Ultrasound Assisted Green Synthesis of 2-Ethylhexyl Stearate: A Cosmetic Bio-lubricant

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Abstract: The 2-ethylhexyl stearate is used as a bio-lubricant in various cosmetic products. The present study is focused on the biocatalyzed esterification of 2-ethylhexanol and stearic acid to form 2-ethylhexyl stearate catalyzed by Fermase CALB 10000 in the presence of ultrasound treatment. The maximum conversion (95.87%) was obtained at molar ratio of 2-ethylhexanol to stearic acid 2:1, enzyme amount of 2 % (w/w), power 80 W, duty cycle 50 % and temperature 50°C in comparatively short reaction time (3 h) in the presence of Fermase as a catalyst. At optimum conditions, it is observed that in the presence of ultrasound; the reaction time minimizes up to 4 h as compared to mechanical stirring method (7 h). The physiochemical properties for the 2-ethylhexyl palmitate were also evaluated.

Key words: bio-lubricant, 2-ethylhexyl stearate, cosmetics, lipase, ultrasound

1 Introduction

Nowadays, there is a huge demand for the synthesis of sustainable products from natural resources. It is equally of major concern to investigate natural raw resources to feature the bio-based lubricants^{1,2}. In recent years, the bio-lubricants have been used widely in the industrial sectors because of their distinctive harmfulness and biodegradable nature. The ethylhexyl esters are oily in nature and therefore regularly used in the beautifying formulations as an oil component and as a solvent for active substances³. It is popularly used in the formulations of skin-care products and eye makeup. With the consistently developing interest for bio-lubricants in various industries like food, pharmaceuticals, and cosmetics, different chemical and enzymatic methods have been evolved for the synthesis of fatty acid ethylhexyl esters^{4,5}.

Several lubricants were obtained from vegetable oils, waste cooking oils and their methyl esters. Zheng *et al.* developed 2-ethylhexyl palmitate by transesterification method by using powdered potassium carbonate as a catalyst⁵. But this method requires a high temperature of 180°C. Hence, enzyme catalyzed production of bio-lubricant is in high demand, as enzymatic synthesis requires ambient conditions. Recently, enzymatic synthesis of 2-ethylhexyl oleate by transesterification of 2-ethylhexanol and biodiesel was reported by Kleinaité *et al.*⁶. Habib *et al.* discussed the transesterification of palm oil methyl ester with 2-ethylhexanol⁷. Richetti *et al.* explained the solvent

free synthesis of 2-ethylhexyl palmitate by using response surface methodology in the presence of Novozym 435⁴. In the literature, there are numerous esterification and transesterification methods reported for the synthesis of 2-ethylhexyl stearate. The esterification method involves reaction with 2-ethyl hexanol and stearic acid while the transesterification method involves reaction between rapeseed oil or palm oil with 2-ethyl hexanol⁸⁻¹⁰. However, all these reports required long reaction time to achieve the desired conversion.

The slow reaction time is a major drawback of enzymatic synthesis, which limits the enzymatic production. For this reason, new techniques such as ultrasound, microwave, and supercritical fluids are used for the process intensification of enzyme catalyzed reactions¹¹⁻¹⁴. Ultrasound may enhance the activity of the enzyme under mild operation conditions and significantly used in the biotechnology and food processes. The ultrasound irradiation progresses the mass transfer in the reaction mixture, which enhances the interaction between the enzyme and substrate molecules^{15,16}. The excess irradiation can denature the enzyme; therefore, it is essential to optimize the parameters to achieve the maximum conversion of the reaction. The present work is based on the ultrasound assisted synthesis of 2-ethylhexyl stearate from stearic acid and 2-ethylhexyl alcohol by esterification method. The Fermase CALB 10000, a commercial lipase has been used for the synthesis. The process was optimized with one factor at a time method consider-

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Accepted April 24, 2020 (received for review December 10, 2019)

Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online

<http://www.jstage.jst.go.jp/browse/jos/> <http://mc.manuscriptcentral.com/jjocs>

ing the parameters such as; molar ratio, enzyme loading, ultrasound power, duty cycle, and temperature.

2 Material and Methods

2.1 Materials

The reactants used in the esterification reactions were stearic acid and 2-ethyl hexanol (99% purity) obtained from Sigma Aldrich, Mumbai. The commercial enzyme Fermase CALB 10000 from *Candida antarctica* immobilized on a macroporous polyacrylate resin was kindly supplied by Fermenta Biotech, Mumbai, India. The potassium hydroxide, sodium hydroxide, ethanol, and phenolphthalein indicator were procured from S. D. Fine chemicals, Pvt. Ltd., Mumbai, India.

2.2 Ultrasound assisted esterification

Ultrasound assisted 2-ethylhexyl stearate using Fermase as a catalyst was carried out in an ultrasonic thermostated water bath (manufactured by Dakshin India Pvt. Ltd., 4.5 L capacity) with 4 transducers located at the bottom of the bath. The reactions were performed in a glass reactor (50 mL capacity) having a flat bottom. The reaction system is heterogeneous in nature; therefore, the overhead stirrer was used to mix the reaction mixture. The reaction assembly was kept in such a manner that it is obtained the extreme gravitational intensity as detected in the former mapping work¹⁷. Ultrasound irradiation was started in the reaction mixture with stirring, and reaction samples were taken out at a definite time intervals. The reaction progress was evaluated by titrating a reaction sample with 0.05 N KOH solution to decide the amount of unconverted stearic acid in terms of the acid value of the mixture. The reaction was conducted until the completion of equilibrium conditions.

2.3 Titrimetric analysis

The production of 2-ethylhexyl stearate ester was calculated by determining the unreacted stearic acid with 0.05 N potassium hydroxide. The phenolphthalein was used as an indicator. The % conversion of ester was determined by the quantity of remaining acid. The ester conversion was calculated by the following equation:

$$\text{Conversion (\%)} = \frac{(\text{Vol. of KOH utilised (without enzyme)} - \text{Vol. of KOH utilised (with enzyme)})}{\text{Vol. of KOH utilised (without enzyme)}} \times 100 \quad (1)$$

2.4 Purification of 2-ethylhexyl stearate

The 2-ethylhexyl stearate was purified by a simple distillation method. The immobilized enzymes in the reaction mixture were removed through Whatman paper. The unre-

acted stearic acid in the reaction mixture was neutralized with Na_2CO_3 and the traces of formed salt were removed by several water washings. Anhydrous MgSO_4 was added to the remaining mixture as a drying agent to remove the water from the mixture. The residual 2-ethylhexanol was removed by distillation.

2.5 Evaluation of bio-lubricant properties

The bio-lubricant properties of 2-ethylhexyl stearate (2-EHS) was tested using standard methods, density: ASTM, 2016a; kinematic viscosity: ASTM, 2017a; moisture content: ASTM, 2016b; namely acid number: AOCS, 2009; pour point: ASTM, 2017b; and Refractive index: AOCS. Official Method Tp 1a-64.

3 Results and Discussion

3.1 Effect of molar ratio

In the solvent free esterification reaction, the ratio of stearic acid to 2-ethylhexyl alcohol can affect the physical and chemical properties of a reaction system. The stearic acid shows solubility in different alcohols like ethanol, propanol, butanol, acetone, ethyl acetate, etc.^{18, 19}. In the present study, the stearic acid is sparingly soluble in the 2-ethylhexyl ethanol. To shift the equilibrium towards the forward direction, it is essential to increase the amount of alcohol in the reaction mixture. Hence it is crucial to optimize the molar ratio of the reactant as the reaction is carried out in the absence of a solvent²⁰. Figure 1 illustrates the molar conversion of ester under the molar ratio of acid to alcohol from 1:1 to 1:3 at 50°C for 3 h of reaction time, 2% enzyme amount (w/w of total substrate) with 200 RPM speed. The ultrasound frequency was kept constant at 25 kHz, power 60 W, and 50% duty cycle. From Fig. 1 it can be seen that when the molar ratio of acid to alcohol was varied from 1:1 to 1:2, the reaction conversion increased from 72% to 84%. The increase in the amount of alcohol may reduce the viscosity of the reaction mixture, and more cavitation bubbles are created because cavitation actions become easier, and successively improves the percent conversion of the reaction. However, the percentage conversion reduces with an increase in the amount of alcohol. The initial increase in the amount of alcohol attributes to improve the reaction rate and the conversion of ester. This may be because of rapid formation of enzyme-substrate complexes. While a decrease in conversion after addition of excess alcohol maybe because of denaturation of the enzyme²¹. Simultaneously, a higher amount of alcohol may be a restraint to the reaction between the enzyme and substrate molecule. Therefore, the results show a 1:2 acid to alcohol ratio which is an optimum molar ratio for further experiment.

Fig. 1 Effect of alcohol to acid molar ratio on conversion of stearic acid in ultrasound assisted enzymatic synthesis of 2-ethylhexyl stearate (catalyst loading 2% (w/w), temperature 60°C, frequency 25 kHz, power input 60 W, W duty cycle 50% and agitation speed 200 rpm).

3.2 Effect of enzyme loading

The total amount of enzyme required for the highest conversion is the key economic factor in the enzymatic esterification study for its industrial application. To select the ideal amount of enzyme required for the esterification reaction, experiments were performed in the range of 1% – 3% (w/w) enzyme loading, temperature 60°C, keeping power 60 W with duty cycle 50% at frequency 25 kHz with 200 RPM speed. With distinctive enzyme amounts, the conversion results achieved are shown in **Fig. 2**. The conversion increased from 81% to the maximum level of 93% with the increase of enzyme amount from 1% to 2% (w/w). However, a further increase of enzyme (above 2%) amount declined conversion to 86%. The effect of ultrasound cavitation is directed by ultrasound power and the properties of the reaction medium. The viscosity of the reaction medium increases as the immobilized enzyme amount is increased, and this has an unfavorable effect on cavitation at higher enzyme loading. This is because of the loss of ultrasound intensity from the absorption and weakening of ultrasound waves at higher enzyme loading in the reaction medium²². Hence, ultrasound dissipation in the reaction mixture was little to generate effective cavitation. It led to creating weak turbulence to impart systematic mixing of reactants, and consequently, conversion decreases^{23, 24}.

Fig. 2 Effect of enzyme on conversion of stearic acid in ultrasound assisted enzymatic synthesis of 2-ethylhexyl stearate (acid to alcohol molar ratio 1:2, temperature 60°C, frequency 25 kHz, power input 60 W, W duty cycle 50% and agitation speed 200 rpm).

3.3 Effect of temperature

2-ethylhexyl stearate production was carried out with 2% enzyme amount, over the range of 40-60°C temperature at acid to alcohol molar ratio of 1:2 and 3 h reaction time. **Figure 3** shows that the conversion of ester increases from 79% to 93% by increasing the temperature from 40°C to 50°C respectively. The rise in temperature shows a positive effect on the progress of reaction due to enhancing the free energy and enzyme activity. Besides, an increase in temperature decreases the viscosity of the reaction, which influences the cavitation bubble formation and its subsequent collapse. However, an increase in temperature up to 60°C dropped the conversion to 84% because at a higher temperature, the enzyme structure gets disrupted and leads to loss of activity of the enzyme. At higher temperatures, more bubbles are produced through nonstop collision with decreased cavitation intensity. This causes the limitation of transporting reactant species from the reaction mixture to the active sites of enzyme surface. This prompts no remarkable change in the percentage conversion of product²⁴. Further, at higher temperatures, the vapor pressure of the reactants increases, and cavitation bubbles are filled with more vapor of the reactants causing small force cavitation. This shows that any change in temperature causes the change in the amount of conversion²⁵. Thus, for further experiments, 50°C was elected as the ideal temperature.

Fig. 3 Effect of temperature on conversion of stearic acid in ultrasound assisted enzymatic synthesis of 2-ethylhexyl stearate (acid to alcohol molar ratio 1:2, enzyme loading 2% (w/w), frequency 25 kHz, power input 60 W, W duty cycle 50% and agitation speed 200 rpm).

3.4 Effect of irradiation power and frequency

The effect of ultrasound power has been studied on the esterification reaction by changing rated power from 60 to 100 W at 25 kHz frequency and keeping other experimental parameters constant. As the ultrasound power increases from 60 W to 80 W with reaction progress of 180 min, the esterification of 2-ethylhexyl ester was increased from 93 to 95% (Fig. 4). With an increase in power, the larger amplitude was created in the solvent medium, which produces more bubbles and collapses. The produce shocked wave accelerates the mass transfer, which improves the enzyme-substrate complex and alters an ester formation. Khan *et al.* found similar results in the production of *n*-butyl palmitate²⁴. But, when ultrasound power was increased from 80 W to 100 W, the conversion was reduced gradually due to enzyme denaturation by high irradiation. Therefore, the ultrasonic power of 80 W was selected as the input power for ultrasound assisted synthesis of 2-ethylhexyl stearate.

Figure 5 shows the effect of two types of ultrasound frequencies, i.e., 25 kHz and 40 kHz for the esterification reaction. It was observed that the conversion of 2-ethylhexyl stearate is higher at 25 kHz than that of 40 kHz. The power dissipation values were calculated by the calorimetric method. The power dissipation values for 25 kHz and 40 kHz were obtained as 85% and 93%, respectively. When a high frequency of ultrasound is applied, an abundant number of cavitation bubbles are created in the mixture of reaction. The excess cavitation bubbles are expected to

Fig. 4 Effect of ultrasound power on conversion of stearic acid in ultrasound assisted enzymatic synthesis of 2-ethylhexyl stearate (acid to alcohol molar ratio 1:2, enzyme loading 2% (w/w), temperature 50°C, frequency 25 kHz, duty cycle 50% and agitation speed 200 rpm).

Fig. 5 Effect of ultrasound frequency on conversion of stearic acid in ultrasound assisted enzymatic synthesis of 2-ethylhexyl stearate (acid to alcohol molar ratio 1:2, enzyme loading 2% (w/w), temperature 50°C, power input 80 W, duty cycle 50% and agitation speed 200 rpm).

Fig. 6 Effect of ultrasound duty cycle on conversion of stearic acid in ultrasound assisted enzymatic synthesis of 2-ethylhexyl stearate (acid to alcohol molar ratio 1:2, enzyme loading 2% (w/w), temperature 50°C, frequency 25 kHz, power input 80 W, and agitation speed 200 rpm).

combine and form bigger and more steady bubbles and, consequently, they create a limit to acoustic energy transfer²⁶⁾. This results in lesser conversion at 40 kHz as compared to 25 kHz. Hence, 25 kHz was selected as the optimal frequency for the formation of 2-EHS.

3.5 Effect of duty cycle

The ultrasound assisted enzymatic esterification reactions are strongly affected by the ultrasound irradiation time. The cavitation produces the higher local temperature, and subsequently, continuous irradiation may denature the enzyme. Additionally, the application of the constant irradiation will impair the transducers and can lead to charring of reactants because of high temperatures accomplished. Consequently, for better results of cavitation; it is used in a specific interval of time²³⁾. Consequently, the effect of various duty cycles on the esterification reaction was studied. **Figure 6** demonstrates that 30% duty cycle (3 min ON and 7 min OFF) gives 81% conversion, that might be the result of the lack of the energy of corresponding reaction; whereas at 70% duty cycle (7 min ON and 3 min OFF) gave only 72% conversion which may be due to the overabundance energy. This noticeably demonstrated that extra exposure of ultrasound irradiation to the reaction mixture might deactivate the enzyme, consequently giving a lesser conversion²⁴⁾. Henceforth, the duty

Table 1 Physicochemical evaluation of 2-EHS.

Property	2-Ethylhexyl stearate
Acid value (mg KOH g ⁻¹)	0.75
Density at 25°C (gcm ⁻³)	859.2
Kinematic viscosity at 40°C (mm ² s ⁻¹)	8.85
Moisture content (wt%)	0.58
Pour point (°C)	-4.0
Refractive index	1.451

cycle of 50% (5 min ON and 5 min OFF) was chosen as an ideal duty cycle for the given reaction, which contributed 95.87% conversion.

3.6 Evaluation of bio-lubricant properties

The bio-lubricant properties, namely an acid number, density; kinematic viscosity; moisture content; pour point; refractive index were evaluated, and the observed results for 2-EHS are listed in **Table 1**. The acid value of 2-EHS is less than 1, indicating the low corrosive effect and kinematic viscosity was 8.85 mm²s⁻¹ at 40°C, suggesting good spreadability. The pour point of 2-EHS was -4°C determines the low-temperature flow properties. The moisture content was measured as 0.58(wt%), which is in the range of lubricant properties. The refractive index of 1.451 indicates good luster imparting properties. The evaluated properties reported in **Table 1**, met the requirement for an emollient.

3.7 Reusability of the catalyst

The economic significance of the enzyme catalyst was decided by its reusability. The immobilization of the enzyme is known to improve the recyclability. In any case, the ultrasound cavitation may cause conformational changes and make compound inactivate to some degree. Hence successive reaction cycles were led to check the reusability of the ferriase enzyme. After the individual cycle, the used enzyme was washed with acetone, dehydrated at 35-40°C for 6 h, and reused again to investigate the reusability of enzyme. **Figure 7** reveals that the ferriase enzyme was successfully used up to 7 successive cycles. The percentage conversion, acquired at the end of the 5th and 7th cycle was 85.54% and 72.49%, respectively. It was detected that the initial activity of the enzyme was reduced to 35% up to 5th cycle and 60% at 7th cycle. Thus, ferriase can be effectively reused up to 5-6th cycles. The reduction in the enzyme activity was credited to predictable contact of the enzyme with ultrasonic cavitation, which may hurt the nearby conformational versatility of the enzyme. Additionally, the nonstop introduction of alcoholic substrate may also cause the deactivation of enzymes. Subsequently, the present examination conveys the utilization of the

Fig. 7 Reusability of immobilized ferulase enzyme for successive cycles.

enzyme and ultrasound as greener innovation for the reasonable future and enzymatic process.

3.8 Comparative study between the ultrasound assisted reaction and conventional synthesis of 2-ethylhexyl stearate

The enzyme catalyzed esterification was performed in ultrasound with stirring (UIS), ultrasound without stirring (UI) and mechanical stirring (MS) under optimized conditions. It is observed that to attain the 95% conversion of 2-EHS, the time required for conventional synthesis was 7 h at temperature 50°C, 2% enzyme loading and acid to alcohol molar ratio 1:2 at 200 RPM speed. However, in the presence of ultrasound with stirring and ultrasound alone gives higher conversion than that of the mechanical stirring method. **Figure 8** depicted the comparative results of the ultrasound with stirring (UIS), ultrasound without stirring (UI), and conventional stirring (CS) method. The reaction conditions for UI were: temperature 50°C, acid: alcohol 1:2, enzyme loading 2% (w/w), frequency 25 kHz, duty cycle 50%, and power 80 W. While in the case of UIS, the reaction was carried out in a similar condition as in UI with 200 RPM stirring speed. It has been detected that the application of ultrasound irradiation may enhance the conversion from 53.36% to 95.85% due to the presence of ultrasound with a stirring method. The cavitation phenomena of ultrasound irradiation can eliminate the mass transfer limitation and alters the reaction towards desired products^{23, 26}. The ultrasound in combination with a stirrer also helps in even mixing the reaction mixture, especially the solid enzyme

Fig. 8 The comparison for synthesis of 2-ethylhexyl stearate through mechanical stirring (MS), ultrasound irradiation without stirring (UI) and ultrasound irradiation with stirring (UIS).

particle with cavitation by irradiation and turbulence by stirrer. Therefore, ultrasound alone reduces the conversion to 82.53% due to the absence of an agitation. The ultrasound with stirrer can enhance the rate of reaction because of uniform mixing of reactant and may reduce the possibility of the enzyme to settle down in the reactor and also the agglomeration of the enzyme. Overall, ultrasound with stirring may provide the highest conversion as compared to other methods^{24, 26}. Thus, the disadvantage of enzyme catalyzed reaction, i.e., the moderate reaction rate, can be overcome by the utilization of ultrasound.

4 Conclusion

For the first time, the ultrasound supported enzymatic synthesis of a bio-lubricant 2-ethylhexyl stearate using Ferulase as a catalyst was studied. The optimization of numerous parameters like acid to alcohol molar ratio, temperature, enzyme loading, duty cycle, ultrasound power, and frequency was completed effectively. With several optimized factors, the conversion of 2-ethylhexyl stearate achieved was 95.87% in a solvent free condition. Correlation of ultrasound assisted enzymatic synthesis with conventional systems realizes the significance of ultrasound for maximum percentage conversion of ester at a lesser reaction time. Overall, improved conversion with less time has an appealing candidature for ultrasound assisted enzymatic esterification, which can be further utilized for different cosmetic products.

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