of limited practical importance on physio-chemical traits of ham.

ACKNOWLEDGEMENT

This project was supported by the National Integrated Food Safety Initiative (Grant no. 2006-51110-03609) of the Cooperative State Research, Education and Extension Service, U.S. Department of Agriculture, the National Pork Board (Grant no. 06-008) and the Iowa State University Food Safety Consortium

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Table 1. Ham formulations

TRT*	Ham kg	Water kg	Salt kg	Sugar kg	$NaNO_2$ ppm	Sodium Eryth[1] ppm	Natural NO_2[1] g	Natural NO_3[1] g	Starter Culture[1] g	LA\ DIA[1] g	Antim A[1] g	Antim B[1] g
A	18.14	3.74	0.5	0.3	-	-	-	-	-	-	-	-
B	18.14	3.72	0.5	0.3	-	-	68.1	-	-	-	-	-
C	18.14	3.52	0.5	0.3	-	-	68.1	-	-	-	158.9	-
D	18.14	3.72	0.5	0.3	-	-	-	68.1	5.0	-	-	-
E	18.14	3.52	0.5	0.3	-	-	-	68.1	5.0	-	158.9	-
F	18.14	3.13	0.5	0.3	-	-	68.1	-	-	-	-	540.0
G	18.14	3.13	0.5	0.3	-	-	-	68.1	5.0	-	-	540.0
H	18.14	3.17	0.5	0.3	156	550	-	-	-	570.0	-	-

*Treatment discription: A = Uncured control: B= Natural nitrite, no antimicrobial: C=Natural nitrite, Antimicrobial A: D=Natural nitrate, no anitmicrobial: E=Natural nitrate antimicrobial A: F=Natrual nitrite, antimicrobial B: G=Natural nitrate, antimicrobial B: H=Traditionally cured control

[1] Sodium Eryth = Sodium Erythorbate; Natural NO_2= Vegstable 504 (Flordia Food Products, Inc.); Natural NO_3 = Vegestable 502 (Flordia Food Products); Starter Culture = CS-Starter Culture299 Bactoferm (*Staphlococcus carnosus*, Chr. Hansen, Inc); LA/DIA = Purasal Opti.Form PD.4 (Purac America); Antim A = Natural Animicrobial A (vinegar, lemon and cherry powder blend); Antim B = Natural Anitimcrobial B (Cultured corn sugar and vinegar blend);

Table 2. Effect of curing treatment and antimicrobial on means of physiochemical properties of ham products during production and storage

	After Mixing			After Fermentation			During Stroage						
TRT	Resid. Nitrite ppm	Resid. Nitrate ppm	pH	Resid. Nitrite ppm	Resid. Nitrate ppm	pH	Resid. Nitrite ppm	Resid. Nitrate ppm	pH	L*	a*	b*	water activity
A	0.8^{c}	0.0^{c}	6.04	-	-	-	2.3^{e}	0.6^{e}	6.13^{de}	68.5^{a}	8.6^{d}	11.1^{a}	0.977
B	24.8^{b}	8.7^{c}	6.12	-	-	-	23.5^{cd}	12.4^{bc}	6.21^{bc}	66.9^{bc}	14.1^{c}	9.5^{cd}	0.975
C	24.4^{b}	13.9^{bc}	6.21	-	-	-	22.3^{d}	13.7^{bc}	6.32^{a}	64.4^{d}	14.7^{b}	11.0^{a}	0.974
D	0.9^{c}	99.0^{a}	6.11	41.2	14.3	6.03	45.6^{a}	7.8^{d}	6.19^{bcd}	66.7^{bc}	14.4^{bc}	9.4^{d}	0.974
E	0.7^{c}	94.7^{a}	6.07	40.4	15.1	6.11	41.7^{ab}	12.7^{bc}	6.25^{ab}	64.3^{d}	14.8^{ab}	10.8^{a}	0.962
F	24.2^{b}	10.0^{c}	6.00	-	-	-	20.2^{d}	11.0^{cd}	6.16^{cde}	66.1^{c}	14.6^{b}	10.0^{b}	0.962
G	0.8^{c}	94.8^{a}	5.95	51.0	16.5	5.92	37.7^{b}	15.7^{b}	6.11^{e}	66.5^{c}	14.8^{ab}	10.2^{b}	0.969
H	68.1^{a}	30.8^{b}	5.91	-	-	-	29.4^{c}	21.9^{a}	6.09^{e}	67.7^{ab}	15.2^{a}	9.8^{bc}	0.967

*Treatment discription: A = Uncured control: B= Natural nitrite, no antimicrobial: C=Natural nitrite, Antimicrobial A: D=Natural nitrate, no anitmicrobial: E=Natural nitrate antimicrobial A: F=Natrual nitrite, antimicrobial B: G=Natural nitrate, antimicrobial B: H=Traditionally cured control, lactate and diacetate

[a-e]Means with a common superscript within same column do not differ significantly (P > 0.05).

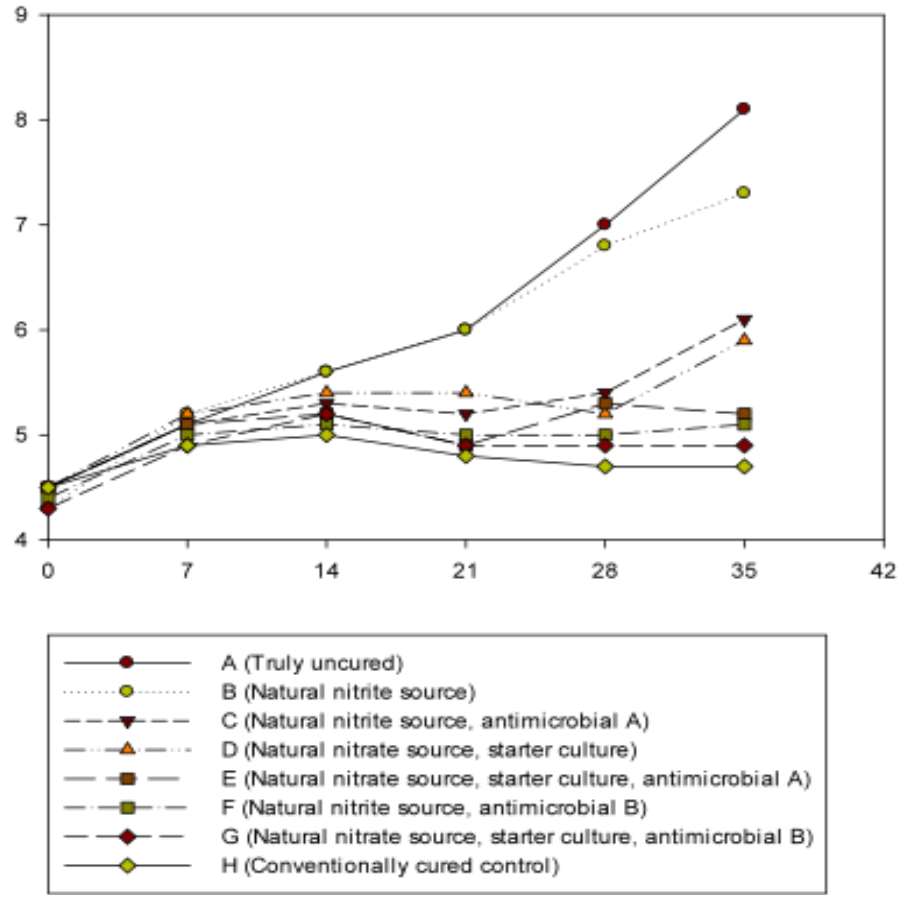

Figure 1. Effect of curing treatments and antimicrobial ingredients on growth of *L. monocytogenes* in ham during storage at room temperature. Days on x-axis and log growth on y-axis.