Immobilized *Staphylococcus xylosus* lipase-catalysed synthesis of ricinoleic acid esters

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ABSTRACT

Staphylococcus xylosus lipase 2 (SXL2) was immobilized by physical adsorption onto $CaCO_3$. A high immobilization yield was obtained ($82 \pm 3.7\%$) corresponding to the loading of 3250 ± 150 IU/g of support. Thermal stability of the $CaCO_3$-immobilized lipase was remarkably enhanced compared with that of the free one. Therefore, the immobilized SXL2 was stable at $80\,°C$ after 60 min-incubation, while the free one was completely inactivated above $50\,°C$.

The immobilized lipase was used to catalyse the enzymatic synthesis of ricinoleic acid estolides using two systems (in presence or absence of organic solvent). The effects of various reactions parameters such as the amount of lipase, the temperature and the molecular sieves were investigated. A conversion yield of about $65 \pm 4\%$ was achieved in a solvent free system using 400 IU of immobilized SXL2 at $55\,°C$, in the presence of 1 g of molecular sieves. The structure of synthesised biopolymer was analyzed by LC–MS, FT-IR and ^{13}C NMR spectroscopy.

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1. Introduction

Estolide is a generic name for linear oligomeric polyesters of hydroxyl fatty acids. Normal estolides are formed when the carboxylic acid functionality of one fatty acid links to the site of unsaturation of another fatty acid to form oligomeric esters. The fatty acids of lesquerella and castor oil have hydroxyl function that provides a site for esterification to take place to produce estolides. Estolides have been reported to occur in nature [1] or to be synthesised at high pressure and at elevated temperature from castor oil or its hydroxylated ricinoleic acid ($18:1^9\text{-OH}^{12}$) [2]. The estolide made from ricinoleic acid and lesqueroleic acid is a useful substance with many applications in industry. For example, it is used as a viscosity controller for chocolate and an emulsifier in margarine, as cutting oil base in metal processing, as pigment dispersant in paint, ink, cosmetics [3] and as a biodegradable lubricants and functional fluids [4].

Many reasons exist for the synthesis of estolides from ricinoleic acid, a major one being that vegetable oil-based lubricants and derivatives have excellent lubricity and biodegradability properties for which they are being examined as a base stock for lubricants and functional fluids [5–7]. Two major problems are encountered with vegetable oils as functional fluids: low resistance to thermal oxidative stability [8] and poor low temperature performance [9,10]. However, with the addition of additive packages, these properties sometimes can be improved, but only at the expense of biodegradability, toxicity and cost.

The chemically synthesised estolides present problems of coloration and odour due to the high reaction temperatures needed. Such products are therefore unsuitable for the food industry. As an alternative, some researchers have investigated the enzymatic synthesis of ricinoleic acid estolides by lipase (E.C. 3.1.1.3) which acts in mild reaction conditions, for example, low temperatures and pressures and neutral pH [11–13].

The formation of estolides by lipase-catalysed reactions depends strongly on the position of the hydroxyl moiety and slightly on the chain length and concentration of the hydroxyl acid. Reactions involving estolide formation from ricinoleic acid or hydrolysis of estolides have been catalysed successfully by "non specific lipases", i.e., those that lack 1,3-positional selectivity (e.g., *Candida rugosa*, *Chromobacterium viscosum*, *Pseudomonas* sp. and *Geotrichum candidum*), but have not been catalysed by 1,3-specific lipases (e.g., *Rhizopus* sp., *Rhizomucor miehei*, porcine pancreatic

Table 1
Immobilization yield of SXL2 and other microbial lipases into carbonate of calcium.

Lipases	Optimal conditions	Immobilization yield (%)	Load (IU/g)	References
SXL2	55 °C, pH 8.5	82 ± 3.7	3250 ± 150	Present study
SSL	37 °C, pH 8.5	52 ± 4	1300 ± 100	[24]
SXL1	45 °C, pH 8.2	47 ± 3.8	1450 ± 75	[25]
SAL3	45 °C, pH 8.2	79 ± 2.6	1500 ± 50	[20]
ROL	55 °C, pH 9.5	95 ± 3.6	2570 ± 100	[23]
PSL	nd	57.8	nd	[26]
CRL	37 °C, pH 7	3.5	nd	[27]

nd, not determined.

lipases, . . .). This may be related to the inability of several 1,3-selective lipases to attack secondary alcohols [1,14]. A review on *Candida antarctica* lipase (CALA) illustrates the increasing attention being paid to this unique biocatalyst and the many applications which have been developed, especially in the synthesis of structured triacylglycerol [15]. Recently we have shown that, like *C. antarctica* A lipase, the staphylococcal lipases efficiently hydrolyse the sn-2 position of triolein [16]. Thus, it might be interesting to test the performance of these lipases in the synthesis of some molecules with high added values by direct esterification in solvent free system.

Here we present the ability of a non commercial immobilized *Staphylococcus xylosus* lipase 2 to synthesise ricinoleic acid estolides. The esterification was carried out in two systems: organic solvent and in solvent free-system. The influence of some variables (lipase amount, temperature and molecular sieves) was studied to optimize the experimental conditions of the estolides synthesis.

2. Experimental

2.1. Materials

Ricinoleic acid (80% of purity) was purchased from Fluka. The hexane, chloroform, acetone were purchased from Prolabo (Paris, France). The carbonate of calcium ($CaCO_3$) was obtained from Pharmacia (Uppsala, Sweden). Molecular sieve dehydrate with indicator for drying solvents 3 Å were purchased from Fluka (Buchs, Switzerland). The pH-stat was from Metrohm (Herisan, Switzerland). The shaker (certomat H/HK) was from B. Braun Biotech (Germany, Melsungen).

2.2. Lipases

S. xylosus (SXL2), *Staphylococcus aureus* lipase (SAL3) and *Rhizopus oryzae* lipase (ROL) have been isolated in our laboratory from Tunisian biotopes [17–19].

2.3. Production and immobilization of lipase

S. xylosus lipase 2 was produced as described by [17]. After 24 h of culture, cells were removed by centrifugation and the lipase, in supernatant, was precipitated by addition of ammonium sulphate up to 65% of saturation followed by centrifugation at 8000 rpm at 4 °C for 30 min. The pellet was dissolved in 20 mM sodium acetate buffer pH 5.4 containing 20 mM NaCl and 2 mM Benzamidine. Then, the solution was centrifuged at 8000 rpm for 10 min and the supernatant containing the lipase was used for immobilization. The crude lipase preparation was immobilized by simple adsorption onto $CaCO_3$ support. The immobilization yield was defined as the ratio of the adsorbed activity recovered at the end of the immobilization period divided by the soluble lipase activity initially added to 1 g of the support. The enzyme immobilization was made onto $CaCO_3$ as described by [20]. For the sake of comparison, SSL, SXL1, SAL3 and

ROL were produced and immobilized into $CaCO_3$ using the same protocol.

2.4. Lipase hydrolytic activity

The activities of the free and immobilized lipases were assayed by continuously measuring the free fatty acid released from mechanically stirred emulsion of olive oil, using 0.1 N NaOH with a pH-stat, adjusted to a constant end point value.

The olive oil emulsion was obtained by mixing (3 × 30 s in a Warring blender) 10 ml of olive oil in 90 ml of 10% GA.

Each kinetic assay was performed at optimal conditions of each lipase (Table 1) in a thermostated vessel in a final assay volume of 30 ml containing 10 ml of olive oil emulsion and 20 ml of 0.25 mM Tris–HCl buffer, 150 mM NaCl, and 2 mM $CaCl_2$. Activity was expressed as units per ml of enzymatic solution. One international unit (IU) of lipase activity was defined as the amount of lipase that catalyses the liberation of 1 μmol of fatty acid from olive oil per minute.

2.5. Thermal stability of free and immobilized lipase

The free and immobilized SXL2, SAL3 or ROL were incubated at various temperatures (40–80 °C) for 24 h of incubation time. The residual activity was then determined under the optimal conditions of each lipase using the standard assay method [21].

2.6. Esterification of ricinoleic acid

Two systems were used to optimize the conversion yield of ricinoleic acid to estolide esters. In the first system, the esterification was performed in the absence of any organic solvent (solvent-free system); however in the second system, the esterification was performed in the presence of organic solvent.

2.6.1. In solvent-free system

The esterification reactions were carried out in open flasks containing 3 g of ricinoleic acid using different amounts of immobilized lipases. The reaction mixture was incubated in an orbital shaker (Certomat H/HK, Germany, Melsungen) at various temperatures (45–55 °C) and 220 rpm.

2.6.2. In organic solvent system

The esterification reactions were performed in screw-capped flasks with a volume of 2 ml containing different concentrations of ricinoleic acid dissolved in various organic solvents. The reaction mixture was incubated in an orbital shaker (Certomat H/HK, Germany, Melsungen) at 55 °C in presence of 400 IU of immobilized lipase with shaking (220 rpm).

For each essay, a control without enzyme was run in parallel under the same conditions. Aliquots of the reaction mixture were periodically withdrawn. The immobilized enzyme was removed by centrifugation at 8000 rpm for 5 min.

The residual acid content was determined by titrating with 0.07 N sodium hydroxide using phenolphthalein solution as an indicator and 3 ml of ethanol. The conversion yield of ricinoleic acid ester was calculated on the basis of the amount of the consumed acid [22].

2.7. Reusability of the immobilized S. xylosus lipase

The esterification of ricinoleic acid was conducted under the optimal conditions of the solvent free system (3 g of ricinoleic acid, 1 g of molecular sieves, stirring 220 and reaction time of 10 h) using *S. xylosus* lipase immobilized onto $CaCO_3$ as catalyst. The iSXL2 was reused many times for consecutive cycles. At the end of each batch, the immobilized lipase was removed from the reaction medium, washed with *n*-hexane in order to remove any substrate or product retained in the support and dried at room temperature. Then, the immobilized lipase was used again for another reaction cycle using fresh substrates.

2.8. Reaction product analysis

2.8.1. HPLC and LC–MS analysis

The identification of ricinoleic acid and ricinoleic acid estolides was carried out by an analytical Shimadzu a C18 column LichroCart with 5 μm particle size, 250 mm × 4 mm (Merck) attached to an LC-10ATvp pump and a UV detector (SPD-10Avp). Detector resolution was set at 1 nm and the absorbance was measured at 205 nm. The ricinoleic acid and ricinoleic acid estolides were dissolved in absolute ethanol. An aliquot (50 μg) was injected into the column and samples were eluted with acetonitrile phosphoric acid (80:20, v/v) at 30 °C, at a flow rate of 1.5 ml/min.

The LC–MS experiments were carried out with an Agilent 1100 LC system consisting of degasser, binary pump, auto sampler and column heater. The column outlet was coupled to an Agilent MSD Ion Trap XCT mass spectrometer equipped with an ESI ion source. Data acquisition and mass spectrometric evaluation was carried out on a personal computer with Data Analysis software (Chemstations).

2.8.2. FT-IR and NMR spectra

In order to check the esterification process, we used FT-IR NEXUS spectrophotometer (Nicolet, Madison, WI, USA) showing the shift of the carbonyl of carboxylic acid group to the carbonyl of ester group. The sample was mixed with KBr. FTIR spectra were acquired after 32 scans between 4000 and 400 cm^{-1} with spectral resolution of 4 cm^{-1}.

The structure of the estolide formed after esterification was also confirmed by ^{13}C NMR and H NMR (Madison, USA). Samples were dissolved in CDCl3 containing trace amounts of tetramethylsilane which was used as an internal chemical shift reference to indicate, in parts per million (ppm), the difference of the resonance frequency.

2.8.3. Intrinsic viscosity

Intrinsec viscosities of ricinoleic acid before and after esterification were determined in triplicate with a viscometer (Brookfield, UK) at different shear rates. The viscosity is expressed in mPa s.

3. Results and discussion

3.1. Immobilization and thermostability of free and immobilized lipase

Immobilization of enzymes plays an important role within applied biotechnology. The main reason for immobilizing enzymes is the ability to isolate the biocatalyst from the reaction
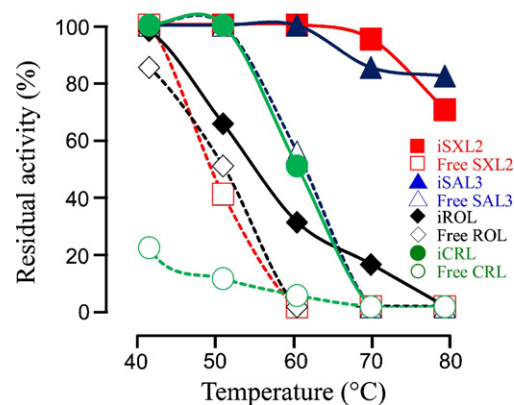

Fig. 1. Thermal stability of free and immobilized *Staphylococcus xylosus*, *Staphylococcus aureus*, *Rhizopus oryzae* and *Candida rugosa* lipases. Data corresponding to the thermal stability of *Candida rugosa* lipase are derived from De oliveira et al. [29].

product to increase its stability and to reuse it in order to increase its productivity. Adsorption is still the most commonly used approach because of its easy use and being the least expensive.

To determine the best conditions immobilization, the influence of the lipase amount (in range of 1000–8500 IU) to be adsorbed onto 1 g of $CaCO_3$ used as support was tested and compared to other microbial lipases [23–27] (Table 1). Our results showed that, the maximum immobilization yield was obtained with SXL2 with a maximum enzyme loading value of 3250 IU/g of support. This value is higher than those obtained with other staphylococcal lipases (SSL, SXL1, SAL3) with a load that not exceed 1500 IU/g of support. At low enzyme loading, the lipase molecules seemed to the contact with the surface, which may result in a change of conformation and consequently in reduced activities. However, for a loading amount higher than 3250 IU, multilayer adsorption might have occurred and effectively inhibited access to the enzyme active sites. These results are in agreement with previous studies [23], which showed that immobilized *R. oryzae* lipase displays low efficiency when adsorbed onto $CaCO_3$ at lipase loading higher than 2570 IU/g support.

To investigate the effect of immobilization on the thermostability of lipase, free and immobilized SXL2 were incubated separately at various temperatures during 60 min and the residual activity was determined and compared to those of the other microbial lipases (Fig. 1). One can note that after immobilization, the stability of all lipases tested was highly improved. Furthermore, the immobilized SXL2 displayed a residual activity of 95% or 70% after 60 min of incubation at 70 °C, 80 °C, respectively; in contrast to free lipase which is fully inactive beyond 55 °C. This is in line with De Oliveira [28], who reported that after 1 h of heat treatment at 60 °C, the immobilized *C. rugosa* lipase retained 50% of its initial activity, and that under the same conditions, the free one lost its full activity. The resistance of immobilized SXL2 to temperature is a potential advantage for practical applications of this enzyme.

3.2. Optimization of conversion yield of synthesis of estolide esters

3.2.1. Preliminary study

We studied the ability of $CaCO_3$-immobilized SXL2 to synthesise the estolide esters by esterification of the hydroxyl and carboxyl groupment of riconelic acid. The conversion yield obtained was compared to those obtained using other lipases (SAL3, ROL) produced in our laboratory and commercial Novozyme 435 (*Candida antarctica* lipase immobilized on macroporous acrylic resin). A preliminary study carried out with these above mentioned lipases
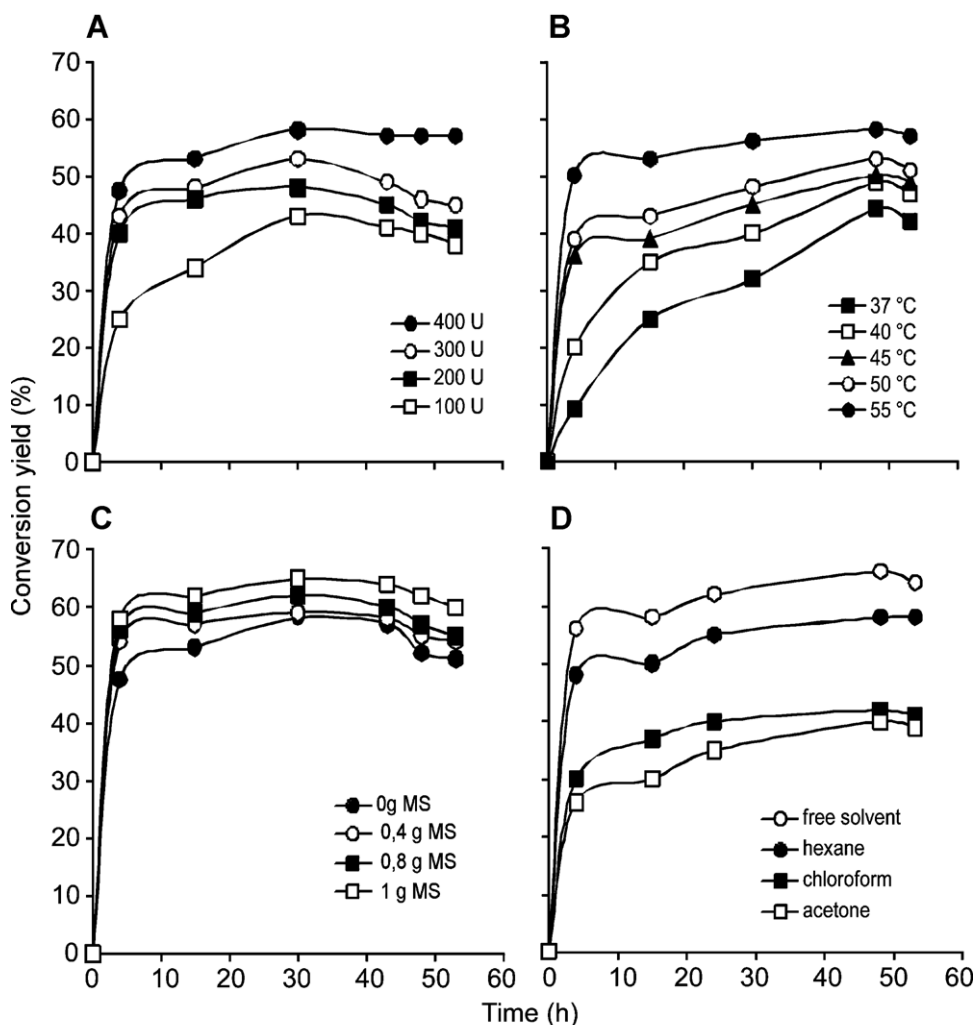
Fig. 2. (A) Influence of different amounts of *Staphylococcus xylosus* 2 lipase on the conversion yield (%). Reactions were carried out using 3 g ricinoleic acid at 55 °C and stirred at 220 rpm. (B) Influence of temperature on the conversion yield (%). Reaction were carried out using 400 IU of immobilized lipase, 3 g of ricinoleic acid and stirred at 220 rpm. (C) Influence of organic solvents on the conversion yield (%). Reactions were carried out using 400 IU of immobilized lipase incubated at 55 °C and stirred at 220 rpm. (D) Influence of molecular sieve dehydrate on the conversion yield (%). Reactions were carried out using 400 IU of immobilized lipase, 3 g of ricinoleic acid and stirred at 220 rpm.

showed that the high conversion yield was obtained with SXL2. Therefore, this lipase was chosen as biocatalyst in this study to catalyse the esterification of ricinoleic acid to produce estolide esters. The effect of other variables on the conversion yield was also checked and showed that only three variables had significant effects on the conversion yield. These variables were the reaction temperature, the amount of immobilized lipase and the molecular sieve dehydrate. The levels of the three other variables, having only a small effect on the esterification yield, were fixed as follows: reaction time: 8 h, stirring speed: 220 rpm; quantity of ricinoleic acid, 3 g. When the esterification reaction was carried out in organic solvent system, 2 ml of hexane are sufficient to riconelic acid solubilization.

3.2.2. Production of ricinoleic acid estolides in a solvent free system
3.2.2.1. Effect of amount of lipase.
In order to determine the optimal amount of lipase used during the esterification reaction, different quantities of immobilized *S. xylosus* lipase 2 corresponding to hydrolytic activity ranging from 100 to 500 IU as determined by pH-stat technique were used. Fig. 2A showed that the conversion increased with increasing the lipase amounts and reached the maximum with 400 IU of lipase. In the presence of high amount of lipase

(beyond 400 IU), a decrease in the conversion yield was observed which can be explained by the fact that in the presence of many molecules of lipases, the active site cannot be exposed to the substrates and the molecules can aggregate together.

3.2.2.2. Effect of temperature on the conversion yield.
To elucidate the impact of the temperature on the synthesis of ricinoleic acid estolides the reaction was carried out at 37, 40, 45, 50 and 55 °C (Fig. 2A). The highest conversion yield ($65 \pm 4\%$) was obtained at 55 °C. Since the synthesis of estolide esters was carried out in open flasks, the increase of the temperature was accompanied by the spontaneous evaporation of water present in the reaction medium. This finding was in line with the results obtained by Bódalo et al. [12] who have observed that the remove of the excess water content during the reaction course by spontaneous evaporation had a positive effect on the synthesis estolides of ricinoleic acid by commercial lipase from *C. rugosa*.

3.2.2.3. Effect of molecular sieve dehydrate.
In the esterification reactions, water constitutes an important factor for two reasons. First, a small amount of water coats the enzyme and probably reduces its contact with substrates which are known as an inhibitor of the synthesis activity. Second, it has an effect on the
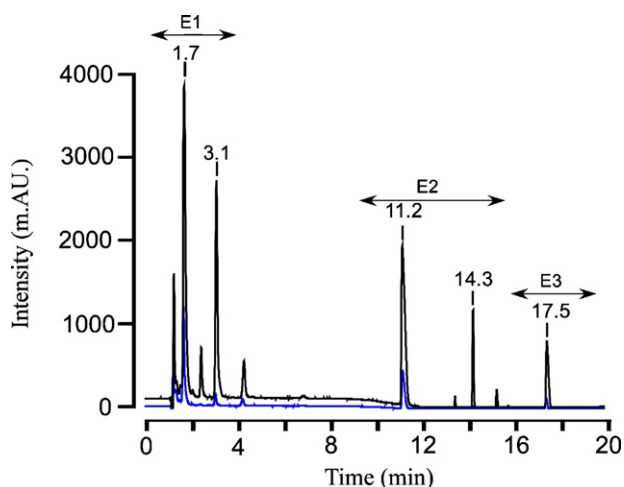
Fig. 3. LC–MS chromatogram of the ricinoleic acid before (black) and after (blue) esterification. E1 correspond to the ricinoleic acid and linoleic acid; E2 corresponds to the dimmers of ricinoleic acid and E3 corresponds to the trimmers and tetramers of ricinoleic acid. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

thermodynamic balance of the reaction. Indeed, a large amount of water favours the hydrolysis reaction and inhibits the esterification reaction. In this study, the effect of the addition of the molecular sieve dehydrate on the esterification yield was investigated. The reaction was carried out with various amounts of added molecular sieve ranging from 0.2 to 1 g. As shown in Fig. 3C, the conversion yield was found to increase with the increase of amount molecular sieve dehydrate. Moreover, the maximum of conversion yield ($65 \pm 4\%$) was obtained in the presence of 1 g of molecular sieve dehydrate. Bódalo et al. [11] was used different dehydratation methods (spontaneous evaporation, a vaccum aspiration and hot air current); hot air was found to be the best way of removing excess water and to obtain estolides with a high degree of condensation.

3.3. Production of ricinoleic acid estolides in the presence of organic solvent

The production of ricinoleic acid estolides by immobilized *S. xylosus* lipase 2 was also studied in the presence of organic solvent (Fig. 2D). For further comparison, we reported in the same figure, the results obtained under the same conditions in a solvent-free system. As we can see from the data presented in Fig. 2D, the maximum conversion yield ($65 \pm 4\%$) was obtained in the absence of organic solvents. Lower conversion yield of 58%, 41% or 39%, were obtained when using hexane, chloroform and acetone, respectively. The esterification reaction in a solvent-free systems offer greater safety, reduction in solvent extraction costs, increased reactant concentrations and consequently volume productivity. The estolide ester obtained by such method is therefore suitable for the food industry.

3.4. Effect of the repeated use of immobilized S. xylosus lipase

The reuse of the enzyme constitutes the main advantage of the process of enzymatic synthesis. It is an important parameter for repeated applications in batch reactors or continuously. The reuse of the lipase for several reactions allows to the reduction of the reaction cost and makes an economically feasible process. However, the idea of enzyme reuse implicitly means that the stability of the immobilized lipase should be high enough to permit this reutilization.
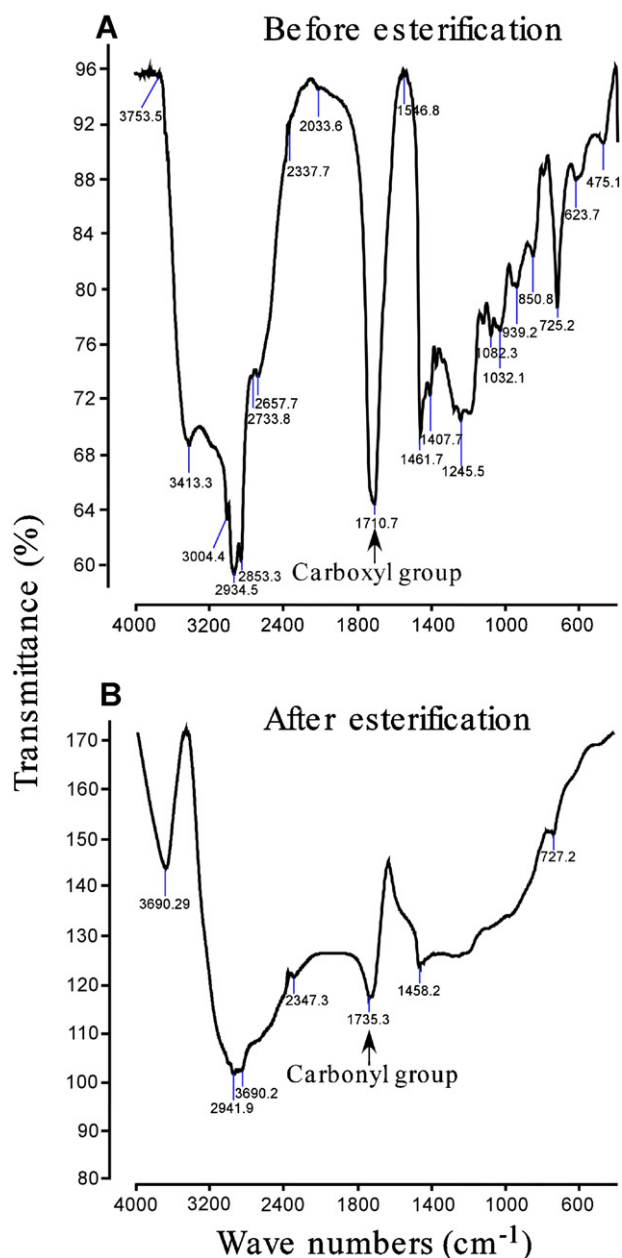

Fig. 4. FT-IR spectrum of ricinoleic acid before (A) and after (B) esterification. The arrow indicates the peak of the new product appeared at 1735 cm corresponding to the presence of the carbonyl group of the ester.

To check this parameter, $CaCO_3$-adsorbed SXL2 lipase was used in subsequent cycles in the esterification reaction of ricinoleic acid at the optimal reaction conditions as previously described in the Experimental section. At the end of the reaction, the immobilized lipase was filtered, washed with *n*-hexane in order to remove any residual substrate or product retained in the support. Then, the immobilized lipase was consecutively reused after each reaction cycle. No decrease in the yield of the ricinoleic acid ester synthesis was observed for 13 cycles of use of the immobilized lipase (data not shown). However, approximately 50% of the ricinoleic acid ester synthesis activity was retained after 15 cycles of use. This could be a result of the denaturation of the enzyme by *n*-hexane or ricinoleic acid after many cycles of use. This phenomenon was previously observed during the repeated use of *R. oryzae* lipase immobilized to $CaCO_3$ or silica aerogels as supports for the synthesis of ethyl valerate or butyl oleate [29,30].
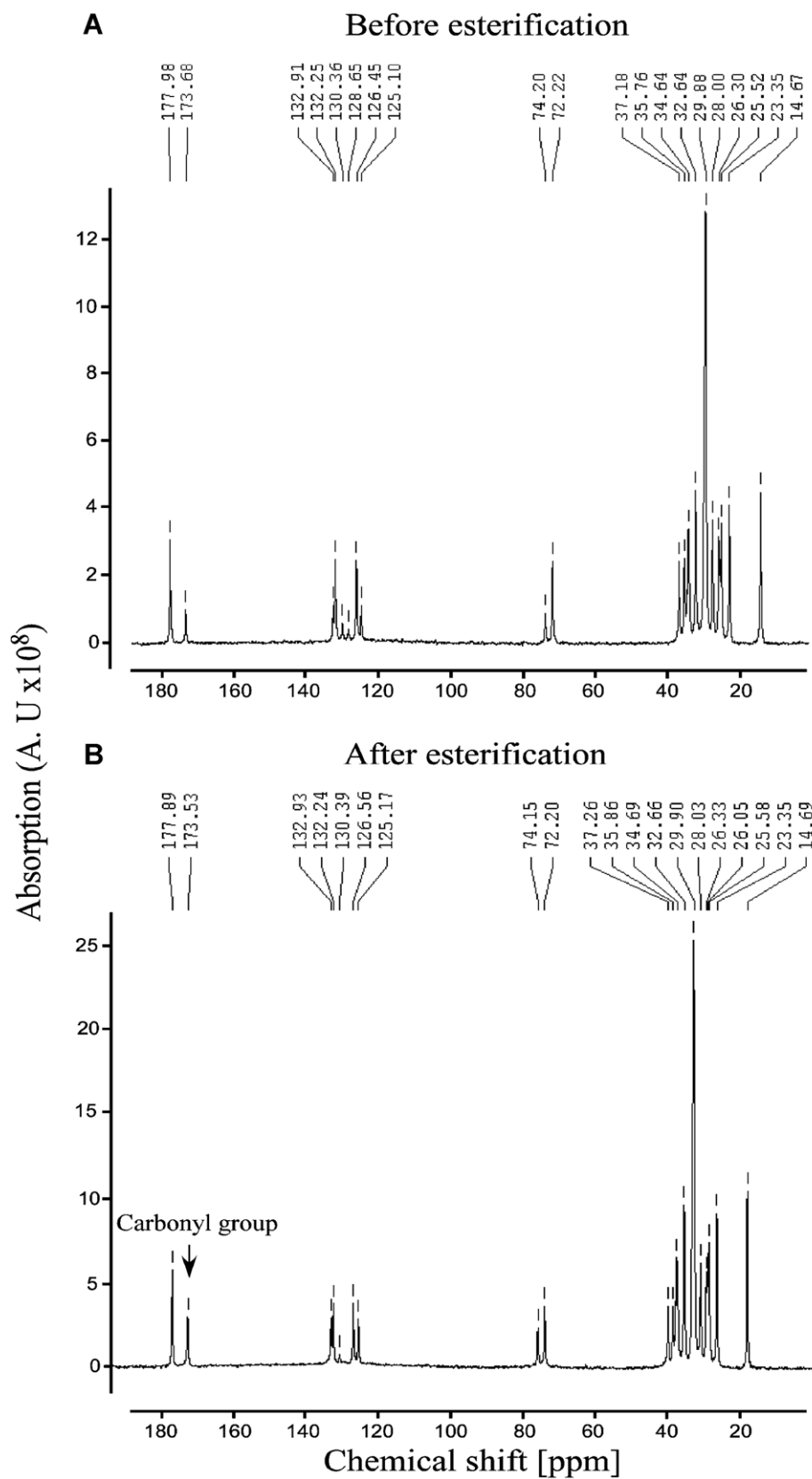
Fig. 5. ^{13}C NMR spectrum of ricinoleic acid before (A) and after (B) esterification. The apparition of the carbonyl group of the ester was marked by the arrow.

3.5. Characterization of estolides

3.5.1. HPLC and LC–MS analysis

HPLC analysis of ricinoleic before and after esterification (Fig. 3) showed different peak which corresponds to the different types of estolide esters formed. One can note from this figure the decrease of the intensity of the peak corresponding to the ricinoleic acid. This data is in agreement with the decrease of the acidity measured by titrating with sodium hydroxide and phenolphthalein solution as color indiacator. Peak assignments were based in the MS spectra considering the possible types of estolides that can be found in the optimal condition of the esterification reactions. The chromatogram presented in Fig. 3 showed three sections: The first section of the chromatogram (elution time between 1.7 and 3.1 min) corresponded to ricinoleic acid and linoleic acid with a molecular weight of $m/z = 297$ and $m/z = 279$, respectively. The second part of the chromatogram (elution time between 11.2 and 14.3 min) corresponds to the dimers of oleic acid (molecular weight of m/z 558.4) and ricinoleic acid (molecular weight of 577.6). In the third section of chromatogram, we have observed the presence of trimers and tetramers with a molecular weight of $m/z = 857.8$ and $m/z = 1134$, respectively. One can also note from Fig. 3 that the intensity of the different peak observed significantly decreased after esterification. This result is in favour of the formation of estolide esters with the higher molecular weight which cannot be detected by LC–MS and confirms the reduction of the acidity observed during the esterification reaction.

3.5.2. FT-IR and NMR

Fig. 4 presents the FT-IR spectra of ricinoleic acid before (Fig. 4A) and after (Fig. 4B) esterification. As shown in this figure, the reacted alcoholic groups, shown by a strong peak between 3000 and 3500 cm^{-1} in the spectrum of ricinoleic acid, decreased in intensity following esterification which is an indication that the major O–H groups of the initial ricinoleic acid are reacted. In comparison with spectra of the ricinoleic acid before esterification, the major change is the presence of the peak at 1735 cm^{-1} corresponding to carbonyl group of the ester formed after esterification of ricinoleic acid (Fig. 4B). The appearance of the band above 1735 cm^{-1} could be considered as an argument for the esterification of ricinoleic acid because the carbonyl groups from the ester are located in this wavelength region. In contrast, the intensity of the strong absorption band at 1710 cm^{-1} (Fig. 4A) due to the stretching of the carbonyl of the acid group was decreased after esterification.

The evidence of esterification of ricinoleic acid was also confirmed by the NMR spectra presented in Fig. 5. The comparison of ^{13}C NMR spectra of ricinoleic acid before (Fig. 5A) and after (Fig. 5B) esterification showed some key features of the formation of the estolide esters. With regard to ^{13}C NMR data, one can note that the intensity of the bands at 173.53 ppm and 177.89 ppm corresponding to the carbony group of the ester and the carboxylic acid, respectively, is different before and after esterification. The ratio between the intensity of the carbonyl group of the ricinoleic acid and the carbonyl group of the ester decreases from 2.1 before esterification to 1.4 after esterification. The formation of ricinoleic acid esters was also confirmed by ^{1}H NMR. One can note the appearance of a new bond at 4.87 ppm which is an indicative of an estolide linkage. All the evidence from the spectra of FT-IR and NMR prove that estolide ester was synthesised successfully.

3.5.3. Viscosity measurement

The introduction of an ester group to ricinoleic acid constitutes an important synthetic task, as it modifies its initial properties such as solubility and viscosity. After esterification, the ricinoleic acid exhibited a decrease in viscosity due to the polymerization of fatty acids, rendering the product highly hydrophobic. The viscosity
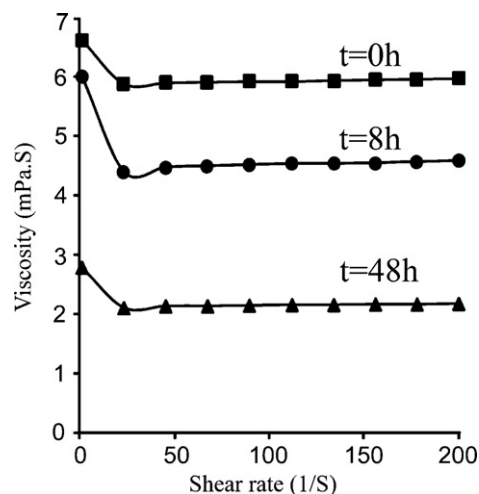

Fig. 6. Viscosity profile of ricinoleic acid before and after esterification.

profiles of ricinoleic acid before and after esterification are shown in Fig. 6. As shown in this figure, the ricinoleic acid as expected had an initial viscosity higher than the estolide esters, with an approximate range of 5.87 mPa s caused by hydrogen bonding of the carboxylate functionality. Capping of the hydroxyl moiety reduces or eliminates the hydrogen bonding and consequently reduces the viscosity which reaches its minimum value (2.1 mPa s) after 48 h of incubation time. This same general trend was also observed by Cermak et al. [31]. The viscosity range of the estolide esters formed after esterification could be useful for numerous applications: lubricants, coating agents, inks, cosmetics, surfactants.

4. Conclusion

This work has attempted to describe the enzymatic esterification of estolide esters with a non-commercial immobilized lipase from S. xylosus lipase 2. This may have an important impact on the cost of the ester production and encourage more use of natural compounds such as vegetable oil. The optimized condition for the synthesis of the estolides was determined to 400 IU of immobilized lipase, an 3 g of ricinoleic acid at 55 °C and 220 rpm in presence of 1 g of molecular sieves dehydrate. In such conditions, the reached conversion yield was about 65 ± 4% during 48 h of incubation time. The product obtained under these conditions could be useful for numerous applications: lubricants, coating agents, inks, cosmetics, surfactants.

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