Journal of Agriculture and Food Research 16 (2024) 101186

Contents lists available at ScienceDirect

Journal of Agriculture and Food Research

journal homepage: www.sciencedirect.com/journal/journal-of-agriculture-and-food-research

Simultaneous geraniol and citronellol transesterification using *Pseudomonas fluorescens* lipase for the production of fragrance and flavour esters: A kinetic study

Alisa S. Wikaputri [a], Derek J. Irvine [a], Robert A. Stockman [b], Parimala Shivaprasad [a,*]

[a] Department of Chemical and Environmental Engineering, University of Nottingham, Nottingham, NG7 2RD, United Kingdom
[b] Department of Chemistry, University of Nottingham, Nottingham, NG7 2RD, United Kingdom

ARTICLE INFO

Keywords:
Lipase
Geraniol
Citronellol
Terpene ester
Transesterification
Kinetic model

ABSTRACT

This study aims to develop, for the very first time, a kinetic model for the simultaneous transesterification of geraniol and citronellol using *Pseudomonas fluorescens* lipase to produce their respective esters. This allows us to test the feasibility of the bioprocess and comprehend the enzyme behaviour, fine-tune process efficiency, and predict process performance. Geranyl and citronellyl esters are key ingredients in fragrance and flavour formulations in the food sector, as well as bioactive compounds for pest and insect repellents in the agricultural sector. Unlocking a greener and sustainable production of these terpene esters directly from plant-derived essential oil is crucial to meet the 2050 net zero targets to produce bio-based chemicals. The simultaneous transesterification of geraniol and citronellol in a batch reactor successfully achieved conversions of 66 % for citronellol and 94 % for geraniol within 5 h. Key optimum conditions were 1:8 molar ratio (geraniol:vinyl acetate), an enzyme loading of 10 wt % of geraniol, temperature of 40 °C, and a stirring speed of 250 RPM. The results indicated that simultaneous transesterification followed a ternary complex mechanism with competitive inhibition by alcohol. Initial testing of rose geranium oil under optimised conditions yielded 32 % citronellol and 50 % geraniol conversion after 30 h. Overall, this study shows promise as a green pathway utilising enzyme catalysis to selectively produce esters from diverse terpene mixtures and can be extended to terpene alcohols derived from flower essential oils.

1. Introduction

Terpene esters, specifically acetate, propionate, and butyrate esters of geraniol and citronellol are commercially significant building blocks for the flavour and bioactive compounds in food and agriculture sectors, respectively [1–3]. They are utilised for the sensory experience and functionality in food additive formulations and have demonstrated potential as an environment-friendly insect and pest repellents [4–7]. Terpene esters are commercially produced through fractional distillation of natural essential oils [9] or synthetically through modification of their respective terpene alcohols [10]. However, both routes are limited by low product yield, harsh operating conditions, high solvent footprint and resource intensive downstream processing for product purification [11]. Hence, there is a need to identify 1) new alternative sources of natural extracts and 2) a green and sustainable pathway for producing flavour, fragrance and bioactive compounds [12].

Flower biomass is an attractive alternative feedstock as it is rich in monoterpenes and is easy to process because of its low lignin content compared to other agricultural biomass. Geraniol and citronellol are the two major terpene alcohols found in flower essential oils [12–14], allowing for further structural modification into their esters. The alternative enzyme biocatalysis to chemocatalysis for synthesising individual geranyl or citronellyl esters by means of esterification or transesterification has been extensively researched over recent decades [16–23]. Enzyme biocatalysis offers several advantages, including mild reaction conditions, high regioselectivity, minimum downstream processing, as well and the capability to generate products of purity equivalent to or surpassing those obtained through fractional distillation [24]. As a result, there is a growing interest, both in research and commercial spheres, in leveraging enzyme biocatalysis as a safer, more sustainable, and greener alternative to chemo-catalysis routes [25]. However, there exist additional considerations in the determination of

* Corresponding author.
E-mail address: parimala.shivaprasad@nottingham.ac.uk (P. Shivaprasad).

https://doi.org/10.1016/j.jafr.2024.101186
Received 7 February 2024; Received in revised form 15 April 2024; Accepted 30 April 2024
Available online 1 May 2024

the most suitable enzymatic pathway, encompassing the selection of specific enzymes, solvents, reaction conditions, and related parameters.

Lipases from *Pseudomonas fluorescens* (PFL), have been effectively employed in the production of terpene esters in both esterification [9] and transesterification [17,18] yielding up to 100 % conversions, which is the choice of enzyme in this study. It is a relatively more economical alternative to *Candida antarctica* lipase B, offering very similar efficiency for terpene ester synthesis [27]. This aspect holds significant importance in techno-economic evaluations for process scale-up [28]. The transesterification pathway of producing terpene esters is the more preferred route, because during esterification, the presence of acid (acyl donor) around enzymes reduces the pH of the surrounding environment and disrupts the aqueous layers of enzymes [29]. An advantage of using a vinyl ester as the acyl donor in transesterification, as opposed to acids, is that the carboxylic group of vinyl ester does not have a pH effect on the enzyme [30]. Furthermore, vinyl acetate has been extensively studied as an activated acyl donor with high reaction conversions for enzymatic synthesis of terpene esters and acetamides [31,32]. Additionally, esterification generates water as a reversible reaction, necessitating continuous water removal to favour the forward reaction. Transesterification eliminates this issue as it forms a vinyl alcohol as a by-product which instantly tautomerises into acetaldehyde, promoting the forward direction [33]. Therefore, vinyl acetate was selected as the acyl donor in this study. It is vital that every aspect in the production of esters align with environmentally friendly practices, such as for the choice of solvent. Organic, non-polar solvents like hexane [34] and toluene [35] are essential to maintain the lipase activity but are not favourable due to high toxicity. Ethyl acetate is a green and a safe alternative solvent which has shown minimum disruption to lipase activity [36]. Hence, greener solvents such as ethyl acetate are increasingly becoming solvent of choice [23,24] and has been used as the solvent in the current study.

Reports on the lipase-catalysed transesterification of terpene alcohol mixtures derived from plant and flower extracts are limited. For instance, enzymatic transesterification of palmarosa oil [37] has been reported, wherein vinyl esters of propionate, acetate, and crotonate were selected as acyl donors. The results showed 20–100 % conversion within 27–168 h of reaction time. Lipase-catalysed transesterification of various essential oils using vinyl acetate, such as citronella oil, was studied by Amaral et al. [38]. A 90–100 % conversion of primary terpene alcohols in various essential oils was achieved for a reaction time of 24 h. In the present study, only the concentration of citronellol was varied as it is present at a higher percentage in rose and geranium essential oils than geraniol [14,15]. This allows the simulation of the heterogeneous composition of terpene alcohols present in rose essential oil and a more realistic application of the reaction kinetics. However, none of the above studies have reported the enzymatic kinetic modelling of a mixture of terpene alcohols.

Kinetic modelling is vital for process scale-up, reactor design, and product quality control. The kinetics and mechanisms of enzyme-assisted single alcohol transesterification have been reported in the literature [22–27]. The ping-pong bi-bi (PPBB) and ternary complex (TC) mechanisms are mostly used to describe the two-substrate reactions. Kinetic constants are generally evaluated by assessing initial rate measurements [45]. Competitive inhibition by either a substrate [46] or product [26] has been well-reported for enzyme-assisted transesterification reactions. However, in another case, a more complex model described by Mitchell and Krieger [47] expresses the PPBB model in terms of specificity constants.

This study aims to demonstrate, for the first time, a systematic kinetic modelling governing the simultaneous transesterification of geraniol and citronellol using PFL. This work reports the optimisation of the simultaneous synthesis of geranyl and citronellyl acetate with the help of previous individual transesterification assays, which was then used to evaluate the kinetics of the simultaneous transesterification reaction. PPBB and TC mechanisms have been used and validated with root mean square error (RMSE) values. Preliminary results from the successful transesterification of rose geranium oil as a model feedstock under the optimised conditions are also presented.

Fig. 1. Annotated experimental setup for transesterification of terpene alcohol(s).

2. Materials and methods

2.1. Materials

All chemicals used were procured from commercial suppliers and used as received without further purification. Citronellol (95 %) was supplied by Acros Organics, geraniol (98 %) and vinyl acetate (99 %) from Sigma-Aldrich and ethyl acetate (99.9 %) from VWR Chemicals. Amano lipase from *Pseudomonas fluorescens* (activity 20,000 U/g) was obtained from Sigma-Aldrich as the biocatalyst for all reactions.

2.2. Enzyme-assisted transesterification

2.2.1. Reactor setup

The experimental setup is illustrated in Fig. 1. The reactions were conducted in a Radley-insulated reactor carousel consisting of 20 cm^3 sealed glass tubes and a stirring hotplate. Ethyl acetate (10 mL) was initially added to the tubes by using magnetic stirrers and heated to 40 °C at a stirring speed of 250 rpm for 5 min. A previous study by the group (unpublished work) has optimised the reaction temperature (40 °C) and stirring speed (250 rpm), and these conditions were used for the rest of the reactions in the study. Additionally, previous research by Shivaprasad et al. [36] illustrated that the transesterification of alcohol with ethyl acetate alone showed no reaction conversion.

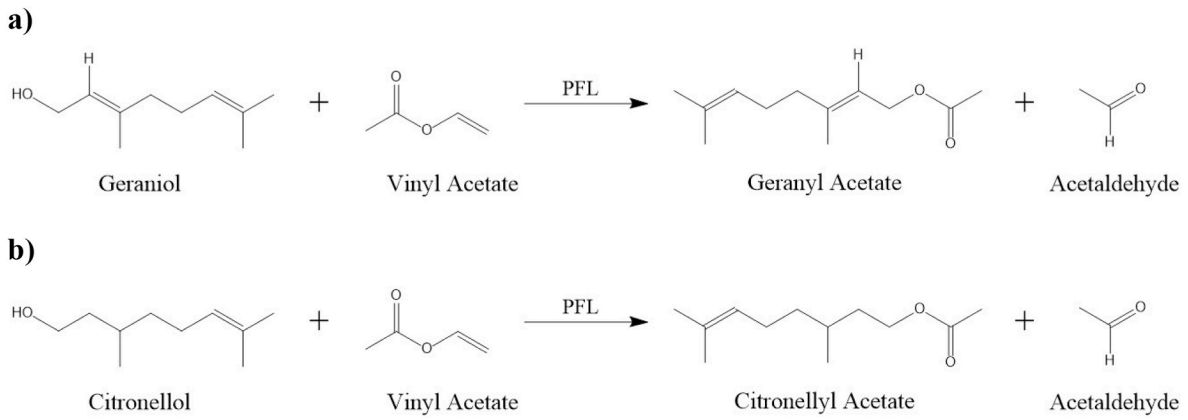

Fig. 2. Overall chemical equations for synthesising geranyl **(a)** and citronellyl **(b)** acetate using vinyl acetate created with ChemDraw.

2.2.2. Single alcohol transesterification

For single alcohol transesterification, the alcohol concentration is kept constant at 0.1 M and the vinyl acetate is varied from 0.1 to 0.4 M to ensure a 1:1, 1:2, 1:3 and 1:4 M ratio (alcohol:vinyl acetate). Then, 10 wt % lipase catalyst (in relation to the mass of alcohol) was added. The overall equations for the transesterification of geraniol and citronellol with vinyl acetate are shown in Fig. 2a and **b**.

2.2.3. Simultaneous geraniol and citronellol transesterification

Similarly, the simultaneous synthesis of geranyl and citronellyl acetate was carried out in carousel tubes by first heating 10 mL of solvent at 40 °C. Equimolar concentrations of geraniol and citronellol (0.1 M) were then added. In other tubes, various concentrations of citronellol in the solvent was studied from 0.1 M to 0.4 M with an increment of 0.1 M and fixed vinyl acetate concentration. This simulates the composition of the major terpene alcohols in rose essential oils. To investigate the influence of acyl donor concentration on the rate of ester production, the reactions were studied at varying molar ratios of geraniol to vinyl acetate (1:4, 1:8, 1:12, and 1:16), that is, vinyl acetate concentrations of 0.4, 0.8, 1.2, and 1.6 M. An enzyme loading of 10 wt. % for geraniol, and all other conditions were identical to those of the single transesterification procedure. Aliquots of 100 μL were withdrawn periodically for the first 30 min and then every hour until the 6th hour. The samples were diluted with 900 μL ethyl acetate solvent for analysis. The final sample was collected 24 h after starting the reaction.

2.3. Sample analysis

The extent of conversion of the terpene alcohols was analysed using a gas chromatography-mass spectrometry (GC-MS) system. Agilent 7920A gas chromatograph (GC) was coupled with an Agilent 5975C mass spectrometer (MS), with a capillary column of 5 % phenyl methylpolysiloxane (30 m × 250 μm x 0.25 μm). The method was previously developed by the research group which will be adopted in this study. Helium is used as the carrier gas at 3 mL/min with a split ratio of 1:100, injection volume of 1 μL and inlet detector temperature of 250 °C. The column oven program temperature began at 70 °C held for 1 min, then increased to 180 °C at a ramp speed of 10 °C/min and held at 180 °C for 1 min. A solvent delay of 4 min was set to drive off the ethyl acetate solvent. Agilent Enhanced Data Analysis and its NIST library was used to identify and confirm the presence of terpene esters. The conversion is acquired from the percentage of each component's peak area. The concentration of each substrate is then calculated using their respective stoichiometric equation.

2.4. Kinetic studies

A transesterification reaction occurs when the alcohol reacts with vinyl acetate to produce an ester, and the vinyl alcohol instantly tautomerises into acetaldehyde. The reaction mechanism can be predicted by performing the reaction at various experimental conditions, and the mathematical model can be derived. A concentration vs. time graph was plotted for different concentrations of citronellol at a specific molar ratio, and a polynomial curve was fitted. The polynomial is then derived to find the initial rates at t = 0 [43]. For a reaction involving two substrates and subsequently two products, the two most common mechanisms proposed to model the synthesis of esters are the non-sequential PPBB and the ordered TC mechanisms [48]. The distinguishing feature of the PPBB mechanism involves temporarily modifying the enzyme into an intermediate form during the process. The reaction then progresses by releasing one or two products while adding the two substrates. In case of the TC mechanism, two of the substrates bind to the enzyme before a reaction begins and subsequently proceeds to form the products [49].

Eqs. 1 and 2 illustrate Cleland's overall PPBB and TC mechanism equations, respectively [50]. The variable **r** is the rate of reaction (M/h), $\mathbf{r_{max}}$ is the maximum rate (M/h), $\mathbf{C_A}$ and $\mathbf{C_B}$ are the concentrations of substrate A and B (M), respectively, $\mathbf{K_{mA}}$ and $\mathbf{K_{mB}}$ are their Michaelis–Menten constant (M), and lastly $\mathbf{K_{iA}}$ and $\mathbf{K_{iB}}$ are their respective competitive inhibition constants (M). SPSS Statistics was utilised to perform a nonlinear regression analysis to determine the constants. The initial rates found from the concentration profile graph is compared with the theoretical values calculated using the two models. A nonlinear regression analysis with 95 % confidence interval was performed for each set of molar ratio in SPSS for kinetic modelling purposes.

$$r = \frac{r_{max} C_A C_B}{K_{mA} C_B \left(1 + \frac{C_B}{K_{iB}}\right) + K_{mB} C_A \left(1 + \frac{C_A}{K_{iA}}\right) + C_A C_B} \quad (1)$$

$$r = \frac{r_{max} C_A C_B}{K_{iA} K_{mB} + K_{mA} C_B + K_{mB} C_A + C_A C_B} \quad (2)$$

The kinetic mechanism of the enzyme assisted simultaneous transesterification was investigated using initial rate measurements, in which the concentrations of both substrates (alcohol and vinyl acetate) were varied systematically, and the results were analysed. The initial rates, V, as a function of the terpene alcohol or the vinyl acetate concentration, were fitted to the two models using nonlinear regression. A constraint was set wherein each variable is equal to or greater than 10^{-16} to prevent negative output values.

3. Results and discussions

3.1. Single alcohol transesterifications

3.1.1. Effect of mole ratio on the conversion of geraniol and citronellol

The molar ratio of the substrates plays an important role in

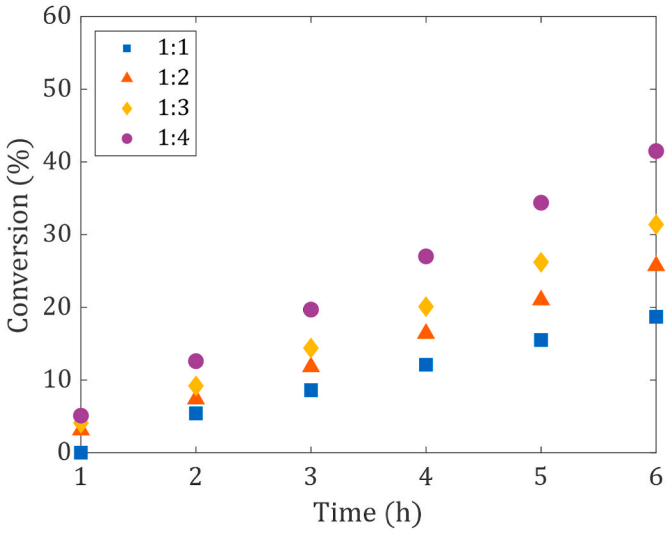

Fig. 3. Changes in the percentage conversion of geraniol over time at reaction conditions: geraniol, 0.1 M; vinyl acetate, 0.1–0.4 M; enzyme loading, 10 wt% of geraniol; temperature, 40 °C; agitation speed, 250 rpm.

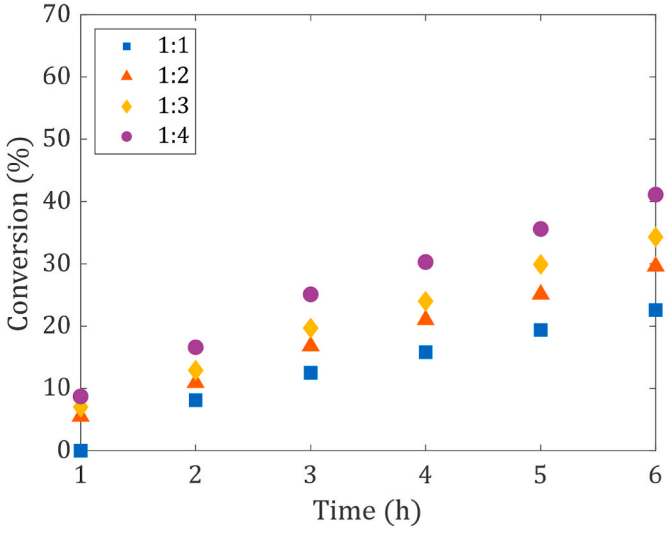

Fig. 4. Changes in the percentage conversion of citronellol over time at reaction conditions: citronellol, 0.1 M; vinyl acetate, 0.1–0.4 M; enzyme loading, 10 wt% of citronellol; temperature, 40 °C; agitation speed, 250 rpm.

transesterification [51]. The present study showed a strong dependence on the concentration of alcohol in the acyl donor for the transesterification of geraniol (Fig. 3). Under the tested conditions, the conversion of geraniol increased with increasing acyl donor concentration, with the highest conversion (42 %) obtained within 6 h at a 1:4 molar ratio of geraniol:vinyl acetate. After 24 h, 95 % conversion was achieved, and a full conversion was observed after 48 h (Supplementary Information Fig. A2). A previous study by Lisa et al. [17] for a similar reaction using an immobilized *Pseudomonas* sp. lipase, achieved similar conversions of 70–78 % after 24 h.

The increase in conversion with elevating vinyl acetate concentrations indicated that vinyl acetate had no inhibitory effect. Devi et al. [34] observed a similar trend in their study using ethanol transesterification with butyric acid, wherein the acyl donor does not deactivate or disturb the water layer surrounding the lipase surface. The full conversion was feasible as the vinyl alcohol produced concomitantly tautomerised to acetaldehyde, which is the more stable form. Acetaldehyde is no longer a substrate for the reverse reaction, resulting in an irreversible transesterification system [52].

Similarly, the effect of acyl donor concentration was studied for the same concentration range for the transesterification of citronellol. From Fig. 4, the citronellyl acetate yield increased from 23 % to 41 % at the 6th hour with an increase in the molar ratio of citronellol to vinyl acetate (1:1 to 1:4). After 24 h, the maximum conversion (88 %) with a molar ratio of 1:4 was observed, which later reached 100 % after 48 h (Supplementary Information Fig. B2). The results showed that increasing the vinyl acetate concentration facilitated the forward reaction, and no competitive inhibition by the acyl donor was observed within the range studied. A similar study conducted by Dhake et al. [31] on citronellol transesterification with vinyl acetate showed that increasing the molar ratio significantly improved the conversion rates up to a 1:6 ratio of citronellol to vinyl acetate. They achieved a maximum citronellol conversion of 91 % after 12 h.

3.1.2. *Kinetic modelling of single alcohol transesterifications*

The effect of substrate concentration on the enzymatic synthesis of terpene esters has been the focus of numerous studies, as it is an important factor in kinetic modelling [53]. From Figs. 3 and 4, the initial reaction rate is shown to increase with increasing concentration of vinyl acetate. Likewise, the rate of reaction increased with increasing geraniol and citronellol concentrations; however, it started to decrease from 3:1 onwards, which may indicate that there was not enough vinyl acetate present for the stoichiometric chemical reaction and that the alcohol hindered the catalytic site of the enzyme. Compounds that slow down enzyme-catalysed reactions are called inhibitors [54]. Competitive inhibition can occur in the transesterification reaction by one or both of the substrates by reversibly binding with lipase, which causes the enzyme to function at reduced rates [55]. Because the inhibitor binds reversibly, the substrate can compete with the other complexes at high concentrations. Therefore, at higher concentrations of geraniol above 0.3 M, it could inhibit lipase activity, thereby reducing the reaction rate. The kinetic models PPBB and TC mechanisms were validated using nonlinear regression based on the initial rates:

$$r_0 = \frac{r_{max} C_{A0} C_{B0}}{K_{mA} C_{B0}\left(1 + \frac{C_{B0}}{K_{iB}}\right) + K_{mB} C_{A0}\left(1 + \frac{C_{A0}}{K_{iA}}\right) + C_{A0} C_{B0}} \quad (3)$$

$$r_0 = \frac{r_{max} C_{A0} C_{B0}}{K_{iA} K_{mB} + K_{mA} C_{B0} + K_{mB} C_{A0} + C_{A0} C_{B0}} \quad (4)$$

For the transesterification of geraniol, the results of the curve fitting of the TC model showed the best fit with an RMSE of 4.7×10^{-4} (Supplementary Information Fig. A1). RMSE calculates the standard deviation of the residuals (prediction errors) between the predicted values from the model and actual observed values. The kinetic constants for the TC model were: $r_{max} = 0.229$ M/h, $K_{mA} = 1.847$ M, $K_{mB} = 0.831$ M, and $K_{iA} = 0.839$ M. It was observed that the Michaelis-Menten constant (K_m) for vinyl acetate was much lower than that of geraniol, suggesting a greater affinity towards the enzyme, indicating that vinyl acetate first binds to the lipase. The present results agree with previous geraniol transesterification study using *Pseudomonas cepacia* (bacterial) lipase by Badgujar and Bhanage [56], who observed a TC mechanism with geraniol inhibition. However, a previous study by Chulalaksananukul et al. [39] observed a PPBB mechanism with geraniol inhibition when immobilised *Rhizomucor miehei* (fungal) lipase was used. These results suggest that the enzyme mechanism depends on the source of the lipase, for example, from bacteria.

According to reports in the literature, vinyl acetate first binds to lipase to form a lipase-vinyl acetate complex, which is then followed by a nucleophilic attack by terpene alcohol, forming a ternary complex. This complex isomerises, releasing the first product (vinyl alcohol) with the concomitant tautomerisation of the alcohol to acetaldehyde. Then, the complex containing lipase and geranyl or citronellyl acetate breaks down to release ester. An illustration of this mechanism is presented in

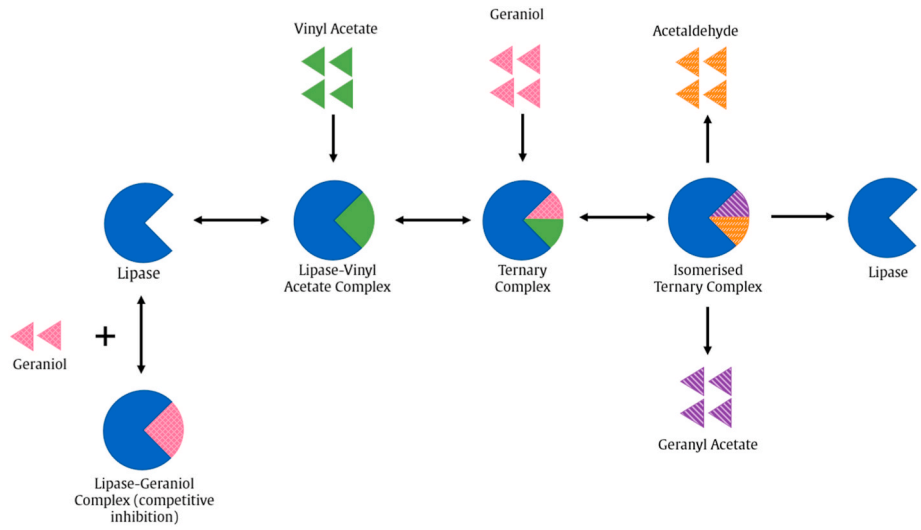

Fig. 5. Illustration of the ternary complex mechanism for the lipase-catalysed geraniol transesterification (number of lipases and substrates does not reflect the actual number present for the reaction).

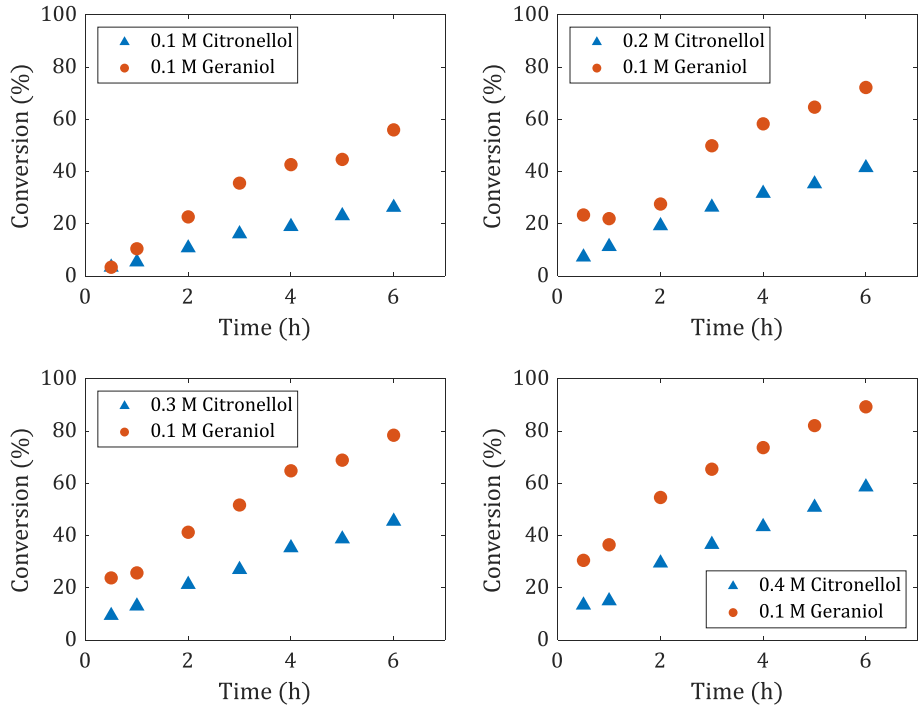

Fig. 6. Effect of alcohol concentration on the conversion to terpene esters (Reaction conditions: citronellol, 0.1–0.4 M; geraniol, 0.1 M; molar ratio, 1:4; enzyme loading, 10 % wt. of geraniol; temperature, 40 °C; agitation speed, 250 rpm).

Fig. 5.

Likewise, both mechanisms were also tested and validated for citronellol transesterification. The results of the nonlinear regression from Supplementary Information Fig. B1 showed a better fit for the TC mechanism however with only 8 % lower RMSE (r_{max} = 0.277 M/h, K_{mA} = 1.933 M, K_{mB} = 0.626 M, and K_{iA} = 0.641 M). This may suggest that the reaction may fit either mechanism. We predict that the ordered bi-bi i.e., the TC complex may have evolved into random bi-bi (PPBB) mechanism as the evolution of a completely specific active site is difficult, therefore promiscuous activities commonly occur [57]. This is consistent with what has been found in previous findings by Xiong et al. [42] using the same lipase (PFL), which reported a PPBB model for synthesising citronellyl ester. Additionally, a study on the transesterification of citronellol with vinyl esters by You et al. [45] and Yadav and Borkar [43] found evidence of a PPBB mechanism with citronellol inhibition.

3.2. Simultaneous geraniol and citronellol transesterification

3.2.1. Effect of alcohol concentration and type

The highlight of this study is the simultaneous transesterification of geraniol and citronellol. Using the optimised conditions for the enzyme loading (10 wt% alcohol), temperature (40 °C) and stirring speed (250 rpm), the simultaneous transesterification was evaluated to determine the best molar ratio of alcohols to acyl donor as there are now two different alcohols present in the mixture. Fig. 6 illustrates the conversion

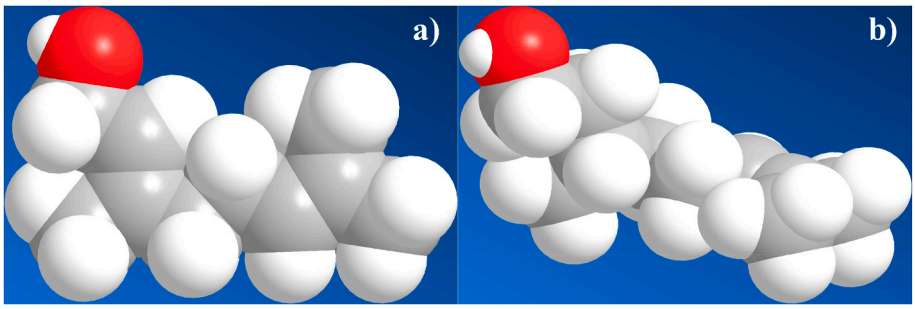

Fig. 7. Space-filling structure of **a)** geraniol and **b)** citronellol created with Chem3D.

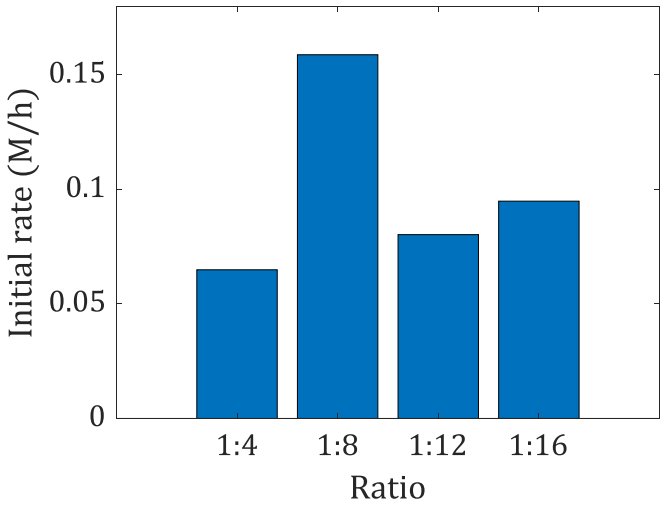

Fig. 8. Comparison of the effect of molar ratio (geraniol:vinyl acetate) towards the initial rates of the reaction at: 0.1 M geraniol, 0.4 M citronellol, enzyme loading of 10 % wt. of total alcohol, temperature of 40 °C, and agitation speed of 250 rpm.

vs. time graph for the conversion of citronellol and geraniol at varying citronellol concentration with a fixed 1:4 molar ratio of alcohols to vinyl acetate.

The results show that increasing the concentration of citronellol increased the conversion rate for both alcohols, such as when citronellol is at 0.1 M (5.4 % conversion) and at 0.4 M (13 % conversion) after 1 h. This is consistent with what has been found in previous study by Yadav and Lathi [11] for the synthesis of citronellyl laurate. Transesterification occurs when the enzyme's adsorbed water layer meets the organic phase. As organic substrates have poor solubility in aqueous medium, geraniol and citronellol will have difficulty diffusing through the water layer of the enzyme. Accordingly, with low concentration of citronellol, the molecules slowly diffuse from the bulk solution to the enzyme water layer, resulting in a slower initial rate. Hence, an increase in the citronellol concentration reduces the diffusion limitation. There appears to be a threshold value in which the transesterification is not diffusion limited, wherein there is no longer diffusion limitation at 0.4 M concentration and above, although this can also have an enzyme-inhibiting impact [26].

Additionally, the results show that geraniol consistently produced a greater conversion than citronellol. For example, at equimolar concentrations of alcohols (0.1 M), the conversion is 56 % and 26 % for geraniol and citronellol, respectively. When the concentration of citronellol is increased to 0.4 M, its conversion (59 %) is still much lower than geraniol conversion (89 %) after 6 h. Identical trends can be seen at different molar ratios 1:8, 1:12 and 1:16 shown in the Supplementary Information Fig. C1 to C3. The maximum conversion reached was found at molar ratio of 1:8, 0.1 M of geraniol and 0.4 M citronellol, with 66 % of citronellol and 94 % for geraniol within 5 h. After 24 h, geraniol reached a full conversion whereas citronellol reached 87 %.

Their chemical structures can explain the difference in the conversions of geraniol and citronellol. The structures of geraniol and citronellol differ in the number of double bonds, wherein geraniol has one extra double bond between the 2nd and 3rd carbon atoms. As seen in Fig. 7, the structure for geraniol is more linear, hence sterically less encumbered than citronellol. Therefore, it is expected that geraniol is more reactive. Similarly, Paiva et al. [58] previously observed that increasing the number of double bonds in the hydrocarbon backbone increase the rate of attack by lipase for the same chain length.

3.2.2. *Effect of vinyl acetate concentration*

The result of increasing the molar ratio of geraniol to vinyl acetate from 1:4 to 1:8 found evidence of increased initial reaction rates (Fig. 8). Specifically, the initial rate at a molar ratio of 1:4 was measured at 0.07 M/h, indicating a 56 % reduction compared to the rate of 0.16 M/h observed at a ratio of 1:8. However, further increase of the molar ratio, exceeding 1:8, resulted in a decline in the reaction rate. The reaction mechanism may explain this: acetaldehyde is produced during the lipase-catalysed transesterification of vinyl esters and alcohols, which is known to deactivate microbial lipases [59]. This mechanism involves the formation of α,β-unsaturated polyphenols, which subsequently form stable Michael-adducts with the enzyme. As such, the presence of vinyl acetate in large amounts leads to the formation of excess acetaldehyde, which may inhibit the enzyme. However, Franken et al. [60] conducted a study to determine the extent of deactivation of microbial lipases from acetaldehyde, where PFL was one of the studied lipases. Their findings indicated that PFL exhibits a notable degree of stability, with only moderate reductions in enantioselectivity and activity observed in the presence of acetaldehyde. Consequently, the inhibition effect of vinyl acetate on the enzyme may not be as pronounced as that of geraniol or citronellol, however this is further investigated in **3.2.4**. The highest rate in the present study (0.16 M/h) was found at a ratio of 1:8, hence implying an optimum ratio for the reaction.

3.2.3. *Effect of alcohol substrate*

It is understood that the initial reaction rate also depends on the structural characteristics of the alcohol substrate. Alcohols with increased branching becomes more sterically encumbered, thereby less likely to bind to the enzyme active site. The Novozym 435 enzyme has been studied by Bauwelinck et al. [61] which aimed to test different primary and secondary alcohols for transesterification of methyl esters. Novozym 435 has a catalytic triad of asp-his-ser in its active site [62] which is the same as that of PFL [63]. Notably, they observed that Novozym 435 demonstrates stringent positional regiospecificity and highlighted the dependence of reaction rate on alcohol linearity, length, and positional isomerism. Consequently, bulky alcohol molecules may encounter steric hindrance. However, both geraniol and citronellol are primary alcohols, suggesting that limitations in reaching complete conversion due to steric hindrance are unlikely.

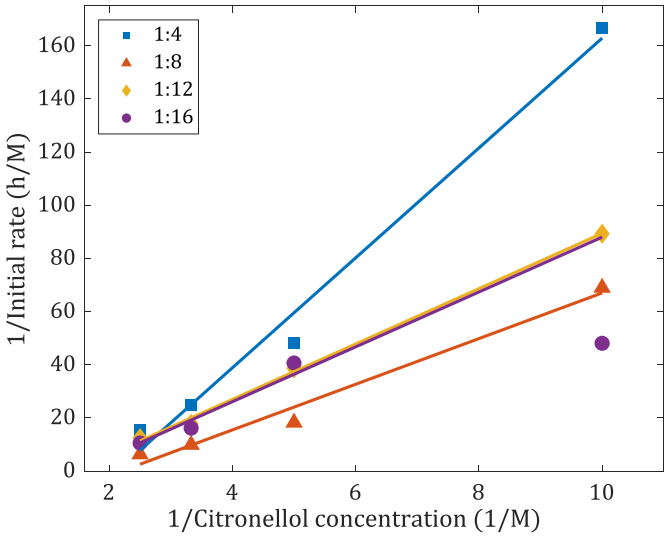

Fig. 9. Lineweaver-Burk Plot for different molar ratio of geraniol to vinyl acetate: (■) 1:4, (▲) 1:8, (◆) 1:12, (●) 1:16 at varied citronellol concentration.

3.2.4. Kinetic modelling

This study focused on determining a suitable kinetic model for the simultaneous transesterification of geraniol and citronellol. The kinetic model established for the transesterification system was based on evaluating the initial rates calculated by changing the reactants' concentrations [64]. The result is then plotted as a Lineweaver-Burk graph (double inverse plot, 1/rate vs. 1/concentration) illustrated in Fig. 9, showing that the line group is converging towards one point. Therefore, using Cleland's general rule [65], the reaction does not follow a ping pong bi-bi mechanism, i.e. no parallel lines observed. Rather, it follows a sequential mechanism i.e., a ternary complex.

From Fig. 9, it is seen that an increase in molar ratio of geraniol to vinyl acetate from 1:4 to 1:8 cause an increase in the initial rates. However, no significant effect was observed beyond 1:8 molar ratio. As discussed in **3.3.2**, an increase beyond 1:8 caused a reduced conversion which may be due to diffusion limitations and not kinetically limited. The nonlinear regression analyses were produced to compare the validity of the proposed mechanisms: TC mechanism with alcohol inhibition and PPBB. From Fig. 10, the result of the analysis showed that the reaction relatively fit the TC model with alcohol inhibition, which is the same as that of the single alcohol transesterification.

The values of the Michaelis-Menten constant (K_m) for vinyl acetate in Supplementary Information Table D1 were consistently lower than that of the alcohol, showing that vinyl acetate has a higher affinity towards the PFL to first form the lipase-acyl donor complex. According to the TC mechanism, vinyl acetate with a lower K_m value demonstrates a stronger binding affinity for the enzyme. Initially, vinyl acetate binds with the enzyme, forming a lipase-vinyl acetate complex. Then, citronellol or geraniol latches on the active site to form a ternary complex. This process resembles the mechanism observed in single alcohol transesterifications, however there is now two nucleophiles competing for the lipase-vinyl acetate complex, as shown in Fig. 11. Thus, the model provides a working conceptual basis for describing the simultaneous citronellol and geraniol transesterification mechanism.

3.3. Rose geranium oil transesterification

Preliminary studies were conducted on the transesterification of rose oil, which is an important finding for understanding the reaction kinetics. The applicability of the results in **3.2** were then tested on commercially available rose geranium oil, as shown in Fig. 12. It is evident from the figure that the conversion of both citronellol and geraniol increased with time. After 30 h of reaction, the maximum conversion of citronellol reached 32 %, which was lower than that of geraniol (50 %). This finding suggests that geraniol is more reactive owing to the extra double bond, as discussed in **3.2.1**. Similar observations were reported by Claon and Akoh [66], where the yield of geranyl esters was found to be higher than that of citronellyl esters under identical operating conditions. Subsequently, they also reported that all

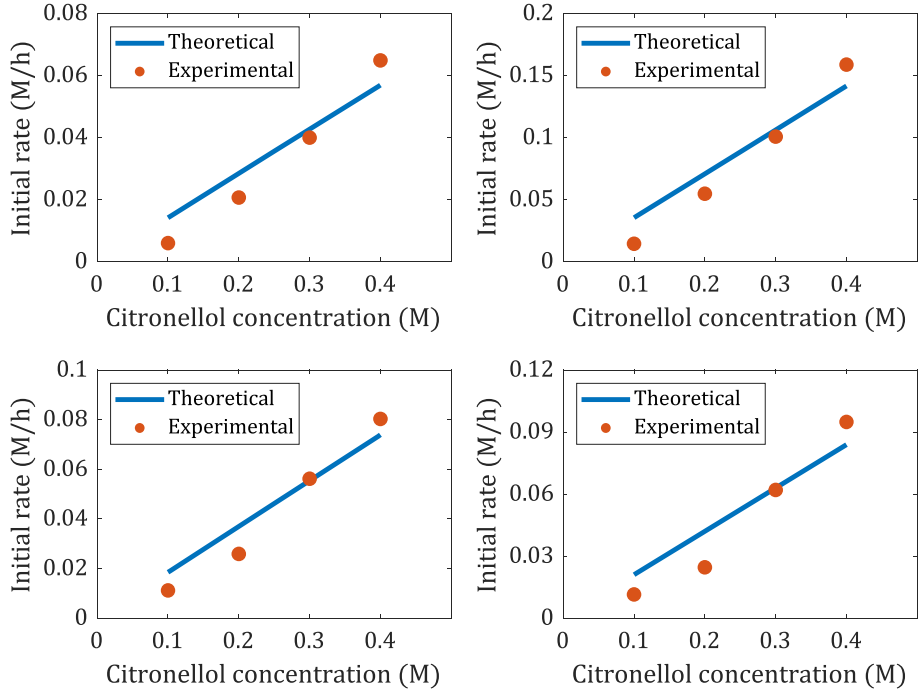

Fig. 10. Curve fitting for ternary complex mechanism at different (geraniol:vinyl acetate) molar ratios wherein: (●) experimental values, (−) simulated values at **a)** 1:4 (RMSE = 0.007), **b)** 1:8 (RMSE = 0.016), **c)** 1:12 (RMSE = 0.007) and **d)** 1:16 (RMSE = 0.011). Confidence interval of 95 %.

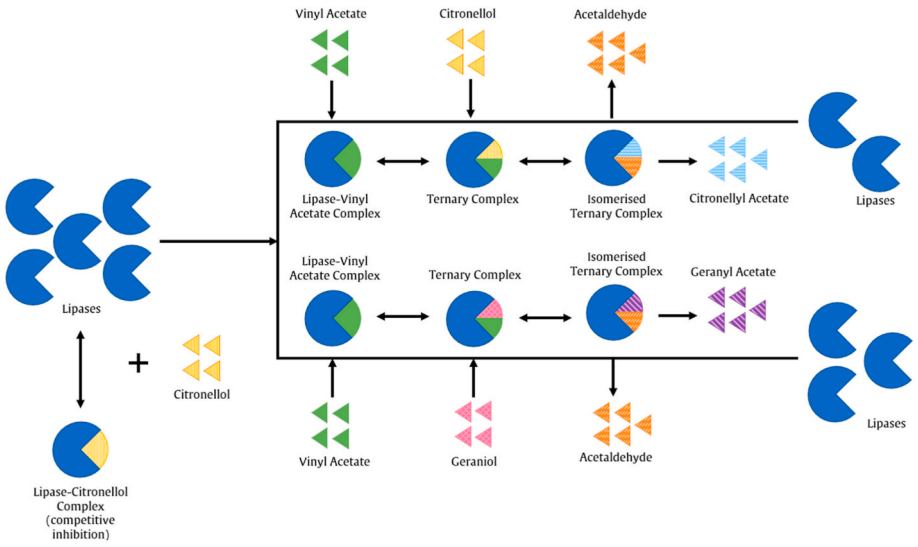

Fig. 11. Ternary complex mechanism for the simultaneous synthesis of geranyl and citronellyl acetate using PFL (number of lipases and substrates does not reflect the actual number present for the reactions).

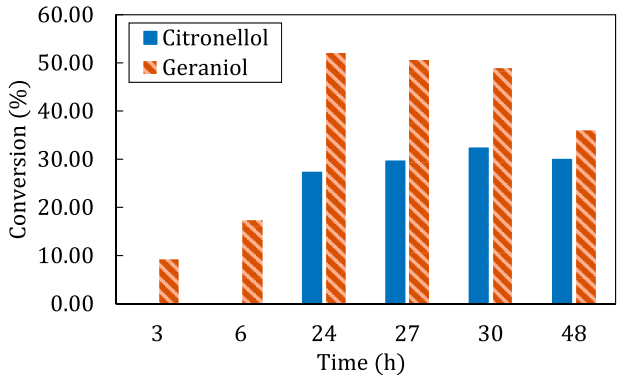

Fig. 12. Conversion at different times for the PFL-assisted rose oil transesterification at 1:8 (rose oil:vinyl acetate), 40 °C temperature and 250 rpm stirring speed.

four lipases studied for producing terpene esters, including PFL, showed greater affinity for geraniol than for citronellol.

Citronellol exists in nature in two enantiomeric forms, (R)-(+) and (S)-(−), and both forms can be present in rose oil [67]. Previous studies by Baser and Ozek [68] indicated that most essential oils contain enantiomeric compounds. Additionally, Chanotiya and Yadav [69] reported that the chiral molecule linalool is present in various essential oils with different enantiomeric distributions. A possible explanation for the lower yields of citronellyl acetate compared with geranyl acetate is the difference in the rate of reaction between the two enantiomers of citronellol. This is also in line with the previous finding by Agustian et al. [70], which demonstrated that lipase showed better conversion towards one enantiomer for the transesterification of racemic atenolol. Wang and Linko [71] also showed that lipases show enantioselectivity towards (R)-(+)-substrates for the synthesis of citronellyl butyrate.

The two enantiomers of citronellol exhibit different characteristics. The (S)-(−)-enantiomer has a delicate fresh rose smell and a sweet peachy flavour [72]. In contrast, the (R)-(+)-enantiomer has a stronger floral smell and a bitter taste. These characteristics play a key role in the flavour and fragrance industries. Therefore, further research in this area may include the study of the enantiomeric composition of citronellol in rose oils, as well as its kinetic resolution.

4. Conclusion

Flower waste is a promising alternative source of sustainable terpene-derived chemicals for the fragrance and flavour industries. Due to the heterogeneous composition of the flowers, it is crucial to evaluate the reaction kinetics to facilitate its valorisation. This study provides an investigative kinetic study of the simultaneous transesterification of citronellol and geraniol using Pseudomonas Fluorescens lipase (PFL). The highlight of the study is the simultaneous production of geraniol and citronellol esters with a maximum yield of 66 % citronellyl acetate and 94 % geranyl acetate after 5 h. The results were attained under the conditions: 0.4 M citronellol, 0.1 M geraniol, 1:8 molar ratio of geraniol to vinyl acetate, a temperature of 40 °C, a stirring speed of 250 rpm and an enzyme loading of 10 wt % of geraniol. Geraniol exhibited higher conversion than citronellol under all conditions, which can be attributed to the difference in their chemical structures. An additional double bond in geraniol likely contributes to its increased reactivity compared to that of citronellol. The present kinetic modelling findings indicated that the simultaneous transesterification reaction followed a ternary complex model with competitive alcohol inhibition.

Preliminary transesterification of commercially available rose geranium oil was performed to test the optimised protocol. The results showed that geraniol exhibited greater reactivity than citronellol, reaching 50 % conversion after 30 h in comparison to 32 % for citronellol. The intricacies of the enantiomeric composition of citronellol, commonly found in most rose oils, suggest a potential influence on the overall reaction kinetics, likely resulting in a comparatively slower rate relative to geraniol. To deepen our understanding, the enantiomeric composition of citronellol and the kinetic resolution of rose oils should be investigated. To the best of our knowledge, this is the first study on the simultaneous transesterification of geraniol and citronellol, which provides key knowledge for future research aimed at optimising and scaling up transesterification reaction of the terpene alcohol mixture. This approach can be applied to combinations of terpene alcohols and the wide range of profiles encountered across the many varieties of flower essential oils.

CRediT authorship contribution statement

Alisa S. Wikaputri: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Derek J. Irvine:** Writing – review & editing, Supervision,

Methodology. **Robert A. Stockman:** Writing – review & editing, Supervision, Methodology. **Parimala Shivaprasad:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgement

PS would like to thank UKRI and the University of Nottingham for AW's PhD studentship. A part of this work has received funding from the Royal Academy of Engineering under the Frontiers of Development scheme.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jafr.2024.101186.

References

[1] A. Masyita, et al., Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives, Food Chem. X 13 (Mar. 2022) 100217, https://doi.org/10.1016/j.fochx.2022.100217.
[2] M. Fan, et al., Application of terpenoid compounds in food and pharmaceutical products, Fermentation 9 (2) (2023), https://doi.org/10.3390/fermentation9020119.
[3] R. K R, S. Gopi, P. Balakrishnan, "Introduction to flavor and fragrance in food processing," in *Flavors and Fragrances in food processing: Preparation and characterization methods*, ACS (Am. Chem. Soc.) Symp. Ser. 1433 (2022) 1, https://doi.org/10.1021/bk-2022-1433.ch001. American Chemical Society.
[4] Y. Liu, X. WeiZhuo, X. Wei, A review on lipase-catalyzed synthesis of geranyl esters as flavor additives for food, pharmaceutical and cosmetic applications, Food Chem. Adv 1 (2022) 100052, https://doi.org/10.1016/j.focha.2022.100052.
[5] A. Plata-Rueda, et al., Insecticidal and repellent activities of Cymbopogon citratus (Poaceae) essential oil and its terpenoids (citral and geranyl acetate) against Ulomoides dermestoides, Crop Protect. 137 (June) (2020), https://doi.org/10.1016/j.cropro.2020.105299.
[6] A. Plata-Rueda, G.D.S. Rolim, C.F. Wilcken, J.C. Zanuncio, J.E. Serrão, L. C. Martínez, Acute toxicity and sublethal effects of lemongrass essential oil and their components against the granary weevil, Sitophilus granarius, Insects 11 (6) (2020) 1–13, https://doi.org/10.3390/insects11060379.
[7] M.B. Isman, S. Miresmailli, C. MacHial, Commercial opportunities for pesticides based on plant essential oils in agriculture, industry and consumer products, Phytochemistry Rev. 10 (2) (2011) 197–204, https://doi.org/10.1007/s11101-010-9170-4.
[9] C.T. Hou, Y. Shimada, Lipases, Encycl. Microbiol (2009) 385–392, https://doi.org/10.1016/B978-012373944-5.00153-X. Third Ed.
[10] C.C.C.R. De Carvalho, M.M.R. Da Fonseca, Biotransformation of terpenes, Biotechnol. Adv. 24 (2) (2006) 134–142, https://doi.org/10.1016/j.biotechadv.2005.08.004.
[11] G.D. Yadav, P.S. Lathi, Synthesis of citronellol laurate in organic media catalyzed by immobilized lipases: kinetic studies, J. Mol. Catal. B Enzym. 27 (2) (2004) 113–119, https://doi.org/10.1016/j.molcatb.2003.10.004.
[12] A.S. Manina, F. Forlani, Biotechnologies in perfume manufacturing: metabolic engineering of terpenoid biosynthesis, Int. J. Mol. Sci. 24 (9) (2023), https://doi.org/10.3390/ijms24097874.
[13] M.N. Boukhatem, A. Kameli, M.A. Ferhat, F. Saidi, M. Mekarnia, Rose geranium essential oil as a source of new and safe anti-inflammatory drugs, Libyan J. Med. 8 (1) (2013) 1–7, https://doi.org/10.3402/ljm.v8i0.22520.
[14] S. Ulusoy, G. Boşgelmez-Tinaz, H. Seçilmiş-Canbay, Tocopherol, carotene, phenolic contents and antibacterial properties of rose essential oil, hydrosol and absolute, Curr. Microbiol. 59 (5) (2009) 554–558, https://doi.org/10.1007/s00284-009-9475-y.
[15] M. Katsukawa, R. Nakata, S. Koeji, K. Hori, S. Takahashi, H. Inoue, Citronellol and geraniol, components of rose oil, activate peroxisome proliferator-activated receptor α and γ and suppress cyclooxygenase-2 expression, Biosci. Biotechnol. Biochem. 75 (5) (2011) 1010–1012, https://doi.org/10.1271/bbb.110039.
[16] L.N. Yee, C.C. Akoh, R.S. Phillips, Terpene ester synthesis by lipase-catalyzed transesterification, Biotechnol. Lett. 17 (1) (1995) 67–70, https://doi.org/10.1007/BF00134198.
[17] L.N. Yee, C.C. Akoh, R.S. Phillips, Pseudomonas sp. lipase-catalyzed synthesis of geranyl esters by transesterification, J. Am. Oil Chem. Soc. 72 (11) (1995) 1407–1408, https://doi.org/10.1007/BF02546220.
[18] C.C. Akoh, L.N. Yee, Lipase-catalyzed transesterification of primary terpene alcohols with vinyl esters in organic media, J. Mol. Catal. B Enzym. 4 (3) (1998) 149–153, https://doi.org/10.1016/S1381-1177(97)00029-5.
[19] L.I.R. Ferraz, et al., Application of home-made lipase in the production of geranyl propionate by esterification of geraniol and propionic acid in solvent-free system, Biocatal. Agric. Biotechnol. 4 (1) (2015) 44–48, https://doi.org/10.1016/j.bcab.2014.07.003.
[20] P. Lozano, R. Piamtongkam, K. Kohns, T. De Diego, M. Vaultier, J.L. Iborra, Ionic liquids improve citronellyl ester synthesis catalyzed by immobilized candida Antarctica lipase B in solvent-free media, Green Chem. 9 (7) (2007) 780, https://doi.org/10.1039/b617444b, 78.
[21] J. Xiong, Y.J. Huang, H. Zhang, Lipase-catalyzed transesterification synthesis of citronellyl acetate in a solvent-free system and its reaction kinetics, Eur. Food Res. Technol. 235 (5) (2012) 907–914, https://doi.org/10.1007/s00217-012-1819-3.
[22] A. Galgali, S.D. Gawas, V.K. Rathod, Ultrasound assisted synthesis of citronellol laurate by using Novozym 435, Catal. Today 309 (April 2017) (2018) 133–139, https://doi.org/10.1016/j.cattod.2017.08.052.
[23] D. Weber, M. da Graça Nascimento, A.L. Parize, Immobilization of Burkholderia cepacia lipase on crosslinked chitosan-based support for the synthesis of geranyl acetate, Biocatal. Agric. Biotechnol. 19 (March) (2019) 101133, https://doi.org/10.1016/j.bcab.2019.101133.
[24] M.D. Murcia, et al., Kinetic modelling and kinetic parameters calculation in the lipase-catalysed synthesis of geranyl acetate, Chem. Eng. Res. Des. 138 (2018) 135–143, https://doi.org/10.1016/j.cherd.2018.08.025.
[25] G. Molina, M.R. Pimentel, G.M. Pastore, Pseudomonas: a promising biocatalyst for the bioconversion of terpenes, Appl. Microbiol. Biotechnol. 97 (5) (2013) 1851–1864, https://doi.org/10.1007/s00253-013-4701-8.
[26] J. Xiong, Y. Huang, H. Zhang, L. Hou, Lipase-catalyzed transesterification synthesis of geranyl acetate in organic solvents and its kinetics, Food Sci. Technol. Res. 20 (2) (2014) 207–216, https://doi.org/10.3136/fstr.20.207.
[27] Y. Zhai, et al., Melia azedarach leaf powder stabilizing Pseudomonas fluorescens lipase to catalyze synthesis of geranyl acetate, Biocatal. Agric. Biotechnol. 37 (September) (2021) 102170, https://doi.org/10.1016/j.bcab.2021.102170.
[28] A.E. Polloni, et al., Polyesters from macrolactones using commercial lipase NS 88011 and Novozym 435 as biocatalysts, Appl. Biochem. Biotechnol. 184 (2) (2018) 659–672, https://doi.org/10.1007/s12010-017-2583-4.
[29] A. Güvenç, N. Kapucu, Ü. Mehmetoğlu, The production of isoamyl acetate using immobilized lipases in a solvent-free system, Process Biochem. 38 (3) (2002) 379–386, https://doi.org/10.1016/S0032-9592(02)00099-7.
[30] M. Nordblad, P. Adlercreutz, Effects of acid concentration and solvent choice on enzymatic acrylation by Candida Antarctica lipase B, J. Biotechnol. 133 (1) (2008) 127–133, https://doi.org/10.1016/j.jbiotec.2007.09.002.
[31] K.P. Dhake, K.M. Deshmukh, Y.P. Patil, R.S. Singhal, B.M. Bhanage, Improved activity and stability of Rhizopus oryzae lipase via immobilization for citronellol ester synthesis in supercritical carbon dioxide, J. Biotechnol. 156 (1) (2011) 46–51, https://doi.org/10.1016/j.jbiotec.2011.08.019.
[32] K.P. Dhake, Z.S. Qureshi, R.S. Singhal, B.M. Bhanage, Candida Antarctica lipase B-catalyzed synthesis of acetamides using [BMIm(PF6)] as a reaction medium, Tetrahedron Lett. 50 (23) (2009) 2811–2814, https://doi.org/10.1016/j.tetlet.2009.03.170.
[33] K.P. Dhake, D.D. Thakare, B.M. Bhanage, Lipase: a potential biocatalyst for the synthesis of valuable flavour and fragrance ester compounds, Flavour Fragrance J. 28 (2) (2013) 71–83, https://doi.org/10.1002/ffj.3140.
[34] N.A. Devi, G.B. Radhika, R.J. Bhargavi, Lipase catalyzed transesterification of ethyl butyrate synthesis in n-hexane— a kinetic study, J. Food Sci. Technol. 54 (9) (2017) 2871–2877, https://doi.org/10.1007/s13197-017-2725-2.
[35] G.D. Yadav, S. Devendran, Lipase catalyzed synthesis of cinnamyl acetate via transesterification in non-aqueous medium, Process Biochem. 47 (3) (2012) 496–502, https://doi.org/10.1016/j.procbio.2011.12.008.
[36] P. Shivaprasad, M.D. Jones, D.A. Patterson, E.A.C. Emanuelsson, Kinetic resolution of 1-phenylethanol in the spinning mesh disc reactor: investigating the reactor performance using immobilised lipase catalyst, Chem. Eng. Process. - Process Intensif. 132 (July) (2018) 56–64, https://doi.org/10.1016/j.cep.2018.08.012.
[37] J. Ramilijaona, E. Raynaud, C. Bouhlel, E. Sarrazin, X. Fernandez, S. Antoniotti, Enzymatic modification of palmarosa essential oil: chemical analysis and olfactory evaluation of acylated products, Chem. Biodivers. 10 (12) (2013) 2291–2301, https://doi.org/10.1002/cbdv.201300241.
[38] M.S.S. Amaral, M.T.W. Hearn, P.J. Marriott, Lipase-catalysed changes in essential oils revealed by comprehensive two-dimensional gas chromatography, Anal. Bioanal. Chem. (2023) 0123456789, https://doi.org/10.1007/s00216-023-04729-0.
[39] W. Chulalaksananukul, J.S. Condoret, D. Combes, Kinetics of geranyl acetate synthesis by lipase-catalysed transesterification in n-hexane, Enzym. Microb. Technol. 14 (4) (1992) 293–298, https://doi.org/10.1016/0141-0229(92)90154-G.
[42] J. Xiong, et al., Synthesis of citronella acetate by lipase catalyzed transesterification in ionic liquid and its kinetics, Food Sci. Technol. 42 (2022) 1–9, https://doi.org/10.1590/fst.91922.

[43] G.D. Yadav, I.V. Borkar, Kinetic and mechanistic investigation of microwave-assisted lipase catalyzed synthesis of citronellyl Acetate, Ind. Eng. Chem. Res. 48 (17) (2009) 7915–7922, https://doi.org/10.1021/ie800591c.
[45] P. You, E. Su, X. Yang, D. Mao, D. Wei, Carica papaya lipase-catalyzed synthesis of terpene esters, J. Mol. Catal. B Enzym. 71 (3–4) (2011) 152–158, https://doi.org/10.1016/j.molcatb.2011.04.012.
[46] M.D. Murcia, et al., Kinetic modelling and kinetic parameters calculation in the lipase-catalysed synthesis of geranyl acetate, Chem. Eng. Res. Des. 138 (Oct. 2018) 135–143, https://doi.org/10.1016/J.CHERD.2018.08.025.
[47] D.A. Mitchell, N. Krieger, Looking through a new lens: expressing the Ping Pong bi bi equation in terms of specificity constants, Biochem. Eng. J. 178 (2022) 108276, https://doi.org/10.1016/j.bej.2021.108276.
[48] P. Pérez Galende, et al., Kinetics of Spanish broom peroxidase obeys a Ping-Pong Bi-Bi mechanism with competitive inhibition by substrates, Int. J. Biol. Macromol. 81 (2015) 1005–1011, https://doi.org/10.1016/j.ijbiomac.2015.09.042.
[49] N.N. Ulusu, Evolution of enzyme kinetic mechanisms, J. Mol. Evol. 80 (5–6) (2015) 251–257, https://doi.org/10.1007/s00239-015-9681-0.
[50] W.W. Cleland, The kinetics of enzyme-catalyzed reactions with two or more substrates or products. II. Inhibition: nomenclature and theory, Biochim. Biophys. Acta (BBA) - Spec. Sect. Enzymol. Subj. 67 (C) (1963) 173–187, https://doi.org/10.1016/0006-3002(63)91815-8.
[51] N.R. Khan, V.K. Rathod, Enzyme catalyzed synthesis of cosmetic esters and its intensification: a review, Process Biochem. 50 (11) (2015) 1793–1806, https://doi.org/10.1016/j.procbio.2015.07.014.
[52] R. Chenevert, N. Pelchat, F. Jacques, Stereoselective enzymatic acylations (transesterifications), Curr. Org. Chem. 10 (10) (2006) 1067–1094, https://doi.org/10.2174/138527206777698093.
[53] G.D. Yadav, I.V. Borkar, Kinetic Modeling of Immobilized Lipase Catalysis in Synthesis of N -Butyl, 2008, pp. 3358–3363.
[54] M. Kuddus, in: F.B. Kuddus (Ed.), Chapter 1 - Introduction to Food Enzymes, Academic Press, 2019, pp. 1–18, https://doi.org/10.1016/B978-0-12-813280-7.00001-3. M. B. T.-E.
[55] P.V. Jawale, B.M. Bhanage, Synthesis of propyl benzoate by solvent-free immobilized lipase-catalyzed transesterification: optimization and kinetic modeling, Bioproc. Biosyst. Eng. 44 (2) (2021) 369–378, https://doi.org/10.1007/s00449-020-02448-9.
[56] K.C. Badgujar, B.M. Bhanage, Synthesis of geranyl acetate in non-aqueous media using immobilized Pseudomonas cepacia lipase on biodegradable polymer film: kinetic modelling and chain length effect study, Process Biochem. 49 (8) (2014) 1304–1313, https://doi.org/10.1016/j.procbio.2014.04.014.
[57] S.D. Copley, An evolutionary biochemist's perspective on promiscuity, Trends Biochem. Sci. 40 (2) (2015) 72–78, https://doi.org/10.1016/j.tibs.2014.12.004.
[58] A.L. Paiva, V.M. Balcão, F.X. Malcata, Kinetics and mechanisms of reactions catalyzed by immobilized lipases, Enzym. Microb. Technol. 27 (3–5) (2000) 187–204, https://doi.org/10.1016/S0141-0229(00)00206-4.
[59] H.K. Weber, H. Stecher, K. Faber, Sensitivity of microbial lipases to acetaldehyde formed by acyl-transfer reactions from vinyl esters, Biotechnol. Lett. 17 (8) (1995) 803–808, https://doi.org/10.1007/BF00129008.
[60] B. Franken, T. Eggert, K.E. Jaeger, M. Pohl, Mechanism of acetaldehyde-induced deactivation of microbial lipases, BMC Biochem. 12 (1) (2011), https://doi.org/10.1186/1471-2091-12-10.
[61] J. Bauwelinck, I. Cornet, M. Wijnants, R. Dams, S. Tavernier, Investigation of the enzyme-catalysed transesterification of methyl acrylate and sterically hindered alcohol substrates, ChemistrySelect 3 (18) (2018) 5169–5175, https://doi.org/10.1002/slct.201703136.
[62] C. Ortiz, et al., Novozym 435: the 'perfect' lipase immobilized biocatalyst? Catal. Sci. Technol. 9 (10) (2019) 2380–2420, https://doi.org/10.1039/c9cy00415g.
[63] K.E. Jaeger, S. Ransac, B.W. Dijkstra, C. Colson, M. van Heuvel, O. Misset, Bacterial lipases, FEMS Microbiol. Rev. 15 (1) (1994) 29–63, https://doi.org/10.1016/0168-6445(94)90025-6.
[64] M.D. Romero, L. Calvo, C. Alba, A. Daneshfar, H.S. Ghaziaskar, Enzymatic synthesis of isoamyl acetate with immobilized Candida Antarctica lipase in n-hexane, Enzym. Microb. Technol. 37 (1) (2005) 42–48, https://doi.org/10.1016/j.enzmictec.2004.12.033.
[65] W.W. Cleland, The kinetics of enzyme-catalyzed reactions with two or more substrates or products: III. Prediction of initial velocity and inhibition patterns by inspection, Biochim. Biophys. Acta (BBA) - Spec. Sect. Enzymol. Subj. 67 (1963) 188–196, https://doi.org/10.1016/0926-6569(63)90227-X.
[66] P.A. Claon, C.C. Akoh, Enzymatic synthesis of geraniol and citronellol esters by direct esterification in n-hexane, Biotechnol. Lett. 15 (12) (1993) 1211–1216, https://doi.org/10.1007/BF00130299.
[67] A. Mannschreck, E. Von Angerer, The scent of roses and beyond: molecular structures, analysis, and practical applications of odorants, J. Chem. Educ. 88 (11) (2011) 1501–1506, https://doi.org/10.1021/ed100629v.
[68] K.H. Can Başer, T. Özek, Analysis of Essential Oils and Fragrances by Gas Chromatography, Gas Chromatogr., 2012, pp. 519–527, https://doi.org/10.1016/B978-0-12-385540-4.00022-5.
[69] C.S. Chanotiya, A. Yadav, Natural Product Communications Enantioselective Capillary Gas Chromatography-Flame, 2009, pp. 7–10.
[70] J. Agustian, A.H. Kamaruddin, H.Y. Aboul-Enein, Enantio-conversion and -selectivity of racemic atenolol kinetic resolution using free Pseudomonas fluorescens lipase (Amano) conducted via transesterification reaction, RSC Adv. 6 (31) (2016) 26077–26085, https://doi.org/10.1039/c6ra01942k.
[71] Y. Wang, Y.-Y. Linko, Lipase-catalyzed enantiomeric synthesis of citronellyl butyrate, J. Ferment. Bioeng. 80 (5) (1995) 473–477, https://doi.org/10.1016/0922-338X(96)80922-6.
[72] U. Ravid, E. Putievsky, I. Katzir, R. Ikan, V. Weinstein, Determination of the enantiomeric composition of citronellol in essential oils by chiral GC analysis on a modified γ-cyclodextrin phase, Flavour Fragrance J. 7 (4) (1992) 235–238, https://doi.org/10.1002/ffj.2730070413.