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Review

Plant latex lipase as biocatalysts for biodiesel production

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Industrial-scale processes currently developed make use of chemical catalysis processes that are highly efficient but require very complex product purification steps. Enzymatic catalysis through plant lipases as biocatalysts is an alternative which, in contrast to chemical catalysis processes, appeared simple to perform, and can be done at low investment cost. Although microbial lipases have been extensively studied, little research has been focused on the use of plant lipases namely plant latex lipases. The present article outlines the most advanced knowledge concerning plant latex characterization in order to show how plant latex can be a promising alternative to catalyze transesterification for biodiesel production. This paper provides an overview regarding the main aspects of latex, such as the reactions catalyzed, physiological functions, specificities, sources and their industrial applications.

Key words: Plant latex, lipase, Transesterification, purification, biodiesel.

INTRODUCTION

Interest in the production of biodiesel a clean renewable fuel is increasing worldwide due to the excessive increase of petroleum prices and the importance of taking environmental concerns into consideration (Mounguengui et al., 2013; Sadeghinezhad et al., 2013). Bio-fuel or biodiesel is usually identified as ester based fuels produced from animal fats or from vegetable oils by using an effective transesterification method. Biodiesel carries 4.5 units of energy against each unit of fossil fuel (Pradhan et al., 2009). Besides this, biodiesel is safer, biodegradable and nontoxic in nature (Mc Carthy et al., 2011). The mixtures obtained after transesterification are composed of fatty acid alkyl monoesters (Robles-Medina et al., 2009; Meher et al., 2006) and to be classified as "biodiesel", it must achieve minimum purity and fulfil the specifications of international standards (Graboski et al., 1998), the European standard EN14214 (FAME, 2003) and the American standard ASTM 6751-09 (ASTM,

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2009). Fatty acid alkyl monoesters may be methyl or ethyl esters respectively if the alcohol used is methanol or ethanol. Conventional biodiesel production methods involve the use of acid or base chemical catalysts *via* homogenous or heterogeneous processes (Marchetti et al., 2007; Brunschwig et al., 2011). Downstream processing costs and environmental problems associated with biodiesel production and byproducts recovery have investigated many research teams to develop new alternative catalysis processes to replace the homogenous catalysis processes generally used in industries (Akoh et al., 2007). In contrast, enzymes (lipases) allow the production of specific alkyl esters, easy recovery of the glycerol, and transesterification of TAG with high free fatty acid content (Soumanou et al., 2012).

Lipases have become more and more prominent on the enzyme biotechnology scenario due to their versatility for hydrolysis and synthesis, their catalytic reactions often being chemo-selective, regio-selective or enantio-selective. Lipases are used in many sectors such as the food, pharmaceutical, fine chemical, oil chemical, biodiesel and industrial detergent industries (Alonso et al., 2005). Lipases act, by definition, at the organic-aqueous interface, catalyzing the hydrolysis of estercarboxylate bonds and releasing fatty acids and organic alcohols (Pereira et al., 2003; Leal et al., 2002; Kamimura et al., 1999; Merçon et al., 1997). However, as Pottevin showed for the first time in 1906, in water-restricted environments, the reverse reaction (esterification) or even various transesterification reactions can occur (Castro et al., 2000).

Lipases can be of animal (pancreatic, hepatic and gastric), microbial (bacterial, fungal and yeast) or vegetable origin, with variations in their catalytic properties (Mukherjee and Hills, 1994). To date, microbial lipases are the most studied. In fact around 58% of the publications of the whole of lipases are devoted to microbial lipases, plant lipases are around 42% of publications and only 11% of the publications are devoted to latex lipases (Google Scholar, 2014). Nevertheless, despite the extensive range of microbial lipases, the use of these enzymes on an industrial scale is still restricted due to high production costs, favoring the search for other sources of these enzymes (Parques and Macedo, 2006).

The modification of fats and oils by transesterification, for instance, can be performed by both chemical and enzymatic catalysis. The industrial transesterification process is currently performed by chemical means, using high temperatures and alkaline metals (KOH, NaOH, HCl, H_3PO_4, H_2SO_4… etc) as the reaction catalyst (Ribeiro et al., 2009; de Araújo et al., 2013). In the enzymatic process, lipases can be used as biocatalysts to promote the exchange of triacylglycerols, showing greater efficiency and leaving no residues (Xu, 2000). For example immobilized *Candida antarctica* lipase has been used for ethyl esterification of docosahexanoic acid and later used to effect over 98.5% fatty acid methyl ester conversion (Fjerbaek et al., 2009). Latex lipases present certain advantages since they do not necessarily have to be purified in order to perform this and other processes (Cambon, 2008). However, several studies have indicated that such processes are very expensive due to the high cost of purification step (de Castro et al., 2004; Noor et al., 2003). Recently, latex lipases have been the focus of much attention as biocatalysts. In some cases, these enzymes present advantages over animal and microbial lipases due to some quite interesting features such as specificity, low cost, availability and ease of purification, representing a great alternative for potential commercial exploitation as industrial enzymes (Villeneuve, 2003).

Although microbial lipases have been extensively studied (Kilcawley et al., 2002; Mendes et al., 2012), little research has been focused on the use of plant lipases in biodiesel production. However, the major drawback for plant lipases implementation at large scale is the low content of enzyme in the post-germination seeds. However, *Caricaceae* or *Euphorbiceae* overcome this disadvantage as their enzymes are available in large amounts stored in their latex (Paques and Macedo, 2006).

The aim of this review was to highlight the potential and current limitations to the use of plant latex lipases for low-cost enzymatic catalysis alkyls esters production and therefore for a possible application for biodiesel production.

LATEX SOURCES AND PLANT-BASED LATEX

Latex is an aqueous emulsion or a milky fluid (Mounguengui et al., 2013) found in the vacuole of specialized secretory cells known as ‘‘laticifers’’ (Fahn, 1982). Some family plants known as laticifers are *Apocynaceae, Asclepiadaceae, Euphorbiaceae, Moraceae* and *Sapotaceae* (Moulin et al., 1994; Palocci et al., 2003; Giordani et al., 1991; Villeneuve et al., 2005; Dhuique-Mayer et al., 2001, 2003). The laticifers plants show intense metabolic activity, with the latex containing lipids, rubbers, resins, and sugars, as well as several proteins and many different enzymes (that is, peroxidases, proteases, esterases, and phosphatases) (Fiorillo et al., 2007; El Moussaoui et al., 2001; Lynn and Clevette-Radford, 1987). The laticifers plants also show secondary metabolic activity directed toward the production of defence-related molecules, which accumulate in appreciable amounts in the latex. Many toxic substances known to be stored in the latex (that is, alkaloids, sterols and terpenoids) have been shown to have a negative impact on insect feeding and phytophage fitness (El Moussaoui et al., 2001). The latex thus represents a chemical defence, and the physiological

role of its constituents, including lipolytic enzymes, could be related to defence mechanisms (El Moussaoui et al., 2001).

The water insoluble fraction of latex shows lipase activity. It is now known that lipases contained in latex from some plants have catalytic properties and numerous industrial applications (Paques and Macedo, 2006). For instance, papaya (*Carica papaya*) latex has already been described in the modification of fats and oils (Villeneuve, 2003; Foglia and Villeneuve, 1997a), in esterification and transesterification reactions (Caro et al., 2000), and more recently in the resolution of racemic mixtures (Cheng and Tsai, 2004). Studies carried out on *C. papaya* showed that its crude latex has a very strong activity on short-chain triacylglycerol (TAG) and *sn3* stereoselectivity in hydrolysis reactions of chiral TAG substrates (Villeneuve et al., 1995). These observations led to industrial use of this latex in particular applications, such as the synthesis of low-calorie triacylglycerols (Foglia and Villeneuve, 1997a) or medium-chain TAG (Caro et al., 2004). These results have prompted interest in other latex plant extracts, in particular, the unripe fruit of the babaco plant (*Vasconcellea* X *Heilbornii* cv., ex *Carica pentagona Heilbornii*) (Kyndt et al., 2005), a member of the papaya family native to the subtropical mountains of Ecuador, which contains a latex similar to the one in *C. papaya*. In common with the latter, babaco has also been shown to exhibit biocatalytic activities in lipolysis and acyl transfer reactions (Dhuique-Mayer et al., 2001, 2003). Latex lipases from plants in the *Euphorbiaceae* (Moulin et al., 1994; Palocci et al., 2003; Giordani et al., 1991; Villeneuve et al., 2005), *Asclepiadaceae* (Giordani et al., 1991) or *Caricaceae* (Giordani et al., 1991; Dhuique-Mayer et al., 2001, 2003) families have also been described as useful biocatalysts for several synthesis applications in the food, pharmaceutical and detergent industries.

Chemical composition and molecular structure of plant-based latex

Most plants from families like *Euphorbiaceae* (Giordani et al., 1991; Moulin et al., 1994; Palocci et al., 2003; Villeneuve et al., 2005), *Asclepiadaceae* (Giordani et al., 1991), *Brassicaceae* (Hills et al., 1990), or *Caricaceae* (Giordani et al., 1991; Dhuique-Mayer et al., 2001, 2003) contain latex and have been described as useful biocatalysts for several applications. Here, a summary of chemical composition of some plant-based latex was performed.

Caricaceae

Within the *Caricaceae* family, *C. papaya* is a soft-stemmed and unbranched tree able to grow up to 20 m in height. As a native to the Central America, the *papaya* tree has successfully established in many ecological niches in tropical and subtropical climates (El Moussaoui et al., 2001). All aerial parts of the female and hermaphrodite plants, including unripe fruits, present laticifers (El Moussaoui et al., 2001) that create a dense network of articulated and anastomosing structures (Roth and Clausnitzer, 1972). Consequently, if incisions are made in those aerial parts, especially in the unripe fruits, an abrupt release of latex (the so-called *C. papaya* latex) is observed. This latex is a thixotropic fluid with a milky appearance, which contains around 15% of dry matter, 85% water, and a great variety of hydrolytic enzymes, mainly proteases (El Moussaoui et al., 2001; Azarkan et al., 2003; Campillo-Alvarado and Tovar-Miranda, 2013). Furthermore, different protein compositions have been reported depending on the age or sex of the tree, as well as on the time of day in which the tapping is carried out (Luis Madrigal et al., 1980; Caro et al., 2000). The mixture of different enzymes present in the latex is supposed to play a defensive role in the plant (El Moussaoui et al., 2001).

Babaco (*C. pentagona Heilb*.) is a tropical plant from a mountain climate, native to Ecuador, appreciated for its flavor. Babaco is a perennial shrub of the Caricaceae family. This natural hybrid grows between 1500 and 2500 m in Ecuador. Annual yields are between 60 and 80 fruits per plant. It is a large seedless fruit, yellow when ripe. Its special aroma is described as having overtones of pineapple, lemon and papaya. When the fruit is green, it exudes latex which has proteolytic characteristics similar to the papaya latex. The fruit is directly consumed when it is fully ripe. It is also possible to produce juice concentrate, jam or dehydrated fruit. The extraction of proteolytic enzymes of the latex from green babaco could be a new industrial application for this fruit. Babaco has economical potential thanks to its sensorial properties, and as a source of proteolytic enzymes.

Apocynaceae

In addition to *Apocynaceae* family, *Plumeria rubra* is also studied. It's growing in tropical and sub-tropical regions of the world (Ye et al., 2009; Coppen and Cobb, 1983) and is grown for ornamental purposes (Perry and Metzger, 1980). Plants of genus *Plumeria*, had their origin from Central America. Different species are now found widely distributed in the warmer regions of the world (Krishnamurthi, 1969) and reputed for their medicinal properties, e.g., antifouling (Coppen et al., 1983), anticancer (Fujimoto and Made, 1988), algicidal (Coppen, 1983). The aqueous extract of *P. rubra* showed antimicrobial (Gupta et al., 2007) anti-inflammatory activities (Dubois and Rezzonico, 2007) and used for the treatment of respiratory ailments (Frei et al., 1998; Case et al., 2006). Plumericin, an iridoid isolated from *P. rubra*

is used as antimicrobial agent (Little and Johnstone, 1951).

The average composition of carbon, hydrogen and nitrogen (%) of *P. rubra* was respectively 44.89, 6.72 and 1.26%. Indeed, some studies have reported that a_w conditions the lipase structure and thus its enzymatic activity both in hydrolysis and in synthesis reactions (Cambon et al., 2006).

Moraceae

Ficus carica, (*Moraceae* family), is one of the earliest cultivated fruit trees and an important crop worldwide for both dry and fresh consumption. It is a native of the Mediterranean coast. In these countries, figs are an important constituent of the Mediterranean diet, which is considered to be one of the healthiest and is associated with longevity (Trichopoulou et al., 2006). Oliveira et al. (2009) compared *F. carica* leaves, pulps and peels. Data obtained indicate that chemical composition and bioactivity are dependent on the variety (Oliveira et al., 2009). 3-O-Caffeoylquinic acid and quercetin 3-O-glucoside were described for the first time, which adds to the knowledge of this species. Leaves may constitute an excellent dietary and economical source of bioactive compounds, namely, phenolic compounds.

F. carica latex is essentially constituted by saturated fatty acids (ca. 86.4% of total fatty acids), whilst dried and fresh fruits show predominantly polyunsaturated fatty acids (ca. 84 and 69% of total fatty acids, respectively) (Jeong and Lachance, 2001; Pande and Akoh, 2010). With respect to monounsaturated fatty acids, oleic acid is presented as the most abundant one in latex (Oliveira et al., 2010), which is in agreement with data found for *F. carica* fruit (Jeong and Lachance, 2001; Pande and Akoh, 2010). Concerning polyunsaturated fatty acids, linoleic acid was the only compound identified (ca. 9.9% of total fatty acids) (Oliveira et al., 2010), which was already described in dried and fresh fig fruits (Jeong and Lachance, 2001; Pande and Akoh, 2010). Regarding protein composition in latex fluid, it is known that lattices of *F. carica* contain multiple forms of proteolytic enzymes (Liener and Friedenson, 1970).

Euphorbiaceae

Euphorbia characias L. (*Euphorbiaceae*) is one of the oldest known medicinal plants of the Western tradition. It is described in most ancient treatises of Greek and Latin medicine (Baumann, 1993), and was held in great esteem up to the development of modern medicine, which made obsolete its use as a powerful cathartic and emetic. *E. characias* is nowadays best known as a garden plant (Appendino et al., 2000). It is one of the most widespread ornamental spurges, and several varieties have been developed, substantially expanding the habitat of this Mediterranean species (Turner, 1995). Two geographical varieties are known, the subsp. *characias* found in the western Mediterranean region, and the subsp. *wulfenii*, which grows in the Balkans, Greece, and Turkey (Turner, 1995). Both subspecies were found active in the mouse ear erythema assay (Evans and Kinghorn 1977). This and the use of the plant as a fish poison (Turner, 1995) suggest the presence of phorbol-type diterpenes.

LATEX LIPASES

Lipases, also known as triacylglycerol ester hydrolases (EC 3.1.1.3), are one of the most versatile biocatalyst with a remarkable ability to achieve a wide range of bioconversion reactions using a variety of substrates. Moreover, lipases possess the unique property of working at a lipid/water interface mainly in organic media (Gupta et al., 2003). In most instances, commercial lipases are generally produced from animals or microorganisms (Kilcawley et al., 2002, Mendes et al., 2012). Nonetheless, most of plant lipases are relatively inexpensive due to their wide availability from natural sources. Plant lipases are mostly found in energy reserve tissues, for example, oilseeds. They act as biocatalysts which are attractive due to their high substrate specificity, low production cost and easy pharmacological acceptance due to their eukaryotic origin. Hence plant lipases represent better potential for commercial applications in organic synthesis, food, detergent and pharmacological industries (Seth et al., 2014). As a result, plant lipases are generally more accepted for food or medicinal applications. However, low expression, uneconomical fold purity and the plethora of difficulties related to their recombinant expression has been limiting their commercial applicability and posing challenges to many researchers (Seth et al., 2014). In addition, the major impediment for its implementation at large scale is the low content of enzyme in the post-germination seeds, bran portion of the grain and wheat gem. Noticeably, *Caricaceae* or *Euphorbiceae* overcome this disadvantage as their enzymes are available in large amounts stored in their latex (Villeneuve, 2003; Paques and Macedo, 2006). In this context, the lipolytic complex of enzymes present in *C. papaya* which, otherwise stated, are referred to as CPL when they are found in the crude latex without pretreatment or pCPL when they are in a crude lipase preparation, hold several advantages over their microbial and animal counterparts such as: (i) good stability to a wide range of pH, temperature, organic solvents and to the presence of other catalysts such as lysozyme, amylase, pectine esterase, thioglucosidase, phosphatase acide, invertase, catalase, peroxidase, lipoxydase,… (Abdelkafi et al., 2011); (ii) relatively inexpensive, e.g. the price is approximately about one third that of crude

Candida rugosa lipase (CRL) (Gandhi et al., 2001; Campillo-Alvarado and Tovar-Miranda, 2013); (iii) can be considered "self-immobilized" enzymes since they are naturally bound to a non-water soluble matrix and thus, do not require further support and can be both recovered and reused (Abdelkafi et al., 2011); (iv) the active sites do not require interfacial activation prompted by detergents such as the pancreatic lipase (Mendes et al., 2012; Giordani et al., 1991); (v) the regio-, stereo-, typo- and substrate selectivities offer high versatility in diverse biochemical reactions; (vi) can be sustainably collected from the industrial papaya agro-waste of sick and unripe fruits (Mendes et al., 2012).

ENZYMATIC (LIPASE) PROPERTIES OF SOME LATEX

The optimum parameters that influence the lipolytic activity of some latex are summaries at Table 1. The lipolytic activities of babaco (Vasconcellea x Heilbornii cv.) and *Plumeria* latex were first measured using sunflower oil as substrate at pH 8 and at temperatures varying from 30 to 70°C by Cambon et al. (2006). Maximum activity was observed at 50°C for babaco (260 IU/g). At 55 and 60°C, significant thermal deactivation was observed for babaco, with 49 and 56% losses of activity, respectively. *Plumeria* appeared to be less sensitive to thermal denaturation and was shown to express its maximum lipolytic activity at 55°C (1400 IU/g), (Table 1). The optimum pH for babaco latex was 7, whereas for *Plumeria* latex, two optimal pH values (4 and 7) were observed. With regard to esterification and acyl transfer reactions, the influence of thermodynamic water activity on reaction yields was determined and correlated with water sorption and desorption isotherms. When babaco latex is used as a biocatalyst, optimal synthesis reaction yields are obtained when the enzymatic extract is stabilized at a water activity (a_w) value of 0.38, which corresponds to a water content of 5.7%. This optimal level of hydration is located on the linear portion of the biocatalyst's sorption isotherm, where the water molecules exhibit high-energy interactions with the protein network (Cambon et al., 2006). In synthesis reactions (esterification, alcoholysis, and interesterification) biocatalyzed by *Plumeria* latex, correlation between best reaction yields and water activity cannot be done. Indeed, the sorption isotherm plot has an atypical shape, indicating that water might be trapped in the latex matrix and, consequently, that the water content of the biocatalyst is highly dependent on the hydration history of the latex (Cambon et al., 2006).

Using tributyrin as substrate, a high level of lipase activity reaching 2,000±185 U/g of CPL was measured using a 10% w/v dispersion of CPL powder in deionized water (Table 1). The lipolytic activity of *C. papaya* latex on the short chain triglyceride tributyrin was described several years ago (Giordani et al., 1991). However, tributyrin is partly soluble in water and some esterases which are active on this substrate did not show any activity on a true lipase substrate such as olive oil. It was observed in a more recent study, however, that CPL could hydrolyze the long chain triglycerides present in test meals and could therefore be considered as a source of true lipase activity (Abdelkafi et al., 2009). It was confirmed here (Table 1) that CPL is active on olive oil (256±8 U/g) as well as on trioctanoin (983±29 U/g). CPL is much more active on short and medium chain TAGs than on long chain TAGs, as occurs with most lipases (Ngando et al., 2006). It is worth noting here that the specific activity of CPL on olive oil was similar to that detected in the dry mesocarp of oil palm fruit (250±14 U/g) (Ngando et al., 2006). CPL was also found to hydrolyze phosphatidylcholine with a specific activity of 65±3 U/g, but showed no activity on cholesterol oleate. Several TAG lipases have been found to show a dual TAG lipase/ phospholipase A1 activity (Simons et al., 1998; Thirstrup et al., 1994) but only if a pure CPL enzyme is obtained it will be possible to determine whether the phospholipase and lipase activities measured with crude CPL are due to the same enzyme.

A a_w affect the reaction rate, enantioselectivity and equilibrium of *C. papaya* lipolytic enzymes, as was reported in the resolution of different (R,S)-2-methylalkanoic acids; CPL showed a maximum initial rate for the (S)-enantiomers (VS) at low water activity (aw = 0.03), whereas the maximum E (enantiomeric ratio, defined as the ratio of initial rates, VS/VR or VR/VS) was achieved by increasing aw to 0.33, further separating VR from VS, although with the penalty of triggering hydrolysis instead of esterification (Chang and Ho, 2011). One of the most advantageous characteristics of the lipolytic enzymes of *C. papaya* is their ability to work efficiently under a broad range of pH and temperature. The good thermostability of the enzymes is attributed to the lipase immobilization in the non-soluble matrix of the latex (Ng and Tsai, 2005). When olive oil was used as the substrate, CPL activity was found to be optimum at pH levels ranging between 9 and 9.5 (Abdelkafi et al., 2001), and the kinetics of fatty acid release were linear for at least 5 min when the pH value was equal to or below 9. At pH values above 9, the kinetics were linear for only 1 to 2 min. These data suggest that CPL is less stable at high pH levels. Optimum conditions for assaying CPL activity on olive oil were therefore set at pH 9. No significant activity could be detected at pH 6 or less (Table 1). The optimum pH range for CPL activity is similar to that determined in the case of other plant lipases from palm oil fruit (Ngando et al., 2006) and babaco (Cambon et al., 2008). When tributyrin and trioctanoin were used as substrates, the maximum activity of CPL was recorded at pH 8 and 9, respectively.

As can be expected, temperature plays a major role in the reaction kinetics; an increase could facilitate the diffusion coefficients of substrates migrating to enzyme-

Table 1. Optimum parameters that influence reaction kinetics of enzymatic properties of some latex and their specificity.

Family	Plants	Optimum parameters				MA[3] (IU/g)	Yield[4] (%)	Alcohols substrates	Type of coordination-site location	Reaction medium	Specificity	Specific activity[a] (IU/g)	Lipids substrates	References
		T[1] (°C)	pH	a_w	WC[2] (%)									
Caricaceae	*C. pentagona* (Babaco)	45 to 50	7	0.38 or < 0.5	5.7	260 to 275	14	Butanol	NA	Solvent-free	*Sn-1,3*	109[e]	Sunflower oil or pure monolein or diolein	Cambon et al., 2006; Chen et al., 2005; Cambon, 2008
Apocynaceae	*P. rubra*	55	4 and 7	0.44	NA	1400	25 to 32	Butanol	NA	Solvent-free	*sn-3*	7400[e]	Sunflower oil or pure monolein or diolein	Cambon et al., 2006; Cambon et al., 2008
Caricaceae	*C. papaya*	50	4 and 10	< 0.11	2	814±38	75	Methanol	Surface	Solvent-free	*Sn-1,3/ sn-3*	256±8[d]	Olive oil[b]	Chang and Ho, 2011; Campillo-Alvarado and Ricardo Tovar-Miranda, 2013; Abdelkafi et al., 2011; Villeneuve et al., 1995; Foglia and Villeneuve, 1997; Villeneuve et al., 1997a; Gonzalo Campillo-Alvarado and Ricardo Tovar-Miranda, 2013; Cambon et al., 2008
												983±29[d]	Trioctanoin[b]	
												2,00±185[d]	Tributyrin[b]	
												65±3[d]	Phosphatidylcoline[c]	
												0	Cholesterol oleate	
Euphorbiaceae	*E. characias*	37 or 50	5 and 8	NA	NA	2909±29	78	Methanol	NA	Mechanically stirred medium of water and oil	*sn-3*	1589±40	Triacetin	Paloccia et al., 2003; Caro et al., 2000; Giordani et al., 1991
						6739±10						3379±2	Tributyrin	
						13369±10						5459±13	Tricaprilin	
						4869±39						3159±5	Linseed oil	
						9259±25						7359±3	Sunflower seed oil	
Euphorbiaceae	*E. wulfenii*	NA	5 and 8	NA	NA	2299±11	80	NA	NA	NA	NA	209±1	Triacetin	Palocci et al., 2003
						7199±1						1379±8	Tributyrin	
						10079±10						1809±13	Tricaprilin	
						3919±21						879±2	Linseed oil	
						4439±40						2509±2	Sunflower seed oil	

[1] temperatures; [2] water content; [3] Maximum activity; [4] % of FFAs released after 1 h; NA: Not available; SA: Specific activity; [a]: Values are means ± SD (n=3); b = Assays with triacylglycerols were performed at 37°C in 2.5 mM Tris-HCl buffer, 150 mM NaCl and at pH 9; c = Assays with phospholipids were performed at 37°C in 7.5 mM $CaCl_2$, 13.3 mM NaTDC and at pH 8; [d]: U/g; [e]: IU/mg of lipases.

active sites, thus enhancing the reaction rate (Varma et al*.,* 2008). This was observed in the increment of the activity of several reactions carried out above room temperature (Tecelão et al., 2012; Lee and Foglia, 2000). A temperature screening of the lipolytic activity on olive oil showed its peak at 50°C, although above 37°C the residual activity of pCPL started to decrease after 1 or 2 min, suggesting that the activity improvement at high temperatures is accompanied by a loss in its stability (Abdelkafi et al., 2011). On the other hand, pCPL was found stable at pH's from 4 to 10, preserving the 75% of its activity after 24 h of incubation at pH 10, whereas short inactivation times were observed out of this range

(Abdelkafi et al., 2011). It is worth noting that only a few microbial lipases, such as that of *Thermomyces lanuginosus* (*Humicola lanuginosa*) (Boel and Huge-Jensen, 1998) have shown similar levels of resistance so far in a large pH range up to pH 10. Concerning lipase activity, the majority of reactions are often carried out in nearly neutral to alkaline pH (Tecelão et al., 2012; Caro et al., 2004; Lee and Foglia, 2000). For example, the CPL mediated hydrolysis in olive oil found its optimum at pH levels between 9 and 9.5 (Abdelkafi et al., 2011), while the SA in the hydrolysis of TAGs present in human diet was better at pH 6 (Abdelkafi et al., 2009). These two pH values account for the observations of (Paques and Macedo, 2006) who by an ammonium sulfate pretreatment enabled the enzyme to work efficiently at pH 6. Alternatively, delipidation with acetone provided an improvement in the lipase activity profile at pH levels close to 9.5 (Paques and Macedo, 2006).

STRUCTURE AND MECHANISM OF THE LIPOLYTIC ENZYMES OF PLANT LATEX LIPASES

Experimental studies have been carried out in order to analyze the ability of lipases to hydrolyse vegetable oils and phosphatides under different conditions (Hara et al., 1997; Mustranta et al., 1995). Marked differences were observed in lipase hydrolytic activity in terms of source, degree of purity, state (free or immobilized), substrate, and reaction medium (solvent-free or biphasic).

A recent screening on latexes of *C. papaya* made it possible to count non-proteic molecular species such as saturated and unsaturated fatty acids, tocopherols, the tocotrienols, alcohols triterpenic, sterols and the possible presence of polyisoprene chains covalently bonded to phospholipid molecules forming a polymeric matrix (Barouh et al., 2010). The proteins quoted above bound once, confers a colloidal stability on latex, which makes the purification of the lipolytic enzymes present in the latex hard to achieve with common separation techniques (Azarkan et al., 2003; Dhouib et al., 2011). According to the literature, only three proteins with lipolytic properties present in the latex of *C. papaya* have been characterized through the aid of the recent sequencing of the *C. papaya* genome (Ming et al., 2008; Campillo-Alvarado and Tovar-Miranda, 2013), although without being purified to homogeneity. Among them GDSL-motif carboxylester hydrolase (CpEst) whose activity was found responsible for the hydrolysis of tributyrin and vinyl esters tested during the analysis. Besides, CpEst did not show a considerable specific activity (SA) towards long chain and medium chain TAGs, in contrast to the whole latex activity, acting as an esterase rather than a true lipase, which strongly suggested that total lipolytic activity in the crude latex could not be attributed to one enzyme (Abdelkafi et al., 2009). Another protein extracted from *C. papaya* is CpLip1 that is also likely to code for the *C. papaya* protein. CpLip1 was identified as a member of the castor bean acid lipase structural family and showed SA towards both short and long TAGs (Dhouib et al., 2011). Results from the literature showed that both CpLip1 and CpEst share a catalytic triad which is similar to that of serine proteases (Pleiss et al., 1998; Brady et al., 1990) - a nucleophilic serine (Ser) residue activated by a hydrogen bond in relay with histidine (His) and aspartate (Asp) in addition to a relatively hydrophilic oxyanion hole that forms hydrogen bonds to the tetrahedral intermediate. However, the active site varies in the amino acids location in the protein (Dodson and Wlodawer, 1998). A major difference between the two enzymes lies on the sequence that forms a “lid” of surface loop that surrounds the catalytic Ser that needs to undergo a conformational change before accessing the whole active domain (Dhouib et al., 2011). On the other hand, the putative structure model of CpEst suggested that the catalytic triad is exposed at the surface of the molecule without a “lid” domain and a binding site for long chain fatty acids (Abdelkafi et al., 2009).

C. papaya lipase (CPL) represents an emerging and versatile biocatalyst (Domínguez de María et al., 2006). This fact is confirmed from the high number of applications described in recent years (Foglia and Villeneuve, 1997a; Mangos et al., 1999; Campillo-Alvarado and Tovar-Miranda, 2013). Its availability as a “natural immobilized” catalyst, combined with a competitive price, makes CPL a promising catalyst in the biotransformations field. In fact, a study of the selectivity of different fatty acid ethyl esters in the CPL-catalysed interesterification of tripalmitin has been reported (Gandhi and Mukherjee, 2000a, b). Interestingly, the use of fatty acid ethyl esters as acyl donors led to a higher CPL selectivity towards the medium-/long-chain derivatives, as well as sn-1 selection. These results seem to be at odds with the other works developed with free fatty acids. A reactant-dependent positional specificity of lipases has been suggested for the explanation (Gandhi and Mukherjee, 2000a, b). Finally, the enzymatic transesterification of tricaprylin with various lauric acid derivatives as acyl donors gave good yields in terms of transesterified triacylglycerols. Vinyl laurate as the best acyl donor (Villeneuve et al., 1997b) presumably resulted from the irreversibility of that reaction, derived from the formation of vinyl alcohol which rapidly tautomerises to acetaldehyde, thus shifting the enzymatic reaction toward the products formation (Weber et al., 1997). Currently, applications regarding fats and oils modification, esterification in organic media, and asymmetric resolution of several chiral acids, as well as non-natural α-amino acids, have been also reported (Mukherjee and Kiewitt, 1996; Borgdorf and Warwel, 1999; Gandhi and Mukherjee, 2000a; Villeneuve et al., 2005).

Like *C. papaya* lipase a study on the catalytic properties of frangipani (*P. rubra*) latex lipase revealed that this latter lipase has a high capacity to catalyze fatty

acid esterification in solvent-free medium in less than an hour, with over 90% yield (Cambon et al*.,* 2006). Due to this capacity, this lipase could be used in a two-step biodiesel production process (hydrolysis and esterification) in association with a second lipase, such as that extracted from *Jatropha curcas*, that is highly active in TAG hydrolysis. Sousaa et al*.* (2010) recently showed that lipase from germinated *J. curcas* seeds could be used for TAG hydrolysis in a hydroesterification process, with 98% yields achieved after 2 h of reaction, without specificity with respect to the fat source used. Moreover, the catalytic activity of the lipase in crude babaco (*C. pentagona*) latex has been studied in transesterification and esterification reactions (Cambon et al., 2009; Dhuique-Mayer et al*.,* 2003). Cambon et al*.* (2009) showed that babaco lipase has catalytic activity during alcoholysis of sunflower seed oil with highly excessive amounts of various primary alcohols in a solvent-free system. Despite its sensitivity to short-chain alcohols such as methanol, the stepwise addition method curbs the inhibitory effect of methanol and enables transesterification yields of around 70% at 30°C after 15 h (Shimada et al*.*, 1999; Shimada et al., 2002).

Preliminary screening on *Euphorbia* species latex showed high lipolytic activity in *E. wulfenii*. For both *E. characias* and *E. wulfenii* latex it was find high lipolytic activity toward medium and long acylic chain triglycerides, but no hydrolytic activity on monoesters and phospholipids was detected (Palocci et al*.,* 2003). Moreover, no synthetic activity was pointed out using as substrates natural and endogenous terpenols (Palocci et al., 2003). The presence of esterase activity in latex of two *Euphorbia* species (*E. pulcherrima* and *E. lathirys*) was also described (Warnaar, 1987) suggesting that, *in vivo*, this activity could be involved in the hydrolysis reaction of triterpenol esters and the subsequent storage of free triterpenols inside the lipidic particles of latex. However, Palocci et al*.* (2003) demonstrated that such enzymes cannot be related to the terpenic metabolism. In fact the enzymes responsible for lipolytic activity in the latex of *E. characias* and *wulfenii* described in the work of Palocci et al*.* (2003) are not able to hydrolyse monoester or to synthesise terpenol esters starting from natural and endogenous terpenols. Moreover, in agreement with Warnaar's hypothesis (Warnaar, 1987), the relative terpenol ratio was constant during the biological cycle and the esterified terpenol fraction was present in negligible quantities for both species studied. On this basis lipolytic activity recovered in latex seems to be due to ''true lipases'' (Huang Anthony, 1984) acting on triglycerides probably present in latex (Hasma and Subramanian, 1986).

PLANT LATEX LIPASES PURIFICATION

Lipases have been purified from various plant parts (Seth et al., 2014). Many plant parts such as leaves of *Triticum* L. species (Kharazian et al., 2009), whole plant parts of *Ricinus communis* (Shahwar et al., 2010), oat bran, etc., are rich in phenolic content. This makes the purification step laborious and the yield is also very low when seed, leaf or latex is used for direct extraction. Plant seeds also contain high amount of lipids which is another associated major problem in plant lipase purification as such lipids interfere in SDS–PAGE. The consequence is a smeared discontinuous gel. Therefore, delipidation step becomes compulsory for extracting plant lipase prior to any other purification steps. This adds to the production cost and time-consuming.

As reported by Seth et al. (2014) a few exception most of the purification involves chromatography techniques. It is also visible that the yield is very low. Ben-Hamida and Mazliak (1985) reported that some of the traditional procedures such as clarification, precipitation, ultrafiltration, differential and density gradient centrifugations results in a low final yield of purified plant lipase. Alternatively, ion exchange and gel filtration chromatography used for purification of plant lipases results in good yield. Moreover, Lazreg-Aref et al. (2012) are recently purified lipase to homogeneity from *F. carica* L. latex of the Zidi variety from *Moraceae* family using silica gel chromatography (Table 2).

BIODIESEL PRODUCTION

The frequent and future scarcity of fossil fuels, combined with concerns over the consequences of dependency on this type of energy source, in terms of changes in the Earth's climate, has forced the world to find alternatives that are less harmful to the environment (de Araújo et al*.*, 2013). Renewable energy sources, especially vegetable fuel, have appeared as an important alternative (Santana et al*.*, 2010).

Biodiesel is made from renewable biological sources and it does not produce sulfur oxide and may reduce soot discharge by one third that of existing petroleum-based products (Ranganathan et al*.*, 2008). Biodiesel in industrial applications may be produced by chemical-catalyzed or enzyme-catalyzed methods. The biodiesel produced by chemical catalyst has several drawbacks such as difficulty in removal of acid or base catalysts from product, high energy requirements, difficulties in the recovery of the catalyst and potential pollution to the environment (Winayanuwattikun et al*.*, 2011; Tan et al*.*, 2010). Enzyme-catalyzed biodiesel production has received more attention because of its advantages, such as low energy consumption, mild operating conditions, nontoxicity, and environment friendly processes, as compared with the chemical-catalyzed method (Dwiarti et al*.*, 2010; Lee et al*.*, 2011). However, the enzyme-catalyzed method is not favored for industrial use because the high cost and low stability of lipases limit its potential application (Chen and Wu, 2003; Soumanou and Bornscheuer, 2003).

Table 2. Purification strategies for plant latex lipases.

Family	Plant sources	Purification steps	Fold increase/ yield	References
Euphorbiaceae	*E. characias*	Acetone/H_2O and silica column	NA	Palocci et al., 2003; Padiglia et al., 1998
Euphorbiaceae	E. *Wulfenii*	Acetone/H_2O and silica column	NA	Palocci et al., 2003; Padiglia et al., 1998
Moraceae	*Ficus carica L.*	Silica gel chromatography	8.5-fold/68.5%	Lazreg-Aref et al., 2012
Caricaceae	*Carica papaya*	Extraction in aqueous two-phase system	NA	Nitsawang et al., 2006
Caricaceae	Babaco or *Carica pentagona (Vasconcellea* x *Heilbornii Cv.)*	Extraction in aqueous two-phase system	15-fold/ NA	Chen et al., 2005
Apocynaceae	*Plumeria rubra*	Hexane/Steric exclusion chromatography	NA	Cambon, 2008

NA: Not available. Fold increase is the ratio of specific activity of the final purified product to the initial specific activity; and yield is the ratio of initial enzyme titer to the final titer obtained after the purification process.

Catalytic conversion techniques for transesterification

Alkalis used for transesterification of oil include NaOH, KOH, carbonates, and alkoxides such as sodium methoxide, sodium ethoxide, sodium propoxide, and sodium butoxide. Alkali-catalyzed transesterification proceeds approximately 4000 times faster than that catalyzed by the same amount of an acidic catalyst (Formo, 1954; de Araújo et al., 2013), and is thus most often used commercially. Potassium hydroxide (KOH) and sodium hydroxide (NaOH) are high sensitive to the purity of the reaction being affected by the water and free fatty acids contents (Marchetti et al., 2007). The presence of water may cause the ester saponification under alkaline conditions. Thus, the glycerides and alcohol must be substantially anhydrous because water causes a partial reaction change to saponification, which produces soap (Wright et al., 1944). Moreover, the free fatty acids can also react with the alkaline catalyst producing soaps and water. The saponification does not only use up the catalyst, but also causes the formation of emulsions which impair the biodiesel separation, recuperation and purification. Therefore, dehydrated vegetable oil with free fatty acids content lower than 1%, anhydrous catalysts and anhydrous alcohol are essential for the commercial feasibility of alkaline catalyst systems (Enweremadu and Mbarawa, 2009). Ma et al. (1998) suggested that the free fatty acid content of the refined oil should be as low as possible, below 0.5%, and Feuge and Grose (1949) also stressed the importance of oils being dry and free of free fatty acids. Freedman et al. (1984) reported that ester yields were significantly reduced if the reactants did not meet these requirements; sodium hydroxide or sodium metboxide reacted with moisture and carbon dioxide in the air, diminishing their effectiveness.

Studies report that acid catalysts are insensitive to the acidity value and are better than alkaline catalysts for vegetable oils with acidity value higher than 1% (Freedman et al., 1984).

Acids used for transesterification include sulfuric, phosphoric, hydrochloric, and organic sulfonic acids. Although transesterification by acid catalysis is much slower than that by alkali catalysis (Freedman et al., 1984; Ma and Hanna 1999; Srivastava and Prasad, 2000), acid-catalyzed transesterification is more suitable for glycerides that have relatively high free fatty acid contents and more water (Freedman et al., 1984; Aksoy et al., 1988). Aksoy et al. (1988) reported that it was necessary to perform transesterification under an acidic condition when the oil component was a low grade material such as sulphur olive oil. In general, the ethyl esters of monounsaturated or short-chain fatty acids with 2% sulfuric acid should make good alternative fuels (Klopfenstein and Walker, 1983). The transesterification by acid catalysis starts by mixing the oil directly with acidified alcohol, in a way that separation and transesterification can occur in a single step, being alcohol the esterification solvent and reagent (Cervero et al., 2008).

Researches on biodiesel have focused on the use of solid acid catalysts known as heterogeneous catalysts. Sulfonic resins, such as Nafion NR50, sulfated zirconia and tungstated zirconia may catalyze transesterification reactions as effectively as sulfuric acid (de Araújo et al., 2013). Studies report that the solid acid catalyst ideal to the transesterification of used cooking oil is expected to have features such as interconnected system of large pores, moderate and high concentrations of strong acids sites and hydrophobic surface (Lotero et al., 2005).

The advantages of using solid acids catalysts are insensitivity to acidity value; esterification and

transesterification may be carried out simultaneously; the catalyst is easy to be recovered; water washing biodiesel is unnecessary; generally high performance in esters; much lower catalyst requirements per tons of biodiesel produced than in other processes; and catalysts may be used for a longer period of time and are environmentally friendly. However, these systems operate under high temperature and pressure.

Although chemical transesterification using an alkali-catalysis process gives high conversion levels of triglycerides to their corresponding methyl esters in short reaction times, the reaction has several drawbacks: it is energy intensive, recovery of glycerol is difficult, the acidic or alkaline catalyst has to be removed from the product, alkaline wastewater requires treatment, and free fatty acids and water interfere with the reaction (Table 4).

Lipases are also able to effectively catalyze the transesteritication of triglycerides in either aqueous or nonaqueous systems, and as shown in Table 4, enzymatic tranesterification methods can overcome the problems mentioned above. In particular, it should be noted that the by-product, glycerol, can be easily recovered without any complex process, and also that free fatty acids contained in waste oils and fats can be completely converted to methyl esters. On the other hand, in general the production cost of a lipase catalyst is significantly greater than that of an alkaline one (Wu et al., 1999).

Moreover, the following advantages for the use of lipases can be mentioned (Marchetti et al., 2007).

i) Possibility of regeneration and reuse of immobilized waste, as it can be left in the reactor if reactivity is kept low.
ii) Higher enzyme thermal stability due to its inactive state.
iii) Easier separation from the product.

Some disadvantages include,

i) Loss of some initial activity due to the volume of oil molecule.
ii) Number of support enzymes is not uniform.
iii) Biocatalysts are more expensive than natural enzymes.

Non-catalytic conversion techniques for transesterification

To overcome delays in the initial reaction time caused by the low solubility of alcohol in the triglyceride phase the non-catalytic options are designed. A common approach consists in the use of a solvent soluble in methanol and oil. The result is a fast reaction, on the order of 5 to 10 min with no catalyst residues, in any phase. One of these cosolvents is the tetrahydrofuran, chosen, partially, due to its boiling point near that of methanol and the need of a very low operational temperature, around 301°C.

A second approach was developed by Saka and Kusdiana (Saka and Kusdiana, 2001) who made a fundamental study of biodiesel production in supercritical methanol. They demonstrated that preheating to a temperature of 350°C and treatment for 240 s in supercritical methanol was sufficient to convert rapeseed oil to methyl esters. Moreover, while the methyl esters produced were basically the same as those obtained in the conventional method with a basic catalyst, the methyl ester yield of the supercritical methanol method was higher. Kinetic analyses of the reactions in subcritical and supercritical methanol revealed that the rate of rapeseed oil conversion to methyl esters increased dramatically in the supercritical state. A reaction temperature of 350°C and a molar ratio of methanol to rapeseed oil of 42 to 1 were considered to be the best conditions (Kusdiana and Saka, 2001). Some advantages of its application are (Balat and Balat, 2008):

i) Glycerides and free fatty acids react with equivalent rates.
ii) The homogeneous phase eliminates diffusive problems.
iii) The process tolerates great percentages of water in the catalytic process of the feedstock requiring periodical removal of water or an intermediary phase to prevent catalyst deactivation.
iv) The catalyst removal phase is eliminated.
v) If a high methanol/oil ratio is used, the total oil conversion can be achieved in few minutes.

Despite all the above mentioned advantages, the supercritical methanol method has serious disadvantages, such as:

i) The process operates at extremely high pressures (25 to 40 MPa);
ii) The high temperatures (350 to 4001°C) result proportionally in high heating and cooling costs;
iii) High methanol: oil ratios (generally established at 42:1) involve high costs for the evaporation of the unreacted methanol.

POTENTIAL LATEX FOR BIODIESEL IN BENIN

Since last few years many laticifers plants have been identified all over the world and in Benin. These plants are distributed on all extent of the Benin territory. They often belong to the family of *Apocynaceae*, *Asclepiadaceae*, *Euphorbiaceae* or *Moraceae* (Table 3). Hence, focus needs to be shifted to lipases plants available in Benin and the details of such potential lipase plant are shown in Table 3. The listed species are used at various ends. They constitute a food, medicinal source as well that of wood for the populations (Table 3). The vegetable near total of the species of cover of Benin is used in traditional medicine by the local populations to fight against diseases (Agbahungba et al., 2001). Various

Table 3. Potential plant latex lipases in Benin.

Family	Botanical name	Common name	Distribution	Latex color	Measured parameters	Use	Part used	References
	Alstonia congensis	Afatin	Sèmè – Dangbo	White	Extraction of proteins; Acute toxicity test; Subacute test; Search for polyphenols by the reaction to ferric chloride; Search for alkaloids ; Search for flavonoïdes by the reaction to the cyanidine; Search for tanins (tanins cathechic by the reagent of STIASNY; tanins gallic); Search for substances quinoniques free and combined by the reaction of BORNTRAEGER; Search for polyterpenes and stéroïdes by the reaction of Libermann-buchard; Search for saponosides; Description of the macromolecules (Search for proteins by the method of LOWRY; Search for polysaccharides)	Treatment of diabetes, interior of plywood, lathed panel, packing-box factory, matches, light boats, moulding, piece of furniture running or elements, interior wood finishing, pencils	Bark, leaf	Ogbonnia et al., 2008; Fofana, 2004
Apocynaceae	*Holarrhena floribunda*	lètin wiwi ou akoyixè ou lengbagbé.	Calavi – Bohicon	White	Toxicity study; Crude protein, fat, crude fiber and total ash contents in the dried leaves were determined using the methods described by Association of Official Analytical Chemists (AOAC, 1990). Carbohydrate (nitrogen free extract) was determined by difference; that is, the sum of the crude protein, fat, crude fibre and total ash deducted from 100. Phytochemical analysis (Mayer, Dragendoff, Wagner and picric reagents were used to test for Alkaloid. Frothing test for saponin, ferric chloride test for tannin while Salkowski test for cardiac glycosides) DPPH radical assay; Lipid peroxidation and thiobarbituric acid reactions; Hydroxyl radical scavenging assay; Nitric oxide radical inhibition activity; Determination of total antioxidant capacity; Determination of total phenol; Reducing power	Veterinary healers, antioxidant, anti-dysenteric, diuretic and febrifuge, intestinal parasitoses, the ascite and sterility	Bark, leaves and roots	Tamboura et al., 2005; Badmus et al., 2010 ; Medecine douce - Medecine africaine, 2014
	Rauvolfia vomitoria	lètin, klanklan tin.	Calavi – Bohicon	White	Acute oral toxicity test; Phytochemical test; Phytochemical screening	Anticonvulsant, insomnia, depression and madness.	Leafs and roots	Amole et al., 2009 ; Medecine douce - Medecine africaine, 2014
	Saba comorensis	NA	Bassila	White	NA	Food, traditional medicine, oedema generalized	Fruit and Leafs	Olivier et al., 2012
	Thevetia peruviana	Tantohu (Fon) ; Olomiojo (Yoruba et Nagot) ; Batonè (Bariba),	Calavi	White	NA	Medicinal plant, laxative, emetic, look after the intermittent fevers	Bark, Leafs	Arbonnier, 2002, Schmelzer and Gurib-Fakim, 2006
Euphorbiaceae	*Anthostema aubryanum*	NA	Sakété	White	Diversity of the woody settlement of a dense forest in sub-wet zone; Cartography and floristic characterization of the marshy forest	Latex = poison; strong vermifuge activity counters the larvae of *Haemonchus contortus* in vitro;	Latex; bark; stem	Hecketsweiler, 1991; Nkeoua and Boundzanga, 1999; Adjakpa et al., 2011
	Euphorbia heterophylla	NA	Calavi	White	Chromatography (The crude methanolic and aqueous extracts were subjected to phytochemical screening); Anti-inflammatory activity	Medicinal use; treatment of constipation, bronchitis and asthma	Leaves; fruits; flowers	Falodun et al., 2004; Falodun et al., 2003; Falodun et al., 2006

Table 3. Contd.

	Milicia excelsa	Iroko/Lokotin	Calavi – Bohicon	White	NA	Exterior wood finishing: Parquet floor Shipbuilding (bordered and bridge);Cabinet work (piece of furniture of luxury); Interior wood finishing: Distinct plating; Skirting; Light frame		CIRAD, 2011
Sapotaceae	*Vitellania poradoxa*	NA	Dan	White	NA	Food, medicinal and cosmetic	Fruits, bark	Medecine douce - Medecine africaine 2014
	Manilkara multinervis	NA	Natitingou	White	NA	Piles in the construction of the houses on pile, Clothes industry of the frame of the houses and the attics	Wood	Medecine douce - Medecine africaine 2014; Agbahungba et al., 1998

Table 4. Comparison between alkali-catalysis and lipase-catalysis methods for biodiesel fuel production (Fukuda et al., 2001; Mounguengui et al., 2013).

Composition	Alkali-catalysis process	Lipase-catalysis process
Reaction temperature	60-70°C	30-40°C
Free fatty acids in raw materials	Saponified products	Methyl esters
Water in raw materials	Interference with the reaction	No influence
Yield of methyl esters	Normal	Higher
Recovery of glycerol	Difficult	Easy
Purification of methyl esters	Repeated washing	None
Production cost of catalyst	Cheap	Relatively expensive

bodies of the plants are used for this purpose to know: sheets, fruits, flowers, barks and roots (Azonkponon, 2001). 92.86% of the species are used as wood energy. With the exception of the species taboos which vary according to the various ethnos groups, all the species of trees are used as and charcoal firewood. 39.29% are used as edible plants (Houngnon, 1981; Sokpon and Lejoly, 1996), 35.21% like work wood, 17.87% as service wood (return in this category all wood which are used in the clothes industry of the frame of the houses and the attics) and 3.57% in local arts and crafts (Dossou et al., 2012).

Conclusion

Plants produce a diverse range of bioactive molecules, making them rich source of different types of bio-catalyst. It is the case of lipases which are now widely used in various industry sectors such as in detergency applications, fatty wastes treatments, pharmaceutical syntheses or oils and fats modifications.

The lipolytic enzymes of laticifers plants have demonstrated to be versatile biocatalysts with the ability to discriminate a wide number of substrates from various applications. In addition, this lipase aggregate holds important advantages over other microbial, animal and plant lipases being its sustainable availability from agro-waste, its "self-immobilized" nature which conferring the ability to work under a broad range of environments and its easy pretreatments being the most remarkable features. Notwithstanding the enormous potential of the lipases present in laticifers plants, the lack of experimentation carried out at the industrial scale prevents its implementation in various bioprocesses, such as the production of high value lipids with improved properties, the manufacture of medical articles and biodiesel engineering.

Till date, a very large majority of lipases that are used in these processes are obtained from microbial sources. Comparatively, the use of plant lipases is much less developed. However, plant enzymes can be also envisaged as biocatalysts for lipids bioconversions. Especially, high activities in hydrolysis and synthesis reactions have been found in some laticifer plants like *C. papaya* and *E. characias*. Concerning the former, favourable applications in the synthesis of low-calorie TAGs,

medium chain TAGs or for the production of conjugated linoleic acids enriched TAGs were reported. Among the *Caricaceae* family, it was shown recently that the unripe fruit of the babaco plant (*Vasconcellea* x *heilbornii*; ex. *C. pentagona*), native to the subtropical mountains of Equator, contains a latex similar to that in *C. papaya*. This latex also displays a strong lipolytic activity which was characterized in terms of biocatalytic activity and selectivity.

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Conflict of Interests

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