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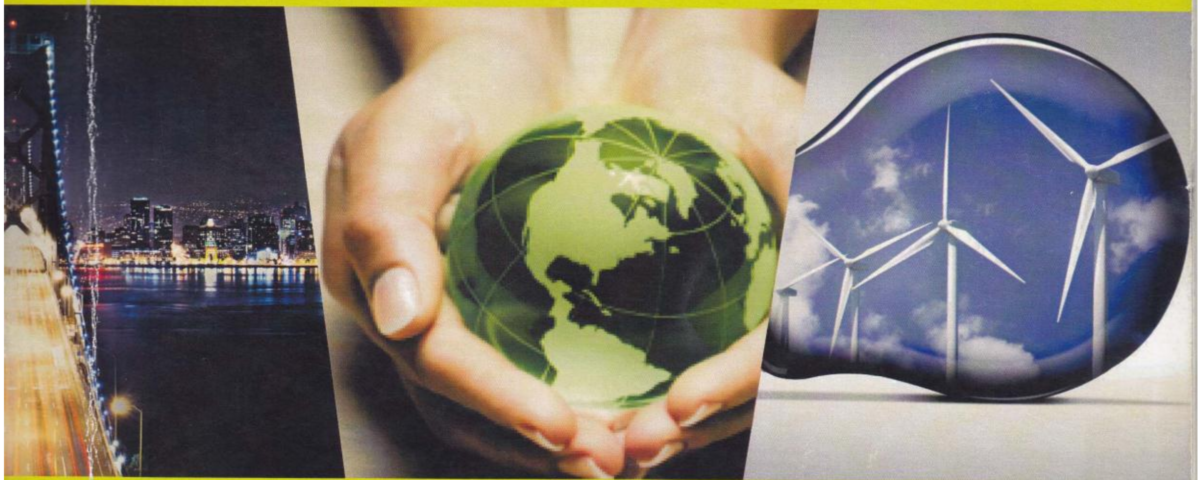

"Intellectual Property Right Based on Green Social Dynamics, Business and Science-Tech"

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"Intellectual Property Right Based on Green Social Dynamics, Business and Science-TechIntellectual Property Right Based on Green Social Dynamics, Business and Science-Tech"

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Kinetics Evaluation on Oleic Acid Ethyl Ester Synthesis Using Lipase From Rice Bran (*Oryza sativa*) and Germinated Jatropha Seeds (*Jatropha curcas.* L)

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Abstract. Recently, lipase has attracted some interests because of its usage in catalyzing esterification reaction for the production of biodiesel (Fatty Acid Ethyl Ester). In this research, lipase from rice bran (Oryza sativa) and germinated jatropha seeds (Jatropha curcas. L) were observed as industry needs low-priced sources of lipase. Kinetics evaluation is very important to understand and further predict the synthesis rate of Oleic Acid Ethyl Ester (OAEE) at certain period. The objective of this study was to evaluate the kinetics of OAEE synthesis catalyzed by lipase from rice bran (Oryza sativa) and germinated jatropha seeds (Jatropha curcas. L). The maximum synthesis rate (Vmax) for lipase from rice bran (Oryza sativa) and germinated jatropha seeds (Jatropha curcas. L) were 60.98 µmol/min and 49.5 µmol/min, respectively. Meanwhile, the Michaelis-Menten constant (Km) for lipase from rice bran and germinated jatropha seeds were 0.073 M and 0.124 M, respectively.

***Keywords**: OAEE; kinetics evaluation; lipase; rice bran (Oryza sativa); germinated jatropha seeds (Jatropha curcas. L).*

1 Introduction.

Lipase (triacylglycerol ester hydrolase, EC 3.1.1.3) is an enzyme that catalyzes both: the hydrolysis of triacilglycerols and the esterification of fatty acid with alcohol (methanol, ethanol, etc) (Olivera et al., 2001; Ganesan et al., 2009; Bisen et al., 2010; Watanabe et al., 2007; Salis et al., 2008). Recently, the usage of biocatalyst, such as lipase, is preferable for the synthesis of biodiesel (Fatty Acid Alkyl Ester) since the usage of chemical catalyst may not be friendly for environment (Halim et al., 2008; Chesterfield et al., 2012; Sotoft et al., 2010; Ghaly et al., 2010). Moreover, the enzymatic synthesis offers several advantages such as mild condition, low energy requirement, minimal waste disposal, and etc (Petersson et al., 2005).
The explorations of low-priced lipase sources have been carried out for industrial applications. It was reported that lipase was produced from microbial sources (*Rhizomucor miehei, Candida rugosa, Candida antartica, Aspergillus niger, Bacillus sp, Pseudomonas sp,* etc) (Ganesan et al., 2009; Bisen et al., 2010; Watanabe et al., 2007; Salis et al., 2008) and plant sources (germinated seeds of *Jatropha curcas* and rice bran) (Abigor et al., 2002; Enujiugha et al., 2004; Tuter et al., 2003; Haas et al., 2001; Natarajan *et al*, 2010; Chuang *et al.*, 2010; Bhardwaj et al., 2001). Germinated seeds of *Jatropha curcas* and rice bran may be

potential as low-priced sources of lipase since they are abundant in some tropical countries, including in Indonesia. On the other hand, the usage of crude lipase is preferable for biodiesel/Fatty Acid Alkyl Ester (FAAE) production.
In this research, the kinetics parameter of Oleic Acid Ethyl Ester (OAEE) synthesis that was catalyzed using crude lipase was evaluated. Most of the kinetics studies of enzymatic esterification in solvents system are frequently based on the application of Michăelis–Menten assumption, which is only valid for simple, controlled or optimized enzymatic reactions (Cabral et al., 2009; Goddard et al., 1999; Khrisna and Karanth., 2001). Therefore, the Michăelis–Menten assumption was applied in this research since the reaction was conducted at optimum condition as obtained in previous research (Prastowo et al., 2012).

2 Material and Methods.

2.1 Material.

Jatropha seeds were obtained from Forestry Department Office of Gunung Kidul Regency, The Special District of Yogyakarta. The fresh rice bran from the rice variety IR-4, the most grown rice in Indonesia, was obtained from local supplier in The Special District of Yogyakarta, Indonesia. Pyridine, oleic acid, acetone, isooctane, $NaH_2PO_4.H_2O$, $NaH_2PO_4.2H_2O$, ethanol and cupri-acetate were obtained from Merck KGaA (Darmstadt, Germany). Fungicide was obtained from local supplier.

2.2 Crude Enzyme Preparation.

Jatropha seeds were selected and further soaked in the mixture of 0.1 M phosphate buffer pH 6 and fungicide (1.5 g/l) for 12 h at the room temperature. After removing the excess water, the seeds were aerated for 1 h prior to be re-hydrated at relative humidity of 90% for 24 h. The seeds were germinated in incubator at room temperature until the length of shoots reached 2 – 2.5 cm. The germinated jatropha seeds were stored at – 20 °C directly after harvesting. Meanwhile, the fresh rice bran was also stored at – 20 °C to prevent protein denaturation.
Twenty gram of peeled germinated jatropha seeds and rice bran were homogenized in 35 mL cold acetone (-20 °C) using homogenizer (IKA T-50, Germany). Then, the feedstock were further defatted in Soxhlet using acetone for 30 min prior to be dried at room temperature and stored at – 20 °C until used.

3 Experimental Procedure.

The enzymatic reaction was conducted at the optimum condition as obtained from previous research (Prastowo *et al.*, 2012). Defatted rice bran (29.58 g) and germinated jatropha seeds (31.02 g) were added into 100-mL oleic acid solution in various concentrations (0.1 M, 0.2 M, 0.3 M, 0.4 M, 0.5 M). Subsequently, ethanol was added into the mixture to obtain molar ratios (oleic acid/ethanol) of 1 : 2.05 and 1 : 1.82, for rice bran and germinated jatropha seeds, respectively. Samples were analyzed every 10 minutes during reaction (60 minutes).

3.1 Maximum Reaction Rate (Vmax) and Michalelis-Menten Constant (Km) Determination.

Reaction rate (v) was determined as the amount of produced ester per minute during early reaction (0 - 10 min), in which enzyme catalyzes the reaction in a constant rate. The Michaelis-Menten constant (Km) and the maximum reaction rate (Vmax) were determined by plotting reciprocal values of reaction rate (1/V) and substrate concentration (1/S) as shown in Table 1, on Lineweaver-Burk plot following eq.1.

$$\frac{1}{v} = \frac{K_M}{V_{\max}[S]} + \frac{1}{V_{\max}} \quad (1)$$

Table 1 Table 1. Reaction rate (V), substrate concentration (S), and their reciprocal values of esterification reaction using lipase from rice bran and germinated jatropha seeds.

Rice Bran				Germinated Jatropha Seeds			
V (μmol/min)	S (M)	1/V (min/μmol)	1/S (1/M)	V (μmol/min)	S (M)	1/V (min/μmol)	1/S (1/M)
35.85	0.1	0.028	10	22.69	0.1	0.044	10
45.15	0.2	0.022	5	29.46	0.2	0.034	5
48.23	0.3	0.021	3.33	33.46	0.3	0.030	3.33
52.92	0.4	0.019	2.5	39.31	0.4	0.025	2.5
53.62	0.5	0.019	2	42.08	0.5	0.024	2

The y-intercept is equivalent to the reciprocal value of maximum reaction rate (Vmax) and the slope represents Km/Vmax. By reciprocating y-intercept and multiplying slope by the reciprocal value of y-intercept, Vmax and Km were obtained.

4 Analysis

The amount of OAEE was determined according to Prastowo *et al* (2012). The amount of OAEE was expressed as the amount of FFA reacting with ethanol to form OAEE. Sample (200 μL) was added into the mixture of isooctane (1.8 mL) and cupri-acetate pyridin (0.4 mL). Subsequently, it was incubated at 30 °C for 10 minutes, and the absorbance of mixture was determined at 715 nm using spectrophotometer (Genesys-20, USA). FFA was determined by difference of absorbance. The amount of OAEE was determined by the difference between the amount of FFA at initial period (without crude enzyme) and the amount of FFA at distinctive period (using crude enzyme).

5 Result and Discussion

The synthesis of OAEE using crude lipase from rice bran and germinated jatropha seeds increased rapidly in the first 10 minutes of reaction, a short period after the reaction started (Fig. 1). The synthesis rate of esterification reaction (v) was determined as the production of ester in the first 10 minutes of reaction, in which lipase catalyzes an esterification reaction in constant rate during this period.

Furthermore, the reaction became saturated and its rate decreased gradually from the first 10 minutes of reaction to the end of reaction. It is suggested that the saturation was probably caused by the production of the water as by product of the esterification reaction during the reaction. The presence of water may lead lipase to hydrolyze the forming ester (Tongboriboon et al., 2010; Salis et al., 2005; Adlercreutz., 2000; Hsu et al., 2002; Polaina and MacCabe., 2007).

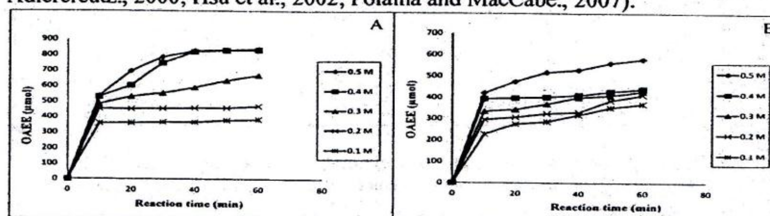


Figure 1 . OAEE synthesis in various substrate concentrations using lipase from rice bran (A) and germinated jatropha seeds (B).

Fig. 2 shows that an increase in substrates concentration (oleic acid) resulted in an increase in synthesis rate of OAEE (v). It is suggested that an increase in substrate concentration may increase the production of OAEE in high concentration.

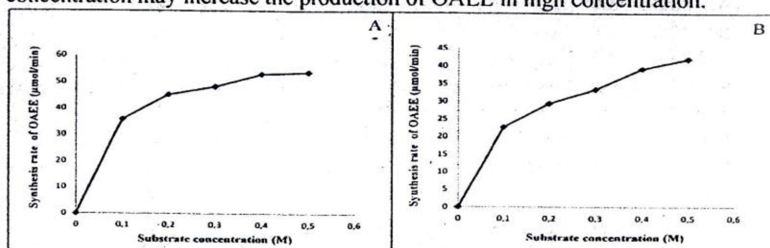


Figure 2 OAEE synthesis rate in an increase of substrate concentrations using lipase from rice bran (A) and germinated jatropha seeds (B).

Fig. 3 shows the plotting of the reciprocal value of synthesis rate (1/V) and substrate concentration (1/S) on Lineweaver-Burk plot. The linear equations ($y = 0,0012x + 0,0164$ and $y = 0,0025x + 0,0202$) were obtained in Fig. 3 for rice bran lipase and germinated jatropha seeds lipase, respectively. The Vmax and Km were thus obtained by reciprocating y-intercept and multiplying slope by the inverse of y-intercept, as shown in Table 2.

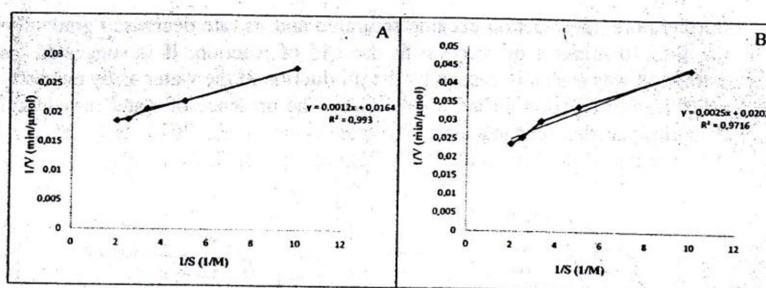
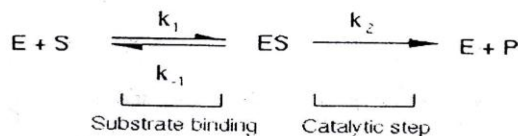


Figure 3 Lineweaver-Burk plot of reciprocal values of reaction rate (V) and substrate concentration (S) using lipase from rice bran (A) and germinated jatropha seeds (B).

Table 2 Maximum synthesis rate (Vmax) and Michaelis-Menten constant (Km) for lipase from rice bran (A) and germinated jatropha seeds (B).

Rice Bran		Germinated Jatropha Seeds	
Vmax (µmol/min)	Km (M)	Vmax (µmol/min)	Km (M)
60.98	0.073	49.50	0.124

Michaelis-Menten constant (Km) for lipase from rice bran and germinated jatropha seeds were 0.073 M and 0.124 M, respectively. Michaelis-Menten constant (Km) is a constant that explains the binding of substrate into the active site of enzyme (Walsh et al., 2007). The substrate may tightly be bound into the active site of enzyme as the Km is low. In contrast, the substrate may weakly be bound into the active site of enzyme as the Km is high (Walsh et al., 2007). This suggestions can be further explained by the eq. 2 and eq. 3 as described below :



(2)

$$Km = \frac{K_2 + K_{-1}}{K_1} \quad (3)$$

K_{-1} denotes the constant of Enzyme-Substrate complex [ES] dissociation, K_1 denotes the constant of Enzyme-Substrate complex [ES] formation, and K_2 denotes the constant of catalytic. In the high Km, the constant of Enzyme-Substrate complex [ES] dissociation (K_{-1}) is higher than the constant of Enzyme-Substrate complex [ES] formation (K_1) while the K_2 is constant. In this condition, substrate which is bound into active site of enzyme may be easily dissociated. It means that the affinity of enzyme is weak. Meanwhile in the low Km, the constant of Enzyme-Substrate complex [ES] dissociation (K_{-1}) is smaller than the constant of Enzyme-Substrate complex [ES] formation (K_1). In this condition, substrate

which is bound into active site of enzyme may not be easily dissociated and furthermore, enzyme may catalyze substrate into product (Walsh et al., 2007).
In this study, the Km of rice bran lipase was 0.59 times lower than the Km of germinated jatropha seeds lipase. It is suggested that the affinity of rice bran lipase to substrate is higher than that of germinated jatropha seeds lipase. As the affinity of rice bran lipase to substrate is higher, OAEE synthesis rate is higher. The Vmax is defined as the maximum rate of OAEE synthesis at certain period (Walsh et al., 2007). In this study, Vmax of rice bran lipase was 1.23 times higher than that of germinated jatropha seeds lipase. It is suggested that rice bran lipase may synthesize ester higher than germinated jatropha seeds lipase.

6 References.

Abigor,R; Uadia, P; Foglia, T; Haas, M; Scott, K; Savary, B. 2002. Partial Purification and Properties of Lipase from Germinating Seeds of *Jatropha curcas L.*, JAOCS, Vol. 79, no. 11 (2002).

Adlercreutz, P. Biocatalysis in non-conventional media. In: Applied Biocatalysis, A.J.J. Straathof and P. Adlercreutz. (eds.), Harwood Academic Publishers, (2000) pp. 295–316.

Al-Zuhair, S, F.W. Ling, L.S. Jun. Proposed kinetic mechanism of the production of biodiesel from palm oil using lipase, Process Biochem. 42 (2007) 951–960.

Bamdad, F, J. Wu, L. Chen. Effects of enzymatic hydrolysis on molecular structure a antioxidant activity of barley hordein, J.Cereal.Sci. 54 (2011) 20-28.

Bhardwaj, K; Raju, A; Rajasekharan, R. 2001. Identification, Purification, and Characterization of a Thermally Stable Lipase from Rice Bran. A New Member of the (Phospho) Lipase Family, Plant Physiology, December 2001, Vol. 127, pp. 1728–1738

Bisen, P.S., Sanodiya, B.S., Thakur, G.S., Baghel, R.K., dan Prasad, G. B. K. S. 2010. Biodiesel production with special emphasis on lipase-catalyzed transesterification. Biotechnol. Lett. vol 32:1019–1030.

Budic, M; Kidric, M; Meglic, V; Cigig, B. A quantitative technique for determining proteases and their substrate specificities and pH optima in crude enzyme extracts. Analytical Biochemistry 388 (2009) 56–62.

Cabral, PP, da Fonseca, MMR, Farriera-Dias, S, 2009, Synthesis of ethyl butyrate in organic media catalyzed by *Candida rugosa* lipase immobilized in polyurethane foams: A kinetic study. Biochemical Engineering Journal 43 (2009) 327–332.

Chesterfield, DM, PL. Rogers, EO. Al-Zaini, AA. Adesina. Production of biodiesel via ethanolysis of waste cooking oil using immobilised lipase. Chem.Eng. (2012).

Chuang, HH; Chen,PT; Wang, WN; Chen, YT; Shaw, JF.2010. Functional Proteomic Analysis of Rice Bran Esterases/Lipases and Characterization of a Novel Recombinant Esterase. J. Agric. Food Chem. 2011, 59, 2019–2025.

Devappa, RK; Makkar HPS; Bekker, K; 2010, Nutritional, Biochemical, and Pharmaceutical Potential of Proteins and Peptides from Jatropha: Review. J. Agric. Food Chem. 2010, 58, 6543–6555.

Enujiugha VN, Thani FA, Sanni TM and Abigor RD. 2004. Lipase activity in dormant seeds of the african oil bean *(Pentaclethra macrophylla* Benth*). Food Chem* **88**: 405-410.

Fukuda, H, A. Kondo and H. Noda. Biodiesel fuel production by transesterification of oil. J. Biosci. Bioeng. (2001) *92*(5), 405–416.

Ganesan, D; Rajendran, A; Thangavelu, V. 2009. An overview on the recent advances in the transesterification of vegetable oils for biodiesel production using chemical and biocatalysts, Rev Environ Sci Biotechnol (2009) 8:367–394 DOI 10.1007/s11157-009-9176-9.

Ghaly, AE, D. Dave, M.S. Brooks, S. Budge. Production of biodiesel by enzymatic transesterification: review, Am. J. Biochem. Biotechnol. 6 (2010) 54–76.

Goddard, R, Bosley, J, Al-Duri, B, 1999, Esterification of oleic acid and ethanol in plug flow (packed bed) reactor under supercritical conditions investigation of kinetics. Journal of Supercritical Fluids 18 (2000) 121–130.

Haas MJ, Cichowicz DJ and Dierov JK. 2001. Lipolytic activity of california-laurel (*Umbellularia californica*) seeds. *J Am Oil Chem Soc* **78**: 1067–1071.

Halim, SFA, A. Harun Kamaruddin, Catalytic studies of lipase on FAME production from waste cooking palm oil in a tert-butanol system, Process Biochem. 43 (2008) 1436–1439.

Hidayat, C., M. Kuntoro, M.D.P, Hastuti, P., Sumangat, D., and Hidayat, T. 2008. Optimasi Sintesis Metil Oleat Menggunakan Biokatalis Lipase dari Kecambah Biji *Jatropha Curcas* L. J. Penelitian Pascapanen Pertanian 5: 1-9

Hsu, AF, K. Jones, T.A. Foglia and W.N. Marmer. Immobilized lipase-catalysed production of alkyl esters of restaurant grease as biodiesel. Biotechnol. Appl. Biochem. (2002) *36*, 181–186.

Prastowo, I, C. Hidayat, P. Hastuti. Production and Optimization of Oleic Acid Ethyl Ester Synthesis Using Lipase From Rice Bran (*Oryza sativa*) and Germinated Jatropha Seeds (*Jatropha curcas.*L) by Response Surface Methodology, Indonesian Journal of Biotechnology (2012), 17 : 17-26.

Khrisna, S, Karanth, NG, 2001, Lipase-catalyzed synthesis of isoamyl butyrate : A kinetic study. Biochimica et Biophysica Acta 1547 (2001) 262-267

Lee, KT, T.A. Foglia and K.-S. Chang. Production of alkyl ester as biodiesel from fractionated lard and restaurant grease. J. Am. Oil Chem. Soc. (2002) *79*, 191–195.

Mahmud, MS, T. Safinski, M.I. Nelson, H.S. Sidhu, A.A. Adesina. Kinetic analysis of oleic acid esterification using lipase as catalyst in a microaqueous environment, Ind. Eng. Chem. Res. 49 (2009) 1071–1078.

Medina, AR, P.A. González-Moreno, L. Esteban-Cerdán, E. Molina-Grima. Biocatalysis: towards ever greener biodiesel production, Biotechnol. Adv. 27 (2009) 398–408.

Natarajan, P; Kanagasabapathy, D; Gunadayalan, G; Panchalingam, J; shree, N; Sugantham, PA; Singh, KK; Madasamy, P. 2010. Gene discovery from Jatropha curcas by sequencing of ESTs from normalized and full-length enriched cDNA library from developing seeds, BMC Genomics 2010, 11:606.

Nurikasari, M, 2011, Optimasi Kondisi Purifikasi Parsial, Karakterisasi dan Kinetika Esterifikasi Lipase *Aspergillus niger* 65I6 dari *Solid State Fermentation* pada Medium Bungkil Jarak. Thesis Program Pasca Sarjana, Universitas Gadjah Mada.

Oliveira AC, Rosa MF, Aires-Barros, MR, Cabral JMS. 2001. Enzymatic esterification of ethanol and oleic acid : A kinetic study. Journal of Molecular Catalysis B: Enzymatic 11. 2001. 999–1005.

Ophart, CE. 2003. Virtual Chemical Handbook, Elmhurst College, http://www.elmhurst.edu/~chm/vchembook/568denaturation.html, retrieved on 15 November 2011

Páez, BC, A.R. Medina,- F.C. Rubio, P.G. Moreno, E.M. Grima. Modeling the effect of free water on enzyme activity in immobilized lipase-catalyzed reactions in organic solvents, Enzyme Microb. Technol. 33 (2003) 845–853.

Petersson, AEV, LM. Gustafsson, M. Nordblad, P. Borjesson, B. Mattiasson, P. Adlercreutz. Waxesters produced by solvent-free energy-efficient enzymatic synthesis and their applicability as wood coatings. Green Chem. (2005) 7, 837–843.

Polaina, J, A.P. MacCabe. Industrial Enzymes : Structure, Function and Applications, Springer, Dordrecht, The Netherlands 653 pp (2007).

Salis, A, M. Monduzzi and V. Solinas. Enzymes for biocatalysis in non aqueous media. Biocatalysis: Chemistry and Biology. A. Trincone. Kerala, Research Signpost. (2005) 29–53.

Salis A, Pinna M, Monduzzi M, Solinas V. 2008. Comparison among immobilized lipases on macroporous polypropylene toward biodiesel synthesis. J Mol Catal B: Enzym 54(1–2):19–26.

Shi, H, L. Xiong, KY. Yang, CQ. Tang, TY. Kuang, NM Zhao. Protein secondary structure and conformational changes of photosystem II during heat denaturation studied by Fourier transforminfrared spectroscopy, J.Mol.Struc. 446 (1998) 137-147.

Shimada, Y, Y. Watanabe, T. Samukawa, A. Sugihara, H. Noda, H. Fukuda, Y. Tominaga. Conversion of vegetable oil to biodiesel using immobilized Candida antarctica lipase, J. Am. Oil. Chem. Soc. 76 (1999) 789–793.

Sotoft, LF, B.G. Rong, K.V. Christensen, B. Norddahl, Process simulation and economical evaluation of enzymatic biodiesel production plant, Bioresour. Technol. 101 (2010) 5266–5274.

Tongboriboon, K, B. Cheirsilp, AH. Kittikun. Mixed lipases for efficient enzymatic synthesis of biodiesel from used palm oil and ethanol in a solvent-free system, J.Mol.Cat.B: Enz. 67 (2010) 52–59.

Tuter M, Secundo F, Riva S, Aksoy HA and Ustun G. 2003. Partial purification of *Nigella sativa* L. seed lipase and its application in trans-esterification reactions. *J Am Oil Chem Soc* **80**: 43 – 48.

Walsh R, Martin E, Darvesh S. A versatile equation to describe reversible enzyme inhibition and activation kinetics: modeling beta-galactosidase and butyrylcholinesterase. Biochim Biophys Acta. 2007 1770:733-46.

Watanabe, Y., T. Nagao, Y. Nishida, Y. Takagi, dan Y. Shimada. 2007. Enzymatic Production of Fatty Acid Methyl Esters by Hydrolysis of Acid Oil Followed by Esterification. J. Am. Oil Chem. Soc. vol 84 : 1015-1021.