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The effect of pH and nitrite concentration on the antimicrobial impact of celery juice concentrate compared with conventional sodium nitrite on *Listeria monocytogenes*

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ABSTRACT

The objectives of this study were to evaluate the impact of pH and nitrite from celery juice concentrate (CJ) on the growth of *Listeria monocytogenes* in broth and on ham slices, and to evaluate the impact of pH and nitrite from CJ on quality attributes of the ham. The pH of both broth and ham were increased by the addition of CJ. The CJ was less effective than conventional nitrite at 100 mg/kg nitrite in broth, but in ham, the CJ treatments at both 100 and 200 mg/kg resulted in growth of *L. monocytogenes* ($p > 0.05$) similar to that of the conventional nitrite at the same concentrations. Reducing the pH of CJ before addition to the ham had greater impact on *L. monocytogenes* growth at 200 mg/kg nitrite than at 100 mg/kg. Celery juice concentrate may increase meat product pH which could have implications for the antimicrobial impact of nitrite in some products.

1. Introduction

For centuries nitrate and nitrite have been used extensively in preserving meat products (Jensen, 1953). Nitrate and, more specifically, nitrite, create the distinctive cured meat color and other characteristics such as distinct flavors, decreased lipid oxidation, and inhibition of bacterial growth, all of which contribute to the uniqueness of cured products (Pegg & Shahidi, 2000; Sindelar & Milkowski, 2011, 2012).

Regardless, consumers have become apprehensive about the use of chemical preservatives, such as nitrate and nitrite, and this is driving the 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 concentrate that contained high concentrations of nitrate or nitrite as an alternative to conventional nitrite. This allowed 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 concentrate was first available in its nitrate form. Before final thermal processing of the processed meat product, the celery concentrate would have to undergo a time-consuming incubation step where a nitrate-reducing starter culture was included to reduce nitrate to nitrite. Further developments by celery concentrate suppliers created a pre-converted celery concentrate containing nitrite that eliminated the wait time of the incubation step and allowed direct addition of pre-converted nitrite during product manufacture. Currently available pre-converted celery concentrates contain 10,000–15,000 mg/kg sodium nitrite and are commonly used today (Sindelar, Sebranek, & Bacus, 2010). However, the amount of celery concentrate added to processed meat is generally limited to 0.2%–0.4% of the formulation because of potential flavor effects (Sindelar, Cordray, Sebranek, Love, & Ahn, 2007). As a result, the added nitrite concentration is significantly less than in conventional products and questions of safety have been raised (Sebranek & Bacus, 2007).

Reduced antimicrobial effectiveness is of particular concern relative to *Listeria monocytogenes*, because this organism has been shown to be prevalent in the environment and can easily contaminate ready-to-eat processed meats (Lungu, Ricke, & Johnson, 2009). Even though this organism is not the most prevalent of the foodborne pathogens

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(Scallan et al., 2011), it has devastating consequences, since 20–30% of those contracting listeriosis die (Doganay, 2003).

To evaluate the potential risk of *L. monocytogenes* growth in natural and organic processed meats made with celery concentrate, Schrader (2010) analyzed eight commercial brands of no-nitrate-or-nitrite-added frankfurters and found that ingredients in five of the brands were less effective in controlling *L. monocytogenes* growth than conventionally cured brands. Myers (2012) also observed greater growth of *L. monocytogenes* on frankfurters and hams manufactured with celery concentrate and speculated that it could be attributed to the elevated pH, observed to be as much as 0.3 pH units in these products compared to products manufactured with conventional sodium nitrite. Typically, celery concentrate has a pH ranging from 8.5 to 10 as provided by suppliers and may impact meat product pH as a result. It is important to note that nitrite's effectiveness as an antimicrobial for many pathogens relies heavily on pH (Tompkin, 2005). For example, reducing the pH typically results in more nitrite reactions during meat curing (Sebranek & Bacus, 2007), and increased *L. monocytogenes* suppression has been observed for nitrite at a reduced pH (McClure, Kelly, & Roberts, 1991). Celery concentrate contains numerous organic and inorganic components including proteins, carbohydrates, minerals and other compounds derived from concentration of raw celery (Djeri, 2010). These compounds may or may not affect pH and nitrite reactions in a meat product. Consequently, the objectives of this study were to evaluate the impact that pH of celery concentrate has on the effectiveness of nitrite for the suppression of *L. monocytogenes* growth, and to compare the effectiveness of nitrite from celery concentrate to conventional sodium nitrite on *L. monocytogenes*. Trypticase soy broth was utilized to first assess the hypothesis that the elevated pH of celery concentrate would decrease the antimicrobial impact of nitrite. Restructured hams were then utilized to evaluate the effects observed in broth in a processed meat product. In addition, the celery concentrate was compared to conventional nitrite using the same nitrite concentrations and the same pH to determine whether the various components present in the celery concentrate (proteins, carbohydrates, minerals, etc.) might affect the impact of nitrite on *L. monocytogenes*, independent of pH.

2. Materials and methods

2.1. Broth study

To study the effects of celery concentrate in a simplified system, trypticase soy broth with yeast extract (TSBYE) was used. Because TSBYE is typically about pH 7, both the TSYBE and the celery concentrate were evaluated at both their inherent pH and at a pH adjusted close to that of a meat product.

2.1.1. Broth preparation

Trypticase soy broth containing 0.6% yeast extract (TSBYE) (Difco, Becton, Dickinson and Company, Sparks, MD., U.S.A.) was chosen for its neutral pH (~7.2) and its ability to support *L. monocytogenes* growth. Two different batches of TSBYE were prepared. One received a pH adjustment using 1 M hydrochloric acid to reduce the pH of the broth to a target of 5.8. The target pH of 5.8 was chosen because it best represents a typical meat system pH of 5.8–6.2. Subsequent measurement of pH following addition of all components including the *L. monocytogenes* inoculation showed that the final pH was 6.09–6.20. The other batch of TSBYE 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).

2.1.2. Sample preparation

Two controls were created for each TSBYE batch (unadjusted pH = ~7.2, adjusted pH = ~5.8) by adding distilled water as a treatment. The treatments with celery concentrate (VegStable 504, Florida Food Products, Eustis, FL) consisted of two 100 mg/kg sodium nitrite treatments, one unadjusted from pH 9.2 and one adjusted to pH 6.0 before addition to the broth. Ten grams of the celery concentrate was first added to 140 ml of distilled water and 10 ml of a 10% solution of citric acid (in distilled water) (Fisher Scientific, Waltham, MA) was then added to reduce the pH of the celery concentrate treatment to ~5.8–6.0. Two ml of each celery concentrate treatment containing 1000 mg/kg sodium nitrite concentration, along with 2 ml of the *L. monocytogenes* inoculum were added to 16 ml of each corresponding TSBYE preparation to create the treatments with the target sodium nitrite concentration of 100 mg/kg. Treatments were stored in dark conditions at 10 °C.

Table 1
Broth experiment treatments.

Treatment	Description	pH
A	Control (TSBYE + H_2O)	7.32
B[a]	Adjusted control (adjusted TSBYE + H_2O)	6.10
C	TSBYE + 100 mg/kg nitrite from celery concentrate	7.60
D[a,b]	Adjusted TSBYE + adjusted 100 mg/kg nitrite from celery concentrate	6.20
E[a]	Adjusted TSBYE + 100 mg/kg sodium nitrite	6.10
F[a]	Adjusted TSBYE + 200 mg/kg sodium nitrite	6.09

[a] Hydrochloric acid used to adjust TSBYE pH to target of 5.8–6.0.
[b] Citric acid used to adjust pH of celery concentrate to 6.0.

2.1.3. Inoculum preparation and sample inoculation

Five strains of *L. 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 h transfers into TSBYE and were incubated at 35 °C. After 48 h 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 was added to each TSBYE preparation.

2.1.4. 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 h. Because MOX is a selective media for *L. monocytogenes*, black colonies that grew on the plates following incubation were counted and recorded as surviving *L. monocytogenes*.

2.1.5. 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 of pH 4.0 and 7.0. Measurements were taken on days 0, 2, 4, 6, 8, 10, and 12.

2.2. Ham study

2.2.1. Product manufacture

Seven ham treatments (Table 2) were produced to determine if the pH of the celery concentrate and concentration of nitrite impacted the growth of *L. monocytogenes* in natural and conventional cured ham products. Celery concentrate (VegStable 504, Florida Food Products, Eustis, FL) was used as the natural source of nitrite. After the celery concentrate was added to distilled water, a 10% solution of citric acid (Fisher Scientific, Waltham, MA) was added for treatments 3 and 5 to lower the celery concentrate pH from 9.2 to the target pH of 5.8–6.0,