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PROPERTIES OF IMMOBILIZED AND FREE LIPASE FROM *RHIZOPUS COHNII*

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Key words: lipase, *Rhizopus cohnii*, immobilization, chitosan polyphosphate, catalytic properties, products of hydrolysis

Rhizopus cohnii lipase was immobilized by adsorption on porous chitosan polyphosphate beads. The obtained preparation, active against fatty acids esters of p-nitrophenol and against olive oil, was characterised and compared with the free enzyme. Both lipase forms displayed the highest activity at pH 8–9. The optimum activity temperature of free and immobilized lipase was 37°C and 30°C, respectively. Immobilization did not significantly change the thermal stability of lipase in comparison with the free enzyme. Both forms of the enzyme were stable at 30°C, they lost half of their activity within 45–50 min at 50°C, and at 60°C they were fully inactivated within 10 min. Affinity to p-nitrophenylpalmitate (pNPP) for both the free and immobilized lipase was identical and amounted K_m=0.2 mmol/L. With the use of HPLC, enzymatic hydrolysis of olive oil by the free and immobilized lipase was compared with the chemical hydrolysis of this substrate. It was found that both forms of the enzyme were active only against emulsified substrate, and the chromatograms of products of reactions catalysed by free and immobilized lipase showed no qualitative differences. Among the products of olive oil degradation by both forms of the enzyme, fractions identified as palmitic and linoleic acids were detected, whereas oleic acid, normally generated in the largest amount after chemical hydrolysis, was not detected.

INTRODUCTION

Enzyme immobilization on insoluble carriers is a widely applied technique. The main purpose of this procedure is to ensure the repeated use of enzymes in the processes of stationary or continuous catalysis, to identify the change of properties in comparison with free enzymes, and to lower the costs of their use. When free enzymes are used, the phenomenon of the so-called "mass transfer limitation" can occur in an organic medium. This problem can be eliminated by applying immobilized enzymes [Persson *et al.*, 2000]. Enzymes can be immobilized on solid carriers by using a variety of methods. A widely applied technique is their adsorption [Akova & Ustun, 2000; Gill *et al.*, 1999; Indrati *et al.*, 1999; Kermasha & Bisakowski, 1998; Kumar--Khare & Nakajima, 2000; Mateo *et al.*, 2000; Persson *et al.*, 2000]. One of many various carriers used for enzyme immobilization by adsorption is chitosan [Pereira *et al.*, 2001; Trzcińska, 1998] and chitosan derivative called chitoxan [Magnin *et al.*, 2001]. Both of these carriers have a number of advantages, *e.g.* they are devoid of toxic properties and chemically neutral, the enzyme penetrates easily into their porous structure and remains strongly bound with the carrier, and furthermore they are readily available, easy to prepare for immobilization, and cheap.

Following immobilization, the enzyme often changes its sensitivity to the temperature and pH value of the medium in comparison with the respective free enzyme. Moreover, thermal stability of the enzyme, the degree of enzyme reactivity with the substrate, and enzyme stability during storage may all vary. Immobilization often changes the Michaelis constant as a result of changes in affinity: enzyme-substrate [Arica *et al.*, 2001; Paiva *et al.*, 2000; Pencreac'h *et al.*, 1997, 1999; Pereira *et al.*, 2001; Soares *et al.*, 1999; Triantafyllou *et al.*, 1997].

The objective of this study was to immobilize *Rhizopus cohnii* lipase on a solid carrier – chitosan polyphosphate, to examine the catalytic properties of the preparation obtained, and to compare its properties with the properties of the free enzyme.

MATERIALS AND METHODS

Preparation of the carrier. Chitosan polyphosphate beads with a diameter of 0.4 to 0.5 cm were used as the carrier for lipase immobilization. The beads were obtained by dropping an intensely aerated solution of 1% chitosan in 1% acetic acid into a mixture of equal quantities of ethanol and 1% NaOH. The beads thus formed, after being rinsed with distilled water, were suspended for 24 h in 0.2 mol/L polyphosphate acid with 0.2 mol/L sodium polyphosphate, and then rinsed with distilled water [Sieliwanowicz *et al.*, 1995].

Lipase immobilization. Concentrated and partly purified by ultrafiltration post-culture liquid was used as the sorption solution [Trzcińska *et al.*, 2000]. The carrier was added into the solution in a proportion of 1:20 (V/V) and

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gently mixed at 20°C. The total time of contact of the carrier with the sorption solution was 12 h. Mixing was interrupted after 2 h, and then after subsequent 4 h of sorption the carrier was separated from the sorption solution and rinsed with 0.05 mol/L phosphate buffer with pH 8.0. Then it was once again combined with the sorption liquid and mixing was continued. After sorption had ended, the carrier with immobilized enzyme was rinsed with 0.05 mol/L phosphate buffer with pH 8.0, until activity against p-nitrophenylpalmitate (pNPP) was no longer detected in the rinsing fluid.

Characteristics of the immobilized lipase preparation. The properties of the obtained preparation, namely the kinetics of the catalysed reactions, stability, and the effect of pH and temperature on enzymatic activity, were studied with the use of hydrolysis of pNPP as the substrate. Standard tests were performed in the 0.05 mol/L phosphate buffer medium, with pH 8.0, at 30°C. In the studies of pH effect on the activity of the preparations, 0.05 mol/L phosphate buffers (with pH ranging from 6.5 to 8.0) and 0.05 mol/L Tris-HCl buffers (with pH ranging from 8.0 to 9.5) at 20°C were used. In spite of the lowered reaction temperature, at pH higher than 9.5 decomposition of pNPP was observed. In order to prevent decomposition of esters of various fatty acids with p-nitrophenol (pNP), the comparison of kinetics of these esters was performed at 25°C.

Lipase activity determination. Lipase activity against esters of fatty acids with p-nitrophenol was determined by a spectrophotometric analysis of p-nitrophenol (pNP) released during a 15-min reaction at λ=400 nm. The concentrations of pNPP, p-nitrophenyllaurate (pNPL), p-nitrophenylbutyrate (pNPB), and p-nitrophenylacetate (pNPA) were 0.4 mmol/L, 0.75 mmol/L, 4.0 mmol/L, and 5.0 mmol/L, respectively [Trzcińska *et al.,* 2000]. The substrates were prepared immediately before use by mixing the ester dissolved in isopropanol with 0.05 mol/L phosphate buffer with pH 8.0, so that the final concentration of isopropanol was 5%. When lipase activity was determined with the use of pNPP, pNPL and pNPB as substrates, 0.1% sodium deoxycholate and 0.05% arabic gum were added to the buffer as emulsifiers. A unit of activity (UpNP) was expressed as the quantity of enzyme preparation that released 1 mmol of p--nitrophenol per 1 min in the reaction conditions. Standard assays were performed at 30°C, using pNPP as the substrate.

Lipase activity against olive oil was measured by titrating, with 0.05 mol/L NaOH, fatty acids released by the preparations tested during 1 h of incubation at 37°C in the reaction mixture, which contained 2.5 mL of Mac Ilvaine's buffer with pH 7 and 2.5 mL of emulsion prepared from olive oil and 2% polyvinyl alcohol mixed in the proportion of 2:3 (V/V). Enzymatic reaction was stopped by adding 15 mL of ethanol. A unit of activity (U) was expressed as the quantity of enzyme preparation releasing 1 μmol of fatty acids per 1 min in the reaction conditions.

Alkaline hydrolysis of olive oil. 20 mL of 0.3 mol/L NaOH solution in 90% methanol was added to 100 mg of olive oil (Sigma). The mixture was maintained at boiling point in a reflux condenser for 90 min. After cooling down, 40 mL of water were added to the hydrolysate and three extractions were performed with 10-mL portions of n-hexane. Organic phases were discarded and 6 mol/L HCl was added to the water phase so as to adjust the pH of the mixture to the value between 3 and 4. Free fatty acids were also extracted with four 10-mL portions of n-hexane. The combined extracts were rinsed 3 to 4 times with water, and 5 g of anhydrous sodium sulphate were added to the organic phase, with the liquid then being left for 3 h, agitated from time to time. The extract was filtrated through filter paper, and the filter was rinsed with n-hexane. The solvent was removed by distillation, and free fatty acids were weighed and analysed with HPLC.

Enzymatic hydrolysis of olive oil. The emulsion consisting of olive oil (20%) in 0.05 M/L phosphate buffer with pH 8, containing 0.1% sodium deoxycholate (DCA-Na) as emulsifier, was gently mixed with the preparation of free or immobilized lipase for 20 h at 20°C. Then chitosan was separated, and the samples were centrifuged and fatty acids were assayed in the collected aqueous phase with the use of HPLC.

Segregation of free fatty acids. Before chromatographic testing, samples of reaction mixtures were purified on 0.5 mL Waters SepPak Silica 0.45 μm syringe columns, while free fatty acids were eluted with a mixture of acetonitrile/tetrahydrofuran at a ratio of 1/1. To segregate free fatty acids, the HPLC Waters 510 chromatograph with a Waters 410 refractometric detector, controlled by the Millennium^{32} chromatographic software, equipped with a thermostat for columns and MK1 injection port with 10-μL loop was used. The column: Waters Free Fatty Acids 3.9 × 150 mm with pre-column of guard type was employed. Elution was performed with acetonitrile/tetrahydrofuran/water 45/20/35 system at 35°C, with rate of 1.5 mL/min.

RESULTS AND DISCUSSION

Obtaining the immobilized *R. cohnii* lipase preparation

R. cohnii lipase was immobilized by adsorption on porous beads of chitosan polyphosphate. For immobilization, concentrated post-culture liquid from 5-day culture (Trzcińska *et al.* 2000), partly purified by ultrafiltration, was used. Initial purification of the preparation was necessary in order to avoid damage of the carrier following more than ten-hour contact with the concentrated post-culture liquid. Sorption conditions were determined experimentally. It was found that the time of sorption should be 10–12 h. It was also shown that the activity of the obtained preparations was *ca.* 30% higher if, instead of the enzyme being in permanent contact with the carrier, the process of sorption was interrupted by washing of the carrier with the buffer solution.

The preparation obtained with the use of the methodology developed showed satisfactory activity against pNPP. It also hydrolysed olive oil, although in the case of this substrate, immobilization limited the reaction rate to a greater extent than in the case of pNPP hydrolysis. One mL of immobilized lipase preparation showed activity against pNPP almost twice as low as the free enzyme, and olive oil was hydrolysed almost 45 times slower than by free lipase (Table 1).

TABLE 1. Activity of free and immobilized lipase preparations.

Preparation	Activity	
	against pNPP	against olive oil
Free enzyme	23.85 UpNP/mL	167 U/mL
Enzyme immobilized on chitosan polyphosphate	13.21 UpNP/mL	3.7 U/mL

Characteristics of immobilized lipase preparation

The preparation of lipase immobilized on chitosan polyphosphate stored in 0.05 mol/L polyphosphate buffer with pH 8.0 at 4°C demonstrated slightly higher stability than free lipase. The half-periods of losses of activity for both forms of the enzyme were 21 and 13 days, respectively (Figure 1).

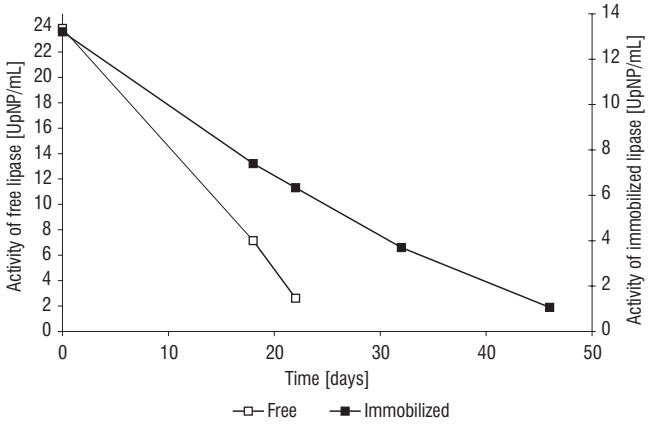

FIGURE 1. Stability of free and immobilized lipase preparations at 4°C.

The tests showed that lipase immobilization on chitosan polyphosphate did not significantly change the sensitivity of the enzyme tested to the pH of the medium. Free and immobilized preparations hydrolysed pNPP at pH higher than 6.5. Both preparations showed the maximum activity within the pH range of 8 to 9 (Figure 2).

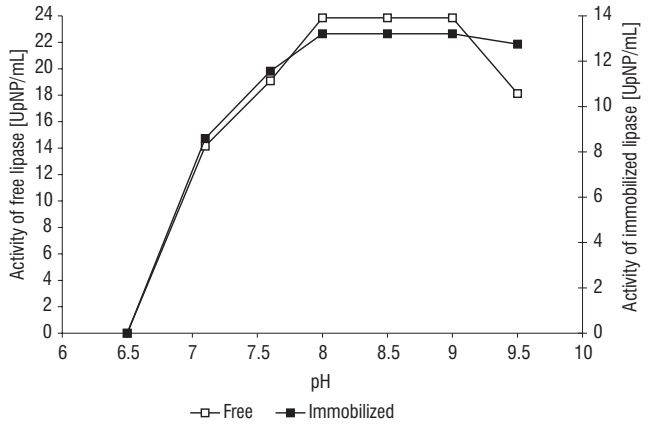

FIGURE 2. Effect of pH on the activity of free and immobilized lipase preparations.

After immobilization, a slight lowering of the temperature optimum of the lipase preparation was observed. While the free enzyme showed maximum activity at 37°C, the tested preparation of lipase hydrolysed pNPP most rapidly at 30°C (Figure 3). Immobilization did not significantly change the thermal stability of the lipase preparation. At 60°C both forms of the enzyme tested were unstable, and after

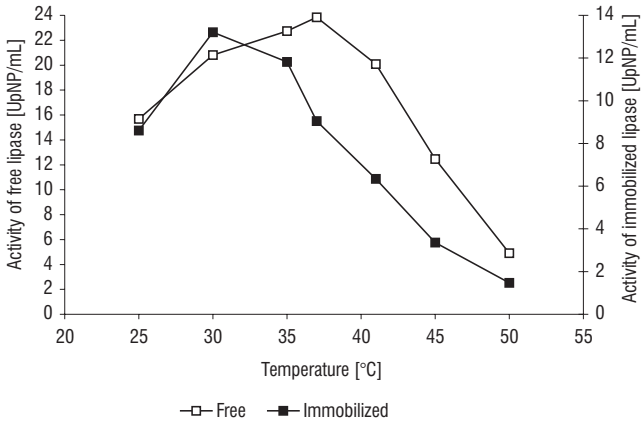

FIGURE 3. Effect of temperature on the activity of free and immobilized lipase preparations.

10-min incubation, no lipase activity in the preparation was detected. At 50°C, loss of half lipase activity was observed for the free and immobilized lipase after 50 min and 45 min, respectively. At the temperature optimum for the immobilized lipase activity (30°C), the tested enzyme preparation was stable, and the lowering of activity after 43-h incubation at this temperature was *ca*. 10%.

R. cohnii lipase immobilized on chitosan polyphosphate, similar to the free enzyme, hydrolysed fatty acid esters of p-nitrophenol. The affinity of both forms of the enzyme to the substrates concerned depended on the carbon chain length of the acid contained in the ester hydrolysed. The tested enzyme forms showed markedly higher affinity to substrates containing a fatty acid with a longer carbon chain. Michaelis constants for the reaction of palmitic acid (C_{16}) ester hydrolysis by both preparations were 0.2 mmol/L, however, for butyric acid (C_4) ester hydrolysis these constants were considerably lower and equalled 2.5 mmol/L and 1.4 mmol/L for free and immobilized lipase, respectively. The affinity of both enzymatic preparations to p-nitrophenol acetate, an ester containing a fatty acid with the shortest carbon chain, was even lower. Michaelis constants were 3 mmol/L and 4 mmol/L, respectively (Table 2). Lipase immobilization on chitosan polyphosphate considerably changed the correlation between the maximum rate of hydrolysis of various pNP esters (Figure 4). The reactions catalysed by free enzyme were most rapid when palmitic (C_{16}) or lauric (C_{14}) acid ester was hydrolysed. If the substrate was butyric acid (C_4) or acetic acid (C_2) esters, the reaction was 2.5 to 3.3 times slower. Furthermore, after immobilization on chitosan polyphosphate, the enzyme tested hydrolysed more rapidly esters containing an acid with a shorter carbon chain, in spite of the fact that, as mentioned above, enzyme affinity to those substrates was markedly lower. A similar but even more marked pattern of changes was observed by other authors when bacterial

TABLE 2. Kinetics of hydrolysis of p-nitrophenol esters of selected fatty acids by free and immobilized lipase preparations.

Preparation	Michaelis constant – K_m [mmol/L]		
	pNPP	pNPB	pNPA
Free enzyme	0.2	2.5	3.0
Enzyme immobilized on chitosan polyphosphate	0.2	1.4	4.0

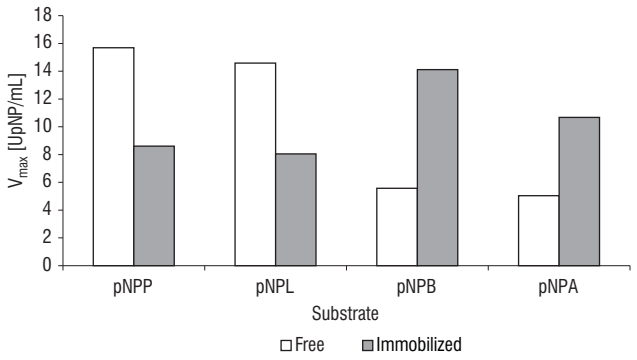

FIGURE 4. Maximum rate of hydrolysis of esters of fatty acids with carbon chain of various length by free and immobilized lipase preparations at 25°C.

lipase was immobilized on a polyethylene carrier [Pancreac'h *et al.,* 1997]. In these tests, it was found that the preparation of immobilized enzyme lost over 99% of activity against pNPP in comparison with the free enzyme, however, its activity against pNPA did not change. In both cases, such direction of changes in activity may be explained by the change of diffusion conditions following immobilization, and consequently, the change in conditions of contact between the enzyme and the substrate. Access to an enzyme immobilised on a carrier is particularly difficult for large molecules of non-dissolved substrates present in the reaction mixture in the form of emulsion (*e.g.* palmitate). This is why immobilization causes such a substantial lowering of activity against pNPP.

With the use of HPLC, products of the olive oil hydrolysis by free and immobilized *R. cohnii* lipase were determined. The results were compared with the products of alkaline hydrolysis of the same substrate. The tests performed initially showed that *R. cohnii* lipase, similar to other lipases described in existing literature, acts exclusively on substrates in the form of emulsion. In an intensively mixed biphasic system in which in aqueous phase enzymatic preparation of *R. cohnii* lipase was present, after lipase extraction with ether no traces of hydrolysis of olive oil glycerides were detected. It was shown that among the products of the hydrolysis of olive oil (prepared in the form of emulsion), obtained with using of two forms of lipase, the fractions identified as palmitic and linoleic acids were present at concentrations of 54% and 38%, respectively, and oleic acid was not found. The absence of this major acid of olive oil (*ca*. 83%) in hydrolysates can be explained in two ways: by the specificity of the action of *R. cohnii* lipase in the place of glycerol esterification, or, equally common among lipases phenomenon of trans-esterification, a reaction reverse to the hydrolysis process. In the latter case, oleic acid most frequently released from olive oil glycerides would usually be directed to glyceride re-synthesis reaction than to the acids more rarely found in olive oil. In the authors' opinion, the weak point of this second explanation is the observed complete absence of oleic acid in hydrolysis products. The trans-esterification, as other enzymatic reactions, should continue only to a certain equilibrium state, and the remains of oleic acid should be found in the reaction medium. The hypothesis of the specificity of the action of *R. cohnii* lipase seems more plausible, all the more so that it is confirmed by existing literature [Adamczak *et al.,* 2000].

Interesting data concerning the affinity of both forms of lipase (free and immobilized) to the natural substrate – olive oil, were provided by quantitative interpretations of the performed chromatographic separations. These results are summarised in Table 3. The preparation of free lipase, used in the experiment, displayed *ca*. 3.5 times less activity against pNPP in comparison with the preparation of the immobilized enzyme. In spite of this difference of activity against pNPP, the quantities of free fatty acids released by the preparations tested from 1 mL of olive oil emulsion were similar and equalled 1.6 mg and 1.4 mg, respectively.

TABLE 3. Comparison of the activity against pNPP of free and immobilized lipase preparations and their effect on the degree of olive oil hydrolysis.

Preparation	Lipase activity UpNP	Released free fatty acids [mg/mL of emulsion]
Free lipase	0.069	1.6
Immobilized lipase on chitosan polyphosphate	0.237	1.4

In the authors' opinion, this difference resulted from more difficult access of the substrate to the micropores of chitosan polyphosphate with the adsorbed enzyme. Consequently, enzymatic catalysis took place most probably only on the surface of the beads. This is a frequently occurring phenomenon during catalysis with the use of immobilised enzymes, which is also often incorrectly considered as the loss of enzymatic activity during the immobilisation process. Difficult access to the enzyme bound with the carrier is most probably also the explanation for a more marked limitation of the rate of hydrolysis of olive oil than that of pNPP observed after immobilization (Table 1). The differences in activity of free and immobilized lipase against various pNP esters were explained analogically (Figure 4).

CONCLUSIONS

1. Chitosan polyphosphate is a suitable carrier for the immobilization of *R. cohnii* lipase. The enzyme immobilized in this carrier showed a high level of activity and had properties close to the properties of the free enzyme.

2. Immobilization on chitosan polyphosphate did not change the pH optimum for activity and thermal stability of *R. cohnii* lipase. On the other hand, after immobilization, the lipase tested had a slightly lower optimum activity temperature in comparison with the free form.

3. Immobilization of *R. cohnii* lipase on chitosan polyphosphate did not affect the kinetics of pNPP hydrolysis. Michaelis constants for reactions catalysed by free enzyme or enzyme immobilized in the carrier discussed were 0.2 mmol/L.

4. No differences in the specificity of the action of free and immobilized lipase were found; in both cases a prior emulsification of olive oil was required for the hydrolysis, and as a result of the reaction, identical natural products were obtained: mainly palmitic and linoleic acids.

5. Among the products of olive oil hydrolysis by *R. cohnii* lipase, oleic acid (contained in olive oil of over 80%) was not detected. The most probable explanation of this fact was considered to be the specificity of the action of the tested lipase with respect to the type of acid in glyceride.

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REFERENCES

1. Adamczak M., Bednarski W., Sawicka-Żukowska R., Krakowiak A., Jędrychowska B., The application of lipase preparation from *Rhizopus cohnii* to animal waste fat biodegradation. Med. Fac. Landbouww. Univ. Gent., 2000, 65/3b, 623–626.
2. Akova A., Usun G., Activity and adsorption of lipase from *Nigella sativa* seeds on celite at different pH values. Biotechnol. Lett., 2000, 22 (5), 355–359.
3. Arica M.Y., Kacar Y., Ergene A., Denizli A., Reversible immobilization of lipase on containing hydrogel membranes. Process Biochem., 2001, 36 (8/9), 846–854.
4. Gill I., Pastor E., Ballesteros A., Lipase-silicone biocomposites: efficient and versatile immobilized biocatalysts. J. Amer. Chem. Soc., 1999, 121 (41), 9487–9496.
5. Indrati R., Marseno D.W., Ohta Y., Immobilization of lipase from *Rhizopus delemar* on polyethylene membrane. Indonesian Food Nutr. Progress, 1999, 6 (11), 19–245.
6. Kermasha S., Bisakowski B., The effect of immobilization on the hydrolytic/interestrification activity of lipase from *Rhizopus niveus*. J. Amer. Oil Chem. Soc., 1998, 75 (12), 1791–1799.
7. Kumar-Khare S., Nakajima M., Immobilization of *Rhizopus japonicus* lipase on celite and its application for enrichment of docosahexaenoic acid in soybean oil. Food Chem., 2000, 68 (2), 153-157.
8. Magnin D., Dumitrin S., Magny P., Chornet E., Lipase immobilization into porous chitoxan beads: activities in aqueous and organic media and lipase localization. Biotechnol. Progr., 2001, 17 (4), 734–737.
9. Mateo C., Abian O., Fernandez-Lafuente R, Guisan J.M., Reversible enzyme immobilization *via* a very strong and nondistorting ionic adsorption on support-polyethylenimine composites. Biotechnol. Bioeng., 2000, 68 (1), 98–105.
10. Paiva A.L., Balcao V.M., Malcata F.X., Kinetics and mechanisms of reactions catalyzed by immobilized lipases. Enzyme. Microb. Technol., 2000, 27 (3–5), 187–204.
11. Pancreac`h G., Leullier M., Baratti J.C., Properties of free and immobilized lipase from *Pseudomonas cepacia*. Biotechnol. Bioeneg., 1997, 56, (2), 181–189.
12. Pancreac`h G., Baratti J.C., Properties of free and immobilized lipase from *Burkholderia cepacia* in organic media. Appl. Microbiol. Biotechnol., 1999, 52 (2), 276–280.
13. Pereira E.B., Castro H.F., Moraes F.F., Zanin G.M., Kinetic studies of lipase from *Candida rugosa*. A comperative study between free and immobilized enzyme onto porous chitosan beads. Appl. Biochem. Biotechnol., 2001, 91/93, 739–752.
14. Persson M., Wehtje E., Adlercreutz P., Immobilization of lipases by adsorption and deposition: high protein loading gives lower water activity optimum. Biotechnol. Lett., 2000, 22 (19), 1571–1575.
15. Sieliwanowicz B., Komorowska A., Jędrzejczak R., Adsorption of iron by polyphosphate derivatives of chitin and chitosan. Polish J. Food Nutr. Sci., 1995, 4 (45), 1, 53–59.
16. Soares C.M.F., Castro H.F., Moraes F.F., Zanin G.M., Characterization and utilization of *Candida rugosa* lipase immobilized on controlled pore silica. Appl. Biochem. Biotechnol., 1999, 77 (79), 745–757.
17. Trzcińska M, Sieliwanowicz B., Sawicka-Żukowska R., Krakowiak A., Ajzenberg V., Some properties of lipase preparation from *Rhizopus cohnii*. Pol. J. Food Nutr. Sci., 2000, 9/50, (3), 15–20.
18. Trzcińska M., β-Glucosidase from *Aspergillus niger* 33/20/K. Isolation, properties, potential application. Acta Acad. Agricult. Tech. Olst., Technologia Alimentorum 29, Supplementum B, 1998.
19. Triantafyllou A.D., Wehtje E., Adlercreutz P., Mattiasson B., How do additives affect enzyme activity and stability in nonaqueous media? Biotechnol. Bioeng., 1997, 54 (1), 67–76.

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WŁAŚCIWOŚCI UNIERUCHOMIONEJ I WOLNEJ LIPAZY Z *RHIZOPUS COHNII*

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Lipazę otrzymaną z grzyba *Rhizopus cohnii* unieruchamiano metodą adsorpcji na porowatych perełkach polifosforanu chitozanu. Otrzymany kompleks, aktywny wobec estrów p-nitrofenolu i oliwy z oliwek, porównywano z analogicznym enzymem wolnym. Unieruchomiona i wolna lipaza wykazywała najwyższą aktywność w pH 8–9 (rys. 2). Optymalna temperatura działania lipazy unieruchomionej wynosiła 30°C zaś lipazy wolnej 37°C. Unieruchomienie nie zmieniało istotnie stabilności termicznej lipazy. Obie formy enzymu wykazywały stabilność w temperaturze 30°C, w temperaturze 50°C połowę aktywności traciły po 45–50 minutach, a w 60°C po 10 minutach następowała całkowita jej inaktywacja. Powinowactwo do p-nitrofenylopalmitynianu lipazy unieruchomionej i wolnej było identyczne i osiągało wartość K_m=0,2 mmol/L (tab. 3). Wykorzystując technikę HPLC porównano enzymatyczną hydrolizę oliwy z oliwek stosując unieruchomioną i wolną lipazę. Stwierdzono, że obie formy enzymu działały wyłącznie na zemulgowany substrat, a chromatogramy obrazujące produkty reakcji katalizowane przez unieruchomioną i wolną lipazę nie wykazywały różnic jakościowych. Wśród produktów degradacji oliwy z oliwek dokonanej przez obie formy enzymu wykryto frakcje identyfikowane jako kwas palmitynowy i linolenowy, nie wykryto natomiast kwasu oleinowego zwykle pojawiającego się w znacznych ilościach po hydrolizie chemicznej.