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INVESTIGATION OF ENZYME-CATALYZED TRANSESTERIFICATION OF USED FRYING OILS

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Investigation of the possibility to convert used frying oils to less harmful but more valuable products is driven by the protection of environment and human health as well as economical reasons. One solution could be the conversion of these oils to transportation fuels and their application in Diesel engines in "pure" form or as blend stocks of diesel fuels. The conversion to biodiesel can be realized by transesterification with various catalysts. This paper presents the results of some experiments made by applying used frying oils and a process which is studied less intensively in the literature. The main goal of our experiments was to compare the transesterification efficiency of the three commercially available immobilized lipases [*Candida antarctica* (Novozym 435), *Rhizomucor miehei* (Lipozyme RM IM) and *Thermomyces lanuginosus* (Lipozyme TL IM)] which were applied under the same conditions and using the same feed. Based on our experimental results we established that we achieved the highest methyl ester content (>94%) approaching well the theoretical yield when we applied *Candida antarctica* (Novozym 435) among the investigated lipase enzymes.

Keywords: lipase, enzyme-catalyzed transesterification, used frying oil, biodiesel

Introduction

Nowadays the amount of vegetable oils used for human consumption has increased significantly. This causes the increase of quantity of the used frying oils and cooking greases which can no more be used in the food industry. The gathering, deposition, recycle or treatment of this high amount of used frying oils are becoming more important in these days. This is caused by the need for decreasing the quantity of wastes, saving our resources, lowering the load of the sewages and dumps, and economical aspects as well [1].

Currently many applications of the used frying oils which can no more be used for edible purposes are known. The appropriately pre-treated used frying oil is an important feedstock for the colour industry, cosmetic industry and road-building industry. Additionally it was used as a feed additive for animals, but now it is forbidden [2, 3, 4, 5].

Beside the above mentioned areas the pre-treatment and purification of used frying oils and their use as fuels (or heating oils) with or without conversion are very important research areas nowadays. The application of used frying oils as fuels is favored by the European Union.

By 2005 1% of the fuels consummated by the EU was biomass derived, thanks to the 2003/30/EC directive of the European Union which helped to merge the use of biofuels [6].

The „EU Strategy for Biofuels" [7] was a milestone in the application of used frying oils, because the European Union declared the need for using new kind of feedstock [7]. According to the latest aims of the European Union the quantity of the biofuels used should be 10% by 2020 [8]. This can also help the application and conversion of used frying oils.

This proposed value can be accomplished by utilization of different vegetable oils, used frying oils and its derivatives as fuels. The utilization options of triglycerides as fuels can be the following:

- direct blending into diesel fuels,
- transesterification to biodiesel fuels,
- production of fuel blending components by different cracking processes (engine gasoline, jet, diesel fuel).

Recently, among these methods the use of biodiesels obtained the transesterification of triglycerides with methanol is the most preferred.

Chemical transformation of used frying oils is not possible by the conventional method (alkali catalyst), because of its high free fatty acid content (5–35%). The adequate amount of alkali catalyst immediately reacts with the free fatty acids found in used frying oils resulting in soap formation and it is not able to catalyze the reaction. A possible way is the conversion of the used frying oils with acid catalyst (hydrochloric acid, sulphuric acid, acid ion-exchange resin). Substantial amount of acid catalyst and significantly higher reaction time is

necessary for the transesterification, compared to the alkali catalyzed method [9, 10].

Another option is the conversion of used frying oils by combined acid and alkali catalyzed transesterification. In this process the free fatty acid content of the used frying oils are first pre-esterified in the presence of acid catalyst, then the transesterification is completed by alkali catalyst [10, 11].

Another possible way is the enzyme-catalyzed transesterification of used frying oils, because lipase enzymes can transform free fatty acids into esters. The application of enzyme catalysts compared to alkali catalysts has several advantages: it is carried out under mild temperature-, pressure-, pH-conditions and no hazardous by-products or wastes are formed (e.g. waste water, soaps), furthermore methyl esters are formed from also the free fatty acids of the raw materials.

However, in all cases it is very practical to separate all undesired components present in the used frying oils before their use or conversion. These undesired components are for example the solid oxidation compounds which form during frying (oxidized triglycerides, epoxides, etc.), oxidized oligomers, non-polar dimers and non-polar polymers, etc [12].

Many processes are known to eliminate these undesired components, thus to clean the used frying oils [13, 14]. For example adsorbents (eg: calcite, sepiolite, montmorillonite, attapulgite), supercritical carbon-dioxide, ozone, water and inert gases can be used.

After adequate pre-treatments the used frying oils can be converted with the similar method as the vegetable oils. This gives many advantages. The most important is that valuable product can be produced of a material that is concerned as waste, so the load of the dumps and the environment decreases. From economical point of view it can be attractive that the price of the used frying oils and that of the methyl-ester produced from them is lower than the price of the vegetable oils and of the vegetable oil fatty acid methyl-esters [13].

Experimental

The main goal of our experiments was to compare the transesterification efficiency of the three commercially available immobilized lipases [*Candida antarctica* (Novozym 435), *Rhizomucor miehei* (Lipozyme RM IM) and *Thermomyces lanuginosus* (Lipozyme TL IM)] which were applied under the same conditions and using the same feed. The operational parameters during our experimental work were based on our previous results [15-18].

Experimental apparatus

The enzyme catalysed transesterification was carried out in a heated shaker equipment with a capacity of 9 Erlenmeyer flasks (New Brunswick G24). Simultaneously all feedstocks can be put into the shaker, so the same parameters can be assured. The temperature in the shaker equipment was controlled manually with a precision of ±1 °C.

Materials and their preparation

Durig our experimental work the feedstocks were Hungarian sunflower oil with high oleic acid content (HOSO), used frying oil (UFO) and the 50-50% mixture (MIX) of the previous materials. The main characteristics of the different feeds is given in *Table 1* and their fatty acid composition in *Table 2.*

Table 1: The main properties of the feeds

Properties	**UFO**	**HOSO**	**MIX**
Density, 15°C, g/cm^3	0.9216	0.9145	0.9195
Kinematic viscosity, 40°C, mm^2/s	39.8	33.7	35.6
Sulphur content, mg/kg	10	5	8
Nitrogen content, mg/kg	12	6	8
CFPP, °C	42	36	39
Acid value, mg KOH/g	2.3	0.5	1.5
Iodine number, g I_2/100g	132	89	103

UFO: used frying oil
HOSO: sunflover oil with high oleic acid content
MIX: 50-50% mixture of the previous
CFPP: Cold Filter Plugging Point

Table 2: The fatty acid composition of the feeds

Fatty acid composition, %*	**UFO**	**HOSO**	**MIX**
C14:0	0.1	0.0	0.1
C16:0	7.9	3.3	4.8
C16:1	0.2	0.1	0.1
C18:0	3.8	3.3	3.5
C18:1	26.3	87.4	59.7
C18:2	60.3	4.2	30.2
C18:3	0.2	0.0	0.1
C20:0	0.2	0.3	0.3
C20:1	0.2	0.2	0.2
C22:0	0.6	0.9	0.8
C22:1	0.0	0.0	0.0
C24:0	0.2	0.3	0.2
C24:1	0.1	0.0	0.1

*The first number represents the number of carbon atoms and the second means the number of double bonds in the molecule
UFO: used frying oil
HOSO: sunflover oil with high oleic acid content
MIX: 50-50% mixture of the previous

During the transesterification reactions analitycal grade methanol (SPEKTRUM 3D) was used.

The investigated enzyme catalysts were the macroporous resin immobilized lipase *Candida antarctica* (Novozym 435) (activity: 7000 PLU/g), acrylic resin

immobilized *Thermomyces lanuginosus* (Lipozyme TL IM) (activity: 250 IUN/g) and anion-exchange resin immobilized *Rhizomucor miehei* (Lipozyme RM IM) (activity: 150 IUN/g) received as a kind gift from Novozymes A/S (Bagsvaerd, Denmark).

Before the transesterification the first step was the pretreatment of the used frying oils and vegetable oils with Tonsil® adsorption clay and adequate volume of pertfil filter aid.

Test method

The methyl ester content of the products were determined according to the EN 14103: 2004 standard [Fat and oil derivatives – Fatty Acid Methyl Esters (FAME) – Determination of ester and linolenic acid methyl ester contents]. During the measurements we used gas chromatograph and we applied methyl heptadecanoate as an internal standard. The conditions of the gas chromatograhic measurements are summarized in *Table 3.*

Table 3: The conditions of the gas chromatograhic measurements

Injector	Split/Splitless injector, 260 °C, 200 ml/min
Column	Supelco Omegawax-250 capillary column, 30 m x 0.25 mm x 0.25 μm
Furnace program	120 °C (1 min) initial temperature 240 °C (10 min) final temperature 5 °C/min
Detector	FID detector, 260 °C
Amount of sample	1 μl

Experimental method

The feeds in Erlenmeyer flasks were shaken by the shaker equipment in the presence of immobilized enzyme catalyst at 50±1 °C, atmospheric pressure for a defined time. Every flask contained 44 g of vegetable/used frying oil and 6g of immobilized lipase (12% of the total amount of reactants). Methanol was added to the reaction mixture in 8 parts by applying a methanol-to-triglyceride molar ratio of 4:1 (6.4 g methanol) instead of the stochiometric ratio of 3:1, considering that excess methanol favors the progress of the reaction. The stepwise addition is necessary to prevent the inhibiting effect of the methanol.

All transesterification reactions were carried out under the same conditions, the reaction times were 4, 8, 12, 16 hours. After the reactions the ester containing phase was separated and the excess of methanol was removed by vacuum destillation. Thereafter, the amount of the product and the methyl ester content of the ester phase obtained through the enzymatic transesterification were determined.

Results and disscussion

In case of all feedstock the yield of the methyl-ester phase was 96–99% of the theoretical value using *Candida antarctica* (Novozym 435) immobilized lipase enzyme. Methyl ester content of the products as a fuction of the reaction time is shown in *Fig. 1.* It can be seen that methyl ester contents in case of a given reaction time differ only by few percents. After 16 hours reaction time methyl ester content of the product obtained form used frying oil (UFO) was the smallest (94.1%), is probably caused the oxided compounds present in the used frying oil, can not be converted by *Candida antarctica*. Yield of the product prepared from high oleic sunflower oil (HOSO) approached the theoretical value by 99.8%, its methyl ester content was 99.0%, meanwhile methyl ester content of the mixture (MIX) was 96.9% after 16 hours reaction time. Methyl ester content of these two products fulfilled the requirements (>96.5%) of the EN 14214:2004 standard.

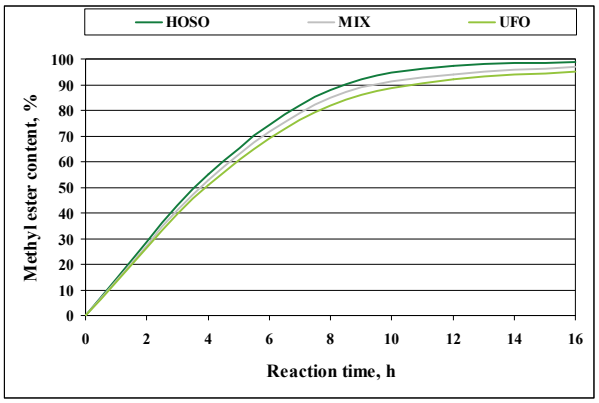

Figure 1: Methyl ester content of products as a function of transesterification time (catalyst Candida antarctica (Novozym 435))

In case of the transesterification carried out in the presence of *Thermomyces lanuginosus* (Lipozyme TL IM) immobilized lipase enzyme methyl ester content of the products differed by only few percents *(Fig. 2)* as a function of the reaction time. However, methyl ester contents at a given reaction time were much lower than in case of *Candida antarctica* (Novozym 435). After 16 hours reaction time methyl ester content of the product prepared from high oleic sunflower oil (HOSO) was the highest (77.3%), that of the mixture (MIX) was 74.4%, menawhile that of the used frying oil (UFO) was only 71.1%.

Methyl ester content as a function of reaction time in case of *Rhizomucor miehei* (Lipozyme RM IM) is shown in *Fig. 4.* After 16 hours reaction time methyl ester content of the product prepared from used frying oil (UFO) was the smallest (63.8%), that of the high oilec sunflower oil (HOSO) was 74.8%, meanwhile that of the mixture (MIX) was 70.2%. Methyl ester content of the mixture was between the results of the high oleic sunlfower oil and the used frying oil *(Fig. 3).*

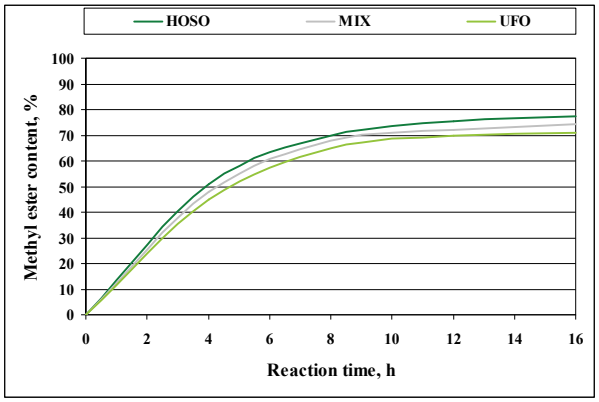

Figure 2: Methyl ester content of products as a function of transesterification time (catalyst Thermomyces lanuginosus (Lipozyme TL IM))

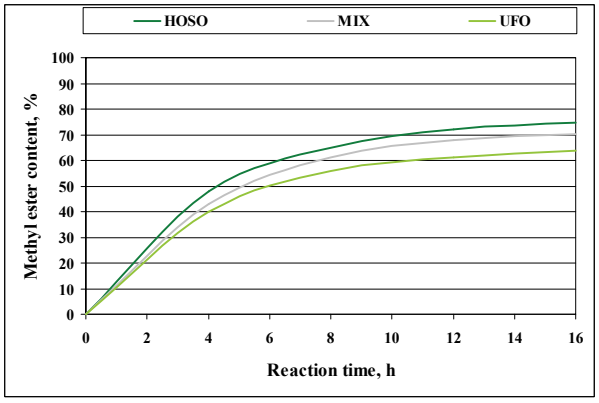

Figure 3: Methyl ester content of products as a function of transesterification time (catalyst Rhizomucor miehei (Lipozyme RM IM))

Methyl ester content of the products after 16 hours reaction time prepared by the three different immobilized lipase enzymes is summarized in Fig. 4.

Based on the results shown in the Figure it can be established that there is significant difference between the methyl ester contents of the products prepared from the same feedstock but with different lipases. Methyl ester content (94–99%) was the highest in case of *Candida antarctica* (Novozym 435) immobilized lipase enzyme in case of all three feedstocks. The lowest values (63–75%) were obtained by applying *Rhizomucor miehei* (Lipozyme RM IM) in all case. Methyl ester content of the products (71–78%) prepared by *Thermomyces lanuginosus* (Lipozyme TL IM) was between that of the previously mentioned two enzymes, but it is closer to the results obtained by applying *Rhizomucor miehei* (Lipozyme RM IM).

Based on our results it was found that in case of all three enzyme catalysts the highest methyl ester contents were achieved from high oleic sunflower oil (HOSO) and the lowest in case of the used frying oil (UFO).

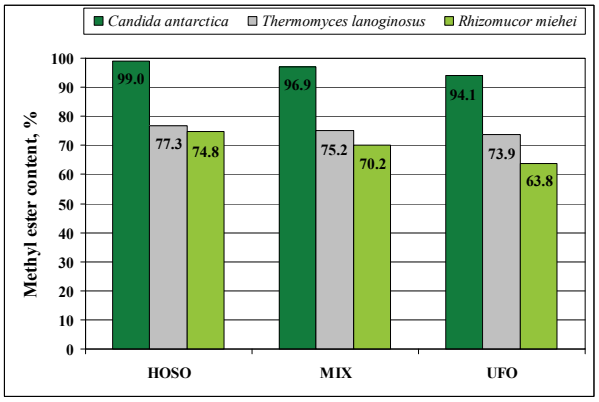

Figure 4: Methyl ester content in case of different feeds and enzymes after 16 hours reaction)

Summary

After the transesterifications carried out at the presence of three different immobilized enzyme catalysts we found that methyl ester content of the products prepared by *Candida antarctica* (Novozym 435) was the highest in all case. Methyl ester content of the products prepared from high oleic sunflower oil (HOSO) and from the 50-50% mixture (MIX) of high oleic sunflower oil and used frying oil satisfied the requirements (≥96.5%) of the standard (EN 14214:2004). However, the products prepared from used frying oil (UFO) did not reach this limit. Theoretical yield of the methyl ester containing phase was approached by 96–99%.

By the application of *Rhizomucor miehei* (Lipozyme RM IM) and *Thermomyces lanuginosus* (Lipozyme TL IM) immobilized lipases methyl ester content of the products was significantly lower, thus it did not satisfy the limit of the standard.

In case of all three enzymes methyl ester content of the products prepared from high oleic sunflower oil (HOSO) was the highest, meanwhile that of used frying oil (UFO) was the lowest. Methyl ester content of the product prepared from the 50-50% mixture (MIX) of high oleic sunflower oil and used frying oil was between the results of the previous two. Methyl ester content of the products clearly depended on the used frying oil content of the feedstock.

REFERENCES

1. GAIO T., CORDEIRO J.: Report of the OILPRODIESEL Project, (2006)
2. CANAKI M.: Bioresource Technology 98 (2007) 183-190

3. KULKARNI M. G., DALAI A. K.: Ind. Eng. Chem. Res. 45 (2006) 2901-2913
4. KEÖVES S., SÁRFALVI N., LAKATOS Á., GUBICZA L., BÉLAFI-BAKÓ K.: Proc. 2nd Int.Conf. Env. Eng. Veszprém, (1999) 248-250
5. NEMESTÓTHY N., LAKATOS G., BÉLAFI-BAKÓ K., GUBICZA L.: Proc. 29th Int. Conf. SSCHE, Tatranska Matliare, (Slovakia), 2002, CD-rom
6. Commission of the European Communities, COM(2006) 845 final, (2007)
7. Commission of the European Communities, COM(2006) 34 final, (2006)
8. Commission of the European Communities, COM(2006) 848 final, (2006)
9. CANAKCI M., GERPEN J.V.: Transactions of ASAE 42 (1999) 1203-1210
10. HANCSÓK J., KOVÁCS F., KRÁR M.: Petroleum & Coal 46 (2004) 36-47
11. BOOCOCK D. G. B.: 53rd Canadian Chem. Eng. Conf. (2003)
12. RIERA J. B., CODONY R., RAFECAS M., GUARDIOLA F.: Working document for the STOA Panel (2000)
13. CVENGROŠ J., CVENGOŠOVA Z.: Biomass and Bioenergy 27 (2004) 172-181
14. CANAKCI M., VAN GERPEN J.: Transactions of the ASAE 44 (2001) 1429
15. KRÁR M., HANCSÓK J., KOVÁCS F., HOLLÓ A., BODA L.: in Proceedings of INTERFACES'05 (2005) 17-24
16. KOVÁCS F., HANCSÓK J., BÉLAFI-BAKÓ K.: in Proceedings of 4th International Colloquium on Fuels (2003) 147-154
17. BÉLAFI-BAKÓ K., KOVÁCS F., GUBICZA G., HANCSÓK J.: Biocatalysis and Biotransformation 20 (2002) 437-439
18. KOVÁCS S., KRÁR M., BECK Á, HANCSÓK J.: 15th European Biomass Conference & Exhibition. Biomass for Energy, Industry and Climate Protection, In Proceedings (ISBN 978-88-89407-59-X) (2007) 1747-1750