The object of the study is a two-stage combined transesterification of a fat composition to enrich the latter with ω-3 polyunsaturated fatty acids (PUFA). The problem was the need to create a stable fat system enriched with ω-3 PUFA, which is minimally oxidized during the production process. The rational parameters of the enzymatic stage of transesterification of fat substrates were substantiated (process temperature 65°C, fat substrate composition: refined deodorized soybean oil: intermediate fat system PMS-MF). The proposed conditions ensure the degree of transesterification of the fat composition at the level of 74.2% with the formation of 0.6% free fatty acids. The oxidative stability of the resulting product is maximum at 4°C, when in 30 days the peroxide value increases to 15.3 mmol ½O_2/kg with losses of ω-3 PUFA of 5.1%. The results of the research are explained by the combination of two stages of transesterification: chemical modification of the fat matrix (product – PMS-MF) and enzymatic using Lipozyme TL IM, which minimizes hydrolytic and oxidative processes of labile components. The key distinguishing feature of the research is the combination of a high degree of enzymatic transesterification (74.2%) with a low level of by-products (0.6% free fatty acids). This was achieved due to the rational ratio of substrates and the temperature of enzymatic transesterification, at which ω-3 PUFA are introduced into the fat system. The obtained results open up prospects for the industrial production of health-promoting fat products with an increased content of ω-3 PUFA and specialized food systems. The technology is effective for fat raw materials with a content of ω-3 PUFA of 7±1% and a peroxide value of less than 1 mmol ½O_2/kg

Keywords: two-step transesterification, ω-3 polyunsaturated fatty acids, Lipozyme TL IM, oxidative stability, fat compositions

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DEVELOPMENT OF BIOTECHNOLOGICALLY MODIFIED FAT COMPOSITIONS ENRICHED WITH OMEGA-3 FATTY ACIDS USING A TWO-STAGE TRANSESTERIFICATION METHOD

Serhii Stankevych
Corresponding author
PhD
Department of Zoology, Entomology, Phytopathology, Integrated Plant Protection and Quarantine named after B. M. Litvinova*
E-mail: sergejstankevich1986@btu.kharkiv.ua
Viktoriia Kalyna
PhD, Associate Professor
Department of Food Technologies
Dnipro State Agrarian and Economic University
Serhiya Yefremova str., 25, Dnipro, Ukraine, 49600
Iryna Mezentseva
PhD, Associate Professor
Department of Occupational and Environmental Safety**
Oleg Kolontaievskyi
PhD
Department of Tourism and Hospitality Management
O.M. Beketov National University of Urban Economy in Kharkiv
Chornoglazivska str., 17, Kharkiv, Ukraine, 61002
Maryna Ponomarova
PhD, Associate Professor
Department of UNESCO "Philosophy of Human Communication and Social and Humanitarian Disciplines"*
Anna Belinska
PhD, Associate Professor
Department of Biotechnology, Biophysics and Analytical Chemistry**
Department of Studies of Technology for Processing Oils And Fats
Ukrainian Research Institute of Oils and Fats of the National academy of agrarian sciences of Ukraine
Dziuba ave., 2 A, Kharkiv, Ukraine, 61019
Yana Svishchova
PhD, Associate Professor
Department of Agrochemistry*
Viktoriia Novikova
PhD, Associate Professor
Department of Chemistry, Biochemistry, Microbiology and Food Hygiene*
Yevhen Barkar
PhD, Associate Professor
Departament Biotechnology and Bioengineering
Mykolaiv National Agrarian University
Georgiy Gongadze str., 9, Mykolayiv, Ukraine, 54008
Yana Honcharenko
PhD
Department of Special Chemistry and Chemical Technology
National University of Civil Protection of Ukraine
Onoprienko str., 8, Cherkasy, Ukraine, 18034
*State Biotechnological University
Alchevskykh str., 44, Kharkiv, Ukraine, 61002
**National Technical University "Kharkiv Polytechnic Institute"
Kyrpychova str., 2, Kharkiv, Ukraine, 61002

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1. Introduction

Current trends in the food industry are focused on the development of health-promoting fat products enriched with essential polyunsaturated fatty acids (PUFA), in particular ω-3 [1, 2]. Despite their critical importance for health, the modern diet is often characterized by an imbalance between ω-3 and ω-6 PUFA, which necessitates the creation of spe-

cialized fat systems with a rationalized fatty acid profile [3]. However, the integration of ω-3 PUFA into food systems is complicated by their high sensitivity to oxidation, which requires the use of innovative technological approaches [4].

A promising method for modifying fats is two-stage transesterification (chemical and enzymatic), which allows for a targeted change in the distribution of fatty acids in triacylglycerols, increasing their functional stability and biological activity [5]. This process can provide selective incorporation of ω-3 PUFAs into the *sn*-2 position of the triglyceride (TAG) molecule, which improves their absorption and reduces the risk of oxidation [6]. At the same time, an important aspect is the stabilization of the resulting compositions by introducing natural antioxidants, such as polyphenol extracts, which inhibit radical processes without affecting the organoleptic properties of the product [7].

Thus, the study of aspects of the biotechnological component of the two-stage transesterification of fat compositions with the aim of enriching *ω*-3 PUFAs is a relevant scientific direction that combines technological efficiency with increasing the nutritional value of fat products. Such studies open up prospects for the creation of innovative fat systems with improved functional properties, long shelf life and compliance with the requirements of modern healthy nutrition.

2. Literature review and problem statement

Modern studies on the creation of structured lipids based on palm oil [8] demonstrate the potential of enzymatic modification for obtaining special-purpose fats with increased bioavailability. However, the authors do not reveal the mechanisms of rationalization of the positional specificity of lipases for the targeted incorporation of *ω*-3 PUFA into the *sn*-2 position of the TAG molecule, which limits the possibilities of creating breast milk fat (BMF) analogues. This is due to the lack of systematic data on the influence of different types of lipases on the selectivity of transesterification of complex fat matrices. This limitation was partially overcome in the work [9], which proposed combining enzymatic modification with microencapsulation to stabilize *ω*-3 PUFA in baby food products. However, the described approach does not take into account the need for synchronous control of oxidative stability and fatty acid profile during two-stage transesterification, which is due to technological difficulties in integrating different processing methods into a single technological process.

The next step in solving this problem was taken in [10], where the effectiveness of ultrasonic treatment for accelerating the synthesis of OPO-structured lipids was proven. Despite the high conversion rates, the proposed two-stage chemoenzymatic method remains energy-intensive and requires further improvement of the catalyst recycling system, especially when working with oxidation-sensitive *ω*-3 PUFA. This limitation arises from the lack of universal protocols for enzyme stabilization under conditions of combined mechanical-enzymatic processing. At the same time, [11] emphasized the need for complete exclusion of trans isomers as a result of transesterification. However, the described analysis of the results does not take into account the specifics of the enrichment of fat compositions with *ω*-3 PUFA, where quality criteria include not only the absence of harmful isomers, but also the preservation of the biological activity of unsaturated acids. This gap in knowledge is due to the difficulty of simultaneously observing the conditions of oxidative stability and nutritional value of fat systems in industrial production.

Studies [12] demonstrate the potential of using oil refining waste for the synthesis of special-purpose fat systems, in particular diglycerides. However, the identified limiting effect of molecular sieves on the selectivity of the process indicates the need for further improvement of catalytic systems for working with complex secondary raw material systems. This limitation is associated with the lack of universal methods for controlling the spatial orientation of molecules during solvent-free esterification, which is especially relevant for processes involving PUFAs. The problem of stabilizing sensitive *ω*-3 acids is partially solved by the approach proposed in [13], which proves the effectiveness of microencapsulation for preserving the biological activity of eicosapentaenoic and docosahexaenoic PUFAs. The issue of compatibility of emulsification methods with subsequent enzymatic modification of lipids also remains unresolved, since the presence of emulsifiers can inhibit the activity of lipases. This limitation is particularly pronounced in studies of transesterification in ionic liquids, as shown in [14]. The results show that, despite the successful use of ionic liquids for the concentration of PUFA, the effect of residual ions on the stability of the finished products during long-term storage was not taken into account. This aspect was partially resolved by the researchers [15] thanks to the two-stage transesterification technology using Novozyme 435, which provided a high yield of the target TAGs. However, the method proposed by them requires additional stages of purification of the finished product and is characterized by high energy consumption, which limits its industrial application.

An important step in overcoming these limitations was made in [16], where the effectiveness of *F. solani* lipase for the enrichment of fish oils was proven. However, despite the high stability of the enzyme preparation, the study does not provide sufficient information about its selectivity for the *sn*-2 position of the TAG molecule, which is key for the creation of structural analogues of LHM.

Of particular interest is the study of the influence of fat-soluble dyes on the oxidative stability of fat systems. The induction period of accelerated oxidation of chlorophyll A and beta-carotene solutions in sunflower oil was studied [17]. It was found that chlorophyll A at a concentration of up to 0.05 g/l practically does not affect stability, while at 0.30 g/l it reduces the induction period by almost 48%. In contrast, beta-carotene at the same concentration extends this period by 54%. The joint presence of both compounds demonstrates a compensatory effect, which allows predicting the shelf life of dye compositions and optimizing their protective properties.

Studies [18] confirmed the effectiveness of direct transesterification for obtaining special-purpose lipids from microbial sources, but revealed limitations in controlling the positional distribution of fatty acids in TAG. This emphasizes the need to combine chemical and enzymatic transesterification, as proposed in [18], to achieve optimal characteristics of structured lipids: high content of *ω*-3 PUFA, oxidative stability and low level of hydrolysis. The chemical stage provides high conversion and cost-effectiveness, while the enzymatic stage provides selectivity and preservation of biological activity of PUFA. This combination of methods allows to overcome the limitations associated with low stability to oxidative degradation of *ω*-3 PUFA. In addition, the described technological approach meets modern requirements for environmental friendliness and resource conservation, as it allows to minimize the use of toxic catalysts and reduce energy

consumption due to a rational combination of stages. Thus, further research in this direction has significant practical potential for the food industry, in particular for the development of innovative products with an increased content of ω-3 PUFA, extended shelf life and optimized consumer qualities.

3. The aim and objectives of the study

The aim of the study is to develop biotechnologically modified fat compositions enriched with omega-3 PUFAs from a previously chemically modified fat system. This will allow obtaining a special-purpose fat composition that can be used in the production of health-promoting food products, such as spreads, bakery products, and specialized lipid additives.

To achieve this aim, the following objectives were accomplished:

– rationalize the parameters of enzymatic transesterification, namely the temperature and ratio of raw material components, increase the efficiency of the process while reducing the intensity of hydrolytic processes for maximum inclusion of ω-3 PUFAs in the fat matrix;

– assess the oxidative stability of the modified fat composition after a two-stage process.

4. Materials and methods of the study

4. 1. The object and hypothesis of the study

The object of the study is the process of enzymatic transesterification of a previously chemically modified fat system with the participation of immobilized lipase Lipozyme TL IM, aimed at incorporating ω-3 PUFA into the TAG matrix. The main hypothesis of the study is the assumption that two-stage transesterification using immobilized lipase Lipozyme TL IM with rational process parameters will allow achieving a degree of incorporation of ω-3 PUFA into the fat matrix of at least 70%, which exceeds the indicators of traditional one-stage methods (40–50%) and provides a sufficient level of bioavailability of these acids. The proposed approach should ensure the preservation of the biological activity of polyunsaturated fatty acids by minimizing oxidative processes during the enzymatic transesterification stage. The implementation of this hypothesis opens up prospects for the creation of special-purpose fat systems with increased nutritional value and specified technological properties, which is relevant for the production of health-promoting products and specialized lipid supplements.

The following assumption was made in the study: the pre-chemically modified fat system is characterized by sufficient homogeneity of composition, which allows standardizing the enzymatic transesterification stage and generalizing the results obtained.

The study adopted a methodological simplification that assumes the constancy of the main process parameters for all experimental series regardless of minor variations in the fatty acid composition of chemically pre-modified fats. This assumption allows to focus on the analysis of the influence of key factors on the efficiency of ω-3 PUFA inclusion, excluding the influence of side changes in the properties of the starting material. Deviations in the fat composition are considered insignificant for interpreting the main regularities of the two-stage transesterification process.

4. 2. Materials used in the experiment

The following materials were used during the research:

1) intermediate fat system *PMS-MF*, obtained by chemical transesterification of the following refined, bleached and deodorized components:

– palm stearin according to CAS 91079-14-0 (manufactured by Malaysia, IOI Group) – 45%;

– coconut oil according to CAS 8001-31-8 (manufactured by Malaysia, Wilmar International) – 30%;

– high-oleic sunflower oil according to CAS 8001-21-6 (manufactured by Ukraine, Kernel) – 25%;

2) refined deodorized soybean oil (manufactured by Ukraine, Cargill), according to CAS 8001-22-7;

3) immobilized enzyme preparation Lipozyme TL IM, which is immobilized on silica gel lipase *Thermomyces lanuginosus* (Denmark, Novozymes A/S).

The intermediate fat system PMS-MF is characterized by physicochemical indicators, appropriate for the subsequent stage of enzymatic modification:

– melting point of the fat system – 34–37°C provides plasticity at room temperature and rapid melting in the oral cavity;

– hardness index at 20°C – 120–140 g/cm^2 indicates the structuredness of the fat matrix for technological application;

– low moisture content (≤0.1%) confirms the effectiveness of preliminary refining and deodorization of the initial components;

– fatty acid composition has a balanced ratio of saturated (50–55%), monounsaturated (35–40%) and polyunsaturated (5–8%) fatty acids.

The technological properties of PMS-MF confirm its suitability for further processing. The system retains oxidative stability when heated to 180°C for 120 min. without significant degradation, which is critical for industrial applications. These characteristics make PMS-MF a promising basis for creating fat products with a balanced composition.

4. 3. Method of biotechnological transesterification of fat composition

Enzymatic transesterification was carried out using immobilized Lipozyme TL IM preparation in a laboratory reactor with mechanical stirring. Before loading into the reactor, the enzyme preparation was activated by moistening with 0.1 M sodium bicarbonate solution (pH 7.5) in a ratio of 1:2 (mass of enzyme:volume of solution) with subsequent holding for 15 minutes at room temperature according to [7]. The reaction mixture was formed from the intermediate fat system PMS-MF and refined soybean oil in varying ratios. The process was carried out at a temperature of 55–70°C under vacuum with a stirring intensity of 500 rpm. The enzyme concentration was 10% of the total mass of the fat system. The reaction time was controlled by sampling every 30 min to analyze the degree of enzymatic transesterification by thin-layer chromatography. After the process was completed, the enzyme was separated by filtration through a glass cloth filter, and the reaction mixture was washed with hot water (60°C) to remove residual free fatty acids. The final product was dried in a vacuum oven at 80°C until the moisture content was ≤0.1%.

4. 4. Method for analyzing the degree of enzymatic transesterification

The degree of enzymatic transesterification was estimated by thin-layer chromatography (Silica Gel 60 F254)

in the hexane: diethyl ether: acetic acid system (70:30:1). The main criterion was the decrease in the intensity of the spots of the original TAGs (*Rf* 0.6–0.8) relative to the newly formed ones (*Rf* 0.4–0.6) after spraying with a phosphomolybdate solution. The ratio of the areas of the spots was used for quantitative assessment, calculating the degree of conversion. For PMS-MF, the focus is on PPP/POP dynamics, for soybean oil, on LnLL/LnLnLn. The method allows for rapid tracking of acyl redistribution with an error of 3–5%.

4. 5. Methods for determining the physicochemical and technological indicators of fat substrates and compositions

The determination of the mass fraction of moisture of fat substrates and compositions was carried out by the gravimetric method according to DSTU 4603. The determination of the acid number (AN) and peroxide number (PN) of fat substrates, reaction mixture and compositions was carried out by the titrimetric method according to DSTU ISO 660 and DSTU ISO 3960.

Methyl esters of fatty acids were obtained according to DSTU ISO 5509. The fatty acid composition was determined by gas-liquid chromatography (Shimadzu, Japan) according to DSTU ISO 5508. Identification of acids was carried out by retention time relative to standards, the quantitative content was calculated as a percentage of the total amount of fatty acids.

Determination of the melting point of fat substrates, reaction mixture and compositions was carried out according to DSTU EN ISO 6321. Assessment of oxidative stability of the obtained fat compositions was determined using accelerated oxidation at 90±1°C for 20 h, determining the dynamics of peroxide accumulation. The induction period was set graphically by the inflection point of the peroxide value change curve, which correlates with the shelf life of the product.

4. 6. Research planning and results processing

In studies on the enrichment of the fat composition of ω-3 PUFA by the method of two-stage transesterification, two- and one-factor experiments were used. In each of the experiments, three repetitions were carried out to ensure the statistical reliability of the results. The approximation of the experimental data was performed by constructing a trend surface, obtaining regression equations (1) and (2). The statistical significance of the equations was confirmed by calculating the Fisher test, based on the assumption (null hypothesis) that the equation is statistically insignificant. The obtained values $F(2,9) = 14.852$ for dependence (1) and $F(2,9) = 11.384$ for dependence (2) significantly exceed the critical tabular value $F_{table}(2,9) = 4.26$ ($p = 0.05$). The obtained result allows to reject the null hypothesis with a probability of 95%. High coefficients of determination ($R^2 = 0.941$ and $R^2 = 0.960$, respectively) confirm the adequacy of the obtained models. The processing of experimental data and the construction of graphical relationships were performed using the Stat Soft Statistica v 6.0 package (USA).

5. Results of enrichment of the fat composition of ω-3 fatty acids by the two-stage transesterification method

5. 1. Rationalization of enzymatic transesterification parameters for maximum incorporation of ω-3 fatty acids into the fat matrix

The initial content of ω-3 PUFA (α-linolenic) in soybean oil was 7.5%, and in the intermediate fat system PMS-MF – 0.0%. Enzymatic transesterification of fat raw materials was carried out under conditions that varied according to the following parameters:

– reaction temperature – from 55°C to 70°C (variation interval 5°C);

– soybean oil content in the mixture with PMS-MF – from 40 to 60% (variation interval 10%).

Rational conditions are determined by the degree of enzymatic transesterification and the minimum formation of free fatty acids in the transesterified fat composition.

Approximate dependences of the degree of enzymatic transesterification (D_{ET}, %) and the formation of free fatty acids (FA_F, %) on the above process parameters are represented by equations (1) and (2), respectively:

$$D_{ET} = -307.9875 + 8.994 \cdot T + 2.93 \cdot C_{SbO} - \\ -0.0577 \cdot T^2 - 0.0189 \cdot T \cdot C_{SbO} - 0.0161 \cdot C_{SbO}^2, \quad (1)$$

$$FA_F = 9.0208 - 0.2863 \cdot T - 0.0363 \cdot C_{SbO} + \\ +0.0027 \cdot T^2 - 0.0001 \cdot T \cdot C_{SbO} + 0.0005 \cdot C_{SbO}^2, \quad (2)$$

where T – the reaction temperature,°C;

C_{SbO} – the content of soybean oil in the mixture with PMS-MF, %.

Fig. 1 shows the graphical dependences of the degree of enzymatic transesterification (*a*) and the formation of free fatty acids (*b*) in the fat composition on the temperature and the content of soybean oil in the mixture with PMS-MF.

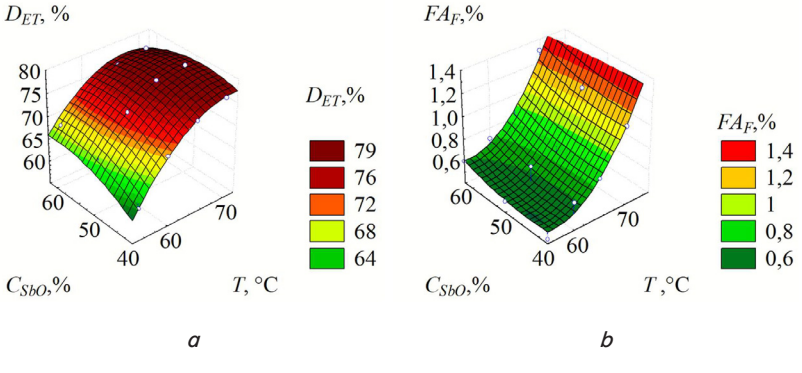

Fig. 1. Dependences of the process of modification of the fat composition: *a* – the degree of enzymatic transesterification depending on the temperature and the content of soybean oil in the mixture with PMS-MF; *b* – the content of free fatty acids depending on the temperature and composition of the mixture

Analysis of the influence of temperature and the ratio of substrates allowed to establish rational parameters of the process of enzymatic transesterification of the fat composition. As can be seen from the obtained data (Fig. 1), the degree of transesterification (D_{ET}) increases with increasing temperature from 55 to 70°C at all studied ratios of substrates. However, analysis of the formation of free fatty acids (FA_F) showed that at a temperature of 70°C there is a significant increase in

FA_F (up to 1.3%), which indicates an intensification of hydrolysis processes. Reasonable process parameters – temperature 65°C and the ratio of soybean oil to PMS-MF 50% – were selected based on a comprehensive analysis of experimental data. Under these conditions, a high degree of transesterification (78.3%) is achieved with minimal free fatty acid formation (0.6%). Although a slightly higher D_{ET} (79%) is observed at 70°C, the FA_F content increases significantly (1.2%), making these conditions less suitable for industrial applications.

It should be noted that the choice of the content of soybean oil in the fat system at the level of 50% is justified by the fact that it allows to achieve an optimal balance between high efficiency of the process (D_{ET} 78.3%) and the minimum degree of hydrolytic processes (FA_F 0.6%). Increasing the proportion of soybean oil to 60% leads to a slight decrease in D_{ET} (76.5%) and an increase in FA_F (0.8%).

Thus, it was established that the combination of a temperature of 65°C and a substrate ratio of 50 : 50 is rational for achieving high efficiency of the transesterification process with minimal losses due to hydrolysis. These parameters provide a degree of enzymatic transesterification of 74.2% with the formation of only 0.6% of free fatty acids in the fat composition.

5. 2. Assessment of the oxidative stability of a modified fat composition after a two-stage transesterification process

To assess the oxidative stability of the modified fat composition enriched with ω-3 PUFA, a series of experiments were conducted to study its resistance to oxidation under different conditions. The resulting product was stored at 4°C and 25°C for 30 days in sealed containers. Quality control included determination of the peroxide value and ω-3 PUFA content. The results of studies on the dependence of the PF of samples of the modified fat composition on the storage temperature are shown in the graph (Fig. 2).

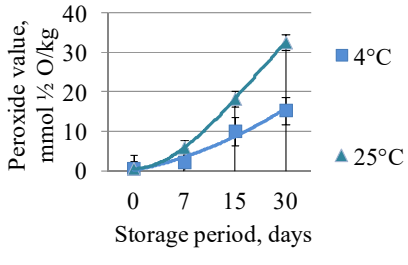

Fig. 2. Dependence of the peroxide value of samples of the modified fat composition on storage temperature

The results of studies on the dependence of the content of ω-3 PUFA in samples of the modified fat composition on storage temperature are shown in the graph (Fig. 3).

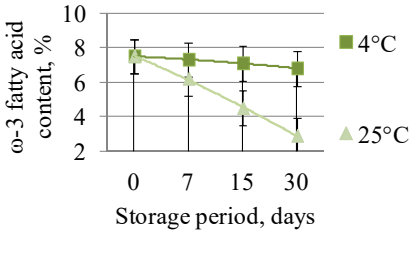

Fig. 3. Dependence of the content of ω-3 fatty acids in samples of the modified fat composition on the storage temperature

The graphs demonstrate the dependence between the accumulation of peroxides (PV) in the modified fat composition and the losses of ω-3 PUFA. At 4°C, after 30 days, PV increases to 15.3 mmol ½O2/kg with losses of ω-3 acids of 5.1%, which confirms the effectiveness of cooling the product to inhibit oxidation. In contrast, at 25°C, a sharp increase in PV to 18.2 mmol ½ O_2 /kg with significant losses of ω-3 PUFA (up to 13.3%) is recorded already on the 15th day. At the same time, by the 30th day, these indicators reach 32.5 mmol ½ O_2 /kg and losses of ω-3 PUFA to 61.0%, respectively.

6. Discussion of the results of enriching the fat composition with ω-3 PUFAs by the two-stage transesterification method

The obtained data (equations (1), (2), Fig. 1, *a*, *b*) demonstrate a clear dependence of the degree of transesterification (D_{ET}) and the formation of free fatty acids in the fat composition (F_{AF}) on the reaction temperature and the ratio of substrates. At a temperature of 55°C, the lowest degree of transesterification (62.1–68.7%) is observed, which is explained by the insufficient activity of Lipozyme TL IM. Increasing the temperature to 60–65°C leads to an increase in D_{ET} to 74.2–78.3% due to optimization of the kinetics of the enzymatic reaction, while a further increase to 70°C causes an increase in F_{AF} (1.1–1.3%) due to thermally induced hydrolysis. The highest efficiency of the process is achieved at 65°C and 50% soybean oil content in the mixture (D_{ET} 78.3%, F_{AF} 0.6%), which is associated with a rational balance between the availability of ω-3 PUFA and the stability of the fat matrix. The decrease in D_{ET} at a soybean oil content of 60% (even at high temperature) may be a consequence of the excess of polyunsaturated acids competing for the active sites of lipase.

The obtained results can be explained by the complex interaction of two main factors. The temperature regime showed a decisive influence on the activity of the enzyme Lipozyme TL IM – its maximum catalytic activity is observed at 65°C. However, with a further increase in temperature to 70°C, the process of hydrolysis begins to prevail over the transesterification process, which is accompanied by thermal degradation of ω-3 acids and an increase in the content of free fatty acids. The rational ratio of substrates (soybean oil : PMS-MF as 1: 1) provides a balance between the availability of ω-3 PUFA and the preservation of the structural integrity of the PMS-MF fat matrix. At the same time, an excess of soybean oil of more than 50% disrupts the micellar organization of the system, reducing the overall efficiency of the enzymatic reaction. In addition, the selectivity of Lipozyme TL IM plays an important role, manifested in the preferential catalysis of transesterification at the sn-1,3 positions of triglycerides, which significantly limits the process of hydrolysis and the formation of free fatty acids. An additional factor that contributes to minimizing hydrolysis is the use of vacuum conditions, which effectively remove water – a by-product of the reaction.

The obtained results of the studies of the PV of samples of the modified fat composition on the storage temperature (Fig. 2, 3) demonstrate the dependence between the storage conditions and the oxidative stability of the fat composition. During storage at 4°C, a relatively slow increase in PF is observed – in 30 days it reaches 15.3 mmol ½ O_2 /kg, which is accompanied by insignificant losses of ω-3 PUFA (5.1%).

This is explained by the low rate of free radical processes at low temperatures. In contrast, storage at 25°C leads to a significant acceleration of oxidation - in 15 days the PF reaches 18.2 mmol ½ O_2 /kg, and the losses of ω-3 PUFA are 13.3%. Such dynamics are due to several key factors. First, an increase in temperature leads to an increase in the kinetic energy of molecules, which contributes to the initiation of chain reactions of autoxidation. Secondly, ω-3 PUFAs, in particular α-linolenic acid, contain bis-allyl methylene groups, which are particularly susceptible to radical oxidation. The critical value of the PV (10 mmol ½ O_2 /kg) is reached already on the 15th day at 4°C, while at 25°C this figure is exceeded already on the 7th day, which indicates the exponential nature of the dependence of the oxidation rate on temperature.

Cascade oxidation at 25°C, which is manifested by a sharp increase in PV after 15 days (up to 32.5 mmol ½ O_2 /kg on day 30), is explained by the accumulation of secondary oxidation products – aldehydes and ketones, which themselves catalyze further lipid decomposition. This is confirmed by the simultaneous decrease in the content of ω-3 PUFA in the fat composition to 5.9% on day 30 of storage, which indicates a deep degradation of PUFA. Thus, the obtained results confirm the critical effect of storage temperature on the stability of the fat composition enriched with ω-3 PUFA, and the need to use low temperatures to preserve its quality.

The results obtained in this work on the biotechnological enrichment of the fat composition of ω-3 PUFA by the two-stage transesterification method demonstrate significant progress compared to existing approaches described in the literature. While in works [8–12] the main limitation was the lack of control over the degree of transesterification, the proposed method of combining chemical and enzymatic transesterification allows achieving a degree of 74.2%. This indicator significantly exceeds the results obtained in [10] using ultrasonic treatment (degree of transesterification 65–70%), and is especially important for the creation of milk fat analogues.

An important difference of the described study is a comprehensive approach to solving the problem of oxidative stability of lipids. In contrast to the works [13–15], which ignored the influence of lipid oxidation on the quality of the final product, this study proved the effectiveness of a rational reaction temperature (65°C) and vacuum conditions. This solution allowed to maintain the content of free fatty acids in the finished product at 0.6%, which is significantly lower than such indicators in the works [10] (2.8%) and [15] (3.5%). It is advisable to pay special attention to the stability of the resulting fat composition. The results obtained showed that during storage at 4°C, the loss of ω-3 PUFA is 5.1% for 30 days, which is significantly better than such indicators given in [13] (12–15%) and [17] (18–20%). This is achieved by combining a rational composition of fat substrates and the use of reasonable technological parameters during the enzymatic transesterification process. Thus, the proposed two-stage transesterification method not only eliminates the main limitations of existing approaches, but also offers a comprehensive solution for the industrial production of stable ω-3 enriched fat compositions. This opens up new opportunities for the creation of functional food products with increased nutritional value.

The presented data reveal key limitations of the transesterification technology, caused by the variability of the properties of the starting raw materials. The fatty acid composition of the components used – the intermediate fat system PMS-MF and soybean oil – can show significant inter-batch differences. In particular, the palmitic acid content in palm stearin ranges from 40% to 55%, and the ω-3/ω-6 ratio in soybean oil depends on the region of origin of the raw materials, which can affect the final product characteristics. In addition, the enzymatic stage of the process shows high sensitivity to the technological parameters of the substrate. The activity of Lipozyme TL IM is significantly reduced in the presence of more than 1% free fatty acids, which leads to a decrease in the degree of transesterification by 15–20%. The stability of the final fat composition also directly depends on the quality of the starting raw materials. An increased PV of soybean oil above 1 mmol ½ O_2 /kg leads to a reduction in the shelf life of the finished product by approximately 30%. In addition, the variability of the content of natural tocopherols between different batches of raw materials can reach 3–5 times, which further complicates the process of standardization of the finished product. To overcome the identified limitations, the use of adaptive approaches is promising, in particular, the correction of technological parameters based on operational gas chromatographic analysis of the composition of the input raw materials. Operational monitoring of the PV during transesterification should also provide additional quality control.

The results obtained reveal certain shortcomings, in particular the lack of detailed data on the influence of various antioxidant systems on the stability of the modified fat composition. Although vacuum conditions and temperature control during enzymatic transesterification have proven their effectiveness, the issue of optimal selection of antioxidants for long-term storage of the product remains unresolved. Particular attention should be paid to the possibility of a synergistic effect between natural tocopherols and synthetic antioxidants, which could increase oxidative stability without changing organoleptic properties.

In addition, the results of the study indicate the prospects for further improvement of the two-stage transesterification technology. An important aspect of further research should be the development of universal protocols for adapting technological parameters to the changing composition of the raw material. In addition, it is worth investigating the effectiveness of antioxidant systems for stabilizing ω-3 PUFA during the transesterification process. These areas of research will allow expanding the application of the technology for different types of fatty raw materials while maintaining high quality indicators of the final product.

7. Conclusions

1. Rational conditions for enzymatic transesterification of fat substrates (intermediate fat system PMS-MF based on palm stearin, coconut and high-oleic sunflower oils and refined deodorized soybean oil) have been established for maximum inclusion of ω-3 PUFAs into the fat matrix. It has been proven that a temperature of 65±1°C and a ratio of soybean oil to PMS-MF of 1:1 ensure the specified efficiency of the process. Under these conditions, a transesterification degree of 74.2% is achieved with minimal formation of free fatty acids (0.6%).

2. The oxidative stability of the modified fat composition was assessed after a two-stage transesterification process at

different temperatures. It was established that the justified parameters of the enzymatic transesterification process allow to achieve an increase in the peroxide value of the fat composition to 15.3 mmol ½ O_2 /kg after 30 days of storage at 4°C. This is accompanied by losses of ω-3 PUFA in the product at the level of 5.1%.

Conflict of interest

The authors declare that they have no conflict of interest regarding this study, including financial, personal, authorship or other, that could influence the study and its results presented in this article.

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Data availability

The manuscript has no linked data.

Use of artificial intelligence tools

The authors confirm that they did not use artificial intelligence technologies when creating the presented work.

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