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Combinatorial Synthesis of Chiral Esters from Fruit Aroma in Continuous-Flow Reactors by Chemical and Enzymatic Catalysis

Calionara Waleska B. Melo,[a] Yasmin O. Santana,[a] Rodrigo M. V. Silva,[a] Marco Antônio M. Bezerra,[b] Humberto R. Bizzo,[c] Rodrigo Octavio M. A. Souza,[b] Raquel Ana C. Leão[b] and Claudia M. Rezende[*,a]

[a]*Laboratório de Análise de Aroma, Instituto de Química, Universidade Federal do Rio de Janeiro, 21941-909 Rio de Janeiro-RJ, Brazil*

[b]*Grupo de Biocatálise e Síntese Orgânica, Instituto de Química, Universidade Federal do Rio de Janeiro, 21941-909 Rio de Janeiro-RJ, Brazil*

[c]*Embrapa Agroindústria de Alimentos, 70770-901 Rio de Janeiro-RJ, Brazil*

Short-chain chiral esters are important constituents in natural fruit flavors, as well as in food and pharmaceutical industries. Different chemical and bio-catalytic routes are used for their synthesis, but the use of simultaneous techniques has not been much explored, as combinatorial synthesis and bio-catalysis in continuous-flow systems. Here, the objective was to synthesize chiral esters typically found in fruit flavors, to obtain chemical libraries with high conversion and enantiomeric excess, using combinations of these three techniques. Combinatorial synthesis in continuous-flow coil-type reactors with fix bed was used in the esterification of the secondary alcohols 2-butanol (**1a**), 2-pentanol (**1b**), 3-hexanol (**1c**), 2-heptanol (**1d**) and 2-octanol (**1e**) with acetic (**2**) and propanoic (**3**) acids. A surface-response method was applied to optimize the reaction conditions. Conversions were above 88% after 60 min for the reaction with chemical catalysts in continuous-flow reactors compared to 85%, but after 120 min, when conventional batch processes were used. By applying a biocatalyst, a chemical library of *R* acetates was prepared, with conversions over 48% after 48 h for batch and 40% after only 70 min for continuous flow reactions. It was observed that both enzymatic and homogeneous chemical catalysis showed to be viable for the chiral esters.

Key words: fruity esters, enantioselectivity, chiral GC, flow chemistry, rotational central composite design

Introduction

Volatile, low-chain esters are part of the natural flavor of fruits and are important additives in foods, beverages, and pharmaceuticals flavoring. The global market of food flavors was estimated in US$14.3 billion in 2020 and an annual growth of 4.6% is expected until 2028.[1]

Many of these esters are chiral and can occur as racemates or as specific enantiomeric proportions, as a result of the high stereoselectivity of enzymatic reactions in plants.[2] The volatile esters are usually synthesized by Fischer esterification or transesterification, both equilibrium reactions. The use of biocatalysts is an important alternative and frequently applied, whenever stereoselective is needed, and due to environmentally friendly protocols.[3] The use of continuous-flow processes is increasing in agrochemical and pharmaceutical industries, pushed by intensification processes to higher conversions in shorter reactional times and its association with biocatalysis proved to be an interesting alternative for the synthesis of natural products in general.[4]

Combinatorial chemistry has long been explored by the pharmaceutical industry.[5] For flavor applications, although described as a very promising approach, either for finding or identify contaminants,[6,7] quite little was published. Collin *et al.*[8,9] used it to synthesize libraries of polyfunctional thiols, as well as mercaptan compounds with aldehydes,[10] esters,[11] and primary alcohols,[12] compounds commonly found in food and beverage products.

One interesting and quite innovative strategy is the association of continuous-flow reactors to combinatorial

*e-mail: claudia.rezendeufrj@gmail.com
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synthesis, producing chemical libraries to different applications. To the best of our knowledge, no work on flow reaction and combinatorial synthesis of food and flavors has been published yet.

Herein, we aimed the application of combinatorial chemistry, biocatalysts and continuous-flow reactions to obtain chemical libraries of chiral esters, with high conversion and enantioselectivity.

Experimental

Materials

All reagents and materials were supplied by Sigma-Aldrich (São Paulo, Brazil). The biocatalyst used was Novozyme 435® (lipase B from *Candida antartica*, Sigma-Aldrich, São Paulo, Brazil) immobilized in polyacrylic macroporous resin, specific activity of 10,000 propyl laurate units (PLU g^{-1}). Molecular sieves of 4 Å (Sigma-Aldrich, São Paulo, Brazil). As starting materials, 2-butanol 99% (**1a**), 2-pentanol 98% (**1b**), 3-hexanol 97% (**1c**), 2-heptanol 97% (**1d**), 2-octanol 99.5% (**1e**); acetic acid 99% (**2**) propanoic acid 99% (**3**), and trifluoroacetic anhydride 98% were used (all from Sigma-Aldrich, São Paulo, Brazil).

Chromatographic analysis

Products were analyzed in an Agilent 6890 gas chromatograph (Agilent, Wilmington, USA) equipped with a flame ionization detector (GC-FID) and in an Agilent 6890 gas chromatograph coupled to a 5975C mass spectrometer detector (GC-MS) (Agilent, Wilmington, USA). For the chiral analysis, a Cyclodex HP β-Chiral-HP (Agilent, Wilmington, USA) with 20% permethylated cyclodextrin (30 m × 0.25 mm × 0.25 μm) capillary column was used. Injector was kept at 250 °C and operated in split mode (1:20).

For the GC-FID (Agilent, Wilmington, USA), the detector was maintained at 250 °C, and hydrogen (0.5 mL min^{-1}) was used as carrier gas. The analyses of the alcohols **1a-1e** were run in isothermal mode at 40 ºC for 35 min. For the conversion (c) calculation (Supplementary Information (SI) section, "Conversion (c)" section), the oven temperature was raised from 70 °C (5 min) to 100 °C at 1.0 ºC min^{-1} and kept at this temperature for 5 min.

For the GC-MS (Agilent, Wilmington, USA), the carrier gas used was helium (1.0 mL min^{-1}), and the oven temperature was programmed from 40 ºC (5 min) to 100 ºC (5 min), at 1 ºC min^{-1}. The mass spectrometer was operated in electron ionization (70 eV), interface and ion source at 230 °C, and analyser (quadrupole) at 150 °C. For the identification of the compounds, the NIST14 spectral library was used.

Determination of the conversion (c), separation factor (α) and enantiomeric excess (e.e.) of alcohols **1a-1e**

Individual esterification reactions for determination of the conversion (c, %), separation factor (α) and enantiomeric excess (e.e., %) were performed with trifluoroacetic anhydride and the alcohols **1a-1e**. Experimental details and results are in the SI section ("Conversion (c)", "Enantiomeric excess (e.e.)" and "Enantioseletivity (E)" sections).

Combinatorial synthesis by batch chemical catalysis

Combinatorial synthesis synthesis of the racemic acetates was done according to the conditions of Jyoti *et al.*[13] with modifications. A stoichiometric mix of the alcohols **1a-1e** (10 mmol) with acetic acid (35 mmol) and sulfuric acid (195 μL) were heated under reflux for 120 min.

Combinatorial synthesis by batch enzymatic catalysis

The biocatalytic synthesis was done according to the protocol of Larios *et al.*[7] for establish the elution order of the *R* and *S* enantiomers in the Cyclodex HP β-Chiral column (Agilent, Wilmington, USA). The lipase activity was previously determined (SI, "Lipase activity" section).[14] For the synthesis, 50 mg of the immobilized Novozyme N435® (Sigma-Aldrich, São Paulo, Brazil) were added over a mixture of 25 mmol of each alcohol **1a-1e** and 25 mmol of acetic acid in 4 mL of hexane (all from Agilent, Wilmington, USA), containing 100 mg of the 4 Å molecular sieve. The mixture was agitated at 180 rpm and incubated at 35 °C for 48 h, being monitored by chiral GC-FID. Based on the conversion values (c, %, SI, "Lipase activity" section) and enantiomeric excess (e.e., %, SI, "Enantiomeric excess (e.e.)" section) obtained, the conditions were transferred to the continuous flow system.

Continuous flow combinatorial synthesis by chemical catalysis

The initial conditions, based on the batch process, were transferred to a homemade continuous flow system, using a polytetrafluoroethylene (PTFE) tubular reactor with 1/8 inches outside diameter (OD), 1/16 inches internal diameter (ID) and volume of 30 mL (Figure 1).

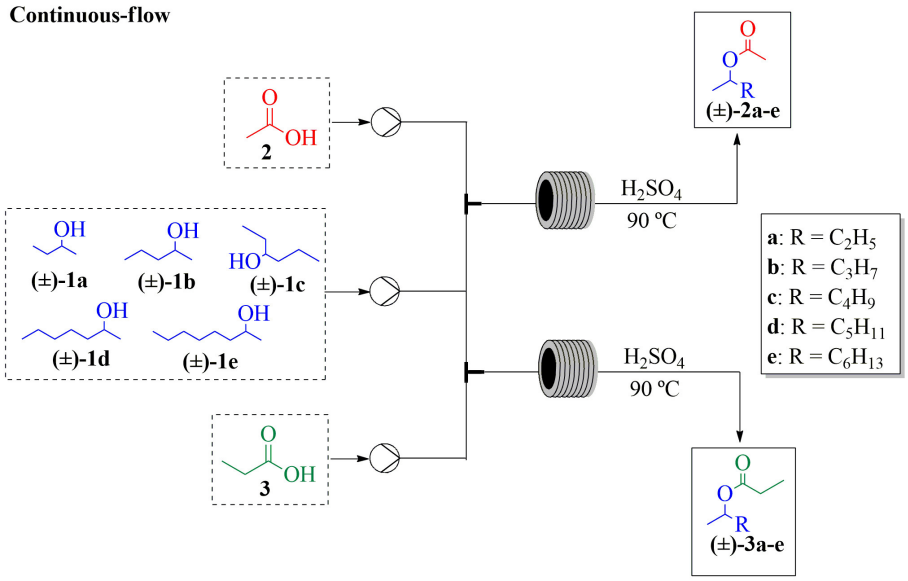


Figure 1. Illustrative scheme for the combinatorial synthesis of acetate and propanoate esters by chemical catalysis in continuous flow.

The temperature of the reaction media was controlled by a heating system added to the reactor. For the reaction, 10 mmol of the alcohols **1a-1e** and 35 mmol of acetic acid were used.

A rotational central composite design (RCCD) was done to identify the important variables to maximize the conversion, using an experimental planning with 2 factors and 2 levels, including a duplicate for the central point and four axial points ($\alpha = \pm 1.414$), resulting in 11 experiments. The levels of the variables were chosen according to preliminary studies of our group.[15] Variables tested were temperature and reactor flow rate (in mL min^{-1}), as affecting the conversion.

The experimental design and data analysis were performed with Statistica 7.0 software.[16] The significance level was set at 95% for the mathematic model and response surface. The decodified matrix of the experiments is shown in Table 1.

Table 1. Decodified values of the variables studied in the rotational central composite design (RCCD) for the optimization of the combinatorial synthesis of acetates in continuous flow by chemical catalysis

Variable	−1	0	1	$-\alpha^a$	α^a
Temperature / °C	85.0	87.5	90.0	83.9	91.0
Flow rate / (mL min^{-1})	0.5	1.0	1.8	0.16	2.4

[a] Distance of each axial point from the center in the central composite design.

After that, the condition with a lower flow rate and higher c was used for the combinatorial synthesis of the alcohols **1a-1e** with propanoic acid (**3**).

Continuous flow combinatorial synthesis by enzymatic catalysis

The study of the enzymatic catalysis under continuous flow conditions was done in a system with a syringe pump connected to the reactor by a PTFE canula. The reactor was previously packed with 2.362 g of lipase N435® (internal volume 7.5 mL), and the reaction temperature was controlled by a heating system. A mixture of 25 mmol from the alcohols **1a-1e** with 25 mmol of acetic acid in 20 mL of hexane was pumped through the reactor.

Based on the preliminary results from the batch process and to optimize the protocol of kinetic resolution catalyzed by the lipase N435®, a RCCD planning was proposed to identify the important variables (temperature and flow rate) and maximize the conversion. The study of the effect of reaction conditions in esterification was based on the work of Thomas *et al.*[17] The experimental design and data analysis were performed with Statistica 7.0 software.[16] The decodified matrix of the experiments is shown in Table 2.

Table 2. Decodified values of the rotational central composite design (RCCD) variables studied for optimization of enzymatic combinatorial synthesis

Variable	−1	0	1	$-\alpha^a$	α^a
Temperature / °C	35	40	45	32.9	47.1
Flow rate / (mL min^{-1})	0.10	0.50	0.25	0.05	0.60

[a]Distance of each axial point from the center in the central composite design.

Results and Discussion

Determination of the e.e. for the alcohols **1a-1e**

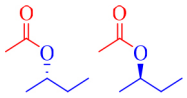
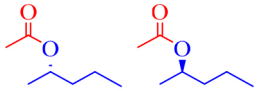
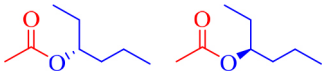
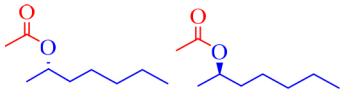
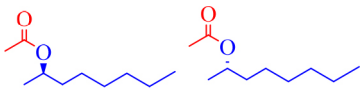
First, as the usual protocol, it was necessary to determine the α_Q and the e.e. for the starting material, the secondary alcohols **1a-1e** (SI, "Determination of e.e. and α_Q of alcohols **1a**-**1e**" and "Enantiomeric excess (e.e.)" sections). The trifluoroacetic derivatives were obtained and their enantioseparation was favored due to the protection of the alcohols, which leads to a reduced polarity and, therefore, to a greater retention. The chromatograms of the trifluoroacetic derivatives are presented in Figure S1 (SI section), using a Cyclodex β-Chiral column, confirming they were present as a racemic mixture. The values for α_Q were between 1.01 and 1.10. Oromí-Farrús *et al.*[18] investigated the separation of these derivatized alcohols using a γ-CD phase at 35 °C and found α_Q similar or lower to those obtained in β-CD phases.

Combinatorial synthesis of racemic acetates by batch chemical catalysis

From the combinatorial synthesis, a chemotheque of racemic esters (**2a-2e**) was prepared and ten esters (5 enantiomeric pairs) were obtained (Figure 2).

Under the chromatographic conditions set, a good separation was observed for the enantiomers. The conversions were high (87.8 to 90.0%, Table 3) although not complete, and peaks for the residual alcohols could be seen in the chromatogram (Figure S2, SI section).

Table 3. Racemic acetates (**2a-2e**) obtained in combinatorial synthesis catalyzed by sulfuric acid

Alcohol	Ester	c / %	α_Q
1a	 **2a**	90.0	1.04
1b	 **2b**	87.6	1.11
1c	 **2c**	85.7	1.04
1d	 **2d**	87.1	1.10
1e	 **2e**	87.3	1.10

c: conversion; α_Q: separation factor for the column Cyclodex HP β-Chiral.

Combinatorial enantiomeric synthesis of acetates by batch enzymatic catalysis (N435®)

A second chemotheque was built with the enantiomers from alcohols **1a-1e** and acetic acid (**2**), using the immobilized Novozyme N435® (Figure 2). The chromatogram of the acetate esters can be seen in Figure S3 (SI section).

The activity of the biocatalyst, the lipase Novozyme N435®, was determined to be higher than 265 U g^{-1} (SI, "Lipase activity' section). This result evidences a high activity for the intended reactions, and it is in agreement with literature data for reactions also carried out at pH 7.0, such as in the work of Wang *et al.*[19] who found an activity of 293.8 U g^{-1}.

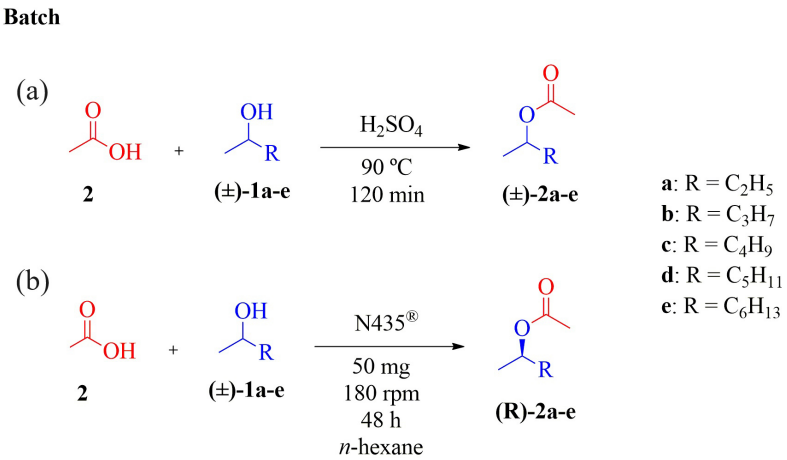
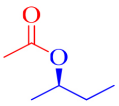
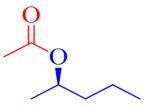
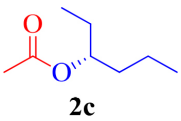
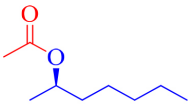
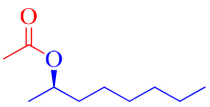


Figure 2. Illustrative scheme for the combinatorial synthesis of racemic acetates by chemical and enzymatic catalysis.

As expected, due to enzyme specificity, only *R* enantiomers were obtained.[20] The results are presented in Table 4. Differences were observed for the values of c (%), e.e. (%) and E (SI, "Conversion (c)", "Enantiomeric excess (e.e.)" and "Enantioseletivity (E)" sections) for the esters, regarding the substrates used. Enantiomeric excesses were higher than 99.0%, except for the compound **1a** (81.6%). This approach is a valuable path for the improvement of synthetic routes of commercial compounds as well as for the development of new substances.[21,22]

Stevenson *et al.*[23] used the lipase N435® for the esterification of phenylethanol, tirosol and methyltyrosol with cinnamic, coumaric (*o*, *m* and *p*), caffeic, dimethylcaffeic, synaptic and methyl *p*-coumaric acids, using 3 different combinations: 3 alcohols plus 8 acids, 3 alcohols plus the 4 more polar acids and 3 alcohols plus the 4 less polar acids, in each case to obtain 24 possible products. The authors observed, after 7 days of reaction, that all expected products were formed in high conversion, as all substrates were consumed, leading to a group of new esters which exhibit antimicrobial activity. The use of combinatorial synthesis and biocatalysis make the discovery of new bioactives faster and greener.

Table 4. Combinatorial batch synthesis of chiral acetates (**2a-2c**) catalyzed by lipase N435®

Ester	c / %	e.e. / %	E
 **2a**	48.8	*R* = 81.6	47
 **2b**	50.8	*R* = 99.0	198
 **2c**	49.8	*R* = 99.9	> 200
 **2d**	49.8	*R* = 99.9	> 200
 **2e**	49.7	*R* = > 99.9	> 200

c: conversion; e.e.: enantiomeric excess; E: enantioselectivity.

Combinatorial synthesis in continuous flow by chemical catalysis

The mixture of racemic acetates **2a-2e** was prepared from the corresponding alcohols **1a-1e** using acetic acid (**2**) and H_2SO_4 as the catalyst in continuous flow mode (Figure 1). The chromatogram can be seen in Figure S4 (SI section).

From the RCCD planning of the process variables, it was possible to identify the quadratic term flow ratio as the significant parameter ($p < 0.05$) with a variation (R^2) of 96.02% explained by the model (Figure S5, Table S1). The value of 24.14 for $F_{calculated}$ was significative, meaning that the model was well adjusted to the experimental data, leading to the response surface and contour graphics for the analysis of the results (Figures 3a and 3b).

From Figures 3a and 3b, it is also possible to note that with a flow of 0.5 mL min^{-1} and a temperature of 90 °C it was possible to obtain higher conversion values. A reduction in c (%) would be expected with an increase in the flow ratio to 1 mL min^{-1}, and this effect could be related

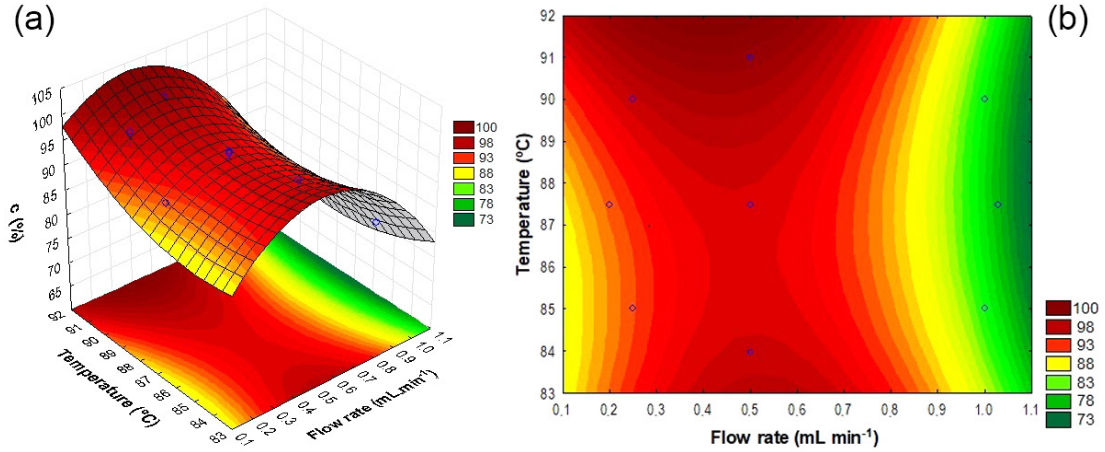
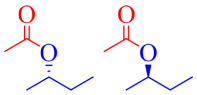
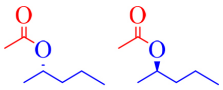
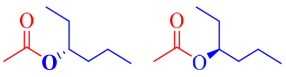
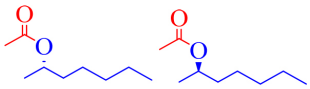
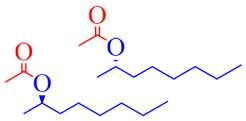


Figure 3. Graphics for the response curve (a) and contour curve (b) for the combinatorial synthesis of acetate esters by chemical catalysis in continuous flow.

to the necessary time for the reagents to circulate and have contact with the catalyst inside the reactor. Therefore, a reduction in the reaction time in the continuous flow system could be obtained, while keeping the high conversion values. The results obtained are present in Table 5.

Table 5. Combinatorial synthesis by chemical catalysis of acetate esters in continuous flow

Alcohol	Ester	c / %
1a	 **2a**	> 99.0
1b	 **2b**	98.5
1c	 **2c**	88.3
1d	 **2d**	93.7
1e	 **2e**	94.7

Residence time: 60 min; flow: 0.5 mL min^{-1}; temperature 90 °C. c: conversion.

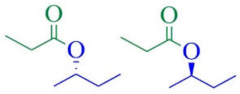
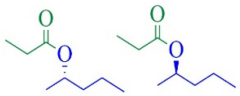
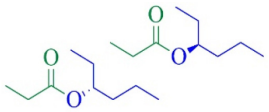
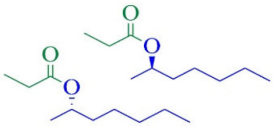
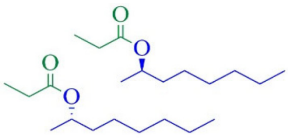
Conditions for optimal conversion were taken from the experimental planning (90 °C; flow of 0.5 mL min^{-1}) and applied to the synthesis of propanoates **3a-3e** (Figure 1, Table 6). The chromatogram can be seen in Figure S6 (SI section).

After a reaction period of 60 min, conversions obtained in flow chemistry varied from 88.3 to 99% for the acetate esters in comparison to 85.7% for the batch protocol in a longer time (120 min). For propanoate esters, conversions were also higher, up to 99% (Table 6).

Thus, the combinatorial synthesis combined with continuous flow favored the synthesis of volatile aroma esters at high conversions, reinforcing that the application of process intensification tools, such as continuous flow, increases the efficiency of chemical reactions due to greater product recovery, less formation of by-products and lower energy consumption.

Enantiomers can have different odors, which is paramount to the flavor and fragrance industry.[2,24] Acetates

Table 6. Combinatorial synthesis of propanoates in continuous flow

Alcohol	Ester	c / %
1a	 **3a**	> 99.0
1b	 **3b**	> 99.0
1c	 **3c**	> 99.0
1d	 **3d**	99.0
1e	 **3e**	99.0

Residence time: 60 min; flow: 0.5 mL min^{-1}; temperature: 90 °C. c: conversion.

are largely used as flavoring compounds. Both enantiomers of 2-heptyl acetate (**2d**), for example, are described to have a fruit character; the *S*-enantiomer is more fruity and fine, while the *R*-enantiomer is herbaceous and has a weaker aroma. For 3-hexyl acetate (**2c**), the *R*-enantiomer has sour, fruit cherry, plum and strawberry notes, while the *S*-enantiomer resembles sweaty, plum, and nectarine. For the enantiomers of **2a**, 2-butyl acetate, both have moldy character, plus herbaceous (*R*) and fruity (*S*).[25] For the 2-pentyl acetate (**2b**), the *R*-enantiomer is described as fruity, green and metallic; the *S* is reported as apple and plum-like.[26]

Brenna *et al.*[25] described the aroma of *R*-enantiomers to be more intense than the corresponding *S*-enantiomers, and that the difference in aroma strength is reduced as the number of carbon atoms increases. After synthesizing chemotheques of ethyl, propyl and isobutyl propanoates, Layton and Trinh[27] described the esters as fruity and having low solubility in water. However, most of them were described in the literature as racemates, and their sensorial descriptors were not fully elucidated. Frölich *et al.*[28] described the importance of chiral alcohols as 2-pentanol

and 2-heptanol for fruit aroma, such as banana, as detected using multidimensional gas chromatography.

As described here, combinatorial synthesis in continuous flow was successfully used to synthesize racemic mixtures of chiral acetates **2c-2e** and propanoates **3a-3e** using secondary alcohols as substrates with conversions from 88.3 to 99.0%. Besides the reduction in the reaction time, the method described here seems to be an alternative to the synthesis of libraries of chiral esters, as a tool in the formulation of aroma blends largely used in the flavor industry, as well as to be used as authentic standards for co-injection, calculation of retention indices, and in gas chromatography with olfactometry detection (GC-O), during the analytical studies for the identification of volatile compounds.

Combinatory synthesis in continuous flow by enzymatic catalysis

The mixture of (*R*)-acetates **2a-2e** was prepared from the corresponding alcohols **1a-1e** using the immobilized Novozyme N435® (Figure 4). The chromatogram can be seen in Figure S7 (SI section).

For the experimental planning, amidst the studied variables, the quadratic term flow ratio and the linear residence time were the parameters statistically significant ($p < 0.05$, Figure S8). Up to 87.86% of the variation (R^2) were explained by the model and the $F_{calculated}$ value was 7.24 (Figure S8, Table S2, SI section). The graphics of response surface and contour curve are presented in Figures 5a and 5b, respectively. From the data it can be inferred that with a slower flow (less than 0.1 mL min^{-1}) and lower temperature (35 ºC), higher conversions can be obtained.

Cvjetko *et al.*[29] used a microreactor at 25 °C with a catalytic fixed bed to the enzymatic synthesis of isoamyl acetate and reported that an increase in the flow, with the consequent reduction of the residence time, led to a reduction of the conversion (70 µL min^{-1}, 3.2 min, 29%, respectively). Reduction in the flow to 5 µL min^{-1} elevated the conversion to 92% (residence time: 45 min). The authors also verified that increasing the temperature to 55 °C, the same 92% conversion could be obtained in only 15 min.

The biocatalyzed esterification with lipase N435® in continuous flow afforded conversions from 40.2 to 49.8%,

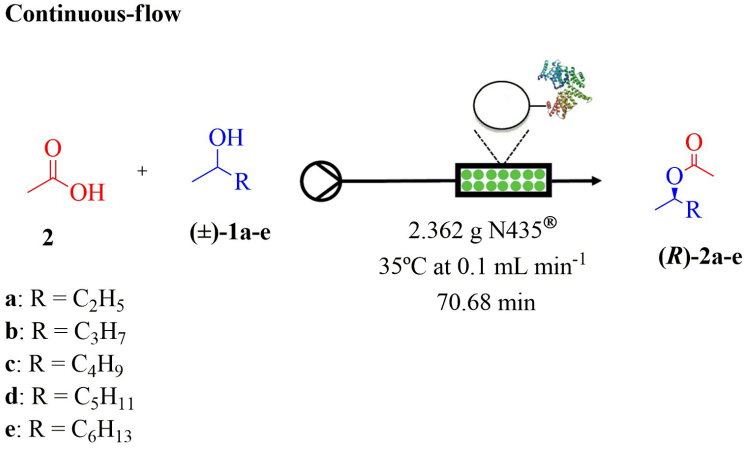


Figure 4. Illustrative scheme for the combinatorial synthesis of acetates by enzymatic catalysis in continuous flow.

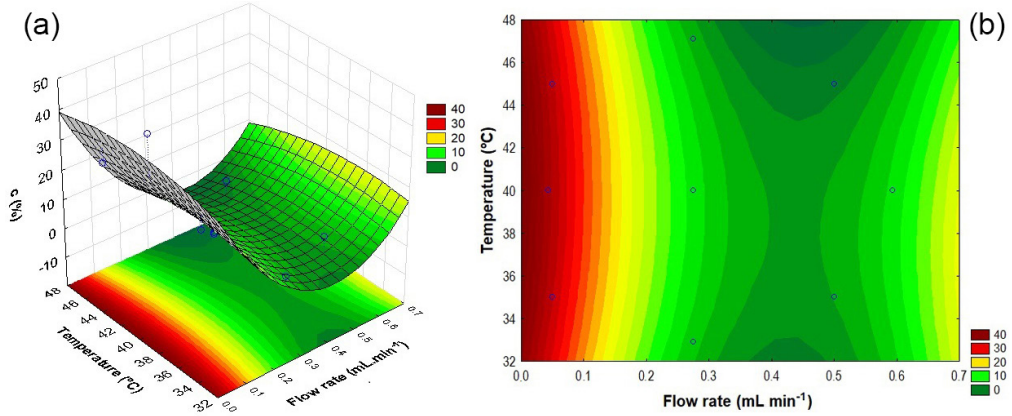
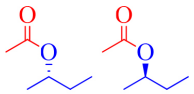
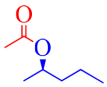
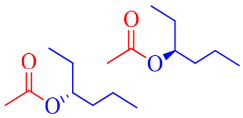
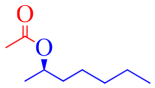
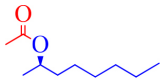


Figure 5. Graphics for the response surface (a) and contour curve (b) for the combinatorial synthesis of acetates by enzymatic catalysis in continuous flow.

with e.e. of the *R* ester ranging from 54.2 to 99.0%, as depicted in Table 7 (Figure S7, SI section).

Table 7. Combinatory synthesis of acetates in continuous flow with enzymatic catalysis

Alcohol	Ester	c / %	e.e. / %	E
1a	 **2a**	40.24	*R* = 54.2	< 10
1b	 **2b**	49.35	*R* = 99.0	> 200
1c	 **2c**	44.46	*R* = 94.6	34.5
1d	 **2d**	49.84	*R* = > 99.0	> 200
1e	 **2e**	49.34	*R* = > 99.0	> 200

c: conversion; ee_{ester}: ester e.e.; (*R*); E: enantioselectivity.

For all the alcohols tested (**1a-1e**), a flow of 0.1 mL min^{-1} was enough to moderate conversion and to obtain high enantiomeric excess, except for the 2-methylbutanol, with only a small e.e. (%). This behavior has been previously observed.[15]

Aguillón *et al.*[14] used a fixed bed reactor for the kinetic resolution of (±)-1,2-propanediol and obtained e.e. from 77.0 to 99.0%, with $E > 200$.[14] A reactor packed with an immobilized transferase obtained from *Mycobacterium smegmatis* was applied by Contente *et al.*[30] to achieve several aroma acetates, such as 2-phenetyl, cinnamyl, geranyl, hexyl and isoamyl acetates, with conversions above 78%.

Similar examples were described to phenolic esters by Annunziata *et al.*,[31] with conversions from 24.0 to 90.0% using a fixed bed reactor with lipase N435®.[30] A similar enzymatic continuous-flow strategy was used by our research group for the synthesis of (*R*)-propylene carbonate from glycerol, also showing excellent conversion and enantioselectivity.[32]

All these studies reinforce that the use of continuous flow systems in organic synthesis for aroma compounds is associated to high reproducibility, efficient control of reaction parameters, homogeneous and fast heating, and cost reduction. Regarding conversion, continuous flow systems are more efficient due to shorter diffusion paths withing the fluid flow and, therefore, to more efficient mass transfer, as previously pointed in the literature.[30]

The esterification of higher acids, namely hexanoic, octanoic and dodecanoic with methanol, ethanol and butanol, using a fixed bed reactor packed with lipase N435®, showed high conversions and enantioselectivities. For the methyl esters formed, conversion was a little lower, 91 to 92%. It was also observed that the enzyme did not catalyze the formation of ethyl dodecanoate in the same extension of ethyl hexanoate and ethyl octanoate (80, 92 and 95%, respectively).[33]

Layton *et al.*[27] produced a chemotheque of 16 esters, including acetates, propanoates, butanoates, pentanoates and hexanoates from yeasts and plasmids (ATF1 from *Saccharomyces cerevisiae*, SAAT from *Fragaria ananassa* and VAAT from *F. vesca*) with high conversions after 24 h of reaction.

Another factor to be considered in these reactions is water production, which leads to hydrolysis and, consequently, to competition with the esterification, resulting in reduced conversions. However, when continuous flow reactions are used, the water generated is continually removed from the reaction site and, therefore, the reaction equilibrium inside the reactor is controlled, without the need of additional procedures for water removal.

Conclusions

Herein, some versatile methods for the synthesis of several chiral esters, usually found in fruit aroma and largely used by the food, cosmetic and pharmaceutical industries, were presented.

Chemotheques were prepared with 20 esters (10 acetates and 10 propionates), synthesized as racemates in a continuous flow tubular reactor, under chemical catalysis. Using a fixed bed reactor packed with lipase N435® high conversions and enantioselectivities were observed, with preferential formation of the *R* isomers.

Regarding combinatorial synthesis by chemical catalysis, racemic mixtures were obtained in high conversions for both acetates (85.8 to 90%) and propanoates (> 99.0%). The enzymatic reactions, on the other hand, lead preferably to the *R* enantiomers after 48 h of reaction (e.e. from 63.2 to 99.8%), which was useful for the identification of the elution order of the enantiomers.

Supplementary Information

All spectra data are available as supplementary data to this article free of charge at http://jbcs.sbq.org.br as PDF file.

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Author Contributions

Claudia M. Rezende, Rodrigo O. M. A. Souza, Humberto R. Bizzo and Calionara W. B. Melo were responsible for conceptualization; Calionara Waleska B. Melo, Yasmin O. Santana, Rodrigo M. V. Silva and Marco Antônio M. Bezerra for investigation, analysis, and data interpretation; Claudia M. Rezende, Rodrigo O. M. A. Souza, Humberto R. Bizzo, Raquel A. C. Leão and Calionara W. B. Melo for writing original draft, writing-review and editing; Claudia M. Rezende and Rodrigo O. M. A. Souza for resources, funding acquisition and project administration. All the authors have participated in drafting and revising the manuscript.

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