This work research object was fat systems interesterification biotechnology using the Lipozyme TL IM immobilized enzyme preparation. The problem of enzyme preparation activation by moistening with sodium bicarbonate aqueous solution with 7.4 ... 7.7 (3 % wt.) pH was solved in the work. The obtained results made it possible to minimize the interesterification process duration with high-quality product obtaining. The proposed enzyme preparation processing made it possible to reduce the duration of the biointeresterification process in a model fat mixture (palm stearin, coconut and soybean oils in a ratio of 1:1:1, respectively) to 3.5...3.7 hours. The product with high quality indicators, namely up to 0.26 mg KOH/g acid number, up to 0.60 mmol ½ O/kg peroxide number and 1.70 c.u. anisidine number, was obtained as a result. The obtained data can be explained by a fact that effective biocatalysis with lipolytic enzymes as the protein molecules requires the existence of two phases – lipid and water. This fact was provided by the activation parameters justified in the study. The obtained results feature was possibility of enzyme preparation activation, which is not provided under industrial conditions due to the threat of raw materials and finished products hydrolytic processes, which leads to the finished product quality deterioration. The research results made it possible to minimize hydrolytic processes in fat system during interesterification with simultaneous process efficiency increase. From a practical point of view, the discovered activation mechanism made it possible to adjust the enzyme preparation processing conditions in fat systems interesterification technology. The applied aspect of scientific result using was the possibility of improving the typical technological process of fat interesterification

Keywords: biotechnological interesterification, immobilized enzyme preparation, interesterification duration, interesterified fat quality indicators

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IMPROVEMENT OF FATTY SYSTEMS BIOTECHNOLOGICAL INTERESTERIFICATION WITH IMMOBILIZED ENZYME PREPARATION USAGE

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1. Introduction

The main direction of vegetable oils modification by nutritional value increasing is associated with use of enzymatic catalysis, namely, the interesterification reaction. Enzymatic interesterification allows the global restructuring of oil and fat industry, replacing the existing method of vegetable oils hydrogenation. The hydrogenation process is accompanied by an increased formation of trans-fatty acids hazardous to human health. In addition, it is associated with an increased

content of nickel, which is used as a process catalyst, in the reaction products [1]. Interesterification is a promising technology for obtaining special fats intended for the confectionery, bakery, dairy and other food industries [2, 3].

The enzymatic properties of lipases, both hydrolytic enzymes and enzymes that catalyze the interesterification reaction, are being actively studied. The use of lipases with different types of specificity makes it possible to carry out the process of directed interesterification. The result of fatty systems interesterification process can be a wide range of products with desired properties and acylglycerol composition without changing the fatty acid composition of the initial mixture. In addition, the method is safe, environmentally friendly, does not lead to trans-isomers accumulation in fat system and is practically waste-free [4].

However, there are certain difficulties associated with process specifics when implementing this method. Interesterification has a special feature in comparison with the most other industrial enzymatic processes, namely the presence of a two-phase reaction system. The substrate and product of enzymatic reaction are located in such a system in a liquid phase immiscible with water, and the enzyme is active at the interface. In addition, the technological aspects include significant enzyme preparation consumption with insufficient technology rationalization, using the hydrolytic enzyme ability to catalyze a reaction reversed its natural reaction, and so on [4, 5].

It is important to increase lipases enzymatic activity and reduce the enzyme preparations consumption in connection with wide possibilities of using lipases enzyme preparations [4, 6]. These problems can be solved by preparation catalytic activity and thermal stability increasing. Technology improvement will allow reducing the production waste volume, rationalizing processing modes, and reasonably using the available material resources.

Thus, biotechnological interesterification technology improvement is advisable in order to obtain safe fatty products with controlled composition, high nutritional and biological value, rationalize the technological process and reduce the amount of fat and oil production waste. This study results introduction into the technological process is necessary due to high-quality special fats needs for food production.

2. Literature review and problem statement

The property of intensively developing biotechnology is the widespread production and use of enzyme preparations produced by various microorganisms and differed in substrate specificity and mechanism of action. Enzyme preparations of microbiological origin are increasingly replacing conventional chemical catalysts in a number of industrial processes. Such preparations have a number of advantages over enzymatic preparations of plant and animal origin in addition to environmental friendliness and high activity. The following advantages can be shown [7]:

– microbiological enzymes production in bioreactors is easily controlled and predictable;

– excreted microbiological enzymes are more stable compared to intracellular animal and plant analogues;

– microorganisms genetic diversity allows production of enzyme preparations with a wide range of specificity;

– enzymes microbiological synthesis can last all year round, in contrast to the production of plant enzymes, which is often seasonal.

Interesterification is carried out using lipases in aqueous-organic media. The use of organic solvents significantly expands the number of reactions carried out using enzymatic catalysis especially with lipolytic enzymes due to the fact of hydrophobic compounds substrate of this enzymes group [8]. Significant shifts in reactions equilibrium can be achieved in two-phase water-organic systems by two phases' ratio optimizing and adjusting the value of the coefficients of components distribution between them. Development and improvement of this method go in two ways:

– new lipases producers search in order to obtain highly efficient and specific catalysts;

– new materials and enzyme immobilization methods development. Lipases enzyme preparations immobilization makes it possible to increase by a factor of 5–12 times their lipolytic activity compared to the initial enzymes [8, 9].

The principle underlying all methods for determining enzyme activity is to record of substrate loss rate (i.e., the substance on which the enzyme acts) or reaction products biosynthesis rate. A feature of enzymatic reactions is the dependence of enzymatic reaction rate on temperature in a rather narrow temperature range, which is characterized by the so-called reaction temperature optimum [8, 9].

Possibility of activating the *Amilorizin P10x* enzyme preparation (*Aspergillus oryzae* was a producer) was tested in [10] using acoustic sound exposure of a certain frequency in the range of 20–20000 Hz. It has been established that acoustic treatment can both decrease and increase the enzyme product amylolytic activity. The question of negative consequences of such acoustic impact on health of personnel serving the production remains unresolved.

There is a method for activating an immobilized heterogeneous enzyme preparation (*Geobacillus stearothermophilus G3* is a producer, aminated silica gel is a carrier) [11]. The influence of the following factors on fatty acid methyl esters yield in the reaction of sunflower oil methanolysis was studied in the work:

– nature of a solvent;

– process temperature;

– methanol : oil molar ratio;

– water and catalyst amount.

The high biocatalyst stability in the reaction was shown under justified optimal conditions: more than 50 % of its initial activity was retained after 480 hours of operation (20 cycles). This makes the indicated biocatalyst promising for use in production of fatty acid esters being a feedstock for biodiesel fuel production. However there are no data of this enzyme preparation activation during the intermolecular interesterification reaction.

Effect of activation of the enzyme preparation (*Candida antarctica* was a producer, chitosan was a carrier) was evaluated in the study [12]. Chitosan as a carrier was activated with divinyl sulfone at various pH values. After immobilization, the enzyme preparation was incubated under alkaline conditions in buffer, followed by incubation in ethylenediamine to block the remaining reactive groups. High thermal lipases stability was obtained upon activation with divinyl sulfone at pH 10.0 as experiments result. It was shown that obtained activation results were better than those obtained using glutaraldehyde as a reagent that activates the chitosan carrier. The disadvantage of the study was the use not desirable for food production chemicals.

There is a method [13] of activating organophosphorus hydrolase (*Flavobacterium* is producer; cellulose modified

with epoxy resin is carrier). Activation was carried out by two different methods using 1,4-butanediol diglycidyl ether and 1,1'-carbonyldiimidazole. Rational conditions of influencing the immobilized enzyme activity parameters in both methods of activation were substantiated. The study disadvantages were the 1,1'-carbonyldiimidazole high sensitivity to moisture, which made it difficult use in biocatalysis, and butanediol diglycidyl ether moderate toxicity to humans. An option to overcome the corresponding disadvantages may be additional stages of product purification, but this is not economically feasible.

The effect of a number of compounds on pancreatic lipase enzyme preparation (cells of the pancreas of cattle were producers) activity were examined in the promising study [14]. The carried out studies made it possible to establish reduced enzyme preparation activity with Ca^{2+} ions absence. At the same time, the calcium chloride adding to the solution led to its significant activation. It was shown the Mg^{2+} ions influence on lipase activity increase, but to a lesser extent then Ca^{2+} ions. Moreover, chlorides had a more pronounced effect than sulfates. It was established the level of influence of inorganic compounds on the reaction rate dependence on the degree. But, there were no data of these activators effect on immobilized form of lipolytic enzyme preparations among the results of the study.

Water presence necessity in composition of *Lipozyme RM IM* immobilized enzyme preparation (*Rhizomucor miehei* was a producer of the enzyme preparation, ion-exchange resin was a carrier) during the interesterification between coconut oil and high oleic rapeseed oil was substantiated in [15]. Water molecules manifestation a certain stabilizing effect on lipase in Lipozyme RM IM composition using hydrogen bonds was noted in a molecular dynamics simulation system with a low water content (5 %). This helped to fix the active sites of the enzyme, which in turn led to enzyme preparation activity increase. But there were no data of activation of lipolytic enzyme preparations immobilized on other carriers, in particular on silica gel.

A study of activation of *Lipozyme TL IM* enzyme preparation (*Thermomyces lanuginosus* is a producer, silica gel is a carrier) is advisable to conduct according to the analysis of specialized scientific publications. The enzyme preparation is widely used in fatty systems biotechnological interesterification and is a mixture of hydrolytic enzymes, mainly lipases, immobilized on silica gel. Silica gel, as a carrier, unlike a number of other analogs, must contain a certain amount of water to maintain lipase activity on phase distribution surface [16]. However, there is a certain lack of data of activating such enzyme preparations or enzymes immobilized on silica gel. Taking this into account, it is important to study the effect on the efficiency of fatty systems biotechnological interesterification using lipases immobilized on silica gel, aqueous solutions pre-moistening with different pH values. Accordingly, it is important to substantiate rational pH values of aqueous solution and preliminary moistening value of enzyme preparation immobilized on silica gel. This will increase process efficiency of biotechnological interesterification of a wide range of fat systems.

3. The aim and objectives of the study

The aim of the study is to improve fat systems biotechnological interesterification using immobilized enzyme preparation. This will allow minimizing interesterification process duration with obtaining a high quality interesterified fat product.

The following tasks were solved to achieve the set aim:

– to determine the physico-chemical parameters and fatty acid composition of fatty raw materials for biotechnological interesterification;

– to investigate the dependence of biotechnological interesterification efficiency from preliminary moistening of the immobilized enzyme preparation and pH of aqueous solution;

– to determine the physico-chemical parameters and fatty acid composition of the interesterified fat product obtained according to improved technology, to compare it with the analog made according to traditional technology.

4. Materials and methods

4. 1. Object and hypothesis of the study

The object of the study is biotechnology of fat systems interesterification using *Lipozyme TL IM* immobilized enzyme preparation. The main hypothesis of the study is possibility of activating *Lipozyme TL IM* immobilized enzyme preparation by aqueous solution pre-moistening with optimal pH value.

The following assumptions were made in the study:

– the pH optimum of the immobilized enzyme might vary slightly compared to the pH optimum of an enzyme in a free state;

– the activated immobilized enzyme preparation had a similar efficiency during interesterification of raw materials that differ in fatty acid composition from the studied raw materials;

– the activated immobilized enzyme preparation had a similar efficiency during raw materials interesterification in order to obtain products with a different melting point.

The following simplifications were adopted in the study:

– raw materials for enzymatic interesterification had similar physical and chemical parameters, in particular, moisture and volatile substances content, mass fraction of phosphorus-containing substances, acid, peroxide and anisidine numbers, as the investigated raw materials.

4. 2. Investigated materials and equipment used in the experiment

The following materials and reagents were used during the research:

– *Lipozyme TL IM* immobilized enzyme preparation, which is the *Thermomyces lanuginosus* lipase immobilized on silica gel (manufactured by Novozymes A/S, Denmark), according to CAS 9001-62-1;

– refined, bleached and deodorized palm stearin (made in Malaysia), according to DSTU 4439/CAS 91079-14-0;

– refined, bleached and deodorized coconut oil (made in Malaysia), according to DSTU 4562/CAS 8001-31-8;

– refined, bleached and deodorized soybean oil (made in Ukraine), according to DSTU 4534/CAS 8001-22-7.

4. 3. Methods of biotechnological interesterification in fatty systems

The process of biotechnological interesterification was carried out under vacuum at the temperature of 70±1 °C with stirring at a speed of 500 rpm. *Lipozyme TL IM* en-

zyme preparation content in reaction mixture was 10 % from fat system mass in accordance with researcher's substantiation [17, 18]. For the purpose of activation *Lipozyme TL IM* immobilized enzyme preparation was pre-moistened with aqueous solutions of citric acid or sodium bicarbonate with 6.5...8.3 pH. Samples were taken from the reaction mixture at certain intervals, their physicochemical parameters were analyzed according to the methods described in 4.3.

PH of aqueous solutions of citric acid and sodium bicarbonate for enzyme preparation moistening was determined by the potentiometric method.

4. 4. Methods of determining the physico-chemical parameters of fatty raw materials, fatty reaction mixture and interesterified fats

Determination the acid and peroxide numbers of fatty raw materials, fatty reaction mixture and interesterified fats was carried out by titrimetric methods in accordance with DSTU ISO 690 and DSTU ISO 3960. Determination the anisidine number of fatty raw materials and transesterified fats was carried out by the colorimetric method according to DSTU ISO 6885. Determination the phospholipids content in fatty raw materials was carried out by the colorimetric method according to DSTU EN ISO 11701. Determination the mass fraction of moisture in fatty raw materials and interesterified fats was carried out by gravimetric method in accordance with DSTU 4603. Determination the melting temperature of fatty raw materials, fatty reaction mixture and interesterified fats was carried out according to DSTU EN ISO 6321. Determination the soap in the fat reaction mixture and interesterified fats was carried out according to DSTU 6048 (ISO 10539).

4. 5. Method of determining the fatty acid composition of fatty raw materials and interesterified fats

Preparation of methyl esters of fatty acids of fatty raw materials and interesterified fats was carried out in accordance with DSTU ISO 5509. Determination the fatty acid composition of fatty raw materials by gas-liquid chromatography was carried out in accordance with DSTU ISO 5508 on a Shimadzu chromatograph (Japan). The process parameters were as follows:

– 160 °C column temperature;
– exposure at 160 °C for 2 minutes;
– 3 °C/min temperature rise;
– 225 °C maximum temperature;
– exposure at 225 °C for 15 minutes.

The evaporator temperature was 240°C; detector temperature – 250 °C; carrier gas (hydrogen) was supplied at a rate of 1.0 ml/min. Fatty acids were identified by comparing the obtained retention time with the retention time of standards. The fatty acids content was calculated as a percentage of their total amount.

4. 6. Research planning and results processing

Two-factor experiments were used in the studies. Each experiment was repeated three times. The processing of received data and construction of graphic dependencies were performed using the Stat Soft Statistica v 6.0 package (USA). Statistical models of dependencies were determined through experimental data approximation by constructing a trend line according to commonly used methods.

Verification of approximation dependences coefficients significance was done by testing the hypothesis of equation zero parameters equality. The absolute value of Student's t-test was calculated, and then compared with the critical theoretical value of this criterion at a given significance level and a given number of freedom degrees for multiple regressions. Influence degree of enzyme preparation preliminary moistening and aqueous solution pH on the following indicators was estimated using R2 determination coefficient:

– interesterification process duration;
– acid number of fat system;
– peroxide number of fat system.

A conclusion about influence degree of selected factors on experimental plans function response was made based on obtained data.

Approximation models significance was determined by comparing calculated Fisher criterion with its critical tabular value at p=0.05 significance level and corresponding freedom degrees number.

5. Results of studies of improvement of fat systems biotechnological interesterification using the immobilized enzyme preparation

5. 1. Determination of physical and chemical parameters of fatty raw materials samples for biotechnological interesterification

The physico-chemical parameters of fatty raw materials experimental samples for biotechnological interesterification were determined. The study results are given in Table 1.

Table 1

Physico-chemical parameters of experimental samples of fatty raw materials for biotechnological interesterification

Physico-chemical characteristics	Samples of refined, bleached and deodorized vegetable oils		
	palm stearin	coconut oil	soybean oil
Acid number, mg KOH/g	0.12	0.20	0.18
Peroxide number, mmol O/kg	0.34	0.52	0.75
Anisidine number, c. u.	1.50	1.75	1.80
Mass fraction of phosphorus-containing substances in terms of stearoleolecithin, %	absence		
Mass fraction of moisture and volatile substances, %	0.03	0.02	0.01
Melting point, °C	47.4	24.3	–16.4

According to the research results, fatty raw materials experimental samples met the requirements established in the relevant regulatory documentation – DSTU 4439/CAS 91079-14-0, DSTU 4562/CAS 8001-31-8, DSTU 4534/CAS 8001-22-7.

The fatty acid composition of experimental samples of fatty raw materials for biotechnological interesterification was determined. The study results are given in Table 2.

According to the research results, the selected raw materials do not contain trans-fatty acids, content of which is regulated for food products. The fatty raw material was chosen for reasons of maximization of acyl species with different molecular weights and different saturation degrees for a comprehensive assessment of physico-chemical parameters of the obtained interesterified fatty products.

Table 2

Fatty acid composition of experimental samples of fatty raw materials for biotechnological interesterification

Fatty acids	Samples of refined, bleached and deodorized vegetable oils		
	palm stearin	coconut oil	soybean oil
$C_{8:0}$	–	8.17	–
$C_{10:0}$	–	6.24	–
$C_{12:0}$	0.23	49.02	–
$C_{14:0}$	1.25	19.76	–
$C_{16:0}$	67.14	8.15	10.78
$C_{16:1}$	0.02	–	5.68
$C_{18:0}$	4.36	1.83	24.16
$C_{18:1}$	21.23	5.36	1.09
$C_{18:2}$	5.25	1.47	49.51
$C_{18:3}$	0.14	–	7.68
$C_{20:0}$	0.38	–	0.45
$C_{20:1}$	–	–	0.23
$C_{22:0}$	–	–	0.42
Total	100.00	100.00	100.00

5. 2. Investigation of dependence of biotechnological interesterification efficiency from preliminary moistening of the immobilized enzyme preparation and pH of aqueous solutions in reaction mass

In accordance with the goal of improving the fat systems biotechnological interesterification using the immobilized enzyme preparation, process efficiency dependence from the following factors was studied:

– preliminary moistening of the enzyme preparation, $C_{w.s.}$, %;

– pH of aqueous solution, $pH_{w.s.}$, un.

The criteria of biotechnological interesterification effectiveness were:

– process duration – time after which the melting point of the fatty product of the reaction practically did not change, $\tau_{f.p.}$, hour;

– acid number of interesterified fat, $AN_{f.p.}$, mg KOH/g;

– peroxide number of interesterified fat, $PN_{f.p.}$, mmol ½ O / kg.

The model fat mixture for biotech interesterification consisted of palm stearin, coconut oil and soybean oil in a ratio of 1:1:1, respectively. The method of multivariate regression with construction of response surfaces was chosen to determine the dependence of biotechnological interesterification process duration, acid number, peroxide number of the fatty product from preliminary moistening of the immobilized enzyme preparation and pH of aqueous solution. The model was built using the method of full factorial experiment.

Preliminary moistening of the immobilized enzyme preparation was varied in the 0...10 % range with the 2 % step. The pH of aqueous solution (citric acid or sodium bicarbonate) was varied in the range of 6.5...8.3 with the step of 0.3. It must be said that these acidic and alkaline reagents were chosen because of their rather mild effect on triglycerides of fat reaction mixture in previous biotechnological interesterification experiments compared to such reagents as hydrochloric acid and sodium hydroxide.

Exposure time of the moistened enzyme preparation was 1 hour. Melting temperature of interesterified fat was 32.3...32.6 °C depending on the enzyme preparation moisturizing method.

The obtained values of biotechnological interesterification process duration were in the range of 3.2...5.0 hours; acid number of interesterified fats – 0.20 ..0.72 mg KOH/g; peroxide number of interesterified fats – 0.55...0.85 mmol ½ O / kg. The surfaces of obtained dependencies are shown in Fig. 1, *a–c*.

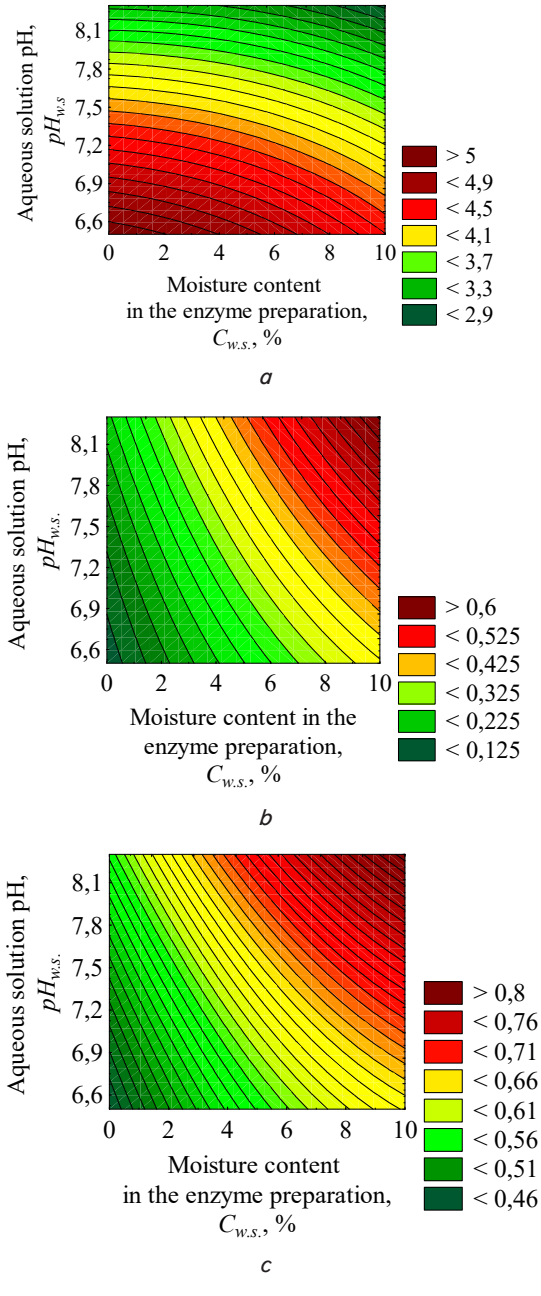

Fig. 1. Dependence of the following parameters from the preliminary moistening value of the immobilized enzyme preparation and the pH of aqueous solution: *a* – biotechnological interesterification process duration; *b* – acid number of interesterified fats; *c* – peroxide number of interesterified fats

The approximation dependences of the efficiency parameters of biotechnological interesterification process are presented with the help of equations (1)–(3), namely:

– process duration ($\tau_{f.p.}$);

– product acid number ($AN_{f.p.}$);

– product peroxide number ($PN_{f.p.}$).

From the factors:
– enzyme preparation preliminary moistening ($C_{w.s.}$);
– pH of aqueous solution for humidification ($pH_{w.s.}$).

$$\tau_{f.p.}(C_{w.s.}, \mathrm{pH}_{w.s.}) = 5.0358 \cdot 0.1331 \cdot C_{w.s.} + \\ +0.8006 \cdot \mathrm{pH}_{w.s.} - 0.0037 \cdot C^2_{w.s.} + \\ +0.0158 \cdot C_{w.s.} \cdot \mathrm{pH}_{w.s.} - 0.1221 \cdot \mathrm{pH}^2_{w.s.}; \quad (1)$$

$$AN_{f.p..}(C_{w.s.}, \mathrm{pH}_{w.s.}) = 0.2132 - 0.0328 \cdot C_{w.s.} - \\ -0.0763 \cdot \mathrm{pH}_{w.s.} + 0.0004 \cdot C^2_{w.s.} + \\ +0.0096 \cdot C_{w.s.} \cdot \mathrm{pH}_{w.s.} + 0.0093 \cdot \mathrm{pH}^2_{w.s.}; \quad (2)$$

$$PN_{f.p.}(C_{w.s.}, \mathrm{pH}_{w.s.}) = 0.5533 + 0.0011 \cdot C_{w.s.} - \\ -0.0751 \cdot \mathrm{pH}_{w.s.} - 0.0008 \cdot C^2_{w.s.} + \\ +0.0039 \cdot C_{w.s.} \cdot \mathrm{pH}_{w.s.} + 0.0093 \cdot \mathrm{pH}^2_{w.s.}. \quad (3)$$

It should be noted that the given approximation dependences describe the real process adequately in the intervals of 0...10 % moistening of immobilized enzyme preparation and 6.5...8.3 pH of aqueous solution for moistening. So, the range of studied factors was determined, namely:

– enzyme preparation moistening amount with an aqueous solution of sodium bicarbonate (3 ... 4 % wt.);

– moistening solution pH (7.4...7.7);

– in which interesterification process efficiency had increased, namely:

– process duration – 3.5...3.7 hours;

– acid number of interesterified fats – 0.15...0.20 mg KOH/g;

– peroxide number of the product – 0.20...0.40 mmol ½ O/kg.

It should be noted that enzyme preparation moistening with aqueous solutions in amount of more than 3...4 % wt. in the studied pH range led to deterioration of physico-chemical parameters of interesterified fats. The fatty mixture hydrolysis reaction and, accordingly, the oxidative spoilage reaction flew in addition to the interesterification reaction during moistening with solutions with less than 7.3 and more than 7.8 pH. This was identified through a positive test for soap (under condition of alkaline solutions action in the amount of more than 3...4 % by mass.). Also, under these conditions, the processes of accumulation of free fatty acids and primary oxidation products (increase in acid and peroxide numbers) flew, which reduced the final product quality indicators.

Conditions for carrying out biotechnological interesterification were adjusted based on obtained research results (according to 4.2). Immobilized enzyme preparation moistening with sodium bicarbonate aqueous solution (3 % wt. amount; 7.4...7.7 pH;15 min moistened enzyme preparation exposure) was proposed.

5. 3. Determination of physico-chemical parameters and fatty acid composition of interesterified fat obtained by improved technology

The physico-chemical parameters of interesterified fat obtained by improved technology were determined. The study results in comparison with the physico-chemical parameters of interesterified fat obtained by generally accepted technology (enzyme preparation moistening – 0 %) are shown in Table 3.

Table 3

Physico-chemical parameters of interesterified fat obtained by improved technology

Physico-chemical characteristics	Samples of interesterified fat obtained by technology	
	improved	generally accepted (control sample)
Acid number, mg KOH/g	0.24	0.26
Peroxide number, mmol O/kg	0.58	0.60
Anisidine number, c. u.	1.70	1.70
Mass fraction of moisture and volatile substances, %	0.02	0.04
Melting point, °C	33.4	33.2
Biointeresterification process duration, hour	3.5	5.0

According to the results of the research (Table 3), a sample of interesterified fat obtained by improved technology practically did not differ from a sample of interesterified fat obtained by generally accepted technology. The difference between the studied samples was only in biotechnological interesterification process duration. It was 3.5 hours according to the improved technology and was 30 % less than process duration of generally accepted technology. It should be added that the obtained product sample met the requirements of DSTU 4336 in terms of physical and chemical parameters.

The fatty acid composition of interesterified fat obtained by improved technology was determined. The study results are given in Table 4.

Table 4

Fatty acid composition of interesterified fat obtained by improved technology

Fatty acid content, %													
$C_{8:0}$	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:1}$	$C_{22:0}$	Total
2.81	2.11	16.40	6.98	28.67	1.91	10.11	9.22	18.73	2.59	0.26	0.07	0.14	100.00

According to the research results, the interesterification process was fully completed (in accordance with the data in Table 2), the selected raw material did not contain trans-fatty acids, content of which is regulated for food products.

6. Discussion of the results of study of interesterification biotechnology using the immobilized enzyme preparation

Improvement of fat systems biotechnological interesterification using *Lipozyme TL IM* immobilized enzyme preparation (Novozymes A/S, Denmark) was studied. Improvement provided immobilized enzyme preparation preliminary moistening with sodium bicarbonate aqueous solution (3...4 % wt., 7.4...7.7 pH) and exposure of the moistened enzyme preparation for 15 minutes. According to the data in Fig. 1 and equations (1)–(3), activation of the enzyme preparation made it possible to reduce process duration to 3.5–3.7 hours in the model fat mixture (palm stearin, coconut and soybean oils in the ratio of 1:1:1, respectively). At the same time, complete interesterification

was preserved (according to the data given in Tables 2, 4) and the product with high quality indicators was synthesized (according to the data given in Tables 1, 3):

– acid number (up to 0.26 mg KOH/g);

– peroxide number (up to 0.60 mmol ½ O/kg);

– anisidine number (1.70 c.u.).

This work differs from existing scientific studies [17, 18] in the search of ways to activate *Lipozyme TL IM* immobilized enzyme preparation before interesterification by moistening with sodium bicarbonate aqueous solution in the pH range of 6.5...8.3. It should be noted that this enzyme preparation is not expected to be activated by moistening with aqueous solutions during the industrial use. But the proposed stage of biotechnological interesterification made it possible to significantly reduce process duration (about 30 %) and practically did not affect such important physico-chemical parameters of the finished product as acid and peroxide numbers (Table 3).

The results of studies made it possible to carry out the process of fatty raw materials biotechnological interesterification using *Lipozyme TL IM* immobilized enzyme preparation more efficiently and quickly. The obtained results of the work (including approximation dependences 1–3) are a scientific legacy that should contribute to more rational use of indicated enzyme preparation in biotechnological interesterification.

Work results use limitation is using the fatty raw materials with certain characteristics in the study:

– physico-chemical parameters (in particular, acid, peroxide and anisidine numbers – according to the data in Table 1);

– fatty acid composition (according to the data in Table 2).

Therefore, when using fatty raw materials with different composition and physico-chemical characteristics in biointeresterification process, these indicators must be taken into account in order to adjust the specified characteristics and physico-chemical parameters of the finished product.

The disadvantage of the study is the lack of data of the effect of moisturizing with aqueous solutions (citric acid or sodium bicarbonate) on other types of immobilized enzyme preparations that are used in fats biotechnological interesterification. However, in addition to *Lipozyme TL IM*, such enzyme preparations for fats interesterification as *Novozymes A/S Novozym 435*, *Lipozyme RM* and others, are in great demand on the market.

It should be noted the promising directions of this work on the improvement of fatty systems biotechnological interesterification to obtain the oxidation-stable product. This is, first of all, creation of fatty raw materials samples with a wide range of physico-chemical parameters and fatty acid composition, followed by a study of influence of the characteristics of fatty systems initial components on interesterified fat quality.

7. Conclusions

1. Physical and chemical indicators (acid, peroxide, anisidine number, mass fraction of phosphorus-containing substances, mass fraction of moisture, melting point) and fatty acid composition of fatty raw materials (palm stearin, coconut, soybean oil) for biotechnological interesterification were determined. The specified fatty raw materials met the requirements of DSTU 4439, DSTU 4562, DSTU 4534, respectively.

2. Dependences of biotechnological interesterification process duration, acid and peroxide numbers of the fatty product from preliminary moistening of the immobilized enzyme preparation and pH of aqueous solution were established. This made it possible to reduce the duration of biotechnological interesterification by approximately 30 %. Rational values of the parameters were: content of sodium bicarbonate aqueous solution – 3...4 % from immobilized enzyme preparation mass; pH of sodium bicarbonate solution – 7.4...7.7. Within reasonable ranges of parameters, content of moisture and alkaline agent did not affect the value of acid and peroxide numbers of the fat product. Exposure time of the moistened enzyme preparation for 15 minutes was effective for activation process of the enzyme preparation. Under these conditions, acid number of the interesterified product did not exceed 0.24 mg KOH/g, peroxide number was 0.60 mmol ½ O/kg, and anisidine number was 1.70 c.u.

3. The physico-chemical parameters of the interesterified fat sample obtained by improved technology corresponded to the parameters of the interesterified fat sample obtained by generally accepted technology without immobilized enzyme moistening with alkaline solution according to DSTU 4336. Characteristics of the obtained interesterified fat sample were: acid number – 0.26 mg KOH/g; peroxide number – 0.60 mmol ½ *O*/kg anisidine number – 1.70 c.u.; mass fraction of moisture and volatile substances – 0.04 %; melting point – 33.2 °C.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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Data Availability

The manuscript has no associated data.

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