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INVESTIGATION OF THE BASIC LAWS OF THE KINETICS OF BIOCATALYTIC HYDROLYSIS OF VEGETABLE OIL

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Abstract

Since the day a person is born, nutrition plays a key part in maintaining his or her health by shaping the longevity potential. Different kinds of nutrients, ingested through food and transformed into structural elements of cells through complex biochemical processes, provide the body with building blocks and energy, determining health, physical, and mental activity, as well as life expectancy. Among such substances are polyunsaturated fatty acids, known to exert an active effect on blood plasma lipids. In particular, they reduce excess low-density cholesterol and significantly reduce the risk of developing and aggravating diseases caused by atherosclerosis. The biocatalytic hydrolysis of vegetable oils is a promising process for the production of polyunsaturated fatty acids. A comprehensive study of the kinetics and thermodynamics of the above process was carried out, which allowed us to develop a relevant mathematical model and identify the basic laws of kinetics. The rate constants of the direct and reverse reactions running in the reaction systems, as well as the equilibrium constants of each of them, were determined. The data obtained were used to compute the thermodynamic parameters and draw conclusions about the contribution of each reaction to the overall process. The results of this investigation will be a scientific basis for the development of an industrial biocatalytic technology for the hydrolysis of vegetable oils to produce polyunsaturated fatty acids, highly demanded in the food industry.

Keywords: hydrolysis; lipase; biocatalysis; kinetics; thermodynamics.

ДОСЛІДЖЕННЯ ОСНОВНИХ ЗАКОНОМІРНОСТЕЙ КІНЕТИКИ БІОКАТАЛІТИЧНОГО ГІДРОЛІЗУ РОСЛИННОЇ ОЛІЇ

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Анотація

Харчування із самого моменту народження людини відіграє ключову роль у збереженні його здоров'я, формуючи потенціал, що забезпечує довголіття. Різні поживні речовини, надходячи з їжею в організм і переутворюючись у ході метаболізму в результаті складних біохімічних процесів у структурні елементи клітин, забезпечують його пластичним матеріалом і енергією, визначаючи здоров'я, фізичну і розумову активність та тривалість життя людини. Одними із таких речовин є поліненасичені жирні кислоти, які активно впливають на ліпіди плазми крові, а саме зменшують надлишок холестерину низької щільності, дозволяють значно знизити ризик розвитку та прогресування захворювань, спричинених атеросклерозом. Перспективним процесом виробництва поліненасичених жирних кислот є біокаталітичний гідроліз рослинних олій. В роботі здійснено комплексне дослідження кінетики і термодинаміки вказаного процесу, в результаті якого було розроблено відповідну математичну модель та встановлено основні закономірності кінетики. Визначено константи швидкостей прямих і зворотних реакцій, що протікають у реакційних системах, а також константи рівноваги кожної з них. На основі отриманих даних розраховано термодинамічні параметри та зроблено висновки про внесок кожної з реакцій в загальний процес у цілому. Результати дослідження слугуватимуть науковим фундаментом для розробки промислової біокаталітичної технології гідролізу рослинних олій, спрямованої на виробництво поліненасичених жирних кислот, які мають великий попит у харчовій галузі.

Ключові слова: гідроліз; ліпаза; біокаталіз; кінетика; термодинаміка.

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Introduction

Raw materials and production base in the food industry are largely based on biotechnology advances. Biocatalytic processes underpin such industries as brewing, wine making, cheese making, dairy production, baking, juice, and beverage production, as well as the production of ethanol and edible organic acids [1–6]. Over the past decades, entirely new enzyme-based technological processes have been invented, such as the production of glucose-fructose syrups from starch, glucose-galactose syrups from whey, and ethanol from cellulose-containing raw materials [7–10].

The fat and oil industry holds a particular position here. The advances of food biotechnology in other industries cannot be directly applied to fat technology, as the enzymatic processes in fats and oil raw materials are significantly different from those in aqueous systems, for example, when making fermentation products. Complicated biocatalysis in heterogeneous systems and the separation of phases at the final stages of this technology were the reasons why major achievements in applying enzymatic processes in fat and oil production emerged only in recent decades [11–14].

Lipases (triacylglycerol acyl hydrolases) are the main enzymes involved in the fat and oil industry. These biocatalysts differ from other hydrolases in the way they act on substrates (acylglycerols, fatty acids, etc.) that are almost insoluble in water. Similarly to other enzymes, lipases are water-soluble and require water to increase their activity. For this reason, enzymatic reactions take place at the interface boundary between the aqueous and organic phases. Lipases also operate in systems with such solvents as hexane, petroleum ether, etc. [15; 16].

Lipases, more than any other hydrolases, can actually catalyze various types of reactions, namely interesterification [17–20], acidolysis [21], alcoholysis [22], esterification, and lipid hydrolysis [23, 24].

Lipase-mediated modification of fats is currently being researched both abroad and in Ukraine. In particular, biocatalytic technologies are being developed for the production of fats with a minimum content of trans-isomers [25–27], preparation of lipid systems for health purposes [28; 29], and hydrolysis of vegetable oils [30; 31].

The biocatalytic hydrolysis of fats is of particular interest because, unlike the chemical process, it requires no advanced hardware and can be used in small and medium-sized enterprises. Besides, enzymatic hydrolysis of fats is more environmentally friendly than chemical hydrolysis [32].

Lipase-mediated hydrolysis provides a rather simple way to solve complex technological and analytical issues. These include both technological solutions for separating and purifying nutrients (e.g., conjugated linoleic acid isomers) and determining the exact triacylglycerol composition of fats [32].

Another area of application for enzymatic hydrolysis of fats is to add flavor and aroma to food and beverages [33]. Flavoring is produced by the release of C_4–C_{10} fatty acids. For instance, cheese flavors are produced from milk fat using pancreatic esterase. Butter is hydrolyzed by about 60–70 %. For flavoring food products, hydrolyzed butter is added in the amount of 0.2–0.5 % by weight. When making Japanese sake, rice is pretreated with an aqueous lipase solution, which adds flavor and color to the finished product [33].

A promising area for using biocatalytic hydrolysis of oils is the production of polyunsaturated fatty acids. Such acids exert a strong effect on blood plasma lipids. They reduce excess low-density cholesterol and significantly decrease the risk of developing and worsening diseases caused by atherosclerosis, in particular coronary heart disease, myocardial infarction, and stroke due to antithrombotic effects. They are also used for the complex treatment of hyperlipidemia and obesity [34].

Nevertheless, we currently lack systematic knowledge of the kinetics of the biocatalytic hydrolysis of vegetable oils, being one of the main prerequisites for its effective industrial implementation. Therefore, this research is aimed at conducting a comprehensive study of the kinetics and thermodynamics of this process.

Experimental procedures

The model mixtures consisted of sunflower oil (produced by Bunge Limited) and water. The biocatalysis of the hydrolysis was conducted using the enzyme agent NovoCor AD L (produced by Novozymes), which is a solution of microbial nonspecific lipase A Candida antarctica. The molar ratio of water to oil was 3 : 1. The procedure was carried out in two parallels at 50 °C, 60 °C, and 70 °C for 10 hours with constant stirring under a layer of nitrogen. At regular intervals, samples were collected to determine the water content according to the method [35] and to analyze the lipid composition by high-temperature gas-liquid chromatography according to AOCS Official

Method Cd 11b-91 [36]. A Shimadzu GC-2010 Gas Chromatography device (Shimadzu Corporation) equipped with a flame ionization detector (FID) was used for the analysis. The capillary column was HP-5. Geometric parameters of the column: 30 m length, 0.25 mm internal diameter, and 0.25 μm thickness of the stationary phase. The stationary phase was 5 % biphenyl – 95 % dimethylpolysiloxane. Temperature program was 80 °C (0 min.), 10 °C/min. up to 320 °C (0 min.), 5 °C/min. up to 360 °C (15 min.) Injector temperature was 320 °C, detector temperature was 370 °C. Carrier gas was helium. Carrier gas flow rate was 3 cm^3/min. Split 1 : 50. The air flow rate for FID was 450 cm^3/min and the hydrogen flow rate for FID was 45 cm^3/min. The injected sample volume was 0.5 μL.

The experimental data obtained (the average value of two parallel studies) were used as input for modeling the kinetics of biocatalytic hydrolysis of vegetable oil.

The mathematical modeling was supported by the results of our previous studies [31], indicating that the process of oil hydrolysis is driven by three simultaneous reactions:

$$TAG + W \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} DAG + FA$$

$$DAG + W \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} MAG + FA$$

$$MAG + W \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} GL + FA,$$

where TAG means triacylglycerols, the main component of vegetable oil; W means water; FA means fatty acids; DAG means diacylglycerols; MAG means monoacylglycerols; GL means glycerol;

k_1, k_2, and k_3 are the rate constants of the direct reactions; k_{-1}, k_{-2}, k_{-3} are the corresponding values for the reverse reactions.

The above reactions were used to develop a system of nonlinear differential equations describing the time-varying concentrations of the initial substrates and reaction products:

$$\begin{cases} \frac{\partial[TAG]}{\partial\tau} = -k_1[TAG][W] + k_{-1}[DAG][FA] \\ \frac{\partial[DAG]}{\partial\tau} = k_1[TAG][W] - k_{-1}[DAG][FA] - k_2[DAG][W] + k_{-2}[MAG][FA] \\ \frac{\partial[MAG]}{\partial\tau} = k_2[DAG][W] - k_{-2}[MAG][FA] - k_3[MAG][W] + k_{-3}[GL][FA] \\ \frac{\partial[FA]}{\partial\tau} = k_1[TAG][W] - k_{-1}[DAG][FA] + k_2[DAG][W] - k_{-2}[MAG][FA] + k_3[MAG][W] - k_{-3}[GL][FA] \\ \frac{\partial[GL]}{\partial\tau} = k_3[MAG][W] - k_{-3}[GL][FA] \\ \frac{\partial[W]}{\partial\tau} = -k_1[TAG][W] + k_{-1}[DAG][FA] - k_2[DAG][W] + k_{-2}[MAG][FA] - k_3[MAG][W] + k_{-3}[GL][FA] \end{cases}$$

The modeling process was performed in the Mathcad environment (Parametric Technology Corporation) and involved identifying the parameters of the developed model using the optimization method to minimize errors between experimental and model data.

The Runge-Kutta method with a fourth-order accuracy of variable step was used as a numerical procedure for modeling differential equations. The data obtained with this method were used to compute the objective function *J(k)*, which actually represented the error between the experimental and model data. It looked like this:

$$J(k) = \sum_{i=0}^{m} \|x_i - \bar{x}(t_i, x^0, k)\|^2,$$

where x_i means the values of the vector of system states obtained experimentally at time t_i; $\bar{x}(t_i, x^0, k)$ means the values of the vector of system states computed with the selected

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simulation modeling procedure at time t_i with initial conditions $x^0 = x_0 = \bar{x}(t_i, x^0, k)$; k means the vector of system parameters; and m means the number of experimental data sets.

A random multidimensional search method, the method of complexes, was used for optimization.

The following condition was used as a termination criterion:

$$\frac{1}{z}\sqrt{\sum_{i=1}^{z}(J(k)_i - J(k)_c)^2} \leq \varepsilon,$$

where $J(k)_c$ means the value of the minimized function in the center of the complex; z means the number of vertices of the hypersphere; and ε means the solution accuracy ($\varepsilon=10^{-3}$).

Results and discussion

Processing the experimental data mathematically in the Mathcad environment allowed us to determine the numerical values of the rate constants of the direct and reverse reactions running during the oil hydrolysis, as well as their equilibrium constants at temperatures of 50 °C, 60 °C, and 70 °C (Table 1 and Table 2, respectively).

Table 1

Rate constants (mole fraction^{-1}·hour^{-1})

Reaction	Values of rate constants at different temperatures					
	50 °C		60 °C		70 °C	
	Direct	Reverse	Direct	Reverse	Direct	Reverse
TAG + W $\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}}$ DAG + FA	0.0305	0.0023	0.0794	0.0042	0.1296	0.0052
DAG + W $\underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}}$ MAG + FA	0.0195	0.0018	0.0413	0.0031	0.0653	0.0038
MAG + W $\underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}}$ GL + FA	0.0086	0.0011	0.0181	0.0019	0.0247	0.0021

Table 2

Equilibrium constants (K)

Reaction	Values of equilibrium constants at different temperatures		
	50 °C	60 °C	70 °C
TAG + W $\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}}$ DAG + FA	13.26	18.90	24.92
DAG + W $\underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}}$ MAG + FA	10.83	13.32	17.18
MAG + W $\underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}}$ GL + FA	7.82	9.53	11.76

The data presented in Table 1 and Table 2 made it possible to compute the thermodynamic parameters of the reactions of vegetable oil hydrolysis according to the Arrhenius method [37], as well as the values of the activation energy for direct and reverse reactions, which are presented in Table 3 and Table 4, respectively.

Table 3

Thermodynamic parameters

Reaction	ΔH, kJ/mol	ΔG, kJ/mol			ΔS, kJ/(mol·K)		
		50 °C	60 °C	70 °C	50 °C	60 °C	70 °C
TAG + W $\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}}$ DAG + FA	29.09	-6.94	–8.14	–9.17	0.11	0.11	0.11
DAG + W $\underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}}$ MAG + FA	21.22	-6.40	–7.17	–8.11	0.09	0.09	0.09
MAG + W $\underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}}$ GL + FA	18.78	-5.52	–6.24	–7.03	0.08	0.08	0.08

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Table 4

Activation energy, E_a (kJ/mol)

Reaction	Direct reaction	Reverse reaction
TAG + W $\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}}$ DAG + FA	46.53	20.28
DAG + W $\underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}}$ MAG + FA	43.51	19.34
MAG + W $\underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}}$ GL + FA	29.52	9.50

When analyzing the data presented in Table 3 and Table 4, it can be deduced that all reactions are endothermic, i.e., they involve heat absorption, as evidenced by the positive values of the thermal effect ΔH. The values of ΔG are negative for all reaction stages at the temperatures in question, indicating their thermodynamic advantage. As the temperature increases for the given reactions, the values of ΔG decrease, i.e., reactions are more favorable towards the creation of products. The fact that the activation energies of direct reactions prevail over those of reverse reactions for all three stages is additional proof of the kinetic disequilibrium in favor of direct reactions. Furthermore, the first reaction is far more complicated since, compared to the second and third reactions, it has the largest activation energy, i.e., it is the limiting one for the entire hydrolysis process.

Conclusions

A comprehensive study of the kinetics and thermodynamics of biocatalytic hydrolysis of vegetable oil was carried out, which allowed us to develop a relevant mathematical model and identify the basic laws of the kinetics of this process. The rate constants of the direct and reverse reactions running in reaction systems, as well as the equilibrium constants of each of them, were determined. The data obtained were used to compute the thermodynamic parameters and make conclusions about the contribution of each reaction to the overall process. The results of this investigation will be a scientific basis for the development of an industrial biocatalytic technology for the hydrolysis of vegetable oils to produce polyunsaturated fatty acids, highly demanded in the food industry.

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