Journal of Molecular Catalysis B: Enzymatic 57 (2009) 104–108

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Solvent-free enzymatic synthesis of feruloylated diacylglycerols and kinetic study

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ARTICLE INFO

Article history:
Received 24 March 2008
Received in revised form 25 July 2008
Accepted 25 July 2008
Available online 5 August 2008

Keywords:
Feruloylated diacylglycerols (FDAG)
Glyceryl ferulate (FG)
Oleic acid (OA)
Lipase-catalyzed esterification
Kinetic model

ABSTRACT

The enzymatic esterification of glyceryl ferulate (FG) and oleic acid (OA) for feruloylated diacylglycerols (FDAG) synthesis in a solvent-free system was studied in this work. The reactions were catalyzed by different commercially available lipases, among which Novozym 435 was found to be the most active biocatalyst. The effects of glycerol in the reaction mixture and various synthesis parameters on yield of FDAG and the initial reaction rate were studied. The optimum synthesis conditions were as follows: temperature, 65 °C; enzyme load, 7.5%; substrate ratio, 7.5:1 (OA/(FG + glycerol), w/w); and reaction time, 12 h. Under the optimum conditions, the conversion of FG and yield of FDAG reached 98.0 ± 1.0% and 82.6 ± 2.2%, respectively. A linear relationship was established between the initial reaction rate and enzyme load up to 10%, which demonstrated that the influence of external mass transfer limitations on the reaction could be eliminated. The relationship between initial reaction rate and temperature was also established, based on the Arrhenius law. Novozym 435 in the present work can be used 18 times under the optimum conditions without essential losses in activity. The reaction kinetics agrees with the Ping-Pong Bi-Bi mechanism characterized by V_m and K'_m values of 5.26×10^{-4} mol/(L min) and 0.26 mol/L, respectively.

1. Introduction

Phenolic acids are natural hydrophilic antioxidants, which ubiquitously occur in the plant kingdom. Ferulic acid (4-hydroxy-3-methoxy cinnamic acid, FA) is a phenolic acid widely distributed in most higher plant. FA has been reported to have many physiological functions, including antioxidant, antimicrobial, anti-inflammatory, antiallergic, antiviral, anticarcinogenic, free radical scavenging, and UV filter properties, which made FA widely used in the food, health, cosmetics, and pharmaceutical industries [1–5].

However, FA exhibits low solubility and stability in hydrophobic media, which limits its applications in oil-based food processing, cosmetics, and other corresponding industries. Thus, lipophilic derivatives of FA, as exemplified by feruloylated diacylglycerols (FDAG) [6–17], glycosylated, and alkylated derivatives [18,19], have been reported. FA is heat- and oxidation-sensitive, which makes enzymatic synthesis of FDAG an attractive alternative to chemical synthesis [9,10,12]. But previous reports regarding the enzymatic synthesis of FDAG have many disadvantages including low yields, long reaction time, solvent requirements, etc. Recently we have reported a novel solvent-free enzymatic route for synthesis of FDAG (Fig. 1A), consisting of two consecutive steps, namely, transesterification of ethyl ferulate (EF) and excessive glycerol to produce glyceryl ferulate (FG) (Fig. 1B), followed by esterification of FG with oleic acid (OA) [20]. This route can produce higher yield of FDAG in a shorter reaction time. An alternative method to synthesize FG using feruloyl esterase from *Aspergillus niger* catalyzed esterification of FA with glycerol has also been reported [21,22].

The purpose of the present study was to investigate the influence of several synthesis parameters, including reaction time, reaction temperature, enzyme load, and substrate ratio (OA/(FG + glycerol), w/w), on the percent molar yield of FDAG and the initial reaction rate in the esterification of FG with OA. Enzyme screening, the effects of glycerol and the external mass transfer on the esterification reaction, and enzyme durability were also evaluated. The reaction kinetic was studied. Values of V_m and K'_m were obtained.

2. Materials and methods

2.1. Biological and chemical materials

Immobilized lipase Lipozyme RM IM (from *Rhizomucor miehei*, RML, 25 BIU/g), Lipozyme TL IM (from *Thermomyces lanuginosus*, TLL, 175 IUN/g), and Novozym 435 (lipase B from *Candida antarctica*, CAL, 10,000 PLU/g) were purchased from Novozymes A/S (Bagsvaerd, Denmark). Lipase (from *Candida lipolytica*, 5000 units/g) powder was purchased from Xueyan Enzyme Company (Wuxi, China). Pancreatin (Porcine pancreas, 141 IU/g) powder was purchased from Sigma Corporation (USA). Ethyl ferulate (purity >99%) was purchased from Suzhou Changtong Chemical Co., Ltd.

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doi:10.1016/j.molcatb.2008.07.010

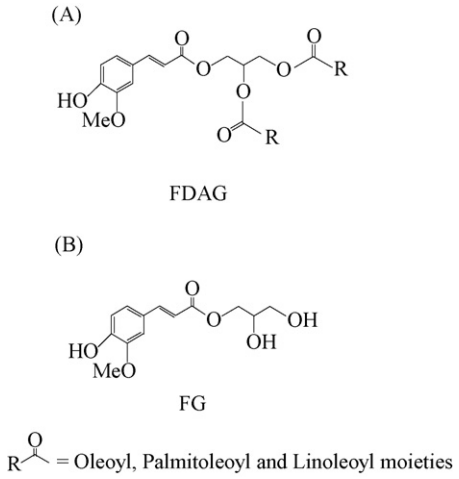

Fig. 1. Chemical structures of FDAG and FG.

(Suzhou, China). Glycerol (purity >99%) and oleic acid (purity >65%) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). OA was fractionated before use to a purity of >90% (GC results: OA 91.2 ± 1.0%, linoleic acid 5.7 ± 1.2%, palmitoleic acid 2.1 ± 0.6%, other fatty acids 1.0 ± 0.5%). Methanol and glacial acetic acid were of HPLC purity. All other reagents were of analytical grade.

2.2. General procedure for the enzymatic synthesis

Transesterification was conducted as described previously [23]: 1.5 mmol EF was reacted with 15 mmol glycerol and 170 mg Novozym 435 at 60 °C and 1.33×10^3 Pa for 10 h in 25 mL round-bottom flasks on a vacuum-rotary evaporator rotating at 100 rpm.

Esterification was performed in 25 mL round-bottom flasks on a vacuum-rotary evaporator rotating at 100 rpm using the following conditions: 0.30 g (glyceryl ferulate + glycerol, glycerol solution of FG obtained after transesterification) or 0.075 g chromatographically purified FG, 2.22 g OA, 0.25 g Novozym 435, 65 °C, 1.33×10^3 Pa.

2.3. HPLC analysis and structural identification of the reaction products

Reactants and products were analyzed as described previously [20]. The products were isolated and identified as described previously [23,24].

3. Results and discussion

3.1. Enzyme screening

The esterification of OA with FG in a solvent-free system, respectively, by five lipases is compared in Table 1. These lipases catalyzed OA incorporation into FG to various extents. Lipozyme TL IM and *Candida lipolytica* lipase had nearly no activity on FDAG synthesis, and pancreatin could give lower activity of FDAG yield; and these lipases could only give lower activities of FG conversion. Lipozyme RM IM gave higher activity of FG conversion and FDAG yield. Novozym 435 gave the highest activity of FG conversion and FDAG yield, and thus was selected for use in subsequent experiments.

Table 1
Enzymatic esterification of FG and OA for FDAG synthesis catalyzed by different biocatalysts[a]

Enzyme	Activity (μmol/(min g))[b]	Yield of FDAG (%)
Novozym 435	1.53 ± 0.02	84.0 ± 1.8
Lipozyme RM IM	1.48 ± 0.03	67.0 ± 2.0
Lipozyme TL IM	0.41 ± 0.03	tr[c]
Candida lipolytica lipase	0.32 ± 0.03	nd[d]
Pancreatin	0.35 ± 0.03	13.6 ± 1.1

[a] Esterifications were performed using different biocatalysts at 65 °C, 1.33×10^3 Pa, and OA/(FG + glycerol) 7.5:1 (w/w) for 12 h. Reactions were carried out in 25 mL round-bottom flasks on a rotary evaporator rotating at 100 rpm.

[b] Enzyme activities (μmol/(min g)) were defined as millimoles of FG converted to FMAG and FDAG combined per minute per gram at the assay conditions stated above.

[c] tr = trace (<0.02).

[d] nd = not detected.

3.2. The effect of glycerol on the esterification

The transesterification product (FG + glycerol) was approximately composed of 75% glycerol and 25% FG. During the early phase of the esterification (~2 h), it was observed that FDAG formation rate (3.2×10^{-5} mol/h) in the presence of glycerol in the reaction mixture was approximately 10-fold faster than that (2.9×10^{-6} mol/h) in the absence of glycerol, and the conversion of FG (98.1 ± 1.5%) and yield of FDAG (84.0 ± 1.8%) in the presence of glycerol were higher than that (79.2 ± 2.0% and 50.1 ± 2.3%) in the absence of glycerol after 12 h. There are two major reasons for this phenomenon: (i) glycerol in larger amounts can provide enough solvents for FG solids; and (ii) excess of glycerol reacts with OA to produce monoacylglycerols (MAG) and diacylglycerols (DAG), which was, e.g., quantified by HPLC profile as 30 ± 3.2% DAG and 15 ± 1.8% MAG at 12 h. Both MAG and DAG are good emulsifiers that led to emulsification phenomena and thus can make the reactants (FG and OA) mix well. Both reasons improve the accessibility of FG to OA and hence facilitate the reaction process. Similar results that glycerol can enhance production of feruloylated species have also been reported [24,25]. Thus, residual glycerol was maintained in the transesterification product when esterification of FG and OA was performed in the following experiments.

3.3. Effect of reaction time

Time course studies are useful in identifying product (FDAG) formation and reactant (FG) disappearance. Fig. 2 shows the reaction progress versus the reaction time. The conversion of FG showed sharp increase until 2 h to reach 94.5 ± 1.5%, and then maintained essentially constant until 16 h. The yield of FMAG showed an increasing–decreasing pattern, while that of FDAG showed an ever increasing one, both with the increasing of reaction time. The results indicate that more amounts of fatty acyls have been transferred to FG with the increasing of reaction time. The FDAG yield increased significantly with increases in reaction time until 12 h to reach 83.5 ± 2.0%, and then maintained essentially constant until 16 h. The reaction time of the present study is thus chosen as 12 h, which guarantee both better FDAG yield and time efficiency than reported results [9,10,12].

3.4. Effect of reaction temperature

Experiments at six different temperatures were carried out to assess the influence of temperature on the esterification reaction. The lowest temperature chosen was fixed at 55 °C, below which the stirring of the reaction mixture becomes difficult because of the high melting point of FG. Fig. 3A shows the yield of FDAG ver-

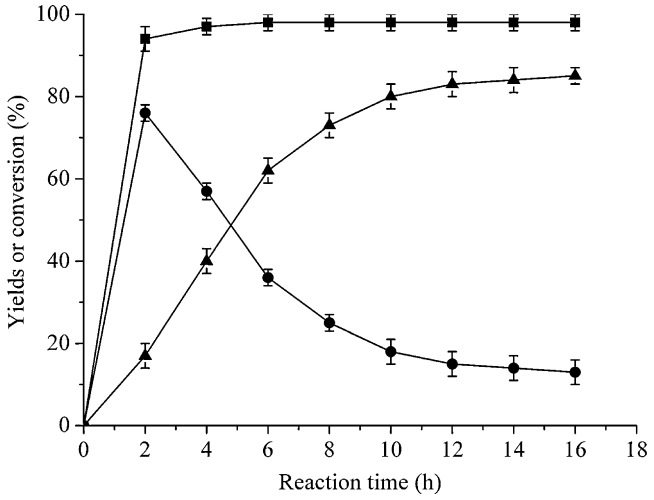

Fig. 2. Effect of reaction time on conversion of FG and yields of FDAG and FMAG. Esterifications were performed at 60 °C, 1.33×10^3 Pa, enzyme load (relative to the weight of total substrates) 10%, and OA/(FG + glycerol) 7.5:1 (w/w). Reactions were carried out in 25 mL round-bottom flasks on a rotary evaporator rotating at 100 rpm. Conversion of FG (■), yield of FMAG (●), yield of FDAG (▲).

sus reaction time at the six temperatures studied. It is obvious that the temperature has a considerable influence on the yield of FDAG; in fact, the reaction time required to reach 76% yield of FDAG was reduced from 16 to 4 h when the temperature rises from 55 °C to 80 °C, which is the result of increasing fatty acyls transfer at high temperature. Similar results have also been reported [26,27]. However, too high temperatures will lead to higher lipase deactivation rates. To obtain good reaction rates and equilibrium yield without thermally deactivating the lipase in reuses, the temperature should be kept around 65 °C.

The initial reaction rates, defined as the initial FDAG yield per unit time (V_0, mol/(L min)), were calculated from six experimental points of the yield–time profile corresponding to the first 0.5 h of the reaction (15.0% or less FDAG yield), where the profiles were found to be approximately linear. So the Arrhenius law can be described as follows:

$$lnV_0 = \ln A - \frac{E_a}{RT} \quad (1)$$

where A = the Arrhenius constant, E_a = the activation energy, R = the gas constant, and T = absolute temperature (K). Fig. 3B shows the values of $\ln V_0$ versus the reciprocal of the absolute temperature T. From the slope of a straight line passing through these points, it is possible to calculate the activation energy. The value of E_a obtained by means of the above-mentioned Eq. (1) is 67.4 kJ/mol, which is higher than other E_a values (0.97–34.5 kJ/mol) determined for other lipase-catalyzed esterification reactions [28]. This difference can be attributed to that the feruloyl of FG has electron-donating and steric hindrance effects, which lead to lower reaction rate than other lipase-catalyzed esterification reactions.

Thus the Arrhenius law equation about the initial reaction rate and the reaction temperature (55–80 °C) can be written into Eq. (2):

$$\ln V_0 = 15.415 - \frac{8109}{T}. \quad (2)$$

3.5. Effect of enzyme load

Fig. 4 shows the dependency between the FDAG yield and enzyme load. The FDAG yield increased with enzyme loads between 2.5% and 10%; and then decreased with enzyme loads exceeding 10%, which could be due to biocatalyst agglomeration and possible

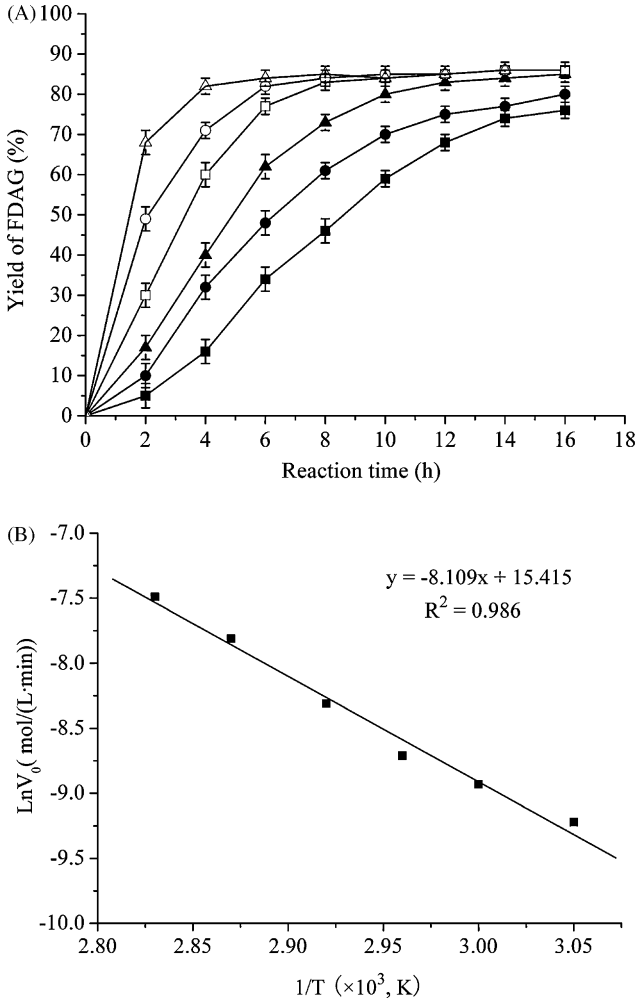

Fig. 3. (A) Effect of reaction temperature on the yield of FDAG. Esterifications were performed at different temperature, 1.33×10^3 Pa, enzyme load (relative to the weight of total substrates) 10%, and OA/(FG + glycerol) 7.5:1 (w/w). Reactions were carried out in 25 mL round-bottom flasks on a rotary evaporator rotating at 100 rpm. 55 °C (■), 60 °C (●), 65 °C (▲), 70 °C (□), 75 °C (○), 80 °C (△). (B) Relationship between the initial reaction rate and reaction temperature (K) during the esterification of OA and FG. Reaction conditions see (A).

diffusional problems. Similar results that excess enzyme present in the reaction decreases the efficiency per mass unit of biocatalyst in other enzymatic reactions have also been reported [29,30]. The equilibrium FDAG yield (83.5 ± 2.0%) at 10% enzyme load is close to that (82.6 ± 2.2%) at 7.5%. Considering the efficiency of biocatalyst, 7.5% enzyme load was selected for the next experiments.

As shown in Fig. 4, the initial reaction rate was drastically raised with the increasing of the enzyme load. The relationship between initial reaction rate and enzyme load (<10%) shows linear behavior, apparently indicating a kinetically controlled enzymatic reaction without external mass transfer limitations. The rotation speed between 20 rpm and 115 rpm had little effects on the conversion of FG and yield of FDAG (data not shown), again indicating that external mass transfer limitations is eliminated in this reaction strategy. Similar reaction characteristics have been observed in other lipase-catalyzed esterification systems [29,31–33].

3.6. Effect of substrate ratio

The yield of FDAG showed an increasing pattern with increasing of substrate ratio (OA/(FG + glycerol), w/w) (Fig. 5A). The FDAG

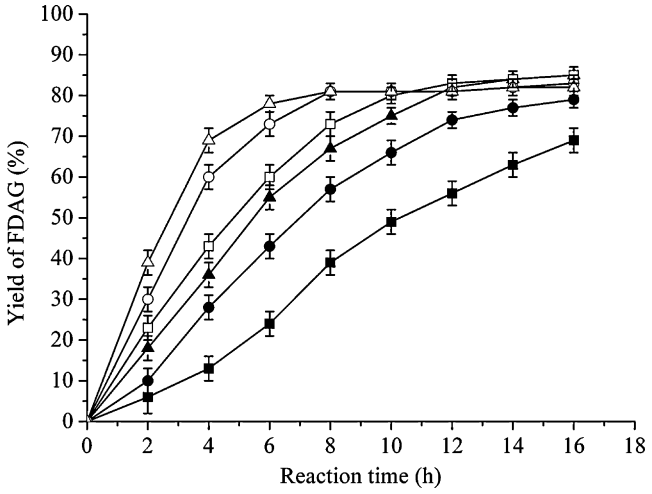

Fig. 4. Effect of enzyme load (relative to the weight of total substrates) on the yield of FDAG. Esterifications were performed at 65 °C, 1.33 × 10^3 Pa, and OA/(FG + glycerol) 7.5:1 (w/w). Reactions were carried out in 25 mL round-bottom flasks on a rotary evaporator rotating at 100 rpm. 2.5% (■), 5.0% (●), 7.5% (▲), 10% (□), 12.5% (○), 15% (△).

yield reached its peak (83.4 ± 2.1%) at a 12.5:1 substrate ratio 12 h, which is close to that (82.6 ± 2.2%) at 7.5:1, 12 h. The initial reaction rate shows increasing–decreasing patterns with the increasing of FG mass concentration (FG/OA, w/w) (Fig. 5B). The maximum initial reaction rate was obtained at a FG mass concentration of 0.04 (FG/OA, w/w) corresponding to a substrate ratio of 6.25:1. Higher FG concentration could cause higher viscosity of the reaction mixture and in turn a considerable mass transfer resistance; besides, due to its electron-donating and steric hindrance, FG at too high concentrations prevents itself from entering the active site of lipases to act as acyl acceptors. Both effects combined account for the overall esterification reaction rate. Similar inhibiting and steric hindrance effects of the electron-donating substituent conjugated to the carboxylic group in phenolic acids have also been reported [6,9,13]. From the standpoint of reaction efficiency, the ideal substrate ratio for the higher FDAG yield was 7.5:1 for this synthesis conditions, although the highest initial reaction rate was obtained at a substrate ratio of 6.25:1.

3.7. Stability of enzyme

Fig. 6 shows the reusability and stability of Novozym 435 under the above-mentioned optimum conditions. The immobilized lipase can be used at least 18 times without essential loss of activity. The FDAG yield was slightly decreased to 74.0 ± 2.0%, and the FG conversion was still maintained above 98.0% at 19 times. Excellent performance of Novozym 435 might benefit from immobilization of lipase and the relatively mild reaction conditions used. The occurrence of OA in the solvent-free system and co-product water-deprived reaction media seem to help the enzyme to retain its activity.

3.8. Kinetic model

Most of the kinetic studies on lipase-catalyzed synthesis of esters have considered the direct esterification of short-chain alcohols with acids using a Ping-Pong Bi-Bi kinetic mechanism with inhibition by substrates, and the corresponding expression for initial reaction rate in the reaction has been proposed assuming rather low product concentrations and removal of the minor amount of water formed in the lipase-catalyzed esterification [34–36]. The

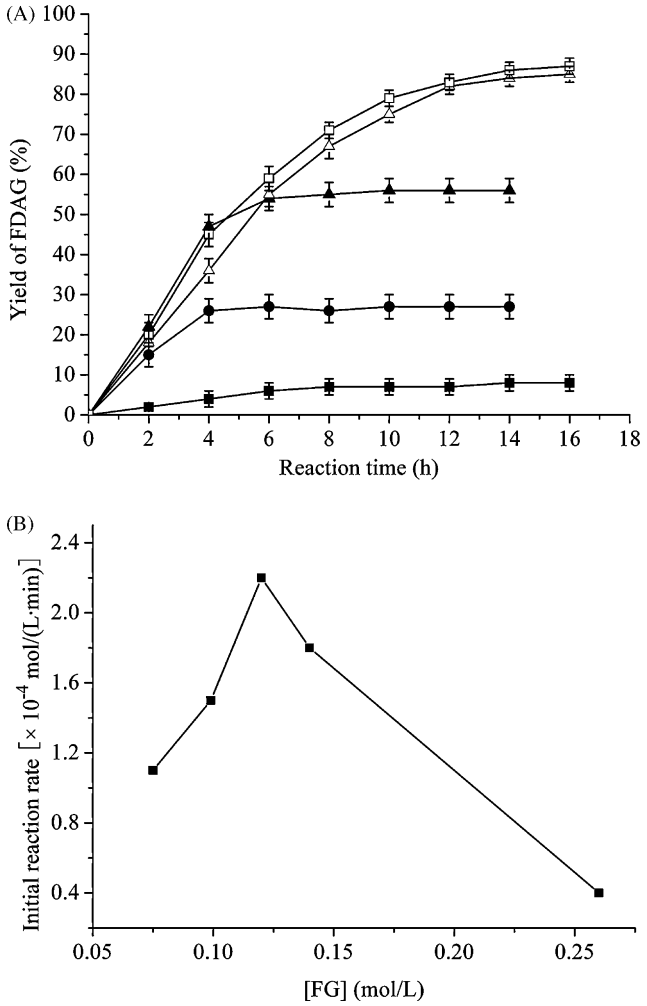

Fig. 5. (A) Effect of substrate ratio (OA/(FG + glycerol), (w/w)) on the yield of FDAG. Esterifications were performed at 65 °C, 1.33 × 10^3 Pa, and enzyme load (relative to the weight of total substrates) 7.5%. Reactions were carried out in 25 mL round-bottom flasks on a rotary evaporator rotating at 100 rpm. 2.5:1 (■), 5.0:1 (●), 6.25:1 (▲), 7.5:1 (△), and 12.5:1 (□). (B) Relationship between the initial reaction rate and FG mass concentration (FG/OA, w/w) during the esterification of OA and FG. Reaction conditions were same as (A).

expression for initial reaction rate in the case of this study, following the Ping-Pong Bi-Bi kinetic mechanism as proposed, is described

$$V_0 = \frac{V_m}{1 + K_A/[A] + K_B/[B](1 + [A]/K_{IA})} \quad (3)$$

where V_0 is the initial reaction rate, V_m the maximum reaction rate, [A] and [B] are the concentrations of FG and OA, K_A and K_B the Ping-Pong constants for FG and OA, K_{IA} is the inhibition constant for FG [37]. Since OA in the reaction system are in significant excess, its concentration can be regarded as a constant. So the initial reaction rate equation can be expressed simply as

$$V_0 = \frac{V_m[A]}{[A] + K'_m} \quad (4)$$

where V_0 is the initial reaction rate, V_m the maximum initial reaction rate, K'_m the apparent Michaelis constant. The Lineweaver–Burk plot of the reciprocal initial rate versus the reciprocal concentration of FG shows good linearity at lower FG concentration (Fig. 7). The values for V_m and K'_m are obtained by non-linear regression: $V_m = 5.26 \times 10^{-4}$ mol/(L min), $K'_m = 0.26$ mol/L.

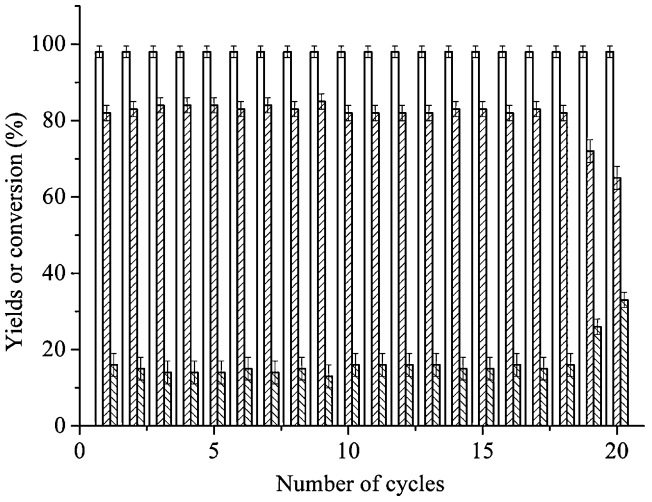

Fig. 6. Effect of enzyme reuses on conversion of FG and yields of FDAG and FMAG. Esterifications were performed at 65 °C, 1.33×10^3 Pa, enzyme load (relative to the weight of total substrates) 7.5%, and OA/(FG + glycerol) 7.5:1 (w/w) for 12 h. Reactions were carried out in 25 mL round-bottom flasks on a rotary evaporator rotating at 100 rpm. Conversion of FG (□), yield of FDAG (▨), and yield of FMAG (▧).

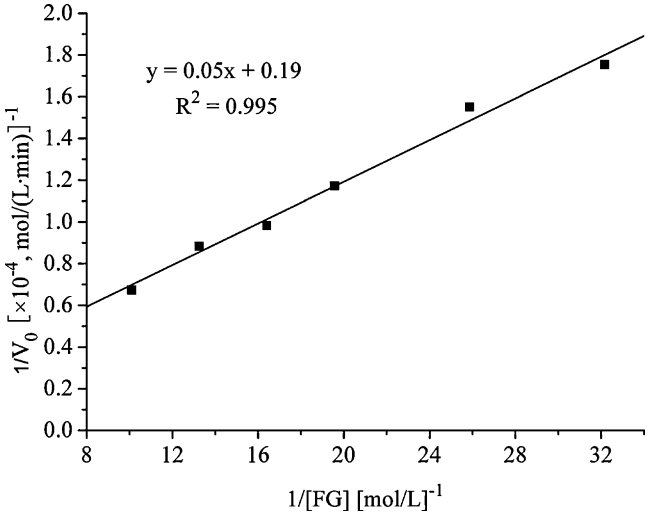

Fig. 7. Reciprocal initial reaction rate versus reciprocal FG concentration at lower FG concentrations. Reaction conditions were same as Fig. 5(A).

4. Conclusion

Esterification of FG and OA for FDAG synthesis was conducted by employing different lipases, among which Novozym 435 was found to be the most active biocatalyst. The presence of glycerol in the reaction mixture can enhance the initial reaction rate and the yield of FDAG. The optimum conditions were as following: temperature, 65 °C; enzyme load, 7.5%; substrate ratio, 7.5:1 (OA/(FG + glycerol), w/w), and reaction time, 12 h. Under the optimum conditions, the conversion of FG and yield of FDAG reached 98.8 ± 1.0% and 82.6 ± 2.2%, respectively, and the external mass transfer limitations can be eliminated in this reaction strategy. Novozym 435 in the present work can be used 18 times. The reaction kinetic was agreed with Ping-Pong mechanism, and the constants values of the kinetic model were obtained.

Acknowledgements

The authors express their gratitude to the RFDP (The Research Fund for the Doctoral Program of Higher Education), for its financial support (Contract No.: 20070295004). We also thank the Testing & Analytical Center of Jiangnan University.

References

[1] E. Graf, Free Radical Biol. Med. 13 (1992) 435–448.
[2] A. Saija, A. Tomaino, R.L. Cascio, D. Trombetta, A. Proteggente, A.D. Pasquale, N. Uccella, F. Bonina, J. Sci. Food Agric. 79 (1999) 476–480.
[3] C. Anselmi, M. Centini, P. Granata, A. Sega, A. Buonocore, A. Bernini, R. Maffei Facino, J. Agric. Food Chem. 52 (2004) 6425–6432.
[4] H. Kikuzaki, M. Hisamoto, K. Hirose, K. Akiyama, J. Agric. Food Chem. 50 (2002) 2161–2168.
[5] N. Nenadis, H.Y. Zhang, M.Z. Tsimidou, J. Agric. Food Chem. 51 (2003) 1874–1879.
[6] B. Guyot, B. Bosquette, M. Pina, J. Graille, Biotechnol. Lett. 19 (1997) 529–532.
[7] C. Vafiadi, E. Topakas, P. Christakopoulos, J. Mol. Catal. B: Enzym. 54 (2008) 35–41.
[8] K. Vosmann, P. Weitkamp, N. Weber, J. Agric. Food Chem. 54 (2006) 2969–2976.
[9] D.L. Compton, J.A. Laszlo, M.A. Berhow, J. Am. Oil Chem. Soc. 77 (2000) 513–519.
[10] J.A. Laszlo, D.L. Compton, F.J. Eller, S.L. Taylor, T.A. Isbell, Green Chem. 5 (2003) 382–386.
[11] S. Karboune, R. St-Louis, S. Kermasha, J. Mol. Catal. B: Enzym. 52–53 (2008) 96–105.
[12] K. Sabally, S. Karboune, R. St-Louis, S. Kermasha, J. Am. Oil Chem. Soc. 83 (2006) 101–107.
[13] H. Stamatis, V. Sereti, F.N. Kolisis, J. Mol. Catal. B: Enzym. 11 (2001) 323–328.
[14] S. Giuliani, C. Piana, L. Setti, A. Hochkoeppler, P.G. Pifferi, G. Williamson, C.B. Faulds, Biotechnol. Lett. 23 (2001) 325–330.
[15] E. Topakas, H. Stamatis, P. Biely, D. Kekos, B.J. Macris, P. Christakopoulos, J. Biotechnol. 102 (2003) 33–44.
[16] M. Safari, S. Karboune, R. St-Louis, S. Kermacha, Biocatal. Biotransform. 24 (2006) 272–279.
[17] D.L. Compton, J.A. Kenar, J.A. Laszlo, F.C. Felker, Ind. Crop. Prod. 25 (2007) 17–23.
[18] E. Topakas, C. Vafiadi, H. Stamatis, P. Christakopoulos, Enzyme Microb. Technol. 36 (2005) 729–736.
[19] C. Vafiadi, E. Topakas, A. Alissandratos, C.B. Faulds, P. Christakopoulos, J. Biotechnol. 133 (2008) 497–504.
[20] S.D. Sun, L. Shan, Y.F. Liu, Q.Z. Jin, X.G. Wang, Z.M. Wang, Biotechnol. Lett. 29 (2007) 1947–1950.
[21] M. Tsuchiyama, T. Sakamoto, T. Fujita, S. Murata, H. Kawasaki, Biochim. Biophys. Acta 1760 (2006) 1071–1079.
[22] M. Tsuchiyama, T. Sakamoto, S. Tanimori, S. Murata, H. Kawasaki, Biosci. Biotechnol. Biochem. 71 (2007) 2606–2609.
[23] S.D. Sun, L. Shan, Q.Z. Jin, Y.F. Liu, X.G. Wang, Biotechnol. Lett. 29 (2007) 945–949.
[24] D.L. Compton, J.A. Laszlo, M.A. Berhow, J. Am. Oil Chem. Soc. 83 (2006) 753–758.
[25] D.L. Compton, J.A. Laszlo, J. Am. Oil Chem. Soc. 83 (2006) 765–770.
[26] T.K. Yang, M.B. Freukidle, X.B. Xu, Food Chem. 92 (2005) 101–107.
[27] X.B. Xu, A.R.H. Skands, C.-E. Høy, H.L. Mu, S. Balchen, J. Adler-Nissen, J. Am. Oil Chem. Soc. 75 (1998) 1179–1183.
[28] V.M. Balcão, A.L. Paiva, F.X. Malcata, Enzyme Microb. Technol. 18 (1996) 392–416.
[29] J.A. Arcos, C.G. Hill, C. Otero, Biotechnol. Bioeng. 73 (2001) 104–110.
[30] D.Z. Wei, P. Zou, M.B. Tu, H. Zheng, J. Mol. Catal. B: Enzym. 18 (2002) 273–278.
[31] G.D. Yadav, K.M. Devi, Biochem. Eng. J. 10 (2002) 93–101.
[32] Z. Guo, Y. Sun, Food Chem. 100 (2007) 1076–1084.
[33] S. Sabeder, M. Habulin, Z. Knez, J. Food Eng. 77 (2006) 880–886.
[34] A.L. Paiva, V.M. Balcao, F.X. Malcata, Enzyme Microb. Technol. 27 (2000) 187–204.
[35] S. Hari Krishna, N.G. Karanth, Biochim. Biophys. Acta 1547 (2001) 262–267.
[36] A. Zaidi, J.L. Gainer, G. Carta, A. Mrani, T. Kadiri, Y. Belardi, A. Mir, J. Biotechnol. 93 (2002) 209–216.
[37] A.E.M. Janssen, B.J. Sjursnes, A.V. Vakurov, P.J. Halling, Enzyme Microb. Technol. 24 (1999) 463–470.