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Thermal inactivation of pectin methylesterase, polygalacturonase, α-mannosidase, α-galactosidase and β-galactosidase activities in papaya (*Carica papaya* L. cv solo 8) pericarp

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Key words: Thermal inactivation, Kinetic parameters, *Carica papaya*, Hydrolases activities

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Abstract

The effect of heat treatment on papaya (*Carica papaya* cv solo 8) pericarp Pectin methylesterase (PME), Polygalacturonase (PG), α-mannosidase (α-MAN), α-galactosidase (α-GAL) and β-galactosidase (β-GAL) activities were studied over a range of 40 to 65 °C. Denaturation of these enzymes, measured by the loss in activity, could be described as a first-order reaction with k-values between 0.0011 and 0.0303 min^{-1}. D- and k-values decreased and increased, respectively, with increasing temperature, indicating fast enzymatic inactivation at higher temperatures. Results suggested that PME, PG, α-MAN, α-GAL and β-GAL are the relatively thermostables enzymes with a Z-value of 34.84, 19.12, 31.54, 42.69 and 17.69 °C respectively and Ea value of 39.90, 105.93, 64.38, 47.28 and 91.46 kJ mol^{-1} respectively. The Gibbs free energy ΔG values range from 89.21 for α-GAL to 102.95 kJ/mol for α-MAN at 40-65 °C. The results of the thermodynamic investigations indicated that the hydrolytic reactions were: (1) not spontaneous (ΔG > 0), (2) slightly endothermic (ΔH > 0) and (3) reversible (ΔS < 0). However, Positive values of entropy (ΔS > 0) for PG indicated that this enzyme is found in a chaotic state at the end of the reaction. The high value obtained for the variation in enthalpy indicated that a high amount of energy was required to initiate denaturation, probably due to the molecular conformation of these enzymes.

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Introduction

The presence of residual endogenous enzymes in either raw or processed fruit and vegetables products may cause a loss of quality during storage. These changes can affect the texture, colour, flavour, and nutritional quality of the product (Adams, 1991). Some examples of this include the enzymes polygalacturonase (PG) and pectin methylesterase (PME), which are involved in the degradation of pectins and therefore affect product viscosity and texture. PME activity has been shown to cause cloud loss in juices (Versteeg *et al.*, 1980). PG activity may further contribute to the degradation of pectin, leading to thinning of purees and loss of particulate texture (Luh et Daouf, 1971). Other examples of enzymes that affect quality include β-Galactosidases of a number of fruit types were reported to possess β-galactanase activities, functioning possibly, as exo-glycanases (Lazan *et al.*, 2004).

Another seemingly unique β-galactanase that has wall-modifying capabilities is papaya α-galactosidase 2 (Soh, 2002). The β-galactosidase of tomato and strawberry fruit was reported to comprise several isoforms encoded by a multigene family, however, not all of the genes were ripened related (Smith and Gross, 2000; Trainotti *et al.*, 2001). Likewise, papaya contained a number of β-galactosidase isoforms, notably β-gal I, II and III (Ali *et al.*, 1998).

These β- galactosidase isoforms were shown to have β-galactanase activities. Immunoblot analysis suggested that accumulation of papaya β-gal I occurs only through ripening, whereas β-gal II protein was detectable in developing fruit and its level decreased as the fruit ripened (Ali *et al.*, 1998). While β-galactosidase total activity in tomato remained unchanged, the activity in papaya increased with ripening (Carey *et al.*, 1995; Lazan *et al.*, 1993; Lazan *et al.*, 1995). The α-mannosidases play an essential role in the process of N-glycans to complex and hybrid oligosaccharides. These enzymes are glycosyl hydrolases that cleave specific α-linked mannose residues (Bédikou *et al.*, 2009). To prevent unwanted changes during storage, fruit and vegetables are generally subjected to some type of treatment during processing in order to inactivate these enzymes.

A heat treatment, such as blanching, pasteurization, or commercial sterilization, is most commonly used. However, other processes such as high pressure or pulsed electric fields have also been proposed.

The effectiveness of these other processes on enzyme inactivation is controversial (Hendrickx *et al.*, 1998; Van Loey *et al.*, 2001). Heat treatment of vegetables can also lead to the loss of desirable characteristics such as color, texture, flavor, and nutrients such as ascorbic acid. For this reason, it is desirable to keep the heat treatment to a minimum yet still have it be sufficient to completely inactivate t

he deleterious enzymes (Anthon and Barrett, 2002). The objective of this study was to determine the effect of heat treatment over a range of temperatures from 40 to 65 °C, on Pectin methylesterase, Polygalacturonase, α-mannosidase, α-galactosidase and β-galactosidase activity. This method permits accurate determination of heating time and temperature to delay softening, ripening and preserve the quality of papaya and consequently, accurate calculations of kinetic and thermodynamic parameters.

Material and methods

Collection of fruit and sampling

The papayas (*Carica papaya* L. var solo 8) were harvested from a farm near Tomassé (Azaguié), a village located about 50 km, in the north of Felix Houphouet Boigny Airport, Abidjan, Cote d'Ivoire. The fruit were transported directly to the Laboratory of Food Biochemistry and Tropical Products Technology, Nangui Abrogoua University.

The green mature stage (the fruit shows 1/ 32 of yellow skin) was selected for this study. The fruit were washed with water, sorted according to the shape, the size and the weight, and packed in boxes then stored immediately at 15 °C. The next day, three boxes (36 fruit) were pulled out for testing. We recorded the enzymatic activities (pectin methylesterase, polygalacturonase, α-mannosidase, α-galactosidase and β-galactosidase) of the fruit.

Measurement of the enzymatic activities

Extraction of enzymes

In order to obtain the enzymatic extract, we ground 10 g of papaya pericarp in 10 ml of 0.9 % (w/v) NaCl. The mixture was centrifuged (centrifuge Jouan multifunction B4i-BR4i, Germany) at 6000 rpm for 30 min at 4 °C and the pellet discarded. The supernatant obtained contained the enzymes.

pNP-glycosidase (α-mannosidase, α-galactosidase and β-galactosidase) activity.

The *p*-nitrophenol (*p*NP)-glycosidase activity was obtained by mixing 50 µl of enzyme extract and 125 µl of 100 mM sodium acetate buffer (pH 5.6), 75 µl of 5 mM *p*NP-α- or β-D-glycoside (Amersham Pharmacia Biotech RPN 1064, Paris, France) as substrate of the enzyme. After the incubation of the mixture at 37 °C for 10 min, the reaction was stopped by adding 2 ml of 2 % (w/v) sodium carbonate. The appearance of a yellow color means the presence of an enzymatic activity, which is the result of the hydrolysis of *p*-nitrophenyl-α- or β-glycoside by the enzyme. The quantification of *p*-nitrophenol (*p*NP) produced was obtained through a spectrophotometer (Spectronic Genesys5, Madison, SA) at 410 nm with a negative control (reagents without the enzyme). The optical density was converted into micromole of *p*NP/min. The specific activity was expressed in micromole of *p*-nitrophenol per min or per mg of protein (µmol/min/mg).

Polygalacturonase activity

The polygalacturonase (PG) activity was determined using the method of Gross (1982). The subtract was mixed (1 % of polygalacturonic acid washed with 80 % ethanol) with 100 mM of acetate buffer (pH 5.6) containing 0.1 M NaCl and 50 µl of enzymatic extract for a total of 200 µl. Then the mixture was incubated at 37 °C for 2 h under continuous agitation. The reaction was stopped by adding 1 ml of sodium borate (pH 9). Then 200 µl of 0.1 % 2-cyanoacetamide were added before the whole mixture was placed in a boiling water bath for 10 min. Finally, the mixture was let to cool down at ambient temperature and the absorbance was read at 276 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 methylesterase activity

The enzymatic activity was determined using the method of Mehri-Kamoun (2001). 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 subtract (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).

Determination of proteins

The proteins concentration of the different enzymatic samples was measured using the Lowry method (Lowry *et al.*, 1951) with serum albumin bovine (SAB) as protein standard.

Thermal inactivation

The thermal inactivation of each enzyme (pectin methylesterase, polygalacturonase, α-mannosidase, α-galactosidase and β-galactosidase) was investigated on the standard condition at various constant temperatures from 40 to 65°C after exposure to each temperature for a period of 5 to 180 min. Each enzyme was incubated in 100 mM sodium acetate buffer (pH 5.6). 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.

Kinetic data analysis

Thermal inactivation of α-mannosidase, α-galactosidase, β-galactosidase, Polygalacturonase, and Pectin methyl esterase can be described by a first-order kinetic model (Terebiznik *et al.*, 1997; Guiavarc'h *et al.*, 2002). The integral effect of an inactivation process at constant temperature, where the inactivation rate constant is independent of time, is given in Eq.1:

$$\ln (At/A0) = -kt \quad (1)$$

where; At is the residual enzyme activity at time t, Ao 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 is defined as a time required, at a constant temperature, to reduce the initial enzyme activity (Ao) by 90 %. For first-order reactions, the D-value is directly related to the rate constant k (Eq. 2) (Stumbo, 1973):

$$D=2.303/k \quad (2)$$

Z (°C) is the temperature increase necessary to induce a 10-fold reduction in D-value and follows the Eq 3:

$$\log (D1/D2] = (T2-T1)/Z \quad (3)$$

where; T1 and T2 are the lower and higher temperatures in °C or °K; D1 and D2 are D-values at the lower and higher temperatures in min. The Z-values were determined from the linear regression of logD and temperature (T). The temperature of treatment and the rate constant in a denaturation process was related according to the Arrhenius equation (Eq 4 or 5):

$$k = Ae (-Ea/RT) \quad (4)$$

or

$$\ln k = \ln A - Ea/R \times T \quad (5)$$

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 $Jmol^{-1}K^{-1}$), T is the absolute temperature in °K. When lnk is plotted versus 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 (Dogan *et al.*, 2002). The values of the activation energy (Ea) and Arrhenius constant (A) allowed the determination of different thermodynamic parameters38 such as variations in enthalpy, entropy and Gibbs free energy, ΔH#, ΔS# and ΔG#, respectively, according to the following equations ((Eq. 6; 7; 8) 39

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

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

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

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) and T is the absolute temperature.

Statistical analysis

The statistical analysis was performed on the results using SPSS (version 10.0) software. The comparison of the variables measured during this study was done using the analysis of variance (ANOVA) and Duncan test. The differences were considered significant if $p \leq 0.05$. All the experiments were conducted in triplicate.

Results and discussion

Heat-denaturation of papaya (*Carica papaya* L. Cv solo 8) pericarp enzyme occurred after 15 min pre-incubation (Table 1). However, the heating had different effects on these enzymes.

Thus, heating at 65 °C of α-mannosidase for 90 min resulted, partial inactivation (49.09 %) of this enzyme activity (Table 1).

Table 1. Effect of treatment temperature and time of the inactivation of papaya (*carica papaya* cv solo 8) Pectin methylesterase (PME). Polygalacturonase (PG). α-mannosidase (α- MAN). α-galactosidase (α-GAL) and β-galactosidase (β-GAL).

	Residual activity (%) at each temperature (°C) of heat treatment														
	45	55	65	45	55	65	45	55	65	45	55	65	45	55	65
Treatment time (min)	α-MAN			α-GAL			β-GAL			PME			PG		
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
15	95.73	91.77	85.67	85.87	72.28	47.28	97.90	67.83	50.00	92.50	87.00	80.50	88.44	74.35	51.38
30	93.29	86.89	77.44	73.37	56.52	34.78	91.26	48.95	10.84	85.50	77.00	63.50	83.97	63.72	41.64
45	90.55	83.23	69.21	65.76	45.65	28.80	87.76	42.31	10.84	80.00	70.00	57.50	73.46	60.15	27.24
60	86.28	79.27	61.59	58.15	41.30	23.37	84.27	37.41	9.44	76.00	66.50	51.00	72.18	56.46	18.28
75	83.23	75.61	55.49	55.98	35.33	19.57	81.47	37.41	9.44	70.50	56.00	42.00	69.42	46.06	10.32
90	79.88	70.73	49.09	50.54	27.72	15.22	79.39	34.97	8.74	67.50	48.50	35.00	68.30	43.15	7.49
105	77.44	66.77	45.12	45.11	23.91	10.33	77.97	34.97	5.24	62.00	43.00	29.00	65.81	35.93	5.08
120	75.00	63.72	39.02	41.30	19.02	7.07	75.52	33.22	2.10	56.00	36.50	23.50	61.47	28.72	3.37
135	68.90	59.15	35.67	34.24	15.22	4.89	73.08	33.22	1.75	52.00	33.50	19.00	58.48	28.25	1.63
150	67.07	54.27	32.93	26.09	9.78	2.72	64.69	30.77	1.40	47.00	27.00	15.00	55.65	27.78	0.97
165	64.02	49.70	28.66	20.65	6.52	1.63	62.24	27.27	1.05	43.00	25.00	13.00	48.08	24.10	0.54
180	61.89	47.56	26.52	11.41	3.80	0.54	59.09	20.63	0.70	37.00	21.00	11.00	46.99	21.34	0.35

At the same temperature, α-galactosidase and β-galactosidase lose about 50 % of their activity in 15 min (Table 1). Furthermore, PME and PG lose about 60 % of their activity at the same temperature for 75 and 30 min, respectively (Table 1).

The linear regressions between the papaya pericarp enzymes activity and heat treatment time at the different temperatures indicate that the inactivation of these enzymes followed a first-order model (Figure 1) for α-MAN, α-GAL and β-GAL and (Figure 2) for PME and PG.

Table 2. K-, D-, Z-and Ea-values for thermal inactivation of papaya pericarp (*Carica papaya* cv solo 8) pectin methylesterase (PME). Polygalacturonase (PG). α-mannosidase (α-MAN). α-galactosidase (α-GAL) and β-galactosidase) (β-GAL) range (4 0-65 °c.

	α-MAN			α-GAL			β-GAL			PME			PG		
Temperature (°C)	D-values (min)	R²	k-values (min⁻¹)	D-values (min)	R²	k-values (min⁻¹)	D-values (min)	R²	k-values (min⁻¹)	D-values (min)	R²	k-values (min⁻¹)	D-values (min)	R²	k-values (min⁻¹)
40	1439.38	0.994	0.0016	383.83	0.991	0.0060	2088.64	0.995	0.0011	1354.71	0.992	0.0017	1354.71	0.992	0.0017
45	885.77	0.991	0.0026	277.47	0.991	0.0083	852.96	0.970	0.0027	460.60	0.991	0.0050	622.43	0.974	0.0037
50	719.69	0.996	0.0032	232.63	0.992	0.0099	719.69	0.992	0.0032	354.31	0.991	0.0065	383.83	0.996	0.0060
55	595.75	0.992	0.0040	154.56	0.991	0.0149	258.76	0.992	0.0089	274.17	0.992	0.0084	291.52	0.992	0.0079
60	460.60	0.993	0.0050	129.38	0.995	0.0178	193.53	0.992	0.0119	239.90	0.991	0.0096	202.02	0.992	0.0114
65	308.08	0.996	0.0076	59.27	0.992	0.0232	81.09	0.998	0.0284	187.24	0.995	0.0123	76.01	0.995	0.0303
Z-value (°C)		31.54			42.73			17.69			34.84			19.12	
Ea (kJ/mol)		64.38			47.28			91.46			39.90			105.93	

This result was consistent with those reported for PME and PG in Carrots, Potatoes and tomato juice (Anthon and Barrett, 2002; Anthon *et al.,* 2002) and was also consistent with the relationships found in earlier studies on fruits and vegetables (Dogan *et al.*, 2005; Ditchfield *et al.*, 2006; Rapeanu *et al.*, 2006). 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.

Table 3. Thermodynamic parameters for papaya (*carica papaya* cv solo 8) pericarp pectin methylesterase (PME), ploygalacturonase (PG), α -mamosidase (α-MAN), α -galactosidase (α -GAL) and β-galactosidase (β-GAL) range (40-65 °C.

Thermodinamic parameters															
	α-MAN			α-GAL			β-GAL			PME			PG		
Temperature (°C)	ΔH* (kJ/mol)	ΔS* (J mol⁻¹ K⁻¹)	ΔG* (kJ/mol)	ΔH* (kJ/mol)	ΔS* (J mol⁻¹ K⁻¹)	ΔG* (kJ/mol)	ΔH* (kJ/mol)	ΔS* (J mol⁻¹ K⁻¹)	ΔG* (kJ/mol)	ΔH* (kJ/mol)	ΔS* (J mol⁻¹ K⁻¹)	ΔG* (kJ/mol)	ΔH* (kJ/mol)	ΔS* (J mol⁻¹ K⁻¹)	ΔG* (kJ/mol)
40	64.77	-117.29	101.46	46.67	-135.83	87.50	81.14	-28.59	91.09	37.29	-163.97	88.61	103.32	37.78	91.49
45	64.73	-117.42	102.07	46.63	-135.95	88.18	81.10	-28.72	91.24	37.25	-164.10	89.40	103.28	37.65	91.31
50	64.69	-117.55	102.66	46.59	-137.09	88.87	81.06	-28.85	91.38	37.21	-164.23	90.25	103.24	37.52	91.12
55	64.65	-117.68	103.25	46.54	-137.22	89.55	81.02	-28.98	91.52	37.17	-164.35	91.07	103.20	37.39	90.93
60	64.60	-117.80	103.83	46.50	-137.34	90.24	80.98	-29.11	91.67	37.12	-164.48	91.89	103.15	37.27	90.74
65	64.56	-117.93	104.42	46.46	-137.47	90.93	80.93	-29.23	91.82	37.08	-164.60	92.72	103.11	37.14	90.55
mean	64.67	-117.61	102.95	46.56	-137.15	89.21	81.04	-28.91	91.45	37.19	-164.28	90.66	103.22	37.46	91.02

The rate of inactivation after logarithmic transformation of α-MAN, α-GAL and β-GAL (Figure 3), then PME and PG (Figure 4) decreased linearly with the inverse of temperature.

This relationship was described by the equation: ln k = -6872 (1/T) + 15.39 (R^2 = 0.99) for α-MAN, ln k = -5689 (1/T) + 13.03 (R^2 = 0.98) for α-GAL, ln k = -13743 (1/T) + 36.94 (R^2 = 0.98) for β-GAL, ln k = -4801 (1/T) + 9.76 (R^2 = 0.99) for PME and ln k = -12747 (1/T) + 34.05 (R^2 = 0.95) for PG, where T represents absolute temperature (°K).

The thermal inactivation of these enzymes showed that the rate constants (k values) (Table 2) increase with increasing temperature. Arrhenius (1889) showed that most of the reactions are faster when the temperature rises. Consequently, if the temperature increases, the rate constant increases.

The D-values (Table 2) obtained at the temperature of pre-incubation of 40 °C, gradually decreases when the temperature increases to 65 °C. These values were used to determine the values of the activation energies (Ea). So, from 40 to 65 °C, the activation energy values for thermal inactivation of these enzymes were calculated to be 64.38, 47.28, 91.46, 39.90 and 105.93 kJ/mol for α-MAN, α-GAL, β-GAL, PME and PG respectively (Table 2). These values are high for all enzymes when compared to the activation energy obtained during the thermal degradation of the carotenoid of papaya puree. 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 (Timasheff, 1993). The effect of temperature on D-values is shown in Figure 5 for α-MAN, α-GAL and β-GAL, then Figure 6 for PME and PG.

From this representation, the Z-value was calculated and found to be 17.69, 19.12, 31.54, 34.84 and 42.73 °C for β-GAL, PG, α-MAN, PME and α-GAL respectively (Table 2).

The high values of the different Z obtained show that these enzymes are heat stable at temperatures between 40 and 65 °C (Barrett *et al.*, 1999).

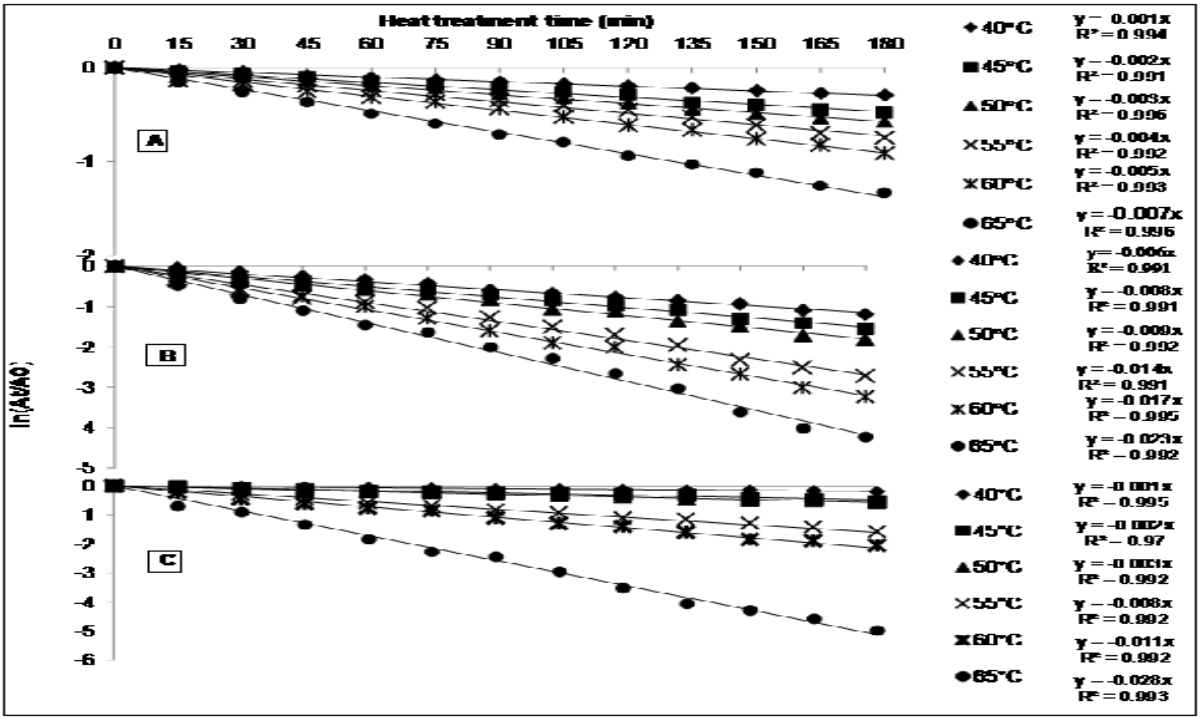

Fig. 1. Thermal inactivation of α-mannosidase (A), α-galactosidase (B) and β-galactosidase (C) in the temperature ranged from 45 to 65 °C. A0 is the initial enzymatic activity and At the activity at each holding time.

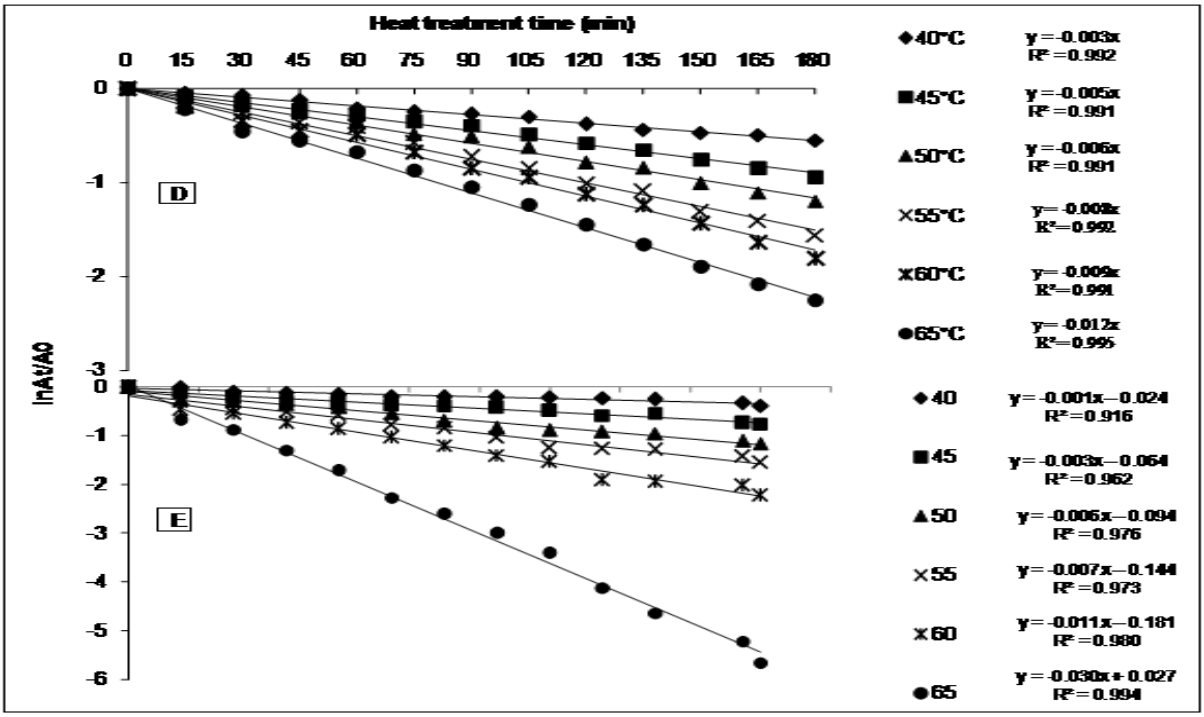

Fig. 2. Thermal inactivation of PME (D), PG (E) at the temperature ranged from 40 to 65 °C. A0 is the initial enzymatic activity and At the activity at each holding time.

Ohtani & Misaki (1983) showed that the α-mannosidase extract of papaya was stable below 60 °C. This work confirms our results that indicate that after 30 min of exposure α-mannosidase to heat (60 °C), it still retains about 80 % of its residual activity. Furthermore, it has been reported that when inactivation of PME of the tomato, the value of Z is between 4.5 and 32 °C (Anthon *et al*., 2002; Nath *et al*., 1983; Raviyan *et al*., 2005). In our study, the value of Z obtained which is 34.84 °C for the PME of the pericarp of papaya is similar to the value indicated by these authors.

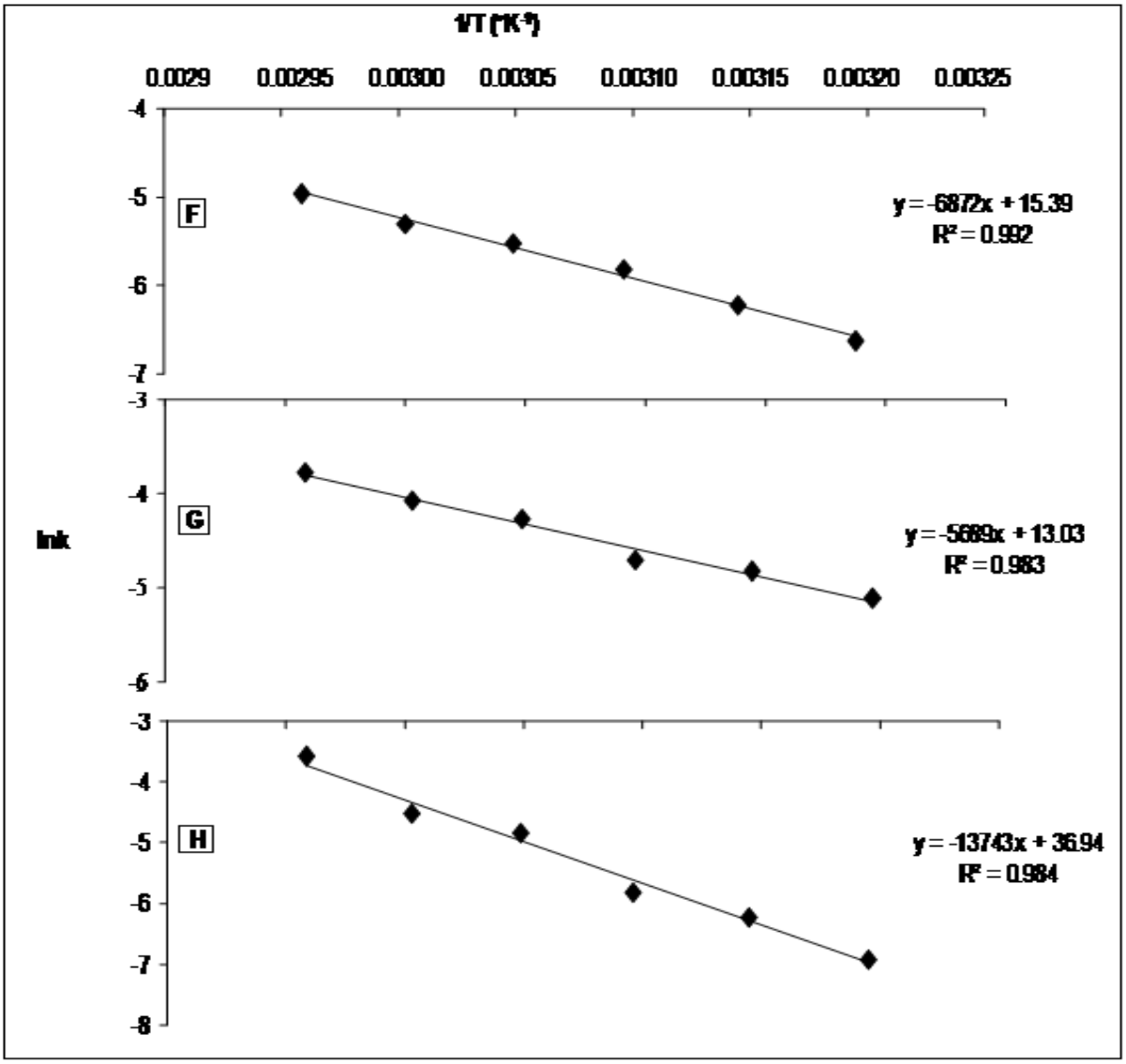

Fig. 3. Temperature dependence of inactivation rate constant for thermal inactivation of α-mannosidase (F), α-α-galactosidase (G) and β-galactosidase (H). 1/T represents the reciprocal of the absolue temperature.

This shows that the PME of the pericarp of papaya Solo 8 has a similar thermal resistance as PME of tomato. Terefe *et al*. (2009) showed that the heat treatment at 50 °C had no significant effect on the activity of polygalacturonase of tomato juice. However, at temperatures between 50 and 75 °C, the heat treatment had resulted in the partial inactivation (37.5 %) of the PG.

This result is in agreement with our results showing that exposure to heat (65 °C) for 30 min of PG causes partial inactivation (40 %) of the enzyme.

The values of enthalpy (ΔH#) and free energy (ΔG#) are positive, while those of entropy (ΔS#) are negative for α-mannosidase, α-galactosidase, β-galactosidase and PME (Table 3).

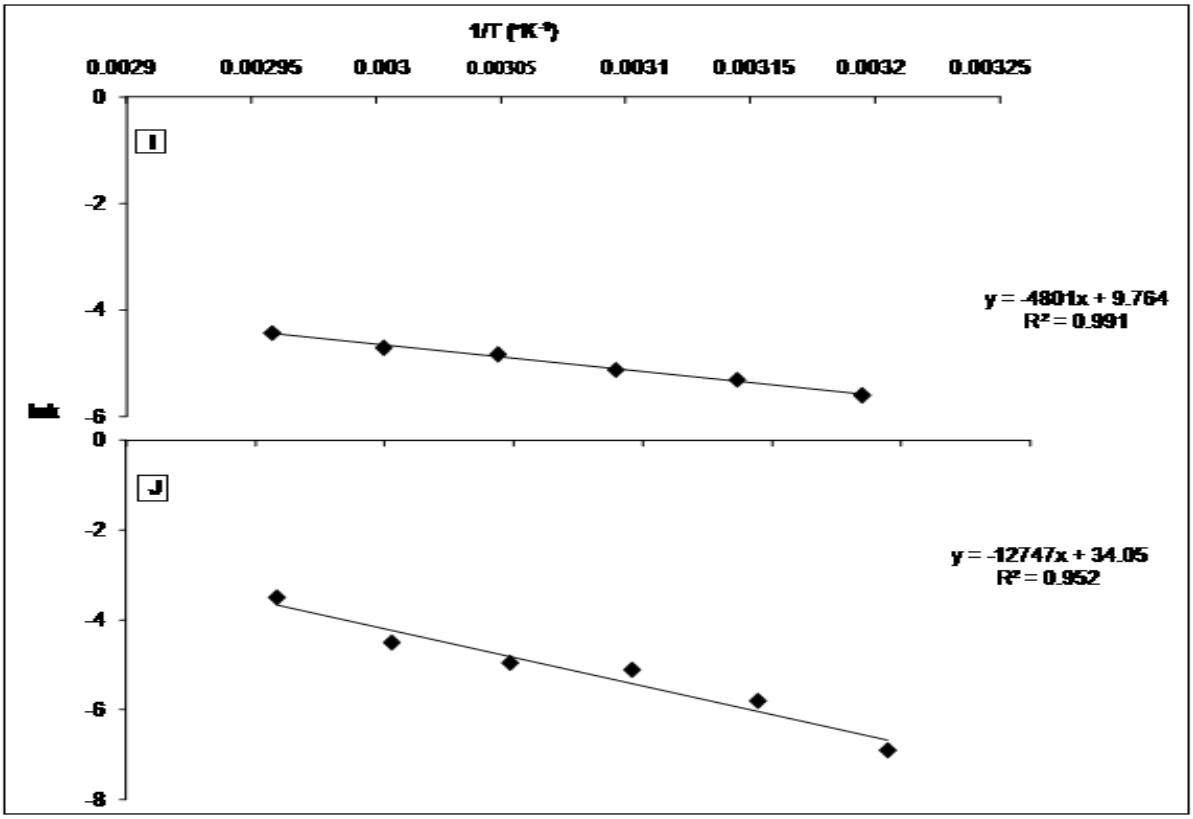

Fig. 4. Temperature dependence of inactivation rate constant for thermal inactivation of PME (I) and PG (J). 1/T represents the reciprocal of the absolue temperature.

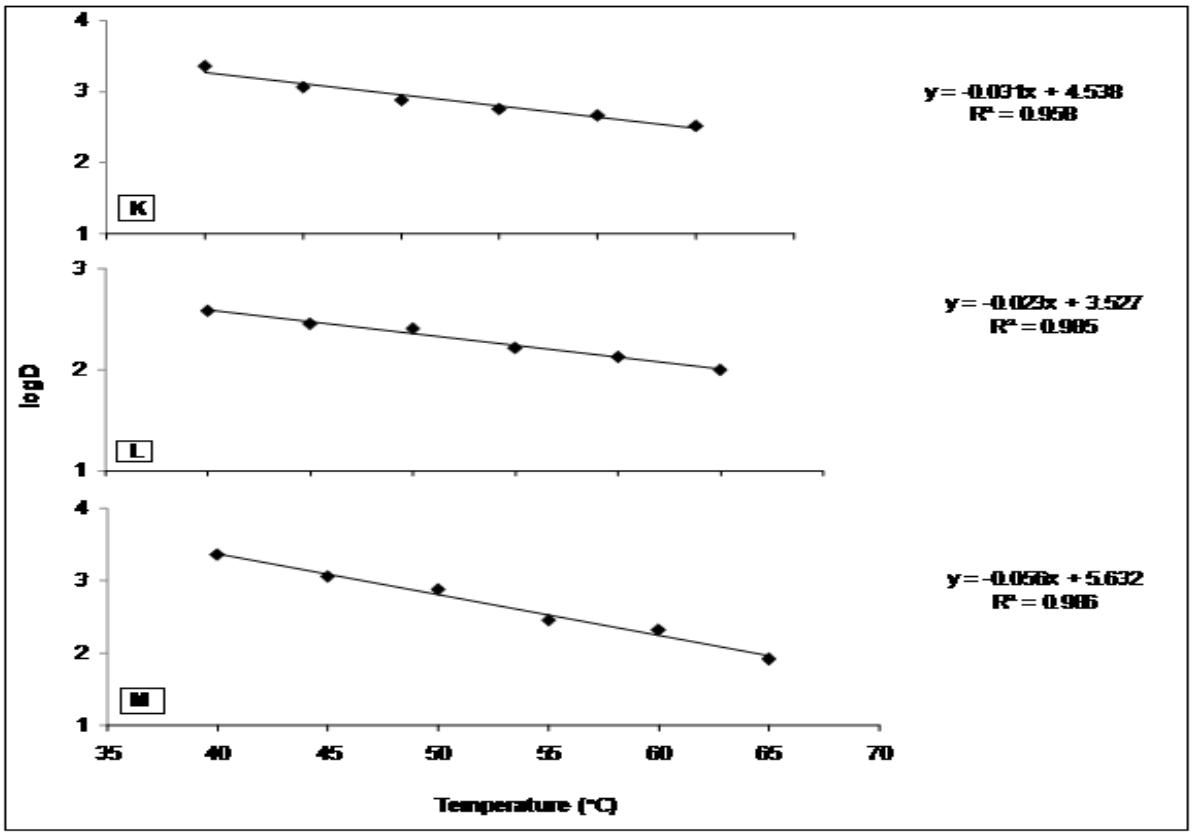

Fig. 5. Effect of temperature on D-Values for inactivation of α-mannosidase (K), α-galactosidase (L) and β-galactosidase (M) activities.

The high values of enthalpy obtained during thermal inactivation of these enzymes from the pericarp of papaya solo 8 indicate that these enzymes undergo substantial conformational changes to be under their activated forms during the heat treatment (Gnangui *et al.*, 2009). Moreover, positive values of the enthalpies suggest that the endothermic nature of the thermal inactivation reactions (Ozdes *et al.*, 2009). However, the negative values observed for the variation in entropy indicate that there are no significant processes of aggregation, since, if this would happen, the values of entropy would be positive (Anema and McKenna, 1996). They also indicate that these reactions are reversible (Gnangui *et al.*, 2009).

Negative values of entropy (ΔS#<0) obtained during thermal inactivation, naturally leads to higher values of free energy (ΔG#). These values are positive, indicating that thermal inactivation of these reactions do not occur spontaneously. These reactions must be initiated by adding energy in the form of heat, hence the high activation energy in these reactions. For cons, the values of enthalpy (ΔH#), entropy (ΔS#) and free energy (ΔG#) are positive for PG. Positive values of entropy (ΔS#) indicate that these enzymes are found in a chaotic state at the end of the reaction. This shows that they have suffered a distortion pronounced between 40 and 65 °C (Anema and McKenna, 1996).

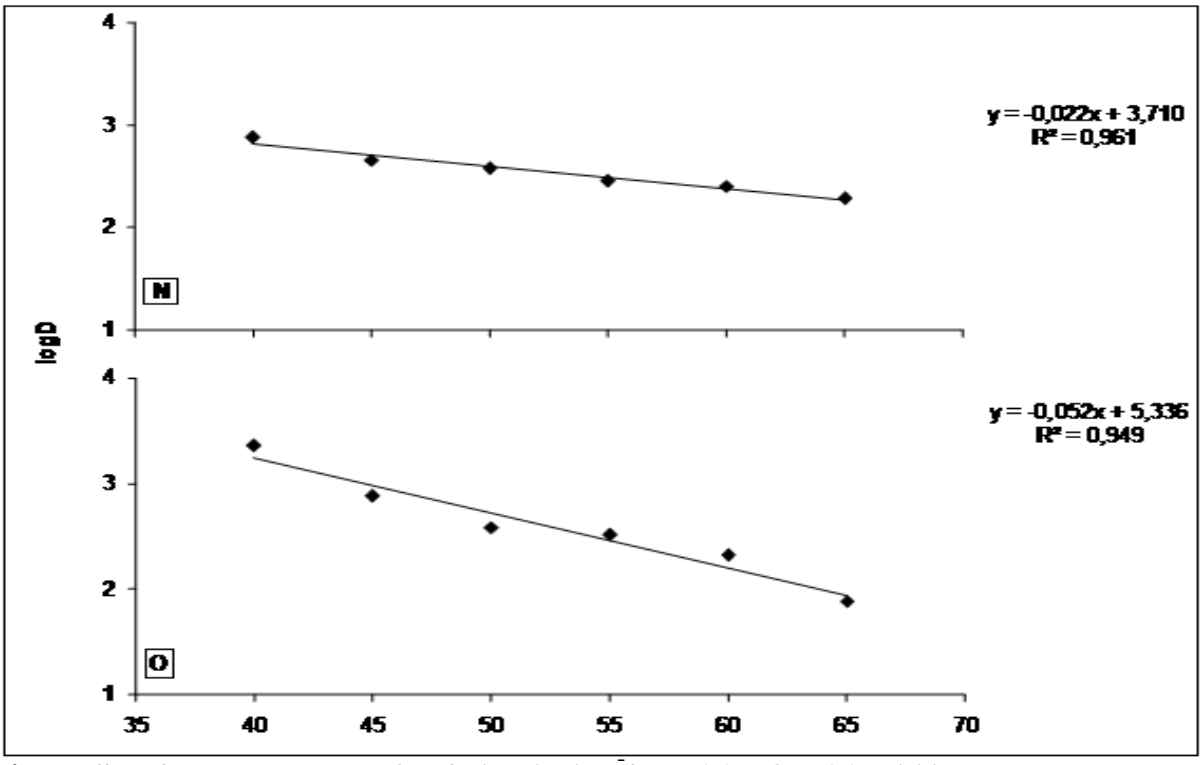

Fig. 6. Effect of temperature on D-Values for inactivation of PME (N) and PG (O) activities.

Hydrolytic activities of all enzymes decrease in thermal inactivation between 40 and 65 °C. However, at 49 °C for 90 min and at 55 °C for 60 min, about 50 % hydrolytic activities are destroyed. Beyond these temperatures, they decrease drastically. Under these conditions, it is up to choose between the processing enzyme crude extract of papaya solo 8 to 49 °C for 90 min and treating the extract at 55 °C for 60 min.

These temperatures destroy about half of the enzyme activity during the time of treatment. These results meet the target that was partially destroying the enzyme activity to slow the ripening process and not completely stop this process. Therefore, treatment of enzyme crude extract at 49 °C for 90 min could meet the target. This result is confirmed by the work of Djioua (2010).

He showed that, for the inactivation of the enzymes, exposing them to a high temperature for a short time is less effective than exposure to a mean temperature (46-55 °C) for a long time.

Conclusion

The enzymatic activities of papaya pericarp enzymes are slightly denatured at 40 and 45 °C. At 49 °C for 90 min and at 55 °C for 60 min, about 50 % of the activities are destroyed. Beyond these temperatures, they decrease drastically. In addition, the values of the thermodynamic parameters (enthalpy ($\Delta H^{\neq}$), entropy ($\Delta S^{\neq}$) and free energy ($\Delta G^{\neq}$) obtained during thermal inactivation of these enzymes demonstrate that they have undergone denaturation pronounced between 49 and 65 °C. However, α-mannosidase and β-galactosidase activity are thermostable, they maintain about 60 % of their hydrolytic activity at 49 °C for 120 min. The objective of this study was to follow the kinetics of thermal inactivation of various enzymes to determine a temperature that may partially inhibit the hydrolytic activity. Therefore, the results of this study show that immersion papayas in a water bath at 49 °C for 90 min treatment can be adapted to delay softening, ripening and preserve fruit quality.

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