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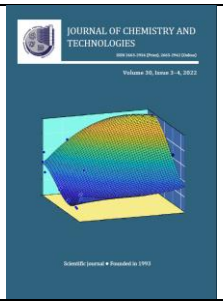

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STUDY OF BIOCATALYTIC SYNTHESIS OF PHYTOSTEROL ESTERS AS FORMULATION COMPONENTS OF NUTRITIONAL SYSTEMS FOR HEALTH PURPOSES

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Abstract

The development of new domestic food technologies aimed at protecting and preserving human health is among the most urgent social challenges of our time. Phytosterols and their derivatives, in particular esters, are the perspective formulation components of food products for health purposes. These compounds regulate plasma cholesterol levels, prevent diseases associated with atherosclerosis, reduce cancer risk, and have anti-inflammatory, antifungal, and antibacterial effects. Currently, phytosterol esters are mainly produced by chemical esterification and transesterification, which involve several drawbacks, in particular, excessive energy consumption, by-product emission, product darkening, low selectivity, and the necessity to remove the catalyst from the product. All the listed drawbacks of chemical technologies are excluded when synthesizing phytosterol esters by biocatalytic esterification, establishing the rational parameters of which was the purpose of this study. The study objective was solved through the response surface methodology. The unknown values of the parameters were determined by applying regression model algorithms. Minimization of the deviation potential was performed by finding appropriate combinations of the predictor experimental series. The Statistica 10 program was used for modeling, experimental data processing, and statistical calculations. A mathematical model was developed to predict the degree of feedstock conversion into products based on the process conditions. The conducted studies made it possible to establish the rational values for biocatalytic esterification aimed at synthesizing esters of phytosterol and fatty acids with a temperature of 58 °C and reaction time of 435 minutes.

Keywords: phytosterol; lipase; biocatalysis; esterification; ester; mathematical modeling.

ДОСЛІДЖЕННЯ БІОКАТАЛІТИЧНОГО СИНТЕЗУ СКЛАДНИХ ЕФІРІВ ФІТОСТЕРОЛІВ ЯК РЕЦЕПТУРНИХ КОМПОНЕНТІВ ХАРЧОВИХ СИСТЕМ ОЗДОРОВЧОГО ПРИЗНАЧЕННЯ

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Анотація

Перспективними рецептурними компонентами харчових продуктів оздоровчого призначення є фітостероли та їх похідні, зокрема складні ефіри. Вони нормалізують рівень холестерину в плазмі крові, запобігають виникненню хвороб, пов'язаних з атеросклерозом, знижують ризик раку і мають протизапальну, протигрибкову та антибактеріальну активність. У теперішній час естери фітостеролів отримують здебільшого шляхом хімічної етерифікації та трансетерифікації, які мають низку вад, зокрема надмірне споживання енергії, утворення побічних речовин, потемніння продуктів, низька селективність, необхідність видалення каталізатора з продукту. Перелічені вади виключаються у випадку синтезу естерів фітостеролів методом біокаталітичного етерифікування, встановленню раціональних параметрів якого було метою представленого дослідження. Задача дослідження вирішувалась з використанням методології поверхні відклику. Визначення невідомих значень вектора параметрів здійснювали шляхом застосування алгоритмів регресійного аналізу. Мінімізацію функціоналу відхилу виконували шляхом знаходження відповідних комбінацій експериментальних рядів предикторів. Розроблено математичну модель, яка дозволяє на основі даних щодо умов процесу прогнозувати ступінь перетворення вихідної сировини в продукти реакції. Проведені дослідження дозволили встановити раціональні значення основних параметрів біокаталітичного етерифікування, спрямованого на синтез складних ефірів фітостеролів та жирних кислот: температура 58 °С, час реакції 435 хвилин.

Ключові слова: фітостерол; ліпаза; біокаталіз; етерифікування; складний ефір; математичне моделювання.

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Introduction

The modern world is facing health crises that particularly threaten the most vulnerable populations, such as people with weakened immune systems, as well as metabolic and cardiovascular diseases. Additional risk factors in today's urbanized society include a sedentary lifestyle combined with the consumption of so-called low-quality fast food.

The development of food products and ingredients containing substances that have a positive effect on physiological processes in the human body is one of the ways to solve this issue.

Lately, Ukraine has been researching the production of prebiotic and symbiotic fat emulsion systems for health purposes [1], as well as developing biocatalytic technologies for the synthesis of lipid systems enriched with omega-3 polyunsaturated fatty acids [2]. One more way to improve food products in terms of quality and safety is to solve the issue of minimizing the content of trans fatty acid isomers, which are harmful to human health. This issue is suggested to be solved through technologies that do not lead to the trans isomerization of fatty acyls [3; 4], as well as the new generation of fatty systems for special purposes known as the oleogels [5].

Phytosterols and their derivatives are the perspective formulation components of food products for health purposes [6–8]. These compounds regulate plasma cholesterol levels, both total and low-density lipoprotein-bound ones [9–11]. Meanwhile, the decrease in absorption of both bile (endogenous) and dietary (exogenous) cholesterol does not affect the level of high-density lipoproteins. These combined actions improve the control of blood cholesterol fractions [12–14]. Owing to this hypocholesterolemic effect, the consumption of phytosterols prevents the occurrence of atherosclerosis-related diseases, such as cardiovascular diseases, hyperlipidemia, and obesity [15–19]. Besides, phytosterols reduce cancer risk and have anti-inflammatory, antifungal, and antibacterial effects [20–23].

Extensive preclinical and clinical studies have confirmed the safety and positive effects of phytosterols and related esters on the body [24; 25]. Based on this evidence, many countries such as the European Union, the United States of America, Canada, China, and Japan have approved these substances in food. However, the widespread use of phytosterols in the food industry is hampered because they are poorly soluble in oils. In addition, free phytosterols have low bioavailability in the human body [26].

It is suggested to use appropriate esters to expand the potential of phytosterols as formulation components of fatty food systems and improve their bioavailability [27].

Currently, phytosterol esters are mainly produced by chemical esterification and transesterification [28]. However, chemical synthesis methods involve several drawbacks, including excessive energy consumption, by-product emission, product darkening, low selectivity, and the necessity to remove the catalyst from the product.

The production of phytosterol esters by biocatalysis is an eco-friendly technology with great potential for use in the industrial manufacture of these products.

The study was aimed at determining the rational parameters of the biocatalytic esterification for the synthesis of esters of phytosterol and fatty acids, which would be further used as formulation components of health food products.

Experimental procedure

The model mixtures consisted of fatty acids of sunflower oil (produced by Cargill) and β-sitosterol (produced by Sigma-Aldrich) in a molar ratio of 1 : 1.

The esterification process was carried out with constant stirring at a speed of 400 rpm. The isooctane was used as a solvent to produce a homogeneous mixture of substrates. The resulting water was collected using molecular sieve UOP Type 3 Å (manufactured by Fluka). The Novozym 435 enzyme preparation (Candida antarctica lipase B immobilized on a macroporous resin) manufactured by Novozymes, Denmark, was used as a biocatalyst. The content of the preparation in the reaction mixture was equal to 10 % by the weight of the substrates.

The optimization criterion was the degree of conversion (DC, %) of fatty acids into the corresponding esters, calculated according to equation (1):

$$DC = 100\% - \frac{AV}{AV_0} \cdot 100, \qquad (1)$$

where AV – the acid value of the mixture after esterification, mg KOH/g; AV_0 – the acid value of the initial mixture, mg KOH/g. Acid values were determined according to the method [29].

Temperature and time of the esterification process were chosen as independent factors that were varied.

The response surface methodology [30] was used to optimize the esterification process, which refers to a set of mathematical and statistical techniques aimed at modeling processes and finding combinations of predictor experimental series to optimize the response function, which is generally described by the following polynomial:

$$\hat{y}(x,a) = a_0 + \sum_{l=1}^{n} a_l x_l + \sum_{k=1}^{n} a_k x_k^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} a_{ij} x_i x_j, \quad (2)$$

where $x \in R^n$ – variable vector, a – parameter vector.

The unknown values of the a parameter vector were determined by applying regression model algorithms and optimization (minimization) of the deviation potential (3):

$$J(x) = \sum_{i=0}^{m} \|y_i - \hat{y}(x,a)\|^2, \quad (3)$$

where m – the amount of y experimental data.

The Statistica 10 (StatSoft, Inc.) program was used for modeling, experimental data processing, and statistical calculations.

Results and discussion

The central composite design was used in the study. Regarding the process under study, the response function (4) was chosen, which is a two-stage polynomial:

$$DC = b_0 + b_1 \cdot t + b_{11} \cdot t^2 + b_2 \cdot \tau + b_{22} \cdot \tau^2 + b_{12} \cdot t \cdot \tau, \quad (4)$$

where DC – degree of conversion, %; b_0 – constant; t – process temperature, °C; τ – process time, minutes; b_1, b_{11}, b_2, b_{22}, b_{12} – coefficients for each element of the polynomial.

The choice of the variation levels and intervals was based on the previous experiments: the process temperature was varied from 40 to 70 °C, and the time was varied from 180 to 480 minutes. To reduce the impact of systematic errors caused by external conditions, the sequence of experiments was randomized.

See Table 1 for the design matrix and experimental values of the response function (average results of three parallel studies).

Table 1

Design matrix and response function values

Experiment number	Temperature, t		Time, τ		Degree of conversion (DC), %
	Coded level	°C	Coded level	Minutes	
1	0	55	$-\sqrt{2}$	180	51.5
2	0	55	0	330	84.3
3	−1	44	−1	224	49.0
4	+1	66	−1	224	68.1
5	0	55	$+\sqrt{2}$	480	93.0
6	$-\sqrt{2}$	40	0	330	58.2
7	0	55	0	330	85.8
8	+1	66	+1	436	87.5
9	$+\sqrt{2}$	70	0	330	81.0
10	0	55	0	330	86.0
11	−1	44	+1	436	79.3

Processing experimental data with Statistica 10 provided the model equation with the calculated coefficients:

$$DC = -306.322_0 + 8.998 \cdot t - 0.069 \cdot t^2 + 0.632 \cdot \tau - 0.001 \cdot \tau^2 - 0.002 \cdot t \cdot \tau, \quad (5)$$

To test the significance of the regression coefficients (5), a Pareto Chart was drawn, which is presented in Fig. 1 (L – linear effect, Q – quadratic effect).

The Pareto Chart (Fig. 1) shows the standardized coefficients ordered by absolute values. The data analysis reveals that all columns cross the vertical line, which is a 95 % confidence level. Thus, we can conclude that all regression coefficients (5) are significant.

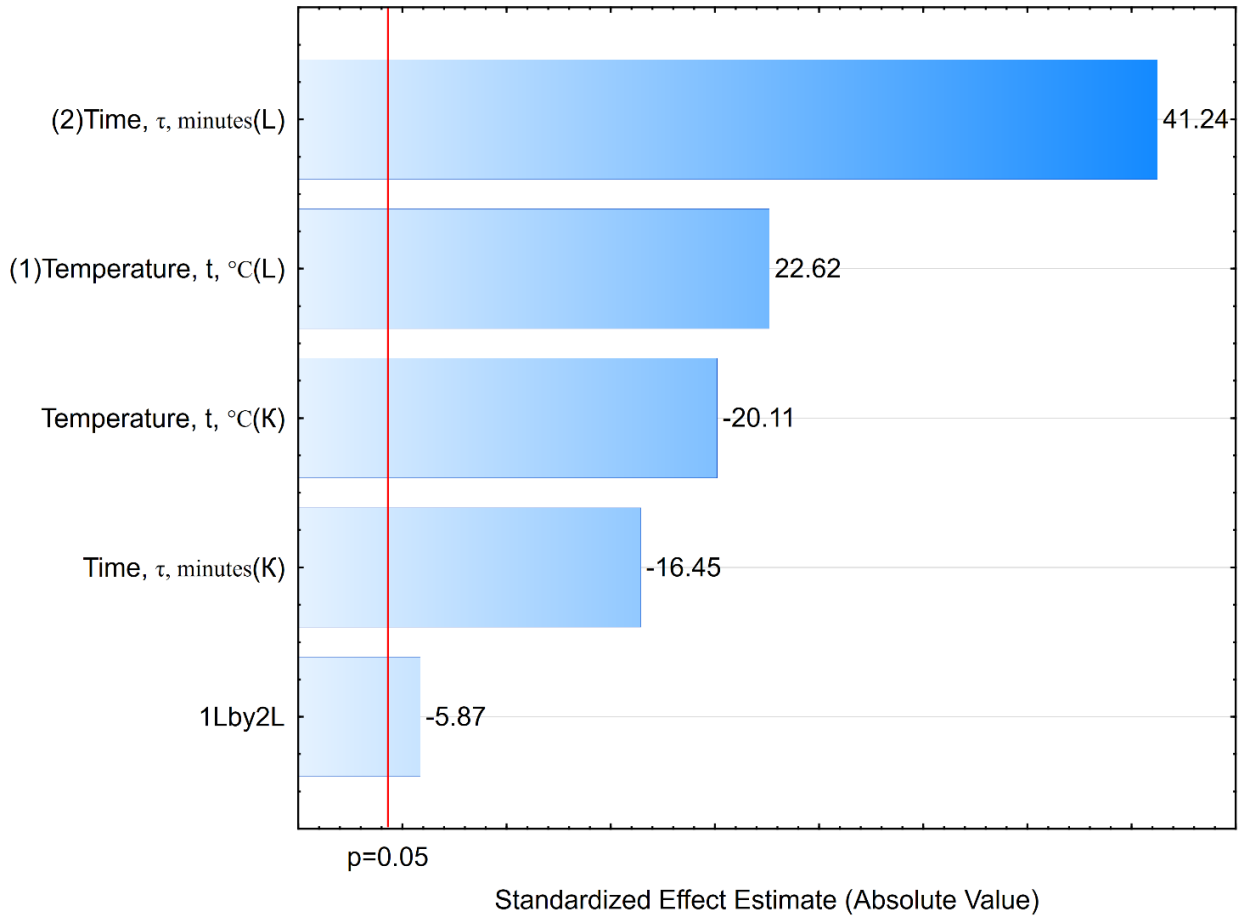

Fig. 1. Pareto chart

The suitability of the obtained model was tested by analysis of variance. See Table 2 for the results.

Table 2

Model analysis of variance

Factor	Sum of squares, SS	Degrees of freedom, df	Mean square value, MS	F-value	The level of significance, p-value
(1) Temperature (L)	441.702	1	441.702	511.623	0.001949
Temperature (Q)	349.175	1	349.175	404.450	0.002463
2) Time (L)	1468.621	1	1468.621	1701.105	0.000587
Time (Q)	233.487	1	233.487	270.448	0.003677
1L by 2L	29.703	1	29.703	34.404	0.027857
Lack of fit	15.349	3	5.116	5.926	0.147774
Pure error	1.727	2	0.863		
The total sum of squares	2416.816	10			
Determination coefficient $R^2 = 0.993$					
Adjusted determination coefficient $R^2_{adj} = 0.986$					

The data presented in Table 2, in particular, the non-significant lack of fit (significance level $p > 0.05$) and the values of determination coefficients (R^2 and R^2_{adj}) close to unity, allowed concluding that the obtained model adequately describes the response.

See Fig. 2 for the cumulative effect of predictors on response.

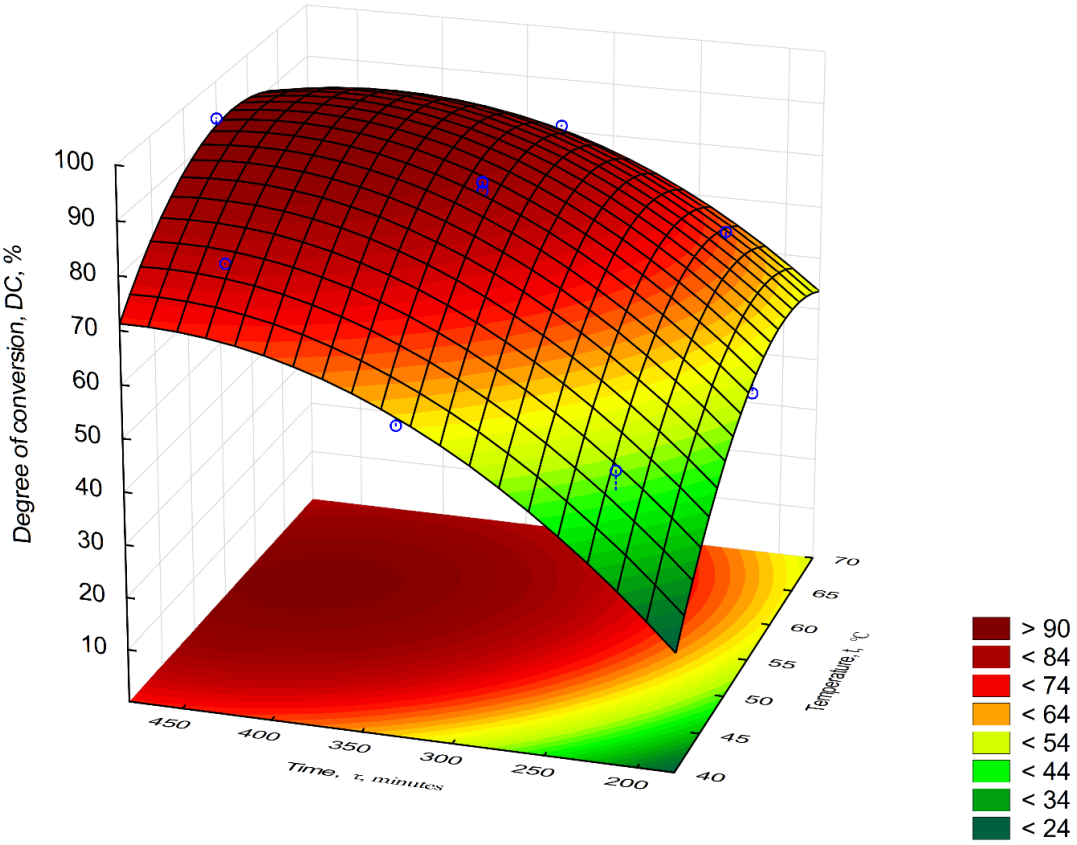

Fig. 2. Dependence of the degree of conversion on time and temperature of the esterification process

As can be noticed (Fig. 2), the values of the degree of conversion, which exceed 90 %, are in the temperature range of 55–62 °C and reaction time of more than 390 minutes.

Analysis of the critical points of regression (5) performed by Statistica 10 made it possible to establish rational parameters that correspond to the maximum degree of conversion with a temperature of 58 °C and reaction time of 435 minutes.

Exceeding the rational values of temperature and process time leads to a decrease in the efficiency of biocatalytic esterification due to partial denaturation of the apoenzyme and inhibition of lipase by the products.

Conclusions

The obtained results showed the possibility of synthesizing phytosterol esters by biocatalytic esterification of fats under the B-type lipase of Candida antarctica. A mathematical model was developed to predict the degree of feedstock conversion into products based on the process conditions. The rational values of biocatalytic esterification, which correspond to the maximal degree of conversion with a temperature of 58 °C and reaction time of 435 minutes were established. The study will provide a scientific basis for the technology to manufacture a new generation of food products for health purposes.

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