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Enzymatic Synthesis of Citronellyl Palmitate in Organic Media: Process Optimization and Kinetic Evaluation

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Kinetics of citronellyl palmitate ester formation from citronellol-palmitic acid was studied by using immobilized lipase Novozym 435 *Candida antarctica*. Lipase immobilized onto macroporous acrylic resin gave the best results. Different parameters like reaction medium, temperature, substrate concentration were optimized for the enzymatic synthesis of citronellyl palmitate. The optimum temperature was 30 °C, optimum molar ratio of alcohol to acid was 1:1. The best solvent for maximum esterification was *n*-hexane. At higher concentration of alcohol enzyme inhibition took place.

Keywords: Lipase Novozyme 435 by *Candida antarctica*, Citronellol, palmitic acid, *n*-Hexane.

INTRODUCTION

The monoterpenoid dihydrogeraniol commonly known as citronellol, is naturally present in essential oils of plants. Citronellol has two enantiomeric isomers; the (+) citronellol is a constituent of citronella oil and (-)-citronellol is found in rose oil [1].

Both geraniol and citronellol are members of acyclic terpenes and have got commercial significance [2]. Esters of citronellol alcohol have often rose like fragrance and have applications in soap, pharmaceutical, cosmetics, food and flavouring industries [3]. Biochemical methods have immense importance due to difficulty in obtaining pure ester of terpene alcohols by chemical method or isolation by dry or steam distillation and solvent extraction from natural sources.

Lipase catalyzed esterification got importance at laboratory and commercial levels over other methods of esterificaiton because these can be carried out easily at ambient conditions [4]. Lipases are basically hydrolyze ester bonds in aqueous environment. These can catalyze synthetic reactions only in micro-aqueous environment in organic reaction media. Moreover the organic reactants have low solubility in water and hence organic solvents become suitable to suppress hydrolysis and enhance esterification [4].

Lipase catalyzed reactions can be explained by sequential and non-sequential mechanisms. Non-sequential mechanism is also known as ping-pong bi-bi mechanism. For two substrates reaction the non-sequential mechanism can be applied as bi-bi mechanism such as the kinetic model of Yadav *et al.* and others for butyl isobutyrate ester formation with inhibition by *n*-butanol [5-10], Ping-Pong kinetic model with competitive inhibition by alcohol [11], by acid [12-14] or by both substrates [6] was suggested in lipase-catalyzed esterification reactions.

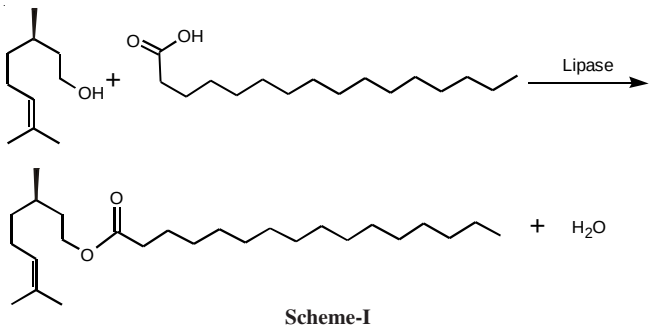
The aim of the work was to investigate the effect of selected parameters using orbital shaker. The esterification of citronellol and palimitic acid which produced citronellyl palmitate was evaluated to study the kinetic of reaction and it mechanism. The ester has a typical odour and can be used in food industry for flavouring and in perfume industry as fragrant.

EXPERIMENTAL

Biocatalyst: Different fungal lipases were produced by fermentation and used as biocatalysts after freeze dying or immobilization. Another biocatalyst used during the study was Novozyme 435 (Lipase from *Candida antarctica*, supported on macropororous acrylic beads 0.2-0.5 mm in diameter) purchased from Sigma Aldrich.

Synthesis of citronellyl palmitate: Different concentrations of citronellol and palimitc acid like 0.1, 0.3, 0.5, 0.7, 1.0 mol per cm^3 were prepared by dissolving calculated quantities in 20 mL *n*-hexane solvent in 100 mL quick fit glass flask. Then 10 mg lipase enzyme was added in each flask. Samples were

placed in the orbital shaker for agitation at 100 rpm at different temperatures like 10, 30, 40 °C. Sample were drawn after 4 h initially and then after 24 h regularly for 3 days. The mixture obtained was cooled by freezing mixture to stop reaction and for further analysis to calculate the percentage yield (**Scheme-I**) [15-19]. After the completion of reaction, the reaction mixture was filtered for the removal of insoluble impurities like enzyme lipase particles. Then evaporated the solvent to get pure product (ester) for further analysis like IR and TLC.



Scheme-I

Lipase assay: Activity of different lipases was determined on the basis of titration of free fatty acid produced by lipase catalyzed hydrolysis of emulsified olive oil [20].

Analysis of esterification mixture: The rate and yield of esterification was determined by titration of residual palmitic acid at any time point using 0.1 mol/dm^3 ethanolic potassium hydroxide [21].

RESULTS AND DISCUSSION

Selection of biocatalyst for the synthesis of citronellyl palmitate: Different lipases were used to catalyze the reaction between citronellol and palmitic acid. Table-1 shows that among the six biocatalyst, Novozym 435 was the best with an esterification yield of 76 % in 72 h.

TABLE-1

Biocatalyst	Esterification yield (%)
Free lipase *Rhizopus arrhizus*	3.0
Rhizopus arrhizus lipase immobilized on celite	35
Rhizopus arrhizus lipase immobilized on alumina	0.0
Rhizopus arrhizus lipase immobilized on silica gel	10
Rhizopus arrhizus lipase immobilized on microporous ceramic particles	41
Novozym 435	76

Effect of citronellol concentration on the reaction rates: It was revealed from the results shown in the Fig. 1 that the rate of reaction was increased on increasing citronellol concentration and achieved highest values at 500 mmol L^{-1}. A further increase in the concentration of alcohol could not enhance the esterification rates at any fixed concentration of the acid. However overall maximum rate of 1.818 mmol L^{-1} min^{-1} was obtained when concentrations of both acid and alcohol were 500 mmol L^{-1}, each. At higher concentrations inhibition took place and reaction rate and percentage acylation were decreased.

Double reciprocal plot of initial esterification rates by varying citronellol concentrations were plotted. Fig. 2 shows

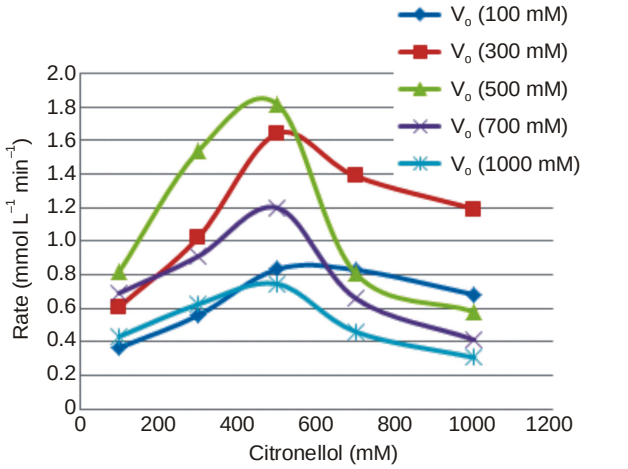


Fig. 1. Effect of citronellol concentration on the rate at various initial palmitic acid concentrations

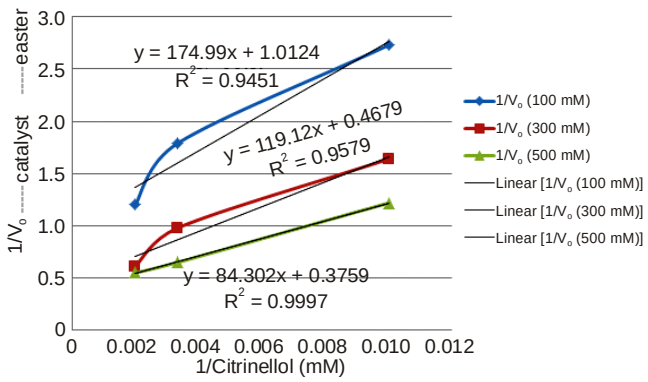


Fig. 2. Double reciprocal plot of initial acylation rate by varying citronellol concentrations

straight lines obtained by ploting graphs between reciprocals of concentrations of citronellol on x-axis and reciprocals of rates of reaction on y-axis. Each line represent relationship between two reciprocals at a fixed concentrations of palmitic acid. The double reciprocal graph was used to calculate Michaelis constant (K_m) with reference to citronellol as under and it was found to be 229.25 mmol L^{-1}.

Effect of palmitic acid concentrations on the reaction rates: It is evident from the Fig. 3 that rates of acylation reactions were affected by changes in palmitic acid concentration. The rates were increased on increasing the concentrations of palmitic acid at various fixed concentrations of citronellol. At all fixed concentrations of citronellol maximum rates of acylation were obtained when the concentration of palmitic acid was 500 mmol L^{-1}. At higher concentrations of palmitic acid inhibition took place and percentage acylation as well as reaction rate were decreased.

Double reciprocal plot of initial esterification rates by varying palmitic acid concentrations were plotted. Fig. 4 shows straight lines obtained by ploting graphs between reciprocals of concentrations of palmitic acid on x-axis and reciprocals of rates of reaction on y-axis. Each line represent relationship between two reciprocals at a fixed concentrations of alcohol. The double reciprocal graph was used to calculate Michaelis constant (K_m) with reference to palmitic acid as under and it was found to be 242.27 mmol L^{-1}.

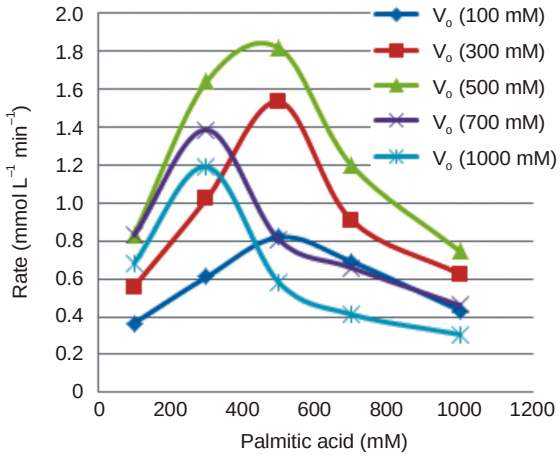


Fig. 3. Effect of palmitic acid concentration on the rate at various citronellol concentrations

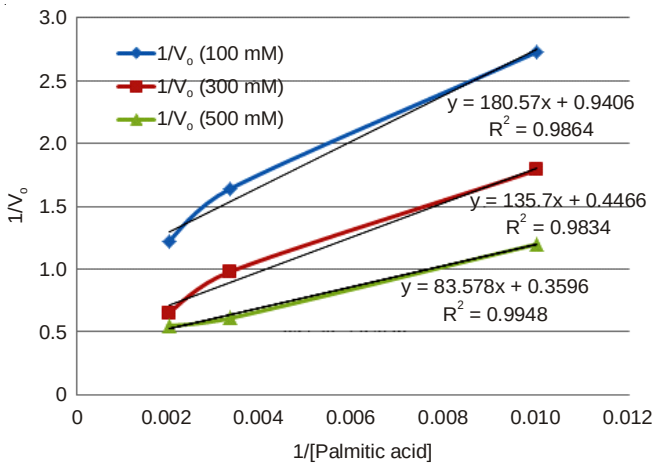


Fig. 4. Double reciprocal plot of initial esterification rate at various palmitic acid concentrations

Effect of temperature on percentage esterification: Esterification of equivalent millimolar concentrations of citronellol/palmitic acid (0.1/0.1, 0.5/0.5) was carried out at 10, 30 and 40 °C for 24 h. At 10 °C esterification yield was quite low. It was noticed that on increasing temperature up to 30 °C maximum esterificaiton was done and with further increase in temperature rate of esterification was decreased. So the optimum temperature for esterification was 30 °C (Fig. 5).

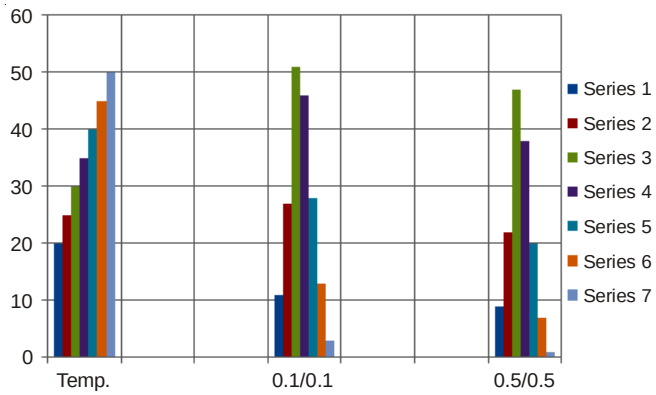


Fig. 5. Effect of temperature on percentage esterification

Effect of solvents on percentage esterification: Percentage esterification was calculated in different solvents (acetone, diethyl ether, benzene and *n*-hexane) of 0.1 mM reaction mixture at 30 °C. *n*-Hexane was the best solvent with 92.65 percentage esterification (Fig. 6). Similar results have already been reported by Tahir *et al.* [22].

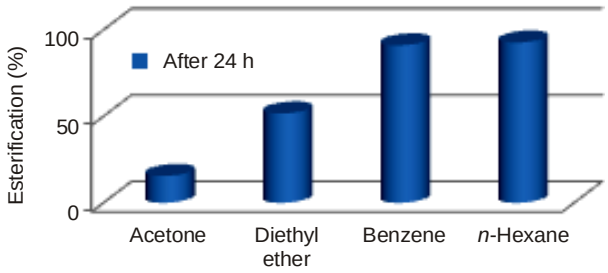


Fig. 6. Effect of different solvents on % yield of citronellyl palmitate

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