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Comparative Study of Acetylation Reactions to Mentol Compounds Using Lipase from Candida Antarctica Recombined Aspergillus oryzae with Rhizomucor miehei

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Abstract

Research about comparison an enzyme lipase of Rhizomucor miehei and Candida antarctica recombined Aspergillus oryzae to catalyzes reaction acetylation compound menthol by the difference of activity of the enzyme. Reaction done on condition temperature 50oc use up acetyl acetic anhydride in a solvent n-hexane in a variety time. The results that are reaction acetylation menthol use a lipase enzyme of Candida antarctica recombined Aspergillus oryzae and Rhizomucor miehei by a unit activity different do not have a significant difference. The difference of the results the final product that is mentil acetate of these enzymes changed as from time to time produce the difference of the results at to 24 hours. The use of lipase of Candida antarctica recombined Aspergillus oryzae have a selectivity higher (96,93 %) in forming mentil acetic than lipase of Rhizomucor miehei with have the ability convert of 77,42%.

Kata kunci— *Acetylation mentol, Lipase Enzyme, Rhizomucor miehei, Candida antarctica*

1. INTRODUCTION

Today industry an enzyme developed rapidly and had the important in for industry. Public awareness of environmental problems higher and the pressure of the experts made technology an enzyme as one alternative to replace several process chemically in for industry. In a chemical reaction catalyst choice that are supposed to be reduce the impact of pollution the environment as the reaction was is specific, and not poisonous[1]. So as to diminish and elimination of chemical compounds dangerous and can and apply the concept of environmentally friendly that is green chemistry. Based on the principles of green chemistry article 9 the use of biocatalyzed in a chemical reaction to reduce pollutants of chemical dangerous, one example of often used as biocatalyzed is an enzyme[2].

The enzyme is a protein molecules that acts as catalyst in a reaction biological (biocatalyst). A working enzyme as a catalyst with form a compound complex enzyme-substrate. Next of a compound complex it will form products chill, and in the end of the reaction the enzyme will be separate back[1]. An enzyme have some excellence of them having the nature of a typical and can active in concentration low, very selective and work on the state of the reaction light (without temperature and without pressure high. An enzyme can be classified after the reaction was one example is an enzyme lipase[3].

An enzyme lipase is one type of of an enzyme hidrolase had played a role in food industry. Lipase who derived from microorganisms are thermophilic, an organism that often used as producer lipase is Candida and Rhizopus. Example of kind of an lipase enzyme based on microorganism is *Rhizomucor miehei* made of mildew, while *Candida antarctica* recombined *Aspergillus oryzae* of leavened.The activity of an enzyme u/g affect a working enzyme to produce the product of one gram an enzyme per minute. Lipase can catalyze various

such a reaction reaction hydrolysis, esterification, and transesterification. But of the three the repercussions an enzyme lipase having activity esterification reaction high[4].

Mentil acetate is a compound of ester derivative of a compound menthol who have benefit to scent, taste and medicines. Compound mentil acetic contained in plants mentha pipperita. Mentil content acetic in plants the width fraction of (1-9,5 %), while compound menthol greater around (30-47 %). So that needs to be done synthesis of a compound menthol be mentil acetic[5].

Reaction esterfication is a reaction the formation of compound ester. The use of a solvent non polar as n-hexane, both in reaction esterfication because it is able to produce results maximum without affecting the other reactions. Reaction esterification with enzymes lipase as biocatalyzed can be done by change compound menthol (starting material) into a compound Its derivatives mentil acetate that in reaction with an acids us donor contributor acetyl. Roji (2001), Have done research the use of an enzyme lipase of *Candida rugosa* to the reaction acetylation menthol and solvent used is n-heptane by producing mentil acetic with rendemen of 29,57 % at the temperature 30^0C for 48 hours[6]. According to Sri (2016), reaction esterification isopulegol use an enzyme lipase of *Rhizomucor miehei* having the activity of an enzyme by 300 u/g with a solvent n-hexane and source of acyl acetic anhidrid produce compound derivative isopulegil acetic of 20,69 % at the temperature 50^0C to the time reaction to 8 hours[7].

After research conducted by Sri (2016), reaction esterification by using temperature 500c better yield the product of 10,16 % percent compared with the at room temperature to the product obtained of 3,68 % and the use of biocatalyzed an enzyme lipase prove even more good with the results of the final product of 13,95 % than with without the use of an enzyme of 7,97 %. While to the method reaction by using the method reflux not produce results maximum. This is different when reaction esterification without method reflux by using magnetig stirrer accompanied warming produce results products is greater than with the methods reflux[7].

Therefore, in this research will be conducted study about comparison the activity of an enzyme lipase of *Candida antarctica* recombined *Aspergillus oryzae* with the activity of an enzyme of 1800 u/g and *Rhizomucor miehei* immobilized having enzyme activity by 300 u/g to acetylation reaction to compounds menthol to meet the concept of green chemistry in a chemical reaction.The use of variation an enzyme lipase intend to knows the difference of the activity of an enzyme lipase of *Candida antarctica* recombined *Aspergillus oryzae* and *Rhizomucor miehei* immobilized to structure menthol to the reaction acetylation with temperature 50^0C**.**

2. MATERIALS AND METHODS

Materials

The materials used in study between other an enzyme lipase *Candida antarctica* recombined *Aspergillus oryzae* and *Rhizomuchor miehei* immobilized immobead 150 from sigma aldrich, menthol, acetic anhydride, and n-hexane.

Enzimatic Acetylation Procedure

Weighed an enzyme lipase of *Candida antarctica* recombined *Aspergillus oryzae* and *Rhizomuchor miehei* as many as 0,15 grams put into erlenmeyer with screw stamp (the volume 100 ml) as distinct. N-hexane as many as 20 ml and acetic anhydride as many as 5 ml put into erlenmeyer with screw stamp (the volume 100 ml) as distinct. A mixture of stirred use magnetic stirrer, then added menthol as many as 4,68 grams of being dissolved into 5 ml n-hexane slowly (drops by drops into a container). The process stirring carried out for 24 hours in temperature 50^0C. Done sampling solution in the four hours , 8 hours, 12 hours, 16 hours, 20 hours, and 24 hours. After that, a mixture of enzyme and a substrate the results of reaction separated by means of centrifuge use mikrocup or eppendorf 3ml. Results in can be analyzed use GC-MS and FTIR.

Analytical Procedure

GC-MS Analytical Procedure

Products acetylation reaction analyzed by inject sample 0,2 use syringe on an instrument GC-MS shimadzu QP2010S. The top which detected at cromatogram in analysis using mass spectra, so that the result of the end of analysis obtained cromatogram and mass spectra of products. The results from the analysis GC - MS of mass cromatogram. Of cromatogram was found the number of of compounds formed from the repercussions. In addition, of a pattern fragmentation compound that exists in cromatogram mass, it is estimated structure a compound formed.

FT-IR Analitycal Procedure

Acetylation products with the spectrophotometer FTIR will yield of spectra showing the relation between % transmitation to the number of a wave that ranged from 4000-400 cm^{-1}. Analysis done by making pellet KBr. Next sample pellet drops on KBr.This sample put in space sample to spectrofotometer FTIR .

The result of analysis using FTIR beneficial to find out a cluster of the function that was found in a compound formed. In addition can be seen change a cluster of function of the conflict between the substrate by the products of the reaction.

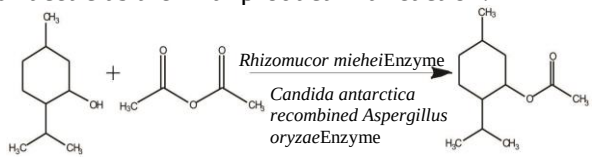
Data Analysis

Characterizing the results of reaction used by gas chromatography -mass spectroscopy (GC-MS) so that obtained data of spectrogram of GC and the pattern fragmentation or from the MS. Based on profile GC-MS products acetylation menthol it can be seen % conversion and % a selectivity an enzyme lipase to mentil acetic.

3. RESULT

The Influence of The Difference of The Results Reaction Acetylation Compound Menthol with Enzymes Lipase of *Rhizomuchor miehei* and *Candida antarctica* recombined *Aspergillus oryzae*

The influence of the difference an enzyme lipase by virtue of the activity of the enzyme used in reaction to compound acetylation menthol determined by do reaction acetylation employing variations an enzyme lipase that is an enzyme of *Candida antarctica* recombined *Aspergillus oryzae* and *Rhizomucor miehei* which each having unit the activity of an enzyme by 300 u /g and 1800 u/g , and variation time reaction. Reaction acetylation enzimatis would be held on temperature 50^0C with a solvent n - hexane and a substrate menthol synthesis for 24 hours the reaction sampled once in four hours. Estimates reaction acetylation i was in this **figure 1.** happened where a substrate used is menthol to the reaction the addition of an acetyl group from a source acetyl that is acetic anhidrid in a cluster of hidroxyl that is menthol. L-mentol that react can produce mentil acetic as the final product in a reaction.



l-Menthol Acetate Anhydrid Menthil

Figure 1. Estimates reaction acetilation enzimatic l-mentol be mentil acetate.

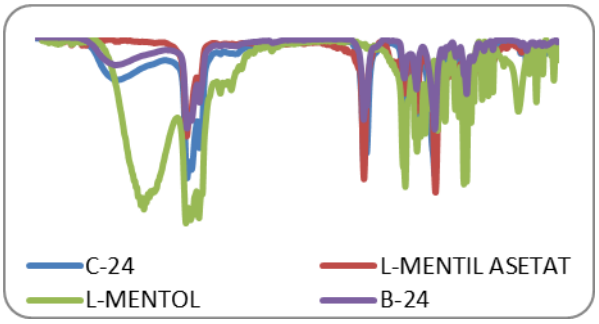


Figure 2. *Overlay* spectra FTIR the results of reaction acetylation menthol with enzymes lipase of *Candida antarctica* recombined *Aspergillus oryzae*(C-24) and *Rhizomucor miehei* (B-24) in to24 hours.

The reaction results analyzed with FTIR spectra and FTIR produce presented in **figure 2.** On the spectra, read 3447.32 on the origin of the existence of cm^{-1} for the FTIR spectra of samples that use the enzyme lipase from *Candida antarctica* on skis, recombined *Aspergillus oryzae* (CAL) on the reaction, while the sample with FTIR spectra and on the reactions of the enzyme *Rhizomucor miehei* from (RML) there are absorption on the 3449.25 cm^{-1} indicating the presence of functional origin (-OH) possibly derived from menthol which still react. Then the existence of the absorption area of 1736.58 cm^{-1} for the enzyme from CAL and 1736.38 cm^{-1} for the enzyme from RML who marked the origin of the cluster (C = O) Carbonyl. The existence of a cluster of absorption (C-O) in the area of 1246.69 cm^{-1}, which shows the possibility of absorption of C-O ester compound proves the target form acetic mentil have formed on both the spectra of the reaction of acetylation with enzyme lipase from *Candida antarctica* on recombined *Aspergillus oryzae* with *Rhizomucor miehei*. Then done overlay to tell the difference of menthol spectra, mentil acetic and the other.

In samples mentil acetic estimated to have a function resembling mentil acetate standard so can be assumed the final product or sample mentil acetic is a compound. But there are still a cluster of O-H alcohol from menthol remaining. This is consistent with the characterization GC - MS that there is still a compound menthol in samples as a product undesirable side. Comparison spectra FTIR in reaction acetylation use of an enzyme lipase *Rhizomucor miehei* and *Candida antarctica* recombined *Aspergillus oryzae*, spectra FTIR enzyme lipase of *Candida antarctica* recombined *Aspergillus oryzae* more resemble with spectra mentil acetic standards. So from the analysis by FTIR it can be seen more results maximum using an enzyme lipase of *Candida antarctica* recombined *Aspergillus oryzae* compared with *Rhizomucor miehei*.

But the difference the activity of used can affect the main product in the form of mentil acetic assuming % area obtained from the analysis GCMS different and difference activity and source microorganism from their for the enzyme lipase. Based on analysis GCMS obtained result in **table 1**.

Table 1. The reaction acetylation l-mentol with variations an enzyme lipase and time

t	Product*Rhizomucor miehei*		Product*Candida antarctica* recombined *Aspergillus oryzae*	
	Compound	% Area (%)	Compound	% Area (%)
4	L-menthol	62,34	L-menthol	62,34
	Mentil acetate	25,73	Mentil acetate	24,97

	Isopulegol	0,41	Isopulegol	3,66
			Cyclohexan	3,34
8	L-menthol	51,75	L-mentol	52,24
	Mentil acetate	42,76	Mentil acetate	42,46
12	L-mentol	27,25	L-menthol	23,33
	Mentil acetate	71,91	Mentil acetate	27,25
16	L-mentol	51,18	-	-
	Mentil acetate	43,39		
	Isopulegol	2,04		
	Dihydrocarveol	0,32		
	Cyclohexan	1,24		
20	L-menthol	51,75	L-mentol	47,91
	Mentil asetate	46,59	Mentil acetate	51,10
	Cyclohexan	0,88	Cyclohexan	0,65
	Isopulegol	0,35	Isopulegol	0,34
24	L-menthol	27,31	L-menthol	22,56
	Mentil acetate	53,07	Mentil acetate	75,04
	Cyclohexan	2,58	Cyclohexan	2,37

Based on the **table 1.** The reaction acetylation menthol with a difference of the activity of the lipase *Rhizomucor miehei* and *Candida antarctica* recombined *Aspergillus oryzae* can affect results, where reaction Acetylation compound menthol will produce the final product mentil acetate do not differ significantly so that it can be assumed of both enzyme lipase has both active same will happen the same reaction.According to research by Ariefin (2016), about the mechanism to reaction between alcohol with carboxylic acid, be brought the reaction between menthol (alcohol) with acetate anhidrid (carboxylic acid) to produce mentil acetate is presented in figure 3.

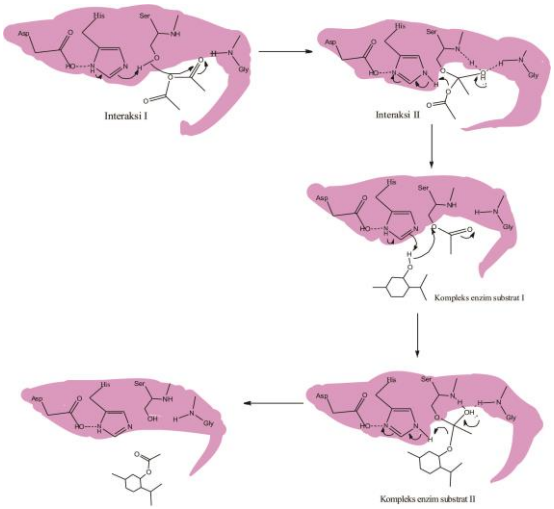


Figure 3. Mechanism reaction acetylation menthol with acetic anhidrid form mentil acetic

Based on **figure 3.** Mechanism reaction begins with the interaction between acetic acid anhidrid with serin in amino acid .The interaction seen in **Interaction I**. The **Interaction I** produce **Interaction II** between acetic anhidrid with enzymes lipase. **Interaction II** release ions acetic as a result after him. Then, an alcohol group of menthol strike **Interaction II** as seen on a **complex of enzymes a substrate I**. The result of a **complex of enzymes a substrate I** it produces a **complex of enzymes a substrate II** and further produces product ester of mentil acetic.

Comparison a Selectivity and of Conversion to Long Reaction to Variation The Activity of an Enzyme Lipase of *Rhizomucor miehei* and *Candida antarctica* Recombined *Aspergillus oryzae* in a Reaction Acetylation Compound Menthol

To know the difference the activity of the in reaction acetylation enzimatis menthol against variation time, conducted reaction acetylation with enzymes lipase of candida antarctica recombined aspergillus oryzae and rhizomucor miehei. Acetylation reaction was conducted using solvent n-hexane with temperatures 50^0C. Influence time or long reaction acetylation menthol observed for 24 hours. Every 4 hours, the reaction sampled and analysis by instrumentation GC-MS. The observation focused on peak compound the main mentil acetic.

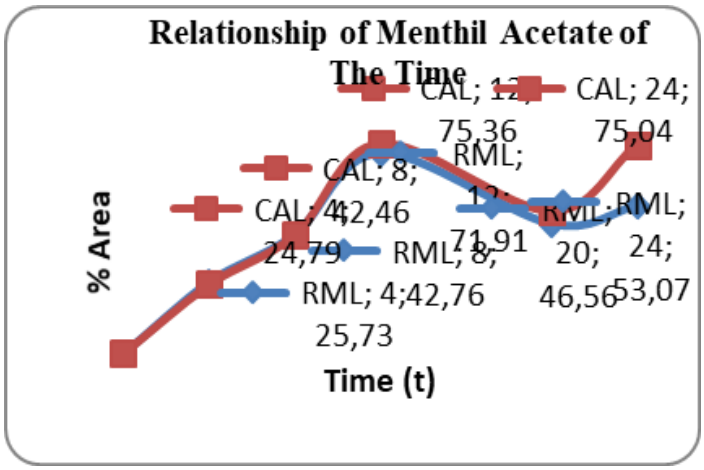


Figure 4. Charts the area acetic % mentil of time on reaction aetylation with enzymes lipase

Charts the relationship between time with % area acetic mentil is presented in **figure 4.** On a chart seen from hours 4th century to 4 hours to12 hours, % area mentil acetic has increased from 4 hours to 12 hours, and decreased back from 12 hours to 20 hours and at to 24 hours increased back. Be seen that the difference of the results the final product that is mentil acetate of these enzymes changed as from time to time produce the difference of the results at to 24 hours.Of the magnitude of % area obtained be seen that menthol as starting material there has been convertible be mentil acetic.This indicates some menthol have responded with acetic anhydride form mentil acetic. Next to know efficient the activity of an enzyme working produce mentil acetic in a reaction acetylation menthol it can be seen by counting value % conversion and % a selectivity of % area mentil acetic and menthol.

Table 2. Comparison % conversion menthol and % a selectivity mentil acetic in reaction acetylation menthol for 24 hours

Time (t)	% Conversion Mentol (%)		% Selectivity Mentil Asetat (%)	
	CAL (1800 U/g)	RML (300 U/g)	CAL	RML
4	37.66	26.14	66.3	98.43
8	42.76	48.25	99.3	88.62
12	76.67	72.25	98.29	98.85
20	52.09	48.33	98.1	96.34
24	77.42	72.69	96.93	73.01

The conversion % showing the number of menthol which has become mentil acetic convertible. From the **table 2.** from the compound acetylation reaction to menthol use of an enzyme lipase*Rhizomucor miehei* with the activity of 300 u/g at 24 hours to having % highest conversion of 72,69 % during the reaction 24 hours .While at the same time with the activity of the 1800 u /g much larger 6 times of an enzyme previous usingenzyme of lipase *Candida antarctica* recombined *Aspergillus oryzae* get % conversion of 77,42 % only 5 %. This shows that with enzymes of *Candida antarctica* recombined *Aspergillus oryzae* less convert compound menthol be mentil acetate though with greater activity. But in terms of % selectivity with enzymes of *Candida antarctica* recombined *Aspergillus oryzae* was higher with 96,93 % compared with enzymes of *Rhizomucor miehei* with % selectivity of 73,01 %. At the same time having the difference 24 %. From the % a selectivity reported those a substrate that react to produce intended products that is mentil acetic with enzymes of *Candida antarctica* recombined *Aspergillus oryzae* can be more selective with a significant difference between these enzymes lipase.

4. CONCLUSION

The acetylation reaction menthol with enzymes lipase of *Rhizomucor miehei* and *Candida antarctica* recombined *Aspergillus oryzae* did not experience distinction significantly. The difference of the results the final product that is mentil acetate of these enzymes changed as from time to time produce the difference of the results at to 24 hours, with the reaction use an enzyme lipase of *Candida antarctica* recombined *Aspergillus oryzae* are more dominant than enzyme of *Rhizomucor miehei*. The use of lipase of *Candida antarctica* recombined *Aspergillus oryzae* have a selectivity higher (96,93 %) in forming mentil acetic than lipase of *Rhizomucor miehei*, with the ability convert of 77,42 %.

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