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Study of starch hydrolysis by *α*–amylasefrom porcine pancreas with deactivation of enzyme

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Abstract. The demand of energy and the search for alternative energy sources are the reason why scientists are interested in starch hydrolysis. The aim of the work was to experimental study of the hydrolysis of starch by α–amylase from porcine pancreas with *α*–amylase deactivation. Based on the experiments data, the parameters of starch hydrolysis by *α*–amylase with deactivation of enzyme was estimated. A mathematical model of temperature impact on the activity of *α*–amylase from porcine pancreas was used. It has been estimated that the activation energy E_a and the deactivation energy E_d were equal to 66 ± 4 kJ/mol and

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161 ± 12 kJ/mol, respectively. Additionally, specific constant of starch hydrolysis k_0 and specific constant of α–amylase deactivation k_{d0} were calculated. The optimum temperature T_{opt} equal to 318 ± 0.5 K was obtained from mathematical model. The obtained values of E_a, E_d, k_0 and k_{d0} parameters were used to the model starch hydrolysis by α–amylase from porcine pancreas at 310 K and 333 K.

Keywords: α–amylase from porcine pancreas, starch hydrolysis, activation energy, deactivation energy

1. INTRODUCTION

Starch is naturally present in vegetables and cereals, which in turn are one of the most important nutrients. The importance of starch and starch products in the food industry is significant. Amylases are hydrolyzing enzymes that hydrolyze the glycosidic bonds present in starch molecules and produce dextrin and oligosaccharides. α-amylases (E.C. 3.2.1.1) are endo–hydrolases which cleave α–1,4 glycosidic bonds of starch, glycogen and other related carbohydrates to low molecular weight products, such as maltose, dextrin and other reduced sugars (Maalej et al., 2021). α–amylase can be isolated from microbial sources, plants and animals and has extensive applications in the food industry, baking and brewing during the occurred starch hydrolysis (Balakrishnanet al., 2019). Native and modified starches are used as texturizing and stabilizers agents in a variety of processed foods (Zinck et al., 2023). Therefore, enzymatic hydrolysis of starch is environmentally friendly technology, and it is important to understand the processes that occur during starch hydrolysis. Additionally, the constant increase in the demand for energy has driven research into alternative energy source in recent years. Technologies that convert biogenic waste into green fuels and chemicals, such as enzymatic hydrolysis are proving to be viable alternatives. Among the enzymatic hydrolysis, a particularly promising technology for release of monosaccharide and oligosaccharides is the hydrolysis of starch raw materials. Worthy of note, the starch can be a product in itself or can be further transformed into a range of products, including biofuels (Albani, 2007; Ledesma-Amaro et al. 2015; Marques et al., 2018).

In our study, α–amylase obtained from porcine pancreas (*Sus scrofa*) was used. Importantly, it is extremely similar to human pancreatic α–amylase (Gopal and Muralikrishna, 2009) and therefore has several applications in health food medicine (El-latif et al., 2020; Oszmiański et al., 2021) and in medical diagnostics (Ademakinwa et al., 2019; Wang et al. 2022).

It is essential to mention that the kinetic parameters of the hydrolysis process are used in the design of appropriate volume of bioreactors, flow rate and processing time. However, during starch hydrolysis complex processes occur, hence the investigation of a kinetic enzymatic model is agreat challenge (Mitchell et al. 2021). Starch used as a substrate is composed of linear amylose molecules and branched amylopectin molecules. Several factors, such as substrate complexity, variability over time, the behavior of intermediate products as well as the production of many final products make it difficult to describe the process of starch hydrolysis. For this reason, a detailed literature review on the kinetics of starch hydrolysis was conducted. Consequently, it has been found thatin order to describe starch hydrolysis process many kinetics models may be applied (Mitchell et al. 2021). For instance, Wojciechowski et al. (2001) described both multienzymatic and multisubstrate reactions simulating the "real" concentrations of all components as a function of time. The above-mentioned authors fitted the model to experimental data on the concentration ofreducing sugar obtained as a results of starch hydrolysis by an α–amylase. The model adequately predicted starch hydrolysis results beyond the conditions that were used to demonstrate this method. The times for productive attack, non–productiveattack and inhibition have been scaled to corresponding real times. In addition, it has been noted that competitive inhibition of enzyme kinetics has not been demonstrated. Besselink et al. (2008) were used the same model with modifications. Murthy et al. (2011) described the hydrolysis of starch by α–amylase, which liquefies the starch, and then the malto–ligosaccharides are saccharified by glucoamylase. The model presented included effects of temperature, pH and enzyme activity with enzyme dose. Problematic starch heterogeneity, gelation and product inhibition were taken into account. Model predictions for glucose were characterized by low determination of regression coefficients R^2 ranged from 0.69 to 0.80, hence it can be concluded that the model is described as highly empirical. Moreira et al. (2021) analyzed the Michaelis-Menten kinetics with product inhibition and were obtained starch hydrolysis time according to an analytical equation. This time corresponded to actual process time, i.e., the model of Moreiraet al. (2021) has an advantage over the models of Wojciechowski et al. (2001) and Besselink et al. (2008), which used hypothetical time that is later scaled empirically. It should be noted that the researchers, taking into account the kinetics of starch hydrolysis, did not present the effect of α–amylase deactivation affecting the starch hydrolysis. In most cases, deactivation of α–amylase has been presented as a temperature effect without starch hydrolysis (Apar and Özbek, 2004) or enzyme concentration on starch hydrolysis (Apar and Özbek, 2004a, 2005; Koyama et al., 2013; Rodríguez et al., 2006;

Presečki et al., 2013). The results of studies on starch hydrolysis with α–amylase deactivation, according to the first order deactivation of α–amylase model were presented by Apar and Özbek (2004a, 2005), where α–amylase was derived from *Bacillus* spp. Rodriguez et al. (2006) described starch hydrolysis using the Michealis-Menten equation with a second-order deactivation model for a commercial α–amylase from *Bacillus licheniformis* (Termamyl 300 L). Presecki et al. (2013) to applied the Michealis-Menten equation with the inhibition of non-competitive products (maltose and glucose) to describe hydrolysis of starch by commercial α–amylase from *Bacillus licheniformis* (Liquozyme Supra and Termamyl 120 L). In this work the scientistsindicated that the products of the reaction are not strong inhibitors. However, when considering the kinetics of starch hydrolysis, the researchers did not analyze the effect of α–amylase deactivation from porcine pancreas on enzyme kinetics.

It is necessary to mention that in the previously published work (Miłek, 2021a) the values of the activation energy E_a, the deactivation energy E_d and the optimum temperature T_{opt} have been determined for starch hydrolysis by α–amylase from porcine pancreas for results obtained via experimental studies by others researchers (Akhond et al., 2016; Aksoy et al., 1998; Gopal and Muralikrishna, 2009; Louati et al., 2010; Guo et al., 2016). In order to make model calculations and thus to design the process, it necessary to know an additional parameter, which is the specific pre-exponential rate constant k_0. Experimental data allowed to determine the k_0 with parameters E_a, E_d and k_{d0}.Therefore, our own study was carried out, the basic on which the above-mentioned parameters were determined. To be complete, it should be noted that studying of starch hydrolysis by porcine pancreas α–amylase with simultaneous deactivation of α–amylase is important to provide a better understanding the investigated process.

2. EXPERIMENTAL AND MATHEMATICAL MODEL

2.1. Materials

α–amylase from porcine pancreas (EC 3.4.21.4) (Typ VI-B, > 10 U/mg protein), DNS –dinitrosalicylic acid and soluble starch from potato have been purchased from Sigma–Aldrich (Poznań, Poland). Monosodium phosphate (NaH_2PO_4), disodium phosphate heptahydrate ($Na_2HPO_4 \cdot 7H_2O$) were obtained from Avantor Performance Materials Poland S. A. (Gliwice, Poland). All chemicals used were of analytical grade.

2.2. Kinetic rate equations for starch hydrolysis with deactivation of α–amylase

Starch hydrolysis kinetics can be described by Michaelis–Menten kinetic model (Rodríguez

et al., 2006; Gopal and Muralikrishna, 2009; Koyama et al., 2013). The Michaelis–Menten constant K_m assumed was much less than the concentration of starch (K_m<<S) thus, the change of starch concentrations S in time t an isothermal of in starch hydrolysis by α–amylase can be described by the following equation

$$\frac{dS}{dt} = -kE \tag{1}$$

in which k is enzymatic reaction rate constant (1/min), whereas E is enzyme concentration (M). The change of dimensionless activity α–amylase a (Ademakinwa et al., 2019; Apar and Özbek, 2004a, 2005; Koyama et al., 2013; Miłek, 2021a) in time t in starch hydrolysis by α–amylase was presented equation

$$\frac{da}{dt} - k_d a \tag{2}$$

in which k_d is rate constant of enzyme deactivation(1/min).

The solution to Eq. (2) for initial conditions $a(t = 0) = 1$ is dependence

$$a = \exp(-k_d t) \tag{3}$$

The rate constant of enzymatic reaction k and rate constant of enzyme deactivation k_d depends on temperature T according to the Arrhenius equation:

$$k(T) = k_0 \exp\left(-\frac{E_a}{RT}\right) \tag{4}$$

$$k_d(T) = k_{d0} \exp\left(-\frac{E_d}{RT}\right) \tag{5}$$

in which k_0 and k_{d0} are specifics constants for starch hydrolysis and for deactivation α–amylase (1/min), respectively; E_a is activation energy (kJ/mol) whereas E_d is deactivation energy (kJ/mol), R is gas constant 8.314 J/(mol·K) and T is temperature (K).

The solution of Eq. (1)–Eq. (5) has been presented in several previously published papers (Miłek and Wójcik, 2009; Miłek, 2011; Wojcik and Miłek, 2016). It describes the change in dimensionless enzyme activity depending on temperature T

$$a(T) = \frac{\exp\left(\frac{(T_{opt}-T)}{RTT_{opt}} \frac{E_d \beta}{(\exp\beta - 1)}\right)\left\{1 - \exp\left[-\beta \exp\left(\frac{E_d (T - T_{opt})}{RTT_{opt}}\right)\right]\right\}}{1 - \exp(-\beta)} \tag{6}$$

where T_{opt} (K) is optimum temperature in which activity of α–amylase has the maximum activity and dimensionless parameter β determined by the following relationship

$$\beta = k_{d0} t_a \exp\left(-\frac{E_d}{RT_{opt}}\right) = t_a k_d(T_{opt}) \tag{7}$$

in which t_a is time of starch hydrolysis (min).

The value of activation energy E_a is determined by the equation

$$E_a = E_d - \frac{E_d \cdot \beta}{\exp\beta - 1} \tag{8}$$

Equations (6)–(8) were applied to determine the parameters E_a, E_d and T_{opt} for inulin

hydrolysis by recombinant exo–inulinase from *Aspergillus niger* (Miłek, 2022), endo–inulinase from *A. niger* no recombinant (Miłek, 2020) and recombinant (Miłek, 2023), as well as for olive oil hydrolysis by lipase from porcine pancreas (Miłek, 2021b).

The software SigmaPlot 15.0 was used to estimate parameters occurring in Eq. (6) by non-linear regression of Levenberg–Margurdt method already used in several previous studies Miłek (2021b, 2022, 2023).

2.3 Effect of temperature on activity α–amylase from porcine pancreas

The activity of *α*–amylase was assayed according to Miller methods (Miller, 1959; Maalej et al., 2021; Merck, 2023) with using dinitrosalicylic acid (DNS). The reaction mixture consisted of 1ml 1% starch solution dissolved in phosphate buffer 0.1 M (pH 6.9) was incubated with 1ml of *α*–amylase solution (2.3 U/ml) at various temperature values (298 K, 303 K, 308 K, 313 K, 318 K, 323 K, 328 K and 333 K) for 3 min. The reaction was stopped by added 1 ml of 0.5% DNS. The mixture was then heated in boiling water bath for 15 min. Next, the contents were cooled and the concentration of reducing sugars, were measurement spectrophotomerically at 540 nm using UV-Vis Jasco 530. All assays were performed in triplicate and the obtained results were presented ± SD. One units of *α*–amylase was defined as the amount of *α*–amylase which produced 1 *μ*mol of reducing sugar in 1 min under specified condition.

Subsequently, in order to determine kinetics parameters of *α*–amylase deactivation, the activity was determined by incubating the reaction mixture for at 298 K, 303 K, 308 K, 313 K, 318 K, 323 K, 328 K, and 333 K for 15 min.

2.4. Modeling of the starch hydrolysis by α–amylase from porcine pancreas

Mathematical model described by Eq. (1) and Eq. (2) for starch hydrolysis by *α*–amylase from porcine pancreas was studied. It has been assumed that the enzyme concentration is equalled $E=aE_0$ and knowledge of dimensionless activity *α*–amylase a described by Eq. (3), allowed to transform the Eq. (1) to the following form

$$\frac{dS}{dt} = -kE_0\exp(-k_d t) \tag{9}$$

Transformed Eq. (9) as function conversion of starch hydrolysis X is as follows:

$$\frac{dX}{dt} = \frac{kE_0}{S_0}\exp(-k_d t) \tag{10}$$

An important point which should be noted is that in the current study, the modelling of the starch hydrolysis was investigated at 310 K and 333 K. Firstly, it is related to the potential use of porcine pancreas *α*–amylase in health food medicine and medical diagnostics. Secondly,

the obtained results would be useful for the process of saccharification starch.

3. RESULTS AND DISCUSSION

3.1. Kinetic parameters of starch hydrolysis

To determine the enzymatic reaction rate constant*k*starch hydrolysis, *α*–amylase activity was determined by incubating the reaction mixture for 3 min at specified temperature in the range from 298 K to 333 K. However, according to the data presented in Figure 1, at 328 K and 333 K the effect of deactivation was significant. Hence, to determine the enzymatic reaction rate constant k, the values of temperature mentioned-above were not taken into account. Indeed, the quantity of reducing sugars were analysed at 298 K, 303 K, 308 K, 313 K, 318 K, 323 K. The Eq. (4) was transformed to linear equation and from Arrhenius plot (Figure 2) the values of E_a and k_0 were estimated. It has been found that the above-mentioned parameters were equal to 50.6 ± 2 kJ/mol and $1.93 \cdot 10^{12}$ 1/min, respectively. The regression coefficient R^2 was higher than 0.99, and the sum of errors squared *SSE* was smaller than 0.056. In turn, the value of the Fisher test F was higher than 559 and the value of probability P was smaller than 0.0001.

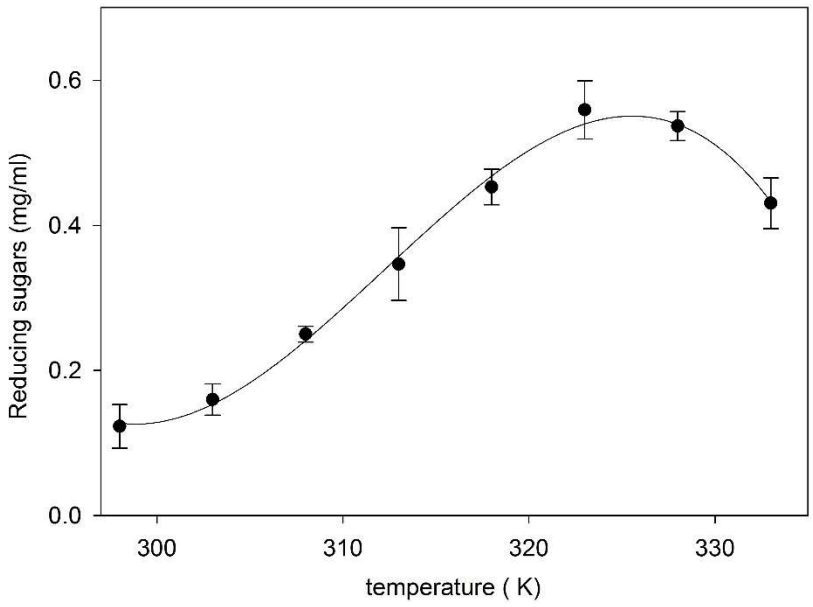

Figure 1. The effect of temperature on the concentration of reducing sugars produced during the starch hydrolysis by *α*–amylase from porcine pancreas at 3 min.

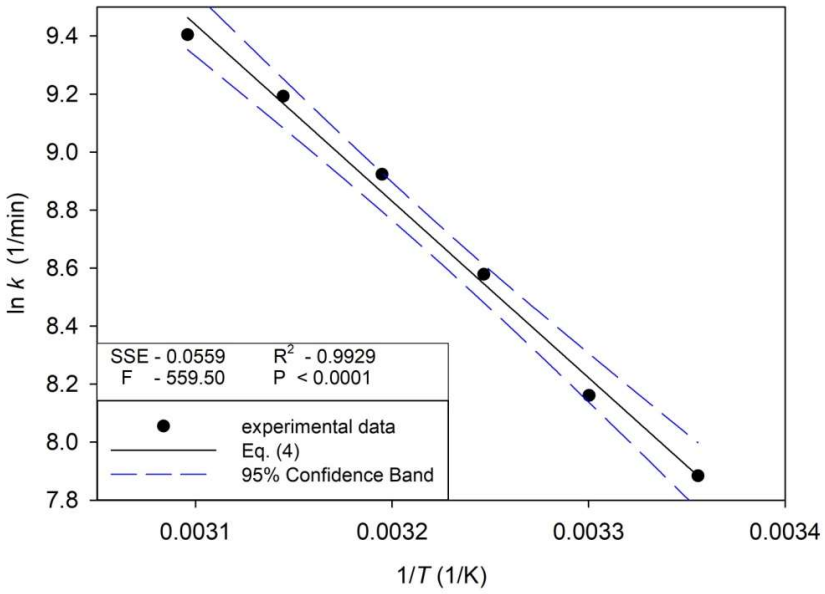

Figure 2. Arrhenius plot to determinevalues of the activation energy E_a and specific constant k_0.

According to the afore mentioned data it can be clearly indicated that the statistical data are highly significant. The parameters E_a and k_0 were determined using a non-linear regression in software SigmaPlot version 15.0 (Gambit, Poland).

3.2. Effect of temperature on activity α–amylase from porcine pancreas

The effect of temperature on the activity of α–amylase from porcine pancreas with statistical data is presented in Figure 3.

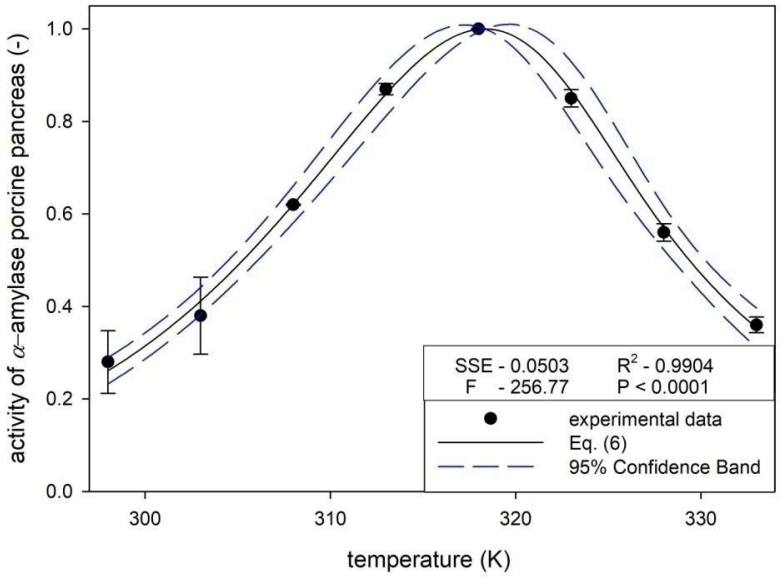

Figure 3. The effect temperature on activity of α–amylase from porcine pancreas with starch hydrolysis for 15 min.

Table 1 shows the values of deactivation energy E_d and optimum temperature T_{opt} as well as parameter β predicted based on the Eq. (6). Then, the knowledge of the E_d and β values allowed to calculate the value of the activation energy E_a from the Eq. (8). Remarkably, the E_a was found to be approximately 30% higher when starch hydrolysis was measurements after 15 minutes and deactivation of α–amylase occurred, compared to starch hydrolysis which was measured after 3 minutes and no deactivation occurred. The values of regression coefficient R^2 was higher than 0.99 and the sum of squared errors *SSE* was smaller than 0.05. Finally, the value of the Fisher test F was higher than 256 and the value of probability P was smaller than 0.0001. The key highlight is therefore that the obtained statistical data are highly significant. The comparison of the obtained values of E_a, E_d, k_0 and T_{opt} for α–amylase from porcine pancreas with those available in the literature are presented in Table 1.

Table 1. Comparison of the obtained values of E_a, E_d, k_0 and T_{opt} parameters for α–amylase from porcine pancreas with literature data.

Source porcine pancreas α–amylase	E_a, kJ/mol	k_0, 1/min	T_{opt}, K	E_d, kJ/mol	References
Sigma–Aldrich	50 ± 2	$1.93 \cdot 10^{12}$	–	–	this study[1)]
Sigma–Aldrich	66 ± 4	$7.80 \cdot 10^{14}$	318 ± 0.5	161 ± 12	this study
Model*	63 ± 0.6	–	–	–	Sočan et al. (2020)
Sigma-Aldrich (St. Louis, MO, USA)	92 ±23	–	311 ± 1	165 ± 19	Miłek, (2021a)[2)]
Merck AG (Germany)	129 ± 9	–	313 ± 0.6	209 ± 5	Miłek, (2021a)[3)]
Sigma Chemical Company	55 ± 17	–	318 ± 1	153 ± 11	Miłek, (2021a)[4)]
Sigma	54 ± 16	–	321 ± 1	163 ± 19	Miłek, (2021a)[5)]
Shanghai Kaiyang Biological Technology Co., Ltd. (Shanghai, China)	20 ± 7	–	326 ± 2	124 ± 14	Miłek, (2021a)[6)]

* for simulation on basic on structural information from Protein Data Bank
[1)]from Arrhenius equation (Fig. 2)
Parameters were determined based on the experimental data: [2)]Akhondet al. 2016 [3)]Aksoy et al., 1998 [4)]Gopal and Muralikrishna, 2009 [5)]Louati et al., 2010 [6)]Guo et al., 2016

In previous work (Miłek 2021a) the values of E_a, E_d and T_{opt} parameters for α–amylase from porcine pancreas were determined for the experimental results of other researchers (Akhond et al., 2016; Aksoy et al., 1998; Gopal and Muralikrishna, 2009; Louati et al., 2010; Guo et al., 2016). It should be emphasized that according to the literature (Table 2), there has been no investigation on the deactivation of α–amylase from porcine pancreas. Notably, determined values of T_{opt} and β and t_a allowed to investigate the rate constant of enzyme deactivation in optimum temperature $k_d(T_{opt})$. Subsequently, the Eq. (7) was transformed and specific constant for α–amylase deactivation k_{d0} was obtained (Table 2).

Table 2. Comparison of the obtained values of E_a, k_0, E_d and k_{d0} parameters for α–amylase from porcine pancreas with literaturevalues for α–amylase from various origin.

Source α–amylase	E_a, kJ/mol	k_0, 1/min	E_d, kJ/mol	k_{d0}, 1/min	References
porcine pancreas Sigma–Aldrich	66 ± 4	$7.80 \cdot 10^{14}$	161 ± 12	$2 \cdot 10^{25}$	this study
salivary human[1)]	–	–	3600	$4.7 \cdot 10^{61}$	Koyama et al. (2013)
salivary human[2)]	–	–	2400	$2.9 \cdot 10^{41}$	
Bacillus licheniformis	42	$1.74 \cdot 10^{8}$	172	$2 \cdot 10^{25}$*	Rodríguez et al. (2006)

*the thermal deactivation was presented by the second–order equation (L/(g·min)) [1)]0.1% starch suspension [2)]3% starch suspension

3.3. Modeling of starch hydrolysis by α–amylase from porcine pancreas

The obtained values of E_a, k_0, E_d and k_{d0} for starch hydrolysis with deactivation of α–amylase from porcine pancreas were used for modeling first–order the conversion of starch for the initial concentration equal to 5%, 10% and 20% at temperature of 310 K and for the initial concentration of 20%, 30% and 40% at 333 K.

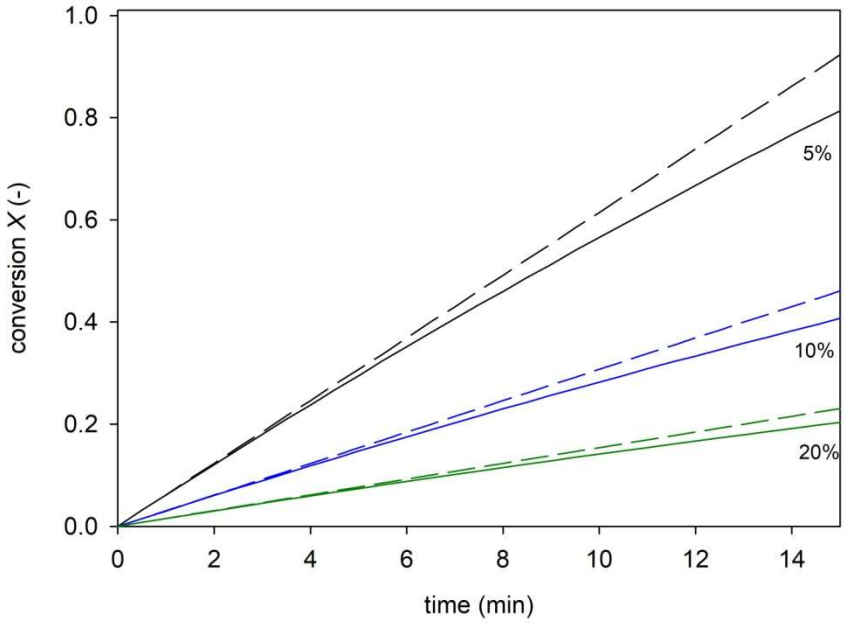

Figure 4. The conversion of starch hydrolysis by α–amylase from porcine pancreas (black line – 5% starch, blue line – 10% starch, green line – 20% starch with deactivation of α–amylase and dash line – without deactivation of α–amylase) at 310 K for15 min.

Based on the results presented in Figure 4, it was found that the conversion of starch hydrolysis decreased correspondingly with increasing starch concentration for the same quantities of the enzyme. Through detailed studies, we showed that for the model without deactivation of α–amylase at the starch concentration of 5%, 10% and 20%, the values of conversion were higher by 11%, 5.4% and 2.7%, respectively. It must be stressed that the data presented in Fig. 4 can be used in the medical diagnosis of patients with hyperamylasemia. Exceeded norms of amylase levels were observed in a group of patients during hospitalization in Covid 19. It should be pointed out that the high values were found in 196 out of patients 1,515, i.e. in 12.9% (Li et al., 2021).

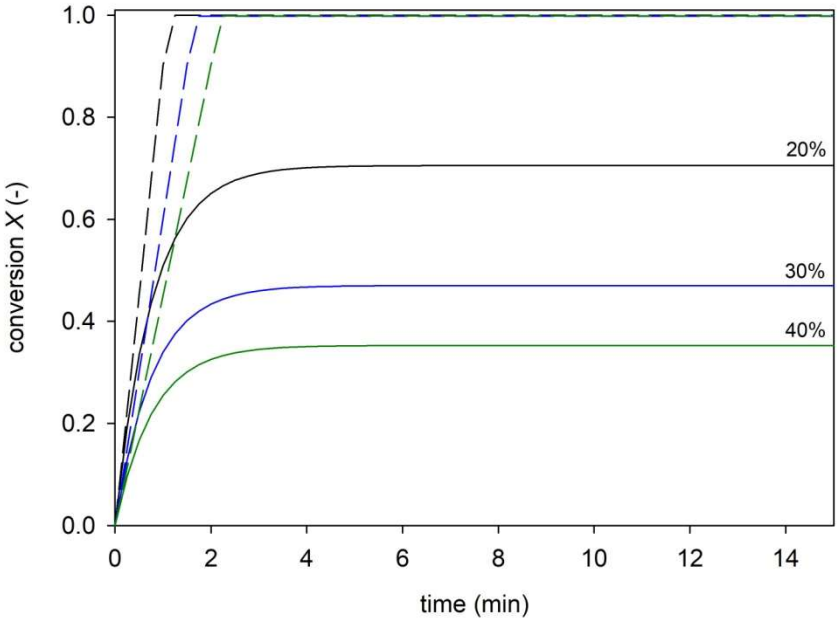

Figure 5. The conversion of starch hydrolysis by *α*–amylase from porcine pancreas (black line – 20% starch, blue line – 30% starch, green line – 40% starch with deactivation of *α*–amylase and dash line – without deactivation of *α*–amylase) at 333 K in time 15 min.

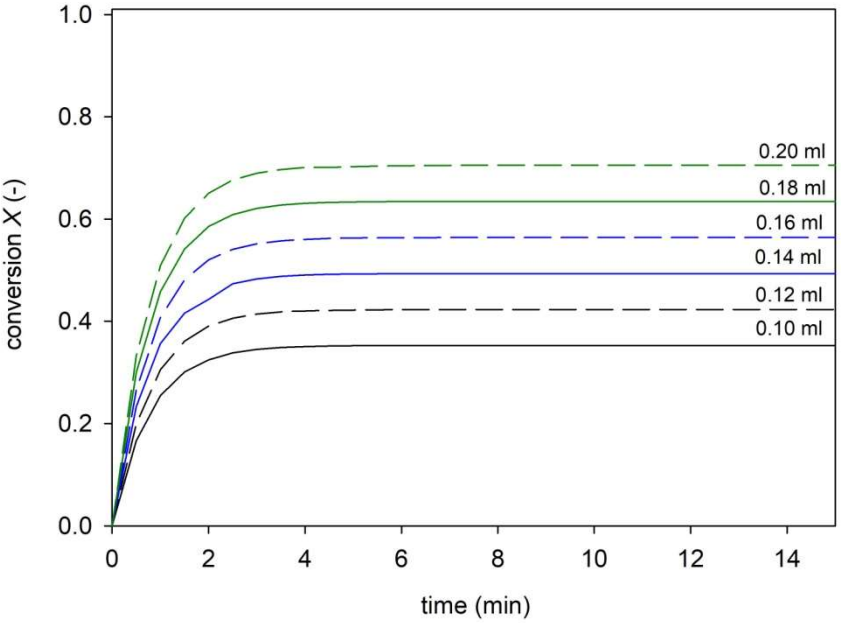

Figure 6. The conversion of starch hydrolysis by *α*–amylase from porcine pancreas at 333 K, starch concentration 40% and different quantities of the enzyme.

It must be recognized that the results presented in Figure 5 have confirmed that the conversion of starch hydrolysis decreased correspondingly with increasing starch concentration for the same quantities of the enzyme. However, using the model without

deactivation of α–amylase for 20%, 30% and 40 % starch concentration, the values of conversion were higher by 29.4%, 53% and 65%, respectively. In turn, results presented in Figure 6 showed that the conversion of starch hydrolysis could be doubled when the amounts of enzymes were also doubled. Importantly, the data presented in Figures5 and 6 can be used in the saccharification of starch.

What is important to point out is that the obtained parameters for starch hydrolysis with deactivation of α–amylase from porcine pancreas (E_a, $k_0$and E_d, k_{d0}) were used to optimize the process along with the optimal amount of enzyme used.

4. CONCLUSIONS

In the current work, the values of E_a, E_d, k_0 and k_{d0} for starch hydrolysis by α–amylase with deactivating enzyme were determined based on the experimental data of the temperature impact on the activity of α–amylase. Additionally, based on the parameters the starch hydrolysis modeling for the initial concentration equal to 5%, 10% and 20% at temperature of 310 K and for the initial concentration of 20%, 30% and 40% at 333 K were presented.

Undoubtedly, the obtained values allowed to better understand the starch hydrolysis with deactivation of α–amylase and can be used to design, modelling and optimizing the investigated process.

SYMBOLS

a dimensionless enzyme activity, -

$a_{\exp}$ α–amylase from porcine pancreas activity determined experimentally, -

$a_{cal}\left(E_d, \beta, T, T_{opt}\right)$ α–amylase from porcine pancreas activity calculated from Eq. (6), -

E the enzyme concentration, M

E_a activation energy, J/mol

E_d activation energy of the deactivation process, J/mol

F Fisher test values,

k enzymatic reaction rate constant, (1/min)

k_0 specific constant enzymatic reaction, (1/min)

k_d rate constant of enzyme deactivation, (1/min)

$k_d(T_{opt})$ rate constant of enzyme deactivation in optimum temperature, (1/min)

k_{d0} specific constant for α–amylase deactivation, (1/min)

P probability value, -

R gas constant, 8.314 (J/(mol K)

R^2 regression coefficient, -

S the starch concentration, M

SSE the sum of squared of errors

t time of starch hydrolysis, min

T temperature, K

T_{opt} optimum temperature, K

Greek symbols

β dimensionless parameter, -

REFERENCES

Ademakinwa A., Agunbiade M., Ayinla Z., Agboola F. 2019. Optimization of aqueous two-phase partitioning of *Aureobasidium pullulans* *a*–amylase via response surface methodology and investigation of its thermodynamic and kinetic properties. *Int. J. Biol. Macromol.*, 140, 833–841. DOI:10.1016/j.ijbiomac.2019.08.159.

Akhond M., Pashangeh Kh., Karbalaei–Heidari H.R., Absalan, G. 2016. Efficient immobilization of porcine pancreatic *α*–amylase on amino–functionalized magnetite nanoparticles: Characterization and stability evaluation of the immobilized enzyme. *Appl. Biochem. Biotechnol*., 180, 954–968. DOI 10.1007/s12010-016-2145-1.

Aksoy S., Tumturk H., Hasirci N. 1998. Stability of *α*–amylase immobilized on poly(methylmethacrylate–acrylic acid) microspheres. *J. Biotechnol*., 60, 37–46. DOI: 10.1016/s0168-1659(97)00179-x.

Albani J.R. 2007. *Starch hydrolysis by amylase*. In *Principles and applications of fluorescence spectroscopy*. Blackwell Publishing, Oxford, UK, 59–78.

Apar D.K., Özbek B. 2004. *α*–Amylase inactivation by temperature during starch hydrolysis. *Process Biochem.*, 39, 9, 1137–1144. DOI: 10.1016/S0032-9592(03)00224-3.

Apar D.K., Özbek B. 2004a. *α*–Amylase inactivation during corn starch hydrolysis process. *Process Biochem.*, 39, 12, 1877–1892. DOI: 10.1016/j.procbio.2003.09.014.

Apar D.K., Özbek B. 2005. *α*–Amylase inactivation during rice starch hydrolysis. *Process Biochem.*, 40, 3–4, 1367–1379. DOI: 10.1016/j.procbio.2004.06.006.

Balakrishnan D., Kumar S.S., Sugathan, S. 2019. *Chapter 11 Amylases for food application supdated information*. In: Parameswaran, B., Raveendran, S., Varjani, S. (Eds) *Green bio-processes. Enzymes in industrial food processing*. Springer, Singapore , 199–228.

Besselink T., Baks T., Janssen A.E.M., Boom R.M., 2008. A stochastic model for predicting dextrose equivalent and saccharide composition during hydrolysis of starch by *α*–amylase. *Biotechnol. Bioeng.*, 100 (4), 684–697. DOI: 10.1002/bit.21799.

El-latif A.O.A., Mohieldeen N., Salman A.M.A., Elpidina E.N. 2020. Isolation and purification of *α*–amylase inhibitors and their in vitro and in vivo effects on *Tribolium castenuem* (Herbst) and *Callosbruchus maculatus* (F.). J. Plant Protec. Research 60(4), 377–387. DOI: 10.24425/jppr.2020.134911.

Gopal B.A., Muralikrishna G. 2009. Porcine pancreatic *α*–amylase and its isoforms: Purification and kinetic studies. *Int. J. Food Prop.*, 12, 571–586. DOI:10.1080/10942910801947755.

GuoH., Tang Y., Yu Y., Xue L., Qian J. 2016. Covalent immobilization of *α*–amylase on magnetic particles ascatalyst for hydrolysis of high-amylose starch. *Int. J. Biol. Macromol.*, 87, 537–544. DOI: 10.1016/j.ijbiomac.2016.02.080.

Ledesmo-Amaro R., Dulermo T., Nicaud J.M. 2015. Engineering *Yarrowia lipolytica* to produce biodiesel from raw starch. *Biotechnol. Biofuels Bioprod.*, 8, 148, 1–12. DOI: 10.1186/s13068-015-0335-7.

Li G., Liu T., Jin G., Li T., Liang J., Chen Q., Chen L., Wang W., Wang Y., Song J., Liang H., Zhang C., Zhu P. Zhang W., Ding Z., Chen X., Zhang B. 2021. Serum amylase elevation is associated with adverse clinical outcomes in patients with coronavirus disease 2019. *Aging* 13 (20), 23442-23458. DOI: 10.18632/aging.203653.

Louati H., Zouari N., Fendri A., Gargouri Y. 2010. Digestive amylase of a primitive animal, the scorpion: Purification and biochemical characterization. *J. Chromatog. B.*,878, 853–860. DOI: 10.1016/j.jchromb.2010.01.047.

Koyama K., Shono J., Taguchi H., Toriba A., Hayakawa K. 2013. Effect of starch on the inactivation of amylase in starch-containing foods. *Food Sci. Technol. Res.*, 19 (6), 989 – 993. https://www.jstage.jst.go.jp/article/fstr/19/6/19_989/_pdf

Maalej H., Maalej A., Affes S., Hmidet N., Nasri, M.A. 2021. Novel digestive *α*–amylase from blue crab (*Portunus segnis*) viscera: purification, biochemical characterization and application for the improvement of antioxidant potential of oat flour. *Int. J. Mol. Sci.*, 22, 1070, 1–16. https://doi.org/10.3390/ijms22031070.

Marques S., Moreno A. D., Ballesteros M., Gírio F. 2018. *Chapter 4 Starch biomass for biofuels, biomaterials, and chemicals*. In Sílvio Vaz Jr. (Ed) *Biomass and green chemistry building a renewable pathway*. Springer International Publishing, Cham, Switzerland, 76–94.

Merck 2023. Enzymatic assay *α*–amylase. Available at: https://www.sigmaaldrich.com/PL/pl/technical-documents/protocol/protein-biology/enzyme-activity-assays/enzymatic-assay-of-a-amylase

Miller G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.*,31, 426–428.

Miłek J., Wójcik M. 2009. Wyznaczanie parametrów termicznej dezaktywacji enzymów. *Inż. Ap. Chem.*, 48, 3, 69–70. https://bibliotekanauki.pl/articles/2070468.pdf

Miłek J. 2011. Studying and modeling of deactivation of catalase, PhD thesis ZUT Szczecin. http://zbc.ksiaznica.szczecin.pl/Content/21119/PDF/Praca%20dokt.%20J.%20Mi%C5%82ek.pdf

Miłek J. 2020. Determination of the optimum temperature and activation energies for the hydrolysis of inulin hydrolysis by endo–inulinase *Aspergillusniger*. *Chem. Process Eng.*, 41 (2),229–236. DOI:10.24425/CPE.2020.132545.

Miłek J. 2021a. Determination of activation energies and the optimum temperatures of hydrolysis starch by *α*–amylase from porcine pancreas. *Molecules*, 26, 4117, 1–9. DOI:10.3390/molecules26144117.

Miłek J. 2021b. The activation energies and optimum temperatures of olive oil hydrolysis by lipase porcine pancreas. *Ecol. Chem. Eng. S*, 28(3), 389–398. DOI:10.2478/eces-2021-0026.

Miłek J. 2022. The inulin hydrolysis by recombinant exo–inulinases: determination of the optimum temperatures and activation energies. *J. Therm. Anal. Calorim*.,147, 8061–8067. DOI:10.1007/s10973-020-10495-3.

Miłek J. 2023. Recombinant endo–inulinases: Determination of the activation, deactivation energies and optimum temperatures in inulin hydrolysis. *J. Therm. Anal. Calorim*., 148, 859–866. DOI: 10.1007/s10973-022-11809-3.

Mitchell D. A., Moreira I., Krieger N. 2021.Potential of time-stepping stochastic models as tools for guiding the design and operation of processes for the enzymatic hydrolysis of polysaccharides – A review. *Bioresour. Technol.*, 323, 124559, 1–12. DOI: 10.1016/j.biortech.2020.124559.

Moreira I., Krieger N., Mitchell D.A. 2021. Time is of the essence: a new strategy for time-stepping in stochastic models describing the enzymatic hydrolysis of colloidal suspensions of polysaccharides. *Chem. Eng. J.*,405, 126672. DOI: 10.1016/j.cej.2020.126672.

Murthy G.S., Johnston D.B., Rausch K.D., Tumbleson M.E., Singh V. 2011. Starch

hydrolysis modeling: application to fuel ethanol production. *Bioprocess Biosyst. Eng.*,34 (7), 879–890. DOI:10.1007/s00449-011-0539-6.

Oszmiański J., Lachowicz S., Nowicka P., Rubiński P., Cebulak T. 2021. Evaluation of innovative dried purée from Jerusalem artichoke - In vitro studies of its physicochemical and health-promoting properties. *Molecules*, 26, 2644. DOI: 10.3390/molecules26092644.

Presečki A.V., Blažević Z.F., Vasić-Rački Đ. 2013. Mathematical modeling of maize starch liquefaction catalyzed by *α*–amylases from *Bacillus licheniformis*: effect of calcium, pH and temperature. *Bioprocess Biosyst. Eng.,* 36(1), 117–126. DOI:10.1007/s00449-012-0767-4.

Rodríguez V.B., Alameda E.J., Gallegos J.F.M., Requena A.R., López, A.I.G. 2006. Enzymatic hydrolysis of soluble starch with an *α*–amylase from *Bacillus licheniformis*. *Biotechnol. Progress,* 22, 718–722. DOI:10.1021/bp060057a.

Sočan J., Purg M., Åqvist, J. 2020. Computer simulations explain the anomalous temperature optimum in a cold–adapted enzyme. *Nat. Commun.*, 11, 2644, 1–11. https://doi.org/10.1038/s41467-020-16341-2

Wang Y., Ral J.-P., Saulnier L., Kansou K. 2022. How does starch structure impact amylolysis? Review of current strategies for starch digestibility study. *Foods*, 11(9), 1223, 1–19. DOI: 10.3390/foods11091223.

Wojciechowski P.M., Koziol A., Noworyta A. 2001. Iteration model of starchhydrolysis by amylolytic enzymes. *Biotechnol. Bioeng.*,75 (5), 530–539. DOI:10.1002/bit.10092.

Wojcik M., Miłek J. 2016. A new method to determineoptimum temperature and activation energies for enzymatic reactions. *Bioprocess Biosyst. Eng.*,39, 1319–1323. DOI:10.1007/s00449-016-1596-7.

Zinck S.S., Christensen S.J., Sørensen O.B., Svensson B., Meyer A.S. 2023. Importance of inactivation methodology in enzymatic processing of raw potato starch: NaOCl as efficient *α*–amylase inactivation agent. *Molecules*, 28, 2947, 1–11. https://doi.org/10.3390/molecules28072947