1 **Effect of moderate electric fields on inactivation kinetics of pectin**
2 **methylesterase in tomatoes: the roles of electric field strength and**
3 **temperature**

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Abstract

The objective of this study was to investigate the effect of Moderate Electric Fields (MEF) on pectin methylesterase (PME) activity in tomatoes. Inactivation kinetics parameters of MEF and conventional thermal treatments were determined for samples of identical prior source and pretreatment history, subjected to nearly identical linearly increasing temperature histories. The presence of electric field (60 Hz, sine wave) resulted in activation of PME at $70 (\pm 1) ^\circ\text{C}$, and accelerated inactivation at higher temperatures. This effect increased with field strength. The results may be interpreted in light of molecular motion calculations for PME, which suggest that enzyme molecules subjected to an oscillating electric field experience electrophoretic motion, and behave as if at a slightly higher temperature than that of the bulk, resulting in an activating effect at lower temperatures, but an inactivation effect at higher temperatures.

Keywords: moderate electric fields, tomato, kinetics, electric field strength, pectin methylesterase,

1. Introduction

Tomatoes are thermally processed in the industrial production process of tomato-based products such as juice, soup, ketchup, and sauces. The desired final consistency of each product varies and is achieved by controlling thermal treatments (Anthon et al., 2002). These treatments, hot-break and cold-break processes, primarily control the enzyme catalyzed reactions related to tomato pectins, which maintain structural functionality (texture, rheology) in tomato-based products.

It is well known that pectin methylesterase (PME) and polygalacturonase (PG), are involved in rapid pectin breakdown leading to a loss in viscosity. Thus, these two enzymes are considered as important quality indicators because their activities directly relate to the structural quality attributes of tomato-based products. Consequently, a number of studies have been reported in the literature to examine and control PME and PG activities. In particular, Anthon et al. (2002) studied the thermal inactivation kinetics of PME and PG in tomato juice, with the aim of fine-tuning the hot-break and cold-break processes. Their results show increasingly rapid (first-order) inactivation with increasing temperature, from 65 to 93 °C. They further explained high and low viscosities of the tomato-based products, respectively, resulting by the hot-break (95 °C) and cold-break (60 °C) processes. However, since these conventional thermal treatments are often accompanied by sensory and nutritional quality damage, alternative and/or complementary treatment processes have been investigated in recent years to control enzyme activities.

The effect of pressure on inactivation of tomato PME and PG during high pressure processing treatments at specific temperatures (Van den Broeck [et al.](#), 2000; Crelier [et al.](#), 2001; Rodrigo [et al.](#), 2006; Houben [et al.](#), 2013) has been studied. Furthermore, Moderate Electric Fields (MEF) applied during ohmic heating have shown nonthermal effects on inactivation of lipoxygenase in buffer solutions (Castro [et al.](#), 2004). Also, a similar electric field-induced effect on denaturation of whey proteins has been observed upon application of MEF treatments (Pereira [et al.](#), 2011).

All matter consists of electrically charged subatomic particles, which from the principles of basic physics, must respond to an externally imposed electric field. Enzymes possess net charges and dipole moments, and will undergo translation and rotation in response to an electric field (indeed, gel electrophoresis works precisely on the principle of translational motion). Further, it is possible for conformational structures to be altered by external fields (Pereira [et al.](#), 2011). Hence the basic principles of physics dictate that electric fields affect enzymes to some extent. Whether or not such effects are significant within actual food matrices is worthy of investigation. In a separate work, [Samaranayake & Sastry \(2016\)](#) have investigated the use of frequency control in MEF treatments to control the PME and PG activities in tomato homogenate. [This](#) prior work (Samaranayake & Sastry, 2016) has shown the efficacy of MEF frequencies at or below 60 Hz either to stimulate or to inhibit the PME activity in tomatoes, and also suggested that frequencies greater than 60 Hz had no effect on enzyme activity at 65°C. However, that work was restricted to a low electric field strength. The objective of the present work was to investigate the role of MEF field strength and temperature

on enzyme activity. Given the variability observed in PG measurement observed by our prior work, we focused on PME for this study.

2. Materials and methods

2.1 Rationale for Experimental Methodology:

Since the application of an electric field to a conductive medium inherently results in ohmic heating, it is necessary either to simultaneously cool the sample to maintain temperature constant; or to regulate the electric field at low (but variable) levels to maintain a constant temperature. The former approach is possible only in specific cases, wherein the heating effects are small, thus the latter approach has often been used during kinetic studies. Unfortunately, this approach results in varying electric fields, and high variability in the results (De la Torre, 2009; Somavat, 2011).

We have noted, however, that significant inactivation occurred during the come-up period of MEF samples during previous trials with various enzymes, suggesting that it might be possible to use a variable temperature approach, wherein the electric field could be maintained at constant levels, and temperature effects would be corrected using control samples exposed to the same thermal history but without an electric field. We have also noted in many of the previous enzyme studies, that the come-up period showed a linearly increasing temperature history.

The idea of deriving kinetic data using a linearly increasing temperature approach has been previously reported by Rhim et al. (1989) and in integral form by Nunes et al. (1991). The

present study uses an approach adapted from these investigators to compare inactivation kinetic parameters between MEF and conventional (control) treatments. A further advantage of this approach is the ability to meaningfully use data during the heating. Given that inactivation of enzymes occurs over a narrow temperature range; the characterization of inactivation kinetics at various temperatures in the activation range in constant temperature experiments is difficult because at the higher temperatures of this range, excessive inactivation occurs during the come-up time, leaving little data to collect. Our approach is intended to obviate such problems.

2.2 Theory of linearly increasing temperature kinetics:

From first-order kinetic relations, for a constant temperature, enzyme inactivation may be represented as:

$$\ln\left(\frac{a_0}{a}\right) = kt \quad (1)$$

When the temperature is a function of time:

$$\ln\left(\frac{a_0}{a}\right) = \int_0^t k \, dt \quad (2)$$

where the dependence of the inactivation rate constant, k , on temperature is given by the Arrhenius equation.

$$k = k_0 e^{-\left(\frac{E_a}{RT}\right)} \quad (3)$$

121 For a linearly increasing temperature program:

122 $T = mt + C$ (4)

123 Substituting Eqs. (3) and (4) into (2) yields:

124 $\ln\left(\frac{a_0}{a}\right) = k_0 \int_0^t e^{-\left(\frac{E_a}{R(mt+C)}\right)} dt$ (5)

125 For a given E_a value, the integral in Eq.(5), $\int_0^t e^{\left(\frac{-E_a}{R(mt+C)}\right)}$, may be determined numerically by

126 Gaussian quadrature for various values of the upper integral limit (t). Thus, the pre-exponential

127 factor, k_0 , may be found by fitting $\ln\left(\frac{a_0}{a}\right)$ vs. $\int_0^t e^{\left(\frac{-E_a}{R(mt+C)}\right)}$ data into a straight line, $y = k_0 x$. The

128 knowledge of k_0 enables the determination of rate constants at various temperatures by the

129 Arrhenius equation (Eq. (3)).

130 This particular kinetic approach can be further extended to determine D- and Z-values as:

131 $D = \frac{2.303}{k}$ (6)

132 and

133 $\frac{d(\log_{10} D)}{dT} = -\frac{1}{Z}$ (7)

134

135 *2.3 Tomato homogenate:*

Tomato homogenate was prepared by blending Roma tomatoes of bright red color purchased from a local grocery store (The Kroger Co., Columbus, OH). The homogenate served as the source of PME and PG, as well as the sample study medium. The preparation procedure was as described below.

In each batch, approximately four tomatoes were washed, peeled with minimal use of heat, and then cut into half-inch dices. About 200 g of the diced tomatoes were rapidly frozen and stored in the freezer until used. Prior to the kinetic studies (see below), the frozen diced tomatoes were thawed, and then subjected to blending, using a 12-speed Osterizer blender (Sunbeam-Oster Company, FL, U.S.A), for 1 min at the maximum blender speed (18,500 rpm, without load). The homogenate was passed through a fine metal screen to remove any remaining pieces of skin and seeds, tested for its pH value (4.3 ± 0.1 , 25 °C), and held on ice. At the time of use, the same sample was divided between MEF and control treatment cells. Since both MEF and control cells were treated simultaneously, this ensured identical source and prior handling histories for both samples.

2.4 Internal and external treatment cells:

The homogenate was subjected to MEF treatments simultaneously with corresponding thermal controls from the same homogenate. Both MEF and control treatments were carried out in glass tubes (length: 90 mm, internal diameter: 4 mm, wall thickness: 1 mm), holding about 1 ml of sample. The MEF treatment cells were equipped with titanium electrodes at the ends, whereas the control treatment cells were closed with non-conductive end caps isolating them from the electric field (Fig. 1). All internal treatment cells, whether MEF or control, were

then placed in an external MEF treatment cell shaped like a tub (length: 14.5 cm, width: 7 cm, depth: 9.5 cm) as shown in Fig. 2.

The external treatment cell (Fig. 2), was filled with a NaCl solution (0.2% (w/v); 800 ml) that was iso-conductive with the tomato homogenate at 25 °C. The NaCl solution was preheated (see below) prior to immersing the internal treatment cells. All internal treatment cells, whether MEF or control, were oriented with their ends facing the electrodes of the external treatment cell (i.e. parallel to the current path when an electric field was applied). With each batch of internal treatment cells, there were two specially made thermocouple cells, prepared similar to the ones shown Fig. 1, but inserted with T-type fine-gage wire thermocouples. In making both the MEF and control thermocouple cells, a tiny hole was drilled in the longitudinal midpoint of the glass tube, and the thermocouple was inserted into the tube, extending to the central axis. The hole was then sealed with non-conductive epoxy. These thermocouple cells were designed to constantly monitor temperature throughout the experiment, representing the temperatures of MEF and control treatments.

2.5 Special Precautions:

Per the above, it is important to note that all trials were conducted with MEF and control thermocouple cells from the same sample at the same time; thus sample-to-sample variability was eliminated. Secondly, temperature data were collected for each replicate, to ensure that data were not artifacts of a sloppy temperature control procedure. Signal conditioning was used to ensure that no current leakage occurred to compromise temperature readings in the MEF tubes. Finally, a heat transfer analysis (described later) **was conducted** to map the

expected temperature distributions in each sample to reconstruct the history of each sample in its entirety.

2.6 Kinetic studies:

Kinetic studies were conducted at MEF field strengths of 5, 8, and 10.5 V/cm using 60 Hz (sine wave) alternating field. The choice of this particular frequency was based on a previous experiment on MEF frequencies, wherein the frequencies of 1-60 Hz had shown to reduce residual PME activities in tomato homogenate. Furthermore, 60 Hz is the frequency of readily available power in the U.S. and it is possible to operate with minimal electrochemical reactions with careful selection of electrodes, *even in pilot-scale ohmic heating operations* (Samaranayake & Sastry, 2005) *and in industrial scale operations*. All the studies were started at 65 °C as PME activity in tomato is known to increase with temperature, peaking at 65 °C (Anthon & Barrett, 2006).

In each experimental run, a batch of 13 internal treatment cells consisting of 5 MEF, 6 control, and the 2 thermocouple cells filled with the tomato homogenate held on ice (at 0 °C) were immersed in the external treatment cell (Fig. 2), as described above, without turning on the electric field. Prior to the immersion, the iso-conductive NaCl solution was pre-heated to 72 °C, in order to start the experiment at 65 °C with a short come-up time. The NaCl solution was stirred magnetically throughout the experiment for uniform heat distribution. The temperature of the NaCl solution was also recorded along with that of the thermocouple cells. As soon as the internal treatment cells reached 65 °C, one of the control cells was readily pulled out of the external treatment cell and rapidly cooled by dropping into ice-water (0 °C). This particular

sample served as the t=0 sample. Immediately after withdrawing the t=0 sample, the electric field was turned on starting both MEF and control treatments. The treatments were performed in the temperature range of 65-95°C (65-90 °C for 5 V/cm), while simultaneously pulling out MEF-control cell pairs at five different time/temperature points. A majority of cell pairs (3/5) were withdrawn when the temperature was ≤ 80 °C, anticipating complete loss of PME activity at higher temperatures (Anthon et al., 2002). The treatment cells were pulled out from the electric field with the help of nylon threads attached to them. Then they were rapidly cooled in ice-water (0 °C) as the t=0 sample. The experiments at each MEF field strength were conducted in triplicate; and residual PME and PG activities of the samples were measured by the established methods given below.

2.7 PME assay:

PME activity was assayed by the spectrophotometric analysis of methanol, released by the activity of PME, according to the 96-well plate procedure recently described by Held et al. (2015). In this procedure 50 μ l aliquots of the tomato supernatants were pipetted into the plate wells. Assays were started by adding 150 μ l the reagent mixture, consisted of Fluoral-P and Bovine serum albumin, to each well. After incubating the plates at room temperature for 45 minutes, absorbances were measured at 412 nm using Multiskan FC plate reader (Thermo Fisher Scientific, Waltham, MA). Methanol concentration in each plate well was calculated by means of the respective absorbance reading and a standard curve of methanol standards.

2.8 Heat Transfer Analysis:

Sample temperatures were measured at the centers, which represent the cold spot for the conventional and hot spot for the MEF samples. However, enzyme activities represent the totality of the samples, thus average temperatures are necessary. To determine average temperatures of samples, the following heat transfer analysis was conducted.

The temperature distribution within a sample cell containing homogenate is assumed to be given by thermal conduction (reasonable, since convection is unlikely, and only serves to minimize the nonuniformities – this approach considers a worst-case of nonuniformity):

$$\nabla \cdot (k \nabla T) + u''' = \rho C_p \frac{\partial T}{\partial t} \quad (8)$$

This equation applies to two parts of the system; the tomato homogenate, and the glass of the tube. At their interface, heat flow continuity applies; i.e.

$$k_{sample} \nabla T_{sample} \cdot \vec{n} \Big|_s = k_{glass} \nabla T_{glass} \cdot \vec{n} \Big|_s \quad (9)$$

At the outer layer of the tube, the equation is subject to the convective boundary condition:

$$k \nabla T \cdot \vec{n} \Big|_s = h_c A (T_s - T_{ext}) \quad (10)$$

T_{ext} is the temperature of the external heating medium, which undergoes a linear temperature increase, given by:

$$T_{ext} = at + b \quad (11)$$

235 The internal energy generation term u''' in Eq. 1 is zero for the glass layer, and for the sample
 236 during conventional heating. For the samples undergoing MEF treatments, however, it is
 237 determined from:

$$238 \quad u''' = |\nabla V|^2 \sigma \quad (12)$$

239 The local electrical potential V is determined from the Laplace equation:

$$240 \quad \nabla \cdot (\sigma \nabla V) = 0 \quad (13)$$

241 Which is subject to the insulating condition at the sample-glass interface:

$$242 \quad \nabla \cdot V|_{sample-glass} = 0 \quad (14)$$

243 and constant potential boundary conditions at the sample-electrode interfaces:

$$244 \quad V|_{electrode-1} = V_1 \quad (15)$$

$$245 \quad V|_{electrode-2} = V_2 \quad (16)$$

246 Finally, the electrical conductivity (σ) of the sample is a function of temperature, given by:

$$247 \quad \sigma = \sigma_{ref} [1 + m(T - T_{ref})] \quad (17)$$

248 where the subscript ref implies the value of the property at a reference temperature (T_{ref}).

249 Electrical conductivity-temperature data were obtained experimentally (data not shown) for all

250 samples using methods established in our laboratory, and used in the simulation. The above

system of equations were solved using the finite element approach using the COMSOL code, using a physics-controlled mesh of normal size.

3. Results and discussion

3.1 Thermal histories of internal and external treatment cells:

Fig. 3 shows typical thermal histories of internal and external treatment cells at different MEF field strengths. It can be seen that nearly identical thermal histories were registered for both internal MEF and external MEF treatment cells during application of electric field. The pre-heating of the iso-conductive NaCl solution in the external treatment cell enabled the restriction of the come-up time (from 0 to 65 °C) of about 28 s. It is also seen that all treatment cells have the same thermal history from 65-90 °C, when the field strength is 5 V/cm. The thermal histories at 8 and 10.5 V/cm (from 65 to 95 °C) show a slight elevation of temperature in the internal MEF treatment cells compared to control cells during application of the electric field. This temperature difference is ≤ 1.5 °C at 8 V/cm, and ≤ 2.0 °C at 10.5 V/cm. This clearly indicates ohmic heating of the homogenate by passage of electric current through the internal MEF treatment cells. The measured temperatures shown in Fig. 3, however, relate to the coldest spot of the control (conventionally heated) treatment cells. Consequently, in order to calculate the kinetic parameters, the average temperature distributions in the internal treatment cells were determined by the modeling approach described earlier.

Representative results of the above studies are detailed in Figs. 4(a) and (b). It is clear that the MEF treatment results in a practically uniform temperature throughout the interior. Further, the control treatment also is of fairly uniform temperature, with only minor variations.

Notably, the average temperature in the controls is slightly higher than the minimum temperatures by a small amount (Fig. 5), and this correction may reasonably be applied to these samples. Thus, for the MEF treatment cells the average temperature is the same as the measured temperature at all the field strengths; whereas the average temperatures of control cells are 0.6 °C and 1 °C higher than the corresponding measured temperatures of the control treatment cells in the cases of 8 V/cm and 10.5 V/cm, respectively.

Linear regression of the average thermal histories for the internal treatment cells, from 65 to 95 °C (or 65-90 °C for 5 V/cm), after correcting for the average temperature, is shown in Fig. 6. It is clearly seen that the temperature rise by ohmic heating is quite linear with time at all the field strengths. The average temperature difference between MEF and control treatment cells was always ≤ 1 °C for all the field strengths. The treatment times during application of the electric field at 5, 8, and 10.5 V/cm were 2100, 280, and 158 s, respectively.

3.2 Effect of electric field on PME activity:

In general, Fig. 7 shows inactivation of PME with increasing temperature in both MEF and control treatments. In particular, at 70 (± 1) °C, there is a PME activation effect by MEF. This so-called activation effect is pronounced, even statistically significant ($P < 0.05$) at 8 V/cm, with increasing field strength.

These results are unexpected. As shown, the temperature of the MEF samples are slightly higher than controls; thus it would be expected that if purely thermal effects existed, that the MEF samples would always show slightly lower activity than the controls, even at the lower

temperatures. However, as shown, there is evidence for an activation effect at 70°C, which suggests that effects other than thermal are taking place. Further, since we monitored temperatures for every replicate, and verified agreement with established heat transfer models, it is clear that an activation effect exists, and cannot be due to any artifact.

As the temperature increases, however, MEF treatments comparatively inactivate PME more rapidly than controls. The effect is statistically significant ($P < 0.05$) with increasing field strengths.

3.3 Kinetic data analysis for PME:

For the temperature range of 65-80 °C, Eq. (5) was numerically integrated by 64-point Gaussian Quadrature using various E_a values, ranging from 50 to 550 KJ/mol. The analysis of data shows that, when E_a is below 100 KJ/mol, a particularly poor straight line fit of $\ln\left(\frac{a_0}{a}\right)$ vs. $\int_0^t e^{\left(\frac{-E_a}{R(mt+C)}\right)}$ data for both MEF and control treatments is obtained, especially at 8 and 10.5 V/cm. The $\ln\left(\frac{a_0}{a}\right)$ vs. $\int_0^t e^{\left(\frac{-E_a}{R(mt+C)}\right)}$ data become quite linear ($R^2 > 0.96$) for any E_a value greater than 200 KJ/mol. Therefore, mathematically, there can be an infinite family of k_0 and E_a values for PME inactivation obeying Arrhenius kinetics. Furthermore, interestingly enough, MEF and control treatments yield distinctive straight lines, which become widely separated particularly at 8 and 10.5 V/cm (Fig. 8). This observation indicates the difference in inactivation kinetics between MEF and control treatments, as well as the efficacy of electric field with increasing field strength. We do note that while our R^2 values were consistently high (at least 0.986), the

straight lines in Fig. 8 were constructed with three points of data, which is the bare minimum number for a straight line fit. Nevertheless, the difference in inactivation kinetics suggests a nonthermal effect of electric field, which may be tuned to control enzymatic activity during processing of food products containing PME.

In the literature, the inactivation kinetics of PME has been studied under numerous sets of conditions. Anthon et al. (2002) have briefly reviewed the kinetic parameters and their respective study conditions reported in literature. Particularly, those studies reported various E_a values depending on the nature of the analyte sample (whether tomato juice or PME in pH buffer), temperature range of the study, and the assay method (whether titrimetric or spectrophotometric, assay pH, and temperature). Since we cannot identify a single absolute value for E_a , the values reported by Anthon et. al. (2002), $E_a = 440$ and 477 KJ/mol, were chosen to calculate k values. The choice of these E_a values was merely due to the fairly comparable experimental conditions in both Anthon et al. (2002) and our kinetic study, and does not reflect our preference for any particular study. The k values calculated at both 440 and 477 KJ/mol, at 70°C , (Table 1) show a significant difference at 10.5 V/cm, a marginal, but not significant difference at 8 V/cm, and no significant difference between MEF and control treatments at 5 V/cm.

3.4 Molecular motion calculations for PME:

To gain further insight on the influence of electric fields on the motion of enzyme molecules, it is worthwhile modeling the dynamics of the enzyme molecule under the field. Due

to the extreme computational effort involved, prior attempts using molecular dynamics have typically involved maximum time scales of the order of one to ten nanoseconds (Budi et al., 2007; Astrakas et al., 2012; Singh et al., 2013). This timescale is too short to enable a simulation of motion of the molecule even for the duration of a single cycle in our frequency range. Instead, our approach is to model the rigid body dynamics of a PME molecule to gain insight into its motion, following a similar approach used by Astrakas et al. (2012) in their stochastic simulations on chignolin, wherein this approach had good accuracy compared to the more detailed molecular dynamics simulations. The principal difference between our approach and theirs is that we consider the electric-field driven displacement only, whereas their computation includes a Brownian motion component. We have chosen to consider the Brownian component separately. Our models involve the following relations (Eqs. (18)-(24)).

Translational Motion: The electrophoretic driving force (F_e) is given by:

$$\mathbf{F}_e = q_{net} \mathbf{E} \quad (18)$$

where the electric field strength E shows sinusoidal variation as:

$$\mathbf{E}(t) = \mathbf{E}_{max} e^{j2\pi ft} \quad (19)$$

The equation for linear motion is then:

$$M \frac{d\mathbf{v}}{dt} = q_{net} \mathbf{E}_{max} e^{j2\pi ft} - 6\pi\eta r \mathbf{v} \quad (20)$$

351 where the second term on the right hand side represents the viscous drag on the molecule
 352 (assumed spherical, Johansson et al., 2002, Table 2). We may determine the displacement ($\mathbf{s}_e$)
 353 as $\mathbf{s}_e = \int \frac{d\mathbf{v}}{dt}$, yielding:

$$354 \quad M \frac{d^2 \mathbf{s}_e}{dt^2} = q_{net} \mathbf{E}_{max} e^{j2\pi ft} - 6\pi\eta r \frac{d\mathbf{s}_e}{dt} \quad (21)$$

355 Rotational motion: The torque ($\boldsymbol{\tau}_e$) on the PME molecule is given by:

$$356 \quad \boldsymbol{\tau}_e = \mathbf{p} \times \mathbf{E} \quad (22)$$

357 The equation for angular motion is:

$$358 \quad I \frac{d\boldsymbol{\omega}}{dt} = \mathbf{p} \times \mathbf{E}_{max} e^{j2\pi ft} - 8\pi\eta r^3 \boldsymbol{\omega} \quad (23)$$

359 The relation for angular displacement ($\boldsymbol{\theta}$) is then found by integrating the above relation:

$$360 \quad I \frac{d^2 \boldsymbol{\theta}}{dt^2} = \mathbf{p} \times \mathbf{E}_{max} e^{j2\pi ft} - 8\pi\eta r^3 \frac{d\boldsymbol{\theta}}{dt} \quad (24)$$

361 Eq. (21) was solved in Matlab, using the ode15s solver for stiff systems of ordinary
 362 differential equations. The enzyme molecules were treated as rigid bodies (when calculating
 363 the viscous drag/torque); and molecular structural information for carrot PME available in the
 364 Protein Data Bank (PDB ID 1gq8) (Johansson et al., 2002) was used in the computations (data
 365 for tomato PME were unavailable). The computational modeling was performed using MATLAB
 366 R2012a (The MathWorks, Inc., MA). The input parameters and their sources are as listed in
 367 Table 2.

Since the mean square translational Brownian displacement of a spherical particle (Duplantier, 2006) over a specified time period, t is given by:

$$\langle s_B^2 \rangle = \frac{RT}{N} \frac{1}{3\pi\eta r} t \quad (25)$$

For the PME molecule, the Brownian displacement (s_B , Eq. (25)) was calculated at various temperatures (65 – 80 °C) for time periods corresponding to one half-cycle of the electric field. Since PME molecules are subjected to both Brownian and electrophoretic displacements in the presence of an electric field, the total mean square displacement was calculated as the sum of squares of Brownian and electrophoretic displacements during the same time period, thus:

$$\langle s_{total}^2 \rangle = \langle s_B^2 \rangle + \langle s_e^2 \rangle \quad (26)$$

This total displacement was then used in Eq. (27) to determine the effective (Brownian + electrical) temperature experienced by a PME molecule.

$$T_{eff} = \frac{3\langle s_{total}^2 \rangle N\pi\eta r}{Rt} \quad (27)$$

The above calculations show “electrical temperature” increases of 0.10, 0.27, and 0.46 °C at 5, 8, and 10.5 V/cm, respectively. These suggest that the additional kinetic energy by the electrophoretic motion represents elevation of temperature of enzyme molecules. In general, when the bulk temperature is below the enzyme’s peak activity temperature (i.e. thermal inactivation threshold temperature), an application of electric field may enhance the enzyme activity because the enzyme molecules operate at an apparently higher temperature than that

386 of the bulk. On the other hand, for this very reason, an electric field may cause rapid
387 inactivation of the enzyme if the bulk temperature is above the enzyme's peak activity
388 temperature. The latter qualitatively explains our observation beyond 70 °C, as described in the
389 preceding section. While the current analysis is for a free-floating enzyme molecule in an
390 aqueous medium, and represents an approximate analysis, the trend is in qualitative
391 agreement with experimental observations.

392 It is worth asking the question whether electric fields result in conformational changes to
393 enzymes. To this point, the literature on molecular dynamics simulation, although conducted
394 over far higher frequencies and field strengths, and far shorter time scales, is still instructive.
395 Singh et al. (2013) found that field strengths in the range of 20-40 kV/cm (in the range for PEF
396 processing) had no major effect on structure and surface properties of soybean hydrophobic
397 protein. Indeed, it took (theoretical) field strengths on the order of 30000 kV/cm to affect
398 protein conformation and solvent-accessible surface area. Given that even field strengths of 20-
399 40 kV/cm are too high to sustain except in pulse mode, and requires separate sample cooling to
400 mitigate ohmic heating effects, field strengths of 30000 kV/cm are not currently achievable,
401 and can only be studied in theory. A similar finding is expressed by Astrakas et al. (2012),
402 working at a field strength of 10 kV/cm on chignolin. These works are useful in the present
403 context, suggesting that at low field strengths, conformational changes are negligible, thus our
404 approach of rigid body simulations is justifiable. The concept that the enzyme experiences
405 motion that is greater than the bulk has also been observed by us in separate studies on the
406 activity of cellulase on a cellulose substrate.

4. Conclusions

The results of our kinetic studies show a significant nonthermal effect of electric field on PME activity, which depend on MEF field strength and temperature. At lower temperatures, an activation effect is observed with MEF in comparison to controls, while at higher temperatures, an inactivation effect is observed. The efficacy of electric field on PME inactivation increases with increasing field strength. Calculations of Brownian and electrophoretic motions suggest that enzyme molecules behave as if at a higher temperature than that of the bulk in the presence of an electric field. This observation is in qualitative agreement with experimental observations. Unlike classical isothermal kinetic methods, where electric field effects are subordinated to thermal effects, the linearly increasing temperature approach allows the study of the inactivation of enzymes during MEF treatment over a temperature range, while maintaining a constant electric field.

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428

Nomenclature

429 a Catalytic activity of enzyme (nkat/ml) at $t=t$; constant in Eq. 11, obtained by regression

430 a_0 Catalytic activity of enzyme (nkat/ml) at $t=0$

431 b Constant in Eq. 11, obtained by regression

432 C Intercept of the linearly increasing temperature program (K)

433 C_p Specific heat (J/kg $^{\circ}$ K)

434 D D - value (decimal reduction time) (min)

435 E_a Activation energy (J/mol)

436 E Electric field strength (V/m)

437 E_{max} Peak value of the electric field strength (V/m)

438 f Electric field frequency (Hz)

439 F_e Electrophoretic driving force (N)

440 h_c Convective heat transfer coefficient (W/m 2 $^{\circ}$ K)

441 I Moment of inertia of molecule (kg m 2)

442 j Complex constant

443 k First-order inactivation rate constant (s $^{-1}$); thermal conductivity in heat transfer model

444 (W/m $^{\circ}$ K)

445	k_0	Pre-exponential or frequency factor (s^{-1})
446	M	Molecular mass (Kg)
447	m	Slope of the linearly increasing temperature program ($^{\circ}K/s$); temperature coefficient of
448		electrical conductivity in Eq. 17 ($^{\circ}K^{-1}$)
449	N	Avogadro's number (6.022×10^{23})
450	$\vec{n}$	Unit normal vector
451	P	Probability
452	$\mathbf{p}$	Dipole moment of molecule (C m)
453	q_{net}	Net molecular charge (C)
454	R	Universal gas constant (8.314 J/mol K)
455	R^2	Goodness-of-fit of the linear regression model
456	$\mathbf{s}$	Displacement vector (m)
457	s	Linear displacement, brownian and electrophoretic (m)
458	r	Radius of the molecule (m)
459	T	Temperature ($^{\circ}C$ or $^{\circ}K$)
460	t	time (s); time for a half-cycle of the electric field ($t = 0.0166$ s) in Eq. (25)
461	u'''	Internal energy generation rate (W/m^3)

462	V	Electrical potential (V)
463	$\mathbf{v}$	Velocity vector (m/s)
464	Z	Z-value (temperature change that results in a 10-fold change in D value) (°C)
465		
466	Greek Letters	
467	η	viscosity of tomato juice (0.22 Pa.s; Rao, A.M. (2007))
468	θ	Angular displacement vector (rad)
469	τ_e	Electrophoretic torque (N m)
470	ρ	Density (kg/m ³)
471	σ	Electrical conductivity (S/m)
472	ω	Angular velocity vector (rad/s)
473		
474	Subscripts or superscripts not described elsewhere	
475	B	Brownian
476	e	Electrophoretic
477	electrode-1	Electrode 1

478	electrode-2	Electrode 2
479	ext	External heating medium (i.e. fluid in external cell)
480	glass	Glass
481	ref	Value at reference temperature
482	s	Surface value
483	sample	Sample

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Table 1: $k \times 10^3$ (s^{-1}) values calculated at 440 and 477 KJ/mol, at 70 °C, for MEF and control treatments.

E _a (KJ/mol)		$k \times 10^3$ (s^{-1})		
		5 V/cm	8 V/cm	10.5 V/cm
440	MEF	15.7 ^a ($\pm$ 1.5)	6.5 ^a ($\pm$ 1.3)	8.2 ^a ($\pm$ 2.8)
	Control	16.8 ^a ($\pm$ 2.2)	4.7 ^a ($\pm$ 0.4)	2.0 ^b ($\pm$ 0.4)
477	MEF	16.3 ^a ($\pm$ 1.6)	5.2 ^a ($\pm$ 1.0)	7.0 ^a ($\pm$ 3.0)
	Control	17.6 ^a ($\pm$ 2.3)	3.8 ^a ($\pm$ 0.4)	1.8 ^b ($\pm$ 0.6)

The values are shown as the mean ($\pm$ standard deviation) of three replicates.

At each activation energy and field strength, the MEF and control treatments with the same superscript letters (*a*, *b*) are not significantly different ($P > 0.05$).

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580 Table 2. Values of parameters used in molecular motion calculations.^a

581	Parameter	Value for PME
582		(PDB ID 1gq8)
583		
584	q_{net} (C)	-4.005×10^{-20}
585	M (Kg)	6.755×10^{-23}
586	r (m)	2.00149×10^{-9}
587	η^b (Pa.s)	0.22

588 ^a The values were obtained by HyperProtein 1.0 (Hypercube, Inc., FL) using the Protein Data
 589 Bank structures (PDB ID 1gq8: Johansson et al., 2002).

590 ^b For tomato juice, 32.2 °C, pH = 4.3, 5.8% solids (Rao, 2007).

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List of Figure Captions

593 Fig. 1. Internal treatment cells. A: MEF; B: control.

594 Fig. 2. External MEF treatment cell. a : magnetic stirrer; b : the treatment cell; c : T-type
595 thermocouple; d : titanium electrodes.

596 Fig. 3 Typical thermal histories of the internal (as measured in the coldest spot) and external
597 treatment cells at 5, 8, and 10.5 V/cm.

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599 heating with a linearly increasing temperature gradient.

600 Fig. 5. Representative plot of medium temperature, and average and minimum sample
601 temperatures for control samples.

602 Fig. 6 Linear regression of the average thermal histories for the internal treatment cells during
603 application of the electric field.

604 Fig. 7 Retention (%) of PME activities in MEF and control treatments at various temperatures.
605 The presence of asterisk (*) at some temperatures indicates significant differences ($P < 0.05$) of
606 mean % retention ($n = 3$) between the two treatments.

607 Fig. 8 Typical $\ln\left(\frac{a_0}{a}\right)$ vs. $\int_0^t e^{\left(\frac{-E_a}{R(mt+C)}\right)}$ data (see Eq. (5)) for E_a values greater than 200 KJ/mol at
608 various field strengths.

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 669 various field strengths.