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Biocatalytic preparation of alkyl esters of citrus flavanone glucoside prunin in organic media

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ABSTRACT

The biocatalytic preparation of alkyl esters of prunin, a flavanone glucoside derivative of citric flavonoid naringin, was performed in organic media using two commercial immobilized lipases: Novozym 435 (*Candida antarctica* lipase B immobilized on an acrylic resin) and Lipozyme RM IM (*Rhizomucor miehei* lipase immobilized on an anion exchange resin). The influence of several reaction parameters on the performance and regioselectivity of the biocatalytic process was investigated using four solvents (acetone, acetonitrile, tetrahydrofurane and t-butanol) and vinyl laurate as a donor. The biocatalytic modification of prunin was strongly dependant on the solvent, the molar ratio of the substrates, the enzyme and the chain length of the donor. The acylation was highly regioselective, obtaining 6″-O-acyl monoesters except when the donor was vinyl acetate in which case a diester seemed to be formed. Novozym 435 proved to be a more efficient biocatalyst than Lipozyme RM IM. In acetone and acetonitrile, derivatives with a 100% conversion yield were synthesized after only 6 h of reaction at 50 °C. The modified derivatives preserved the antiradical activity of the flavanone glucoside and their solubility notably increased in the hydrophobic medium 1-octanol.

1. Introduction

There is a subclass of flavonoids in citrus fruits, called flavanone glycosides. Due to their properties and biological activity, these substances and some of their derivatives have important applications in the pharmaceutical and food industries [1–4].

The Argentine Northeast region is a citrus producer. In particular, the province of Salta is the main grapefruit producer of Argentina. Both in the peal and immature aborted fruits abundant levels of the flavanone glycoside naringin (4′,5,7-trihydroxiflavanone-7-β-D-α-L-rhamnosyl(1→2)-β-D-glucoside) can be found.

In the food industry, naringin can be used as a flavouring substance to provide bitterness in foods like marmalades and tonic water or grapefruit flavoured beverages. Besides these applications, other valuable derivatives of naringin can be obtained. In this laboratory a very efficient biocatalytic method was developed to obtain rhamnose from supersaturated solutions of naringin [5]. The enzymatic hydrolysis of naringin catalysed by α-rhamnosidases produces rhamnose and prunin (4′,5,7-trihydroxiflavanone-β-D-glucoside). The potential manufacture of rhamnose, a valuable substance in food industry, would leave prunin as a by-product. Prunin could have applications in the pharmaceutical and food industries on its own due to its biological activities [6–12]. However, its application could be limited by its low solubility in lipophilic media.

A viable alternative is the lipase-catalysed process in order to synthesize new lipophilic derivatives. By modifying the hydrophilic/lipophilic character through the acylation of prunin, new compounds could be obtained that may not only have different solubility properties but also probably possess new biological activities.

Various groups have reported the feasibility of the enzymatic acylation of flavonoid glycosides catalysed by lipases and proteases in organic media [13–15], ionic liquids [16,17] and in highly concentrated homogeneous solutions [18]. The enzymatic acylation of naringin in particular, has been studied by various authors [15–30]. However, to our knowledge, enzymatic synthesis of prunin esters has not been described as yet.

In this work, the enzymatic acylation of prunin was studied using alkyl vinyl esters with different chain lengths as donors and *Candida antarctica* lipase B (Novozym 435) and *Rhizomucor miehei* lipase (Lipozyme RM IM) as biocatalysts. Purification processes, antiradical activities and solubility measurements in water and 1-octanol of the obtained compounds are also described.

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2. Materials and methods

2.1. Materials

Molecular sieves (sodium aluminosilicate) with pore diameter 4 Å, DPPH and naringenin (4′,5,7-trihydroxiflavanone) were from SIGMA (USA). Vinyl acetate, vinyl butyrate and vinyl laurate were from Fluka (USA). Vinyl decanoate and vinyl stearate were from Aldrich (Germany). All other reagents were analytical grade. Naringin was obtained according to the method described by Geronazzo et al. [31] from immature aborted grapefruits. Prunin was obtained by enzymatic hydrolysis of naringin according to the method described by Soria and Ellenrieder [32]. Novozym 435 (*C. antarctica* lipase B immobilized on an acrylic resin) and Lipozyme RM IM (*R. miehei* lipase immobilized on an anion exchange resin) were a gift from Novozymes Latin America Limited (Brazil).

2.2. Synthetic reactions

Water was removed from the solvents and vinyl esters used in the synthesis by adding molecular sieves (30 mg ml^{-1}), shaking the resulting mixture for 24 h at room temperature and keeping them in the presence of the adsorbent.

60 μmol of prunin or naringin and 200 mg of molecular sieves were placed in a screw-capped vial with a Teflon seal. 3 ml of an appropriate mixture of vinyl ester and solvent were added and the resulting reaction medium was incubated with gentle shaking at the chosen temperature. The reaction was started by adding 50 mg of immobilized enzyme and run with shaking (200 rpm). At regular time intervals, 50 μl aliquots were extracted and appropriate dilutions of these samples were analysed by HPLC. All experiments were carried out in duplicate.

2.3. Analytical methods

Substrate and product concentrations were determined by HPLC using a RP-8 LiChrospher 100 Merk column (25 cm long, 4 mm internal diameter and 5 mm particle size). Samples (20 μl) containing prunin and prunin esters were eluted isocratically with the following mobile phases and flow-rates: (a) prunin acetates, 50/50 acetonitrile/water at 0.6 ml min^{-1}; (b) prunin butyrate, 65/35 acetonitrile/water at 0.7 ml min^{-1}; (c) prunin decanoate, prunin laurate and prunin stearate, 80/20 acetonitrile/water at 0.8 ml min^{-1}. Samples (20 μl) containing naringin and naringin laurate were eluted with acetonitrile/water 80/20. Elution profiles were monitored at 280 nm. Calibration curves for prunin, prunin esters, naringin and naringin laurate were obtained using purified samples. Conversion yields were expressed by the molar ratio of ester formed to flavanone glucoside (prunin or naringin) introduced at the beginning of the reaction. Initial rates were calculated from the slope of the linear portion of plots of the product concentration versus time. Samples were taking at 10, 20, 30, 40 and 60 min.

2.4. Lipase operational stability

Operational stability of Novozym 435 was tested by reusing the enzyme in the synthesis of prunin laurate in various solvents. In each cycle of the assay, 20 mM prunin, 200 mM vinyl laurate, 67 mg ml^{-1} molecular sieves and 17 mg ml^{-1} Novozym 435 were incubated 6 h at 50 °C. After this time, the enzyme was separated from the reaction medium, washed with the used solvent and added to a fresh medium preheated at 50 °C. For each cycle, the conversion rate of prunin after 6 h of reaction was measured.

2.5. Purification of prunin esters

Prunin acetate, prunin butyrate, prunin decanoate, prunin laurate and prunin stearate were obtained on a gram scale using Novozym 435 and acetone as the solvent. In a typical experiment, 190 ml of acetone, 5 g of molecular sieves, 1.75 g of prunin and the appropriate amount of vinyl ester to achieve a vinyl ester/prunin molar ratio of 10 were placed into screw-capped recipients. The temperature was set at 50 °C prior to starting the reaction with 1 g of Novozym 435. After achieving a near 100% yield, the immobilized enzyme and the molecular sieves were separated from the reaction medium by filtration and the acetone was recovered in a rotating evaporator at 40 °C. The residual mixture was then washed three times with hexane, centrifugating each time at 2000 rpm for 5 min. Prunin ester was obtained from the solid residue by redissolving it in DMSO and evaporating the solvent at 50 °C. Purity was checked by HPLC.

2.6. Prunin laurate structural identification

^{1}H NMR and 2D NMR experiments were measured on a Bruker Avance DMX-500 NMR spectrometer operating at 500.13 MHz (^{1}H) and 125.76 MHz (^{13}C) using standard Bruker software.

^{1}H (500.13 MHz, DMSO-d_6): δ 5.33 (1H, m, H-2), 3.10 (1H, m, H-3-ax), 2.75 (1H, m, H-3-eq), 6.17 (1H, sbr, H-6), 6,18 (1H, sbr, H-8), 7.27 (2H, d, 8.4 Hz, H-2′, H-6′), 6.83 (2H, d, 8.4 Hz, H-3′, H-5′), 4.88 (1H, dd, 5.0, 7.3 Hz, H-1″), 3.50 (1H, dd, 7.5, 8.0 Hz, H-2″), 3.46 (1H, dd, 8.0, 8.5 Hz, H-3″), 3.33 (1H, m, H-4″, superposable to solvent signal), 3.63 (1H, ddd, 2.0, 8.8, 9.2 Hz, H-5″), 4.18 (1H, m, H-6$_a$″), 4.42 (1H, dd, 2.0, 11.7 Hz, H-6$_b$″), 2.27 (2H, m, H$_\alpha$ to C=O), 1.52 (2H, m, H$_\beta$ to C=O), 1,21 (XH, m, CH$_2$), 0.85 (3H, t, terminal CH$_3$).

^{13}C (125.76 MHz): δ 80.0 (d, C-2), 43.9 (t, C-3), 197.7 (s, C-4), 163.4 (s, C-5), 98.8 (d, C-6), 165.4 (s, C-7), 96.9 (d, C-8), 163.0 (s, C-9), 103.9 (s, C-10), 129.1 (s, C-1′), 128.6 (d, C-2′, C-6′), 116.0 (d, C-3′, C-5′), 157.7 (s, C-4′), 100.2 (d, C-1″), 73.5 (d, C-2″), 76.7 (d, C-3″), 70.9 (d, C-4″), 75.1 (d, C-5″), 64.3 (t, C-6″), 35.0 (t, C$_\alpha$ to C=O), 27.7 (t, C$_\beta$ to C=O), 174.8 (C=O).

2.7. Radical scavenging activity

The antioxidant activities of prunin and prunin esters were determined by its ability to scavenge DPPH [33]. 50 μl of the solutions of the samples in DMSO at different concentrations were each added to 950 μl of DPPH 60 μM methanolic solutions. The blank sample consisted of 50 μl of DMSO added to 950 μl of 60 μM DPPH. The absorbance was measured at 518 nm as a function of the time taken for the reaction to reach the steady state. The tests were carried out in triplicate. The results of the assays are presented as percentages of the remaining DPPH, calculated as follows: % DPPH = [DPPH]ss/[DPPH]o) × 100, where ([DPPH]ss was the concentration of DPPH at steady state and [DPPH]o was the initial concentration.

2.8. Water and 1-octanol solubilities

In order to determine the solubility of prunin and prunin esters in water and 1-octanol, saturated solutions were prepared by equilibrating the compounds at 20 °C overnight. After centrifugation, the concentration of each compound in the supernatant was measured by HPLC.

2.9. Data analysis

Statistical analysis and graphics were carried out using GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego, California, USA, www.graphpad.com.

3. Results and discussion

The hydrolysis of naringin catalysed by α-rhamnosidase produces rhamnose and prunin (Fig. 1). Rhamnose is a very valuable sugar in the food industry and with the aim of exploiting this reaction to the maximum, an enzymatic method to produce lipophilic derivates of prunin was investigated in this work.

From the commercially available immobilized lipases we selected Novozym 435, *C. antarctica lipase B* (CALB) and Lipozyme RM IM, *R. miehei* lipase (RML). Among the numerous acyl donors used during transesterification, vinyl esters are the most efficient since the vinyl alcohol formed during the process tautomerizes to the low-boiling-point acetaldehyde, thus shifting the equilibrium towards ester formation [34]. Because of this, vinyl esters were chosen for this work. For studying the influence of different parameters, vinyl laurate was selected as a donor because of its intermediate chain length.

3.1. Effect of the solvent

The time course of the synthesis of prunin laurate catalysed by CALB and RML using different solvents at 50 °C, is shown in Fig. 2. Solvents of intermediate hydrophobicity were chosen as reaction media, such as acetonitrile (log *P* −0.33), acetone (log *P* −0.23), tetrahydrofuran (log *P* 0.49) and t-butanol (log *P* 0.89). In all cases, both prunin and the produced ester were completely solubilized. The reactions were performed in the presence of molecular sieves and, except the enzymes, all the reaction components were previously dried. Chromatograms of HPLC with detection at 280 nm showed only 2 peaks, prunin and that of the reaction product which always appeared in the same retention time, indicating that the same product was obtained for all solvents and both enzymes. The product was identified as prunin 6″-O-laurate (Fig. 1; Section 4).

A notable reverse correlation between the solvents hydrophobicity, measured by the value of log *P* and the reaction rates, was observed for both enzymes. This correlation has been found in other reactions also catalysed by lipases [35–37]. The best solvents were acetone and acetonitrile for both enzymes. Other

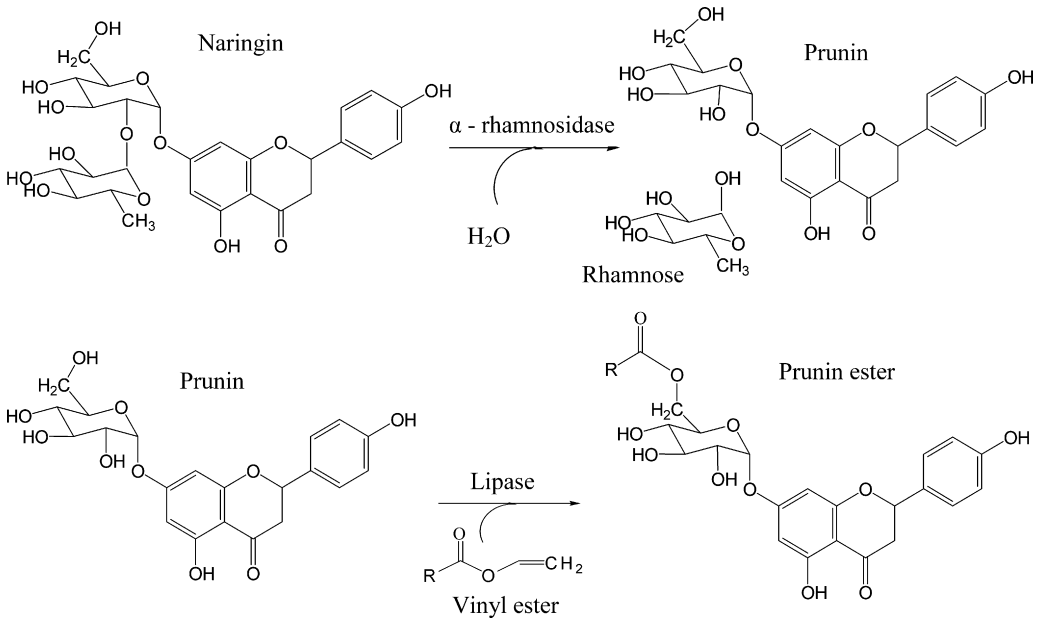

Fig. 1. Scheme of enzymatic hydrolysis of naringin catalysed by α-rhamnosidase and enzymatic synthesis of prunin 6″-O-acyl ester catalysed by lipase.

authors have also reported that acetone and acetonitrile are very good reaction solvents in flavonoid glycoside esters synthesis [21,23,26,28–29,36–38]. In both solvents, the total conversion of prunin in its ester was achieved in just 6 h, when CALB was used as the biocatalyst and with a conversion yield of 97,3% in acetone and 87,3% in acetonitrile in 24 h when using RML.

3.2. Effect of the enzyme

Both CALB and RML were capable of catalysing the prunin 6″-O-laurate synthesis with appreciable yields and reaction rates in the four solvents. However CALB proved to be more efficient than RML and all subsequent studies were performed using the former.

While most of the authors who studied similar reactions used CALB to synthesize flavonoid glycoside esters, only a few of them used RML [16,22], reporting that CALB was a more effective biocatalyst than RML, in agreement with observed results in this paper.

3.3. Effect of the vinyl laurate/prunin molar ratio

In order to find the minimum vinyl laurate/prunin molar ratio necessary to achieve higher conversions in short reaction times, the vinyl laurate/prunin molar ratio was tested from 1 to 40 at 50 °C using CALB as the biocatalyst. The conversion yields obtained for each solvent varying the molar ratio are shown in Fig. 3.

In acetone and acetonitrile, a molar ratio of 10 was enough to achieve yields of almost 100% in just 6 h of reaction and no substantial improvement was observed by increasing the molar ratio. However, in THF a molar ratio of 40 was necessary in order to reach such a yield and a longer reaction time was needed (24 h). In t-butanol, a yield of almost 80% was achieved in 24 h of reaction using a molar ratio of 40.

3.4. Structural identification of prunin laurate

The HMBC experiment for the only reaction product obtained from prunin and vinyl laurate in the four tested solvents showed

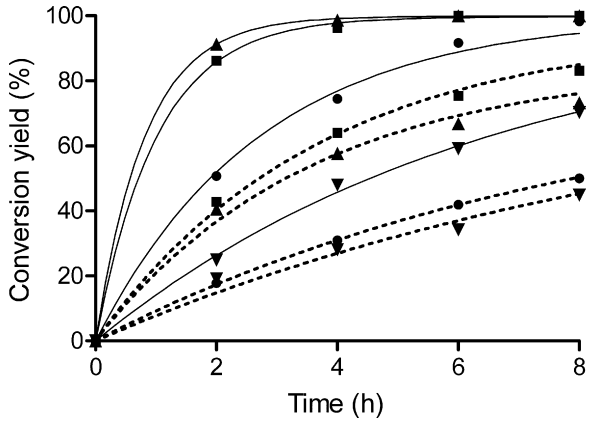

Fig. 2. Time course of prunin 6″-O-laurate synthesis catalysed by CALB (Novozym 435) (——) and RML (Lipozyme RM IM) (-----) in acetonitrile (▲),acetone (■), tetrahydrofurane (●) and t-butanol (▼). Conditions: 20 mM prunin, 400 mM vinyl laurate, 67 mg ml^{-1} molecular sieves and 17 mg ml^{-1} enzyme at 50 °C. Data are expressed as the mean of duplicate assays. All standard deviations were below 5%.

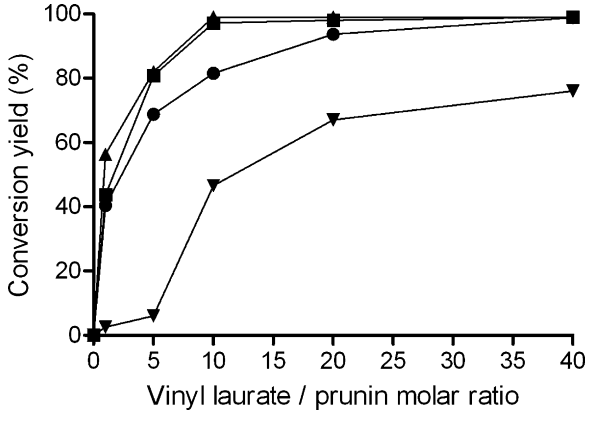

Fig. 3. Effect of vinyl laurate/prunin molar ratio on the conversion yields of prunin 6″-O-laurate synthesis catalysed by CALB (Novozym 435) after 6 h in acetonitrile (▲) and acetone (■), and after 24 h in tetrahydrofurane (●) and t-butanol (▼). Conditions: 20 mM prunin, 67 mg ml^{-1} molecular sieves and 17 mg ml^{-1} enzyme at 50 °C. Data are expressed as the mean of duplicate assays. All standard deviations were below 5%.

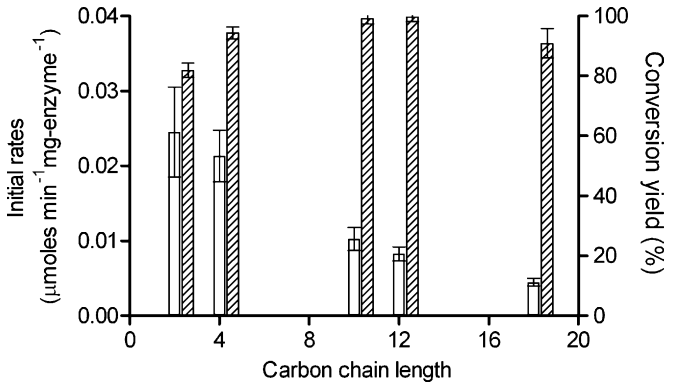

Fig. 4. Effect of acyl donor chain length on initial rates (white bars) and conversion yields at 24 h (patterned bars) of prunin esters synthesis catalysed by CALB (Novozym 435) in acetone. Conditions: 20 mM prunin, 100 mM vinyl ester, 67 mg ml^{-1} molecular sieves, 3.3, 6.7 and 17 mg ml^{-1} (white bars) and 17 mg ml^{-1} (patterned bars) enzyme at 50 °C. Data are expressed as the mean of duplicate assays ± standard deviation.

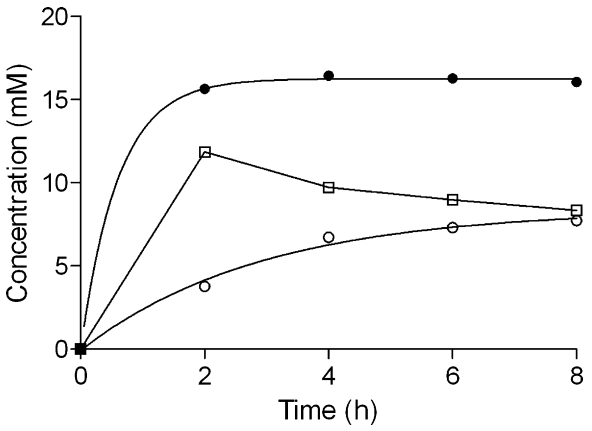

Fig. 5. Time course of prunin acetate synthesis catalysed by CALB (Novozym 435) in acetone. P1 (□), P2 (○) and P1+P2 (●). Conditions: 20 mM prunin, 100 mM vinyl acetate and 17 mg ml^{-1} enzyme at 50 °C. Data are expressed as the mean of duplicate assays. All standard deviations were below 3%.

correlations for carbonyl groups at δ_C 174.8 with H-6″a and H-6′b for both enzymes, clearly indicating that the lauryl was attached to C-6″. These data unequivocally lead to the proposal that esterification occurs at the 6″-hydroxyl group of the sugar moiety (Fig. 1). The acylation proceeded in a highly regioselective manner.

The selectivity of CALB for the C-6″ of glucose from naringin has been reported using a wide variety of donors [16,22,26]. The binding pocket of CALB is an elliptical step funnel of 9.5 × 4.5 Å wide and 12 Å deep. This narrow and deep substrate binding pocket has, compared to other lipases, very limited available space in the active site pocket and so can be expected to exhibit a high degree of selectivity [39–41]. The binding pocket of RML is a shallow bowl with a long axis of 18 Å and a width of 4.5 Å at its base and 6 Å at the protein surface [41]. Despite having a more spacious binding pocket, under these experimental conditions RML has the same regioselectivity that CALB.

3.5. Effect of acyl donor chain length

Prunin ester synthesis reactions were performed using linear acyl donors with different chain lengths (vinyl acetate, vinyl butyrate, vinyl decanoate, vinyl laurate and vinyl stearate) in acetone at 50 °C. Assays were performed with 3.3, 6.7 and 17 mg ml^{-1} of CALB enzyme. Given that when the chain length of the donor increases its hydrophobicity does the same, a low vinyl ester/prunin molar ratio was used in the assays to prevent excessive variations in the hydrophobicity of the media. Prunin and its esters were solubilized in all the tested reaction mixture. The relationship between initial rates and the amount of enzyme were linear. The initial rates per mg of enzyme are shown in Fig. 4. In addition, the conversion yields at 24 h with a quantity of enzyme fixed in 17 mg ml^{-1}, are reported. It is clear that the longer the chain length, the slower the initial reaction rate is. However, the yields achieved after 24 h exceeded 80% regardless of the chain length of the donor used.

The binding pocket of CALB consists of an elliptical and steep funnel whose walls predominantly constitute hydrophobic aliphatic amino acid residues. The active site is located at the bottom of the pocket, which is a more hydrophilic region and is constituted by the triad Ser 105 Asp 187 His 224. CALB has a large hydrophobic surface surrounding the entrance of the active site channel. The active site pocket can be partitioned into two sides, an acyl side and alcohol side, where the corresponding parts of the substrate will be located during catalysis. The proposed acyl side of the active site pocket is more spacious than the alcohol side [42]. Pleiss et al. [41] suggested that the lipases have a long, hydrophobic scissile acyl binding site located inside the binding pocket (at the wall of a binding funnel) and that the length and hydrophobicity of this binding site should correlate with the acyl chain length profile of the respective lipase. According to the binding site characteristics described by these authors, CALB has a high activity on short and medium acyl donors and a minor one when donors present an alkyl long chain. This would explain the effect of the chain length observed in this paper. However, the scissile acyl binding site is not the only determinant of chain length specificity. The lipase activity and specificity can be influenced by many factors such as water activity, solvent, substrates, immobilization support and donor/acceptor molar ratio. The effect of the acyl donor chain length in synthesis reactions of flavonoid glycoside esters has been investigated and discussed in several papers [13–14]. The results obtained by various authors showed some contradictions, which could be explained by the differences in the substrates and the operational conditions used.

Only one reaction product, which was expected to be prunin 6″-O-acyl ester, was observed in all cases by HPLC, except in the chromatograms of prunin acetate synthesis, where two products eluted with different retention times (P1 and P2). The kinetic profiles (Fig. 5) show that at the beginning of the reaction, the concentration corresponding to P1 increases faster than that of P2, followed by a decrease of the concentration of P1 accompanied by a rise of P2. It is thought that prunin 6″-O-acetate is formed first, and given that the acyl group introduced in the prunin molecule is small, the obtained derivative is still a substrate for the enzyme and another hydroxyl of sugar moiety is acetylated. Given the small size of the binding pocket, the esters with larger acyl groups are not substrates for the enzyme.

These results are consistent with studies of the acylation of isoquercitrin, a flavonol glucoside, in experimental conditions similar to this paper. Danieli et al. [37] studied the transesterification reaction of isoquercitrin using vinyl acetate as a donor and Novozym 435 as the biocatalyst in acetone at 45 °C and reported the formation of 3″,6″-O-diacetate. Chebil et al. [36], using a donor/acceptor molar ratio four times higher than that used by Danieli et al. [37], obtained 3″,6″-O-diacetate and 2″,3″,6″-O-triacetate and did not observe monoacetate. Other authors have reported the acylation of isoquercitrin with more bulky donors such as vinyl cinamate [21–22], vinyl ferulate, vinyl coumarate [22] and several aro-

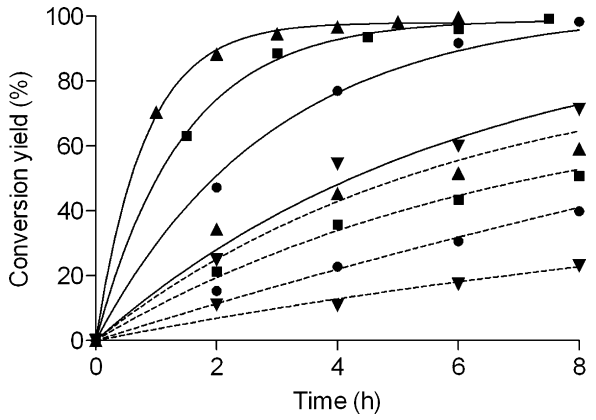

Fig. 6. Time course of prunin 6″-O-laurate (——) and naringin 6″-O-laurate (- - - - -) synthesis catalysed by CALB (Novozym 435) in acetonitrile (▲), acetone (■), tetrahydrofurane (●) and t-butanol (▼). Conditions: 20 mM prunin, 200 mM vinyl laurate, 67 mg ml^{-1} molecular sieves and 17 mg ml^{-1} enzyme at 50 °C. Data are expressed as the mean of duplicate assays. All standard deviations were below 3%.

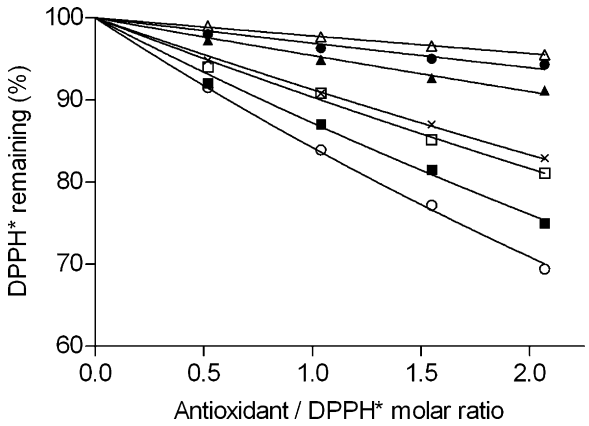

Fig. 7. DPPH remaining in the steady state versus compound/DPPH molar ratio for prunin (△), naringin (●), naringenin (▲), prunin 6″-O-butyrate (○), prunin 6″-O-decanoate (□), prunin 6″-O-laurate (■) and prunin 6″-O-stearate (×). Data are expressed as the mean of duplicate assays. All standard deviations were below 3%.

matic acid vinyl esters [38] obtaining the corresponding 6″-O-acyl monoesters. CALB acylates only position 6 and the more bulky derivatives are no longer substrates for a further acylation, probably due to steric hindrance.

3.6. Effect of glycoside flavanone

The acylation of flavanone diglycoside naringin, from which prunin is derived, has been reported by various authors [15–30] but it was carried out under different operational conditions. In order to compare the synthesis reactions of naringin 6″-O-laurate and prunin 6″-O-laurate, this study was performed under the same conditions using CALB. In Fig. 6 the transesterification reaction profiles of prunin and naringin with vinyl laurate in the four solvents are shown. It was observed that the formation rate of naringin 6″-O-laurate was much lower than that of prunin 6″-O-laurate. This can be explained by the larger size and decreased hydrophobicity of naringin, whose sugar moiety is the disaccharide neohesperidose whereas the sugar moiety of prunin is glucose. Gao et al. [22] also observed that the acylation rate of a flavonoid carrying a disaccharide moiety was noticeably reduced compared to those with a monosaccharide aglycon. However, they did not compare flavonoid glycoside derivatives of the same aglycon. Pedersen et al. [43] suggested that when the chain length of the donor or the size of the flavonoid is increased, the steric hindrance, that may occur on the binding pocket, increases too, reducing the probability of formation of the product.

3.7. Lipase operational stability

The operational stability of CALB was evaluated in the synthesis of prunin 6″-O-laurate at 50 °C in the four solvents employed with a vinyl laurate/prunin molar ratio of 10. After 12 runs, the conversion yields remained unchanged in all solvents.

3.8. Purification of prunin esters

The prunin 6″-O-butyrate, prunin 6″-O-decanoate, prunin 6″-O-laurate and prunin 6″-O-stearate esters were synthesized on gram scale in acetone using CALB as the biocatalyst. Considering that in all cases it was possible to achieve the total conversion of prunin in a few hours of reaction, it was possible to purify them using a simple method. The ester purification method consisted basically of evaporating the acetone first and then eliminating the remaining vinyl ester by extracting it with hexane. Once the hexane was evaporated the only remaining solid was the prunin ester. In the case the conversion yield would have been lower than 100%, various prunin separation processes were tested, looking for solvents capable of dissolving or the prunin ester or the prunin, but not both. For example, in the case of prunin and prunin laurate, both ethyl acetate and diethyl ether were capable of performing the separation. The separation of prunin estearate and prunin was achieved using 1-octanol.

3.9. Radical scavenging activity

Even though various methods to measure antioxidant activities have been found [44], the DPPH method is widely used for quantifying the free radical scavenging activity. Experiments under the same conditions were undertaken in order to measure the radical scavenging activity, not only of the obtained esters, but also of prunin, the unmodified flavanone glucoside. Flavonoids have the ability to scavenge free radicals and many of their biological properties are associated with this ability. This activity of phenolic compounds depends on the structure, in particular the number and positions of the hydroxyl groups, and the nature of substitutions on the aromatic rings [45,46]. Because of this, a modification of the structure might drastically alter its biological properties. This study examined whether or not the introduction of the alkyl chains in the prunin molecule alters its antiradical activity.

In Fig. 7 the results of the remaining DPPH percentage versus compound/DPPH molar ratio are shown. It can be observed that diglycoside naringin and glucoside prunin possessed an identical capacity to capture free radicals, even though this is a little below that of the aglycon naringenin from which they are derived. The introduction of the hydrophobic chain in prunin not only did not alter its capacity to capture free radicals, but it seemed to be slightly increased. No correlation was observed in this experiment between the chain length and the free radical scavenging activity. This result suggests that maybe these compounds could be used as antioxidant.

3.10. Solubility

The solubility data of prunin and its esters in water and in 1-octanol are shown in Table 1. An increase of the solubility in 1-octanol and a decrease of the solubility in water were clearly observed when the chain length of the acyl group introduced in the prunin molecule was increased. The obtained 6″-O-acyl deriva-

Table 1
Water and 1-octanol solubilities of prunin and prunin 6″-O-acyl esters at 20 °C. nd: not detected. Data are expressed as the mean of duplicate assays. All standard deviations were below 5%.

	Solubility (mM)	
	Water	1-Octanol
Prunin	1.07	1.11
Prunin butyrate	0.64	26.0
Prunin decanoate	nd	66.0
Prunin laurate	nd	66.7
Prunin stearate	nd	116

tives were more lipophilic than the parent prunin, what makes them suitable for taking advantage of its antioxidant properties in hydrophobic matrices.

4. Conclusions

From the results of this study it can be concluded that hydrophobic derivatives of prunin can be easily obtained through lipase catalysed transesterification under mild reaction conditions. The best conditions involve the use of a solvent with low toxicity (acetone), a relatively low vinyl ester/prunin molar ratio (10), and an immobilized enzyme (CALB) which is very stable and can be reused many times. The total conversions of prunin are achieved in a few hours and the products can be easily purified. Prunin esters preserved the antiradical activity of the flavanone glucoside parent and their solubility notably increased in the hydrophobic medium 1-octanol, making them suitable for use in hydrophobic matrices in the food, cosmetic and pharmaceutical industries. Certainly, these possible applications are subordinated to toxicological studies.

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