A STUDY ON THERMAL INACTIVATION PARAMETERS OF PECTINMETHYL ESTERASE FROM TURKISH HACIHALILOĞLU APRICOT (PRUNUSARMENIACAL)

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Abstract: This study was carried out to determine thermal inactivation parameters of pectin methylesterase (PME) from Hacıhaliloğlu apricot which is an important variety grown in Malatya region of Turkey. The first order inactivation constants (k_D) increased with increasing temperature, indicating that the enzyme was less thermostable at higher temperatures. Increasing the temperature from 60°C to 70°C resulted in a decrease in $t_{1/2}$ values. D values obtained in this study ranged between 6.0 and 30.9 min at the temperatures studied. Energy of activation (E_a) and Z values were found to be 155.5 kj mol^{-1} (r^2=0.9887) and 14.1 °C (r^2=0.9869).

Keywords: Apricot, Pectin Methylesterase, Kinetics, Thermal Inactivation Kinetics

I. INTRODUCTION

One of the main problems in the fruit and vegetable product industry is maintenance of turbidity in fruit and vegetable juices and of consistency of whole or diced products during processing and storage. Research on non-thermally treated products demonstrated that cloud loss in fruit juices is mainly due to the cooperative action of pectin methylesterase (PME, EC 3.1.1.11) and polygalacturonase (PG). PMEs play an important role in determining the extent to which demethylesterified pectic polysaccharides are accessible to degradation by PG. Demethylation of pectin results in a juice that separates in clear serum and a sediment, which arises from low methoxyl pectin that complexes with calcium ions. Therefore, control of PME activity is crucial for the cloud stability of juices. Cloud loss problem can be easily overcome with thermal processes (Fischer and Bennett, 1991; Castaldo et al., 1997).

PMEs are cell wall-bound enzymes and catalyse the hydrolytic cleavage of the methylester moieties on pectin molecules, resulting in the release of methanol and partially de-esterified pectin. The control of PME activity has been a common subject of study because of the implications in the modification of the texture of fruit and vegetables and as a destabilizing agent of pectin materials in fruit juices and concentrates (Sila et al., 2007).

Turkey is the leading apricot producer in the world with an annual production of 390000 tons in 2005, which amounted to 13% of the world production. Malatya region of Turkey is particularly important for cultivation, production, and processing of apricots, as around 50% of the fresh apricots and 90% of the dried apricots in Turkey are produced in this region. The most cultivated apricot varieties in Malatya region are, Hacıhaliloglu, Hasanbey, Sogancı, Kabaası, Alyanak, Cataloglu, and Cologlu (Asma, 2000; Akin et al., 2008).

There has been numerous research on PME from different sources, e.g. carrot (Unal and Bellur, 2009), green beans (Laats et al., 1997), orange juice (Lee et al., 2003), pepper (Castro et al., 2006). This study was aimed at determining thermal inactivation parameters of PME from Hacıhaliloğlu apricot

II. MATERIALS AND METHODS

2.1.Materials

The apricots used in this study were obtained from Malatya region of Turkey and frozen at -25°C until used.

Apple pectin (70-75% esterification degree), trisma base, dialysis bag (cellulose membrane: 76mm×49mm) were purchased from Sigma (St.louis, USA). Sodium hydroxide, ethanol (99%), ammonium sulphate, hydrochloric acid, sodium disulfite, polyvinylpolypyrrolidone (PVPP) and sodium chloride were purchased from Merck (Darmstadt, Germany). All the reagents were analytical grade.

2.2.Extraction of PME

PME is ionically bound to cell wall and therefore a buffer with a high ionic strength (Tris, NaCl) is needed to extract it from cell wall (Nunes et al., 2006).

The extraction of Hacıhaliloğlu apricot PME was performed according to the method ofDenes et al. (2000) with modifications. All extraction steps were performed at 4°C to prevent enzyme inactivation. The deseeded apricots in batches of 300 g were chopped and then homogenized in 300 ml of cold distilled water containing 500 mg/L sodium disulphite for two minutes at maximum speed by using Waring blender (Model HGB2WTS3, Torrington, Connecticut, USA). The homogenate was centrifuged (10000 x g for 30

min at 4°C) and the supernatant was discarded. The pellet was mixed with 200 mL of cold distilled water containing 500 mg/L sodium disulphite and then centrifuged at 10000 x g for 30 min at 4°C. Mixing and centrifuging were performed 3 times. The last pellet was resuspended in 200 mL of cold buffer solution of 20 mM Tris-HCl (pH 7.5) buffer containing 1 M NaCl and 500 mg/L $Na_2O_5S_2$, followed by centrifugation at 10000 x g for 30 min at 4°C. 1% (w/v) PVPP (polyvinylpolypyrrolidone) was added to the supernatant and magnetically stirred for 30 min at 4°C, followed by centrifugation at 5000 x g for 10 min at 4°C. This procedure was repeated until a clear, colourless supernatant was obtained. Altogether, a total of 1.2 kg of apricots was used in the extraction of PME.

All the supernatants obtained at each extraction step were combined and then subjected to 80% ammonium sulphate precipitation. The precipitate containing PME was collected by centrifugation at 10000 x g for 60 min at 4°C and dissolved in 3 mL 10 mM Tris-HCl (pH 7.0). The extract was then dialysed overnight in Tris buffer (pH 7.0) at 4°C. The extract was used as PME source in the following experiments.

2.3.Assay of PME Activity

PME activity was measured titrimetrically by determining free carboxyl groups formed as a result of enzyme action on pectin. The reaction mixture was composed of 10 mL of 0.5% apple pectin solution containing 0.1 M NaCl and 0.5 mL of PME extract. The reaction was carried out at 30°C in a water-jacketed reaction beaker. The volume of NaOH required to maintain the pH of the reaction mixture at 7.5 for 10 min was measured. One unit of PME was defined as the amount of enzyme that released 1 µmol of carboxyl groups/min, under the aforementioned assay conditions (Denes et al., 2000).

PME activity was calculated using the following formula:

E (units/mL) = (mL of NaOH)(molarity of NaOH)(1000) /(time)(mL of enzyme)

2.4.Thermal Inactivation Kinetics

The enzyme samples were incubated for 2, 5 and 8 min at 60ºC and 2, 4 and 6 min at 65ºC and 70ºC in screw-cap tubes. The screw-cap tubes were pre-heated to the selected temperature to prevent temperature lag before the addition of enzyme solution. The enzyme samples were removed from water bath after pre-set times and were immediately transferred to ice bath to stop thermal inactivation. After the enzyme sample was cooled in ice bath, the residual activity (A) was determined within 60 min as described previously. A non-heated enzyme sample was used as blank (A_o). The percentage residual activity was calculated by comparison with the unheated sample. First order inactivation constant (k_D) was calculated from the slope of the natural logarithm (ln) of A/A_o vs. time graph. Half-life of the enzyme ($t_{1/2}$) was calculated by using the following equation: $t_{1/2} = 0.693/ k_D$.

Decimal reduction time (D value) was estimated from the relationship between k_D and D value: $D = \ln (10)/ k_D$. The Z value, which is the temperature increase required for a one-log10 reduction (90% decrease) in D value, was determined from a plot of $\log_{10}^{D}$ versus temperature. The slope of the graph is equal to $1/Z$ value. The energy of activation of denaturation (E_a) was calculated by multiplying the slope of Arrhenius plot (i.e. natural logarithm of k_D values vs. reciprocal of absolute temperatures ($1/T$)) with universal gas constant, R (kJ/molK) (Marangoni, 2003).

III. RESULTS AND DISCUSSION

3.1.Thermal Inactivation Kinetics

The thermal inactivation parameters of Hacıhaliloğlu apricot PME are presented in Table 1. The first order inactivation constants (k_D) increased with increasing temperature, indicating that the enzyme was less thermostable at higher temperatures.The half-life ($t_{1/2}$) is another important parameter used in the characterization of enzyme stability. Increasing the temperature from 60°C to 70°C resulted in a decrease in $t_{1/2}$ values (Table 1). Unal and Bellur (2009) who investigated the thermal inactivation of black carrot PME reported k_D values of 0.040, 0.133 and 0.337 min at 55, 60 and 65°C, respectively, which are similar to those obtained in this study.

The decimal reduction time (D value) is the time, at a given temperature and pressure, needed for 90% reduction of the initial activity. D values obtained in this study ranged between 6.0 and 30.9 min at the temperatures studied (Table 1). Unal and Bellur (2009) reported D values of 57.7 min at 55°C and 6.8 min at 65°C for black carrot PME, which are similar to those obtained in this study.

The temperature dependence of the decimal reduction time is characterized by Z value, which is the temperature increase needed for a one $\log_{10}$ reduction (90% decrease) in the D value.The Z value obtained in this study was14.1 °C (0.9869). E_a value for thermal inactivation of Hacıhaliloğlu apricot PME was found to be155.5 kj/mol (0.9887). Unal and Bellur (2009) reported an Ea value of 196.8 kJ/molfor black carrot PME, which is close to the one calculated in this study.

Table 1

Thermal inactivation parameters of Hacıhaliloğlu apricot PME

Temperature (ºC)	k_D (1/min)	r^2	$t_{1/2}$ (min)	D (min)
60	0.0746	0.9999	9.3	30.9
65	0.199	0.9877	3.5	11.6
70	0.3829	0.9265	1.8	6.0

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