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# APPLICATION OF USED FOR FRYING OIL FOR BIODIESEL FUEL PRODUCTION

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**Abstract**

*Because the production of biodiesel from food crops may cause negative economic, social, and environmental effects, alternatives are sought to satisfy the raw material demand for biodiesel production*

*The aim of research is to evaluate the possibilities of application of used for frying oil and butanol for biodiesel production by applying biotechnological methods.*

*For the investigations biocatalyst - lipase Lipozyme RM IM was selected. The optimal conditions for the production of biodiesel fuel were determined: 20 ℃ temperature, butanol and oil molar ratio - 3:1, 13% of lipase, 5.18 % of water and 30 hours duration of the process. The quality analysis of the received product was performed*

**Key words:** *biodiesel, frying oil, butanol, lipase.*

## INTRODUCTION

Increasing prices of fossil energy sources and their depletion as well as environmental pollution associated with the use of mineral fuel drives the search for ways to make better use of local renewable energy resources, including waste ones. The application of non-food oil for biodiesel production can reduce the cost price of biodiesel fuel production by 60% to 90% (Demirbas 2009). Vegetable oil after food preparation is no longer suitable for cooking. High temperature and water promotes the hydrolysis of triglycerides and occurrence of free fatty acids (Marmesat 2007). Water and free fatty acids have a negative effect on the transesterification process. Alkaline transesterification can be applied when the concentration of free fatty acids is less than 2%.

In the production of fatty esters from oil or fat with higher acidity, the catalyst is consumed for the production of soaps, and the resultant soaps form emulsion and inhibit further transesterification. Therefore, by studying the possibilities of the use of waste oils or fats containing more than 2 % of free fatty acids for biodiesel production, it was found that it should be applied esterification process by using acid catalysts for reduction of free fatty acid content before the transesterification of triglycerides. Sulphuric, phosphoric, hydrochloric acid or sulphonic acid are used as catalysts for free fatty acids esterification (Ma & Hanna 1999; Srivastava & Prasad 2000; Ruiz-Mendes 2008). However, the reaction proceeds much slower than when using an alkaline catalyst (Talebian-Kiakalaieh et al. 2013). In addition to the aforementioned traditional methods, attention is drawn recently to the biotechnological method where enzymic preparations – lipases are used as catalysts in biodiesel synthesis. In this case, it is easier to separate the by-product and catalyst. Since both transesterification and esterification take place at the same time during the synthesis, free fatty acids contained in raw materials of poor quality such as used for cooking oil become irrelevant (Haigh et al. 2013). Water contained in used for cooking oil increase the activity of some enzymes and accelerates the biodiesel production process.

In the industrial production of biodiesel, vegetable oil or fat are usually transesterified with methanol; however, research is also conducted with the use of other alcohols (Iso et al. 2001; Abigor et al. 2000). Butanol and ethanol have many advantages compared with the methanol produced from mineral resources (natural gas). These alcohols can be obtained from renewable sources; therefore, the share of biofuels in biodiesel will increase and environmental pollution with greenhouse gas emissions will decrease and a higher concentration of oxygen in the fuel would allow the reduction of emissions of solid particles (Laza & Bereczky 2011). The transesterification of rapeseed oil by using sodium metal or sodium hydroxide catalysts proceeds less effectively with butanol than with methanol (Atabani et al. 2012). In the case of the transesterification of flaxseed oil with butanol with the use of sodium metal, a yield of around 96% is obtained (Nimcevic et al. 2000).

Some authors (Hossain et al.) note that the yield of fatty acid butyl esters obtained from waste soybean oil is lower than that of methyl and ethyl esters (the yield of butyl esters is about 60%, ethyl esters – about 65%, and methyl esters – about 70%) when using alkaline catalysts (NaOH and KOH) (Hossain et al. 2010). In addition, they studied the production of biodiesel from waste rapeseed (canola) oil with transesterification with methanol, ethanol and butanol and using sodium and potassium alkoxides as the catalyst. The results showed that the same trend remains: the lowest yield was obtained for butyl esters, and the highest yield – for methyl esters.

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The application of biotechnological methods for biodiesel production was also studied (Abigor 2000; Iso, 2001). It was found that in transesterification reactions catalysed with lipases, the highest yields of biodiesel are obtained when using butanol and ethanol. Nevertheless, data on the transesterification of oil with a higher content of free fatty acids with butanol using biotechnological methods could not be found. The aim of this study is to evaluate the possibilities of the application of used for cooking oil and butanol in biodiesel production process by applying the biotechnological methods.

## MATERIALS AND METHODS

The oil used for the studies was prepared in laboratory conditions, i.e. pure oleic acid (analytically pure, Ekros) was added to rapeseed oil meeting the requirements of the standard LST 1959. The oil had initial acidity of 4.12%.

The biodiesel fuel production experiments were performed in a conical flask, which was connected to a condenser, a thermometer with a temperature controller, and a mixer (with a constant rotation speed of 200 $min^{-1}$). The required amount of rapeseed oil was poured into the flask and it is continuously stirred and thermostatically controlled. When the oil heated up to the required temperature, butanol (99.5% purity, Chempur) was poured into the flask and catalyst was added.

At the initial stage, in order to select the most efficient biocatalyst for the process of the transesterification of used for cooking oil with butanol, 6 lipases were tested: Novozyme 435, Lipozyme TL IM, Lipozyme RM IM, Novozyme resinase, Novozyme Lipalase, and Novozyme Lipex. The tests were performed under the following conditions:

- temperature – 40 °C;
- enzyme content – 5% (of the amount of oil);
- alcohol (butanol and oil molar ratio) – 3:1,
- reaction time – 5 hours.

The samples obtained after the synthesis were filtered and washed with water, then water and butanol were removed by means of a vacuum rotary evaporator (temperature – 90 °C; vacuum – 30 mbar). The resultant rapeseed oil butyl esters (RBE) were analysed by the thin-layer chromatography method. RBE (50 ml) was dissolved in 500µl of diethyl ether and the resultant mixture was homogenised. 2µl of the prepared mixture was dripped with a syringe on thin-layer chromatography plates.

Solutions of rapeseed oil, rapeseed oil methyl esters, and monoglycerides of fatty acids in diethyl ether prepared in the same manner were applied as reference. Then the plates were immersed in a system of solvents (petroleum ether, diethyl ether, and acetic acid were mixed in portions of 70:30:1 by volume, respectively) (Surinėnaitė, 2001) so that the edge of the plate would be submerged but the solvent would be below the start line. The plates were held until the eluent reached 1-1.5 cm to the top of plates. Then the plates were removed, dried, and held in iodine vapour. When the picture emerged, the plates were analysed – the spots of the test samples were compared with those of the reference samples (the position, brightness, and area of the spots were considered).

After selecting the most active biocatalysts for the process of the transesterification of used for cooking oil with butanol, the optimal conditions of the biotechnological method with the application of the reaction surface methodology were selected (studied) using Design-Expert 9.0 Trial software. The studies were carried out under the following conditions: temperature – 20 to 60°C; butanol-to-oil molar ratio – 3 to 20; enzyme content – 0.5% to 20%; water content – 0% to 20%; synthesis duration – 0.5 to 30 hours.

The compliance of the quality of the resultant product with the requirements defined for the quality of biodiesel in the standard EN 14214 was studied.

Taking into account the fact that the content of butyl esters in the initial stages of studies was low, it was evaluated according to the content of glycerides in the sample. Glycerides (glycerol, monoglycerides, diglycerides, and triglycerides) were analysed with a gas chromatograph Perkin Elmer Clarus 500 (detector – FID; column – Restek MXT-Biodiesel TG (0.15 m – 0.32 mm – 0.10µm) according to the standard LST EN 14105.

On the basis of the determined amount of glycerides, the amount of esters was calculated according to the following formula (Bailer, 1994):

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$\eta E=100\cdot(1-(0.2411\cdot MG+0.1426\cdot DG+0/1012\cdot TG)/10.441)$,

where: ηE – the amount of esters, %; MG, DG, and TG – the concentration of mono-, di-, and triglycerides (determined by the gas chromatography method), %; 0.2411, 0.1426, and 0.1012 – the conversion indicators for the respective glycerides; 10.441 – the amount of glycerol obtained from 1 kg of rapeseed oil.

When the content of esters in biodiesel was more than 80%, the gas chromatography method specified in the standard EN 14103 was applied to determine the ester content in the product. The study was conducted with a gas chromatograph Perkin Elmer Clarus 500 (detector – FID): a capillary column Alltech AT-FAME (30 m – 0.25 mm – 0.25 μm). The gas carrier gas (hydrogen) flow – steady, 3 ml/min.

Density and viscosity of products were measured with a viscometer Stabinger SVM 3000. Water content was measured with a Karl Fischer titrator. *Density* was measured in accordance with the requirements of the standard EN ISO 12185; *iodine number* was measured in accordance with the requirements of the standard EN ISO 3961. *Viscosity* was measured in accordance with the requirements of the standard EN ISO 3104. *Cetane number* was calculated with the use of Omega 1.5 software (Petroprogram company). Oxidation stability at 110°C was determined with a RANCIMAT 743 device in accordance with the standard EN 14112 (sample weight – 3 ± 0.001 g; blowback air flow – 10 l $h^{-1}$). For conducting cold filter plugging point (CFPP) tests, a thermostatic bath PROLINE RP-845 (LAUDA, Germany) was used and analysis was performed in accordance with the requirements of the standard EN 116.

## RESULTS AND DISCUSSIONS

### *Selection of biocatalyst*

At the first stage, the most effective biocatalyst suitable for the synthesis of fatty acid methyl esters from rapeseed oil with a higher content of free acids (4.12%) with the use of butanol was selected. The study involved six lipases: Novozyme 435, Lipozyme TL IM, Lipozyme RM IM, Novozyme resinase, Novozyme Lipalase, and Novozyme Lipex. The samples obtained after the synthesis were analysed by the thin-layer chromatography method (Fig. 1).

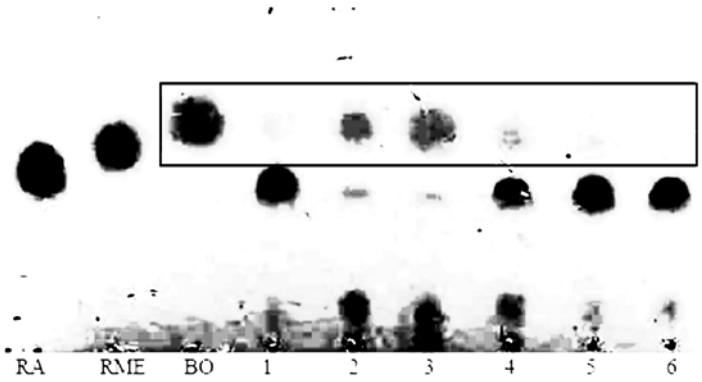



**Fig. 1.** The efficiency of lipases in the synthesis of rapeseed oil butyl esters

RA – rapeseed oil, RME – rapeseed oil methyl esters, BO- butyl oleate, 1 – Novozyme 435,

2 – Lipozyme TL IM, 3 – Lipozyme RM IM, 4 - Novozyme resinase, 5 – Novozyme Lipalase, 6 – Novozyme Lipex

The position, brightness, and area of the resultant spots were compared with those of the spots of the reference sample. The reference samples included the solutions of pure RA, RME, and BO in diethyl ether.

As can be seen from the chromatograms, the lipases Novozyme Lipalase and Novozyme Lipex are ineffective in the RBE production process. When catalysing the synthesis with other enzymes, RBE does form; however, some non-reacted oil also remains. By their efficiency, lipases can be ranked as follows (in the ascending order of the efficiency of the enzyme preparations): Novozyme 435, Novozyme resinase, Lipozyme TL IM, Lipozyme RM IM, and Lipozyme RM IM (most efficient). Therefore, the enzyme Lipozyme RM IM was selected for further studies.

***Optimal conditions for the synthesis of rapeseed oil butyl esters with the use of biocatalyst***

In order to determine the optimum conditions for the process of transesterification of rapeseed oil with a higher content of free acids (4.12%), a factorial experiment was conducted. For this purpose, an experimental plan was prepared with the use of Design-Expert 9.0 software. 31 tests were performed; the experimental data are presented in Table 1.

As can be seen from the results of the tests, the yield of rapeseed oil butyl ester (RBE) ranges from 10.82 to 98.67%.

The lowest yield was found under the following reaction conditions: temperature – 43 °C; butanol and oil molar ratio – 20:1; amount of catalyst – 20.51% of the amount of oil; reaction time – 0.5 hours. The highest yield was found under the following reaction conditions: temperature – 20 °C, butanol and oil molar ratio – 3:1; amount of catalyst – 17.51% of the amount of oil; reaction time – 30 hours.

**Table 1**. The experimental plan matrix with the biodiesel (RBE) yield experiment results

| No. | Temperature,°C | Butanol and oil molar ratio | Lipases, % | Water, % | Reaction time, h | Ester content % |
|---|---|---|---|---|---|---|
| 1 | 28.40 | 20.00 | 4.79 | 18.80 | 0.50 | 50,57 |
| 2 | 38.60 | 10.82 | 7.62 | 0.00 | 14.51 | 55,01 |
| 3 | 20.00 | 13.88 | 17.56 | 7.60 | 9.20 | 71,46 |
| 4 | 59.60 | 12.93 | 20.00 | 16.37 | 23.39 | 27,57 |
| 5 | 60.00 | 20.00 | 11.62 | 10.10 | 15.55 | 74,12 |
| 6 | 60.00 | 3.00 | 13.76 | 20.00 | 30.00 | 31,42 |
| 7 | 42.00 | 3.00 | 20.00 | 10.50 | 15.55 | 88,13 |
| 8 | 20.00 | 3.00 | 15.42 | 20.00 | 0.50 | 33,13 |
| 9 | 60.00 | 13.46 | 20.00 | 0.00 | 30.00 | 58,23 |
| 10 | 20.00 | 20.00 | 0.50 | 0.00 | 30.00 | 44,00 |
| 11 | 20.00 | 13.88 | 17.56 | 7.60 | 9.20 | 71,46 |
| 12 | 43.00 | 20.00 | 20.00 | 0.00 | 0.50 | 10,82 |
| 13 | 20.00 | 19.07 | 5.77 | 9.50 | 16.12 | 49,88 |
| 14 | 20.00 | 3.00 | 7.42 | 8.78 | 17.76 | 95,71 |
| 15 | 38.60 | 11.24 | 8.11 | 10.20 | 30.00 | 86,01 |
| 16 | 60.00 | 3.00 | 0.50 | 0.00 | 30.00 | 26,82 |
| 17 | 20.00 | 3.00 | 17.51 | 0.00 | 30.00 | 98,67 |
| 18 | 42.00 | 3.00 | 20.00 | 10.50 | 15.55 | 82,85 |
| 19 | 58.40 | 12.69 | 20.00 | 20.00 | 0.50 | 52,16 |
| 20 | 38.60 | 11.24 | 8.11 | 10.20 | 30.00 | 86,10 |
| 21 | 38.60 | 10.82 | 7.62 | 0.00 | 14.51 | 93,71 |
| 22 | 60.00 | 15.98 | 0.50 | 2.70 | 0.50 | 24,94 |
| 23 | 20.00 | 3.00 | 0.50 | 4.10 | 0.50 | 25,09 |
| 24 | 60.00 | 20.00 | 0.50 | 20.00 | 30.00 | 31,58 |
| 25 | 51.40 | 3.59 | 1.47 | 18.31 | 4.78 | 19,99 |
| 26 | 60.00 | 3.00 | 13.71 | 0.90 | 0.50 | 54,45 |
| 27 | 28.00 | 20.00 | 20.00 | 20.00 | 28.67 | 90,03 |
| 28 | 20.00 | 7.85 | 0.50 | 20.00 | 22.49 | 18,49 |
| 29 | 40.94 | 11.75 | 9.91 | 19.30 | 12.89 | 21,26 |
| 30 | 20.00 | 6.57 | 20.00 | 19.70 | 30.00 | 83,16 |
| 31 | 38.60 | 11.24 | 8.11 | 10.20 | 30.00 | 84,94 |

The butanol and oil molar ratio and lipase amount are among the key indicators that have the greatest influence on the formation of butyl esters. The studies were performed while changing the oil and butanol molar ratio of from 3:1 to 20:1 and changing the amount of lipase as well as evaluating the yield of butyl esters when the water content is 0 and the process temperature is 20 °C (Fig. 2). The molar ratio is evaluated within a wide range because, according to the data of other researchers who analysed the synthesis of butyl esters of various oils, the molar ratio changed from 1:6 to 1:30 (Chotowichien et al. 2009; Warabi, et al. 2004; Freedman et al. 1984).

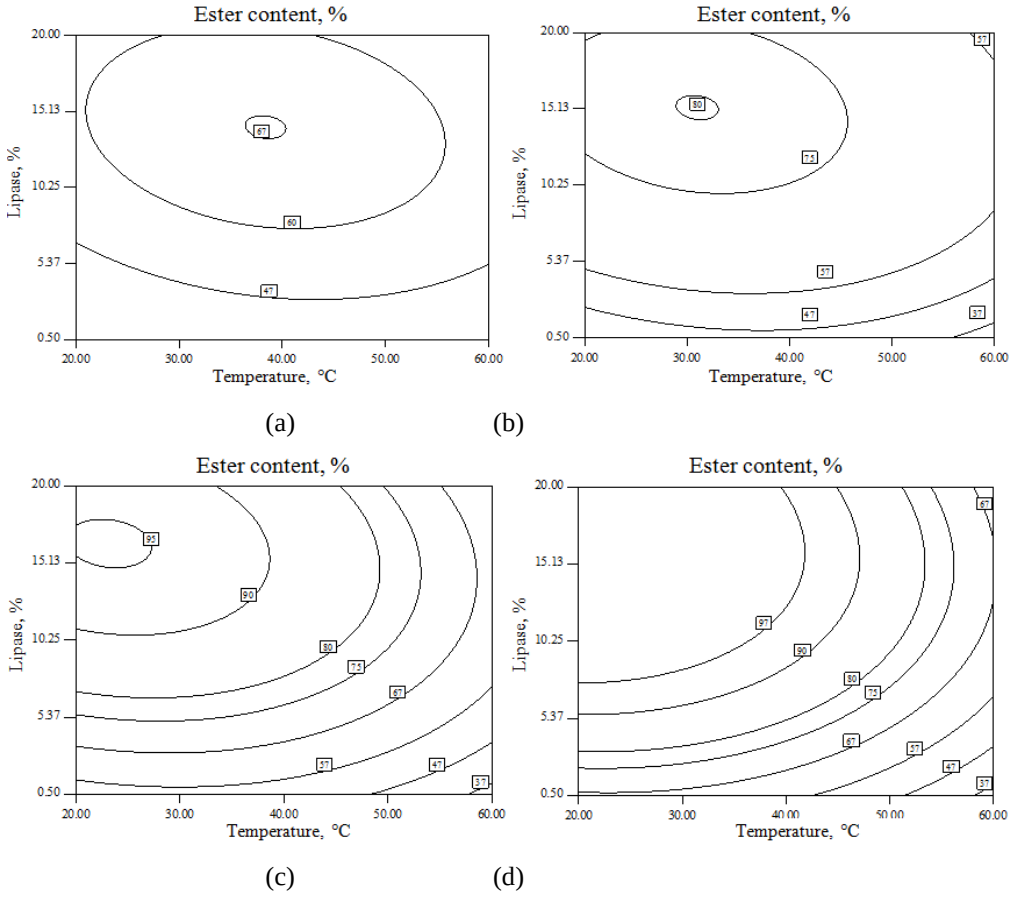



**Fig. 2**. The impact of the catalyst amount and butanol-to-oil molar ratio on the transesterification yield when the process duration is: (a) – 0.5 h; (b) – 10 h; (c) – 20 h; (d) – 30 h

Results presented in the chart show that under the same conditions (temperature, lipase amount, and duration), the increase in butanol content in reaction media negatively influence on transesterification yield. The ester content of up to 100% is obtained when the process duration is 30 hours (Fig. 2d), catalyst concentration is ≥9.6 %; butanol and oil molar ratio is 3:1; under the same conditions and duration of 20 hours, the yield of butyl esters is 86%. It could be stated that when using biocatalysts, the optimal butanol and oil molar ratio is 3:1 and the duration is 30 hours.

In order to utilize the used for cooking oil, which contains not only free fatty acids but also water, in the synthesis of biodiesel, the impact of water on the transesterification yield was determined. Also, in the synthesis of biodiesel catalysed by enzymes, other than in transesterification by chemical methods, water contained in the reaction medium has a positive effect on the transesterification yield. The water additive causes conformational changes of lipase in an oil-water environments. However, lipases are hydrolases, while the excess of water leads to hydrolysis, which reduces the amount of esters (Gog et al. 2012; Rodrigues et al. 2008; Linko et al. 1995).

The influence of water in ester formation processes was analyzed (Fig. 3), assuming the process duration of 20 hours and butanol-to-oil molar ratio of 3:1. When the process involves no water, the yield of butyl esters higher than 95% was obtained when the process temperature was 20-28°C and catalyst concentration was 15–17.2% (Fig. 3a).

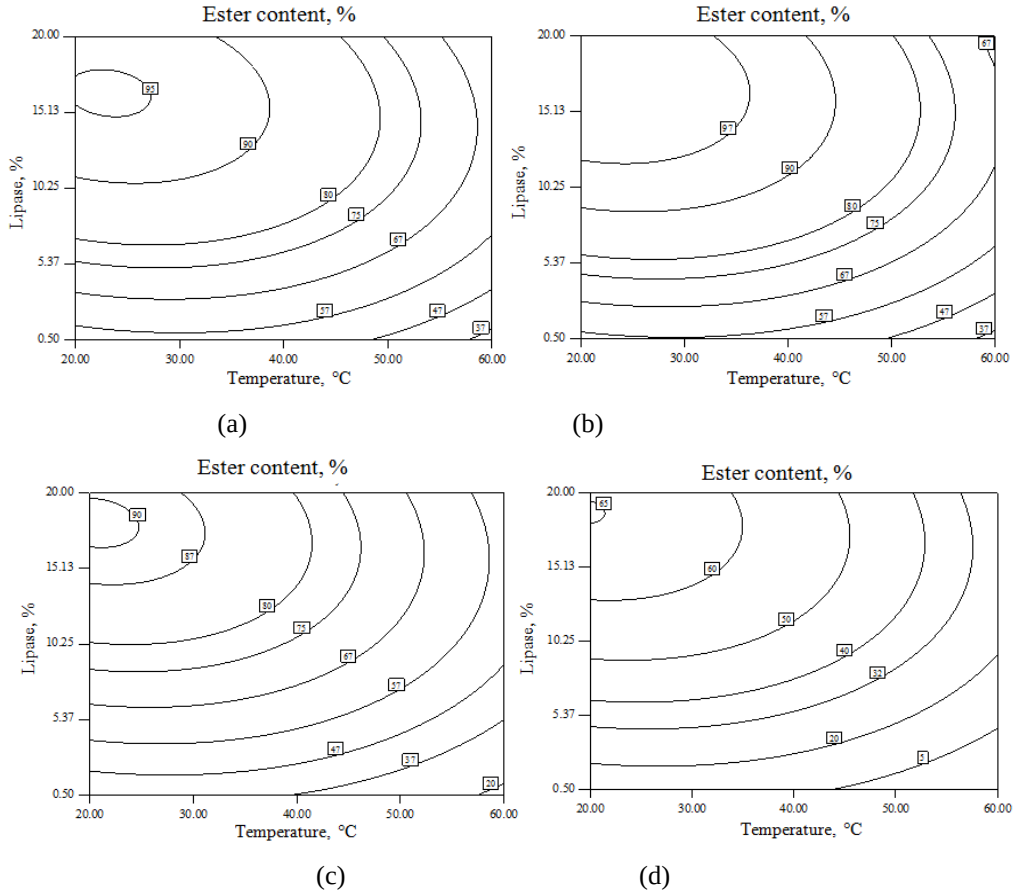



**Fig. 3.** The impact of the amount of catalyst and process temperature on the yield of esters when the amount of water is (a) – 0%; (b) – 7%; (c) – 14%; (d) – 20%.

The ester content (≥ 97%), which complies with the requirements of the standard EN 14214, was obtained when the water content was 7% (Fig. 3 b.), process temperature was 20-35 °C, and catalyst concentration was 12–20%. As water content increased to more than 7%, the yield of esters decreased, which can be seen from Graphs 4c and 4d: the lowest ester content (77%) was obtained when the content of water was 20%. On the basis of the model, it is predicted that the optimum water content in biochemical rapeseed oil butyl esters synthesis 5.18%.

The Figure 4 shows the dependence of the ester content on the catalyst concentration and process temperature. The product yield (≥ 96.5%) complying with the requirements of EN 14214 was obtained with the following values of the indicators: process temperature – ≤ 30°C; catalyst concentration – ≥ 15.5%. Other process indicators were recorded as follows: butanol and oil molar ratio – 3:1; water content – 5.18%; reaction time – 30 hours.

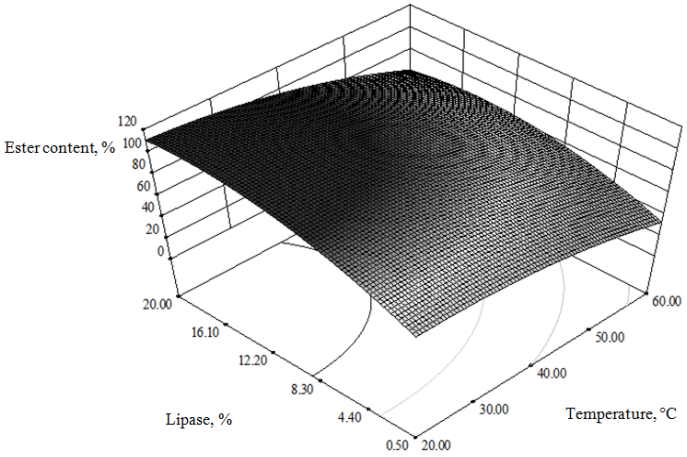



**Fig. 4.** The dependence of the ester content on the lipase amount and process temperature

Based on the model, the following optimal process conditions were determined: temperature – 20°C; butanol and oil molar ratio – 3:1; lipase content – 13%; water content – 5.18%; reaction time – 30 hours. It appears from the experimental plan that the predicted values of the yield of butyl esters reach 100%. Under these process conditions, a yield of 96.6% of rapeseed oil fatty acid butyl esters was obtained in laboratory conditions.

To summarise, it can be stated that the yield of rapeseed oil butyl esters (≥ 96.6 %) complies with the requirements of the standard EN 14214 when the product is obtained under the following reaction conditions:

- temperature – 20°C, butanol and oil molar ratio – 3:1; amount of catalyst – 17.51% of the amount of oil; reaction time – 30 hours;
- temperature – 20°C, butanol and oil molar ratio – 3:1; amount of catalyst – 13% of the amount of oil; water content – 5.18%; reaction time – 30 hours.

***The physical and chemical properties of rapeseed oil butyl esters***

In order to determine how the physical and chemical properties of rapeseed oil butyl esters comply with the requirements of EN 14214 and to compare them with the respective properties of conventional biodiesel – rapeseed oil methyl ester (RME), the physical and chemical properties of rapeseed oil butyl esters were determined. These are presented in Table 2.

**Table 2.** The physical and chemical properties of RBE

| **Parameter** | **EN 14214 requirements** | | **RME** | **RBE** |
|---|---|---|---|---|
| | **min.** | **max.** | | |
| Ester content, % | 96.5 | - | 97.0 | 98.67 |
| Density at 15 °C, kg/m$^3$ | 860 | 900 | 875 | 880 |
| Viscosity at 40 °C, mm$^2$/s | 3.50 | 5.00 | 4.6 | 5.1 |
| Monoglyceride content, % | - | 0.80 | 0.49 | 0.12 |
| Diglyceride content, % | - | 0.20 | 0.16 | 0.19 |
| Triglyceride content, % | - | 0.20 | 0.04 | 0.07 |
| Cetane number | 51.0 | - | 54.1 | 59.7 |
| Iodine value, g $J_2$ /100 g | - | 120 | 112 | 111 |
| Moisture content, mg/kg | - | 500 | 150 | 130 |
| Oxidation stability 110 °C, h | 6 | | 6.32 | 1.02 |
| Cold filter plugging point, °C | -5 °C (in summer)<br>-26 °C, -32 °C ( in winter) | | -10 | -14 |

As can be seen from Table 2, RBE meets the requirements of the standard, except for oxidation stability. Oxidation resistance can be increased by adding antioxidants.

It appears that RBE has such advantages as a higher cetane number compared to RME (59.7 and 54.1, respectively) and low cold filter plugging (-14°C). However, RBE is slightly more viscous and denser than RME. It can be attributed to a bigger length of the carbon chain of the fatty acid butyl ester.

In accordance with the requirements of the standard EN 14214, the oxidation stability of biodiesel (induction period – IP) must be at least 6 hours. The studies showed that RBE has a very low induction period (1.02 h). In order to increase it, RBE stabilisation studies were performed with the use of the industrial antioxidants Ionol, Baynox, and Kerobit which are used to enhance the oxidation stability of conventional biodiesel. The results of the studies are presented in Fig. 5.

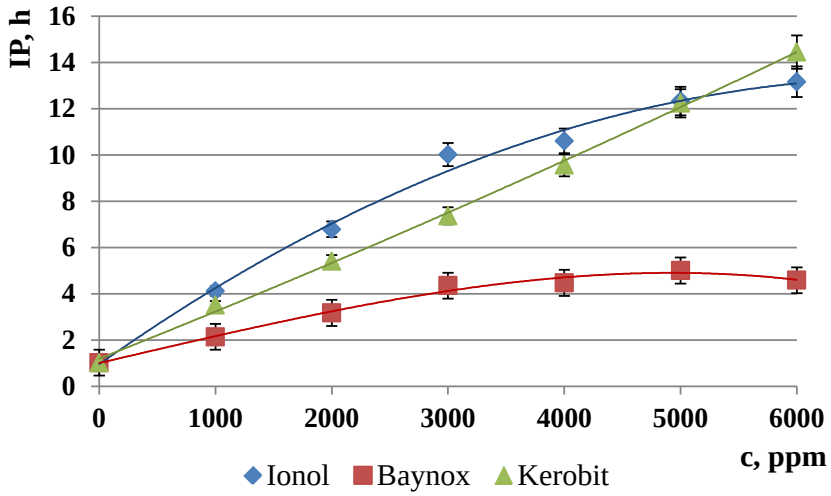



**Fig. 5.** The dependence of the induction period on the antioxidant amount

As can be seen from the presented data, the antioxidant Ionol enhances the oxidation stability of RBE most efficiently. For the induction period to reach (and exceed) 6 hours (as required by the standard EN 14214), a 1800 ppm antioxidant additive Ionol is required. As the amount of this additive is increased further, the induction period increases to 13 hours. Kerobit is another sufficiently effective antioxidant. When using 2500 ppm of this additive, the induction period reaches 6 hours; when adding more than 4,500 ppm of antioxidant Kerobit, the induction period value increases more rapidly; with 6000 ppm, a value of 14.2 hours is obtained. Antioxidant Baynox did not show good stabilization: even when using 6000 ppm of antioxidant Baynox, the induction period increased only to 5 hours.

## CONCLUSIONS

1. Lipozyme RM IM is the most effective biocatalyst suitable for the synthesis of fatty acid butyl esters of rapeseed oil with acidity of 4.12%.
2. The highest yield of rapeseed oil butyl esters with the use of biocatalyst is obtained under the following conditions: 13% of lipase Lipozyme TL IM (of the weight of oil); butanol and oil molar ratio – 3:1; temperature – 20°C; reaction time – 30 hours; water content – 5.18%. When the process does not involves any water, the conditions are the same, only the content of lipase is 17.5%.
3. By almost all physical and chemical properties, RBE complies with the requirements of the standard EN 14214 and can be used as fuel for diesel engines. In order to enhance oxidation stability, it is appropriate to use the industrial antioxidant Ionol (optimal dosage – 1800 ppm) or Kerobit (optimal dosage – 2500 ppm).

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