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Kinetic Modelling of Thermal Inactivation of a Keratinase from *Purpureocillium lilacinum* LPSC # 876 and the Influence of Some additives on Its Thermal Stability

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Abstract Thermal inactivation of a keratinase produced by *Purpureocillium lilacinum* LPSC #876 was kinetically investigated using several enzyme inactivation models at the temperature range of 50–65 °C. Among the models studied, the Weibull distribution was the best model that describes the residual activity of *P. lilacinum* keratinase after heat treatment over the selected temperatures. The stabilising effect of metal ions (Ca^{2+} or Mg^{2+}, 5 mmol l^{-1}) or polyols (propylene glycol and glycerol, 10 % v/v) was investigated, showing that the presence of Ca^{2+} increases the enzyme stability significantly. Conforming to the increased Ca^{2+} concentration, thermal stability of the enzyme also increased, with 10 mM of Ca^{2+} being the concentration of metal in which the enzyme retained 100 % of its original activity after being incubated for 1 h at 55 °C. The effects of temperature on Weibull equation parameters and on the characteristics of the inactivation curves were evaluated. In the absence of any additives (control), the reliable time (t_R) of the keratinase, analogous to D value, ranged from 484.16 to 63.67 min, while in the presence of Ca^{2+} the t_R values ranged from 6,221 to 414.95 min at 50–65 °C. *P. lilacinum* keratinase is a potentially useful biocatalyst, and therefore, kinetic modelling of thermal inactivation addresses an important topic for its application in various industrial processes.

Keywords Keratinase · Thermal stability · Kinetic modelling · Weibull distribution · Calcium and polyols

Introduction

Keratinases are a special type of proteases which have the ability to attack “hard-to-degrade”, insoluble keratin substrates. They are robust enzymes displaying a great diversity in their

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biochemical characteristics. They differ from conventional proteases because of their broad substrate specificity towards a variety of insoluble, keratin-rich substrates including feather, wool, nail and hair (1).

Keratinases from several microorganisms have been extensively studied and biochemical properties like pH and temperature optima, molecular mass, catalytic type, and their stability in the presence of various detergents and organic solvents have been described in detail in earlier reports (2–4). As mentioned above, besides these properties, the most important which demarcates them from other proteases is their substrate specificity. Owing to this ability, keratinases find immense applications in various environmental and biotechnological sectors where conventional proteases lag behind. Increasing interest has been focused on their application for the bioconversion of keratin-rich wastes, producing protein hydrolysates for utilization as nitrogen fertilizers and animal feed; also, they are investigated in prion degradation, as a component in detergent and feed formulations and as dehairing agent in leather industry (5–7).

Enzyme activity and stability are topics directly linked with enzyme applications. Various phenomena could lead to enzyme inactivation including autolysis, aggregation, coagulation, denaturation due to exposure to solvents, surfactants, salts and extremes of temperatures and pH (8, 9). Enzyme stabilization techniques have been investigated to counteract these deleterious mechanisms, providing biocatalysts with higher efficiency, increasing the biotechnological and economical potential of enzyme-based processes (10). This is why predicting enzyme inactivation is essential for enzyme characterization from both scientific and technological perspectives. Mathematical models, consisting of equations that provide an output based on a set input data, represent a powerful and concise way to express physical behavior in mathematical terms (11). Therefore, adequate kinetic models and thermodynamic studies may enable a better knowledge of the enzyme behavior during inactivation.

Purpureocillium lilacinum LPSC #876, a keratin-degrading fungus isolated from La Plata alkaline forest soils, is reported to produce extracellular proteases with biotechnological potential (12). A keratinolytic protease, produced by this fungus during submerged cultivation on hair waste medium was purified from cultured supernatants. In order to characterize *P. lilacinum*'s keratinase, the present work evaluates the thermal stability data obtained when the enzyme was submitted to a heat treatment in a temperature range between 50 °C and 65 °C. Various mathematical models proposed to predict enzyme residual activity as a function of time were statistically analyzed and the kinetic model that better explains the thermal inactivation was selected. The effect of metal ions (Ca^{2+} or Mg^{2+}) or polyols (propylene glycol and glycerol) on thermal stability of the enzyme was also investigated.

Materials and Methods

Microorganism, Keratinase Production and Purification

For keratinase production, *P. lilacinum* (formerly *Paecilomyces lilacinus*) LPSC #876 belonging to Spegazzini Institute fungal culture collection (La Plata National University, Argentina) was used. It was cultivated in a hair waste medium for 110 h at 28 °C and 200 rpm (12). After 110 h of cultivation, all the contents of each flask were withdrawn and centrifuged at 5,000 × *g* and 4 °C for 20 min in order to precipitate the fungal biomass. The cell-free supernatant was submitted to a purification protocol comprising (1) keratinase concentration with ammonium sulphate (0–85 % saturation), (2) gel-filtration chromatography in a Sephadex G-25 column (General Electric, Little Chalfont, UK), (3) anion-exchange chromatography in a DEAE-

Sephadex column followed by cation-exchange chromatography in a Sp-Sepharose-FF column (General Electric) and (4) gel-filtration chromatography in a Superdex-75 column. All chromatographic steps were carried out on an Amersham FPLC-U900 system (General Electric). After each purification step, keratinolytic and proteolytic activities were both measured as described below, with the latter activity determination being used for enzyme characterization (13).

Assay of Enzyme Activity

Keratinolytic activity was determined as described elsewhere (12) using azokeratin as substrate. Substrate solution containing 30 mg of azokeratin and 0.8 ml of 0.1 M Tris–HCl buffer (pH 9.0) was stirred for 15 min until azokeratin was completely suspended. An appropriate dilution of the enzyme preparation (0.1 ml) was added and the admixture was incubated for 25 min at 37 °C. The reaction was stopped by the addition of 0.2 ml of trichloroacetic acid (10 %, w/v) and centrifuged (5,000×*g*, 10 min). The absorbance of the supernatant was measured at 440 nm. One unit of keratinolytic activity (U_K) was defined as the amount of enzyme that, under the above mentioned reaction conditions, causes an increase of 0.01 units in the absorbance at 440 nm per minute.

Proteolytic activity was measured with azocasein as substrate as described elsewhere (12). An aliquot of 0.1 ml of the enzyme preparation, suitably diluted, was mixed with 0.250 ml of Tris–HCl buffer (100 mM, pH 9.0) containing 1 % (w/v) azocasein, and incubated for 30 min at 37 °C. The reaction was stopped by the addition of 1.0 ml of trichloroacetic acid (10 %, w/v). After a further 15 min at room temperature, the admixture was centrifuged at 5,000×*g* for 10 min. A reaction blank was performed with 0.1 ml of heat-inactivated enzyme. One millilitre of 1 M NaOH was then added to 1 ml of the supernatant and the absorbance measured at 440 nm. One unit of protease activity was defined as an increase of 0.1 unit per minute in the absorbance at 440 nm under those experimental conditions. Azocasein and azokeratin were synthesized as described by Riffel and Brandelli (14).

Heat Inactivation Experiments — Effect of Metal Ions and Polyols on Enzyme Thermostability

Kinetics of thermal inactivation of *P. lilacinum* keratinase was determined by incubating the enzyme in the absence (control) or presence of metal ions (Ca^{2+} or Mg^{2+}, 5 mmol l^{-1} final concentration) or polyols (propylene glycol and glycerol, 10 % v/v final concentration) at 50, 55 °C, 60 °C or 65 °C. Sealed tubes containing 1 ml of the enzyme with and without additives were immersed and heated in a thermostatically controlled water bath at temperatures ranging from 50 °C to 65 °C. Samples of each tube were withdrawing at fixed time intervals and the residual activity was determined as described earlier, with azocasein as substrate. The enzyme activity after 1 min of heating-up time was considered to be the initial activity (A_0), eliminating the effects of heating up. Assays were done in triplicate.

Effects of Calcium, Propylene Glycol and Glycerol on Enzyme Activity at Different Concentrations

The effects of Ca^{2+}, propylene glycol and glycerol were tested by adding different concentrations of this additives to the enzyme (0–10 mM final concentration for Ca^{2+} and 0–50 % w/v in the case of polyols). The admixtures were incubated for 1 h at 55 °C and the residual activity

was measured under standard assay conditions as it was described above. Experiments were performed at least in triplicate and data were expressed as means±standard deviations.

Kinetic Models of Enzyme Inactivation

Considering the complexity of the structure of an enzyme and the variety of different phenomena involved in the inactivation, Lencki et al. (8) proposed that inactivation phenomena proceeds through a network of reactions including dissociation (for multimeric enzymes), denaturation, aggregation, coagulation and chemical decomposition. Due to the complexity of reaction that may happen in the molecule during heat inactivation, several inactivation equations have been proposed to model this kinetics behaviour.

In this work several kinetics models based on different thermal inactivation mechanisms were tested (Table 1). These models cover first-order, parallel reactions, and consecutive reactions. In the equations, A/A_0 represents the residual proteolytic activity at time t (min), and k (min^{-1}) is the reaction rate constant at a given temperature.

First-order kinetics is one of the most used models employed to describe thermal inactivation and have been reported to model heat degradation of several enzymes (15–17) (Eq. 1). The Weibull distribution model (Eq. 2) is based on the assumption that, under the conditions examined, the momentary rate of thermal sensitivity to heat is only a factor of the transient heating intensity and residual activity, but not of the rate at which the residual activity has been reached (18). This model is characterized by two parameters: n and b. The parameter n determines the shape of the distribution curve, whereas b emulates the thermal reaction rate (19). On the other hand, parallel models (Eqs. 3–5) indicate the existence of a mixture of enzymes with different heat sensitivities and/or catalytic properties, suggesting that residual activity could be described by the summation of two exponential decays. In the distinct isoenzymes model (Eq. 3) and in Two-fraction model, residual activities for the "labile" and "stable" isoenzymes are represented by A_L and A_S, respectively. k_L and k_R are the correspondent first-order reaction rate constant for each fraction, respectively (20). Finally, fractional conversion model (Eq. 5) refers to a first-order inactivation process, and takes into account of the non-zero enzyme activity upon prolonged heating because of the presence of an extremely heat-resistant enzyme fraction (A_r) (21).

Comparison of Kinetics Models and Statistical Treatment

Residual proteolytic activities obtained as describes in the section "Heat Inactivation Experiments" were fitted according to the models presented in section "Kinetic Models of Enzyme Inactivation" using nonlinear regression by Statistica 7.0 (StatSoft Inc., Tulsa, OK, USA).

Table 1 Kinetic models used to analyze thermal inactivation of *P. lilacinum* keratinase

Kinetic model	Equation (no.)
First-order	$A/A_0=\exp(-kt)$ (1)
Weibull distribution	$A/A_0=\exp(-bt^n)$ (2)
Distinct isoenzymes	$A/A_0=A_L \exp(-k_L t)+A_S \exp(-k_R t)$ (3)
Two-fraction	$A/A_0=a\times \exp(-k_L t) + (1-a) \times \exp(-k_R t)$ (4)
Fractional conversion	$A/A_0 = A_r + (A_0 - A_r) \times \exp(-kt)$ (5)

Coefficient of correlation (r^2), chi-square (χ^2) and standard error of means (SEM) were the statistical criteria for comparison of kinetic models (19, 22).

χ^2 is given by Eq. 6:

$$\chi^2 = \frac{\sum \left(a_{\text{measured}} - a_{\text{predicted}}\right)^2}{(m-p)} \tag{6}$$

while the error of means (SEM) is given by Eq. (7):

$$\text{SEM} = \frac{\sum \left(a_{\text{measured}} - a_{\text{predicted}}\right)^2}{\sqrt{m}}, \tag{7}$$

where m is the number of observations and p is the number of parameters. Estimation of negative kinetic parameter at a given temperature is a physical criterion for rejection of an inactivation model. The model with the lowest χ^2 and SEM, and higher r^2 for the residual proteolytic activity is the best choice from a statistical point of view (22).

Results and Discussion

A keratinolytic serine protease secreted by *P. lilacinum* LPSC # 876 growing on a minimal basal medium containing 1 % (w/v) hair waste as carbon and nitrogen source was purified as described elsewhere with an overall purification factor of 19.8-fold with a final yield of 1.3 % (Table S1). The molecular mass of the purified keratinase determined by SDS-PAGE was 37 kDa (Fig. S1). Its biochemical features such as pH and temperature stability, stability in the presence of detergents and organic solvents, suggest the suitability of this enzyme as a valuable biocatalyst for industrial processes (13). The operational stability of this kind of catalysts is one of the most important characteristics and high stability is always looked for to ensure the economical and technical feasibility of the industrial process. Therefore, as an essential stage for enzyme characterization, kinetic modelling of thermal inactivation as well as the improvement of the kinetic thermal stability of *P. lilacinum* protease was investigated. Several studies dealing with mathematical modelling of kinetic thermal inactivation of enzymes just assume first-order kinetics from semi logarithmic plots of activity versus time (15, 23–25). The choice of the best equation for process modelling is essential from both engineering and economical points of view, minimising errors and improving the effectiveness of the process that finally results in lower costs. Several mathematical models have been proposed to explain thermal inactivation of biocompounds (11). In this work, five inactivation kinetic models, listed in Table 1, were tested in order to fit the experimental data obtained from heat treatments of *P. lilacinum* keratinase.

When first-order inactivation kinetics model was tested for its applicability to the thermal inactivation data, it did not yield good r^2 values at all temperatures tested (Table 2), and neither did other models like distinct isoenzymes, two-fraction and fractional conversion models — all models based on the presence of two isoenzyme populations inactivating by first-order mechanism. Some of them could not give a good fit at all the temperatures and were not physically feasible as negative parameter values were obtained. Therefore, it may be concluded that none of the models based on preconceived mechanism could adequately explain thermal inactivation pattern of *P. lilacinum* keratinase. When Weibull distribution was tested, it was observed that it yielded a better fit of experimental data (Table 2). The low SEM and χ^2 values and the high r^2 value indicate that the Weibull distribution model satisfactorily described the

Table 2 Performance of kinetic models to describe the thermal inactivation of *P. lilacinum* keratinase

Model (eq.)	r^2	χ^2	SEM	Remarks
First-order	[0.893; 0.981]	[0.0025; 0.0092]	[0.0051; 0.0227]	Rejected: low r^2 and high SEM and χ^2
Weibull distribution	[0.987; 0.999]	[4.8E−5; 0.00041]	[0.0001; 0.0013]	Accepted: higher r^2 and lower SEM and χ^2
Distinct isoenzymes	[0.921; 0.981]	[0.0024; 0.0133]	[0.0034; 0.0189]	Rejected: negative parameters estimates
Two-fraction	[0.914; 0.998]	[0.0002; 0.0073]	[0.00041; 0.012]	Rejected: negative parameters estimates
Fractional conversion	[0.956; 0.991]	[0.0012; 0.0022]	[0.0021; 0.0039]	Rejected: low $r2$ and high SEM and χ^2

kinetics of *P. lilacinum* keratinase inactivation in the range of temperatures tested. When the experimental data were fitting to the tendency curves estimated by first-order and by Weibull model, respectively, it could be seen that the data fitted better to the last model than to first-order model (Fig. S2). Moreover, in Fig. 1a there is a linear behaviour between experimental data and values calculated by the Weibull distribution that demonstrates the high quality of the predicted values by the equation. Weibull pattern has been scarce used to model thermal inactivation of enzymes: it was used to model thermal inactivation of a peroxidase (26), a pectin methyl esterase (27) and a protease (22); and it seems to be a helpful alternative to the

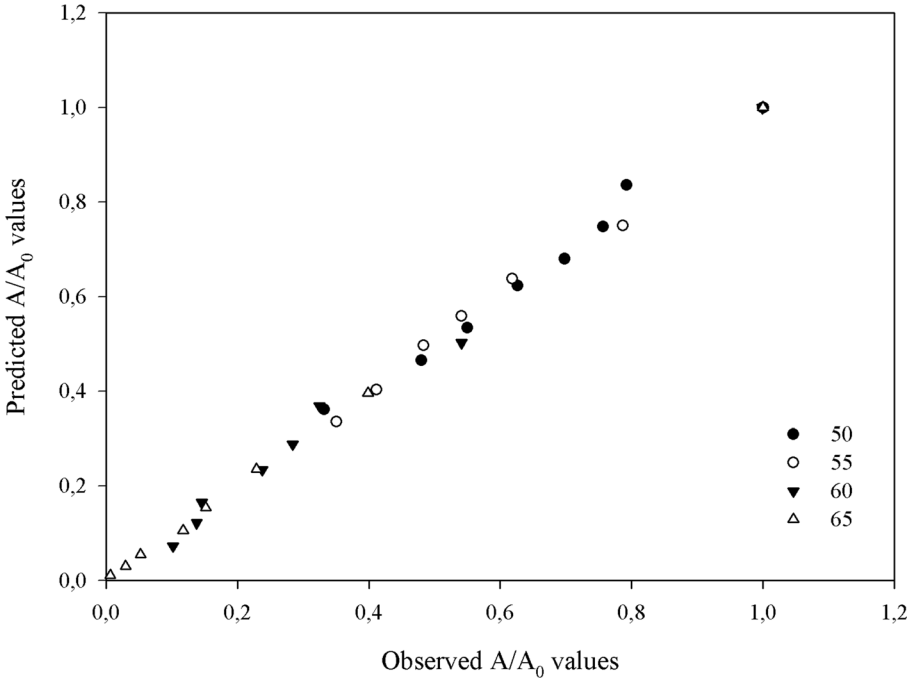

Fig. 1 Observed and predicted values of residual proteolytic activity of *P. lilacinum* keratinase at temperature range 50–65 °C

usual kinetics equations used to model thermal inactivation like first-order, two fractions or distinct isoenzymes.

Weibull distribution is characterized by two parameters b and n. The parameter n determines the shape of the distribution curve while b determines its scaling. Consequently, b and n are called the scale and shape factors, respectively. The Weibull rate parameter b is characteristic of each reaction and emulates thermal reaction rate. The exact nature of the underlying distribution is usually unknown and cannot be assumed a priori (28). A Weibull distribution with $n>1$ indicates that the semi-logarithmic inactivation curve has a downward concavity, $n<1$ indicates an upward concavity and we have an exponential distribution when $n=1$ (29). The values for the shape factor n obtained at different temperatures (50–65 °C) are presented in Table 3, it can be seen at in all cases n values obtained for *P. lilacinum* keratinase are less than 1 and indicate the "tailing" phenomena, suggesting that enzymes molecules showed different inactivation susceptibilities during heat treatment (22). Moreover, Fig. 2 shows how the n depends with temperature, inferring that in this case the value of the shape parameter (n) is independent on the treatment temperature. This observation is in agreement with Fernández et al. (30) data concerning the influence of heating temperature and medium pH on the n values for *Bacillus* spores. van Boekel (29) reported a study on the influence of heating temperature on the shape (n) and scale (b) factors for inactivation kinetics of different vegetative bacteria and yeast species survival kinetics. In most cases, the shape parameter was clearly independent of heating temperature, however, in some, dependencies appear significant. Here, the results of a one-way ANOVA confirmed that n values obtained from different temperature treatments were not statistically different, yielding a mean value of 0.6265±0.068. As indicated by Cunha et al. (31), van Boekel (29), Fernández et al. (30) and Couvert et al. (32) the parameter n should be consider as a behavior index, indicating the kinetic pattern of the mechanism controlling the process studied, and therefore, it should be independent of external factors like temperature, pH and water activity. Considering the shape parameter constant and equal to the mean value 0.6265, a new set of b parameters was re-estimated (Table 3).

As it was mentioned above, parameter b reflects the thermal reaction rate. It can be seen in Table 2 that b values increased as temperature increased, ranging between 0.0371 and 0.1715 min^{-1} in the temperature interval of 50–65 °C. The dependence of the parameter b with temperature can be expressed algebraically by log-logistic equation (19). This equation is described by

$$b(T) = \ln(1 + \exp[k'(T - Tc)]), \tag{8}$$

Table 3 Kinetic parameter value n for thermal inactivation of *P. lilacinum* keratinase and estimation of the parameter b obtained by fitting the Weibull distribution to the experimental data, keeping n constant (0.6562)

Temperature (°C)	r^2	n
50	0.9875	0.699
55	0.9918	0.653
60	0.9922	0.537
65	0.9996	0.617
	r^2	b (with n=0.6265)
50	0.9830	0.0371
55	0.9918	0.0538
60	0.9922	0.1145
65	0.9996	0.1715

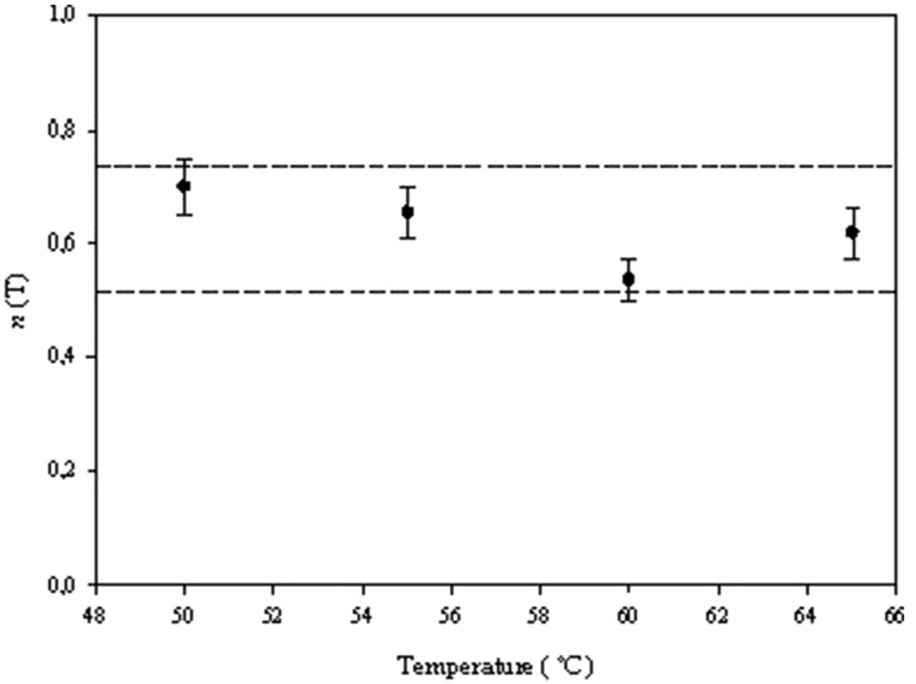

Fig. 2 Graph of the shape parameter n±95 % confidence intervals as function of temperature for inactivation of *P. lilacinum* keratinase

where T_c is a marker of the temperature level where the inactivation occurs at a significant rate, and k' is the steepness of the b(T) increase once this temperature has been exceeded (19). High k' values and low T_c may indicate that the biocompound is more susceptible to thermal degradation (33). Figure 3 shows how b (T) fits to a log-logistic equation. The temperature where the inactivation is accelerated (T_c) was estimated and resulted to be 80.23 °C, this temperature is higher than the T_c reported by San'tAnna et al. (22) for protease P7 (T_c= 70.358 °C), the k' was 0.107 °C^{-1}. According to this data, *P. lilacinum* keratinase can be considered stable in the temperature interval evaluated, and can be employed at a process that works with temperatures up to 75 °C.

For the time needed to reduce the enzyme activity by a factor of 10 (analogous to D value), one could use the concept of reliable life (t_R), which is in fact the 90 % percentile of the failure time distribution. In the case of enzymes, the term "failure" corresponds to enzyme inactivation and is calculated as follows:

$$t_R = \left[\frac{2.303}{b}\right]^{1/n} \tag{9}$$

In order to study the effects of different additives on *P. lilacinum* keratinase thermal stability, t_R values were calculated in the absence or presence of this compounds. Assuming that any factor which increases enzyme heat stability will increase t_R, it can be inferred from Table 4 and Fig. 4, that at all temperatures tested, enzyme stability was highly increased in the presence of Ca^{2+}, followed by propylene glycol and glycerol; meanwhile, Mg^{2+} has no effect on enzyme stability and at some temperatures (50 °C and 60 °C) this metal ion even decrease

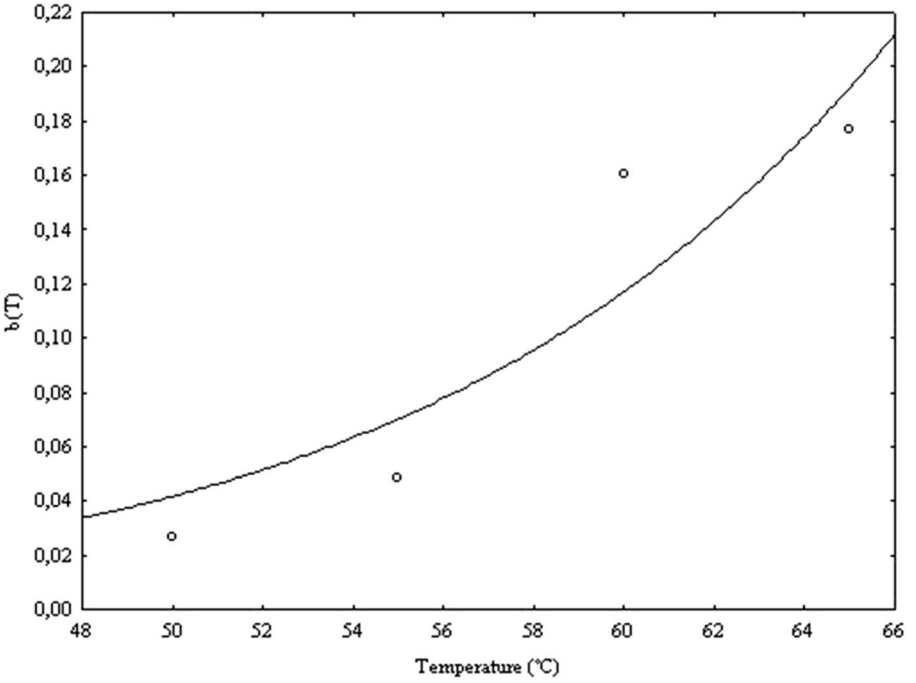

Fig. 3 Temperature dependence of Weibull distribution parameters. The b values were fitted to log-logistic equation. This equation was determined as $b(T)=\ln(1+\exp[0.107(T-79.482)]$ ($r^2=0.8404$)

it. Although the increased thermostability of proteases in the presence of calcium ions and polyols is described elsewhere, the few studies dealing with the mathematical modelling of kinetic thermal inactivation of enzymes in the presence of this additives, just assume first-order kinetics from semi-logarithmic plots of activity versus time (23, 24, 34). Here, Weibull distribution was used to model thermal inactivation of *P. lilacinum* keratinase in the presence of metal ions or polyols. It was found that b values decreased when the additive has a protective effect on enzyme thermal denaturation as well as t_R increased (Table 3). In the absence of any additives (control), the t_R values ranged from 484.16 to 63.67 min in the temperature range of 50–65 °C, while in the presence of Ca^{2+} the values ranged from 6,221 to 414.95 min. San'tAnna et al. (22) reported t_R values ranging from 164.41 to 8.287 min in the same range of temperatures for the protease P7 from *Bacillus* sp. without additives. These values of t_R are lower than those reported here, inferring that *P. lilacinum* keratinase is more stable than protease P7.

By plotting t_R values on a log scale against the corresponding temperatures, an exponential relationship is observed ($r^2=0.949$) (Fig. S3). van Boekel (29) defined a z' value for Weibull distribution (analogous to the classical z value). For *P. lilacinum* keratinase, the z' value was estimated in 15.9 min while for *Bacillus* sp. protease P7 was 8.81 °C, meaning that keratinase from *P. lilacinum* turned out to be more robust than the one reported by San'tAnna et al. (22).

In order to study the effect of the concentration on enzyme activity of those additives which exerts thermo protection, calcium, propylene glycol and glycerol were chosen and added at different concentrations to the enzyme. After 1 h of incubation at 55 °C, residual protease activity of the admixtures was measured under standard assay conditions. Ca^{2+} concentrations were varied between 0 and 15 mM while the concentration of the polyols varied between 0 %

Table 4 Kinetic parameter values for thermal inactivation of *P. lilacinum* keratinase in the presence of additives

	T (°C)	b (min^{-1})	t_R (min)
Control	50	0.0269	484.2
	55	0.0483	368.7
	60	0.1603	112.5
	65	0.1770	63.7
Propylene glycol	50	0.0197	671.7
	55	0.0269	1,257.6
	60	0.2195	284.2
	65	0.1220	84.4
Glycerol	50	0.0218	626.9
	55	0.0104	546.5
	60	0.1589	153.5
	65	0.1432	70.7
Ca^{2+}	50	0.0139	6,221.1
	55	0.0111	3,217.5
	60	0.0424	667.8
	65	0.0425	414.9
Mg^{2+}	50	0.0229	387.7
	55	0.0417	458.1
	60	0.4665	71.6
	65	0.1698	70.0

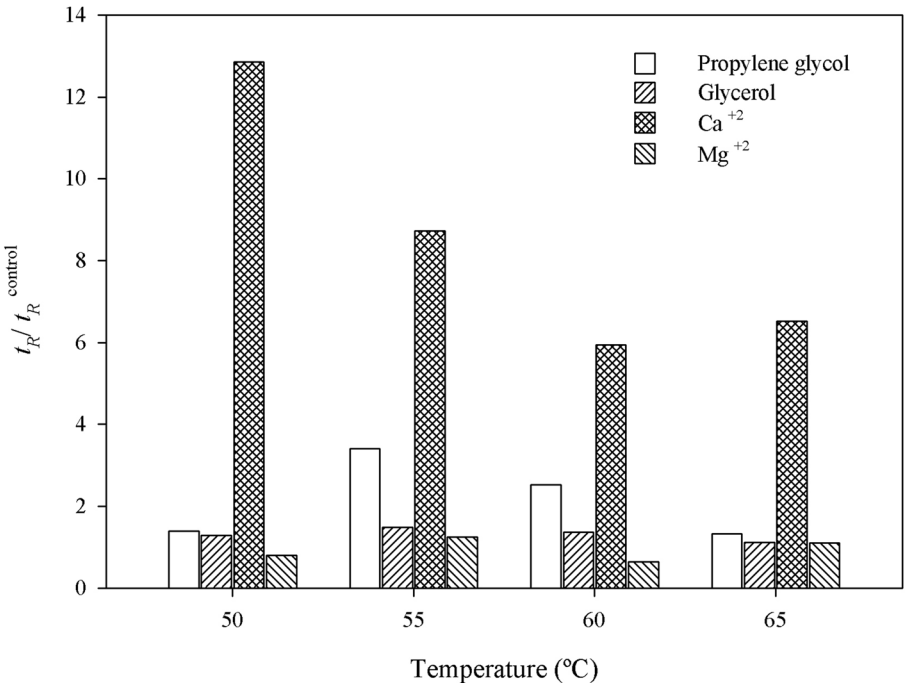

Fig. 4 Relative reliable life ($t_R/t_R^{control}$) in the presence of additives at different temperatures

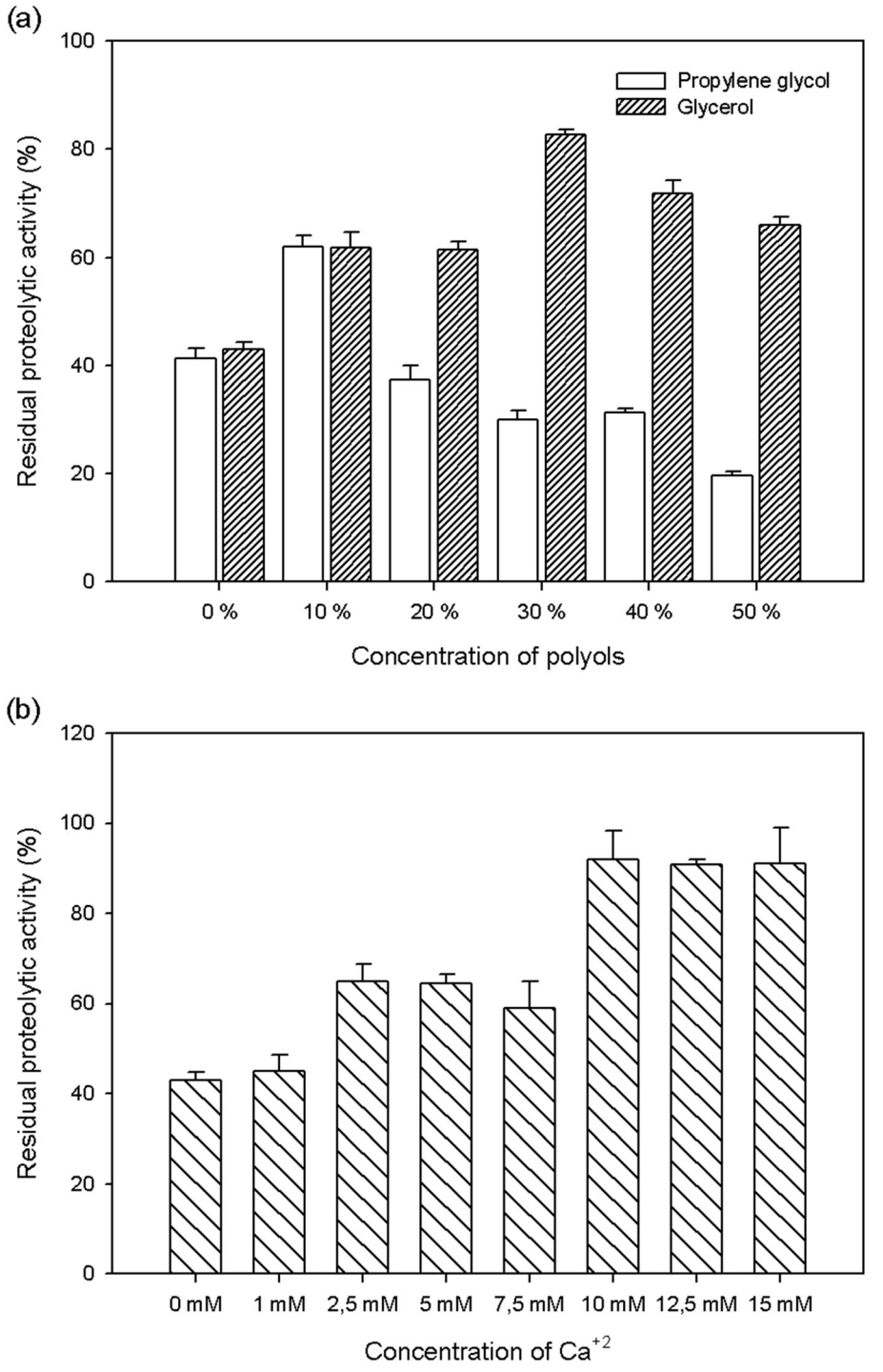

Fig. 5 Effect of different concentration of polyols (**a**) and Ca^{2+} (**b**) on enzyme stability at 55 °C

and 50 % v/v. After 1 h of incubation, the enzyme without any additives retained around 45 % of its proteolytic activity. When calcium and glycerol was used as protective agent, it was observed that the protective effect increased as their concentration increased, reaching a maximum residual activity at a particular concentration of 10 mM and 30 % v/v, respectively. For calcium concentration, it was observed that at values from 10 mM hereinafter, the enzyme retained 100 % of its activity, whereas in the case of glycerol and propylene glycol, at concentrations higher than 30 % v/v and 10 % v/v, respectively, the residual proteolytic activity began to decrease (Fig. 5a, b). The enzyme retained 83 % of its proteolytic activity in the presence of 30 % v/v of glycerol and 62 % in the presence of propylene glycol. Compared with the control (enzyme without additives), proteolytic activity increased 1.84- and 1.4-fold when glycerol and propylene glycol were added, respectively. Similar results were reported by Daroit et al. (16) for a keratinase from *Bacillus* sp. P 45 in the presence of increasing concentration of Ca^{2+} and Mg^{2+}. Maximum activity was observed with 3 mM Ca^{2+} and 4 mM Mg^{2+} in comparison to the control and tended to decrease with higher concentrations of the metal ions. Bhosale et al. (35) studied the effect of the concentration of Ca^{2+} on the stability of an alkaline protease from *Conidiobolus coronatus* (NCL 86.8.20) and found that at low concentrations (5 and 10 mM) this metal ion did not exert any protective effect, while at higher concentrations (25 and 250 mM) it exerted a significant protective effect, increasing from 0 % to 23 % the residual activity of this protease after 1 h of incubation at 50 °C.

Keratinase from *P. lilacinum* LPSC # 876 is considered a promising biocatalyst for enzyme-based processes and the knowledge of it thermal stability is essential for it application. Weibull distribution provided the best mathematical equation to model heat inactivation of this keratinase. *P. lilacinum* keratinase may be considered stable to be used up to 79 °C. In the absence of any additives, decimal reducing times ranged from 484.16 to 63.67 min in the temperature interval of 50–65 °C; in the presence of Ca^{2+}, propylene glycol and glycerol, these values increased, showing a protective effect of these additives against thermal inactivation. When thermal inactivation data obtained from *P. lilacinum* keratinase was fixed to first-order model, *D* values — obtained at different temperatures — turned out to be quite different from those parameters obtained with Weibull distribution. *D* values (analogous to t_R) obtained were 333.76, 211.17, 76.76 and 45.9 min at 50 °C, 55 °C, 60 °C and 65 °C, respectively. The differences between these values give us an idea of the errors that can be committed when the kinetics studies are made just assuming first-order behaviour without testing other thermal inactivation models. Summarising, the Weibull distribution has been found to be a powerful tool to assess the adequacy of existing and planned thermal processes. This distribution turned out to be a stochastic model sufficiently flexible to account for enzyme inactivation at different processing conditions. Information of Weibull model parameters for enzymes is scarce, as this is the first report on kinetics data for thermal denaturation of a keratinase from *P. lilacinum* LPSC #876.

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