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Analysis of nitrite and nitrate concentrations present in locally produced ham labeled cured and uncured

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ABSTRACT

For many years, consumer concerns have grown regarding the health and safety of foods. One such concern involves the use of certain preservatives, such as sodium nitrite, in meat products. In this study, the concentration of nitrite and nitrate as preservatives in cured and uncured ham were determined from a local producer, Krehbiels Specialty Meats in McPherson, Kansas. Nitrite and nitrate determinations were carried out spectroscopically using the method outlined in Merino, 2009. The results of this research project indicate that the nitrate concentrations between the cured and uncured ham analyzed were not statistically significantly different with a difference of 0.38 ppm. The nitrite concentrations between the cured and uncured ham analyzed were statistically significantly different with a difference of 4.73 ppm. However, the difference in nitrite concentration between the two types of ham was not necessarily large enough to be considered a major health concern.

Keywords: *cured, ham, meat products, nitrate, nitrite, uncured*

INTRODUCTION

For many years, consumer concerns have been growing regarding the health and safety of foods available on the market. Some of these concerns are focused on the preservatives that are added to foods, including those used in the curing of certain meat products. Two of the most common preservatives used in the production of traditionally cured meat products are sodium nitrite and sodium nitrate (Rivera, et al. 2019). These preservatives work to inhibit the growth of bacteria in addition to helping maintain the color and flavor of meat products. However, nitrite in these preservatives can react with secondary amines in the acidic environment of the stomach to form carcinogenic nitrosamines within the human body (Rivera, et al. 2019; Van den Bran, et al. 2020). As a result of rising health concerns, many consumers have turned to alternative options available on the market such as those bearing natural, organic, or no-nitrites-or-nitrates-added labeling. Often these products will bear the general label of “uncured.”

The direct addition of nitrite or nitrate – such as with sodium nitrite – in uncured meat products is not allowed by the United States Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS), as these additives are considered chemical preservatives (Jackson, et al. 2011). Therefore, manufacturers have turned to alternative sources such as plant-based additives for the preservation of these products to mimic the results of traditionally cured meat products (Rivera, et al. 2019) Plant-based alternatives include natural nitrate sources, such as green leafy vegetables that are rich in nitrates, for example: beets, lettuce, arugula, watercress, celery, spinach and chard (Martinez, et al. 2019).

Though the use of plant-based alternatives may seem appealing for health-conscious consumers, in the past, these products may not have been as safe as their traditional counterparts. Studies regarding the growth of bacterial pathogens have suggested that natural, alternative meat curing processes may not have the same antimicrobial impact as traditional meat curing processes do, increasing the risk of contracting foodborne illnesses (Jackson, et al. 2011).

Due to concerns regarding the safety of these uncured products, the USDA-FSIS began regulating the minimum concentrations of nitrite and nitrate in uncured labeled products in 2018 under the FSIS Directive 7120.1 (Rivera, et al. 2019). The concentrations of nitrite and nitrate in the samples tested should reflect the enactment of these new regulations, as the concentrations should be higher than they were previously.

A study similar to this research project was conducted in five major U.S cities from September 2008 to March 2009, in conjunction with several universities, including the Department of Animal Science at Texas A&M University (Nuñez De González, et al. 2012). The results of this major survey provide a benchmark for various meat products that are conventionally cured in comparison with organic, naturally, uncured, and indirectly cured meats. Regarding conventionally cured meat products, the levels of nitrites were determined to be lower by two to 17-fold in comparison with studies making up the National Academy of Sciences (NAS) database from 1981 (Nuñez De González, et al. 2012). This change is likely the result of regulatory limits being put into place by the USDA after 1981 that placed maximum limits on the allowable concentrations of nitrites used in meat products (Nuñez De González, et al. 2012). If past regulatory

changes have been shown to have such a significant impact on the concentrations of nitrite and nitrate in traditionally cured meat products, it may be inferred that the 2018 regulations put in place by the USDA-FSIS may have a perceivable impact as well. After determining the nitrite and nitrate ion concentrations in ham labeled cured and uncured, the results were compared with those from the survey published in 2012 to see whether such a change has occurred.

MATERIALS AND METHODS

The procedure carried out is based on the procedure outlined in Merino 2009 (Merino, 2009).

Sample Preparation:

Ham used in this research project was obtained from a local meat producer, Krehbiels Specialty Meats, located in McPherson, Kansas. Preparation of the test samples began by taking approximately half of a pack of cured or uncured ham and cutting off the harder outside edges. The samples were diced as finely as possible by hand before being placed in a blender. Samples were then blended on high for ten seconds before being pulsed until partially homogenized. Three 8 g portions of the partially homogenized test sample were massed. Each portion of the test sample was added to the blender with 60 mL of hot deionized water (50-60°C) and was blended and pulsed until well blended. The portions were then quantitatively transferred to Erlenmeyer flasks in preparation for the clarification process.

Protein Precipitation and Clarification:

Clarification was performed to remove unwanted particles in the solution, such as fat and proteins. To carry out the clarification process, 4.0 mL of Carrez solution 1 followed by 4.0 mL of Carrez solution 2 were added, with swirling after each addition. Carrez solution 1 was prepared by dissolving 150 g of potassium hexacyanoferrate (II) trihydrate in water and diluting to 1,000 mL. The solution was stored in an aluminum wrapped bottle to keep out light. Carrez solution 2 was prepared by dissolving 230 g of zinc acetate dihydrate in water and diluting to 1,000 mL.

After addition of the two Carrez solutions, the test solutions were each transferred to centrifuge cups and were centrifuged at 4,000 rpm at 25°C for ten minutes. The supernatant liquid was filtered quantitatively through fine filter paper into 100-mL volumetric flasks and diluted to volume with deionized water.

Nitrite Determination:

Three 20.0 mL aliquots of the test solutions were added to 100-mL volumetric flasks for the determination of nitrite. 10.0 mL of ammonia buffer with a pH 11 were then added to each. The ammonia buffer was prepared by adding 75 mL of ammonia to 825 mL of water, adjusting the pH to 11.0 using 1.0 M HCl and diluting to volume in a 1,000 mL volumetric flask. For the color development, to each flask, 2.0 mL of color reagent 1 were added, mixed, and left to stand for five minutes at room temperature. Then, 2.0 mL of color reagent 2 were added, and mixed. The colored solutions were diluted to volume with deionized water. Color reagent 1 was prepared by dissolving 2.0 g of sulphanilamide in water, adding 105 mL of concentrated HCl and diluting to 200 mL with water. Color reagent 2 was prepared by dissolving 0.2 g of N-(1-naphthyl)-ethylene diamine dihydrochloride in water and diluting to 200 mL. The solution of coloring reagent 2 was stored in a brown bottle and was discarded monthly or when a dark purple to brown color developed. The absorbance of each solution was measured at 540 nm after dilution, adjusted against water.

Nitrate Determination:

For the reduction of nitrate to nitrite, three 20.0 mL aliquots of the three test solutions were added to glass bottles. 10.0 mL of the pH 11 ammonia buffer were added to each, followed by 0.1 g of zinc dust. Each glass bottle was capped and shaken manually for five minutes. The clear supernatant was filtered quantitatively through fine filter paper and the precipitate was washed twice during filtering. The filtrate was collected in 100-mL volumetric flasks. The same color development and measurement steps were carried out on the reduced nitrate solutions as was carried out for the nitrite solutions. The concentration of nitrate was later calculated as the difference between the nitrites in the reduced nitrate solutions and the nitrites in the unreduced solutions.

Nitrite and Nitrate Calibration Curves:

Standard nitrite and nitrate solutions were used in making calibration curves. The nitrite stock solution was prepared by dissolving 0.6023 g of analytical grade sodium nitrite in water and diluting to 200 mL in a volumetric flask to make a 2.008 g nitrite/L solution. The nitrate stock solution was prepared by dissolving 0.6526 g of analytical grade potassium nitrate in water and diluting to 200 mL in a volumetric flask to make a 2.001 g nitrate/L solution. The standard solutions were refrigerated to maintain stability.

Nitrite and nitrate standard solutions were prepared daily by diluting 5 mL of the stock solutions in separate 100 mL volumetric flasks. Six aliquots of varying volumes were taken from each working solution. For the nitrite calibration curve, 0, 1, 2, 3, 4, and 6 mL volumes of standard nitrite solution were used. For the nitrate calibration curve, 0, 1, 3, 4, 6, and 8 mL volumes of standard nitrate solution were used.

Each of these aliquots was then added to an Erlenmeyer flask followed by 60 mL of hot deionized water (50-60°C). The clarification, reduction, color

development and measurement procedures were carried out in the same manner as the test samples.

RESULTS

Regarding the uncured ham, the mean concentration of nitrite was 1.15 ppm (SD = 0.16), and the mean concentration of nitrate was 7.17 ppm (SD = 3.43). Regarding the cured ham, the mean concentration of nitrite was 5.87 ppm (SD = 0.93), and the mean concentration of nitrate was 7.55 ppm (SD = 2.69). For each type of ham, two packages were analyzed.

Two-sample t-tests assuming unequal variances for the nitrate concentrations and equal variances for the nitrite concentrations were performed to determine if a statistically significant difference between the mean concentrations of nitrite and nitrate in both types of ham was present. The difference of the mean concentration of nitrite in the two types of ham was statistically significant, with a difference of 4.73 ppm ($p < 0.05$). The difference in the mean concentration of nitrate in the two types of ham was not statistically significant, with a difference of 0.38 ppm ($p > 0.25$)

DISCUSSION

In comparing concentrations of nitrite and nitrate in cured and uncured ham from a local producer, there is a statistically significant difference in the mean concentration of nitrite, but not in the mean concentration of nitrate. This is somewhat consistent with findings from a prior survey by Texas A&M University (Nuñez De González, et al. 2012). In the survey published in 2012, the authors concluded that there was not a significant difference in the nitrite or nitrate concentrations of the products making up the category that included ham. The results of this study agree with this 2012 survey regarding the differences in nitrate concentrations but disagrees regarding the differences in nitrite concentrations. The disagreement in results could be the result of regional differences as the previous study consisted of samples from across the United States, while this research project focused solely on results from a local producer. The disagreement in results could also be the result of the use of a broader category of products in the survey published in 2012, instead of only focusing on one specific meat product – in this case, ham.

The results from this research project indicate that there is a statistically significant difference in the nitrite concentrations of cured and uncured ham from a local producer. However, this difference is relatively small with a difference of 4.73 ppm. The reason why the difference in nitrite concentration is 4.73 ppm and there is not significant difference in the concentration of nitrate between the two types of ham may be the result of regulations regarding these preservatives. Due to concerns of pathogenic growth in uncured meat products, the minimum allowable concentration of nitrite and nitrate in these products is now regulated and is comparable to the minimum allowable concentrations of nitrite and nitrate in cured meat products.

While the results from this research project indicate that there is a difference in the nitrite concentrations of cured and uncured ham, a difference of 4.73 ppm may not be a large enough difference to be of statistical importance. However, this difference may be significant enough to influence consumer choices when considering the consumption of cured or uncured meat products.

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LITERATURE CITED

Jackson, AL, GA Sullivan, C Kulchaiyawat, JG Sebranek, and JS Dickson. 2011. Survival and Growth of Clostridium Perfringens in Commercial No-Nitrate-or-Nitrite-Added (Natural and Organic) Frankfurters, Hams, and Bacon. Journal of Food Protection 74:410-416.

Martinez, L, P Bastida, J Castillo, G Ros, and G Nieto. 2019. Green Alternatives to Synthetic Antioxidants, Antimicrobials, Nitrates, and Nitrites in Clean Label Spanish Chorizo. Antioxidants 8:184.

Merino, L. 2009. Development and Validation of a Method for Determination of Residual Nitrite/Nitrate in Foodstuffs and Water After Zinc Reduction. Food Analytical Methods 2: 212-220.

Nuñez De González, MT, WN Osburn, MD Hardin, M Longnecker, HK Garg, NS Bryan, and JT Keeton. 2012. Survey of Residual Nitrite and Nitrate in Conventional and Organic/Natural/Uncured/Indirectly Cured Meats Available at Retail in the United States. Journal of Agricultural and Food Chemistry 60:3981-3990.

Rivera, N, M Bunning, and J Martin. 2019. Uncured-Labeled Meat Products Produced Using Plant-Derived Nitrates and Nitrites: Chemistry, Safety, and Regulatory Considerations. Journal of Agricultural and Food Chemistry 67:8074-8084.

Van den Brand, AD, M Beukers, M Niekerk, G van Donkersgoed, M van der Aa, B van de Ven, A

Bulder, H van der Voet, and CR Sprong. 2020. Assessment of the combined nitrate and nitrite exposure from food and drinking water: application of uncertainty around the nitrate to nitrite conversion factor. Food Additives and Contaminants: Part A 37:568-582.