Comparison of three methods for rendering plant fat transesterification

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Abstract

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Three most frequently used methods for fat transesterification were compared using rendering plant fat (RPF) as model. Acid-catalysed transesterification was found to be the most effective (conversion 90%) at optimum conditions (fat: methanol ratio 1:10, sulphuric acid amount of 2% v/v, temperature 95°C). Base-catalysed transesterification of RPF on the other hand, results in much lower conversion (45%) at optimum conditions (fat:methanol ratio 1:20, NaOH amount 8% w/v, optimum temperature 95°C). The difference is done (among others) by the fact that RPF has high concentration of free acid (high acidity number) compared with the fats usually used for transesterification and that free acids are not esterified in alkaline media. Enzyme-(lipase) catalysed reaction could lead to partial esterification of free fatty acids, but with much lower reaction velocity. This fact leads to higher conversion in the case of enzyme-catalysed transesterification of RPF compared with base-catalysed reaction; nevertheless, even in this case the conversion is much lower in comparison with acid-catalysed reaction. The optimum conversion in enzyme-catalysed reaction was 55%.

Keywords: biofuel; biodiesel; animal waste fat; esterification; methanol

Starting from the first oil crisis (1974) continuously increasing demand for energy and decreasing sources of petroleum resources has led to the search for alternative renewable and sustainable fuel. Biodiesel is one of the promising substitutes for petro-diesel. Biodiesel prepared by transesterification of any material non-utilizable for food is to be preferred, as such material does not compete with food production. Rendering plant fat is of advantage. This fat not only does not compete with food production but even does not occupy agricultural area (Atadashi et al. 2010). Fats of plant as well as animal origin were used for transesterification (Altun 2011). Animal fat mostly used for transesterification is beef tallow (Araujo et al. 2010; Thamsiriroj, Murphy 2010). Production of biofuel competes with the needs of fat for food (Stein 2007). This situation leads to the search for non-food fats or plants cultivated only for the technical oil production for transesterification. The most popular in this sense is jatropa (*Jatropa curcas*) (Berchmans et al. 2010; Chen et al. 2010; Corro et al. 2010). Very attractive sources of fats for transesterification are also different waste fats, like restaurant waste oil (Canakci 2007), municipal savage sludge (Cydzik-Kwiatkowska et al. 2010; Kargbo 2010) etc.

One of the animal waste fats is rendering plant fat (RPF), which is relatively cheap, is available at large quantities in localized plants and could be transes-

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terified like any other animal fat. RPF has relatively high acidity number (as high as 24 mg KOH/g), which is hindrance to the application of base-catalysed transesterification, as esterification of free acid is impossible in alkaline media. Other conditions for transesterification of RPF are very similar to the transesterification of tallow or other animal fats.

Different methods could be used for transesterification. Acid-catalysed transesterification is most frequently used one with sulphuric acid as catalyst (Deng et al. 2010), second is base-catalyzed transesterification with sodium hydroxide as catalyst, less frequently potassium hydroxide (Berchmans et al. 2010; Deng et al. 2010). Recently the heterogeneous catalysis was studied, using bases or acids like calcium oxide, SrO, TiO_2 or ZrO_2 (Qian et al. 2010; Yoo et al. 2010). Interesting is also the application of quail and chicken egg-shells as catalyst for transesterification (Cho, Seo 2010).

Lipases are also convenient catalysts for transesterification of plant oils and animal fat (Calabro et al. 2010; Raita et al. 2010). Lipase is able to catalyze oil transesterification with comparable efficiency like other methods but the esterification process is much slower compared with acid-catalysed one. Really, enzyme-catalysed esterification of free fatty acid is too slow to have a practical significance. Lipases are also destroyed with organic solvent (even methanol) at slightly elevated temperature. In present work we compare three methods of transesterification, acid-catalysed, base-catalysed, and enzyme-catalysed reaction.

MATERIAL AND METHODS

RPF was obtained from the Czech University of Life Sciences Prague. This fat was produced mainly from pork and cattle with only small amount of others. Separation of this fat includes expansive drying, separation by elevated temperature under pressure, and cooling. Acidity number of RPF is usually in the range of 20 to 40 mg KOH/g depending on the time of storage and the method of preparation. In our case acidity number was 23.63 mg KOH/g.

Fatty acids methyl esters (FAME) content in reaction mixture was measured by gas liquid chromatography (GLC) according to the method of Bannon et al. (1985) with the use of capillary gas chromatograph Hewlett-Packard, model 6890N (San Francisco, USA) with flame ionization detector and a polar capillary column. According to the content of fatty acids in RPF the theoretical yield of methyl esters was calculated to be 808 mg FAME from 1 g of RPF.

Acidity number was determined according to the ČSN 58 0130. Transesterification was accomplished in 500 ml round-bottom flasks at different temperatures and different amount of catalyst (sulfuric acid, sodium hydroxide) and different excess of methanol under reflux. The excess of methanol was from twice the amount of RPF to thirty times, reaction time was from 2 to 9 h, reaction temperature from 70 to 95°C, and load of sulphuric acid from 1 to 5%, of sodium hydroxide 1 to 12%.

Different procedure was used for enzyme-catalysed transesterification. Enzyme-catalysed transesterification was carried-out at optimum pH of the enzyme on the shaker (GFL 1083, GFL, Burgwedel, Germany). Temperature range for transesterification catalyzed with lipase (Novozym 435, Novozym, Bagsvaerd, Denmark) was lower than for acid or base-catalyzed reaction due to low stability of enzyme at higher temperature.

Lipase (acylglycerol acylhydrolase E.C. 3.1.1.3) used in this work were products of the Novozymes (Bagsvaerd, Denmark) produced of *Candida antarctica* recombinant in *Aspergillus niger* under commercial name Novozym 435 for immobilized enzyme. Novozym 435 is Lipozyme CALB L immobilized by sorption on cross-linked poly (methyl methacrylate) (Pierre et al. 2006).

Lipase activity was measured according to Humbert et al. (1997) by incubation of samples with 50-mM-*p*-nitrophenyl butyrate in acetonitrile as substrate at 37°C. After adding inhibiting mixture the absorbance at 420 nm was read. Values for absorbance were converted to μmol *p*-nitrophenol using a standard curve.

Optimization was carried-out in the order methanol excess, catalyst amount (see the difference in the case of enzyme), reaction temperature, reaction time. In each next step, the optimum conditions from previous step were used.

RESULTS AND DISCUSION

In the first step we optimized the excess of methanol. The starting conditions (other than methanol excess) were selected arbitrarily on the base of literature data. The course of all three reactions is different and, as a consequence, different are also the starting conditions. In the case of enzyme-catalysed reaction the conditions were selected considering the low stability

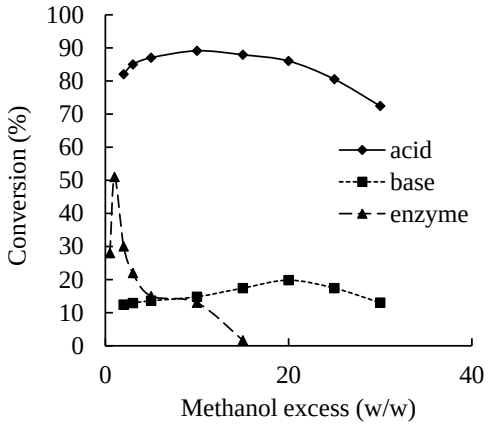

Fig. 1. Relation between methanol excess and conversion in rendering plant fat transesterification
Acid-catalysed: temperature 95°C, sulphuric acid concentration 1%, reaction time 7 h; base-catalysed: temperature 80°C, sodium hydroxide concentration 6%, reaction time 4 h; enzyme-catalysed: temperature 30°C, enzyme dose 600 units/g RPF, reaction time 5 h

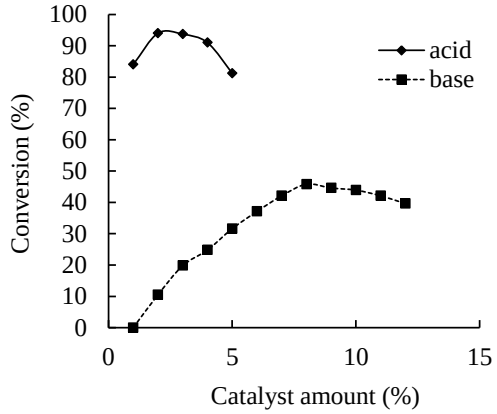

Fig. 2. Relation between catalyst (acid or base) doses and conversion in rendering plant fat transesterification
Acid-catalysed: temperature 95°C, methanol excess 10-fold, reaction time 7 h; base-catalysed: temperature 80°C, methanol excess 20-fold, reaction time 4 h

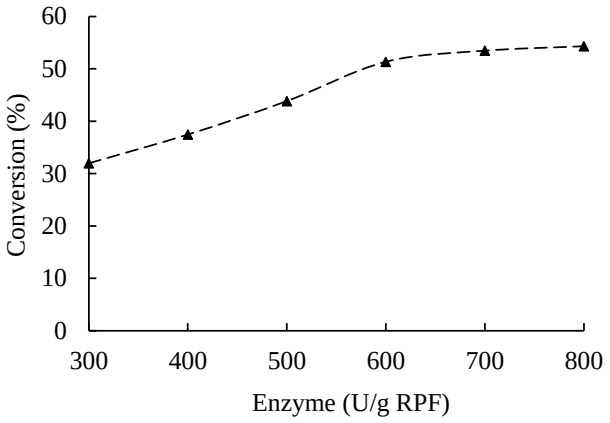

Fig. 3. Relation between enzyme dose and conversion in rendering plant fat transesterification
Enzyme-catalysed: temperature 30°C, methanol relation 1:1, reaction time 7 h

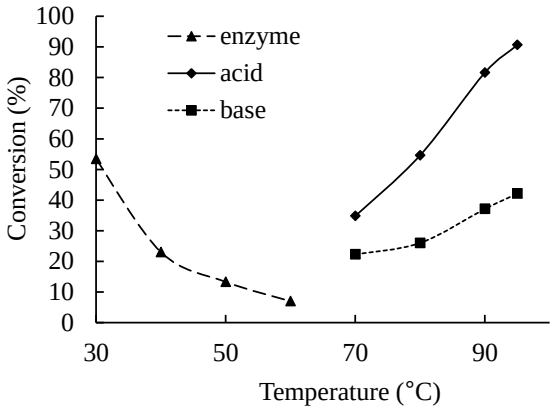

Fig. 4. Influence of reaction temperature in rendering plant fat transesterification
Acid-catalysed: methanol excess 10-fold, sulfuric acid concentration 2%, reaction time 7 h; base-catalysed: methanol excess 20-fold, sodium hydroxide concentration 8%, reaction time 4 h; enzyme-catalyzed: methanol:RPF ratio 1:1, enzyme dose 700 units/g RPF, reaction time 7 h

of enzyme in organic media, involving methanol. Reaction temperature was 30°C, reaction time 5 h and enzyme dose 1000 U/g RPF. Starting conditions for base-catalysed reaction were different, which is done by the fact, that in the first instance it is necessary to neutralize free fatty acids (the catalyst concentration was as high as 6%), temperature was raised to 80°C and reaction time was shortened to 4 hours. Acid-catalysed transesterification of RPF represents two reactions, the first is esterification of free fatty acids, and the second one is true transesterification. For this, the stronger conditions are needed; the temperature was raised to 95°C. In acid-catalyzed transesterification the acid is true catalyst and is not consumed during reaction. If such, the concentration of acid could be lower compared with the concentration of base in base-catalysed reaction. The concentration of sulphuric acid was lowered to 1%. The reaction time was for acid-catalysed reaction 7 hours. At these conditions the conversion of RPF to FAME was measured in the range of methanol excess 1 to 30-fold of the weight of RPF. 0.5 % was the minimum concentration methanol

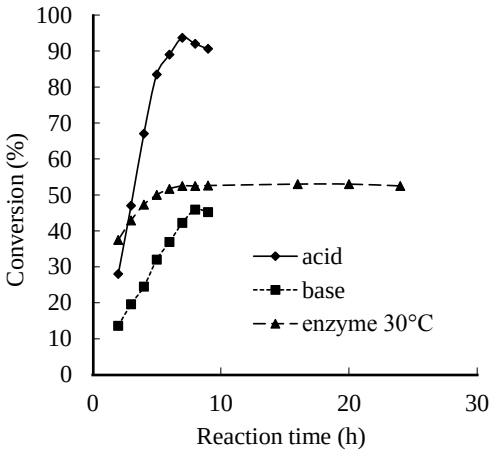

Fig. 5. Influence of reaction time in rendering plant fat transesterification

Acid-catalysed: methanol excess 10-fold, sulfuric acid concentration 2%, reaction temperature 95°C; acid-catalysed: methanol excess 20-fold, sodium hydroxide concentration 8%, reaction temperature 80°C; enzyme-catalysed: methanol:RPF ratio 1:1, enzyme 700 units/g RPF, reaction temperature 30°C

used for enzyme-catalysed reaction. The course of reaction is shown in Fig. 1.

The curves in Fig. 1 are very different. Acid-catalyzed reaction gives high conversion in all methanol concentration, while conversion in both base-catalyzed as well as enzyme-catalyzed reaction is low. Acid-catalysed reaction shows flat maximum approximately at 10-fold excess of methanol, base-catalysed reaction has flat maximum at 20-fold excess and enzyme-catalysed transesterification shows maximum at 1:1 relation of RPF:methanol. The last relation is done preferentially by the low enzyme stability in higher concentration of methanol. These concentrations of methanol were used for the next step.

One problem appeared in the study of catalyst concentration. Concentration of acid or base could be calculated as percentage, but concentration of enzyme is necessary to be calculated in enzyme units per substrate mass. The figure, as a consequence, must be different for acid- and base-catalysed reaction and enzyme-catalysed reaction. Due to the first step of optimization (methanol excess optimization) the conversion was raised. Acid-catalysed reaction gives conversion as high as 94% at acid concentration of 2%. Conversion of 46% was achieved in base-catalysed reaction at sodium hydroxide concentration 8%, and even enzyme-catalysed transesterification give higher result, 51% at enzyme dose 600 U/g RPF. The results are shown in Figs 2 and 3. The next step was the optimization of reaction temperature. Again, the remarkable differences must be adopted in acid-base catalysis and enzyme catalysis due to the enzyme stability. Optimized parameters from previous experiments were used. Fig. 4 shows the results. Conversion higher than 50% was achieved in enzyme transesterification, conversion more than 40% in the case of base-catalysed reaction and 92% in acid-catalysed reaction.

The last optimization step concerned the reaction time. The result shows in this case only the minimum reaction time needed for maximum conversion and, as a consequence, is not crucial. Nevertheless, some improvements were also attained, as shown in Fig. 5.

Rendering plant fat is one of the most promising sources of primary material for biodiesel production. This material fulfils two crucial requirements for base material for this process. RPF is available in large quantities in localized plants and, secondly, this material is neither use as food nor use the farmland dedicated for production of food. RPF can be transesterified using acid-catalysed, base-catalysed or enzyme-catalysed reaction, which has different requirements for the reaction conditions, but has closely similar application. The optimum conditions, as well as the conversion at these conditions are summarized in Table 1.

CONCLUSION

We compared three methods and we are able to draw some common conclusions. In the first instance, the acid-catalysed method is the most universal. As RPF suffers partial hydrolysis during extraction and stocking, the resulting fat contains free acid. Only at acid-catalysed reaction free fatty acids can be esterified. They are esterified neither during base-catalyzed reaction nor during enzyme-cata-

Table 1. Optimum conditions and conversion in rendering plant fat transesterification

Catalyst	Optimum conditions				Conversion (%)
	methanol excess (x-fold)	catalyst amount (%)	reaction temperature (°C)	reaction time (h)	
Acid	10	2	95	7	94
Base	20	8	80	7	53
Enzyme	1	800 U/g	30	8	54

lysed reaction. For this fact, the acid-catalysed reaction gives much higher conversion compared with both others. Very promising is the possibility of direct transesterification of slaughter-house waste fat without extraction (Prošková, Kučera 2010).

Biodiesel (fatty acid methyl esters) produced from slaughterhouses waste have some advantages (relatively cheap, does not occupy agricultural area) but also some disadvantages (bad cold flow properties and oxidation stability). FAME from these sources have worse cold flow properties compared with petrochemical diesel, so it cannot be used in many parts of world in winter months. Very often it also shows poor oxidative stability that causes the biodiesel molecules to react with oxygen and produce sediments. From this point of view the presence of synthetic antioxidants is necessary to improve oxidative stability. The best way to stabilize biodiesel is to blend it with petroleum diesel.

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