Acta of Bioengineering and Biomechanics
Vol. 23, No. 3, 2021

Original paper
DOI: 10.37190/ABB-01827-2021-02

The effect of pH on determination of activation energies and the optimum temperatures of hydrolysis of olive oil by lipase from porcine pancreas

JUSTYNA MIŁEK*

Department of Chemical and Biochemical Engineering, Faculty of Chemical Technology and Engineering, University of Science and Technology in Bydgoszcz, Bydgoszcz, Poland.

Purpose: The present paper reports the determination of the activation energies and the optimum temperatures of olive oil hydrolysis by porcine pancreas lipase with simultaneous effect of pH. *Methods*: The parameters were estimated based on the literature data on the activity curves versus temperature for olive oil hydrolysis by lipase obtained from porcine pancreas. It was assumed that both the hydrolysis reaction process and the deactivation process of lipase were first-order reactions by the enzyme concentration. A mathematical model describing the effect of temperature on porcine pancreas lipase activity was used. *Results*: The determinate activation energies E_a were from 31.37 ± 5.38 kJ/mol to 61.60 ± 11.46 kJ/mol, the optimum temperatures T_{opt} were obtained in the range from 305.46 ± 1.26 K to 313.23 ± 1.18 K and the values of deactivation energies E_d were in the range from 65.18 ± 3.19 kJ/mol to 109.27 ± 6.79 kJ/mol. *Conclusions*: The obtained results (E_a, E_d, T_{opt}) might find application in research on the prognosis of pancreatic cancer.

Key words: *pancreatic cancer, activation energy, deactivation energy, optimum temperature, lipase porcine pancreas*

1. Introduction

The pancreatic cancer is an exceptionally aggressive tumor with high mortality. Performing a comprehensive literature review shows that there are no effective diagnostic methods allowing for the prognosis of this type of cancer. Lipase (triacylglycerol esters hydrolase, EC 3.1.1.3) is one of the most important pancreatic enzymes. This enzyme catalyses the hydrolysis of long-chain triacylglycerols to glycerol, free fatty acids and mono- and diacylglycerols [11]. Stotz et al. [24] hypothesized that the increased level of lipase quantity in the peripheral blood can be a diagnosis for trauma to the pancreas (in the case of acute pancreatitis). Indeed, low lipase levels may make a prognosis for a pancreatic tumor.

It is well documented that porcine materials are often used in bioengineering and biomedical research [3], [5], [26], [28]. For instance, porcine pancreas lipase is used for medical purposes as a medicine for patients with lipase deficiency [6]. Moreover, it is suitable for the treatment of acute pancreatitis and any conditions associated with pancreatic exocrine atrophy, including chronic pancreatitis and cystic fibrosis [19].

It must be recognized that processes involving lipase cannot be designed without knowing the kinetic parameters of the process. Therefore, studies on the effect of temperature on lipase activity are required. Hydrolysis with porcine pancreas lipase is usually carried out at temperatures higher than 33 °C [1], [4], [6]–[9], [13], [23], thus, a significant inactivation of the enzyme may occur. Therefore, it is necessary to deter-

* Corresponding author: Justyna Miłek, Department of Chemical and Biochemical Engineering, Faculty of Chemical Technology and Engineering, University of Science and Technology in Bydgoszcz, ul. Seminaryjna 3, 85-326 Bydgoszcz, Poland. Phone: +48 52 374 90 49, e-mail: jmilek@utp.edu.pl

Received: March 16th, 2021
Accepted for publication: May 26th, 2021

mine the activation energy E_a, the activation energy of the deactivation process E_d and the optimum temperature T_{opt} for porcine pancreas lipase. The method of determining the kinetic parameters based on experimental data on the effect of temperature on the activity of lipase from porcine pancreas has been presented in several previous studies [4], [7]–[9].

The purpose of the present work was to estimate parameters of the activation energies E_a, the deactivation energies E_d and the optimum temperatures of oil olive hydrolysis by pancreas lipase, whose obtained values can be used in works focused on prognosis for a pancreatic tumor.

2. Materials and methods

2.1. Assay of lipase activity and effect of pH

Lipase activity is most often determined by the titration method [4], [7]–[9], [23]. It is based on sodium hydroxide determination of fatty acids formed during hydrolysis of triacylglycerol by lipase. The unit of lipolytic activity (U) for the titration method is defined as the amount of enzyme that releases of 1 μmol fatty acid from olive oil in 1 minute under the conditions of determination, usually 37 °C and pH in the range from 6.0 to 7.5 [4], [7]–[9].

Parameters: optimum temperatures T_{opt}, activation energies E_a and activation energies of the deactivation process E_d of olive oil hydrolysis by porcine pancreas lipases were estimated from activity change curves at temperature effect [4], [7]–[9].

2.2. The effect of temperature on lipase activity

Values of activation energies, E_a and E_d can be determined from the curves of the dependence of the logarithm of the reaction rate (ln v) on the reciprocal of temperature ($1/T$), the so-called Arrhenius dependence [10]. However, the determined values of E_a and E_d by application of the traditional method is burdened with an error.

When studying lipase activity during the hydrolysis of olive oil, it is assumed that the change in substrate concentration S during reaction time t is described by the first-order equations due to the concentration of the enzyme

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

where k is the kinetic constant of the enzymatic reaction [1/min] and E is the concentration of the active enzyme [M]. Substrate specifity of pancreatic lipase is a parameter insignificantly influence on lipase kinetics.

The change in lipase dimensionless activity a is also described by the first-order kinetics [1], [13], [20], using the following equation

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

where k_d is the kinetic constant of the enzymatic reaction [1/min].

The solution of Eq. (2) for the initial condition a (t = 0) = 1 is

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

Kinetic constants k and deactivation constant k_d depend on temperature T and are described by the Arrhenius equations as:

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

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

where A, B are pre-exponential factors of the hydrolysis reaction rate or deactivation process of lipase [1/min], E_a is the activation energy for the enzymatic reaction [kJ/mol] while E_d is the activation energy of the deactivation process [kJ/mol], R is the gas constant 8.315 J/(mol·K), and T is the absolute temperature [K].

Equations (1)–(5) are the basis for the derived dependence of the change in the dimensionless activity of the enzyme on the temperature measurement T as follows:

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

where T_{opt} is the temperature at which lipase shows maximum activity [K] and dimensionless parameter β determines the relationship

$$\beta = Bt_a \exp\left(-\frac{E_d}{RT_{opt}}\right), \quad (7)$$

where t_a is reaction time of olive oil hydrolysis by lipase from porcine pancreas [min]. It means that knowing the value of the activation energy of the deactivation reaction E_d and the parameter β, the activation energy E_a is determined by the following equation

$$E_a = E_d - \frac{E_d \cdot \beta}{\exp\beta - 1}. \quad (8)$$

The full analysis of the solution of Eq. (6) was presented in an earlier publication of Wojcik and Miłek [27].

Equations (6)–(8) were used to determine the kinetic parameters of inulin hydrolysis by exo-inulinases *Aspergillus niger* [12], olive oil hydrolysis (pH 8.9) by porcine pancreas lipase [13], *p*-nitrophenyl palmitate hydrolysis by lipases from *Rhizopus oryzae* 3562 and *Enterobacter aerogenes* [14], hydrolysis of starch by α-amylase *Bacillus licheniformis* [15], inulin hydrolysis by endo-inulinase *A. niger* [16] and inulin hydrolysis by inulinase *K. marxianus* [27].

Based on Eq. (6), the kinetic parameters E_d and T_{opt} were estimated by non-linear regression according to the methods of least squares [17], [18], [27], determining the residual sum of squared (*RSS*) from the equation:

$$RSS(E_d, \beta, T_{opt})$$

$$= \sum_{i=0}^{n} \frac{1}{a_{exp}^2}(a_{exp} - a_{cal}(E_d, \beta, T_i, T_{opt}))^2 = \min, \quad (9)$$

where a_{exp} is lipase dimensionless activity determined experimentally and $a_{cal}(E_d, \beta, T, T_{opt})$ is lipase dimensionless activity calculated from Eq. (6).

3. Results

Literature data [4], [7]–[9] for porcine pancreas lipase from Sigma-Aldrich (or Sigma) was analyzed. In most cases, type II porcine pancreas lipase with an activity of 177.3 U/mg were used. In Table 1, the conditions for measuring lipase activity during the hydrolysis of olive oil with the various buffer pH, the various duration of measurement and the used the initial concentration of olive oil [4], [7]–[9] are presented.

Table 1. Measurement conditions of porcine pancreas lipase activity used to olive oil hydrolysis

pH phosphate buffer	*t* [min]	Oil olives concentration	Ref.
6.0	20	25% (vol.)	[4]
6.9	30	25% (vol.)	[7]
7.0	30	25% (vol.)	[8]
7.5	15	20% (vol.)	[9]

t is reaction time of olive oil hydrolysis by lipase from porcine pancreas.

The concentration of used buffers was 0.1 M and polyvinyl alcohol was used as the emulsifier. The activity of lipase at a specified temperature was determined in the pH range of 6.0–7.5.

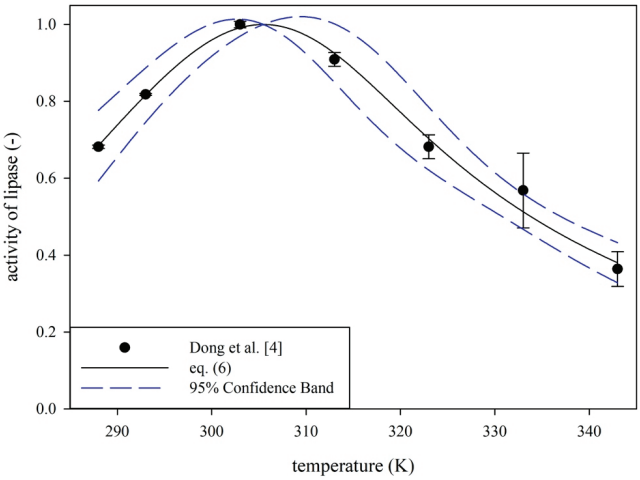

Fig. 1. The activity of lipase from porcine pancreas [4]

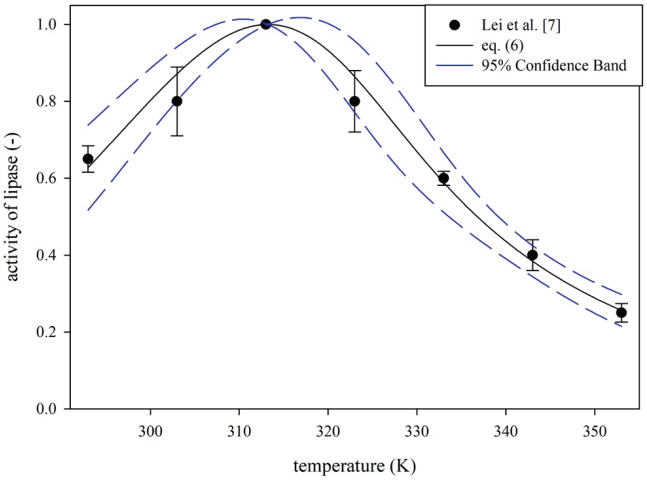

Fig. 2. The activity of lipase from porcine pancreas [7]

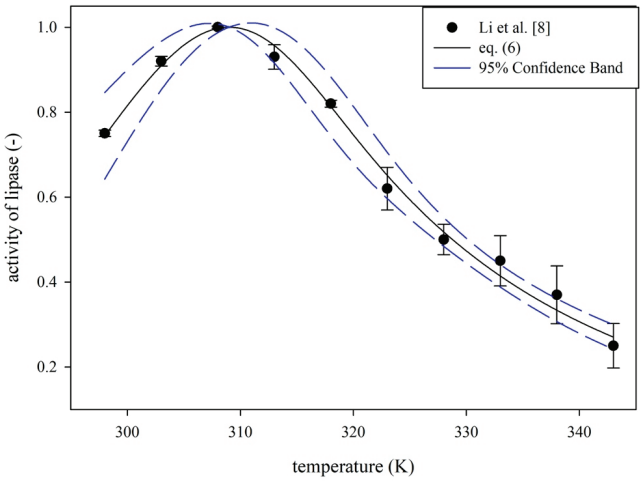

Fig. 3. The activity of lipase from porcine pancreas [8]

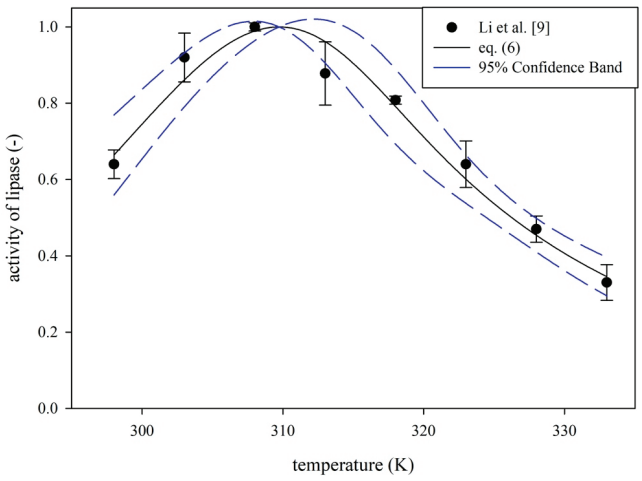

Fig. 4. The activity of lipase from porcine pancreas [9]

Table 2. The values of parameters estimated for lipases from porcine pancreas

Fig.	T_{opt} [K]	β	E_d [kJ/mol]	E_a [kJ/mol]	E_d/E_a	Ref.
1	305.46 ± 1.26	1.48 ± 0.22	65.18 ± 3.19	36.75 ± 5.64	1.77	[4]
2	313.23 ± 1.18	1.01 ± 0.17	74.45 ± 2.86	31.37 ± 5.38	2.37	[7]
3	308.71 ± 0.78	1.52 ± 0.19	93.44 ± 6.23	53.68 ± 8 .13	1.74	[8]
4	309.45 ± 0.96	1.48 ± 0.26	109.27 ± 6.79	61.60 ± 11.46	1.77	[9]

T_{opt} – the temperature at which lipase shows maximum activity, β – dimensionless parameter determines the Eq. (7), E_d – the activation energy of the deactivation reaction of olive oil hydrolysis by lipase porcine pancreas, E_a – the activation energy of olive oil hydrolysis by lipase porcine pancreas.

Table 3. Statistical data obtained by determining the kinetic parameters of lipase from porcine pancreas

Fig.	R^2	RSS	p			F	P	Ref.
			E_d [kJ/mol]	T_{opt} [K]	β [–]			
1	0.9832	0.0568	<0.0001	<0.0001	0.0030	120.46	0.0003	[4]
2	0.9877	0.0672	<0.0001	<0.0001	0.0027	160.39	0.0002	[7]
3	0.9863	0.0621	<0.0001	<0.0001	<0.0001	251.81	<0.0001	[8]
4	0.9732	0.0760	<0.0001	<0.0001	0.0024	90.90	<0.0001	[9]

R^2 – regression coefficient, RSS – standard errors of estimation, F – Fisher test value, p-value – probability value for single parameter (E_d or T_{opt} or β), P-value – probability value for value parameters E_d, T_{opt} and β, T_{opt} – the temperature at which lipase shows maximum activity, β – dimensionless parameter determines the Eq. (7), E_d – the activation energy of the deactivation reaction of olive oil hydrolysis by lipase porcine pancreas, E_a – the activation energy of olive oil hydrolysis by lipase porcine pancreas.

Based on experimental data on the change in activity of lipase from the porcine pancreas [4], [7]–[9] vs. temperature, values of deactivation energies E_d, β parameters and temperatures optimal T_{opt} were determined on the basis of Eq. (6). In Figures 1–4, experimental data on lipase activity by hydrolysis of olive oil as a substrate are shown, along with activity curves plotted based on Eq. (6) for the values of the specified parameters E_d, T_{opt}, β listed in Table 2.

The obtained parameters T_{opt}, β, E_d for lipases from porcine pancreas are presented in Table 2, according to the increasing value of the activation energy of the deactivation reaction. With the values of the activation energy parameter for the deactivation reaction E_d and the parameter β, the activation energy value E_a was calculated based on equation (8). The obtained E_a values are presented in Table 2.

In Table 3, statistical data obtained during the determination of the kinetic parameters of porcine pancreatic lipase are presented. High values of regression coefficient R^2 above 0.97, and standard errors of estimation RSS below 0.076 were obtained; while statistical variability of E_d and T_{opt} parameters in most of the analyzed cases was $p < 0.0001$.

Fisher test values were in the range of 90.90–251.80 with a low probability value [$P \leq 0.0003$], which confirmed that it was appropriated to apply Eq. (6) when determining parameters. Also, in Figs. 1−4, standard deviation errors for experimental points are presented, while the 95% confidence limits were marked for the obtained curves.

4. Discussion

This work aimed to identify the activation energy E_a, the activation energy of the deactivation process E_d and the optimum temperature T_{opt} of oil olive hydrolysis by porcine pancreas lipase. Knowing the obtained values can be used in works focused on prognosis for a pancreatic tumor.

4.1. The activation energy E_a

The obtained values of the activation energy E_a of olive oil hydrolysis by lipase obtained from porcine pancreas were in the range from 31.37 ± 5.38 kJ/mol to 61.60 ±11.46 kJ/mol. The value of the activation energy E_a determined in an earlier paper [13] for the hydrolysis of olive oil (pH 8.9) by a lipase from porcine pancres was higher and amounted to 86.75 ± 11.47 kJ/mol. Dong et al. [4] determined from Arrhenius plot the value of E_a activation energy of the hydrolysis (pH 7.0) equal to 29.50 kJ/mol. This value was by about 20% lower than value determined and presented in Table 2.

The data collected in Table 2 show that there is a correlation between the values of the deactivation energy E_d and the activation energy E_a (Eq. 6). It has been reported that for most of analyzed cases, as the value of E_d increases, the value of E_a increases. According to the calculations for the measurement of Li et al. [9], the energy value E_a was twice as high compared to the E_a values obtained by Lei et al. [7]. The observed reason may be due to the different time in which of the lipase activity determination. Indeed, the measurements time of hydrolysis olive oil time was equal to 15 minutes and 30 minutes in papers [7] and [9], respectively. Importantly, the differences in the pH of the hydrolyzed olive oil as well as the concentration of the hydrolyzed olive oil may influence the activation energy value E_a. Schmitke et al. [22] have indicated that the increase in the amount of water in the environment during the ester synthesis reaction is thermodynamically unfavorable due to the shifting of the hydrolysis balance. The resulting amounts of water forms an increasing layer around the enzyme and hinders access to the catalytic enzyme for hydrophobic synthesis [22].

4.2. The activation energy of the deactivation process E_d

The obtained values of the activation energy of the deactivation process were in the range from 65.18 ± 3.19 kJ/mol to 109.27 ± 6.79 kJ/mol (Table 2). It has been found that the deactivation energy values increase along with increasing pH of the olive oil solution used in hydrolysis (Fig. 5).

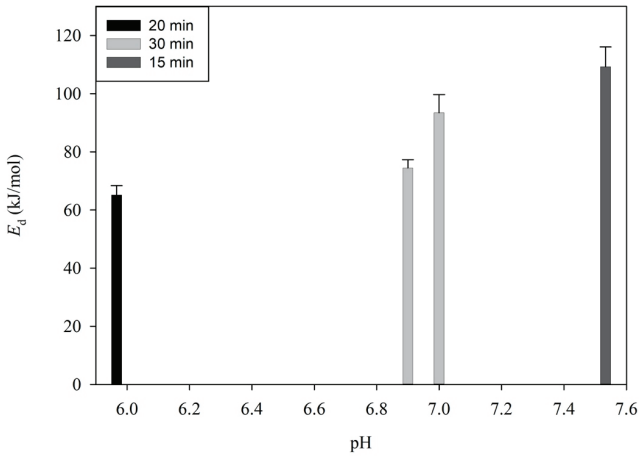

Fig. 5. The values of activation energy of the deactivation process of E_d lipase from porcine pancreas versus on the pH of hydrolyzed olive oil

In an earlier work [13], the E_d value of hydrolysis of olive oil (pH 8.9) by lipase from porcine pancreas was found as equal to 122.12 ± 7.26 kJ/mol. In turn, Tsuita and Okuda [25] determined the value of E_d deactivation energy in the hydrolysis of tributyrin (pH 7.0) equal to 140.87 kJ/mol. The differences in the obtained activation energy value of the deactivation process E_d can be caused by the use of various substrates with different pH for hydrolysis.

4.3. Optimum temperature T_{opt}

The determined values of the optimum temperature T_{opt} of olive oil hydrolysis by lipase from porcine pancreas were different by about six degrees and are in the range from 305.46 ± 1.26 K to 313.23 ± 1.18 K (Table 2). In an earlier work [13], a T_{opt} of olive oil hydrolysis (pH 8.9) by porcine pancreatic lipase was

determined and equal 306.78 ± 0.54 K for the measurements presented by Bagi et al. [1].

Olive oil has been used as the substrate. Determining the parameters for the hydrolysis of another used substrate or a different kinds of lipase [2], [20] requires analysis in terms of the reaction course model and changes in enzyme activity.

The determined values of T_{opt}, E_a, E_d parameters may be comparable to those presented in the analogous studies focused on porcine and human pancreas lipase. However, the careful analysis of experimental design of the pancreas, must be carried out before interpolating the results to other theoretical, clinical, biomaterial studies.

5. Conclusions

The following method of determining parameters was used: the optimum temperatures T_{opt}, activation energies E_a and deactivation energies E_d of olive oil hydrolysis by lipases from porcine pancreas reaction based on four curves of changes of activity of lipases from porcine pancreas depending on the temperature of hydrolysis.

For the optimum temperatures T_{opt}, the difference between the obtained values was six degrees. The differences in the calculated values of the activation energy of the deactivation reaction E_d are equal to about 45 kJ/mol, for the activation energy of the reaction E_a equal to about 30 kJ/mol. It is essential to mention that the noted differences in values of parameters can be caused by the various duration of the lipase activity assay, different pH values of the hydrolyzed olive oil used to test the lipase activity as well as different concentrations of the olive oil.

The obtained parameters of the activation energies E_a, the deactivation energies E_d and the optimum temperatures of hydrolysis of oil olive by pancreas lipase can be particularly important for studies focused on prognosis of a pancreatic tumor.

References

[1] BAGI K., SIMON L.M., SZAJÁNI B., *Immobilization and characterization of porcine pancreas lipase*, Enzyme Microb. Tech., 1997, 20, 531–535.

[2] CASAS-GODOY L., DUQUESNE S., BORDES F., SANDOVAL G., MARTY A., *Lipases: An Overview*, [in:] G. Sandoval (Eds.), *Lipases and Phospholipases. Methods in Molecular Biology (Methods and Protocols)*, Humana Press, 2012, 861.

[3] CHLADEK W., CZERWIK I., *Mechanical properties of temporomandibular joint disc on the basis of porcine preparation investigations*, Acta Bioeng. Biomech., 2008, 10 (4), 15–20.

[4] DONG H., LI J., LI Y., HU L., LUO D., *Improvement of catalytic activity and stability of lipase by immobilization on organobentonite*, Chem. Eng. J., 2012, 181–182, 590–596.

[5] ELSHEIKH A., KASSEM W., JONES S.W., *Strain-rate sensitivity of porcine and ovine corneas*, Acta Bioeng. Biomech., 2011, 13 (2), 25–36.

[6] GUERRAND D., *Lipases industrial applications: focus on food and agroindustries*, OCL 2017, 24 (4), D403.

[7] LEI L., BAI Y., LI Y., YI L., YAN Y., XIA C., *Study on immobilization of lipase onto magnetic microspheres with epoxy groups*, J. Magn. Magn. Mater., 2009, 321, 252–258.

[8] LI X., ZHU H., FENG J., ZHANG J., DENG X., ZHOU B., ZHANG H., XUE D., LI F., NIGEL J.M., LI Y, PENG Y., *One-pot polylol synthesis of graphene decorated with size- and density-tunable Fe_3O_4 nanoparticles for porcine pancreatic lipase immobilization*, Carbon, 2013, 60, 488–497.

[9] LI Y., JING T., XU G., TIAN J., DONG M., SHAO Q., WANG B., WANG Z., ZHENG Y., YANG C., GUO Z., *3-D magnetic graphene oxide-magnetite poly(vinyl alcohol) nanocomposite substrates for immobilizing enzyme*, Polymer, 2018, 149, 13–22.

[10] LYKIDIS A., MOUGIOS V., ARZOGLOU P., *Kinetics of the two-step hydrolysis of triacylglycerol by pancreatic lipases*, Eur. J. Biochem., 1995, 230, 892–898.

[11] MENDES A.A., OLIVEIRA P.C., DE CASTRO H.F., *Properties and biotechnological applications of porcine pancreatic lipase*, J. Mol. Catal. B: Enzym., 2012, 78, 119–134.

[12] MIŁEK J., *Application of the new method to determine of the kinetic parameters of inulin hydrolysis by exo-inulinase Aspergillus niger*, J. Therm. Anal. Calorim., 2021, DOI: 10.1007/s10973-020-10495-3.

[13] MIŁEK J., *Calculation of temperature optimum as well as activation and deactivation energy for the olive oil hydrolysis with porcine pancreas lipase*, Przem. Chem., 2020, 99 (4), 585–587.

[14] MIŁEK J., *Determination of the activation energies and optimum temperature for the hydrolysis of p-nitrophenyl palmitate catalyzed by lipases*, Przem. Chem., 2021, 100 (1), 103–104.

[15] MIŁEK J., *Determination the optimum temperature and activation energy for the hydrolysis of starch catalyzed by α-amylase Bacillus licheniformis*, Przem. Chem., 2020, 99 (6), 880–881.

[16] MIŁEK J., *Determination the optimum temperatures and activation energies of inulin hydrolysis by endo-inulinase Aspergillus niger*, Chem. Proc. Eng., 2020, 41 (3), 229–236.

[17] MIŁEK J., *Estimation of the kinetic parameters for H_2O_2 enzymatic decomposition and for catalase deactivation*, Braz. J. Chem. Eng., 2018, 35 (3), 995–1004.

[18] MIŁEK J., *Thermodynamics and kinetics of thermal deactivation of catalase Aspergillus niger*, Pol. J. Chem. Technol., 2020, 22 (2), 67–72.

[19] NOZAWA F., YALNIZ M., SARUC M., STANDOP J., EGAMI H., POUR P.M., *Effects of fungal pancreatic enzymes on the function of islet cells in syrian golden hamsters*, JOP. J. Pancreas (Online), 2013, 14 (3), 228–236.

[20] OLUSESAN A.T., AZURA L.K., FORGHANI B., BAKAR F.A., MOHAMED A.K.S., RADU S., MANAP M.Y.A., SAARI N., *Purification, characterization and thermal inactivation kinetics of a non-regioselective thermostable lipase from a genotypically identified extremophilic Bacillus subtilis NS 8*, New Biotechnology, 2011, 28, 738–745.

[21] PILAREK M, SZEWCZYK K.W., *Kinetic model of 1,3-specific triacylglycerols alcoholysis catalyzed by lipases*, J. Biotech., 2007, 127, 736–744.

[22] SCHMITKE J.L., WESCOTT C.R., KLIBANOW A.M., *The mechanistic dissection of the plunge in enzymatic activity upon transition from water to anhydrous solvents*, J. Am. Chem. Soc., 1996, 118, 3360–3365.

[23] STOYTCHEVA M., MONTERO G., ZLATEV R., LEÓN J.Á., GOCHEV V., *Analytical methods for lipases activity determination: A review*, Curr. Anal. Chem., 2012, 8, 400–407.

[24] STOTZ M., BARTH D.A., RIEDL J.M., ASAMER E., KLOCKER E.V., KORNPRAT P., HUTTERER G.C., PRINZ F., LACKNER K., STÖGER H., GERGER A., PICHLER M., *The lipase/amylase ratio (LAR) in peripheral blood might represent a novel prognostic marker in patients with surgically resectable pancreatic cancer*, Cancers, 2020, 12, 1798, 1–10.

[25] TSUJITA T., OKUDA H., *Effect of bile salts on the interfacial inactivation of pancreatic carboxylester lipase*, J. Lipid Research, 1990, 31, 831–838.

[26] VELJKOVIĆ D.Ž., RANKOVIĆ V.J., PANKOVIĆ S.B., ROSIĆ M.A., KOJIĆ M.R., *Hyperelastic behavior of porcine aorta segment under extension-inflation tests fitted with various phenomenological models*, Acta Bioeng. Biomech., 2014, 16 (3), 37–45.

[27] WOJCIK M., MIŁEK J., *A new method to determine optimum temperature and activation energies for enzymatic reactions*, Bioprocess Biosyst. Eng., 2016, 39, 1319–1323.

[28] WYCHOWAŃSKI M., OBRĘBSKI M., RĄPAŁA K., WIT A., GAJEWSKI J., MARCZAK K., *Strength of proximal humeral fraction fixation employing implants of various types – a study of porcine bones*, Acta Bioeng. Biomech., 2008, 10 (3), 29–35.