University of Pardubice

Faculty of Chemical Technology

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Alkali catalysed transesterification of rapeseed oil

Doctoral thesis

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Annotation

The subject of this thesis was a study of the preparation of ester with a low content of free fatty acids by alkali catalysed transesterification. The transesterification of rapeseed oil was carried out under homogeneous catalyst (batch reactor) and heterogeneous catalyst (fixed bed reactor). For the homogeneous catalysis (potassium hydroxide), the attention was paid to (i) the monitoring of transesterification by pH and conductivity, (ii) the preparation of esters with a low content of free fatty acids by two methods: one method is purifying the esters with a high content of free fatty acids by adding calcium hydroxide and second method is stopping of reaction by addition of inorganic acids, (iii) the detailed description of side product of transesterification (the glycerol phase), including ester losses after separation of the ester phase from the glycerol phase. Butanol as another alcohol, which is possible to use in transesterification of rapeseed oil, was used and the transesterification including the various types of stopping reaction and separation of products were described. The obtained data were statistically evaluated in programs STATISTICA and QC Expert.

In the case of the heterogeneous catalysis, the possibility of using mixed oxides in a fixed-bed reactor was studied. Two types of mixed oxides (Mg-Al and Mg-Fe) were used in the transesterification with methanol and obtained results were compared. Moreover, the long-term catalyst test (300 h) was carried out to study the stability of mixed oxides.

Keywords: Transesterification, alkali catalyst, free fatty acids, esters

Annotation in Czech

Předmětem dizertační práce bylo studium přípravy esterů s nízkým obsahem volných mastných kyselin pomocí alkalicky katalyzované transesterifikace. Reakce byla provedena jak pomocí homogenního katalyzátoru ve vsádkovém reaktoru tak i heterogenního v kontinuálním reaktoru. V případě homogenní katalýzy (hydroxid draselný), byla pozornost zaměřena na (i) sledování průběhu transesterifikace pomocí pH and specifické vodivosti: (ii) přípravu esterů s nízkým obsahem mastných kyselin dvěma metodami: čištěním esterů se zvýšeným obsahem mastných kyselin pomocí přídavku hydroxidu vápenatého, nebo zastavením reakce neutralizací katalyzátoru přesným přídavkem anorganické kyseliny, (iii) detailní popis vedlejšího produktu transesterifikace (glycerolové fáze), včetně ztrát esterů po separaci reakčních produktů. Butanol jako nízkomolekulární alkohol lze také použít v transesterifikaci rostlinného oleje. Reakce s ním byla popsána včetně zastavení pomocí různých anorganických kyselin a také separací reakčních produktů (glycerolové fáze od esterové). Získaná data byla statisticky vyhodnocena v programech STASTICA a QC Expert.

V heterogenní katalýze byla studována možnost použití směsných oxidů v reaktoru s pevným ložem (průtočném uspořádaní). Jako katalyzátor byly použity směsné oxidy Mg-Al a Mg-Fe, které byly použity v transesterifikaci s methanolem a získané výsledky byly porovnány. Pro ověření stability katalyzátorů byl proveden dlouhodobý test, který trval 300 hodin.

Klíčová slova: Transesterifikace, alkalicky katalyzátor, volné mastné kyseliny, estery

List of papers:

This doctoral thesis is written in the form of a commented set of scientific publications. The publications results were obtained in years 2015-2019 at the Department of Physical Chemistry, Faculty of Technology, University of Pardubice.

1) **Vávra A.**, Hájek M., Skopal F., *The removal of free fatty acids from methyl ester*, Renewable Energy 103 (2017), 695-700 (IF = 4.36)

2) **Vávra A.**, Hájek M., Skopal F., *Biodiesel Production: Acceleration and Simplification of Separation by Addition of Inorganic Acid*, Journal of Cleaner Production 192 (2018), 390-395 (IF = 6.39)

3) **Vávra A.**, Hájek M., Skopal F., Měkotová M., *Biodiesel: the study of methyl esters loss in the glycerol phase at various conditions*, Journal of Cleaner Production 197 (2018), 1573-1578 (IF = 6.39)

4) Hájek M., Skopal F., **Vávra A.**, Kocík J., *Transesterification of rapeseed oil by butanol and separation of butyl ester*, Journal of Cleaner Production 155 (2017), 28-33 (IF = 5.72)

5) Frolich K., **Vávra A.**, Kocík J., Hájek M., Jílková A., *The long-term catalytic performance of mixed oxides in fixed-bed reactors in transesterification,* Renewable Energy 143 (2019), 1259-1267 (IF = 5.44)

Further publications (published during the doctoral study, but not including the doctoral thesis)

6) Hájek M., Kocík J., Frolich K., **Vávra A.**, *Mg-Fe mixed oxides and their rehydrated mixed oxides as catalysts for transesterification*, Journal of Cleaner Production 161 (2017), 1423-1431 (IF = 5.72)

7) Musil M., Skopal F., Hájek M., **Vávra A.**, *Butanolysis: Comparison of potassium hydroxide and potassium tert-butoxide as catalyst for biodiesel preparing from rapeseed oil*, Journal of Environmental Management 218 (2018), 555-561 (IF = 4.87)

8) Musil M., Hájek M., Skopal F., **Vávra A.**, *Improved method of water removal from vegetable oil,* Chemical paper 73 (2019), 767-769 (IF = 1.24)

9) Hájek M., Muck J., Kozáček P., Kocík J., Lederer J., **Vávra A.**, *The synthesis of Zn-Al hydrotalcites under different conditions and testing in aldol condensation*, Scientific Papers of the University of Pardubice, Series A, 23 (2017), 129-144 (without IF)

Traineeships:

- UniCRE Litvínov, doc. Ing. Martin Hájek, Ing. Jaroslav Kocík, Ph.D., 3. – 14. 9. 2017
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- European Industrial Doctoral School Summer Workshop 2019, University of Pardubice, Jamie McDonald, University of Pardubice, 19. – 24. 5. 2019

Presentations:

The results of our research were presented personally by 6 oral and 3 posters. Other 16 contributions were in proceedings.

Oral:

1) **Vávra A.**, Skopal F., Hájek M., *Odstraňovaní volných mastných kyselin z bionafty*, 16. Študentská vedecká konferencia, Slovakia Republic, Bratislava, 12. 11. 2014

2) Hájek M., Skopal F., **Vávra A.**, Kocík J., *Transesterification of rapeseed oil and separation of butyl ester*, 10th Conference on Sustainable Development of Energy, Water and Environment Systems, Dubrovnik, Croatia, 27. 9. – 2. 10. 2015

3) **Vávra A.**, Hájek M., Skopal F., *Biodiesel: the influence of various conditions on the solubility of esters in the glycerol phase*, 12th Conference on Sustainable Development of Energy, Water and Environment Systems, Dubrovnik, Croatia, 4. – 8. 10. 2017

4) **Vávra A.**, Skopal F., Hájek M., *Biodiesel production: acceleration and simplification of separation by addition of inorganic acid*, International Conference on Chemical Technology, Mikulov, Czech Republic, 10. – 12. 4. 2017

5) **Vávra A.**, Hájek M., Frolich K., Kocík J., Jílková A., *The catalytic performance of Mg-Al and Mg-Fe mixed oxides in transesterification in flow reactor*, 13th Conference on Sustainable Development of Energy, Water and Environment Systems, Palermo, Italy, 30. 9. – 4. 10. 2018

6) **Vávra A.**, Hájek M., Skopal F., Kocík J., *The Preparation of esters by homogeneous and heterogeneous catalysts*, European Industrial Doctoral School Summer Workshop, Pardubice, Czech Republic, 19. – 24. 5. 2019

Poster:

7) **Vávra A.**, Skopal F., Hájek M., *The removing of free fatty acids from biodiesel*, The 24th European Biomass Conference and Exhibition, Amsterdam, Nederland, 6. – 9. 6. 2016

8) **Vávra A.**, Hájek M., Jílková A., Frolich K., Kocík J., *Using of Mg-Al and Mg-Fe mixed catalyst in transesterification in fixed bed-reactor*, 51. Symposium on catalysis, Prague, Czech Republic, 4. – 5. 11. 2019

9) **Vávra A.**, Hájek M., Skopal F., Kocík J., *Preparation of esters by homogeneous and heterogeneous catalysts*, European Industrial Doctoral School Summer Workshop, Pardubice, Czech Republic, 19. – 24. 5. 2019

The aim of the thesis

The aim is the description of various possibilities of preparation of esters with a low content of free fatty acids by alkali catalysed transesterification with alcohols (methanol and butanol). The studied preparation of esters with a low content of free fatty acids is important because the increasing content of free fatty acids in esters can cause corrosion of fuel system. Moreover, the free fatty acids can react with parts of the fuel system and formed soaps, which plug up filters in diesel engines. The free fatty acids are possible to eliminate by (i) their neutralisation from the ester phase or by (ii) precise addition of inorganic acids after the transesterification so that the soaps are not converted to free fatty acids. Both approaches for the preparation of ester with a low content of free fatty acids are described in detail and statistically evaluated. The ester losses occur during the transesterification of rapeseed oils and the separation of products, which significantly decreases the ester yield. Therefore, the attention is also paid to a detailed description and explanation of the causes of esters losses. The transesterification is carried out under homogeneous catalysts (potassium hydroxide) in a batch reactor and under heterogeneous catalyst (the mixed oxides Mg-Al and Mg-Fe) in a fixed-bed reactor.

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Abbreviations and symbols

Abbreviations

A	alcohols (methanol, ethanol, butanol, etc.)
CWP	component weight plot
DG	diacylglycerols(s)
E	ester(s)
EP	the ester phase
FAME	fatty acids methyl esters
FFA	free fatty acid(s)
G	glycerol
GP	the glycerol phase
MG	monoacylglycerols(s)
TG	triacylglycerol(s)
TOFA	tall oil fatty acids

Symbols

an_{in}	acid number of the input ester phase, mg KOH g^{-1}
an_{out}	acid number of the output ester phase, mg KOH g^{-1}
Ca^{2+}	calcium content in the ester phase, mg kg^{-1}
f	stirring speed of reaction mixture, rpm
MR	molar ratio of methanol to input oil, -
t	reaction time, min
P	catalyst amount, wt%
T	the reaction temperature, °C
V_w	amount of additional water into the ester phase, µl
w_E	ester content in the glycerol phase, wt%
w_G	glycerol content in the glycerol phase, wt%
w_{H_2O}	water content in the ester phase, ppm
w_{salts}	salts content in the glycerol phase, wt%
w_{soaps}	soaps content in the glycerol phase, wt%
w_{water}	water content in the glycerol phase, wt%

1   Introduction

The world's energy consumption is ceaselessly rising in the last few years. Many scientists around the whole world are trying to find new sources of renewable energy, which could replace fossil fuels [1]. Non-renewable resources including fossil fuels (coal, oil, natural gas, etc.) are found in limited reserves and can be depleted, unlike renewable resources which cannot be depleted. Renewable energy belongs to the natural resources that have can naturally recover in a human time scale. They provide several key objectives: (i) security of energy supply, (ii) the reduction of greenhouse gases, (iii) the reduction of energy price, (iv) the possibility of new jobs and general economic growth [2]. Primarily, these are resources of energy gained from thermonuclear hydrogen combustion within the Sun. In their drawing can theoretically continue for other millions of years – until the Sun shines. This kind of energy can be obtained from sunlight, wind and hydropower. Among the less common renewable resources that can be used only in certain regions of the world include the tidal (that comes from the inertia of system the Earth with the Moon) and geothermal energy (that comes from the heat of the Earth's interior). The definition of renewable resources under the Czech Environmental act is as follows: Renewable natural resources can recover partially or completely in gradual consumption, either alone or with a human contribution [3].

Humans used renewable resources (wood) since prehistoric times, but the population growth has led to an increasing need for deforestation. Paradoxically this big issue was resolved by fossil fuels, which allowed to raise energy other than growing crops (need a large area of land). The fear of depletion of coal reserves has first appeared in the USA in 1873. Increasing interest in renewable resources dates back to the 1970s following the oil crisis [4].

In recent years, biofuels gain more attention of the public and scientific community, which is due to rising oil prices, the need to increase energy security, concern over greenhouse gases from fossil fuels and equally important is the support of renewable fuel by government subsidies [5].

The first generation biofuels (mainly produced from food crops) have a limited ability to replace fossil oil, reduce climate change and economic growth. Biofuels include bioethanol (produced from grain, sugar beet, sugar cane, corn, starch, etc.), rapeseed oil methyl ester (produced from pressed rapeseed oil) and furthermore fatty acid methyl ester (FAME) (produced from pressed

oil plants – palm oil, sunflower oil, soybean oil, etc.) [6]. Bioethanol is widely used in the USA and Brazil, which utilize an excess of sugar cane. In Europe, biodiesel is primarily used [7].

Problems related to the first generation biofuels have increased interest in the development of biofuels produced from non-edible biomass, such as forest biomass including mining residues, agricultural waste (straw, hay, corn, rapeseed and others), fast-growing grasses and wood or biological waste from households. These fuels include bioethanol, diesel oil as a product of Fisher-Tropsch synthesis, biobutanol from bioethanol, etc. The second generation energy crops have a great potential to replace fossil oil than those of the first generation, but the technological process is incomparably more complicated than the formation production of bioethanol and the transesterification of vegetable oils. Although significant progress has been made, there are still many technical issues that have to be eliminated and overcome so that these crops could be used commercially [8, 9].

There is also the third generation biofuels using microorganisms (algae, cyanobacteria) that are relatively undemanding to the environment and the area. This category biofuel includes microalgae and various types of bacteria that can produce hydrocarbon-based fuel from CO_2 and sunlight. Large companies around the world are trying to solve problems associated with using these fuels. The fact is that microalgae have a huge potential and are up to 100 times more profitable per hectare than corn, soy and sugar cane [10, 11].

2 Biodiesel

Biodiesel (FAME) is a fuel based on methyl esters of unsaturated and saturated fatty acids of vegetable or animal origin and belongs to the ecological fuels. It is produced by a reaction called transesterification, in which methanol reacts with oil in the presence of a catalyst [12]. In diesel engines, biodiesel can be used without special modifications as a fuel separately or in a mixture with fossil diesel [6]. In the European Union, its importance and consumption are constantly growing, as currently 7 wt%, had to be blended into diesel by producers [13]. Recently, more and more attention has been paid to the second generation biofuels (non-edible resources), which are widely supported by the European Union and should replace first generation biofuels (edible resources) in the future [14].

The quality of fatty acid methyl esters has to conform with the values given by the European standard EN 14214. The standard specifies a total of 23 different parameters, e.g. density, viscosity, flash point, acid number (free fatty acids content – FFAs), methanol content, water content, content of reaction components: free glycerol (content of pure glycerol in the ester phase – EP), MG, DG, TG, content of group I metals (Na, K), group II metals (Ca, Mg), phosphorus content, etc., which as a final product has to conform with and necessary to determine. The standards methods for analysing parameters are recommended [15].

2.1 Advantages and disadvantages

Biodiesel belongs to the renewable resources of energy and it can be used in existing diesel engines without any modification. The carbon balance of biodiesel is zero because carbon dioxide releases during its combustion is removed from the atmosphere by photosynthesis of plants and converted back into oil. However, it should be noted that the whole process of biodiesel production, i.e. seed sowing, plant growth, harvesting, extracting of oil from crops, transesterification, finishing a final product preparation and transport of all substances significantly influence the carbon balance (increasing carbon dioxide emission). There is an extensive discussion about the environmental benefits of biodiesel [16-19]. Another discussed factor is that rape cultivation occupies the ground and competes with crops for the food purpose and thus increases their cost. This statement is questionable because approximately 6.3% of arable land in Europe is used for biodiesel production. In 2018, in the Czech Republic rape was grown on 412 thousand hectares, i.e. approximately 16% of arable land which is similar to 2013. Oil obtained from rape is used not only for biodiesel production but also for food purposes, e.g. in the Czech Republic about 30% of rape harvest was used for biodiesel

production (the maximum was in 2011 46.7%), about 70% was used for other purposes, especially for a food purpose [20].

Biodiesel is a nontoxic ecological fuel, which does not contain sulphur or aromatic hydrocarbons and it is also biodegradable. Its biodegradability in water is significantly shorter than diesel (95% of biodiesel degrades compared to 40% of diesel after 28 days). In water, it does not cause microbiological load up to a concentration of 10 g l^{-1} [21]. Biodiesel has a higher lubricity than diesel and therefore reduces the engine wear [22, 23]. It is a good solvent, therefore it can dissolve the dirt from the engine, which can plug a fuel filter. It can damage parts of rubber in some engines [24]. An important property of biodiesel is its viscosity, which is comparable to diesel [25, 26]. Unlike vegetable oil including rapeseed oil has higher viscosity and therefore transesterification of oil to biodiesel is required [27]. Biodiesel viscosity increases with decreasing temperature (e.g. biodiesel from rapeseed oil solidifies at about –5 °C) and cannot be used at low temperatures [28, 29]. Biodiesel density is 2-7% higher than diesel fuel density [30].

One of the disadvantages is the economic cost of the production process. The most expensive input is vegetable oil (as a source of triacylglycerol), which accounts for 85-90% of the biodiesel cost. Therefore, the use of waste cooking oil is considered as an attractive source of triacylglycerol. However, it should be noted that the use of waste cooking oils is mainly associated with the problem of increased the FFAs content [31, 32]. Other disadvantages are long-term storage and oxidative stability of biodiesel. When biodiesel comes into contact with a significant amount of water (e.g. humidity) then the esters are hydrolysed to the FFAs. It contains double-bound and therefore aldehydes, ketones and acid can be formed by the gradual oxidation process [33]. The advantage is that the preparation of biodiesel by transesterification is almost a waste-free technology since all by-products (including glycerol waste) can be used [34].

Biodiesel has lower caloric value than diesel (biodiesel has $37.5 - 39 \text{ MJ kg}^{-1}$ in comparison with diesel 42.5 MJ kg^{-1}), which causes lower performance and higher fuel consumption [35]. Conversely, biodiesel burns better than diesel fuel, thus reducing the engine smoke. It was found that biodiesel has lower emissions of carbon dioxide, sulphur, and air pollution [36-38]. On the other hand, the emissions of NO_2 are significantly increasing, but emissions of NO are decreasing compared to diesel fuel. For biodiesel, combustion of the total amount of NOx is slightly higher than for diesel fuel combustion [39, 40]. It should be mentioned that the

emissions of biodiesel depend on the oxygen amount during the process of biodiesel combustion.

2.2 Feedstock

The raw materials for biodiesel production are resources of triacylglycerols and alcohols. Between resources of triacylglycerols belong vegetable and animal fats and recently rapidly expanding raw materials such as algae and cyanobacteria (oil extracting from them) or waste cooking oils [34].

In transesterification, various type of alcohols includes methanol, ethanol, propanol, butanol and amyl alcohol can be used. Methanol and ethanol are the most commonly used. Methanol is mainly used due to its lower cost and its appropriate physical and chemical properties. Methanol reacts with triglyceride quickly and the alkali catalyst dissolves readily. The higher number of carbons in the alcohol chain means a lower reactivity and higher boiling point, i.e. under the same pressure, the transesterification could be carried out at higher temperatures. Simultaneously with an increasing carbon number increases the caloric value of the esters. Methanol and ethanol are almost immiscible with oil and form a two-phase system. The reaction proceeds at the interface and the intense stirring is necessary. On the other hand, butanol is miscible with oil and the reaction takes place in the whole volume [41, 42].

The main part of vegetable oils are triacylglycerols (ester of higher fatty acids and glycerol) and the further matter such as the FFAs, DG, water etc. The vegetable oils have various profiles of higher fatty acids. Table 1 is shown the composition of some types of vegetable oils as well as the comparisons with animal fat. The fatty acids are abbreviated according to that the first number indicates the number of carbon atoms and the second number indicates a number of double bonds – for example abbreviation 18:1 is oleic acid. The most abundant saturated fatty acids are palmitic and stearic. Unsaturated acids mainly represent oleic, linoleic and linolenic acid. Other important components of the oil are the FFAs which are not bound through glycerol and they are not part of triacylglycerols. These FFAs determine the acid number of the oil. The composition of the oil is variable (various plant varieties and various growing conditions), e.g. the content of oleic acid in rapeseed oil ranges from 45% to 61%, therefore only the average content of fatty acids are shown in Table 1.

Table 1: *The content of fatty acids in various type of vegetable oils and animal fats* [43]

Oil / fat	Content of major fatty acid %				
	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)
Palm	45	8	38	10	0.5
Soybean	10	4	21	56	8
Rapeseed	4.5	1.5	56	21	10
Sunflower	6.5	5	24	63	0.3
Olive	11.5	2.5	74	9.5	1.5
Beef tallow	26	20	40	4.5	0.5
Mutton tallow	27	32	32	1.6	0.2

The vegetable oils can be obtained by different ways, either from pulps of fruits (palm, olive), from seeds of fruits (rapeseed, sunflower) or also from beans (soy). Pulp oil is obtained by pressing, which has to be carried out at the place of production, for palm oil it is necessary to carry it out at the place of palm plantation. The reason for this is very low durability of the fruit with pulp. By contrast, oilseeds and beans can be stored and transport almost unlimitedly. Two technologies are the most widespread worldwide: pressing on continuous screw presses and extracting of hydrocarbons (usually fraction C6). A combination of pressing followed by solvent extraction is used to obtain large volumes when it is possible to achieve a yield of 98% of the oil contained in raw materials. On a local scale, the producers mainly use a pressing technology to obtain a maximum of 85% of the contained oil in raw materials. The content of phospholipids and slimes components are removed from raw materials by degumming. The content FFAs are variable, for example in rapeseed oil in the range of 0.5-1.0 wt%, but in comparison with tropical oil which usually contains 2-5 wt%, it is also possible that some kinds of vegetable oil contain more than 90% of the FFA (e.g. TOFA [44]). For the food industry, oils have to be refined to get the correct yellow colour and neutral aroma. It is also necessary to refine the crude oil at least partially for the production of fatty acid methyl esters [43, 45, 46].

3 Transesterification

One possibility of biodiesel preparation is transesterification (re-esterification) (1), which is generally defined as a chemical reaction between an ester and alcohol (A), whose product is esters (E) again and glycerol (G). Reacting alcohol (R'') replaces the original alcoholic group in the ester (R').

$$RCOOR' + R''OH \leftrightarrows RCOOR'' + R'OH \tag{1}$$
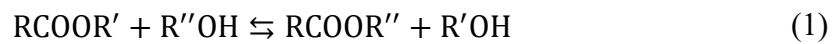
When the reaction mixture contains triacylglycerols (TG) from vegetable oils or animal fats than alkyl ester of higher fatty acids and glycerol are formed by transesterification (2).

$$TG + 3\,A \leftrightarrows G + 3\,E \tag{2}$$

This reaction proceeds in three consecutive steps during which undesirable compounds such as diacylglycerols (DG) and monoacylglycerols (MG)[1] are formed (3).

$$
\begin{aligned}
TG + A &\leftrightarrows DG + E \\
DG + A &\leftrightarrows MG + E \\
MG + A &\leftrightarrows G + E
\end{aligned}
\tag{3}
$$

If the oil with alcohol (e.g. methanol and ethanol) is immiscible then the three reactions proceed in parallel in the first minutes of the reaction, where a mass transfer is an important factor. To avoid the effects of mass transfer, the reaction is carried out at intensive stirring. After the first few minutes, the consecutive character of the reaction predominates, it means that the TG are converted into the DG and immediately after that the DG into the MG and finally MG into the esters and glycerol. The forming esters increase the miscibility between the compounds of the reaction mixture and act as a solvent for alcohol and acylglycerols. Therefore the reaction between vegetable oil and alcohol (the rate-determining step) is aided by the presence of esters and the vegetable oil reacts gradually in large amounts [47-49].

The transesterification is an equilibrium reaction and esters are the main product of interest, transesterification is carried out with an excess of alcohol to shift the direction of reaction towards the products [50]. There are two partially miscible phases obtained, lower glycerol-rich and upper ester-rich phases. Each phase has different content of other compounds present in the reaction mixture [51].

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[1] The abbreviations MG, DG, TG and E are not mean a chemical individuum, but the mixture of various acylglycerols or esters of higher fatty acids.

The increasing temperature increases the reaction rate until the temperature reaches the boiling point. The enthalpy of transesterification of rapeseed oil and methanol at 40 °C is $-9,8 \pm 0,9$ kJ mol^{-1}, for rapeseed oil and ethanol at the same temperature is the enthalpy of reaction $-9,3 \pm 0,7$ kJ mol^{-1}. For using rapeseed oil the transesterification is a slight exothermic reaction [46].

3.1 Catalysis in the transesterification

The transesterification can be carried out without a catalyst only at high temperatures and pressures (alcohol in supercritical stage), otherwise, it is necessary to use a catalyst [52, 53]. A catalyst is a substance that participates in a chemical reaction but remains chemically unchanged after the reaction. It is only able to influence reaction kinetics (reaction rate), but not the reaction thermodynamics (reaction feasibility). Conversely, substances that decrease the reaction are called inhibitors. Generally, catalysis of transesterification can be divided into two categories – chemical and enzymatic. Chemical catalysis is sorted to homogeneous and heterogeneous which are further divided into alkali and acid. If the catalyst is present in the same phase (usually liquid) as the reactant, it is homogeneous. Otherwise, if the catalyst is in a different phase (i.e. solid, immiscible liquid or gaseous) than the reactants, it is heterogeneous [54, 55]. The properties of each type of catalyst are shown in Table 2.

Nowadays, enzymatic catalysis is getting a lot of attention. Various types of lipases are used as a catalyst, moreover, lipases are very often immobilized on different carries. Their main advantages are a lower reaction temperature (usually 25-40 °C), i.e. it means lower energy consumption and the immobilized enzyme can be reused. Enzyme catalysts are insensitive to the content of the FFAs and water in the input oil, i.e. can be used oil with a high content of the FFA and water. Another great advantage is an easy recovery of glycerol from esters because the saponification reaction does not proceed. The enzymatic catalyst has also some limitations such as high cost of the enzyme, a long reaction time (up to ten hours), low yield and the enzyme can be deactivated by alcohol [56-58].

Alkaline homogeneous catalysts are most commonly used in transesterification, because of low cost and high efficiency [59]. The most popular are NaOH and KOH or their alcoholates which are well soluble in alcohols. They are used in an industrial scale as the only ones. The disadvantages of homogeneous alkali catalysts are that they cannot be easily recovered from the reaction mixture and reused. They are lost either by the side reaction (saponification of acylglycerols or esters) or they have to be neutralised after the reaction time. The low-quality

triacylglycerols resources with a high content of the FFAs (up to 3 wt%) and water cannot be used for alkaline catalysts because the presence of water causes hydrolysis of oils to the FFAs [60]. The usual use of alkaline catalyst is 0.9-1.2 wt% to oil [61].

Table 2: *Overview of the catalysts used in transesterification*

| Type of catalyst	Advantages	Disadvantages
Homogeneous alkali catalyst	- mild reaction conditions and less energy required - easy availability of catalyst - the high reaction rate (4000 times faster than acid ones) - high yields of reaction (99%)	- using only quality oils (if the FFA content in oil is more than 2 wt% the saponification takes place - irreversible loss of catalyst and decreasing of yield reaction - produce more wastewater from purification
Heterogeneous alkali catalyst	- environmentally friendly - reusable - easy to separate from the reaction mixture - the possibility of continuous operation	- increase energy requirements for the reaction (temperature, pressure) - challenging catalyst synthesis - catalyst instability - lower activity in compare with homogeneous ones
Homogeneous acid catalyst	- insensitive to the FFA and water content in the oil - esterification and transesterification occur simultaneously - easy availability of catalyst - saponification can be avoided	- low reaction rate - can lead to equipment corrosion - irreversible loss of catalyst
Heterogeneous acid catalyst	- insensitive to FFA and water content in the oil - esterification and transesterification occur simultaneously - recyclable -	- low reaction rate - high reaction conditions and longer reaction times - more energy requirement - catalyst instability
Enzyme catalyst	- mild reaction conditions - environmental friendly and non-polluting - reusable	- the very slow reaction rate - high cost - sensitive to alcohol (methanol can deactivate the enzyme)

Acid catalysts are suitable for oils with a high content of the FFAs as they can simultaneously transesterify oils and esterify the FFAs, but their reaction rates are slow. Acid catalysts are almost 4000 times slower than alkaline catalysts [62, 63]. As acid homogenous catalysts can be

used sulphuric acid, hydrochloric acid, sulfonic acid, etc. [64-66]. Due to the reaction rate limitation, a two-step reaction is used, firstly the FFAs are esterified and subsequently transesterification is carried out with an alkaline catalyst. This approach could provide a high biodiesel conversion (up to 98%) [67].

The problems with separation of catalysts and their reuse can be solved by heterogeneous catalysts, which are considered as more ecological due to decreasing water usage during the production of esters and separation of esters and glycerol is easily to recover from the reaction mixture. Moreover, heterogeneous catalysts do not produce wastewater, as in the case of homogeneous catalysts. Another indisputable advantage is that they can be reusable, which could facilitate a biodiesel production in a continuous process. The main disadvantages are technical demands of transesterification (a higher molar ratio of alcohol and usually high pressure – a pressure reactor is necessary), complicated catalyst synthesis and leaching of metals into the reaction products [68, 69]. The metallic oxides (alkaline earth metals, transition metals) [70], mixed oxides (Ca-Al, Mg-Al, Mg-Fe, etc.) [71-73], supported metal oxides [74].

4 Homogeneous catalysis

As mentioned previously, strong hydroxides (KOH, NaOH) or alcoholates of metals (CH$_3$OK, CH$_3$ONa) are used as homogeneous alkaline catalysts. In this work, KOH was exclusively used. In the case of alkaline catalysis, the reaction particle is alcoholate of corresponding metal formed by the dissolving of strong hydroxide in the alcohol (e.g. for methanol and KOH (4))

$$CH_3OH + K^+OH^- \leftrightarrows CH_3O^-K^+ + H_2O \qquad (4)$$
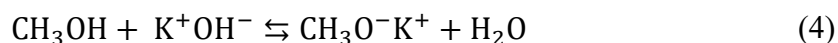
This is an equilibrium reaction and therefore it is suitable to use feedstock with the lowest water content since water presence water shifts the equilibrium towards the alcohol and hydroxide which leads to saponification. Triacylglycerols, the FFAs contained in oil and the main product – esters of higher fatty acids can be saponified. A simplified reaction scheme of alkali catalysed transesterification (A) including saponification of TG (B), ester (C) and neutralisation of the FFAs (D) are shown in Figure 1, where R_i is a chain from higher fatty acids.
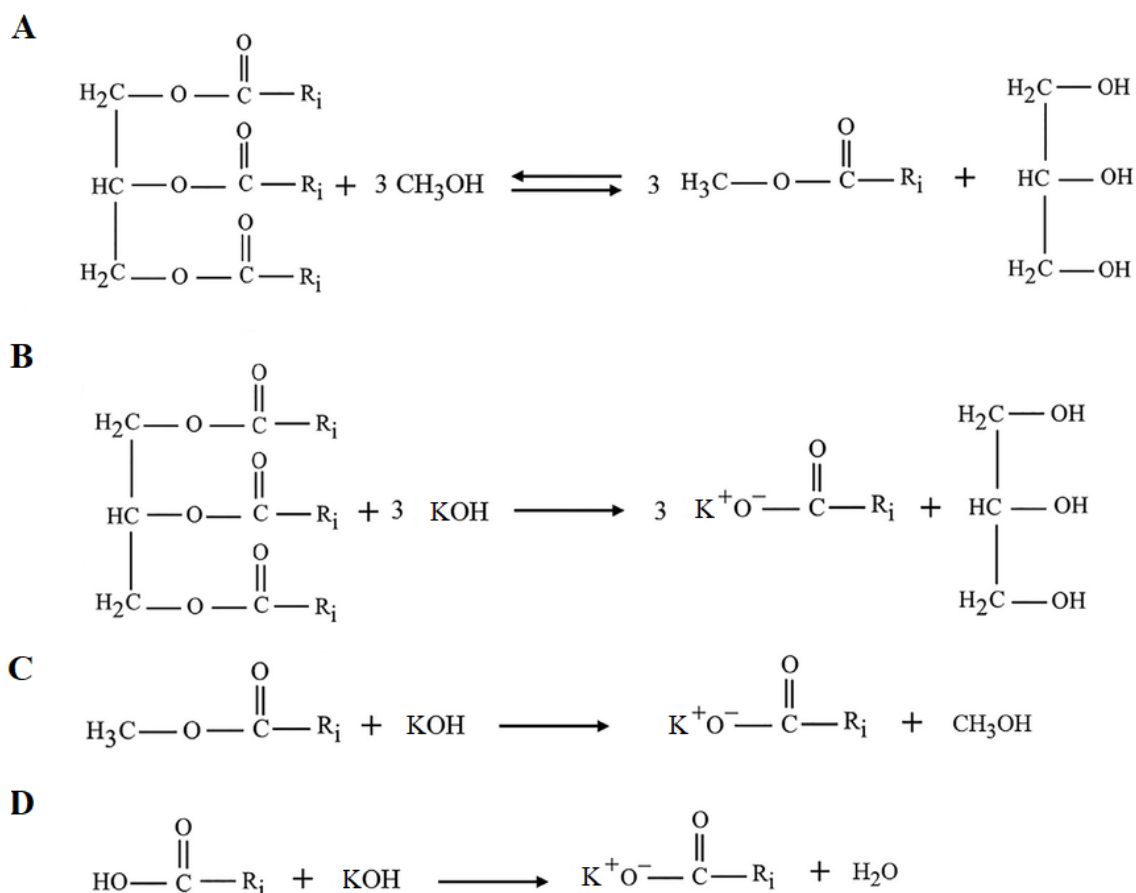
Figure 1: *Simplified scheme of alkali catalysed transesterification*

4.1 Monitoring of transesterification by various method

The course of the reaction can be monitored by various methods using changes of properties during transesterification. As shown (4), dissociations of KOH are formed OH$^-$ ions which allow the formation of methanolate and therefore the course can be monitored by pH. The measuring of pH during the transesterification process is complicated by the fact that this reaction is carried out in the non-aqueous environment and therefore the special electrode (with internal ethanolic electrolyte LiCl) designed for the non-aqueous titration is necessary to use. Since the formation of methanolate results in dissociation of hydroxide and methanol to ions, the presence of the ions can be monitored by the specific conductivity. The course of transesterification monitored by pH value and specific conductivity is shown in Figure 2.
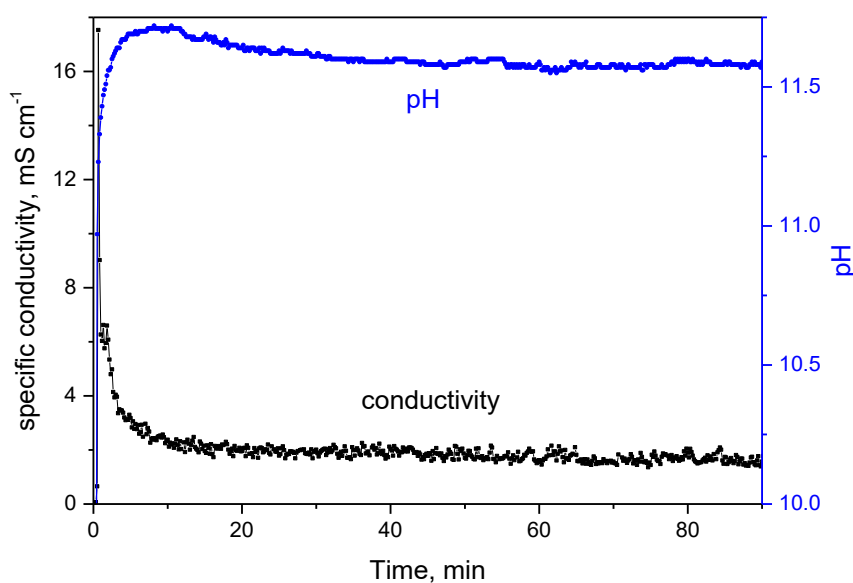
Figure 2: *The course of transesterification (pH and conductivity)*

At the beginning of the reaction, the pH value is stabilised at a relatively constant (pH value is approximately 12) because hydroxide ions are formed by dissociation in the reaction mixture. The pH value slight decreased, which was caused by the side reaction (saponification), because saponification consumed OH$^-$ ions and formed soaps was not dissociate. Unlike pH value, the conductivity firstly sharply increased (near to value 16 μS cm^{-1}) after addition of methanolate into the oil and almost immediately decreased to a constant value (approximately 2 μS cm^{-1}) and subsequently decreases very slowly similar as the pH value. Thus, the ions are not almost present in the reaction mixture. However, in the reaction mixture, both methoxide and hydroxide ions, as well as soaps, were found by titration of hydrochloride acid. This incongruity

can be explained by formation of revers micelles that surround the ions immediately at the start of the reaction and ions cannot be detected by conductivity and are likely cannot actively take part in the reaction. Moreover, it was found that the amount of unreacted catalyst (ions of OH^- and CH_3O^-) is in the range of 40-60% for a different initial amount of catalyst, i.e. uncreated catalyst remains in the reaction mixture. The formed micelles can be destroyed by an additional of methanol to the reaction mixture and the ions are again released, which can be also monitored by specific conductivity. Firstly, the conductivity gradually increased until the conductivity sharply increased (from $\mu S\ cm^{-1}$ to $mS\ cm^{-1}$), in this point micelles were destroyed. It was found that the amount of added methanol to the reaction mixture is dependent on the reaction temperature and increased with the decreasing temperature. Very similar behaviour was found for the viscosity of reaction mixture. In contrast, the pH was not significantly influenced by the addition methanol into the reaction mixture, the pH only gradually decreases due to dilution of the reaction mixture by methanol. These findings confirmed that during the transesterification reaction micelle is formed, immediately after mixing the components [75].

4.2   The catalyst neutralisation after transesterification

Since transesterification is a reversible reaction, therefore it is mostly carried out in excess alcohol to the stoichiometric amount (methanolysis 2x, butanolysis 6x). During the process of removal unreacted alcohol, a reverse reaction may occur (from the esters and glycerol are formed successively MG, DG and TG) and therefore the reaction has to be stopped [76]. The reaction can be finished by stopping of the mixing and subsequent the separation of formed the EP from the glycerol phase (GP), whereby the catalyst as a polar substance passes into the GP and the reaction does not proceed further. The obtained EP is usually known as crude biodiesel, which contains many impurities such as remaining acylglycerols, glycerol, FFAs, methanol, vegetable dyes, soaps and salts. The glycerol phase is usually low quality since it also contains many impurities such as water, soaps and remaining catalyst. Both phases have to be purified, i.e. a further technological treatment is necessary, which increases the energy consumption of biodiesel production. This approach is traditionally used in the industry [50].

Another approach is to neutralise a catalyst after the reaction time. The weak acid (e.g. gaseous CO_2) or inorganic acid (e.g. H_3PO_4, HCl) can be used for neutralisation of the catalyst. The carbon dioxide added into the reaction mixture has the advantage that it behaves as a weak acid and neutralised only alkaline catalysts (KOH) and cannot convert the soaps into the FFAs, i.e. does not increase the acid number. Conversely, the amount of potassium ions and free glycerol in the EP is mostly higher than allowed by the European standard EN 14214. Moreover, the

separation of the EP from the GP usually takes longer than 24 h [77]. Therefore, after removing of excess alcohol from the whole reaction mixture, it is possible to add to the reaction mixture a defined amount of water (determined according to the photometric titration [78]), which accelerates the product separation and improves the properties of the EP. The added water as a polar substance passes into the GP and dilutes it, i.e. decreasing glycerol content in the GP [79].

In others works, inorganic (strong) acids are usually applied for the kinetics, wherein the samples are taken at a different time and subsequently, the ester content in the EP is determined by GC, i.e. this procedure is not part of biodiesel production [80]. The inorganic acids such as HCl and H_3PO_4 are also possible to use for the stopping of transesterification. There are two options (i) acid addition into the whole reaction mixture until pH falls to 2-3, which means that soaps are converted into the FFAs (increase the acid number of the ester phase), the formed FFAs is necessary to remove from the EP (in publications 1 a process for the removal of the FFAs by using a strong alkaline has been described), (ii) if the acid is added precisely, then only catalytically active methoxide ions are neutralised, the FFAs are not formed and acid number remains almost zero (in publication 2 a new approach for simplification of biodiesel preparation has been described)

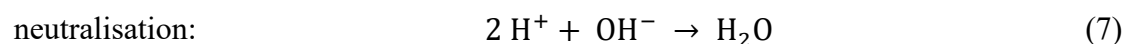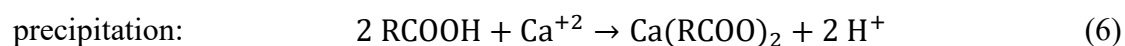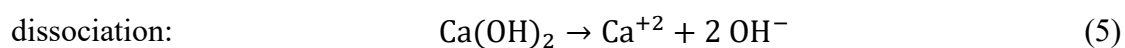**Neutralisation by inorganic acid and removing free fatty acids**

For the first option, when soaps are formed from the FFAs, the product of neutralisation by phosphoric acid is solid potassium phosphate (KH_2PO_4), which can be removed from the reaction mixture by filtration. The advantages of this approach are that content of potassium, water and free glycerol in the EP conforms with the standard, i.e. the maximum content for potassium ions is 5 mg kg^{-1}, for water is 500 ppm and for free glycerol in the EP is 0.02 wt%. Moreover, the GP does not contain any soaps, which improve separation of the GP from the EP and the glycerol content in the GP is higher in comparison with the approach used in industry (i.e. separation of both phases after transesterification). The disadvantage of this approach is that the FFAs are formed and thus the acid number of the EP does not conform with the standard. The FFAs can be removed from the EP by neutralisation. The publication 1 is focused on neutralisation of the FFAs from the EP. The calcium hydroxide, which reacts with the FFAs and forms calcium soaps, can be used for neutralisation. The calcium soaps are insoluble in the EP unlike potassium soaps, therefore the solid calcium soaps can be removed from the EP. Calcium soaps form an insoluble precipitate that cannot be filtered, therefore it can be removed by centrifugation. The calcium soaps formation was monitored by transmittance, where at first transmittance increased (the calcium hydroxide was solvated and dissolved) and after

approximately 10 min solid calcium soaps are begun to form, which is associated with a decreased in the transmittance to a constant value (Figure 3).
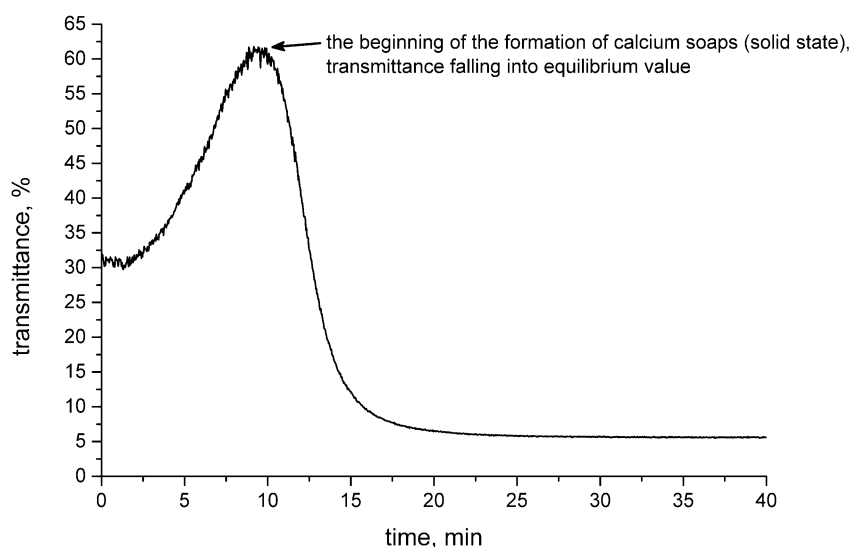
Figure 3: *Transmittance record of precipitation*

The removal of FFAs from the EP consisted of three steps: (i) the dissolution and dissociation of $Ca(OH)_2$ to ions (5), (ii) the reaction of calcium ions with the FFAs to form insoluble calcium soaps (6), the neutralisation (7) and (iii) the last step removal of soaps by centrifugation

$$\text{dissociation:} \qquad Ca(OH)_2 \rightarrow Ca^{+2} + 2\ OH^- \qquad\qquad (5)$$

$$\text{precipitation:} \qquad 2\ RCOOH + Ca^{+2} \rightarrow Ca(RCOO)_2 + 2\ H^+ \qquad\qquad (6)$$

$$\text{neutralisation:} \qquad 2\ H^+ + OH^- \rightarrow H_2O \qquad\qquad (7)$$

The dissociation of $Ca(OH)_2$ was important for removal of the FFAs because only calcium cations reacted with the FFAs (i.e. no reaction with FFAs without dissociation). The calcium hydroxide was practically insoluble in the EP and remained undissolved. The calcium hydroxide, as well as, are polar molecules, therefore the small amount of water improved the dissociation of $Ca(OH)_2$. Firstly the dissociation of $Ca(OH)_2$ with the different amount of water addition (0.6, 0.22, 0.38 and 0.63 wt%) and the stirring time (2, 5, 7, 9, 25 and 119 h) were tested (solubility test). It was found that the dissociation increased with the increasing amount of water addition and the stirring time. The solubility test was used to determine limits of the amount water addition and the stirring time for the experiment planning the amount of water

addition 0.63 wt% and the stirring time 6 h were determined. The longer stirring time then 6 h only had a negative influence on the removal process, because it caused the increased calcium content in the EP. The independent variables (such as the acid number of the acid number of input the ester phase – an_{in}, mg KOH g^{-1}; the molar ratio of calcium to the FFAs – MR, –; water addition – V_w, µl; the reaction time – t, h; the stirring speed – f, rpm and the reaction temperature – T, °C) were chosen that influenced the dependent variables (such as acid number of output the ester phase – an_{out}, mg KOH g^{-1}, the content of calcium ions – Ca^{2+}, mg kg^{-1} and water in the ester phase – w_{H_2O}, ppm). The experiments were designed according to the Placket-Burman designs and linear models were used to describe the relationship between the dependent and independent variables. The statistical significant independent variables were determined by statistical testing (significance of parameters, exclusion of outliers, regression triplet, etc., according to [81]). The programs STATISTICA and QC Expert were used for statistical analysis. From the equations obtained for the dependent variable: an_{out} (8), Ca^{2+} (9) and w_{H_2O} (10) were determined optimal conditions so that it conformed with the standard (detailed calculation procedures of the dependent variables are also described in publication 1).

$$an_{out} = 1.64 + 0.57\ an_{in} - 0.19\ t$$
$$(R^2 = 0.885) \tag{8}$$

$$Ca^{2+} = 46.4 - 20.4\ an_{in} - 10.3\ V_w + 19.7\ t + 32.5\ T$$
$$(R^2 = 0.829) \tag{9}$$

$$w_{H_2O} = 605.7 + 149.1\ MR + 105.1\ V_w - 76.6\ f - 109.6 - 124\ T$$
$$(R^2 = 0.919) \tag{10}$$

The optimal conditions were found: a molar ratio of calcium soaps to the FFAs of 3.33:1, the water addition of 0.14 wt% and time reaction of 3 h. The predicted (calculated) and experimental values of dependent variables were compared. There was found a good correlation between predicted and measured values for output acid number and calcium content, which were very similar to the predicted value. The water content was higher than the predicted value and did not conform with the standard. The advantages of this process are that no washing water is necessary and obtained ester does not need to be purified. Furthermore, this process is practically waste less because all side products are also usable. The product of catalyst neutralisation (solid phosphate) can be used as a fertilizer the same as calcium soaps.

Precision addition of inorganic acid

Another approach for neutralisation of remaining catalyst (methoxide ions) after transesterification is the precise addition of inorganic acid (H_3PO_4 and HCl) so that only methoxide ions are neutralised. In publication 2, this new method of acceleration and simplification of separation in biodiesel production is described in detail. The effect of varying amount of concentrated acid on the acid number and potassium content of the EP was studied. Therefore, the dependence of potassium content and an acid number of the EP on the amount of hydrochloric acid was determined (Figure 4).
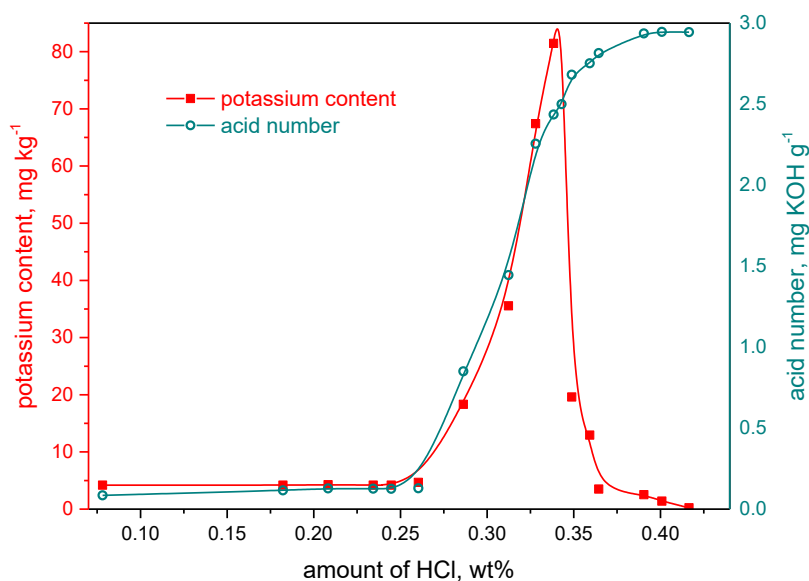
Figure 4: *The relation between potassium content and acid number in the EP on the amount of added hydrochloric acid.*
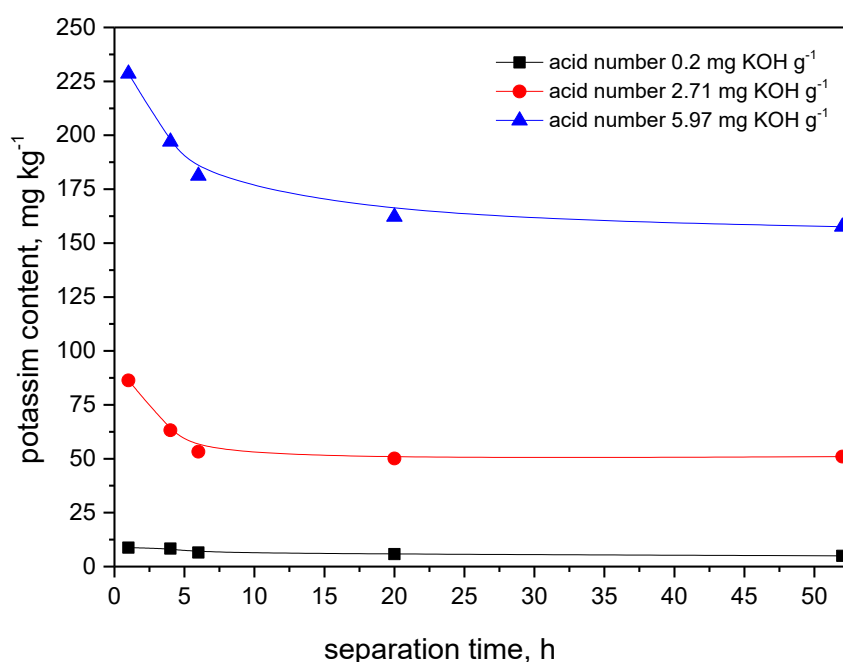
The potassium content and acid number of the EP were relatively constant up to about 0.25 wt% of HCl (the amount of acid is related to the reaction mixture). This was explained as the methoxide ions (formed from KOH and CH_3OH (4)) were neutralised by HCl to form chloride ions and methanol (11). The methoxide ions are more alkali than other substances in the reaction mixture and therefore are titrated as first. The potassium chloride is a polar substance and therefore potassium chloride passes into the polar GP.

$$CH_3O^- + HCl \rightarrow Cl^- + CH_3OH \tag{11}$$

Further addition of hydrochloric acid leads to an increase of the acid number because the soaps are converted into the FFAs, which reminds in the EP (12).

$$RCOOK + HCl \rightarrow K^+ + Cl^- + RCOOH \tag{12}$$

The acid number of the EP reached a constant value (3 mg g^{-1}) and further increasing was not possible, because all soaps were converted into the FFAs. The dependence of the acid number of the EP on the amount of HCl was expected, unlike the dependence of potassium content, which showed an unusual peak. The potassium content in the EP was increased sharply from 0.25 to 0.35 wt% of HCl addition (the maximum of potassium was approximately 80 mg kg^{-1}). When the additional amount of HCl was added, then the potassium content sharply decreased below 5 mg kg^{-1} (limit of EN 14214). This was explained as the formed FFAs (non-polar substances) are mainly contained in the EP (also non-polar substances) and the FFAs increased the dissolution of potassium soaps in the EP and increased the potassium content. When all the soaps were converted to the FFAs, the potassium content decreased almost zero because KCl as a polar substance remained in the GP. Besides, this finding was confirmed by independent experiments in which the oleic acid was artificially added to the raw reaction mixture before separation. The dependence of the potassium content on the separation time was measured for two various acid number (i.e. two various amounts of oleic acid) and it was compared with the dependence without any addition of oleic acid (Figure 5). It was found that the potassium content increased with an increased acid number of the EP, thereby confirming that FFAs increase solubility of soaps in the EP.

Figure 5: *The relationship between potassium content for a different acid number of the EP*

Furthermore, it was found that the pH dependence on the amount of acid during the addition of acids to the reaction mixture after transesterification was different (Figure 6). The hydrochloric acid curve exhibits one inflexion point (indicating neutralisation of methoxide ions), unlike the phosphoric acid curve. The phosphoric acid curve did not have an inflexion point (the pH value gradually decreased), and therefore the pH dependence cannot be used as an indication. The reason for this behaviour was consecutive changes formation of phosphoric acid derivatives. Therefore, it was necessary to determine the exact amount of methoxide ions by potentiometric titration of HCl before the addition of phosphoric acid.
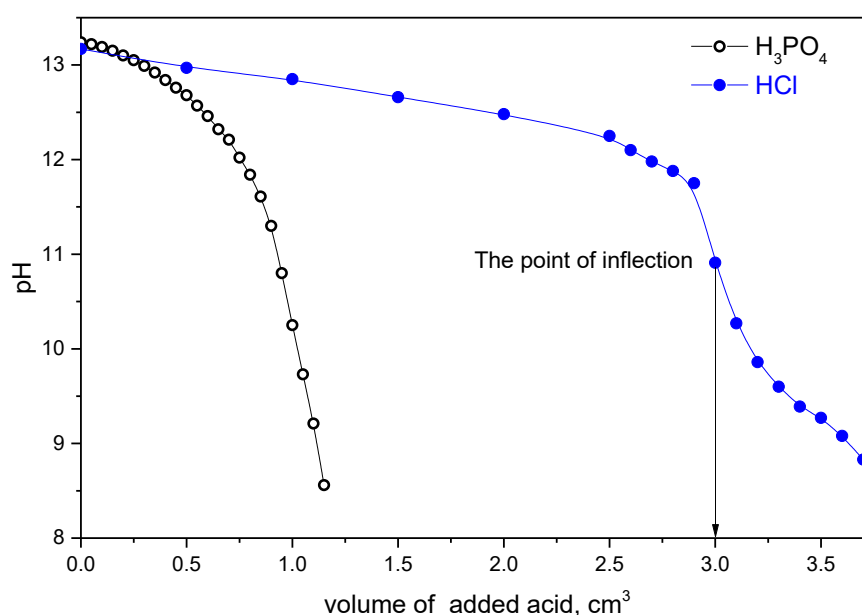
Figure 6: *The relation between pH on the volume of added HCl and H₃PO₄ added into the reaction mixture*

The experiments for both acids (HCl and H_3PO_4) were repeated ten times and important properties of the EP and GP were determined. For each property, mean values were calculated as well as the standard deviation of measurement by Horn's procedure for small selection (according to [81]). The emphasis was put on the acid number and the potassium content in the EP, which can be influenced by the amount of acid added when methoxide ions are neutralised. The best results were obtained by using concentrated phosphoric acid when the prepared methyl esters conformed with the EN 14214. The separation of the EP from the GP took approximately 5 h and separation could be accelerated by centrifugation, which took only 5 min and obtained methyl esters conformed with the EN 14214. The approach significantly simplifies the process of methyl esters (biodiesel) production and improves an economic and environmental balance of the total biodiesel production, because (i) it eliminates the need for purification steps (such

as wet and dry washing) (ii) biodiesel conforms to the standard including water content. Therefore no drying process of biodiesel is needed (iii) as the glycerol content in the GP is relatively high.

4.3   Methyl esters losses in the glycerol phase

In the previous section, the attention was mainly paid to the EP, whereas the following text will be mainly focused on the GP and especially the study of methyl ester losses after the transesterification. The glycerol phase is an integral part of the esters production process. Although it is mostly neglected and it is considered as a waste product of transesterification that is not further analysed. The detailed description and understanding of the GP composition, especially the esters content are important for (i) the explanation of the causes of esters losses (i.e. increasing ester yield) (ii) easier further treatment and usage of the GP. The GP usually contains 30-60 wt% of glycerol and further matter: soaps, water, salts, alcohol, remaining catalyst also esters. The exact composition of the GP generally depends on the transesterification process used and the method of separation and purification of the obtained esters. Moreover, ester content in the GP depends on the type of alcohol used: the ester content is in the range 6-15 wt% for methanol [82] and is the range 13-29 wt% for ethanol [83]. The esters content in the GP corresponds to the esters losses during the transesterification and decreasing the methyl esters yield [84]. The description and explanation of methyl esters content in the GP during the production of methyl esters allows to minimize their losses and make a production of methyl esters more economically and ecologically suitable.

The third publication is focused on the study of methyl esters content in the GP for three types of transesterification stopping by catalyst neutralisation by (i) gaseous carbon dioxide (CO_2), (ii) gaseous carbon dioxide with water (CO_2 with water) addition to accelerate the separation (iii) precisely addition of phosphoric acid (H_3PO_4), so that only methoxide ions were neutralised. Moreover, the methyl esters distribution according to fatty acids was also studied, because vegetable oil is composed of several types of fatty acids (Table 1). In the case of used rapeseed oil, the following fatty acids are mainly represented: oleic, linoleic, linolenic and palmitic acids. It was found that the ester losses for transesterification with methanol were in the range of 6-8 wt%, which complies with the previous publications [82]. The ester losses were almost similar for the stopping reaction by CO_2 and H_3PO_4, unlike CO_2 with water addition. In this case, the highest esters losses approximately about 15% higher than for types of stopping (without water) were found, because water increased the solubility of the esters in the GP. Water probably increases the dissociation of soaps in the GP, which then increases the esters content

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in the GP. As confirmed by other authors, the soap behaves as emulsifiers, which makes the separation of EP from GP difficult.

Different distribution of methyl esters of fatty acids in the GP for three types of stopping transesterification (CO_2, CO_2 with water and H_3PO_4) and input oil were found. For CO_2 was found, that the most abundant was methyl ester of oleic acid, following by linolenic, linoleic and palmitic acid of methyl esters. For stopping of reaction by CO_2 with water addition was found the same profile of methyl esters according to the FFA as CO_2 without water. For using H_3PO_4, the most abundant was methyl ester of linolenic acid, followed by methyl esters of oleic and linoleic acid, the methyl ester of palmitic acid was not present. The stopping of transesterification by phosphoric acid was chosen for the detailed analysis of methyl esters content in the GP, because (i) the obtained methyl esters conformed with the EN 14214, (ii) the most different distribution of methyl esters in the GP according to the higher fatty acids, (iii) the relatively fast separation (less than 6 h). Four independent variables: the reaction time – t, min; the molar ratio of methanol to oil – MR, –; the catalyst amount – P, wt% and the reaction temperature – T, °C; were chosen which influenced dependent variables (focused on the GP) i.e. the content of glycerol – w_G, wt%; esters – w_E, wt%; soaps – w_{soaps}, wt%; salts – w_{salts}, wt% and water – w_{water}, wt%. The factorial designed experiments were carried out to plan experiments. The main attention was paid to the influence of esters losses by changing independent variables and the relationship between a dependent (GP properties) and independent variables (reaction conditions) was described. The chosen properties of the GP were statistically evaluated by the linear model, which described the relationship between variables. Equations for each property of the GP were obtained. The obtained equations confirmed that the increasing temperature and catalyst amount supported the side reaction – saponification and form polar soaps that remain in the polar GP. Generally, saponification of oil takes place at a higher temperature due to higher activation energy of saponification (approximately 69.1 kJ mol⁻¹) [85] than the activation energy of transesterification (26.7-61.5 kJ mol⁻¹) [80]. Moreover, the soaps content decreases with higher molar ratio, because the higher molar ratio shifts the reaction towards the main products [86].

However, the main attention was paid to describe the methyl esters losses in the GP, i.e. methyl esters content in the GP. The relationship between the esters content and reaction conditions was evaluated by the linear model (13) and also by the non-linear model (14) (quadratic model), which had the higher regression coefficient and described the relationship more precisely.

$$w_E = 12.22 - 7.58\,MR + 2.54P + 2.79\,T$$
$$(R^2 = 0.92) \tag{13}$$

$$w_E = -4.86 + 5.51\,P + 3.13\,T + 9.28\,(MR)^2 - 4.59\,(MR) - 2.37\,(MR)T \tag{14}$$
$$(R^2 = 0.99)$$

The linear model is clear and confirms the previous finding that the increasing molar ratio shifts the reaction towards products (esters and glycerol), i.e. fewer soaps are formed and the separation of the obtained products is easier. The increasing catalyst amount and reaction temperature increased methyl ester loss because both variables increased soaps content (supported saponification). Since more soaps mean more methyl ester loss, which was also confirmed by principal component analysis [81].

The explanation of the quadratic model is more complicated, especially influenced by the molar ratio methanol to oil, because many equation members contain a molar ratio. The member $(MR)^2$ is always positive and therefore always increases the methyl ester loss. The reason could be that methanol increases the solubility of the esters in the GP, i.e. increases the methyl ester losses. The other members are a combination of molar ratio with the catalyst amount and temperature.
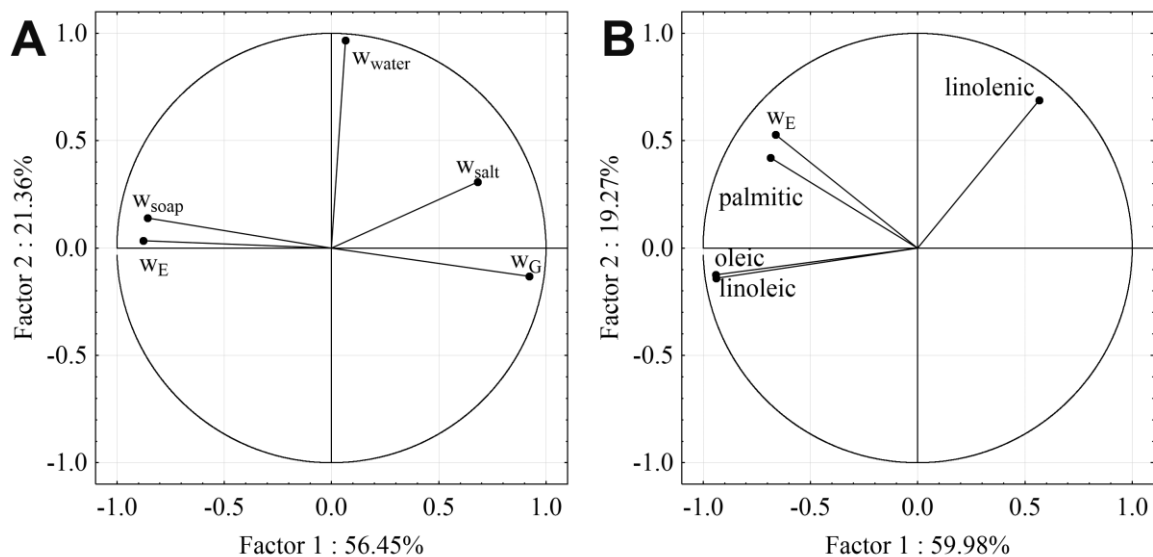
Figure 7: *The CWP for (A) properties of the GP and (B) methyl esters of higher fatty acids*

The principal component analysis was evaluated for important properties of the GP (dependent variables) and also for the distribution of methyl esters according to higher fatty acids (Figure 7). The principle weight plot (CWP) for dependent variables confirmed results obtained from the linear regression as well as the quadratic model (i.e. soaps and methyl ester content had a very strong positive correlation). Both of these variables had a negative correlation with

glycerol content, which is quite clear because more soaps and esters in the GP mean decreased glycerol content in the GP. The soaps are an emulsifier and make the separation more complicated.

The distribution of methyl esters according to higher fatty acids in the GP was determined for an experiment by the factorial design. The distribution was very different for each run, therefore the distribution depends on the independent variables (reaction conditions). Any significant relationship between methyl esters and reaction conditions was not found. Therefore, relations (correlation) for dependent variables were studied by the CWP. There were found two strong positive correlations between methyl esters of oleic acid and linolenic acid and methyl ester of palmitic acid and ester content in the GP. The positive correlation between methyl esters of oleic and linolenic acid can be caused by their relative similarity because both methyl esters content 18 carbons with one or two double bonds. If the GP contained increasing content of methyl ester of palmitic acid than the esters content in the GP was also increased, i.e. which meant more methyl ester of palmitic acid more total ester content in the GP.

4.4 Transesterification of vegetable oil by butanol

As mentioned before, the transesterification can be carried out with various types of alcohols, such as methanol (transesterification with methanol was described in the previous text), ethanol (not part of this work) and butanol (publication 4 is focused on using butanol as alcohol in transesterification of rapeseed oil). Butanol is hydrophobic, less corrosive and it has a higher boiling point (n-butanol 118 °C) in comparison with methanol (65 °C). Due to the higher boiling point, the higher reaction temperature can be used, but the higher temperature supported saponification. Butanol is miscible with oil (the homogenous system is formed), unlike methanol and ethanol, which are immiscible (the heterogeneous system is formed). It is also reported in the literature that the butyl esters have a higher caloric value than the methyl esters [42].

The butyl ester was prepared by the transesterification of vegetable oil in the presence of KOH as the catalyst. The catalyst was neutralised after reaction by CO_2 and inorganic acid (phosphoric acid, hydrochloric acid and sulphuric acid) added into the whole reaction mixture. As an acid was added until pH fell to 2-3, the FFAs were formed from soaps and the acid number was increased. Moreover, the separation was carried out with addition or without the addition of water, and both ways were compared. Six independent variables (the molar ratio of butanol, the catalyst amount, the reaction temperature and the time and the addition method)

were chosen, which influenced the chemical reaction (the concentration of acylglycerols, the yield of the EP, content of potassium and free glycerol in the EP) were chosen. These variables influence both the transesterification and also the separation and the experiments were designed according to the Placket-Burman design. The relationship between the dependent and independent variables was described by the linear models for transesterification and separation after transesterification was also studied. After the reaction time, the content of acylglycerols was determined in the reaction mixture without separation. It was found that the reaction mixture was homogeneous (mixture not separated) for molar ratio butanol to oil 6:1 and contained a higher content of acylglycerols. The results from the linear model correspond to the previous findings, i.e. the increasing molar ratio of butanol shifts the reaction towards product and increasing catalyst amount and temperature and they both supported saponification.

Strong and weak acids were used for the catalyst neutralisation. As inorganic acids (phosphoric, sulphuric and hydrochloric), the separation took only minutes, because glycerol content in the GP was about 90 wt%, which caused a high density of the GP, unlike the EP. The EP did not contain potassium and free glycerol, but the acid number was increased (soaps were converted into the FFAs). Conversely, weak acid gaseous CO_2 cannot convert soaps into FFAs, but in this case, potassium content and free glycerol content were usually increased. For separation without water addition, the separation took out more than 24 h and potassium content and free glycerol was high, i.e. 100-200 mg kg^{-1} and 0.2-04 wt% of free glycerol. If water was added (according to [78]) than potassium content and free glycerol were significantly decreased by approximately 4 and 10 times. The acylglycerols content was also decreased but only slightly. On the other hand, water content was higher compared to no water addition and therefore the EP had to be dried (because the water content is one of the parameters of the standard). The GP was also studied, but unfortunately, the ester content in the GP was not determined. However, the strong relationship between butanol content and the flashpoint was found, because the flashpoint depends only on the butanol content.

The chosen properties were compared with methyl esters and diesel produces from crude oil. Formed butyl ester has the density very similar to methyl ester and both have it slightly higher than diesel. Butyl ester has a higher kinematic viscosity than methyl ester and diesel. The butyl ester has a caloric value of approximately about 7.5% higher than methyl ester which is similar or slightly lower than diesel, according to the different sources of value for diesel. Conversely, from density and viscosity, the caloric value is not given by the standard and probably depends on the type of ester and diesel.

5 Heterogeneous catalysis

The issue of the use of heterogeneous catalysts in transesterification has been described in the section catalysis in transesterification (3.1 Catalysis in the transesterification), including comparisons with homogeneous and enzymatic catalysts. Generally, the heterogeneous catalysts are divided into oxidation-reduction and acid-alkaline catalysts. Oxidative-reduction is not used in the biodiesel production since the transesterification is a catalysed acid-alkaline reaction. Thus, acid-alkaline catalysts are suitable for the biodiesel production. This part is focused on mixed metal oxides (Mg-Al and Mg-Fe) prepared from hydrotalcites-like precursors by thermal pre-treatment – calcination. Hydrotalcites are ionic clays having a layered structure with a general formula $[M_{1-x}^{II} M_x^{III}(OH)_2^{x+}](A_{x/n}^{n-}) \cdot m\ H_2O$, where M represents bivalent (Mg, Ca, Zn, etc.) or trivalent metallic cations (Al and Fe), x is the molar ratio of M^{3+}, A^{n-} is anion (such as nitrates, carbonates, etc.) and m is the number of molecules water. The structure is derived from the brucite, which is magnesium hydroxide $Mg(OH)_2$, but part of the magnesium cations is replaced by another cation (e.g. Ca, Al, Fe). By this substitution, the cation-containing layer obtains a positive charge, and keep the electroneutrality, this charge is compensated by the negative charge of anions in the anionic layer. The anionic layer is located between two positively charged cationic layers and also water is bounded in it. The mixed oxide is then formed by the thermal pre-treatment of hydrotalcites to remove water and layered structure is destroyed [87].

The transesterification catalysed by mixed oxides is usually carried out in a batch reactor and many more publications are focused on the study of Mg-Al then Mg-Fe mixed oxides. Only a few publications are focused on the use of mixed oxides in a fixed-bed reactor, especially if mixed oxides are used as a catalyst. In a batch reactor, the mixed oxides Mg-Al and Mg-Fe were studied by many authors and Mg-Al mixed oxides achieve greater activity compared to Mg-Fe mixed oxides. For Mg-Al mixed oxides, Hájek et al. achieved up to 80% ester yield at reaction conditions: molar ratio of Mg:Al 7.2:1, molar ratio methanol to rapeseed oil 24:1, 4 wt% of catalyst to oil, intensity stirring at 320 rpm, reaction temperature 117 °C and reaction time 8 h [88]. Compared to the Mg-Fe mixes oxides using in transesterification, when also Hájek et al. achieved lower esters yield approximately 70% at reaction conditions very similar as in the case of using Mg-Al mixed oxides, i.e. at the same molar ratio methanol to oil 24:1, catalyst amount 4 wt%, reaction temperature 120 °C and reaction time 8 h, but at different molar ratio Mg to Fe 4-5:1 and stirring 400 rpm [89].

In publication 5 Mg-Al and Mg-Fe mixed oxides in the fixed-bed reactor were compared. The transesterification was studied at different weight hour space velocity (WHSVs) and temperature, which is defined as the ratio of the rapeseed oil mass rate to the catalyst amount. Moreover, both catalysts were tested in a long-term catalyst test, which takes 300 h (i.e. 12.5 days), including the stability of the catalysts in transesterification of rapeseed oil. The both mixed oxides were prepared by the co-precipitation method at constant pH (9.5 for Mg-Al and 10 for Mg-Fe) from the solution of cations (from nitrate of Mg, Al and Fe) at the temperature of 60 °C for Mg-Al and room temperature for Mg-Fe. The solution of cations was prepared by the dissolution of nitrate in deionized water to achieved total cations concertation of 1 mol dm^{-3} and the molar ratio of both catalysts was 3:1. The solution of cations was continuously added (8 ml min^{-1}) into the glass reactor under intensive stirring 400 rpm and the pH was kept at the constant value by addition KOH 2 mol dm^{-3} solutions during the precipitation. After addition of a whole solution of cations into the reactor, the product was left to stir at 60 °C for 1 h. The product of suspension hydrotalcites was filtered out by deionized water until pH fell to 7 and all alkaline solution was washed off. The obtained washed hydrotalcites were dried (at 65 °C for 12 h and then dried hydrotalcites were calcined at 450 °C for 3 h with temperature ramp of 5 min^{-1}) to achieve mixed oxides (catalyst activity form). Both catalysts were characterized by various methods (XRD, TGA, XRF, etc.). The catalysts structure was confirmed by the XRD analysis, when typical diffraction lines were observed for hydrotalcites and also for mixed oxides, including calculation of the MgO crystal size. It was found that the crystal size and specific surface area of the Mg-Fe were higher than those properties of the Mg-Al for hydrotalcite and the mixed oxides. Unlike the total concertation of basic sites (determined by temperature-programmed desorption of CO_2), which were for the Mg-Al had two times higher than Mg-Fe. Temperature stability of catalysts was studied by thermogravimetric analysis, where was found that Mg-Al is stable at a higher temperature than Mg-Fe and a real molar ratio of both Mg-Al and Mg-Fe mixed oxides were determined by X-ray fluorescence analysis.

The catalysts activity was tested in the transesterification reaction of rapeseed oil by methanol in a fixed-bed reactor. The molar ratio of methanol to oil 24:1 was the same in all experiments and the influences of temperature and WHSVs on the catalyst activity for both catalyst (Mg-Al and Mg-Fe) were studied. Three WHSVs values of 1, 2 and 3 h^{-1} at reaction time 32 h for each WHSVs was tested and the WHSVs value 1 h^{-1} was repeated at the end to verify catalyst stability. It was found that the ester yield and triacylglycerols conversion (which were

determined for samples taken every 4 h) decrease with increasing WHSVs, therefore the increasing WHSVs means decreasing contact time. The Mg-Al mixed oxide achieved the higher esters yield and triacylglycerols conversion in comparison with Mg-Fe mixed oxides, which correspond to the concentration of basic sites. The influence of temperature on the activity of both catalysts was also tested. The three temperatures 115, 150 and 200 °C at a constant WHSV (1 h^{-1}) were tested and at the end temperature 115 °C was repeated to verify the stability of the catalysts, and the reaction time was also 32 h for each temperature. The triacylglycerols conversion and ester yield increased with increasing temperature for both catalysts. The Mg-Al had higher catalyst activity for temperature 115 and 150 °C because the Mg-Al contained a higher amount of basic sites than the Mg-Fe catalyst. At the highest temperature 200 °C, the catalyst activity for both catalysts was almost the same because at this temperature the reaction rate was high (according to the Arrhenius equations).
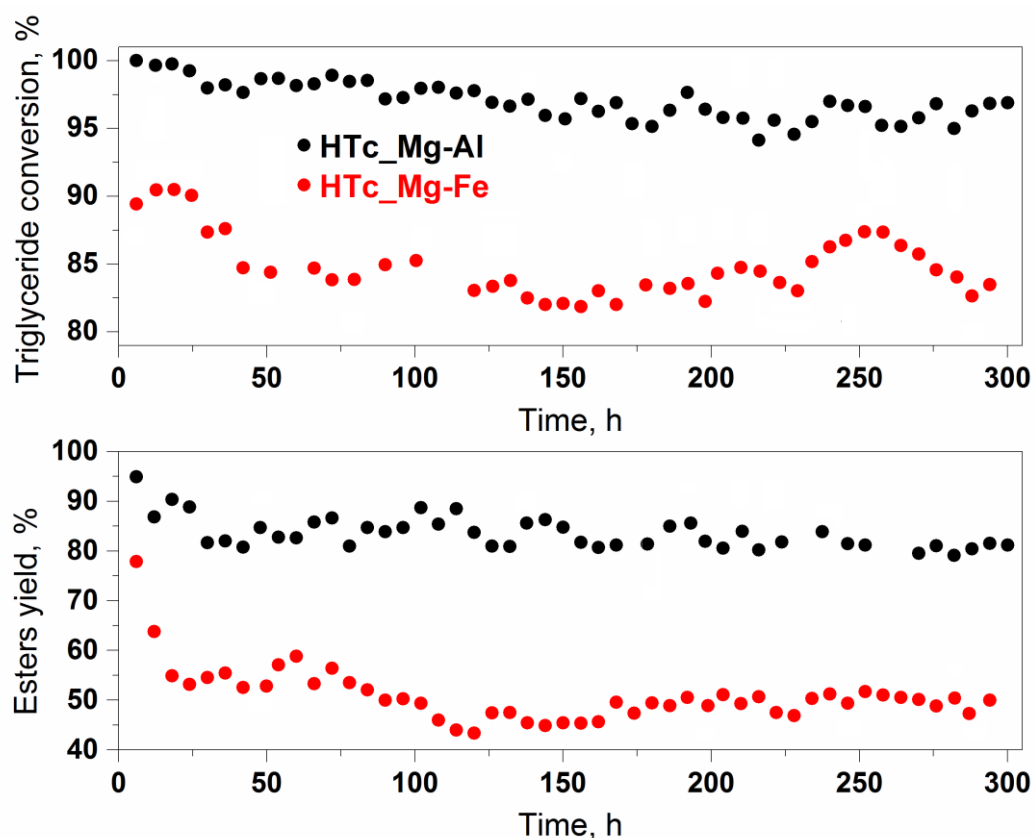
Figure 8*:* The long-term catalyst test of Mg-Al and Mg-Fe mixed oxides

Finally, the catalyst lifetime and stability of catalysts were tested by a long-term catalyst test, which takes 300 h and samples were taken every 6 h for determination ester yield and triacylglycerols conversion. The long-term test was carried out at WHSV 1 h^{-1} and temperature

150 °C (Figure 8). It was found that both catalysts are stable during the test, the catalyst activity of both catalysts slightly decreased: for Mg-Al triglyceride conversion from 99% to 97% and ester yield from 89% to 81% and Mg-Fe triglyceride conversion from 92% to 84% and ester yield from 53% to 50%. The Mg-Fe mixed oxide had a lower catalyst activity because it had to twice less basic sites. Moreover, the total leaching of the catalyst was determined for all experiments and was found as insignificant.

6 Conclusions

The dissertation thesis was focused on the transesterification of rapeseed oils with alcohols (methanol and butanol) catalysed by alkaline catalysts – homogenous (potassium hydroxide) and heterogeneous (mixed oxides Mg-Al and Mg-Fe). Obtained data presented in this thesis can be summarised as follows:

- The methyl esters with a low content of free fatty acids were prepared by stopping the reaction by neutralisation of the catalyst by the addition of inorganic acid. Two methods of neutralisation catalyst by inorganic acid were used:

- The inorganic acids were added until pH value felt to 2-3, which means that soaps were converted into free fatty acids. The advantages of this method are a relatively fast separation of the ester phase from the glycerol phase and the ester phase does not contain potassium ions and free glycerol. The disadvantage is the formation of free fatty acids from soaps, which had to be removed from the ester phase by neutralisation of free fatty acid by calcium hydroxide.

- Inorganic acids were added precisely so that the only catalyst was neutralised, and free fatty acids were not formed. Obtained ester phase was conformed with the European standard for biodiesel (EN 14214) without other purification processes.

- The glycerol phase (side product of transesterification) was thoroughly described. The focus was mainly on methyl ester losses after the different methods of stopping reaction (by weak acid gaseous carbon dioxide or inorganic acid). Moreover, the distribution of esters according to higher fatty acids (such as linolenic, linoleic, oleic a palmitic) was determined for all methods of stopping the reaction. The description and understanding of methyl esters content in the glycerol phase during the preparation of biodiesel can enable to minimize their losses and make the biodiesel preparation more ecological and economically friendly.

- The ester was prepared by using butanol as a reactant with potassium hydroxide as a catalyst. The butyl ester preparation and separation of the reaction product after the reaction were described.

  Transesterification of rapeseed oil with methanol was carried out in a fixed-bed reactor catalysed by mixed oxides Mg-Al and Mg-Fe as a catalyst. The stability of the catalyst was described by a long-term catalyst test.

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