Kinetics and Thermodynamics of Thermal Inactivation of Cellulase from Salivary Glands of *Macrotermes subhyalinus* Little Soldier

Fagbohoun Jean Bedel[1]✎ , Yapi Jocelyn Constant[2], Deffan Zranseu Bénédicte[2]

[1]Department of Biochemistry-Genetics, University Peleforo Gon Coulibaly, Korhogo, Côte d'Ivoire
[2]Department on Biochemestry-Microbiologie, University Lorougnon Guede, Daloa, Côte d'Ivoire

Abstract: For optimization of biochemical processes in food and pharmaceutical industries, the evaluation of enzyme inactivation kinetic models is necessary to allow their adequate use. kinetics and thermodynamic analysis of cellulase (GS-CX) from salivary glands of *Macrotermes subhyalinus* little soldier were studied, using carboxymethylcellulose as a substrate. Optimal conditions for enzymatic studies were determined to be pH 5.0 and 60 °C. Thermal inactivation of GS-CX was examined in more detail between 50 and 65 °C and in relation to exposure time. The investigation suggests the existence of a non-sensitive heat fraction on the enzyme structure, which is relatively stable up to temperatures close to 55 °C. Denaturation of this enzyme, measured by loss in activity, could be described as a first-order model, with k-values between 0.0052 and 0.0337 min^{-1}. D- and k-values decreased and increased, respectively, with increasing temperature, indicating faster cellulase (GS-CX) inactivation at higher temperatures. Results suggested that GS-CX is a relatively thermostable enzyme with a Z-value of 18.08 °C and Ea of 115.81 kJmol^{-1}. The results of the thermodynamic investigations indicated that the hydrolytic reactions were: (1) not spontaneous ($\Delta G > 0$) and (2) slightly endothermic ($\Delta H > 0$). Positive values of entropy ($\Delta S > 0$) for GS-CX 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 this enzymes. Results shown that the enzyme is quite stable for biotechnological applications.

Keywords: Carboxymethylcellulose, Kinetics And Thermodynamic Parameters, *Macrotermes Subhyalinus*, Thermal Stability, Salivary Glands, Cellulase

Introduction

The major nutritional component for wood-feeding termites is cellulose, which is plant's main structural constituent. Apart from being structural pests, termites have a huge impact on terrestrial ecological processes, as they are part of the main terrestrial cellulose recyclers (Inoue *et al*., 1997). Cellulase activity in the midgut of these insects is very high (Hogan *et al*., 1988), and studies have shown that they rely solely on their own cellulases (Slaytor, 1992; Slaytor, 2000) rather than the cellulases of symbiotic bacteria present in their lower intestine (Brune and Stingl, 2005). In nature, cellulose degradation requires the synergistic action of three types of glycoside hydrolases (GH): endo-*β*-1,4-glucanases (EG; EC. 3.2.1.4), exo-*β*-1, 4-cellobiohydrolases (CBH; EC. 3.2.1.91), and *β*-glucosidases (EC. 3.2.1.21) (Clarke,1997). Cellulase is a synergistic enzyme that is used to break up cellulose into glucose or other oligosaccharide compounds (Parry *et al.,* 2001). Based on their mode of action, three varieties of cellulase exist which any highly cellulolytic organism must possess (Breznak and Brune 1994). Exoglucanases (EC 3.2.1.91) are the first class of cellulases. The second class of cellulases consists of the endoglucanases (EC 3.2.1.4) while the third class is the beta-glucosidases (EC 3.2.1.21). Cellulases contribute to 8% of the worldwide industrial enzyme demands (Elba and Maria, 2007). The cellulase market is expected to expand dramatically when cellulases are used to hydrolyzed pretreated cellulosic material to sugars, which can be fermented to bioethanol and biobased products on large scales. Thus, the use of cellulase in various industrial processes is indispensable. It is used in plants and agricultural waste processing (Mswaka and Magan, 1998; Lu *et al*., 2004), biofuels (Vaithanomsat *et al*., 2009), triphase biomethanation (Chakraborty *et al*., 2000), chiral separation and ligand binding studies (Nutt *et al*., 1998). In addition to this, the major industrial application of cellulases are in textile industry for bio- polishing of fabrics and

✎ **Fagbohoun Jean Bedel (Correspondence)**
☎ + 225 07383758

producing stonewashed look of denims, as well as in household laundry detergents for improving fabric softness and brightness (Hill *et al.*, 2006). Besides, they are used in animal feeds for improving the nutritional quality and digestibility, in processing of fruit juice and in baking, while de-inking of paper is yet another emerging application. A potential challenging area where cellulases would have a central role is the bioconversion of renewable cellulosic biomass to commodity chemicals (Lynd *et al.*, 2005). Based on the importance of lignocellulose-degrading enzymes, t is necessary to know optimal conditions of enzyme active sites functioning and the effect of different physical-chemical factors on their activity. Hus, any process that enhances the structural stability and reaction rate of enzymes has a favorable impact on their industrial application (Sousa, 1995; Matsumoto *et al.*, 1997). However, there is no report concerning thermal stability of these cellulases. Thus, the knowledge on kinetics of thermal inactivation of cellulase GS-CX is important to allow their suitable utilization as biocatalysts in industry. The present study focus on the investigation of kinetic and thermodynamic parameters of cellulase (GS-CX) from salivary glands of *Macrotermes subhyalinus* little.

Materials and Methods
Enzyme sources
Little soldiers of the termite *Macrotermes subhyalinus* were from the savannah of Lamto (Abidjan, Côte d'Ivoire). They were collected directly from their nests and then stored frozen at -20°C. The cellulase substrate carboxymethylcellulose was obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). All other chemicals and reagents were of analytical grade.

Extraction and purification procedures
Salivary glands (10 g) were dissected and homogenized with 20 mL 0.9 % NaCl (w/v) solution using a blender (Ultra-Turrax) and then sonicated as previously described by Rouland *et al.* (1988a). The homogenate was centrifuged at 20,000 x g for 15 min. The collected supernatant constituted the crude extract. After freezing at -180°C in liquid nitrogen, the crude extract was stored at - 20°C (Kouamé *et al.*, 2005).

The purification procedure was carried out in the cold room (4°C). The crude extract of the termite salivary glands was loaded onto an anion-exchange chromatography using a DEAE-Sepharose Fast Flow column (2.5 cm x 4.5 cm), equilibrated with 20 mM sodium acetate buffer (pH 5.0). The column was washed at a flow rate of 90 mL/h with two bed volumes of equilibration buffer to remove unbound proteins. Bound proteins were then eluted with a stepwise salt gradient (0.1, 0.2, 0.4 and 2 M) of NaCl in 20 mM sodium acetate buffer (pH 5.0), and fractions of 2 mL were collected. One peak of enzyme possessing endo-xylanase/endo-cellulase activity was obtained and the active fractions were pooled. The pooled active fractions were loaded onto a cation-exchange chromatography using a CM-Sepharose CL-6B column (2.6 cm x 4.0 cm), equilibrated with 20 mM sodium acetate buffer (pH 5.0). The column was washed with the same buffer at a flow rate of 90 mL/h. Cellulase activity was eluted with a stepwise salt gradient (0.1, 0.2, 0.4 and 2 M) NaCl in 20 mM sodium acetate buffer (pH 5.0). Fractions of 2 mL were collected and, to the pooled active fractions, solid sodium thiosulphate was slowly added to give a final concentration of 1.7 M and the resulting enzyme solution was subsequently applied on a Phenyl Sepharose 6 Fast Flow column (1.5 cm x 3.2 cm) previously equilibrated with 20 mM sodium acetate buffer (pH 5.0) containing 1.7 M of sodium thiosulphate salt. The column was washed with a reverse stepwise gradient of sodium thiosulphate concentrations (from 1.7-0 M) dissolved in the same sodium acetate buffer at a flow rate of 78 mL/h and fractions of 1 mL were collected. The pooled active fractions were dialyzed overnight against 20 mM sodium acetate buffer (pH 5.0) and constituted the purified enzyme solution.

Enzyme assay and protein determination
Under the standard test conditions, cellulase activity was assayed spectrophotometrically by measuring the release of reducing sugars from Birchwood carboxyméthylcellulose (CMC). The reaction mixture (0.38 ml) contained 0.2 ml of 0.5% CMC (w/v) dissolved in 20 mM acetate buffer (pH 5.0) and 0.1 ml enzyme solution. After 30 min of incubation at 45°C, the reaction was terminated by adding 0.3 ml of dinitrosalicylic acid solution followed by 5 min incubation in a boiling water bath. The product was analysed by measuring the optical density at 540 nm. One unit (U) of enzyme activity was defined as the amount of enzyme capable of releasing one μmol of reducing sugar per min under the defined reaction conditions. Specific activity was expressed as units per mg of protein (U/mg of protein). Protein concentrations were determined spectrophotometrically at 660 nm by method of **Lowry *et al*. (1951)** using bovine serum albumin as a standard.

Thermal inactivation
Thermal inactivation of the enzyme was investigated at various constant temperatures from 50 to 65°C after exposure to each temperature for a period of 5 to 60 min. The enzyme was heated in sealed tubes, which was incubated in 100 mM sodium acetate buffer (pH 5.0) in a thermostatically controlled water

bath. Tubes were withdrawn at each time intervals and immediately immersed in an ice bath, in order to stop heat inactivation. 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

First-order kinetic has been reported to describe thermal inactivation of cellulase (GS-CX) (Guiavarc'h *et al.*, 2002). The integral effect of inactivation process at constant temperature, where the inactivation rate constant is independent of time, is given in Eq. 1:

$$Ln\ [A_t/A_0] = -kt \qquad (Eq.\ 1)$$

where;

A_t is the residual enzyme activity at time t (min),

A_0 is the initial enzyme activity,

k (min^{-1}) is the inactivation rate constant at a given condition.

k-values were obtained from the regression line of ln (At/Ao) versus time as slope.

D-value is defined as the time needed, 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; Espachs-Barroso *et al.*, 2006):

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

The Z-value (°C) is the temperature increase needed to induce a 10-fold reduction in D-value (Stumbo, 1973). This Z-value follows the Eq. 3:

$$Log\ [D_1/D_2] = [T_2-T_1]/Z_T \qquad (Eq.\ 3)$$

where,

T1 and T2 are the lower and higher temperatures in °C or K.

Then, D1 and D2 are D-values at the lower and higher temperatures in min, respectively.

The Z-values were determined from the linear regression of log (D) and temperature (T).

Thermodynamic Analysis

The Arrhenius equation is usually utilized to describe the temperature effect on the inactivation rate constants and the dependence is given by (Eq. 4 or 5):

$$k = Ae^{(-Ea/RT)} \qquad (Eq.\ 4)$$

Eq. 4 can be transformed to: ln k = ln A – Ea/R × T. (Eq. 5)

where,

k is the reaction rate constant value,

A the Arrhenius constant,

Ea (kJ.mol^{-1}) the activation energy,

R (8.31 J.mol^{-1}K^{-1}) the universal gas constant

T is the absolute temperature in Kelvin (K).

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 (Dogan *et al.*, 2000 and 2002).

The values of the activation energy (Ea) and Arrhenius constant (A) allowed the determination of different thermodynamic parameters (Marin *et al.*, 2003) such as variations in enthalpy, entropy and Gibbs free energy, ΔH, ΔS and ΔG, respectively, according to the following expressions (Galani and Owusu, 1997):

$$\Delta H^{\#} = Ea - RT \qquad (Eq.\ 6)$$
$$\Delta S^{\#} = R\ (lnA - ln\ K_B/h_P - ln\ T) \qquad (Eq.\ 7)$$
$$\Delta G^{\#} = \Delta H^{\#} - T\ \Delta S^{\#} \qquad (Eq.\ 8)$$

Where;

K_B is the Boltzmann constant (1.38 x 10^{-23} J/K),

h_P is the Planck constant (6.626 x 10^{-34} J.s),

T is the absolute temperature.

Statistical Analyses

All determinations reported in this study were carried out in triplicate. Results were expressed as means ± standard deviation.

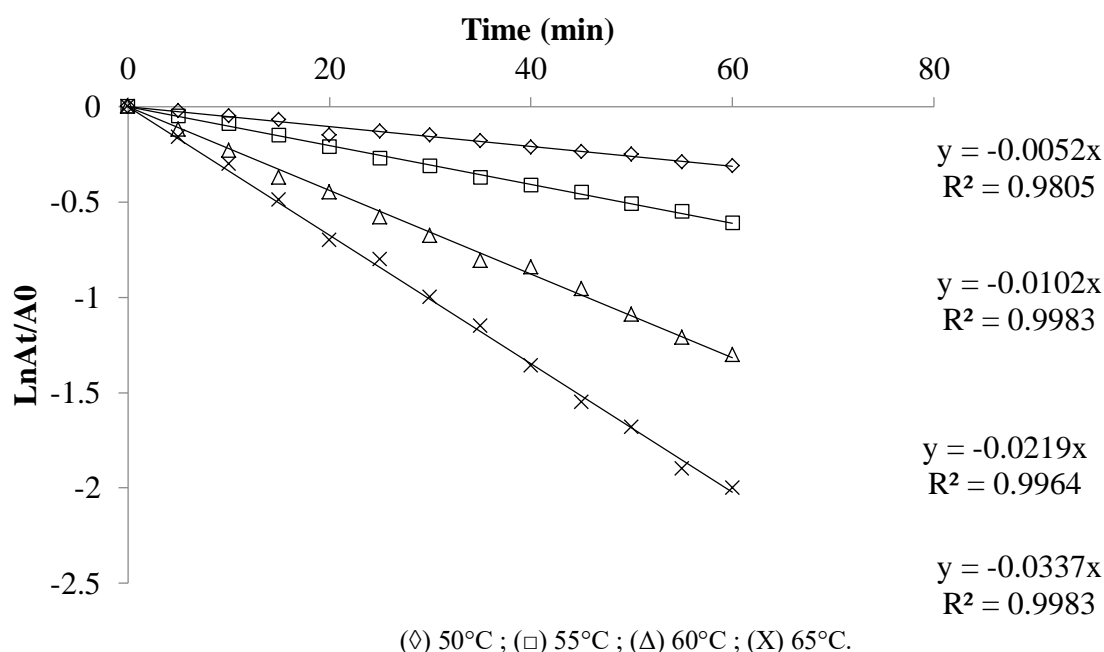## Results and Discussion

Kinetic Analysis of Thermal Denaturation

The optimum temperature of cellulase (GS-CX) purified from salivary glands of Macrotermes subhyalinus little soldier was 60 °C (Fagohoun *et al.*, 2014). In this study, thermal inactivation of Macrotermes subhyalinus little soldier GS-CX was screened at different temperatures ranged from 50 to 65 °C by determining the residual enzymatic activity (Table 1). The cellulase (GS-CX) activity was decreased with increasing heating time (5 - 60 min) and temperature (50 – 65 °C). Indeed, at temperatures between 50 and 65 °C, heat-denaturation of cellulase (GS-CX) occurred after 5 min of incubation (97.73 to 85.21 %). Although heating at 60°C for 30 min resulted in partial (50.76 %) inactivation. However, a strong inactivation of both enzyme activities was obtained after 60 min of heat treatment at 65°C (13.53 %). On one hand, the decrease of percentage residual activity at temperatures higher than 50 °C 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 (Tabatabai 1982). It has been noted that heat stability of the enzyme may be related to molecular forms of enzyme (Zhou and Feng 1991).

The logarithmic linear relationship between the cellulase activity and heat treatment time for the temperature ranged from 50 to 65 °C followed first-order kinetics (Figure 1). This result agrees with those reported for cellulase from fungi trichoderma

viride (Abdul, 2015) and in rice Grasshopper *hieroglyphus banian* (Haloi *et al*., 2012)

Table 1: Effect of treatment temperature and time on the inactivation of cellulase from salivary glands of *Macrotermes subhyalinus* little soldier.

Temperature (°C)	Relative activity (%) at each treatment time (min)											
	5*	10	15	20	25	30	35	40	45	50	55	60
50	97.73± 0.8	95.12± 1.9	93.24± 1.0	86.07± 0.9	87.80± 0.6	86 .07± 1.1	83.52± 0.0	80.65± 0.4	78.89± 0.1	77.88± 0.2	74.82± 0.4	73.34± 0.3
55	95.12± 0.6	91.39± 0.9	86.07± 0.4	81.06± 0.4	76.33± 0.2	76.33± 0.6	69.07± 0.3	66.36± 0.7	63.76± 0.7	60.04± 0.2	57.69± 0.7	54.33± 0.2
60	88.69± 0.7	79.45± 1.0	69.07± 0.4	63.76± 0.8	55.98± 1.0	50.76± 0.4	44.61± 0.4	43.07± 0.7	38.46± 1.1	33.62± 0.3	29.81± 0.5	27.25± 0.3
65	85.21± 0.8	74.08± 1.6	61.26± 0.2	49.65± 1.3	44.93± 0.5	36.78± 0.2	31.66± 0.2	25.66± 0.5	21.22± 1.0	18.64± 0.6	14.95± 0.9	13.53± 0.6

Figure 1: Thermal inactivation curves of cellulase (GS-CX) from salivary glands of *Macrotermes subhyalinus* little soldier in sodium acetate buffer (pH 5.0) in the temperature range 50-65 °C. A_0 is the initial enzymatic activity and A_t the activity at each holding time. Each data point is the mean of three determinations

$$y = -0.0052x \quad R^2 = 0.9805$$
$$y = -0.0102x \quad R^2 = 0.9983$$
$$y = -0.0219x \quad R^2 = 0.9964$$
$$y = -0.0337x \quad R^2 = 0.9983$$

(◊) 50°C ; (□) 55°C ; (Δ) 60°C ; (X) 65°C.

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 cellulase (GS-CX) from salivary glands of *Macrotermes subhyalinus* little soldier is less thermostable at higher temperatures (60-65 °C). The dependence of the k-values with temperature was adequately fitted by the Arrhenius equation ($R^2 = 0.990$) (Figure 2). This linearity is an indication that the inactivation in salivary glands GS-CX occurs through a unique mechanism dependent on temperature, such as protein unfolding (Gnangui *et al*., 2009; Waliszewski *et al*., 2009).
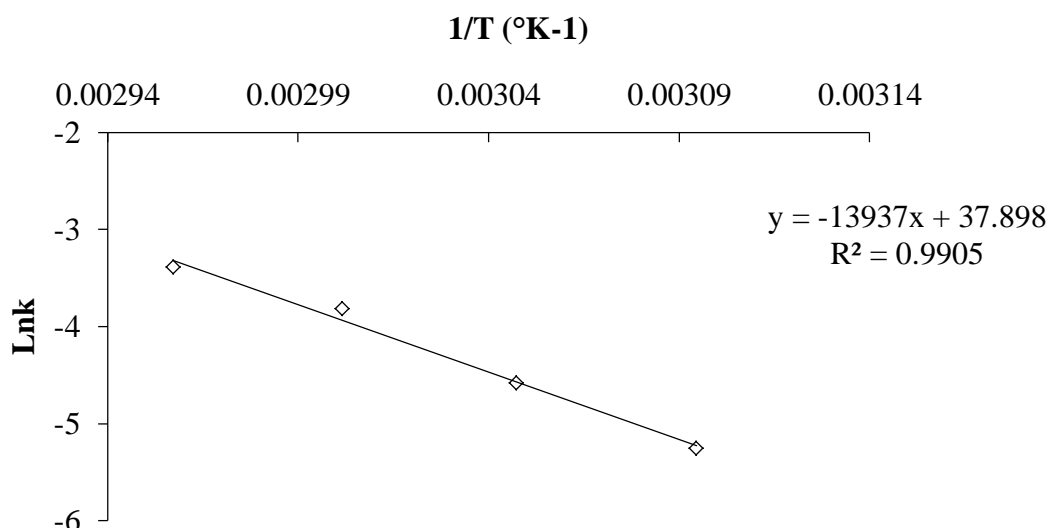
Figure 2: Arrhenius plot showing the effect of temperature on the rate constant for the thermal inactivation of cellulase (GS-CX) from salivary glands of *Macrotermes subhyalinus* little soldier. 1/T represents the reciprocal of the absolute temperature. Each data point is the mean of three determinations

The half-life ($t_{1/2}$) and the decimal reduction time (D-value) are other important parameters commonly used in the characterization of enzyme stability. Increasing the temperature from 50 to 65 °C resulted in a decrease in $t_{1/2}$ and D-values (Table 2). At the same temperature (65 °C), cellulase (GS-CX) from salivary glands of *Macrotermes subhyalinus* little soldier ($t_{1/2}$ = 20.56 min) was less thermostable than those of other vegetal sources such as exo-PG from pela *Linhagem Fungica Thermomucor indicae-seudaticae* (t1/2 = 58.3 min) (Martin, 2010) and exo-polygalacturonase (exo-PG) from Thermophilic Fungus *Rhizomucor pusillus* ($t_{1/2}$ = 96.3 min) (Trindade *et al*., 2016).

From 50 to 65 °C, the activation energy (Ea) value for thermal inactivation of cellulase (GS-CX) was calculated to be 115.81 kJ.mol^{-1} (Table 2). High

activation energy reflects a greater sensitivity of cellulase to temperature change

(Chutintrasri and Noommhorm 2006). This suggests that the denaturation process requires a high-energy input to the enzyme-substrate complex to initiate denaturation, probably due to a possible compact structure of enzymes and the strength of the thiol groups (SH) or disulfide bond at the active site (Bjorck and Farkye 1992). The Ea-value from salivary glands of *Macrotermes subhyalinus* little soldier cellulase was much higher than those reported for horseradish cellulase (30.1 kJ.mol-1) and glucose oxydase from horseradish (60.2 kJ.mol-1) (Giannakopoulou *et al*., 2019), but lower than that for Thermophilic Fungus *Rhizomucor pusillus* (125.5 kJ.mol-1) (Trindade *et al*., 2016), exo-PG from A. sojae (286.2 kJ.mol-1) (Dogan and Tari, 2008) and the endoglucanases from A. fumigatus obtained by submerged cultivation (154.7 kJ.mol-1) (Saqib *et al*., 2010). On this basis, cellulase in salivary glands of Macrotermes subhyalinus little soldier can be classified as heat-sensitive cellulase in terms of inactivation kinetics.

Table 2: k, D-, $t_{1/2}$, Z- and Ea-values for thermal inactivation of cellulase from salivary glands of *Macrotermes subhyalinus* little soldier. in the 50-65 °C temperature range.

Temperature (°C)	Kinetic parameters					
	K (min^{-1})		D (min)	$t_{1/2}$ (min)	Z (°C)	Ea (kJ/mol)
	Value	R^2				
50	0.0052 ± 0.001[a]	0.980	442.88 ± 25	133.27 ± 8		
55	0.0102 ± 0.002	0.998	225.78 ± 17	67.94 ± 5	18.08 ± 0.1	115.81 ± 0.16
60	0.0219 ± 0.001	0.996	105.15 ± 15	31.64 ± 3		
65	0.0337 ± 0.001	0.998	68.33 ± 17	20.56 ± 5		

(Figure 3) presents the relationship between decimal reduction time and temperature, where the slope of the curve represents -1/Z$_T$. The estimated value of

Macrotermes subhyalinus little soldier cellulase was 18.08 °C (R^2 = 0.9885). The Z-values for cooking

and nutrients degradation (25-45°C) are generally greater than microbial inactivation (7-12°C) (Awuah *et al*., 2007). In fact, differences between the D- and Z-values of enzyme and nutrients are exploited to optimize thermal processes and can be exploited also to maintain carboxymethylcellulase activity after treatment. This indicates that any change in temperature processing affects more intensely the stability of cellulase (GS-CX). In this study, D-, Z- and k-values indicate that carboxylmethylcellulase is heat stable and then can be used in high temperature short time and low temperature long time industrial processes such as pasteurization, where values of 65°C for 3-5 min and 55°C for 30 min, respectively, are generally considered. In fact, the D- and Z-values of carboxylmethylcellulase is exploited to optimize thermal processes and to preserve enzyme activity after treatment. According to Barrett *et al*. (1999), high Z-values indicate more sensitivity to the heat treatment time and low Z-values indicate more sensitivity to increasing temperature.
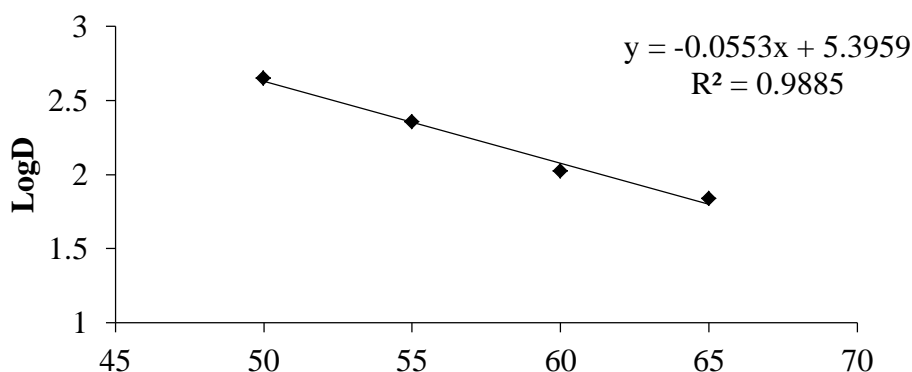

$$y = -0.0553x + 5.3959$$
$$R^2 = 0.9885$$

Figure 3: Variation of decimal reduction times with temperature for of cellulase (GS-CX) from salivary glands of *Macrotermes subhyalinus* little soldier. Each data point is the mean of three determinations

Thermodynamic Analysis of Thermal Denaturation

The thermodynamic parameters provide 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 (Longo and Combes 1999). These parameters include the Gibbs free energy ($\Delta G^{\#}$) change considered as the energy barrier for enzyme inactivation, the enthalpy ($\Delta H^{\#}$) change measuring of the number of bonds broken during inactivation, and the entropy ($\Delta S^{\#}$) change that indicates the net enzyme and solvent disorder. In this study, they were calculated in the temperature range from 50 to 65 °C (Table 3).

Within the error range of our measurements, results showed that the enthalpy ($\Delta H^{\#}$) is independent of temperature; thus, there is no change in enzyme heat capacity. The $\Delta H^{\#}$ value of cellulase (89.94 kJ.mol^{-1}) was highly than those reported for other cellulase: 23 kJ.mol-1 for horseradish (Giannakopoulou *et al*., 2019) and 13.4 kJ.mol-1 for fungi trichoderma viride (Abdul, 2015). The high $\Delta H^{\#}$ change obtained indicates that cellulase of Macrotermes subhyalinus little soldier undergoes a significant conformational change in order to find in its activated during heat treatment and high amount of energy was required to initiate denaturation, probably due to the molecular conformation of cellulase (GS-CX) (Marin *et al*., 2003). Positive $\Delta H^{\#}$ values indicate the endothermic nature of the hydrolysis reaction. Our results would therefore suggest that cellulase from salivary glands of Macrotermes subhyalinus little soldier is more structurally robust than the others enzymes.

In contrast, the Gibbs free energy ($\Delta G^{\#}$) value is directly related to protein stability: the higher the ΔG is, the higher will be the enzyme stability. Results showed that the $\Delta G^{\#}$ values the cellulase (GS-CX) slightly increased from 109.36 kJ.mol^{-1} to 110.27 kJ.mol^{-1}, when the incubation temperature was elevated (50 - 65 °C) indicating that the inactivation processes were not spontaneous. Since $\Delta G^{\#}$ decreases with increasing temperature whereas $\Delta H^{\#}$ is overall constant, one could expect a significant contribution of entropy changes to the thermodynamics of the considered system. Indeed, it was already demonstrated that activation entropy has a dominant role in thermal inactivation of proteins in aqueous solutions (Bromberg *et al*., 2008).

According to *Anema and Mckenna (1996)*, the positive values of entropy ($\Delta S^{\#}$) for the hydrolysis reaction of carboxymethylcellulose indicate that the reaction proceeds with less speed and is characterized by low regularity. Small changes in the values of $\Delta S^{\#}$ indicates a preferential destruction of weak bonds (hydrogen and electrostatic), resulting in a lower loss of catalytic activity. The positive values for change in $\Delta S^{\#}$ also indicate that there are no significant processes of aggregation for carboxylmethylcellulase.

Furthermore, the high values obtained for $\Delta S^{\#}$ variation probably reflect an increased disorder of the active site or the structure of cellulase, which is the main driving force of heat denaturation (D'amico *et al*., 2003). The cellulase GS-CX from salivary glands of Macrotermes subhyalinus little soldier studied here therefore involves a structurally robust but temperature-sensitive enzymatic system, whose thermal denaturation is mainly under entropic control.

Table 3: Thermodynamic parameters for the Thermal Inactivation of cellulase from salivary glands of *Macrotermes subhyalinus* little soldier at different Temperatures

Temperature (°C)	Thermodynamic parameters		
	$\Delta H^{\#}$ (kJ/mol)	$\Delta S^{\#}$ (J mol^{-1} K^{-1})	$\Delta G^{\#}$ (kJ/mol)
50	113.13 ± 0.6[a]	126.23 ± 1.6	72.33 ± 0.3
55	113.08 ± 0.5	126.10 ± 1.7	71.70 ± 0.4
60	113.04 ± 0.6	125.98 ± 0.3	71.07 ± 0.1
65	113.00 ± 0.4	125.85 ± 0.6	70.44 ± 0.1
Mean	113.06 ± 0.5	126.04 ± 0.4	71.39 ± 0.3

[a] Mean (±SD) for triplicate experiments

Conclusion
The results of this study reveals that thermal inactivation of cellulase (GS-CX) from salivary glands of *Macrotermes subhyalinus* little soldier could be described by a first-order kinetic model. The D-, Z-, k-values, indicate that GS-CX is heat stable and then could be utilized in pasteurization conditions, maintaining part of their biological activity. The high values obtained for activation energy (Ea) and change in enthalpy (ΔH#) indicated that a high amount of energy was needed to initiate denaturation of this carboxylmethylcellulase, most likely due to its stable molecular conformation. Therefore, cellulase involves a structurally robust but temperature-sensitive enzymatic system, whose thermal denaturation is mainly under entropic control.

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