FIRMNESS OF CARROT SLICES SUBMERGED IN BRINES: EXPERIMENTAL DATA AND MATHEMATICAL MODEL

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ABSTRACT

The firmness of carrot slices was measured at various temperatures for several concentrations of brines in sodium chloride and lactic acid. A model assuming that textural decay is due to irreversible, pseudo first-order enzyme-catalyzed degradation of the carrot cell wall polysaccharides in the presence of two alternative paths for first order enzyme deactivation was fitted to the aforementioned data sets. The physicochemical and statistical significance of the model and fits were evaluated and discussed. The model is useful for predictions of the textural decay based on reasonable physicochemical considerations in a wide range of brine specifications with practical interest.

INTRODUCTION

Most commercial processes of long term preservation of vegetables via pickling are based on the use of an edible acid in a salted brine, where the acid (e.g. acetic acid) may be deliberately added *per se* or produced *in situ* by bacterium inocula (e.g. lactic acid). As expected, the final texture of the pickled vegetables depends on the processing conditions selected; although a crisp texture of pickles is a desirable characteristic (Bell *et al.* 1972) most vegetables

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tend to soften during the pickling process. Native enzymes of the vegetable tissue, or enzymes excreted by the native microflora, have been implicated with such decay (Fleming 1982) viz. by postulation that softening of vegetables may occur as a result of pectinolytic activity of endogenous or exogenous enzymes which is affected by the presence of calcium cations (Howard and Buescher 1990; McFeeters and Fleming 1990; Chang *et al.* 1993; Fleming *et al.* 1993) or as a result of specific binding of Ca^{2+} to the cell wall which may prevent extensive disruption of its polysaccharide network depending on the pH and concentration of sodium chloride of the brine (Fleming *et al.* 1983).

Cell walls of carrot roots are a multicomponent, heterogeneous material with a composition that varies with the age of the carrots; the most important components of the immature carrot tissue are pectic polysaccharides complexed with proteins and polyphenolic materials, but as maturation progresses parenchymatous and lignified tissues build up (Barry *et al.* 1984). As with other higher plants, two main tissue types can be considered: (1) the parenchyma (commonly designated by cortex), which is a storage tissue rich in starch and sucrose; and (2) the vascular system, which comprises the xylem (upward direction, inner portion of the core), which is rich in salts, and the phloem (downward direction, outer portion of the core), which is rich in reducing sugars (Mayer *et al.* 1965, Soddu and Gioia 1979). Therefore, detailed technological studies should, in principle, consider each tissue type separately.

Some studies were reported on vegetable texture modification during pickling processes (Buesher *et al.* 1979; McFeeters and Fleming 1990; Fleming *et al.* 1993; Moreira 1994), but the mathematical prediction of that set of physical properties was in all cases a difficult task especially in wide ranges of processing temperatures. The present work has attempted to develop a mechanistic model that may aid in the simulation of the dynamic evolution of the firmness of fresh carrots as pickling progresses using, in addition to processing time and temperature, the concentration of salt and the concentration of acid as manipulated variables.

MATERIALS AND METHODS

Raw Material

Fresh carrots (cultivar: Nantes) were bought at random at local markets in Portugal.

Chemicals

Sodium chloride, lactic acid, copper sulfate, and potassium permanganate (all reagent grade) were purchased from Merck (Germany).

Equipments

Isothermal and shaking conditions were obtained using a Kottermann shaker water bath 3047 (Labortechnik, Germany). Sterilization was accomplished using a laboratory retort Austester 437 G (Selecta, Spain). The texture of the carrots was evaluated with a Texture Analyzer (Stevens, UK) fitted with a TA 9 probe (a perforating needle with 1.7 mm diameter) connected to a chart recorder, using an isothermal chamber B-80 (Memmert, Germany) to damp temperature variations during the texture assays.

Brine Preparation

Brines were prepared at room temperature using the required amounts of solid sodium chloride (previously dried overnight at 60C) or liquid lactic acid, and diluting them with deionized water to the final desired concentrations. The final brine concentrations were 50, 75, 100, 150, and 200 g/L of sodium chloride, and 5, 10, and 20 g/L of lactic acid, respectively. The prepared solutions were poured into 250-mL glass flasks and sterilized for 15 min. Control brines (corresponding to 0 g/L of salt and 0 g/L of lactic acid) were prepared with sterilized plain water.

Experimental Protocol

Prior to every experiment, fresh carrots with tops cut off were thoroughly washed with tap water, submerged for 15 min in a 20 g/L solution of copper sulfate, and then for 15 min in a 20 g/L solution of potassium permanganate. The carrots were then sliced normally to the longitudinal axis into pieces ca. 1.0 cm-high and ca. 3.0 cm in diameter. Each carrot slice was stuck with a sterilized pin made of stainless steel, dropped into a 250-mL flask previously filled with sterilized brine with the desired acid or salt concentration, and gently stirred (orbital velocity setting: 5) at a selected temperature (20, 30, 40, and 50C) for ca. 72 h. The ratio of the volume of the brine to the mass of the carrots utilized was 7:1 (mL/g), corresponding to ca. 50 mL of brine per slice of carrot. At predefined times, one or more carrot slices were slightly dried with

tissue paper, placed in a sterilized Petri dish, and incubated at 25C for 2 h prior to texture assaying.

Texture Assay

The texture of each dried carrot slice was evaluated by puncture normal to the cross section of the slice in both the core and the cortex portions. The firmness of each portion of the carrot slice was evaluated using the texture analyzer (potential sensitivity: 2 V, perforation speed: 0.05 mm/s, penetration depth: 5 mm, and recorder paper speed: 10 cm/min). Each sample was assayed at least three consecutive times, and the average of the measurements was used as the value for the firmness.

RESULTS AND DISCUSSION

Physical Analyses

The texture analyzer ensures a constant rate of penetration of the perforating probe and a virtually instantaneous stop at the end of the preselected penetration depth; therefore, a typical texture profile, or penetrogram (see Fig. 1), yields continuous information pertaining to force versus deformation of the carrot material. The mechanical information conveyed by such plot has traditionally been summarized via seven discrete numerical values classically termed General Food Texture Parameters (Bourne 1968): brittleness, firmness, cohesiveness, adhesiveness, elasticity, gumminess, and chewiness. Although for some foods they may not correlate at all with the sensory parameters suggested by their names, significant correlations have been reported for cooked potatoes (Leung *et al.* 1983) and pickled peppers (Fleming *et al.* 1993) in terms of firmness. Preliminary experimental values of texture parameters on treated carrot slices indicated the absence of a brittleness peak along the whole pickling period selected, and a constant adhesiveness value irrespective of submersion time. Since firmness was clearly defined with low variability, we selected firmness obtained in the first cycle of the penetrogram as a measure of the textural quality of carrot pickles. The experimental data on the effect of sodium chloride at various temperatures on firmness are in Fig. 2. Normalization of firmness by its initial value reduced considerably the scatter of the data and allowed an efficient mechanistic model of texture dynamics to be postulated and fitted. Although (as shown by a *posteriori* statistical analyses) the initial texture

values of the cortex and the core of carrot slices were not statistically different at the 5% level of significance, both portions were not expected to behave identically upon submersion in lactic acid or sodium chloride brines.

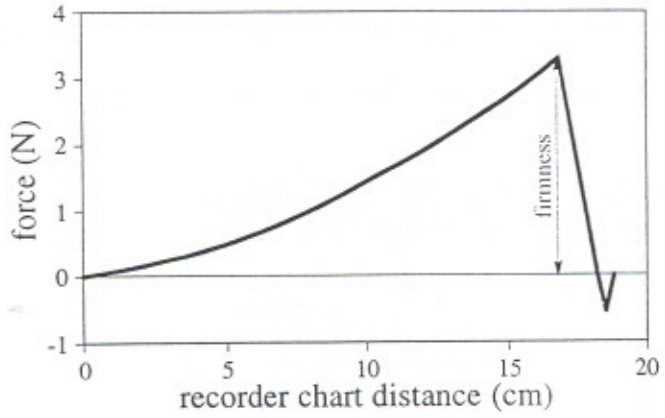


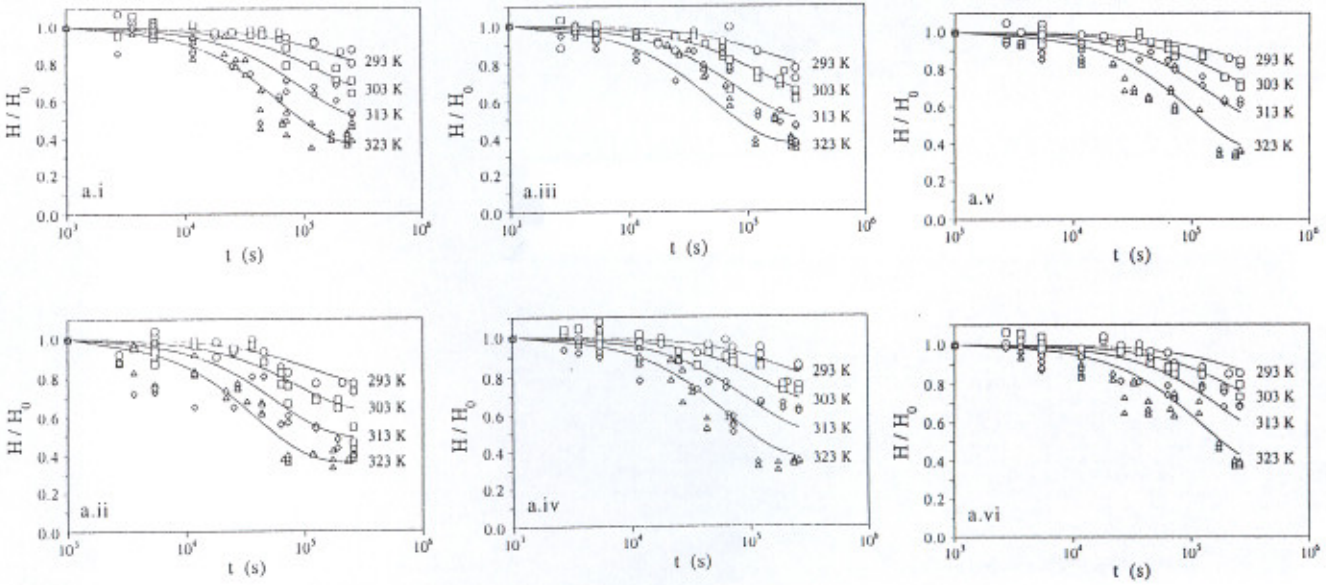
FIG. 1. TYPICAL PENETROGRAM FOR TEXTURAL ASSESSMENT OF A CARROT SLICE (FIRST CYCLE)

Statistical Analyses

Mathematical derivation of models for the normalized firmness of carrot slices is included in the Appendix. For the unitemperature, uniconcentration model the resulting equation is

$$\ln\left\{\frac{H}{H_0}\right\} = \frac{v_{max,0}}{K_m\, k_1\,(1 + K)}\left(\exp\{-\,k_1\,(1 + K)\,t\} - 1\right) \qquad (1)$$

where the mathematically independent parameters to be fitted to data taken at each temperature and concentration of salt or acid ($v_{max,0}/K_m$ and $k_1\,(1 + K)$) are defined in the Appendix. Equation (1) was fitted to each data set independently using the unweighted, nonlinear regression analysis package GREG (Caracotsios *et al.* 1985): the estimated parameters are in Table 1.


a.i
a.ii
a.iii
a.iv
a.v
a.vi
H / H0
t (s)
293 K
303 K
313 K
323 K

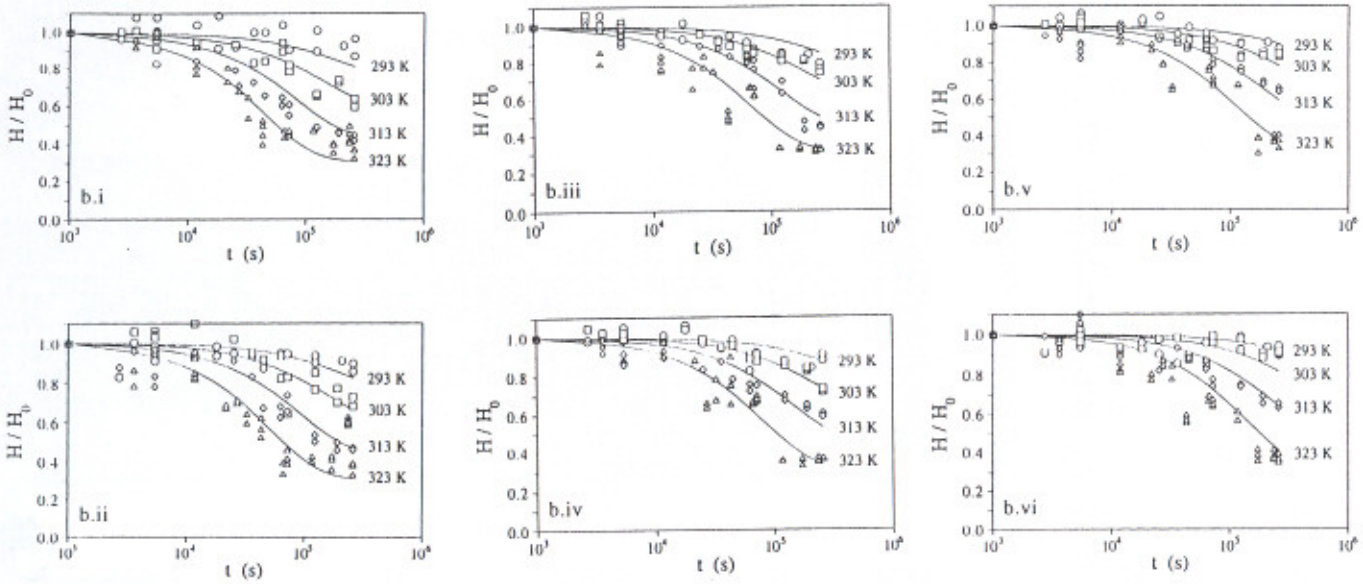


FIG. 2. EVOLUTION OF THE NORMALIZED FIRMNESS OF THE (a) CORE AND THE (b) CORTEX OF CARROT SLICES, H/H_0, WITH TIME OF SUBMERSION, t, FOR (i) 0.0, (ii) 50.0, (iii) 75.0, (iv) 100.0, (v) 150.0, AND (vi) 200.0 G/L OF SODIUM CHLORIDE AND VARIOUS TEMPERATURES: EXPERIMENTAL DATA TAKEN AT 293 (○), 303 (□), 313 (◇), AND 323 K (Δ), AND THEORETICAL FIT (__)

TABLE 1.

PARAMETER ESTIMATES FOR $\frac{v_{max,0}}{K_m}$ (S^{-1}, FIRST COLUMN) AND $k_1(1+K)$ (S^{-1}, SECOND COLUMN) OBTAINED FROM INDEPENDENT FITS OF EACH EXPERIMENTAL DATA SET OBTAINED AT GIVEN BRINE CONCENTRATION AND TEMPERATURE TO THE LUMPED MODEL DEFINED BY EQ.(1)

concentration (g/L)	temperature (K)	salt effect				acid effect			
		core		cortex		core		cortex	
		($x10^{-6}$)	($x10^{-6}$)	($x10^{-6}$)	($x10^{-6}$)	($x10^{-6}$)	($x10^{-6}$)	($x10^{-6}$)	($x10^{-6}$)
0	293	0.00390	0.0138	0.360	1.07	0.053	0.0689	2.10	3.07
	303	1.50	3.53	1.10	2.17	3.20	4.01	1.80	3.06
	313	12.0	21.6	2.60	4.55	13.0	20.5	18.0	23.4
	323	15.0	26.6	3.20	5.39	12.0	17.7	18.1	26.8
5	293	-	-	-	-	3.60	6.34	4.50	7.20
	303	-	-	-	-	45.0	73.9	23.0	37.9
	313	-	-	-	-	76.0	112	71.0	107
	323	-	-	-	-	74.0	89.1	60.0	72.9
10	293	-	-	-	-	30.0	41.3	35.0	49.9
	303	-	-	-	-	39.0	53.8	49.0	68.6
	313	-	-	-	-	53.0	71.3	49.0	59.7
	323	-	-	-	-	69.0	79.4	95.0	111
20	293	-	-	-	-	34.0	45.3	46.0	59.6
	303	-	-	-	-	40.0	52.8	56.0	83.5
	313	-	-	-	-	130	158	57.0	70.6
	323	-	-	-	-	68.0	77.7	93.0	108
50	293	55.0	19.9	12.0	49.2	-	-	-	-
	303	4.60	10.2	3.00	6.06	-	-	-	-
	313	100	206	59.0	120	-	-	-	-
	323	75.0	120	54.0	89.1	-	-	-	-
75	293	7.10	26.2	1.50	5.73	-	-	-	-
	303	3.00	6.53	3.00	6.53	-	-	-	-
	313	15.0	28.7	5.90	9.03	-	-	-	-
	323	11.0	16.6	20.0	28.8	-	-	-	-
100	293	1.30	4.29	1.30	3.95	-	-	-	-
	303	1.40	3.77	1.50	3.67	-	-	-	-
	313	18.0	32.4	13.0	22.4	-	-	-	-
	323	15.0	22.6	16.0	24.6	-	-	-	-
150	293	0.810	3.65	0.570	1.70	-	-	-	-
	303	4.20	11.4	2.30	6.46	-	-	-	-
	313	1.60	28.1	14.0	23.0	-	-	-	-
	323	15.0	22.7	13.0	20.2	-	-	-	-
200	293	0.830	2.78	4.70	17.6	-	-	-	-
	303	3.30	8.33	3.30	6.90	-	-	-	-
	313	11.0	18.5	13.0	22.4	-	-	-	-
	323	16.0	25.5	16.0	25.6	-	-	-	-

The assumption $K_m >> C_{p,o}$ prior to derivation of Eq. A.4 in the Appendix is reasonable because the molar concentration of cell wall polysaccharides is expected to be low. The assumption $v_{max,0}/K_m k_1 \sim 1$ may be expected in view of the facts that (1) the time scale for an enzyme-catalyzed reaction ($C_{P,0}/v_{max,0}$) is smaller than the time scale for the corresponding

enzyme-deactivation reaction ($1/k_1$), and (2) a large value for the ratio $K_m/C_{P,0}$ (as assumed above) makes the product of $C_{P,0}/v_{max,0}$ by $K_m/C_{P,0}$ approach the order of magnitude of $1/k_1$. Further proof of the assertion that $v_{max,0}/K_m k_1 \sim 1$ was obtained *a posteriori* via use of the information conveyed by the independent fits of parameters $k_1(1+K)$ and $v_{max,0}/K_m$ in Eq. 1 to each data set in Table 2 coupled with the information conveyed by the simultaneous fits of parameter K as given by Eq. [A.10] and [A.12] to all data sets; typical results thus obtained for the estimated value of the ratio $V_{max,0}/K_m K_1$ are plotted in Fig. 3 for the data sets encompassing all temperatures and salt concentrations tested. It is apparent that such ratio does not deviate considerably from unity in any situation, which backs up the tentative transformation of Eq. A.3 into Eq. A.4.

TABLE 2.
PARAMETER ESTIMATES (AND ASSOCIATED CONFIDENCE INTERVALS, CI) OBTAINED BY NONLINEAR REGRESSION ANALYSIS OF THE FIT OF THE THEORETICAL MODELS GIVEN BY EQS. (2) AND (3) TO THE EXPERIMENTAL DATA OBTAINED AT VARIOUS BRINE CONCENTRATIONS AND TEMPERATURES

	salt effect		acid effect	
	core	cortex	core	cortex
identification	parameter ±95% CI	parameter ±95% CI	parameter ±95% CI	parameter ±95% CI
$L_{2/1}$	-17.12 ± 4.08	-17.57 ± 4.73	-8.69 ± 3.21	-7.65 ± 3.17
$\Delta E_{act,2/1}$ (J.mol^{-1})	-4.44×10^4 $\pm 1.07\times10^4$	-4.56×10^4 $\pm 1.25\times10^4$	-1.967×10^4 $\pm 8.15\times10^3$	-1.700×10^4 $\pm 8.10\times10^3$
pE_{act} (J.mol^{-1})	-4.7357 ± 0.0812	-4.8261 ± 0.0672	-4.6831 ± 0.0815	-4.7071 ± 0.0827
$p(k_{s,i,0}\ C_{E,tot})$ (kg.m^{-3}.hr^{-1})	-4.39 ± 1.69	-6.33 ± 1.71	-	-
$p(k_{a,i,0}\ C_{E,tot})$ (kg.m^{-3}.hr^{-1})	-	-	-3.99 ± 1.54	-4.75 ± 1.72
$pK_{s,0}$ (kg.m^{-3})	2.647 ± 0.395	3.292 ± 0.465	-	-
$p(K_{a,0}/\sqrt{K_{LA}})$ (kg$^{0.5}$.m$^{-1.5}$)	-	-	0.798 ± 0.154	0.226 ± 0.207
$pK_{s,1}$ (kg.m^{-3})	-0.318 ± 0.294	-0.563 ± 0.415	-	-

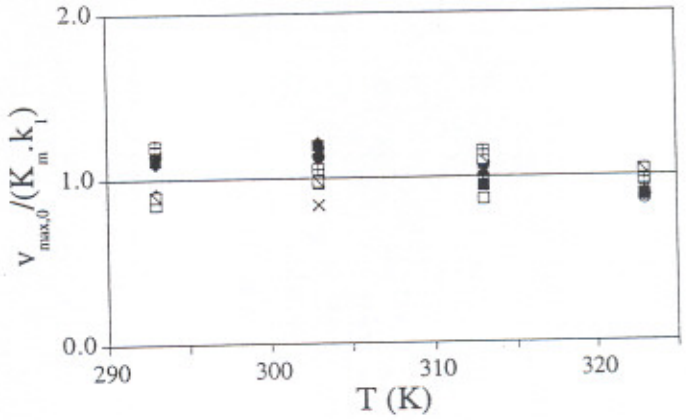


FIG. 3. VARIATION OF THE RATIO $v_{max,0}/(K_m.k_1)$ WITH TEMPERATURE USING THE ESTIMATES FOR PARAMETERS $v_{max,0}/K_m$ AND $k_1(1+K)$ GIVEN IN TABLE 1 AND THE ESTIMATES FOR PARAMETER K COMPUTED FROM TABLE 2 ENCOMPASSING THE CORTEX OF THE CARROT SLICES SUBMERGED IN BRINES AT (○) 0.0, (□) 50.0, (◇) 75.0 (Δ) 100.0, (X), 150.0, and (+) 200.0 G/L OF SODIUM CHLORIDE AND THE CORE OF THE CARROT SLICES SUBMERGED IN BRINES AT (●) 0.0, (■) 50.0, (◆) 75.0, (▲) 100.0, (⊠), 150.0, AND (⊞) 200.0 G/L OF SODIUM CHLORIDE

We note that Eq. A.4 is a simplification of an equation recently proposed elsewhere (Moreira 1994) encompassing the softening of turnips brought about by acetic acid, viz. $H = H_1 \exp\{-k_1 t\} + H_2 \exp\{- k_2 t\}$, where H_1 and H_2 are the initial firmness in two hypothetical sequential degradative processes, and k_1 and k_2 are two first order rate constants (where it is expected that $k_1 >> k_2$). This equation seemed to be generally adequate to fit experimental data pertaining to the dynamic evolution of the firmness of a vegetable material submerged in a brine under isothermal conditions. However, in addition to the empirical nature of such model, validation was achieved only for relatively high temperatures. Our model differed because (1) it had a physicochemical basis, and (2) it was tested over a wide range of temperatures of interest. Relatively low temperatures were chosen in order to assess the possible effects of fermentation in the so-called cold-packing process (which naturally takes place at low temperatures), since it has been claimed that firmness retention is improved in some vegetable products if pasteurization is not required (Fleming *et al.* 1993). Nevertheless, the time scale corresponding to halving the initial firmness of the carrot tissue is of the same order of magnitude as that found for turnips (Moreira 1994).

For the multitemperature, multiconcentration models, the resulting equations (see Appendix) are

$$\frac{H}{H_0} = \frac{\exp\left\{L_{2/1} - \frac{\Delta E_{act,2/1}}{RT}\right\} + \ldots}{1 + \exp\left\{L_{2/1} - \frac{\Delta E_{act,2/1}}{RT}\right\}} \ldots \exp\left\{-\frac{10\text{-}p(k_{a,i,0}\, C_{E,tot})}{\frac{10\text{-}p(K_{a,0}/\sqrt{K_{LA}})}{\sqrt{C_{LA}}} + 1} \exp\left\{-\frac{10\text{-}pE_{act}}{RT}\right\}\left(1 + \exp\left\{L_{2/1} - \frac{\Delta E_{act,2/1}}{RT}\right\}\right)t\right\} \quad (2)$$

and

$$\frac{H}{H_0} = \frac{\exp\left\{L_{2/1} - \frac{\Delta E_{act,2/1}}{RT}\right\} + \ldots}{1 + \exp\left\{L_{2/1} - \frac{\Delta E_{act,2/1}}{RT}\right\}} \ldots \exp\left\{-\frac{10\text{-}p(k_{s,i,0}\, C_{E,tot})}{\frac{10\text{-}pK_{s,0}}{C_{NaCl}} + 1 + \frac{C_{NaCl}}{10\text{-}pK_{s,1}}} \exp\left\{-\frac{10\text{-}pE_{act}}{RT}\right\}\left(1 + \exp\left\{L_{2/1} - \frac{\Delta E_{act,2/1}}{RT}\right\}\right)t\right\} \quad (3)$$

where the mathematically independent parameters to be fitted to data taken at various temperatures and concentrations of salt ($L_{2/1}$, $\Delta E_{act,2/1}$, pE_{act}, $p(k_{s,i,0}\, C_{E,tot})$, $pK_{s,0}$, and $pK_{s,1}$) and to data taken at various temperatures and concentrations of lactic acid ($L_{2/1}$, $\Delta E_{act,2/1}$, pE_{act}, $p(k_{a,i,0}\, C_{E,tot})$ and $p(K_{a,0}\sqrt{K_{LA}})$) are defined in the Appendix. Equations 2 and 3 were fitted to all data sets simultaneously using the unweighted, nonlinear regression analysis package GREG (Caracotsios *et al.* 1985). Plots of the model given by Eq. 3 as applied to the core and the cortex are depicted in Fig. 2 for six concentrations of sodium chloride. Similar results (not shown) were obtained on application of Eq. 2 for all concentrations of lactic acid for both the core and cortex of the carrots.

Although the actual concentrations of total lactic acid and sodium chloride in the carrot tissue itself (rather than in the brine) should have been used in Eq. 2 and 3, their measurement is a difficult task in practical terms; furthermore, previous experimental work has indicated that the partition coefficients of both solutes between carrot and brine are approximately unity, and the variations of the concentrations of such compounds in the brine in the time frame of interest are small.

Incremental sum of squares analyses were implemented (Bates and Watts 1980) in order to assess the use of the simpler model given by Eq. 1 for each data set at a given temperature and concentration of acid or salt, as appropriate, instead of the complex model denoted by Eq. 2 or Eq. 3, respectively, for all data sets considered at the same time. These tests allowed one to assess the statistical usefulness of computing 16 or 24 estimations of both parameters

$k_1(1+K)$ and $v_{max,0}/ K_m k_1$ for the case of acid dependence or salt dependence, respectively, when compared with computing one estimation of all parameters $L_{2/1}$,$\Delta E_{act,2/1}$, E_{act}, $k_{a,i,0} C_{E,tot}$, and $K_{a,0}/\sqrt{K_{LA}}$, or one estimation of all parameters $L_{2/1}$, $\Delta E_{act,2/1}$, E_{act}, $k_{s,i,0} C_{E,tot}$, $K_{s,0}$, and $K_{s,1}$, respectively. Such incremental sum of squares analyses have indicated that there is statistical reason on the 5% level of significance to select the multitemperature, multiconcentration model (Eq. 2 for acid or Eq. 3 for salt) for all sets of data considered simultaneously and thus reject the unitemperature, uniconcentration model (Eq. 1) for each set of data; therefore, Eq. 2 and 3 should be selected for technological modelling of carrots (or other similar vegetables) despite their complexity arising from a considerable number of parameters.

Since several experiments were replicated, estimators of the intrinsic experimental variability were available and were used in lack of fit analyses for the multitemperature, multiconcentration models (Bates and Watts 1980). The results of these analyses indicated that there was no statistically valid reason (at the 5% level of significance) to doubt the form of the multitemperature, multiconcentration models in view of the experimental scatter of the data generated in our experiments. Furthermore, the *t*-ratio associated with every parameter estimate was above unity (Table 2), which suggests that convergence to the true minima of the residual sum of squares objective functions based on Eqs. 2 and 3 was achieved.

Immersion of carrot slices in salted brines or acid brines caused softening effects which: (1) were higher as temperature increased (an observation that is consistent with the decrease of K with temperature); such effect was lower in the cortex than in the core in salt brines, and approximately the same in the core and in the cortex in lactic acid brines; (2) were faster as the concentration of lactic acid increased (consistent with the increase of $v_{max,0}/K_m$ with lactic acid concentration); this effect was more pronounced in the cortex than in the core; (3) passed through a rate maximum located at ca. 0.7 g/L (for the core) and 0.5 g/L (for the cortex) as the concentration of sodium chloride increased (an observation which is consistent with the bell-shaped variation of $v_{max,0}/K_m$ with C_{NaCl}); such effect was less pronounced in the cortex than in the core; and (4) were slower as time elapsed until a plateau was eventually reached. The maximum of $v_{max,0}/K_m$ on salt concentration but not on acid concentration was expected in view of the forms of Eq. A.6 and A.7, respectively; attempts to rewrite the denominator of Eq. A.5 as $K_{a,0}/C_H^+ + 1 + K_{a,1} C_H^+$ were pointless because, in view of the narrow experimental range selected for the acid concentrations, parameter $K_{a,1}$ remained undeterminate in all fits.

The parameter 95% confidence intervals pertaining to the cortex and pertaining to the core partially superimpose for all parameters except pE_{act} for both the acid brines and the salt brines, and $p(K_{a,0}/\sqrt{K_{LA}})$ for the acid brines (Table 2). The nul hypothesis (i.e., there is no difference between core and

cortex behavior) is rejected at the 5% level of significance for pE_{act} for both brine qualitative compositions. Consequently, the firmness of the cortex was significantly more labile to temperature than that of the core, and such comparative observation does not seem to depend on the nature of the brine due to the different composition of these two tissues. The same differences in microenvironment may also account for the (significant at the 5% level) increase in parameter $p(K_{a.0}/\sqrt{K_{LA}})$ when going from the core to the cortex of the carrot: if the lactic acid power remained essentially unchanged, formation of form E_0 from form E_1 would be easier for enzyme existing in the cortex than in the core of the carrot.

The numerical values of the parameter estimates are reasonable from a physicochemical point of view. For the experiments where the salt concentration in the brine was varied, the activation energies associated with the enzyme-catalyzed reactions were ca. 54.4 and 67.0 kJ/mol for the core and the cortex, respectively; for the experiments where the acid concentration in the brine was varied, the activation energies were 48.2 and 50.0 kJ/mol. These values are well within the range encountered for most hydrolytic enzymes (Bailey and Ollis 1986). In the experiments where the salt concentration of the brine was varied, the differences between the activation energies associated with kinetic constants k_1 and k_2, i.e. $\Delta E_{act.2/1}$ was 44.4 and 45.6 kJ/mol for the core and the cortex, respectively; in the experiments where the acid concentration of the brine was varied, the differences were 19.7 and 17.0 kJ/mol. All these values are acceptable in view of the range of activation energies of thermal denaturation of enzymes (which is a nonenzymatic reaction) (Bailey and Ollis 1986).

The pK values associated with breakage of the ionic bonds between enzyme molecule E^1 and salt ions are 2.65 and 3.29 for the core and the cortex, respectively, and -0.318 and -0.563 for the enzyme form E^2. The fact that the former values are relatively high suggests that forms of enzyme with few intramolecular salt bridges are thermodynamically favored with respect to forms of enzyme with several intramolecular salt bridges. By the same token, the fact that the latter values are considerably low suggests that forms of enzyme with few intramolecular salt bridges are thermodynamically favored with respect to forms of enzyme without intramolecular salt bridges.

Assuming that the ratio of the square root of the molecular weight of lactic acid to the molecular weight of enzyme is of the order of magnitude of 10^{-4}, and combining the dissociation constant of lactic acid (ca. 1.4×10^{-4}M) with the pK values associated with the acid dissociation constant of the enzyme form E_1 (see Table 2), one concludes that the pK of protolysis of the enzyme (on a molar basis) is 6.1 for the core and 6.7 for the cortex. Both these values are reasonable in view of the pK values found for most enzymes (Segel 1975) and suggest a (putative) Histidine residue involved in maintenance of a proper enzyme conformation for catalysis as happens with several known hydrolases.

APPENDIX

Firmness of a material can be viewed as the resistance offered to structural breakage due to a thin penetration device. Although a direct mechanistic relationship may not exist between firmness (H) and concentration of cell wall polysaccharides (C_p), firmness decay is often accompanied by hydrolysis of cell wall polysaccharides, and the effect of hydrolysis of cell wall polysaccharides on firmness increases with extent of hydrolytic reactions. One empirical relationship valid for a limited period of time and able to simulate such observations is

$$\frac{H}{H_0} = 1 + \ln\left\{\frac{C_P}{C_{P,0}}\right\} \qquad (A.1)$$

where H_0 and $C_{P,0}$) are the initial values of the firmness and the concentration of cell wall polysaccharides, respectively. In addition to cellulose, pectic compounds which have been identified in carrot tissues include polysaccharides of galactose, arabinose, and uronic acid, which may be complexed with proteins and polyphenolics (Barry *et al.* 1984).

In principle hydrolases act reversibly on substrates, but the action of carbohydratases (e.g., pectinases, cellulases, and hemicellulases) on cell wall polysaccharides has no significance, and if it occurs at all only oligosaccharides (with a very limited contribution to firmness) would be formed. Therefore, the action of hydrolases upon the cell wall polysaccharides was assumed to follow the irreversible Michaelis-Menten mechanism. Hydrolases are labile to thermal degradation with several possibilities of conformational changes in the proteinaceous backbone that deactivate the enzymes. Two major first order, irreversible routes were assumed, and the pathways were described by the kinetic constants k_1 and k_2. Coupling the mass balance to active enzyme molecules to the mass balance to cell wall polysaccharides, one obtains (Bailey and Ollis 1986)

$$-\frac{dC_P}{dt} = \frac{v_{max,0}\exp\{-k_1\{1+K\}t\}C_P}{K_m + C_P} \qquad (A.2)$$
$$t = 0\ ,\ C_P = C_{P,0}$$

where t is time of carrot submersion in the brine, $v_{max,0}$ is reaction rate under saturation conditions of the enzyme with substrate, K_m is Michaelis-Menten parameter, and K is ratio of k_2 to k_1.

This model implicitly assumes that hydrolytic enzymes exist and they can move more or less freely in the intercellular fluid of the vegetable tissue. Despite the observation that enzymatic activity is absent in carrot tissues at high temperatures (Massiot *et al.* 1992), active β-galactosidase was identified in aqueous suspensions of carrot cells (Konno and Katoh 1992), and evidence for intercellular movement of native enzymes has been reported for enzymes secreted by aleurone cells of cereal grains in the germinating seed (Hillmer *et al.* 1992), α-amylase in germinating barley seeds (Gibbons 1985) and in pea stem tissue (Beers and Duke 1988), and cell-wall-degrading enzymes in tobacco roots (Benhamou and Cote 1994) and soybean tissues (Baron-Epel *et al.* 1992).

Assuming that $K_m >> C_{P,0}$, integration (Stephenson 1973) of Eq. A.2 yields

$$\ln\left\{\frac{C_P}{C_{P,0}}\right\} = \frac{v_{max,0}}{K_m\, k_l\, (1 + K)}\{\exp[-k_l\,(1 + K)\,t] - 1\} \qquad (A.3)$$

which corresponds to Eq. 1 in the main text. Further assuming that $\frac{v_{max,0}}{K_m\, k_l} \sim 1$, combining Eqs. A.1 and A.3 gives

$$\frac{H}{H_0} = \frac{K}{1 + K} + \frac{1}{1 + K}\exp\left\{-\frac{v_{max,0}\,(1 + K)}{K_m}\,t\right\} \qquad (A.4)$$

Due to the proteinaceous nature of enzymes, both the pH and the ionic strength of the solution affect their three-dimensional structure, and, consequently, their conformation in the vicinity and at the active site; such alterations are likely to lead to changes in the affinity of the active site for binding the substrate (Segel 1975).

For the case of pH dependence, a general Dixon-Webb form of pH dependence was assumed, i.e., (1) the enzyme can exist in two protonated forms, E_0 and E_1 (where form E_1 possesses one more bound proton than form E_0) which correspond to as many different enzyme conformations, but (2) only form E_1 is able to bind the substrate and catalytically transform it into product. Combination of these assumptions with the Michaelis-Menten mechanism implies that (Segel 1975)

$$v_{max,0} = \frac{k_{a,i}\, C_{E,tot}}{\frac{K_{a,0}}{C_{H^+}} + 1} \qquad (A.5)$$

where $k_{a,i}$ is first order (intrinsic) rate constant associated with the E_i form of the enzyme, $C_{E,tot}$ total concentration of enzyme, $K_{a,0}$ dissociation constant of the E_i form of enzyme, and C_H^+ concentration of hydrogen ions. If the dissociation of lactic acid as a weak acid is also taken into account, and if this dissociation is described by constant K_{LA}, then Eq. A.5 becomes

$$v_{max,0} = \frac{k_{a,i}\, C_{E,tot}}{\frac{K_{a,0}}{\sqrt{K_{LA}}\,\sqrt{C_{LA}}} + 1} \qquad (A.6)$$

where C_{LA} is the total concentration of lactic acid.

For the case of ionic strength dependence, it was assumed that (1) the enzyme can exist in three charged forms, E^0, E^1, and E^2 (where form E^1 possesses one more ionic bond with a salt counter ion than form E^0, and form E^2 possesses one more ionic bond with a salt counter ion than form E^1) which correspond to three different enzyme conformations (because each extra salt counter ion that participates in an ionic bond to a polar side group of an amino acid residue prevents one intramolecular salt bridge), but (2) only form E^1 is able to bind the substrate and hence catalytically transform it into product. In a similar fashion to Dixon-Webb models (Segel 1975), this implies that

$$v_{max,0} = \frac{k_{s,i}\, C_{E,tot}}{\frac{K_{s,0}}{C_{NaCl}} + 1 + \frac{C_{NaCl}}{K_{s,1}}} \qquad (A.7)$$

where $k_{s,i}$ is first order (intrinsic) rate constant associated with the form of the enzyme with the intermediate number of intramolecular salt bridges (or intermediately charged enzyme form), and $K_{s,0}$ and $K_{s,1}$ constants which describe the formation of ionic bonds between the enzyme and the salt counter ions.

Assuming the Arrhenius relationship for temperature dependence one gets (Bailey and Ollis 1986)

$$k_{a,i} = k_{a,i,0} \exp\left\{-\frac{E_{act}}{R\,T}\right\} \qquad (A.8)$$

where $k_{a,i,0}$ is pre-exponential factor, R is ideal gas constant, T is absolute temperature, and E_{act} is activation energy. Similarly for salt concentration dependence one gets

$$k_{s,i} = k_{s,i,0} \exp\left\{-\frac{E_{act}}{RT}\right\} \qquad (A.9)$$

where $k_{s,i,0}$ denotes pre-exponential factor. If all binding constants (i.e., $K_{a,0}$, $K_{s,0}$, $K_{s,1}$) are essentially constant within the temperature range chosen, the constant K satisfies the relationship

$$K = k_{2/1} \exp\left\{-\frac{\Delta E_{act,2/1}}{RT}\right\} \qquad (A.10)$$

where $k_{2/1}$ denotes ratio of the pre-exponential factors and $\Delta E_{act,2/1}$ difference between the activation energies associated with the two enzyme deactivation processes. This differential parameter plays a role similar to that of the enthalpy change of reaction in van't Hoff law if K were considered as a (pseudo) equilibrium constant (Smith and van Ness 1987).

Combination of Eq. A.4, A.6, A.8, and A.10 yields

$$\frac{H}{H_0} = \frac{\exp\left\{L_{2/1} - \frac{\Delta E_{act,2/1}}{RT}\right\} + \ldots}{1 + \exp\left\{L_{2/1} - \frac{\Delta E_{act,2/1}}{RT}\right\}} \qquad \ldots \exp\left\{-\frac{10^{-p(k_{a,i,0}\, C_{E,tot})}}{\frac{10^{-p(K_{a,0}/\sqrt{K_{LA}})}}{\sqrt{C_{LA}}} + 1} \exp\left\{-\frac{10^{-pE_{act}}}{RT}\right\}\left(1 + \exp\left\{L_{2/1} - \frac{\Delta E_{act,2/1}}{RT}\right\}\right) t\right\}$$

(A.11)

whereas combination of Eq. A.4, A.7, A.9 and A.10 yields

$$\frac{H}{H_0} = \frac{\exp\left\{L_{2/1} - \frac{\Delta E_{act,2/1}}{RT}\right\} + \ldots}{1 + \exp\left\{L_{2/1} - \frac{\Delta E_{act,2/1}}{RT}\right\}} \qquad \ldots \exp\left\{-\frac{10^{-p(k_{s,i,0}\, C_{E,tot})}}{\frac{10^{-pK_{s,0}}}{C_{NaCl}} + 1 + \frac{C_{NaCl}}{10^{-pK_{s,1}}}} \exp\left\{-\frac{10^{-pE_{act}}}{RT}\right\}\left(1 + \exp\left\{L_{2/1} - \frac{\Delta E_{act,2/1}}{RT}\right\}\right) t\right\}$$

(A.12)

which correspond to Eq. 2 and 3 in the main text.

NOMENCLATURE

$C_{E,tot}$	total concentration of enzyme
C_H^+	concentration of hydrogen ions
C_{LA}	total concentration of lactic acid
C_{NaCl}	concentration of sodium chloride
C_P	concentration of cell wall polysaccharides
$C_{P,0}$	initial concentration of cell wall polysaccharides
E_{act}	activation energy of $k_{a,i}$
E_0	less protonated form of enzyme
E_1	more protonated form of enzyme
E^0	most charged enzyme form
E^1	intermediately charged enzyme form
E^2	least charged enzyme form
H	firmness of carrot tissue
k_1	first order deactivation constant of hydrolytic enzyme(s)
k_2	first order deactivation constant of hydrolytic enzyme(s)
$k_{2/1}$	ratio of pre-exponential factors for alternative deactivations paths
$k_{a,i}$	first order (intrinsic) rate constant associated with E
$k_{a,i,0}$	pre-exponential factor of $k_{a,i}$
$K_{a,0}$	acid dissociation constant of E^1
K	ratio of first order deactivation constants
K_{LA}	dissociation constant of lactic acid
K_m	Michaelis-Menten constant
$k_{s,i}$	first order (intrinsic) rate constant associated with E^1
$k_{s,i,0}$	pre-exponential factor of $k_{s,i}$
$K_{s,0}$	ion dissociation constant of E°
$K_{s,1}$	ion dissociation constant of E^1
$L_{2/1}$	natural logarithm of $k_{2/1}$
pE_{act}	negative decimal logarithm of E_{act}
$pK_{a,o}$	negative decimal logarithm of $K_{a,0}$
$p(k_{a,i,o}\ C_{E,tot})$	negative decimal logarithm of $(k_{a,i,o}\ C_{E,tot})$
$pK_{s,0}$	negative decimal logarithm of $K_{s,0}$
$pK_{s,1}$	negative decimal logarithm of $K_{s,1}$
$p(k_{s,i,o}\ C_{E,tot})$	negative decimal logarithm of $(k_{s,i,0}\ C_{E,tot})$
R	ideal gas constant
t	time elapsed
T	absolute temperature
$v_{max,0}$	initial enzyme-catalyzed reaction rate under saturation by substrate
$\Delta E_{act,2/1}$	difference between activation energies of alternative deactivation paths

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