

# Catalytic behavior of *Carica papaya* latex in transesterification reactions

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The *Carica papaya* latex-catalyzed transesterification of tricaprylin with various acyl donors gave good yields of transesterified triacylglycerols with vinyl laurate and lauric acid whereas alkyl laurates reacted poorly. Using dried or water washed latex as the catalyst resulted in improved yields in shorter reaction times with the free acid and vinyl ester but not with the alkyl esters

## Introduction

Processes that use plant enzymes may have an advantage over microbial enzymes because of their availability and lower cost. In this regard, several publications dealing with the application of plant lipases in oil and fat biotransformations have appeared (Piazza *et al.*, 1989, Parmar and Hammond, 1994, Mukherjee, 1994). Recently, lipase activity in *Carica papaya* latex (CPL) was reported (Giordani *et al.*, 1991) and its substrate specificity characterized (Mukherjee and Kiewitt, 1996, Villeneuve *et al.*, 1996, Villeneuve *et al.*, 1997). CPL extract is principally known for containing papain, a protease widely used in the food industry. However, because of its fatty acyl and 1,3-positional lipase selectivities, CPL has potential as a biocatalyst in lipid transformations as demonstrated in a recently patented process on milk fat modification (Graille *et al.*, 1996). In this paper, we have assessed the potential of CPL as a biocatalyst by studying the CPL-catalyzed transesterification reaction of tricaprylin with vinyl esters, fatty acids, and alkyl esters.

## Materials and methods

### Enzyme

The crude dried extract from *Carica papaya* latex (CPL) was obtained from Sigma Chemical (St. Louis, MO). The granular material was ground before use.

### Substrates

Tricaprylin, lauric acid, methyl laurate, ethyl laurate, vinyl laurate and 1-octanol were purchased from Sigma Chemical. Octyl laurate was synthesized by the  $\text{BF}_3$ -catalyzed esterification of lauric acid with octyl alcohol. Solvents were purchased from Burdick and Jackson (Muskegon, MI) and were HPLC grade. Bis-(trimethylsilyl)trifluoroacetamide (BSTFA) was obtained from Regis Chemical Co. (Morton Grove, IL).

## Transesterification reactions

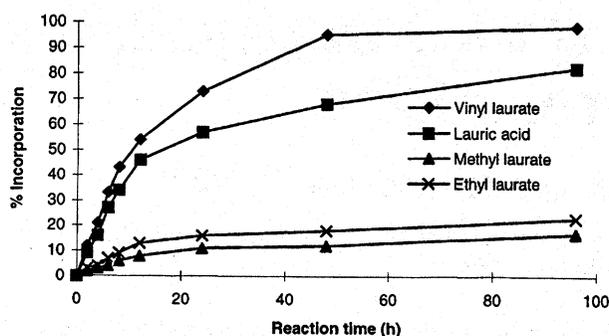
Into a screw-top vial was placed 0.1 mmole of either vinyl laurate, lauric acid, or an alkyl laurate, 0.2 mmole of tricaprylin and CPL (10 wt% of total weight of substrates). The tube was placed in a water jacketed beaker and 60EC water was circulated through the jacket from a constant temperature bath. The reaction mixture was stirred magnetically at 200 rpm throughout the reaction.

## Analysis of reaction mixtures

Over the time course of the above reaction, samples containing approximately 5 mg of lipid were removed periodically to which was added 50  $\mu\text{l}$  of dry pyridine and 100  $\mu\text{l}$  of BSTFA. The samples were heated at 100EC for 10 min to convert partial glycerides and free fatty acids to their silylated derivatives. After dilution in 5 ml of hexane the samples were analyzed by gas chromatography (GC) as follows: Hewlett Packard 5890 gas chromatograph (Wilmington, DE) equipped with a cold on-column capillary injector, a DB1-HT capillary column (J&W Scientific, Folsom, CA), 15 m H 0.32 mm i. d., film thickness 0.1 mm. The chromatographic conditions were: on column injection, flame ionization detector at 370EC, He carrier gas at 5.5 ml  $\text{min}^{-1}$ . Separations were made using the following oven temperature profile: initial temperature 70EC, programmed to a final temperature of 350EC at 20EC  $\text{min}^{-1}$ , hold at final temp 4 min. Retention times for triacylglycerols of interest are: tricaprylin, 9.3 min; 1-lauroyl-2,3-dicapryloylglycerol, 10.4 min; and 1,3-dilauroyl-2-capryloylglycerol, 11.9 min.

## Measurement of lipase activity

Lipase activity was measured titrimetrically using a VIT 90 Video Titrator (Radiometer, Copenhagen, Denmark) with a tricaprylin emulsion (200 mM of tricaprylin,



**Figure 1** Percentage of triglycerides that contain lauroyl groups formed in the reaction of tricapyrylin with various acyl donors using crude CPL.

15 mM  $\text{CaCl}_2$  and 4.2% (wt/v) gum arabic). Hydrolysis reactions were conducted at 26EC at pH 7.5. Assays were run in triplicate.

#### Isolation of the water insoluble fraction of CPL

CPL (20 g) was suspended in 150 ml of water and centrifuged at 10,000 rpm for 10 min. The water was decanted, the pellet was resuspended in water and the process repeated. After four water washes, the insoluble latex pellet was stored in a desiccator over anhydrous calcium sulfate. Water activity ( $a_w$ ) of the washed CPL powder was determined with an Aqualab CX-2 (Decagon Devices Inc., Pullman, WA) instrument. The washed latex powder was equilibrated in a desiccator until the desired  $a_w$  is obtained.

#### Results and discussion

Lipase-catalyzed transesterification reactions of triacylglycerols can be carried out using various types of acylating agents. Accordingly, the course of a given lipase-catalyzed transesterification reaction will depend upon the acylating agent used, e.g., either a carboxylic acid, an alkyl ester or a vinyl ester. To determine if such differences are exhibited by the lipase in *Carica papaya* latex we studied the CPL-catalyzed transesterification of tricapyrylin with several acyl donors. The reactions studied were carried out using vinyl laurate, lauric acid, and methyl or ethyl laurate in hexane solution with 10% w/w of crude CPL as biocatalyst. The time course of formation of transesterified TAGs having one or two lauroyl residues was measured by GC. With the crude CPL powder, the use of vinyl laurate as the acyl donor gave the best incorporation of lauroyl residues (>90%) onto the TAG backbone after 96 h reaction (figure 1). This result arose probably because the trans-

**Table 1** Time course of formation of lauroyl containing triacylglycerols<sup>a</sup> in the *Carica papaya* latex-catalyzed interesterification of tricapyrylin with various acyl donors

catalyst ( $a_w$ )	time (h)	acyl donor			
		vinyl laurate	lauric acid	methyl laurate	ethyl laurate
crude CPL (0.56)	24	70	55	9	12
	48	95	77	17	23
dried CPL (> 0.10)	24	92	90	10	14
	48	96	94	11	15
ww-CPL (0.26)	24	96	65	22	30
	48	96	82	33	40

<sup>a</sup>Sum of new triacylglycerols: 1,2-dicapryloyl-3-lauroylglycerol and 1,3-dilauroyl-2-capryloylglycerol as determined by gas chromatography.

esterification reaction using this acyl donor is an irreversible reaction (Faber and Riva, 1992). For the reactions with the other acyl donors, the following differences were observed; transesterification of tricapyrylin with lauric acid gave better yields of mixed TAGs (>80%) than the reactions using methyl or ethyl laurate (figure 1). For the latter esters, yields of newly formed TAGs were only 17% and 23%, respectively, after 96 h reaction.

We initially thought that the slower reactions observed with the alkyl esters might be because of a competing hydrolysis of tricapyrylin (formation of diacylglycerols was observed) as a result of the water content of crude CPL (6% w/w,  $a_w$  0.56). The above reactions, therefore, were repeated with dried biocatalyst. With the dried CPL ( $a_w < 0.10$ ), there was a significant increase in the incorporation (>25%) of lauroyl residues into tricapyrylin with vinyl laurate and lauric acid within 24 h compared to the crude latex, Table 1. However, increased incorporation of lauroyl residues into tricapyrylin was not observed for the methyl or ethyl laurate reactions. In addition, when the CPL  $a_w$  was adjusted to its optimum ( $a_w$  0.26, Ozenne, 1993) the yields of new triacylglycerols were similar to those obtained with the crude latex, Table 1.

Giordani *et al.* indicated that for *Carica papaya* latex the proteolytic enzymes are water soluble, whereas the lipase activity is associated with the particulate fraction of the latex. We decided to use the water insoluble fraction of CPL (ww-CPL) in the transesterification reaction between tricapyrylin and methyl and ethyl laurate. The crude powder was water washed several times and the insoluble fraction recovered and dried to a given  $a_w$ .

The ww-CPL retained about 90% of the lipolytic activity originally present in the crude CPL (69.9 U mg<sup>-1</sup> for ww-CPL and 76.2 U mg<sup>-1</sup> for the crude CPL). Two experiments were carried out: the first used ww-CPL with the same  $a_w$  as the crude CPL (0.56); in the second reaction the  $a_w$  of this biocatalyst was adjusted to its optimal  $a_w$  (0.26). Results were similar in both instances in that there was increased yields of lauroyl containing TAGs with these acyl donors compared to reactions with the crude latex. However, conversions still were not comparable to those from vinyl laurate and lauric acid, Table 1.

We conclude from this study that vinyl esters and free fatty acids are preferred acylating agents over alkyl esters in CPL-catalyzed transesterification reactions. Use of the dried or water washed latex in these reactions gave improved yields of transesterified TAGs in shorter reaction times with lauric acid and vinyl laurate but not for the alkyl laurates. It appears that there may a water

soluble material present in the crude CPL that inhibits its lipase activity with alkyl esters.

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