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**RESEARCH ARTICLE**

# *Carica papaya* by-products as new biocatalysts for the synthesis of oleic acid esters

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**Abstract**

*Carica papaya* lipase is a versatile biocatalyst that is employed for many biotechnological purposes. Its lipase activity was first observed to be tightly linked to the insoluble fraction of latex. Nevertheless, recent studies have shown that this activity is also present in the fruit peel and seeds, suggesting that the lipase activity occurs in other parts of the plant. In the present work, the hydrolytic activity on trioctanoin was determined in various plant by-products, including latex, leafs, petioles, meristems, fruits, and the stem. The most hydrolytic activity was found in the latex (11 U/mL), followed by the petioles (1.7 U/mL). The hydrolytic selectivity was determined using triacetin, tripropionin, tributyrin, and trioctanoin. The enzymes present in the latex showed a higher rate of hydrolysis of tributyrin, while those present in the petioles had a preference for tripropionin, possibly indicating the occurrence of at least two different triacylglycerol hydrolases. Five self-immobilized biocatalysts were obtained: lyophilized latex (LL), lyophilized petioles (LP), bagasse from petioles (BP), and, after a simple cold water washing treatment, treated lyophilized latex (TLL), and treated lyophilized petioles (TLP). This procedure yielded a 5- and 10-fold increase in the latex and petiole activity, respectively, on tributyrin. The selected biocatalysts, TLL and BP, were tested for the synthesis of oleic acid esters (OAE), reaching conversions over 80%. Unexpectedly, only BP preferentially synthesized dodecyl oleate (DO) and showed the highest thermostability. Therefore, BP was further assayed for DO synthesis in a packed bed reactor (PBR), achieving 96% conversion over 40 h. This study shows the great potential of *C. papaya* by-products, particularly BP, as biocatalysts for the synthesis of OAE.

**Keywords:** *Biocatalyst*, *byproducts*, Carica papaya, *esters*, *lipase*, *petioles*

## Introduction

*Carica papaya* is a palm-like plant in the Caricaceae family that possesses a hollow, unbranched stem that sometimes reaches 20 m height (el Moussaoui et al. 2001). It is cultivated in tropical and sub-tropical regions around the world for its commercialized fruit. Worldwide, *C. papaya* production reached 12.4 million tons of fruit in 2012. It should be noted that the planting cycle lasts for only two years (FAO 2012). As a result of this production, approximately 120 million tons of by-products are generated each year, including leaves, meristem, petioles, stem, and unripe fruits, all of which have no commercial value. In particular, the unripe fruits are rich in latex-producing cells called *laticifers*. Latex is a white thixiotropic fluid, the main function of which is believed to be a defensive role against pathogens and insects (el Moussaoui et al. 2001). It contains a wide range of hydrolytic enzymes, including, but not limited to, cysteine proteases, chitinases, esterases,

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and lipases. Latex is composed of a water-miscible phase that contains water soluble enzymes (cysteine proteases and chitinases); while the hydrophobic enzymes, mainly esterases and lipases, are tightly bound to the solid polyisoprenoid matrix. To date, their separation, purification, and molecular characterization have been impossible (Giordani et al. 1991; el Moussaoui et al. 2001; Abdelkafi et al. 2009).

Esterases (EC 3.1.1.1) and lipases (EC 3.1.1.3) are carboxylic ester hydrolases that hydrolyze soluble short chain (<C4) and non-soluble long chain (>C8) triglycerides (TAGs), respectively (Lortie 1997). Both are known for their regio-, topo-, and enantioselectivity, as well as for the ability to accept a wide range of natural and synthetic substrates (Schmid and Verger 1998). Many esterases and lipases are highly active in the presence of organic solvents (Bornscheuer and Kazlauskas 2006). In the absence of water, they can perform esterification, transesterification, and other reactions. All of these treatments make esterases and lipases the most employed biocatalysts in organic chemistry, leading to the synthesis of a wide range of valuable products, such as esters (Schmid and Verger 1998). Short acyl chain esters (<C4) are used as additives in the food industry (Liu et al. 2004), while large acyl chain esters (>C8) are widely used in the cosmetic, pharmaceutical, and lubricant industries (Villa et al. 2003; von Scala et al. 2005). Immobilized esterases or lipases are ideal biocatalysts because it is possible to reuse them, and they can be used in packed bed reactors (PBRs), which considerably reduces the process cost (Malcata et al. 1992; Chen and Wu 2003). This type of biocatalyst can be obtained from *C. papaya*.

Latex from *C. papaya* has been widely studied. Its hydrolytic activity, which generates medium-long chain TAGs, such as tributyrin, were formerly attributed to one lipase, which is commonly referred to as *C. papaya* Lipase (CPL). Currently, it is known that there is more than one enzyme responsible for this activity on the polymeric network; there is at least one esterase and one lipase (Abdelkafi et al. 2009; Dhouib et al. 2011). The insoluble fraction of latex has been largely employed as a biocatalyst in the organic synthesis because its hydrolytic enzymes are considered to be naturally immobilized (Giordani et al. 1991; Gandhi and Mukherjee 2000, 2001). Previous publications in which CPL was employed mainly focused on its strict *sn*-1,3 selectivity and enantioselectivity. Giordani and Gandhi used CPL for the synthesis of TAGs, which possess short acyl chains at the *sn*-1,3 positions and are considered to be hypocaloric (Giordani et al. 1991; Gandhi and Mukherjee 2001). Moreover, Chen successfully employed this biocatalyst for the resolution of a (*R*,*S*)-naproxen-2,2,2-trifluoroethyl ester (Chen and Tsai 2005). There are only a few reports available regarding the use of the by-products of *C. papaya*. Paques et al. carried out a partial purification and characterization of the lipase activity found in the seeds and fruit peels (Paques et al. 2008). In previous work performed in our laboratory, Galindo demonstrated that the proteolytic activity is found in different proportions in *C. papaya* by-products (e.g., leaves, petiole, and stem) (Galindo-Estrella et al. 2009). Thus, the esterase/lipase activity might also differ in various by-products, and therefore, the by-products can be used as non-toxic and cheap biocatalysts. Furthermore, the high intrinsic hydrophobic character of *C. papaya* esterases and lipases suggests that these enzymes may be strongly attached to the matrices of the by-products, and thus, they can be used as naturally immobilized biocatalysts for the synthesis of several esters.

In this work, for the first time, it is shown that esterase/lipase activity is present in other *C. papaya* by-products (i.e., petioles). Moreover, it was easy to obtain different biocatalysts from latex and *C. papaya* by-products that are useful for the synthesis of oleic acid esters, in particular dodecyl oleate (DO). Finally, bagasse from petioles (BP), the most thermostable biocatalyst, was successfully used in a PBR for the synthesis of DO, demonstrating that BP is a very interesting and attractive by-product that should be considered for industrial processes involving the continuous synthesis of long chain esters.

## Materials and methods

### *Materials*

Triton X100, sodium hydroxide (NaOH), sodium chloride (NaCl), hydrochloric acid (HCl), oleic acid, triacetin (TC2), tripropionin (TC3), tributyrin (TC4), trioctanoin (TC8), methanol, *n*-propanol, dodecanol, *tert*-butanol, diisopropyl ether, toluene, isooctane, molecular sieves (nominal pore diameter 3 Å), and casein were obtained from Sigma-Aldrich®. *Tris*(hydroxymethyl aminomethane) was purchased from Research Organic®. Dibasic sodium phosphate and pyridine were procured from Karal®, while copper acetate was purchased from Caledon®. Anhydrous ethanol and *n*-butanol were obtained from the best quality local suppliers.

### *Sample collection*

Fresh latex was extracted directly from the unripe fruits from the plants, which were donated by Papaya

Peninsular S.A. Yucatán, México, by making four vertical incisions and collecting the fluid in 500-mL polyethylene-terephthalate jars. Then, the different parts of the plant were separated into the by-products mentioned above. All of the samples were immediately stored in sealed polyethylene bags at −20 °C until treatment.

*The treatment of samples to obtain biocatalysts*

The latex samples were treated using a method similar to that described by Giordani et al. (1991). The most hydrophilic compounds were removed by washing them thoroughly from the solid fraction of the crude latex with distilled water at 4 °C. The mixture was shaken vigorously with a vortex at a ratio of 1:7 (v:v) crude latex:water and centrifuged at 8600*g* and 4 °C for 20 min. The esterase/lipase activity was measured in both the supernatant and sediment during the treatment. This treatment was repeated until no significant protease activity was detected in the supernatant.

The liquid extracts were obtained by milling each by-product with an industrial disk mill (Exmex). The solid residues or bagasses were dried at 25 °C, milled, and sieved (sieve #30). The liquid extract from the petioles received the same treatment as described above for the latex. All of the solids obtained after this washing treatment with cold water were lyophilized and labeled as ''Treated Lyophilized''. The powders obtained were designated as ''biocatalysts'' and preserved at −20 °C.

*Protease activity*

The proteolytic activity was measured on casein using the spectrophotometric method reported by Anson et al. (1938) that was modified for use in a 96-well microplate format. A 100 μL of the different liquid extracts or 100 μL of a suspension of lyophilized biocatalyst powder at 100 g/L was used for the assay. The protease activity was expressed in IU per mL of liquid extract or mg of lyophilized powder. One IU corresponds to 1 μmol of tyrosine equivalents released per minute. Each assay was performed in triplicate.

*Esterase/lipase activity assay*

The esterase/lipase activity was assayed by measuring the fatty acids released from mechanically stirred TC2 (354 mM), TC3 (208 mM), TC4 (114 mM), and TC8 (39 mM) emulsions, using 0.1 N NaOH with a pH-Stat GPT Titrino (Metrohm®) set at a pH end point. Each assay was performed at 25 °C in a thermostated vessel containing 2.5 mM Tris–HCl buffer and 150 mM NaCl at pH 7. The rate of spontaneous hydrolysis of the substrate was recorded 5 min prior to sample injection. A 500 μL of the different liquid extracts or 100 μL of a suspension of lyophilized biocatalyst powder at 100 g/L was used for the assay. The esterase/lipase activity was expressed in IU per milliliter of liquid extract or milligram of lyophilized powder. One IU corresponds to 1 μmol of fatty acid released per minute. Each assay was performed in triplicate.

*The synthesis of oleic acid esters*

The synthesis of oleic acid esters was performed in 1.5 mL vials with an equimolar (200 mM) solution of oleic acid and the corresponding alcohol in 1 mL of isooctane. All the reactions contained molecular sieves and 25 mg of biocatalyst, except those in which BP (37.5 mg) was used. The samples were agitated at 300 rpm and 50 °C. Each assay was performed in triplicate.

*Monitoring of the synthesis reactions*

The concentration of oleic acid was used to calculate the initial rate and progress of the synthesis reactions. A spectrophotometric method based on the formation of a colored copper-fatty acid complex was employed to quantify the concentration of oleic acid using an oleic acid standard curve (Kwon and Rhee 1986). A 50 μL of the reaction mixture was diluted 10-fold in isooctane and 250 μL of a 5% (w/v) copper acetate–pyridine solution pH 6.1 was added to the diluted sample and mixed in a vortex for 30 s and was centrifuged at 15,900*g* for 5 min. A 200 μL of the organic phase was carefully collected and placed in a 96-well microplate, and the optical density was measured at 690 nm in an X-Mark Bio Rad® spectrophotometer. Each assay was performed in triplicate.

*The effects of various reaction solvents and alkyl chain lengths on the synthesis reactions*

The effects of *tert*-butanol, diisopropyl ether, toluene, and isooctane on the synthesis of ethyl oleate were evaluated. The effect of the alkyl chain length on esterification was determined by carrying out the synthesis of methyl, ethyl, propyl, butyl, and dodecyl oleate. All of the reactions were performed as described in the ''Synthesis of oleic acid esters'' section.

*Determination of the thermostability of the C. papaya biocatalysts*

Each biocatalyst was incubated for 4 h at 45 °C. After the incubation period, the thermostability was evaluated by measuring the residual hydrolytic activity on TC8 at 30 °C and pH 7.0. Each assay was performed in triplicate.

*Continuous dodecyl oleate synthesis*

The bagasse from petioles was used in a PBR for the continuous synthesis of DO. The PBR (500 mL, 2.5 cm diameter, and 25 cm high) was filled with 72 g of bagasse. The substrates, oleic acid and dodecanol, were mixed in an equimolar (200 mM) solution with isooctane. The flow rate and the temperature were kept at 1 mL/min and 50 °C, respectively. The progress of the synthesis reaction was determined in triplicate.

## Results and discussion

*Proportion of C. papaya by-products and esterase/lipase activity of the liquid extracts*

The proportion in humid weight (%HW) and the hydrolytic activity on TC4 and TC8 for the latex and liquid extracts from each *C. papaya* by-product are shown in Table 1. Although, latex was very difficult to collect and was obtained in a very small amount (0.1%HW), its hydrolytic activity was the highest among the by-products (TC4: 98.76; TC8: 10.88 U/mL). The stems were by far the most abundant (89%HW) among the by-products, followed by the fruits (5%HW). Nonetheless, the hydrolytic activity on TC8 (lipase activity) of the stems and fruits combined with the meristems and leaves was less than 1 U/mL (Table 1). For this reason, we decided to discard these by-products from further studies.

Table 1. Proportion of *Carica papaya* by-products and hydrolytic activity on tributyrin and trioctanoin.

| By-products | Proportion in humid weight (%HW) | TC4 | TC8 | Relative activity towards TC8 (%)[a] |
|---|---|---|---|---|
| Latex | 0.1 | 98.76 | 10.88 | 11 |
| Leaves | 0.9 | 19.37 | 0.67 | 3 |
| Meristems | 2 | 2.15 | 0.02 | 1 |
| Petioles | 3 | 4.95 | 1.67 | 34 |
| Fruits | 5 | 2.00 | 0.02 | 1 |
| Stems | 89 | 0.16 | 0.01 | 6 |

Esterase/lipase activity (U/mL) was measured at 25 °C and pH 7.0, using tributyrin (TC4) and trioctanoin (TC8) as substrate. Values are the average of three independent assays with SD less than 5%.

[a]Expressed as percentage with respect to TC4.

However, even though the petioles have a moderate weight proportion in plants (3%HM), their hydrolytic activity on TC8 was the highest out of all the by-products (1.67 U/mL). Thus, petioles were selected as the most promising by-product because it contains a greater proportion of lipase activity than latex, as observed by the relative activity towards TC8 with respect to TC4, which was 3-fold higher in the petioles (Table 1). Furthermore, the petioles contain a low specific protease activity (0.63 U/mg protein) compared to the other by-products (1.22–10 U/mg protein), as described by Galindo-Estrella et al. (2009). This activity facilitates its removal from the insoluble fraction. Therefore, latex and petioles were selected for the generation of biocatalysts with esterase/lipase activity because they are the most promising by-products in terms of their hydrolytic activity and availability.

*The esterase/lipase activity of C. papaya biocatalysts*

The lyophilized latex (*LL*), treated lyophilized latex (*TLL*), lyophilized petiole (*LP*), and treated lyophilized petiole (*TLP*) biocatalysts were obtained by washing away most of the soluble compounds, including proteases (see ''Materials and methods'' section). Bagasse obtained by a petiole milling process was dried, and its capacity as a biocatalyst (Bagasse from Petioles: *BP*) was evaluated. The activities of the five biocatalysts towards triglycerides with different chain lengths are shown in Figure 1. The latex biocatalysts showed higher hydrolytic activity (up to 517-fold, Figure 1A) in all of the substrates tested compared to those attained from petioles (Figure 1B and C). It should be noted that the maximum activity of the latex biocatalysts was with tributyrin, as previously observed by the Verger group (Giordani et al. 1991), whereas in the case of petiole biocatalysts, the highest hydrolysis rate was measured with tripropionin. These results strongly suggest that the esterases and lipases present in the latex biocatalysts are different than those present in petioles. After a cold wash, the activity per gram of the biocatalyst powder towards the partially soluble substrates, TC2 through TC4, was increased approximately 5- and 10-fold in *TLL* and *TLP*, respectively. In no case, esterase/lipase activity was found in the washing water.

As previously discussed, the relative activity towards TC8 with respect to TC4 was higher in petioles than in latex. Thus, during the processing of the petiole biocatalyst, the proportion of lipase activity was evaluated (Table 2). The petiole juice has the highest proportion of lipase activity. However, in ester synthesis it is necessary to

remove water from the reaction mixture. After lyophilization, the proportion of lipase activity was reduced 6-fold in *LP*. When the drying treatment was performed at room temperature, as is the case for generating *BP*, the proportion of the lipase activity was reduced only 1.7-fold compared to the lipase activity of the petiole juice. These results suggest that the reduction in the lipase activity is due to freezing. This phenomenon has already been reported for other enzymes (Privalov 1990). In *TLP*, both activities were reduced between 52% and 98%. This result suggests that the hydrolases responsible for each activity in petioles were possibly stabilized by metal ions ($Mg^{2+}$, $Ca^{2+}$, $Mn^{2+}$, $Fe^{2+}$, $Cu^{2+}$, and $Zn^{2+}$) that could have been removed by washing (Migliaccio et al. 2010). Similarly, it has been reported that cationic metals, particularly divalent cations, can modulate the lipase activity in some plants. For example, the lipase from a *C. papaya* close relative, *Carica pentagona*, is dependent on $Ca^{2+}$ (Dhouib et al. 2011). Similarly, plant lipases from different classes found in the seed of *Prunus dulcis* and latex from *Ficus carica* increase their activity in the presence of $Ca^{2+}$, $Mn^{2+}$, $Fe^{2+}$, $Co^{2+}$, and $Ca^{2+}$, $Cu^{2+}$, respectively (Yeşiloğlu and Başkurt 2008; Lazreg-Aref et al. 2012). We selected BP for further studies because its lipase/esterase activity proportion was the greatest and obtaining it was the most cost effective and easiest to acquire of those collected. There is a large potential for the application of BP in organic synthesis reactions.

### *Kinetics of the synthesis of oleic acid esters*

Ethyl oleate synthesis was used as a reaction model to evaluate the effect of solvent hydrophobicity on the reaction yield (Table 3). The best reaction solvent was isooctane, a solvent with high hydrophobicity (Log *P* 4.4). This result was consistent with previous work in which a solvent with similar hydrophobicity, heptane (Log *P* 4.3), was used in the alcoholysis of different oils, using latex as a biocatalyst (Su and Wei 2014). Thus, the synthesis of different alkyl chain length oleic acid esters using *TLL* and *BP* as biocatalysts was studied (Figure 2). As shown in Figure 2(A), *TLL* showed a 3- to 44-fold higher synthesis activity than *BP* (Figure 2B) in all of the tested alcohols. Indeed, the average productivity of *TLL* (1357 μmol/g h) is higher than those values calculated using the reported data for the synthesis of ethyl (441 μmol/g h) and butyl oleate (603 μmol/g h) and 4-fold less for the synthesis of propyl (5723 μmol/g h), employing the *Rhizopus* sp.,

Table 2. Variation of esterase/lipase units during petioles process.

| | TC4 (U) | TC8 (U) | Relative activity towards TC8 (%)[a] |
|---|---|---|---|
| Petioles (juice) | 1981 | 668 | 34 |
| Bagasse from petioles | 24,849 | 4938 | 20 |
| Lyophilized petioles | 2139 | 118 | 5.5 |
| Treated lyophilized petioles | 941 | 16 | 1.7 |

From a petioles kg U (total units) of esterase/lipase activity measured at 25 °C and pH 7.0, using tributyrin (TC4) and trioctanoin (TC8) as substrate, respectively.
[a]Expressed as percentage with respect to TC4.

Table 3. Effect of different reaction solvents using TLL and BP as biocatalysts in the ethyl oleate synthesis.

| | | (%) conversion of ethyl oleate | |
|---|---|---|---|
| Solvent | Log *P* | TLL[a] | BP[b] |
| Isooctane | 4.4 | 82.3 ± 2.8 | 80.5 ± 0.2 |
| Toluene | 2.5 | 12.0 ± 2.0 | 11.6 ± 0.1 |
| Diisopropyl ether | 1.5 | 15.0 ± 2.0 | 15.0 ± 1.5 |
| *tert*-Butanol | 0.8 | 7.7 ± 2.1 | 6.7 ± 0.7 |

TLL, treated lyophilized latex; BP, bagasse from petioles. % of conversion after 1 h[a] and 48 h[b]. Reaction was performed at 200 mM of oleic acid and ethanol, 50 °C and 300 rpm.

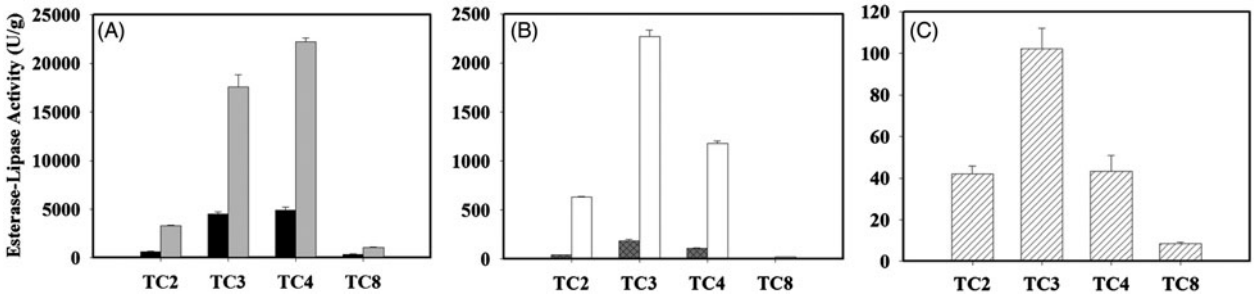


Figure 1. Esterase/lipase activities of the biocatalysts on homogenous triglycerides with different acyl chain lengths. (A) Black bars: lyophilized latex. Grey bars: treated lyophilized latex. (B) Dark gray bars: lyophilized petioles. White bars: treated lyophilized petioles. (C) White striped bars: bagasse from petioles. These experiments were carried out at 25 °C in 2.5 mM Tris–HCl buffer (30 mL, final volume) containing 150 mM of NaCl at pH 7.

*Candida antarctica* (Novozym 435), and *Rhizomucor miehei* (Lipozyme IM) lipases, respectively (Habulin et al. 1996; Salis et al. 2005; Martinez-Ruiz et al. 2008). Nonetheless, all these productivity data were obtained at different conditions and comparison should be taken carefully.

The kinetic profile for the synthesis of oleic acid esters using *TLL* does not show a clear alkyl chain preference (Figure 2A) compared to that observed by Caro et al. during the alcoholysis of trilaurin with primary alcohols (C1–C12; Caro et al. 2004). In contrast to the literature, TLL does not show significant inhibition when methanol and ethanol were used as alkyl chain donors (Caro et al. 2004; Su and Wei 2014). Therefore, it should be considered to be a robust biocatalyst with a potential application for the synthesis of short chain alkyl esters. Nevertheless, *BP* showed a synthesis preference towards medium-long chain esters (C4–C12; Figure 2B). The initial rate ratio for dodecyl and methyl oleate (C12/C1) revealed that dodecyl oleate (C12) was synthesized three times faster than methyl oleate (C1) by *BP*. These outcomes demonstrate that *BP* has a preferential activity regarding long chain alcohols, a typical behavior of classic lipases in organic synthesis (Habulin et al. 1996). Moreover, this suggests that the binding interactions between lipase activity and the petiole solid matrix are stronger than the interaction with other hydrolases, such as proteases and esterases, present in this by-product. The latter would explain why the extraction process (see ''Materials and methods'' section) generates an enriched lipase activity biocatalyst, as demonstrated in the previous section.

To evaluate the ability of the biocatalysts to perform synthesis versus hydrolysis, the ratio of DO synthesis ($C12_S$) and TC8 hydrolysis ($TC8_H$) was calculated for *TLL* and *BP*. The $C12_S/TC8_H$ ratio for *BP* (0.46) was 3-fold higher than that obtained for *TLL*, indicating that *BP* is better suited as a biocatalyst for the synthesis of long chain alkyl esters in organic solvents than it is for their hydrolysis. Furthermore, it should be noted that bagasse, as a fibrous by-product, has the ability to absorb the reaction solvent, generating serious diffusion problems and limiting the amount of the biocatalyst that could be used in batch systems. Therefore, it was not possible to reduce the reaction times in batch systems (48 h). However, *BP* was active during a long reaction time (Figure 2B), suggesting that this biocatalyst is more resistant to thermal inactivation and better suited for use in continuous systems. An attractive system is a PBR because the catalyst bed is positioned with a fixed height after a substrate mixture is fed into it at a controlled flow rate, leading to the continuous recovery of a product with a high conversion yield at the reactor outlet. Thus, we postulate that a continuous PBR system could be a better option for the synthesis application of *BP*.

### *Evaluation of the thermostability of the biocatalysts from C. papaya*

The thermostability of a biocatalyst is important to assess because thermal enzymatic denaturation is one of the critical factors for the successful operation of a PBR. Accordingly, the thermostability of the papaya biocatalysts (*LL*, *TLL*, *TPL*, and *BP*) was evaluated after incubation at 45 °C, and the residual activity was determined using TC8 as the substrate (Figure 3). As expected, the thermal inactivation profiles followed first-order kinetics. The petiole biocatalysts showed a higher thermostability, with a half-life approximately 7-fold higher than the latex biocatalysts. This result could also be due to an effect of the matrix or that the lipase activity of the petiole corresponds to a different enzyme than that presents in the latex and is, presumably, more stable. For the

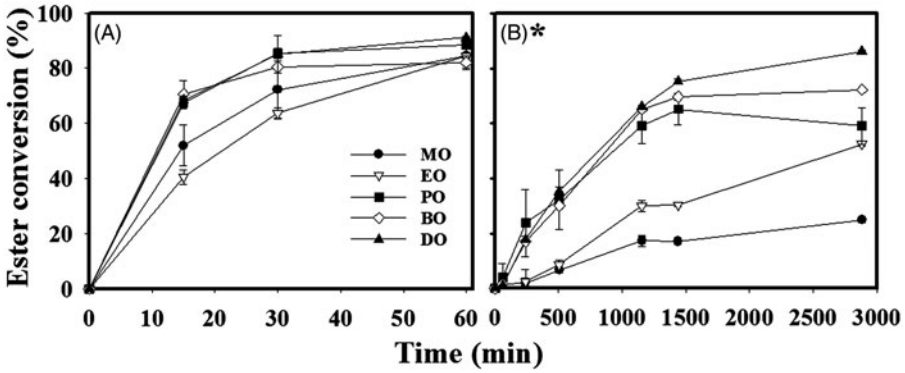


Figure 2. Kinetics for the synthesis of oleic acid esters. (A) Treated lyophilized latex, TLL; (B) bagasse from petioles, BP. Synthesis was performed at 50 °C using isooctane as the reaction solvent. *37.5 mg BP/mL. MO – methyl oleate; EO – ethyl oleate; PO – propyl oleate; BO – butyl oleate; DO – dodecyl oleate.

latex biocatalysts, the washing treatment slightly improved the biocatalyst thermostability, leading to a 1.6-fold increase in its half-life (26 min), probably due to the lack of proteases in the *TLL* following the washing treatment. Likewise, among petioles biocatalysts, *BP* was the most thermostable, with a half-life of 3-fold higher than *TLP*, suggesting that the support (bagasse) confers enhanced stability to the enzyme responsible for the lipase activity (Cao 2006). In general, plant lipases exhibit a lower thermostability than microbial lipases; some, such as *Thermomyces lanuginosus* and type A from *C. antarctica*, have half-lives of 20 and 1400 h at 60 and 70 °C, respectively (Heldt-Hansen et al. 1989). However, the residual activity of *BP* after one hour of incubation at 45 °C (65%) was similar to that reported for other plant lipases, such as wheat germ (70%), rice bran (90%), or a precipitate of *C. papaya* seeds and fruit peel (80%) (Bhardwaj et al. 2001; Kapranchikov et al. 2004; Paques et al. 2008). *BP* could be a good candidate for the continuous synthesis in a PBR.

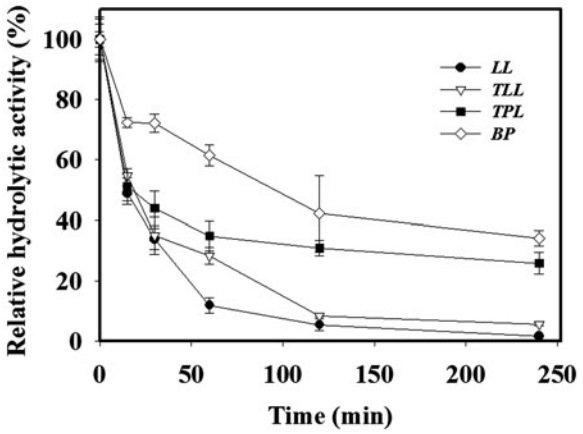


Figure 3. Kinetics of the thermal inactivation of the *Carica papaya* biocatalysts. The optimal conditions for each biocatalyst were determined by the residual hydrolytic activity on trioctanoin at 45 °C for four hours. The data are the means ± SD ($n = 3$).

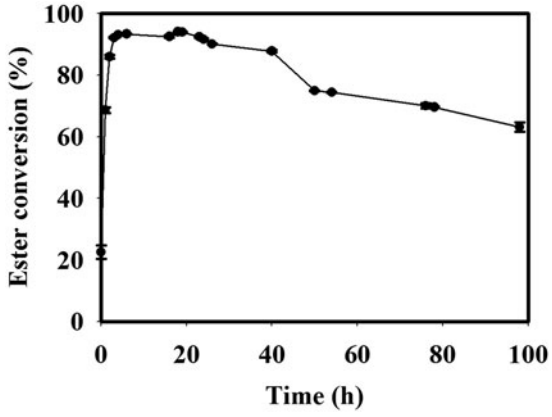


Figure 4. The continuous synthesis of dodecyl oleate in a packed bed reactor. The synthesis was catalyzed by bagasse from petioles using isooctane as the reaction solvent and a 0.2 M final substrate concentration (molar ratio 1:1) at 50 °C.

*The continuous synthesis of dodecyl oleate in a PBR*

The continuous synthesis of dodecyl oleate was carried out in a PBR using *BP* as a biocatalyst (Figure 4). The PBR reached the steady-state only after 2 h of operation, and the reaction conversion (96%) remained stable for at least 40 h. After this time, the conversion gradually declined to 65% at 60 h, and this result could be a consequence of water accumulation in the bed of the reactor during the reaction progress. Although, the steady-state BP–PBR (40 h) was not as long as reported for the transesterification synthesis of butyl oleate (150 h) using *C. papaya* latex in a PBR (Su and Wei 2014), we must emphasize that the amount of petioles in the plant is much greater than the amount of latex in the plant. Additionally, the process that is used to obtain biocatalysts from the petioles is much simpler than the process that is used to obtain them from latex. Consequently, *BP* is a much more attractive biocatalyst than latex.

Moreover, the productivity attained in the steady-state BP–PBR was 11,202 mol/h, which is 23 times greater than the productivity achieved by the immobilized lipase of *R. miehei* (Lipozyme®) during the transesterification synthesis of butyl oleate using a similar system and 20 mM as the initial substrate concentration (Dossat et al. 1999). This result could be attributed to the higher substrate concentration employed in our experiment (200 mM). Therefore, the BP–PBR system seems to be a competitive alternative choice for the synthesis of additional valuable organic compounds, such as dodecyl oleate, which is widely used in the cosmetics industry.

## Conclusions

In this work, it was demonstrated, for the first time, that it is possible to obtain new biocatalysts with lipase/esterase activity from the by-products of *C. papaya* that have different properties for hydrolysis and synthesis than those previously reported for latex (mainly thermostability and selectivity). The latter suggests the presence of different enzymes in the *C. papaya* by-products. To confirm this, more studies are required. However, we proved that these biocatalysts can be obtained, leading to a new outlook for catalytic studies in other *C. papaya* by-products, especially petioles.

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## Declaration of interest

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The authors report no conflicts of interest.



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