

Diffusion of Nitrite and Nitrate Salts in Pork Tissue in the Presence of Sodium Chloride

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ABSTRACT:The objective of the present work was to analyze the effect of NaCl concentration on the diffusion coefficients of NaNO_2 and KNO_3 in pork tissue using unsteady techniques. Concentrations of nitrite and nitrate as a function of immersion time were determined in cylinders of meat using spectrophotometric techniques; experiments were performed at 4 °C and 20 °C. The unsteady state mathematical formulation was applied considering radial mass transfer. The diffusion coefficients were obtained fitting the theoretical curves to experimental data. An increase of the salt diffusivities in the tissue was observed when the diffusion occurred at high NaCl concentrations. This fact could not be explained by the Maxwell-Stefan theory. Penetration of high NaCl concentration in a tissue may affect the cellular structure leading to higher diffusion coefficients.

Keywords: diffusion, nitrite, nitrate, sodium chloride, meat curing

Introduction

CURING PROCESSES TRADITIONALLY UTILIZE sodium chloride, nitrite, and nitrate salts. Nitrite affects the flavor, has antimicrobial action, and it is responsible for the red color in cured meat due to the combination of nitrogen oxide with myoglobin. Nitrite shows a toxic action affecting blood pigments and vitamin A; regulations controlling the use of curing agents were established in the United States in 1926 (USDA CFR 9.318.7), and the same rules are in effect at present, with slight modifications. The critical feature of these rules is that a maximum use level of sodium nitrite is defined, but meat processor may use less. Basically, no more than 0.25 oz (7.1 g) may be used per 100 lb (45.4 kg) of meat (resulting in 156 mg/kg or 156 ppm) (Borchert and Cassens 1998).

Nitrate was incorporated to the brine as it was found (Grau 1965) that nitrite is formed by bacterial reduction of nitrate. Sodium chloride acts as preservative and modifies water-holding capacity of the proteins.

Curing salts may be used in different ways, according to the desired final product. One of the methods used by the industry is the immersion of the meat cut in brines. In this case, salt penetration is related to the equilibrium between the salt concentration in the interior of the meat piece and the external brine solution. Diffusion of salt in solid foods such as pork, beef, fish, and cheese has been studied by many workers (Fox 1980; Djelveh and Gros 1988; Dussap and Gros 1980; Wood 1966). For the controlled manufacture of these products, it is important to know the factors influencing salt penetration and to be able to predict the

diffusion rate. Salt penetration is largely governed by the concentration gradient and the temperature (Djelveh and others 1988). Deviations from Fick's law are observed for systems with and without electrolytes, when multicomponent diffusion is produced (Crank 1957). The diffusive flux of each component is affected by the other components of the solution. The effective diffusivities of the ionic species can be calculated by using Nernst-Planck equation as a particular case of the Maxwell-Stefan (Taylor and Krishna 1983).

The objectives of the present work were: a) to determine the diffusion coefficient of sodium nitrite and potassium nitrate in pork tissue (*Longissimus dorsi*) using brine solutions at 4°C and 20°C; b) to establish the effect of sodium chloride on the diffusion coefficients and on tissue microstructure; c) to apply the Stefan-Maxwell theory in electrolyte solutions to calculate diffusion coefficients in the presence of NaCl; and d) to relate the diffusion coefficients obtained with those predicted by Stefan-Maxwell theory.

Materials & Methods

RADIAL (UNIDIRECTIONAL) DIFFUSION system was adopted using long cylinders of *Longissimus dorsi* pork tissue (10 cm length and approximately 1.5 cm diameter) that were immersed in brines of different composition containing sodium chloride, sodium nitrite, and potassium nitrate (Table 1). The solutions were vigorously stirred to assure constant solute concentration at the solid-fluid interface. At different times, 2 cylinders were taken from the brine; the end zones of the cylinders were eliminated to

avoid border effects; only the central zone of each cylinder (2.5 cm length) was retained. The content of nitrite or nitrate present in the samples was determined at different contact times. Each run lasted about 3 h and experiments were performed at 4 °C and at 20 °C in thermostatic chambers. In each test, the water content of the tissue was measured in duplicate, first initially and at again the end of the experiment.

Equilibrium concentrations of salts were obtained by immersion of small tissue samples in the different brines for at least 48 h.

Nitrite determination

Meat tissue was homogenized in an Omni-mixer equipment (Sorvall Omni-Mixer 17106, DuPont Instruments, Newtown, Conn., U.S.A.); bidistilled water at 90 °C and 10 ml HgCl_2 saturated solution were added to denature proteins. The suspension was stirred for 10 min, diluted with water, and filtered; 25 ml of the final solution was taken and Hach kit Nitrivier 3, (method 371) for nitrite determination was used. After reacting for 15 min, nitrite concentration was measured at 507 nm in a Hach spectrophotometer (Hach Co., Loveland, Colo., U.S.A.) (DR/2000).

Nitrite in the sample reacts with sulfanilic acid, forming an intermediate diazonium salt. This couples with chromotropic acid to produce a pink colored complex (Griess reaction) directly proportional to the amount of nitrite present (Girard 1991).

Nitrate determination

Similar extraction procedures were fol-

lowed for nitrate determination. The nitrate in the final solution was reduced to nitrite using cadmium (Hach kit Nitrover 6, method 351), and then nitrite was measured as explained previously. In this case, mercuric chloride was omitted because it interfered with the determination.

Electron microscopy

Small pieces of tissue (0.5 cm in diameter and 0.2-0.3 cm thick) were used for electron microscopy analysis. The tissue samples were immersed in different brines (G, H, I, J on Table 1) during at least 48 h, until equilibrium concentrations were obtained. The samples were fixed with Carnoy fluid (60% ethyl alcohol, 30% chloroform, glacial 10% acetic acid, v/v) at 4 °C for 24 h. Samples were dehydrated using gradually increasing concentrations of ethyl alcohol: 70 % (12 h), 95 % (2 h) and 100% (2 h). Samples were mounted on aluminium stubs using double-sided tape and then coated with a layer of gold (40-50 nm), allowing surface and cross-section visualization. Micrographs of the samples were obtained with an electron microscope (SEM 505, Philips, Eindhoven, The Netherlands).

Mathematical Model

Mass transport of a solute from the surface towards the center of food tissue can occur through the extracellular space and/or through the cell walls and membranes. Due to the complex nature of food tissues, it is difficult to predict which of these transport mechanisms take place, or which are the parameters that control the transport rate in each case. Besides, foodstuffs usually have a strongly heterogeneous structure and exact analytical solutions cannot always be found because of wide variations of the diffusivity of the penetrating substance in the various parts of multilayer cellular systems. Moreover, it is sometimes difficult to have a precise idea of the volume into which the solute can diffuse and of the diffusion path it takes (Gros and others 1984). That is the reason why an effective diffusion coefficient which can be determined experimentally is normally used an effective diffusion coefficient, which can be determined experimentally (Stahl and Loncin 1979; Schwartzberg and Chao 1982; Zartitzky and Califano 1999).

Meat tissue can be considered as an insoluble matrix and an aqueous phase through which the solute diffuses. For an infinite cylinder, the diffusion mechanism in symmetric porous materials can be expressed in the general form of the microscopic mass balance in terms of an effective matrix diffusivity D_m :

$$\frac{\partial C}{\partial t} = \frac{1}{r} \frac{\partial}{\partial r} \left(r D_m \frac{\partial C}{\partial r} \right) \quad (1)$$

where: C is the solute concentration in the solid as a whole expressed as the weight of solute per unit weight of tissue; r , the radius of the cylinder; D_m , the effective diffusion coefficient in the tissue (m^2/s) and t time (s). The solution of this equation depends on the boundary conditions. When the solid material, initially containing C_0 concentration of the solute (expressed as weight of solute per unit weight of tissue), is brought suddenly (at $r = R$) into contact with a well-stirred solution of constant solute concentration C_f at the interface, the boundary conditions are as follows:

$$\begin{aligned} t = 0 \quad C = C_0 \quad 0 \leq r \leq R \\ t > 0 \quad C = C_f = \varepsilon \cdot C_f \quad r = R \\ t > 0 \quad \frac{\partial C}{\partial r} = 0 \quad r = 0 \end{aligned} \quad (2)$$

where C_f is the bulk concentration of the solute in the dipping solution (weight of solute per unit volume of solution), ε is the ratio of the equilibrium distribution between the solute concentration in the dipping solution (C_f) and the solute concentration in the solid (C_f) at the interface. If the solute is diffusing in the solid matrix through the aqueous liquid phase, the value of ε is related to the tissue water content (vol water/weight of tissue).

The following analytical solution for Equation 1 is obtained given the boundary conditions expressed in (2) (Crank 1957):

$$\frac{C - C_f}{C_0 - C_f} = 1 - 2 \sum_{n=1}^{\infty} \frac{\exp(-D_m \alpha_n^2 t) J_0(\alpha_n R)}{\alpha_n J_1(\alpha_n R)} \quad (3)$$

$R \cdot \alpha_n$ are the n -roots of equation $J_0(R \alpha_n) = 0$ being J_0 the zero order and J_1 the 1st order Bessel's functions.

The total uptake at a given time t , $M(t)$ is calculated by integrating Eq. 3 between 0 and R .

$$M(t) = M(\infty) \left(1 - \sum_{n=1}^{\infty} \frac{4 \exp(-D_m \alpha_n^2 t)}{R^2 \alpha_n^2} \right) \quad (4)$$

where $M(t)$ is the mass of solute that enters in the tissue during time t expressed as g

solute/g meat tissue; $M(\infty)$ is the maximum amount of solute that could enter at infinite time and corresponds to the concentration of salt in equilibrium with the external solution

A computer program (Mathcad 7 Professional, MathSoft Engineering & Education, Inc., Cambridge, Mass., U.S.A.) was used to calculate diffusion coefficients of nitrite and nitrate in the presence of NaCl. The mass that entered the tissue at a given time $M(t)$, and the maximum amount of solute that would be entered at infinite time $M(\infty)$, were used to determine the experimental values of the dimensionless total uptake $M^* = M(t)/M(\infty)$. Diffusion coefficients were proposed and predicted values of M^* were obtained. In the numerical simulation the diameter of each sample at the end of the experiment was considered. The value of diffusivity that lead to a minimum sum of the residues and of the square residues was selected by an iterative procedure. Residue was considered as the difference between the experimental and the calculated values of M^* .

Estimation of the effective diffusivity of ionic species in multicomponent electrolyte solution by the Maxwell-Stefan theory

When multicomponent diffusion occurs, deviations of Fick's law are observed; the diffusive flux of each component is affected by the other components of the solution. To analyze the effect of NaCl on the diffusion coefficient of sodium nitrite and potassium nitrate, the Maxwell-Stefan's theory was applied (Taylor and Krishna 1983). Mass transfer in electrolytic solutions requires a description of the movement of mobile ionic species, material balances, current flow, electroneutrality, and fluid mechanics. Solutes do not diffuse independently; a diffusion potential is established and ions interact with it.

In mixtures of electrolytes, the generalized Maxwell-Stefan equation is written for each of the ionic and non-ionic (that is, solvent) species in the system.

$$d_i = \sum_{j=1}^n \frac{x_i x_j (u_i - u_j)}{D_{ij}} \quad (5)$$

$$= \sum_{j=1}^n \frac{(x_i N_j - x_j N_i)}{c_t D_{ij}}$$

where d_i is the generalized driving force; n is the number of components; x_i, x_j denote ionic mole fractions; u_i, u_j are the velocities of components i, j ; c_t total concentration; N_i, N_j

Table 1—Effective diffusion coefficients of sodium nitrite and potassium nitrate ($D_{m_{\text{salt-tissue}}}$) obtained from experiments using pork tissue immersed in saline solutions of different compositions

Key to solution composition	Immersion solutions	NaNO_2 $D_{m_{\text{salt-tissue}}}$ $\cdot 10^9 \text{ m}^2/\text{s}$	
		4 °C	20 °C
A	NaNO_2 3 g/l	0.38 ^a	0.41 ^a
B	NaNO_2 3g/l + KNO_3 2.5g/l	0.35 ^a	0.45 ^a
C	NaNO_2 3g/l + NaCl 10g/l	0.40 ^a	0.56 ^b
D	NaNO_2 3g/l + NaCl 70g/l	0.54 ^b	0.74 ^c
E	NaNO_2 3g/l + NaCl 140g/l	0.65 ^c	0.85 ^d
F	NaNO_2 3g/l + NaCl 140g/l + KNO_3 2.5g/l	0.70 ^c	0.78 ^{c,d}

Key to solution composition	Immersion solutions	KNO_2 $D_{m_{\text{salt-tissue}}}$ $\cdot 10^9 \text{ m}^2/\text{s}$	
		4 °C	20 °C
G	KNO_3 2.5g/l	0.41 ^a	0.42 ^a
H	KNO_3 2.5g/l + NaCl 10g/l	0.40 ^a	0.45 ^a
I	KNO_3 2.5g/l + NaCl 70g/l	0.69 ^c	0.82 ^d
J	KNO_3 2.5g/l + NaCl 140g/l	0.88 ^d	0.95 ^e

a, b, c, d, e: means within columns without common superscript letters differ ($p < 0.05$) as calculated by Least Significant Difference test (LSD)

molar fluxes; D_{ij} Maxwell–Stefan’s diffusivities.

The external electrical force per mole acting on species i , F_i , is given by:

$$F_i = -z_i F \nabla \phi \quad (6)$$

where z_i is the ionic charge of the species, F is Faraday’s constant = $9.65 \cdot 10^4$ Coulomb/mol and ϕ is the electrical potential measured in volts. The generalized driving force, when the condition of electroneutrality is met, becomes (Newman 1991)

$$c_i RT d_i = c_i \nabla_{T,P} \mu_i + c_i z_i F \nabla \phi \quad (7)$$

where c_i is the molar concentration of component i , μ_i the chemical potential, R the ideal gas constant and T absolute temperature.

Neglecting friction between ions and considering interaction with water, in dilute electrolyte systems, the driving force reduces to:

$$d_i = \nabla x_i + x_i z_i \frac{F}{RT} \nabla \phi \quad (8)$$

that leads to the Nernst–Planck equation in the absence of convection:

$$N_i = -D_i^0 \nabla c_i + c_i z_i D_i^0 \frac{F}{RT} \nabla \phi \quad (9)$$

where D_i^0 is the ionic diffusion at infinite dilution in water (Taylor and Krishna 1983). The ionic diffusivity in the solution can be calculated as follows:

$$D_{i,\text{solution}} =$$

$$D_i^0 - \frac{t_i}{z_i} \sum_{j=1}^{n-1} z_j D_{j,n}^0 \frac{\nabla c_j}{\nabla c_i} \quad (10)$$

where: the maximum concentration differences $\Delta c_j / \Delta c_i$ was used to estimate $\nabla c_j / \nabla c_i$; t_i is the transference number ($t_i = K_i / K$) with K_i equivalent electric conductivity of species i and $K = \sum K_i$

The equivalent conductivity of the ionic species can be calculated as follows:

$$K_i = \frac{F^2}{RT} c_i z_i^2 D_i^0 \quad i = 1, \dots, n \quad (11)$$

The diffusion coefficient of a salt in water (binary electrolyte solution, at infinite dilution) becomes:

$$D_{\text{salt}} = \frac{(z_+ - z_-) D_+ D_-}{z_+ D_+ - z_- D_-} \quad (12)$$

where D_+ and D_- are the ionic diffusivities in water at infinite dilution (D_i^0) for each ion, normally available in literature.

To estimate the diffusion coefficient of a salt in a solution containing other ions (in conditions different from an infinite dilution), D_+ and D_- correspond to the ionic diffusivities of such ions in the solution ($D_{i,\text{solution}}$) and have to be calculated using Eq. 10.

Results & Discussion

THE WATER CONTENT OF THE SAMPLE DID not change significantly with increasing times (approximately 3 h); the mean water content in the tissue ranged between 72 and 74 %.

In previous works (Pinotti and others 2000), it was verified that the maximum NaCl concentration (140 g/l) did not interfere with the determination of nitrite and nitrate in model solutions and in solutions extracted from meat tissue. Equilibrium concentrations of the salts in the tissue (C_f) were determined after immersion of small tissue samples in the tested solutions at different concentrations C' . The ratio between C_f and C' was given by ε , considering ε as the water content in the tissue.

Diffusion coefficients of nitrite and nitrate

Figure 1 a and 1 b shows the experimental and predicted values of the dimensionless nitrite concentration in the tissue as a function of time for brine solutions containing: a) NaNO_2 3g/l (A) and b) NaNO_2 3g/l + NaCl 140 g/l (E) at 20 °C. Figure 2 a and 2 b corresponds to nitrate diffusion in brine solutions containing: KNO_3 2.5 g/l + NaCl 10 g/l (H) and KNO_3 2.5 g/l + NaCl 70 g/l (I) at 4 °C. A satisfactory agreement between experimental and predicted dimensionless total uptake values (M^*) was observed.

Diffusion coefficients in meat tissue for different solutions tested are shown in Table 1. At each temperature, differences between diffusivities were nonsignificant between solutions A, B, C, G, and H. The absence or a low NaCl concentration did not affect curing salts diffusivities in the tissue.

A marked increase of the curing salt diffusivities in the tissue was observed when the diffusion occurred at high NaCl concentrations (70 and 140 g/l). The presence of high NaCl concentrations in the curing solutions increased the effective diffusivities of nitrite and nitrate in comparison to the solution without sodium chloride. At 20 °C the diffusion coefficients of the curing salts in the tissue ($D_{m_{\text{salt-tissue}}}$) were higher than at 4 °C, however nonsignificant differences were observed for solution A and G.

Diffusivities calculated by Maxwell-Stefan equation:

To verify if the effect of NaCl on nitrite and nitrate diffusion coefficients can be explained by the Maxwell-Stefan theory, the diffusivities of the ions and of the salts in water and in the ionic solutions were calculated (Taylor and Krishna 1983). For the calculations, code numbers were assigned to

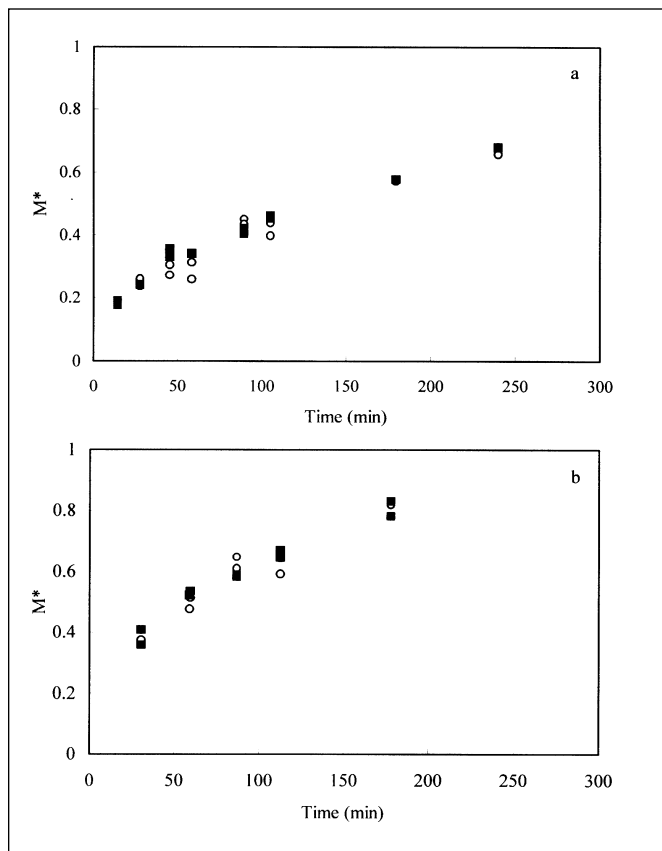


Figure 1—Dimensionless nitrite concentration in the pork tissue as a function of time for a brine solution containing: a) NaNO₂ 3g/l and b) NaNO₂ 3g/l + NaCl 140 g/l at 20 °C (O = experimental and ■ = predicted values)

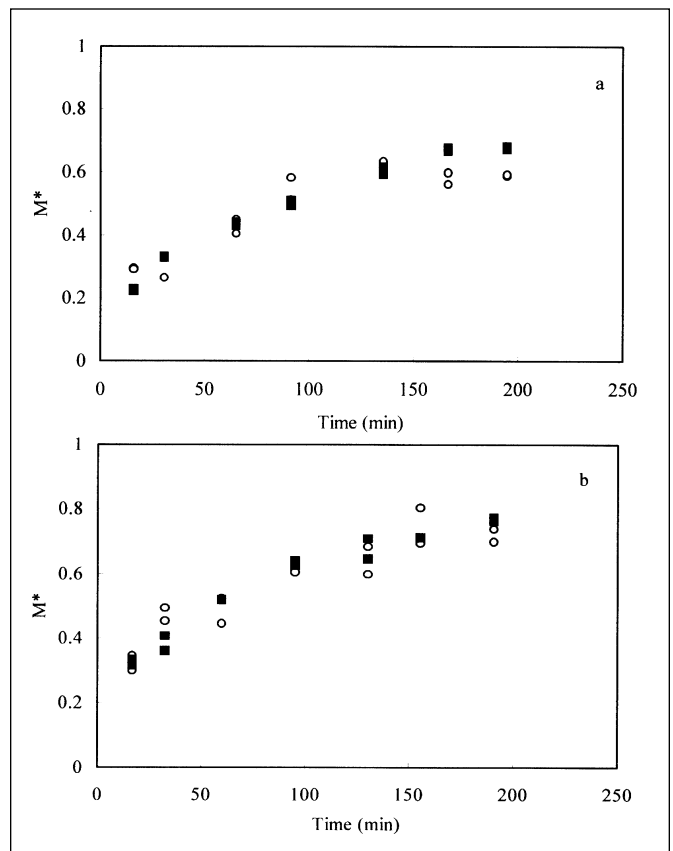


Figure 2—Dimensionless nitrate concentration in the pork tissue as a function of time for a brine solution containing: a) KNO₃ 2.5 g/l + NaCl 10 g/l and b) KNO₃ 2.5 g/l + NaCl 70 g/l at 4 °C (O = experimental and ■ = predicted values)

each component of the system: NO₂⁻ = 1, Na⁺ = 2, NO₃⁻ = 3, K⁺ = 4, Cl⁻ = 5, H₂O = 6.

As an example, we describe the estimation of the diffusion coefficient of sodium nitrite in water at 25 °C. Applying Eq. 12, this diffusion coefficient was calculated using reported data of D₀ⁱ values in water (at infinite dilution) at 25 °C (Lide 1997) (Table 2, left column); the obtained value was: D_{NaNO₂-H₂O} = 1.571.10⁻⁹ m²/s. Similarly for potassium nitrate in water the D_{KNO₃-H₂O} = 1.929.10⁻⁹ m²/s

To calculate the diffusion coefficient of sodium nitrite in a solution containing NaNO₂ 3 g/l and NaCl 140 g/l (solution E, Table 1), Eq. 12 was also applied, however D_{i,solution} values are not available in literature and have to be calculated using Eq. 10. Thus, the previous calculation of the maximum concentration differences, equivalent conductivities, and transference numbers is required.

In the selected example, for each ion (NO₂⁻, Na⁺, Cl⁻) the maximum differences between the concentration in the external solution and in the diffusing medium (Δc)

were calculated considering that initially the medium had zero ion concentration, thus:

$$\Delta c_1 = 0.043 \text{ mol/l}; \Delta c_2 = 2.441 \text{ mol/l}; \Delta c_5 = 2.393 \text{ mol/l}$$

The equivalent conductivity values K_i calculated by Eq. 11 were: K₁ = 0.3123 mho/m; K₂ = 12.232 mho/m; K₅ = 18.300 mho/m; then K = Σ K_i = 30.843 mho/m.

The transference numbers (t_i = K_i/K) for each ion were:

$$t_1 = 0.0102, t_2 = 0.3965; t_5 = 0.5933$$

Applying Eq. 10, the following results (shown in Table 2) were obtained.

$$D_{\text{NO}_2^- \text{-solution E}} = D_{1 \text{-solution E}} = D_{1-6}^0 \cdot (t_1 / z_1 Dc_1) (z_1 D_{1-6}^0 Dc_1 + z_2 D_{2/6}^0 \Delta c_2 + z_5 D_{5-6}^0 Dc_5) = 1.517 \cdot 10^{-9} \text{ m}^2/\text{s}$$

In a similar way, D_{Na⁺-solution E} = D_{2-solution E} was calculated obtaining 1.612.10⁻⁹ m²/s.

Applying Eq. 12, the diffusion coefficient

of sodium nitrite in the solution E was calculated (Table 3), using the previously calculated ionic diffusivities in solution (Table 2, right column); being z₊ = 1, z₋ = 1, D₊ = 1.612.10⁻⁹ m²/s, D₋ = 1.517.10⁻⁹ m²/s, then:

D_{NaNO₂-solution E} = 1.563.10⁻⁹ m²/s, that is lower than the value 1.571.10⁻⁹ m²/s calculated previously for diffusion of the salt in water at the same temperature.

Following the same procedure, the diffusion coefficient of potassium nitrate in a solution containing KNO₃ 2.5 g/l and NaCl 140 g/l (solution J) was D_{KNO₃-solution J} = 1.843.10⁻⁹ m²/s (lower than D_{KNO₃-H₂O} = 1.929.10⁻⁹ m²/s).

The obtained results show that the diffusion coefficients of the salts in the solution containing NaCl were lower than the diffusion coefficients at infinite dilution in water at the same temperature, thus the consideration of ion migration by the Maxwell-Stefan theory did not explain the higher effective diffusivities of nitrite and nitrate when NaCl is present in the system.

Table 2—Diffusion coefficients of ions in water at infinite dilution (D°) and ionic diffusivities in solution ($D_{i, \text{solution}}$) at 25 °C.

Diffusion coefficient of ions in water at infinite dilution $D^\circ \cdot 10^9 \text{ (m}^2/\text{s)}$ (Lide, 1997)	Effective ionic diffusivity $D_{i, \text{solution}} \cdot 10^9 \text{ (m}^2/\text{s)}$ (calculated by Eq. 10)
$\text{NO}_2^- \text{-H}_2\text{O}$: 1.912	$\text{NO}_2^- \text{- solutionE}$: 1.517
$\text{Na}^+ \text{-H}_2\text{O}$: 1.334	$\text{Na}^+ \text{- solutionE}$: 1.612
$\text{NO}_3^- \text{-H}_2\text{O}$: 1.902	$\text{NO}_3^- \text{- solutionJ}$: 1.513
$\text{K}^+ \text{-H}_2\text{O}$: 1.957	$\text{K}^+ \text{- solutionJ}$: 2.358
$\text{Cl}^- \text{-H}_2\text{O}$: 2.032	

SolutionE: NaNO_2 3 g/l + NaCl 140 g/l
 SolutionJ: KNO_3 2.5 g/l + NaCl 140 g/l

Effect of NaCl on the tissue microstructure

The higher values of the diffusion coefficients of nitrite and nitrate when NaCl also diffuses in the meat tissue could be attributed to the influence that NaCl has on fiber microstructure. Experiments using cylinders of pork tissue, which had been previously immersed for 48 h in aqueous solutions containing 0, 10, and 70 g/l NaCl and then immersed in solutions containing sodium nitrite and sodium chloride (Table 4) showed high values of the diffusion coefficients at both 4 °C and 20 °C. These results confirm that not only the simultaneous diffusion of NaCl and nitrite or nitrate lead to higher diffusion rates of the curing salts, but the same phenomenon was observed when the meat was previously equilibrated with NaCl solutions and then submitted to curing solutions containing NaNO_2 and NaCl. In this experiment, when the tissue was submitted twice to NaCl, the diffusion coefficients of sodium nitrite (Table 4) were higher than the corresponding values in Table 1.

The penetration of a chemical preservative in a tissue may affect its microstructure leading to diffusion coefficients that depend on the concentration of the diffusing solute. Related to this last aspect, it was reported that especially high levels of NaCl alter animal and vegetable tissues, producing higher diffusion coefficients (Drusas and others 1988; Cho and others 1988).

The effective diffusion coefficient in a complex matrix such as the meat tissue ($D_{\text{salt-tissue}}$) is proportional to the molecular diffusion coefficient in water ($D_{\text{salt-H}_2\text{O}}$). Different coefficients have to be considered in the following relationship:

$$D_{\text{salt-H}_2\text{O}}/D_{\text{salt-tissue}} = f(\varepsilon, \xi, \psi) \quad (13)$$

where ε is the fraction of the solvent in the tissue, ξ is the degree of obstruction in the matrix, and ψ is the interactions between matrix-solvent-and solute (Díaz and others 1993).

In Table 5, the values of $D_{\text{salt-H}_2\text{O}}$ at 20 °C are shown in the 1st column. They were calculated using data at 25 °C (Table 3, 1st column) and applying the Stokes-Einstein equation: $D \cdot \mu/T = \text{constant}$ (where m is the water viscosity and T the absolute temperature) in order to make comparisons with $D_{\text{m-salt-tissue}}$ obtained in the present work from the experiments at 20 °C (Table 1).

Relating the diffusivity of sodium nitrite in the meat tissue, ($D_{\text{NaNO}_2\text{-tissue}} = 0.41 \cdot 10^{-9} \text{ m}^2/\text{s}$, Table 1) with the molecular diffusivity in water ($D_{\text{NaNO}_2\text{-H}_2\text{O}} = 1.373 \cdot 10^{-9} \text{ m}^2/\text{s}$), both at 20 °C the obtained f value was 3.35. A value of $f = 3.4$ was reported by Dussap and Gros (1980) for fresh meat at 2 °C.

In the presence of NaCl 140 g/l (solution E) and considering data of Table 5, the ratio of the diffusivities was lower ($f = 1.62$). In the case of potassium nitrate, the values of f were 4.01 without NaCl (solution G) and 1.77 in the presence of NaCl 140 g/l (solution J). As can be observed, the value of f decreases when NaCl is present in the tissue; this fact could be attributed to a decrease of the obstruction degree in the meat tissue.

The effect of NaCl in meat tissue was extensively addressed in literature and it strongly depends on the concentration levels of the salt (Grau 1965; Offer and Trinick 1983; Cheftel and others 1993). Meat tissue treated with increasing concentrations of NaCl shows important changes. At low NaCl concentrations, swelling of the fibers, and high values of water holding capacity are observed. The amount of water bound to proteins increases with increasing NaCl concentration up to 6-9 g/l (salting-in). Concentrations between 45-60 g/l NaCl produce maximum fiber-swelling in meat tissue (Offer and Trinick 1983). The increase in water holding capacity might be attributed to the lateral expansion of myofibrils, which is coupled to protein solubilization. These levels of NaCl irreversibly alter cell structure affecting water binding by proteins because of their effects on electrostatic interactions.

Table 3—Diffusion coefficients of salts in water ($D_{\text{salt-H}_2\text{O}}$) and diffusion coefficients of salts in solutions ($D_{\text{salt-solution}}$) calculated by Stefan-Maxwell theory at 25 °C.

$D_{\text{salt-H}_2\text{O}} \cdot 10^9 \text{ (m}^2/\text{s)}$	$D_{\text{salt-solution}} \cdot 10^9 \text{ (m}^2/\text{s)}$
$D_{\text{NaNO}_2\text{-H}_2\text{O}}$: 1.571	$D_{\text{NaNO}_2\text{-solutionE}}$: 1.563
$D_{\text{KNO}_3\text{-H}_2\text{O}}$: 1.929	$D_{\text{KNO}_3\text{-solutionJ}}$: 1.843

When NaCl concentrations ranged between 100-120 g/l the phenomenon is reversed, fiber volume decreased, the tissue lost its own water and proteins precipitated causing disruption in the matrix. At high salt concentrations, the water holding capacity decreases due to the salting-out effect which implies a strong bond between water and salt and the subsequent protein dehydration. The juice that is released by the meat tissue when NaCl concentrations are very high contains water soluble mineral salts, non protein nitrogen compounds, albumine, and globuline dissolved in the sarcoplasm and myoglobin pigments (Grau 1965); the electrical double layer surrounding the protein molecules is suppressed. Higher NaCl concentration causes important modifications of the proteins and fibers reached the maximum water losses.

SEM micrographs of pork tissue treated with different solutions were compared to analyze microstructural changes due to the presence of NaCl. Figure 3a corresponds to a sample without treatment (control); Figure 3b, c, d show the tissue treated with solution G (KNO_3 2.5 g/l), solution I (KNO_3 2.5 g/l + NaCl 70 g/l) and solution J (KNO_3 2.5 g/l + NaCl 140 g/l) respectively. The samples treated with KNO_3 2.5 g/l did not show significant differences with respect to the control. Fibers in Figure 3c submitted to KNO_3 2.5 g/l + NaCl 70 g/l show swelling. NaCl 140 g/l (Figure 3d) produced fragmented and dehydrated fibers; in that condition of high NaCl concentration the highest diffusion coefficients of potassium nitrate were measured in the tissue because of the decrease in the degree of obstruction in the matrix. These results agree with the findings previously reported in literature.

In industrial processes, diffusion of curing salts occurs in meat cuts of considerable size, where a NaCl concentration gradient in the tissue progressively affects its microstructure increasing the diffusivity of the salts. If meat cuts are left in the brine for the time predicted using the nitrite diffusion

coefficient calculated in the absence of NaCl, the actual concentration attained by the meat, would be higher than the maximum permitted values for nitrite.

Conclusions

THE HIGHER DIFFUSION COEFFICIENTS OF curing salts in the presence of sodium chloride could not be explained on the basis of the electrical forces due to the ions present in the system. The consideration of ions migration by application of Stefan-Maxwell theory did not explain the higher effective diffusivity values in the tissue. However the microstructural changes due to a decrease of the obstruction degree in the meat tissue, as shown by the SEM micrographs, could explain the rise of nitrite and nitrate diffusion coefficients in the presence of sodium chloride. These results can be used by the industry to optimize the curing process in brine solutions.

References

Borchert L, Cassens R. 1998. Chemical hazard analysis for sodium nitrite in meat curing. <http://www.ag.ohio-state.edu/~meatsci/borca2.htm>. Accessed May 2, 2001.
 Crank J. 1957. The Mathematics of Diffusion. London: Oxford University Press. 66 p.

Table 4—Effective diffusion coefficients of sodium nitrite in pork tissue ($D_{m_{NaNO_2-tissue}}$) that was previously immersed during 48 hours in aqueous solutions containing sodium chloride (1° step) and afterwards immersed in brines of different compositions (2° step) at 4 °C and 20 °C.

Previous immersion solution (1° step)	Brine solution (2° step)	$D_{m_{NaNO_2-tissue}} \cdot 10^9 (m^2/s)$	
		4 °C	20°C
Pure water	$NaNO_2$ 3 g/l	0.39 ^a	0.42 ^a
NaCl 10 g/l	$NaNO_2$ 3 g/l +NaCl 10 g/l	0.59 ^b	0.76 ^b
NaCl 70 g/l	$NaNO_2$ 3 g/l +NaCl 70 g/l	0.59 ^b	0.78 ^b

.a, b, c, d: means within columns without common superscript letters differ ($p < 0.05$) as calculated by Least Significant Difference test (LSD)

Table 5—Diffusion coefficients of salts in water at infinite dilution (D_{salt-H_2O}) and effective diffusion coefficients of sodium nitrite and potassium nitrate ($D_{m_{salt-tissue}}$) in pork tissue, both at 20 °C

$D_{NaNO_2-H_2O} \cdot 10^9 (m^2/s) *$	$D_{m_{NaNO_2-tissue}} \cdot 10^9 (m^2/s) **$	Ratio of diffusivities f (Eq. 13)
$D_{NaNO_2-H_2O}: 1.373$	$D_{NaNO_2-tissue}$ immersed in solutionA: 0.41	3.35
	$D_{NaNO_2-tissue}$ immersed in solutionE: 0.85	1.62
$D_{KNO_3-H_2O}: 1.685$	$D_{KNO_3-tissue}$ immersed in solutionG: 0.42	4.01
	$D_{KNO_3-tissue}$ immersed in solutionJ: 0.95	1.77

* values obtained converting data of Table 3 (first column) from 25 °C to 20 °C using Stokes-Einstein equation

** data correspond to experimental values shown in Table 1

Cho HY, Kim JB, Pyun YR. 1988. Diffusion of sodium chloride in Chinese cabbage during salting. Korean J Food Sci Technol 20:711-716.
 Diaz G, Wolf A, Kostaropoulos A, Spiess W. 1993. Dif-

fusion of low molecular compounds in food model systems. J Food Process Pres 17:437-454.
 Djelveh G, Gros JB. 1988. Measurement of effective diffusivities of ionic and non-ionic solutes through

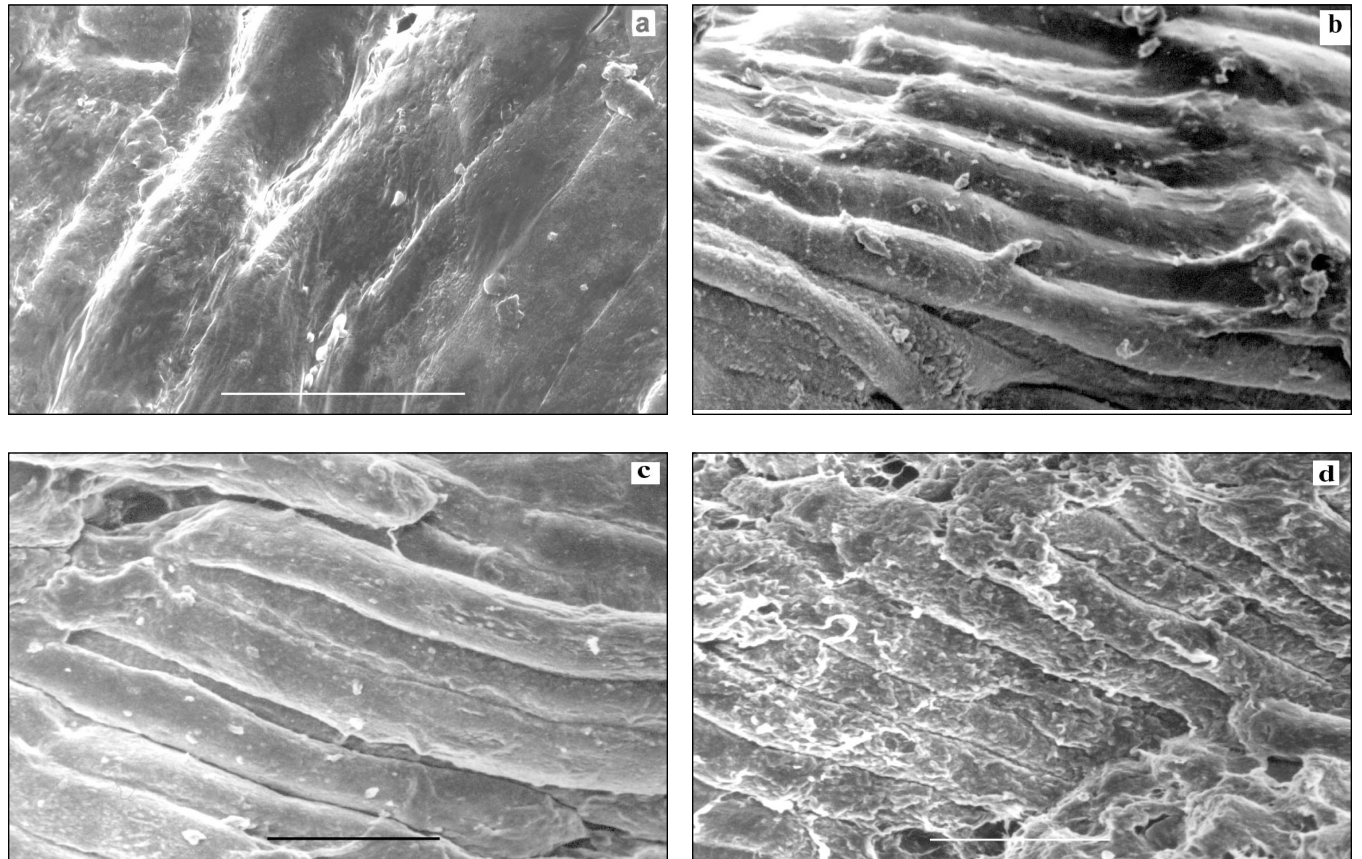


Figure 3—Scanning Electron Micrographs of pork tissue: (a) without treatment, (b) treated with KNO_3 2.5 g/l, (c) KNO_3 2.5 g/l + NaCl 70g/l, and (d) KNO_3 2.5 g/l + NaCl 140g/l. Scale = 100 mm between marks.

- beef and pork muscles using a diffusion cell. *Meat Sci* 23:11-20.
- Djelveh G, Petit M, Gros JB. 1988. Influence of sodium chloride concentration, potassium nitrate and temperature on the apparent diffusion coefficient of chloride ions through agar gels. *Lebensm-Wiss U-Technol* 21:103-107.
- Drusas A, Vagenas G, Saravacos GD. 1988. Diffusion of sodium chloride in green olives. *J Food Eng* 7:211-222.
- Dussap CG, Gros JB. 1980. Diffusion-sorption model for the penetration of salt in pork and beef muscle. In: *Food Process Engineering*, Vol I. Linko P, Malkii Y, Olkku J, Larinkari J, Editors. London: Elsevier Applied Science. p 407-411.
- Cheftel JC, Cuq JL, Lorient D. 1993. Aminoácidos, Péptidos y Proteínas. In: *Química de los alimentos*. Fennema O, Director. Zaragoza, España: Editorial Acribia. p 275-414.
- Fox JB. 1980. Diffusion of chloride, nitrite and nitrate in beef and pork. *J Food Sci* 45: 1740-1744.
- Girard JP. 1991. Tecnología de la Carne y de los Productos Cárnicos. Zaragoza, España: Editorial Acribia S.A.
- Gros JB, Dussap CG, González-Méndez N. 1984. *Engineering and Food*. Vol 1, Engineering Sciences in the Food Industry, BM McKenna, Editor. London: Elsevier Applied Science. p 287-297.
- Grau R. 1965. *Carne y Productos Cárnicos*. Zaragoza, España: Editorial Acribia. p 124-143.
- Lide DR. 1997-1998. *Handbook of Chemistry and Physics*. Edition 78th. Florida: CRC Press.
- Newman JS. 1991. *Electrochemical Systems*. 2nd Edition. New Jersey: Prentice Hall.
- Offer G, Trinick J. 1983. On the mechanism of water holding in meat: the swelling and shrinking of myofibrils. *Meat Sci* 8: 245-281.
- Pinotti A, Califano A, Zaritzky N. 2000. Diffusion of curing salts in meat: effect of sodium chloride on tissue microstructure. *Scanning* 22 (2):137-138.
- Price J, Schweigert B. 1976. *Ciencia de la Carne y de los Productos Cárnicos*. Zaragoza, España: Editorial Acribia S.A.
- Schwartzberg H, Chao RY. 1982. Solute diffusivities in leaching process. *Food Technol*: 36:73-86.
- Stahl R, Loncin M. 1979. Prediction of diffusion in solid foodstuffs. *J Food Proc Pres* 3: 213-223.
- Taylor R, Krishna R. 1983. *Multicomponent Mass Transfer*. New York: John Wiley & Sons, Inc. P 37-43.
- Wood FW. 1966. The diffusion of salt in pork muscle and fat tissue. *J Sci Food Agric* 17: 138-140.
- Zaritzky N, Califano A. 1999. Effective diffusion Coefficients of chemical preservatives in Food Tissues. In: *Trends in Heat, Mass & Momentum Transfer Vol 5*, Trivandrum, India: Research Trends. p 127-139.

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