Lipase-catalyzed transesterification of rendering plant fat – Short communication

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Abstract

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Soluble lipase (Lipozyme CALB L) was immobilized by covalent bond to chitosan pellets prepared from *Aspergillus niger* mycelium. This immobilized enzyme was compared with commercial immobilized lipase of the same origin (Novozym 435). Novozym 435 is also lipase CALB L commercially immobilized by sorption on poly-(methyl acrylate). Novozym 435 shows much higher conversion of rendering plant fat in methanol under optimum conditions, having, at the same time, lower optimum temperature and lower stability at higher temperature. Lipozyme CALB L immobilized on chitosan leads to a low conversion, regardless its higher thermal stability. Novozym 435 gives conversion of about 50% of theoretical value, which is in good accordance with basically catalyzed transesterification of rendering plant fat described elsewhere. Lipozyme CALB L immobilized on chitosan gives conversion of about 10% of theoretical value only. The use of Novozym 435 in two-step system (enzyme-acid) seems to be more convenient compared with traditional two-step system (base-acid).

Keywords: biofuel; biodiesel; rendering plant fat; transesterification; lipase

Transesterification of vegetable oils can be used to produce fatty acid esters with properties similar to petroleum-based diesel fuel, and this process is increasingly researched as a means of producing an environmentally acceptable alternative fuel (biodiesel) from renewable resources (Mittelbach 1990; Basri et al. 1997; Selmi, Thomas 1998). Different vegetable oils are used for this purpose, including sunflower oil (Mittelbach 1990; Selmi, Thomas 1998), soybean oil (Myniyappa et al. 1996), or palm oil (Basri et al. 1997; Carter et al. 2007). Competition between food and biofuel (Stein 2007) leads to the search for fat sources which are not used as food, such as restaurant waste lipids (Canakci 2007), rice bran oil (Zullaikah et al. 2005) or damaged coffee beans (Oliveira et al. 2008). One of the potential and not fully utilized sources of fat for conversion to biodiesel is also rendering plant fat (Prošková et al. 2009a, b).

Acid-catalyzed or base-catalyzed transesterification are used most frequently (Prošková et al. 2009a, b), but enzymatic transesterification could bring some advantages, substantially the lower energy consumption. In the present work we have studied the transesterification of rendering plant fat (RPF) using two types of immobilized lipase.

MATERIAL AND METHODS

RPF was obtained by means of the Czech University of Life Sciences Prague, Prague, the Czech

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Republic. This fat was produced mainly from pork and cattle fat with the presence of small amount of some others. The separation of this fat includes expansive drying, separation by elevated temperature under pressure, and cooling. Fatty acid composition was published in detail elsewhere (Prošková et al. 2009a).

Enzyme-catalyzed transesterification was carried out at optimum pH of the enzyme on the shaker.

Lipases (acylglycerol acylhydrolase E.C. 3.1.1.3) used in this work were products of Novozyms Company produced by *Candida antarctica* recombinant in *Aspergillus niger* under commercial name Lipozyme CALB L for soluble and Novozym 435 for immobilized enzyme. Novozym 435 is Lipozyme CALB L immobilized by sorption on cross-linked poly-(methyl methacrylate) (Pierre et al. 2006). Soluble lipase Lipozyme CALB L was immobilized on chitosan pellets principally according to the method described by Kayastha and Srivastava (2001). Chitosan pellets were prepared by the method described earlier (Kučera 2004).

Lipase activity was measured according to Humbert et al. (1997).

Protein concentration was measured by the method of Lowry in the modification of Hartree (1972).

Determination of methylesters was carried-out by gas liquid chromatography (GLC) according to the method of Bannon (1985) with the use of capillary gas chromatograph Hewlett-Packard model 6890N.

Theoretical yield of transesterification was calculated on the basis of fatty acid composition of rendering plant fat. The determined theoretical yield was 808.3 mg of methylesters from 1 g of RPF.

Methanol, sulfuric acid and all solvents were obtained from Lach-Ner, Ltd., the Czech Republic.

All standards were obtained from the SIGMA Fine Chemicals, the Czech Republic.

RESULTS AND DISCUSSION

Enzyme-catalyzed reaction was carried-out in round-bottom flask (volume 100 ml) in water bath on shaker. Selected amount of enzyme was added to 1 g of fat in appropriate volume of methanol under selected temperature and shaken for selected time. The reaction was finished by cooling in ice bath. Immobilized enzyme was then separated by filtration.

As methanol causes inactivation of enzyme, we started with the study of the effect of methanol:RPF on the reaction course. Both immobilized enzymes were compared. The commercially available lipase immobilized on partially hydrophobic support (Novozym 435) and enzyme immobilized in our laboratory by covalent binding of commercial lipase, Lipozyme CALB L, on chitosan prepared from pellets of *Aspergillus niger* as described elsewhere (Kučera 2004) (Lipozyme CALB L-CVB chitosan). Starting activity of both immobilized enzymes was almost the same (1,042 U/g for Novozym 435 and 1,020 U/g for Lipozyme CALB L-CVB chitosan). In the transesterification reaction these two enzymes behave differently as evident from the Fig. 1. The reaction in this case was carried out at 30°C for 7 h with the enzyme activity 600 U/g of RPF.

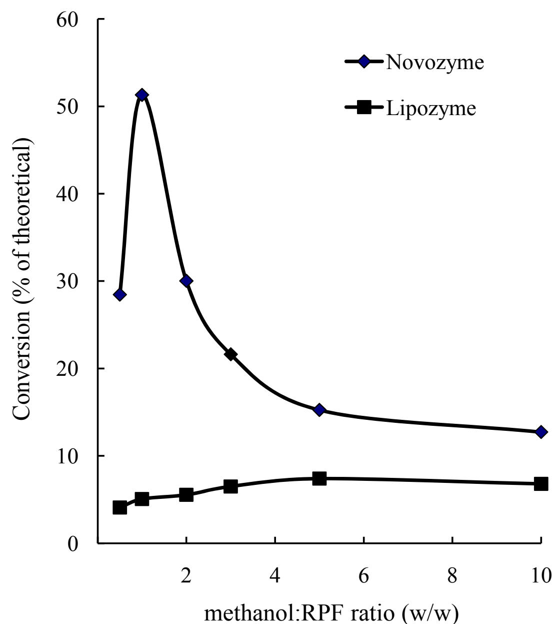

Fig. 1. Influence of methanol:RPF ratio on the RPF transesterification (Conditions: enzyme activity – 600 U/g RPF, temperature 30°C, reaction time – 7 h)

Fig. 1 shows lipase immobilized on hydrophobic material (Novozym 435) to give higher conversion in these conditions compared with that immobilized on hydrophilic one (Lipozyme CALB L bound to chitosan). Higher concentration of methanol leads to a strong decrease of conversion with Novozym 435, while Lipozyme-immobilized on hydrophilic material is relatively stable at higher concentration of methanol. Nevertheless, the conversion is more convenient in the case of Novozym 435 enzyme.

We have, therefore, studied the effect of different doses of enzyme (in relation with RPF amount) on the conversion. Fig. 2 shows this effect for both

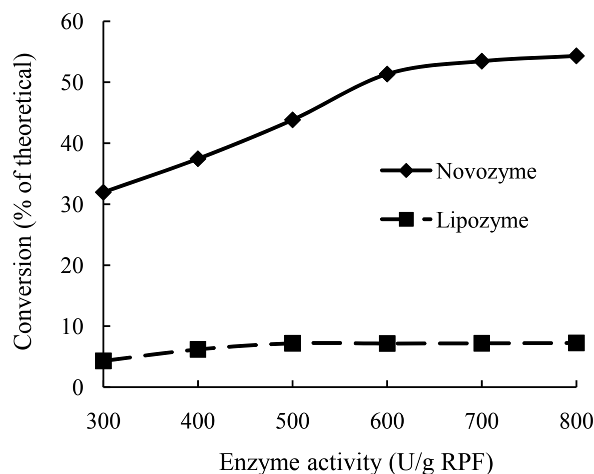

Fig. 2. Effect of enzyme doses on transesterification of RPF (Conditions:temperature 30°C, reaction time – 7 h, methanol:RPF ratio – 1:1)

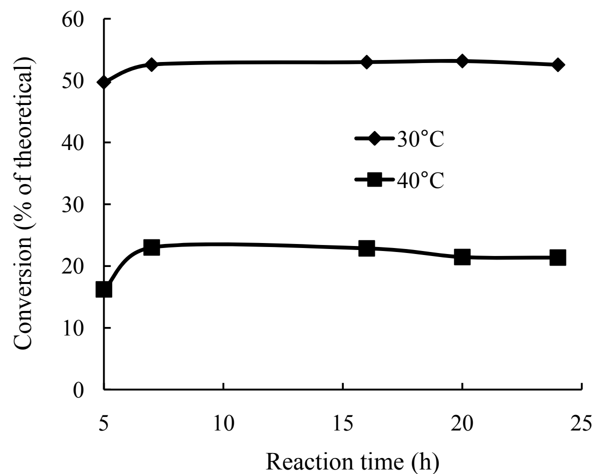

Fig. 4. Effect of prolonged time on transesterification of RPF catalyzed with Novozym 435 (Conditions:enzyme activity 700 U/g RPF, methanol:RPF ratio – 1:1)

enzymes. The results in Fig. 2 indicate slightly better results, but still the conversion is low compared with the data published for transesterification of plant oil. This experiment also shows much lower conversion in the case of the reaction catalyzed with lipase covalently bound to chitosan. The conversion achieved with Novozym 435 is comparable with the data published for the basically catalyzed transesterification of RPF (Prošková et al. 2009b). Similarly as in the basically catalyzed transesterification we suppose that glycerides are transesterified but free fatty acid is not.

In the next experiments we studied the influence of reaction temperature on the conversion for both immobilized enzymes at optimum doses of enzyme (700 U/g RPF) and optimum relation of methanol: RPF. The result is summarized in Fig. 3. As is demonstrated by this figure, in no case the lipase on hydrophilic support does give the conversion higher than 10% while the enzyme bound to hydrophobic support gives the result as high as 55%. The hydrophilic enzyme on the other hand has higher temperature optimum (40°C) compared with hydrophobic one (30°C) which is undoubtedly done by the more convenient microenvironment in hydrophilic support.

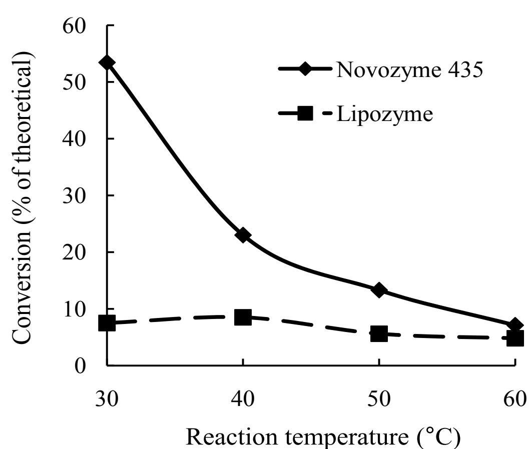

Fig. 3. Effect of temperature in transesterification of RPF (Conditions: methanol:RPF ratio – 1:1, enzyme 700 U/g RPF, reaction time – 7 h)

The lipase immobilized with hydrophilic support gives lower conversion in all experiments compared with hydrophobic one. These results are done by the microenvironment of the enzyme in hydrophobic support, which is more convenient for reaction of hydrophobic substrate. This effect is also proved by higher stability of lipase immobilized with hydrophilic support, which is more convenient for enzyme stability.

Reaction time was established arbitrarily. In the last experiment we proved the effect of reaction time on transesterification of RPF. As Lipozyme bound to chitosan in newer experiments gives acceptable results, we tested the time dependence for Novozym 435 only. The results are shown in Fig. 4. Fig. 4 shows 7 h to be enough for the reaction to be finished.

Comparing the results obtained in this work with the transesterification of RPF catalyzed with acid (Prošková et al. 2009a) or base (Prošková et al. 2009b), one can find the reaction with the lipase immobilized with hydrophobic support (Novozym 435) closely similar to basically catalyzed transesterification of this fat. Lipase immobilized

with hydrophilic support (chitosan) gives only poor results. As enzyme-catalyzed reaction is energetically less demanding, compared with basically catalyzed reaction, the two-step transesterification enzyme-acid is to be preferred to base-acid one.

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