

Lipase activity in alcoholysis and esterification reactions of crude latex from babaco fruit (*Carica pentagona*)

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Résumé : L'activité lipasique du latex brut de la plante subtropicale de Babaco (*Carica pentagona*) est étudiée dans des réactions d'alcoololyse et d'estérification. Les résultats indiquent que le latex brut de *Carica pentagona* présente des propriétés biocatalytiques équivalentes au latex brut de *Carica papaya* dans des réactions de synthèse telles que l'alcoololyse de triacylglycérols et la réaction d'estérification (les rendements de réaction sont respectivement 72,3 % et 70,2 % après 3 h pour l'alcoololyse et 31,8 % et 33,8 % après 24 h pour la réaction d'estérification). Par contre, la papaine brute commerciale donne des rendements de réaction beaucoup plus bas que les latex bruts des deux espèces de *Carica* (28,9 % en alcoololyse et 5,4 % en estérification).

Summary : Lipase activity in alcoholysis and esterification reactions were studied for crude latex from the subtropical Babaco plant (*Carica pentagona*). The results reveal that crude *Carica pentagona* latex exhibits equivalent biocatalytic properties in synthesis reaction via triacylglycerol alcoholysis and in esterification reaction compared to the crude latex from *Carica papaya* (the reaction yields are 72.3% and 70.2% respectively after 3 hrs for alcoholysis and 31.8% and 33.8% for esterification after 24 hrs). In contrast, commercially crude papain expresses lower reaction yield than crude latices from the two *Carica* species (28.9% in alcoholysis and 5.4% in esterification).

Mots-clés : Babaco, *Carica pentagona*, *Carica papaya*, activité lipasique, alcoololyse, estérification

Keywords : Babaco, *Carica pentagona*, *Carica papaya*, lipase activity, alcoholysis, esterification

ARTICLE

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The Babaco (*Carica pentagona* Heilborn) plant is a member of the papaya family (*Caricaceae*) native to the subtropical mountains of Ecuador. The unripe fruit of this natural hybrid (*Carica stipulata* Badillo and *Carica pubescens* Lenne and Koch [1]) exudes a latex similar to those in *Carica papaya* [2]. As in *Carica papaya*, biocatalytic activities of this latex are attributed to the presence of lipases [3, 4]. In fact, these crude latex are an enzymatic cocktail with several proteases (such as papain - EC.3.4.22.2 - and chymopapain - EC.3.4.22.6 - in *Carica papaya*), lipases and probably other enzymes. Recent studies showed that crude latex from *Carica papaya* was an efficient catalyst for various enzymatic processes involving oil and fat and demonstrated that this crude latex exhibit good lipase activity in hydrolysis, interesterification and esterification reactions ([5, 4, 6, 6bis, 7]. In a previous work, biocatalytic properties of crude latex from babaco fruit were demonstrated in proteolysis, lipolysis, and interesterification reactions [8]. In the present paper, we extended the study of crude latex from *Carica pentagona* in triacylglycerols alcoholysis reaction and esterification reactions performed in solvent free system. In order, to promote babaco culture in Ecuador and to find others sources of plant lipases, this work underlines the lipase activity in crude latex from babaco fruit. A comparison is made between commercially-available crude papain (dried latex from *Carica papaya* commercially available as crude papain), crude latex from *Carica papaya* and crude latex from *Carica pentagona*.

Materials and methods

Enzymes

Commercially available crude papain (EC 3.4.22.2) preparation is purchased from Sigma with reference P-3375. *Carica pentagona* latex was collected and dried near Quito in Combaya province, Ecuador. *Carica papaya* latex was collected in Uganda. These fresh latices were collected in the early morning by tapping the green fruits following the optimal collection procedure. [9]. Fresh latices were obtained by making three longitudinal incisions (depth 2-3 mm) on the green fruit epidermis using a wood blade. Umbrella-like devices were attached around the trunks to collect the exuded latex, which was then dried or freeze-dried.

Substrates

Trilaurin and lauric acid were purchased from Sigma (grade > 99 % purity).

Chemicals

All solvents used were reagent grade and purchased from Sigma, St Louis, MO, USA. The 1-butanol was HPLC grade (> 99.8 % purity) and purchased from Sigma.

Alcoholysis reactions

These reactions were investigated using an homogeneous TAG : trilaurin and 1-butanol as alcohol in molar ratio 40 :1. Reactions were initiated by the addition of 150 mg *Carica papaya* latex or *Carica pentagona* latex preparation (approx. 8 % w/w of total substrates) in solvent free system. The sealed vials (25 ml flask) were placed in an oven at 55 °C, and reaction mixtures were agitated by magnetic stirring. Over the course of the reactions, samples (25 µl) were removed periodically from the reaction medium. After dilution with 1 ml *n*-hexane, the reaction was stopped by filtering of the catalyst (Millex 0.5 µm, Millipore, Bedford, MA). An aliquot (100 µl) was added in a tube containing 1 mL *n*-hexane. Then, the composition of the mixture was analysed by gas chromatography (GC). The butyl laurate obtained and the non esterified products were separated with an on-column injector and a Rtx-1 dimethyl polysiloxane capillary column (3 m × 0.32 mm i.d. x film thickness 0.25 µm, Restek). The chromatography conditions were flame-ionization detection at 370 °C and Helium carrier gas at 5.5 ml·min⁻¹. Separations were made using the following oven temperature profile : initial temperature 90 °C for 1 min, then heating by 20 °C·min⁻¹ to 280°C, and holding for 6 min.

Esterification reactions

The reaction was carried out between lauric acid and 1-butanol in a molar ratio 1 :12. To performed reaction, the reaction medium was stirred at 200 rpm in a sealed vial (25 mL flask) at 55 °C. The reaction was started by adding to the mixture an amount of 150 mg of biocatalyst (8 % w/w of total substrates). Over the time course of these reactions, samples (25 µL) were removed from the reaction medium. After dilution with 1 mL *n*-hexane they were filtered (Millex 0.45 µm, Millipore) An aliquot (100 µl) was added in a tube containing 1 ml *n*-hexane and 0.5 µl of this mixture was analysed by GC as described above. The reference esterification reaction were carried out in the same conditions without the biocatalyst.

Results

Comparaison between commercially available crude papain and crude latices from Carica papaya and carica pentagona in alcoholysis reactions. Table 1 reports the lipase activity in alcoholysis reaction of *Carica pentagona* latex compared to the one of *Carica papaya* latex and commercially available preparation of crude papain. Alcoholysis reaction reached equilibrium after 3 h with a reaction yield of 72.3 % when crude latex of *Carica pentagona* was used as biocatalyst. Similar yield was obtained with crude latex of *Carica papaya* (70.2 %). In contrast, when alcoholysis reaction was biocatalysed by preparation of crude papain the reaction rate was slower and with only 48.4 % after 7 h. Finally, trilaurin was almost completely converted into butyl laurate and partial acylglycerols in 24 h with a reaction yield of 82.5 % and 84 % respectively for crude latex from *Carica pentagona* and *Carica papaya* as biocatalyst. Only 60.2 % was obtained when alcoholysis reaction was carried out with

commercially crude papain. These results underline and confirm the important lipase activity of crude latex from babaco fruit. Indeed, lipase activity of crude latex from babaco fruit was higher than the tested crude papain preparation. Due to this new biocatalytic property of this crude latex, it could be considered as a supplementary source of plant lipase. Because of its strong activity, this raw material could be advantageously exploited on an industrial scale for classical enzymatic reactions, such as the ones carried out using crude papain preparation.

Table 1. Lipase activity in alcoholysis reaction of crude *Carica pentagona* latex in comparison with commercially preparation of crude papain.

| Synthesis yield of Butyl laurate | | | | | |
|----------------------------------|------|------|------|------|------|
| (Moles % * to total lipids) | | | | | |
| Enzyme preparation | | | | | |
| (Crude latex) | Time | 1 h | 3 h | 7 h | 24 h |
| <i>Carica pentagona</i> | | 57.1 | 72.3 | 73.9 | 82.5 |
| <i>Carica papaya</i> | | 53.4 | 70.2 | 76.5 | 84.0 |
| Commercially crude papain | | 16.5 | 28.9 | 48.4 | 60.2 |

*Mean of three determinations. Relative standard deviation < 2 %.

Lipase activities of crude Carica pentagona latex compared with crude Carica papaya latex, and commercially available crude preparation of papain in esterification reactions.

Data given in *table 2* shows lipase activity in esterification reactions of *Carica pentagona* latex as compared to *Carica papaya* latex and commercially available preparation of crude papain. According to Caro *et al.* 2001 [10], it is important to take into account the thermal catalysis of the non-biological reference esterification reaction between lauric acid and 1-butanol at 55 °C. Indeed, in these conditions corrected values (obtained with reference results subtracted) are reported in *table 3*. Similar results are observed for esterification reaction biocatalyzed by *Carica papaya* and *Carica pentagona* crude latex. The reaction reached equilibrium after 30 h and the reaction yields were 39.4 % et 36.6 % respectively for crude latex from *Carica papaya* and *Carica pentagona*. On the other hand (*table 2*), a tiny difference is observed between reference reactions (11.7 % at 30 h) and those biocatalysed by commercially crude papain (16.9 % at 30 h) where the yield variation is about 5 %. These results indicate that commercially crude papain show very weak lipase activity in these esterification reactions. Furthermore, these results corroborate the fact that commercially crude papain shows generally lower lipase activity than crude latex from *Carica species*. These results were in accordance with those recently obtained in a previous study [8]. In this work, it was shown in interesterification reactions that activities of commercially crude papain were twice lower than these of crude latex from *Carica pentagona*.

Table 3. Lipase activity in esterification reaction of crude *Carica pentagona* latex in comparison with crude *Carica papaya* latex and commercially preparation of crude papain.

| Synthesis yield of Butyl laurate | | | | | | |
|----------------------------------|------|------|------|------|------|------|
| (Moles % * to total lipids) | | | | | | |
| Enzyme preparation | | | | | | |
| (Crude latex) | Time | 8 h | 24 h | 30 h | 48 h | 72 h |
| <i>Carica pentagona</i> | | 19.8 | 31.8 | 36.6 | 39.2 | 40.4 |
| <i>Carica papaya</i> | | 19.1 | 33.6 | 39.4 | 50.1 | 51.9 |
| Commercially crude papain | | 0.3 | 5.4 | 5.5 | 5.5 | 5.6 |

*Mean of three determinations. Relative standard deviation < 2 %.

Conclusion

In addition to confirm our previous results demonstrating interesting lipase properties of crude latex from *Carica pentagona*, this paper shows a great potential in synthesis activity for this plant extract. The use of this new biocatalyst can be successfully exploited for lipid biotransformations in interesterification reactions but also via triacylglycerol alcoholysis and in esterification reactions. To the best of your knowledge, it is the first time that a study evaluates lipase activity in alcoholysis and esterification reactions of crude latex from babaco fruit. Furthermore, one of the advantages of this raw material is its direct application as biocatalyst without any purification step prior to their use.

RÉFÉRENCES

1. Kempler C, Kabaluk T. Babaco (*Carica pentagona* Heilb.) : A possible crop for the Greenhouse. HortScience 1996 ; 31 : 785-8.
2. Viteri P. El cultivo del babaco en el Ecuador, Manual N19, Programa de Frutales, Instituto Nacional de investigaciones Agropecuarias INIAP, Ecuador 1992.
3. Giordani R, Moulin A, Verger R. Tributylglycerol hydrolase activity in *Carica papaya* and other latices. Phytochemistry 1991 ; 30 : 1069-72.
4. Villeneuve P, Pina M, Sharbek A, Graille J, Foglia TA. Specificity of *Carica papaya* latex in lipase-catalysed interesterification reactions. Biotech Tech 1997 ; 11 : 91-4.
5. Mukherjee KD, Kiewitt I. Specificity of *Carica papaya* latex as biocatalyst in the esterification of fatty acids with 1-butanol. J Agric Food Chem 1996 ; 44 : 1948-52.

- 6.** Caro Y, Villeneuve P, Pina M, Reynes M, Graille J. Lipase activity and fatty acid typoselectivities of plant extracts in hydrolysis and interesterification. *J Am Oil Chem Soc* 2000 ; 77 : 349-54.
- 6bis.** Caro Y, Villeneuve P, Pina M, Reynes M, Graille J. Investigation of crude latex from various *Carica papaya* varieties for lipid bioconversion. *J Am Oil Chem Soc* 2000 ; 77 : 891-901.
- 7.** Caro Y, Pina M, Turon F, Guilbert S, Mougeot E, Fetsch D, Attwool P, Graille J. Plant lipases : Biocatalyst aqueous environment in relation to optimal catalytic activity in lipase-catalyzed synthesis reactions. *Biotechnology and bioengineering* 2002 ; 77 : 693-703.
- 8.** Dhuique-Mayer C, Caro Y, Pina M, Ruales J, M. Dornier, Graille J. Biocatalytic properties of lipase in crude latex from babaco fruit (*Carica pentagona*). *Biotechnology letters* 2001 ; 23 : 1021-4.
- 9.** Madrigal LS, Ortiz AN, Cooke RD, Fernandez RH. The dependence of crude papain yields on different Collection ("Tapping") procedures for papaya latex. *J Sci Food Agric* 1980 ; 31 : 279-85.
- 10.** Caro Y, Dhuique-Mayer C, Turon F, Pina M, Reynes M, Graille J. The biocatalytic activity of bromelain in ester synthesis reaction : difference between the intrinsic lipase activity and thermal catalysis. *Biotechnology letters* 2001 ; 23 : 2035-9.