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Can supplemental nitrate in cured meats be used as a means of increasing residual and dietary nitrate and subsequent potential for physiological nitric oxide without affecting product properties?

Emily L. Usinger [a], Elaine M. Larson [a], Steven E. Niebuhr [a], Christine A. Fedler [b], Kenneth J. Prusa [a,b], James S. Dickson [a], Rodrigo Tarté [a], Joseph G. Sebranek [a,b,*]

[a] Department of Animal Science, Iowa State University, Ames, IA 50011, United States
[b] Department of Food Science and Human Nutrition, Iowa State University, Ames, IA 50011, United States

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ABSTRACT

The effects of formulated sodium nitrate plus supplemental nitrate (SN) from celery juice powder on residual nitrite, residual nitrate, rancidity, microbial growth, color, sensory properties, and proximate composition of frankfurters, cotto salami and boneless ham during storage (1 °C) were studied. The products were assigned one of two treatments, which were each replicated twice: control (156 ppm sodium nitrite) or SN (156 ppm sodium nitrite and 1718 ppm sodium nitrate in combination with 2% VegStable 502). Sensory parameters and proximate composition were measured once for each replication. All other analytical measurements were conducted at regular intervals for 97–98 days. The SN showed no increase in residual nitrite compared to the control. No changes ($P > 0.05$) were observed in residual nitrate during storage for any of the products. The results showed that addition of SN did not significantly alter most physical, chemical or microbial properties of cured meat products during refrigerated storage, but some product dependent sensory effects were observed.

1. Introduction

Sodium nitrate and sodium nitrite have been used in meat products as curing agents and preservatives for centuries. However, nitrate is seldom used today because it must be converted to nitrite to be effective, which is a slow process achieved by microbial reductase. Nitrite is used in cured meats because it provides these products with improved quality characteristics including color, shelf life, and flavor. The addition of sodium nitrite also contributes to meat product safety by reducing the potential for outgrowth of several microbial pathogens including *Clostridium botulinum* and *Listeria monocytogenes* in cured meat products (Cammack et al., 1999; Sebranek & Bacus, 2007). Sodium nitrite provides these benefits to cured meat by undergoing reactions within the meat system to form nitric oxide. Nitric oxide production from nitrite is the necessary step to achieve cured characteristics (Sebranek, 2009).

The use of nitrite for meat curing became a major concern in the 1960s when it was discovered that nitrite has potential to form carcinogenic nitrosamines when combined with secondary amines in a suitable environment (Butler, 2015). However, changes in meat curing formulations and procedures have significantly improved the control and greatly reduced nitrite concentrations in finished cured meats (Cassens, 1997; Bedale, Sindelar, & Milkowski, 2016). Thus, human exposure to nitrite from cured meat is very limited. Further, it is important to note that nitrate is inert in cooked meat products because nitrate-reducing bacteria have been eliminated (Honikel, 2008). Nitrate, by itself, is considered a benign, inactive ingredient (Butler, 2015).

On the other hand, in the 1980s, it was discovered that nitric oxide, derived from nitrite and nitrate, is very important to many human physiological functions, and dietary nitrate as a source of nitric oxide has been shown in several human clinical studies to have significant health benefits (Bedale et al., 2016). Butler (2015), for example, concluded that "The presence of nitrite in food is free of danger and a diet high in nitrate is beneficial to the health".

While cured meats contribute a very small portion of human dietary intake of nitrate and nitrite, the human body derives nitrate and nitrite through two methods: endogenously through the nitric oxide synthase (NOS) pathway and exogenously through dietary consumption (Bryan, 2009). In the diet, nitrate and nitrite can be found in vegetables, water and some meats (Archer, 2002). The ingestion of nitrate from food leads to the conversion of nitrate to nitrite through bacteria in the mouth and subsequently to nitric oxide. Consequently, once a product containing nitrite or nitrate is ingested, the body's nitric oxide levels have been shown to increase as a result, provided the ingested amount is sufficient (Lundberg & Weitzberg, 2010). The discovery of nitric oxide, along with follow-up research, has made it clear that nitric oxide is one

* Corresponding author at: 215 Meat Laboratory, Iowa State University, Ames, IA 50011, United States.
E-mail address: sebranek@iastate.edu (J.G. Sebranek).

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of the most important signaling molecules in the human body for regulation of physiological functions such as blood flow to the tissues and organs (Bryan, 2009).

The ability to form nitric oxide and the important physiological role of this molecule, has established a new context for nitrate and nitrite. Incorporating sufficient nitrate concentration with nitrite in the meat system has potential to provide consumers with a meat product that could have a physiological impact similar to leafy green vegetables, derived from the increase in dietary nitrate that is provided by vegetable products. Manufacturing a product that contains nitrate in combination with nitrite will allow a typical curing reaction by nitrite to provide the necessary cured meat characteristics. At the same time, because nitrate is inert in cooked meat (Honikel, 2008; Sebranek, 2009), it is not typically depleted in a cooked meat system during storage time and distribution and, thus, can act as a source of dietary nitrate. This dietary source of nitrate could ultimately lead to increased nitric oxide production in vivo and contribute to reduced risk of cardiovascular diseases, such as heart attack and stroke (Bryan, 2006). Recent published literature has showcased the benefits of dietary nitrate and nitrite as well as the potential effects on human health (Butler, 2015; Bedale et al., 2016). However, it is clear that current negative perceptions of nitrate and nitrite in cured meat regarding health and safety need to be overcome before the use of supplemental nitrate (SN) in food can be accepted.

The United States Department of Agriculture-Food Safety Inspection Service (USDA-FSIS) currently limits ingoing (added) nitrate (as sodium nitrate) in chopped meat and poultry to 1718 parts per million (ppm) (USDA, 1995), which is equivalent to 137 mg of nitrate ion in a 112 g portion of finished meat product. Because significantly greater concentrations of dietary nitrate (over 200 mg) have been studied in human clinical trials (Larsen, Ekblom, Sahlin, Lundberg, & Weitzberg, 2006; Webb et al., 2008; Bryan, 2009; Liu et al., 2013; Kapil, Khambata, Robertson, Caulfield, & Ahluwalia, 2015; Velmurugan et al., 2016) with several positive health effects including blood pressure reduction, it is likely that SN would be necessary to achieve a comparable level of dietary nitrate from cured meat. Utilizing celery powder concentrate, as currently approved without restriction by USDA-FSIS (Sebranek, Jackson-Davis, Myers, & Lavieri, 2012), provides a means of adding SN. A practical limitation, however, could be the impact, at the necessary concentration, of the added celery concentrate on product quality characteristics.

This study was initiated to test the hypothesis that the addition of SN to cured meat, utilizing celery powder in addition to formulated sodium nitrate to achieve a nitrate concentration that could potentially impact nitric oxide concentrations in consumers, will introduce no significant changes in meat product quality or microbial characteristics. Therefore, the objectives of the present study were to evaluate the physical, chemical and microbial effects of SN in cured meat products, when manufactured to contain 220 mg or more nitrate per 112 g serving of cured meat.

2. Materials and methods

2.1. Experimental design

The experimental design consisted of two treatments of three different products, each of which were replicated twice. A control and a SN treatment were manufactured using boneless ham, cotto salami and frankfurter products. All products were manufactured in the Iowa State Meat Laboratory under USDA inspection. A.C. Legg (A.C. Legg, Inc., Calera, AL, U.S.A.) provided spices, and celery powder (VegStable 502) was provided by Florida Food Products, Inc. (Florida Food Products, Inc., Eustis, FL, U.S.A.) to be used as a SN source. The SN treatment products were formulated to achieve a target of 220 mg or more nitrate per 112 g serving (1964 ppm as nitrate ion) of cured meat. This concentration was achieved by including sodium nitrate at 1718 ppm as permitted by the USDA as well as including 2% celery powder containing 30,000 ppm nitrate according to the supplier. However, the combination must not result in >200 ppm of nitrite, calculated as sodium nitrite, in the finished product (USDA, 1995). For the SN products, potassium chloride was substituted for 8% of the sodium chloride used in the control treatment to compensate for the additional sodium content of the sodium nitrate and to keep the sodium content of the two treatments similar. Two percent celery powder as used in this study is a higher concentration than typically used in processed meats, but was chosen to achieve the desired amount of added nitrate.

2.1.1. Product manufacturing for ham treatments

Boneless ham (semitendinosus, semimembranosus, biceps femoris, and gracilis) was obtained from and processed in the Iowa State University Meat Laboratory. Control ham treatments were manufactured with salt (2.2%), sodium tripolyphosphate (0.28%), sugar (1.3%), modern cure (0.25%) (6.25% nitrite, 156 ppm sodium nitrite), and sodium erythorbate (0.05%, 547 ppm). The SN treatments included identical ingredients as the control with the addition of VegStable 502 (2%), sodium nitrate (0.17%, 1718 ppm), salt (2%) and potassium chloride (0.17%). The replications of the control and SN treatments were processed separately, but in the same manner. Boneless ham was ground through a Biro® grinder (Model 7.5 424852, The Biro® Manufacturing Co., Marblehead, OH, U.S.A.) fitted with a 0.95 cm plate. The ground ham was mixed with water/ice and nonmeat ingredients in a Higashimoto Kikai paddle mixer (Model 90.3.3, Nava, Japan) for 5 min. The mixture was ground a second time in the Biro® grinder with a 0.64 cm grinder plate to provide product uniformity. The product was loaded into a vacuum filler (RS 1040C, Risco U.S.A. Corp., South Eaton, MA, U.S.A.) and stuffed into 23 × 66 cm clear, fibrous casings (Kalle, Gurnee, IL, U.S.A.). The stuffed ham was hung on a smoke truck and placed into a Maurer (Maurer AG, Reichenau, Germany) oven with a natural smoke generator (Raucherzeuger Goliath 11, Reichenau, Germany), for conventional thermal processing. The two treatments in each replication were thermally processed together in the same oven for approximately 5 h using a standardized smokehouse processing schedule for hams until reaching an internal temperature of 71 °C. After cooking was completed, the products were cooled at 1 °C for 12 h overnight and subsequently stored at 1 °C. Ham was sliced (3.7 mm thickness) the following day using a Bizerba slicer (Model No. 10191442, Piscataway, NJ, U.S.A.) and vacuum packaged into half-pound packages using high barrier bags (Cryovac Sealed Air Corporation, 6 × 12, Duncan, SC, U.S.A.) with an oxygen transmission rate of 3–6 cm^3 at 23 °C (m^2, 24 h atm @ 23 °C, 0 RH) and a water vapor transmission rate of 0.5–0.6 g at 38 °C (100% RH, 645 cm^2, 24 h), with a Ultravac Model UV 2100 packaging machine (Koch, Kansas City, MO, U.S.A.). All ham treatments were stored in boxes at 1 °C.

2.1.2. Product manufacturing for cotto salami treatments

Beef 90 trim (60% of meat block) and pork 50 trim (40% of meat block) were obtained from and processed in the Iowa State University Meat Laboratory. Control treatments were manufactured with caseinate (3.5%), salt (2.7%), garlic (0.09%), black pepper (0.25%), cracked black pepper (0.19%), cardamom (0.12%), modern cure (0.25%) (6.25% nitrite, 156 ppm sodium nitrite), and sodium erythorbate (0.05%, 547 ppm). The SN treatments included identical ingredients as the control with the addition of VegStable 502 (2%), sodium nitrate (0.17%, 1718 ppm), salt (2.5%) and potassium chloride (0.22%). The two replications of the cotto salami were processed separately, similar to the hams. Beef and pork were ground separately through a Biro® grinder (Model 7.5 424852, The Biro® Manufacturing Co., Marblehead, OH, U.S.A.) fitted with a 0.95 cm plate. The ground beef was mixed with the salt and half of the water/ice in a Higashimoto Kikai paddle mixer (Model 90.3.3, Nava, Japan) for 5 min. The pork and the rest of the water and nonmeat ingredients were added to the mixer and mixed for 5 min. The mixture was then ground a second time in the Biro® grinder with a 0.64 cm grinder plate, loaded into a vacuum filler (RS 1040C, Risco