6:8

Rate of formation of nitric oxide myoglobin: Effects of chloride ion concentrations.

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Salt (sodium chloride) and nitrite (either sodium or potassium) are universally used for manufacturing what are known as "cured" meat products. There have been occasional investigations of potential interactive effects of these two compounds but the results have been inconclusive. It has been observed that choride will increase the apparent nitrite concentrations determined by the Griess method when sulfanilic acid is used (Hildrum, 1979). When sulfanilamide was used for the Griess reagent, no chloride effect was observed (Fox et al., 1981). These observations indicate that some of the differential results in the literature concerning the effect of salt on nitrite may have been due to the particular variation of the Griess method chosen. For example, reports have indicated that sodium chloride resulted in increased residual nitrite (Fujimaki et al., 1975), no change (Olsman and Krol, 1972) or decreased residual nitrite (Lee and Cassens, 1980). If these results were the extent of the evidence any observed change in nitrite concentrations with changes in salt might be interpreted as simply due to the effects observed on the Griess reagent.
It has also been reported however, that the presence of chloride may suppress nitrosation reactions in meat or model systems (Hildrum et al., 1975; Theiler et al., 1981) which would imply some specific interaction between these compounds under conditions similar to those in meat products. It has been known for some time that nitrosyl chloride (NOCl) can be formed from nitrite and chloride under very acidic conditions (Challis and Shuker, 1978) and that nitrosyl chloride serves as an active, effective nitrosating intermediate. These results indicate that a specific interaction between sodium chloride and nitrite may occur in meat systems. This work was initiated to determine whether salt concentrations play a role in nitrite reactions in cured meat systems. The objectives were to first, determine the effect of salt or nitrite under conditions similar to cured meat and second to determine whether any observed effects were of practical significance in a cured meat product.

Methods:
The initial part of the work utilized a model system consisting of an acetate buffer solution containing bovine serum albumin, metmyoglobin, sodium ascorbate, sodium nitrite and sodium chloride. By purging all solutions of oxygen and then holding them under nitrogen, this system served as a good means of measuring the rate of formation of nitric oxide from the mixture. Since the nitric oxide-metmyoglobin reaction is known to be extremely fast, the formation of the red nitric oxide pigment form provides an accurate indication of nitric oxide formation. Scanning the visible spectra in the range of 470-560 nm demonstrates the change with time from metmyoglobin (505 nm) to nitric oxide myoglobin (545 nm).
The solutions were prepared by first preparing a 0.1 M acetate buffer at pH 5.6 which was then held under nitrogen in a glovebox. Sodium chloride was weighed into erlenmeyer flasks in amounts that corresponded to 0, 0.5, 1.0, 2.0 and 4.0% of the final solution (weight/volume) and placed in the glovebox under nitrogen. Myoglobin (0.3 mM) and bovine serum albumin (2%) were dissolved in the acetate after which 23 milliliters were pipetted to each of the flasks, allowing the salt to dissolve. Since salt alters solution pH, each erlenmeyer was individually adjusted back to the pH of the 0% salt sample (pH = 5.6) by adding 0.1 N NaOH. Following adjustment, stock solutions of nitrite and ascorbate were added (1 ml of each) to start the reaction and scanning (470-560 nm) immediately initiated. All handling and transfers were done in the glovebox under nitrogen and cuvettes transferred to a spectrophotometer sample compartment which was under a constant stream of nitrogen. The sample compartment held 5 cuvettes in a rotating holder thus further transfers were not needed. Scans were repeated as frequently as necessary to measure the reaction rates.
Several different nitrite and ascorbate concentrations were evaluated for the effect of salt concentration. These included nitrite at levels of 0.23, 0.46, 0.93, 1.36, 1.80 and 2.26 mM and ascorbate at levels of 0.31, 0.64, 1.28 and 3.13 mM. Each combination of nitrite/ascorbate concentration was evaluated for the rate of nitric oxide formation as a result of salt concentrations.
The study of meat systems was designed to evaluate several of the usual variables encountered when dealing with raw materials for meat curing. These included pigment content, product pH, addition of reductant (erythorbate) and internal product (cooked) temperature. A large diameter bologna was manufactured for all comparisons and formulated to include 0.5, 1.0, 2.0 or 4.0% sodium chloride. Nitrite was included at either 75 or 156 ppm and erythorbate when used, at 550 ppm. Product variables were achieved as follows; the pigment content variable was studied in a standard 50% beef/50% pork trim mixture and a turkey white meat mixture, product pH was manipulated by addition of glucono delta lactone (GDL) to achieve pH levels of 4.9, 5.7 and 5.8, reductant (erythorbate) was either included at 550 ppm or not at all. Finally, the product was finished at temperatures of 52°C, 60°C or 68°C.
The product ingredients were chopped in a bowl chopper following the ISU Meat Laboratory bologna emulsion procedure. Following the chopping, the emulsion was stuffed into 51 mm diameter fibrous casings, smokehouse processed to appropriate internal temperature, chilled overnight and vacuum packaged in a high barrier film. Evaluations of each product treatment included pH, salt content (Orion, 1980), cooked yields, nitroso pigment, total pigment (Hornsey, 1956) and residual nitrite (Fox et al., 1981). Measurements were made on the raw mixture before stuffing, immediately after processing and at 15 days and 30 days after manufacture.
All treatment combinations in both the model system studies and the meat mixture were replicated 3 times. Data analysis was done using analysis of variance and Duncan's Multiple Range Test (Steel and Torrie, 1980).

Results and Conclusions:
The addition of chloride to a metmyoglobin-ascorbate-nitrite mixture resulted in a significantly greater production of nitric oxide as measured by nitric oxide pigment formation. The reaction was determined to be zero order and the reaction rate constants ($mM \cdot min^{-1}$) are shown in table 1 for all nitrite levels with

Table 1: Reaction Rate Constants (k in $mM \cdot min^{-1} \times 10^{-3}$) for the Formation of Nitric Oxide.

Nitrite (mM)	Ascorbate (mM)	Chloride %				
		0	0.5	1.0	2.0	4.0
2.26	3.13	2.8	3.2	3.2	3.5	4.4
2.26	1.28	1.9	2.2	2.6	3.0	3.5
2.26	0.64	1.1	1.4	1.5	2.1	3.1
2.26	0.31	0.9	0.8	0.8	0.8	0.9
1.80	3.13	1.8	2.0	2.2	2.2	2.3
1.36	3.13	3.3	3.3	3.3	4.1	4.1
0.93	3.13	2.0	2.5	2.8	2.8	2.8
0.46	3.13	1.1	1.1	1.1	1.4	1.7
0.23	3.13	1.2	1.2	1.2	1.2	1.5

sodium ascorbate. There is a consistent increase in the reaction rate constants as chloride increases within any one combination of nitrite/ascorbate concentrations. The reaction rates generally decrease with lower nitrite or ascorbate levels as would be expected and are more consistent in the case of ascorbate than of nitrite. It seems clear that chloride concentrations exert some influence on production of nitric oxide from nitrite in this system. This would imply that NOCl may indeed be a part of nitrite reaction sequences at a pH similar to meat. A reaction sequence has been proposed (Fox et al., 1986) which results in a rate expression that includes nitrite, ascorbate, chloride and pH terms as rate factors.

$$\frac{d[NO]}{dt} = \frac{K_5}{K_4}\left(\frac{[NO_2^-]\,[A^-]\,[H^+]}{K_A+[H^+](K_N+[H^+]}\right)^{\frac{1}{2}} \times (K_1[H^+] + K_3K_N)^{\frac{1}{2}} + K_2\frac{[NO_2^-]\,[H^+]\,[Cl^-]}{K_N + [H^+]}$$

This expression was used to calculate expected rate constants which are as shown in table 2. The calculated constants correspond quite well to the measured constants also shown in table 2.

Table 2: Calculated Rate Constants for Formation of NO (extremes of treatment combinations).

Nitrite (mM)	Ascorbate (mM)	Chloride %			
		0%		4%	
		calculated	measured	calculated	measured
2.26	3.13	2.7	2.8	4.5	4.4
2.26	0.64	1.4	1.1	3.7	3.1
0.23	3.13	1.3	1.2	1.4	1.5

While these results are of interest, the carefully controlled conditions do not reflect all influences present in a meat system. Consequently, the meat product study was done to assess the practical significance of the above observations. Generally, the influence of chloride in the meat system was found to be non-significant for those characteristics measured. Table 3 demonstrates a few of the many treatment combinations and it can be observed that while there is often a trend toward higher cured pigment and less residual nitrite with increasing chloride concentrations, the differences are very small.

Table 3: Some Comparisons of Meat Emulsions for Nitrite Related Characteristics as a Result of Different Chloride Concentrations.

Product	Target pH	Chloride %				
		0	0.5	1.0	2.0	4.0
Beef/pork mixture						
nitrosyl heme pigment (ppm)	5.8	67	68	69	68	76
	4.9	103	107	107	111	116
residual nitrite (ppm)	5.8	44	49	48	47	44
	4.9	22	20	21	20	19
measured pH (cooked samples)	5.8	5.7	5.8	5.9	5.9	6.0
measured pH (cooked with GDL)	4.9	4.9	5.0	5.1	5.1	5.2
Turkey white muscle						
measured pH	5.8	5.8	5.9	6.0	6.0	6.1
nitrosyl heme pigment	5.8	14	14	14	13	14
residual nitrite	5.8	61	65	61	58	54

Only pH showed a significant increase with increasing chloride levels. It appears that the complexity and extent of nitrite reactions in a meat system largely overrides any potential influence on nitrite reactions by the chloride ion.

It would seem, therefore, that while chloride has a significant influence on nitrite reactions in a model system, the effects in meat are too small to be of any practical importance. This is consistent with the observation that in a model system, the effect of chloride was on the rate of the reaction measured, not on the amount of end product, which in all cases reached the same final quantity. However, it should be noted that some very subtle changes in nitrite could be of significance to cured product characteristics that were not assessed in this study. One example is the potential interaction of nitrite with microbial cells, a phenomenon that is not well understood but generally conceded to involve relatively small amounts of "active" nitrite or a nitrite reaction intermediate. Changing quantities of intermediate reactive species formed from nitrite as a result of chloride concentrations could be important in cured meat when other factors (such as time of heat processing) are also altered. These are areas that deserve further study.

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