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Effects of controlled-frequency moderate electric fields on pectin methylesterase and polygalacturonase activities in tomato homogenate

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ABSTRACT

The effect of controlled-frequency moderate electric field treatments on pectin methylesterase and polygalcturonase activities in tomato homogenate was investigated by subjecting identically treated control and electrically-treated samples to the same temperature history. Additionally, a model was developed for the motion of the enzyme molecules subjected to an electric field. Results show that the application of electric fields at a low field strength (0.4 V/cm) constant temperature (65 °C) has a statistically significant effect on pectin methylesterase activity, typically at or lower than 60 Hz. At higher frequencies, the effects are negligible. Molecular motion simulations suggest that the efficacy at low frequencies may be due to the amplitude of motion being of the order of the intermolecular distance for water. Higher frequencies result in small overall displacements due to rapid reversals in the direction of motion.

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1. Introduction

Endogenous enzymes naturally found in fruits and vegetables are known to be responsible for changes in postharvest food quality. Changes in functional properties, particularly texture (rheology) in fruits and vegetables as well as their preprocessed intermediates and end products, are directly related to enzyme-catalyzed reactions. Among several catalytic reactions activated by various enzymes, the disintegration of pectin structure by pectin methylesterase (PME) and polygalcturonase (PG) is considered to play a significant role in the structural functionality (Anthon, Sekine, Watanabe, & Barrett, 2002; Crelier, Robert, Claude, & Juillerat, 2001; Fachin et al., 2002). Controlling the retention (or breakdown) of pectins is thus of great importance particularly during processing of fruits and vegetables. Consequently, there have been a number of studies reported in the literature over the decades to examine and control the activities of these enzymes.

Alternative and/or complementary processing technologies have been investigated in recent years for controlling enzyme activities. Some, for instance, high pressure processing, have shown to cause both activation and inactivation of tomato PME and PG at specific pressure–temperature combinations (Broeck, Ludikhuyze, Loey, & Hendrickx, 2000; Crelier et al., 2001). Inactivation of various enzymes has been reported in pulsed electric field processing at relatively low temperatures (<50 °C) (Leong & Oey, 2014; Yang, Li, & Zhang, 2004), although the occurrence of hot spots created at electrode edges may have had significant thermal effects (Meneses et al., 2013; Salengke, Sastry, & Zhang, 2012). The Moderate Electric Fields (MEF) applied during ohmic heating have been shown to exhibit a nonthermal inactivation effect on lipoxygenase and polyphenoloxidase in buffer solutions (Castro, Macedo, Teixeira, & Vicente, 2004). A recent study with whey proteins treated by conventional and ohmic heating shows reduced (statistically significant levels at some temperatures) protein denaturation upon application of MEF (Pereira, Teixeira, & Vicente, 2011).

It is worth considering what an electric field might do to enzymes. In principle, all matter consists of electrically charged subatomic particles, which from basic physics, must respond individually to an externally imposed electric field. For molecules, it is the net charge that is important. Enzymes, which are proteins, possess net charges and dipole moments when in an aqueous environment, and will move in response to an electric field (indeed, well-known techniques of gel electrophoresis are used to separate proteins precisely on this principle). 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.

One of the fundamental difficulties in determining if electric fields have nonthermal effects on enzymes is that temperature control is challenging – electric fields in conductive media result in currents and the inevitable ohmic heating. The higher the field

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Nomenclature

C_R	constant (=3 in the DSE theory, but often used as a fitting parameter)
$\mathbf{E}$	electric field strength
f	frequency
$\mathbf{F}_e$	electrophoretic driving force
I	moment of inertia of molecule
j	complex constant
k_B	Boltzmann constant
m	mass of molecule
$\mathbf{p}$	dipole moment of molecule
q_{net}	net charge on molecule
r	radius of molecule
$\mathbf{s}$	displacement vector
t	time
T	absolute temperature
$\mathbf{v}$	velocity vector
v_s	volume of molecule

Greek letters

η	viscosity of medium
$\boldsymbol{\theta}$	angular displacement vector
$\boldsymbol{\tau}_e$	electrophoretic torque
τ_R	rotational self-diffusion correlation time
$\boldsymbol{\omega}$	angular velocity vector

Subscripts or Superscripts not mentioned elsewhere

max	maximum

strength, $\mathbf{E}$ the greater the ohmic heating by a factor of $\mathbf{E}^2$ – thus studies at high electric field strengths (as for example, with pulsed electric fields) are conducted under highly transient field and temperature, not typically well-controlled conditions. This may compromise or complicate data analysis, and necessitate the use of complex, sometimes unverified models to untangle the various effects (Meneses et al., 2013).

At the present time, information relating to nonthermal effects of MEF treatments on food proteins and enzymes is rare; and the existing studies have been mainly concerned with electric field effects on biological cells (Kulshrestha & Sastry, 2003; Loghavi, Sastry, & Yousef, 2008, 2009; Somavat, Mohamed, Chung, Yousef, & Sastry, 2012). Therefore, the objective of this study was to investigate the effect of electric field frequency on PME and PG activities upon application of controlled-frequency moderate electric fields. However, unlike the enzyme studies that are often conducted in model buffer systems, our study used freshly prepared tomato homogenate as the source of PME and PG, as well as their study medium. The use of this realistic study medium accounts for the possible food matrix effects, such as shielding of enzyme molecules from the electric field by the insulating nature of the insoluble solids. An additional objective was to develop mathematical models for motion of enzymes under MEF, in order to gain further insight into possible effects.

2. Materials and methods

2.1. Preliminary considerations for the food matrix

Enzymes are charged molecules and thus respond to external electric fields. While the effects of electric field may be clearly observable in model buffer systems, there is evidence, from pulsed electric field processing that the food matrix significantly influences the electric field effect on enzymes (Oey, 2010). Furthermore, uneven distribution of the electric field due to nonuniformity of food matrices, is well-known in ohmic heating (Zareifard, Marcotte, Ramaswamy, & Karimi, 2014). For instance, enzyme molecules attached to or trapped in membrane segments (electrical insulator) and other debris (e.g. tomato skin, seeds, insoluble fibers) may be shielded from the electric field, whereas free-floating enzyme molecules may be highly responsive to the electric field. Therefore, we paid careful attention to sample preparation and controlled the operations in the sample preparation procedure (see below) to minimize potential shielding effects leading to sample-to-sample variability.

Moreover, since PME- and PG-catalyzed reactions take place as soon as the tomato tissues are torn, the entire sample preparation procedure was carried out well below room temperature or with minimal exposure to heat, in order to preserve the enzyme activities. Finally, all control and MEF samples were tested simultaneously, with identical preparation and prior handling histories, being subsets of the same larger sample. This ensured elimination of any history variables.

2.2. Sample preparation procedure

Roma type tomatoes of bright red color were obtained from a local grocery store (The Kroger Co., Columbus, OH). In each batch, approximately four tomatoes were washed and then peeled with minimal use of heat, first by immersing each individual tomato in boiling water (⩾98 °C) for 10 s and then by instantly dropping into ice-water (0 °C). The loosened tomato skin was removed by hand after about 10 min in the ice-water bath. The peeled tomatoes were sliced in half to remove the seeds and locular gel, and then cut into half-inch dices using a kitchen dicer. About 200 g of the diced tomatoes were weighed into a polyethylene freezer bag and placed in a −20 °C freezer laying the bag flat for rapid freezing. The frozen tomatoes were stored in the freezer until used.

2.3. Preliminary blending experiments

We conducted some preliminary experiments to identify a sufficient blending time, speed (rpm), the resulting solid and serum (liquid) contents, and the enzyme distribution in the different phases. Prior to blending, the frozen diced tomatoes were thawed to about 1 °C by keeping the bag at room temperature with constant temperature monitoring inside the bag. It is important to note that, though enzyme-catalyzed reactions start with thawing, the reaction rates are insignificant at lower temperatures (⩽1 °C) compared to room temperature (25 °C) (Terefe, Delele, Loey, & Hendrickx, 2005). Also, performing the blending operation at a lower temperature (about 1 °C), would be advantageous in preserving enzyme activities of the original sample.

Homogenate was prepared by blending the entire content of the thawed diced tomato in the bag (200 g), using a 12-speed Osterizer blender (Sunbeam-Oster Company, FL, U.S.A). The blending time was varied as: 0.5, 1, 2, 3, and 4 min at the maximum blender speed (18,500 rpm, without load). These blending times and speed (i.e. high-speed short time blending) were chosen after several trial and error runs, such that to minimize the homogenate from warming up during blending. The homogenate was passed through a fine metal screen to remove any remaining pieces of skin and seeds, and then held on ice until centrifugation. Furthermore, a sample

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