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Research Article

Transesterification reaction for palm based wax esters by immobilized lipase EQ3 isolated from wastewater of fish canning industry

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Abstract

Palm olein based wax esters were synthesized by tranesterification for increasing value of palm olein and applications as well as reducing chemical usage. The immobilized lipase EQ3 from *Burkholderia* sp. EQ3 which was isolated from wastewater of fish canning factory was used as biocatalyst in liquid wax esters synthesis reaction. The optimal reaction conditions for palm olein based wax esters synthesis were studied for immobilized lipase EQ3 dosages, temperatures, the molar ratios of palm olein to oleyl alcohol, substrate concentrations and organic solvents. All of these factors had an effect on efficiencies of enzymatic transesterification reaction. The optimal conditions were 10 U of immobilized lipase loading, temperature of 37°C, the molar ratio of palm olein to oleyl alcohol at 1:3 (mol/mol) and the substrate concentration of 0.33% (w/v) in isooctane, and 12 h of the reaction time producing the palm olein based wax esters reached the highest synthesis more than 80%.

Keywords: *Burkholderia* sp. EQ3, lipase EQ3, transesterification, wax esters

Introduction

Lipases are one of the most usage in industrial applications that are also growing rapidly (Schmid et al., 2001). The microbial lipases produce the great variety of catalytic activities, rapid growth of microorganisms on inexpensive media with independent seasonal fluctuations. *Burkholderia* lipases have been widely researched in terms of their commercial potential in a variety of biotechnological applications such as in food, dairy, detergent and pharmaceutical industries. Indeed, the *Burkholderia* genus is one of the most important lipase-producing microorganism (Mullen et al., 2007). Generally, the enzymatic reactions provide for an environmentally friendlier, more energy efficient and cost-effective techniques due to low-energy demanding operation and simpler downstream processing. However, using native enzyme as a biocatalyst presents some drawbacks, such as poor stability under operational conditions, difficulty of product recovery, and impossibility of multiple reuses in industrial process. The enzyme immobilization techniques have been improved their functionality, stabilized catalytic properties and reusability of enzyme, for example, the enzyme immobilized on carriers via covalent binding (Yemul & Imae, 2005; Yong et al., 2008), entrapment (Ge et al., 2009), or adsorption (Sörensen et al., 2010). Moreover, the immobilization enzyme provides

Vol. 17 Special issue: 9-17 [2018]
doi: 10.14416/j.appsci.2018.02.S02

for its repeated use as well as it is easy separation from the reaction medium (Minovska et al., 2005).

Wax esters can be extracted from animal and plant materials such as beeswax, jojoba oil and carnauba wax. They are widely used in plasticizer, lubricant, pharmaceutical and cosmetic industries (Gunawan et al., 2004). Wax esters composed of long chain fatty acids from fat or oil and fatty alcohol (Petersson et al., 2005). For wax esters synthesis, the chemical-catalyzed method has many disadvantages, such as corrosive acids required, hazards in handling corrosive chemicals, high energy consumption and degradation of synthesized esters (Knox & Cliffe, 1984; Yadav & Lathi, 2003). On the other hand, lipase-catalyzed process has been increasingly used for wax esters synthesis because of mild reaction condition and environmental friendly process. Gunawan et al. (Gunawan et al., 2004) synthesized palm-based wax esters from palm oil and oleyl alcohol by Lipozyme IM. Decagny et al. (Decagny et al., 1998) studied alcoholysis between triolein and stearyl alcohol to produce wax esters. Moreover, Radzi et al. (Radzi et al., 2005) studied large scale production of liquid wax ester from oleic acid and oleyl alcohol using Novozyme 435. From the above information, the immobilized lipases are widely used, and the immobilized forms make them achieving the stability for industrial applications.

The aims of this research were to investigate the liquid wax esters synthesis from palm olein and oleyl alcohol catalyzed by immobilized lipase EQ3 with optimization the transesterification reaction.

Materials and methods

Bacterial strain and immobilized lipase EQ3

Burkholderia sp. EQ3 producing an extracellular lipase was isolated from wastewater treatment system of the tuna canning factory in southern of Thailand on basal medium containing 1% fish oil at 37°C for 12 h (Ungcharoenwiwat & H-Kittikun, 2013). The crude lipase EQ3 from acetone precipitation was immobilized onto Accurel MP-100 as hydrophobic carrier obtained from Akzo Nobel Membrana (Obernburg, Germany). The immobilized lipase EQ3 was prepared with various enzyme activities by adsorbtion technique (Ungcharoenwiwat et al., 2016). The immobilized lipase EQ3 was stored at 4°C until use.

Chemicals

Palm olein was supplied from the local market (Hat Yai, Thailand). Oleyl alcohol was supplied by Sigma-Aldrich Corp. (St. Louis, Missouri, USA). All other chemicals used were of analytical grade.

Analyses

Lipase activity

The lipase activity was assayed in a two-phase system according to Lee and Rhee (Lee & Rhee, 1993) using 10% (w/v) palm olein dissolved in isooctane as a substrate. One unit of enzyme activity was defined as the enzyme necessary to release 1 μmol of palmitic acid per minute at the specified condition.

Wax esters analysis

Wax esters synthesis through transesterification reaction was analyzed on silica-coated quartz rods, chromarods S III (Silica gel powder coated) from Mitsubishi Chemical Medicine Corporation (Tokyo, Japan) using a thin layer chromatography-flame ionization detection

The Journal of Applied Science **Vol. 17 Special issue: 9-17 [2018]**
วารสารวิทยาศาสตร์ประยุกต์ doi: 10.14416/j.appsci.2018.02.S02

system (TLC-FID) (IATROSCAN MK5, Iatron Laboratories Inc. (Tokyo, Japan)) (Ungcharoenwiwat & H-Kittikun, 2013).

The factors effecting on transesterification reaction for palm oil based wax esters synthesis by immobilized lipase EQ3

The reaction mixture consisted of palm olein (150 μmol, 142.8 mg), oleyl alcohol (450 μmol, 120.8 mg), immobilized lipase and hexane (1 mL) in screwed-cap tube. The reaction was incubated at 37°C and 150 rpm for 72 h. All experiments were assayed in duplicate. The samples were collected and diluted with chloroform (1:2 v/v). The percentage of wax esters synthesis for each reaction was determined using TLC-FID analysis. The factors following below were studied while the other conditions were fixed.

1. *Effect of enzyme concentration:* 2 U, 5 U and 10 U
2. *Effect of temperature:* 30°C, 37°C and 45°C
3. *Effect of substrate molar ratio:* 1:1, 1:2, 1:3 and 1:4 mol/mol (palm olein/oleyl alcohol)
4. *Effect of substrate concentration:* the optimum molar ratio of palm olein and oleyl alcohol (1:3 mol/mol, 1.0X). The substrate concentration levels at 0.33X, 0.66X and 1.0X were investigated.
5. *Effect of organic solvent:* toluene (log P=2.5), hexane (log P=3.5), heptane (log P=4.0) and isooctane (log P=4.5)

Results and discussion

Factors effecting on palm olein based wax esters synthesis by immobilized lipase EQ3

Amount of immobilized lipase EQ3

The amount of enzyme is one of the most important factors for wax esters synthesis. It can increase the reaction rate at appropriate amount of enzyme. For all cases studied, the reaction rate of palm olein based wax esters synthesis enhanced rapidly when increasing the immobilized enzyme dosage from 2 (56%) to 10 U (87%) (Figure 1). Moreover, the immobilized lipase EQ3 obtained the highest percentage of wax esters when using 10 U of enzyme with highest wax esters for 24 h (84.31%). The wax esters synthesis had been slightly increased after 24 to 72 h. This result was agreement with Gunawan and Suhendra (Gunawan & Suhendra, 2008), the production of palm kernel oil based wax esters was not increased beyond the time that might due to the mass-transfer limitations and the reaction rate of forward and reverse equilibrium. Thus, the immobilized lipase at 10 U was selected for wax esters synthesis in the subsequent experiments.

Temperature

The temperature effects on the substrate solubility as well as its influencing on the enzyme molecule and substrate solubility. The temperatures in reaction mixture were investigated in ranges at 30-45°C, and the reaction time was maintained at 24 h (Figure 2). The temperature had slightly affected on transesterification, and the palm olein based wax esters were found to be a maximum of 85.60% at 45°C for 24 h. In the previous work, the optimum temperature for wax esters synthesis of crude fish fat and cetyl alcohol using the free lipase EQ3 from acetone precipitation was at 37°C (Ungcharoenwiwat & H-Kittikun, 2013), and

Vol. 17 Special issue: 9-17 [2018]
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the immobilized form of lipase EQ3 on Accurel could improve the thermal stability of tranesterification.

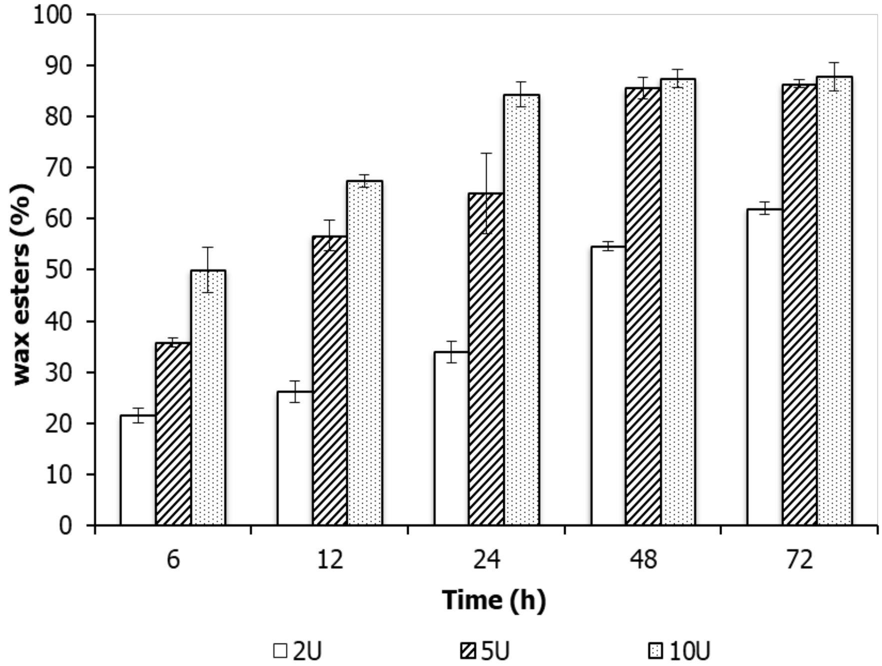

Figure 1. Effect of enzyme concentrations on palm olein based wax esters synthesis by immobilized lipase EQ3 (reaction mixture: substrate molar ratio 1:3, hexane 1 ml, at 37°C and 150 rpm).

Substrate molar ratio

The substrate molar ratio influences the product yield. The results given in Figure 3, show that the increasing the palm olein to oleyl alcohol molar ratio from 1:1 to 1:3 (mol/mol) led to increase the percentage of wax esters. The optimum molar ratio of palm olein and oleyl alcohol was 1:3 (mol/mol) with the highest wax esters of 80.27% for 24 h. Moreover, the rate of wax esters synthesis was reduced at 1:4 (mol/mol). Gunawan and Suhendra (Gunawan & Suhendra, 2008) also found the highest palm kernel oil alcoholysis at 1:3 (mmol of palm kernel oil/mmol of oleyl alcohol) with 86.4% wax esters. The decreasing of tranesterification reaction indicated that oleyl alcohol might inhibit the lipase activity. Due to the access alcohol could distort the essential water layer around the enzyme and then obstruct the oil diffusion and reduce the catalytic activity (Gunawan & Suhendra, 2008; Ungcharoenwiwat & H-Kittikun, 2013).

The Journal of Applied Science
วารสารวิทยาศาสตร์ประยุกต์

Vol. 17 Special issue: 9-17 [2018]
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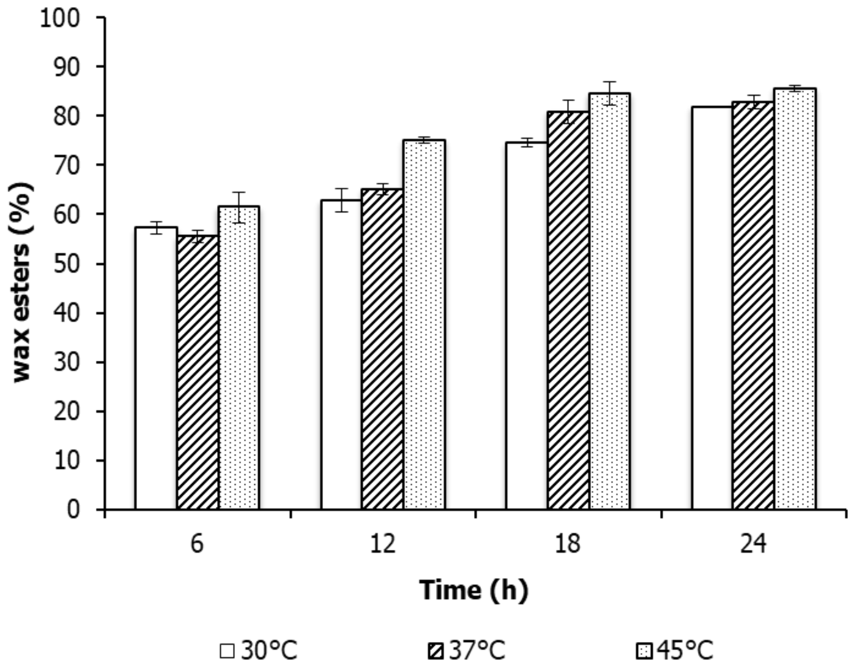

Figure 2. Effect of temperature on the synthesis of palm olein based wax esters by immobilized lipase EQ3 (reaction condition: enzyme 10 U, substrate molar ratio 1:3, hexane 1 ml and 150 rpm).

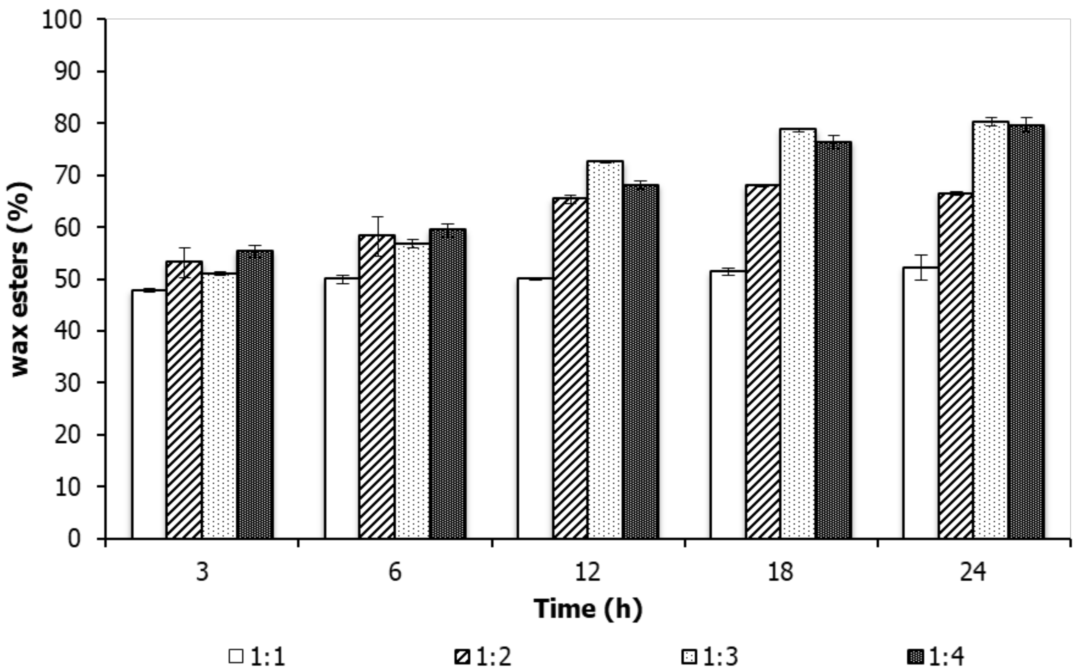

Figure 3. Effect of molar ratio of palm olein and oleyl alcohol on wax esters synthesis by immobilized lipase EQ3 (reaction condition: enzyme 10 U at 45°C, hexane 1 ml and 150 rpm).

Vol. 17 Special issue: 9-17 [2018]
doi: 10.14416/j.appsci.2018.02.S02

Substrate concentration

The substrate concentrations seem to play an important role for increasing the synthesis yield of wax esters and the reaction rate. The result was shown in Figure 4. The three levels of substrate concentration were varied at 0.33X, 0.66X and 1.0X of the optimum molar ratio of substrate. The palm olein based wax esters synthesis by the immobilized lipase EQ3 was exhibited in minimum substrate concentration (0.33X) with the highest alcoholysis level. At 0.33X, the wax esters synthesis was rapidly increased to 87.59% for 12 h. Moreover, the wax esters synthesis was slowed down when increasing the substrate concentration. At high substrate concentration had negative effect on attachment of enzyme and substrate (Al-Zuhair, 2005).

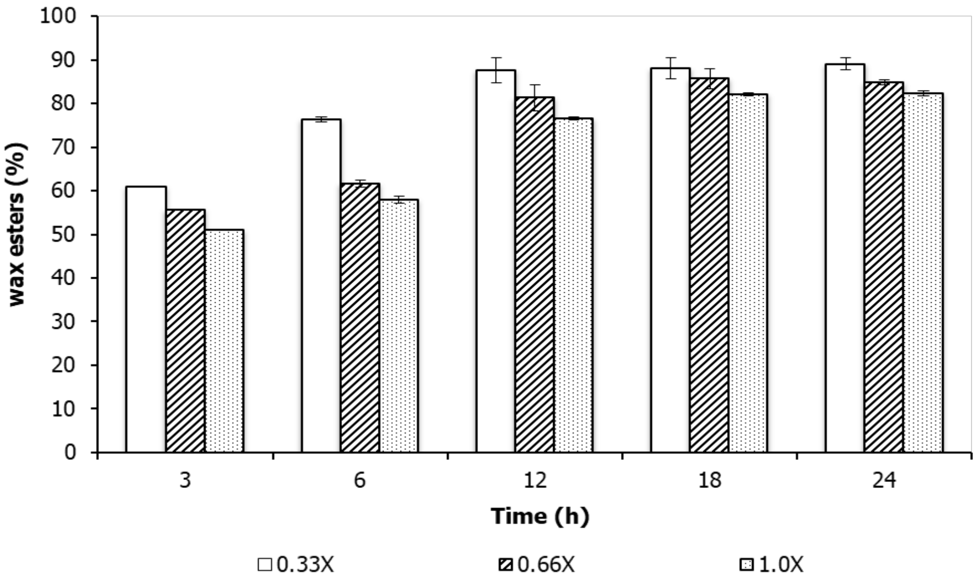

Figure 4. Effect of substrate concentration on palm olein based wax esters synthesis by immobilized lipase EQ3 (reaction condition: enzyme 10 U, substrate molar ratio 1:3, at 45°C, hexane 1 ml and 150 rpm).

Various organic solvents

The palm olein based wax esters synthesis by transesterification had been performed in various organic solvents with immobilized lipases. The synthesis with organic solvent system displayed more efficiency of enzymatic process. In this study, the polarity of organic solvents in terms log P value had an influence on alcoholysis reaction (Figure 5). The result showed that the immobilized lipase EQ3 exhibited high wax esters synthesis in non-polar organic solvents (log P $\geq$ 3.5). The wax esters synthesis was the highest of 89.05% at 12 h in isooctane. Moreover, the immobilized lipase EQ3 synthesized low amount of wax esters in higher polarity of organic solvent due to they could strip off the essential water layer around the enzyme molecule that preserved its active conformation (Gunawan & Suhendra, 2008).

The Journal of Applied Science **Vol. 17 Special issue: 9-17 [2018]**
วารสารวิทยาศาสตร์ประยุกต์ doi: 10.14416/j.appsci.2018.02.S02

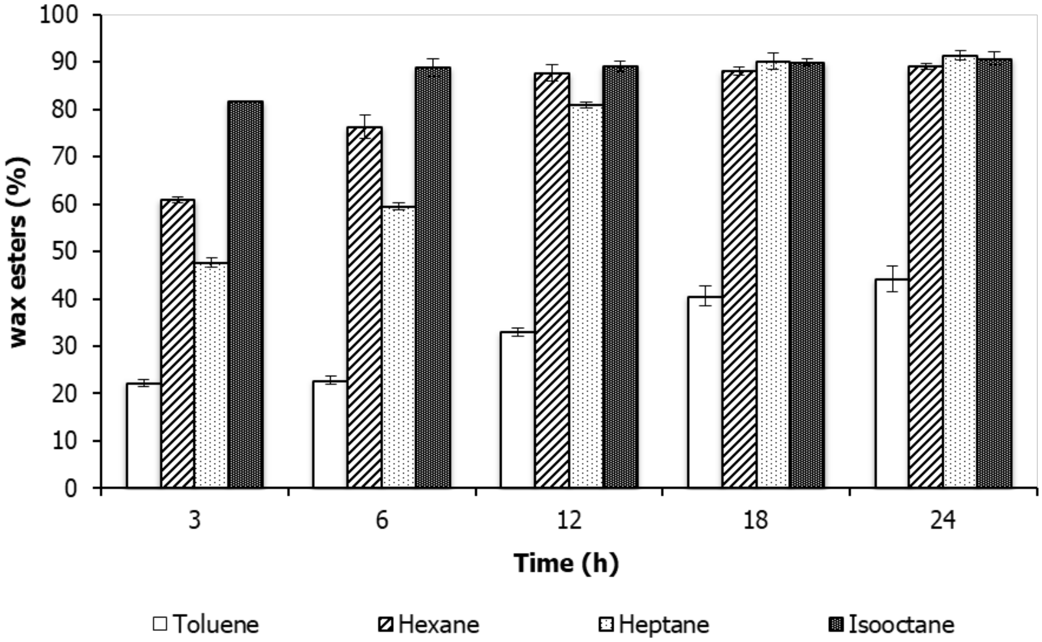

Figure 5. Effect of organic solvents on palm olein based wax esters synthesis by immobilized lipase EQ3 (reaction condition: enzyme 10 U, substrate molar ratio 1:3 (0.33X), at 45°C and 150 rpm).

Conclusion

In this study, the immobilized lipase EQ3 on Accurel MP-100 has enable to catalyst the wax esters synthesis reaction by tranesterification of palm olein and oleyl alcohol with reasonable yield. The optimum condition for wax esters synthesis was 10 U of immobilized lipase EQ3, 45°C and substrate molar ratio 1:3 (mol/mol) dissolved in isooctane with 89% at 12 h. In this research, the study in physicochemical properties of palm olein based wax esters is continued interestingly to know its application. The palm olein based wax esters was long chain liquid wax esters that could be potentially applied in cosmetics, pharmaceuticals or lubricants industries.

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The Journal of Applied Science **Vol. 17 Special issue: 9-17 [2018]**
วารสารวิทยาศาสตร์ประยุกต์ doi: 10.14416/j.appsci.2018.02.S02

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Vol. 17 Special issue: 9-17 [2018]
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