Reaktor

p-ISSN 0852 – 0798
e-ISSN 2407 – 5973

Acredited: SK No.: 60/E/KPT/2016
Website : http://ejournal.undip.ac.id/index.php/reaktor/

Reaktor, Vol. 18 No. 2, June Year 2018, pp. 71-75

Performance of Various Organic Solvents as Reaction Media in Plant Oil Lipolysis with Plant Lipase

Astri Nur Istyami[1,*)], Tatang Hernas Soerawidjaja[1)], Tirto Prakoso[1)], and Made Tri Ari Penia Kresnowati[2)]

[1)]Department of Bioenergy and Chemical Engineering, Faculty of Industrial Technology, Institut Teknologi Bandung
Jl. Ganesa No. 10 Bandung, Phone/Fax. (022) 2500989/(022) 2501438
[2)]Department of Food Engineering, Faculty of Industrial Technology, Institut Teknologi Bandung
Jl. Ganesa No. 10 Bandung, Phone/Fax. (022) 2500989/(022) 2501438

[*)]Coresponding author: anistyami@che.itb.ac.id

(Received: December 04, 2017; Accepted: June 14, 2018)

Abstract

Fatty acids are intermediate substances in synthesis of oleochemical products. Enzymatic technology of fatty acids production (also known as lipolysis) is now developing as potential substitution for the conventional production of fatty acid, i.e. thermal hydrolysis of triglyceride. It offers more economical process condition, low energy consumption, and minimal product degradation compared to the conventional process. This research aims to evaluate performance of various organic solvents as reaction media in lipolysis with plant latex lipase. Organic solvents observed were chloroform, n-hexane, diethyl ether, benzene, acetone, ethanol, methanol, n-heptane, and isooctane. Analysis of each organic solvent effect on lipolysis was described based on solvents properties. Conversion of lipolysis with organic solvents is 0.10-1.25 times fold compared to conversion of non-solvent lipolysis. We suggest that dielectric constant and viscosity are the two main organic solvent properties affecting lipase performance in lipolysis. Overall, n-hexane, n-heptane, and isooctane are recommended to be used as reaction media in lipolysis with plant lipase because their effects to degree of lipolysis are positive.

Keywords: *frangipani; lipase; lipolysis; organic solvent*

How to Cite This Article: Istyami, A.N., Soerawidjaja, T.H., Prakoso, T., and Kresnowati, M.T.A.P., (2018), Performance of Various Organic Solvents as Reaction Media in Plant Oil Lipolysis with Plant Lipase, Reaktor, 18(2), 71-75, http://dx.doi.org/10.14710/reaktor.18.2.71-75

INTRODUCTION

As the largest producer of palm oil around the world, Indonesia has a large potential to produce various oils and fats derivatives. Nevertheless, 39% palm oils produced is exported to other country in crude state, because plant oil processing into valuable oleochemical products in Indonesia has not been maximized.

Conversion of triglyceride into free fatty acids is one of the most important step in oils and fats processing into valuable oleochemical products. Aside from being used in food and non-food industry, fatty acid is also found to be precursor for hydrocarbons through decarboxylation process (Kubičková *et al.*, 2005). It opens the fatty acid utilization into a whole

new industry sector of biofuel, and fatty acid demand is expected to dramatically increase in the future.

Production of fatty acids is established through triglyceride hydrolysis into fatty acids and glycerol. Current technology of this process is noncatalytic thermal hydrolysis of triglyceride (also known as Colgate-Emery process), which operates at 240-260ºC, 50 bar (Barnebey and Brown, 1948). The high temperature causes high energy demand and undesired side reactions such as multiple bonds polymerization and thermal degradation (Mounguengui *et al.*, 2013). To overcome this drawbacks, enzymatic triglyceride hydrolysis technology is developed. This process utilizes lipase enzyme and is also known as lipolysis. Lipase enzymes are active in room temperature, making lipolysis an energy-efficient alternative process to produce fatty acids. High specificity of lipase prevents side reactions. Lipase has been commercially produced from microorganism, but its industrial application is limited by the high production cost (Seth *et al.*, 2013).

Aside from microorganism, plant seeds and plant latex are potential sources of lipase due to their high availability, low production cost and ease of handling (Avelar *et al.*, 2013). Plant-based lipases that have been reported are castor seeds (Avelar *et al.*, 2013), physic nuts (Barros *et al.*, 2010), oatseeds (Piazza dan Haas, 1999), sunflower seeds (Barros *et al.*, 2010), ricebran (Loeb *et al.*, 1949), papaya latex (Domínguez de María *et al.*, 2006), frangipani latex (Cambon *et al.*, 2006), *Euphorbia characias latex* (Fiorillo *et al.*, 2007), etc. In other work, we reported frangipani latex as the most active lipase sources compared to other plant lipase sources such as castor seeds, rice bran, and papaya latex. The effects of process variables in lipolysis has been reported, such as effects of temperature and pH (Cambon *et al.*, 2006), water amount (Mounguengui *et al.*, 2013), presence of activator and inhibitor ions (Barros *et al.*, 2010), and utilization of organic solvents (Kumar *et al.*, 2016). Investigation of these variables are necessary to develop plant lipase utilization with good performance and economic feasibility.

Kumar *et al*. (2016) reported that organic solvents utilization gives several advantages, such as increasing substrate solubility and making immobilization, recycling, and separation step easier. Nevertheless, utilization technique of organic solvents is important to be investigated because they can also decrease enzyme activity (Nakatani *et al.*, 1992).

In reactions involving lipase enzyme, organic solvents takes role in several ways. Organic solvents opens the lipase enzyme 'lid' due to presence of interfacial activation (Maraite *et al.*, 2013) with different efficiency between the solvent types (Adlercreutz, 2013). On the other hand, organic solvents increases substrate solubility, thus decreases free energy and reaction conversion (Ryu and Dordick, 1992). It also may work as lipolysis reaction inhibitor (Graber *et al.*, 2007).

The effect of solvents on lipase is a cumulative effect of various parameters such as dipole moment, hydrogen bonds (Khmelnitsky *et al.*, 1988) and polarity index (Gupta *et al.*, 2004). Increasing of lipase activity in organic solvents utilization was reported in lipolysis with oat seed (Piazza and Haas, 1999), which uses trimethylpentane (isooctane) with 39:1 ratio to oil substrate to attain 98.7% conversion in 22.5 hours. In development of enzymatic process technology, solvents utilization needs to be evaluated casuistically, especially when different lipase source or substrates are used.

This study aims to determine performance of various organic solvents as reaction media in plant oil lipolysis with frangipani (*Plumeria rubra*) latex lipase. The determination of suitable solvent is expected to increase enzymatic lipolysis performance, thus make it comparable with conventional triglyceride hydrolysis technology.

METHODS

Latex Preparation

Frangipani latex was collected from local tree in Bandung, Indonesia. Latex was settled for 5 days in 7ºC refrigerator. Particulate settled was decantated and dried in a container with silica gel in 7ºC for 7 days.

Lipolysis

Palm oil (Bimoli, produced by PT Salim Ivomas Pratama Tbk.) was mixed with latex particulate, ammonia-$NHCl_4$ buffer (pH 8), and water with 10:1:1:2 ratio, respectively. Organic solvents was added with ratio 1:1 (mL/gram oil). The reaction mixture was agitated for 24 hours in 950 rpm. All solvents used are technical grade.

Degree of Lipolysis Determination

Sampling for analysis was taken on the 4th and 24th hour. Lipolysis product was settled for 15 minutes and two layers are formed. Sample was pipetted from upper layer for ±1 gram, solved in ethanol:choloform solution (50:50 volume) and titrated with 0.1 N ethanolic KOH. Degree of lipolysis was determined with Equation (1) (Rooney dan Weatherley, 2001).

$$Degree\ of\ hydrolisis\ (\%) = \frac{V_{KOH} x 10^3 x M_{KOH} x MM}{W_t\ x\ f} x100 \quad (1)$$

V_{KOH} is volume of ethanolic KOH needed to neutralize sample (mL), M_{KOH} is concentration of KOH solution (±0.1 M), MM is relative molecular mass of palm oil fatty acids, W_t is sample mass (gr) and f is mass fraction of oil in mixture.

Determination of KOH solution concentration was precisely done with standardization procedure. Potassium hydrogen phtalate (KHF) was precisely weighted (±0.001 gr) and solved in demineralized water. After addition of phenolphtalein indicator, KHF aqueous solution was titrated with ethanolic KOH solution. Concentration of ethanolic KOH was determined with Equation (2).

$$M_{KOH} = \frac{w_{KHF} \, x \, 10^3}{V_{KOH} \, x \, Mr_{KHF}} \quad (2)$$

w_{KHF} is the mass of KHF (gr), v_{KOH} is volume of ethanolic KOH needed in titration (mL), Mr_{KHF} is relative molecular mass of KHF (204.2).

RESULTS AND DISCUSSION

Figure 1 shows degree of lipolysis attained with utilization of various organic solvents as lipolysis reaction media. Table 1 shows solvents properties, i.e. dielectric constant and viscosity of each solvents.

Lipolysis with most solvents shows lower conversion compared to the nonsolvent lipolysis. The decreasing performance might be due to enzyme denaturation (Nakatani *et al.*, 1992; Ryu and Dordick, 1992). Exception was showed in utilization of n-hexane, n-heptane, and isooctane, which results in higher conversion compared to nonsolvent lipolysis. It also shows tendency of decreasing degree of lipolysis as the solvent polarity increases. Exceptional performance of n-hexane, n-heptane, and isooctane as lipolysis reaction media might be due to several causes. First, low polarity of those solvents increases oil solubility in system and contact with enzymes. In *Candida antartica* lipase utilization, ketone groups in solvents was reported to inhibit lipase performance, while hydrocarbon solvents with no oxygenated group do not show this tendency (Graber *et al.*, 2007). Second, the enzyme active site is easier to be accessed by substrate when the site active 'lid' was contacted with organic solvents, as stated by Maraite *et al.* (2013) about the effects of tetrahydrofuran solvent on lipase active site. Third, enzyme specificity that is influenced by three dimensional conformation of enzyme amino acid (Maraite *et al.*, 2013) was changed due to pronotaion by solvents. This is similar phenomenon to enzyme conformational change by pH and temperature which affects its activity.

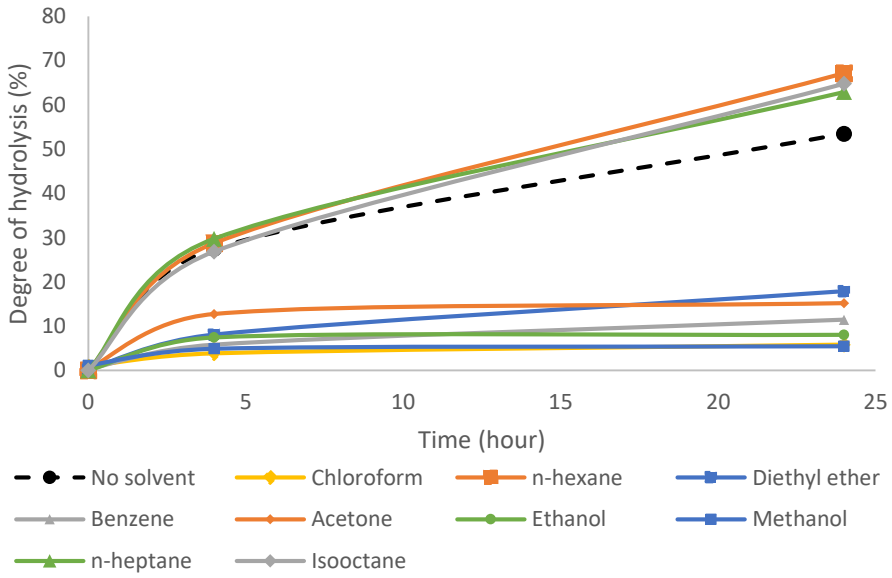

Figure 1. Degree of lipolysis with frangipani latex with various organic solvents as reaction media

Table 1. Dielectric constant, viscosity of organic solvents and the degree of lipolysis resulted

Solvents	Dielectric constant	Viscosity (10^{-3} Pa.s)	Degree of lipolysis resulted in experiment	
			4 hours	24 hours
n-hexane	1.88 [a]	0.31 [a]	28.7	67.1
n-heptane	1.92 [a]	0.42 [a]	29.8	62.9
Isooctane	1.94 [a]	0.50 [a]	26.9	64.8
Benzene	2.28 [b]	0.60 [c]	5.8	11.5
Diethyl ether	4.34 [b]	0.22 [c]	8.1	17.9
Chloroform	4.81 [a]	0.57 [a]	3.9	5.8
Ethanol	24.55 [a]	1.1 [a]	7.5	8.0
Acetone	20.7 [a]	0.36 [a]	12.8	15.2
Methanol	32.70 [a]	0.59 [a]	4.9	5.5

Sources:
[a] http://macro.lsu.edu
[b] http://showme.physics.drexel.edu
[c] https://www.accudynetest.com

Aside from polarity, low viscosity was predicted to increase lipolytic activity. It was concluded from degree of lipolysis involving diethyl ether that was higher than lipolysis with benzene, and also degree of lipolysis with acetone that was higher than using chloroform or ethanol. Low viscosity increases mixing quality of reaction system and contact surface area between oil and water. According to this analysis. We suggest that polarity index and viscosity are two main factors affecting organic solvents performance as lipolysis reaction media.

Low processing cost is an advantage offered by lipolysis with plant-based lipase. Lipase from frangipani latex shows good performance in crude condition without necessity of complicated and high-cost purification. At the end of reaction. Lipolysis products tend to be thickened and solidified due to high content of free fatty acids. Utilization of solvent makes the product handling and separation easier. This step needs low cost solvent to keep the overall process economically feasible. In this work. Utilization of solvents with low cost and high availability was proved not to decrease activity of lipase instead it strengthen the lipolysis performance.

Aside from determination of suitable solvent. pH controlling is also important to keep the enzyme protonated (Adlercreutz. 2013). Future challenges in lipolysis with plant lipase are increasing lipolysis conversion to make it competitive with microbial lipase (with process variable optimization) and fatty acid and glycerol purification (through efficient product separation between fatty acids and glycerol, and also from the enzyme).

ACKNOWLEDGEMENTS

The authors are grateful to Ministry of Research, Technology and Higher Education of the Republic of Indonesia for research grant awarded in scheme Doctoral Dissertation Research 2017 for experimental funding.

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