

DIFFUSION OF CHLORIDE, NITRITE, AND NITRATE IN BEEF AND PORK

JAY B. FOX, JR.

ABSTRACT

The diffusion of sodium chloride, nitrite, and nitrate into beef, pork, and pork fat was studied by the porous disc technique. Total salt uptake and boundary concentration analyses were performed on beef. Various salt combinations and concentrations and various muscles were studied. Chloride diffusion was normal by all three methods of analysis, but skew boundaries and decreased uptake of nitrite were observed in the boundary measurements; diffusion rates of nitrite were constant in the porous disc method. Nitrite diffused slower than chloride in meat, but the rate of nitrate diffusion was sometimes greater than that of chloride. Comparison of the results of the steady state diffusion with the total uptake and boundary concentration showed the diffusion of nitrite to be governed by both physical and chemical factors.

INTRODUCTION

THE RATES of diffusion of curing salts in meats determine the length of time required for processing and the uniformity of cure distribution. This is as true for rapid ham curing by stitch pumping and tumbling or massaging as it is for long term brine and dry salt cures. A thorough understanding of the diffusion process is necessary to the interpretation of the effects and interactions of the various salts used in curing and the effects of techniques such as tumbling and massaging. The basic data for this understanding are the diffusion constants of the curing salt anions. Although the two most important anions are chloride and nitrite, determinations of diffusion constants for curing salts have been limited to sodium chloride in pork (Wistreich et al., 1959, 1960; Kőrmendy and Gantner, 1958; Wood, 1966). The determinations were made with cylinders of meat enclosed in glass tubes so that diffusion proceeded in one plane. While the technique is suitable for the study of single diffusing solutes, if more than one solute is used and interactions occur, it is difficult or impossible to separate individual effects. Chemical reactions in the tissue may take place; nitrite disappears in meat during curing through reaction with tissue components (Mirna and Hofmann, 1969; Fox and Nicholas, 1974). Nitrate has been said to increase the permeability of muscle tissues to water and to affect the penetration of other salts (Jensen, 1942). These effects may be separated operationally by a steady state diffusion system in which a concentration gradient is established across a membrane or porous disc (Crank, 1967; Bull, 1951). Under these conditions, the effect of one solute may be observed by varying its concentration in a series of experiments in which the concentration of the other solutes remain constant. We therefore undertook a study of the diffusion of sodium chloride, nitrite, and nitrate into both beef and pork, using the porous disc technique to determine the effect of salt-salt and salt-tissue component interactions. For purposes of comparison, we ran diffusion experiments in cylinders as previously described (Wood, 1966), analyzing both for total uptake and the shape of the diffusing boundary.

EXPERIMENTAL

THE VARIOUS CUTS and/or muscles as indicated were purchased from local suppliers. For the preparation of either cylinders or discs, the meat was vacuum packaged and immersed in an alcohol bath at -3 to -4°C until it was firm. For cylinders, the meat was cut into 7-cm lengths and cores were cut out parallel to the fiber with a 1.4-cm cork borer. The cylinders so formed were aspirated into 1.4-cm i.d. glass tubes 10 cm long until one surface of the meat was flush with the end of the glass tube. The exact dimensions were such that a close fit was obtained. Bundles of five to six of the glass tubes were suspended in 500 ml of cure solution with the flush surface of meat immersed half way down in the cure solution. The solutions were stirred occasionally, although the absence of light refraction gradients due to concentration variation indicated stirring was not necessary (cf. Wood, 1966).

For discs, the firmed meat was sliced into 1-cm thick sections perpendicular to the fibers. A stainless steel cutter was used to cut out 5.0-cm discs. The discs were then placed into 5.0 cm i.d. \times 1 cm thick Lucite rings, and cellulose film was smoothed over both surfaces. The ring was incorporated as the center member of a 3-component cell (Fig. 1). The lower compartment, enclosed on the bottom, was of the same diameter as the ring, had a filling tube on the side, and held 140 ml of cure solution. A magnetic stir bar was placed in the cell before it was assembled. The upper compartment of the cell was an open cylinder of the same diameter as the ring. The joining surfaces of the upper and lower compartments with the center ring were beveled so they would not slip. The cellulose membranes acted as gaskets to seal the beveled joints. The upper and lower compartments were fitted on the outside with retainer rings so the assembly could be bolted together. After the cell was assembled, the lower compartment was filled with the appropriate cure solution and the filling tube was plugged. The upper cell was filled with water. The cell was placed on a magnetic mixer and a

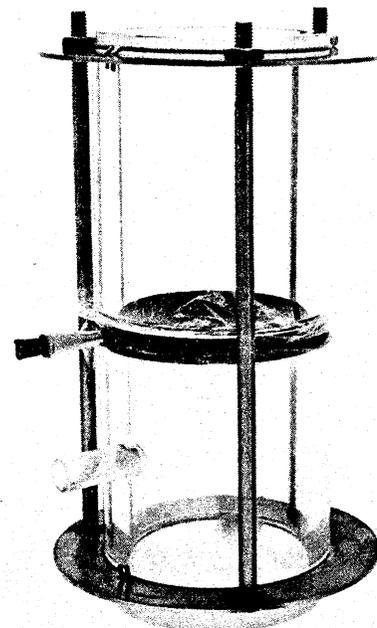


Fig. 1—Perspective view of diffusion cell.

glass stirrer rotated by a 60-rpm motor was placed in the upper compartment. Samples were withdrawn as desired from the upper compartment. All experiments were run at 4–5°C. To determine the rate of diffusion through the cellulose membranes the two sheets of cellulose were set in place to seal the beveled joints, and then the top membrane was cut out.

Chloride concentration was measured with an Orion solid state chloride electrode. Nitrite concentration was determined by the AOAC method of analysis (AOAC, 1976). Nitrate concentration was measured with an Orion nitrate electrode. Standard for chloride, nitrite, and nitrate were run with each set of samples. The curing salt concentrations were those used by a commercial dried beef manufacturer (4.27M Cl⁻) and a ham cure brine suggested by Kramlich et al. (1973).

Calculation of diffusion constants

The defining equations for the diffusion constant, D, for the three methods of measurement are:

Total uptake (Wood, 1966)

$$S_t = 2C_o(Dt/\pi)^{1/2} \quad (1)$$

where S_t is the total salt uptake in time, t, per unit area of exposed surface. The slope of a plot of S/C_o as a function of $t^{1/2}$ is $2\sqrt{D/\pi}$.

Diffusing boundary (Crank, 1967)

$$C_{(d,t)} = \frac{1}{2}C_o \operatorname{erfc} \frac{d}{\sqrt{Dt}} \quad (2)$$

The error function, $\operatorname{erfc} x$ ($x = \frac{d}{\sqrt{Dt}}$), is calculated from the equation for a given value of concentration, C, at d, the distance from the meat surface. The value of x is determined from tabular values of the $\operatorname{erfc} x$ (Crank, 1967; WPA, 1941; Milne-Thomson and Comrie, 1944). The diffusion constant was then calculated for given values of d and t.

Porous disc (Crank, 1967)

$$M_t = \frac{D(C_o - C_h)At}{h} \quad (3)$$

where M_t is the total amount of salt diffusing through the disc in time t, h is the thickness of the disc, A is the area of the disc, C_o is the initial concentration of the salt in the lower cell, and $C_o - C_h$ is the concentration difference between the two sides of the disc. Because in the early stages of the diffusion process C_o changes minimally and $C_h \approx 0$, C_o is used in the equation (Bull, 1951).

To convert diffusion constants from one temperature to another, the following equation was used (Bull, 1951).

$$D_{T_1} = D_{T_2} \frac{T_1}{T_2} \times \frac{\eta_{T_2}}{\eta_{T_1}} \quad (4)$$

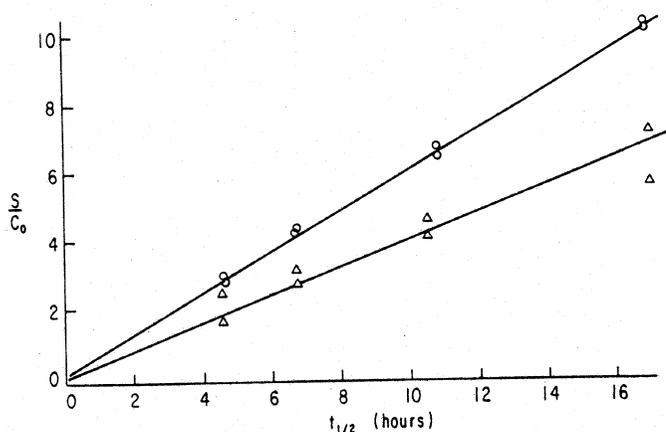


Fig. 2—Total uptake of sodium chloride and sodium nitrite in cylinders of beef semitendinosus at 5°C. [NaCl] = 4.27M, [NaNO₂] = 32.6 mM. ○, Cl⁻; △, NO₂⁻.

where $T = ^\circ\text{K}$ and η is viscosity of the medium. Since the viscosity term is relative, we assumed that the relative viscosities of water in muscle are the same as those in free water.

Relative flow resistance

Because cellulose membranes were used to hold the meat discs in place, we needed an assessment of their effect on the diffusion rates. According to Crank, the resistance of individual sheets to flow is given by the equation:

$$\frac{Fh_1}{D_1} + \frac{Fh_2}{D_2} + \dots + \frac{Fh_n}{D_n} = \left[\frac{h_1}{D_1} + \frac{h_2}{D_2} + \dots + \frac{h_n}{D_n} \right] F \quad (5)$$

The flow, F, is constant across each sheet in the steady state, and h_i/D_i is the resistance to flow of each sheet. The total resistance to flow is the sum in brackets, so the total resistance, R, may be calculated from the known values of h, the thickness of the sheets, and the diffusion constants, D. For chloride, the values are:

$$R_t = \frac{2 \times 0.0025}{0.024} + \frac{1.00}{0.28} \quad (6)$$

$$= (0.20 + 3.57)$$

$$= 3.77$$

The first term is for the two cellulose membranes, where 0.0025 is the thickness of each in cm and D is the diffusion constant of chloride through the membrane. The second term is the resistance to flow through the 1.0 cm meat sample. For chloride, and nitrite and nitrate as well, this meant a reduction of 4 to 5.5% between the measured value and the true value. All reported values were corrected for the cellulose membrane resistance.

RESULTS

FIGURE 2 shows the results of measuring total uptake of both chloride and nitrite in cylinders of beef semitendinosus. The diffusion constants were calculated from the slopes of the curves according to Eq (1), giving values of $(0.26 \pm .02)$ and $(0.11 \pm 0.035) \times 10^{-5} \text{ cm}^2 \text{ sec}^{-1}$ for chloride and nitrite, respectively. To obtain further insight into the process, we analyzed the cores in sections across the diffusing boundary for chloride nitrate and nitrite, with the results shown in Figure 3. The diffusion constants for the sample shown in Figure 3 and a second sample are given in Table 1. The coefficients of variation for the individual chloride diffusion constants for both methods of determination were 8, 9, and 15% compared with a value of 15% reported by Wood (1966) and a range of 0.3 to 21.6% reported by Körmödy and Gantner (1958).

The nitrate and chloride curves of Figure 2 and the data of Table 1 show an anomaly in that the concentrations of both salts are too high near the meat surface, giving abnormally high diffusion constants. Apparently, the concentration of the salts approaches a near maximal value which does not stay at the meat surface $X = 0$, but moves into the cylinder with time. Except for these high values near the cut surface, the diffusion constants did not vary significantly across the diffusing boundary for chloride and nitrate. The nitrite boundaries, however, diffused faster at the leading edge. The concentration of nitrite was relatively higher than expected from a normal diffusion curve at low nitrite levels and relatively lower at high nitrite concentrations. A chemical reaction, monomolecular in nitrite, may be ruled out because it results in a uniform decrease in the measured diffusion constant (Crank, 1967). On the other hand, a chemical reaction, 2nd order or higher in nitrite or chloride, would result in the relatively lower nitrite values observed at higher nitrite and salt concentrations. The differences at 1.05 and 1.75 cm between the nitrite diffusion constants for the two samples may represent a difference in chemical reactivity of the two pieces of meat. Alternatively, since the diffusion of nitrite takes place in the presence of a

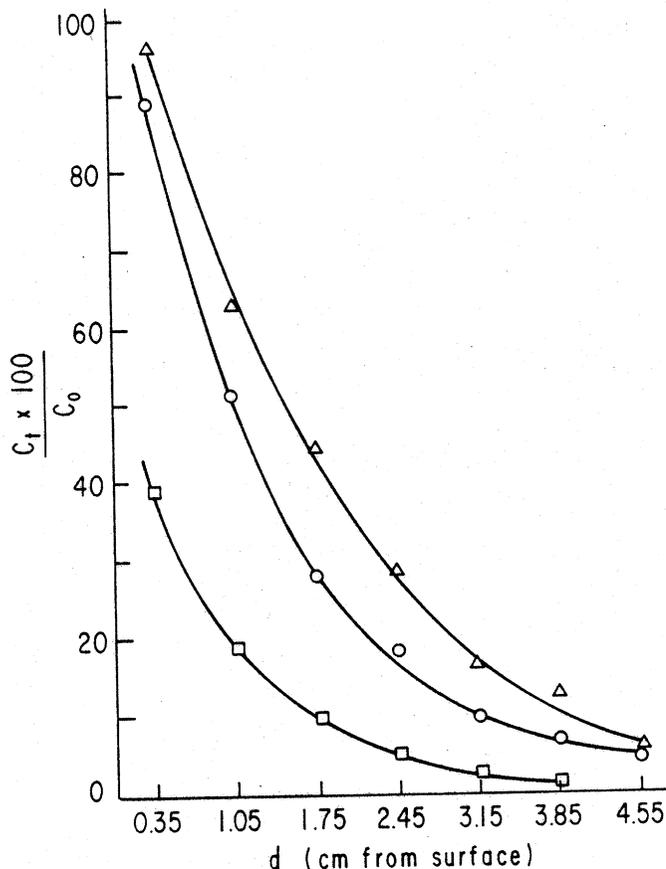


Fig. 3— $C_t \times 100/C_0$ for nitrite, chloride, and nitrate as a function of the distance from the surface of beef semitendinosus exposed to a cure composed of 4.27M sodium chloride, 36 mM sodium nitrite, and 103 mM sodium nitrate. Δ , Cl^- ; \square , NO_2^- ; \circ , NO_3^- .

Table 1—Diffusion constants at 5°C calculated from the concentration of anions at distance d from the meat surface measured across the diffusing boundary

d cm	$D \times 10^5 \text{ cm}^2 \text{ sec}^{-1}$					
	Sample 1			Sample 2		
	Cl^-	NO_2^-	NO_3^-	Cl^-	NO_2^-	NO_3^-
—	0.35	1.80	0.020	0.46	1.80	— ^a
0.12	1.05	0.36	0.048	0.19	0.26	0.43
0.14	1.75	0.41	0.084	0.19	0.23	0.38
0.13	2.45	0.39	0.12	0.24	0.27	0.34
0.15	3.15	0.37	0.15	0.27	0.30	0.37
0.19	3.85	0.45	0.19	0.34	0.32	0.41
0.25	4.55	0.45	—	0.28	0.35	0.42
—	D^b	0.405	—	0.252	0.288	0.392
		± 0.039		$\pm .057$	± 0.044	$\pm .034$

^a Measured concentration was the same as that of the brine.

^b Omitting the 0.35 cm values.

sodium chloride gradient, the nitrite may have diffused slower because of some physical changes induced by the chloride. To separate these effects, we turned to the steady state conditions of the porous disc technique. This technique was also expected to improve the precision of replicate analyses. In total uptake and diffusing boundary analyses, each diffusion constant is the result of only one analysis per meat sample since the sample is homogenized for the analysis. With the porous disc technique, a large number of deter-

Table 2—Diffusion constants at 5°C for chloride, nitrite, and nitrate by porous disc measurements

Exp	Meat	$D \times 10^5 \text{ cm}^2 \text{ sec}^{-1}$					
		Cl^-		NO_2^-		NO_3^-	
		[M]	D	[mM]	D	[mM]	D
1	Beef semi-tendinosus	4.27	0.26	36	0.16	103	0.16
			0.27		0.18		0.18
			0.25		0.16		0.17
2	Beef semi-tendinosus	4.27	0.26	36	0.17	—	—
		3.20	0.26	27	0.18		
		2.14	0.27	18	0.18		
		1.71	0.26	14.4	0.19		
		1.07	0.24	9.0	0.20		
		1.07	0.26	9.0	0.20		
		0.68	0.26	5.8	0.22		
0.34	0.26	2.9	0.25				
0.17	0.26	1.4	0.24				
3	Beef semi-tendinosus	4.27	0.26	14.4	0.17		
			0.26	9.0	0.18		
			0.26	9.0	0.18		
			0.26	4.3	0.17		
			0.26	2.9	0.15		
0.26	1.4	0.17					
4	Beef semi-tendinosus	4.0	0.25	20	0.18	10	0.17
			0.24		0.18	50	0.18
			0.25		0.18	90	0.16
			0.24		0.17	130	0.15
5	Beef semi-tendinosus + 40.7 mM STPP	3.08	0.24	32.6	0.17	26.4	0.16
			0.23		0.17		0.14
			0.24		0.18		0.15
	0.25		0.19		0.16		
6	Beef semi-tendinosus salt grade	3.08	0.28	32.6	0.16	26.4	0.20
			0.27		0.16		0.20
			0.27		0.15		0.18
			0.26		0.15		0.22
			0.26		0.15		0.22
7	Pork semi-membranosus I	3.08	0.19	32.6	0.12	26.4	0.22
			0.22		0.13		0.24
	Semitendinosus II		0.22		0.13		0.24
			0.22		0.13		0.23
	R. femorus		0.20		0.13		0.22
	L. dorsi I		0.22		0.13		0.23
	II		0.22		0.13		0.23
Back fat		0.028		0.019		—	

minations may be made on each disc. Since any set of cylinders or discs are made from one piece of meat, a larger number of determinations may be made for the discs than for the cylinders.

The results of studying a number of curing variables are shown in Table 2. The first set of data are for a triplicate run to test the precision of the technique. The coefficient of variation of the individual diffusion constants was $\pm 11\%$ ($n = 8$) and the variation of the replicate analysis was $\pm 4\%$. This precision was observed for the chloride values determined during the rest of this study. The precision of the nitrite values was somewhat lower, about $\pm 20\%$ for individual diffusion constants and $\pm 7\%$ for the cumulative values. The effect of varying the concentration of cure is shown in Experiment 2, Table 2. The concentration of both chloride and nitrite was intentionally varied simultaneously, as a first approximation to the conditions that take place during diffusion of the salts into meat, only under steady state conditions. The diffusion constant for nitrite increased at

lower nitrite and chloride concentrations. The effect was due to chloride concentration; at constant chloride concentration, the diffusion constant for nitrite remained the same with varying nitrite concentration (Experiment 3). Upon removal of the samples from the cell, swelling was evident depending on salt concentration. As the sodium chloride concentration increased, the samples fit progressively tighter in the retainer ring. At 0.34M Cl⁻, the disc, after the diffusion study, had the same degree of tightness of fit that the original sample had; at 0.17M Cl⁻, the disc was loose and fell out upon removal of the cellulose membranes.

The next three experiments show that nitrate, triphosphosphate (STPP), and different grades of salt had no significant effect on the diffusion rates. The observed differences between the corresponding diffusion constants of the three experiments may be attributed to the muscle tissue itself, since the differences were common to one whole set of samples. The last set of data on pork was determined to compare the results of the porous disc technique with values obtained by us and previous workers using the cylinder technique. Muscle type did not appear to make any difference, and the diffusion constants for chloride in pork by the porous disc method are close to those reported by Wood. Converting his value for the chloride diffusion constant from -2 to 5°C, a value of $0.18 \times 10^{-5} \text{ cm}^2 \text{ sec}^{-1}$ is obtained, compared with our values of $0.19\text{--}0.22 \times 10^{-5} \text{ cm}^2 \text{ sec}^{-1}$.

Since we do not know the variables involved in converting the diffusion constants in fat for temperature, we cannot make a direct comparison of our chloride value with that reported by Wood (1966), $0.007 \times 10^{-5} \text{ cm}^2 \text{ sec}^{-1}$. In Wood's study the rate of diffusion in fat was about 1/20 of that in muscle tissue; in our study it was about 1/10.

DISCUSSION

OUR DIFFUSION CONSTANTS for chloride in pork agree with the data of both Wood (1966) and Kőrmeny and Gantner (1958). However, Wood calculated a value of $0.41 \times 10^5 \text{ cm}^2 \text{ sec}^{-1}$ from the data of Wistreich et al. (1959) and hypothesized that fat content may have been a controlling factor since Wistreich et al. used leg muscles which, presumably, were lower in fat content. Both we and Kőrmeny and Gantner used semitendinosus, a leg muscle, and our disc diffusion data are in agreement with those of Wood. Furthermore, we did not find any difference in rate of diffusion of chloride with different pork muscles. Fat streaks were present in varying quantities in both the discs and cylinders of meat but the observed variation in adipose tissue content had no effect on the calculated diffusion constants. The answer to this point could be obtained only by studying muscle tissues with different intracellular fat contents.

Diffusion constants for chloride and nitrite in pork were lower than the corresponding constants for beef, but the nitrate diffusion constants were higher, especially by comparison with the free diffusion constants. The values of the free diffusion constants for chloride and nitrate at 5°C are 0.84 and $0.79 \times 10^{-5} \text{ cm}^2 \text{ sec}^{-1}$, respectively (International Critical Tables, 1927, corrected for temperature). As discussed below, chemical reaction did not appear to be a factor in the diffusion of nitrite in the disc experiments, hence the high values for nitrate are not the result of conversion of nitrite to nitrate. Diffusion may be hindered (Crank, 1967) or facilitated (Wittenberg, 1965; Margalit and Schejter, 1976) by interaction with specific groups in the substrate. Of the two ions, chloride and nitrate, the latter would be expected to be the least affected by interactions, since from their positions in the Hofmeister series, chloride reacts more strongly with proteins than does nitrate (Jencks, 1969). Because of the muscle-to-muscle variation, it is not

evident which of the possible effects of tissue swelling, protein denaturation, ion binding, and/or chemical reaction cause the variations in the rate of diffusion. If nitrate diffuses with little or no tissue component interaction, then, on an average, its diffusion would be the most uniform and fastest, which may be the basis for Jensen's (1942) statement concerning increased permeability of meat tissues with nitrate. However, our results do not show any effect of nitrate on facilitating the diffusion of chloride or nitrite.

The increase in diffusion rate of nitrite with decreasing chloride concentration in the disc experiments is a physical phenomenon rather than a chemical one. Irreversible chemical reaction of solute with substrate results in a reduction of the measured diffusion rate (Crank, 1967), as observed in the boundary analysis experiments. In the steady state with a sample of finite thickness, chemical reaction during diffusion would result in an upward curving of the diffusion curve with time, due to slowing of the reaction, allowing more nitrite to diffuse. Since the curves were linear with time at all concentrations and the nitrite diffusion constant did not vary with nitrite concentration at constant chloride concentration, we conclude chemical reaction was not the controlling factor for steady state diffusion. The physical change involved is related to tissue swelling. Kőrmeny and Gantner (1958) observed the swelling due to the salt, determined the diffusion rate in tubes of two different radii and concluded that size of tube did not affect the diffusion rate. This conclusion does not mean that swelling of tissue due to salt did not have an effect on the rate of diffusion. The relative amount of tissue compression induced by swelling would be the same in any area size, since the expansion in any dimension is a proportion of the magnitude of the dimension. This is true even in the relatively large surface area of the discs. Our results show that tissue swelling is a factor in the rate of diffusion. Chloride itself did not show the effect of increased resistance to diffusion, since the diffusion constant did not vary with distance. The result was probably due to two factors operating across the boundary, the first being a slowing of diffusion due to chloride interaction with the substrate. After the chloride had saturated the active sites, the diffusion would then be slowed by the increased resistance to flow due to the swelling, the two processes resulting in the rate of diffusion remaining constant across the boundary. The maximal effect was attained at chloride concentrations between 1.5 and 2.0M, which suggests an interaction with the peptide bonds of the muscle proteins because this is the approximate concentration of the bonds. A partial unfolding of the peptide chain by such an interaction would explain the observed swelling of the tissues.

The nitrite diffusion constant calculated for the total uptake experiments fell to $0.12 \times 10^{-5} \text{ cm}^2 \text{ sec}^{-1}$ or lower as measured across the boundary from lower to higher nitrite concentrations. This value is lower than the constant for the steady state, $0.18 \times 10^{-5} \text{ cm}^2 \text{ sec}^{-1}$. Since the former represents diffusion into an infinite substrate with an excess of tissue reactants available, the difference in the diffusion constants represent nitrite reacted with tissue components. Based on calculation of the theoretical diffusion curve for the two experiments of Table 2 with $D = 0.18 \times 10^{-5} \text{ cm}^2 \text{ sec}^{-1}$, the loss of nitrite through reaction with tissue components was calculated to be 50% for sample 1 and 30% for sample 2 at 1.05 and 1.75 cm for each sample. The value of $D = 0.11 \times 10^{-5} \text{ cm}^2 \text{ sec}^{-1}$ for the total uptake experiment indicates a 30% loss of nitrite in that experiment also. These values agree with losses of 25–35% calculated by various authors from total nitrite analysis (Sebranek et al., 1978; Emi-Miwa et al., 1976).

Effect of phosphate

In addition to Experiment 5 in Table 2, other disc dif-

fusion experiments, as well as diffusing boundary analysis, also showed no difference between the rates of diffusion of either chloride or nitrite with or without phosphate. Conversely, Krause et al. (1978) reported that sodium tripolyphosphate increased the rate of diffusion of nitrite. However, there was a high loss of nitrite in their samples without phosphate which, as shown in the diffusing boundary analyses, can result in an apparent lowering of the rate of diffusion. The samples with phosphate showed little loss of nitrite, which may have been the result of raising the pH of the cure. Kőrmeny and Gantner (1958) had reported larger diffusion constants at higher pH values, so we adjusted the pH of our cure solutions to that of the meat to eliminate the effect.

The decrease in the rate of diffusion due to tissue swelling may explain the phenomenon observed by Ockerman and Organisciak (1978). They were following the rate of diffusion by the increase in area of cure penetration from a point source of cure injection, and found that after about 10 hr the rate of diffusion began to increase. This observation may be explained on the basis of an initially slow diffusion due to localized internal swelling caused by high salt concentrations at the site of injection. A lowering of the salt concentration due to diffusion would decrease the swelling and result in faster salt diffusion with time.

SUMMARY

THE RESULTS of this study show that the diffusion of nitrite, as measured by Griess analysis, is governed principally by two factors, one physical, due to changes induced in the tissue by chloride, and the second chemical, caused by reaction of nitrite with endogenous tissue components. The first may be an artifact due to confinement of the tissue sample. From the results of this and other studies, diffusion of salts in meats has been found to be a variable process, dependent upon specific interactions between individual meat samples and the diffusing ions.

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