Lactonization Castor Oil (*Ricinus Communis*) using Lipase B from *Candida Antarctica Recombined Aspergillus oryzae* as Bioflavor

Galuh Alya Stywarni[1], Elvina Dhiaul Iftitah[1] and Arie Srihardyastutie[1]
[1]*Department of Chemistry, Faculty of Mathematics and Science, Brawijaya University, Malang, Indonesia*

Keywords: Castor Oil (*Ricinus communis*), Lactonization, Lipase, Bioflavor.

Abstract: Lactone is a widely flavor that is used in food production. Lactonization using microbial or enzyme has natural labelled products, has a higher economic value than artificial products and is safe for the environment. Lactonization of castor oil (*Ricinus communis*) using lipase B from Candida antarctica recombined Aspergillus oryzae (T = room, 40ºC) for 24, 48 and 72 h were investigated. The lactonization reaction was carried out using a magnetic hotplate stirrer with the reaction system consisting of castor oil, n-hexane solvent, Na2CO3 solution, and lipase biocatalyst. Lactonization castor oil products were analysed using GC-MS. At T = room, the major products were ester: methyl ricinoleate, 53.64% (t = 24 h) and other products were fatty acids and lactone. Lactone: γ-dodecalactone, 1.75% (t = 48 h) was a minor product. Whereas at T = 40ºC, only produced ester, the major product was methyl ricinoleate, 81.33% (t = 72 h).

1 INTRODUCTION

One of the potential sources of natural-based raw materials that are widely used in industry is castor oil. Castor oil consists of thick yellow liquid, has a characteristic odour with a molecular weight of 933.45 g/mol, a density of 0.95 g / cm3 and a boiling point of 313°C (Moradi et al., 2013). The content of castor oil consists of ricinoleic acid, linoleic acid, oleic acid, stearic acid, palmitic acid, dihydroxystearic acid, linoleic acid, and eicosanoic acid (Farbood and Willis, 1985). The main component of castor oil is ricinolein, a glyceride from ricinoleic acid. Ricinoleic acid has three functional groups namely ester linkage, double bonds and hydroxyl groups which are used as sources of renewable raw materials in chemical reactions, modification, and transformation into useful products (Wache et al., 2001). In the food industry, castor oil potential produces bioflavor. Various lipase-producing microbes have been reported as catalyse bioflavor (γ-decalactone) using castor oil substrate or ricinoleic acid (12-hydroxy-9-octadecenoic acid).

γ-Decalactone as a flavouring agent has fruit, creamy, peach, apricot, and fatty taste. In enzymatic biotransformation, the substrate is degraded through α-oxidation to produce 4-hydroxidecanoic acid, then cyclization to γ-decalactone (Gutman et al., 1989). Based on research by Gotz et al. (2013), immobilized B lipase from Candida antarctica able to catalyse the formation of (S) -γ-valerolactone from a substrate (S) -ethyl-4- hydroxy pentanoate with a yield of 90% (Antczak et al., 1991). According to Gutman et al. (1989), the lactonization reaction rate affected by the hydrophobicity of the solvent, n-hexane solvent is two times faster than ether and four times faster than chloroform (Khan and Rathod, 2018).

2 MATERIALS AND METHODS

2.1 Chemicals and Enzymes

Candida antarctica lipase B (recombinant from Aspergillus oryzae) (1800 U/gram), n- hexane, and sodium carbonate were obtained from Sigma-Aldrich. Castor oil were obtained from Organic Supply Co.

2.2 GC-MS Analysis of Castor Oil (*Ricinus communis*)

Transesterification reaction castor oil was carried out

Stywarni, G., Iftitah, E. and Srihardyastutie, A.
Lactonization Castor Oil (Ricinus Communis) using Lipase B from Candida Antarctica Recombined Aspergillus oryzae as Bioflavor.
DOI: 10.5220/0009955000370040
In *Proceedings of the 2nd International Conference of Essential Oils (ICEO 2019)*, pages 37-40
ISBN: 978-989-758-456-5

to determine the components of fatty acids. 50 g castor oil, 38 mL of ethanol and 1 mL of H_2SO_4 1 M were put in 100 mL Erlenmeyer flasks. The mixture was refluxed at 60-70ºC for 2 h. Then, Saturated NaCl was added to separate organic and water phase. The organic phase dehydrated by anhydrous Na_2SO_4 and dissolved in n-hexane (1:40, *v/v*).

Gas Chromatography (GC) equipped with a Mass Spectra (MS) detector and Restrex Rxi®-1MS capillary column. Oven temperature was held at 40 - 250ºC; injection temperature was 250ºC; The carrier gas, helium, was adjusted to a linear velocity 0.7 mL/min and 24.9 kPa. The injection volume into the GC apparatus was 0.5µl.

2.3 Castor Oil Lactonization

The reaction was carried out in 100 mL Erlenmeyer flasks, containing 6 g castor oil, 40 mL of n-hexane solvent,1 mL of Na_2CO_3 solution, and 0.1 g *Candida antarctica* recombined *Aspergillus oryzae*. The reactions were stirred using magnetic hotplate stirrer at room temperature and 40ºC for 24, 48 and 72 h. Then the pH of the mixture was measured. Each sample was centrifuged to separate the enzyme and the oil phase. The samples dissolved in n-hexane (1:20, *v/v*).

Gas Chromatography (GC) equipped with a Mass Spectra (MS) detector and Restrex Rtx®-5MS capillary column. Oven temperature was held at 40 - 250ºC; injection temperature was 250ºC; The carrier gas, helium, was adjusted to a linear velocity 1.01 ml/min and 50 kPa. The injection samples into the GC apparatus was 0.5µl.

3 RESULTS AND DISCUSSION

3.1 Analysis of Castor Oil Substrate (*Ricinus communis*)

Generally, the composition of ricinoleic acid in castor oil comprises approximately 90%, while the composition of other fatty acids: linoleic acid, oleic acid, stearic acid, palmitic acid, dihydroxystearic acid, linolenic acid, and eicosanoic acid less than 5% (Kourist and Hilterhaus, 2015). Based on analysis using GC-MS, the highest % concentration transesterification product castor oil was methyl ricinoleate (88.666%) (Table 1). So that, ricinoleic acid is the major component of castor oil. Some fatty acid components were not found in castor oil substrate, but % concentration of ricinoleic acid as the major component was good quantity.

Table 1: Trans-esterification product of castor oil.

% Cons.	Compound
0.872	Methyl-14-methyl pentadecanoate
4.285	Methyl-9-12-octadecadienoate
4.547	Methyl-11-octadecenoate
1.630	Methyl octadecanoate
88.666	Methyl ricinoleate

2.4 Effect of Temperature and Reaction Time

Lactonization using *Candida antarctica lipase B* recombined *Aspergillus oryzae* not only produce lactone. The lactone was γ- dodecalactone only formed at room temperature for 48 h. (Table 2).

Table 2: Effect temperature and reaction time on lactone formation.

Temperature (ºC)	Reaction Time (h)	Lactone
room	24	-
	48	√
	72	-
40	24	-
	48	-
	72	-

Biotransformation product of castor oil at room temperature were esters, fatty acids and lactone. Whereas at 40ºC only formed esters (Table 3).

Table 3: Biotransformation product of castor oil.

T (ºC)	t (h)	Compound	% Area
Ambient	24	Methyl ricinoleate	53.64
	48	9-octadecenoic acid	4.37
		γ-Dodecalactone	1.75
		Methyl dodecanoate	4.58
		Dodecanoic acid	1.53
		Methyl-9-octadecenoat	1.59
	72	Methyl dodecanoat	1.61
		Methyl ricinoleat	12.77
40	24	Methyl ricinoleat	69.90
	48	Methyl ricinoleat	64.95
	72	Methil ricinoleat	81.33

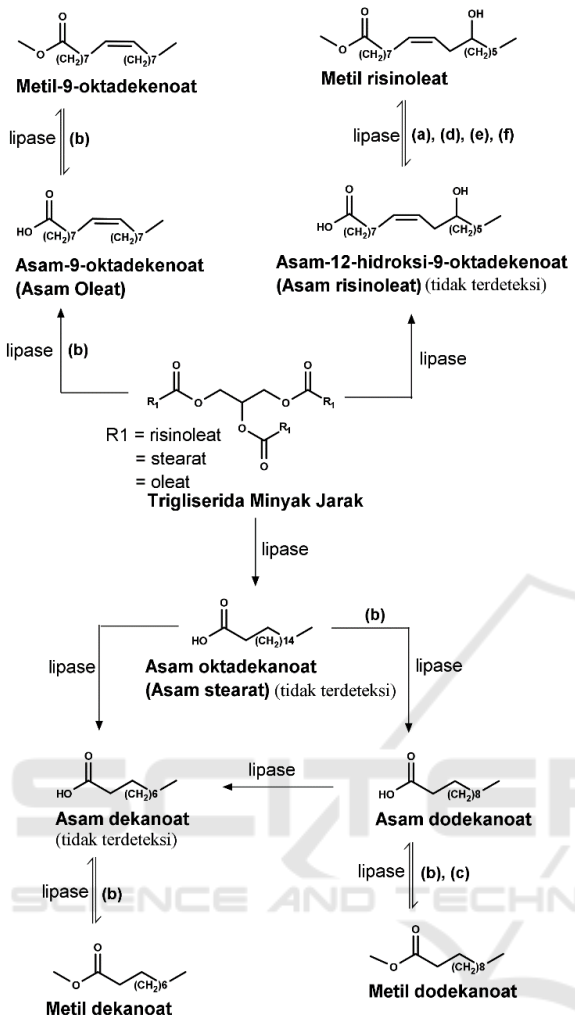

Figure 1. Estimated mechanism formation of fatty acid and ester. Condition of reaction: (a)T= room, t = 24 h; (b)T= room, t = 48 h; (c)T= room, T = 72 h; (d)T= 40ºC, t =24 h; (e)T= 40ºC, t = 48 h; (f) T = 40ºC, t = 72 h.

As the date in Table 3 show, Biotransformation of castor oil at room temperature and 0ºC produces esters (methyl ricinoleate) as the major product. It can be assumed that before the esterification reaction, triglyceride was hydrolysed to fatty acids, ricinoleic acid (undetectable). The optimal yield of methyl ricinoleate at 40ºC for 72 h (81.33%), is higher than the methyl ricinoleate at room temperature.

Formation C_{18} Fatty acid products at room temperature (9-octadecenoic acid, t = 48 h) and C_{12} (dodecanoic acid, t = 48 h) showed that lipase B *Candida antarctica* recombined *Aspergillus oryzae* had ability to hydrolysis triglyceride and shortening fatty acid carbon chain. This is probably hydrolysis reaction because lipase had active side catalytic: serine, histidine, and aspartate (Veld, 2010). The shortening C_{18} fatty acid chain to C_{12} can be assumed at beta carbon position that occur oxidation into carbonyl groups as much as three times. Source water in hydrolysis reaction form added Na_2CO_3 solution as component reaction. Then, fatty acid was probably forming ester. The fatty acid is then probably transformed into ester. Formation reaction of fatty acid and ester showed at Figure 1 9-octadecanoic acid probably to form methyl-9-octadecanoic (t = 48 h), dodecanoic acid probably to form methyl dodecanoate (t = 48 and 72 h). Methyl dodecanoate product (t = 72 h, 1.61%), lower than methyl dodecanoate (t = 48 h, 4.58%).

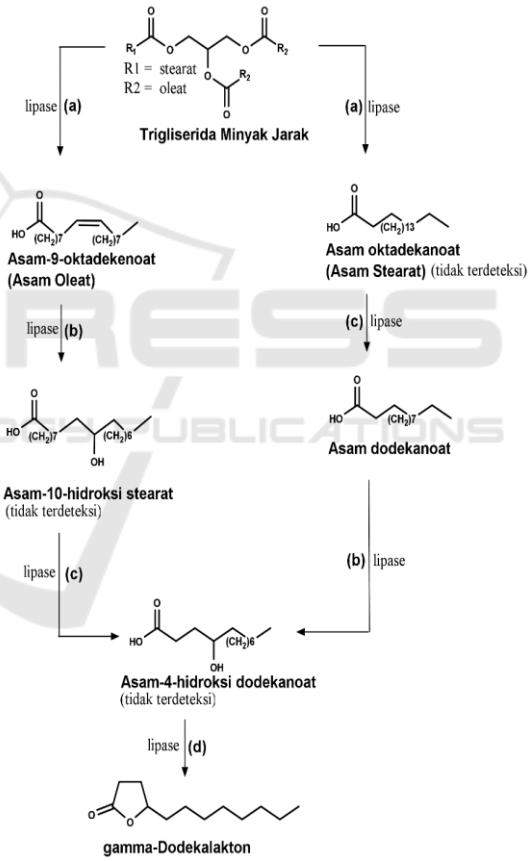

Figure 2. Estimated mechanism formation of gamma-dodecalactone. Reaction: (a) hydrolysis, (b) hydroxylation (c) shortening of carbon chains (d) lactonization.

Lactonization reaction to form γ- dodecalactone is probably from 9- octadecanoic acid, then hydroxylated to form 10-hydroxy octadecanoic acid (undetectable), after that undergoes a carbon chain

shortening to form 4-hydroxy dodecanoic acid (undetectable), and it was occurring lactonization become γ-dodecalactone. Formation of γ-dodecalactone product is also probably from dodecanoic acid (Figure 2). The mechanism of γ-dodecalactone formation (Figure 2) refers to Han et al. (1995) who use *Mortierella isabellina* on dodecanoic acid substrate and Haffner et al. (1996) using the *sporobolomyces odour* on 9-octadecanoic acid (oleic acid) that occur hydroxylation (Goswami et al., 2013) to form γ-dodecalactone

The target compound that is γ-decalactone was not formed in biotransformation of castor oil at room temperature and 40ºC, it is estimated that due to ideal biotransformation reaction conditions for esterification, so that the hydroxylation reaction to form ricinoleic acid (not detected) as substrate probably convert quickly to ester (methyl ricinoleate). The formation of another lactone (γ-dodecalactone, 1.75%) as minor product at room temperature for 48 h probably so because the hydrolysis of 9-octadecenoic acid and dodecanoic acid was formed during the 48 h, so that lactonization (intra-esterification reaction) is possible at these time.

4 CONCLUSIONS

Lactonization of castor oil only produces lactone at room temperature for 48 h. The lactone product was γ-dodekalakton as minor product (1.75%). The major products biotransformation was methyl ricinoleat (T=room, t=24 h: 53.64%); (T=room, t= 72 jam: 12.77%); (T=40ºC, t=24 h: 69.90%); (T=40ºC, t=48 h: 64.95%); (T=40ºC, t=72 h 81.33%).

REFERENCES

Antczak, U., Gora J., Antczak, T., Galas, E., 1991. Enzymatic Lactonization of 15-Hydroxypentadecenoic and 16-Hyhroxyhexadecenoic Acids to Macrocyclic Lactones. E*nzyme Microbial Technology*, 13, 589-593.

Farbood, M, I., Willis B, J., 1985. Production of γ-Decalactone. *US Patent*, 306, 691.

Goswami, D., Basu, J.K., De, Sirhendu., 2013. Lipase Applications in Oil Hydrolysis with a Case Study on Castor Oil: A Review. *Critical Reviews in Biotechnology*, 33(1), 81–96.

Gotz, K., Liase, A., Ansorge-Schumacher M., Hilterhalus, L., 2013. A- Chemo-Enzymatic Route to Synthesize (S)-γ- Valerolactone From Levulinic Acid. *Application Microbial Biotechnology,* 97, 3865-3873.

Gutman, A, L., Zuobi, K., Bravdo, T., 1989. Lipase-Catalyzed Preparation of Optically Active γ-Butyrolactones in Organic Solvent. *Journal Organic Chemistry*, 55, 3546-3552.

Khan, N, R., Rathod, V, R., 2018. Microwave Assisted Enzymatis Synthesis of Specially Esters: A Mini - Review. *Process Biochemistry*, Department of Chemical Engineering, India, Institute of Chemical Technology.

Kourist, R., Hilterhaus, R., 2015. Microbial Lactone Synthesis Based on Renewable Resources, *Microbiology Monographs*, 2, 277-278.

Moradi, H., Asadollahi, M, A., Nahvi, I., 2013. Improved γ-Decalactone Production from Castor Oil by Fed-Batch Cultivation of Yarrowia Lipolytica. *Biocatalytic Agriculture Biotechnology*, 2(1), 64–68.

Wache, Y., Aguedo, M., Choquet, A., Gatfield, IL., Nicaud, J-M., Belin, J-M., 2001. Role of Beta-Oxidation Enzymes in Gamma-Decalactone Production by the Yeast Yarrowia Lipolytica, *Applied And Environmental Microbiology*, 67(12), 5700–5704.

Veld, M, A, J., 2010. *Candida antarctica Lipase B catalysis inorganic, polymer and supramolecular chemistry:Eindhoven University of Technology.*