Chemically Modified Lipase from *Thermomyces lanuginosus* with Enhanced Esterification and Transesterification Activities

Jennifer Noro,[a] Artur Cavaco-Paulo,*[a] and Carla Silva*[a]

Lipase from *Thermomyces lanuginosus* is one of the most explored enzymes for the esterification of several added-value industrial compounds, such as biodiesel, fragrances, and flavors. Its selectivity in these reactions is mostly related with its activity towards small alcohols. In this work, the impact of the chemical modification, with 4 dodecyl chains at its surface, was evaluated regarding its transesterification and esterification activities, comparing with the native form. Linear size-differentiated alcohols (from 1 to 20 carbons in the aliphatic chain) were used to explore for the first time the effect of the chain length in both transesterification and esterification reactions, using *p*-nitrophenyl palmitate and oleic acid as model compounds, respectively. The chemically modified lipase showed an outstanding improvement of its catalytic performance than the native enzyme, being this increase directly proportional to the size of the alcohols chain used as substrates. The enormous potential and remarkable versatility of this novel super catalyst was here demonstrated, where diverse types of esters, differing in their potential applications (biodiesel, cosmetics, fine chemistry), were efficiently synthesized. The produced esters were fully characterized by ^{1}H NMR, GC-MS, and FTIR.

Introduction

Lipase from *Thermomyces lanuginosus* (TL) was the first recombinant lipase produced,[1] and nowadays, it remains one of the most explored enzymes for the synthesis of compounds with industrial value. Besides their natural triacylglycerols hydrolysis function, it also demonstrates esterification and transesterification activity.[2] Ester compounds are present in many daily products, being the major components of flavors, fragrances, polymers, fats, among others.[3] The use of lipases for their synthesis is considered a green and environmentally friendly practice, regarding the high reactional yields and the mild reaction conditions associated.[4]

Among the different lipases, lipase TL is one of the most explored for transesterification and esterification reactions. Ashrafuzzaman and co-workers observed that immobilized lipase TL demonstrated the highest regioselectivity in the acylation of sucrose esters comparing to other lipases.[5] The transesterification reaction was carried out using divinyl esters. A similar approach was undertaken by Chávez-Flores *et al.*,[6] in the regioselective transesterification of vinyl laurate with a probiotic sugar, lactulose, using the same form of enzyme. Corrêa and co-workers[7] studied the esterification of the flavors, geraniol and citronellol, promoted by immobilized lipase TL. The reactions were carried out by coupling oleic, lauric, and stearic acid, being the produced esters isolated in good yields (>60%).[7]

The esterification of oleic acid with isoamyl alcohol was performed by Lage *et al.*.[8] The authors used lipase TL immobilized onto polymethacrylate particles as reaction catalyst. The ester, isoamyl oleate, which can be used as a biolubricant, was successfully synthesized with high conversion (85%).

Another major field of application of this lipase is in the production of biodiesel. The extensive investigation reported in this area stems from the world demand to find green and renewable sources of fuels.[9] Biodiesel can be manufactured through enzymatic catalysis, by the transesterification or esterification of oils/fats with small-length alcohols, such as methanol or ethanol. Countless sources of oils/fats can be used for this purpose. Sunflower, coconut, soybean, palm, and cotton seed oils or even wastes from the food industry, with varied composition, are some of the potential sources.[10]

As described previously, most reports regarding transesterification and esterification reactions describe the use of lipase TL in its immobilized form. The immobilization of enzymes presents several advantages, in comparison to other methodologies, including reusability, thermostability, and others.[11] However, immobilization techniques, besides being a more expensive strategy, it reduces the enzyme mobility and consequently, its catalytic performance.[12] Many reports form literature have been describing the chemical modification of lipases as an efficient methodology to overcome the limitations associated to the immobilization methodologies.[13] However, a lack of practical examples regarding its implementation on the synthesis of industrial added-value products can be found. We have previously reported that the PEGylation of lipase TL improved its polymerase activity comparing with the native

[a] Dr. J. Noro, Prof. A. Cavaco-Paulo, Dr. C. Silva
Department of Biological Engineering
University of Minho
Campus de Gualtar
4710-057, Braga (Portugal)
E-mail: artur@deb.uminho.pt
carla.silva@ceb.uminho.pt

enzyme. Higher degree of polymerization and conversion yield were obtained in the biosynthesis of a polyester, poly(ethylene glutarate).[14]

The chemical modification of lipase TL by grafting small hydrophobic aldehydes and isothiocyanates to the exposed lysine residues at the enzymes' surface, was recently reported by us. Besides higher thermostability, the modified enzyme revealed also improved activity for the hydrolysis of differentiated chain-length substrates.[13b]

Lipases displaying both esterification and transesterification selectivity for short and long alcohols are hard to find, especially when high reactional yields are required. The world demand in finding suitable, cost-effective, and eco-friendly catalysts that can be implemented in numerous fields at the same time, led us to investigate the effect of the chemical modification of lipase TL for this purpose.

In this work, the transesterification and esterification activity of the native and modified (grafted with four dodecyl chains) lipase TL were explored against differentiated chain-length alcohols (Figure 1). *p*-Nitrophenyl palmitate and oleic acid were used as model lipids for the transesterification and esterification reactions, respectively. A broad range of alcohols (from methanol to eicosanol, in a total of 11 alcohols) were used as substrates for the evaluation of the activity of both enzyme forms (native *vs* modified), thus varying the range of possible applications.

Results and Discussion

Chemical modification of lipase TL

Lipases are ubiquitous enzymes with outstanding catalytic properties. Besides their natural function for the hydrolysis of triacylglycerols into glycerol and fatty acids, they can perform the opposite reactions, the biosynthesis of esters. Esters are found in countless type of compounds, and are of great importance in the food industry, cosmetics, pharmaceutics, etc..[7,15]

We previously reported the modification of lipase TL with different aliphatic/aromatic aldehydes and isothiocyanates and tested their hydrolytic activity against 7 differentiated chain-length *p*-nitrophenyl substrates.[13b]

It was observed that the lipase modified with four dodecyl chains (Figure 2A II) showed an improved hydrolytic activity for all tested substrates (up to 2-fold), comparing to the native enzyme. The degree of modification was accessed through MALDI-TOF analysis (Figure 2B) and the TNBSA assay. Given the promising results obtained, herein we aim to explore the catalytic activity of this modified enzyme for the production of industrial added-value esters.

Prior to the evaluation of the transesterification and esterification activity of both enzymes, their half-life time at different temperatures was assessed (25, 37 and 50 °C) (Figure 2C). The temperatures were chosen according to the optimum temperature range of activity of the studied lipases and considering the typical array of temperatures reported for the evaluation of esterification reactions. Higher temperatures were not considered in these experiments, considering that in previous stability studies performed, we observed a drastic decrease of activity for both lipases near 70 °C.[13b] Moreover, lower reactional temperatures are beneficial for the establishment of a more economic process.

The data revealed that at 25 and 37 °C, both enzymes showed remarkable stability until 2 months of incubation. At 50 °C both enzymes displayed high stability, with half-life times near 330 h. In this way, both lipases showed to be suitable catalysts for longer reactional times.

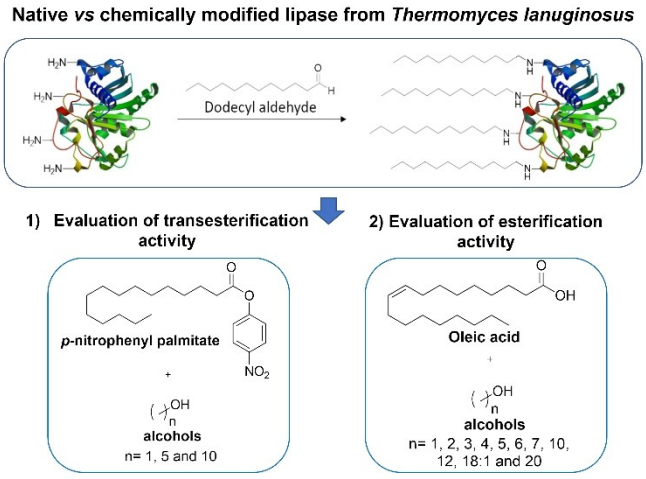


Figure 1. Overall scheme of the work: studying the impact of the chemical modification of lipase from *Thermomyces lanuginosus* on its transesterification and esterification activities.

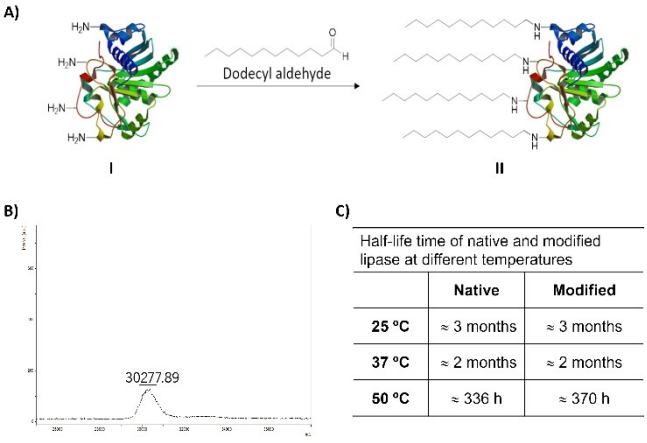


Figure 2. A) Representative scheme for the modification of (**I**) native lipase from *Thermomyces lanuginosus* with dodecyl aldehyde to produce (**II**) modified lipase TL with 4 dodecyl chains; **B**) MALDI-TOF of the modified lipase, confirming the grafting of 4 dodecyl chains (MW of native = 29620.3);[13b] **C**) Half-life time of both enzymes at different temperatures.

Transesterification activity

The transesterification activity of both native and modified lipase TL was assessed using the standard procedure of the literature.[16] The colorimetric assay allows to observe the activity by the release of *p*-nitrophenol after transesterification of *p*-nitrophenyl palmitate with an alcohol.

Typically, this assay is only performed using ethanol, as alcohol source and no data regarding its execution using differentiated alcohols was found. For this reason, three different alcohols were tested: methanol, pentanol and decanol, representatives of small, medium, and long aliphatic chains, respectively (Figure 3A). For the first time, the effect of the chain-length of linear alcohols on the transesterification activity of lipase TL through *p*-NPP was evaluated.

In Figure 3B is depicted the ester formation using native and modified lipase TL, regarding the transesterification of *p*-NPP with the mentioned alcohols. Given the known selectivity of lipase TL, the best results were achieved when the shorter alcohol tested, methanol, was applied. An activity decreasing tendency was observe as the chain length of the alcohols increased.

Despite this trend, the modified enzyme showed superior transesterification activity comparing with the native form, for all the alcohols tested. We may infer from Figure 3B, the increment of ester formation, proportional to the *p*-NPP transesterification.

Comparatively to the native form, the activity of the modified lipase for the transesterification with methanol increased 26%, whereas for pentanol increased 86% and for decanol an increment of 103% was achieved.

For an accurate comparison of the transesterification activity of both lipases, a constant value (*K*) was calculated (*K* = [ester product]/[starting material]) after reaction. This value is directly proportional to the amount of product formed. From Figure 3B it can be perceived that an higher *K* value was obtained for the modified lipase, corresponding to an higher amount of product formed.

GC-MS was performed to validate this assay after 7 h of reaction (Figure 3C). The chromatogram showed two major peaks, one corresponding to the unreacted *p*-NPP (23 min), and the other to the transesterified ester product (methyl ester at 11.2 min, pentyl ester at 15.9 min and decyl ester at 21.3 min.).

Figure 3C depicts the GC chromatograms of the products after reactions with both lipases (native in black, and modified in red), using methanol as alcohol substrate. The peak intensity differences obtained for native and modified lipase was rather evident. The peak intensity of the methyl ester was more pronounced when the modified lipase was used as transesterification catalyst, confirming an higher amount of ester efficiently synthesized. The same trend was observed for the other alcohols tested, pentanol and decanol (data not shown), as previously observed by the colorimetric assay (release of *p*-NP).

The impact of the chemical modification on the lipases' structure was previously carried out using molecular dynamics simulations. The data revealed an enlargement of the active site, ensuring an easier access of the substrates. Moreover, the hydrophobic character of the linker (dodecyl aldehyde) could decrease the need of interfacial activation.[13b]

Considering these previous findings, we may predict that the enlargement of the active pocket promoted by the chemical modification had also a positive effect towards the transesterification activity of the enzyme.

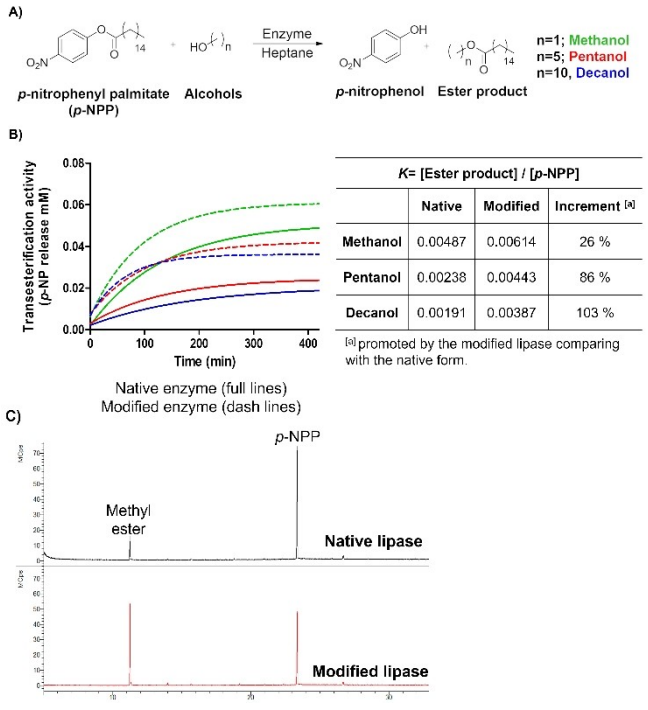


Figure 3. A) Reactional scheme for the transesterification reaction of *p*-nitrophenyl palmitate (*p*-NPP) with differentiated size-alcohols (methanol, pentanol and decanol), to produce *p*-nitrophenol and an aliphatic ester; **B**) Transesterification activity of native (full lines) and modified lipase (dash lines), using methanol (green), pentanol (red) and decanol (blue) as alcohol substrates. *K* values calculated after 7 h of reaction; **C**) GC-MS chromatograms of the products of the transesterification of *p*-NPP with methanol, catalysed by native and modified lipase (after 7 h of reaction).

Esterification activity

Effect of alcohol's chain-size

Oleic acid is a lipophilic compound well explored to test the esterification activity of many lipases, broadening their application for biodiesel production, when esterified with small alcohols,[17] or for lubricants or cosmetical purposes when esterified with longer alcohols.[18]

Besides the many applications reported for lipase TL, most of them describe its use in the immobilized form. Moreover, as far as we know, the effect of the alcohol chain-length on the lipase esterification activity was only recently explored using an immobilized form.[19] For this reason, this subject was herein extensively explored for the first time, using both forms of the enzyme (native and chemically modified), envisaging to open

up new routes for the development of new industrial products where the ester compounds play a crucial role. Furthermore, the lack of practical examples regarding the production of industrial products by chemically modified lipases inspired us to deepen the knowledge about this issue.

In this way, a total of 11 linear alcohols were tested, varying from 1 carbon (methanol) to 20 carbons (eicosanol) in the aliphatic chain, for the esterification of oleic acid (Figure 4A).

From the data obtained (Figure 4B), we may observe that both enzyme forms showed similar catalytic performances for the shorter chain alcohols, containing until 3 carbons (from methanol to propanol), being the esters isolated with excellent yields (≈80%). For the longer chain alcohols, with more than 3 carbons, a remarkable difference of the catalytic behavior was observed between native and modified enzyme. While reactional yields below 40% were obtained when catalysis was performed by the native form, the reactional yields obtained when using the modified form were superior for all the longer alcohols tested. The limited size of the native enzymes' active site may be associated to the activity loss when longer alcohols were used as substrates. Moreover, the alcohol, when in contact with the enzyme, may induce an inhibitory effect.[20] Nonetheless, reactional yields up to 56% were obtained when using modified lipase regardless the alcohol used. Apart from the easier access to the active site, resulting from the enlargement

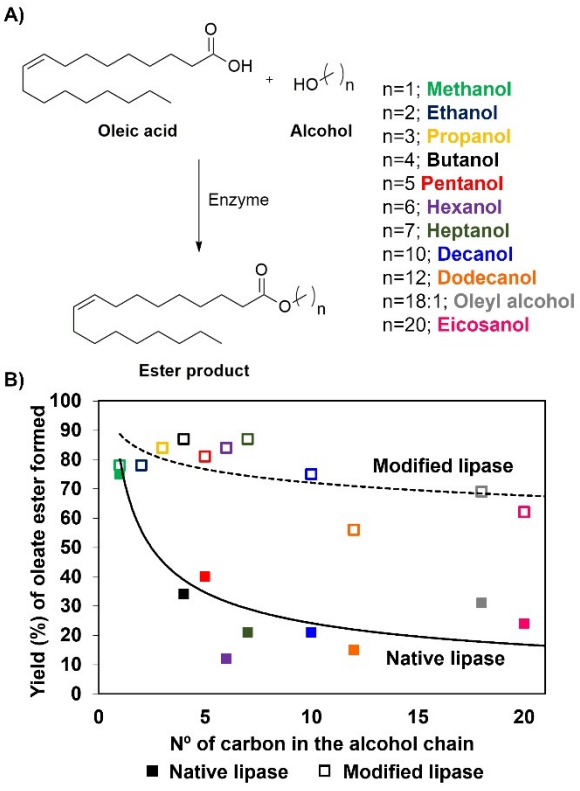


Figure 4. A) Reactional scheme for the esterification of oleic acid with differentiated chain-length alcohols; **B**) Reactional yield (%) of the esterification reaction of oleic acid with different alcohols catalysed by native and modified lipase TL (0.08% w/v). Results obtained after 24 h of reaction, with the reactions performed at 37 °C.

promoted by the grafting with dodecyl chains,[13b] the greater stability acquired against increasing chain-length alcohols may be the differentiating factor for the higher ester production yields achieved. Moreover, the highly hydrophobic linker (dodecyl) may strongly interact with the organic solvent (*n*-heptane) and the substrates used. This feature may improve the catalytic activity of modified lipase at the solid-liquid interface. Additionally, favorable interactions between the dodecyl chain, located at the lipases' lid, and the longer alcohols may occur, driving the substrate closely to the active site for further synthesis.

Kovalenko *et al.*[19] performed a similar study but using an immobilized form of the enzyme, and no comparison with the free enzyme form was reported. The results showed that the highest reactional rate was achieved when heptanoic acid (7C) and butanol (4C) were used as starting materials. In this work, a much longer acid was used as starting material (oleic acid C18:1), and therefore, due to constrains related with the active site access, it may be expected that only small alcohols, like methanol or ethanol, would be efficiently synthesized by the native enzyme.

The chemically modified form of this lipase, revealed to had higher catalytic activity against longer alcohols, demonstrating high potential for industrial applications.

We previously performed other chemical modifications of lipase TL.[13b] This enzyme was also grafted with naphthyl isothiocyanate, naphthaldehyde, butyraldehyde or hexyl aldehyde. However, the esterification activity after these modifications, did not display different values than observed for native enzyme form (data not shown).

Enzyme dosage *vs* temperature of reaction

The reaction temperature, alongside with the amount of catalyst are key factors to control in esterification reactions. The optimum hydrolytic temperature of lipases is 37 °C, being also commonly used for the esterification reactions.

Considering that, lower or higher temperatures may also be applied on these types of reactions. Herein the studies were also carried out at room temperature (25 °C) and at 50 °C, given the great stability of the lipases, previously observed (Figure 2C). Higher temperatures were not considered in this study, given the low stability of the enzymes previously reported by us.[13b]

In Figure 5A are depicted the *K* values obtained after esterification reaction with the lowest enzyme dosage tested (0.08% w/v) at different temperatures (25, 37 and 50 °C). To simplify, only the results of three alcohols, representing a small, medium, and longer alcohol (methanol, 1C, decanol, 10C, and eicosanol, 20C) are presented.

For the lowest temperature tested (25 °C), the native enzyme showed only great catalytic activity when methanol was used as substrate. For the longer alcohols (decanol and eicosanol), no esterification was observed (results similar to the control experiments). At this temperature, the modified lipase revealed considerably higher esterification conversion (higher *K*

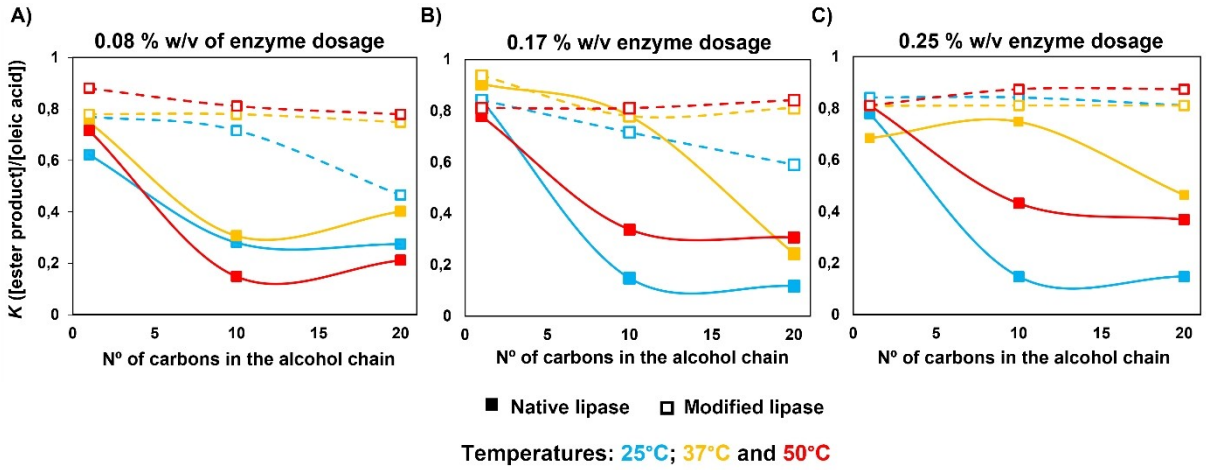


Figure 5. A) *K* values (*K* = [ester product]/[oleic acid]) regarding the effect of temperature (25, 37 and 50 °C), in the esterification of oleic acid with alcohols (methanol, decanol and eicosanol), using native *vs* modified lipase (0.08 % w/v) after 30 h of reaction; **B**) Same as A) but with the enzyme dosage 0.17 % w/v; **C**) Same as A) but with the enzyme dosage 0.25 % w/v.

values) than the native enzyme, regardless the substrate used. The esterification activity of the native form at 37 °C was superior than at 25 °C, especially for eicosanol. The reactional conditions using the optimal temperature of the enzyme may be responsible for this increase. The same trend was observed for the modified enzyme. Low reactional outcomes were observed at 50 °C for the native enzyme, using the longer alcohol substrates (decanol and eicosanol). This activity loss was probably due to some inactivation of the enzyme when exposed to high temperatures.

The modified lipase showed similar *K* values for the reactions carried out at 37 and 50 °C, confirming the thermostability acquired by chemical modification with the dodecyl chains.

Given the known selectivity of the native form for shorter alcohols, such as methanol, the esterification conversion of the modified lipase increased only 1.2-fold when this substrate was applied. The increment observed for the longer alcohols was remarkably higher, 5.5-fold for decanol and 3.7-fold for eicosanol.

The higher stability of the modified enzyme in an hydrophobic environment (*n*-heptane) may contribute for the enhancement of the esterification activity. Moreover, the hydrophobic grafted dodecyl chains around the lipase, in particular the chain located at the lid, may drive the substrates to the active site, favoring the esterification reaction.

Regarding the effect of the enzyme dosage, we may observe that, for both enzyme forms, the esterification activity was directly proportional to the amount of enzyme used (Figure 5B and C). It was also noteworthy that the performance of the modified lipase, for the lowest enzyme dosage (0.08 %) (Figure 5A), surpasses the native outcome for the highest enzyme concentration (0.25 %) (Figure 5C).

Products characterization

^{1}H NMR spectroscopy was accessed for the characterization of all the biosynthesized esters. The spectra of the esters showed a similar profile, where the differences relied on the presence of the peaks of the introduced alcohols. Considering this, Figure 6A depicts, as example, the ^{1}H NMR spectrum of propyl oleate, the ester produced in the reaction between oleic acid and propanol. Regarding the oleate moiety, the protons of the double bond (d) were generally observed at δH 5.3 ppm as a multiplet. The protons adjacent to the allenic bond (c) were detected at δH ≈ 2.0 ppm, in the same signal multiplicity. At δH 2.2 ppm was observed a triplet, attributed to the protons near the carbonyl bond (f). All other signals of the oleate unit were observed below 2 ppm.

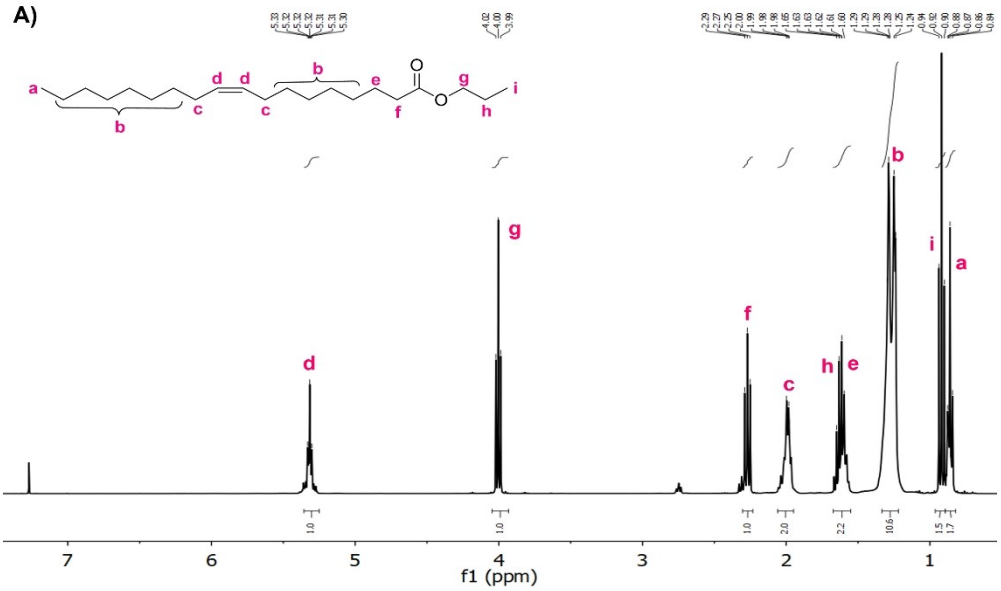
A triplet at δ_H 4.0 ppm was detected in all compounds, which corresponds to the protons located near the oxygen atom (g). This signal confirms the occurrence of the esterification since it was not present in the spectrum of oleic acid. The remaining protons belonging to the alcohol moiety were also observed below 2.0 ppm, which in most cases, appeared overlapped by the oleate signals. Overall, most of the esters herein synthesized are commercially available and the ^{1}H NMR obtained are in accordance with the results reported in databases/literature.[21]

Gas chromatography was also carried out to confirm the synthesis of the esters through their mass fragmentation. In Figure 6B are described the retention time of each ester, their *m/z* after analysis, and their theoretical molecular weight.

From the data obtained, we may assert that all expected esters were successfully obtained.

FTIR analysis was also conducted for the analysis of the ester products. All the biosynthesized compounds showed a similar profile.

In Figure 6C is depicted the spectra of oleic acid and propyl oleate. In the spectrum of the starting material, oleic acid, it was



B)

Biosynthesized ester	Retention time (min)	*m/z*	Theoretical molecular weight
Methyl oleate	13.6	296	296.5
Ethyl oleate	14.5	310	310.5
Propyl oleate	15.7	324	324.5
Butyl oleate	16.8	339	338.6
Pentyl oleate	18.0	352	352.6
Hexyl oleate	19.0	364	366.6
Heptyl oleate	20.1	380	380.6
Decyl oleate	23.3	422	422.7
Dodecyl oleate	26.4	450	450.8
Oleyl oleate	38.3	532	532.9
Eicosyl oleate	30.3	549[a]	563.0

[a]Molecular weight with a methyl cleavage: 563-14=549.

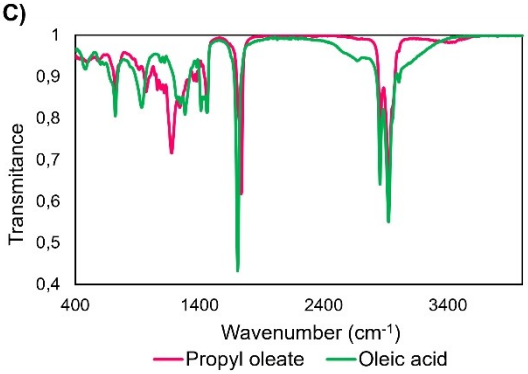


Figure 6. A) ^{1}H NMR spectrum of propyl oleate ($CDCl_3$), synthetized by reaction between oleic acid and propanol catalysed by the modified lipase; **B**) Retention time, *m/z* obtained after GC-MS analysis and theoretical molecular weight of all biosynthesized esters; **C**) FTIR spectra of oleic acid (green) and of propyl oleate (pink) synthetized by the modified lipase.

possible to observe a broad stretching bond between ν 2500 and 3200 cm^{-1} which corresponds to C–H stretching vibration and to the OH group of the carboxylic acid. The carbonyl group (C=O) was observed at 1706 cm^{-1} in the oleic acid, and at 1738 cm^{-1} in the ester. A strong stretching C–O band at 1177 cm^{-1} was observed in all biosynthesized product, which corresponds to the C–O band of the ester bond. From the data obtained, we may conclude that the FTIR analysis clearly indicate the formation of the respective ester compound.

Conclusion

The chemical modification of lipase TL by grafting four dodecyl chains revealed an outstanding improvement of the enzymes' stability and activity towards transesterification and esterification reactions. In both reactions, the increment of the ester synthesized was directly proportional to the size of the alcohol aliphatic chain. A large panoply of esters differing in the final application, from biodiesel to cosmeceutical, revealed to be efficiently biosynthesized using a single enzyme. These results underline the potentiality of the chemical modification approach as a cheap, versatile, and efficient technique for the synthesis of many value-added industrial products.

Experimental Section

Materials and methods

Lipase from *Thermomyces lanuginosus*, butanol, hexanol, oleyl alcohol, *p*-nitrophenol, molecular sieves 4 Å pellets (1.6 mm diameter), were purchased from Merck. *p*-nitrophenyl palmitate was obtained from Santa Cruz Biotechnology. Oleic acid, methanol, ethanol, propanol, pentanol, heptanol, decanol, dodecanol and eicosanol were purchased from TCI Chemicals. *n*-Heptane was acquired from Fischer Chemicals (HPLC grade) and dried over molecular sieves prior to usage.

Chemical modification of lipase TL

The chemical modification of the lipase was performed using the procedure previously reported.[13b] The modified lipase TL was isolated with four grafted dodecyl chains. Native lipase TL was used after ultrafiltration for the removed of any additives. Both enzymes were used in their lyophilized form.

Half-life time of the enzymes ($T_{1/2}$)

The half-life time of the lipases (native and modified) was evaluated at different temperatures (25, 37 and 50 °C). Prior to the evaluation of the transesterification/esterification activity, the $T_{1/2}$ of the enzymes was accessed to ensure that no significant loss of activity occurred at the different temperatures tested. For this, the lipases (1 mg/mL), dissolved in phosphate buffer (pH 7.8, 50 mM), were placed in a water bath, under different temperatures (25, 37 and 50 °C). At different time intervals, the hydrolytic activity was measured against *p*-nitrophenyl hexanoate. The assay was executed as previously described.[13b] Afterwards, the $T_{1/2}$ was calculated as reported.[22] The measurements were performed in a Synergy Mx Multi-Mode Reader from BioTek (USA) in a 96 well plates. One unit of enzyme activity was defined as the amount of enzyme which catalyses the production of 1 μmol *p*-nitrophenol from the initial substrate per minute.

Transesterification activity

The transesterification activity of both enzymes (native and modified) was measured using different alcohols, to evaluate the effect of the chain length on the final activity of the enzymes. The reactions were performed following the procedure reported by Teng and Xu.[16] Briefly, the enzymes (10 mg), were added to a flask containing 10 mL of a 10 mM solution of *p*-nitrophenyl palmitate in dry *n*-heptane. Then, 60 μL of 1 M of the respective alcohol was added to the flask and placed in a water bath at 37 °C, under stirring (150 rpm). Aliquots of 30 μL were withdrawn at different time intervals and quenched with 1 mL of NaOH (0.1 M). Then, 200 μL were placed in a 96-well plate and the *p*-nitrophenol released was read at 400 nm. The enzymes activity was then calculated by plotting the *p*-NP released over time.

Esterification activity

In a flask containing the enzyme (native or modified, 0.08, 0.17 or 0.25 % w/v) was added the oleic acid (300 μL) and the respective alcohol (1 equivalent) in 3 mL of *n*-heptane. The suspension was placed at the desired temperature (25, 37 or 50 °C) under stirring. At different time intervals, 100 μL of the solution was taken, and the volume made up until 5 mL with a solution of ethanol/acetone 1:1. This solution was then titrated with NaOH 20 mM, using phenolphthalein 0.5 % w/v as indicator.

K values were then calculated for both reactions, regarding the following equation:

$$K = [\text{ester product}]/[\text{starting material}]$$

Products characterization

Nuclear Magnetic Resonance spectroscopy (NMR)

After both transesterification and esterification reactions, the *n*-heptane and the volatile alcohols were completely removed in the rotary evaporator (Heidolph, Germany). Chloroform was added, and the solution washed with 5 % $NaHCO_3$ solution (3×) followed by water (2×). The organic layer was dried over $MgSO_4$, filtered and the solvent removed in the rotary evaporator to afford the pure product as a white solid (palmitate esters) and colourless oil (oleate esters). ^{1}H NMR (400 MHz) was then performed dissolving the products in deuterated chloroform ($CDCl_3$), and the samples analysed in a Bruker Avance III.

Gas Chromatography – Mass Spectrometry (GC-MS)

GC was performed using a Bruker SCION 436 system with a split/splitless injector coupled to a mass spectrometer (MS). Injections were carried at 250 °C in the split mode 1:10 using a Rxi-5Sil MS (Restek) column (30 m × 0.25 mm, and 0.25 μm film thickness), with a column-head pressure of 7.3 psi using helium as carrier gas. The oven temperature started at 150 °C and was held for 3 min, and the temperature increased until 280 °C at a rate of 7 °C/min. A full scan mode (50–600 m/z) was applied for the identification of the target compound. The mass spectrometer (MS) was operated in electron ionization (EI) mode at 70 eV with total ion chromatogram detection mode for quantitative determination and *S/N* ratio of 5.

Fourier-transform infrared spectroscopy (FTIR)

Infrared spectra were recorded on a FTIR Platinum-ATR Bruker Alpha II. The samples were analysed over the range 400–4000 cm^{-1}, with a spectral resolution of 4 cm^{-1}. All spectra were an average of over 24 scans.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: lipase from *Thermomyces lanuginosus* · dodecyl chains · activity · transesterification · esterification

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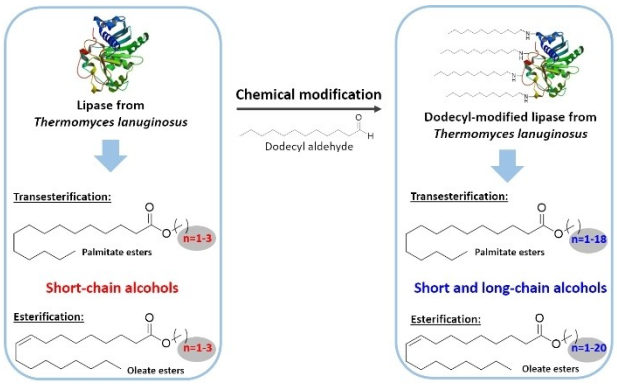

Dr. J. Noro, Prof. A. Cavaco-Paulo, Dr. C. Silva**

The chemical modification of lipase from *Thermomyces lanuginosus* using dodecyl aldehyde as linker, showed to beneficially improve its catalytic activity. The results reported in this paper, showed an outstanding increase of its activity towards esterification and transesterification reactions, comparing with the native form, being the most pronounced improvement obtained when using the longest alcohol chains for both type of reactions.