Lipase Activity and Fatty Acid Typoselectivities of Plant Extracts in Hydrolysis and Interesterification

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ABSTRACT: Lipase fatty acid typoselectivities of *Euphorbia characias* latex and commercially available crude preparation of bromelain were determined in the hydrolysis of homogeneous triacylglycerols (TAG) and natural TAG mixtures. Their activities were compared to a commercially available crude preparation of papain. Under optimal lipolysis conditions at pH 8.0 and 10 min of incubation time, maximal activities were observed at 45, 55, and 50°C, respectively, for *E. characias* latex, crude bromelain, and crude papain. Commercially available crude preparations of bromelain exhibited very poor hydrolysis activity. Latex from *E. characias*, which contained 340 mg of dried material per milliliter of fresh latex, exhibited a high lipase activity and a short-chain fatty acid preference in the hydrolysis of homogeneous TAG. For all substrates, it showed a better activity than crude papain. Lipase fatty acid typoselectivities of crude bromelain and crude papain also were studied in interesterification reactions of tributyrin with a series of homogeneous TAG. Experiments showed that crude bromelain [water activity (A_w): 0.21] had no activity in interesterification. Regarding reactions with crude papain (A_w: 0.55), yields of newly formed TAG decreased with increasing chain length of TAG, except for the reaction with trimargarin. For interesterification of tributyrin with unsaturated TAG, triolein reacted faster than polyunsaturated TAG. During these interesterification reactions, the proportion of new TAG with two butyroyl residues was higher than new TAG with only one butyroyl residue. This phenomenon was more pronounced for reactions with long-chain TAG.

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KEY WORDS: Bromelain, *Euphorbia characias*, hydrolysis, interesterification, papain, plant lipase, typoselectivity.

The use of enzymes is becoming increasingly important in a number of applications in industries such as the pharmaceutical, dairy, and cosmetic industries. Plant enzymes may have an advantage over animal or microbial enzymes owing to their availability, their apparent relative ease of purification, as well as their particular selectivities. For instance, numerous plant enzymes have been studied for their lipase fatty acid typoselectivities, i.e., selectivity for a particular fatty acid (FA) or, more generally, for a type of FA family (1,2). Actually, several plant extracts with already known or just suspected enzymatic activities are coming under increased scrutiny, *Carica papaya* latex being one example, which is principally known to contain papain (3), a protease that is widely used in the food and beverage industries. Crude papain is the commercial name given to the spray-dried latex obtained by tapping the green fruits of *C. papaya*, and crude bromelain is the name given to the crude dried extract of pineapple (*Ananas comosus*) stem, which contains bromelain, a protease used in pharmaceutical industries.

Recently, Mukherjee and Kiewitt (4,5) showed that crude papain and crude bromelain were able to catalyze the esterification of various FA with 1-butanol. The ability of these plant extracts to catalyze the esterification reaction is attributed to the presence of lipases. The short-chain preference of lipase from *C. papaya* latex in hydrolysis of homogeneous TAG has been studied by Giordani *et al.* (6), but the *sn*-3 stereoselectivity of this above lipase was characterized by Villeneuve *et al.* in hydrolysis (7–9) and interesterification (10) reactions of selected TAG. The lipase activity of *Euphorbia characias* latex has been characterized by Giordani *et al.* (6) with tributyrin as substrate.

As a continuation to the above studies and bearing in mind that the activities of a lipase in hydrolysis and esterification reactions are often independent from each other (11), we decided to evaluate the lipase activity and FA typoselectivities of crude bromelain and *E. characias* latex in the hydrolysis of TAG, in comparison to a commercially available crude preparation of papain. The biocatalytic activities of crude bromelain and crude papain also were evaluated in interesterification reactions.

MATERIALS AND METHODS

All solvents of analytical grade were purchased from Sigma (St. Quentin Fallavier, France). *Enzymes.* The following enzymatic preparations were purchased from Sigma: crude papain (EC 3.4.22.2, from *C. papaya* latex, crude powder, Ref. P-3250) and crude bromelain (EC 3.4.22.33, from pineapple stem, crude powder, Ref. B-2252). All dried crude preparations were ground before use. Fresh latex from *E. characias* was collected in the Montpellier countryside (France). Stems were cut with a razor blade, and the latex was allowed to flow into a collection vial. The operation was repeated many times,

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and the collected fresh latex was stored at 4°C until use. Protein contents (%protein) in plant extracts were determined according to the Kjeldahl assay, and the water activity (A_w) was measured at 25°C with an FA-ST/1 instrument (GBX Scientific Instrument, Romans, France). Biocatalyst water content was calculated after the determination of dried materials in the plant extracts (103°C—24 h).

Substrates. The homogeneous TAG (purity >99%), from tributyrin to tri-α-linolenin, were purchased from Sigma. The natural TAG mixtures, such as copra TAG (rich in trilaurin: TriC12:0), olive TAG [rich in triolein: TriC18:1,(*cis*)-9], sunflower TAG [rich in trilinolein: TriC18:2,(*cis*)-9,12], and linseed TAG [rich in tri-α-linolenin: TriC18:3,(*cis*)-9,12,15], were from commercial vegetable oils. Firstly, these TAG mixtures were purified by column chromatography on activated aluminum oxide (150 mesh; Sigma-Aldrich, France) following the procedure described by the Association Française de Normalisation (AFNOR) norm (12): Norme Française n° 6800. Briefly, 10 g of crude oils was dissolved in 50 mL of *n*-hexane and applied on the top of a column containing 30 g of activated aluminum oxide (freshly prepared and hydrated with 5% water). TAG mixtures were eluted with *n*-hexane and recovered by stripping off the solvent in vacuum.

Hydrolysis reactions. Lipases contained in the plant extracts were analyzed for their hydrolytic action on TAG in a mechanically stirred medium of water and oil. Lipase fatty acid typoselectivities were characterized by studying the hydrolysis rates of various substrates containing different types of FA (chain-length, unsaturation, etc.); homogeneous TAG and natural TAG mixtures were used in order to have a wide range of substrates.

TAG (1.5 mmol) were emulsified mechanically in 40 mL of NaCl solution (0.15 M). Hydrolysis reactions were carried out with 10 µL of fresh latex when studying *E. characias* extract or 5 mg for all the other dried plant extracts (crude papain and crude bromelain). This quantity was weighed precisely with a Mettler-type H35AR, fine-precision balance (Mettler, Viroflay, France). Each reaction was carried out at constant pH 8.0 using a pH-Stat (736 GP-Titrino; Metrohm, Switzerland) equipped with a pH glass electrode (KCl/3 M) and a thermostated vial (Ikamag-Ret; Junke&Kunkel, Staufen, Germany). We decided not to add any emulsifier to the mixture in order to prevent any emulsifier/protein interaction and to get the intrinsic enzyme activity.

The released free fatty acids (FFA) were titrated with a 0.1 M NaOH solution, except for hydrolysis reactions with crude bromelain, for which the titration was carried out with a 0.01 M NaOH solution. Lipase activities were derived from the initial slopes of the kinetic curves and expressed as international units (IU) per gram of dried plant extract, or per mL of fresh plant latex. One IU corresponds to one micromole of FFA liberated per minute. Assays were run in triplicate.

Interesterification reactions. To a vial containing tributyrin (100 µmol) in 5 mL of *n*-hexane and another homogeneous TAG (100 µmol) from the series tricaproin to tri-α-linolenin was added. Reactions were carried out with 10% (w/w) of lipase preparation to total TAG. The sealed vials were placed in an oven at 50°C, and reaction mixtures were agitated by magnetic stirring at 300 rpm throughout the reaction.

Over the time course of the above reactions, samples (50 µL) containing *ca.* 1 mg of lipids were removed periodically from the reaction medium. After dilution in 2 mL of *n*-hexane, they were filtered (Millex 0.5 µm; Millipore, Bedford, MA), and aliquots (0.4 µL) were analyzed by gas–liquid chromatography (GLC) as follows: Carlo Erba instrument model HRGC (Erba Science, Paris, France), cold on-column capillary injector, an Rtx-1 dimethyl polysiloxane capillary column (Restek, Bellefonte, PA), 3 mL × 0.32 mm i.d. × 0.25 µm film thickness. The chromatography conditions were: cold on-column injection, flame-ionization detection at 370°C, and He carrier gas at 5.5 mL min^{-1}. Separations were made using the following oven temperature profile: initial temperature 100°C for 1 min, 100 to 200°C at 20°C·min^{-1}, 200 to 330°C at 10°C·min^{-1}, and final time 5 min.

RESULTS AND DISCUSSION

Optimal lipolysis conditions. Firstly, lipase activities of crude papain were evaluated with various amounts of tributyrin as substrate in the assay system: pH 8.0, 50°C, and 10 min of incubation time. Results (Fig. 1) showed that a strong increase in lipase activity only appeared above the limit of solubility of tributyrin in water (3–5 mM). Optimal activities were obtained from 40 mM of tributyrin in the reaction medium (40 mL of NaCl solution 0.15 M).

In order to determine the optimal hydrolysis temperatures, hydrolysis reactions were carried out at pH 8.0, with 10 min of incubation at various temperatures (22, 35, 45, 50, 55, 60, and 68°C). Figure 2 shows the relative lipase activities of *E. characias* latex, crude bromelain, and crude papain at various temperatures, with maximum activity taken as 100%. Optimal activities were observed in the range 45–55°C for all plant extracts, namely, 45°C for *E. characias* latex, 55°C for crude bromelain, and 50°C for crude papain. Consequently, these optimal lipolysis conditions were kept for any further

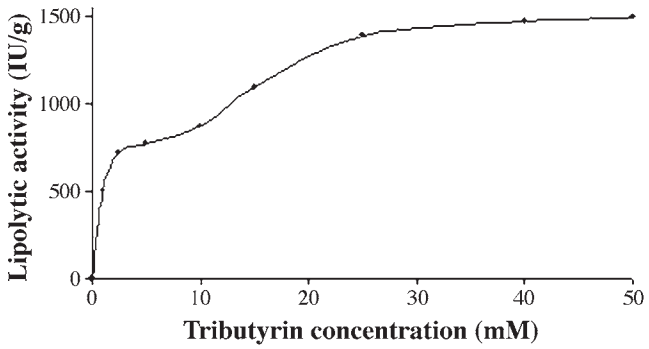

FIG. 1. Lipase activity of crude papain as a function of tributyrin concentration in 40 mL of NaCl solution (0.15 M). Lipase activities were determined at pH 8.0, 50°C, and with 10 min of incubation time. The data were arithmetic means of three determinations.

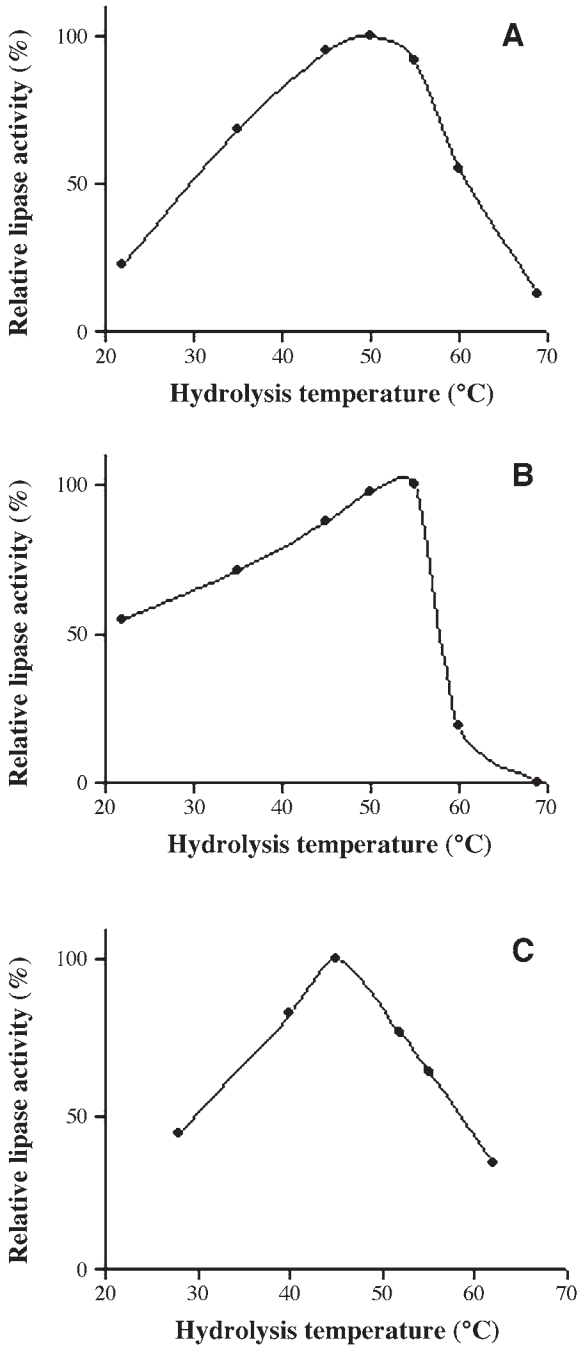

FIG. 2. Relative lipase activities of crude papain (A), crude bromelain (B), and *Euphorbia characias* latex (C) at various temperatures. Lipase activities were determined with tributyrin as substrate (1.5 mmol in 40 mL of 0.15 M NaCl solution) at pH 8.0 and with 10 min of incubation time. The data were arithmetic means of three determinations.

determination. The Arrhenius activation energy (estimated over the range 22–35°C and with 10 min of incubation time) was 11 kcal/mol for *E. characias* latex, 5 kcal/mol for crude bromelain, and 15 kcal/mol for crude papain.

Lipase FA typoselectivities in hydrolysis. The use of homogeneous TAG to detect possible selectivities does have certain drawbacks, since lipase FA typoselectivities in lipolysis reactions are due not only to the enzyme itself but also to the substrate, and more precisely, to the interfacial quality (13). Therefore, it is essential to conduct hydrolysis reactions under conditions where all substrates are in the same physical state (14). So under the chosen operating conditions (pH 8.0; 50°C), we were able to use only those homogeneous TAG that are liquid at this temperature, such as tributyrin, tricaproin, tricaprylin, tricaprin, and triolein. The natural TAG mixtures (copra, olive, sunflower, and linseed) were obviously liquid at this temperature.

The lipase activities of *E. characias* latex, crude bromelain, and crude papain are presented in Table 1. These activities were derived from the initial slopes of each kinetic curve. Results showed that, compared to *E. characias* latex and crude papain, the commercially available crude preparation of bromelain exhibited very poor lipase activity in hydrolysis. For all substrates tested, this activity was optimal for tributyrin (64 IU/g) and linseed TAG (63 IU/g) as substrate. To the best of our knowledge, this is the first time that a study has evaluated the "true" lipase activity of this preparation with authentic TAG, which are natural lipase substrates. Although this activity is low, it does exist and has been quantified.

Our results are in accordance with the ones obtained by Mukherjee and Kiewitt (4,5) in which the biocatalytic activity of a commercially available crude preparation of bromelain was quantified in esterification reactions. In fact, it is indeed important to keep in mind that even with the same lipase, acyltransferase and triacylglycerol hydrolase activity can be very different. For example, a lipase preparation that has high esterification activity may have high or little hydrolytic activity (11). Therefore, we believe that both results are very complementary, since our study was carried out through hydrolysis of TAG. Now, we can assert that the commercially available crude preparation of bromelain contains

TABLE 1
Lipase Fatty Acid Typoselectivities of the Latex from *Euphorbia characias* and the Two Commercially Available Crude Preparations of Bromelain and Papain

	Lipase activity (IU/g)		
	Latex of *E. characias*	Crude bromelain	Crude papain
Tributyrin	3500* (=10295**)	64 ± 3 (4.7%)[a]	1590 ± 33 (2.1%)[a]
Tricaproin	950* (=2795**)	45 ± 3 (6.7%)[a]	1350 ± 29 (2.1%)[a]
Tricaprylin	835* (=2456**)	51 ± 3 (5.9%)[a]	1080 ± 25 (2.3%)[a]
Tricaprin	470* (=1382**)	41 ± 3 (7.3%)[a]	1050 ± 27 (2.6%)[a]
Copra TAG	220* (=647**)	42 ± 3 (7.1%)[a]	590 ± 18 (3.0%)[a]
Triolein	44* (=129**)	42 ± 4 (9.5%)[a]	55 ± 4 (7.2%)[a]
Olive TAG	34* (=100**)	42 ± 3 (7.1%)[a]	68 ± 5 (7.3%)[a]
Sunflower TAG	48* (=141**)	49 ± 4 (8.2%)[a]	56 ± 4 (7.1%)[a]
Linseed TAG	27* (=79**)	63 ± 4 (6.3%)[a]	52 ± 5 (9.6%)[a]

[a]Mean values ± standard deviation (relative standard deviation percentage) of triplicate analyses. Lipase activities were determined by pH-Stat with each triacylglycerol (TAG) at pH 8.0, at 50°C, and with 10 min of incubation time. Activities were expressed as IU/g of crude preparation (or *IU/mL of fresh latex; **IU/g of dried extract of latex). One IU corresponds to 1 µmol of free fatty acids released per minute.

lipases which exhibit high esterification activity (4,5) and very poor hydrolytic activity of TAG in our conditions.

Latex from *E. characias*, which contains 340 mg of dried material per milliliter of fresh latex, exhibited a very high lipase activity in hydrolysis (Table 1). For all substrates tested, this latex showed a better activity than commercially available crude preparation of papain. For example, the activity with tributyrin was 3,500 IU/mL for fresh *E. characias* latex (or 10,295 IU/g for dried extract of latex), whereas *C. papaya* latex exhibited only 1,590 IU/g. Moreover, lipase from *E. characias* latex showed a short-chain preference in hydrolysis of homogeneous TAG; as a matter of fact, activities decrease dramatically with increasing chain length of TAG. The lipase activity was optimal with tributyrin as substrate. With tricaproin and tricaprylin, this latex lipase exhibited 25% activity relative to that with tributyrin. With tricaprin, it exhibited 13% activity relative to that of tributyrin, 6% with copra TAG, and finally, 1% activity with long-chain TAG. However, this particular selectivity of lipase from *E. characias* latex should be confirmed subsequently in hydrolysis reactions with chiral TAG and the corresponding racemic, according to the method developed by Villeneuve *et al.* (7–9).

Lipase FA typoselectivities in interesterification. The selectivity of a lipase for hydrolysis may not be conserved in interesterification reactions. Thus, if a biocatalyst is to be used in interesterification in a nonaqueous medium, its activity and selectivity under these conditions should be evaluated independently. We decided to evaluate the lipase activity and FA typoselectivities of two commercially available crude preparations (crude bromelain and crude papain) in interesterification reactions.

Several homogeneous TAG (saturated, monounsaturated, or polyunsaturated) were reacted separately with tributyrin. Reactions were carried out in *n*-hexane solution with a 10% (w/w) lipase preparation to total TAG. The lipase catalyzes acyl exchange between the two substrates. Thus, each acyl exchange between tributyrin (BuBuBu) and the other homogeneous TAG (XXX) is characterized by the formation of new TAG with one (BuXX) or two (BuBuX) butyroyl residues. The total equivalent carbon number of these abovementioned molecules differs from the two initial substrates. The interesterification activity was quantified by following the appearance of these newly formed TAG by GLC, and the formation of partial glycerides [diacylglycerols (DAG), . . .] in the reaction medium was also monitored by GLC.

Under our conditions, experiments with crude bromelain (38.2% protein; 2.9% water; A_w: 0.21) show that this preparation has no activity in interesterification for all TAG tested with tributyrin in *n*-hexane. Regarding interesterification reactions with crude papain (60.7% protein; 7.8% water; A_w: 0.55), data show that interesterification activity was optimal with tributyrin against tricaproin: 70 mol% of newly formed TAG appearing in the TAG fraction after 24 h of interesterification (Fig. 3). For interesterification reactions with tributyrin against the other saturated TAG, the proportion of newly formed TAG decreased with increasing chain length of TAG (only 21 mol% of new TAG appearing in the TAG fraction after 24 h interesterification between tributyrin and tristearin), except for the interesterification reaction with tributyrin against trimargarin (49 mol% of new TAG). For interesterification reactions with tributyrin against unsaturated TAG, the following differences were observed: triolein, which is a TAG containing a monounsaturated FA, reacted faster (34 mol% of new TAG) than polyunsaturated TAG such as trilinolein or tri-α-linolenin. For the latter substrates, yields of new TAG were only 20 and 23 mol%, respectively, after 24 h interesterification.

During these interesterification reactions with crude papain as biocatalyst, we also monitored the relative proportion of new TAG with one or two butyroyl residues with time (Table 2). Results show that throughout the reactions, the proportion of new TAG with two butyroyl residues was higher than new TAG with only one butyroyl residue. This phenomenon was more pronounced for interesterification reactions with tributyrin against long-chain TAG. Actually, over the first 6 h of reaction, the "BuBuX/BuXX" ratio was 1.2–1.6 in interesterification reactions of tributyrin with TriC6:0, TriC8:0, or TriC10:0. This ratio was 2.1–2.6 in interesterification reactions of tributyrin with TriC12:0 or TriC14:0, 3.0–3.1 in interesterification reactions of tributyrin with TriC16:0, TriC17:0, or TriC18:0, and 3.5 in interesterification reactions of tributyrin with mono- or polyunsaturated TAG (such as triolein, trilinolein, or tri-α-linolenin). The proportion of partial glycerides formed in the reaction medium after 24 h interesterification, from the competing hydrolysis of TAG, remained under 11% (w/w) for all interesterification reactions.

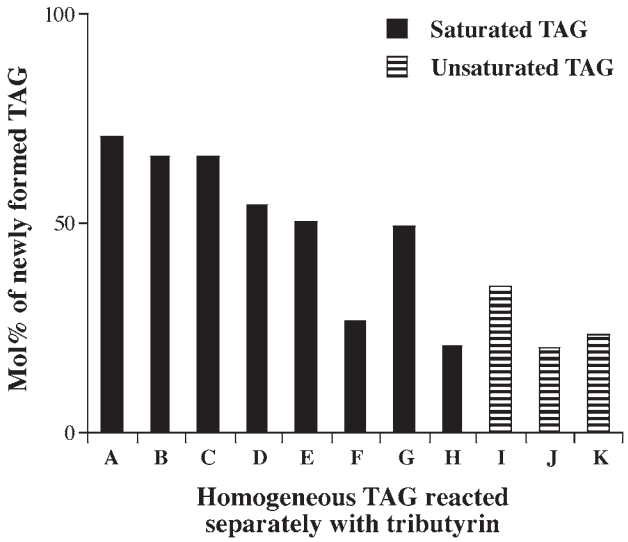

FIG. 3. Proportion of newly formed triacylglycerols (TAG) in the TAG fraction after 24 h interesterification of tributyrin with a series of homogeneous TAG and with crude papain as biocatalyst. Reactions were carried out in *n*-hexane (5 mL) at 50°C with a 10% (w/w) of crude papain to total TAG. Tributyrin (100 µmol) was reacted with 100 µmol of another homogeneous TAG: tricaproin (A), tricaprylin (B), tricaprin (C), trilaurin (D), trimyristin (E), tripalmitin (F), trimargarin (G), tristearin (H), triolein (I), trilinolein (J), tri-α-linolenin (K).

TABLE 2
Time Courses of the Formation of New TAG with One or Two Butyroyl Residues, from Interesterification of Tributyrin with a Series of Homogeneous TAG and with Crude Papain as Biocatalyst[a]

TAG	Reaction time (h)			
(mol% in the TAG fraction)	0	1	6	24
Tributyrin (BuBuBu) + tricaproin (CaCaCa)				
Residual TAG	100.0	81.6	50.5	29.6
New BuBuCa	—	10.1	27.0	36.1
New BuCaCa	—	8.3	22.5	34.3
Tributyrin (BuBuBu) + tricaprylin (CyCyCy)				
Residual TAG	100.0	87.8	59.1	34.1
New BuBuCy	—	8.2	25.2	36.1
New BuCyCy	—	4.0	15.7	29.8
Tributyrin (BuBuBu) + tricaprin (CCC)				
Residual TAG	100.0	85.0	59.8	34.3
New BuBuC	—	9.9	23.6	32.6
New BuCC	—	5.1	16.6	33.1
Tributyrin (BuBuBu) + trilaurin (LaLaLa)				
Residual TAG	100.0	92.5	73.4	45.4
New BuBuLa	—	5.4	18.1	32.5
New BuLaLa	—	2.1	8.5	22.2
Tributyrin (BuBuBu) + trimyristin (MMM)				
Residual TAG	100.0	92.2	72.6	49.5
New BuBuM	—	5.9	19.8	32.5
New BuMM	—	1.9	7.6	18.2
Tributyrin (BuBuBu) + tripalmitin (PPP)				
Residual TAG	100.0	97.8	89.0	73.4
New BuBuP	—	1.7	8.3	19.1
New BuPP	—	0.4	2.7	7.5
Tributyrin (BuBuBu) + trimargarin (MaMaMa)				
Residual TAG	100.0	94.7	78.9	50.9
New BuBuMa	—	3.7	15.8	32.5
New BuMaMa	—	1.6	5.3	16.9
Tributyrin (BuBuBu) + tristearin (SSS)				
Residual TAG	100.0	98.3	91.5	79.1
New BuBuS	—	1.3	6.3	15.1
New BuSS	—	0.4	2.1	5.8
Tributyrin (BuBuBu) + triolein (OOO)				
Residual TAG	100.0	96.5	85.3	65.5
New BuBuO	—	2.5	11.4	24.4
New BuOO	—	1.0	3.3	10.1
Tributyrin (BuBuBu) + trilinolein (LLL)				
Residual TAG	100.0	96.2	86.4	79.9
New BuBuL	—	2.9	10.6	15.6
New BuLL	—	0.8	3.0	4.5
Tributyrin (BuBuBu) + tri-α-linolenin (LnLnLn)				
Residual TAG	100.0	93.4	83.9	76.9
New BuBuLn	—	5.1	12.4	17.7
New BuLnLn	—	1.5	3.6	5.4

[a]Reactions were carried out in *n*-hexane (5 mL) at 50°C and with a 10% (w/w) of commercially available crude preparation of papain in total TAG. See Table 1 for abbreviation.

Thus, during interesterification reactions between a short-chain TAG and a long-chain TAG with crude papain as biocatalyst, the proportion of new TAG with two short-chain FA residues will remain higher than new TAG with two long-chain FA residues. The above results are complementary with the ones obtained by Villeneuve *et al.* (10), in which the specificity of lipase from *C. papaya* latex was determined in interesterification reactions of tricaprylin with another series of TAG. It is important to keep in mind that the same authors removed the ambiguity between short-chain FA typoselectivity and *sn*-3 stereoselectivity of the *C. papaya* lipase (high *sn*-3 stereoselectivity in hydrolysis and interesterification reactions of selected TAG).

Potential industrial uses. The use of lipases in oil and fat bioconversion has many advantages over classical chemical catalysts. It is possible to classify lipases based on their selectivities and to select a particular enzyme for a desired application. For example, the high lipase activity and the particular FA typoselectivity of lipase from *E. characias* latex in hydrolysis reactions could be advantageously exploited on an industrial scale. This biocatalyst may be used in the production of low-calorie structured TAG, or in the dairy industry to obtain specific flavor components by the release of short- or medium-chain FA from milk fat.

Moreover, if this latex lipase exhibits the same activity and FA typoselectivities in the other classical reactions of oil and fat modification, namely esterification, transesterification and interesterification, it could be used also as a good biocatalyst for lipid transformations. For instance, it could be used in inter- and transesterification reactions of TAG to remove selectively the short-chain FA and to replace them by, for example, long-chain saturated FA. This biocatalyst could be used also to modify the FA composition and the distribution of TAG in oils and fats, in order to obtain new products with predetermined physical and chemical properties. This latex lipase may prove useful in interesterification reactions to increase the ratio of medium-chain triacylglycerols in different oils (15).

REFERENCES

1. Jensen, R.G., F.A. Dejong, and R.M. Clarck, Determination of Lipase Selectivity, *Lipids 18*:239–252 (1983).
2. Ncube, I., T. Gitlesen, P. Adlercreutz, J.S. Read, and B. Mattiasson, Fatty Acid Selectivity of a Lipase Purified from *Vernonia galamensis* Seed, *Biochim. Biophys. Acta 1257*:149–156 (1995).
3. Mc Kee, R.A., and H. Smith, Purification of Proteinases from *Carica papaya*, *Phytochemistry 25*:2283–2287 (1986).
4. Mukherjee, K.D., and I. Kiewitt, Specificity of *Carica papaya* Latex as Biocatalyst in the Esterification of Fatty Acids with 1-Butanol, *J. Agric. Food Chem. 44*:1948–1952 (1996).
5. Mukherjee, K.D., and I. Kiewitt, Substrate Specificity of Lipases in Protease Preparations, *Ibid. 46*:2427–2432 (1998).
6. Giordani, R., A. Moulin, and R. Verger, Tributyroylglycerol Hydrolase Activity in *Carica papaya* and Other Latices, *Phytochemistry 30*:1069–1072 (1991).
7. Villeneuve, P., M. Pina, D. Montet, G. Renard, and J. Graille, Chiral Synthesis of a Triglyceride: Example of 1-Butyroyl-2-oleoyl-3-palmitoyl-*sn*-glycerol, *Chem. Phys. Lipids 72*:135–141 (1994).
8. Villeneuve, P., M. Pina, D. Montet, and J. Graille, Determination of Lipase Specificities Through the Use of Chiral Triglycerides and Their Racemics, *Ibid. 76*:109–113 (1995).
9. Villeneuve, P., M. Pina, D. Montet, and J. Graille, *Carica papaya* Latex Lipase: *sn*-3 Stereoselectivity or Short-Chain Selectivity? Model Chiral Triglycerides Are Removing the Ambiguity, *J. Am. Oil Chem. Soc. 72*:753–755 (1995).
10. Villeneuve, P., M. Pina, A. Skarbek, J. Graille, and T.A. Foglia, Specificity of *C. papaya* Latex in Lipase-Catalysed Interesterification Reactions, *Biotech. Tech. 11*:91–94 (1997).

11. Mudherwa, J., M. Pina, and J. Graille, Aptitude à la Transesterification de Quelques Lipases Regioselectives 1–3, *Oleagineaux 43*:385–392 (1988).
12. *Recueil de Normes Françaises 1993—Corps Gras, Graines Oléagineuses, Produits Dérivés*, 5th edn., edited by AFNOR Normes, Paris, 1993, pp. 116–120.
13. Schmid, R.D., and R. Verger, Lipases: Interfacial Enzymes with Attractive Applications, *Angew. Chem. Int. Ed. Engl. 37*:1608–1633 (1998).
14. Villeneuve, P., and T.A. Foglia, Lipase Specificities: Potential Application in Lipid Bioconversions, *INFORM 8*:640–650 (1997).
15. Villeneuve, P., M. Pina, and J. Graille, Mise en Evidence des Sélectivités des Lipases en Interestérification à l'Aide d'un Triglycéride Chiral Modèle, *Oléagineux Corps Gras Lipides 3*:459–464 (1996).

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