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# Enhancing the activity of pectinase using pulsed electric field (PEF) treatment

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ABSTRACT

Pectinase is commercially important in the fruit processing and fiber degumming industries. Emerging technologies that improve its stability and activity could have potential in improving its commercial value further. The effects of pulsed electric field (PEF) treatment on the enzymatic activity, kinetics and conformational structure of pectinase were evaluated. Maximum activity was obtained at 12 kV/cm and a flow velocity of 80 mL/min, and was 21.89 ± 1.67% higher than the untreated enzyme. This enhancing effect could be maintained for 19 h when the treated enzyme was stored at 4 °C. PEF treatment increased the enzyme kinetic constants $V_{max}$, $k_{cat}$, $t_{1/2}$, $\Delta G$, and decreased $K_m$, $k$, $Ea$, $\Delta H$, and $\Delta S$, indicating improved affinity between enzyme and substrate and enhanced catalytic efficiency. PEF treatment did not alter the polypeptide composition of the pectinase molecules, but induced the transfer of the tryptophan microenvironment from the polar surface to the hydrophobic interior, with a 10.28% increase in α-helices and a 17.80% reduction in random coils.



## 1. Introduction

Pectinases constitute a group of enzymes that break down pectic polysaccharides in plant cell walls (Jayani et al., 2005; Kant et al., 2013). They are used commercially in fruit processing and in the degumming of natural fibers (Demir et al., 2001). Whilst they have many useful catalytic properties, the native enzymes are relatively high in cost, exhibit poor stability, and are difficult to recover and re-use (Mateo et al., 2007). To overcome these limitations, immobilization of the enzymes coupled with their chemical or genetic modification have been used as techniques to improve their catalytic features and produce a more economically attractive commercial product (Chiliveri and Linga, 2014; Latifian et al., 2007; Wu et al., 2014). However, these technologies themselves have limitations, such as the production of undesirable chemical residues and long cultivation periods. In order to address these issues, certain non-thermal treatment technologies been developed as alternatives. For example, pulsed electric field (PEF), high hydrostatic pressure (HHP) and ultrasound irradiation, have been proposed as technologies to activate or stabilize enzymes (Balasubramaniam et al., 2008; Ho et al., 1997; Tran and Le, 2011).

In this context, PEF is emerging as a non−thermal food processing technology that delivers short pulses of high voltage across a food material placed between two or more electrodes (Leong and Oey, 2014). Because of its low energy consumption, short timeframe, and the fact that it does not results in chemical residues, PEF has been used to modulate the activity and stability of many enzymes, with significant success (Ho et al., 1997; Zhao and Yang, 2008); (Salvia-Trujillo et al., 2011), showing that low intensity PEF can enhance enzymatic activity. The enzymes whose activities have been increased successfully with PEF include: β-galactosidase, peroxidase, protease, glucoamylase, invertase, enolase, lysozyme and pepsin (Ho et al., 1997; Lu and Yin, 2014; Najim and Aryana, 2013; Ohshima et al., 2007). Although the PEF-enhanced activities of these enzymes have been previously evaluated and reported, the underlying mechanisms have not yet been fully understood. Research to date has suggested two possible explanations for observed PEF-induced enzyme activation, that are not mutually exclusive: (1) the creation of a greater number of active sites and/or an increase in the size of existing active sites on the enzyme molecules (Aguilo-Aguayo et al., 2008), and (2) the alteration of the intermolecular structure of the enzyme (Zhao and Yang, 2008). Thus, PEF is known to be effective in activating various enzymes. However, the application of PEF technology in improving the properties of pectinase has not previously been studied.

In this study, we evaluated the effect of PEF technology on

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**Abbreviations**

| | |
|---|---|
| PEF | pulsed electric field |
| CD | circular dichroism |
| $V_{max}$ | maximum rate of reaction |
| $K_m$ | Michaelis constant |
| $k_{cat}$ | catalytic constant |
| $k$ | thermal inactivation rate constant |
| $t_{1/2}$ | half-life period |
| $Ea$ | activation energy |
| $\Delta H$ | enthalpy |
| $\Delta S$ | entropy |
| $\Delta G$ | free energy |
| Tyr | tyrosine |

pectinase activity, molecular structure, kinetics, and thermodynamic properties. The specific aims of the study were to (1) optimize PEF processing technologies by determining the optimum parameters for pectinase activation, (2) investigate the effects of PEF treatment on pectinase enzyme kinetics and thermodynamic parameters, based on the Michaelis–Menten and the Arrhenius equations, and (3) to determine the molecular structure alterations caused by PEF treatment, using SDS-PAGE, circular dichroism (CD) spectroscopy and fluorescence spectroscopy. Based on our findings, a viable method for the modification of pectinase by PEF treatment was developed, that could inform the future improvement of pectinase-based fruit processing technologies as well as the development of similar technologies for the processing of other enzymes.

## 2. Materials and methods

### 2.1. Materials

The pectinase used in the study was derived from *Aspergillus Niger* lyophilized powder (30,000 U/g) and was acquired from Shanghai Aladdin Industrial, Inc. (Shanghai, China). Pectin was supplied by Sigma Chemical Company. The precision protein standard was obtained from Solarbio Co. (Beijing, China). All other reagents used were of analytical grade. Deionized water was used for all experiments.

### 2.2. PEF treatments

The PEF system employed was self-made by Fujian Agriculture and Forestry University. The working parameters of the PEF equipment are as follows: a co-field continuous processing chamber; pulse frequency, 1–10 kHz; number of pulses, 80; pulse width, 5–20 μs; electric field intensity, 0–50 kV/cm; flow velocity, 50–180 mL/min; electrode diameter, 0.29 cm; and square-wave form, monopolar.

Pectinase was weighed and dissolved in 1000 mL citrate buffer (0.2 M, pH 3.5) and stored at 4 °C. Before being subjected to PEF treatments, 10 mL of this solution was diluted into 1 L deionized water (25 ± 1 °C). The resulting solution was immediately pumped into the PEF system using a Langer BT00-100M Pump (Langer Instruments, China) at an electric field intensity of 3–21 kV/cm and a flow velocity of 50–160 mL/min. The PEF voltage and current were precisely monitored via a digital oscilloscope. Pectinase activity was measured immediately after PEF treatment.

### 2.3. Enzyme assay

To perform the pectinase activity assay, mixed solutions of 900 μL pectin substrate solution (pH 3.5) and 100 μL enzyme solution were maintained at 55 °C in a water bath (Changzhou Ao Hua Instrument Co. Ltd., Jiangshu, China.) for 30 min according to the procedure outlined by Jing *et al.* ((Jing et al., 2006)). The reaction was interrupted by adding 1 mL DNS and heating to 100 °C for 5 min. The reaction tube was then removed and quickly cooled to room temperature. After adding 8 mL deionized water, the sample absorbance was determined at 540 nm with an ultraviolet spectrophotometer (T6, Beijing Purkinje General Instrument Co. Ltd, Beijing, China), and the corresponding *d*–galacturonic content was determined from the *d*–galacturonic standard curve. One enzyme unit (U) was expressed as the amount of pectinase that hydrolyzed pectin to form 1 mg *d*-galacturonic acid per 30 min at 55 °C and pH 3.5.

### 2.4. Pectinase stability during refrigerated storage

PEF-treated pectinase was refrigerated at 4 °C and measured for its residual activity at 1 h intervals. Untreated enzyme was used as the control.

Pectinase after extraction was stored at cold room temperature (±4 °C) for 10 days. Then, the enzymatic activity of the pectinase after storage was measured as earlier mentioned. The ratio of pectinase activity after storage to the activity of pectinase before storage provided the efficiency of pectinase storage stability as shown in equation below:

### 2.5. Enzyme kinetic parameters of PEF-treated pectinase

The effects of PEF on the enzyme kinetic parameters of the pectinase were measured at varying pectin concentrations (0.1–0.8%, w/w) with a constant concentration of enzyme (23.8 U/mL). The affinity of the substrate for the enzyme followed the Michaelis–Menten enzyme kinetic model in accordance with the equation described below:

$$v = \frac{V_{max}[S]}{K_m + [S]} \quad (1)$$

where $v$ is the initial reaction rate, $V_{max}$ is the maximum reaction rate achieved at saturating substrate concentration, [S] is the substrate concentration and $K_m$ is the Michaelis–Menten constant that defines the substrate concentration at which the reaction rate is half of $V_{max}$ (Subhedar and Gogate, 2014).

### 2.6. Thermal stability and inactivation kinetics of PEF-treated pectinase

A sample of pectinase (10 mL, 23.8 U/mL) was placed in a tube and maintained in a water bath at different temperatures between 40 and 60 °C, with enzymatic activity being analyzed every 10 min. Enzyme activity was regarded as 100% when incubated for 0 min ($E_0$). Enzyme thermal inactivation can be expressed by the first-order kinetic equation:

$$\ln\left(\frac{E_t}{E_0}\right) = -k \times t \quad (2)$$

where $E_t$ is the enzymatic activity at time $t$, $k$ is the thermal inactivation rate constant and $t$ is the heating time. From the thermal inactivation curve $k$ is obtained. The half-life ($t_{1/2}$) and activation energy ($Ea$) can then be calculated by the following formulas:

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