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Comparison of the Lipase Activity in Hydrolysis and Acyl Transfer Reactions of Two Latex Plant Extracts from Babaco (*Vasconcellea* × *Heilbornii* Cv.) and *Plumeria rubra*: Effect of the Aqueous Microenvironment

EMMANUELLE CAMBON,† FANNY GOUZOU,† MICHEL PINA,† BRUNO BAREA,† NATHALIE BAROUH,† REGINA LAGO,† JENNY RUALES,§ SHAU-WEI TSAI,# AND PIERRE VILLENEUVE*,†

UMR IATE, Laboratoire de Lipotechnie, CIRAD, TA 40/16, 73 rue Jean François Breton, 34398 Montpellier Cedex 5, France; Departamento de Ciencia de Alimentos y Biotecnología, Escuela Politecnica Nacional, P.O. Box 17 01 2759, Quito, Ecuador; Department of Chemical Engineering, National Cheng Kung University, Tainan 70101, Taiwan

The enzymatic properties of *Plumeria rubra* latex have been evaluated for the first time, showing a high activity in both hydrolysis and synthesis reactions, and compared to the biocatalytic behavior of babaco (*Vasconcellea* × *Heilbornii* cv.) latex. Both biocatalysts have been optimized by studying the various parameters that influence reaction kinetics. The optimum temperatures for hydrolysis reactions were 50 and 55 °C for babaco and *Plumeria*, respectively. 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 such as alcoholysis and interesterification, 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 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. 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.

KEYWORDS: Lipase; latex; thermodynamic water activity; acyl transfer; water sorption/desorption

INTRODUCTION

Lipases, triacylglycerol ester hydrolase (E.C. 3.1.1.3), are enzymes that catalyze the cleavage of triacylglycerol ester bonds, acting at the water/oil interface (*1*). They are used in synthesis reactions and have a number of applications in food products, cosmetics, and pharmaceuticals (*2*). Lipases have several advantages over classical chemical catalysts. For example, enzymatic reactions are carried out under milder conditions, the formation of side products is usually avoided, and kinetic control is easier. Also, owing to their specificities (acyl specificity, regioselectivity, stereoselectivity), it is possible to use them in biocatalytic processes to precisely modify the fatty acid composition and regiodistribution of oils and fats to obtain new products with designed physical and chemical properties (*3*−*6*). The lipases generally used in industry come from microbial or animal sources (*7*, *8*). Nevertheless, although they have been studied to a much lesser degree, it has been shown that plant lipases can exhibit high biocatalytic activities and may have advantages over animal and microbial enzymes due to their availability and relative ease of purification (*5*, *9*). The lipolytic activity of a few plant extracts has been studied. In particular, some laticifer plants such as *Carica papaya* (*10*) and *Euphorbia characias* (*11*) have been found to have high activity in hydrolysis and synthesis reactions. Studies carried out on *C. papaya* showed that its crude latex has a very strong activity on short-chain triacylglycerol (TAG) and *sn*3 stereoselectivity in hydrolysis reactions of chiral TAG substrates (*12*). These observations led to industrial use of this latex in particular applications, such as the synthesis of low-calorie triacylglycerols

* Corresponding author [telephone + 33-(0)4 67 61 55 18; fax +33 (0)4 67 61 55 15; e-mail villeneuve@cirad.fr].
† UMR IATE.
§ Escuela Politecnica Nacional.
# National Cheng Kung University.

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(*13*) or medium-chain TAG (*14*). These results have prompted interest in other latex plant extracts, in particular, the unripe fruit of the babaco plant (*Vasconcellea* × *Heilbornii* cv., ex *Carica pentagona Heilbornii*) (*15*), 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 (*16*, *17*). To control the equilibrium between hydrolysis and synthesis reactions, one of the essential parameters that has to be monitored is the aqueous microenvironment of the biocatalyst through its thermodynamic water activity (a_w) and water sorption/desorption isotherms. Indeed, some studies have reported that a_w conditions the lipase structure and thus its enzymatic activity both in hydrolysis and in synthesis reactions (*18*). For synthesis reactions, the aqueous phase in the reaction medium must be limited to the one necessary for a good enzyme folding; otherwise it is used as a reaction substrate, shifting the equilibrium to the hydrolysis reaction. Studying the changes in synthesis yield as a function of a_w enables the optimum initial a_w value to be established. The latter can then be correlated with biocatalyst water content using water sorption and desorption kinetics (*19*−*22*).

In this study, the lipolytic action of babaco latex was optimized for hydrolysis and synthesis reactions and compared to the plant latex from *Plumeria rubra*. *P. rubra* is a tropical flowering tree, also called *frangipani*, belonging to the Apocynaceae family. In this case the latex is present inside the trunk and branches and, as far as we know, is studied here for the first time. The effect of the aqueous environment on the biocatalytic activity of these plant lipases in acyl transfer reactions was investigated.

MATERIALS AND METHODS

Materials. Chemicals and substrates (purity > 99%) were purchased from Sigma (St. Louis, MO). Butanol (HPLC grade, >99.8% purity) was stabilized and dried over Na_2SO_4 before use. Babaco latex was collected and dried near Quito in Combaya province, Ecuador. The fresh latex was obtained by making three longitudinal incisions on the epidermis of the unripe fruit. *P. rubra* latex was obtained by making incisions on the trunk and branches of bushes found in Taiwan and was dried. Both types of latex were lyophilized and ground before use.

Hydrolysis Reactions. *Measurement of Lipolytic Activity at Different Temperatures.* Sunflower oil or pure monolein or diolein (respectively, 2 and 10 mL for reactions with babaco and *Plumeria* latex) was emulsified mechanically in 40 mL of aqueous NaCl solution (0.15 M) and 10% (w/w) arabic gum with magnetic stirring at 300 rpm. Reactions were carried out at pH 8 and different temperatures using various amounts (from 0.5 to 5% w/w of substrate) of biocatalyst. To know the minimum substrate concentration in the medium corresponding to saturation, reactions were observed at 50 °C with different amounts of oil. The free fatty acids released were titrated automatically with an aqueous NaOH solution (0.1 M) using a pH stat equipped with a pH glass electrode and a thermostated cuvette. Lipolytic activity (based on the weight of latex used) was calculated from the initial velocity; one international unit corresponds to 1 μmol of fatty acids released per minute.

Measurement of Free Fatty Acids after Incubation of Sunflower Oil in Different Buffers with the Tested Latex. Sunflower oil (2 mL) was emulsified with 2 mL of various buffer solutions at different fixed pH values. Latex powder was then added (40 mg of babaco latex or 20 mg of *Plumeria* latex). The reaction medium was stirred in an orbital incubator (300 rpm) at 50 °C for 60 min for reactions with babaco latex and at 60 °C for 30 min with *Plumeria* latex. The medium was centrifuged for 10 min at 2200 rpm at 4 °C. After the addition of 40 mL of diethyl ether/ethanol (50:50, v/v), the organic phase was titrated with NaOH (0.1 M) in the presence of phenolphthalein.

Thin-Layer Chromatography (TLC) Analysis. TLC equipment was used to evaluate the nonpolar lipid composition of hydrolyzed sunflower oil. Aliquot fractions (10 μg) were removed periodically from the reaction mixtures and diluted in 1 mL of hexane. Samples (2 μL) were then applied with an automatic applicator (Linomat IV, Camag Ltd., Muttenz, Switzerland) on preparative silica plates (20 × 10 cm, silica gel F254, 0.25 mm layer thickness, Merck, Darmstadt, Germany). Development was carried out with hexane/diethyl ether/acetic acid 70:30:1 (v/v/v). Spots were visualized by spraying with a solution of copper sulfate/phosphoric acid (50:50, v/v) and exposure at 180 °C for 10 min.

Measurement of Biocatalyst Water Sorption and Desorption Isotherms. The DVS-1 automated moisture sorption analyzer (Surface Measurement Systems Ltd., London, U.K.) was used to evaluate the water sorption and desorption of plant extracts. At 25 °C, the instrument has a working humidity range from 3 to 98%; the required humidity is obtained by mixing dried and saturated vapor gas flows in the right proportions using two mass flow controllers and one vapor humidifier (total flow rate of 200 sccm). For the measurements, 40 mg of dried latex was used. This last one was obtained after incubation of crude latex in a desiccator containing silica. Moreover, the sorption analysis began with an 8-h step with dried air. After that, nine humidity steps with a relative humidity range from 10 to 90% (for sorption) and from 80 to 3% (for desorption) were chosen. A microbalance (sensitivity of 0.1 μg) inside the incubator measured the mass difference between the dried and the humidified latex. These measures are reported as the mass of water (g) incorporated inside 100 g of dried latex.

Synthesis Reactions in Solvent-free Systems Using Biocatalyst Preincubated at Different Relative Humidities. *Equilibration of Biocatalysts at Several a_w Values and Measurements of a_w.* Biocatalyst extracts were preincubated at 25 °C in a desiccator containing different saturated salt solutions for at least 2 weeks. The nature of the salt imposed a specific thermodynamic water activity (a_w). The salts used were LiCl (a_w = 0.12), potassium acetate (a_w = 0.22), $MgCl_2$ (a_w = 0.33), K_2CO_3 (a_w = 0.44), $Mg(NO_3)_2$ (a_w = 0.55), and NaCl (a_w = 0.75). The a_w values of the preincubated enzymatic extracts were verified using an aqua-lab system (FA-st/1, GBX, Romans, France).

Esterification and Alcoholysis Reactions. Synthesis reactions were carried out with butanol (20 mmol) and lauric acid (1 mmol) for esterification reactions or triolein (0.33 mmol) for alcoholysis reactions. Substrates in solvent-free systems were mixed in an orbital stirred incubator (300 rpm) at a fixed temperature depending on the biocatalyst (50 °C for babaco latex and 60 °C for *Plumeria* latex). Reactions were started by adding 10% (w/w of total substrate) of biocatalyst extract pre-equilibrated at various a_w values. At intervals, 25-μL samples were removed, diluted in 1 mL of hexane, and filtered (Millex 0.45 μm, Millipore, Bedford, MA). The resulting solutions (100 μL) were diluted in 1 mL of hexane and 0.5-μL aliquot fractions were analyzed by gas chromatography (GC).

Interesterification Reaction. Reactions, carried out in a solvent-free system with tricaprylin (0.2 mmol) and trimyristin (0.1 mmol), were incubated in an orbital stirred incubator (300 rpm) at 60 °C. Reactions were started by adding 10% (w/w of total substrate) of biocatalyst extract pre-equilibrated at various a_w values. At intervals, 75-μL samples were removed, diluted in 2.5 mL of hexane, and filtered (Millex 0.45 μm, Millipore). Aliquot fractions of 0.5 μL were analyzed by GC.

GC Analysis. GC was performed using a Carlo Erba instrument, model HRGC (Erba Science, Paris, France), with a cold on-column capillary injector and an Rtx-1 dimethyl polysiloxane capillary column (Restek, Bellefonte, PA): length, 3 m; internal diameter, 0.32 mm; film thickness, 0.25 μm. The chromatographic conditions used were as follows: cold on-column injection, flame ionization detection at 350 °C, and helium carrier gas at 5.5 mL min^{-1}. The oven temperature program was as follows: for esterification reactions, 110 °C isotherm; for alcoholysis reactions, initial temperature 100 °C for 1 min, from 100 to 200 °C at 20 °C/min, from 200 to 340 °C at 10 °C/min, 20 min at 340 °C; for interesterification reactions, initial temperature, 200 °C for 1 min, from 200 to 340 °C at 20 °C/min, 4 min at 340 °C.

All of the experiments described were performed in triplicate, and the results given correspond to the mean value of three determinations.

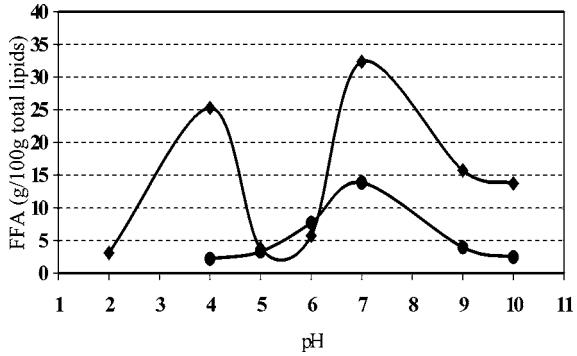

Figure 1. Influence of pH on hydrolysis reactions biocatalyzed by babaco (●) and *Plumeria* (◆) latex. Reactions were carried out using an emulsion of sunflower oil (2 mL) in 2 mL of selected buffers at different pH values with 2% babaco latex at 50 °C or 1% *Plumeria* latex at 60 °C.

RESULTS

Characterization of Babaco and *Plumeria* Latex in Hydrolysis Reactions: the Influence of Temperature and pH. The lipolytic activities of babaco and *Plumeria* latex were first measured using sunflower oil as substrate at pH 8 and at temperatures varying from 30 to 70 °C. 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). The influence of pH was then evaluated by measuring free fatty acids (FFA) released during the hydrolysis of sunflower oil in different buffer solutions with crude dried latex (**Figure 1**). In the case of babaco latex, the highest yield was obtained for reactions carried out at pH 7 (14% of FFAs released after 1 h) showing that, under such conditions, the ionization of the enzyme chemical groups leads to optimal biocatalytic function of the protein. At pH 5 and 9, only 3.5 and 4% fatty acids were released from the glycerol backbone, respectively, corresponding to a 75% loss of hydrolysis activity compared to the optimum pH value. Two optimal pH values (4 and 7) were observed for *Plumeria* latex.

Best conditions are obtained in reactions with an optimized biocatalyst when the enzymes are saturated with substrate and when the quantity of biocatalyst is within the range where the relationship between activity and the amount of enzyme is linear. Otherwise, lipolytic activity would be underestimated. We found that babaco and *Plumeria* latex lipolytic enzymes are saturated with substrate at triacylglycerol concentrations from, respectively, 60 and 200 mM.

Determination of the Lipid Classes Formed during Hydrolysis of Sunflower Oil by Babaco and *Plumeria* Latex. The products formed during partial enzymatic hydrolysis of sunflower oil were analyzed by TLC. For reactions carried out with babaco latex, a classical hydrolysis pattern was observed, with gradual appearance of FFA, diacylglycerols (DAG), and monoacylglycerols (MAG), while the triacylglycerol band intensity decreased as hydrolysis progressed (**Figure 2**). On the other hand, MAG and DAG bands were not clearly visible during hydrolysis with *Plumeria* latex. At first, it was assumed that *Plumeria* latex lipase activity exhibited a strong substrate specificity with a significant preferential activity on partial glycerides (mono- and acylglycerols) compared to TAG. Indeed, such specificity would explain the particular profile of TLC plates obtained for the sunflower oil hydrolysis. In this scenario, as soon as partial glycerides are formed from TAG hydrolysis,

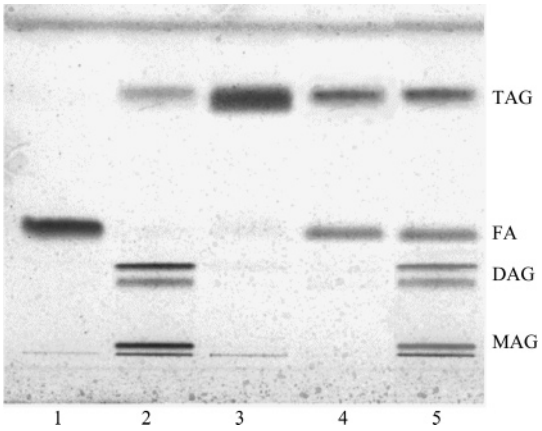

Figure 2. TLC plate to analyze the lipid composition after partial hydrolysis of sunflower oil using babaco and *Plumeria* latex as biocatalysts: lane 1, fatty acid standards (Sigma); lane 2, mono-olein standards (Sigma); lane 3, blank reaction in the absence of biocatalyst; lane 4, *Plumeria*-catalyzed reaction; lane 5, babaco-catalyzed reaction. [Reactions were carried out using a water/sunflower oil emulsion with 1.5% (w/w) biocatalyst; MAG, monoacylglycerols; DAG, diacylglycerols; TAG, triacylglycerols; FA, fatty acids.]

they would be cleaved (competing with the initial TAG substrates). However, when lipase activity on sunflower oil was compared with that on pure mono- or diolein substrates (data not shown), we observed that the kinetics of FFA release were equivalent in terms of lipolytic units (respectively, 8 and 7% of FFA released after 5 min in reactions conducted at 50 °C and pH 7.5). Therefore, these results did not confirm the potential substrate specificity of the *Plumeria* enzymatic powder.

Influence of Initial Thermodynamic Water Activity (a_w) on Fatty Acid Ester Synthesis Reactions Catalyzed by Babaco and *Plumeria* Latex. The influence of thermodynamic water activity on synthesis reactions catalyzed by the two types of crude latex was evaluated. Generally, such experiments can be done by measuring the a_w of the entire reaction medium (*21*, *22*) or by controlling the initial a_w of the biocatalyst in question. In our case, because the reaction medium was composed of only anhydrous reactants (dry butanol and dry oils), with no added solvent, we estimated that the water present in the system could be provided only by the biocatalyst protein matrix itself. Therefore, in light of the previous work on *C. papaya* lipase (*19*), we considered that the effect of thermodynamic water activity on enzyme biocatalytic activity had to be preferentially investigated with latex enzymatic powders preequilibrated at different a_w values. For both reactions catalyzed by crude babaco latex, esterification and alcoholysis, the kinetic curves showing the quantity of fatty acids released as a function of time were typical of enzymatic reactions. The most appropriate a_w values were observed to range from 0.3 to 0.5, with an optimal value of 0.38 (**Figure 3**).

Results obtained with crude *Plumeria* latex were very different from the results with babaco. During esterification experiments, the equilibrium between hydrolysis and esterification was unstable (**Figure 4**). With all of the tested a_w values, the esterification seemed to be very fast during the first minutes, with high production of butyl laurate. However, for prolonged reaction times, all newly synthesized esters were hydrolyzed. Afterward, strong competition was observed between esterification of lauric acid and hydrolysis of butyl laurate as lipase substrates. These surprising results were repeated three times and at two temperatures, 50 and 60 °C. We also checked that the ester seen on GC did not come from the lyophilized latex

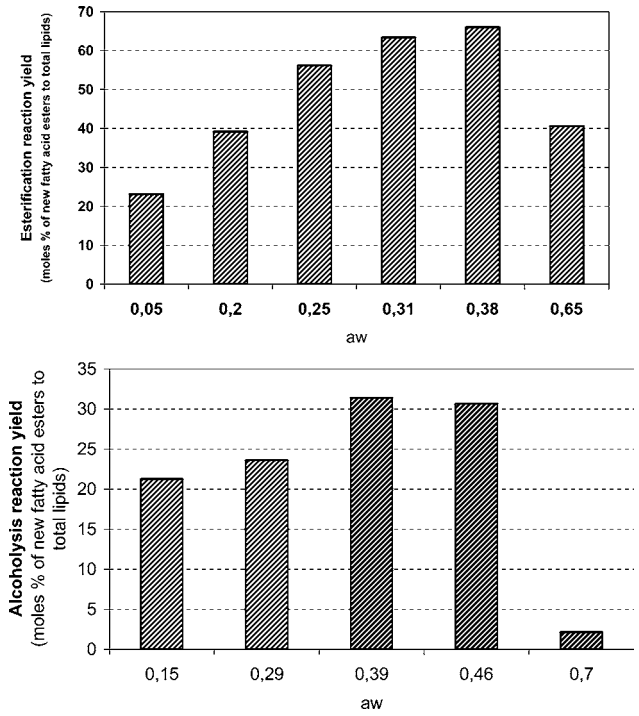

Figure 3. Influence of the thermodynamic water activity on the yield of esterification (**a**) and alcoholysis (**b**) for reactions catalyzed by babaco latex. Reaction conditions: for esterification, lauric acid/butanol 1:20 molar ratio, 50 °C, 7 h, 10% w/w biocatalyst; for alcoholysis, triolein/butanol 0.33:20 molar ratio, 50 °C, 24 h, 10% w/w biocatalyst.

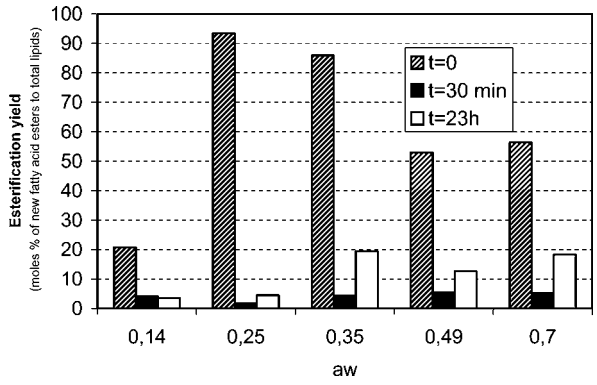

Figure 4. Influence of the thermodynamic water activity on the yield of esterification catalyzed by *Plumeria* latex. Reaction conditions: lauric acid/butanol 1:20 molar ratio, 60 °C, 10% w/w biocatalyst.

used as biocatalyst. This phenomenon did not appear with a non-water-producing acyl transfer reaction such as interesterification between dried tricaprylin and trimyristin. Graphs of synthesized esters plotted against time showed typical reaction kinetics, with final reaction yields of 70% (mole percent of new triacylglycerols to total lipids) (data not shown). The interesterification yields were shown to be strongly dependent on the a_w, with a maximal yield obtained for an a_w of 0.44 (**Figure 5**).

Water Sorption/Desorption Isotherm of Crude Babaco and *Plumeria* Preparations. To correlate these data to the water content of the biocatalysts, sorption and desorption isotherms were obtained with an automated DVS-1. The kinetics of water sorption were measured at increasing relative humidities (RH) (3−90% corresponding to a_w values of 0.03−0.9, respectively) and decreasing RHs (from 80 to 3%, a_w values from 0.8 to 0.03).

During the sorption isotherm, the weight of babaco latex increased and each RH step needed more time to reach

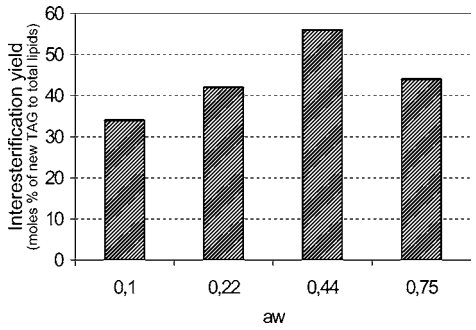

Figure 5. Influence of the thermodynamic water activity on the yield of interesterification catalyzed by *Plumeria* latex. Reaction conditions: tricaprylin/trimyristin 2:1 molar ratio, 60 °C, 7 h 10% w/w biocatalyst.

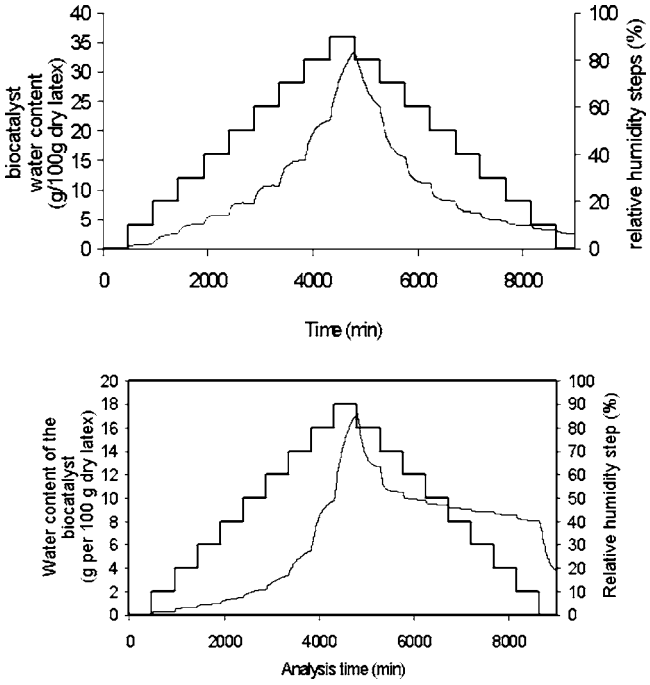

Figure 6. Water sorption and desorption kinetics of crude babaco latex (**a**) and crude *Plumeria* latex (**b**) measured using a DVS-1 automatic sorption analyzer: stippled curve, biocatalyst water sorption and desorption kinetics; solid curve, relative humidity steps.

equilibrium (**Figure 6a**). Eight-hour steps were sufficient until a RH of 70. For the two last steps, equilibrium was not reached. For a given RH, depending on the curve studied (sorption or desorption), the water content can be quite different, highlighting the importance of the hydration history. This indicates that when the latex is incubated up to a relative humidity of 90%, part of the water absorbed becomes irreversibly associated with it. This phenomenon, first observed in the papaya latex, causes a change in the physical state of the latex and a partial loss of its enzymatic activity when it is incubated at high RH (*19*). However, for babaco latex, we observed that the sorption and desorption curves were quite similar and symmetrical, showing that the irreversible association of water with this enzymatic powder is very limited (**Figure 7**).

In contrast, with *Plumeria* latex powder, this irreversible phenomenon did exist and was very marked (**Figure 6b**). Indeed, a very difficult water desorption curve was observed, indicating that water had become entrapped in the latex matrix.

DISCUSSION

It was shown that best hydrolysis activities are obtained at pH 7 at a temperature of 50 °C and at pH 7 at 55 °C for babaco

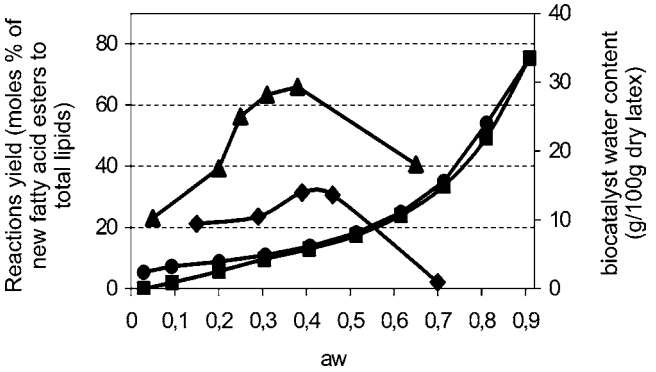

Figure 7. Influence of varying the crude babaco latex aqueous state (a_w and water content) on the yield of esterification (▲) and alcoholysis (◆) reactions: water sorption isotherm of the biocatalyst (■); water desorption isotherm (●).

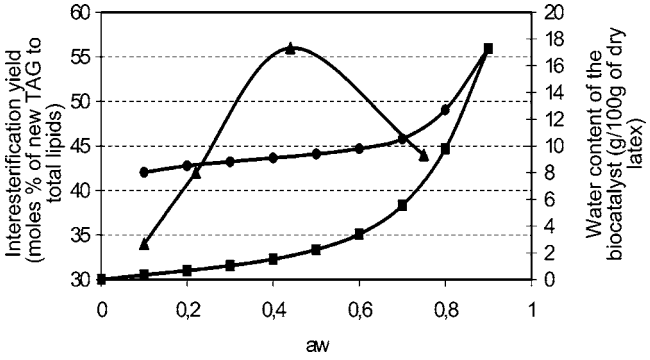

Figure 8. Influence of varying of crude *Plumeria* latex aqueous state (a_w and water content) on the yield of interesterification reaction (▲): water sorption isotherm of the biocatalyst (■); water desorption isotherm (●).

and *Plumeria* latex, respectively. The substrate concentration needed to be minimally 60 and 200 mM triacylglycerol and the maximum quantity of enzyme 2% (g/g substrate). The lipolytic activities of babaco latex follow standard curves for pH and temperature. In contrast, *Plumeria* latex exhibited unusual behavior, with two optimal pH values for hydrolysis (4 and 7). This might be due to the presence of two distinct lipases being active at different pH values.

We obtained water sorption and desorption isotherms from moisture sorption and desorption kinetics, tracing the water content of latex as a function of a_w. These isotherms could be correlated with synthesis yields for latex-catalyzed reactions (**Figures 7** and **8**). To optimize the a_w, two types of reactions were carried out: esterifications between lauric acid and butanol, producing ester and water, and acyl transfer reactions such as alcoholysis, by which an acyl group is transferred from the triacylglycerol backbone to butanol, or interesterifications corresponding to acyl exchanges between two triacylglycerols. In the second reaction type, no water molecules are formed. In reactions catalyzed by babaco latex, the optimal a_w values for the enzymatic powder were approximately the same for both reactions (around 0.4). Thus, we showed that water production did not change the optimal a_w, confirming that for babaco latex the crucial parameter to monitor when using such enzymatic powder is the initial powder hydration state of the biocatalyst and, more precisely, its thermodynamic water activity. Concurrently, babaco latex water sorption /desorption (**Figure 7**) showed two distinct parts: a linear portion (for a_w values between 0 and 0.4) followed by an exponential portion ($a_w >$ 0.4). This kind of plot has already been observed, first on microbial lipase preparations (*Rhizomucor mihei*, Lipozyme TM, *Candida deformans*, and *Rhizopus arrhizus*) (*20*, *23*) and later on *C. papaya* latex (*19*). It was shown that the first part corresponds to high-energy interactions, such as hydrogen bonds, between water and latex molecules. The second part corresponds to lower energy interactions, such as capillary or osmotic interactions. The superposition of plots showing esterification and alcoholysis yields in terms of a_w with sorption isotherms showed that the maximal synthesis yield was obtained for a_w values of around 0.4 (0.38), corresponding to an a_w/water content pair situated at the end of the linear portion of the water sorption curve. The corresponding water content would allow optimal protein folding with a maximum catalytic capacity, without being used as a substrate to hydrolyze newly synthesized fatty acid esters.

This correlation between latex water content and optimal a_w does not apply to *Plumeria* latex (**Figure 8**). In this particular case, the sorption and desorption plots were not symmetrical. This phenomenon could explain the atypical biocatalytic behavior of *Plumeria* latex. Indeed, this water sorption/desorption experiment tends to demonstrate that water molecules formed during the esterification reaction that we described previously were in fact directly absorbed by the *Plumeria* latex through hydrogen or capillary bonds. Those water molecules closely entrapped in the latex matrix could then be preferentially used as substrate molecules to hydrolyze the newly synthesized substrate molecules. Subsequently, equilibrium would establish between fatty acid and fatty acid ester production, with a high rate of hydrolysis compared to synthesis.

Another consequence of water molecules being trapped in the latex is that its a_w and water content change significantly during reactions when water is released, such as esterification. Indeed, at the theoretical completion of the esterification reaction (100% yield), 1 mmol of water would be formed. If one considers that all water molecules produced become trapped inside the latex, the humidity of the plant extract would increase by 10% (g/g latex). For a biocatalyst preincubated at a RH of 40%, its a_w could vary from 0.4 to 0.9. Consequently, the moisture sorption isotherm cannot predict the optimal initial a_w for the best esterification reaction yield. The optimization of this parameter must be done using synthesis reactions that do not produce water, such as interesterification. In this case, the observed behavior of *Plumeria* is more similar to that obtained with babaco and the classical behavior of various latex lipases that have previously been studied (*5*). However, the initial a_w also varies as a function of the hydration history of the latex. As a consequence, the initial a_w value alone is not sufficient to predict the biocatalytic behavior of the enzyme and must be correlated with the true initial water content of the enzymatic powder.

If *Plumeria* latex could trap not only water molecules but, in a more general way, molecules with hydroxyl groups, through hydrogen bonding, the binding that occurs between water and latex molecules could also occur between partial glycerides and the latex matrix. Consequently, the results observed with the TLC analysis to determine the lipid class of the hydrolysis products (**Figure 2**), which showed an absence of MAG and DAG bands on silica plates, could be explained by the fact that such partial glycerides were in fact trapped in the latex matrix.

The phenomenon of water trapping by *Plumeria* latex could explain the nonequilibrium between esterification and hydrolysis. Additionally, it is worth noting that such behavior could also be derived from other properties of *Plumeria* latex, such as the presence of two lipases, as suspected in light of the results obtained for the influence of pH on lipolytic activity. One could

imagine that in the particular conditions used in synthesis reactions, the observed products present in the reaction medium result from different enzymatic activities.

In conclusion, this study highlights the importance of determining crucial biocatalytic parameters, such as water activity optima and water content, when using such plant-derived enzymatic latex powders. A good knowledge of the water sorption and desorption behavior of the biocatalyst that one intends to use is of paramount importance, especially when the sorption and desorption are not symmetrical, as is the case herein with *Plumeria* latex.

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