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Effect of moderate electric fields on inactivation kinetics of pectin methylesterase in tomatoes: The roles of electric field strength and temperature

Chaminda P. Samaranayake, Sudhir K. Sastry*

Department of Food, Agricultural and Biological Engineering, The Ohio State University, 590 Woody Hayes Drive, Columbus, OH, 43210, United States

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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 (±1) °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.

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 polygalcturonase (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

* Corresponding author.
E-mail addresses: samaranayake.3@osu.edu (C.P. Samaranayake), sastry.2@osu.edu (S.K. Sastry).

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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 and 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 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:

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

When the temperature is a function of time:

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

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

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

For a linearly increasing temperature program:

$$T = mt + C \tag{4}$$

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

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

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 Gaussian quadrature for various values of the upper integral limit (t). Thus, the pre-exponential 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 knowledge of k_0 enables the determination of rate constants at various temperatures by the Arrhenius equation (Eq. (3)).

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

$$D = \frac{2.303}{k} \tag{6}$$

and

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

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, 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.