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# Study of thermal stability of two acid phosphatases from breadfruit (*Artocarpus communis*) seeds: kinetic and thermodynamic analysis

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**Abstract:** The thermal stability of acid phosphatases ACP1 and ACP2 from breadfruit (*Artocarpus communis*) seeds was investigated by studying the effect of heat treatment over a range of 55 to 75 °C. Thermal inactivation of each enzyme, evaluated by loss in activity, was apparently followed by first-order kinetics with k-values comprised between 0.0139 ± 0.0005–0.0620 ± 0.0001 and 0.0117 ± 0.0002–0.0362 ± 0.0004 $min^{-1}$ for ACP1 and ACP2, respectively. D and k-values decreased and increased, respectively, with increasing temperature, indicating faster inactivation of ACP1 and ACP2 at higher temperatures. Ea and Z-values were estimated to 69.19 ± 2.71 kJ/mol and 31.55 ± 1.05 °C for ACP1, 52.25 ± 1.94 kJ/mol and 41.84 ± 2.06 °C for ACP2. Thermodynamic parameters were also calculated. All the results suggest that both acid phosphatases are relatively resistant to long heat treatments up to 60°C.

## INTRODUCTION

Acid phosphatases (orthophosphoric-monoester phosphohydrolase, EC 3.1.3.2) (APases) form a group of enzymes catalyzing hydrolysis of a variety of phosphate esters in the acidic environments. APases are believed to increase orthophosphate (Pi) availability under phosphorous deficient conditions (Vance et al., 2003).

In plant roots, acid phosphatases seem to be involved in the solubilization of macromolecular organic phosphates in soils which can then be utilized by plants (Panara et al., 1990). From tubers, Kamenan (1984) and Kouadio (2004) have reported an important role of acid phosphatases concerning the transport of phosphate in the metabolic phenomena taking place during the preservation of yam (*Dioscorea cayenensis rotundata*) and cocoyam (*Xanthosoma* sp.) tubers. From seeds and seedlings, the physiological function of the acid phosphatases is to provide inorganic phosphate to the growing plant during germination and many different phosphate esters of sugars and substrates stored in the seed and seedling need to be hydrolyzed during germination and growth (Hoehamer et al., 2005).

Considering this importance of APases, many authors have attempted to understand the kinetic behaviour of these enzymes when exposed to high temperatures in plant such as root (Eduardo and Gonzalez, 2004), such as seed and seedling (Biswas and Cundiff, 1991; Kaur et al., 2011; Gnanwa et al., 2014). Thus, comparative studies of thermophilic and mesophilic enzymes have demonstrated that weak interactions such as hydrogen bonds (Macedo-Ribeiro et al., 1996), disulfide bonds (Hopfner et al., 1999), ion pairs (Vetriani et al., 1998), salt bridges (Criswell et al., 2003), hydrophobic interactions (Elcock, 1998) and compactness (Russell et al., 1997) are of importance for stability. Therefore, enhancement of the structural stability of enzymes is of great importance for their application in several industrial processes. The mechanism of thermal denaturation depends on various physicochemical parameters of the solution in which the reaction is catalyzed. Any process that enhances the structural stability and rate of reaction of enzymes has a favourable impact on their industrial application (Wasserman, 1984; Timasheff 1993; Matsumoto et al. 1999; Sousa, 2009).

In a previous study, Konan et al. (In press) purified to homogeneity two acid phosphatases from breadfruit (*Artocarpus communis*) seeds. This plant belongs to the family Moraceae. It grows in the Tropics, where the fruits are used in a variety of food preparations (Ragone, 2003). Both phosphatases termed ACP1 and ACP2 showed maximum activity at 55 °C and interesting properties to catalyse the transfer of phosphoryl residues from pyrophosphate onto phenol. These enzymes are also able to catalyse the transphosphorylation of sugars and proteins (Konan, 2009).

Considering these important properties of ACP1 and ACP2, the present work aimed to investigate

the effect of heat treatment over a range of temperatures from 55 to 75°C, on these acid phosphatases. So, determination and analysis of kinetic and thermodynamic parameters were undertaken. The knowledge of kinetic parameters in thermal treatments would enable modulate processes to achieve desirable enzyme activity at the end of the heat operation.

## MATERIALS AND METHODS

### Enzyme

Acid phosphatases (ACP1 and ACP2) used in this study were purified from the breadfruit (*Artocarpus communis*) seeds (Konan et al., in press). Theses enzymes were homogeneous in on polyacrylamide-gel electrophoresis in the absence of sodium dodecyl sulphate (SDS).

### Phosphatase assay

The two enzymes assays were performed as described below (Gnanwa et al., 2014). Briefly, each purified acid phosphatase extract (50 µl) was incubated with 1.5 mM *para*-Nitrophenylphosphate (*p*NPP, 75 µl) on 100 mM acetate buffer pH 5.5 (125 µl) at 37 °C for 10 min. The final volume was 250 µl and the reaction was stopped by adding 2 ml of sodium carbonate (2 %, w/v). Absorbances were measured at 410 nm using a spectrophotometer (SHIMADZU) using *para*-Nitrophenol (pNP) as the standard. Under the above experimental conditions, one unit of the enzyme activity (UI) is defined as the amount of enzyme capable of releasing one µmol of pNP per minute. Specific activity was expressed as units per mg of protein (U/mg of protein).

### Protein determination

Protein was determined according to Lowry method (Lowry et al., 1951) using bovine serum albumin as standard.

### Thermal inactivation

The thermal inactivation of each acid phosphatase (ACP) was investigated at various constant temperatures from 55 to 75°C after exposure to each temperature for a period of 5 to 60 min. Each enzyme was incubated in 100 mM acetate buffer (pH 5.5). 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 each phosphatase 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:

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

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):

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

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

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

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)

$$k = Ae^{(-Ea/RT)} \qquad (4)$$
$$\text{or } \ln k = \ln A - Ea/R \times T \qquad (5)$$

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).

Table 1. Effect of treatment temperature and time on the inactivation of acid phosphatases ACP1 and ACP2. Values represent mean ± SD three independent determinations

| Treatment time (min) | Residual activity (%) at each temperature (°C) of heat treatment | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 55 | | 60 | | 65 | | 70 | | 75 | |
| | ACP1 | ACP2 | ACP1 | ACP2 | ACP1 | ACP2 | ACP1 | ACP2 | ACP1 | ACP2 |
| 5 | 91.41 ± 1.96 | 97.05 ± 0.63 | 78.54 ± 1.95 | 90.54 ± 0.91 | 74.35 ± 1.96 | 81.03 ± 1.86 | 68.78 ± 0.93 | 76.89 ± 1.88 | 58.65 ± 1.95 | 70.65 ± 1.88 |
| 10 | 86.65 ± 2.02 | 91.24 ± 0.88 | 73.13 ± 1.94 | 80.43 ± 1.79 | 70.15 ± 0.51 | 74.24 ± 1.84 | 59.63 ±1.94 | 68.42 ± 0.78 | 48.67 ± 0.91 | 62.43 ± 1.93 |
| 15 | 81.96 ± 0.98 | 84.24 ± 1.76 | 69.25 ± 0.94 | 75.29 ± 0.89 | 60.25 ± 2.98 | 69.24 ± 2.12 | 50.15 ± 1.94 | 60.55 ± 2.78 | 36.18 ± 1.95 | 55.29 ± 1.85 |
| 20 | 77.30 ± 2.93 | 78.56 ± 0.85 | 64.03 ± 1.88 | 69.21 ± 1.84 | 52.33 ± 1.95 | 64.95 ± 2.91 | 42.32 ± 1.95 | 55.03 ± 1.92 | 27.26 ± 1.92 | 45.76 ± 1.05 |
| 25 | 74.33 ± 0.96 | 74.59 ± 1.05 | 60.18 ± 0.93 | 65.85 ± 2.07 | 50.24 ± 0.06 | 57.85 ± 1.85 | 37.25 ± 0.94 | 50.56 ± 1.79 | 20.43 ±1.98 | 41.29 ± 0.87 |
| 30 | 71.50 ± 0.93 | 68.76 ± 1.73 | 55.57 ± 1.95 | 60.39 ± 1.55 | 42.71 ± 1.93 | 51.63 ± 0.85 | 31.01 ± 0.96 | 43.52 ± 2.16 | 16.54 ± 0.92 | 34.59 ± 1.87 |
| 35 | 65.22 ± 1.93 | 65.35 ± 1.91 | 51.08 ± 0.94 | 56.41 ± 2.18 | 38.51 ± 0.93 | 46.94 ± 1.92 | 28.12 ± 0.93 | 38.38 ± 1.81 | 12.52 ± 1.96 | 28.74 ± 1.84 |
| 40 | 57.14 ± 2.93 | 61.49 ± 1.12 | 43.35 ± 2.97 | 52.92 ± 0.48 | 33.12 ± 2.94 | 41.58 ± 1.86 | 21.41 ± 1.91 | 32.36 ± 0.48 | 09.32 ± 0.84 | 23.64 ± 0.13 |
| 45 | 55.43 ± 0.91 | 59.95 ± 1.24 | 41.03 ± 0.97 | 48.62 ± 0.77 | 28.65 ± 0.95 | 38.65 ± 1.89 | 18.04 ± 0.86 | 29.76 ± 0.95 | 07.02 ± 0.05 | 19.85 ± 0.89 |
| 50 | 48.17 ± 1.92 | 56.12 ± 0.82 | 35.87 ± 1.95 | 43.13 ± 0.78 | 25.06 ± 1.88 | 33.49 ± 0.89 | 14.09 ± 0.95 | 24.58 ± 0.06 | 04.45 ± 1.02 | 16.81 ± 1.77 |
| 55 | 46.76 ± 0.94 | 52.46 ± 1.48 | 30.71 ± 1.95 | 40.35 ± 1.83 | 21.18 ± 0.92 | 29.58 ± 1.88 | 10.71 ± 0.96 | 22.80 ± 0.23 | 03.13 ± 0.83 | 13.77 ± 0.05 |
| 60 | 42.15 ± 1.97 | 50.46 ± 1.05 | 28.09 ± 1.95 | 35.75 ± 0.93 | 17.58 ± 1.93 | 26.75 ± 1.93 | 08.12 ± 0.96 | 18.94 ± 0.83 | 02.20 ± 0.01 | 11.25 ± 1.33 |

The values of the activation energy (Ea) and Arrhenius constant (A) allowed the determination of different thermodynamic parameters such as variations in enthalpy, entropy and Gibbs free energy, $\Delta H^{\#}$, $\Delta S^{\#}$ and $\Delta G^{\#}$, respectively, according to the following equations ((Eq. 6; 7; 8)

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

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

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

Where;

KB is the Boltzmann constant ($1.38 \times 10^{-23}$ J/K), hP is the Planck constant ($6.626 \times 10^{-34}$ J.s) and T is the absolute temperature.

**Data analysis**

All the experiments were carried out in triplicates and the results were expressed as means ± standard deviation. The statistical differences among the means of data were calculated using one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT). Significance was set at $P < 0.05$.

## RESULTS AND DISCUSSION

### Thermal inactivation kinetics of acid phosphatases

The thermal stability of acid phosphatases from breadfruit (*Artocarpus communis*) seeds was investigated by studying the effect of heat treatment over a range of 55 °C to 75 °C. The optimum temperature of the acid phosphatases purified from these seeds was 55 °C (Konan et al., in press). Thus, theses acid phosphatases are mesophilic enzymes. In this study, the effect of heat treatment over a range of temperatures from 55 to 75 °C on both acid phosphatases was evaluated by determining the residual enzymatic activity. The thermal stability profiles of acid phosphatases ACP1 and ACP2 presented in the form of the residual percentage activity are shown in Table 1. The activities of acid phosphatases were decreased with increasing heating time (5–60 min) and temperature (55–75°C). Indeed, at temperatures between 60-75°C, heat-denaturation of the enzymes occurred after 5 min of incubation (91.41 ± 1.96 to 58.65 ± 1.95 % and 97.05 ± 0.63 to 70.65 ± 1.88 % for ACP1 and ACP2, respectively). Although the heat treatment during 25 min at 65 °C caused the partial inactivation (50.24 ± 0.06 %) of acid phosphatase ACP1 whereas for acid phosphatase ACP2, the partial inactivation (50.56 ± 1.79 %) during the same period occurred at 70 °C. On one hand, acid phosphatases from breadfruit (*Artocarpus communis*) seeds showed a typical temperature-dependent inactivation profile in the presence of the substrate used. At higher temperature, the enzymes most likely underwent denaturation and lost their activities. Stauffer (1989) states that denaturation is the heat induced spontaneous, irreversible breakdown of the secondary and tertiary structure of the enzyme protein such that the enzyme will no longer function and cannot re-activate. The results of the heat inactivation studies suggest that these enzymes belong to the group of less thermo stable enzymes.

Considering the logarithmic linear relationship between acid phosphatases ACP1 and ACP2 activities and heat treatment time for the temperatures ranged from 55 to 75 °C (Fig. 1), it can be concluded that the thermal inactivation of each acid phosphatase can be adequately described by a first order reaction. These results were consistent with those reported for pyrophosphatase from vacuolar (Yang et al., 2004), for acid phosphatase from cod (Johnsen *et al*., 2007) and for purple acid phosphatase from breadfruit (*Artocarpus communis*) seeds (Gnanwa et al., 2014).

From the slope of the lines in Fig. 1, the inactivation rate constants (k) were calculated. The trend of changes in k values with increasing temperature indicates a faster inactivation of acid phosphatases at higher temperatures. Rate constants of each acid phosphatase thermal inactivation increased with the temperature (Table 2). This result showed clearly that ACP1 and ACP2 were completely unstable at high temperatures (65-70 °C) since a higher rate constant means that the enzyme was less thermostable (Marangoni, 2002).

Table 2. k-values for thermal inactivation of acid phosphatases ACP1 and ACP2 at temperature range (55–75°C). Values represent mean ± SD three independent determinations

| Temperature (°C) | ACP1 | | ACP2 | |
|---|---|---|---|---|
| | k-values ($min^{-1}$) | $R^2$ | k-values ($min^{-1}$) | $R^2$ |
| 55 | $0.0139 \pm 0.0005^{b}$ | 0.98 | $0.0117 \pm 0.0002^{ab}$ | 0.99 |
| 60 | $0.0207 \pm 0.0003^{cd}$ | 0.98 | $0.0167 \pm 0.0003^{cde}$ | 0.99 |
| 65 | $0.0284 \pm 0.0005^{ef}$ | 0.99 | $0.022 \pm 0.004^{fg}$ | 0.99 |
| 70 | $0.0410 \pm 0.0004^{g}$ | 0.98 | $0.0278 \pm 0.0001^{h}$ | 0.99 |
| 75 | $0.0620 \pm 0.0001^{e}$ | 0.99 | $0.0362 \pm 0.0004^{e}$ | 0.99 |

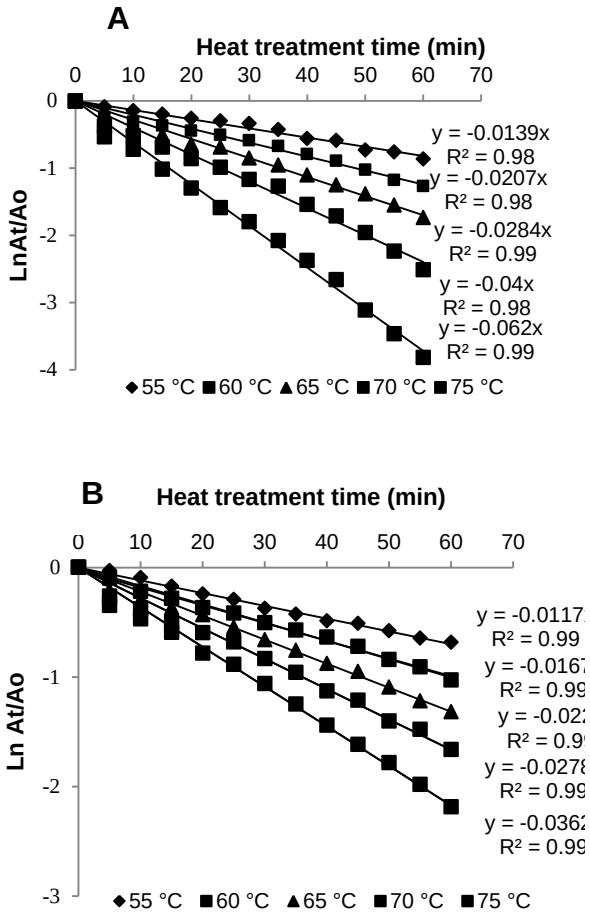


Fig. 1. Thermal inactivation of acid phosphatases ACP1 and ACP2 from breadfruit (*Artocarpus communis*) seeds in sodium acetate buffer pH 5.5 in the temperature ranged from 55 to 75°C. $A_0$ is the initial enzymatic activity and $A_t$ the activity at each holding time. [A]: acid phosphatase ACP1, [B]: acid phosphatase ACP2

Table 3. D, Z and Ea-values for thermal inactivation of acid phosphtases ACP1 and ACP2 at temperature range (55–75°C). Values represent mean ± SD three independent determinations.

| D values (min) | | |
|---|---|---|
| | ACP1 | ACP2 |
| $D_{55}$ | 165.68 ± 2.94$^{k}$ | 196.84 ± 4.06$^{l}$ |
| $D_{60}$ | 111.26 ± 2.65$^{h}$ | 137.90 ± 3.58$^{i}$ |
| $D_{65}$ | 81.09 ± 3.97$^{de}$ | 104.68 ± 5.34$^{f}$ |
| $D_{70}$ | 57.58 ± 4.27$^{c}$ | 82.84 ± 2.68$^{d}$ |
| $D_{75}$ | 37.15 ± 1.03$^{a}$ | 63.62 ± 2.03$^{a}$ |
| Z value (° C) | 31.55 ± 1.05$^{a}$ | 41.84 ± 2.06$^{b}$ |
| Ea (kJ/mol) | 69.19 ± 2.71$^{a}$ | 52.25 ± 1.94$^{b}$ |

In order to establish the link between treatment time and enzyme activity, the D-values were calculated. The decimal reduction time (D value) was calculated according to equation 2. D value is the time, at a given temperature and pressure, needed for 90% reduction of the initial activity.

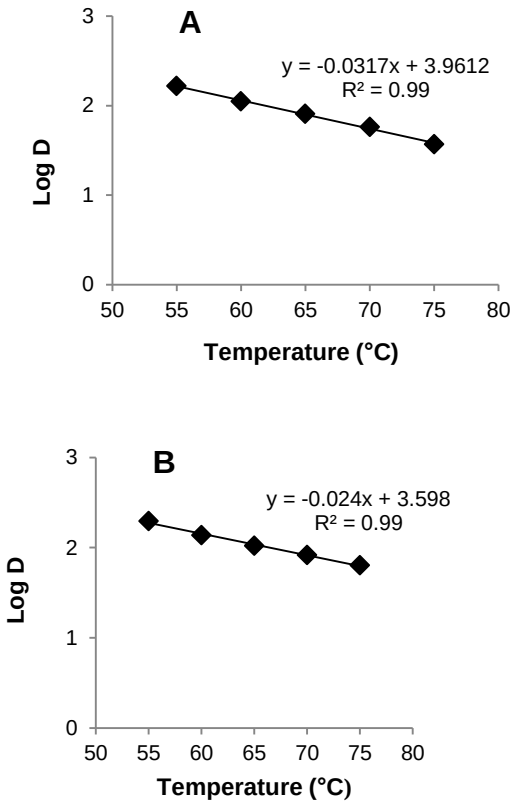


Fig. 2. Effect of temperature on D-values for inactivation of acid phosphatases ACP1 and ACP2 from breadfruit (*Artocarpus communis*) seeds. [A]: acid phosphatase ACP1; [B]: acid phosphatase ACP2

As shown in table 3, D-values decreased by increased at temperature. The D values ranged from 165.68 ± 2.94 min to 37.15 ± 1.03 min for ACP1 inactivation and from 196.84 ± 4.06 min to 63.62 ± 2.03 min for ACP2. It is worth mentioning that at 75 °C, the D value for ACP2 inactivation was 1.71 times higher than the corresponding value for ACP1 inactivation. This remarkable decrease at D-value between 55 and 75°C indicated a potential thermal denaturation of acid phosphatases from breadfruit (*Artocarpus communis*) seeds. The effect of temperature on D-values is shown in Fig. 2, and from this representation, the Z-values were calculated and found to be 31.55 ± 1.05 °C and 41.84 ± 2.06 °C for ACP1 and ACP2, respectively at 55 – 75 °C (Table 3). In general, high Z-values mean more sensitivity to the duration of heat treatment and lower Z-values mean more sensitivity to increase in temperature (Barrett et al., 1999). Therefore, the Z-values for breadfruit (*Artocarpus communis*) seeds acid phosphatises indicated that this enzyme is more to sensitive to increase of temperature than to the extension of treatment time.

The activation energy (Ea) was seen as the energy absorbed or released needed to the molecules be able to react (van Boekel, 2008). In this study, the phosphatases activation energy (Ea) values were

calculated to be 69.19 ± 2.71 kJ/mol and 52.25 ± 1.94 kJ/mol for ACP1 and ACP2, respectively (Table 3). ACP1 displayed relatively higher energy barrier (69.19 ± 2.71 kJ/mol) than ACP2 (52.25 ± 1.94 kJ/mol). Obviously, ACP2 showed a considerably higher thermosensitivity upon heat treatment. These values were similar to pyrophosphatase (70.2 kJ/mol and 54.9 kJ/mol) from vacuolar (Yang et al., 2004). Therefore, the high value found for the activation energy means that a high amount of energy is needed to initiate denaturation of phosphatases, probably due to the compactness of the molecule and disulfide bridges (Björck, 1992). These values of activation energy were much higher than that reported for acid phosphatase from moth bean (9.44 kJ/mol) (Mohamed and Al-Omair, 2010) and purple acid phosphatases from Sweet potato (49.3 kJ/mol) (Kusudo et al., 2003).

**Thermodynamic studies of acid phosphatases**

Thermostability represents the capability of an enzyme molecule to resist against thermal unfolding in the absence of substrate, while thermophilicity is the ability of an enzyme to work at elevated temperatures in the presence of substrate (Georis et al., 2000). Irreversible thermal inactivation occurs in two steps as shown below:

N<—>U —>I

N is the native, U is the unfolded enzyme which could be reversibly refolded upon cooling and I is the inactivated enzyme formed after prolonged exposure to heat, and therefore, cannot be recovered on cooling. The thermal denaturation of enzymes is accompanied by the disruption of non-covalent linkages, including hydrophobic interactions, with concomitant increase in the enthalpy of activation (Daniel, 1996). The opening up of the enzyme structure is accompanied by an increase in the disorder, randomness or entropy of activation (Vieille and Zeikus, 1996).

It is essential to study acid phosphatases in order to determine the thermodynamics and thermostability properties, and hence apply the acid phosphatase obtained from breadfruit (*Artocarpus communis*) seeds that show the most promising industrial potentials (Konan, 2009). It is also important to understand the structure-stability relationships of their enzymes. An estimation of the thermodynamic parameters helps to understand the probable denaturation mechanism, which is very important in enzymatic processes (Ortega et al., 2004). Table 4 shows the changes in enthalpy ($\Delta H^{\#}$), entropy ($\Delta S^{\#}$) and Gibbs free energy change ($\Delta G^{\#}$) occurring during the thermal inactivation of purified acid phosphatases.

The average values of $\Delta H^{\#}$, $\Delta S^{\#}$ and $\Delta G^{\#}$ were respectively 66.38 ± 0.08 kJ/mol, -70.48 ± 0.18 J.mol/K and 90.20 ± 0.52 kJ/mol for ACP1 and 49.44 ± 0.06 kJ/mol, -123.17 ± 0.18 J.mol/K and 91.07 ± 0.9 kJ/mol for ACP2. The enthalpy for ACP1 was higher than that of ACP2. The enthalpy ($\Delta H$) change in the system corresponds to the heat released or absorbed in the transformation (constant pressure). In this study, the positive value of this parameter indicates that the catalytic reaction was endothermic. The heat of reaction extracted from the surroundings in the transformation of the substrates, undergoes a considerable change in conformation during denaturation.

The Gibbs free energy change ($\Delta G$) indicates the spontaneity of the reaction catalyzed under the conditions of temperature and pressure employed. In this study, both values were positive indicating that the processes were endergonic, that is they are not spontaneous.

The negative values for change in entropy were obtained in all temperature measured indicating that there are aggregation processes taking place during thermal inactivation of acid phosphatases (Anema and McKenna 1996; Dumitraşcu et al., 2012).

Based on above results, it is concluded that thermal inactivation of each acid phosphatase ACP1 and ACP2 of breadfruit (*Artocarpus communis*) seeds be described by a firs-order kinetic model. D-, Z-, k-values and the high values obtained for activation energy and change in enthalpy indicated that a high amount of energy was needed to initiate denaturation of these acid phosphatases, most likely due to its stable molecular conformation. This high thermostability may be taken into account when thermal treatments are used to obtain processed products derived from breadfruit (*Artocarpus communis*) seeds.

Table 4. Thermodynamic parameters of acid phosphatases ACP1 and ACP2 under heat treatment between 55 to 75°C (assuming a 1st-order kinetic model). Values represent mean ± SD three independent determinations

| Temperature (°C) | Thermodynamic parameters | | | | | |
|---|---|---|---|---|---|---|
| | $\Delta H^{\#}$ ( kJ/mol) | | $\Delta S^{\#}$ (J $mol^{-1}$ $K^{-1}$ ) | | $\Delta G^{\#}$ ( kJ/mol) | |
| | ACP1 | ACP2 | ACP1 | ACP2 | ACP1 | ACP2 |
| 55 | $66.46 \pm 0.08^{gh}$ | $49.52 \pm 0.02^{d}$ | $-70.23 \pm 0.03^{h}$ | $-122.93 \pm 0.01^{d}$ | $89.50 \pm 0.04^{e}$ | $89.84 \pm 0.02^{d}$ |
| 60 | $66.42 \pm 0.02^{ef}$ | $49.48 \pm 0.01^{c}$ | $-70.36 \pm 0.03^{g}$ | $-123.05 \pm 0.03^{c}$ | $89.85 \pm 0.03^{g}$ | $90.46 \pm 0.04^{f}$ |
| 65 | $66.38 \pm 0.01^{de}$ | $49.44 \pm 0.02^{b}$ | $-70.48 \pm 0.04^{f}$ | $-123.18 \pm 0.03^{b}$ | $90.20 \pm 0.03^{h}$ | $91.07 \pm 0.05^{h}$ |
| 70 | $66.34 \pm 0.03^{d}$ | $49.40 \pm 0.01^{a}$ | $-70.60 \pm 0.03^{e}$ | $-123.30 \pm 0.02^{a}$ | $90.55 \pm 0.03^{i}$ | $91.69 \pm 0.04^{j}$ |
| 75 | $66.30 \pm 0.02^{a}$ | $49.36 \pm 0.02^{a}$ | $-70.72 \pm 0.05^{a}$ | $-123.42 \pm 0.02^{a}$ | $90.91 \pm 0.03^{e}$ | $92.31 \pm 0.05^{e}$ |
| Mean | $66.38 \pm 0.08^{d}$ | $49.44 \pm 0.06^{b}$ | $-70.48 \pm 0.18^{e}$ | $-123.17 \pm 0.18^{a}$ | $90.20 \pm 0.52^{i}$ | $91.07 \pm 0.9^{h}$ |

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