CHAPTER 3. The effect of pH and nitrite concentration on the antimicrobial impact of celery juice compared with sodium nitrite on *Listeria monocytogenes*

A paper to be submitted to *Meat Science*

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Abstract

Increasing consumer concerns of harmful preservatives have intensified consumers' demand for natural and organic alternatives. In response to this demand, uncured or no-nitrate-or-nitrite-added meat products which utilize celery juice concentrates as an alternative to sodium nitrite, have emerged on the market to replace conventional nitrite sources. The objective of this study was to evaluate the effect of celery juice pH for the impact of nitrite on *L. monocytogenes* growth. In addition, equal concentrations of nitrite in celery juice and conventional nitrite were evaluated to determine the impact of nitrite concentration from these sources on *L. monocytogenes* growth. These objectives were assessed using both a broth and ham system. Celery juice (CJ) was less effective than the conventional nitrite in the broth study at 100 ppm nitrite concentration but in the ham experiment the CJ treatments at both 100 and 200 ppm resulted in similar growth of *L. monocytogenes* ($p>0.05$) compared to their counterparts 100 and 200 ppm sodium nitrite. Adjusting the pH of the celery juice proved to be more effective at suppressing *L. monocytogenes* growth at 200 ppm than 100 ppm in the ham. No differences in growth ($p>0.05$) were found between the unadjusted 100

ppm celery juice (pH~9.2) and adjusted 100 ppm celery juice (pH~6.0) in either the broth or ham study. Color measurements of the ham indicated that all the CJ treatments were darker (lower L*) and more yellow (higher b*) than the sodium nitrite treatments. As concentration increased within the CJ treatments the L* became significantly lower ($p<0.05$) and b* values became significantly ($p<0.05$) greater. Overall, similar redness (a*) values were seen in both the CJ and sodium nitrite treatments. Residual nitrite concentrations were similar for both the 100 and 200 ppm treatments in the ham study, except for the adjusted (pH~ 6.3) 200 ppm CJ treatment which had significantly less ($p<0.05$) residual nitrite than the unadjusted (pH~6.6) 200 ppm CJ and 200 ppm sodium nitrite treatments.

Introduction

For centuries nitrate and nitrite have been used extensively in preserving meat products. Accidental discovery of these curing agents probably occurred during the traditional salting of meat dating back to 1600 BC (Jenson, 1953). Specific types of salt that were adulterated with nitrate developed a reddish color, which lead to what is commonly seen in cured meats today (Pegg & Shahidi, 2000). Other characteristics such as distinct flavors, decreased lipid oxidation, and inhibition of bacteria growth also contribute to the uniqueness of cured products (Sindelar & Milkowski, 2011).

However, concerns emerged in the 1950's relating to the safety of nitrate and nitrite inclusion in meat products. Studies indicated that free amines in herring meal were reacting with nitrite to form carcinogenic compounds called nitrosamines (Barnes & Magee, 1954; Magee & Barnes, 1956). In response to the nitrosamine concern, the United States Department of Agriculture (USDA) enforced maximum inclusion concentrations of nitrite in

all cured meat products that are still effective today (USDA, 1995). These maximum levels are strictly adhered to and have reduced the risk of nitrosamine production (Sindelar & Milkowski, 2012). Recently, new research has indicated that nitric oxide homeostasis in the body is critical for maintaining optimal blood pressure levels and controlling the blood flow of cardiac muscles (Bryan & Hord, 2010). This research, along with others, has clearly shown that dietary nitrate can be beneficial to an individual's overall health; especially for aging adults (McKnight et al., 1997; Parthasarathy & Bryan, 2012). Thus, nitrite in food is currently viewed by many in a much more positive light.

Regardless, consumers are apprehensive about the use of chemical preservatives, such as nitrate and nitrite, and this is driving consumers to seek alternative food products in natural and organic markets. In doing so, organic sales alone have risen from $1 billion in 1990 to $26.7 billion in 2010 (Organic Trade Association, 2011). To meet the needs of these consumers meat manufactures have created "no-nitrate-or-nitrite-added" or "uncured" labeled meat products that qualify to be labeled as natural or organic. In order to produce a product with the same characteristics seen in a conventionally cured product, manufacturers began using vegetable juice alternatives that contained high concentrations of nitrate. This allows the manufacturers to comply with the natural and organic labeling regulations (USDA, 2005). Celery juice concentrate is prominently used by the meat industry for this purpose because it has very little vegetable pigment and a mild flavor which minimizes the "vegetable" flavor sometimes perceived in the final meat product (Sebranek & Bacus, 2007). Originally, celery juice powder was first available in its nitrate form. Before processing, the celery juice powders would have to undergo a time-consuming incubation step where a

nitrate-reducing starter culture would be added to reduce nitrate to nitrite. Further developments created a pre-converted celery juice containing nitrite that eliminated the wait time of the incubation step and allowed direct addition during processing. Current pre-converted celery juice powders contain 10,000-15,000 ppm nitrite and are the most commonly used celery juice powder used today (Sindelar et al., 2010).

Listeria monocytogenes has become a hot topic of concern for meat processors recently due to its contamination of ready-to-eat meats and ability to withstand an adverse environment like refrigeration temperatures (Lungu et al., 2009). In 1936, the implications of this bacterium first became evident when its infection, listeriosis, caused abortions in pregnant women and meningitis in adults (Gray & Killinger, 1966). Populations that are immunocompromised such as pregnant women, children, and the elderly are especially prone to listeriosis (Liu, 2008). Even though this organism is not the most prevalent of the foodborne pathogens (Scallan et al., 2011), it has devastating consequences, since 20-30% of those contracting listeriosis result in death (Doganay, 2003). Schrader (2010) analyzed eight commercial brands of no-nitrate-or-nitrite-added frankfurters and found that five were less effective in reducing *L. monocytogenes* growth compared to conventionally cured brands. Myers (2012) also observed an increase in growth of *L. monocytogenes* on the no-nitrate-or-nitrite-added products and speculated that it could be attributed to the elevated pH observed in these products. Typically, celery juice concentrate has a pH ranging from 8.5-10 and may impact meat product pH as a result. It is important to note that nitrite's effectiveness relies heavily on pH (Tompkin, 2005). According to Tarr (1941), a pH at or above 7 inhibits nitrites' microbiological effectiveness. By reducing the pH, more nitric oxide is produced and

results in an increase in *L. monocytogenes* suppression (McClure et al, 1991). Reduced antimicrobial effectiveness is of particular concern relative to *L. monocytogenes,* because this organism has been shown to be prevalent in the environment and can easily contaminate ready-to-eat processed meats. Consequently, the objective of this study was to evaluate the impact of pH on the effectiveness of nitrite in celery juice for the suppression of *L. monocytogenes* growth on restructured ham products. In addition, the celery juice concentrate was compared to conventional nitrite using the same nitrite concentrations to evaluate whether the various components present in the celery juice affect the impact of nitrite on *L. monocytogenes*.

Materials and Methods

Broth Study

Broth preparation

Trypticase soy broth containing 0.6% yeast extract (TSBYE) (Difco, Becton, Dickson and Company, Sparks, MD., U.S.A.) was chosen for its neutral pH (~ 7.2) and its ability to support *Listeria monocytogenes* growth. Two groups of TSBYE were made. One received a pH adjustment using 1M hydrochloric acid to reduce the pH of the broth to 5.8. The pH of 5.8 was chosen because it best represents a typical meat system pH. The other group did not receive a pH adjustment (pH = ~ 7.2). These broths were then used to prepare experimental treatments for incubation with *L. monocytogenes* (Table 1).

Sample preparation

Two controls were created for each TSBYE adjusted group (Unadjusted = ~7.4, Adjusted = ~ 5.8) by adding distilled water as a treatment. The pre-converted celery juice

(VegStable 504, Florida Food Products, Eustis, FL) treatments consisted of two 100 ppm treatments, one unadjusted for pH prior to use and one adjusted (Unadjusted pH = ~9.2, Adjusted pH = ~6.0). The celery juice concentrate was added to distilled water to obtain 100 ppm nitrite concentration. 10 grams of citric acid (Fisher Scientific, Waltham, MA) was mixed with 90 ml of distilled water to obtain a 10% solution and then was added accordingly to reduce the pH of the adjusted celery juice treatment to ~6.0. Two ml of each treatment, along with 2 ml of the *L. monocytogenes* inoculum were added to 16 ml of each corresponding TSBYE treatment. Treatments were stored in dark conditions and held at 10°C.

Table 1

Broth study treatment descriptions.

Treatment	Description
A	Unadjusted control (unadjusted TSBYE + H_2O)
[a]B	Adjusted control (adjusted TSBYE + H_2O)
C	Unadjusted TSBYE + unadjusted 100 ppm celery juice
*[a]D	Adjusted TSBYE + adjusted 100 ppm celery juice
[a]E	Adjusted TSBYE + 100 ppm sodium nitrite
[a]F	Adjusted TSBYE + 200 ppm sodium nitrite

*Citric acid used to adjust pH of celery juice to 6.0.

[a]Hydrochloric acid used to adjust TSBYE pH to 5.8

Inoculum preparation and sample inoculation

5 strains of *Listeria monocytogenes* (Scott A, H7969, H7764, H7769, H7762) were obtained from the Food Safety Research Laboratory (FSRL) at Iowa State University. Each strain received a minimum of two consecutive 24 hour transfers into TSBYE and were incubated at 35°C. After 48 hours all 5 strains were homogenized together to create a cocktail (~10^9 cells per ml). The cocktail was diluted using 0.1% peptone water (Difco, Becton

Dickinson, Sparks, MD) to obtain 10^4 cells per ml. 2 ml of the diluted cocktail were added to each treatment.

Microbiological analysis

Appropriate ten-fold dilutions from each homogenized experimental treatment were made. From each treatment's designated dilutions, 0.1 ml was surface plated in duplicate onto Modified Oxford Medium supplemented with Modified Oxford Antimicrobial Supplement (MOX) (Difco, Becton Dickinson, Sparks, MD) on days 0, 2, 4, 6, 8, 10, and 12. Inoculated plates were incubated at 35°C for 48 hours. After 48 hours inoculated plates were counted.

pH determination

pH analysis was conducted by directly inserting the pH electrode (Fisher Scientific, Accumet 15, Waltham, MA) into the broth for each treatment. The pH meter was calibrated using phosphate buffers 4.0 and 7.0. Measurements were taken on days 0, 2, 4, 6, 8, 10, and 12.

Ham Study

Product manufacture

Seven treatments (Table 2) were produced to determine if pH and concentration of nitrite impacted the growth of *Listeria monocytogenes* in natural and conventional cured ham products. Two replications were conducted. Pre-converted celery juice (VegStable 504, Florida Food Products, Eustis, FL) was used as the natural source of nitrite. 10% solution of citric acid (Fisher Scientific, Waltham, MA) was added to celery juice for treatments 3 and 5 to lower the celery juice pH to approximately 6.

Table 2

Ham study treatment formulations.

Treatment*	Code	Ham Insides (kg)	Water (kg)	Salt (kg)	Sugar (kg)	VegStable 504 (g)	Sodium nitrite (g)	Sodium nitrite (ppm)[b]
1	Control	9.09	1.83	0.24	0.14	-	-	-
2	Unadj 100 ppm CJ	9.09	1.83	0.24	0.14	75.6	-	100
3[a]	Adj 100 ppm CJ	9.09	1.83	0.24	0.14	75.6	-	100
4	Unadj 200 ppm CJ	9.09	1.83	0.24	0.14	151.2	-	200
5[a]	Adj 200 ppm CJ	9.09	1.83	0.24	0.14	151.2	-	200
6	100 ppm $NaNO_2$	9.09	1.83	0.24	0.14	-	1.13	100
7	200 ppm $NaNO_2$	9.09	1.83	0.24	0.14	-	2.27	200

*Treatments: 1, no nitrite source added (Control); 2, unadjusted 100 ppm celery juice; 3, adjusted 100 ppm celery juice; 4, unadjusted 200 ppm celery juice; 5, adjusted 200 ppm celery juice; 6, 100 ppm sodium nitrite; 7, 200 ppm sodium nitrite.

[a]Treatments with addition of citric acid to obtain a pH of 6 in the celery juice.

[b]Total batch weight basis.

All treatments were based on a total of 11.3 kg.

Hams were produced at the Iowa State University (ISU) Meat Laboratory. Pork inside ham muscles (semimembranosus) were received fresh from a local processor and held at 0°C. The ham muscles were course-ground (Biro MFG Co., Model 7.5 424852, Marblehead, Ohio, U.S.A.) using a 9.52 mm plate. Non-meat ingredients were added to a vacuum paddle mixer (Fotosa, SA., Barcelona, Spain) along with the ham muscles according to the formulations found in Table 2. It should be noted that USDA sodium nitrite limits are based on the meat block weight, but to correspond with the concentrations used in the broth experiment, sodium nitrite was formulated on a total batch weight basis for this experiment. No phosphates were included because they are not permitted ingredients for natural and organic labeled meat products. After mixing for 2 minutes, the meat mixture was reground

through a 6.35 mm plate and stuffed into a 50 mm diameter impermeable plastic casing (Nalobar APM 45, Kalle USA, Gurnee, IL) using a vacuum stuffer (Risco vacuum stuffer, Model 1040C, Stoughton Mass., U.S.A.). Impermeable casings were used to minimize the transfer of nitrogen oxide gases during thermal processing. Treatments were then placed into a single truck smokehouse (Thermal Processor, Maruer-Atmos, Reichenau, Germany) for thermal processing. All products reached an internal temperature of 73.9°C. Products were then transported to a 0°C cooler overnight to stabilize. The next day each treatment was sliced (Bizerba, SE 12 D, Piscataway, NJ., USA) into 11 mm thick portions weighing approximately 25 g $\pm$ 0.5 g. For microbiology analysis, individual slices were placed in each bag (Cryovac Sealed Air Corporation, B2470, Duncan, SC) with an oxygen transmission rate of 3-6 cc at 40°F (m^2, 24 hrs atm @ 40°F, 0% RD) and a water vapor transmission rate of 0.5-0.6 g at 100°F (100% RD, 100 in^2, 24 hrs) and vacuumed packaged (UV 2100, Multivac, Inc., Kansas City, MO). For chemical analysis, two 25 gram slices were placed together into one bag (Cryovac Sealed Air Corporation, B2470, Duncan, SC) and vacuum packaged. The microbiology samples were transported to the Food Safety Research Laboratory (FSRL) and stored at 4°C in a dark cooler in the Meat Laboratory. Samples for chemical analysis were transported to a separate 4°C dark storage cooler.

Inoculum preparation

5 strains of *Listeria monocytogenes* (Scott A, H7969, H7764, H7769, H7762) were obtained from the FSRL at Iowa State University. Strains were individually grown in trypticase soy broth containing 0.6% yeast extract (TSBYE) (Difco, Becton, Dickson and Company, Sparks, MD., U.S.A.) and underwent two 24 hour transfers at 35˚C. All 5

transferred stains were combined to create a 50 ml cocktail (~10^9 cells per ml). From this cocktail dilutions were made using 0.1% buffered peptone water (Difco, Becton Dickson and Company, Sparks, MD., U.S.A.) to obtain a target inoculation of 10^4 cells per gram.

Sample inoculation

The packages containing the ham slices were aseptically opened and surface inoculated with 0.25 ml of the *L. monocytogenes* cocktail to obtain target 10^4 cells per gram for each slice of ham. Ham slices were then repackaged using the FSRL vacuum packager (Multivac, Model A-300/52, Kansas City, Mo., USA) and stored in a dark cooler at 4°C.

Microbiological analysis

On days 0, 3, 7, 10, 14, 21, 28, and 35, one inoculated 25 g sample from each treatment was aseptically removed from its packaging and placed into a 7.5 inch x 12 inch WhirlPak™ filter bag (VWR International, Radnor, PA) along with 99 ml of buffered peptone water (Difco, Becton Dickinson, Sparks, MD). It was then homogenized (Stomacher 400, Seward Medical, London, UK) on the normal setting for 60 seconds. Following homogenization, appropriate ten-fold serial dilutions were made using 0.1% buffered peptone water. Designated dilutions of 0.1 ml were surface plated in duplicate on MOX (Difco, Becton Dickinson, Sparks, MD). Inoculated plates were incubated at 35°C for 48 hours. Immediately following incubation the inoculated plates were counted.

pH determination

The pH meter (Inlab Solids Pro probe; MultiSeven pH meter, 92 Metler Toledo Inc, Columbus, OH) was calibrated using 4.0, 7.0, and 10.0 phosphate buffers. A 9:1 water: slurry

was used to determine the pH of the ham samples on days 0, 3, 7, 10, 14, 21, 28, and 35. All measurements were done in duplicate.

Color analysis

Color was analyzed using the HunterLab LabScan XE spectrocolorimeter (HunterLab, Reston, VA). A port size of 3 cm and a viewing area of 2.54 cm were used along with Illuminant A and 10° standard observer. The instrument was standardized by covering the white standard (X= 80.45, Y= 85.37, Z= 90.79) with saran wrap (SC Johnson & Sons, Racine, WI) to account for the saran wrap used on the samples while taking measurements. Four measurements (CIE L*, a*, and b*) were taken randomly for each treatment on days 0, 3, 7, 10, 14, 21, 28, and 35.

Residual nitrite

Samples from color analysis were then ground and homogenized using a food processor (KitchenAid, Model KFP715, St Joseph, MI). Residual nitrite was determined according to AOAC method 973.31 (AOAC, 1990c) on days 0, 3, 7, 10, 14, 21, 28, and 35 and expressed as sodium nitrite. All measurements were done in duplicate.

Water activity

Samples were analyzed with AquaLab 4TE water activity meter (Decagon Devices Inc., Pullman, Wash., U.S.A.) on day 0. The 0.76 and 1.00 standards were used to calibrate the instrument. All measurements were conducted in duplicate.

Proximate analysis

Moisture (AOAC, 1990b), crude protein (AOAC, 1993), and crude fat (AOAC, 1990a) were analyzed in duplicate for each treatment on day 0.

Statistical analysis

For the broth and ham experiments, statistical analysis was conducted using a randomized complete block design including replication, treatment, day and treatment x day in the model as fixed block effects. Measurements were analyzed using the statement proc glimmix with the Statistical Analysis System (SAS 9.2, SAS Institute Inc., Cary, NC, 2008). Due to the significant interaction between treatment and day, treatment means were compared for each day resulting in all pairwise comparisons calculations. Tukey multiple comparison adjustment was used to determine the pairwise comparisons. For moisture, fat, protein and water activity in the ham study, the proc glm statement was used to determine differences amongst means. In both experiments, significant differences were denoted with a $p<0.05$.

Results and Discussion

Broth Study

Listeria monocytogenes growth and pH

Table 3 and Fig. 1 illustrate the differences between treatments found for growth of *L. monocytogenes* in broth over the 12 day period. On days 0 and 2 there were no significant differences ($p>0.05$) amongst treatments. As expected the unadjusted control (pH~7.3) and adjusted control (pH~6.1) broth treatments had similar ($p>0.05$) growth throughout the entire study and resulted in greater growth ($p<0.05$) than all other treatments for days 4-12. This confirms that the addition of nitrite regardless of the source (celery juice or sodium nitrite) significantly affects the growth of *L. monocytogenes*. No differences ($p>0.05$) in growth were found between treatment C (unadjusted TSBYE + unadjusted 100 ppm CJ, pH 7.6) and D

(adjusted TSBYE + adjusted 100 ppm CJ, pH 6.2). In addition, these treatments also had statistically different ($p<0.05$) pH's, where treatment D maintained a lower pH (6.20 – 6.44) than treatment C (7.60 – 6.95) throughout the entire study (Table 4). Because, the pH's are different, this experiment suggests that there is no difference in the antimicrobial effect of nitrite against *L. monocytogenes* within this pH range of 6.2 – 7.6. No differences ($p>0.05$) between treatment D (adjusted TSBYE + adjusted 100 ppm celery juice) and treatment E (adjusted TSBYE + 100 ppm sodium nitrite, pH 6.10 – 6.11) were observed between days 0 and 8. On day 10 and 12, significantly higher numbers of *L. monocytogenes* were observed for the celery juice treatment (treatment D) compared to the sodium nitrite treatment (treatment E). Because the pH's of treatments D and E do not differ (Table 4), it appears that, when compared in broth, the celery juice may be less effective than sodium nitrite at the same nitrite concentration. In this experiment, sodium nitrite at both 100 ppm (treatment E) and 200 ppm (treatment F) were superior to the other treatments for suppressing *L. monocytogenes* growth. Treatment F (200 ppm sodium nitrite) had the lowest growth compared to all other treatments on days 8-12, again confirming that nitrite concentration affects the antimicrobial impact of nitrite against *L. monocytogenes*.

Table 3

Least square means for the interaction of treatment and day for *Listeria monocytogenes*[1] growth in broth study

Treatment*	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12
A	4.00[a]	4.60[a]	6.10[a]	7.35[a]	8.75[a]	9.75[a]	10.15[a]
B	3.90[a]	4.45[a]	5.85[a]	7.15[a]	8.35[a]	9.30[a]	9.65[a]
C	4.00[a]	4.10[a]	4.60[b]	6.05[b]	7.25[b]	8.05[b]	8.45[b]
D	3.95[a]	4.15[a]	4.90[b]	5.60[b]	6.65[bc]	7.60[b]	8.20[b]
E	3.95[a]	4.00[a]	4.65[b]	5.25[bc]	5.85[c]	6.55[c]	7.10[c]
F	3.90[a]	3.95[a]	4.20[b]	4.50[c]	4.75[d]	4.95[d]	5.25[d]

SEM[2] = 0.303

*Treatments: A, unadjusted TSBYE + distilled H_2O (unadjusted control); B, adjusted TSBYE + distilled H_2O (adjusted control); C, unadjusted TSBYE + unadjusted 100 ppm celery juice; D, adjusted TSBYE + adjusted 100 ppm celery juice; E, adjusted TSBYE + 100 ppm sodium nitrite; F, adjusted TSBYE + 200 ppm sodium nitrite.

[1]*Listeria monocytogenes* growth recorded as log CFU/ml.

[2]SEM = standard error of the means.

[a-d]Means in same column that have different superscripts are significantly different ($p<0.05$).

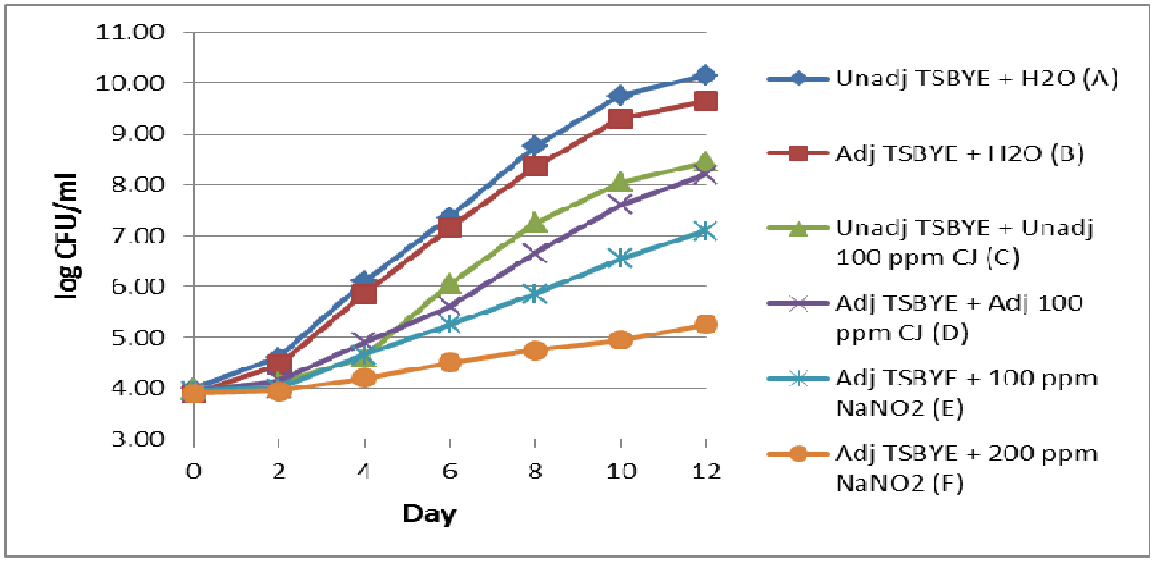

Fig. 1. Least square means of *L. monocytogenes* (log CFU/ml) growth amongst broth treatments after 10^4 log CFU/ml inoculation held at 10˚C for 12 days.

Throughout the 12 days of this experiment, both sodium nitrite (treatments E & F) treatments had statistically similar ($p>0.05$) pH's. This demonstrates that the concentrations of sodium nitrite used in this experiment did not affect the pH of the broth environment for adjusted TSBYE. However, as shown in table 4, treatment C (unadjusted TSBYE + unadjusted 100 ppm celery juice) had a higher pH ($p<0.05$) than all other treatments including treatment A (unadjusted TSBYE control) on days 4-12, which suggests that the growth of the microorganisms in the broth may have decreased the pH in the unadjusted TSBYE without added nitrite. While not statistically different from treatment A at days 0-2, it is noteworthy that treatment C had a numerically higher pH compared to all other treatments on each day.

Table 4

Least square means for the interaction of treatment and day for pH in broth study.

Treatment*	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12
A	7.32^{a}	7.32^{a}	7.31^{b}	7.22^{b}	7.10^{b}	6.78^{b}	6.06^{bc}
B	6.10^{b}	6.12^{b}	6.12^{c}	6.10^{c}	6.17^{c}	5.86^{d}	5.65^{c}
C	7.60^{a}	7.72^{a}	7.79^{a}	7.81^{a}	7.80^{a}	7.54^{a}	6.95^{a}
D	6.20^{b}	6.30^{b}	6.38^{c}	6.41^{c}	6.38^{c}	6.36^{bc}	6.44^{b}
E	6.10^{b}	6.12^{b}	6.13^{c}	6.12^{c}	6.11^{c}	6.05^{cd}	6.02^{bc}
F	6.09^{b}	6.12^{b}	6.13^{c}	6.12^{c}	6.12^{c}	6.11^{cd}	6.11^{b}
SEM[1] = 0.150							

*Treatments: A, unadjusted TSBYE + distilled H_2O (unadjusted control); B, adjusted TSBYE + distilled H_2O (adjusted control); C, unadjusted TSBYE + unadjusted 100 ppm celery juice; D, adjusted TSBYE + adjusted 100 ppm celery juice; E, adjusted TSBYE + 100 ppm sodium nitrite; F, adjusted TSBYE + 200 ppm sodium nitrite.

[1]SEM = standard error of the means.

[a-d]Means in same column that have different superscripts are significantly different ($p<0.05$).

Ham Study

Listeria monocytogenes growth and pH

Table 5 and Fig. 2 show the least square means of *L. monocytogenes* growth for all treatments on each day. Significant differences ($p>0.05$) amongst treatments were not

detected until day 7. As expected, the control (no nitrite source) had significantly ($p<0.05$) greater numbers of *L. monocytogenes* than all other treatments for days 10-35.

Table 5
Least square means for the interaction of treatment and day on *Listeria monocytogenes*[1] growth in ham study

Treatment*	Day 0	Day 3	Day 7	Day 10	Day 14	Day 21	Day 28	Day 35
1	3.60^{a}	3.75^{a}	4.55^{a}	5.30^{a}	6.00^{a}	7.80^{a}	8.45^{a}	9.25^{a}
2	3.75^{a}	3.60^{a}	3.75^{b}	4.30^{b}	4.70^{b}	5.85^{b}	6.45^{b}	7.15^{b}
3	3.75^{a}	3.65^{a}	3.90^{ab}	4.10^{b}	4.55^{b}	5.30^{bc}	6.05^{bc}	6.70^{bc}
4	3.80^{a}	3.80^{a}	3.75^{b}	4.05^{b}	4.20^{bc}	5.10^{c}	5.70^{c}	6.65^{bc}
5	3.55^{a}	3.70^{a}	3.65^{b}	3.75^{b}	3.70^{c}	4.20^{d}	4.75^{d}	5.50^{d}
6	3.80^{a}	3.75^{a}	3.90^{ab}	4.05^{b}	4.30^{bc}	5.10^{c}	6.30^{bc}	7.05^{b}
7	3.55^{a}	3.70^{a}	3.85^{ab}	4.20^{b}	4.20^{bc}	4.85^{cd}	5.70^{c}	6.00^{cd}
SEM[2] = 0.369								

*Treatments: 1, no nitrite source added (Control); 2, unadjusted 100 ppm celery juice; 3, adjusted 100 ppm celery juice; 4, unadjusted 200 ppm celery juice; 5, adjusted 200 ppm celery juice; 6, 100 ppm sodium nitrite; 7, 200 ppm sodium nitrite.
[1]*Listeria monocytogenes* growth recorded as log CFU/g.
[2]SEM = standard error of the means.
[a-d]Means in same column that have different superscripts are significantly different ($p<0.05$).

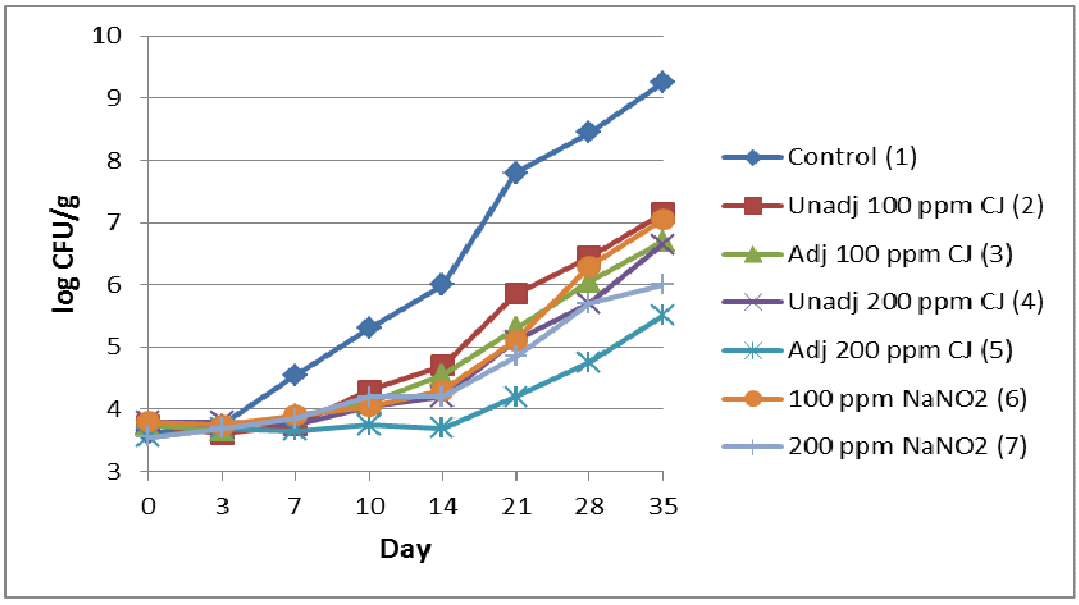

Fig. 2. Least square means of *L. monocytogenes* (log CFU/g) growth amongst ham treatments after 10^4 log CFU/g inoculation held at 4˚C for 35 days.

Other researchers (Duffy et al., 1994; Ngutter & Donnelly, 2003) have shown that nitrite is effective in suppressing *L. monocytogenes* growth in meat products. No differences ($p>0.05$) in growth were observed between the Unadj 100 ppm CJ (treatment 2) and Adj 100 ppm CJ (treatment 3). On days 0, 3, 7, 14, 21, and 35, the Adj 100 ppm CJ (treatment 3) had a significantly lower pH ($p<0.05$) than the Unadj 100 ppm CJ (treatment 2) (Table 6). Even though the pH's were different for the majority of the experiment, the microbiology data indicates that there was no difference in the antimicrobial effect within the pH range observed with the 100 ppm celery juice treatments. Similar results for microbial growth were also noted in the broth experiment. On days 21-35, the Adj 200 ppm CJ (treatment 5) had significantly ($p<0.05$) lower *L. monocytogenes* growth than the Uadj 200 ppm CJ (treatment 4). The pH differences ($p<0.05$) were significant for the duration of the experiment between the Unadj 200 ppm CJ and Adj 200 ppm CJ treatments where the Adj 200 ppm CJ treatment maintained a lower pH (Table 6). Since, the concentration of nitrite for both of these treatments was the same, the pH difference may have affected the microbial growth differences observed at 200 ppm in this experiment. Looking back at the adjusted and unadjusted 100 ppm celery juice treatments (Table 5) where there were no differences in *L. monocytogenes* growth, it is interesting to note that the unadjusted and adjusted 200 ppm celery juice treatments were indeed different ($p<0.05$). This suggests that both pH and concentration of celery juice may have affected the product pH and the subsequent *L. monocytogenes* growth as observed in this experiment.

During the 21 & 28 day time period, the Unadj 100 ppm CJ (treatment 2) resulted in significantly ($p<0.05$) higher numbers of *L. monocytogenes* (Table 5) than the Unadj 200

ppm CJ (treatment 4), but at the end of the study (day 35) both treatments had similar ($p>0.05$) populations. During the entire study, the Unadj 200 ppm CJ treatment maintained a higher pH ($p<0.05$) than the Unadj 100 ppm CJ (Table 6).

Table 6

Least square means for the interaction of treatment and day on pH in ham study.

Treatment*	Day 0	Day 3	Day 7	Day 10	Day 14	Day 21	Day 28	Day 35
1	6.14^{de}	6.14^{de}	6.14^{de}	6.11^{e}	6.12^{d}	6.13^{d}	6.08^{d}	6.12^{d}
2	6.46^{b}	6.45^{b}	6.43^{b}	6.42^{b}	6.44^{b}	6.44^{b}	6.42^{b}	6.28^{bc}
3	6.28^{cd}	6.28^{cd}	6.28^{cd}	6.27^{bcd}	6.27^{cd}	6.28^{c}	6.28^{bc}	6.10^{d}
4	6.68^{a}	6.65^{a}	6.65^{a}	6.63^{a}	6.64^{a}	6.65^{a}	6.65^{a}	6.64^{a}
5	6.35^{bc}	6.37^{bc}	6.36^{bc}	6.36^{bc}	6.36^{bc}	6.37^{bc}	6.37^{b}	6.37^{b}
6	6.11^{e}	6.13^{e}	6.12^{e}	6.13^{de}	6.14^{d}	6.12^{d}	6.12^{d}	6.16^{cd}
7	6.24^{ce}	6.24^{ce}	6.23^{ce}	6.23^{ce}	6.23^{cd}	6.24^{cd}	6.22^{cd}	6.13^{cd}

$SEM^{1} = 0.051$

*Treatments: 1, no nitrite source added (Control); 2, unadjusted 100 ppm celery juice; 3, adjusted 100 ppm celery juice; 4, unadjusted 200 ppm celery juice; 5, adjusted 200 ppm celery juice; 6, 100 ppm sodium nitrite; 7, 200 ppm sodium nitrite.

[1]SEM = standard error of the means.

[a-e]Means in same column that have different superscripts are significantly different ($p<0.05$).

This difference also suggests that the increase in concentration of celery juice may affect the product pH. On days 14-35, the Adj 200 ppm CJ treatment had significantly lower ($p<0.05$) numbers of *L. monocytogenes* growth than that of the Adj 100 ppm CJ treatment (Table 5). This supports the previous observations that nitrite concentration impacts *L. monocytogenes* growth. In addition, 100 ppm $NaNO_2$ resulted in significantly greater populations of *L. monocytogenes* on day 35 compared to 200 ppm $NaNO_2$, which reiterates the impact of nitrite concentration on *L. monocytogenes* growth. On all days except day 14, Unadj 100 ppm CJ, Adj 100 ppm CJ, and 100 ppm sodium nitrite were statistically similar ($p>0.05$). Ultimately, these treatments at the end of the experiment, reached the same population,

which suggests that, at 100 ppm nitrite, celery juice is just as effective as sodium nitrite in reducing *L. monocytogenes* growth when used at that concentration. Previous studies (Schrader, 2010; Jackson et al., 2011) have shown that typical usage levels of celery juice (0.2-0.4% if the batch weight) resulted in 20-60 ppm of ingoing nitrite and have been less effective in reducing *L. monocytogenes* and *Clostridium perfringens* growth than the traditional sodium nitrite ingoing concentrations of 120-156 ppm. The subpar performance of the celery juice has been attributed to the low ingoing nitrite concentrations by numerous other researchers. However, celery juice concentrations used in commercial products have remained low because of the undesirable vegetable flavor perceived at higher concentrations. Sindelar et al. (2007) reported that the concentration of 0.35% celery juice elicited a higher negative response from panelists when compared to a lower concentration of 0.20%. In addition, the Adj 200 ppm celery juice (treatment 5) in this study was statistically similar ($p>0.05$) to 200 ppm $NaNO_2$ (treatment 7) for suppression of *L. monocytogenes* growth on all days except day 28 ($p<0.05$), which supports the previous observations that equal nitrite concentrations elicits a similar antimicrobial impact on *L. monocytogenes*. Because, the Unadj 200 ppm CJ (treatment 4) was different ($p<0.05$) than the Adj 200 ppm CJ (treatment 5), the results suggest that the pH adjustment in treatment 5 (Adj 200 ppm CJ) affected the antimicrobial impact of the celery juice. The Adj 200 ppm CJ (treatment 5) also suppressed growth ($p<0.05$) more effectively than all other treatments except treatment 7 (200 ppm $NaNO_2$) on days 21 and 35. The results from this experiment suggest that at higher concentrations of celery juice, the antimicrobial impact of pH of the celery juice is more prominent, probably due to the pH effect on a greater nitrite concentration. It is likely that

more nitrite in the celery juice when combined with more acidic conditions, increases the impact of the antimicrobial activity of nitrite.

Color analysis

Results for the L* color analysis of the hams across the 35 day experiment are shown in Table 7. On day 0, the control and 100 ppm $NaNO_2$ treatments were similar ($p>0.05$), while all other treatments exhibited significant differences ($p<0.05$) in lightness. All celery juice treatments were darker ($p<0.05$) than both the $NaNO_2$ treatments throughout the entire study. On all days, significant differences ($p<0.05$) were evident between the 100 ppm CJ treatments and 200 ppm CJ treatments. Results indicated that as the concentration of the celery juice increased, there was an increase in darkness (lower L*). This also matches the visual perception seen during the study.

Differences in a* measurements are shown in Table 8. As expected, the control had significantly less ($p<0.05$) redness than all other treatments throughout the 35 day study. On day 0, treatments 2, 4, 5, and 6 (Unadj 100 ppm CJ, Unadj 200 ppm CJ, Adj 200 ppm CJ, and 100 ppm $NaNO_2$. respectively) had statistically similar ($p>0.05$) redness values, while on the same day, 200 ppm $NaNO_2$ (treatment 7) was significantly redder ($p<0.05$) than all other treatments. Both 100 ppm CJ treatments (treatment 2 and 3) had statistically similar redness ($p>0.05$) as 100 ppm $NaNO_2$ (treatment 6) on days 3-35. In addition, both 200 ppm CJ (treatments 4 and 5) had statistically similar redness ($p>0.05$) as the 200 ppm $NaNO_2$ (treatment 7) on days 7-35. The similarities within each concentration for both the natural and conventional nitrite sources demonstrate that celery juice produced the same amount of redness as traditional nitrite for the majority of the storage time in this study.

Table 7

Least square means for the interaction of treatment and day on L* in ham study.

Treatment*	Day 0	Day 3	Day 7	Day 10	Day 14	Day 21	Day 28	Day 35
1	70.67^{a}	70.39^{a}	70.21^{b}	70.30^{ab}	70.33^{a}	70.64^{a}	70.67^{a}	69.76^{ab}
2	67.33^{d}	67.08^{c}	67.75^{c}	66.99^{c}	66.76^{d}	67.14^{c}	67.24^{c}	66.57^{d}
3	68.88^{c}	67.75^{c}	67.35^{c}	67.68^{c}	67.58^{c}	67.40^{c}	67.34^{c}	67.62^{c}
4	65.11^{f}	64.41^{e}	64.11^{e}	64.24^{d}	64.09^{f}	64.29^{e}	63.78^{e}	63.86^{f}
5	66.49^{e}	65.60^{d}	65.71^{d}	64.99^{d}	65.16^{e}	65.36^{d}	65.49^{d}	65.39^{e}
6	71.45^{a}	70.83^{a}	71.08^{a}	70.65^{a}	70.28^{a}	70.57^{a}	70.51^{ab}	70.01^{a}
7	69.78^{b}	69.35^{b}	69.75^{b}	69.84^{b}	69.33^{b}	69.02^{b}	69.86^{b}	69.15^{b}

SEM[1] = 0.281

*Treatments: 1, no nitrite source added (Control); 2, unadjusted 100 ppm celery juice; 3, adjusted 100 ppm celery juice; 4, unadjusted 200 ppm celery juice; 5, adjusted 200 ppm celery juice; 6, 100 ppm sodium nitrite; 7, 200 ppm sodium nitrite.

[1]SEM = standard error of the means.

L* = lightness on scale of 0-100.

[a-f]Means in same column that have different superscripts are significantly different ($p<0.05$).

Table 8

Least square means for the interaction of treatment and day on a* in ham study.

Treatment*	Day 0	Day 3	Day 7	Day 10	Day 14	Day 21	Day 28	Day 35
1	12.14^{d}	10.99^{d}	10.83^{c}	10.71^{c}	10.49^{c}	10.14^{c}	9.94^{b}	10.20^{d}
2	16.50^{b}	16.80^{ac}	16.24^{b}	16.87^{b}	16.93^{ab}	16.56^{b}	16.65^{a}	16.72^{bc}
3	16.00^{c}	16.64^{bc}	16.77^{a}	16.94^{b}	16.77^{b}	16.93^{ab}	17.01^{a}	16.57^{c}
4	16.32^{bc}	16.64^{bc}	16.97^{a}	17.04^{b}	17.05^{ab}	16.89^{ab}	16.88^{a}	16.99^{ac}
5	16.53^{b}	17.06^{ab}	16.89^{a}	17.51^{a}	17.36^{a}	17.24^{a}	16.84^{a}	17.10^{ab}
6	16.56^{b}	16.56^{c}	16.54^{ab}	16.95^{b}	16.96^{ab}	16.78^{b}	16.73^{a}	16.93^{ac}
7	17.05^{a}	17.21^{a}	16.94^{a}	17.10^{ab}	17.24^{a}	17.29^{a}	17.01^{a}	17.28^{a}

SEM[1] = 0.164

*Treatments: 1, no nitrite source added (Control); 2, unadjusted 100 ppm celery juice; 3, adjusted 100 ppm celery juice; 4, unadjusted 200 ppm celery juice; 5, adjusted 200 ppm celery juice; 6, 100 ppm sodium nitrite; 7, 200 ppm sodium nitrite.

[1]SEM = standard error of the means.

a* = redness on scale of 0-100.

[a-d]Means in same column that have different superscripts are significantly different ($p<0.05$).

The yellowness (b*) measurements (Table 9), indicated that the celery juice treatments were significantly more ($p<0.05$) yellow than the conventional nitrite treatments

and the control. Within the celery juice treatments, both the 100 ppm CJ (treatments 2 and 3) had significantly less ($p<0.05$) yellow than the 200 ppm CJ (treatments 4 and 5). This suggests that as the concentration of celery juice increased, there was an increase in yellowness in the final ham product. This is most likely due to the particulates of the plant-derived concentrate that includes plant pigments. During days 3-10, the Adj 200 ppm CJ (treatment 5) was more yellow ($p<0.05$) than the Unadj 200 ppm CJ (treatment 4), but started and ended the study with similar yellow ($p>0.05$) values. In this case, the results suggest that the pH adjustment of the 200 ppm CJ may have impacted the yellowness in the final product at certain time periods. Both $NaNO_2$ treatments elicited the lowest ($p<0.05$) amount of yellowness throughout the entire study when compared to the rest of the treatments.

Table 9

Least square means for the interaction of treatment and day on b* in ham study.

Treatment*	Day 0	Day 3	Day 7	Day 10	Day 14	Day 21	Day 28	Day 35
1	13.40^{d}	13.55^{d}	13.77^{d}	13.85^{d}	13.85^{c}	13.79^{c}	13.76^{c}	13.90^{c}
2	14.06^{c}	14.22^{c}	13.72^{d}	14.18^{cd}	14.39^{b}	14.21^{b}	14.36^{b}	14.29^{bc}
3	14.48^{b}	14.61^{c}	14.44^{c}	14.52^{c}	14.61^{b}	14.46^{b}	14.54^{b}	14.41^{b}
4	17.09^{a}	17.01^{b}	17.07^{b}	17.23^{b}	17.16^{a}	17.18^{a}	17.32^{a}	16.78^{a}
5	16.98^{a}	17.55^{a}	17.52^{a}	17.82^{a}	17.49^{a}	17.54^{a}	17.21^{a}	17.17^{a}
6	11.40^{e}	11.08^{e}	11.24^{e}	11.35^{e}	11.26^{d}	11.30^{d}	11.24^{d}	11.18^{d}
7	11.42^{e}	11.21^{e}	11.04^{e}	11.25^{e}	11.41^{d}	11.41^{d}	11.19^{d}	11.37^{d}
$SEM^{1} = 0.145$								

*Treatments: 1, no nitrite source added (Control); 2, unadjusted 100 ppm celery juice; 3, adjusted 100 ppm celery juice; 4, unadjusted 200 ppm celery juice; 5, adjusted 200 ppm celery juice; 6, 100 ppm sodium nitrite; 7, 200 ppm sodium nitrite.

[1]SEM = standard error of the means.

b* = yellowness on scale of 0-100

[a-e]Means in same column that have different superscripts are significantly different ($p<0.05$).

Residual nitrite

Residual nitrite concentrations for all treatments throughout the shelf life of the ham products are represented in Table 10 and Fig. 3. As expected, the control treatment had

essentially no residual nitrite and was significantly lower ($p<0.05$) than all other treatments. Because it has been suggested that nitric oxide, which is derived from nitrite, may provide an inhibitory effect against microorganisms (Tompkin, 2005); it is no surprise that the control treatment had both low residual nitrite concentrations and high numbers of *L. monocytogenes*. As shown in Table 10, the Adj 200 ppm CJ (treatment 5) had significantly less residual nitrite ($p<0.05$) than that of the Unadj 200 ppm CJ (treatment 4) on all days except day 7. It has been shown that reduced pH speeds up the curing reaction (creates more nitric oxide) and as a result, less residual nitrite can be expected (Cassens et al., 1978). This allows more nitric oxide to become available to act as an antimicrobial. However, when comparing the Unadj 100 ppm CJ and Adj 100 ppm CJ treatments, there was no significant difference ($p>0.05$) found between the residual nitrite concentrations (Table 10). These findings correspond to no differences found between the *L. monocytogenes* growth for these treatments, which could imply that at lower concentrations of celery juice (and nitrite) the pH impact on nitrite effectiveness is less. Unadj 100 ppm CJ, Adj 100 ppm CJ, and 100 ppm NaNO2 treatments all had significantly less residual nitrite ($p<0.05$) than the 200 ppm nitrite treatments (Table 10), which demonstrates that, as the concentration of ingoing nitrite increases, the residual nitrite amounts also increase accordingly. Xi et al. (2011) found the same trend when studying different ingoing sodium nitrite concentrations. Overall, the residual nitrite concentrations decreased gradually during the 35 day storage period. Others have also reported a gradual decline of residual nitrite throughout the shelf life of meat products (Jantawat et al., 1993; Myers et al., 2013). Significantly higher concentrations of residual nitrite ($p<0.05$) were found in the Unadj 200 ppm CJ treatment versus the 200 ppm

$NaNO_2$ treatment (Table 10). Similar results were shown in Myers et al. (2013). Those authors commented that it was unusual to have higher concentrations of residual nitrite that corresponded with increased growth of *L. monocytogenes*. They speculated that the celery juice may have provided beneficial nutrients to *L. monocytogenes* since 97.75% of the celery juice used in the experiment was composed of organic and inorganic constituents.

Table 10

Least square means for the interaction of treatment and day on residual nitrite[1] in ham study.

Treatment*	Day 0	Day 3	Day 7	Day 10	Day 14	Day 21	Day 28	Day 35
1	3.69^{d}	3.56^{d}	2.51^{d}	2.63^{d}	1.68^{e}	2.49^{d}	2.85^{d}	3.32^{d}
2	71.45^{c}	69.24^{c}	67.59^{c}	64.72^{c}	63.69^{c}	57.36^{c}	56.81^{c}	51.50^{c}
3	69.72^{c}	65.18^{c}	60.67^{c}	55.52^{c}	55.25^{cd}	51.09^{c}	49.16^{c}	40.42^{c}
4	151.09^{a}	143.20^{a}	128.89^{a}	123.71^{a}	123.93^{a}	118.38^{a}	115.19^{a}	106.94^{a}
5	133.23^{b}	122.65^{b}	115.68^{ab}	105.15^{b}	103.99^{b}	95.15^{b}	87.67^{b}	79.67^{b}
6	61.56^{c}	62.45^{c}	56.95^{c}	52.34^{c}	50.05^{d}	46.20^{c}	43.93^{c}	39.62^{c}
7	122.08^{b}	114.66^{b}	107.68^{b}	97.04^{b}	95.28^{b}	88.31^{b}	81.15^{b}	71.11^{b}

SEM[2] =4.69

*Treatments: 1, no nitrite source added (Control); 2, unadjusted 100 ppm celery juice; 3, adjusted 100 ppm celery juice; 4, unadjusted 200 ppm celery juice; 5, adjusted 200 ppm celery juice; 6, 100 ppm sodium nitrite; 7, 200 ppm sodium nitrite.

[1]Residual nitrite reported as ppm.

[2]SEM = standard error of the means.

[a-e]Means in same column that have different superscripts are significantly different ($p<0.05$).

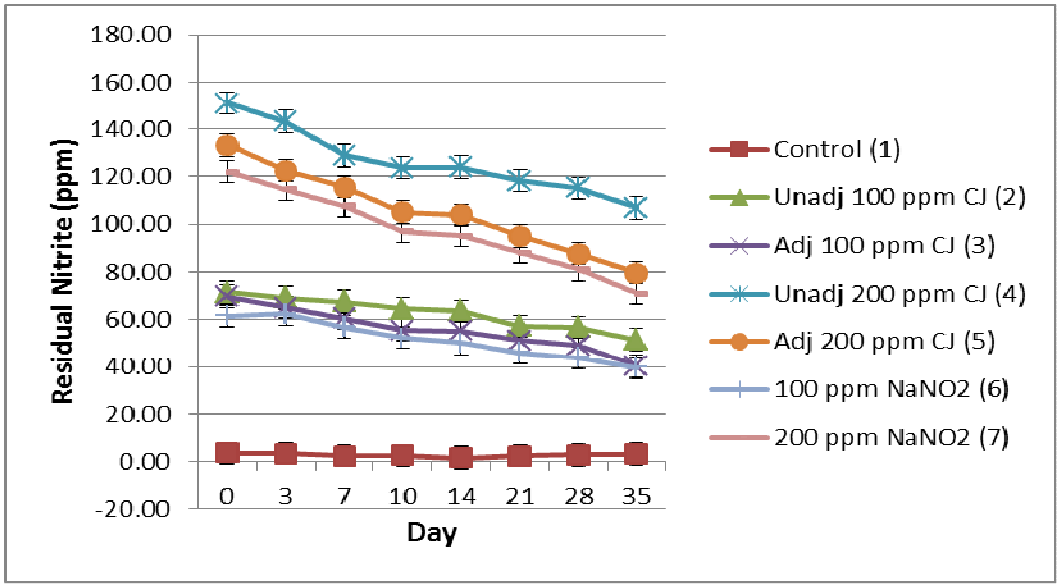

Fig. 3. Least square means of residual nitrite (ppm) for the ham study treatments after 10^4 log CFU/g inoculation held at 4˚C for 35 days.

Proximate analysis and Aw

The least square means of % moisture, % fat, % protein, and Aw are listed in Table 11. No differences ($p>0.05$) were observed for % moisture, % fat, and Aw between treatments. Protein differences ($p<0.05$) were observed between the Adj 200 ppm CJ and both the control and 200 ppm $NaNO_2$ treatments, and may have resulted from raw meat differences in the formulation between treatments or the addition of celery juice plus the citric acid. An explanation for the lower protein content in the Adj 200 ppm CJ treatment is not clear, but is unlikely to be of any practical significance since all other compositional properties did not differ among the treatments.

Table 11

Proximates and water activity measurements for all ham treatments on day 0.

Treatment*	Moisture (%)	Fat (%)	Protein (%)	Aw
1	75.37[a]	2.66[a]	18.85[a]	0.9791[a]
2	75.71[a]	1.98[a]	18.17[ab]	0.9778[a]
3	75.74[a]	1.87[a]	18.27[ab]	0.9768[a]
4	75.36[a]	1.79[a]	18.40[ab]	0.9749[a]
5	75.51[a]	2.19[a]	17.70[b]	0.9753[a]
6	75.64[a]	2.09[a]	18.33[ab]	0.9785[a]
7	75.41[a]	2.41[a]	18.46[a]	0.9781[a]
SEM[1]	0.213	0.214	0.161	0.0007

*Treatments: 1, no nitrite source added (Control); 2, unadjusted 100 ppm celery juice; 3, adjusted 100 ppm celery juice; 4, unadjusted 200 ppm celery juice; 5, adjusted 200 ppm celery juice; 6, 100 ppm sodium nitrite; 7, 200 ppm sodium nitrite.

[1]SEM = standard error of the means.

[a-b]Means in same column that have different superscripts are significantly different ($p<0.05$).

Conclusion

The broth experiment indicated that the pH adjustment that occurred between the two 100 ppm celery juice treatments (unadjusted TSBYE + unadjusted CJ and adjusted TSBYE + adjusted CJ) did not have an antimicrobial effect on *L. monocytogenes* growth. The same results were observed for the unadjusted and adjusted 100 ppm CJ treatments within the ham study. Differences in *L. monocytogenes* growth between the 100 ppm $NaNO_2$ and both the 100 ppm CJ treatments demonstrated that celery juice was less effective than conventional nitrite at the same nitrite concentration for suppressing *L. monocytogenes* in the broth system. However, the results from the ham experiment show that at equal concentrations of nitrite, celery juice was as effective as the sodium nitrite treatments in the meat product. Because the ham experiment represents the practical application of celery juice in the meat industry, it is a more realistic model compared to the broth system. At the same time, the broth experiment

suggested that the pH impact of celery juice concentrate can affect nitrite reactions and could be a consideration for some product applications.

As the concentration of the celery juice concentrate increased within the ham study, the pH of the ham product increased as well. When the pH adjustment was applied to the 200 ppm CJ, there was decreased *L. monocytogenes* growth and lower residual nitrite concentrations. Even though the pH adjustment had an impact on *L. monocytogenes* growth at 200 ppm, the Adj 100 ppm CJ did not show the same effect, which could be due to the lesser nitrite concentration. Similar residual nitrite concentrations and *L. monocytogenes* growth for the Unadj and Adj 100 ppm CJ treatments suggest that a larger pH reduction may need to be used at 100 ppm of nitrite in order to accelerate the nitric oxide production and therefore reduce *L. monocytogenes* growth. Particulates within the celery juice concentrate, such as fibers, sugars, and minerals (Djeri, 2010), could also hinder the reactivity of nitrite, depending on the chemical properties of these components.

The celery juice treatments also affected ham color and as the concentration was increased, the hams became darker (lower L*) and more yellow (higher b*) than conventional treatments. This is most likely due to the particulates (fibers, sugars, and minerals) that are present in the celery juice. Overall, the redness (a*) values were similar for both the celery juice and conventional treatments at equal nitrite concentrations.

Future research efforts on the use of celery juice concentrate as a meat curing agent for natural and organic processed meats should focus on developing a more concentrated form of celery juice that has increased nitrite concentration, lower pH and reduced vegetable off-flavors in order to increase the effectiveness of the ingoing nitrite. Even though this study

shows that celery juice was as effective as conventional nitrite in ham at equal nitrite concentrations, potential pH impact of the celery juice concentrate may be of significance for nitrite reactions in some applications. In addition, flavor strongly impacts consumer acceptability of meat products, and from previous research (Sindelar et al., 2007) sensory panel results indicated that celery juice concentrate can impart an undesirable flavor at high concentrations. This would be a concern for consumer products with concentrations of celery juice comparable to our study which used 0.67% (100 ppm) and 1.33% (200 ppm) to reach the desired nitrite concentrations.

References

AOAC, Association of Official Analytical Chemists. (1990a). Fat (crude) or ether extract in meat. Official method 960.39.*Official Methods of Analysis* (15th ed.). Arlington, VA: AOAC.

AOAC, Association of Official Analytical Chemists. (1990b). Moisture in meat. Official method 950.46. *Official Methods of Analysis* (15th ed.). Arlington, VA: AOAC.

AOAC, Association of Official Analytical Chemists. (1990c). Nitrites in cured meats. Official Method 973.31. *Official Methods of Analysis* (15th ed.). Arlington, VA: AOAC.

AOAC, Association of Official Analytical Chemists. (1993). Crude protein in meat and meat products. Official method 992.15.*Official Methods of Analysis* (15th ed.). Arlington, VA: AOAC.

Barnes, J.M., Magee, P.N. (1954). Some toxic properties of dimethylnitrosamine. *British Journal of Industrial Medicine*, 11(3), 167-174.

Bryan, N.S., & Hord, N.G. (2010). Dietary nitrates and nitrites: the physiological context for potential health benefits in: N.S. Bryan (Ed.), Food, Nutrition and the nitric oxide pathway: Biochemistry and bioactivity, DESTech Publications, Inc., Lancaster, PA, 59-77.

Cassens, R.G., Ito, I., Lee, M., & Buege, D. (1978). The use of nitrite in meat. *Bioscience*, 28(10), 633-637.

Djeri, N. (2010). Evaluation of vegstable 504 celery juice powder for use in processed meat and poultry as a nitrite replacer. Ph.D. Thesis. University of Florida, Gainesville, FL.

Doganay, M. (2003). Listeriosis: clinical presentation. *FEMS Immunology and Medical Microbiology*, 35, 173-175.

Duffy, L.L., Vanderlinde, P.B., Grau, F.H. (1994). Growth of *Listeria monocytogenes* on vacuum-packed cooked meats: effects of pH, aw, nitrite and ascorbate. *International Journal of Food Microbiology*, 23, 377-390.

Gray, M.L. and Killinger A.H. (1966). *Listeria monocytogenes* and listeric infection. *Bacterilogical Reviews*, 30, 309.

Jackson, A.L., Sullivan, G.A., Kulchaiyawat, C., Sebranek, J.G., Dickson, J.S. (2011). Survival and growth of *Clostridium perfringens* in commercial no-nitrate-or-nitrite-added (natural and organic) frankfurters, hams, and bacon. *Journal of Food Protection*, 74(3), 410-416.

Jantawat, P., Runglerdkriangkrai, J., Thunpithayakul, C., & Sanguandeekul, R. (1993). Effects of initial nitrite level, heating and storage on residual nitrite and spoilage of canned ham roll. *Journal of Food Quality*, 16(1), 1-11.

Jensen, L.B. (1953). Early preparations of foods. In man's foods. Nutrition and environments in food gathering times and food producing times. The Garrard Press, Champaign, IL, pp. 159-170.

Liu, D. (2008). Epidemiology, pp. 27-59. In Liu, D. (ed.), Handbook of *Listeria monocytogenes*. Taylor & Francis Group, LLC, Boca Raton, FL.

Lungu, B., Ricke, S.C., Johnson, M.G. (2009). Growth, survival, proliferation and pathogenesis of *Listeria monocytogenes* under low oxygen or anaerobic conditions: A review. *Anaerobe*, 15, 7-17.

Magee, P.N., Barnes, J.M. (1956). The production of malignant primary hepatic tumours in the rat by feeding dimethyinitrosamine. *British Journal of Cancer*, 10(1), 114-122.

McClure, P.J., Kelly, T.M., Roberts, T.A. (1991). The effects of temperature, pH, sodium chloride and sodium nitrite on the growth of *Listeria monocytogenes*. *International Journal of Food Microbiology*, 14(1), 77-91.

McKnight, G.M., Smith, L.M., Drummond, R.S., Duncan, C.W., Golden, M., Benjamin, N. (1997). Chemical synthesis of nitric oxide in the stomach from dietary nitrate in humans. *Gut*, 40 (2), 211-214.

Myers, K. M. (2012). Evaluation of high hydrostatic pressure, meat species, and ingredients to control *Listeria monocytogenes* in ready-to-eat meats. Ph.D. Thesis. Iowa State University, Ames, IA.

Myers, K.M., Cannon, J., Montoya, D., Dickson, J., Lonergan, S., Sebranek, J. (2013). Effects of high hydrostatic pressure and varying concentrations of sodium nitrite from traditional and vegetable-based sources on the growth of *Listeria monocytogenes* on ready-to-eat (RTE) sliced ham. *Meat Science*, 94(1), 69-76.

Ngutter, C.M. and Donnelly, C.W. (2003). Nitrite-induced injury of *Listeria monocytogenes* and the effect of selective versus nonselective recovery procedures on its isolation from frankfurters. *Journal of Food Protection*. 66:2252-2257.

Organic Trade Association. (2011). Industry statistics and projected growth. Available at: http://www.ota.com/organic/mt/business.html. Accessed 20 February 2013.

Parthasarathy, D.K., & Bryan, N.S. (2012). Sodium nitrite: The "cure" for nitric oxide insufficiency. *Meat Science*, 92, 274-279.

Pegg R.B, Shahidi, F. (2000). Nitrite curing of meat: The N-Nitrosamine problem and nitrite alternatives. Food & Nutrition Press, Inc. Trumbull, CT.

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.A., Roy, S.L., Jones, J.L., Griffin, P.M., (2011). Foodborne illness acquired in the United States-major pathogens. *Emerging Infectious Diseases*, Vol 17(1). http://wwwnc.cdc.gov/eid/article/17/1/p1-1101_article.htm. Accessed 21 February 2013.

Schrader, K.D. (2010). Investigating the control of *Listeria monocytogenes* on uncured, no-nitrate-or-nitrite-added meat products. Ph.D. Thesis. Iowa State University, Ames, IA.

Sebranek, J.G., Bacus, J. (2007). Natural and organic cured meat products: Regulatory, manufacturing, marketing, quality, and safety issues. American Meat Science Association White paper series, No. 1.

Sindelar, J.J., Cordray, J.C., Sebranek, J.G., Love, J.A., Ahn, D.U. (2007). Effects of vegetable juice powder and incubation time on color, residual nitrate and nitrite, pigment, pH, and trained sensory attributes of ready-to-eat uncured ham. *Journal of Food Science*, 72(6), S388-S395.

Sindelar, J.J. and Milkowski, A.L. (2012). Human safety controversies surrounding nitrate and nitrite in the diet. *Nitric Oxide*, 26, 259-266.

Sindelar, J.J, and Milkowski, A.L. (2011). Sodium nitrite in processed meat and poultry

meats: A review of curing and examining the risk/benefit of its use. American Meat Science Association. AMSA white paper series, No. 3.

Sindelar, J.J., Sebranek, J.G., Bacus, J.N. (2010). Uncured, natural and organic processed meat products. American Meat Science Association. Meat processing technology series.

Tarr, H.L.A. (1941). The action of nitrites on bacteria. *Journal of the Fisheries Research Board of Canada*, 5(3), 265-275.

Tompkin, R.B. (2005). Nitrite. p. 169-236. *In* P.M. Davidson, J.N. Sofos, and A.L. Branen (ed.), Antimicrobials in food. Taylor & Francis, Boca Raton, FL. <http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7620-3.pdf>. Accessed 12.28.12.

USDA, United States Department of Agriculture. (2005). Food Standards and Labeling Policy Book. http://www.fsis.usda.gov/OPPDE/larc/Policies/Labeling_Policy_Book_082005.pdf. Accessed Jan. 22, 2013.

USDA, United States Department of Agriculture. (1995). Processing Inspector's Calculations Handbook (FSIS Directive 7620.3).

Xi, Y., Sullivan, G.A., Jackson, A.L., Zhou, G.H., Sebranek, J.G. (2011). Use of natural antimicrobials to improve the control of *Listeria monocytogenes* in a cured cooked meat model system. *Meat Science*, 88, 503-511.

CHAPTER 4. GENERAL CONLUSIONS

Natural and organic meat products have become increasingly popular to the general consumer for its ability to provide a preservative-free product. Nitrite is included in preservatives not allowed in meat products labeled natural or organic. To circumnavigate the legalities, manufactures have incorporated celery juice has the nitrite source in these products to obtain the same unique characteristics seen in conventionally cured meat products. However, by substituting conventional sodium nitrite with a celery juice concentrate, there has been less ingoing nitrite observed in the celery juice inclusion percentages used, which causes an increased risk of *Listeria monocytogenes* growth within these products. *L. monocytogenes* is of utmost concern to processors because upon its outbreak, a large percentage of infected individuals have fatal outcomes.

Although the literature indicates that celery juice included at typical levels of 0.2-0.4% has greater growth of *L. monocytogenes*, this study showed that at equal concentrations celery juice is just as effective as sodium nitrite in ham. In addition, when the pH adjustment was applied to the Adj 200 ppm CJ treatment, an increased antimicrobial effect was observed by reduced *L. monocytogenes* growth. However, for both the broth and ham study, the pH adjustment did not have an antimicrobial impact on *L. monocytogenes* when applied to 100 ppm CJ. Color analysis in the ham study indicated that as the concentration of the celery juice increased, the products became darker (lower L*) and more yellow (higher b*).

For future research, emphasis should be focused on developing a more nitrite concentrated form of celery juice that minimizes the vegetable flavor that is currently seen in higher concentrations of celery juice. Since the appearance of the celery juice treated hams

were darker and more yellow, sensory analysis regarding the flavor and color should be considered when developing a more nitrite concentrated celery juice powder.

ACKNOWLEDGEMENTS

First, I would like to thank my major professor, Dr. Joseph Sebranek, for his guidance and expertise over the last couple of years. Words cannot describe how appreciative I am of the opportunity to obtain my degree at Iowa State University. I fully appreciated his patience and understanding throughout the whole process. I am truly blessed to have had him as my major professor and will always be grateful. I would also like to thank Dr. Jim Dickson for lending his expertise when designing the experiment and answering my many microbiology questions. I also greatly appreciated the advice and guidance of my co-major professor, Dr. Lester Wilson. To all my committee members I would like to thank them for serving as members.

I would like to thank Steve Niebuhr, who assisted with the microbiology protocol and answered many questions I had about microbiology. I would also like to thank Elaine Larson for conducting the proximate and color analysis. Her patience and guidance in the laboratory was very much appreciated.

I also wanted to thank Nick Lavieri, Ben Ruther, and Jonathon Campbell (graduate students) who helped with the manufacture of the hams. Also, I would like to thank Alyssa Fitzpatrick and Jennifer Kirk (undergraduates) for their outstanding help in the chemistry and microbiology labs.

Lastly, I would like to thank my family for their support and understanding. Having the support to pursue Graduate School from my fiancé, Cullan, meant the world to me. Thank you to my parents, Gerry and Connie, for their unwavering love and encouragement.

I cannot begin to express how grateful I am to all of those mentioned above, because their help was pivotal to my success here at Iowa State University.