Kinetics and Thermodynamics Analysis of the Thermal Inactivation of Three Partial Purified Pectinases (Pectin Methylesterase, Polygalacturonase and Pectin Lyase) Extracted from Papaya (*Carica papaya* L. cv solo 8) Epicarp

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Abstract— Three pectinases (pectin methylesterase, polygalacturonase and pectin lyase) were partially purified from papaya (*Carica papaya* L. cv solo 8) epicarp grown in Côte d'Ivoire and its activity, the kinetics and thermodynamic analysis of the pectinases were studied, using pectin and polygalacturonic acid as a substrates. Optimal conditions for enzymatic studies were determined to be pH 7.5; 5 and 8 respectively and 60 °C; 45 °C and 50 °C respectively. Thermal inactivations of pectin methylesterase (PME), polygalacturonase (PG) and pectin lyase (PL) were examined in more detail between 45 and 65 °C and in relation to exposure time. Denaturation of these enzymes, measured by the loss in activity, could be described as a first-order reaction with k-values between 0.0025 and 0.0516 min. D- and k-values decreased and increased, respectively, with increasing temperature, indicating faster pectinases inactivation at higher temperatures. Results suggested that PME, PG and PL are the relatively thermostables enzymes with a Z-value of 21.64, 20.32 and 27.07 °C respectively and Ea value of 21.85, 20.86, and 20.57 kJ/mol respectively. Thermodynamic parameters were also calculated. The Gibbs free energy ΔG# values range from 89.20 to 91.38 kJ/mol. All the results suggest that these pectinases were relatively resistant to long heat treatments up to to 55 °C.

Index Terms— *Carica papaya*, Epicarp, Kinetics and thermodynamic analysis, Pectin lyase, Pectin methylesterase, Polygalacturonase, Purification, Thermal inactivation.

1 INTRODUCTION

Pectin is a complex polysaccharide which forms an important component of middle lamella and primary cell wall of higher plant. Pectinases or pectinolytic enzymes can degrade pectic substances [1, 2]. Depending on their mode of action, the enzymes hydrolyzing pectin are broadly known as pectinases, which include pectin methylesterases (E.C.3.1.1.11), polygalacturonase (E.C.3.2.1.15) and pectin lyase (E.C.4.2.2.10) [3, 4]. Pectin methylesterase (PME) activity has been shown to cause cloud loss in juices [5]. PME catalyzes the removal of the methyl groups from the polygalacturonic acid chain, leaving an increased number of free carboxyl groups that can then bind cations and cross-link the pectin chains. The action of PME also makes the pectin susceptible to further degradation by polygalacturonase (PG) because this enzyme acts only on segments of the pectin chain that have been demethylated by PME. PG activity may further contribute to the degradation of pectin, leading to thinning of purees and loss of particulate texture [6]. PG cleaves the polygalacturonic acid backbone of the pectin and reduces the average length of the pectin chains. This degradation of the pectin chains reduces the viscosity of the juice [7]. The pectin lyases (PL) act on pectins, oligomers and polymers of galacturonic acid by catalyzing the rupture between two galacturonic acid units by a β-elimination mechanism. They are involved in the process of maceration of plant vegetation [8].

Interest in enzymes also lies in their usages for various biotechnological purposes [9]. It was pointed out that some pectinases have been exploited biotechnologically in various industries like wine industry, food industry, paper industry for bleaching of pulp and waste paper recycling; in the processing of fruit–vegetables, tea–coffee, animal feed; extraction of vegetable oil and scouring of plant fibres [10, 11, 12, 13]. Whereas most industrial production of pectinases is limited to some species of bacteria, yeast [14, 15, 16]. Due to the extensive applications of enzyme in various industries there is a need to find novel sources of the enzymes. Furthermore, utilization of agro-industrial residues (in the form of peels and rinds) for enzyme production minimizes environmental pollution and allows the production of high value-added products using an economical process.

Fruit peels such *Carica papaya* cv. solo 8 are usually discarded during processing but are richer sources of pectinase than pulp at the mature stage presenting 1/8 of yellow skin [17]. This peel contains a valuable natural enzyme known as pectinase and thus can be used as a rich, natural, and abundant source for the commercial production of this enzyme.

Considering these important properties of pectinases, the present work aimed to investigate the effect of heat treatment over a range of temperatures from 45 to 65°C on these enzymes. So, determination and analysis of kinetic and thermodynamic parameters were undertaken.

2 Material and Methods

2.1 Collection of fruit and sampling

The papayas (*Carica papaya* L. var solo 8) were harvested from a farm near Tomassé (Azaguié), a village located at about 50 km north of Felix Houphouet Boigny Airport, Abidjan, Cote d'Ivoire. The fruit were transported directly to the Biocatalysis and Bioprocessing laboratory of Nangui Abrogoua University (Côte d'Ivoire). They were selected according to the mature stage (1/8 fruit with yellow skin) proposed by N'da et al. [18], average size, and absence of defects. The fruits selected were washed thoroughly to free from mud, ferns and other extraneous material, dried on blotting paper. They were then manually peeled; the peels were cut and washed with running water to remove mucilage. After washing, they were stored at 4 °C.

2.2 Chemicals

Pectin from citrus fruits, bovine serum albumin (BSA) and Bradford reagent were supplied by Sigma-Aldrich (St. Louis, MO, USA). The standard proteins for molecular weight determination and electrophoresis reagents on polyacrylamide gel were supplied by BIO-RAD. All chemicals and reagents used were analytical grade.

2.3 Extraction

The papaya epicarps (50 g) were ground using a blender in 50 mL NaCl solution 0.9 % (w/v). The homogenate was subjected to sonication (4 °C) at 50 - 60 Hz frequency using a BANDELIN SONOPLUS HD 2200 for 10 min and then centrifuged at 10,000 g for 30 min at 4 °C. The supernatant filtered through cotton wool was kept refrigerated and used as the crude extract.

2.4 Purification Procedures

The purification procedure was carried out in the cold room (4 °C).

Sulphate ammonium precipitation

Fifteen (15) mL of the enzymatic crude extract of the papaya (*Carica papaya* L. cv solo 8) epicarps were saturated with a different concentration of ammonium sulphate (20-80 %) and left with gentle stirring for 24 h in a refrigerator at 4 ° C. The mixture was then centrifuged at 6000 g for 30 min at 4 °C with a refrigerated centrifuge (MIKRO 22R, Hettich). The precipitated proteins contained in the pellet were suspended either in 1 ml of sodium acetate buffer (0.1 M pH 5) for PG; 1 mL of Tris-HCl buffer (0.1 M pH 7.5) for PME or in 1 mL of Tris-HCl buffer (0.1 M pH 8) for PL. The fractions containing the pectinase activities are pooled and subjected to dialysis.

Dialysis

The dialysis extracts were immersed in a large volume of buffer (0.1M sodium acetate pH 5 for PG or 0.1M Tris-HCl pH 7.5 for PME or pH 8 for PL) with continuous stirring for 16 h. The membrane having pores allowed small molecules such as ammonium and sulfate ions to cross, while not allowing the large protein molecules to pass through it. Every 4 hours the buffer solution was renewed. This step made it possible to re-

move the excess salt but also the substances of low molecular weight such as the pigments contained in the enzymatic raw extract.

Isoelectric focusing

The dialyzed extracts were subjected to an isoelectric-focusing technique. It consisted in balancing the ion exchange membranes with a solution of phosphoric acid and sodium hydroxide overnight before using the membranes. This operation consisted in immersing the red anode in a solution of H3PO4 (0.1 M), the black cathode in a solution of NaOH (0.1M). Then prepare the focusing chamber. This preparation consists of mixing 100 µl of dialysed extract, 150 µl ampholyte (Bio-lyte® 3/10, Bio-Rad Laboratories Hercules) and 2.75 ml of distilled water. Fill the 3 ml syringe with the sample and slowly charge the focus chamber. Also add 6 ml of H3PO4 (0.1 M) and 6 ml of NaOH (0.1 M) respectively into the ventilation hole of the anodic assembly (red button) and cathode (black button). Isoelectric focusing was then carried out at 350 V, 3 mA, 25 W for 1 h 30 min. Once the IEF race was completed, the fractions were harvested as quickly as possible to avoid diffusion of the separated proteins. The three pectinolytic activities were assayed in each fraction to identify the isoelectric point of pectinases isolated from papaya epicarp cv.solo 8.

2.5 Enzyme Assay and Proteins Determination

Pectin methylesterase activity

The enzymatic activity was determined using the method of Mehri-Kamoun [19]. The principle of the reaction consists to remove the specific methoxyl groups located on the C6 of some galacturonyl groups using a pectin methylesterase (PME) enzyme. The measurement of the activity relies on the pH variation due to the removal of the carboxylic group, which leads to the acidification of the medium. The method used in this experiment involved the mixing of 2 ml of subtracts (1 % pectin in 0.15 % NaCl solution pH 7.0) and 1 ml of enzymatic solution. Then the mixture was incubated at 37 °C for 2 h in a water bath. The pH of the mixture was measured at the beginning of the incubation then 2 h after. One unit of PME activity corresponded to 10 fold the volume (µl) of 0.01 M NaOH added to the mixture to bring the pH value back to initial value at 37 °C. The PME activity was then expressed in unit/mg of protein (U/mg).

Polygalacturonase activity

The polygalacturonase (PG) activity was determined by a standard colorimetric method of Miller [20] using 3.5-dinitrosalicylic acid (DNS). 900 µL of subtract (0.5 % of polygalacturonic acid prepared in 100 mM of acetate buffer pH 5) was mixed with 100 µl of enzymatic extract for a total of 1000 µl. Then the mixture was incubated at 40 °C for 15 min. The reaction was stopped by adding 1.5 mL of DNS. After DNS was added the whole mixture was placed in a boiling water bath for

5 min. Finally, the mixture was let to cool down at ambient temperature and the absorbance was read at 540 nm using a blank (mixture without subtract) and D-galacturonic acid for the standard curve.

The galacturonase activity was expressed as the equivalent of galacturonic acid produced per milligram of protein per minute (µmoL/mg/min).

Pectin lyase activity

The pectin lyase (PL) activity was determined using the method of Preiss & Ashwell [21]. An assay mixture (2.5 mL) consisting of a 100 mM Tris-HCl buffer pH 8.0, 10 mM $CaCl_2$, 0.5 % pectin and enzyme solution was incubated at 40 °C for 1 h. After incubation, the activity was determined by measuring the absorbance of the reaction mixture at 548 nm.

One unit of pectin lyase activity was defined as the amount of enzyme present in 1 ml of enzymatic solution which released 1 µM galacturonic acid for 1 min.

Determination of proteins

Protein concentration was determined by Bradford method using bovine serum albumin (BSA) as a standard [22].

2.5 Thermal Inactivation

The thermal inactivations of the enzymes were determined at constant temperature between 45 and 65 °C after exposure to each temperature for a period of 15 to 120 min. The enzymes were incubated in 100 mM Tris-HCl buffer pH 7.5 for PME or pH 8 for PL or pH 5.0 for PG in 100 mM sodium acetate buffer. Aliquots were drawn at intervals and immediately cooled in ice-cold water. Experiments were performed in triplicate. The residual enzymatic activity, determined in both cases at 37 °C under the standard test conditions, was expressed as percentage activity of zero-time control of the untreated enzyme.

2.6 Kinetic Data Analysis

The temperature dependence of the reaction rate constant for the studied enzyme served as the basis for fitting to the Arrhenius equation [23]: $\ln [At/A\circ] = -kt$ (1)

Where; At is the residual enzyme activity at time t, A0 is the initial enzyme activity; k is the reaction rate constant (min−1) at a given condition. k values were obtained from the regression line of ln [At /A0] versus time as -slope.

The D-value represents the time required to reduce the concentration of the component under examination to 90% of its initial value.

The decimal reduction time (D) was calculated according to Stumbo [24] as:

$D = 2.303/k$ (2)

Z (°C) is temperature increase needed for a 90% reduction in D-value (temperature sensitivity parameter), and follows the equation:

$\text{Log } [D1/D2] = [T2\text{-}T1]/ZT$ (3)

Where; T1 and T2 represent the lower and higher temperatures, °C or °K; D1 and D2 are D-values at the lower and higher temperatures in minutes. The Z-values were estimated from the linear regression of log D and temperature (T).

The temperature of treatment and the rate constant in a denaturation process was related according to the Arrhenius equation [23]: $k = Ae(-Ea/RT)$ (4)

Equation 4 can be transformed to: $\ln k = \ln A - Ea/R \times T$. (5)

Where; k is the reaction rate constant value, A is the Arrhenius constant,

Ea is the activation energy (energy required for the inactivation to occur), R is the gas constant (8.31 Jmol-1K-1), T is the absolute temperature in Kelvin.

When the "ln" of "k" is plotted against the reciprocal of the absolute temperature, a linear relationship should be observed in the temperature range studied. The slope of the line obtained permitted to calculate the activation energy and the ordinate intercept corresponds to ln A [25, 26].

The values of the activation energy (Ea) and Arrhenius constant (A) allowed the determination of different thermodynamic parameters [27] such as variations in enthalpy, entropy and Gibbs free energy, ΔH, ΔS and ΔG, respectively, according to the following expressions [28]:

$\Delta H\# = Ea - RT$ (6)

$\Delta S\# = R (\ln A - \ln KB/hP - \ln T)$ (7)

$\Delta G\# = \Delta H\# - T \Delta S\#$ (8)

Where; KB is the Boltzmann constant (1.38 x 10-23 J/K),

hP is the Planck constant (6.626 x 10-34 J.s), T is the absolute temperature.

3 Results and Discussion

Kinetic Analysis of Thermal Denaturation

The optimum temperature of three pectinases (pectin methylesterase, polygalacturonase and pectin lyase) purified from papaya (*Carica papaya* L. cv solo 8) epicarp were 60, 45 and 50 °C respectively, for PME, PG and PL [29]. In this study the effect of heat treatment over a range of temperatures from 45 to 65 °C, on the pectinases were evaluated by determining the residual enzymatic activity. The thermal stability profil of three pectinases presented in the form of the residual percentage activity is shown in Table 1.

However, the heating had different effects on these enzymes. Thus, the activities of three enzymes were decreased with increasing heating time (15-120 min) and temperature (45–65 °C). Indeed, at temperatures between 45-65 °C, heat-denaturation of the enzymes occurred after 15 min of incubation (98.30 to 81.35 % for PME, 96.88 to 51.21 % for PG and 95.96 to 33.33 % for PL). The heating at 65 °C of PME for 60 min resulted, partial inactivation (49.15 %) of this enzyme activity (Table 1). At the same temperature, polygalacturonase and pectin lyase loss about 50 and 65 % of their activity in 15 min, respectively (Table 1). On one hand, the decrease of percentage residual activity at temperatures higher than 50 and 60 °C respectively, for PG and PL was most likely due to the unfolding of the tertiary structure of the enzyme to form the secondary structure and on other hand; it could be explained by the chemical modification [30]. For example after juices clarification by pectinases, it is necessary to stop the reaction to inhibit the activity of the enzymes.

The logarithmic linear relationship between three pectinases (PME, PG and PL) activity and heat treatment time for the temperature range of 45-65 °C respectively followed first-order kinetics (Fig. 1, 2 and 3).

This result was consistent with those reported for PME and PG of papaya epicarp [31] and PME and PG in tomato juice [7].

The extent of the denaturation of these enzymes increases with temperature and the processing time. This shows that these enzymes are sensitive to heat but at varying degrees.

From the slopes of these lines, the inactivation rate constants (k) were calculated and are given in Table 2. The rate constant increased with the heating temperature, indicating that papaya epicarp (*Carica papaya* L. cv solo 8) pectinases are less thermostable at higher temperatures (50 - 65 °C). The half-life and the decimal reduction time (D-value) are other important parameters commonly used in the characterization of enzyme stability. Increasing the temperature from 45 to 65 °C resulted in a decrease in t1/2 and D-values (Table 2).

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The dependence of the inactivation rate constants with temperature was adequately fitted by the Arrhenius equation (R2 = 0.990) (Fig. 2). This linearity is an indication that the inactivation in papaya epicarp pectinases occurs through a unique mechanism dependent on temperature, such as protein unfolding [33].

From 45 to 65 °C, the activation energy (Ea) values for thermal inactivation of these enzymes were calculated to be 21.85, 20.86 and 20.57 kJ/mol for PME, PG and PL respectively (Table 2). These relative high values of activation energy found for these enzymes mean that an important amount of energy is needed to initiate denaturation of each enzyme. It also indicates the relative stability of these proteins [34].

As shown in table 3, D-values calculated for the three pectinases (PME, PG and PL) from papaya epicarp decreased by increased at temperature. In general, the larger an enzyme and the more complex its structure, the more susceptible it is to high temperature [35].

The effect of temperature on D-values is shown in figure 4, and from this representation, the Z-values were calculated and found to be 21.64, 20.32 and 27.07 °C at 45-65 respectively, for PME, PG and PL (Table 3). The Z-values (temperature increase necessary to reduce D value by 90%) obtained show that these enzymes are thermostable at temperatures between 45 and 65 °C. Our results are similar to those reported by Barrett et al. [36], these authors showed that the lactoperoxidase was stable at temperatures between 40 and 65 °C. These Z-values were much higher than that reported for PME carrot juice (5.73 °C) [37] and for tomato juice (4.8 °C) [7]. Furthermore, it has been reported that when inactivation of PME of the tomato, the value of Z is between 4.5 and 32 °C [7, 38, 39]. In our study, the value of Z obtained which is 21.64 °C for the PME of the epicarp of papaya is included to the value indicated by these authors. The results provide useful information about the temperature that affect the pectinases activities of *Carica papaya* cv. solo 8 epicarp and might be used to facilitate its control during juices transformation.

TABLE 1. EFFECT OF TREATMENT TEMPERATURE AND TIME OF THE INACTIVATION OF PAPAYA EPICARP (*CARICA PAPAYA* CV SOLO 8) PECTIN METHYLESTERASE (PME), POLYGALACTURONASE (PG) AND PECTIN LYASE (PL).

	Relative activity (%) at each Temperature (°C) of heat treatment														
	45	50	55	60	65	45	50	55	60	65	45	50	55	60	65
Treatment time (min)	PME					PG					PL				
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
15	98.30	93.22	89.83	84.74	81.35	96.88	85.81	75.43	72.32	51.21	95.96	87.50	80.83	58.33	33.33
30	94.91	86.44	81.35	74.58	67.80	89.96	77.85	66.43	54.40	37.02	91.67	75	65	41.67	17.5
45	91.52	76.27	71.19	64.41	55.93	80.97	71.97	59.51	44.98	22.49	87.5	62.5	50	33.33	11.67
60	89.83	71.19	66.10	57.63	49.15	75.43	67.13	51.90	32.52	14.53	83.33	55.83	37.5	24.17	2.5
75	88.13	66.10	61.02	47.46	38.98	72.32	56.75	35.98	26.64	7.96	80	49.17	33.33	18.33	1.67
90	86.44	61.02	50.85	40.68	32.20	69.55	46.71	32.52	22.49	5.88	70.83	46.67	27.5	15.17	0.75
105	81.35	55.93	44.07	35.59	27.12	68.17	34.26	26.64	17.99	3.81	58.33	41.67	20	8	0.5
120	69.49	47.46	38.98	28.81	20.34	61.94	31.49	20.41	15.57	2.42	41.67	33.33	16.67	6.67	0.17

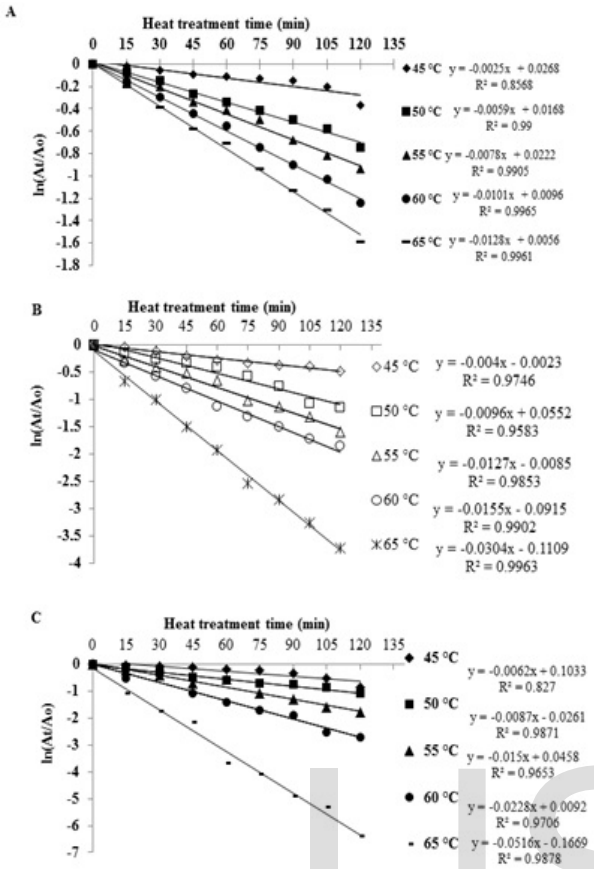

Fig. 1.Thermal inactivation of pectin methylesterase (A), polygalacturonase (B) and pectin lyase (C) in the temperature ranged from 45 to 65 °C. A0 is the initial enzymatic activity and At the activity at each holding time.

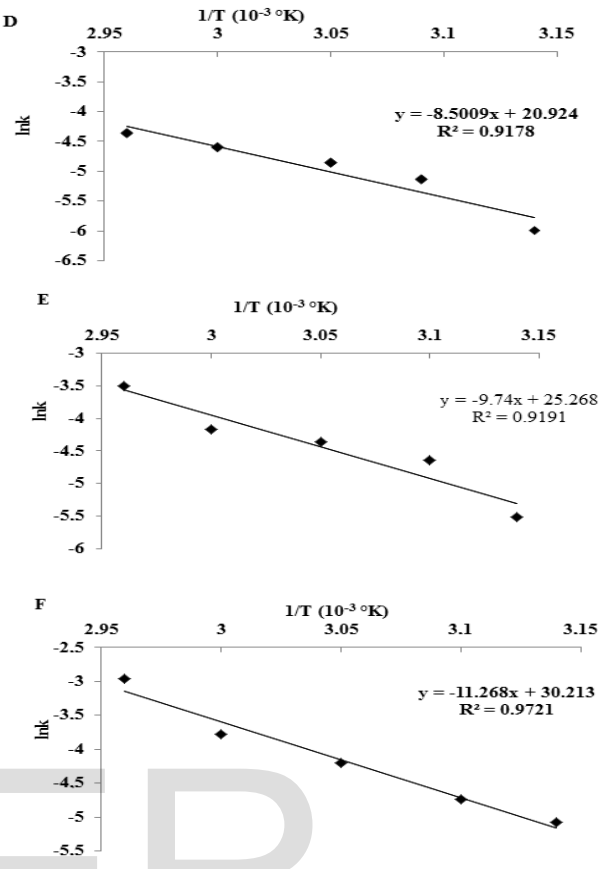

Fig. 2. Arrhenius plot showing the effect of temperature on the rate constant for the thermal inactivation of papaya epicarp (*Carica papaya* cv solo 8) pectin methylesterase (D), polygalacturonase (E) and pectin lyase (F). 1/T represents the reciprocal of the absolute temperature. Each data point is the mean of three determinations.

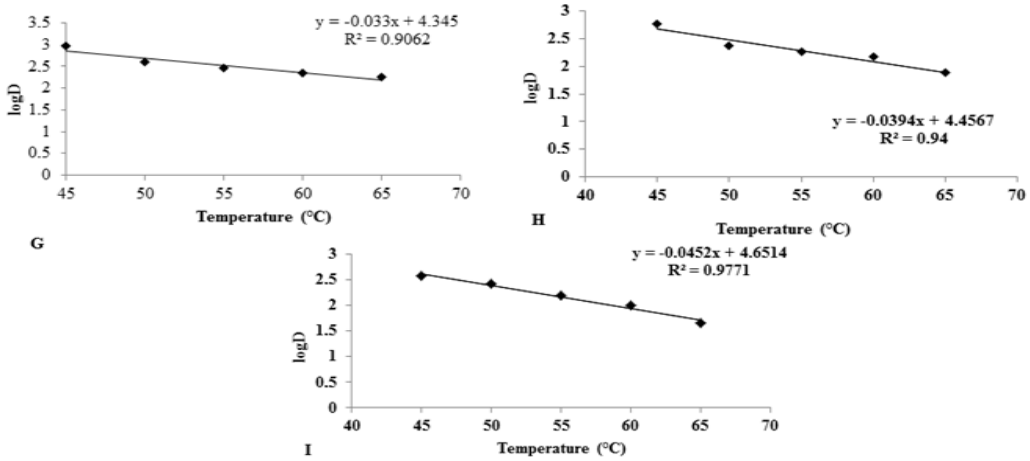

Fig. 3. Variation of decimal reduction times with temperature for the thermal inactivation of papaya epicarp (*Carica papaya* cv solo 8) pectin methylesterase (G), polygalacturonase (H) and pectin lyase (I).

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TABLE 2. K-, D-, Z-AND EA-VALUES FOR THERMAL INACTIVATION OF PAPAYA EPICARP (*CARICA PAPAYA* CV SOLO 8) PECTIN METHYLESTERASE (PME), POLYGALACTURONASE (PG) AND PECTIN LYASE (PL) RANGE (45-65 °C).

Temperature (°C)	Kinetic parameters								
	Pectin methylesterase			Polygalacturonase			Pectin lyase		
	K (min-1)		D (min)	K (min-1)		D (min)	K (min-1)		D (min)
	Value	R2		Value	R2		Value	R2	
45	0.0025	0.86	921.2	0.004	0.97	575.75	0.0062	0.83	371.45
50	0.0059	0.99	390.34	0.0096	0.96	239.89	0.0087	0.98	264.71
55	0.0078	0.99	295.26	0.0127	0.98	181.34	0.015	0.96	153.53
60	0.0101	0.99	228.02	0.0155	0.99	148.58	0.0228	0.97	101.01
65	0.0128	0.99	179.92	0.0304	0.99	75.76	0.0516	0.98	44.63
Z (°C)		21.64			20.32			27.07	
Ea (kJ/mol)		21.85			20.86			20.57	

Thermodynamic Analysis of Thermal Denaturation

The calculation of the thermodynamic parameters of inactivation provides information on the enzyme thermal stability for each step of the heat-induced denaturation process. This could help in detecting any secondary stabilization or destabilization effects that would go unnoticed if only the half-life times were considered [40]. These parameters include ΔG#, the Gibbs free energy change considered as the energy barrier for enzyme inactivation, ΔH#, the enthalpy change measuring of the number of bonds broken during inactivation, and ΔS#, the entropy change that indicates the net enzyme and solvent disorder. They were calculated in the temperature range 45 to 65 °C from experimental data using equations 6-8 (Table 3).

The values of enthalpy (ΔH#) and free energy (ΔG#) are positive, while those of entropy (ΔS#) are negative for the three pectinases (pectin methylesterase, polygalacturonase and pectin lyase) (Table 3). Results for ΔH# show that, within the error range of our measurements, the enthalpy is independent of temperature; thus, there is no change in enzyme heat capacity (Table 3). Moreover, positive values of the enthalpies suggest that the endothermic nature of the thermal inactivation reactions [41]. In general, ΔH# is seen as a measure of the number of noncovalent bonds broken in forming a transition state for enzyme inactivation. Therefore, the higher the ΔH# is, the larger will be the number of noncovalent bonds present in the enzyme molecule, which is going to be more stable. In fact, the stability of a protein is the result of a delicate balance between stabilizing and destabilizing forces, which may be influenced by several factors, e.g., the number of hydrogen and disulfide bridges, the folding degree and hydrophobicity of the molecule, and the amount of ionic and other interactions [42].

In contrast, the ΔG# value is directly related to protein stability: the higher the ΔG# is, the higher will be the enzyme stability. When the incubation temperature was elevated from 45 to 65 °C, there was a slightly increase of ΔG# values for the three enzymes (Table 3), indicating that the inactivation processes were not spontaneous. Since ΔG# decreases with increasing temperature whereas ΔH# is overall constant, one could expect a significant contribution of entropy changes to the thermodynamics of the considered system. In fact, it was already demonstrated that activation entropy has a dominant role in thermal inactivation of proteins in aqueous solutions [43, 44].

As indicated in Table 3, all ΔS# values for thermal inactivation of the three pectinases from papaya (*Carica papaya* L. cv solo 8) epicarp are negative in the temperature range 45 to 65 °C. This suggests that there are no significant processes of aggregation, since, if this would happen, the values of entropy would be positive [45]. The most common cause of the heat inactivation of enzymes is the loss of the native conformation (unfolding of the active tertiary protein structure to a disordered polypeptide), a process identified as thermodenaturation, which takes place as a result of increased molecular mobility at elevated temperature[46]. It is worth noting that previous ΔS# values for pectinases thermodenaturation process were negative, as a result of the commonly lower reported Ea (and therefore ΔH#) values [33, 47, 48]

TABLE 3. THERMODYNAMIC PARAMETERS FOR PAPAYA EPICARP (*CARICA PAPAYA* CV. SOLO 8) PECTIN METHYLESTERASE (PME), POLYGALACTURONASE (PG) AND PECTIN LYASE (PL) RANGE (45-65 °C).

Temperature (°C)	Thermodynamic parameters								
	pectin methylesterase (PME)			ploygalacturonase (PG)			pectin lyase (PL)		
	$\Delta H^{\#}$ (kJ/mol)	$\Delta S^{\#}$ (J $mol^{-1}K^{-1}$)	$\Delta G^{\#}$ (kJ/mol)	$\Delta H^{\#}$ (kJ/mol)	$\Delta S^{\#}$ (J $mol^{-1}K^{-1}$)	$\Delta G^{\#}$ (kJ/mol)	$\Delta H^{\#}$ (kJ/mol)	$\Delta S^{\#}$ (J $mol^{-1}K^{-1}$)	$\Delta G^{\#}$ (kJ/mol)
45	21.23	-220.05	91.203	20.48	-218.49	89.96	19.80	-217.00	88.80
50	19.25	-220.18	90.37	18.45	-218.62	89.07	19.20	-217.13	89.33
55	18.79	-220.31	91.05	17.97	-218.74	89.72	18.01	-217.26	89.27
60	18.36	-220.44	91.77	17.70	-218.87	90.58	17.13	-218.87	90.01
65	17.97	-220.56	92.52	16.07	-218.99	90.09	15.09	-217.51	88.61
Mean	19.12	-220.31	91.38	18.14	-218.74	89.89	17.84	-217.55	89.20

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4 CONCLUSION

The extract of the pectin methylesterase, polygalacturonase and pectin lyase from papaya (*Carica papaya* L. cv solo 8) epicarp studied here therefore comprises a structurally robust but temperature-sensitive enzymatic system, whose thermal denaturation is mainly under entropic control. Their high thermostability must be taken into account in the biotechnological processes of the food and non-food industries for various applications.

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