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Lipase-Catalyzed Synthesis of a Sugar Alcohol-Based Nonionic Surfactant

ATENA ADNANI[1], MAHIRAN BASRI[1,*], NAZ CHAIBAKHSH[1], ABU BAKAR SALLEH[2] and MOHD BASYARUDDIN ABDUL RAHMAN[1]

[1]Faculty of Science, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
[2]Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

*Corresponding author: Fax: +60 3 89432508; Tel: +60 3 89486106; E-mail: mahiran@science.upm.edu.my

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Synthesis of three xylitol fatty acid esters, namely xylitol stearate, palmitate and caprate, was performed in hexane using lipase as the biocatalyst. Enzyme screening results indicated that novozym 435 had the highest catalytic activity in the synthesis of ester. The conversion of substrates was significantly increased by using molecular sieve for water removal. The maximum conversions of three fatty acids, stearic acid, palmitic acid and capric acid to xylitol esters were 96, 92 and 88 %, respectively, at the optimum reaction conditions including temperature, 60 ºC; time, 18 h; substrate molar ratio, 1; enzyme amount, 0.12 g and molecular sieve amount 4 g in 30 mL solvent. Subsequent analysis by GC-MS indicated that the amount of produced diesters was significantly more than mono, tri and tetra esters.

Key Words: Xylitol, Fatty acid, Sugar alcohol ester, Novozym 435.

INTRODUCTION

Surfactants are amphiphilic molecules which are widely used in many industries. Sugar and sugar alcohol fatty acid esters, in the group of nonionic surfactants, have high emulsifying, stabilizing, detergency and other useful effects[1]. They find widespread applications as emulsifiers in food, detergents, cosmetics and pharmaceutical industries[2,3]. Sugar alcohol fatty acid esters are harmless to the environment due to their complete biodegradability under anaerobic and aerobic conditions. They are more favourable to consumers due to low skin irritation and toxicity[4]. Their antimicrobial[5], antitumoral[6] and insecticidal[7] properties have been reported and might open new markets.

The traditional chemical synthesis of sugar fatty acid esters needs corrosive acid catalysts, complex and expensive reaction setup and high pressure and temperature[1,8]. In recent years, lipase- catalyzed synthesis of sugar fatty acid esters has revealed an interesting alternative, due to the general properties and advantages of the biological over the chemical synthesis[9]. Enzymatic sugar ester synthesis is based on esterification reactions catalyzed by hydrolases. Since estrification is a reversible reaction (eqn. 1), the products such as water in the media should be removed to shift the equilibrium of the reaction away from hydrolysis to obtain a maximum yield of sugar alcohol ester[10].

$$\text{Sugar alcohol} + \text{Fatty acid} \rightleftharpoons \text{Ester} + \text{Water} \quad (1)$$

Water removal can be attained by different methods such as using open tube, molecular sieves, evacuation *in vacuo* and pervaporation[11].

In recent years, several researchers have reported enzymatic synthesis of sugar-alcohol based surfactants. Sorbitan oleate was synthesized by Xu *et al.*[12], using immobilized *Candida antarctica* lipase B. Water produced in the reaction was removed by a reduced-pressure system, which increased the production of sorbitan oleate monoester to a maximum conversion of 80 % in a solvent-free system compared to the conversion obtained by chemical synthesis which was about 50 %. No obvious improvement in conversion was observed by adding molecular sieve.

In this paper, we reported the effect of using molecular sieves under reflux system for production of xylitol stearate, palmitate and caprate with immobilized lipase from *Candida antarctica* (novozym 435). The influences of several parameters on xylitol esters production and also reusability of enzyme were studied.

EXPERIMENTAL

A commercials lipase, novozym 435 (*Candida antarctica* lipase B immobilized on macroporous acrylic resin, novozym A/S) and lipases from *Rhizomucor* Miehei (lipozyme RMIM) and *Thermomyces lanuginosus* (lipozyme TLIM) were used as biocatalysts for the sugar alcohol esters synthesis. The acyl donor was stearic acid, palmitic acid and capric acid (Merck, Germany). Xylitol was used as the acyl acceptor (Aldrich,

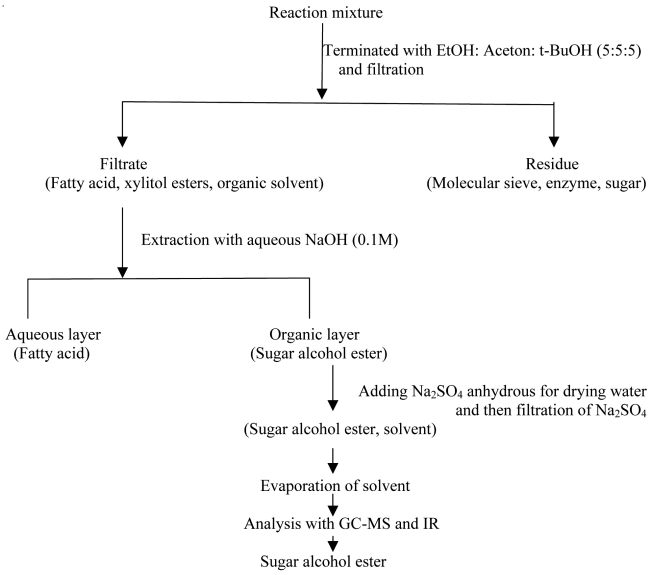
USA). Hexane, t-BuOH, acetone and ethanol were from Merck, Germany. Molecular sieve (3 Å, bead, 4-8 mesh, $K_nNa_{12-n}[(AlO_2)_{12}(SiO_2)_{12}]\cdot xH_2O$) were used as the water removal adsorbent (Aldrich, USA).

Xylitol esters synthesis: Reaction mixture included specified amounts of fatty acid, xylitol and molecular sieves in hexane. After addition of enzyme, the reaction was carried out in a 50 mL flask under reflux system at 200 rpm on magnetic stirring (C-MAG HS7). At the end of the reaction, a mixture of acetone/t-BuOH/EtOH (5:5:5 v/v/v) was added to the reaction mixture. The enzyme and molecular sieves were then separated by filtration and the filtrate solution was titrated with 0.1 M NaOH(aq) in the presence of phenolphthalein. A control without the enzyme for each reaction mixture was treated the same way as the reaction sample. All the experiments were performed in triplicate. The amount of fatty acid in the reaction mixture and control were specified and the conversion percentage was defined as the ratio of converted fatty acid in the reaction mixture to control fatty acid amount (eqn. 2).

$$\text{Conversion}\,(\%) = \left(1 - \frac{A}{B}\right) \times 100 \qquad (2)$$

where A = moles of the acid in the reaction mixture and B = initial moles of the acid.

The produced esters were extracted using a 100 mL separatory funnel. Extraction of xylitol esters after the synthesis has been shown according to **Scheme-I**.



Scheme-I: Extraction scheme of sugar alcohol ester

Identification of reaction product: The products were periodically tested using thin layer chromatography, TLC (Merck type DC-plastic folein Keiselgel 60 F_{254}), Fourier transform infra red spectroscopy, FTIR (Perkin Elmer, model 1650) and GC-MS QPSOSA (Shimadzu, Japan). Preliminary detection and identification of reaction product were performed by two procedures. The first method was performed using silicagel plates eluted with chloroform/methanol/acetic acid/water mixture (80:15:8:2 v/v/v/v). Compounds were identified by spraying the plate with α-naphthol solution (1.5 g of α-naphthol was dissolved in 51 mL of EtOH and then added to 4 mL of water and 6.5 mL of sulfuric acid 18 M). The products (black spots) were obtained by carbonization at 105 ºC (5.5 min)[13]. In the second method, the developing solvent system used was chloroform/methanol/acetic acid/water (70:20:8:2 v/v/v/v). The plates were treated with cerium-reagent (25 g molybdato phosphoric acid, 1 g of cerium-IV-sulphate, 80 mL of sulphuric acid 18 M and 1000 mL distilled water) and visualized by heating (blue spot)[14].

Further identification for ester formation was performed by FT-IR (Perkin-Elmer, model 1650) and gas chromatography/mass spectroscopy GC-MS on a Shimadzu (model GC 17A; model MS QP5050A, Tokyo, Japan) instrument equipped with a non polar column (fused silica capillary column SGE BPXS, 30 m × 0.25 mm i.d. × 0.25 μm thickness).

RESULTS AND DISCUSSION

Identification of reaction product: Products from esterification reaction between fatty acids and xyliol catalyzed by novozym 435 were monitored by TLC. The presence of the products and fatty acid were detected as blue spots with cerium reagent and black spots with α-naphtol reagent.

FT-IR analysis result is shown in Table-1. Absorption bands between 1729-1727 cm^{-1} belongs to stretching C=O (ester) and bands at 1257, 1184 and 1107 cm^{-1} show stretching of C-O acidic and alcoholic parts of the ester.

TABLE-1
FTIR ANALYSIS

Characteristic group	Frequency (cm^{-1})
C-O (stretching)	1107, 1184, 1257
CH_2 (bending)	1470
C=O (stretching)	1727-1729
C-H (stretching)	2851, 2915
O-H (stretching)	3419

Final identification of the esters was performed by GC-MS analysis. Various acyl donor substitutions at hydroxyl groups of xylitol were observed. Table-2 shows the percentage conversion and retention times of xylitol stearate, palmitate and caprate. Formation of xylitol diester was predominant over mono and tri esters. This is due to the fact that *Candida antarctica* lipase B possesses a confirmed regioselectivity for the acylation of primary OH groups of polyhydroxylated molecules[15].

TABLE-2
PERCENTAGE CONVERSION AND RETENTION TIMES OF ESTERS OBTAINED BY GC-MS ANALYSIS

Xylitol ester	Mono (%)	Di (%)	Tri (%)	Tetra (%)
Xylitol stearate	3.27 (17)	95.07 (17.433)	1.66 (18.475)	–
Xylitol palmitate	10.70 (15.89)	88.00 (16.54)	1.30 (17.47)	–
Xylitol caprate	9.59 (15.60)	68.81 (15.97)	17.00 (16.07)	4.6 (17.198)

*Retention time is shown in the parentheses

Screening of enzyme: Before the optimization of all reaction parameters, screening of three commercial lipases including novozym 435, lipozyme RMIM and lipozyme TLIM

for sugar alcohol ester synthesis was carried out. The reaction condition was: substrate molar ratio 0.33, molecular sieve 1 g, enzyme amount 0.3 g, temperature 50 ºC, volume of solvent 30 mL and reaction time 7 h. Fig. 1 shows the enzyme screening results for the xylitol esters synthesis. Novozym 435 exhibited the highest percentage conversion (20, 18 and 12 % for xylitol stearate, xylitol palmitate and xylitol caprate, respectively). Therefore, it was selected for optimization of the reaction parameters in ester synthesis.

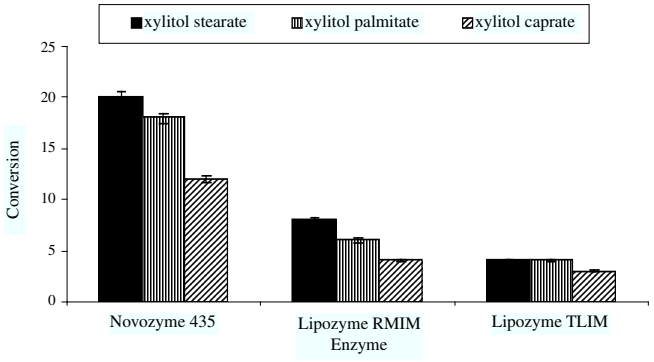


Fig. 1. Screening of enzymes. The reaction mixture consisted of xylitol 1 mmol, fatty acid 3 mmol, hexane 30 mL, molecular sieve 1 g, enzyme 0.3 g, at 50 ºC and 200 rpm for 7 h

Effect of reaction time: Time course study gives an insight into the performance of an enzyme as the reaction progresses. Such progress curves help determine the shortest time necessary for achieving a high yield and so enhance cost-effectiveness of the process[16]. Fig. 2 shows the time course for the novozym 435-catalyzed reactions of xylitol with stearic acid, palmetic acid and capric acid. The effect of time in the sugar alcohol ester synthesis was investigated by varying reaction periods (3, 7, 10, 14, 18, 24 and 48 h) while other conditions were constant. The conversion was increased with increasing reaction time. Novozym 435 gave the highest conversion in reaction period of 18 h. Prolonging the reaction increases the hydrolysis of esters (reverse reaction of esterification) thus the percentage yield decreases[17].

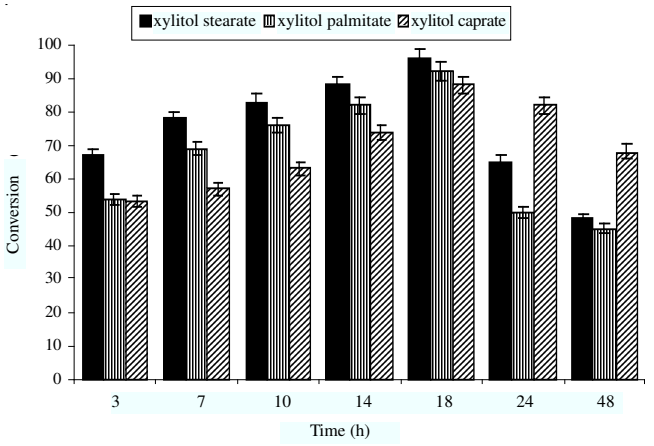


Fig. 2. Time course study for the synthesis of (■) xylitol stearate, (▥) xylitol palmitate and (▨) xylitol caprate: temperature 60 °C, molar ratio of xylitol/fatty acid 1, molecular sieve amount 4 g, enzyme amount 0.12 g, volume of hexane 30 mL, 200 rpm

Effect of temperature: The effect of temperature can be attributed to its effect on substrate solubility as well as its direct influence on the reaction and the enzyme[18]. Although an increase in temperature reduces viscosity and enhances the reaction rate[19], high temperatures may cause enzyme denaturation[20,21]. Effect of temperature on esterification reaction is shown in Fig. 3. The conversion of substrates was increased with increase in temperature from 30-60 ºC before dropping at 70 ºC. This is because beyond a critical temperature enzyme may have been deactivated. The result is similar in the findings by most reviewed papers that novozym 435 was optimally used at 40-60 ºC[22]. The enzyme molecule after 60 ºC unfolds and alters its tertiary and quaternary or globular structure. Consequently, the catalytic power of lipase will reduce, because denaturation process occurs. Change in reaction temperature can affect the activity and stability of the enzyme and thus the rate of reaction[23].

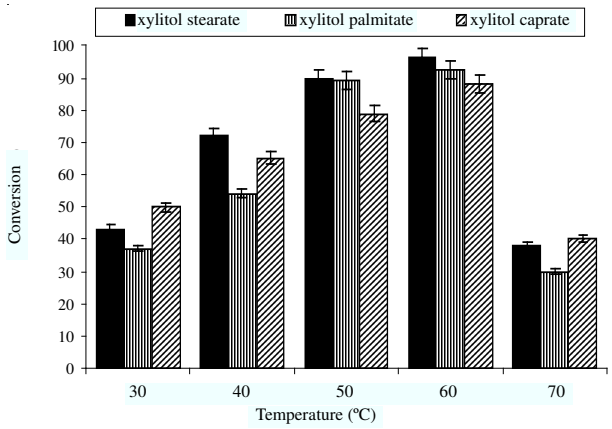


Fig. 3. Effect of temperature on the synthesis of (■) xylitol stearate (▥) xylitol palmitate and (▨) xylitol caprate: reaction time 18 h, molar ratio of xylitol/fatty acid 1, molecular sieve amount 4 g, enzyme amount 0.12 g, volume of hexane 30 mL, 200 rpm

Effect of using molecular sieves: The enzyme-catalyzed reactions in organic media critically depend on the amount of water in the reaction system[4,24]. The water produced during the esterification reaction disturbs the equilibrium and leads to hydrolysis of the ester. To obtain a maximum yield of sugar alcohol ester by shifting the equilibrium of the reaction away from hydrolysis, the water should be removed. In this study, water removal has been done by using molecular sieves. The effect of molecular sieve amount was also analyzed (Fig. 4). Adding 1 g of molecular sieves causes a significant increase (> 5-fold) in the conversion. Fatty acids conversion is increased with increasing the molecular sieve amount to 4 g. Then, decrease in the conversion occurred due to excessive stripping away the essential water for enzyme activity from its vicinity[4].

Effect of the molar ratio of sugar/acyl donor: The enzymatic sugar ester synthesis was studied at different molar ratios of sugar to fatty acids (Fig. 5). For xylitol stearate, the maximum conversion was obtained at the xylitol/stearic acid ratio 0.5-1.0. When the palmitic acid and capric acid were used as acyl donors, the conversion increased up to molar ratio 1:1 and so, equimolar ratios of substrate were adequate to give good relative conversion. Further increase in xylitol:fatty

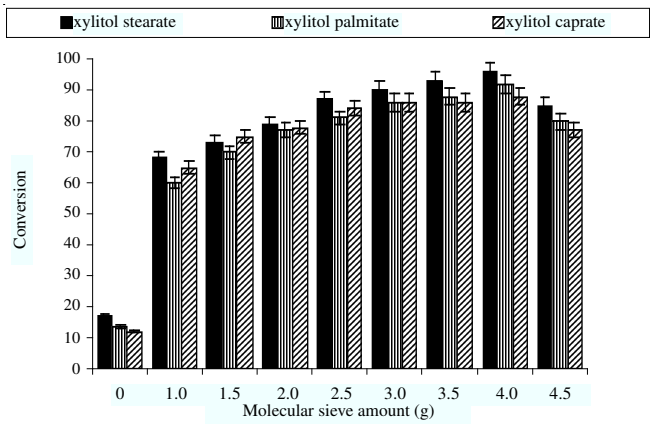


Fig. 4. Effect of molecular sieve amount added (0-4.5 g) on the synthesis of (■) xylitol stearate, (▥) xylitol palmitate and (▨) xylitol caprate: reaction time 18 h, temperature 60 ºC, molar ratio of xylitol/fatty acid 1, enzyme amount 0.12 g, volume of hexane 30 mL, 200 rpm

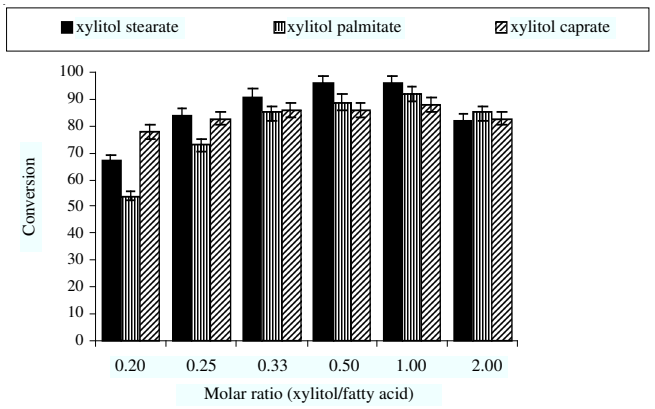


Fig. 5. Effect of substrate molar ratio of xylitol:fatty acid on the synthesis of (■) xylitol stearate, (▥) xylitol palmitate and (▨) xylitol caprate: reaction time 18 h, temperature 60 ºC, molecular sieve 4 g, enzyme amount 0.12 g, volume of hexane 30 mL, 200 rpm

acids molar ratio higher than 1:1, caused decrease in the conversion. These observations agree well with Cao *et al.*[25]. Moreover, increasing the molar ratio would decrease the enzyme activity since competing alcohol binding reduces formation of acyl-enzyme complex[26].

Effect of the quantity of novozym 435: Amount of enzyme plays a crucial economic role in any biocatalytic process. Influence of the enzyme amount on the synthesis of xylitol esters was assessed to determine the minimum amount necessary for achieving high conversions. Fig. 6 shows that the conversion is increased with increasing the amount of novozym 435 from 0.05-0.12 g, but after 0.12 g enzyme amount, decrease in conversion is observed. The result was similar to what was reported by Torres and Otero[27] that an excess of enzyme did not increase the conversion. This can be attributed to mass transfer limitations imposed by the high viscosity of a heterogeneous reaction mixture comprising the biocatalyst, synthesis product and residual substrates[16], so using a low amount of enzyme is important since the cost of enzyme is usually higher than that of substrates.

Effect of solvent: The use of biocatalyst in organic solvents provides many advantages, such as increase in solubility of hydrophobic substrates, avoiding undesired water dependent side reactions, as well as the ability to shift the thermodynamic

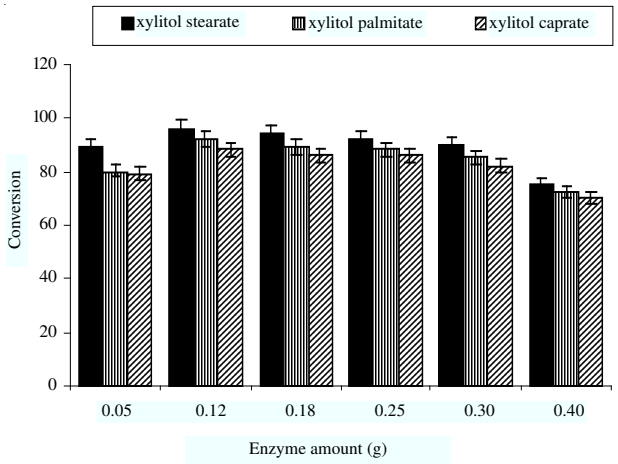


Fig. 6. Effect of novozym 435 amount on the synthesis of (■) xylitol stearate, (▥) xylitol palmitate and (▨) xylitol caprate: reaction time 18 h, temperature 60 ºC, molar ratio of xylitol/fatty acid 1, molecular sieve 4 g, volume of hexane 30 mL, 200 rpm

equilibrium of many processes to the synthetic reaction. The role of solvent contributed to its effect on good mixing and solubility of the substrates in the reaction[16]. As it can be seen in Fig. 7, the conversions of xylitol stearate, xylitol palmitate and xylitol caprate are increased up to 30 mL solvent volume. After that a decrease in the conversion can be seen, which may attributed to inhibition of enzyme by the solvent that can act as competitive inhibitor to the substrates.

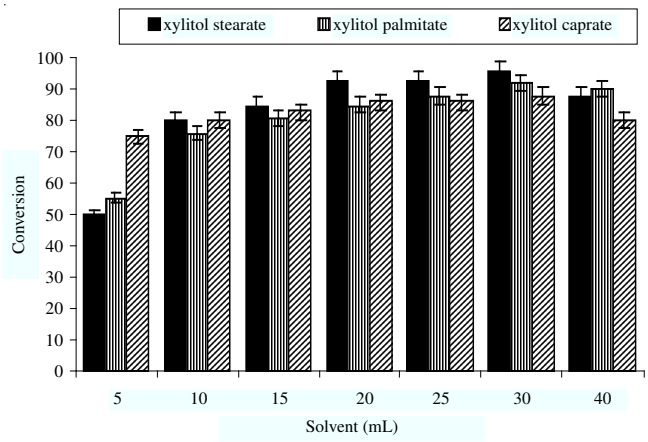


Fig. 7. Effect of volume of solvent on the synthesis of (■) xylitol stearate, (▥) xylitol palmitate and (▨) xylitol caprate: reaction time 18 h, temperature 60 ºC, molar ratio of xylitol/fatty acid 1, enzyme amount 0.12 g, molecular sieve 4 g, 200 rpm

Effect of enzyme reusability: Reuse of enzyme helps to reduce the product cost and makes the process more economical. The ability of immobilized lipase to retain its synthetic activity during recycling was examined. Novozym 435 activity in the sugar alcohol ester synthesis has been tested for four cycle reuse in terms of percentage conversion without adding the molecular sieve. After each reaction, the enzyme was filtrated and reused in a new reaction. Fig. 8 demonstrates the reusability of novozym 435. The activity of enzyme decreased gradually which might be due to leaching of the enzyme molecules into the reaction medium and also loss of enzyme during filtration and drying since no make-up quantities were added.

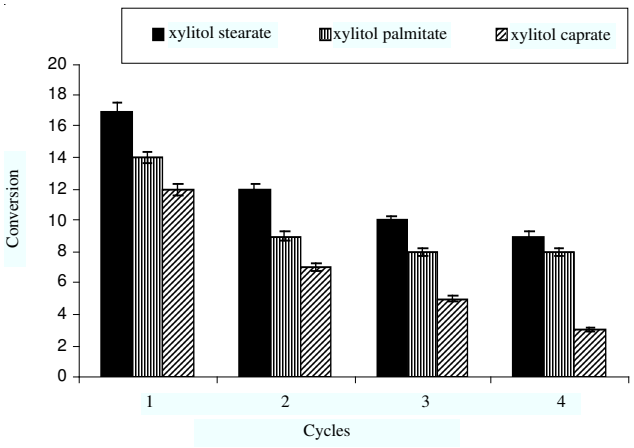


Fig. 8. Operational stability of novozym-435 for the synthesis of (■) xylitol stearate, (▥) xylitol palmitate and (▨) xylitol caprate: reaction time 18 h, temperature 60 ºC, molar ratio of xylitol/fatty acid 1, enzyme amount 0.12 g, volume of hexane 30 mL, 200 rpm

Conclusion

The effects of several parameters affecting the enzymatic synthesis of xylitol esters were studied. Various factors such as substrate properties and reaction equilibria can affect the conversion yield. A high percentage conversion of substrates was obtained at the optimum conditions. The maximum conversions for xylitol stearate, palmitate and caprate were 96, 92 and 88 %, respectively. Water formed during the reaction resulted in a considerable decrease in conversion. Addition of molecular sieve (4 g molecular sieve in 30 mL of solvent) helped to increase the conversion (> 7-fold) by removing of water and pushing the equilibrium to completion.

REFERENCES

1. I.S. Yoo, S.J. Park and H.H. Yoon, *J. Ind. Eng. Chem*., **13**, 1 (2007).
2. S. Piccicuto, C. Blecker, J.C. Brohee, A. Mbampara, G. Lognay, C. Deroanne, M. Paquot and M. Marlier, *Biotech. Agron. Soc. Environ*., **5**, 209 (2001).
3. R.K. Sexena, P.K. Ghosh, R. Gupta, W.S. Bradoo and R. Gulati, *Curr. Sci*., **77**, 101 (1999).
4. S. Tarahomjoo and I. Alemzadeh, *Enzym. Microbiol. Tech*., **33**, 33 (2003).
5. M. Ferrer, J. Soliveri, F.J. Plou, N. Lopez-Cortes, D. Reyes-Duarte, M. Christensen, J.L. Copa-Patino and A. Ballesteros, *Enzym. Microbiol. Tech*., **36**, 391 (2005).
6. S. Okabe, M. Saganuma, Y. Tada, Y. Ochiai, E. Sueoka and H. Kohya, *J. Jpn. Cancer. Res*., **90**, 669 (1999).
7. G.J. Puterka, W. Farone, T. Palmer and A. Barrington, *J. Econo. Entomol*., **93**, 636 (2003).
8. M.B. Abdul Rahman, U.H. Zaidan, M. Basri, M.Z. Hussein, R.N.Z.A. Rahman and A.B. Salleh, *J. Mol. Catal. B: Enzym*., **50**, 33 (2008).
9. E. Castilo, F. Pezzotti, A. Navarro and A. Lopez-Munguia, *J. Biotech*., **102**, 251 (2003).
10. S. Adachi and T. Kobayashi, *J. Biosci. Bioeng*., **99**, 87 (2005).
11. Y. Yan, U.T. Bomscheuer, G. Stadler, S. Lutz-Wahl, M. Reuss and R.D. Schmid, *J. Am. Oil Chem*., **78**, 147 (2001).
12. Y. Xu, D. Wang, X.Q. Mu and Y.Q. Ni, *J. Am. Oil Chem*., **80**, 647 (2003).
13. S. Soultani, J.M. Engassar and M. Ghoul, *J. Mol. Catal. B: Enzym*., **11**, 725 (2001).
14. F. Ganske and U.T. Bornscheuer, *J. Mol. Catal. B: Enzym*., **36**, 40 (2005).
15. S. Gebhardt, S. Bihler, M. Schubert-Zsilavecz, S. Riva, D. Monti, L. Falcone and B. Danieli, *Helv. Chim. Acta*, **85**, 1943 (2002).
16. S. Mat Radzi, M. Basri, A.B. Salleh, A. Ariff, R. Mohammad, M.B.A. Rahman and R.N.Z.R.A. Rahman, *Elect. J. Biotech*., **8**, 290 (2005).
17. P. Villeneuve, B. Barea, P. Sarrazin, F. Davrieux, R. Boulanger, Y. Caro, M.C. Figueroa-Espinoza, M. Pina and J. Graille, *Enzym. Microbiol. Tech*., **33**, 79 (2003).
18. F.N. Lucia and B.A. Daniel, *J. Sci. Food. Agric*., **81**, 1193 (2001).
19. V.V. Yankah and C.C. Akoh, *J. Am. Oil Chem. Soc*., **77**, 495 (2000).
20. S.H. Krishna, T.J. Reddy and G.V. Chowdary, *Bioresour. Tech*., **77**, 193 (2001).
21. A. Illanes, C. Altamirano, M. Fuentes, F. Zamorano and C. Aguirre, *J. Mol. Catal. B: Enzym*., **35**, 45 (2005).
22. P. Lozano, T. De Diego, D. Carrie, M. Vaultier and J. Iborra, *J. Mol. Catal. B: Enzym*., **21**, 9 (2003).
23. R.M. Daniel, M.J. Danson, R. Eisenthal, C.K. Lee and M.E. Peterson, *Extremophiles*, **12**, 51 (2008).
24. F. Chamouleau, D. Coulon, M. Girardin and M. Ghoul, *J. Mol. Catal. B: Enzym*., **11**, 949 (2001).
25. L. Cao, A. Fischer, U.T. Bornscheuer and R.D. Schmid, *Biocatal. Biotransform*., **14**, 269 (1997).
26. E.R. Gunawan, M. Basri, M.B.A. Rahman, A.B. Salleh, R.N.Z.A. Rahman, *Enzym. Microbiol. Tech.*, **37**, 739 (2005).
27. C. Torres and C. Otero, *Enzym. Microbiol, Tech*., **29**, 3 (2001).