Journal of Chemistry and Technologies, 2025, *33*(1), 146-152

Journal of Chemistry and Technologies

pISSN 2663-2934 (Print), ISSN 2663-2942 (Online).

journal homepage: http://chemistry.dnu.dp.ua
editorial e-mail: chem.dnu@gmail.com

UDC 665:664.3

IMPROVING THE EFFICIENCY OF VEGETABLE OIL EXTRACTION BY BIOCATALYTIC TREATMENT OF OILSEED RAW MATERIALS

Pavlo O. Nekrasov*, Tetiana O. Berezka, Oleksandr P. Nekrasov, Olga M. Gudz, Svitlana M. Molchenko
National Technical University «Kharkiv Polytechnic Institute», 2 Kyrpychova Str., Kharkiv, 61002, Ukraine
Received 6 February 2025; accepted 18 March 2025; available online 15 April 2025

Abstract

Conventional technology for processing vegetable oilseeds is typically characterized by exposing the processed product to intense heat and humidity. Such methods have an adverse effect on the beneficial components of oil-containing raw materials, irreversibly degrading the quality and yield of the resulting product, while increasing the cost of edible oil production. The above drawbacks can be eliminated through preliminary enzymatic treatment of oilseed raw materials. Owing to their protein nature, enzymes operate under mild conditions, increasing the yield of finished products, improving their quality and nutritional value. This paper suggests an improved method for preparing sunflower seed meats for pressing by treating it with a complex of enzyme agents such as Alcalase 2.4 L FG and Viscozyme L. To analyze the collected data and optimize the parameters, the response surface methodology was chosen based on the central composite rotational design. This research has shown the feasibility of using a hydrolase complex in preparing sunflower seed meats for oil extraction aimed at increasing the efficiency of the pressing process. A mathematical model has been designed to predict the oil yield based on the parameters of biocatalytic raw material treatment. We found the most suitable parameters for obtaining the maximum oil yield: the enzyme complex content was 0.37 % by the meats weight; the temperature was 57 °C; and the duration of meats treatment was 115 minutes. Under these predictor values, the product yield was 77.3 % ± 1.4 % of the total oil content of the raw material, which is almost 16 % higher than the average value of this figure for control experiments not subjected to enzymatic treatment.

Keywords: meats; hydrolase complex; biocatalysis; optimization; rotational design.

ПІДВИЩЕННЯ ЕФЕКТИВНОСТІ ВИЛУЧЕННЯ РОСЛИННОЇ ОЛІЇ ШЛЯХОМ БІОКАТАЛІТИЧНОЇ ОБРОБКИ ОЛІЙНОЇ СИРОВИНИ

Павло О. Некрасов, Тетяна О. Березка, Олександр П. Некрасов, Ольга М. Гудзь, Світлана М. Мольченко
Національний технічний університет «Харківський політехнічний інститут», вул. Кирпичова, 2, Харків, 61002, Україна

Анотація

Традиційна технологія переробки рослинної олійної сировини характеризується застосуванням інтенсивних теплових і вологотеплових впливів на оброблюваний матеріал. Використання таких методів негативно впливає на корисні компоненти олієвмісної сировини, незворотно знижуючи якість і вихід отриманого продукту поряд із збільшенням витрат на виробництво харчових олій. Зазначені недоліки усуваються у разі попередньої ферментативної обробки олійної сировини. Завдяки своїй білковій природі ферменти працюють у м'яких умовах, збільшуючи вихід готової продукції, підвищуючи її якість і харчову цінність. У роботі запропоновано удосконалений метод підготовки м'ятки насіння соняшнику до пресування шляхом її обробки комплексом ферментних препаратів Alcalase 2.4 L FG та Viscozyme L. Для аналізу отриманих у ході досліджень даних та оптимізації параметрів було обрано методологію поверхні відклику із використанням центрального композиційного ротатабельного плану. У результаті дослідження показана доцільність використання комплексу гідролаз у підготовці м'ятки насіння соняшника до вилучення олії з метою підвищення ефективності процесу пресування. Розроблено математичну модель, яка дозволяє на основі даних щодо параметрів біокаталітичної обробки сировини прогнозувати вихід олії. Встановлено оптимальні параметри, які дають можливість отримати максимальний вихід олії: кількість ферментного комплексу – 0.37 % від маси м'ятки, температура – 57 °C, тривалість обробки м'ятки – 115 хвилин. Вихід продукту за вказаних значень предикторів становив 77.3 % ± 1.4 % від загальної олійності сировини, що майже на 16 % більше, ніж середнє значення даного показника для контрольних дослідів, в яких зразки не піддавали ферментативній обробці.

Ключові слова: м'ятка; комплекс гідролаз; біокаталіз; оптимізація; ротатабельний план.

**Corresponding author: e-mail: nekrasov2007@gmail.com*

doi: 10.15421/jchemtech.v33i1.322422

Journal of Chemistry and Technologies, 2025, *33*(1), 146-152

Introduction

Currently, the issue of resource conservation and optimal utilization of raw materials is getting urgent for Ukrainian food industry enterprises. This is mainly caused by the insufficient functioning of resource-saving techniques and equipment in production, as well as the neglect of energy analysis for existing and new technologies and equipment.

Rising demand for vegetable oils, as well as present-day requirements to improve their quality and safety, prompt the need for new strategies to increase target product yields not involving the use of potentially hazardous solvents and high-cost technologies [1–4].

Using biocatalysts, such as enzymes, is one of the most effective approaches to solving this issue. Enzyme-based technologies provide energy savings, reduce the amount of raw material losses, increase target product yields, their high quality, and enable the development of a flexible process flow chart [5–7].

Recently, developed countries have been researching the use of biocatalytic processes involving certain classes of enzymes, namely hydrolases, in the chemistry and technology of fats [8–11]. Hydrolases are the key mechanism for a whole range of different lipid conversion reactions under mild conditions, from hydrolysis to esterification and transesterification [12–17].

Since oilseeds belong to a complex biological system, this paves the way for increasing the degree of oil extraction through the influence of hydrolases on the structure of raw materials, which is aimed at disrupting the association of lipid globules with cell wall components [18; 19]. That's because lipids are localized in oilseed cells as spherosomes of different sizes. Spherosomes are surrounded by membranes consisting of proteins and polar lipids. The membranes of spherosomes of high-oil cells come into contact with each other, resulting in a continuous system over a large part of the cell bulk. This system includes cell organelles and localized clusters of reserve substances, mainly proteins, the membrane system is connected to through ionic and hydrophobic interactions. To maximize the oil extraction from the cell, it is imperative to destroy the cell wall, disintegrate the cluster of reserve substances and the membrane system [1; 20; 21]. The structure of the oilseed's cell walls is based on a complex of cellulose, hemicellulose, and pectin substances. Cellulose produces microfibrils with linear parts of hemicellulose molecules fixed on their surface by hydrogen bonds. The lateral branches of hemicellulose are covalently bonded to pectin, moreover, each pectin molecule is bonded to several hemicellulose molecules that surround it. The pectin hydrolysis by pectinases causes the wall to swell, as the bonding link between other polymers is removed from its structure. The wall disintegrates into cellulose-hemicellulose complexes, which can slide relative to each other in the space freed from pectin. Additionally, the action of pectolytic enzymes leads to the maceration of plant tissue, as separate cells are also linked by pectin substances. To achieve even deeper destruction of cell walls, it is reasonable to add cellulases and hemicellulases to the hydrolase complex [22]. For instance, the combination of cellulolytic and pectolytic enzymes increased the degree of extraction of rapeseed oil when pressed from 60 % to 65 % [23]. The enzymatic aqueous extraction of pumpkin seed oil by a combination of pectinase and cellulase at pH of 4.0, temperature of 50 °C, and time of 24 hours made it possible to achieve an oil extraction rate of 59 % [24].

The most challenging aspect of oil extraction technology is to overcome protein-lipid interactions in the cell membrane system, as well as the bonds between proteins and lipids released from spherosomes upon disintegration of their membranes. Mainly polar lipids and unsaturated fatty acids of acylglycerols are responsible for the formation of bonds with proteins [25]. Hydrolysis by endo-type proteases, which break down protein into large fragments, is more preferable for disintegrating localized clusters of reserve protein and destabilizing membranes [26]. When using a complex of endo- and exoproteases, low molecular weight hydrolysis products (associated with lipids and complicating their isolation in pure form) accumulate along with the disintegration of the material.

Consequently, to develop an effective biocatalytic technology for vegetable oil production that maximizes target product yield, it is required to use an enzyme complex comprising the widest range of hydrolases, namely pectinase, cellulase, hemicellulase, and protease.

In the process of extracting vegetable oils using the aqueous method, the enzyme agents Alcalase 2.4 L FG and Viscozyme L proved to be highly effective [27–29]. Alcalase 2.4 L FG is a bacterial protease classified as a serine endoprotease and obtained by directed fermentation of a selective strain of Bacillus licheniformis with subsequent purification. Viscozyme L is a multienzyme agent derived from the fungal strain Aspergillus

Journal of Chemistry and Technologies, 2025, *33*(1), 146-152

aculeatus. This agent contains pectinase, endo-beta-glucanase, xylanase, cellulase, and hemicellulase.

Presently, we lack detailed information on the combined effect of the main technological parameters on the processes of enzymatic preparation of raw materials for oil extraction by the press method under the influence of these enzyme agents and the possibility of their optimization. This determines the urgency of conducting research in this area.

This research is aimed at optimizing parameters of the process of preparing plant material for oil extraction by the press method with a complex of enzymes considering the criterion of maximizing final product yield.

Experimental procedure

Sunflower seeds, the most common oilseed in Ukraine, were chosen as the initial raw material.

The sunflower seeds were hulled on a centrifugal dehuller with a capacity of 250 kg/h. To crush the seeds, a laboratory chopper RT-1 (manufactured by the Odesa Experimental Plant) with a maximum rotor speed of 8000 rpm was used. The meats produced after crushing the hulled sunflower seeds were exposed to moisture and heat treatment. Then they were divided into two parts.

The first part, used as a control, was dried and pressed at ambient temperature. The meats were pressed with a laboratory hydraulic press under a pressure of 15 MPa in a 150 mm diameter crushing cell. The oil content in the oilseeds was determined according to the method [30].

The second part of the meats was exposed to enzymatic treatment. A complex of enzyme agents Alcalase 2.4 L FG and Viscozyme L (manufactured by Novozymes, Denmark) in a 1:1 mass ratio was used for biocatalysis. It was treated within the following variation ranges of the main parameters selected as independent variables: enzyme complex content of 0.1-0.5% by the meats weight, temperature of 45-65 °C, and treatment duration of 30-120 minutes. The ratio of enzyme agents in the complex, as well as the levels and intervals of parameter variation, was selected according to the results of preliminary experiments. Following the enzymatic preparation, the oilseeds were dried and pressed under the same conditions as the control samples.

To optimize the parameters of the enzymatic preparation of sunflower seed meats for oil extraction, we used the response surface methodology [31], consisting of a set of mathematical and statistical techniques aimed at modeling processes and finding combinations of experimental series of predictors to optimize the response function, which is generally described by the following polynomial:

$$\hat{y}(x,a) = b_0 + \sum_{l=1}^{n} b_l x_l + \sum_{k=1}^{n} b_k x_k^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} b_{ij} x_i x_j, \quad (1)$$

where $x \in R^n$ stands for a vector of variables, b – a vector of parameters.

The unknown values of the vector of parameters b were defined by applying regression analysis algorithms and optimizing (minimizing) the deviation functions (2):

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

where m stands for an amount of experimental data y.

The central composite rotational design was applied. For the process under study, a response function with the following polynomial was chosen (3):

$$OY = b_0 + b_1 \cdot e + b_{11} \cdot e^2 + b_2 \cdot t + b_{22} \cdot t^2 + b_3 \cdot \tau + b_{33} \cdot \tau^2 + b_{12} \cdot e \cdot t + b_{13} \cdot e \cdot \tau + b_{23} \cdot t \cdot \tau, \quad (3)$$

where OY stands for the oil yield, % of the total content; b_0 – a constant; e – the content of the enzyme complex, % of the meats weight; t – the process temperature, °C; τ – the process duration, minutes; b_1, b_{11}, b_2, b_{22}, b_3, b_{33}, b_{12}, b_{13}, b_{23} – coefficients for each element of the polynomial.

To minimize the impact of systematic errors caused by external conditions, the execution sequence of the experiments was randomized.

Modeling, experimental data processing, and statistical calculations were performed using Statistica 14 (TIBCO Software Inc.).

Results and discussion

These studies revealed that the average oil yield of the control samples was 61.4 % ± 1.2 % of the total oil content of the seeds.

A design matrix with the obtained experimental values of the response function (average results of three parallel studies) was developed for the oilseed raw material that was exposed to enzymatic treatment before pressing (Table 1):

Journal of Chemistry and Technologies, 2025, *33*(1), 146-152

Table 1

Design matrix and response function values

Experiment number	Enzyme complex content, e		Temperature, t		Time, τ		Oil yield, OY, % of total content
	Coded level	% of the meats weight	Coded level	°C	Coded level	Minutes	
1	0	0.30	–1.682	45	0	75	69.2
2	–1	0.18	+1	61	+1	102	71.8
3	–1	0.18	–1	49	+1	102	68.0
4	+1.682	0.50	0	55	0	75	74.1
5	–1	0.18	–1	49	–1	48	64.5
6	0	0.30	0	55	0	75	73.2
7	0	0.30	0	55	–1.682	30	66.0
8	–1.682	0.10	0	55	0	75	63.9
9	–1	0.18	+1	61	–1	48	63.3
10	+1	0.42	–1	49	–1	48	67.8
11	+1	0.42	+1	61	+1	102	77.1
12	0	0.30	0	55	+1.682	120	74.8
13	0	0.30	0	55	0	75	72.0
14	0	0.30	+1.682	65	0	75	68.5
15	0	0.30	0	55	0	75	72.5
16	+1	0.42	+1	61	–1	48	68.1
17	+1	0.42	–1	49	+1	102	72.3

The significance of the regression coefficients and the validity of the model were tested by analysis of variance, presented in Table 2 (where L stands for linear effect, Q – quadratic effect).

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)Enzyme complex content (L)	69.076	1	69.076	190.117	0.005
Enzyme complex content (Q)	22.044	1	22.044	60.672	0.016
(2) Temperature (L)	0.437	1	0.437	1.202	0.387
Temperature (Q)	23.759	1	23.759	65.392	0.015
3) Time (L)	95.961	1	95.961	264.113	0.004
Time (Q)	9.135	1	9.135	25.142	0.038
1L by 2L	0.320	1	0.320	0.881	0.447
1L by 3L	0.845	1	0.845	2.326	0.267
2L by 3L	3.645	1	3.645	10.032	0.087
Lack of fit	11.133	5	2.227	6.128	0.146
Pure error	0.727	2	0.363		
The total sum of squares	218.678	16			
Determination coefficient $R^2 = 0.946$					
Adjusted determination coefficient $R^2_{adj} = 0.876$					

The data presented in Table 2 suggest that that the model adequately describes the response. This is evidenced by the lack of fit (significance level $p > 0.05$) and the values of the determination coefficients (R^2 and R^2_{adj}), which are close to 1. Furthermore, the results of the analysis of variance showed that the linear effect of temperature and the effects of the interaction of factors are insignificant since the significance level for these effects is $p > 0.05$. Hence, these regression terms were excluded from the model. The resulting model equation with the calculated coefficients is as follows (4):

$$OY = 47.234 + 60.166 \cdot e - 68.925 \cdot e^2 + 0.002 \cdot t^2 + 0.199 \cdot \tau - 0.001 \cdot \tau^2 \quad (4)$$

Response surfaces with corresponding level line maps were developed to study the main patterns of predictors' influence on the degree of oil extraction. They reflect the dependence of the final product yield on each pair of factors, assuming that the value of the third factor is fixed at the optimum (Fig.).

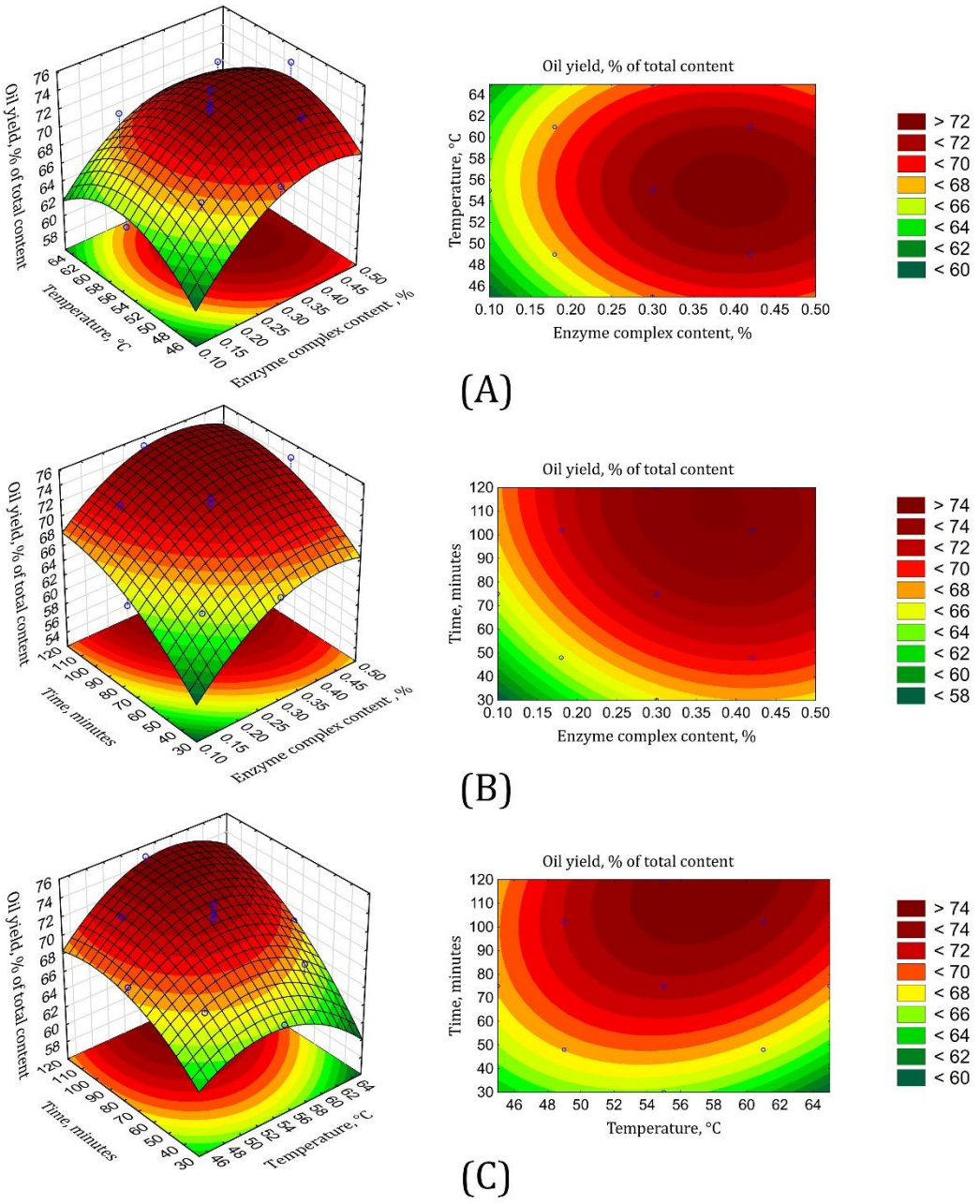

Figure. 3D response surface and contour plots effects: (A) Temperature and Enzyme complex content; (B) Time and Enzyme complex content; (C) Time and Temperature

Analyzing the results shown in Fig. reveals that the reasonable temperature regime for the selected enzyme complex lies in the range of 52–59 °C. A further increase in temperature reduces the efficiency of the process due to thermal denaturation of apoenzymes. The reasonable values of the enzyme complex content and the process duration fall within the range of 0.33–0.46 % by the meats weight and 90–115 minutes, respectively. Extending the process duration and increasing the biocatalyst content above the reasonable values has almost no effect on the target product yield, and in some factor ratios even leads to a slight decrease in oil yield. It is caused by the fact that these conditions result in the release of hydrophobic fragments of proteins and structural polysaccharides of oilseed raw material that were screened in their native form. This results in an increase in the fat-holding capacity of the meats, leading to a decrease in the target product yield.

These findings are highly consistent with the results of the analysis of critical regression points (4) in Statistica 14, allowing us to establish the values of the optimal process parameters: the enzyme complex content was 0.37 % by the meats weight, the temperature was 57 °C, and the duration of meats treatment was 115 minutes. Under the specified parameters, the average value of pressed oil yield within the conditions of an

additional verification experiment, which was carried out in five repetitions, was 77.3 % ± 1.4 % of the total oil content of seeds. Meanwhile, the relative deviation of the value predicted by the model from the experimental one was 2.7 %. These findings suggest that the response values predicted by regression equation (4) are highly consistent with the experiment, and the developed model is highly reliable.

Conclusions

This study has revealed the feasibility of using the enzyme complex Alcalase 2.4 L FG and Viscozyme L to prepare sunflower seed meats for oil extraction with the aim to increase the efficiency of the pressing process. A mathematical model has been designed to predict the oil yield based on the parameters of biocatalytic raw material treatment. Reasonable intervals of their values were established for each of the predictors and the optimal parameters were calculated to obtain the maximum oil yield: the enzyme complex content was 0.37 % by the meats weight, the temperature was 57 °C, and the duration of the meats treatment was 115 minutes. Based on these predictor values, the product yield was 77.3 % ± 1.4 % of the total oil content of the raw material, which is almost 16 % higher than the average value of this indicator for control experiments where the samples were not exposed to enzymatic treatment with hydrolases.

These findings will provide grounds for the development of a new resource-saving technology for the integrated processing of oilseed raw materials.

References

[1] Rani, H., Sharma, S., Bala, M. (2021). Technologies for extraction of oil from oilseeds and other plant sources in retrospect and prospects: A review. *J. Food Process Eng.*, *44*(11), e13851. https://doi.org/10.1111/jfpe.13851

[2] Jiang, F., Yuan, L., Shu, N., Wang, W., Liu, Y., Xu, Y.-J. (2020). Foodomics Revealed the Effects of Extract Methods on the Composition and Nutrition of Peanut Oil. *J. Agric. Food Chem.*, *68*(4), 1147–1156. https://doi.org/10.1021/acs.jafc.9b06819

[3] Hu, Z., Hu, C., Li, Y., Jiang, Q., Li, Q., Fang, C. (2024). Pumpkin seed oil: a comprehensive review of extraction methods, nutritional constituents, and health benefits. *J. Sci. Food Agric.*, *104*(2), 572–582. https://doi.org/10.1002/jsfa.12952

[4] Riaz, T., Iqbal, M.W., Mahmood, S., Yasmin, I., Leghari, A.A., Rehman, A., Mushtaq, A., Ali, K., Azam, M., Bilal, M. (2023). Cottonseed oil: A review of extraction techniques, physicochemical, functional, and nutritional properties. *Crit. Rev. Food Sci. Nutr.*, *63*(9), 1219–1237. https://doi.org/10.1080/10408398.2021.1963206

[5] Ferreira, I.J.B., Alexandre, E.M.C., Saraiva, J.A., Pintado, M. (2022). Green emerging extraction technologies to obtain high-quality vegetable oils from nuts: A review. Innov. Food Sci. Emerg. Technol., 76, 102931. https://doi.org/10.1016/j.ifset.2022.102931

[6] Punia, S., Kumar, M., Siroha, A.K., Purewal, S.S. (2021). Rice Bran Oil: Emerging Trends in Extraction, Health Benefit, and Its Industrial Application. *Rice Sci.*, *28*(3), 217–232. https://doi.org/10.1016/j.rsci.2021.04.002

[7] Ng, Y.J., Tham, P.E., Khoo, K.S., Cheng, C.K., Chew, K.W., Show, P.L. (2021). A comprehensive review on the techniques for coconut oil extraction and its application. *Bioprocess Biosyst. Eng.*, *44*(9), 1807–1818. https://doi.org/10.1007/s00449-021-02577-9

[8] Razak, N.N.A., Cognet, P., Pérès, Y., Aroua, M.K., Gew, L.T. (2024). A decade development of lipase catalysed synthesis of acylglycerols using reactors: a systematic review. *Rev. Chem. Eng.*, *40*(7), 815–837. https://doi.org/10.1515/revce-2023-0050

[9] Xia, S., Lin, J., Sayanjali, S., Shen, C., Cheong, L.-Z. (2024). Lipase-catalyzed production of biodiesel: a critical review on feedstock, enzyme carrier and process factors. *Biofuels Bioprod. Bioref.*, 18 (1), 291–309. https://doi.org/10.1002/bbb.2561

[10] Nekrasov, P.O., Gudz, O.M., Nekrasov, O.P., Berezka, T.O. (2020). Optimizing the parameters of the production process of fat systems with a minimum content of trans-isomers. *Voprosy khimii i khimicheskoi technologii – Issues of Chemistry and Chemical Technology*, (3), 128–133. https://doi.org/10.32434/0321-4095-2020-130-3-128-133

[11] Cozentino, I.D.S.C., Rodrigues, M.D.F., Mazziero, V.T., Cerri, M.O., Cavallini, D.C.U., de Paula, A.V. (2022). Enzymatic synthesis of structured lipids from grape seed (Vitis vinifera L.) oil in associated packed bed reactors. *Biotechnol. Appl. Biochem.*, *69*(1), 101–109. https://doi.org/10.1002/bab.2085

[12] Nekrasov, P.O., Berezka, T.O., Nekrasov, O.P., Gudz, O.M., Molchenko, S.M., Rudneva, S.I. (2023). Optimization of the parameters of biocatalytic hydrolysis of vegetable oil using the methods of neural networks and genetic algorithms. *Journal of Chemistry and Technologies*, *31*(1), 140–146. https://doi.org/10.15421/jchemtech.v31i1.274704

[13] Silva, A.R.D.M., Gonçalves, L.R.B., da Silva, I.J., Jr. (2025). Innovations in packed-bed reactors utilizing immobilized lipase catalysts: A comprehensive technical and scientific review. *Mol. Catal.*, *573*, 114814. https://doi.org/10.1016/j.mcat.2024.114814

[14] Nekrasov, P.O., Berezka, T.O., Nekrasov, O.P., Gudz, O.M., Molchenko, S.M. (2024). Investigation of the basic laws of the kinetics of biocatalytic hydrolysis of vegetable oil. *Journal of Chemistry and Technologies*, *32*(1), 191–197. https://doi.org/10.15421/jchemtech.v32i1.298927

[15] Lee, Y.J., Kim, H.-W., Lee, J., Kim, B.H., Kim, I.-H. (2025). Synthesis of structured lipids using microalgae oil and capric acid via a two-step enzyme reaction. *Process Biochem.*, 150, 213–220. https://doi.org/10.1016/j.procbio.2025.01.009

[16] Nekrasov, P.O., Berezka, T.O., Nekrasov, O.P., Gudz, O.M., Rudneva, S.I., Molchenko, S.M. (2022). Study of biocatalytic synthesis of phytosterol esters as formulation components of nutritional systems for health purposes. *Journal of Chemistry and Technologies*, *30*(3), 404–409. https://doi.org/10.15421/jchemtech.v30i3.265174

[17] Nekrasov, P.O., Gudz, O.M., Nekrasov, O.P., Kishchenko, V.A., Holubets, O.V. (2019). [Fatty systems with reduced content of trans-fatty acids]. *Voprosy khimii i khimicheskoi technologii – Issues of Chemistry and Chemical Technology*, (3), 132–138 (in Ukrainian). https://doi.org/10.32434/0321-4095-2019-124-3-132-138

[18] Hu, B., Li, Y., Song, J., Li, H., Zhou, Q., Li, C., Zhang, Z., Liu, Y., Liu, A., Zhang, Q., Liu, S., Luo, Q. (2020). Oil extraction from tiger nut (Cyperus esculentus L.) using the combination of microwave-ultrasonic assisted aqueous enzymatic method - design, optimization and quality evaluation. *J. Chromatogr. A*, 1627, 461380. https://doi.org/10.1016/j.chroma.2020.461380

[19] Qin, Z., Chang, Y.-L., Chen, Z.-M., Wang, Y.-G., Fan, W., Gu, L.-B., Qin, Z., Liu, H.-M., Zhu, X.-L., Mei, H.-X., Wang, X.-D. (2024) A novel strategy for preparing lignan-rich sesame oil from cold-pressed sesame seed cake by combining enzyme-assisted treatment and subcritical fluid extraction. *Ind. Crops Prod.,* 218, art. no. 119041. https://doi.org/10.1016/j.indcrop.2024.119041

[20] Monica, P., Ranjan, R., Kapoor, M. (2024). Family 3 CBM improves the biochemical properties, substrate hydrolysis and coconut oil extraction by hemicellulolytic and holocellulolytic chimeras. *Enzyme Microb. Technol.*, 174, 110375. https://doi.org/10.1016/j.enzmictec.2023.110375

[21] Valladares-Diestra, K., de Souza Vandenberghe, L.P., Soccol, C.R. (2020). Oilseed Enzymatic Pretreatment for Efficient Oil Recovery in Biodiesel Production Industry: a Review. *Bioenergy Res.*, *13*(4), 1016–1030. https://doi.org/10.1007/s12155-020-10132-9

[22] Zhang, W., Yu, J., Wang, D., Han, X., Wang, T., Yu, D. (2024). Ultrasonic-ethanol pretreatment assisted aqueous enzymatic extraction of hemp seed oil with low Δ9-THC. *Ultrason. Sonochem.*, 103, art. no. 106766. https://doi.org/10.1016/j.ultsonch.2024.106766

[23] Thomsen, K., Raak, N., Gregersen, S.B., Månsson, L., Miquel Becker, E. (2024) Enzyme-assisted extraction of rapeseed oil with minimum water addition: a proof-of-concept study. *Int. J. Food Sci. Technol.*, 59 (5), 3013–3019. https://doi.org/10.1111/ijfs.17030

[24] Atsakou, A.E., Remonatto, D., Júnior, R.H.M., Paz-Cedeno, F.R., Masarin, F., Andrade, G.S.S., de Lucca Gattas, E.A., de Paula, A.V. (2023). Synthesis of dietary lipids from pumpkin (Cucurbita pepo. L) oil obtained by enzymatic extraction: a sustainable approach. *3 Biotech*, 13 (11), art. no. 358. https://doi.org/10.1007/s13205-023-03781-y

[25] Liu, Z., Liao, H., Wei, C., Qi, Y., Zou, Z. (2023). Application of an aqueous enzymatic–ultrasound cavitation method for the separation of Sapium sebiferum seed kernel oil. *Ultrason. Sonochem.*, 101, 106704. https://doi.org/10.1016/j.ultsonch.2023.106704

[26] Piseskul, J., Suttisansanee, U., Chupeerach, C., Khemthong, C., Thangsiri, S., Temviriyanukul, P., Sahasakul, Y., Santivarangkna, C., Chamchan, R., Aursalung, A., On–nom, N. (2023). Optimization of Enzyme-Assisted Mechanical Extraction Process of Hodgsonia heteroclita Oilseeds and Physical, Chemical, and Nutritional Properties of the Oils. *Foods*, 12 (2), art. no. 292. https://doi.org/10.3390/foods12020292

[27] Mehanni, A.E., El-Reffaei, W.H., Akdowa, E.P., Bouba, A.A. (2014). Enzyme aqueous extraction of Moringa oleifera and Canola seed oils and their effect on physiochemical characteristics and stability. *Riv. Ital. Sostanze Grasse*, *91* (3), 187–197.

[28] Peng, L., Ye, Q., Liu, X., Liu, S., Meng, X. (2019). Optimization of aqueous enzymatic method for Camellia sinensis oil extraction and reuse of enzymes in the process. *J. Biosci. Bioeng.*, *128*(6), 716–722. https://doi.org/10.1016/j.jbiosc.2019.05.010

[29] Liu, C., Hao, L.-H., Chen, F.-S., Zhu, T.-W. (2020). The mechanism of extraction of peanut protein and oil bodies by enzymatic hydrolysis of the cell wall. *J. Oleo Sci.*, *69*(11), 1467–1479. https://doi.org/10.5650/jos.ess20148

[30] International Organization for Standardization. (2009). *Oilseeds – Determination of oil content*. (ISO 659:2009). Geneva, Switzerland: International Organization for Standardization.

[31] Wu, C.F.J., Michael S. Hamada, M.S. (2021). *Experiments: Planning, Analysis, and Optimization, 3rd Edition*. Hoboken, USA: John Wiley & Sons Inc.