Specificity of *Carica papaya* Latex as Biocatalyst in the Esterification of Fatty Acids with 1-Butanol

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Papaya (*Carica papaya*) latex, reportedly known to have good lipolytic activity, has been evaluated as biocatalyst in the esterification of various fatty acids with 1-butanol in the presence of myristic acid as the reference standard. *C. papaya* latex strongly discriminates against fatty acids having a *cis*-4 unsaturation, e.g. *all-cis*-4,7,10,13,16,19-docosahexaenoic acid, *cis*-6 unsaturation, e.g. petroselinic (*cis*-6-octadecenoic), γ-linolenic (*all-cis*-6,9,12-octadecatrienoic), and stearidonic (*all-cis*-6,9,12,15-octadecatetraenoic) acids, and *cis*-8 unsaturation, e.g. dihomo-γ-linolenic (*all-cis*-8,-11,14-eicosatrienoic) acid. Fatty acids having *cis*-5 unsaturation, e.g. *all-cis*-5,8,11,14,17-eicosapentaenoic acid, and those having a *cis*-9 unsaturation, e.g. oleic (*cis*-9-octadecenoic) and α-linolenic (*all-cis*-9,12,15-octadecatrienoic) acids are very well accepted as substrates. Fatty acids having hydroxy groups, e.g. ricinoleic (12-hydroxy-*cis*-9-octadecenoic) acid and 12-hydroxystearic acid, epoxy groups, e.g. *trans*-9,10-epoxystearic acid, and cyclopentenyl groups, e.g. hydnocarpic [(11-(2′-cyclopentenyl)undecanoic] acid and chaulmoogric [13-(2′-cyclopentenyl)tridecanoic] acid are also well accepted as substrates. The observed substrate specificities are similar to those reported for lipase preparations from microorganisms, animals, and plants.

Keywords: *Carica papaya latex; plant lipase, biocatalyst; enzymatic esterification; fatty acid specificity*

INTRODUCTION

A major group of lipases from plants consists of triacylglycerol lipases (EC 3.1.1.3) that hydrolyze the ester bonds of storage triacylglycerols of seeds, such as oilseeds and cereal grains. Various sources of lipases from plant tissues, techniques for their isolation and purification, and their properties are well documented (Mukherjee, 1994; Mukherjee and Hills, 1994). Several publications have appeared in recent years on the possible applications of lipases from plants in biotransformations of fats and other lipids (Kadyrova et al., 1983; Hassanien and Mukherjee, 1986; Piazza et al., 1989; Hills and Mukherjee, 1990; Rao et al., 1992; Ayorinde et al., 1993; Dandik et al., 1993; Ncube et al., 1993, 1995; Parmar and Hammond, 1994; Jachmanián et al., 1995; Jachmanián and Mukherjee, 1996). Active preparations of lipases are relatively easy to isolate from plants (Ory et al., 1960; Hassanien and Mukherjee, 1986; Piazza et al., 1989; Rao et al., 1992; Ayorinde et al., 1993; Jachmanián et al., 1995; Jachmanián and Mukherjee, 1996), and pronounced substrate specificities of such biocatalysts (Huang et al., 1988) can be utilized in lipid biotechnology (Mukherjee, 1990).

Latex from papaya (*Carica papaya*)—a well-known commercially available enzyme preparation containing papain that has been employed for many years in food and beverage industries—has also been used as a biocatalyst in esterification reactions (Dordick, 1989; Stevenson and Storer, 1991). *C. papaya* latex also has a good activity in the hydrolysis of tributyroylglycerol (Giordani et al., 1991), and the stereoselectivity of this latex in the hydrolysis of triacylglycerols has been recently published (Villeneuve et al., 1995). We report here the fatty acid selectivity of *C. papaya* latex lipase as biocatalyst in the esterification of 1-butanol with various unsaturated fatty acids and fatty acids having hydroxy, epoxy, and cyclopentenyl groups. The reactions were carried out under competetive conditions in the presence of myristic acid as the reference standard.

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MATERIALS AND METHODS

Materials. The *C. papaya* latex was a purified extract obtained from Sigma, Deisenhofen, Germany. The granular latex preparation was ground in a mortar and pestle to a fine powder to pass a 0.8 mm mesh width sieve. All chemicals of analytical grade were from E. Merck, Darmstadt, Germany. Pure fatty acids were purchased from Sigma, and the cyclopentenyl fatty acids were a generous gift from Prof. Helmut K. Mangold, Münster, Germany.

Esterification. In control experiments myristic acid (50 mM) and 1-butanol (100 mM), dissolved in a total volume of 250 μL of hexane, were reacted in the presence of 5 mg of *C. papaya* latex powder by magnetic stirring at 30 °C for various periods.

For the determination of specificity constants esterification reactions were carried out as described above using 25 mM of each of the fatty acids, individually, together with 25 mM myristic acid, the reference standard, and 100 mM 1-butanol dissolved in a total volume of 250 μL of hexane in the presence of 5 mg of *C. papaya* latex powder. The esterification of 12-hydroxystearic acid and *cis*-9,10-epoxystearic acid was carried out using 250 μL of methyl *tert*-butyl ether and chloroform/hexane (1:1 v/v), respectively, as reaction medium.

Lipid Extraction and Analysis. Lipids were extracted from the reaction products with hexane, and in the case of the reaction products from 12-hydroxystearic and *cis*-9,10-epoxystearic acid with methyl *tert*-butyl ether and chloroform/hexane (1:1 v/v), respectively. The fatty acids contained in the reaction products were converted to methyl esters using diazomethane, and the resulting mixtures of methyl esters and butyl esters were analyzed by gas chromatography (Mukherjee et al., 1993). Data used for kinetic analysis were based on arithmetic means of the composition of unreacted fatty acids and butyl esters in the reaction products determined by two separate analyses of each sample by gas chromatography with established precision and coefficients of variation (Firestone and Horwitz, 1979).

S0021-8561(96)00216-6 CCC: $12.00

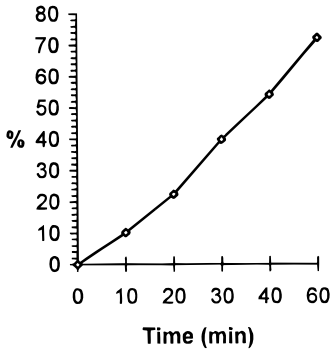

Figure 1. Formation (percentage) of butyl esters during esterification of myristic acid (50 mM) with 100 mM 1-butanol in hexane using *C. papaya* latex as biocatalyst.

Kinetic Analysis. Specificity constants were calculated for each fatty acid according to the method of Rangheard et al. (1989) from the extent (micromoles) of formation of butyl ester of the fatty acid examined and that of myristic acid by reaction with 1-butanol under competitive conditions. For two substrates competing for the enzyme, the ratio of the reaction rates for each substrate (v_1 and v_2) is given by

$$v_1/v_2 = \alpha(\text{Ac1X})/(\text{Ac2X})$$

where Ac1X and Ac2X are the concentrations of the two substrates at time X and α is the competitive factor which is defined by the equation

$$\alpha = [V(\text{Ac1X})/K(\text{Ac1X})]/[V(\text{Ac2X})/K(\text{Ac2X})]$$

where V is maximal velocity and K is Michaelis constant.

The competetive factor was calculated from the substrate concentrations Ac1X0 and Ac2X0 at time zero as follows:

$$\alpha = \log(\text{Ac1X0}/\text{Ac1X})/\log(\text{Ac2X0}/\text{Ac2X})$$

From the competetive factor α, the specificity constant was calculated as 1/α with reference to the specificity constant of myristic acid taken as 1.00.

RESULTS

Figure 1 shows the time course of esterification of the reference compound, myristic acid, with 1-butanol catalyzed by *C. papaya* latex. It is evident that as much as 70% of myristic acid is converted to its butyl ester within a reaction time of 1 h.

The time course of esterification of 1-butanol with individual unsaturated fatty acids, i.e. oleic (*cis*-9-octadecenoic), petroselinic (*cis*-6-octadecenoic), α-linolenic (*all-cis*-9,12,15-octadecatrienoic), γ-linolenic (*all-cis*-6,9,12-octadecatrienoic), stearidonic (*all-cis*-6,9,12,15-octadecatetraenoic), dihomo-γ-linolenic (*all-cis*-8,11,14-eicosatrienoic), eicosapentaenoic (*all-cis*-5,8,11,14,17-eicosapentaenoic), and docosahexaenoic (*all-cis*-4,7,10,13,-16,19-docosahexaenoic) acid, in the presence of the reference compound, myristic acid, is given in Figure 2.

Similarly, Figure 3 shows the time course of esterification of 1-butanol with individual fatty acids having hydroxy, epoxy, and cyclopentenyl groups, i.e. ricinoleic (12-hydroxy-*cis*-9-octadecenoic), 12-hydroxystearic, *cis*-9,10-epoxystearic, *trans*-9,10-epoxystearic, hydnocarpic [(11-(2′-cyclopentenyl)undecanoic], and chaulmoogric [13-(2′-cyclopentenyl)tridecanoic] acid, in the presence of the reference compound, myristic acid.

Figure 4 shows the specificity constants obtained in the esterification of the individual fatty acids with 1-butanol catalyzed by *C. papaya* latex. For most fatty acids the specificity constants were calculated from the composition of the reaction products after about 40−60% of the reference standard, myristic acid, present in the reaction mixture had been converted to butyl esters. For the reactions of 12-hydroxystearic acid and *cis*-9,10-epoxystearic acid that were esterified at rather low overall rates (Figure 3), the specificity constants were calculated after 20 and 4%, respectively, of the reference standard had been converted to butyl ester.

Low overall reaction rates (Figure 2) and low specificity constants (Figure 4) were observed in the esterification of fatty acids having a *cis*-4 unsaturation, e.g. docosahexaenoic acid, *cis*-6 unsaturation, e.g. petroselinic, γ-linolenic, and stearidonic acids, and *cis*-8 unsaturation, e.g. dihomo-γ-linolenic acid. Obviously, the above groups of fatty acids were discriminated against as substrates in esterification with 1-butanol, catalyzed by *C. papaya* latex. In contrast, oleic and α-linolenic acids having a *cis*-9 unsaturation and eicosapentaenoic acid having a *cis*-5 unsaturation gave good overall rates of esterification (Figure 2) and specificity constants close to 1 (Figure 4).

Most of the other less common fatty acids were found to be excellent substrates in the esterification with 1-butanol, catalyzed by *C. papaya* latex, as evident form good overall reaction rates (Figure 3) and the specificity constants, which were similar to that of myristic acid or even substantially higher (Figure 4).

Thus, with both ricinoleic acid and 12-hydroxystearic acid as substrate relatively high overall rates of esterification compared to that of myristic acid were observed (Figure 3). The specificity constant with 12-hydroxystearic acid was somewhat lower than that of ricinoleic acid, which is consistent with recent findings with several lipase preparations from microorganisms, an animal tissue, and a higher plant (Jachmanián et al., 1996).

In the reaction of the mixture of *cis*-9,10-epoxystearic and myristic acids with 1-butanol, catalyzed by *C. papaya* latex, very little esterification was observed (Figure 3); however, the specificity constant (Figure 4) does not show discrimination against *cis*-9,10-epoxystearic acid over myristic acid. With *trans*-9,10-epoxystearic acid as substrate the overall rates of esterification were higher than with the corresponding *cis*-isomer (Figure 3) and the specificity constant was close to 2 (Figure 4), indicating that *trans*-9,10-epoxystearic acid is a better substrate than myristic acid.

The specificity constants obtained for the cyclopentenyl fatty acids having saturated alkyl chains, e.g. hydnocarpic acid and chaulmoogric acid, were close to 1 or higher (Figure 4). Consistent with the findings with other lipase preparations (Jachmanián et al., 1996), specificity constants were always higher with hydnocarpic acid as substrate than with chaulmoogric acid (Figure 4).

DISCUSSION

The ability of the *C. papaya* latex as biocatalyst to discriminate against straight-chain fatty acids having a *cis*-4 unsaturation (docosahexaenoic), *cis*-6 unsaturation (petroselinic, γ-linolenic, and stearidonic), or *cis*-8 unsaturation (dihomo-γ-linolenic) in the esterification with 1-butanol (Figures 2 and 4) is in agreement with earlier findings using lipases from microbial, animal, and plant origin (Hills et al., 1989, 1990a,b; Mukherjee

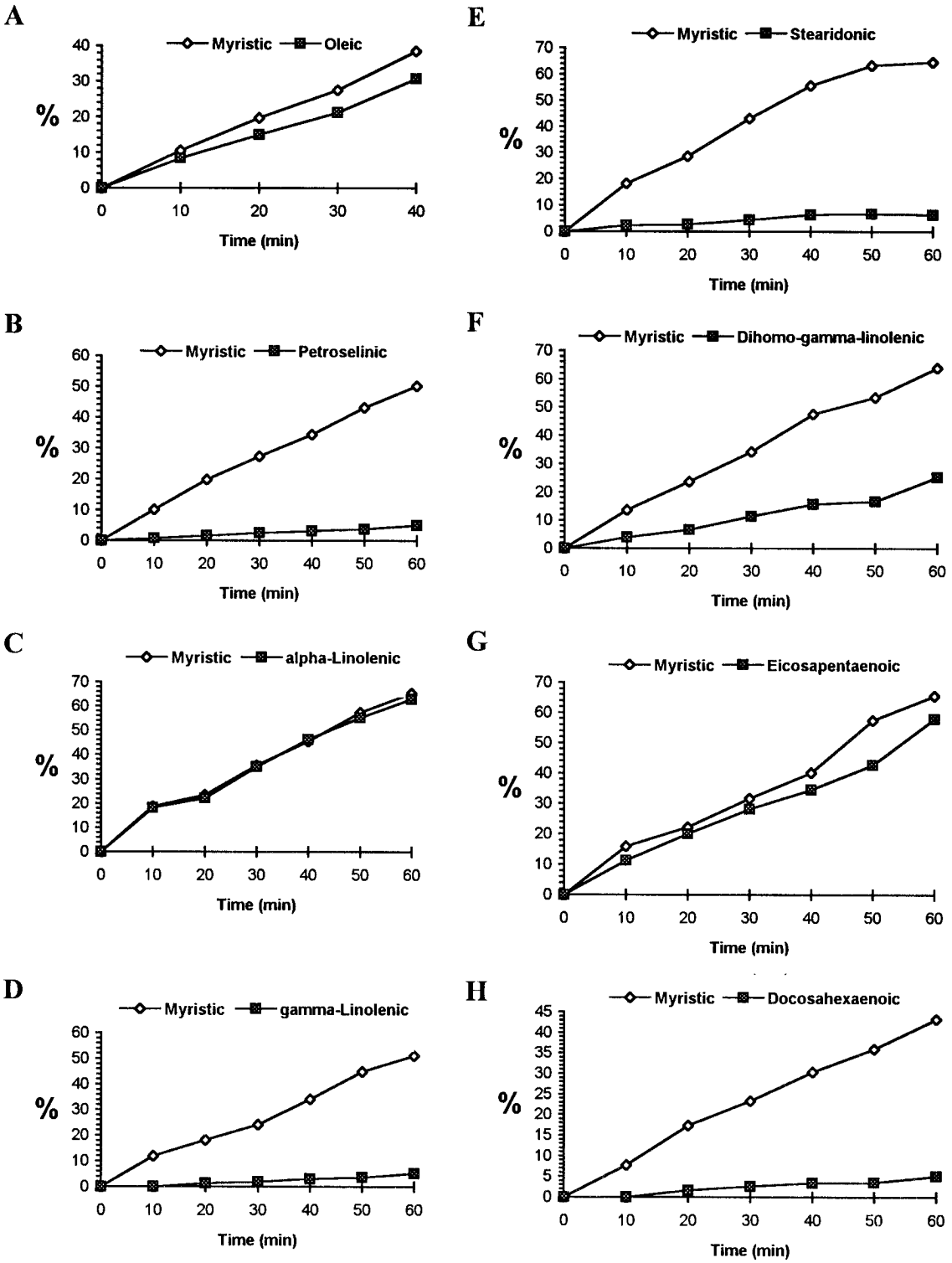

Figure 2. Formation (percentage) of butyl esters during esterification of mixtures (25 mM each) of myristic acid and individual unsaturated fatty acids with 100 mM 1-butanol in hexane using *C. papaya* latex as biocatalyst: (A) oleic; (B) petroselinic; (C) α-linolenic; (D) γ-linolenic; (E) stearidonic; (F) dihomo-γ-linolenic; (G) eicosapentaenoic; (H) docosahexaenoic.

and Kiewitt, 1991; Mukherjee et al., 1993; Syed Rahmatullah et al., 1994a,b; Jachmanián et al., 1995; Jachmanián and Mukherjee, 1996). All of these data strongly suggest that discrimination against fatty acid substrates having the first double bond after the carboxyl group as *cis*-4, *cis*-6, or *cis*-8 is a common feature of most triacylglycerol lipases irrespective of their origin. It was suggested for the lipase from *Brassica napus* that the enzyme discriminates against fatty acids with a double bond at even-number carbons which are known to have *anti*-orientation with respect to the carboxyl group (Hills et al., 1990b). The same is probably the case with most triacylglycerol lipases as is evident from the present data and results of a recent study (Jachmanián et al., 1996).

It is interesting to note that most of the fatty acids with less common structures, such as hydroxy acids, epoxy acids, and cyclopentenyl acids having saturated alkyl chains, are distinctly favored by *C. papaya* latex as substrates for esterification as compared to a straight-chain fatty acid, such as oleic acid (Figures 3 and 4). Similar substrate specificities have been recently re-

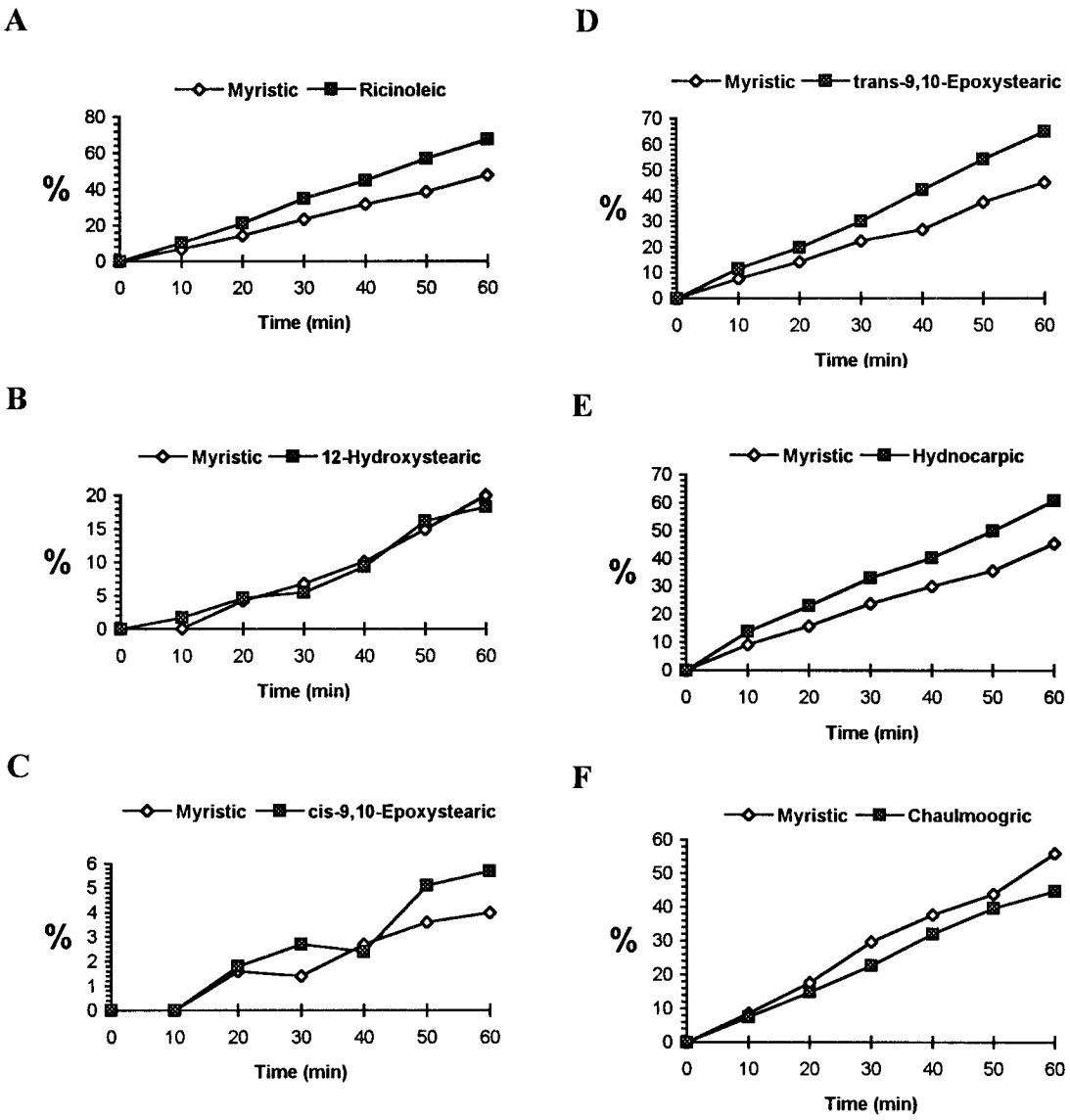

Figure 3. Formation (percentage) of butyl esters during esterification of mixtures (25 mM each) of myristic acid and individual hydroxy, epoxy, and cyclopentenyl fatty acids with 100 mM 1-butanol in hexane using *C. papaya* latex as biocatalyst: (A) ricinoleic; (B) 12-hydroxystearic; (C) *cis*-9,10-epoxystearic; (D) *trans*-9,10-epoxystearic; (E) hydnocarpic; (F) chaulmoogric.

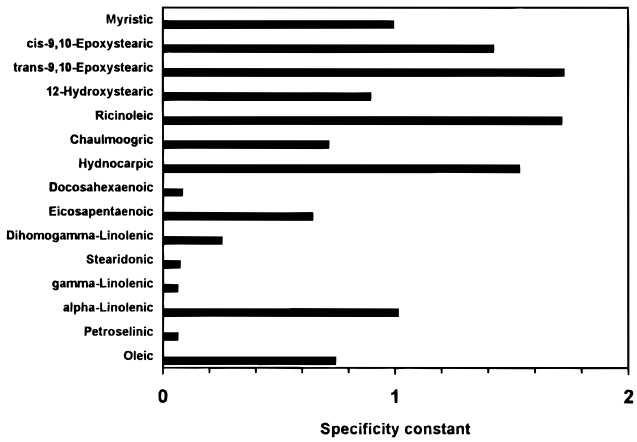

Figure 4. Specificity constant in the esterification of mixtures (25 mM each) of myristic acid and individual unsaturated, hydroxy, epoxy, and cyclopentenyl fatty acids with 100 mM 1-butanol in hexane using *C. papaya* latex as biocatalyst.

ported for several lipases of microbial, animal, and plant origin (Jachmanián et al., 1996).

The selectivities reported here for the *C. papaya* latex as biocatalyst should be useful for enrichment of fatty acids having *cis*-4, *cis*-6, or *cis*-8 unsaturation or those containing hydroxy, epoxy, and saturated cyclopentenyl groups from mixtures with other fatty acids via kinetic resolution, e.g. via selective esterification or selective hydrolysis. As compared to lipases from genetically transformed microorganisms, *C. papaya* latex, which is available as a bulk commercial product and has been used for many years in the food, beverage, pharmaceutical, and cosmetic industries, should find ready acceptance as a cheap biocatalyst of plant origin for modification of natural fats and lipids aimed at preparation of specific added value products, such as nutraceuticals and novel foods. Enzymatically modified fats for use in nutraceuticals and novel foods include structured lipids (Akoh, 1995), e.g. structured triacylglycerols containing medium-chain saturated or long-chain polyunsaturated fatty acids esterified specifically at the *sn*-1,3 or *sn*-2 position that result in improved absorption of such fatty acids and consequently enhanced physiological action when used, for example, in infant feed or clinical nutrition. Such products can be conveniently prepared using concentrates of polyunsaturated fatty acids, such as γ-linolenic acid or docosahexaenoic acid, prepared from fatty acid mixtures obtained from natural

oils and fats via selective esterification catalyzed by *C. papaya* latex.

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Received for review March 27, 1996. Revised manuscript received May 15, 1996. Accepted May 15, 1996.[⊗]

JF960216D

[⊗] Abstract published in *Advance ACS Abstracts,* July 1, 1996.