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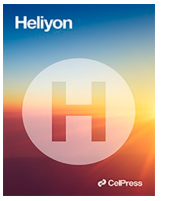

Research article

Enzymatic synthesis of pyridine heterocyclic compounds and their thermal stability

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A R T I C L E I N F O

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Novozym 435
Pyridine esters
GC-MS-O
TG

A B S T R A C T

An efficient method was discovered for catalyzing the esterification under air using Novozym 435 to obtain pyridine esters. The following conditions were found to be optimal: 60 mg of Novozyme 435, 5.0 mL of n-hexane, a molar ratio of 2:1 for nicotinic acids (0.4 mmol) to alcohols (0.2 mmol), 0.25 g of molecular sieve 3A, a revolution speed of 150 rpm, a reaction temperature of 50 °C, and reaction time of 48 h. Under nine cycles of Novozym 435, the 80 % yield was consistently obtained. Optimum conditions were used to synthesize 23 pyridine esters, including five novel compounds. Among them, gas chromatography-mass spectrometry-olfactometry (GC-MS-O) showed phenethyl nicotinate (**3g**), (*E*)-hex-4-en-1-yl nicotinate (**3m**), and octyl nicotinate (**3n**) possessed strong aromas. Thermogravimetric analysis (TG) revealed that the compounds **3g**, **3m** and **3n** exhibited stability at the specified temperature. This finding provides theoretical support for adding pyridine esters fragrance to high-temperature processed food.

1. Introduction

Due to good biological activity and easily modified structure, pyridine derivatives are widely used in flavor [1], medicine [2], food [3], pesticide [4] and other fields. The presence of esters is widespread in nature and they are frequently utilized as additives in beverages, dairy products, and jams owing to their desirable flavor and fragrance properties, such as floral and fruity notes [5]. Pyridine esters have recently attracted more attention in food related fields because of their unique aromatic flavor. Therefore, they are expected to have great development prospects in the field of flavors and fragrances.

Based on recent literature, the approaches utilized for synthesizing pyridine esters primarily encompass metal catalysis [6,7], photocatalysis [8], electrocatalysis [9], base catalysis [10] and Lewis acid catalysis [11]. Currently, several approaches to synthesizing pyridine esters face challenges such as high temperature, low yield, residual catalyst, and heavy metal contamination. For example, it has been reported that CeO_2 was used to catalyze the synthesis of a series of pyridine esters at 160 °C. Although good yields of products were obtained, the reaction temperature was relatively high, and there were issues with residual heavy metals [12]. Furthermore,

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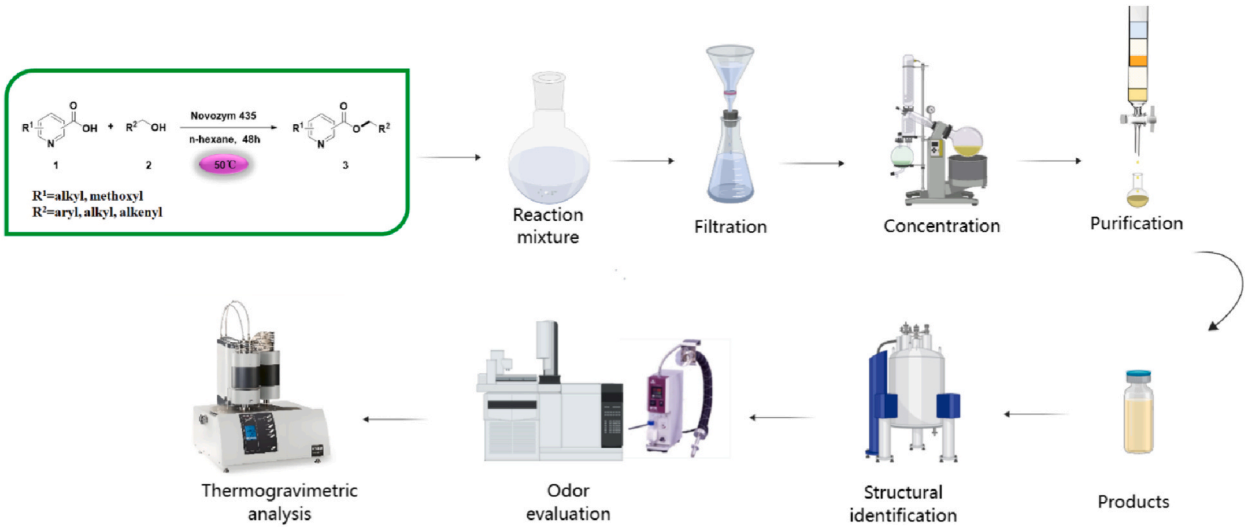


Fig. 1. Flow chart of the synthesis step from preparation until analysis.

employing palladium as a catalyst resulted in the synthesis of pyridine esters at 110 °C, but with a yield of only 31 % [13]. However, our research group has recently discovered that enzymes have great potential in catalyzing the synthesis of pyridine esters [14]. Compared with traditional catalysts, they have the characteristics of high efficiency and specificity which follow the concept of green chemistry [15–17]. Novozym 435 is one of the most used commercial catalysts which is made via immobilizing CaLB (*Candida antarctica* Lipase B) on macroporous acrylic resin [18]. It exhibits high selectivity, mild conditions, and recyclability in organic solvents, which are widely used in esterification [19,20], racemization [21] and acylation [22]. Moreover, gas chromatograph-mass spectrometry-olfactometer (GC-MS-O) is an efficient analytical identification technology which can be used to distinguish the odor characteristics of compoundse [23]. Thermogravimetry (TG), derivative thermogravimetric analysis (DTG) and differential thermal analysis (DTA) can be used to research the thermal stability of compounds at high temperature [24]. Combined with the above analysis techniques, we could evaluate potential of synthesized pyridine esters in the field of flavors and fragrances.

In this research, the esterification of nicotinic acids and alcohols was conducted using Novozym 435 as a catalyst in the presence of n-hexane. To optimize the reaction conditions, a systematic study was conducted to investigate the effects of various parameters on the conversion and rate of reaction. These parameters included the type of lipase, lipase dosage, various solvents, time, amount of molecular sieves, molar ratio, revolution speed, and temperature. Under optimal reaction conditions, various substrates were used to synthesize a range of pyridine esters, and their structure was confirmed by nuclear magnetic resonance (NMR), infrared spectroscopy (IR) and high resolution mass spectrometry (HRMS). Additionally, the odor characteristics of pyridine esters were analyzed using GC-MS-O. The thermal stability of these aromatic compounds was evaluated through TG analysis, offering valuable insights for the development of pyridine esters in the field of flavors and fragrances. Fig. 1 showed the process from synthesis to analysis in this study.

2. Materials and method

2.1. Materials

Novozym 435(*Candida antarctica* Lipase B immobilized on macroporous resin), Lipozyme TLIM (*Thermomyces lanuginosus* lipase immobilized on silica gel) and CRL (*Candida rugosa* lipase immobilized on macroporous resin) were bought from Novozymes A/S. Nicotinic acid, 2-methoxynicotinic acid, 5-methylnicotinic acid and isonicotinic acid were bought from Energy Chemical Co., Ltd. Benzyl alcohol, 3-phenyl-1-propanol, phenethyl alcohol, 2-naphthalenemethanol, 3-phenyl-1-propanol and naphthalen-2-ethanol were bought from Macklin Biochemical Technology Co., Ltd. Methallyl alcohol, 3-chlorobenzyl alcohol, (*E*)-4-hexen-1-ol and geraniol were bought from Meryer Chemical Technology Co., Ltd. 1-pentanol, 3-methyl-1-butanol, 2-ethyl-1-butanol, 1-decanol, 1-hexanol and 1-octanol were purchased from Aladdin Biochemical Technology Co., Ltd. Cyclopropyl carbinol, cyclopentanemethanol, cyclohexanemethanol, cyclohexanol and cycloheptanol were bought from Zhejiang Karry Pharmachem Co., Ltd. Acetonitrile, n-hexane, 2,2,4-trimethylpentane and toluene were purchased from Guangzhou Kangyang Chemical Co., Ltd. DCE (dichloroethane), DMF (*N*,*N*-dimethylformamide) and 1,4-dioxane were from Henan Tianhua Pharmaceutical Co., Ltd. DMSO (dimethyl sulfoxide) and dichloromethane were purchased from Qingdao Dexin Chemical Co., Ltd. Molecular sieve 3A (zeolite 3A, $M(Si0_2)/M(Al_20_3)\approx2$) were bought from Zhengzhou Acme Chemical Co., Ltd.

2.2. Synthesis steps

The parameters evaluated in our study included: the type and dosage of enzymes, the solvent, molar ratio, amount of molecular

Table 1
Effect of lipase types on the yield of **3a**[a].

Lipase	Yield (%)[b]
Novozym 435	46
Lipozyme TLIM	n.d.
Candida rugosa Lipase (CRL)	31

[a] Reaction conditions: 2-methoxynicotinic acid **1a** (1.0 mmol), benzyl alcohol **2a** (0.2 mmol), 60 mg Lipase, 1.00 g molecular sieve 3A, acetone (7.5 mL), 50 °C, 150 rpm, 72 h.
[b] Isolated yields.

sieve, rotation speed, reaction temperature, and reaction time. Under air atmosphere, 2-methoxynicotinic acid **1a** and benzyl alcohol **2a** were utilized in a series of consecutive experiments were conducted to identify the optimum reaction parameters for synthesizing pyridine esters: Novozyme 435 was 60 mg, n-hexane was 5.0 mL, and the amount of molecular sieve 3A was 0.25 g. The reaction was conducted at 150 rpm for 48 h.

After determining the optimal reaction conditions, nicotinic acids (**1a-1d**, 0.4 mmol), alcohols (**2a-2t**, 0.2 mmol), Novozym 435 (60 mg) and molecular sieve 3A (0.25 g) were added to a 30 mL tube that had a magnetic stirring bar at air condition. The mixture was then given 5 mL of *n*-hexane, and it was stirred at 150 rpm for 48 h in a water bath thermostat at 50 °C 10 mL of ethyl acetate was incorporated into the reaction mixture after cooling, and it was then washed with 10 mL of brine. Ethyl acetate was used to extracted the aqueous layer twice. The combined organic solvent was filtered, concentrated in vacuo, and dried over anhydrous Na_2SO_4. The residual was purified to obtain products **3** ranging from 24 % to 99 % yields using flash liquid chromatography on silica gel with petroleum ether-EtOAc as the extraction solvent.

2.3. Structural identification

The identification of compounds involved the utilization of nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR), high resolution mass spectrometry (HRMS) and infrared spectroscopy (IR). The spectra data of ^{1}H NMR and ^{13}C NMR were recorded by a BRUKER AVANCE III 400 MHz spectrometer (^{1}H 400 MHz, ^{13}C 100 MHz). The Thermo Scientific Nicolet 6700 FT-IR Spectrometer was used to collect the data of infrared spectra. The data of HRMS was acquired by using an AB SCIEX Triple TOF 5600+ high resolution mass spectrometer from the United States.

2.4. Odor evaluation

Utilizing the method by Feng [25], the sensory panel was selected. The group consisted of two men and three women who were healthy, non-smoking and between the ages of 23 and 29. They were selected for odor evaluation. These panelists were affiliated with the Henan agriculture university. They had standardized training to recognize and describe aromas, and each possessed over 120 h of sensory experience in odor assessment. During GC analysis (Agilent, 7890B-5977A), team members were requested to verbally characterize the odor at the occurrence of peaks. The supplementary materials provided the analytical conditions of GC-MS-O (Agilent, 7890B-5977A; Gerstel OP3, Germany) in detail. Before the publication of the paper, all participants provided written informed consent.

2.5. Thermogravimetric analysis

Spectrally pure aluminum oxide was utilized as the reference substance. The air flow was 60 mL min^{-1}. The sample, sieving and weighing 10 mg, was placed in an alumina crucible under aerobic conditions. The temperature was controlled between 30 and 800 °C, and the heating rate was 20 °C min^{-1}. To obtain the curves of thermogravimetric (TG), derivative thermogravimetric analysis (DTG), and differential thermal analysis (DTA) for the samples, a simultaneous thermal analyzer (STA 449 F3, Netzsch, Germany) was employed.

3. Results and discussion

3.1. Optimization of reaction conditions

Initially, the synthesis of 2-methoxybenzyl nicotinate (**3a**) was conducted by reacting 2-methoxynicotinic acid (**1a**) with benzyl alcohol (**2a**). The types of lipases were preferentially screened in Table 1, and three different commercially immobilized lipases including Novozym 435, Lipozyme TLIM and CRL were chosen for an investigation into the effect on the synthesis of **3a**. When Novozym 435 was used as biocatalyst, the yield of **3a** could reach 46 %. When CRL was used, the yield of **3a** was 31 %. However, when using lipozyme TLIM, no reaction occurred. As a result, for the subsequent study, Novozym 435 was employed.

In the enzyme-catalyzed reactions, solvents directly interact with substrates and enzymes, and their polarity and the types of functional groups they contain can influence the activity of enzymes[26,27]. Therefore, the type of solvent is an important criterion to

Table 2
Effect of solvent on the yield of **3a**[a].

Solvent	Yield (%)[b]
n-hexane	78
2,2,4-trimethylpentane	32
acetonitrile	46
toluene	55
DCE	28
DMF	n.d.
1,4-dioxane	n.d.
DMSO	n.d.
dichloromethane	n.d.

[a] Reaction conditions: 2-methoxynicotinic acid **1a** (1.0 mmol), benzyl alcohol **2a** (0.2 mmol), 60 mg Novozym 435, 1.00g molecular sieve 3A, solvent (7.5 mL), 50 °C, 150 rpm, 72 h.
[b] Isolated yields.

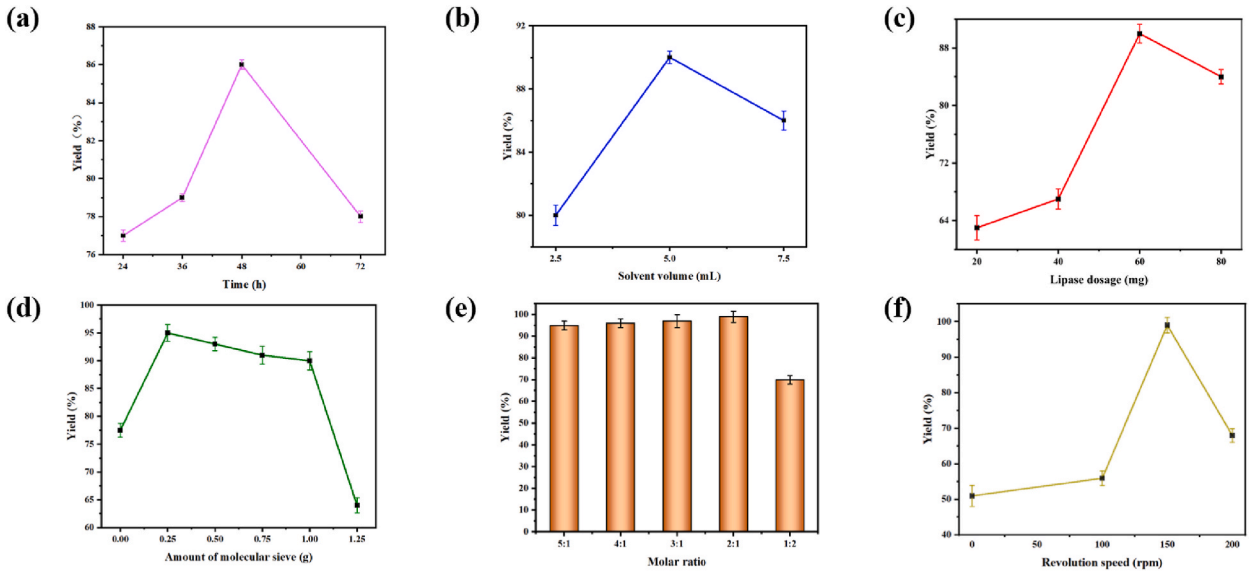


Fig. 2. Optimization of the reaction conditions. (a) reaction time, (b) solvent volume, (c) lipase dosage, (d) amount of molecular, (e) molar ratio, (f) revolution speed.

be screened. Based on lipase types, the impact of different solvent varieties on the yield of **3a** were examined, and the results were presented in Table 2. Moreover, three repetitions of the experiment were conducted. It was shown that the yield of **3a** exhibited considerable variation across the 9 solvents. When DMF, 1,4-dioxaneand, DMSO and dichloromethane were used as the solvents, product 3a was not found. When 2,2,4-trimethylpentane toluene and DCE were solvents, the yields of **3a** reached 32 %, 55 % and 28 %, respectively. When n-hexane was utilized as the solvent, the yield of **3a** was 78 % which reached the maximum yield. n-hexane is a non-polar solvent that has been extensively studied and proven to significantly enhance enzyme activity [28]. Additionally, considering its excellent solubility for substrates, low cost, and low toxicity [29].Therefore, n-hexane was chosen as the solvent in the following experiments.

Reaction conditions: **1a**, **2a**, Novozym 435, molecular sieve 3A, n-hexane, at 50 °C with stirring. Yields are isolated yields.

According to the screening of solvent type, reaction times (24–72 h) were screened. In addition, three repetitions of the experiment were conducted. As shown in Fig. 2 (a), the yield of **3a** reached 86 % at 48 h. When the reaction time was reduced or prolonged, the yield would decrease. Therefore, the optimal reaction time was 48 h.

To examine the influence of solvent volume on the productivity of **3a**, the experiments were conducted using 2.5, 5.0 and 7.5 mL, respectively. Furthermore, three repetitions of the experiment were conducted. Additionally, the highest yield was illustrated in Fig. 2 (b). When the volume of the solvent was 5.0 mL, the yield of 90 % was obtained for **3a**.

In enzyme-catalyzed reactions, the cost factor of enzymes must be considered. Therefore, it is of great significance to achieve high yields at low concentrations [30]. Aiming to determine the optimal dosage for the study, the reaction was conducted by varying the lipase amount in 20 mg increments within the range of 20–80 mg. In addition, three repetitions of the experiment were conducted. As depicted in Fig. 2 (c), the yield of **3a** were 63 %, 67 %, 90 % and 84 % at 20, 40, 60 and 80 mg, respectively. Consequently, the optimal

Table 3
Effect of reaction temperature on the yield of **3a**[a].

Temperature (°C)	Yield (%)[b]
40	83
50	99
60	59

[a] Reaction conditions: 2-methoxynicotinic acid **1a** (0.4 mmol), benzyl alcohol **2a** (0.2 mmol), 60 mg Novozym 435, 0.25 g molecular sieve 3A, n-hexane (5 mL), 150 rpm, 48 h.
[b] Isolated yields.

lipase dosage was determined to be 60 mg, with a yield of 90 %.

The purpose of adding molecular sieves to the reaction mixture is to absorb water, diminishing the inhibitory effects caused by its excess presence and thereby enhancing the conversion rate [31]. However, some studies have indicated that an excessive amount of molecular sieves can negatively impact the yield[32,33]. Therefore, in the following experiments, various amount of molecular sieve (ranging from 0 to 1.25 g) was tested to assess their impact on the reaction. Three repetitions of the experiment were conducted. As depicted in Fig. 2 (d), the yield of **3a** peaked at 95 % when 0.25 g of molecular sieve was used. Consequently, 0.25 g was selected for subsequent experiments in this study.

In enzyme-catalyzed reactions, the substrate molar ratio significantly influenced the reaction rates and enzyme activity, often serving as a crucial evaluation parameter in experimental condition optimization [34–36]. Therefore, based on the results of the amount of molecular sieve screening, the optimization of the molar ratio was further conducted, including ratios of 5:1, 4:1, 3:1, 2:1 and 1:2. The experiment was repeated three times. Fig. 2 (e) clearly indicates that the yield of **3a** increased with an elevated proportion of **2a**, peaking at 2:1, while it gradually reduced with the increase of benzyl alcohol. Consequently, the highest yield of 99 % was achieved at a molar ratio of 2:1, which was identified as the optimal ratio for the subsequent study.

To explore the influence of varying rotational velocity on the catalytic efficiency of Novozym 435 in esterification, the experiments were carried out at revolution velocity varying from 0 to 200 rpm. The trials were repeated three times to ensure reproducibility and accuracy. Significantly, as shown in Fig. 2 (f), the peak performance achieved at a rotation speed of 150 rpm, resulting in a remarkable 99 % yield of **3a**.

The reaction temperature significantly impacts the enzymatic reaction. While increasing the reaction temperature enhances substrate-enzyme collisions and thus increase the reaction rate, excessively high temperatures can result in enzyme inactivation. Conversely, if the reaction temperature is too low, it hinders substrate transformation [37]. Thus, to determine the optimal temperature, experiments were conducted in the temperature range of 40–60 °C with 10 °C intervals. Additionally, three repetitions of the experiment were conducted. Table 3 illustrated that the most effective reaction temperature, yielding 99 % of **3a**, was at 50 °C.

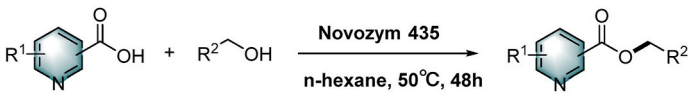
3.2. Recyclability of the Novozym 435 enzyme

Reusability is one of the most significant characteristics of lipase. In this section, the enzyme particles were separated from the reaction medium by filtration under the optimal conditions. Then, the separated lipase particles were washed three times with 10 mL of n-hexane. After drying at 40 °C for 40 min, the recovered Novozym 435 particles were utilized for the synthesis of **3a**. The reutilization of Novozym 435 was investigated in the esterification procedure by reusing it for 9 cycles in n-hexane at a temperature of 50 °C. Additionally, three repetitions of the experiment were conducted.

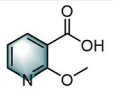
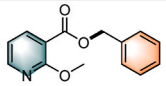
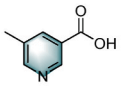
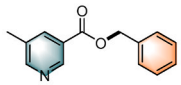
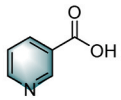
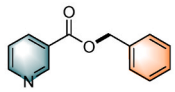
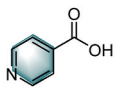
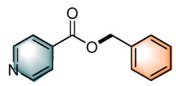
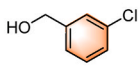
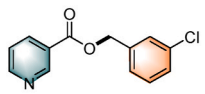
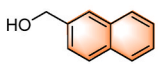
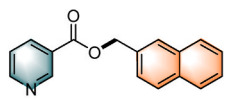
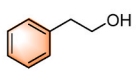
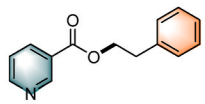
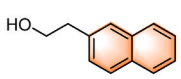
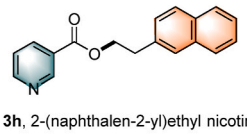
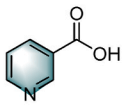
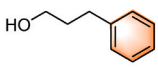
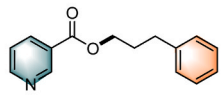
As shown in Fig. S1, the yield of **3a** decreased with the increased using times of Novozym 435. Employing **1a** and **2a** as substrates, the yield of the synthesis of **3a** remained remarkably strong at 90 % after 5 cycles. Interestingly, even Novozym 435 had been used nine cycles, the yield remained at a substantial level of 80 %. The experimental results demonstrate that the catalytic performance of Novozym 435 exhibits remarkable stability even after using for multiple times. Therefore, lipase Novozym 435 exhibits significant economic value and potential for reusing in esterification synthesis.

3.3. Substrate scope

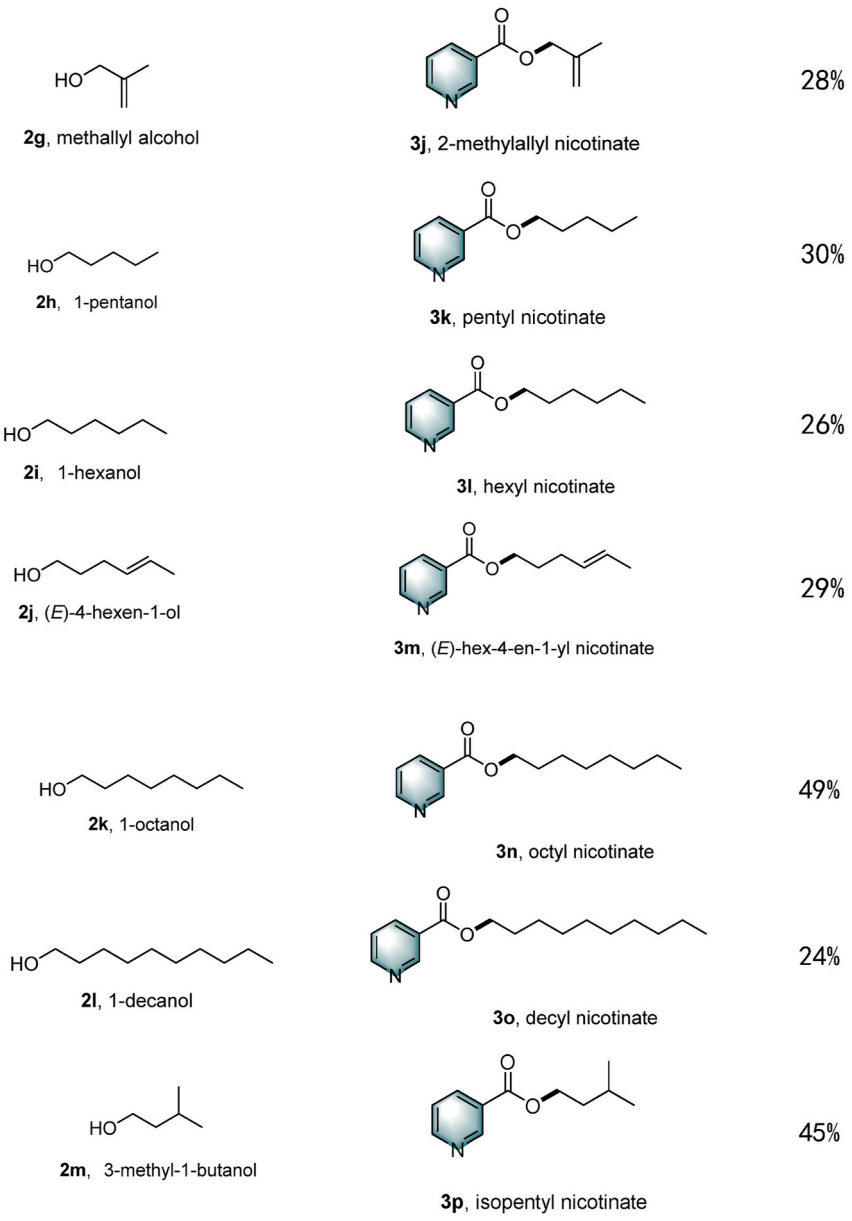
After establishing the optimal conditions, the next objective was to apply this method to various other substrates. Scheme 1 illustrated the efficient production of the desired pyridine ester compounds with moderate to high yields using a series of commercially available nicotinic acids and alcohols. Initially, we utilized benzyl alcohol (**2a**) and various carboxylic acid compounds (2-methoxynicotinic acid, 5-methylnicotinic acid, nicotinic acid and isonicotinic acid) for esterification reactions, yielding corresponding compounds (**3a**-**3d**) at rates ranging from 41 % to 99 %. Remarkably, compound **3a** reached a high yield of 99 %. Following optimization, nicotinic acid (**1c**) was employed as the substrate to investigate the applicability of various other aromatic and aliphatic alcohol compounds in this reaction. When undergoing esterification reactions with aromatic alcohol compounds (2-naphthalenemethanol, 3-phenyl-1-propanol, phenethyl alcohol, naphthalen-2-ethanol), corresponding compounds (**3f**-**3i**) were obtained at yields ranging from 32 % to 52 %. When 3-chlorobenzyl alcohol (**2e**) reacted with **1c**, the yield of the product (**3e**) was 30 %. Next, methallyl alcohol, *(E)*-4-hexen-1-ol, and geraniol were used as substrates to react with **1c**, resulting in corresponding products (**3j**, **3m**,



1a-1d 2a-2t 3a-3w

Nicotinic acids	Alcohols	Pyridine esters	Yields
 **1a**, 2-methoxynicotinic acid	 **2a**, benzyl alcohol	 **3a**, 2-methoxybenzyl nicotinate	99%
 **1b**, 5-methylnicotinic acid		 **3b**, 3-methylbenzyl nicotinate	47%
 **1c**, nicotinic acid		 **3c**,benzyl nicotinate	78%
 **1d**, isonicotinic acid		 **3d**, benzyl isonicotinate	41%
	 **2b**, 3-chlorobenzyl alcohol	 **3e**, 3-chlorobenzyl nicotinate	30%
	 **2c**, 2-naphthalenemethanol	 **3f**, naphthalen-2-ylmethyl nicotinate	44%
	 **2d**, phenethyl alcohol	 **3g**, phenethyl nicotinate	52%
	 **2e**, naphthalen-2-ethanol	 **3h**, 2-(naphthalen-2-yl)ethyl nicotinate	32%
 **1c**, nicotinic acid	 **2f**, 3-phenyl-1-propanol	 **3i**, 3-phenylpropyl nicotinate	50%

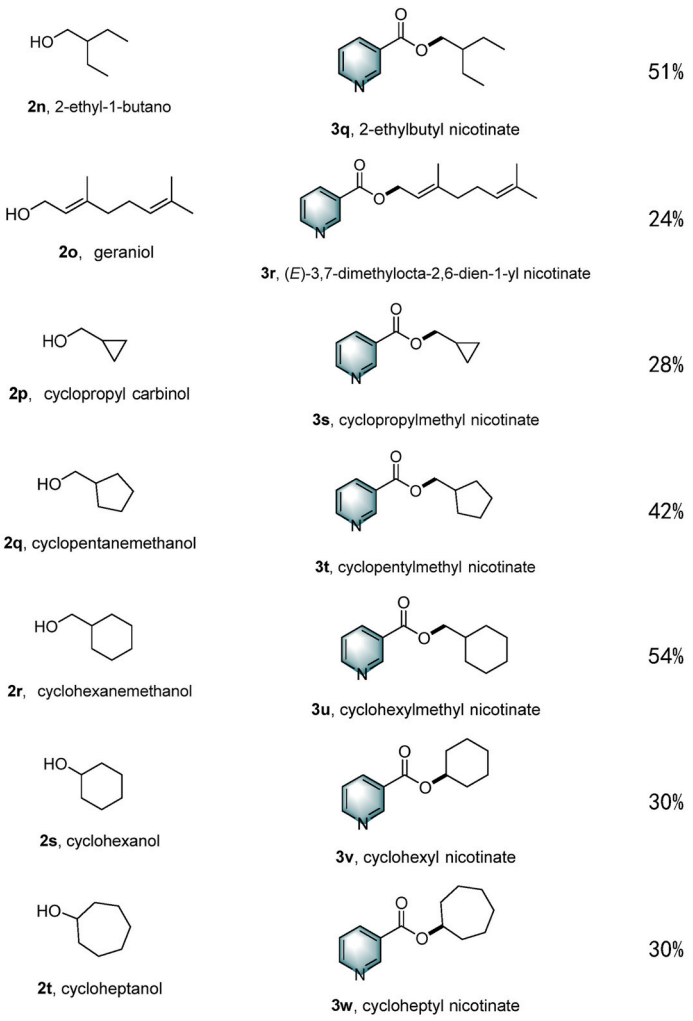
Scheme 1. Substrate expansion of nicotinic acids (**1**) and alcohols (**2**). Reaction conditions: under air, nicotinic acids (**1**, 0.4 mmol), alcohols (**2**, 0.2 mmol), 60 mg Novozym 435, 0.25 mg molecular sieve 3A, n-hexane (5 mL), 150 rpm, 50 °C, 48 h. Yields are isolated yields.



Scheme 1. (*continued*).

and **3r**) with yields of 28 %, 29 %, and 24 %, respectively. Moreover, reactions between fatty alcohol compounds (1-pentanol, 1-hexanol, 1-octanol, 1-decanol, 3-methyl-1-butanol, and 2-ethyl-1-butanol) and **1c** resulted in corresponding products (**3k**, **3l**, **3n**, **3o**, **3p**, and **3q**) with yields ranging between 24 % and 51 %. The reactions of **1c** and **2q** yielded the highest product (**3q**) with a yield reaching 51 %. Additionally, cycloalkanol compounds (cyclopropyl carbinol, cyclopentanemethanol, cyclohexanemethanol, cyclohexanol, and cycloheptanol) were employed as substrates for esterification reactions with **1c**, yielding the respective products (**3s**-**3w**) at rates ranging from 28 % to 54 %. The maximum yield of product (**3u**) was achieved at 54 % when reacting **1c** with **2u**. Using this reaction method, a total of 23 pyridine ester compounds were synthesized, demonstrating its exceptional applicability and universality. William et al. reported a methodology utilizing titanium (Ti) as a catalyst to generate a series of pyridine esters from various nicotinic acids and alcohols at 160 °C, with yields ranging from 58 % to 93 % [38]. In comparison, our method features lower reaction temperatures, broader substrate range, lower cost and decreased pollution, thereby presenting superior prospects for practical application.

In addition, a scale-up experiment was conducted by employing 2-methoxynicotinic acid **1a** (10 mmol, 1.53 g) and benzyl alcohol **2a** (5 mmol, 0.54 g) to investigate the feasibility of scaling up the process. As shown in Supporting information, it can be clearly seen that the high yield of 93 % (1.06 g) was achieved for the synthesis of product **3a**.



Scheme 1. (*continued*).

Table 4
Aroma description.

Synthetic Product	Aroma assessed in this study
Phenethyl nicotinate (**3g**)	Pineapple-like
(E)-hex-4-en-1-yl nicotinate (**3m**)	Bitter almond-like
Octyl nicotinate (**3n**)	Lily flower-like

3.4. Flavour characteristics

The results from GC-MS-O analysis indicated that among the expanded 23 substrates, three compounds (**3g**, **3m** and **3n**) exhibited distinct and unique aromas. The aroma characteristics of compounds **3g**, **3m** and **3n** were shown in Table 4. Products **3g** had a pineapple aroma. Products **3m** possessed a bitter almond aroma. Products **3n** presented a lily flower aroma.

3.5. Thermal analysis

Among the 23 synthesized pyridine esters, the **3g**, **3m**, and **3n** exhibited distinct aromatic fragrances. In the field of flavors and fragrances, the stability and aroma characteristics of compounds are crucial. Therefore, we conducted thermogravimetric analysis on these three compounds to understand their stability and potential volatility at high temperatures, aiming to evaluate their potential application value in the fields of food and cigarette flavoring. The profiles of TG, DTG and DTA for compounds **3g**, **3m** and **3n** were

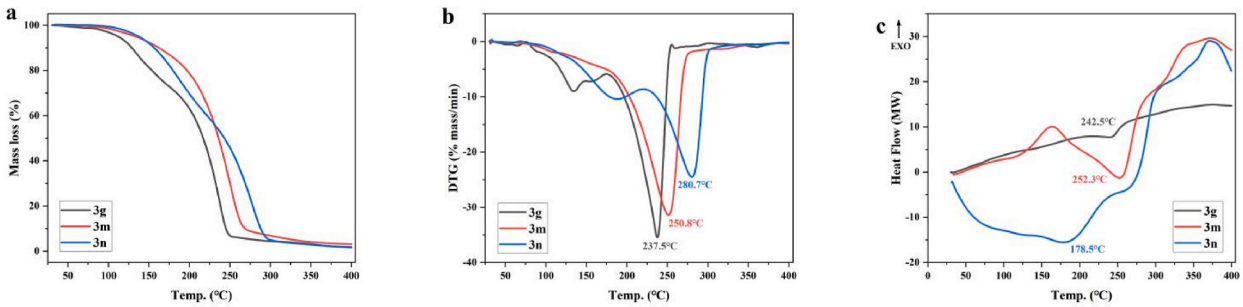


Fig. 3. Thermogravimetric curves for **3g**, **3m and 3n**. (a) TG, (b) DTG, (c) DTA.

Table 5
The data of thermal analysis for compounds **3g**, **3m**, and **3n**.

Compound	DTA			TG-DTG		Mass Loss/%
	T_{onset}/°C	T_{peak}/°C	T_{end}/°C	T_P/°C	T_{range}/°C	
3g	211.0	242.5	256.7	237.5	81.7–255.6	94.08 %
3 m	167.8	252.3	277.6	250.8	92.6–276.3	91.25 %
3n	149.3	178.5	228.6	280.7	98.6–304.4	95.57 %

depicted in Fig. 3 (a, b, c), and they degraded at a heating rate of 20 °C min^{-1}. Products **3g**, **3m** and **3m** disintegrated at temperatures varying from 81.7 to 255.6, 92.6.6 to 276.3, and 98.6–304.4 °C, respectively, as shown in Fig. 3 (a, b). The peak mass loss rates for **3g**, **3m** and **3n** during different phases of the disintegration were 237.5, 250.8 and 280.7 °C, respectively. At the same time, after reaching the temperatures of T_P, the mass of **3g**, **3m** and **3n** decreased by 78.32 %, 70.64 % and 83.37 %, respectively. The total mass reductions of **3g, 3m** and **3n** were 94.08 %, 91.25 % and 95.57 % from the beginning, respectively. Fig. 3 (c) illustrated the DTA curves of samples **3g**, **3m** and **3n** examined in an environment of air. The equipment was used to record the peak temperatures of DTA curves. Analysis of the DTA results revealed that the decomposition processes of products **3g**, **3m**, and **3n** were characterized by endothermic reactions. The information regarding the decomposition temperature were summarized in Table 5. As shown in Table 5 and Fig. 3, the endothermic peaks of **3g**, **3m** and **3n** appeared in the main mass loss areas, which were 242.5, 252.3 and 178.5 °C, respectively. During the endothermic phase, these three samples might have vaporized or degraded. In general, the TG-DTG and DTA results evidenced that these samples exhibited stability at ambient temperature.

4. Conclusions

In conclusion, an efficient enzymatic approach to synthesis pyridine esters was investigated in this study. Compared with traditional chemical methods, the enzymatic reaction using Novozym 435 as catalyst has milder conditions, which avoided the use of organic catalysts. Firstly, continuous experiments were conducted to determine the optimal reaction conditions. The optimum reaction conditions are as follows: 60 mg Novozym 435, molar ratio (2:1) of nicotinic acids (**1**, 0.4 mmol) to alcohols (**2**, 0.2 mmol), 0.25 g molecular sieve 3 A, n-hexane 5 mL, temperature 50 °C, rotational speed 150 rpm in the shaking water bath for 48 h. Products **3a** achieved a great yield, up to 99 %. After 9 cycles, a yield of 80 % was still obtained. The enzyme catalysis approach demonstrated significant potential for the efficient synthesis of pyridine esters, thus it had broad application prospects. Subsequently, a series of nicotinic acids and alcohols produced 23 pyridine esters under optimal conditions, among them, **3g**, **3m** and **3n** identified by GC-MS-O as having pineapple, bitter almond and lily flower aroma, respectively. The TG-DTG and DTA results showed that the main mass of **3g**, **3m** and **3n** varies from 81.7 to 304.4 °C with mass decreased by 94.08 %, 91.25 % and 95.57 %, respectively. The weight loss process of these three compounds is endothermic, mainly caused by evaporation or decomposition, with endothermic peaks of 242.5, 252.3 and 178.5 °C. These three compounds had good thermal stability at room temperature. With the increasing attention of pyridine esters, these three aroma pyridine esters were anticipated to possess significant opportunities for extensive development in food or cigarette flavoring.

Data availability statement

The data associated with this study have not been deposited into a publicly available repository. Data will be made available on request.

CRediT authorship contribution statement

Longxin Wang: Writing – original draft, Methodology. **Qianrui Zhao:** Validation, Conceptualization. **Guangpeng Wu:** Writing –

review & editing. **Pengze Wang:** Writing – review & editing, Methodology. **Meng Zhou:** Investigation, Formal analysis, Data curation. **Zhiyong Wu:** Supervision, Methodology, Formal analysis. **Miao Lai:** Writing – review & editing, Supervision. **Mingqin Zhao:** Supervision, Methodology.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32435.

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