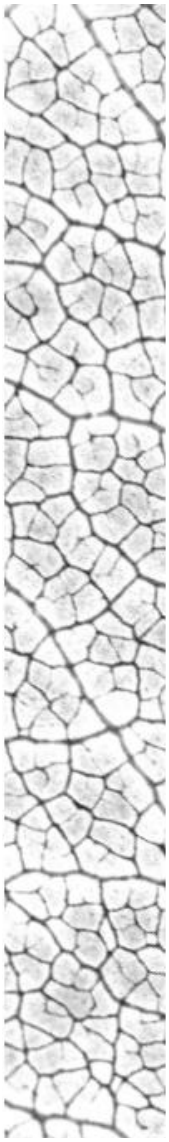

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Interesterification of a three-component fat blend for use in bread baking

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Abstract
Fat products are an important component of additional raw materials used in the production of bakery products. They are used to improve the rheological properties of the dough, improve the taste and nutritional value of bakery products, and improve their digestibility. In this study, chemical interesterification of a three-component blend based on fully hydrogenated oil, palm oil, and rapeseed oil in a ratio of 20/20/60, respectively, was performed to obtain interesterified fat for use in baking. The article presents the results of the study of fatty acid composition, melting point, content of solid triglycerides (STG) and trans-isomers of fatty acids of interesterified fat. To identify the optimal conditions for the process in enlarged experiments, mathematical processing of experimental data was performed to obtain regression equations, on the basis of which the optimization of the chemical interesterification process was carried out, which allowed determining its optimal operating modes: temperature of the process, amount of catalyst, duration of the interesterification process on the optimization criterion – the content of trans-isomers of fatty acids. The interesterified fat obtained in this way had the specified physical and chemical properties, had the necessary range of plasticity, and the content of trans-isomers of fatty acids met the requirements of the current legislation.

Keywords: trans-isomers of fatty acids, interesterification, hydrogenation, interesterified fats

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INTRODUCTION

One of the industries that uses a significant amount of fat up to 50% is baking. Their number can reach in the recipe from 2% to 14 %. At the same time, fat products in the production of bakery products can perform various functions: act as additional raw materials that improve the rheological properties of the dough, form organoleptic and physico-chemical indicators of the quality of bakery products and help preserve the freshness of products, as well as increase their nutritional value.

Due to the limited availability of solid natural fats used in various branches of the food industry, fats that have undergone modification are widely used. Until recently, partial hydrogenation was the main method of fat modification, which allows to obtain products with a high melting point, high hardness and resistance to the oxidation process, but was characterized by the formation of a large number of trans-isomers of fatty acids.

Long-term studies have shown a negative effect of trans-isomers of fatty acids on metabolic processes occurring in the human body. It has been repeatedly confirmed that long-term use of products containing trans-isomers of fatty acids increases the risk of cardiovascular disease due to increased cholesterol and low-density lipoproteins (Tardy *et al.* 2011; Stender *et al.* 2014; Dracheva, 2016; Willett *et al.* 1993).

They cause non-communicable diseases such as type II diabetes and metabolic syndrome (Thompson *et al.* 2011).

Due to the proven harmful effects of trans-isomers of fatty acids, the World Health Organization recommended reducing their consumption to 1% of the daily caloric content of the diet. In many countries, there are legal restrictions on the content of trans-isomers of fatty acids in food, in particular in fat-and-oil products.

Currently, interesterification is a widely used modification method for obtaining fats with the required structural and rheological characteristics (Meng *et al.,* 2010).

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This method changes the physical properties of oils and fats by redistributing fatty acid residues. This results in a change in the glyceride composition of the fat blend without changing their fatty acid composition (Rousseau *et al.,* 2008; Azizkhani *et al.,* 2011).

According to the type of catalyst used, interesterification is divided into chemical and enzymatic (Shelamova *et al.* 2018; Berben *et al.* 2000).

In enzymatic interesterification, the enzyme lipase is used and the process is carried out at a low temperature, since a higher temperature causes the enzyme to be deactivated. The reaction is relatively slow and can be stopped at any time, which allows to get the required degree of interesterification.

It is better to use enzymes for interesterification in an immobilized form, that is, artificially bound to an insoluble carrier. In contrast to free enzymes, immobilized ones have a number of advantages: ease of removal from the reaction medium, repeated use, and continuity of the process (Jala *et al.,* 2012; Kovalenko *et al.,* 2017; Bekbolatova *et al.,* 2018).

However, the disadvantages of enzyme interesterification are the relatively high cost of the enzymes and the careful preparation of the starting fats before interesterification due to the high sensitivity of the enzymes.

The most common type of interesterification is chemical, which occurs when ethylates or methylates of alkaline metals are used as catalysts (Zaiceva, 2011). Due to the use of new portions of the catalyst for each subsequent batch of oil, it allows to get a product with constant physical and chemical characteristics. But it also requires thorough cleaning of the finished product in order to remove the catalyst, which leads to additional product losses.

Currently, instead of partially hydrogenated vegetable oils characterized by a high content of trans-isomers of fatty acids, chemical or enzymatic interesterification of a blend of fully hydrogenated solid oils that do not contain trans-isomers of fatty acids or solid tropical oils with various liquid oils is used in developed countries (Ribeiro *et al.,* 2009; Kim *et al.* 2008; Ribeiro *et al.* 2009; Farmani *et al.,* 2009; Farmani *et al.,* 2007).

Since it is known that with complete hydrogenation in fats, trans-isomers of fatty acids are not formed because all double bonds are saturated with hydrogen atoms. However, the greatest disadvantage of fully hydrogenated oils is the predominance of tristearin, the most refractory fraction of triglycerides (68°C). Studies on the content of this fraction in interesterified and non-interesterified blends with different contents of fully hydrogenated oil (Ahmadi *et al.,* 2008) allowed to conclude that interesterification contributes to a significant decrease in the content of tristearin in blends.

Thus, the modern method of obtaining modified fats without or with a reduced content of trans-isomers of fatty acids and with the required structural and rheological characteristics, including those with optimal profiles of melting curves, is interesterification.

MATERIALS AND METHODS

Raw materials

Refined rapeseed oil of domestic production was purchased at a local supermarket, fully hydrogenated M6 oil and palm oil are presented by the fat-and-oil enterprise.

Sodium methylate (Sigma-Aldrich) (alkali metal alcoholate) is the most widely used low-temperature interesterification catalyst. Sodium methylate is a white, crystalline powder. Mass fraction of alcoholate - 92 %, bulk density - 0.2 g/cm^3, self-melting temperature on air - 80°C, melting temperature – 127°C, decomposition temperature – 400°C, working temperature range 80-130°C, reactivity – dissolved by water, acids, peroxides, O_2, CO_2.

Methods

Blends preparation

For the chemical interesterification process, a three-component fat blend was prepared consisting of fully hydrogenated oil (FHO), palm oil (PO), and rapeseed oil (RSO) in ratios of 20:20:60 (FHO:PO:RSO). Mixing and heating of the blends to the neutralization temperature of 70°C was performed directly in the neutralizer. Alkaline neutralization of fat blends was carried out periodically with an alkali solution of 60 g/dm^3, and the phase separation was performed by centrifugation. After the soap stock was separated, the unwashed fat blends containing no more than 0.1 % soap were sent to dry. Deep drying of fat blends to a moisture of no more than 0.015% was performed in a interesterification reactor at a temperature of 130°C and a residual pressure of no more than 4 kPa. The dried fat blends were cooled for interesterification to 80°C.

Chemical interesterification process

The process of chemical interesterification was carried out under laboratory conditions, using the classical method in a laboratory chemical reactor equipped with a top-drive agitator, with a mixing rate of 500 revolutions per minute. The raw material was previously subjected to neutralization and deep drying. The powdered catalyst was added in an amount of 0.6 to 1.4 g into a reactor under vacuum with a working agitator. To complete the interesterification process, the blend of fats with the catalyst was kept in the reactor under mixing and varying temperatures in the range from 80 to 120°C and the duration of the interesterification process in the range from 30 to 150 minutes until the appearance of a characteristic brown color indicating a sufficient amount of the catalyst and the normal course of the interesterification process. During the reaction using sodium methylate, soaps and

methyl esters of fatty acids are formed. Each mole of sodium methylate added to fat usually yields one mole of fatty acid methyl esters and one mole of soap. As a result, interesterified fat increases the content of SAS-monoglycerides, especially diglycerides, which do not always have a beneficial effect on its quality and create problems at the next stages of fat processing due to emulsification. Therefore, at the end of the interesterification process, the catalyst residue was deactivated by adding hot water, with sedimentation for phase separation. The separated soap solution was merged. The interesterified fat was treated with a 5% solution of citric acid to remove soap residues. Then dried under vacuum.

Fatty acid composition

Determination of the fatty acid composition of fat was carried out on a gas chromatograph model GC 1000 “Chromos” with a flame ionization detector according to GOST 30418-96 Method for determining the fatty acid composition. The method is based on the conversion of triglycerides of fatty acids into methyl esters of fatty acids and gas chromatographic analysis of the latter. The method is applicable in the range of mass fractions of fatty acids 0.1-100%.

Used a stainless steel gas chromatographic column 100 m long, with internal diameter 2.5 mm. Filler column: chromatone N-AW, treated with 10% carbowax 20M. As carrier gas - helium compressed. Electrolysis hydrogen from the SGN-2 type hydrogen generator.

The analysis conditions were as follows:

temperature of the column thermostat - 180-190°C;

evaporator temperature - 250°C;

detector furnace temperature - 200°C;

the flow rate of the carrier gas (helium) - 30-40 cm/min.;

Methyloleate yield time is not more than 15 minutes.

Essence of the method. The sample of the tested oil is mixed well. In a glass tube, take 2-3 drops of oil with a pipette, dissolve them in 1.9 ml of hexane. 0.1 cm of sodium methylate solution in methanol of a concentration of 2 mol/dm^3 is added to the solution. After intensive mixing for 2 minutes, the reaction mix is maintained for 5 minutes and filtered through a paper filter. And add a sample of about 1 mm solution of methyl esters of acids in hexane into the chromatograph.

Analysis of fatty acid trans-isomers

The content of trans-isomers of fatty acids was determined according to ISO 15304: 2002 “Animal and vegetable fats and oils. Determination of the content of trans fatty acid isomers of vegetable fats and oils. Gas chromatographic method”. Performed on a gas chromatograph model GC-1000 “Chromos” with flame ionization detector and temperature programming. Used a stainless steel gas chromatographic column 100 m long, with internal diameter 2.5 mm. Filler column: chromatone N-AW, treated with 10% carbowax 20M. As carrier gas - helium compressed. Electrolysis hydrogen from the SGN-2 type hydrogen generator.

Essence of the method. The methylated fatty acids of the sample are separated by the chain length, saturation level, geometry, and position of the double bonds on a capillary gas chromatographic column using a highly polar stationary phase.

The gas chromatograph is injected with 0.5 to 1.0 μl of methyl esters of the test sample (with a concentration of approximately 7 mg/ml in n-heptane). The obtained result of the chromatogram of the test sample is compared with typical chromatograms of a similar type of sample.

Solid triglyceride content (Solid fat content)

The content of solid triglycerides was determined using the Bruker Minispec mq 20 nuclear magnetic resonance (NMR) analyzer. The AOCS Cd 16b-93 method was used as a direct method with consecutive measurements of samples at temperatures of 10, 20, 30 and 40°C and softening unstabilized fats. The fat sample in the NMR tube was first melted at 70°C for 30 minutes, followed by cooling at 0°C for 90 minutes before measurement. Melting, cooling and tempering of the sample was performed in a pre-balanced thermostat with a water bath. The average values of solid triglycerides were based on three measurements.

Melting point

The melting point was determined using the AOCS Cc 3-25 method in open capillaries. When using this technique, glass capillaries (inner diameter of 1 mm) were filled to a height of 10 mm with molten fat, then the capillaries were kept for 16 hours at 4-10°C. After tempering, the capillaries were heated in a water bath at a rate of 0.5°C per minute, starting from 8-10°C to the expected melting temperature. The moment of the end of the process was determined by the physical movement of the fat column at the set hydrostatic pressure.

Acid and peroxide value

The acid value of the interesterified fat was determined by titration of 0.1 M KOH (AOCS Cd 3d63), and the peroxide value was determined by iodometric method (AOCS Cd 8b - 90).

Optimization of the chemical interesterification process. Development of a mathematical model and adequacy assessment

To obtain the mathematical model of technological process of chemical interesterification of oils, which is a regression equation used rotatable plan of the second order (Box plan) when the number of factors K-3, the number of plan experiments - 20, the number of experiments at the zero point was 6 and the number of coefficients is 10. For process optimization of chemical interesterification of oils, the following factors: temperature of the chemical process of interesterification (T, °C), amount of the catalyst (m, g),

Table 1. Coding of variation intervals and levels of input factors

Factors		Variation levels					Variation intervals
Natural	Coded	-1,68	-1	0	+1	+1,68	
T, °C	x_1	80	90	100	110	120	10
m, g	x_2	0,6	0,8	1	1,2	1,4	0,2
t, min	x_3	30	60	90	120	150	30

Table 2. Matrix of rotatable planning of experimental studies of the oil interesterification process

Coded values			Natural values			Experimental values
x_1	x_2	x_3	T, °C	m, g	t, min	c_{ti}, %
2	3	4	5	6	7	9
-	-	-	90	0,8	60	1,3
-	-	+	90	0,8	120	1,6
-	+	-	90	1,2	60	1,7
-	+	+	90	1,2	120	1,7
+	-	-	110	0,8	60	1,8
+	-	+	110	0,8	120	2
+	+	-	110	1,2	60	1,9
+	+	+	110	1,2	120	2,1
-1,68	0	0	80	1	90	1,2
1,68	0	0	120	1	90	2,2
0	-1,68	0	100	0,6	90	2
0	1,68	0	100	1,4	90	1,9
0	0	-1,68	100	1	30	2
0	0	1,68	100	1	150	1,6
0	0	0	100	1	90	1,7

Table 3. Value of confidence intervals of the optimization criterion

Process of chemical interesterification of oils		Input parameter	Confidence intervals			
			Δb_0	Δb_i	Δb_{ii}	Δb_{ij}
TFA content	c_{ti}, %	y_1	±0,10	±0,06	±0,06	±0,08

duration of the chemical process of interesterification (t, min), which influence the optimization criteria - the content of trans-isomers of fatty acids TFA (c_{ti}, %).

Next, coded the intervals and levels of variation of input parameters, which are presented in **Table 1**. The planning matrix is shown in **Table 2**.

Table 3 shows the values of confidence intervals for optimizing the chemical interesterification process of oils.

The coefficient of the regression equation is significant if its absolute value is greater than the confidence interval ($bi>\Delta bi$). Otherwise, it is considered insignificant and can be excluded from further consideration of the mathematical model.

Comparing the values of confidence intervals with the corresponding regression coefficients, it can be concluded that the interaction effects of input factors are insignificant, and they could be ignored.

Next, searched for the optimal response functions with the highest possible accuracy (solving a compromise problem), while taking into account insignificant coefficients for building a mathematical model that will represent a regression equation:

$$y= b_0+ b_1x_1+ b_2x_2+ b_3x_3 + b_{12}x_1x_2+ b_{13}x_1x_3 + b_{23}x_2x_2 + b_{11}x_1^2+ b_{22}x_2^2+ b_{33}x_3^2$$

The adequacy of the obtained mathematical regression models was evaluated using the Fischer criterion F_p.

RESULTS AND DISCUSSIONS

Fatty acid composition and content of fatty acid trans-isomers

The physical, functional, and organoleptic properties of fats are partly a function of the fatty acid composition, but they also depend on the distribution of fatty acids in the triglycerides that make up them. The consistency, plasticity, ability to emulsify, whipping, spreadability, and properties of finished products are influenced by the ratio of saturated fatty acids and polyunsaturated fatty acids and their position in triglycerides, which determine the rate and temperature range of melting.

Studied the fatty acid compositions of starting fat-and-oil (palm oil, fully hydrogenated oil M6 and rapeseed oil) to the interesterification and finished interesterified fat, which assessed the biological effectiveness of fat-and-oil products and the content of trans-isomers of fatty acids on the level of which is judged on the safety of fat-and-oil products. The results of the research are shown in **Table 4**.

Analysis of **Table 4** data shows that palm oil contains saturated and unsaturated fatty acids in a ratio of about 1:1, belongs to the palmitic group and is characterized by the highest content of palmitic acid 46.0 %, which contributes to the production of polymorphic form of *β'* crystals, increasing the range of plasticity and giving fat-and-oil products a creamy taste. The content of trans-isomers of fatty acids formed as a result of deodorization was 0.9%. In hydrogenated fat M6, 86.6% saturated stearic acid prevails, there are no trans-isomers of fatty

Table 4. Fatty acid composition of the starting fat-and-oil raw materials and interesterified fat

Fatty acid composition, wt.%	Before interesterification			After interesterification
	palm oil	fully hydrogenated oil M6	rapeseed oil	interesterified fat
Lauric C12:0	0,3		-	0,1
Myristic C14:0	1,2	0,1	0,2	0,1
Palmitic C16:0	46,0	7,5	4,8	14,5
Palmitoleic C16:1	0,4	-	-	0,1
Stearic C18:0	3,4	86,6	1,6	15,9
Oleic C18:1	38,4	0,1	61,6	53,8
Linolic C18:2	9,3	0,2	20,0	5,2
Linolenic C18:3	0,2	-	10,1	6,0
Eicosanic C20:0	-	0,8	0,5	0,1
Erucidic C22:1	-	0,5	0,5	0,4
Sum of fatty acid trans-isomers, %	0,9	-	0,6	1,5

Table 5. STG content and melting point of interesterified fat

Name of raw materials	STG content, % at °C				Tm, °C
	10°C	20°C	30°C	40°C	
Interesterified fat	21,6	15,1	7,3	0,9	37,0

acids since all double bonds are saturated with hydrogen atoms. Rapeseed oil contains the largest amount of oleic acid 61.6% and, accordingly, belongs to the oleic group of oils. The content of trans-isomers of fatty acids that appeared as a result of deodorization was 0.6%. It is known that the greatest technological and physiological effect is achieved when high-melting fats and liquid vegetable oils are interesterified. Thus, the resulting interesterified fat is characterized by a balanced fatty acid composition due to a higher concentration of unsaturated oleic acid, the content of which was 53.8%. Adding fat products to the dough with a high content of polyunsaturated fatty acids, which can turn into peroxide compounds under the action of flour lipoxygenase, can increase the oxidation of sulfhydryl groups in the test of the protein-proteinase complex of flour and thereby improve the rheological properties of the test. The presence of palmitic acid in the composition will positively affect the plasticizing properties of the product. The amount of trans-isomers of fatty acids in interesterified fat was 1.5%, which meets the requirements of current legislation (Technical regulations, 2011).

Solid fat content and melting point

The content of solid triglycerides characterizes one of the most important properties of solid fats and oils - the ability to acquire the necessary structure at a certain temperature. The STG content at 10^0C characterizes the hardness of the fat-and-oil product when it is stored in the refrigerator. If the TTG content is less than 32% at this temperature, then this product is well spread immediately after extraction from the refrigerator. The STG content of 15 to 35% indicates good plastic properties of the product at this temperature. The content of STG at 33-38^{0}C is associated with the ability of the fat product to melt in the mouth and, therefore, characterize its taste properties. The high content of STG at these temperatures has an adverse effect on the organoleptic properties of the fat product and its digestibility (Livinsky, 2009).

The characteristics of the melting point and the content of solid triglycerides STG of interesterified fat at different temperatures are presented in **Table 5** and in **Fig. 1**.

STG content at 20^{0}C in the interestrified fat was 15.0%, it has a wide range of plasticity, which is shown in **Fig. 1**, and creates prerequisites for ensuring optimal rheological properties of the test prepared using these fats, as well as a high degree of aeration and uniformity of semi-finished products.

The melting point of the interesterified fat was 37°C, these temperature values are associated with the presence of fully hydrogenated oil in the composition of the interesterified fat.

According to research, interesterified fat is characterized by optimal technological characteristics: melting point, content of solid triglycerides at a temperature of 20^{0}C and at the same time a wide range of plasticity, which meets modern requirements for fats for the production of bakery products.

Acid and peroxide value

In addition to the technological properties and safety of fat-and-oil products, one of the requirements is their resistance to oxidation, due to the physical and chemical properties and fatty acid composition of the fat product. **Table 6** shows the physical and chemical parameters of interesterified fat.

Studies of physical and chemical indicators of the quality of interesterified fat showed that the mass fraction of moisture was 0.3%, which may determine their resistance to oxidative processes during storage. Insignificant values of peroxide (0.5 mol/kg ½ O) and acid numbers (0.1 mg KOH/g) in interesterified fat are grounds to assume a lower rate of change of these indicators during storage.

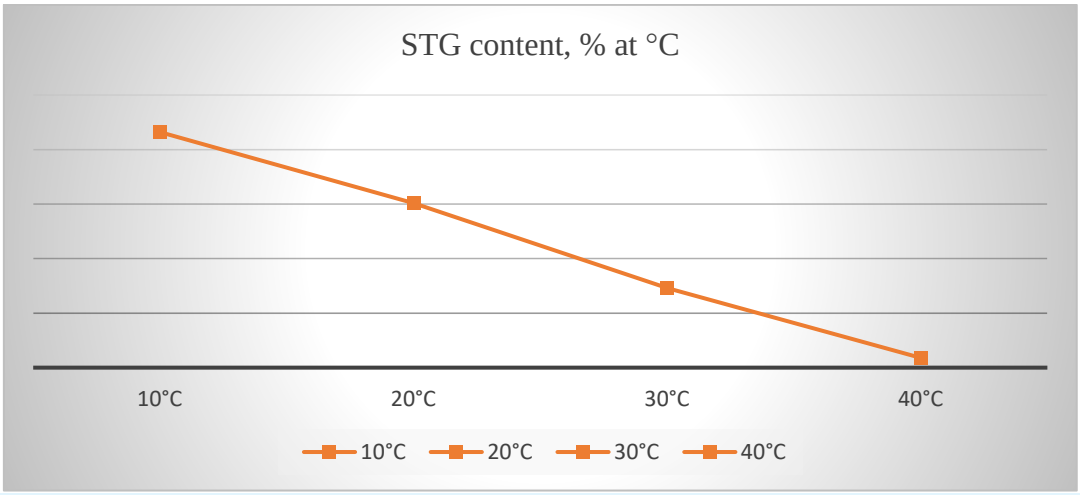

Fig. 1. STG content in interesterified fat

Table 6. Physical and chemical parameters of interesterified fat

Name of indicators	Interesterified fat
Acid value, mg KOH/g	0,1
Peroxide value, mol/kg ½O	0,5

Optimization of the chemical interesterification process parameters

Improving the process of interesterification of vegetable oil blends with three-component blends requires the use of modern methods of mathematical modeling and optimization. The use of such methods in the oil and fat industry allows reducing the time of research, the number of experiments and identifying the best technological modes of the processes under consideration. To identify the optimal conditions for carrying out processes in enlarged tests, mathematical processing of experimental data was performed to obtain regression equations, on the basis of which the process of chemical interesterification was optimized.

After the canonical transformation models of the second order were obtained for the regression equation in canonical form, based on which built the model in three-dimensional space representing a plane, which characterizes the temperature of the process chemical interesterification (T, °C), number of the introduced catalyst (m, g), duration of the chemical process of interesterification (t, min), which influence the optimization criteria – the content of trans-isomers of fatty acids TFA (c_{ti}, %).

Thus, the regression equations for the chemical interesterification process of oils, for coded values, will take the following form:

$$y_1 = 1{,}720345712 + 0{,}232776x_1 + 0{,}038942x_2 + 0{,}00205x_3 - 0{,}0375x_1x_2 + 0{,}0125x_1x_3 - 0{,}0375x_3^2 - 0{,}01989x_2^2 + 0{,}068313x_2^2 + 0{,}015393x_3^2$$

After decoding the independent variables in the equations we get the regression equations for natural values of the factors:

$$c_{ti} = -3{,}0132 + 0{,}078052T - 0{,}78342m - 0{,}00093t - 0{,}01875Tm - 0{,}0000416Tt - 0{,}00625mt - 0{,}0002T^2 + 1{,}707818m^2 + 0{,}0000171t^2$$

Fischer's criterion $F_p = 3.66$.

Thus, given that the $Fp<F_{table}$ model of the technological efficiency of the chemical interesterification process of oils in a chemical reactor can be considered adequate with a 95% confidence probability. **Fig. 2** shows graphical images of dependency graphs.

The analysis of three-dimensional spatial models shows that the necessary values of the optimization criterion are achieved in the search area under consideration. This means that the variation levels of input factors in the planning of experiments are taken quite correctly.

The analysis of the presented graphs showed that on the three-dimensional model in the space there are optimal regions of variable values T (°C), m(g), t(min), at which the technological process of chemical interesterification is carried out with optimal values of c_{ti} STI (%).

Given the dependent variables of the technological chemical interesterification process of oils - T(°C), m(g) t(min) allow with sufficient accuracy to predict the values of the optimization criteria in the studied range of factor values - content of fatty acid trans-isomers (c_{ti}, %). At the same time, it is possible to establish the dominant influence of each studied factor on the process optimization criteria, which allows describing the kinetics of the chemical interesterification process of oils with a sufficient approximation. The results obtained will allow to optimize the process under study by applying the developed mathematical model.

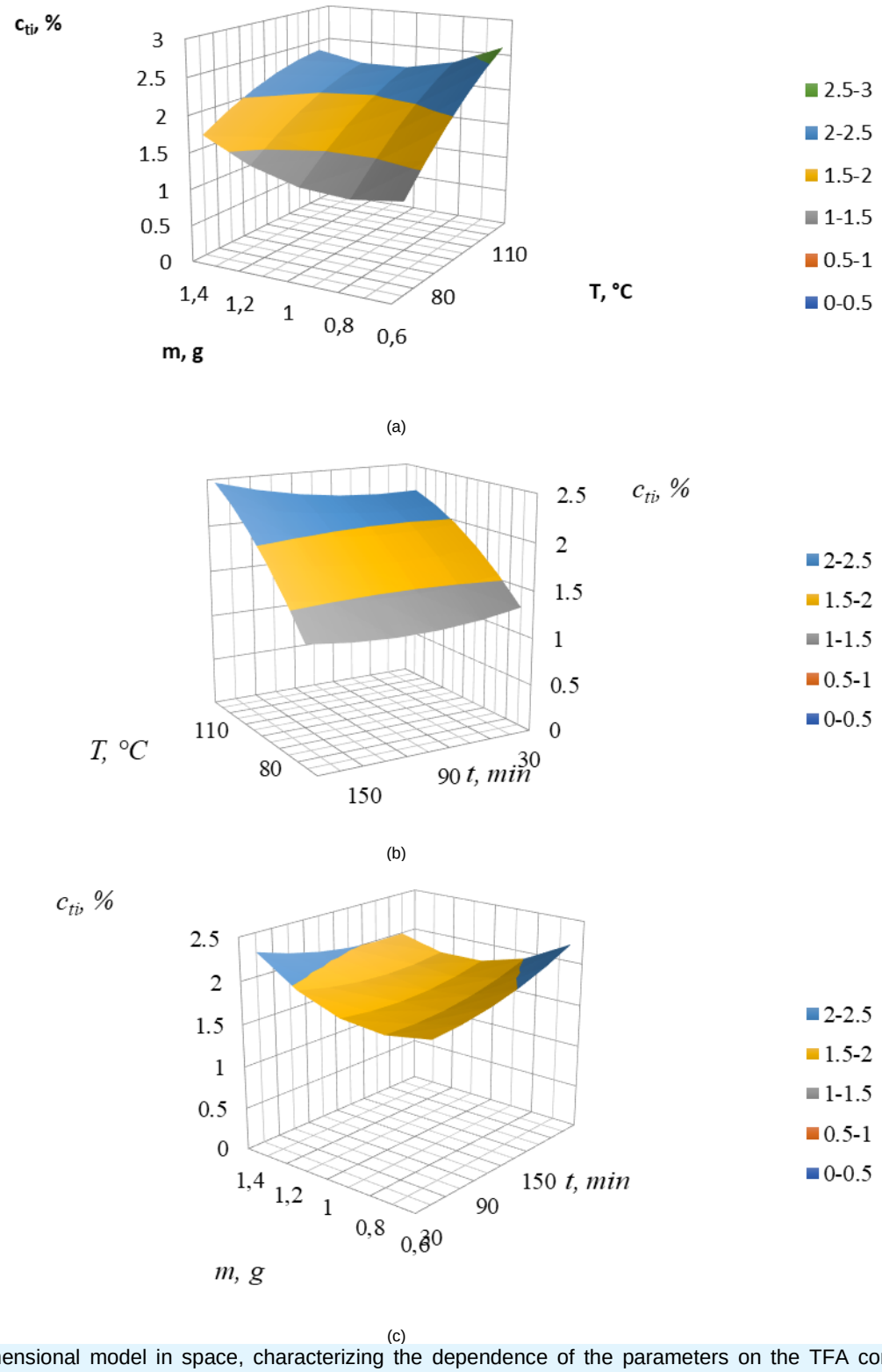

Fig. 2. Three-dimensional model in space, characterizing the dependence of the parameters on the TFA content: a) y_n=f(T,m) the temperature of the chemical interesterification of oils (T, °C) and the amount of the catalyst(m, g); b) y_n=f(T,t) the temperature of the chemical interesterification of oils (T, °C) and the duration of the chemical interesterification process (t, min); C) y_n=f(m,t) the amount of the catalyst(m, g) and the duration of the chemical interesterification process (t, min.)

On the basis of experimental researches and mathematical modeling of the chemical interesterification process of oil blends, the following optimal values: the temperature of the chemical

interesterification process - 110°C, the amount of catalyst - 1.2 g, the duration of the chemical interesterification process - 120 min influencing one of the optimization criteria - the content of trans-isomers to 1.5 %.

Based on experimental data, the influence of various factors on the process of chemical interesterification of obtaining interesterified fats was revealed. The values of parameters at which it is advisable to carry out this process are set.

CONCLUSIONS

Chemically interesterified three-component blend of palm oil, fully hydrogenated oil and rapeseed oil in a mass ratio of 20: 20: 60 is characterized by a balanced fatty acid composition due to a higher concentration of oleic acid and a content of trans-isomers of fatty acids - 1.5, corresponding to current legislation.

According to experimental data and data from mathematical modeling of the process, the production technology of interesterified fat for use in baking from a three-component blend of fats with optimization of technological modes was improved. The established optimal technological conditions for obtaining interesterified fat are as follows: the temperature of the chemical interesterification process is 110°C, the amount of catalyst introduced is 1.2 g, and the duration of the chemical interesterification process is 120 minutes.

The results obtained showed that the interesterified fat is characterized by optimal technological characteristics and more meets the modern requirements for fats for the production of bakery products.

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