



April 4, 2019

Ms. Michelle Arsenault  
National Organic Standards Board  
USDA-AMS-NOP

**Docket:** AMS-NOP-18-0071

**RE: Celery Powder (Sunset 2021)**

Dear Ms. Arsenault:

Thank you for this opportunity to provide comment on the 2021 Sunset Review of celery powder listed on 205.606 of the National List (7 CFR § 205.606 - non-organically produced agricultural products allowed as ingredients in or on process products labeled as organic).

The Organic Trade Association (OTA) is the membership-based business association for organic agriculture and products in North America. OTA is the leading voice for the organic trade in the United States, representing over 9,500 organic businesses across 50 states. Our members include growers, shippers, processors, certifiers, farmers' associations, distributors, importers, exporters, consultants, retailers and others. OTA's mission is to promote and protect organic with a unifying voice that serves and engages its diverse members from farm to marketplace.

The Organic Trade Association supports the continued listing of celery powder on the National List due to the fact that it is an essential ingredient used in processed organic meat products, and an organic alternative is not commercially available. Celery powder has been in use for over a decade as a "curing" agent in certain processed meat products as an alternative to sodium and potassium nitrate and nitrite. Since 2007, conventionally grown celery powder has been allowed for use in certified organic meat products. During this time, the organic processed meat industry has grown to an estimated \$150 million. As the demand for organic processed meats increases, the organic industry wants to replace the use of conventional celery powder with an organic alternative.

The Organic Trade Association is committed to help the industry innovate and proactively take steps to make this happen. In response to the information being requested and to help inform the fall 2019 review and vote on whether to relist celery powder for an additional five years, we offer the following comments.

**NOSB questions:**

**1. *Is non-organic celery powder still essential for the production of ORGANIC processed meats?***

Yes. Celery powder continues to be the only natural source of nitrate allowed as a curing agent in processed certified organic meat. Organic forms of celery powder that meet the required functionality for processed organic meats are not commercially available, and we are not aware of other organic crops that can deliver the same attributes. Celery powder is being used by many organic meat and poultry processors producing organic meat products where the synthetic chemicals, nitrate and nitrite, are not permitted. If celery powder is removed from the National List, organic bacon and other cured organic meats will cease to exist. This would have a devastating impact on an already struggling organic livestock sector and its associated supply chain. Retaining celery

powder on the National List until an organic alternative is commercially available is important to organic livestock producers and it is important for consumers that choose to support organic practices.

Celery powder contains natural forms of nitrate that are converted to nitrite when added to meat, which, in turn, function as a curing agent for products such as organic ham, hot dogs and bacon. Additionally, “pre-converted” forms are used where an incubation with a nitrate reducing bacterium produces celery powders that are high in nitrite. The use of celery powder eliminates the need for conventional purified nitrate and nitrite curing ingredients. The essential function of nitrate/nitrite in processed meats is most importantly related to food safety with antimicrobial properties versus *Clostridium botulinum* and *Listeria monocytogenes* which are very important for protection of public health. Additionally shelf life is improved. Historically, manufacturers struggled to develop traditionally cured products such as ham, bacon and hot dogs that were accepted by consumers without nitrate from either natural or synthetic sources. These products failed the consumer testing, and consumers were not willing to pay more money for lower quality products. Celery powder was placed on the National List to fill a void while the organic sector ramped up organic meat production, and organic forms of celery powder were developed by manufacturers of natural celery powder.

The goal continues to be the commercial availability of organic celery powder. While the organic industry would like to see non-organic celery powder removed from the National List, an appropriate and effective alternative needs to be commercially developed first. The original petition for celery powder foresaw no difficulty in the future production of an organic version. To date, however, a viable, functional version has not been successfully developed. There are several technical and production issues that have proven to be barriers. For example, some of the alternative varieties that achieve the necessary nitrate levels impart too strong of a flavor in the meat products and would not be acceptable to consumers. Other factors include harvest and post-harvest conditions and the time and distance between harvest and processing, and how those variables impact nitrate level retention. The organic meat market also continues to be relatively small.

The greatest barrier perhaps is our ability to secure the additional funding we have been requesting to continue the research needed to address standardization of nitrogenous compounds in appropriate organic celery and/or other crop varieties and the time needed to complete extensive commercial testing on the potential alternatives that are being trialed. See Question #3 below.

- 2. *Compared with growing celery for vegetable production, is increased use of synthetic nitrogen fertilizers required to produce source plants with enough nitrate for celery powder production?***  
The Organic Trade Association does not have this data. Regardless of the answer, we believe that the organic sector should be working toward developing an organic alternative that is consistent with organic principles. Our focus is on finding a solution that works in an organic production system, rather than gathering information on current conventional practices. The research driven by the Organic Celery Powder Working Group is focusing on organic variety selection and understanding the post-harvest impacts. If additional N is needed to produce organic source plants with enough nitrate for meat curing it should be done in an environmentally friendly way that supports organic principles (and complies with organic regulations). Regardless, the fate of excess

N is the same whether it is organic or synthetic. We are no better off unless we find an alternative systems-based strategy rather than input substitution, and that has been our approach.

**3. *Since 2015, what progress has been made on the production of organic celery for powder production?***

In the fall of 2015, the Organic Trade Association in collaboration with The Organic Center (TOC) convened the “National List Innovation Working Group” consisting of members interested in investing in applied research to identify alternatives to materials currently on the National List including organic, natural, or more compatible synthetics. The Working Group topics and participants vary, based on the needs and projects identified by the organic sector. Participants are investors in the development of alternatives, or by invitation of investors working in collaboration with public and private research institutions and extension personnel.

The first project (initiated by the Celery Powder Working Subgroup) was to find an organic alternative to non-organic celery powder. To begin to address the issues, the Working Group focused the first six months on establishing research partners, identifying funding opportunities and working in collaboration with the University of Wisconsin on the submission of a proposal for an Organic Research and Extension Initiative (OREI) planning grant. The planning grant proposal, submitted in early March 2016 and awarded later that year, helped to develop the roadmap of integrated research and extension activities needed to adequately address and overcome production challenges. An additional proposal to Farmers Advocating for Organics (FAFO) was also awarded.

The money from the OREI planning grant was used to identify the needed partners, crops, data and research questions that, in turn, informed the full \$2 million OREI grant that was applied for on January 19, 2017, and again in 2018. It was also used to fund the national stakeholder meeting held at the EcoFarm conference in, Asilomar, CA, in 2017. The FAFO grant money funded initial varietal testing in organic celery crops and broader testing of production-scale organic celery that were harvested in fall 2016. Unfortunately, both OREI funding proposals were not accepted, slowing research progress down in 2018.

Despite the setback, the efforts continue, not only for celery powder but for solutions that could potentially benefit all of agriculture. The working group research project sets out to identify potential varieties of organic crops that would meet the chemical specification needed for curing, while being easily incorporated into current crop rotation systems. It will also identify potential management protocols that need to be developed to achieve target nitrate levels in the curing crop to produce the required shelf life, prevent bacteria in the cured meat, and produce the desired flavor, color and texture in food. The project also aims to identify crops which could act as an incentive for expanding organic acreage, given the economic opportunity to partner with contractors that produce curing agents for organic processed meat products. Additionally, the project is investigating potential challenges and pitfalls associated with the production of a high nitrate crop, such as environmental concerns for run-off and excess nutrient leaching.

Identifying solutions for the organic processed meat industry’s need for a curing powder is extremely complex, and the timeline to develop an effective organic alternative does not happen overnight. It requires a very deliberate and well-researched road forward, it takes a multiregional,

multi-stakeholder coordinated effort, it requires substantial funding and it relies on consumer demand. Although the lack of funding has put the project behind schedule, we believe significant progress is being made and that the commitment and organization of the Celery Powder Working Group and our research partners has presented a solid model on how to best carry out the process for developing alternatives to a National List material. **See Attachment A.**

The Organic Center in partnership with the University of Wisconsin plans to submit an OREI proposal one last time. We will also be looking to other funding avenues and calling on industry to further invest in the development of an organic alternative to natural celery powder.

**4. *Are there strategies to produce organic celery powder that is standardized to consistently meet safety and other requirements of the meat processing industry? If not, is enough organic celery being produced to support the meat industry, why not?***

Yes, innovative strategies are underway. However, more research is needed in order to adequately answer this question. To the best of our knowledge, the organic celery grown in the United States is not grown for use as a natural curing agent. It is grown for fresh vegetable consumption, as a nutritional juice or supplement, or for seasoning. The varieties used for culinary and nutritional consumption are the not the same as the ones used to produce nitrite. The question is whether there is enough organic celery of the correct variety being produced to support the meat industry. As far as we know, very little organic celery is being grown at commercial scale for the meat industry, but research efforts and trials are underway.

**5. *Are there commercially available agriculturally produced alternatives to celery powder? What is your experience with them? Are they organic? Does their use vary by application? Are they more effective in one application compared to another?***

There are other vegetables and minerals which contain natural nitrates including beets, Swiss chard, spinach and sea salt. Although each has its benefits and challenges, none are an identical equivalent to natural celery powder in quality, form and function. The most promising of the potential alternatives that we are aware of is Swiss chard. More research and testing are needed.

**6. *What is the latest information on the human health risks of nitrate and nitrites present in processed meats from either synthetic or plant-based sources?***

To the best of our knowledge, the source of the nitrate/nitrite (synthetic vs. plant-based) does not make a difference. Nitrate and nitrite are simple inorganic ions and the source has no impact on the chemical properties. See the attached research article by King et. al. with respect to on set of food safety parameters (**Attachment B**). We have also attached several other papers representing the latest information about nitrate/nitrite and human physiology (**See the list of references below**). Based on our review, we are unaware of any new information since the last Sunset Review. We would like to defer to the expert panel to answer this question completely.

On behalf of our members across the supply chain and the country, the Organic Trade Association thanks the National Organic Standards Board for the opportunity to comment, and for your commitment to furthering organic agriculture.

Respectfully submitted,



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Vice President, Regulatory and Technical Affairs  
Organic Trade Association

cc: Laura Batcha  
Executive Director/CEO  
Organic Trade Association

**Attachment A:** Developing Natural and Organic Alternatives

**Attachment B:** King, A. M., K. A. Glass, A. L. Milkowski and J. J. Sindelar (2015). "Comparison of the Effect of Curing Ingredients Derived from Purified and Natural Sources on Inhibition of *Clostridium perfringens* Outgrowth during Cooling of Deli-Style Turkey Breast." Journal of Food Protection **78**(8): 1527-1535.

**Additional Helpful References**

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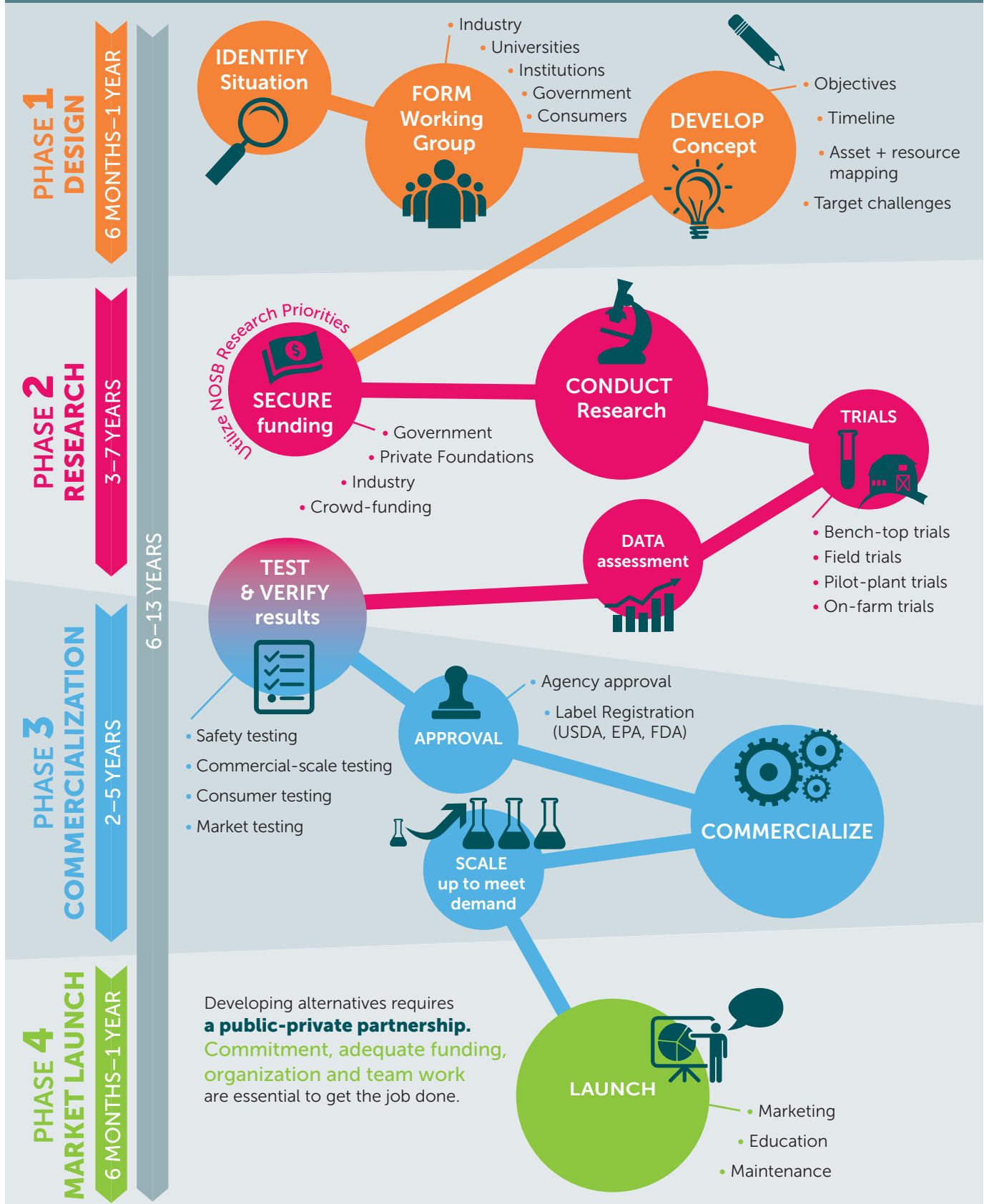
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# A model for developing ORGANIC AND NATURAL INPUTS for use in organic food and farming



## Attachment B

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## Comparison of the Effect of Curing Ingredients Derived from Purified and Natural Sources on Inhibition of *Clostridium perfringens* Outgrowth during Cooling of Deli-Style Turkey Breast

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### ABSTRACT

The antimicrobial impact of purified and natural sources of both nitrite and ascorbate were evaluated against *Clostridium perfringens* during the postthermal processing cooling period of deli-style turkey breast. The objective of phase I was to assess comparable concentrations of nitrite (0 or 100 ppm) and ascorbate (0 or 547 ppm) from both purified and natural sources. Phase II was conducted to investigate concentrations of nitrite (50, 75, or 100 ppm) from cultured celery juice powder and ascorbate (0, 250, or 500 ppm) from cherry powder to simulate alternative curing formulations. Ground turkey breast (75% moisture, 1.2% salt, pH 6.2) treatments were inoculated with *C. perfringens* spores (three-strain mixture) to yield 2.5 log CFU/g. Individual 50-g portions were vacuum packaged, cooked to 71.1°C, and chilled from 54.4 to 26.7°C in 5 h and from 26.7 to 7.2°C in 10 additional hours. Triplicate samples were assayed for growth of *C. perfringens* at predetermined intervals by plating on tryptose-sulfite-cycloserine agar; experiments were replicated three times. In phase I, uncured, purified nitrite, and natural nitrite treatments without ascorbate had 5.3-, 4.2-, and 4.4-log increases in *C. perfringens*, respectively, at 15 h, but <1-log increase was observed at the end of chilling in treatments containing 100 ppm of nitrite and 547 ppm of ascorbate from either source. In phase II, 0, 50, 75, and 100 ppm of nitrite and 50 ppm of nitrite plus 250 ppm of ascorbate supported 4.5-, 3.9-, 3.5-, 2.2-, and 1.5-log increases in *C. perfringens*, respectively. In contrast, <1-log increase was observed after 15 h in the remaining phase II treatments supplemented with 50 ppm of nitrite and 500 ppm of ascorbate or ≥75 ppm of nitrite and ≥250 ppm of ascorbate. These results confirm that equivalent concentrations of nitrite, regardless of the source, provide similar inhibition of *C. perfringens* during chilling and that ascorbate enhances the antimicrobial effect of nitrite on *C. perfringens* at concentrations commonly used in alternative cured meats.

*Clostridium perfringens* is a gram-positive, nonmotile, anaerobic bacillus with square ends that forms heat stable spores (14). This organism is one of the most widely distributed bacteria and has been isolated from soil, water, intestines, food, and air. To develop illness, a person must consume 10<sup>8</sup> to 10<sup>9</sup> vegetative cells. Upon exposure to the gastrointestinal environment, cells sporulate and release an enterotoxin, causing symptoms that include abdominal pain, nausea, and diarrhea but that generally subside after 1 to 2 days (8, 14). Although foodborne illness caused by *C. perfringens* results in very few hospitalizations or deaths, it causes an estimated nearly 1 million cases of illness in the United States each year (27).

As a ubiquitous organism, *C. perfringens* is often a component of the normal intestinal microflora of healthy animals and humans, which can lead to contamination of meat products due to fecal cross-contamination during processing. In one U.S. study (37), 1.6% of raw whole muscle and 48.7% of raw ground or emulsified meats were

positive for the presence of *C. perfringens*, and 5.3% of the ground or emulsified samples were positive for *C. perfringens* spores. Cooked meat provides a very suitable growth environment in which *C. perfringens* can have a remarkably short generation time of less than 10 min at 43 to 47°C; in general, growth can occur at 12 to 50°C (29).

To mitigate the risk of *C. perfringens* germination and outgrowth during cooking and chilling in meat products, the U.S. Department of Agriculture (USDA) Food Safety Inspection Service (FSIS) has established controlled chilling procedures (43), and performance standards have been published in Appendix B of the FSIS *Performance Standards for the Production of Certain Meat and Poultry Products* (42). Appendix B states that maximum internal temperatures of uncured products shall not remain between 54.4°C (130°F) and 26.7°C (80°F) for more than 1.5 h nor between 26.7°C (80°F) and 4.4°C (40°F) for more than 5 h. Cured products, defined as those with a minimum 100 ppm of ingoing nitrite, are allowed a longer chilling period. According to Appendix B, maximum internal temperatures of these products must not remain between 54.4°C (130°F) and 26.7°C (80°F) for greater than 5 h nor between 26.7°C (80°F) and 7.2°C (45°F) for an

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additional 10 h. These chilling requirements were designed to limit outgrowth of *C. perfringens* to a maximum 1-log increase during postthermal processing cooling.

Traditionally, cured meats have been prepared using sodium nitrite, which has been well documented to affect *C. perfringens* outgrowth (22, 25, 26). In one study (26), pork was cured with various concentrations of nitrite, inoculated with 11 spores per gram *C. perfringens*, and held at 1 to 4°C for 2 weeks, 13°C for 2 weeks, and 26°C for 6 weeks, and *C. perfringens* was enumerated after 4, 6, 8, and 10 weeks. Higher concentrations of nitrite decreased the percentage of inoculated spores recovered from the treatments. After 10 weeks, in treatments with 0, 50, 100, 150, and 200 ppm of sodium nitrite, 38.0, 12.0, 5.4, 3.6, and 0.9% of inoculated spores, respectively, were recovered. Thus, currently used concentrations (maximum allowed) of sodium nitrite in the United States, 156 ppm for comminuted products and 200 ppm for immersion cured, massaged, or pumped products regulated by the USDA, are well supported and representative of concentrations needed to provide effective control of *C. perfringens*, and published reports support recommendations for this generally used concentration near the maximum allowable ingoing concentrations (41).

Because of increased consumer demand for preservative-free “clean label” processed meat options, alternative cured meats are widely available and are produced without direct addition of purified (or synthetic) sodium nitrite, which is considered a preservative. Alternative cured meats can be made with natural sources of nitrite, such as that derived by using specific starter cultures, such as *Staphylococcus carnosus*, to reduce naturally occurring nitrate in celery powder (30, 32). However, these processing techniques result in lower concentrations of nitrite in these products than in traditionally cured meats (30). Because of nitrite’s contribution to food safety, these lower concentrations prompt the question of whether alternative cured products are equivalent to their traditionally cured counterparts from a microbiological safety perspective. In a study of commercially prepared alternative cured, natural, and organic samples, Jackson et al. (16) reported decreased *C. perfringens* inhibition in 7 of 10 frankfurter brands, six of seven ham brands, and four of nine bacon brands relative to traditionally cured controls. However, the nitrite concentrations measured at the time of testing were variable (e.g., <1 to >65 ppm of residual nitrite in two brands of commercial frankfurters). This variation could be explained by differences among manufacturers in the initial amount of nitrite added to the formulation or the age of the products at the time of testing, as differences in nitrite concentrations during the storage shelf life would be expected as the nitrite depletes over time. Similar results have been observed for *Listeria monocytogenes* growth and *Clostridium botulinum* growth and toxin production in alternative cured meats compared with controls traditionally cured with purified sodium nitrite (28, 44). In none of these studies was an attempt made to standardize the nitrite concentrations or to add cure accelerators to the formulations incorporating nitrite from natural sources. Currently, products cured with nitrite from natural sources frequently contain less than 100

TABLE 1. Base formulation for manufacture of deli-style turkey breast<sup>a</sup>

Ingredient	%	Amt (g)
Turkey breast	100.0	2,268
Water plus ice (50% + 50%)	20.0	454
Salt	1.4	31.8
Modified food starch	2.0	45.4
Sodium tripolyphosphate	0.4	9.1
Total		2,807.7

<sup>a</sup> Formulated ingredients reported as ingoing percentage based on poultry weight.

ppm of nitrite and do not qualify for the same extended cooling as do meats cured with the direct addition of sodium nitrite (1).

Although recent work suggests that both purified nitrite and nitrite from natural sources have similar antimicrobial activity against *L. monocytogenes* and *C. perfringens* when used at comparable concentrations, equivalency against *C. perfringens* has not been thoroughly evaluated during extended chilling following the guideline in Appendix B (10, 11). The overall objective of this study was to determine the antimicrobial impact of purified nitrite and nitrite from natural sources for the control of *C. perfringens* outgrowth during a 15-h biphasic chilling period in deli-style turkey breast. The objective of phase I was to determine whether purified nitrite and ascorbate and nitrite and ascorbate from natural sources, used at equal concentrations, provided similar inhibition of *C. perfringens*. The objective of phase II was to evaluate the antimicrobial impact of natural nitrite and ascorbate at lower concentrations representative of currently produced alternative cured meats.

## MATERIALS AND METHODS

**Spore preparation.** Three strains of *C. perfringens* (ATCC 13124, 12915, and 12916) were grown individually using a modification of procedures outlined by Kennedy et al. (18) to induce sporulation. To enumerate spores, an aliquot of each strain was heat shocked for 20 min at 75°C to kill vegetative cells, and appropriate serial dilutions were made in 0.1% peptone and plated on tryptose-sulfite-cycloserine agar (TSC; Oxoid Ltd., Basingstoke, UK) without egg yolk. Once agar was solidified, plates were overlaid with 8 to 10 ml of TSC and incubated anaerobically (AnaeroPack System 7.0-liter jar with Pack-Anaero Anaerobic Gas Generating System, Mitsubishi Gas Chemical Co., Tokyo, Japan) for 24 h at 35°C. Spore crops were stored in 0.85% saline at -20°C until spore cocktails were prepared for individual experiments. For each meat inoculation, a fresh inoculum was prepared by mixing equivalent levels of the three strains to provide approximately 2.5 log CFU/g of poultry.

**Meat preparation and inoculation.** Frozen turkey breasts obtained from a commercial supplier were thawed and stored at 2.2 to 4.4°C until used (within 4 days). Turkey was ground through a 4.76-mm-whole-size plate attached to a grinder (model 4732, Hobart Corp., Troy, OH). The base formulation for the model turkey breast is given in Table 1. For each treatment, nonmeat ingredients were dissolved in distilled water. To ensure complete solution in the brine, the ingredients were added in the following order: sodium tripolyphosphate, salt, modified food starch, nitrite, and ascorbate.

TABLE 2. Definitions of treatments used to evaluate the effect of equal concentrations of purified and natural sources of nitrite and ascorbate (cure accelerator) for inhibiting outgrowth of *C. perfringens* during a 15-h cooling period (phase I)

Treatment <sup>a</sup>	Ingredient <sup>b</sup>	
	100 ppm of nitrite	500 ppm of ascorbate
1: uncured	0	0
2: SA	0	0.0547% sodium ascorbate
3: SN	0.16% purified curing salt	0
4: PCN	0.44% cultured celery juice powder	0
5: SN + SA	0.16% purified curing salt	0.0547% sodium ascorbate
6: PCN + CP	0.44% cultured celery juice powder	0.32% cherry powder

<sup>a</sup> Uncured, no nitrite from any source; SN, purified sodium nitrite from curing salt containing 6.25% sodium nitrite; PCN, natural preconverted nitrite from cultured celery juice powder containing 2.25% sodium nitrite equivalent; SA, purified sodium ascorbate from pure chemical source; CP, natural ascorbate from cherry powder containing 12% ascorbate.

<sup>b</sup> Concentration (ppm) added based on concentration provided in ingredient specifications and percentage of formulation on poultry weight basis.

The fresh ground turkey and brine were mixed in a mixer (model AS 200, Hobart Corp.) for 3 min, and then approximately 800 g of batter was removed to be packaged as uninoculated samples to be used for analysis of residual nitrite and proximate composition and temperature monitoring throughout processing. The remaining batter was inoculated with the *C. perfringens* spore mixture to yield approximately 2.5 log CFU/g of poultry.

For each sample, 50-g portions of poultry batter were vacuum sealed in oxygen- and moisture-impermeable bags (3 mil high barrier; oxygen transmission rate of 50 to 70 cm<sup>3</sup>/m<sup>2</sup>, 24 h at 25°C and 60% relative humidity; water transmission rate of 6 to 7.5 g/m<sup>2</sup>, 24 h at 25°C and 90% relative humidity; UltraSource, Kansas City, MO) using a vacuum packaging machine (Multivac AGW, Sepp Hagemuller KG, Wolfertschewenden, Germany). To ensure consistent temperature profiles for all samples, the packages were flattened to approximately 3 mm thickness, similar to procedures reported by Kalinowski et al. (17). Samples were held overnight at 4°C until used for cooking and cooling.

**Ingredients and treatment combinations.** This study was conducted in two phases. Phases I and II comprised 6 and 10 treatments, respectively (Tables 2 and 3). Ingredient concentrations were determined from the manufacturer’s specifications provided for that ingredient and calculated based on a sodium nitrite or sodium ascorbate basis to achieve the target concentration in each treatment. In phase I, purified ingredients were sodium nitrite from curing salt (6.25% sodium nitrite, 93.75% sodium chloride; Sure Cure, Excalibur Seasoning Company, Pekin, IL) and sodium ascorbate (Excalibur Seasoning Company). In both phases, natural nitrite was in the form of cultured celery juice powder (2.25% sodium nitrite equivalent; Accel 2000, Kerry Ingredients and Flavours, Beloit, WI), and natural ascorbate was in the form of cherry powder (12% ascorbic acid; VegStable 515, Florida Food Products, Eustis, FL).

**Cooking, cooling, and sampling.** Before cooking, a thermocouple (digital thermometer and type K probe, Thermo Fisher Scientific, Waltham, MA) was inserted into each of three representative packages through a rubber septum to monitor the internal temperature during cooking and cooling. Temperature data loggers (iButton Temperature Logger DS1922T, Maxim Integrated, San Jose, CA) were placed in the incubator and in four uninoculated packages to continuously record ambient air and meat temperatures during cooking and cooling. Packages were attached to removable incubator racks with small binder clips and immersed

in a 75°C water bath until the internal temperature of representative packages reached 71°C, which heat shocked the spores and killed any vegetative cells. The time to target cook temperature (approximately 5 min) was manually recorded. Cooked samples were immediately placed into a programmable air incubator (model BOD50A16 incubator, Revco, Thermo Electron Corp., Asheville, NC; model UP550 program controller, Yokogawa Electric Corporation, Tokyo, Japan) and held at 60°C until all samples were loaded into the incubator (maximum of 20 min). This temperature is outside the growth range for the organism and was manually monitored until cooling began. The incubator cooling program was set to cool the product in a biphasic design that matched the maximum cooling time and temperatures for cured products outlined in FSIS Appendix B (42) (54.4 to 26.6°C over 5 h and 26.6 to 7.2°C over 10 h) (Figs. 1 and 2).

TABLE 3. Definitions of treatments used to evaluate the effect of combinations of natural sources of nitrite and ascorbate (cure accelerator) for inhibiting outgrowth of *C. perfringens* during a 15-h cooling period (phase II)

Treatment <sup>a</sup>	Ingredient <sup>b</sup>			
	ppm		%	
	Nitrite <sup>c</sup>	Ascorbate <sup>d</sup>	PCN	CP
1: 0/0	0	0	0	0
2: 50/0	50	0	0.22	0
3: 50/250	50	250	0.22	0.21
4: 50/500	50	500	0.22	0.41
5: 75/0	75	0	0.34	0
6: 75/250	75	250	0.34	0.21
7: 75/500	75	500	0.34	0.41
8: 100/0	100	0	0.45	0
9: 100/250	100	250	0.45	0.21
10: 100/500	100	500	0.45	0.41

<sup>a</sup> Treatments defined by concentrations of natural nitrite and ascorbate in formulation (ppm of nitrite/ppm of ascorbate).

<sup>b</sup> Concentration (ppm) added based on concentration provided in ingredient specifications and calculated on poultry weight basis.

<sup>c</sup> Naturally generated nitrite from cultured celery juice powder (PCN) containing 2.25% sodium nitrite equivalent.

<sup>d</sup> Natural ascorbate from cherry powder (CP) containing 12% ascorbate.

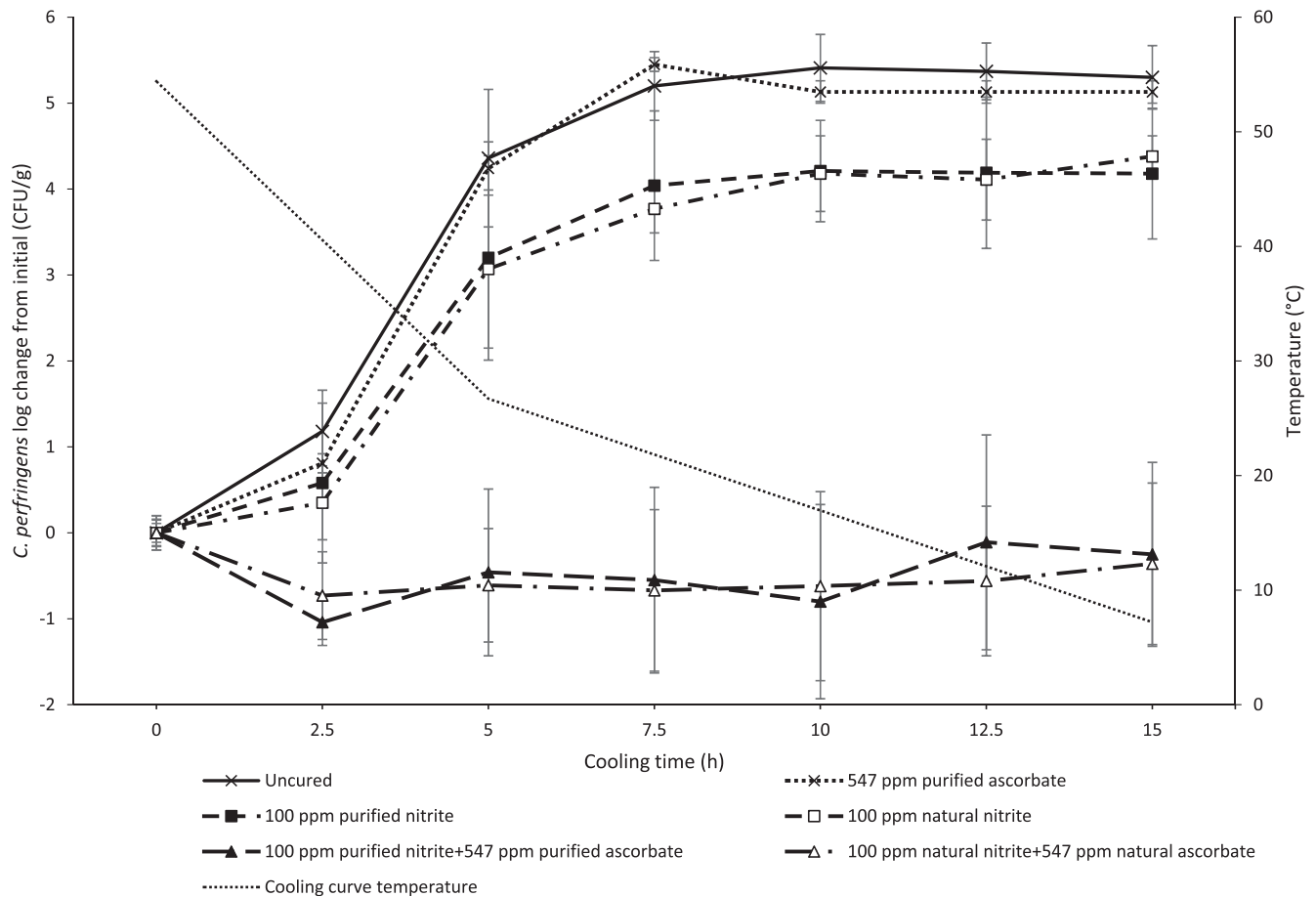


FIGURE 1. Change (sampling point minus initial) in counts of *C. perfringens* during 15 h of cooling of ground turkey breast formulated with purified nitrite or nitrite from cultured celery juice powder plus purified ascorbate or ascorbate from cherry powder (phase I). Data points represent the mean of three independent replications, and error bars represent the standard deviation.

Triplicate samples of each treatment were removed at 0, 2.5, 5, 7.5, 10, 12.5, and 15 h for phase I and 0, 5, 10, and 15 h for phase II. From each 50-g sample, a representative 25-g portion was removed and diluted with 50 ml of Butterfield's phosphate buffer and homogenized for 2 min in a lab blender (Stomacher 400, A. J. Seward, London, UK). Serial dilutions of homogenates were plated onto TSC plates with an 8- to 10-ml TSC agar overlay and incubated anaerobically at 35°C for 24 h. Each set of experiments was replicated three times with different batches of poultry and spore inocula.

**Chemical analysis.** Triplicate uninoculated samples for each treatment were analyzed for moisture (5 h, 100°C vacuum oven method), pH (measured in a slurry prepared by homogenizing 10 g with 90 ml deionized water), NaCl (measured as percentage of  $\text{Cl}^-$ ,  $\text{AgNO}_3$  potentiometric titration; DL22 food and beverage analyzer, Mettler, Columbus, OH), and water activity ( $a_w$ ; AquaLab 4TE water activity meter, Decagon, Pullman, WA) (2, 33). Residual nitrite was analyzed in duplicate uninoculated meat samples from each treatment, which were frozen at -80°C immediately after cooking. A dedicated high-performance liquid chromatography instrument was used to quantify residual nitrite according to modifications of methods previously reported (6, 20). Samples were powdered in liquid nitrogen and stored at -80°C until analysis. Five grams of sample was homogenized with 45 ml of phosphate buffer (100  $\mu\text{M}$ , pH 7.4) and then split into two slurries and centrifuged at 10,000  $\times g$  at 4°C for 5 min (Avanti J-E with JA-25.50 rotor, Beckman Coulter, Indianapolis, IN). After

centrifugation, 400  $\mu\text{l}$  of supernatant from each slurry and 400  $\mu\text{l}$  of methanol were transferred into a 1.5-ml snap cap centrifuge tube. This mixture was vortexed on high speed for 3 to 5 s and allowed to sit at 4°C for at least 10 min to allow the methanol to break down any remaining protein in the sample. The samples were then centrifuged for 8 min at 13,000  $\times g$  at 4°C (Eppendorf model 5424 centrifuge, Brinkmann Instruments, Westburg, NY), and the supernatant was transferred into a new 1.5-ml snap cap tube for quantification using the ENO-20 NOx analyzer (EiCom USA, San Diego, CA). This extraction process yielded four subsamples per treatment, whose residual concentrations were measured using the ENO-20 NOx analyzer and data processor (EPC-500, (EiCom USA), and the data were analyzed with PowerChrom (version 2.3, eDaq, Denistone East, New South Wales, Australia). Analyzer settings were 0.22 ml/min for the reactor pump, 0.66 ml/min for the carrier pump, and an injection volume of 50  $\mu\text{l}$ . Standards were made from purified  $\text{NaNO}_2$  powder diluted with MilliQ water (Millipore, Billerica, MA) into 0, 20, 40, 60, 80, and 100  $\mu\text{M}$   $\text{NaNO}_2$  solutions. Standards and samples were analyzed following the same procedure to determine the concentration of  $\text{NaNO}_2$ .

**Statistical analysis.** Three independent replications were performed for each of the two phases. Data were compared using an analysis of variance and the mixed models procedure of the Statistical Analysis System (SAS Institute, Cary, NC). The model included fixed main effects of treatment (phase I,  $n = 6$ ; phase II,  $n = 10$ ) and replication ( $n = 3$ ). The random effect was the interaction of treatment by replication. All least significant

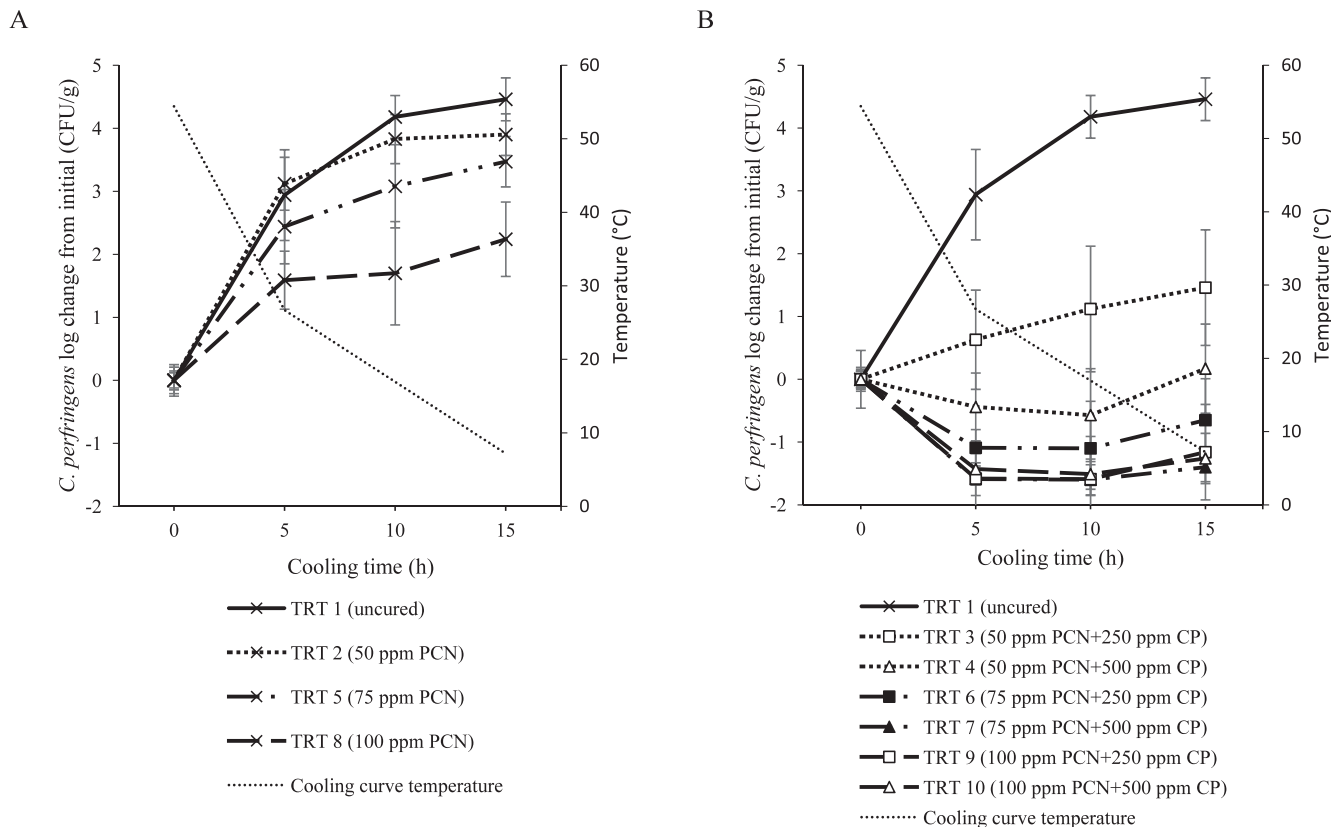


FIGURE 2. Change (sampling point minus initial) in counts of *C. perfringens* during 15 h of cooling of ground turkey breast (phase II). (A) Treatments with natural nitrite from cultured celery juice powder (PCN). (B) Treatments with PCN and natural ascorbate from cherry powder (CP). Data points represent the mean of three independent replications, and error bars represent the standard deviation.

differences were found using the Tukey-Kramer pairwise comparison method with significance determined at  $P < 0.05$ .

### RESULTS AND DISCUSSION

Measurements of moisture, salt, pH, and  $a_w$  confirmed consistency of formulation and manufacturing among treatments. All treatments in phase I had means ( $\pm$  standard deviations) of  $74.2\% \pm 1.8\%$  moisture,  $1.31\% \pm 0.14\%$  NaCl,  $6.31 \pm 0.05$  pH, and  $0.981 \pm 0.003$   $a_w$ . Residual nitrite was analyzed in only the four treatments that included nitrite in the formulations; residual nitrite was  $73.1 \pm 15.7$  and  $78.6 \pm 7.0$  ppm for 100 ppm of ingoing nitrite from purified and natural sources, respectively. When purified or natural sources of ascorbate was added to formulations, the residual nitrite was  $67.8 \pm 17.1$  and  $64.9 \pm 16.3$  ppm, respectively. However, the addition of ascorbate did not reduce ( $P > 0.05$ ) residual nitrite concentrations compared with treatments without ascorbate.

Proximate analyses for phase II treatments were similarly consistent, with  $76.6\% \pm 0.4\%$  moisture,  $1.19\% \pm 0.03\%$  NaCl, pH  $6.22 \pm 0.03$ , and  $0.980 \pm 0.002$   $a_w$ . Phase II results indicated that residual nitrite concentration was dependent upon ingoing nitrite concentration but was not affected by presence or concentration of ascorbate (Table 4). Treatments with 50 ppm of ingoing nitrite had 37.7 to 38.7 ppm of residual nitrite, treatments with 75 ppm of ingoing nitrite had 54.7 to 59.6 ppm of residual nitrite, and treatments with 100 ppm of ingoing nitrite had 77.2 to 80.5 ppm of residual nitrite. The residual nitrite results for

both phases indicated that the cure accelerating function of ascorbate did not have an appreciable effect on nitrite depletion during the time allowed in this product and process. In commercial meat products, less than 50% of the originally formulated nitrite is usually recoverable after thermal processing, and cure accelerators significantly affect residual nitrite concentrations (19, 23, 31). However, in the present study the artificially short time these products were

TABLE 4. Residual nitrite measured in phase II poultry samples containing various concentrations of nitrite from preconverted celery powder and ascorbate from cherry powder

Treatment <sup>a</sup>	Residual nitrite <sup>b</sup>
1: 0/0	NT
2: 50/0	$38.7 \pm 3.2$ C
3: 50/250	$37.9 \pm 3.6$ C
4: 50/500	$37.7 \pm 3.3$ C
5: 75/0	$59.6 \pm 8.9$ B
6: 75/250	$59.1 \pm 6.0$ B
7: 75/500	$54.7 \pm 4.1$ B
8: 100/0	$80.5 \pm 5.0$ A
9: 100/250	$78.9 \pm 6.3$ A
10: 100/500	$77.2 \pm 7.5$ A

<sup>a</sup> Treatments defined by concentrations of natural nitrite from preconverted celery powder and natural ascorbate from cherry powder (ppm of nitrite/ppm of ascorbate).

<sup>b</sup> Values are mean  $\pm$  standard deviation. Means with the same letter are not significantly different ( $P > 0.05$ ). NT, not tested.



exposed to high temperatures for thermal processing (approximately 5 min) limited the extent to which nitrite depletion occurred and minimized the difference in depletion between treatments with ascorbate and those without, which would be more pronounced after a longer commercial cooking process.

Throughout chilling, measurements verified that the maximum range of sample temperatures in the incubator was 3°C, although the temperatures generally were within 1°C. The individual sample temperatures tracked the prescribed chilling curve and target temperature, and all individual samples reached the target temperatures of 26.6°C at  $5.0 \pm 0.26$  h and of 7.2°C at  $15.0 \pm 0.25$  h. Changes in *C. perfringens* populations from the initial level versus temperature over 15 h are shown in Figure 1 (phase D). Approximately 2.5 log CFU/g was recovered after the initial cooking (heat shock) and before chilling. Recovered populations after heat shocking were not different among treatments ( $P > 0.05$ ), indicating the consistency of the inoculation procedure. As expected, the uncured control provided a suitable growth environment for this organism, with a  $4.36 \pm 0.80$ -log increase observed within the first 5 h when the meat temperature was in the known optimal growth range for *C. perfringens*. The treatment containing purified ascorbate in the absence of nitrite supported growth similar to that of the control ( $4.24 \pm 0.31$ -log increase;  $P > 0.05$ ), confirming that ascorbate alone did not inhibit *C. perfringens*. In both treatments containing 100 ppm of nitrite, either purified or from natural sources, but without a cure accelerator, *C. perfringens* grew similarly, and growth was similar to that in the uncured control ( $P > 0.05$ ), with only a slightly greater inhibition (<1-log difference) than in the control. As for the control and ascorbate-only treatments, the majority of growth occurred within the first 7.5 h, with populations remaining relatively unchanged ( $P > 0.05$ ) from 7.5 to 15 h of chilling, when temperatures ranged from 20.2 to 7.2°C according to the chilling cycle. In contrast, the treatments containing 100 ppm of nitrite plus 547 ppm of ascorbate, either purified or from natural sources, limited the outgrowth of *C. perfringens* throughout this chilling period to less than 1 log CFU/g, and final populations were not significantly different from the initial populations ( $P > 0.05$ ). Purified nitrite plus ascorbate and natural nitrite plus ascorbate resulted in decreases of  $0.25 \pm 1.07$  and  $0.36 \pm 0.94$  log CFU/g, respectively. These data confirm that when used at equal concentrations, the purified forms and natural forms of nitrite and ascorbate provided equivalent microbial inhibition ( $P < 0.05$ ). Furthermore, the combination of 100 ppm of nitrite and 547 ppm of ascorbate provided significantly greater inhibition than did 100 ppm of nitrite alone ( $P < 0.05$ ).

Although no differences were observed in the antimicrobial effect of 100 ppm of nitrite and 547 ppm of ascorbate derived from natural or purified sources, further testing was necessary to validate the safety of products made with lower concentrations, which are relevant to current industry practice. Although conventionally cured meats commonly contain both a cure accelerator (e.g., ascorbate or erythorbate) and >100 ppm of sodium nitrite, alternative cured meats

often are made with natural sources of nitrite, which provide <100 ppm of ingoing sodium nitrite, and without a curing accelerator such as ascorbate (32, 34, 35). The hypothesis of phase II of this study was that natural nitrite and ascorbate used at concentrations representative of current alternative curing formulations could inhibit *C. perfringens* outgrowth during the 15-h biphasic chilling process outlined in FSIS Appendix B (42).

Changes in *C. perfringens* populations over time versus temperature are presented in Figure 2, with the 0 value on the y-axis representing the populations recovered after heat shock. Populations of *C. perfringens* after heat shocking were not different among treatments ( $P > 0.05$ ). Results from phase II confirmed a >4-log increase of *C. perfringens* in the uncured control, whereas nitrite alone, although concentration dependent, provided limited additional inhibition of *C. perfringens* outgrowth at 50, 75, or 100 ppm (Fig. 2A). Nitrite had a dose-dependent effect, and as nitrite concentration increased *C. perfringens* growth decreased; however, even 100 ppm of ingoing nitrite alone was insufficient to limit outgrowth to <1 log CFU/g. After 15 h of chilling, growth in the 50 ppm of nitrite treatment was not significantly different from that in the uncured control ( $3.90 \pm 0.33$ -log and  $4.46 \pm 0.34$ -log increases, respectively) ( $P > 0.05$ ). Growth in the 75 ppm of nitrite treatment ( $3.47 \pm 0.40$ -log increase) was significantly less than that in the uncured control ( $P < 0.05$ ) but not different from that in the 50 ppm treatment; whereas growth in the 100 ppm of nitrite treatment ( $2.24 \pm 0.59$ -log increase) was significantly lower than that of the control and the 50 and 75 ppm of nitrite treatments ( $P < 0.05$ ).

Supplementing with 50 ppm of nitrite treatments with 250 ppm of ascorbate increased the inhibition of *C. perfringens* growth compared with the uncured control ( $P < 0.05$ ; Fig. 2B). The only nitrite plus ascorbate treatment that allowed *C. perfringens* growth relative to the initial level was 50 ppm of nitrite plus 250 ppm of ascorbate, which supported a  $1.46 \pm 0.92$ -log increase during the entire chilling period. However, increasing ascorbate concentrations to 500 ppm in combination with 50 ppm of nitrite limited growth to <1 log CFU/g during chilling. The populations of *C. perfringens* decreased in all of the remaining treatments containing  $\geq 75$  ppm of nitrite plus  $\geq 250$  ppm of ascorbate during chilling ( $P < 0.05$ ). *C. perfringens* levels in treatments with 75 ppm of nitrite plus 250 or 500 ppm of ascorbate decreased  $0.65 \pm 0.66$  and  $1.4 \pm 0.23$  log CFU/g, respectively, and levels in treatments with 100 ppm of nitrite plus 250 or 500 ppm of ascorbate decreased  $1.16 \pm 0.76$  and  $1.26 \pm 0.40$  log CFU/g, respectively.

These results confirmed that equivalent concentrations of nitrite, regardless of the source, provided similar inhibition of *C. perfringens* during chilling and that greater inhibition occurred when nitrite was combined with ascorbate. However, these results also suggests that 100 ppm of sodium nitrite alone, excluding a cure accelerator such as ascorbate or erythorbate, may be insufficient to prevent *C. perfringens* outgrowth even when the cooling profile of a high-moisture turkey product meets the extended chilling option of FSIS Appendix B for a cured product.

The Perfringens Predictor model, part of the ComBase online modeling system ([http://modelling.combase.cc/ComBase\\_Predictor.aspx](http://modelling.combase.cc/ComBase_Predictor.aspx)), was used to estimate that an uncured product with pH 6.2 and 1.2% salt at chilling temperatures similar to those used in this study would allow for a 3.8-, 4.7-, and 5-log increase in *C. perfringens* at 5, 7.5, and 15 h of cooling, respectively. Under the same product parameters and chilling profile, the model predicted that a cured product with 100 ppm of ingoing sodium nitrite would support a 2.5-, 3.4-, and 3.6-log increase by 5, 7.5, and 15 h, respectively. The model accurately predicted the observed growth in this study for a product with 6.2 pH, 1.2% salt, and with or without 100 ppm of nitrite but did not take into consideration the synergistic effect of ascorbate during chilling.

The uncured treatment results differed slightly between phase I and phase II, phase I resulting in 0.84-log greater growth than phase II for the uncured product. Similarly, 100 ppm of nitrite alone in phase I treatments permitted 1.94- to 2.14-log greater increases in *C. perfringens* than did 100 ppm of nitrite alone in phase II. Overall, product composition was comparable between the two phases, although a slightly lower pH in phase II (6.2 versus 6.3) could have had a limiting effect on total outgrowth. Gibson and Roberts (9) studied the effects of pH on *C. perfringens* and found that at pH 6.8 and 6.2, growth was observed in 1 week at 15°C in broth, whereas decreasing the pH to 5.6 inhibited growth until 4 weeks. Although chilling procedures in the present study were the same for both phases, heavier loading of the incubator for phase II decreased the rates at which the incubator could remove heat at high temperatures early in the chilling cycle, so that phase II samples took approximately 0.5 h longer to reach 50°C, the reported maximum growth temperature for *C. perfringens* (14). The combination of these two factors of pH and temperature reduction rate may explain the slightly decreased *C. perfringens* growth observed in phase II compared with phase I. However, uncured treatments in both phases allowed substantial *C. perfringens* outgrowth, and 100 ppm of nitrite alone was insufficient in either phase to limit outgrowth to <1 log CFU/g.

Only products cured with the direct addition of purified sodium nitrite currently qualify for the extended cooling option outlined in FSIS Appendix B. Traditional curing formulations normally contain near the maximum allowable ingoing concentration of sodium nitrite (156 to 200 ppm) and almost universally include ascorbate or its stereoisomer, erythorbate, as a cure accelerator. Traditionally, cure accelerators have been utilized for quality impacts, most notably maximizing the usage of added nitrite, increasing the formation of cured pigment, and maintaining cured color during storage (21). Therefore, products that currently qualify for this extended cooling option (15 h) would contain >100 ppm of ingoing nitrite and would likely also include ascorbate or erythorbate near the maximum regulatory limit of 547 ppm; according to the results of the present study, these products could be safely chilled over this 15-h period. However, in alternative cured formulations, ingoing nitrite concentrations are commonly

<100 ppm, and ascorbate (available in a natural form as cherry powder) can often be omitted from products; therefore, formulations without ascorbate may be considered inherently less safe when cooled using this 15-h chilling curve (30).

Although the mechanism of the antimicrobial action of nitrite is not entirely clear, these results confirm previous findings that higher concentrations of nitrite provide a higher protection against pathogen growth (7, 26, 36). However, the minimum nitrite concentration that produces a consistent antimicrobial result is not clearly known. Asan and Solberg (3) reported that nitrite concentrations as low as 25 ppm were inhibitory to *C. perfringens* in broth but only after the combined nitrite and broth were heat sterilized at 121°C for 20 min, a heat treatment that cannot be replicated in a processed meat product to yield a product acceptable to consumers. Redondo-Solano et al. (24) evaluated nitrite concentrations for inhibition of *C. perfringens* in ham during 15 h of exponential chilling between 54.4 and 7.2°C. These authors reported that 100 ppm of sodium nitrite was sufficient to limit *C. perfringens* outgrowth to <1 log CFU/g when packages were heat shocked and cooled 3 h after inoculation, but when held for 24 h before heat shocking and chilling, the same concentrations of nitrite allowed significant outgrowth of approximately 3 log CFU/g. Although product parameters such as moisture, pH, and  $a_w$  were comparable between the ham used by Redondo-Solano et al. and the turkey used in the present study, the difference in cooling methods could explain the contradictory results; exponential cooling would have provided less overall time in the optimum growth range of 43 to 47°C compared with the biphasic linear cooling process used in the present study. To prevent toxin production by *C. botulinum* in frankfurters, Hustad et al. (13) determined that 50 ppm of ingoing nitrite was sufficient. However, Jackson et al. (15) found that *C. perfringens* grew similarly in frankfurters and hams indirectly cured with a natural nitrite source at approximately 50 ppm of nitrite and stored at 20°C for 10 days compared with an uncured control with no nitrite. These results suggest that lower concentrations of nitrite found in alternative cured meat formulations (e.g. 50 or 75 ppm) are effective for inhibiting outgrowth of *C. perfringens* when supplemented with sufficient concentrations of a cure accelerator such as the ascorbate included in the present study.

The synergistic effect of ascorbate and nitrite for inhibiting *Clostridium* growth in meat products is still not clearly understood. In several studies, ascorbate or erythorbate enhanced the antimicrobial effect of nitrite against clostridia (4, 39, 40). Although a direct comparison between treatments with and without nitrite was not made in those studies, in another study (12) large intact hams cured with 200 ppm of sodium nitrite and 540 ppm of sodium erythorbate and then inoculated with *C. perfringens* did not support growth over a chilling curve similar to that used in the present study and even inhibited growth over 23 h of chilling from 54.4 to 7.2°C. In perishable canned cured meats, ascorbate enhanced the antimicrobial impact of both 50 and 156 ppm of sodium nitrite (39). Tompkin (38) investigated the mechanism by which ascorbate enhanced



the effect of nitrite by substituting ingredients that represented each of the individual functions of ascorbate. Specifically, butylated hydroxyanisole and tertiary butylhydroquinone were substituted to test the antioxidant effect, sodium sulfide and cysteine were substituted as reducing agents, and EDTA was tested as a chelating agent. Only EDTA provided inhibition similar to that of ascorbate, leading to the conclusion that the inhibitory effect was not attributed to the reducing capacity or the antioxidant effects of ascorbate. Instead, the hypothesis was that inhibition was due to cell damage by nitric oxide reduced from nitrite, followed by the chelation of a cation essential for recovery by ascorbate. Tompkin et al. (40) reported that 200 ppm of isoascorbate used with 50 ppm of sodium nitrite provided inhibition of *C. botulinum* similar to that provided by 156 ppm of sodium nitrite alone in perishable canned meat. Our results suggest that 50 ppm of sodium nitrite with 250 ppm of ascorbate may be just below the minimum threshold needed to control *C. perfringens* outgrowth in meat products during a 15-h chilling period but emphasize the critical role that ascorbate plays in conjunction with nitrite to prevent *C. perfringens* outgrowth.

Some researchers have concluded that ascorbate and erythorbate do not enhance the antimicrobial activity of nitrite at all (5, 24, 26). Bowen et al. (5) formulated wieners with 0, 15, 30, 50, 100, and 150 ppm of nitrite and 0, 105, and 655 ppm of ascorbate. At 28°C, inoculated *C. botulinum* began to produce toxin by 7 days in wieners with ≤50 ppm of nitrite, whereas wieners with ≥100 ppm did not develop toxin within 56 days of testing. No enhancement or detriment to the nitrite effect on toxin prevention was found when ascorbate was included in formulations. Redondo-Solano et al. (24) reported that ham formulated with 547 ppm of erythorbate plus 50 or 100 ppm of nitrite actually supported greater increases of *C. perfringens* than did the same concentrations of nitrite without erythorbate during exponential cooling over 15 h. Variability in meat formulation, such as salt concentration, pH, and  $a_w$ , and differences in cooling procedures could account for the inconsistent conclusions regarding the effect of ascorbate in cured meats; thus, this issue warrants further investigation. These contradictory published results probably have prevented the general industry-wide acceptance of cure accelerators as contributors to a multiple hurdle approach to food safety.

The study confirmed the importance of the concentration of nitrite, not the source, for impacting the microbiological safety of meat products. At equal nitrite concentrations, purified sodium nitrite and nitrite from cultured celery juice powder had an equivalent antimicrobial effect. Because formulations of alternative cured products often contain lower concentrations of natural nitrite than do products conventionally cured by direct addition of purified sodium nitrite, it is critical to evaluate the complete formulation, including concentrations of all curing ingredients, to ensure utmost product safety. These study results also revealed that the inclusion of ascorbate can greatly contribute to product safety with regard to outgrowth of *C. perfringens*. Because ascorbate and erythorbate function in

identical ways, these ingredients could be used interchangeably. Similar to nitrite, both purified and natural sources of ascorbate, when used at equal concentrations, provide the same enhancement of the antimicrobial activity of nitrite. The interaction of the two ingredients is evident in this study, and the true margin of safety at minimum regulatory limits of 100 ppm of ingoing nitrite as the only point of differentiation for chilling requirements is worth reconsidering, because 100 ppm of ingoing nitrite did not limit growth to <1 log CFU/g when used without ascorbate in this study. In this study, combined usage of concentrations of nitrite and ascorbate of 50 and 500 ppm or as low as 75 and 250 ppm, respectively, were effective for controlling the outgrowth of *C. perfringens* during a 15-h cooling period. Although nitrite and ascorbate concentrations are critical for predicting product safety for chilling processed meat products, overall product composition and chilling times and temperatures must also be considered to ensure safe chilling of cured meats.

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