

# Microwave-Assisted Enzymatic Esterification Production of Emollient Esters

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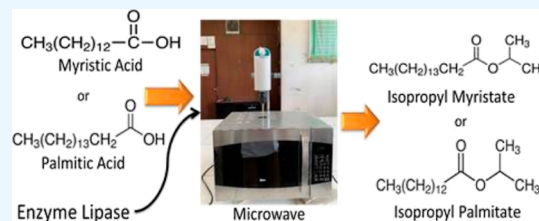
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**ABSTRACT:** Currently, esterification production of isopropyl myristate (IPM) or isopropyl palmitate (IPP) uses a homogeneous or heterogeneous acid substance as a catalyst and is conducted at high temperatures and pressures. Utilization of this type of catalyst requires an additional postproduction process (neutralization and purification), which burdens the production cost. Microwave enzymatic esterification is a simple and fast method. The results showed that reaction time, ratio molar of fatty acids to isopropyl alcohol, catalyst weight, and microwave power have a significant effect on the IPM or IPP conversion. Further, the energy consumption of this process is less than other enzymatic esterification and is certainly more energy efficient, which could save 99 and 29% of processing time.



## INTRODUCTION

Oleochemical products, such as fatty acid esters, have wide applications in cosmetics, food, pharmaceutical, coatings, lubricants, biodiesel, and other industries.<sup>1</sup> The global oleochemicals market was valued at US\$ 20.1 billion in 2019 and is expected to grow at an annual growth rate of 5.8% from 2020 to 2027. Isopropyl myristate (IPM) and isopropyl palmitate (IPP) are among the fatty acid esters that are in great demand in food, cosmetics, and pharmaceuticals as thickeners or lubricants. IPM and IPP are dry, gentle, and nongreasy emollients that are used as excellent solvents for mineral oils, silicones, and lanolin.<sup>2,3</sup> Due to their good absorption characteristics, IPM and IPP are also widely used in cosmetics as oil components, which require excellent distribution properties and good absorption through the skin.<sup>4–6</sup> IPM and IPP can be produced from palm kernel oil-based myristic acid and palm oil-based palmitic acid, respectively, by esterification reaction with isopropyl alcohol (IPA) using an acid catalyst, such as *p*-toluene sulfonic acid (*p*-TSA) or sulfuric acid.<sup>7–9</sup> Even though homogeneous acid catalysts have high catalytic activity, there are some disadvantages, such as unwanted byproducts due to side reactions, equipment corrosion, and environmental pollution.<sup>2</sup> Heterogeneous acid catalysts are proposed to overcome the problems caused by the use of these homogeneous catalysts. The main advantages of heterogeneous catalysts are low equipment corrosion, no environmental pollution, high selectivity, and ease of separation.<sup>10</sup> However, solid acid catalysts also have some drawbacks, such as low thermal stability, low surface area, and solubility in polar media reducing catalytic activity.<sup>11</sup> Lipase enzymes have also been used as catalysts to replace these acid

catalysts and have been shown to catalyze esterification reactions with high yields.<sup>12,13</sup> However, the enzymatic reaction requires a long reaction time to complete and, hence, is insufficient.<sup>14</sup>

The use of microwaves as a tool for chemical reactions has been developed and proven that it could speed up the reactions.<sup>15</sup> The use of microwaves in accelerating reactions has also been categorized as a green reaction due to less waste being produced and the speed at which the desired product is formed.<sup>16–19</sup> The application of microwaves to accelerate the esterification reaction for the manufacture of ethyl laurate using a lipase enzyme catalyst has been explored and is proven to produce emollient ester with high yields and in a short reaction time.<sup>18</sup> Other researchers have shown that the esterification reaction for the production of polyethylene glycol stearate can be accelerated from 360 to 70 min by using microwave radiation and lipase enzyme catalysts.<sup>20</sup> However, based on a literature search, there has been no research on the use of microwaves to accelerate the esterification reaction for the manufacture of IPM and IPP with the enzyme lipase as a catalyst.

Based on this, this present study aims to produce IPM and IPP from the esterification reaction of myristic or palmitic acid with isopropanol using the lipase enzyme as a catalyst under

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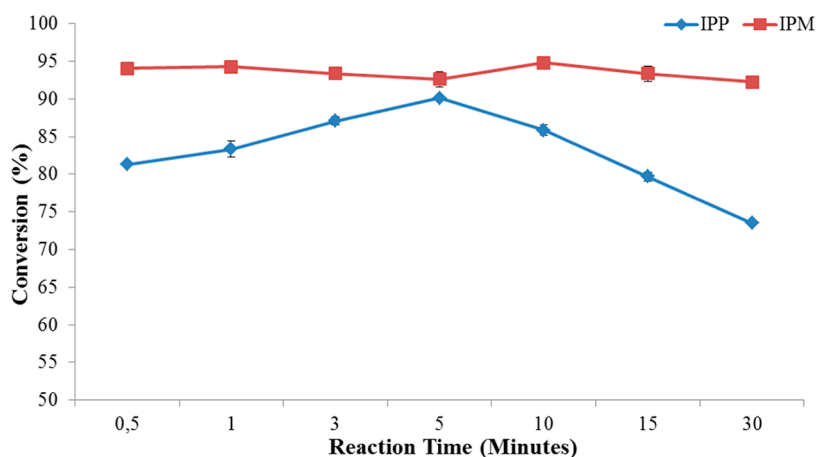


Figure 1. Effect of the reaction time on IPM and IPP conversion.

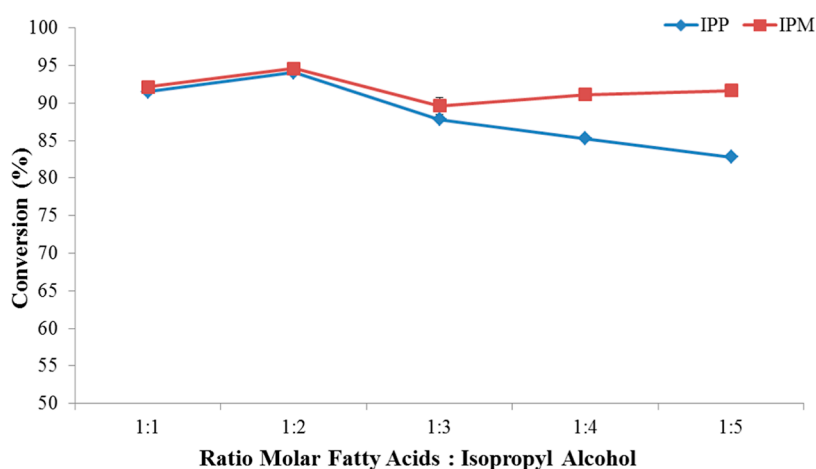


Figure 2. Effect of molar ratio on IPM and IPP conversion.

microwave radiation. The utilization of microwaves to speed up the esterification production of IPM and IPP with lipase enzymes can provide a new repertoire in the process of making emollient ester cosmetic raw materials with a process that is more environmentally friendly and yields high yields. Thus, the problems associated with the use of chemical catalysts, which are high cost and produce unwanted byproducts, can be overcome.

## RESULTS AND DISCUSSION

**Effect of Reaction Time.** Finding an appropriate reaction time is necessary as the incomplete or backward reaction could be achieved in reversible reactions like esterification.<sup>21</sup> Hence, the esterification production of IPM and IPP was conducted under reaction conditions of molar ratio of IPA to myristic or palmitic acid of 1:2, enzyme lipase concentration of 20 wt %, and microwave power of 20%. The IPM and IPP conversions as a function of reaction time for the esterification of myristic or palmitic acid with IPA using lipase enzymes is plotted in Figure 1. As shown in Figure 1, the IPM conversion was slightly higher than the IPP conversion with average conversions of 93.5 and 83%, respectively. Interestingly, the IPP conversion graph pattern showed an increase in the conversion as the length of reaction time was increased and reached a maximum conversion of  $90.1 \pm 0.4\%$  in a reaction time of 5 min. In contrast, the IPM conversion graph depicted

a plateau line with a slightly decreased in reaction time of 5 min. Furthermore, either IPM or IPP conversion exhibits a decreasing slope in the increase of reaction time. This may be attributed to the decreasing enzyme lipase activity due to increasing reaction temperature in the prolonged reaction time.<sup>14</sup> The reaction temperature was observed to have increased from room temperature to 50 °C after 30 min of reaction time. Microwave irradiation-induced thermal toward the reaction mixture, which denatured the protein enzyme rendering losing its catalytic activity.<sup>22</sup> Yadav and Thorat (2012) observed the deactivation of lipase enzyme at a reaction temperature of 60 °C due to thermal denaturation.<sup>23</sup> Similarly, other researchers in microwave-assisted enzymatic esterification of *n*-butyl palmitate noticed a decreasing conversion at reaction temperatures above 60 °C.<sup>19</sup> A one-way analysis of variance (ANOVA) concerning the reaction time on either IPM or IPP conversion revealed a significant effect. The Tukey's posthoc test showed that the significance was driven by all the parameters tested for IPM while it does not confirm the difference for IPP.

**Effect of Ratio Molar.** Stoichiometrically, 1 M of fatty acid is required for an esterification reaction with 1 M of alcohol. However, an excess volume of alcohol usually is used to drive the reaction toward the product.<sup>24</sup> Hence, the effect of the ratio molar of fatty acid to IPA ranges from 1:1 to 1:5 with an increment of 1 molar of alcohol being determined under the reaction condition of catalyst weight of 20 wt %, a reaction

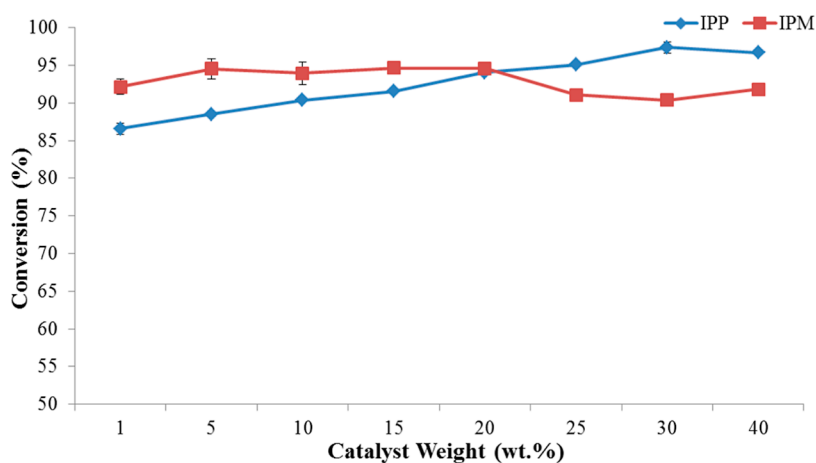


Figure 3. Effect of catalyst weight on IPM and IPP conversion.

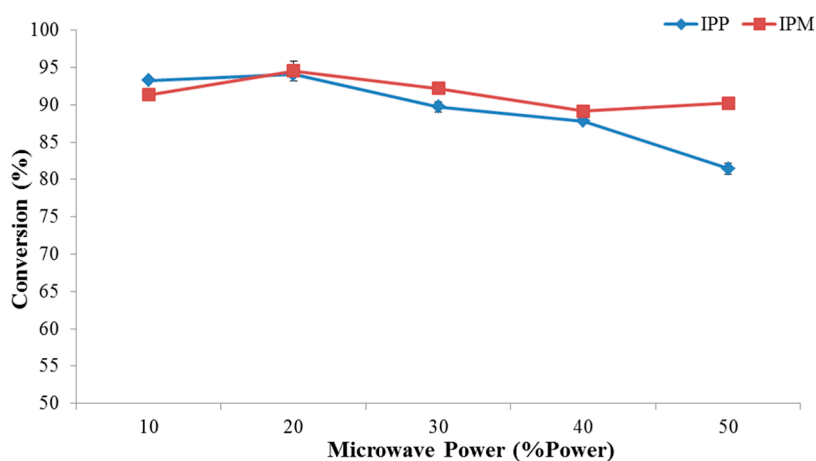


Figure 4. Effect of the microwave power on IPM and IPP conversion.

time of 1 min, and microwave power of 20%. Interestingly, the ester conversion of >90% was achieved using the stoichiometric molar ratio, as shown in Figure 2. As expected, the ratio molar parameter affected the conversion of either IPM or IPP. The ester conversion was increased from  $92.1 \pm 0.0$  to  $91.5 \pm 0.1\%$  at a ratio molar of 1:1 to  $94.6 \pm 0.2$  and  $94.1 \pm 0.2\%$  at a ratio molar of 1:2 for IPM and IPP, respectively. However, the increasing alcohol volume in the reaction mixture did not further elevate the ester conversion. The IPM and IPP conversions were decreased with the addition of alcohol volume. This is presumably due to the enzyme inactivation process and the dilution effect of the alcohol.<sup>18,25</sup> In an excess volume of IPA, the collision frequency of fatty acid and enzyme lipase was reduced which affected the reaction rate and decreased the conversion.<sup>26</sup> This result was in agreement with the result observed for biodiesel production using recombinant lipase<sup>27</sup> and liquid enzyme Eversa transform 2.0 as a catalyst.<sup>26</sup> Khan and Rathod (2020) also achieved a similar result in the enzymatic synthesis of *n*-butyl palmitate. The ester conversion decreased after reaching a maximum conversion of 95.4% at a ratio molar of *n*-butanol to palmitic acid of 1:2 to 60.9% at a ratio molar of 1:3.<sup>19</sup> Further, the ANOVA test showed that all ratio molars of fatty acid to alcohol have significant effects on both IPM and IPP conversion, which were driven by high conversion at a ratio molar of 1:2.

**Effect of Catalyst Weight.** The esterification of fatty acids with IPA was conducted using enzyme lipase as a catalyst with

different enzyme weights of 1, 5, 10, 15, 20, 25, 30, and 40 wt % (based on the weight of fatty acid). The reaction time, ratio molar of fatty acid to IPA, and microwave power were kept constant at 5 min, 1:2, and 20%, respectively. Figure 3 depicts the IPM and IPP conversions when different enzyme lipase weights were used. As shown in this figure, the esters conversion increases with the increase in enzyme lipase dosage to reach the maximum conversion. The IPM and IPP maximum conversion of  $94.7 \pm 0.5$  and  $97.4 \pm 0.7\%$  were achieved using enzyme dosages of 20 and 30 wt %, respectively. However, increasing catalyst weight beyond the culmination point tends to decrease the ester conversion. This phenomenon may be explained by the tendency of enzyme lipase to form an aggregate due to a higher amount of the reactant.<sup>28</sup> Furthermore, another reason is no significant volume of IPA is available in the reactant to fully suspend the enzyme lipase causing less mass transfer.<sup>29</sup> Other researchers suggested that this occurrence is due to the protein–protein interaction in the higher dosage of the enzyme.<sup>30</sup> The significant effect of changing catalyst loading on the conversion of either IPM or IPP was detected by using analysis of variance. Tukey's test emphasizes the significance of IPP conversion being driven by low conversion at a catalyst weight of 1%, while all the catalyst weights tested significantly affected the IPM conversion.

**Effect of Microwave Power.** Microwave irradiation power plays an important role in the chemical reaction as it could

increase the reaction rate, particularly for the molecule that possesses a dipole moment. In this study, the enzyme and the substrate have polar functional groups that constantly try to realign when exposed to microwave irradiation. Hence, rapid friction between molecules producing heat occurred due to continual reorientation of the molecules.<sup>31</sup> The effect of microwave powers of 10–50% on the IPM and IPP conversion was examined under reaction conditions of reaction time of 5 min, molar ratio of 1:2, and catalyst weight of 20 wt %. The esterification of fatty acid with alcohol initially increased with an increase in the microwave power with the maximum conversions of  $94.6 \pm 1.5$  and  $94.1 \pm 0.2\%$  for IPM and IPP, respectively, achieved using a microwave power of 20%. This is consistent with the data from previous reports in the synthesis of 4'-O-acetyl-resveratrol<sup>32</sup> and enzyme-catalyzed biodiesel production.<sup>33</sup> This increment could be attributed to the increase of microwave power creating reorientation molecules that became faster.<sup>33</sup> However, ester conversion decreased with an increase in microwave power. One possible reason for this is enzyme denaturation due to increasing temperature with increasing the microwave power.<sup>32</sup> Further, a ANOVA revealed that microwave power has a significant effect on the conversion of either IPM or IPP. All of the microwave powers tested have a significant effect on IPP conversion, while high conversion drives the significant effect on IPM conversion, which is determined by the Tukey's test (Figure 4).

**Comparison of Esterification Fatty Acid and IPA Methods.** Table 1 presents some studies in the esterification

**Table 1. Summary of Some Esterification Production of IPM or IPP**

product/device	reaction condition	conversion (%)	refs
IPP	Novozyme 435, 4% (w/w), 1:15, 150 min, 60 °C, molecular sieve 10% (w/w)	90	35
water bath shaker			
IPP	<i>p</i> -TSA, 5 wt %, 1:7, 360 min, 82 °C	92.4	8
reflux			
IPM	Novozyme 435, 1:15, 30 min, 60 °C	98.5	5
packed bed reactor (2.5 × 30 cm)			
IPM and IPP	Amberlyst-45, 15 wt %, 1:3, 30 min, 85 °C, 450 W	83.3	17
microwave		83.7	
IPM % IPP	lipase from <i>Aspergillus oryzae</i> , 20 and 30 wt %, 1:2, 1 min, and 35 °C	97.4	this study
microwave		94.5	

of myristic or palmitic acid with IPA to produce IPM or IPP. The esterification is conducted using either homogeneous or heterogeneous acid catalyst or enzyme lipase assisted with different reaction devices, such as packed bed reactor and microwave. In all cases, the ester conversion reached >90% except for a study by Tarigan et al. (2023), which uses heterogeneous acid as a catalyst. This is due to the different phases of the Amberlyst-45 catalyst with other substrates limiting the mass transfer, which affects the conversion.<sup>34</sup> The reaction conditions, such as catalyst weight, ratio molar of fatty acid to alcohol, reaction time, and temperature, do not have a

significant effect on the ester conversion. The highest ester conversion of 98.5% was achieved by using a packed bed reactor. However, the enzyme lipase used in this study was poured into a 2.5 cm × 30 cm tube, hence increasing mass transfer between reactants. The catalyst loading for the other reported results varied from 4–30 wt %. In terms of reaction time, homogeneous acid catalysts required a long reaction time to achieve 92.4% conversion under reflux conditions. In contrast, microwaves could enhance esterification to achieve an IPP conversion of 97.4% in a short reaction time as presented in this study. Interestingly, even though this study uses a low volume of alcohol, the ester conversion is quite similar to other results, which use a high volume of IPA. The reaction temperature used varied from 35 to 85 °C. It is worth noting that the reaction temperature in this study occurs due to rapid collision and friction between molecules.

**Energy and Time Consumption of Enzymatic Esterification Process.** The energy consumption of the device used in the reaction is important to examine for commercialization purposes. Table 2 shows the energy consumption of

**Table 2. Comparison of Energy Consumption and Reaction Time of Enzymatic Esterification**

device	energy consumption (kW h kg <sup>-1</sup> )	reaction time (min)	refs
water bath shaker	283	150	35
packed bed reactor	17.3	30	5
microwave	12.2	1	this study

the enzymatic esterification isopropyl-fatty acid, which was calculated roughly based on the lab scale. Due to no information regarding the electricity consumption of the appliances used in those studies, the power is determined based on the usual laboratory devices. The water bath shaker requires 1700 W of power while the microwave consumes 1400 W. The packed bed reactor itself does not require electricity to perform but to conduct the reaction the device requires other supporting appliances, such as a water bath, peristaltic pump, and magnetic stirrer, which consume 341 W of power in total. The energy consumption for microwave-assisted enzymatic esterification was lower than that for the other appliances. In comparison, the microwave device showed an energy saving of 96% and 42% compared to the water bath shaker and packed-bed reactor, respectively. In addition, the processing time of the enzymatic esterification reaction using a microwave was lower and could save 99 and 29% compared to the other reported published methods.

## EXPERIMENTAL SECTION

**Materials and Methods.** All chemicals used in this study were bought from a local chemicals dealer in Medan—Indonesia and used without any treatment. The lipase enzyme from *Aspergillus oryzae* was purchased from Sigma-Aldrich. The modified household microwave with input and output powers of 1400 and 900 W, respectively, equipped with a water condenser and magnetic stirring was used in this study.

**Esterification Production of IPM and IPP.** The microwave-assisted enzymatic esterification of fatty acids with IPA was conducted in the batch mode on a laboratory scale. The maximum conversion of IPM and IPP was determined by

exploring parameters of reaction times of 0.5, 1, 3, 5, 10, 15, and 30 min, the molar ratio of fatty acids to IPA of 1:1 to 1:5 with increments of 1 molar alcohol, catalyst weights of 1, 5, 10, 15, 20, 25, 30, and 40 wt % and microwave powers of 10, 20, 30, 40, and 50% (based on an output power of 900 W). For each experiment, 2 g of myristic or palmitic acids, 1.19 mL of IPA (for ratio molar of 1:2), and 5 wt % of lipase enzyme (0.1 mL) were placed in a microwave glass reactor and irradiated with power of 20% for 5 min reaction times. After the completion of the reaction process, the product mixture was separated using centrifugation, and the lighter phase which contained IPM or IPP and leftover IPA was transferred to a 50 mL round-bottomed glass for alcohol evaporation. The IPM or IPP was placed in an Erlenmeyer glass for acid value determination.

**Analytical Methods.** The formation of IPM and IPP was identified using Fourier transform infrared spectroscopy, while the conversions were estimated by measuring the acid value of the initial and final samples following AOCS Cd 3d-63. The acid value is expressed as

$$AV = \frac{V \times N \times 56.1}{W}$$

where AV is the acid value (mg KOH/g sample),  $V$  is the consumed volume of KOH (mL),  $N$  is the concentration of KOH (mol/L), and  $W$  is the mass of sample (g). The conversion of IPM or IPP ( $C$ ) was determined by

$$C = \frac{AV_i - AV_f}{AV_i}$$

where  $AV_i$  and  $AV_f$  are the initial and final acid values of the samples, respectively.

**Statistical Analysis.** The single significance effect of reaction time, ratio molar of fatty acids to IPA, catalyst weight, and microwave power on the conversion of either IPM or IPP was statically analyzed using Statistica v13 with a significance level set to  $\alpha = 0.05$ .

## CONCLUSIONS

In the current study, the effect of microwave-assisted esterification production of IPM and IPP was studied by using enzyme lipase as a catalyst. The results showed that all research variables, such as the ratio molar of myristic or palmitic acid to IPA, catalyst weight, reaction time, and microwave power, have a significant effect on the formation of IPM and IPP esters, for which a maximum conversion of IPM of  $97.4 \pm 0.7\%$  was obtained under the reaction conditions of a ratio molar of 1:2, catalyst weight of 30 wt %, 5 min reaction time, and 20% microwave power. The maximum IPP conversion of  $94.5 \pm 1.3\%$  was obtained at a ratio molar of 1:2, 5 wt % of catalyst weight, 1 min reaction time, and 20% microwave power. The results indicate that microwave radiation can facilitate the esterification production of IPM and IPP with a high conversion and fast reaction times. Further, microwave-assisted enzymatic esterification consumes less energy and could save reaction times of 99 and 29% compared to another enzymatic esterification method.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.3c04336>.

ANOVA and Tukey post hoc tests for all parameters tested on ester conversion (PDF)

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### Notes

The authors declare no competing financial interest.

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