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KINETICS AND THERMODYNAMICS OF BIOCATALYTIC GLYCEROLYSIS OF TRIACYLGLYCEROLS ENRICHED WITH OMEGA-3 POLYUNSATURATED FATTY ACIDS

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The enrichment of fats with omega-3 polyunsaturated acids increases the nutritive value of fats and makes them more health promoting by boosting the immune system and reducing blood coagulability, the level of neutral lipids and the risk of coronary heart disease and atherosclerosis. That is why it is important to develop scientific basis of the synthesis of these lipid systems. There have been created the mathematic model of kinetics of enzymatic glycerolysis of triacylglycerols enriched with omega-3 polyunsaturated fatty acids, enabling quantitative and qualitative assessment of the reaction composition. For the mathematic modeling of the process, a system of nonlinear differential equations has been created which describes the change in the content of the resulting substrates and reaction products through time. To determine acylglycerol composition of the reaction systems, the high temperature gas-liquid chromatography method was used. The simulation was performed using Mathcad 15 environment via the identification of the parameters of the model by means of a random multivariable search algorithm being called a method of complexes, in the course of which the errors between experimental and model data were minimized. The Runge–Kutta method with a variable step of the fourth order of accuracy was used as a numerical procedure for differential equations modelling. As a result of the calculations, the numerical values of the constants of direct and reverse reactions rates and the corresponding equilibrium constants were determined. Based on the received constants of homogeneous substrates mixtures the values of thermodynamic parameters and activation energies of the studied process have been calculated. The conclusions have been drawn about the contribution of each reaction, occurring during biocatalytic glycerolysis of triacylglycerols, to the process under consideration.

Keywords: glycerolysis, biocatalysis, triacylglycerol, omega-3 polyunsaturated fatty acid, method of complexes.

Introduction

The development of new techniques of production of health promoting foods, that are aimed at protecting and preserving human health, is one of the current social issues in these latter days [1]. Up-to-date scientific practice in the field of healthy nutrition shows that regular consumption of food compounds reducing the level of neutral fat in the blood prevents atherosclerosis-related diseases such as coronary heart disease, myocardial infarction, hyperlipidemia and obesity [2]. Thus, the innovative direction in the development of the fat and oil industry is the production of fatty foods enriched with diacylglycerols containing acyls of omega-3 polyunsaturated fatty acids. These acids constitute a part of phospholipids of all cell membranes, that ensure impulse transmission and receptors functioning, and are precursors in the synthesis of lipid mediators, which are crucial for the regulation of a number of physiological processes. Additionally, omega-3 fatty acids boost the immune system and reduce blood coagulability, the risk of coronary heart disease and atherosclerosis. They are involved in the early stages of inflammatory process being able to fight inflammation without suppression of the immunodefences and slowing wounds healing [3].

Today, chemical glycerolysis of fats, which are the mixtures of acylglycerols, such as triacylglycerols, with the substances of lipid and non-lipid nature, is the main diacylglycerols production technique. The course of reaction is influenced at high temperatures (over 200^0C) by alkaline catalysts: calcium or sodium

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hydroxides [4]. A low yield of final product, the irreversible loss of the part of catalyst, and the difficulty of its removing from the reaction are the disadvantages of the process. Another drawback is a sufficiently high temperature that increases energy consumption and causes the destruction of thermo labile fatty components, such as acylglycerols containing omega-3 polyunsaturated fatty acids. Biocatalytic process allows preventing these disadvantages. However, the kinetics of this process has not been adequately investigated.

The paper is aimed at a comprehensive study and analysis of kinetics and thermodynamics of biocatalytic glycerolysis of triacylglycerols enriched with omega-3 polyunsaturated fatty acids, targeted at the synthesis of corresponding diacylglycerols.

Experimental

Linseed oil, the triacylglycerols of which contain polyunsaturated α-linolenic omega-3 acid, was chosen as the model triacylglycerols containing acyls of polyunsaturated acids [5].

Model mixtures contained linseed oil and glycerol in a molar ratio of 1:1. To form a homogeneous mixture of substrates, a mixture of tert-butanol and tert-pentanol in a volume ratio of 1:1 was used as a solvent. Biocatalytic activity in the glycerolysis process was investigated using the lipolytic preparation Novozym 435 (Novozymes, Denmark). The amount of biocatalyst was 10 wt.% relative to the mass of substrates. The process was performed at the temperatures of 50, 60 and 70^{0}C for 6 hours with constant stirring under a nitrogen layer. At specified intervals, the samples were taken and analyzed by a high-temperature gas-liquid chromatography method according to AOCS Official Method Cd 11b-91 [6]. Clarus 500 Gas Chromatography Chromatograph (Perkin-Elmer) was used equipped with a flame-ionization detector (FID). Restek Rtx-65TG capillary column was used; its geometric parameters were as follows: the length of 30 m, the inner diameter of 0.25 mm and the stationary phase thickness of 0.2 microns. A mixture of 35% dimethyl and 65% diphenylpolysiloxane (Crossbond) was a stationary phase. The temperature program was as follows: 80^{0}C (0 min), 10^{0}C/min up to 320^{0}C (0 min), 5^{0}C/min up to 360^{0}C (15 min) The injector temperature was 320^{0}C, the detector temperature was 370^{0}C. Helium was a carrier gas. The speed of carrier gas was 3 cm^3/min. The split was 1:50. The air consumption for FID was 450 cm^3/min and the hydrogen consumption for FID was 45 cm^3/min. The volume of the sample was 0.5 μL. Analysis was made in two parallels. The peak identification and calibration were performed according to Sigma-Aldrich standards.

Mathematic modeling of the biocatalytic glycerolysis process

Biocatalytic glycerolysis of triacylglycerols enriched with omega-3 polyunsaturated fatty acids is provided by the simultaneous occurrence of three reactions as follows:

$$\text{TAG + GL} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{DAG +MAG;}$$

$$\text{DAG + GL} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{2MAG;}$$

$$\text{TAG +MAG} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \text{2DAG,}$$

where k_1, k_2 and k_3 are the rate constants of direct reactions, and k_{-1}, k_{-2}, k_{-3} are the rate constants of reverse reactions, correspondingly.

In the equations, the following abbreviations are used: TAG, GL, DAG and MAG represent triacylglycerols, glycerol, diacylglycerols and monoacylglycerols, respectively.

To conduct mathematical simulation of the process of biocatalytic glycerolysis of triacylglycerols enriched with omega-3 polyunsaturated fatty acids, a system of nonlinear differential equations was created which describes the change in the content of the resulting substrates and reaction products through time as follows:

$$\left.\begin{aligned}
\frac{d[TAG]}{d\tau} &= -k_1[GL][TAG] + k_{-1}[DAG][MAG] - \\
&\quad - k_3[TAG][MAG] + k_{-3}[DAG]^2; \\
\frac{d[DAG]}{d\tau} &= k_1[GL][TAG] - k_{-1}[TAG][MAG] - \\
&\quad - k_2[GL][DAG] + k_{-2}[MAG]^2 + \\
&\quad + 2k_3[TAG][MAG] - 2k_{-3}[DAG]^2; \\
\frac{d[MAG]}{d\tau} &= k_1[GL][TAG] - k_{-1}[DAG][MAG] + \\
&\quad + 2k_2[GL][DAG] - 2k_{-2}[MAG]^2 - \\
&\quad - k_3[TAG][MAG] + k_{-3}[DAG]^2; \\
\frac{d[GL]}{d\tau} &= -k_1[GL][TAG] + k_{-1}[DAG][MAG] - \\
&\quad k_2[GL][DAG] + k_{-2}[MAG]^2.
\end{aligned}\right\} \quad (1)$$

The modeling was performed in the Mathcad 15 environment (Parametric Technology Corporation) that implied identifying the parameters of the model using an optimization method, in which the errors between experimental and model data were minimized [7]. The general algorithm scheme is presented in Fig. 1.

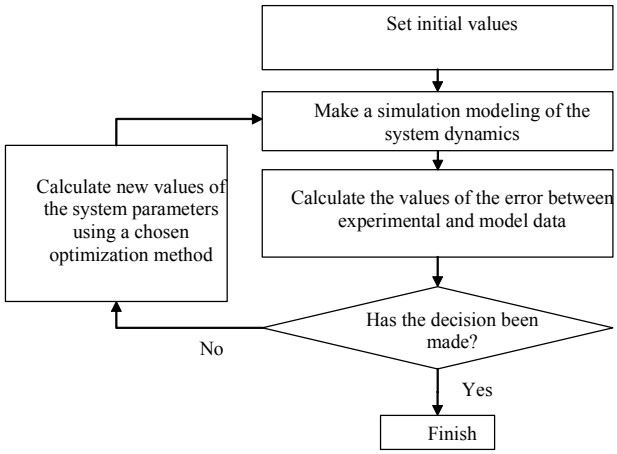

Fig. 1. General scheme of parameters identification algorithm

The Runge-Kutta method with a variable step of the fourth order of accuracy was used as a numerical procedure for differential equations modeling. The data received by means of this method were used to calculate the target J(k) function, actually being an error between the experimental and model data, and was as follows:

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

where x_i is the system states vector value that was received experimentally at the time t_i; $\bar{x}\left(t_i, x^0, k\right)$ is the system states vector value calculated using a chosen procedure of simulation modeling at the time t_i with initial conditions $x^0 = x_0 = \bar{x}\left(t_0, x^0, k\right)$; k is the system parameters vector; and m is the number of sets of experimental data.

An algorithm of random multivariable search was used for optimization, it is called «the method of complexes».

This method allows resolving the main issue of optimization of nonlinear multidimensional systems, including the one being studied, in particular «stalling» into local optimums due to their significant «raving». The solution is provided by the method which makes it possible to randomly select the hyperspace within the range and relatively initial conditions as well as new directions of search of a solution in the case of failure at some current optimization step.

The following condition was used as a criterion for iterations finish:

$$\frac{1}{z}\sqrt{\sum_{i=1}^{z}\left(J(k)_i - J\left(k\right)_c\right)^2} \le \varepsilon , \qquad (3)$$

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

Results and discussions

Using the program developed according to the algorithm given in Fig. 1, the experimental data were processed and the numerical values of the rate constants were determined for the direct and reverse reactions occurring during glycerolysis of triacylglycerols enriched with omega-3 polyunsaturated fatty acids; their equilibrium constants (Tables 1 and 2) at 50, 60 and 70°C under the conditions of the substrate system homogeneity were calculated too.

The data given in Tables 1 and 2 allow calculating the thermodynamic parameters of the reactions of glycerolysis of triacylglycerols enriched

Table 1

The rate constants of the reactions

Reaction	The values of the rate constants at different temperatures (mole fraction^{-1}·hour^{-1})					
	50°C		60°C		70°C	
	direct	reverse	direct	reverse	direct	reverse
TAG + GL $\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}}$ DAG +MAG	0.0761	0.0011	0.1165	0.0014	0.1752	0.0015
DAG + GL $\underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}}$ 2MAG	0.0460	0.0016	0.0655	0.0017	0.1206	0.0020
TAG + MAG $\underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}}$ 2DAG	0.0104	0.0006	0.0202	0.0009	0.0334	0.0011

with omega-3 polyunsaturated fatty acids at specified temperatures. Thus, the dependences lnK=f(1/T) (Fig. 2) were built in half-logarithmic coordinates.

Table 2

The equilibrium constants of the reactions

Reaction	The values of the equilibrium constants at different temperatures		
	50^0C	60^0C	70^0C
TAG + GL $\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}}$ DAG +MAG	69.18	83.21	116.80
DAG + GL $\underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}}$ 2MAG	28.75	38.53	60.30
TAG + MAG $\underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}}$ 2DAG	17.33	22.44	30.36

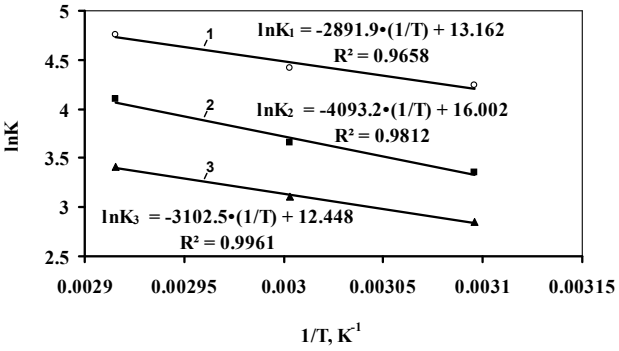

Fig. 2. Plot of the logarithm of equilibrium constants vs. the reciprocal of temperature: 1 – TAG + GL $\rightleftharpoons$ DAG +MAG; 2 – DAG + GL $\rightleftharpoons$ 2MAG; 3 – TAG + MAG $\rightleftharpoons$ 2DAG

The dependences presented in Fig. 2 for each reaction are governed by the straight line equations with the corresponding slope. Thus, the numerical values of the reaction heat (ΔH) can be calculated according to the formula:

$$\Delta H = -tg\alpha \cdot R, \quad (4)$$

where tgα is the slope and R is the universal gas constant (8.314 J mol^{-1} K^{-1}).

The values of Gibbs energy (ΔG) and entropy (ΔS) in the temperature range of 50 to 70^0C (i.e. 323–343 K) were determined according to the formulae (5) and (6), accordingly.

$$\Delta G = -RT \ln K, \quad (5)$$

where K is the equilibrium constant at the temperature T.

$$\Delta S = \frac{\Delta H - \Delta G}{T}. \quad (6)$$

The values of the activation energies (E_a) of direct and reverse reactions in the temperature range of 60 to 70^0C (333–343 K) were calculated according to the following formula:

$$E_a = \frac{RT_1T_2}{T_2 - T_1} \ln \frac{k_{T_2}}{k_{T_1}}, \quad (7)$$

where k_{T_1} and k_{T_2} are the rate constants at the temperatures of T_1 and T_2, respectively.

The numerical values of the thermodynamic characteristics of the studied reactions and their activation energies are summarized in Tables 3 and 4.

Having analyzed the data given in Tables 3 and 4, it has been concluded that all reactions are endothermic, that is, heat absorbing. This is shown by the positive values of the heat ΔH effect and domination of the numerical values of the activation energy in the forward direction over the corresponding values for the reverse ones.

As the temperature for the specified reactions rises, the ΔG value decreases, meaning that reaction equilibrium sifts toward the formation of the reaction

Table 3

The thermodynamic characteristics of the reactions

Reaction	ΔH, kJ/mol	ΔG, kJ/mol			ΔS, kJ/(mol·K)		
		50^0C	60^0C	70^0C	50^0C	60^0C	70^0C
TAG + GL $\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}}$ DAG +MAG	24.04	–11.38	–12.24	–13.58	0.11	0.11	0.11
DAG + GL $\underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}}$ 2MAG	34.03	–9.02	–10.11	–11.69	0.13	0.13	0.13
TAG + MAG $\underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}}$ 2DAG	25.80	–7.66	–8.61	–9.73	0.10	0.10	0.10

products. Additionally, the second and third reactions are hindered to a greater degree than the first one, since the following inequality is valid: $\Delta G_1 < \Delta G_2 < \Delta G_3$. Comparing the values of the activation energies given in Table 4, it may be predicted that the temperature dependence of the diacylglycerols output has a maximum, after which there will be a decrease of this indicator due to the fast increase of the second reaction rate, having the greatest value of the activation energy if compared to the first and third ones.

Table 4

The activation energies of the reactions

Reaction	E_a, kJ/mol	
	direct	reverse
TAG + GL $\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}}$ DAG +MAG	38.75	6.55
DAG + GL $\underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}}$ 2MAG	57.97	15.43
TAG + MAG $\underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}}$ 2DAG	47.76	19.06

Conclusions

1. The main characteristics of the kinetics of biocatalytic glycerolysis of triacylglycerols enriched with omega-3 polyunsaturated fatty acids were determined.

2. The rate constants of the direct and reverse reactions occurring in the reaction systems and the corresponding equilibrium constants were calculated.

3. Based on the obtained data, the thermodynamic parameters were calculated and the conclusions were drawn about the contribution of each reaction to the general processes.

REFERENCES

1. *Tkachenko N.A., Nekrasov P.O., Vikul S.I.* Optimizatsiya retsepturnogo skladu napoyu ozdorovchogo pryznachenn'ya na osnovi syrovatky // Eastern-European Journal of Enterprise Technologies. – 2016. – Vol.79. – No. 1/10. – P.49-57.

2. *Optimization* of formulation composition of the low-calorie emulsion fat systems / Tkachenko N., Nekrasov P., Makovska T., Lanzhenko L. // Eastern-European Journal of Enterprise Technologies. – 2016. – Vol.81. – No. 3/11. – P.20-27.

3. *Hegde M.V., Zanwar A.A., Adekar S.P.* Omega-3 fatty acids: keys to nutritional health. – Switzerland: Springer International Publishing, 2016. – 610 p.

4. *O'Brien R.D.* Fat and oils: formulating and processing for applications. – Boca Raton: Taylor & Francis Group, 2009. – 745 p.

5. *Chemat S.* Edible oils: extraction, processing, and applications. – Boca Raton: CRC Press, 2017. – 263 p.

6. *Firestone D.* Official methods and recommended practices of the American Oil Chemist's Society. – Champaign: American Oil Chemists' Society, 2003. – 1200 p.

7. *Korobov V., Ochkov V.* Chemical kinetics with Mathcad and Maple. – Wien: Springer-Verlag, 2011. – 344 p.

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КІНЕТИКА І ТЕРМОДИНАМІКА БІОКАТАЛІТИЧНОГО ГЛІЦЕРОЛІЗУ ТРИАЦИЛГЛІЦЕРИНІВ, ЗБАГАЧЕНИХ ОМЕГА-3 ПОЛІНЕНАСИЧЕНИМИ ЖИРНИМИ КИСЛОТАМИ

П.О. Некрасов, О.М. Півень, О.П. Некрасов, О.М. Гудзь, Н.О. Кривоніс

Збагачення жирів омега-3 поліненасиченими кислотами збільшує їх харчову цінність і робить більш корисними для здоров'я людини, включаючи покращення імунітету, зменшення згортання крові та рівня в неї нейтральних ліпідів, а також ризику коронарної хвороби серця й атеросклерозу. Тому розробка наукових основ синтезу вказаних ліпідних систем є актуальною задачею. В роботі створено математичну модель кінетики ферментативного гліцеролізу триацилгліцеринів, збагачених омега-3 поліненасиченими жирними кислотами, яка дозволяє здійснювати якісне та кількісне оцінювання складу реакційних сумішей. Для математичного моделювання процесу було складено систему нелінійних диференціальних рівнянь, що описують зміну вмісту вихідних субстратів і продуктів реакцій у часі. Ацилгліцериновий склад реакційних систем визначався методом високотемпературної газорідинної хроматографії. Моделювання здійснювалося в середовищі Mathcad 15 і полягало в ідентифікації параметрів моделі з використанням алгоритму випадкового багатовимірного пошуку – методу комплексів, в ході якого проводилася мінімізація помилок між експериментальними і модельними даними. Як чисельну процедуру моделювання диференціальних рівнянь використано метод Рунге Кутта зі змінним кроком четвертого порядку точності. В результаті було визначено чисельні значення констант швидкостей прямих і зворотних реакцій, а також відповідних констант рівноваги. На основі отриманих констант для гомогенних сумішей субстратів розраховано термодинамічні параметри та значення енергії активації для досліджуваного процесу. Зроблено висновки про внесок кожної з реакцій, що протікають при біокаталітичному гліцеролізі триацилгліцеринів, в перебіг загального процесу у цілому.

Ключові слова: гліцероліз; біокаталіз; триацилгліцерин; омега-3 поліненасичена кислота; метод комплексів.

KINETICS AND THERMODYNAMICS OF BIOCATALYTIC GLYCEROLYSIS OF TRIACYLGLYCEROLS ENRICHED WITH OMEGA-3 POLYUNSATURATED FATTY ACIDS

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REFERENCES

1. Tkachenko N.A., Nekrasov P.O., Vikul S.I. Optimizatsiya retsepturnogo skladu napoyu ozdorovchogo pryznachenn'ya na osnovi syrovatky [Optimization of formulation composition of health whey–based beverage]. *Eastern-European Journal of Enterprise Technologies*, 2016, vol. 79, no. 1/10, pp. 49-57. (*in Ukrainian*).

2. Tkachenko N., Nekrasov P., Makovska T., Lanzhenko L. Optimization of formulation composition of the low-calorie emulsion fat systems. *Eastern-European Journal of Enterprise Technologies*, 2016, vol. 81, no. 3/11, pp. 20-27.

3. Hegde M.V., Zanwar A.A., Adekar S.P., *Omega-3 fatty acids: keys to nutritional health*. Springer International Publishing, Switzerland, 2016. 610 p.

4. O'Brien R.D., *Fat and oils: formulating and processing for applications*, *3rd ed.* Taylor & Francis Group, Boca Raton, USA, 2009. 745 p.

5. Chemat S., *Edible oils: extraction, processing, and applications.* CRC Press, Boca Raton, USA, 2017. 263 p.

6. Firestone D., *Official methods and recommended practices of the American Oil Chemist's Society, 5th ed.,* American Oil Chemists' Society, Champaign, Il, USA, 2003. 1200 p.

7. Korobov V., Ochkov V., *Chemical kinetics with Mathcad and Maple.* Springer-Verlag, Wien, 2011. 344 p.