Effects of Vegetable Juice Powder Concentration and Storage Time on Some Chemical and Sensory Quality Attributes of Uncured, Emulsified Cooked Sausages


ABSTRACT: Uncured, no-nitrate/nitrite-added meat products can be manufactured with vegetable juice powder (VJP) and a starter culture containing *Staphylococcus carnosus*, resulting in quality and sensory attributes similar to traditional cured products. The 1st objective of this study was to determine the effects of varying concentrations of VJP and incubation times (MIN-HOLD) on quality characteristics, including lipid oxidation, color, and cured meat pigment concentrations, of emulsified-frankfurter-style-cooked (EFSC) sausages over a 90-d storage period. The 2nd objective was to compare residual nitrate and nitrite content resulting from different processing treatments and the 3rd objective was to assess sensory properties of finished products. Four EFSC sausage treatments (TRT) (TRT 1: 0.20% VJP, 30 MIN-HOLD; TRT 2: 0.20% VJP, 120 MIN-HOLD; TRT 3: 0.40% VJP, 30 MIN-HOLD; TRT 4: 0.40% VJP, 120 MIN-HOLD) and a sodium nitrite-added control (C) were used for this study. No differences for lipid oxidation (TBARS) between any TRTs and C or over time were observed. No differences (*P > 0.05*) for CIE *L* values were found between TRTs. CIE *a* and reflectance ratio values revealed that TRTs 2, 4, and C were redder than TRTs 1 and 3 at day 0. Trained sensory intensity ratings for cured aroma, cured color, cured flavor, uniform color, and firmness determined that all but TRT 1 were similar to C. These results indicate a longer incubation time (120 compared with 30 min) was found more critical than VJP level (0.20% or 0.40%) to result in products comparable to a sodium nitrite-added control.

Keywords: emulsified, residual nitrate, residual nitrite, uncured, vegetable juice powder

Introduction

Meat curing can be defined as the use of both salt and nitrite (the reduced form of nitrate) to chemically alter the physical, chemical, and often microbiological properties of meat products (Cassens and others 1979). Although used for hundreds of years, sodium nitrite was not approved for meat curing by the USDA until 1925 (Pearson and Tauber 1984). It was not until the turn of 20th century that scientists determined that nitrate did not have a direct role in the curing process and the conversion of nitrate to nitrite was necessary for curing reactions. This step is normally accomplished by the bacterial reduction of nitrate to nitrite (MacDougall and others 1975; Sebranek 1979; Gray and others 1981; Pinotti and others 2001). Bacterial reduction can be accomplished by microorganisms found in the natural flora of meat or by intentional addition of microorganisms with nitrate-reducing properties (Sanz and others 1997).

Nitrite is responsible for the development of cured color and flavor, serves as a strong antioxidant to protect flavor, and acts as a strong antimicrobial to control *Clostridium botulinum* outgrowth (Shahidi and Pegg 1992). Nitrite controls and stabilizes the oxidative state of lipids in meat products (Shahidi and Hong 1991), thus preventing lipid oxidation and subsequent warmed-over flavors (Yun and others 1987; Vasavada and Cornforth 2005). Less nitrite is needed to provide for color development than to control bacteria (Roberts 1975).

The value of meat color to the consumer is extremely important. The 4 determining attributes for consumer purchasing decisions are color, juiciness, flavor, and toughness/tenderness. Of these attributes, color is the first and primarily most important factor of the decision-making process to purchase meat products (Aberle and others 2001). Recent consumer interest for natural, organic, and healthier perceived foods has prompted consumer demands for uncured, no-nitrate/nitrite-added meat and poultry products.

Two classifications of uncured, no-nitrate/nitrite-added meat and poultry products currently exist in the marketplace: those that do not utilize nitrate or nitrite (uncured products) and those that attempt to simulate nitrite-added cured product properties. In order to manufacture cured products without direct addition of sodium nitrite, a nitrate source and reducer must be utilized. Vegetables are well known to contain significant amounts of nitrate (Walker 1990; Fujihara and others 2001) and when added at high enough levels with a nitrate reducer may provide adequate amounts of nitrate to accomplish curing reactions.

Therefore, the objectives of this research were to first determine the effects of varying concentrations of commercial vegetable juice powder (VJP) and incubation times on quality characteristics including lipid oxidation, color, and cured meat pigment concentrations of emulsified-frankfurter-style cooked (EFSC) sausages over an extended storage period, and second, to determine if differences

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exist in finished products as determined by trained sensory analysis. A 3rd objective was to determine the effects of VIP concentration and incubation time on nitrate and nitrite concentrations during product manufacture and over an ensuing storage period.

Materials and Methods

Experimental design and data analysis

Varying concentrations of VIP and incubation (MIN-HOLD) times (30 or 120 min) for the manufacture of EFSC sausage were investigated. Four EFSC sausage treatments (TRT 1: 0.20% VIP; 30 MIN-HOLD; TRT 2: 0.20% VIP; 120 MIN-HOLD; TRT 3: 0.40% VIP; 30 MIN-HOLD; TRT 4: 0.40% VIP; 120 MIN-HOLD) and a sodium nitrite-added control (C) were used for this study.

Statistical analysis was performed for all measurements using the SAS (version 9.1, SAS Institute Inc., Cary, N.C., U.S.A.) mixed model procedure (SAS Inst. 2003). The experimental design was a 2 (VIP level) × 2 (MIN-HOLD time) factorial design. The main plot consisted of 3 blocks (replication) and 4 EFSC sausage treatments and a control, resulting in 15 observations for trained sensory and proximate composition. The model included the fixed main effects of treatment and replication. The random effect was the interaction of treatment × replication.

Within the main factorial design was a split plot for measurements over time. The split plot contained 5 sampling periods (days 0, 14, 28, 56, and 90) and combined with the main plot resulted in a total of 75 observations for color, nitrite, nitrate, pH, cured pigment, total pigment, and lipid oxidation. The model included the fixed main effects of treatment, replication, and day and the interaction of treatment × day. The random effect was the interaction of treatment × replication.

The significant main effect means for all experiments were separated and least significant differences were found using Tukey-Kramer multiple pairwise comparison method. Significance level was determined at P < 0.05. For all other experiments, main effects were tested for significance using a mixed effects model.

Product procurement and manufacture

Ready-to-eat EFSC sausages were manufactured with 85% lean coarse-ground (9.5 mm) fresh beef trimmings and 50% lean fresh pork trimmings obtained from a local supplier. The 50% pork trimmings were ground (Biro MFG Co., Marblehead, Ohio, U.S.A.) using a 1.27-mm plate. Samples (5.90 kg) of both beef and pork trimmings were separated into 5 batches (11.34 kg each). Treatments (TRTs 1 to 4) and control (C) were randomly assigned to the batches. The beef and pork EFSC sausage formulation for TRTs 1 and 2 consisted of the following ingredients: 59.4% beef trimmings, 19.8% pork trimmings, 15.8% ice/water, 1.77% salt, 1.58% dextrose, 1.42% spices (Blend TG-05-405-000, A.C. Legg Packing Co., Calera, Ala., U.S.A.), 0.0436 sodium erythorbate, and 0.0124% sodium nitrite. No phosphates were added to any TRTs or C because the EFSC sausages were intended to be similar to natural or organic products that restrict phosphate usage.

Emulsions were produced using methods described by Rust (1987). The EFSC sausages were manufactured using a vacuum bowl cutter (Krämer & Grebe Model VSM65, Krämer & Grebe GmbH & Co. KG., Biendenkopf-Wallau, Germany). The beef trim was chopped with salt, VIP, or nitrite (depending on treatment), and half of ice/water under vacuum until 3 °C was achieved. The bowl cutter was scraped and the pork, dextrose, spices, starter culture, or sodium erythorbate (depending on treatment) and remaining water were added and chopped under vacuum until 14 °C was achieved. After chopping was completed, the meat batter was transferred to a rotary vane vacuum-filling machine with linking attachment (Risco vacuum stuffer, Model RS 4003-165, Stoughton, Mass., U.S.A.) and stuffed into 33-mm impermeable plastic casings (WP-E Clear 35 Micron, World Pac U.S.A. Intl. Inc., Sturtevant, Wis., U.S.A.). The impermeable casings were used to control cross-contamination effects that any environmentally released nitric oxide gas could have on the TRTs during thermal processing. The casings had an O2 permeability rate of 6 to 7 cm2/m2/24 h at 1 atm and a water vapor permeability of 130 g/m2/24 h.

TRTs were placed on separate smokehouse trucks to allow separate incubation (MIN-HOLD) times (30 or 120 min). The stuffed EFSC sausages were transferred to 2 single truck thermal processing ovens (Mauer, AG, Reichenau, Germany; Alkar, Model MT EVD RSE 4, Alkar Engineering Corp., Lodi, Wis., U.S.A.). Incubation was conducted at 40.6 °C dry bulb and 39.4 °C wet bulb temperatures. MIN-HOLD times started when the internal temperature of the EFSC sausages reached 37.8 °C. The control was added to the thermal processing oven after incubation steps were complete. Cooking was accomplished using a common frankfurter smokehouse schedule reaching an internal temperature of 71.1 °C. After thermal processing, the EFSC sausages were chilled for 12 h at 0 to 2 °C. The EFSC sausages were placed in barrier bags (Cryovac B540, Cryovac Sealed Air Corp., Duncan, S.C., U.S.A.) and vacuum packaged. The packaging film had an O2 transmission rate of 3 to 6 cc/m2/24 h at 1 atm, 4.4 °C, and 0% RH, and a water vapor transmission rate of 0.5 to 0.6 g/645 cm2/24 h and 100% RH.

Color measurements

Color measurements were conducted using a Hunterlab Labscan spectrophotometer (Hunter Assoc. Laboratories Inc., Reston, Va., U.S.A.). Values for the white standard tile were X = 81.72, Y = 86.80, and Z = 91.46. Exterior color of the EFSC sausage was measured immediately after removing from the packaging material and internal color was measured immediately after slicing the EFSC sausage lengthwise. Illuminant A, 10° standard observer with a 1.27-cm viewing area and 1.78-cm port size, was used to analyze EFSC sausage samples. Commission Intl. d’Eclairage (CIE) L* (lightness), a* (redness), and b* (yellowness) and cured meat color were determined by reflectance ratio of wavelengths 650/570 nm (Erdman and Watts 1957; Hunt and others 1991). Measurements were taken at 6 randomly selected areas on the samples (n = 2) and the resulting average was used in data analysis.

Proximate composition

Proximate composition was determined for the EFSC sausage samples including crude fat (AOAC 1990a), moisture (AOAC 1990b), and crude protein (AOAC 1993).
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pH determination
The pH of the EFSC sausage samples was determined by blending the samples with distilled, deionized water in a 1:9 ratio, then measuring the pH with a pH/ion meter (Accumet 925, Fisher Scientific, Fair Lawn, N.J., U.S.A.) equipped with an electrode (Accumet Flat Surface Epoxy Body Ag/AgCl combination Electrode Model 13-620-289, Fisher Scientific, Fair Lawn, N.J., U.S.A.) calibrated with phosphate buffers 4.0 and 7.0, according to the method of Sebranek and others (2001). For each treatment, measurements were made in duplicate.

TBARS analysis
Lipid oxidation was measured by the modified 2-thiobarbituric acid reactive substances (TBARS) test as described for cured meats (Zipser and Watts 1962). TBARS values were reported as mg of malonaldehyde equivalents/kg of meat sample. For each treatment, measurements were made in duplicate.

Total and cured pigment analysis
Mononitrosylhemochrome (cured meat pigment) and total pigment concentrations were measured after extraction in 80% acetone and acidified acetone, respectively (Hornsey 1956). The experiment, including sample preparation, was done in subdued light to reduce pigment fading. Samples were finely ground/chopped using a food processor (Sunbeam-Oskar Model 4817, Sunbeam Products Inc., Delray Beach, Fla., U.S.A.) prior to extraction.

Cured pigment analysis was conducted using a modified method of Hornsey (1956). Duplicate 10-g samples were mixed with 40 mL of acetone and 3 mL of distilled, deionized water with a Polytron mixer (Type PT 10/35, Kinematica GmbH, AG, Switzerland) for 1 min at speed setting 7. The sample was immediately filtered through a Whatman 42 filter paper, and the absorbance (540 nm) measured on the filtrate.

Nitrosylhemochrome concentration was calculated as

\[ \text{Nitrosylhemochrome concentration} = \frac{A_{540}}{\text{sample weight} \times \text{dilution factor}} \]

Total pigment concentration was calculated as

\[ \text{Total pigment concentration} = \frac{A_{680}}{\text{sample weight} \times \text{dilution factor}} \]

Residual nitrite analysis
Residual nitrite was determined by the AOAC method (AOAC 1990c). All residual nitrite assays were done in duplicate and all treatments within a block were analyzed at the same time to minimize variation in the analysis due to time.

Residual nitrate analysis
Sample preparation and nitrate determination methods were modifications of Ahn and Maurer (1987). Five grams of meat product samples were weighed in a 50-mL test tube and homogenized with 20 mL of distilled, deionized water (DDW) using a Polytron homogenizer (Type PT 10/35, Brinkmann Instruments Inc., Westbury, N.Y., U.S.A.) for 10 s at high speed. The homogenate was heated for 1 h at 80 °C water bath. After cooling in cold water for 10 min, 2.5 mL of the homogenate was transferred to a disposable test tube (16 x 100 mm). Carrer II (dissolve 10.6 g potassium ferrocyanide in 100-mL DDW) and Carrer I (dissolve 23.8 g zinc acetate in 50 mL DDW, then add 3 mL glacial acetic acid and dilute to 100 mL with DDW) reagents were added (0.1 mL each) to precipitate proteins. The solution was diluted with 2.3 mL of DDW and mixed well. After precipitation, the supernatant was centrifuged at 10,000 × g for 20 min and the clear upper layer was used for nitrate measurement by high performance liquid chromatography (Agilent 1100 Series HPLC system, Agilent Technologies, Wilmington, Del., U.S.A.). The column used was Agilent Zorbax SAX (analytical 4.6 × 150 mm, 5 µm) (Agilent, Wilmington, Del., U.S.A.) and the elution buffer was 15-mM phosphate buffer, pH 2.35, with isocratic elution. Flow rate was 1.0 mL/min and sample volume was 25 µL. The wavelength used was 210 nm. The area of nitrate peak was used to calculate nitrate concentration (ppm) using a nitrate standard curve.

Trained sensory panel
EFSC sausages were evaluated by a trained sensory panel for color, aroma, flavor, and texture characteristics. Ten trained panelists, made up of Iowa State Univ. students and staff, were used for each session. For training, three 1-h sessions were held using commercial and experimental products to develop descriptive terms for the desired attributes. One evaluation session containing 5 EFSC sausage treatments was held for each replication. During the evaluation session, each panelist evaluated the cured frankfurter aroma, internal cured frankfurter color, uniformity of internal frankfurter color, cured frankfurter flavor, and firmness for each treatment.

Attributes were measured using a line scale (numerical value of 15 units) with graduations from 0 to 15 where 0 represented none (aroma and flavor), not uniform (color), low (color), and soft (firmness) and 15 represented intense (aroma and flavor), high (color), uniform (color), and hard (firmness).

Expectorant cups were provided to prevent taste fatigue and distilled deionized water and unsalted soda crackers were provided to clean the palate between samples. The presentation order was randomized for each session. A computer ballot was constructed and data were collected using a computerized sensory scoring system (COMPUSENSE five, v.4.4, Compusense Inc. Guelph, Ontario, Canada, NIH3N4).

EFSC sausage samples were removed from vacuum packages and added to 2 quarts of boiling water in 3-quart saucepans. The covered pans containing EFSC sausage were immediately removed from the heat source and allowed to rest for 7 min. EFSC sausages were cut into 1.9-cm pieces with 1.27-cm pieces from each end being discarded. Panelists were presented 2, 1.9-cm randomly selected heated pieces per sample in a covered container and asked to determine intensity of aroma, internal cured color, cured flavor, uniformity of internal color, and texture of the EFSC sausage samples.

Results and Discussion

Product processing attributes
Various product and processing parameters were recorded during the manufacture of the EFSC sausages. The means for beef trim characteristics were as follows: 16.5% fat, pH of 5.53, and a temperature of −0.67 °C. The means for pork trim were as follows: 55.8% fat, pH of 6.24, and a temperature of 3.33 °C. The average pH of water used in the formulation was 8.88 with a temperature of 5.0 °C. Temperature and pH of TRTs and C batches were measured after stuffing and before incubation (preincubate). Preincubate pH ranged from 5.44 to 5.46 and no differences were found between any TRTs or C. The pH was also measured after the incubation step but prior to the cooking steps (postincubate). Postincubate pH ranged from 5.41 to 5.54 and no differences were found between the TRTs.

The time needed, at incubation temperatures, for the internal temperature of the EFSC sausage TRTs to reach optimum conditions...
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(37.8 °C) ranged between 21 and 35 min. The difference in time was
due to slight variation in stuffing temperatures between replications
(n = 3) as well as performance differences between the 2 thermal
processing ovens. Total average thermal processing times (including
come-up time to optimum incubation temperature) for TRTs 1 and
3 was 109 min and for TRTs 2 and 4 was 208 min. The C was added
to the thermal processing oven after incubation of the TRTs was
completed and had a total average thermal processing time of 83
min.

Color measurements

Both external and internal color measurements were measured
for EFSC sausage TRTs and C. Since EFSC sausages were cooked
in impermeable casings with no smoke treatment applied, external
color demonstrated color development on the outside surface of un-
cured, no-nitrate/nitrate EFSC sausages relative to the nitrite-added
control. As meat pigment (myoglobin) concentration is increased in
a formulation, the proportion of curred pigment formation near/on
the external surface may lessen in an uncured, no-nitrate/nitrite-
added system as thermal processing conditions and lower concen-
trations of nitrate–converted–to–nitrite may adversely affect the ratio
of cured to uncured pigment formation.

A significant (P < 0.05) interaction was observed between treat-
ment and day for objective external CIE a* values as reported in
Table 1. As incubation times increased at each VJP concentration,
redness values increased (P < 0.05) at days 0 and 14. Although not
significant, this trend was also observed at days 28, 56, and 90. All
TRTs were significantly different (P < 0.05) than C at day 0 except
for TRT 4. Fernández-Ginés and others (2003) reported a decrease in
TRTs and C are reported in Table 2. CIE L* values generally increased
over time with the largest significant (P < 0.05) increase in lightness
occurring between days 0 and 90.

A significant (P < 0.05) interaction was observed for treatment ×
day for external cured color fading as indicated by measurements of
reflectance ratio (Erdman and Watts 1957; Hunt and others 1991)
and are reported in Table 3. TRT 4 and C had significantly (P < 0.05)
higher reflectance ratios than TRTs 1, 2, and 3 at day 0 and higher
(P < 0.05) reflectance ratios than TRTs 1 and 3 at day 14. However,
no differences (P > 0.05) were found between TRT 4 and C on any
day. Interestingly, as incubation times increased, reflectance ratio
values increased, regardless of VIP level. Exterior reflectance ratios
generally increased over time (day) with the exception of the control,
which was unexpected. This may be explained by residual nitrate
present in TRTs acting as a reservoir for nitrite–related reactions
during storage. The phenomenon is supported by Houser and others
(2005) who reported color fading and regeneration occurring in ham
over time.

Internal CIE a* values of EFSC sausages for which a significant
(P < 0.05) treatment × day interaction was observed are listed in
Table 4. TRTs 1 and 3 were significantly (P < 0.05) less red than TRTs
2, 4, and C at day 0. TRT 4 and C were also redder (P < 0.05) than
TRTs 1 and 3 at day 14. These results indicate the development of
a* redness was more dependent on the length of incubation rather
than the concentration of VIP when compared to a nitrite-added
control. Internal CIE L* values for the main effect of day (time) are
reported in Table 2. Combined means for the 90-d storage period
showed an increase (P < 0.05) in lightness between day 0 compared

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Table 1 — Least squares means for the interaction of treat-
m ent combination (TRTs 1 to 4, C) with storage time (days
0, 14, 28, 56, 90) for objective external surface color (a*)
of no-nitrite-added (TRTs 1 to 4) and nitrite-added control
(C) EFSC sausages

<table>
<thead>
<tr>
<th>TRT</th>
<th>Day 0</th>
<th>Day 14</th>
<th>Day 28</th>
<th>Day 56</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13.08</td>
<td>15.62</td>
<td>16.18</td>
<td>15.96</td>
<td>16.83</td>
</tr>
<tr>
<td>2</td>
<td>16.39</td>
<td>17.66</td>
<td>17.52</td>
<td>17.20</td>
<td>17.69</td>
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<td>3</td>
<td>13.86</td>
<td>16.26</td>
<td>16.50</td>
<td>16.56</td>
<td>17.40</td>
</tr>
<tr>
<td>4</td>
<td>18.09</td>
<td>18.22</td>
<td>17.68</td>
<td>17.44</td>
<td>18.30</td>
</tr>
<tr>
<td>C</td>
<td>18.64</td>
<td>18.43</td>
<td>18.11</td>
<td>18.07</td>
<td>18.60</td>
</tr>
</tbody>
</table>

SEM = 0.28

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Table 2 — Least squares means* for the main effects of
storage time (days 0, 14, 28, 56, 90) for objective internal
color (L*), objective external color (L*, a*, b*) and total pig-
m ent* of no-nitrite-added (TRTs 1 to 4) and nitrite-added
control (C) EFSC sausages

<table>
<thead>
<tr>
<th>Day</th>
<th>L* (internal)</th>
<th>L* (external)</th>
<th>a*</th>
<th>b* (external)</th>
<th>Total pigment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>63.38</td>
<td>65.75</td>
<td>1.02</td>
<td>16.36</td>
<td>151.9</td>
</tr>
<tr>
<td>14</td>
<td>65.29</td>
<td>67.99</td>
<td>0.12</td>
<td>15.98</td>
<td>139.8</td>
</tr>
<tr>
<td>28</td>
<td>65.73</td>
<td>70.76</td>
<td>0.12</td>
<td>16.18</td>
<td>139.8</td>
</tr>
<tr>
<td>56</td>
<td>67.99</td>
<td>70.76</td>
<td>0.12</td>
<td>16.18</td>
<td>139.8</td>
</tr>
<tr>
<td>90</td>
<td>67.99</td>
<td>70.76</td>
<td>0.12</td>
<td>16.18</td>
<td>139.8</td>
</tr>
</tbody>
</table>

*Combined means of treatment combinations (TRTs 1 to 4 and C).

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Table 3 — Least squares means for the interaction of treat-
m ent combination (TRTs 1 to 4, C) with storage time (days
0, 14, 28, 56, 90) for external surface reflectance ratio
(Ratio)* of no-nitrite-added (TRTs 1 to 4) and nitrite-added
control (C) EFSC sausages

<table>
<thead>
<tr>
<th>Day</th>
<th>TRT 1</th>
<th>TRT 2</th>
<th>TRT 3</th>
<th>TRT 4</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.61</td>
<td>2.09</td>
<td>2.11</td>
<td>2.07</td>
<td>2.06</td>
</tr>
<tr>
<td>14</td>
<td>1.99</td>
<td>2.09</td>
<td>2.11</td>
<td>2.07</td>
<td>2.06</td>
</tr>
<tr>
<td>28</td>
<td>1.70</td>
<td>1.94</td>
<td>1.99</td>
<td>2.02</td>
<td>2.02</td>
</tr>
<tr>
<td>56</td>
<td>2.21</td>
<td>2.17</td>
<td>2.11</td>
<td>2.12</td>
<td>2.16</td>
</tr>
<tr>
<td>90</td>
<td>2.25</td>
<td>2.20</td>
<td>2.19</td>
<td>2.18</td>
<td>2.18</td>
</tr>
</tbody>
</table>

SEM = 0.03

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*Cured meat color measurement by reflectance ratio of wavelengths 650/570
nm where no cured color = 1.1, moderate fade = 1.6, less intense but
noticeable cured color = 1.7 to 2.0, and excellent cured color = 2.2 to 2.6.

**Treatment combinations: TRT 1 = low VJP + short MIN-HOLD; TRT 2 = low
VJP + long MIN-HOLD; TRT 3 = high VJP + short MIN-HOLD; TRT 4 = high
VJP + long MIN-HOLD; C = 156-ppm (mg/kg) sodium nitrite.

***Day 0, 14, 28, 56, and 90-d vacuum-packaged samples held at 0 to 2 °C.

**SEM = standard error of the means for no-nitrite-added and nitrite-
added EFSC sausages.

*Means within same row with different superscripts are different (P < 0.05).

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to days 14, 28, 56, and 90. The main effects of treatment and day were significant ($P < 0.05$) for internal $b$ values and are reported in Table 5.

Table 4 reports a significant ($P < 0.05$) interaction of treatment and day for internal cured meat color measurements determined by reflectance ratio. Trends similar to external reflectance ratio results were observed. At day 0, a significantly ($P < 0.05$) greater reflectance ratio was observed for TRT 2 compared to TRT 1 and also for TRT 4 compared to TRT 3. Furthermore, no differences at day 0 were found between TRTs 2, 4, and C, indicating that those treatment combinations possessed "excellent cured color" ratings (Hunt and others 1991).

It is worthy to mention that all treatment combinations over all days received at least a “less intense but noticeable cured color” rating and all measurements over time for TRTs 2, 4, and C received an “excellent cured color” rating according to cured color intensity ratings outlined by Hunt and others (1991). This would indicate that length of incubation has more effect on cured meat measurements of reflectance ratio than VIP level.

### Proximate composition

Proximate composition for EFSC sausages for moisture ranged from 61.36% to 61.98% (standard error of the means = 0.11), and C was significantly ($P < 0.05$) higher in moisture than TRTs 2 and 3 (data not shown). Fat ranged from 21.09% to 21.65% (standard error of the means = 0.16) and protein ranged from 13.24% to 13.54% (standard error of the means = 0.23). These results show that treatment combinations were uniform in proximate composition.

### pH determination and TBARS analysis

No significant differences were observed for the treatment × day interaction for pH; however, the main effects of treatment and day were both significant ($P < 0.05$) (data not shown). Combined least squares means of treatment combination (TRTs 1 to 4, C) for pH over time (days 0, 14, 28, 56, and 90) ranged from 5.88 to 6.01. Day 0 showed a significantly ($P < 0.05$) lower pH value than days 14, 22, 56, and 90. Combined least squares means of days (0, 14, 28, 56, and 90) for the pH of treatment combinations (TRTs 1 to 4, C) ranged from 5.94 and 6.04. TRT 1 revealed a significantly ($P < 0.05$) higher pH value than TRTs 2, 3, 4, and C. Actual changes in pH were small so the overall importance relative to this work should be minimal.

No significant differences were observed for any interaction or main effects for lipid oxidation measured by TBARS. Least squares means of treatment × day interaction for TBARS values ranged between 0.208 and 0.285, which are well below detectable levels for lipid oxidation. A TBARS value of 0.5 to 1.0 is considered to be the threshold for oxidized odor and 1.0 to 2.0 for oxidized flavor (Taladgis and others 1960). TBARS values were low for the control, as expected, because sodium nitrite was added. Nitrite has been shown to be an effective antioxidant (Shahidi and others 1991). Higher values and greater differences for TBARS values, especially over time, were expected for TRTs 1, 2, 3, and 4 because sodium nitrate was not added. These expectations, however, were not realized, suggesting that TRTs containing VIP were comparable to the sodium nitrite control for lipid oxidation over time.

Table 6 reports a significant ($P < 0.05$) interaction of treatment and day for internal cured meat color measurements determined by reflectance ratio. Trends similar to external reflectance ratio results were observed. At day 0, a significantly ($P < 0.05$) greater reflectance ratio was observed for TRT 2 compared to TRT 1 and also for TRT 4 compared to TRT 3. Furthermore, no differences at day 0 were found between TRTs 2, 4, and C, indicating that those treatment combinations possessed “excellent cured color” ratings (Hunt and others 1991).

It is worthy to mention that all treatment combinations over all days received at least a “less intense but noticeable cured color” rating and all measurements over time for TRTs 2, 4, and C received an “excellent cured color” rating according to cured color intensity ratings outlined by Hunt and others (1991). This would indicate that length of incubation has more effect on cured meat measurements of reflectance ratio than VIP level.

### Proximate composition

Proximate composition for EFSC sausages for moisture ranged from 61.36% to 61.98% (standard error of the means = 0.11), and C was significantly ($P < 0.05$) higher in moisture than TRTs 2 and 3 (data not shown). Fat ranged from 21.09% to 21.65% (standard error of the means = 0.16) and protein ranged from 13.24% to 13.54% (standard error of the means = 0.23). These results show that treatment combinations were uniform in proximate composition.

### pH determination and TBARS analysis

No significant differences were observed for the treatment × day interaction for pH; however, the main effects of treatment and day were both significant ($P < 0.05$) (data not shown). Combined least squares means of treatment combination (TRTs 1 to 4, C) for pH over time (days 0, 14, 28, 56, and 90) ranged from 5.88 to 6.01. Day 0 showed a significantly ($P < 0.05$) lower pH value than days 14, 22, 56, and 90. Combined least squares means of days (0, 14, 28, 56, and 90) for the pH of treatment combinations (TRTs 1 to 4, C) ranged from 5.94 and 6.04. TRT 1 revealed a significantly ($P < 0.05$) higher pH value than TRTs 2, 3, 4, and C. Actual changes in pH were small so the overall importance relative to this work should be minimal.

No significant differences were observed for any interaction or main effects for lipid oxidation measured by TBARS. Least squares means of treatment × day interaction for TBARS values ranged between 0.208 and 0.285, which are well below detectable levels for lipid oxidation. A TBARS value of 0.5 to 1.0 is considered to be the threshold for oxidized odor and 1.0 to 2.0 for oxidized flavor (Taladgis and others 1960). TBARS values were low for the control, as expected, because sodium nitrite was added. Nitrite has been shown to be an effective antioxidant (Shahidi and others 1991). Higher values and greater differences for TBARS values, especially over time, were expected for TRTs 1, 2, 3, and 4 because sodium nitrate was not added. These expectations, however, were not realized, suggesting that TRTs containing VIP were comparable to the sodium nitrite control for lipid oxidation over time.
Quality attributes of uncured sausage . . .

Total and cured pigment analysis
Least squares means for combined treatment combinations of total pigments are reported in Table 2. Analysis for total pigments revealed that concentrations decreased over time. Because the measurements were conducted on different days (0, 14, 28, 56, and 90) error may have been introduced into the testing procedures because total pigment concentration would not be expected to change with time (day).

A significant (P < 0.05) difference for the interaction of treatment × day was observed for cured pigment concentration (Table 7). TRTs 2, 4, and C had significantly (P < 0.05) greater cured pigment concentrations at days 0 and 14 than TRTs 1 and 3 and slightly though not significantly (P > 0.05) greater at days 56 and 90. However, no differences between TRTs 2, 4, and C were found at any day. Trends indicated that as incubation times increased, cured pigment concentrations also increased regardless of VIP level. Thus, the level of formulated VIP does not appear to be as important as the amount of time allowed for the VIP nitrate-to-nitrite conversion to result in cured pigment development. Trends also showed that cured pigment development generally increased over time for TRTs 1, 2, 3, and 4. This may be explained by residual nitrate present in VIP formulated TRTs with residual nitrates serving as a reservoir for nitrite-related reactions during storage.

Residual nitrite analysis
Residual nitrite in EFSC sausages was determined before incubation (preincubate) and after incubation (postincubate) (Table 8), and after thermal processing (day 0) continuing throughout a 90-d storage period (Table 9). The C compared to all TRTs (1 to 4) was significantly (P < 0.001) higher in residual nitrite at preincubate and no residual nitrite was detected in any TKIs (1 to 4) (Table 8). At postincubate, all TRTs contained residual nitrite and all TRTs had different (P < 0.05) amounts except for TRTs 1 and 3. From this table, the importance of incubation time for the conversion of nitrate to nitrite is clear. As incubation time was increased, residual nitrite levels also increased. It should be noted that residual nitrite was not measured for the control at postincubate since no incubation step was applied to the control.

Significant (P < 0.05) interactions of treatment × day (time) for treatment combinations were present for residual nitrite and are found in Table 9. As expected, residual nitrite levels diminished over time for all treatment combinations. This observation has been well documented by Jantawat and others (1993), who found a decreasing residual nitrite level with increased storage time, relationship, and by Hustad and others (1973), who reported that nitrite concentration was affected by both storage time and storage temperature. Since storage temperature was held constant in this study, storage time would be believed to be the principal factor in nitrite concentration decreases. Another explanation was suggested by Ahn and others (2002), who noted packaging effects in sausage samples stored in vacuum packages compared to aerobic packages. These authors reported that vacuum-packaged sausages had lower residual nitrite than samples stored in aerobic conditions. The authors suggested that this phenomenon was caused by the product environment being in the reduced state thus allowing the conversion of nitrite to nitric oxide and resulting in the lower residual nitrite levels found.

Within each pair of treatment combinations (TRTs 1 and 2; TRTs 3 and 4) where VIP level was held constant, residual nitrite levels were significantly (P < 0.05) higher when the incubation time increased (Table 9). This pattern occurred at all days over the 90-d storage time except for the VIP level tool TRTs 1 and 2 at day 90. Also, TRT 4 resulted in higher (P < 0.05) residual nitrite values than the C on all days. This indicates that either the VIP concentration of 0.40%
Quality attributes of uncured sausage...

resulted in a greater amount of nitrite converted from nitrate during 120 min of incubation compared to the nitrate-added control or a higher proportion of the nitrite in the control was reacted through curing reactions, resulting in the low C nitrite values observed.

Residual nitrate analysis

Residual nitrate in EFSC sausages was also determined before incubation (preincubate) and after incubation (postincubate) (Table 8), and after thermal processing (day 0) continuing throughout a 90-d storage period (Table 10). TRTs 1 and 2 and TRTs 3 and 4 were formulated with VIP levels of 0.20% and 0.40%, respectively. Preincubate values for TRTs 1 and 2 or TRTs 3 and 4 for nitrate would then be expected to be very similar. Both TRTs 1 and 2 were significantly ($P < 0.05$) lower in residual nitrate than TRTs 4, while the control was different ($P < 0.05$) than all TRTs (Table 8). Overall, residual nitrate differences were not surprising. Also, residual nitrate was detected in the control, to which only nitrite was added. Cassens and others (1979) suggest that a portion of nitrite added to meat during the curing process is actually converted to nitrate.

Postincubate values for all TRTs for residual nitrate were significantly ($P < 0.05$) different from one another (Table 8). Again, residual nitrate was not measured in C because it was not incubated. Postincubate values for all TRTs were lower than preincubate values indicating that nitrate-to-nitrite conversion occurred (Table 8). As incubation time was increased (30 to 120 min), postincubate residual nitrate levels were significantly ($P < 0.05$) lower. These values along with the presence of residual nitrite in TRTs at postincubation signify that conversion of nitrate to nitrite occurred. As residual nitrate for each TRT decreased from preincubate to postincubate, residual nitrate increased. These results show that nitrate available in VIP was reduced to nitrite by the starter culture containing $S$. carnosus. Although this action can be accomplished by natural microorganisms found in the natural flora of the meat (MacDougall and others 1975; Sebranek 1979; Sanz and others 1997; Pinotti and others 2001), it can also be more effectively accomplished by intentional addition of microorganisms with nitrate-reducing properties (Sanz and others 1997). This additional reduction step in the curing reaction is critical and necessary for a curing system with nitrate-containing VIP.

A significant ($P < 0.05$) difference for the interaction of treatment $\times$ day was also observed for residual nitrate and the corresponding values are found in Table 10. As incubation time increased, and VIP level remained constant, significantly ($P < 0.05$) less residual nitrate was present at all days for the corresponding TRTs (TRT 1 compared to TRT 2; TRT 2 compared to TRT 4). As could be expected, TRT 3 revealed the highest residual nitrate value because TRT 3 comprised a high VIP level and a low incubation time, resulting in less nitrate-to-nitrite conversion. As with preincubate measurements, the control showed residual nitrate values at each measured day over the 90-d storage period.

Several researchers have reported the presence of nitrate in products to which only nitrite was added. Pérez-Rodríguez and others (1996), monitoring nitrite and nitrate in frankfurters, reported that in nitrate-cured, cooked, and packaged frankfurters, approximately 50% of ingoing nitrite was present while about 10% to 15% of the added nitrite was found as nitrate. Interestingly, one theory is that a secondary oxidation involving nitrous acid could be involved in the conversion of nitrite to nitrate. This theory is also supported by Herrig (cited in Dethmers and Rock 1975), who proposed nitrate formation from the simultaneous oxidation and reduction of nitric acid to yield nitrite oxide and nitrate, and from the oxidation of nitric acid by oxygen to yield nitrite, which could subsequently react with water to yield nitrite and nitrate. Interestingly, little change in residual nitrate over time for the TRTs or C was observed and may be explained by the previous comments. This is further supported by noting that residual nitrite levels generally decreased over time while residual nitrate levels generally remained constant. The report of nitrite-to-nitrate reactions by Hustad and others (1973) may explain the constant nitrate levels found in this study. Instead of both residual nitrite and nitrate levels diminishing over time, a portion of nitrate may have been continually converted to nitrate, helping to maintain the levels of nitrate observed.

The residual nitrate of VIP was measured and found to be 27462 ppm (mg/kg) ($n = 3$) or 2.75% of the VIP (w/w). Therefore, formulation nitrate (when added to bowl cutter) was approximately 69 ppm for TRTs 1 and 2 and 139 ppm for TRTs 3 and 4. Hustad and others (1973) discussed loss of nitrite during processing and reported an average nitrite reduction of 16% after the meat was added to the bowl cutter. Similar trends were found in our results for both residual nitrate (Table 8 and 10) and nitrite (Table 8 and 9), indicating that both nitrate and nitrite were either physically lost during the manufacturing process or unavailable (in reactive form) for measurement due to involvement in curing reactions.

<table>
<thead>
<tr>
<th>Table 10</th>
<th>Least squares means for the interaction of treatment combination (TRTs 1 to 4, C) with storage time (days 0, 14, 28, 56, 90) for residual nitrate (ppm)$^a$ of no-nitrite-added (TRTs 1 to 4) and nitrite-added control (C) EFSC sausages</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRT$^b$</td>
<td>Day$^c$ 0</td>
</tr>
<tr>
<td>1</td>
<td>$25.8^a$</td>
</tr>
<tr>
<td>2</td>
<td>$9.3^b$</td>
</tr>
<tr>
<td>3</td>
<td>$73.6^a$</td>
</tr>
<tr>
<td>4</td>
<td>$12.2^a$</td>
</tr>
<tr>
<td>C</td>
<td>$33.0^a$</td>
</tr>
<tr>
<td>SEM$^d$</td>
<td>2.67</td>
</tr>
</tbody>
</table>

$^a$Residual nitrate determination reported in ppm of sample.

$^b$Treatment combinations: TRT 1 = low VIP + short MIN-HOLD; TRT 2 = low VIP + long MIN-HOLD; TRT 3 = high VIP + short MIN-HOLD; TRT 4 = high VIP + long MIN-HOLD; C = 156-ppm (mg/kg) sodium nitrite.

$^c$Day 0 = 0, 14, 28, 56, and 90 d vacuum packaging samples held at 0 to 2°C.

$^d$SEM = standard error of the means for no-nitrite-added and nitrite-added EFSC sausages.

$^e$Means within same row with different superscripts are different ($P < 0.05$).

$^f$Means within same column with different superscripts are different ($P < 0.05$).

Table 11 | Least squares means for sensory attributes of cured frankfurter aroma (cured aroma), internal cured frankfurter color (cured color), uniformity of internal frankfurter color (uniform color), cured frankfurter color (cured color), cured frankfurter flavor (cured flavor) and firmness for no-nitrite-added (TRTs 1 to 4) and nitrite-added control (C) EFSC sausages

<table>
<thead>
<tr>
<th>Sensory attributes$^a$</th>
<th>TRT$^b$</th>
<th>Day$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Cured aroma</td>
<td>8.41</td>
<td>7.76$^e$</td>
</tr>
<tr>
<td>Cured color</td>
<td>8.77</td>
<td>9.33$^m$</td>
</tr>
<tr>
<td>Uniform color</td>
<td>8.12</td>
<td>8.42$^m$</td>
</tr>
<tr>
<td>Cured flavor</td>
<td>8.45</td>
<td>9.16$^l$</td>
</tr>
<tr>
<td>Firmness</td>
<td>9.33</td>
<td>10.47$^l$</td>
</tr>
<tr>
<td></td>
<td>0.40</td>
<td>0.43</td>
</tr>
</tbody>
</table>

$^a$Treatment combinations: TRT 1 = low VIP + short MIN-HOLD; TRT 2 = low VIP + long MIN-HOLD; TRT 3 = high VIP + short MIN-HOLD; TRT 4 = high VIP + long MIN-HOLD; C = 156-ppm (mg/kg) sodium nitrite.

$^b$Sensory attributes = trained panel scores using a line scale (numerical value of 15 units) with graduations from 0 to 15 where 0 represented none (aroma, color), low (cured color) not uniform (uniformity of color), and soft (firmness) and 15 represented intense (aroma, flavor), high (cured color), uniform (uniformity of color), and hard (firmness).

$^c$SEM = standard error of the means for sensory attributes for nitrite-added and nitrite-added EFSC sausages.

$^d$Means within same column with different superscripts are different ($P < 0.05$).

$^e$Means within same column with different superscripts are different ($P < 0.05$).
Besides storage time and temperature, pH has also been suggested to affect residual nitrite and nitrate levels. Sebranek (1979) noted the importance of pH on residual nitrite and indicated that a pH decrease as little as 0.2 pH units during manufacture can result in a doubling in the rate of color formation due to more favorable nitrite–myoglobin interactions. This effect of pH is supported by Prusa and Kregel (1985) and Kilic and others (2001). However, no differences in pH between any treatment combination were found in the present study, which indicated that pH did not play a role in differences of residual nitrite and nitrate found or in any observed differences in cured characteristics.

Trained sensory panel

Trained sensory analysis was performed on day 14 following product manufacture in order to mimic the approximate time period of initial product availability in a commercial distribution chain. Significant differences (P < 0.05) for the main effects of treatment combinations for sensory attributes are reported as least squares means in Table 1. The control received the highest scores for all sensory attributes. No off-flavor/aroma or vegetable aroma/flavor attributes were included in the sensory evaluation because sensory training did not indicate any objectionable flavors or aromas present in the products used to develop the descriptive terms for the sensory ballot. This may have been due to the frankfurter spices used, which could provide a predominant aroma/flavor and result in flavor masking in the EPSC sausages. No differences for cured aroma were found between any treatment combinations but trends indicated that increasing incubation time improved cured aroma sensory scores.

The control had a significantly (P < 0.05) higher score for cured color than TRTs 1, 3, and 4. TRT 1 was significantly (P < 0.05) lower for cured color than TRTs 2, 4, and C, indicating that visual cured color was affected by incubation time and VIP level. For uniformity of color, TRT 1 was different (P < 0.05) than TRTs 2, 4, and C, while no differences (P > 0.05) were observed between TRT 1 and TRT 3 or between TRTs 2, 3, 4, and C. No differences (P > 0.05) for cured flavor were found between any TRTs; however, C had a higher (P < 0.05) score for cured flavor than TRTs 1, 2, and 3. Additionally, C was firmer (P < 0.05) than all TRTs. It is unclear why these differences were found unless sensory perception of firmness was affected by the other sensory attributes. However, nitrite-curing reactions have been suggested to increase firmness (Pegg and Shahidi 2000).

Conclusions

Treatment combinations containing VIP and starter culture containing S. carnosus were shown to be comparable to a sodium nitrite-added control for color, lipid oxidation, cured pigment, and trained sensory measurements. No differences in TBARS values between any treatment × day combination were observed and all values were below the detectable threshold of lipid oxidation, indicating that all treatment combinations were effective in controlling lipid oxidation. Measurements of color, cured pigment, residual nitrate, and residual nitrite for the TRTs validated that curing reactions occurred. Incubation time was found to be a more critical factor than VIP concentration to produce cured meat properties similar to the control for objectively measured attributes. This was also the case for sensory results; however, sensory differences were not as definitive as the objective measurements, suggesting that differences may not be as easily detected by consumers. It is worthwhile to note that the sodium nitrite-added control had the highest sensory scores for all attributes measured, though differences were not significant in all cases.

The ingoing nitrate levels of 69 ppm (TRTs 1 and 2) and 139 ppm (TRTs 3 and 4) as used in this study would result in less nitrite than the USDA FSIS maximum allowable limit of 156 ppm nitrite, even if the nitrate was 100% converted. Since USDA FSIS requires a minimum of 120 ppm nitrite in cured meat products labeled “Keep Refrigerated” (USDA 1995), the low ingoing levels found in this study could result in no microorganism-related concerns, specifically relative to C. botulinum survival and outgrowth.

The VIP used in this study was an effective replacement for sodium nitrite at the tested levels for the manufacture of uncured, no-nitrate/nitrite-added EFSC sausages. At the VIP concentrations (0.20% and 0.40%) tested, a longer incubation time (120 min) was found to be more critical than the VIP concentration for results comparable to a sodium nitrite-added control.

Further research regarding additional increased or decreased incubation times and VIP levels is needed to generate a better understanding of the relationship and effects, especially at higher VIP concentrations, that may have on the quality characteristics of EFSC sausages. Further research regarding the effects of this technology on microbiological control and shelf life of these products at the concentrations tested in this study is also needed.

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References


