Lipase-Catalyzed Alcoholysis of Crambe Oil and Camelina Oil for the Preparation of Long-Chain Esters[1]

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ABSTRACT: Crambe oil and camelina oil were transesterified with oleyl alcohol, the alcohols derived from crambe and camelina oils, *n*-octanol or isopropanol using Novozym 435 (immobilized lipase B from *Candida antarctica*), Lipozyme IM (immobilized lipase from *Rhizomucor miehei*), and papaya (*Carica papaya*) latex lipase as biocatalysts. The highest conversions to alkyl esters were obtained with Novozym 435 (up to 95%) in most cases, whereas Lipozyme IM and papaya latex lipase gave lower (40 to 50%) conversions. The conversions with long-chain alcohols (oleyl alcohol, crambe alcohols, and camelina alcohols) were higher (40 to 95%) than with medium-chain *n*-octanol (30 to 85%). Isopropyl esters of crambe oil and camelina oil were obtained with rather low conversions using Novozym 435 (<40%) and Lipozyme IM (about 10%) as biocatalysts, whereas with papaya latex lipase no isopropyl esters were formed. The conversions of crambe oil and camelina oil to oleyl and *n*-octyl esters using Novozym 435 as biocatalyst were hardly affected by the ratio of the substrates, but with Lipozyme IM the conversions to alkyl esters distinctly increased with an excess of alcohol substrate.

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KEY WORDS: Bio-esters, camelina oil, crambe oil, jojoba oil analog, lipase-catalyzed alcoholysis, long-chain esters, transesterification.

Interest in lipase-catalyzed preparation of wax and bio-esters has grown in the last few years because of the possibility to obtain a wide variety of high-quality products under mild reaction conditions utilizing the substrate selectivity of such biocatalysts (1–9). The alcoholysis of triacylglycerols from vegetable oils with fatty alcohols, catalyzed by lipases, leads to a mixture of long-chain and very long chain wax esters with properties potentially suitable for applications in cosmetics and lubricants (10–15).

In an accompanying paper the alkali-catalyzed alcoholysis of crambe oil and camelina oil to produce long-chain esters was studied (16). We report here the solvent-free lipase-catalyzed alcoholysis of these oils with long-chain alcohols, such as oleyl alcohol and alcohols derived from crambe oil and camelina oil with the aim to prepare wax esters as substitutes for jojoba oil. Moreover, lipase-catalyzed alcoholysis of crambe oil and camelina oil with *n*-octanol and isopropanol was carried out with the aim to prepare products similar to "bio esters" for cosmetics (17). Novozym 435 (immobilized unspecific lipase-B from *Candida antarctica*) (18–20), Lipozyme IM (immobilized *sn*-1,3-specific lipase from *Rhizomucor miehei*) (21,22), and papaya (*Carica papaya*) latex lipase (23–25) were used as biocatalysts.

EXPERIMENTAL PROCEDURES

Materials. Novozym 435 and Lipozyme IM were kind gifts from Novo Nordisk Biotechnologie GmbH (Mainz, Germany). *Carica papaya* latex was purchased from Sigma-Aldrich-Fluka (Deisenhofen, Germany). The crude latex was ground and sieved to grains <0.8 mm.

Refined oils from crambe (*Crambe abyssinica*) seeds and camelina (*Camelina sativa*) seeds were provided by the Institut für Pflanzenbau der Landesforschungsanstalt für Landwirtschaft und Fischerei Mecklenburg-Vorpommern (Gülzow, Germany). Fatty acid composition of the oils (designated by number of carbon atoms:number of *cis*-double bonds) was as follows. Crambe oil: 16:0 = 2%, 18:0 = 1%, 18:1 = 17%, 18:2 = 9%, 18:3 = 6%, 20:0 = 1%, 20:1 = 4%, 22:0 = 2%, 22:1 = 56%, 24:1 = 1%; camelina oil: 16:0 = 5%, 18:0 = 3%, 18:1 = 14%, 18:2 = 16%, 18:3 = 36%, 20:0 = 1%, 20:1 = 15%, 22:0 = <1%, 22:1 = 3%, 24:1 = <1%. The alcohols from crambe and camelina were prepared from their methyl esters *via* hydrogenolysis by the Deutsche Hydrierwerke (DHW) (Rodleben, Germany). Composition of the alcohols (designated by number of carbon atoms:number of *cis*-double bonds) was as follows. Crambe alcohols: 16:0 = 3%, 18:0 = 2%, 18:1 = 21%, 18:2 = 1%, 18:3 = 2%, 20:0 = 2%, 20:1 = 4%, 22:0 = 5%, 22:1 = 56%, 24:0 = 2%, 24:1 = 2%; camelina alcohols: 16:0 = 10%, 18:0 = 5%, 18:1 = 30%, 18:2 = 10%, 18:3 = 3%, 20:0 = 6%, 20:1 = 27%, 22:1 = 9%. Oleyl alcohol (technical grade, containing 4% stearyl alcohol, 90% oleyl alcohol, 5% linoleyl alcohol, 1% eicosenyl alcohol) was purchased from Sigma-Aldrich-Fluka, and percolated prior to use over alumina under argon. *n*-Octanol and isopropanol were supplied by E. Merck (Darmstadt, Germany).

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All distilled solvents and reagents of analytical grade were obtained from E. Merck. Reference lipid standards were from Sigma-Aldrich-Fluka and Nu-Chek-Prep (Elysian, MN).

Alcoholysis. Reactions were carried out in glass tubes provided with Teflon-lined screw-caps using 0.3 mmol oil (crambe oil or camelina oil) with the stoichiometric amount of alcohol (oleyl alcohol, crambe alcohols, camelina alcohols, *n*-octanol, or isopropanol). The concentration of Novozym 435 or Lipozyme IM used was 5% w/w of the reactants; when papaya latex lipase was used as biocatalyst, the concentration was 10% w/w. All reactions were carried out by magnetic stirring at a temperature of 60°C under nitrogen. Samples were taken at reaction times of 4, 8, and 24 h.

Product isolation and analysis. Samples of 75 μL were taken and about 1 mL isohexane was added. The biocatalyst was removed by centrifugation. After adding about 1 mL of water to remove any glycerol formed by alcoholysis of the oils, the organic phase was separated and dried over anhydrous sodium sulfate.

A known amount of methyl heptadecanoate was added as internal standard to an aliquot of the reaction products, and the mixture was fractionated by thin-layer chromatography on Silica Gel H using isohexane/diethyl ether/acetic acid (80:20:1, vol/vol/vol) as developing solvent. The lipid fractions were made visible by exposing the edges of the chromatoplates to iodine vapor. The fraction containing alkyl esters of fatty acids and the internal standard was scraped off, eluted with water-saturated diethyl ether, and dried. The mixture of alkyl esters was analyzed by gas chromatography in a Hewlett-Packard 5890 instrument (Hewlett-Packard GmbH, Waldbronn, Germany) equipped with flame-ionization detectors. The separations were carried out on a Quadrex 400-5HT column (25 m × 0.25 mm i.d. × 0.1 μm film) (Quadrex Corporation, New Haven, CT) with hydrogen as carrier gas (column pressure 50 kPa). Temperature was programmed from 160 (2 min isothermal) to 310°C, 10°C/min, then to 420°C, 5°C/min, 5 min isothermal. Peaks were identified by comparison with reference standards and wax esters of jojoba oil, and quantitated using HP GC ChemStation Rev. A.06.3 [509] software (Hewlett-Packard) using correction factors that were obtained from mixtures of known composition.

Alcohol compositions were determined by gas chromatography in a Hewlett-Packard 5890 instrument on a HP-1 column (25 m × 0.32 mm i.d. × 0.52 μm film; Hewlett-Packard) with nitrogen as carrier gas (column pressure 85 kPa). Temperature was programmed from 180 (2 min isothermal) to 260°C, 6°C/min (5 min isothermal). Quantitation was made using HP ChemStation B.01.02 software (Hewlett-Packard). Peaks were identified by comparison with reference standards.

RESULTS AND DISCUSSION

Alcoholysis of crambe oil and camelina oil with oleyl alcohol. A commercially available technical-grade oleyl alcohol was investigated as a model long-chain alcohol in the lipase-catalyzed alcoholysis with crambe oil and camelina oil. The results, given in Figure 1 A and B, show that with both oils the conversions using Novozym 435 are high (90–100%) compared to those using Lipozyme IM (50–60%) and papaya latex lipase (30–50%).

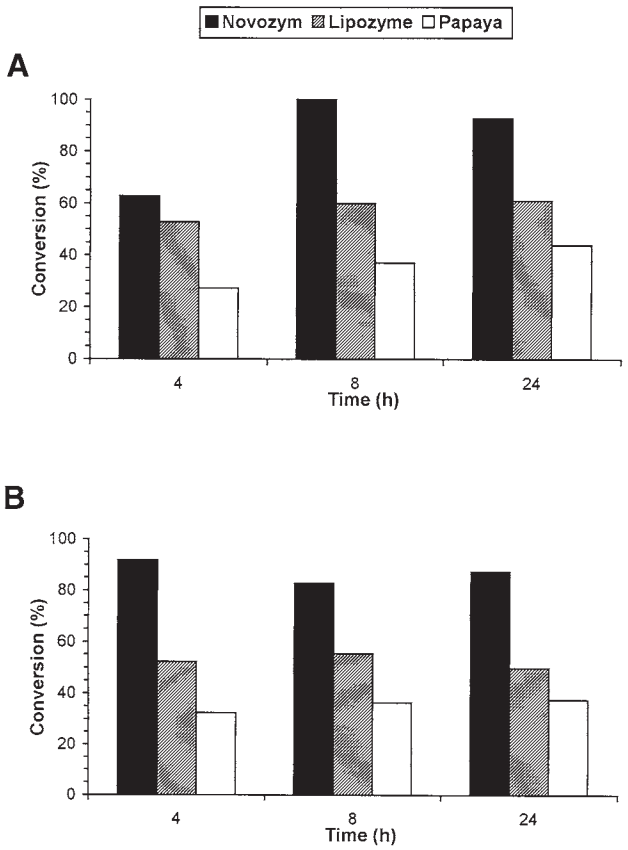

FIG. 1. Time course of lipase-catalyzed alcoholysis of (A) crambe oil and (B) camelina oil with oleyl alcohol (molar ratio of triacylglycerols/alcohol = 1:3). Enzyme sources: Novozyme and Lipozyme, Novo Nordisk Biotechnologie GmbH (Mainz, Germany); papaya latex lipase, Sigma-Aldrich-Fluka (Deisenhofen, Germany).

The compositions of the wax esters formed by alcoholysis of the two oils with oleyl alcohol are as expected (Fig. 2) from the composition of the oils, given in the Experimental Procedures section. The alcoholysis of crambe oil with oleyl alcohol leads mainly to wax esters with the chain lengths C36 and C40 (Fig. 2A) whereas in corresponding products from camellina oil the C36 chain length predominates (>70%) (Fig. 2B).

We further investigated the influence of the substrate ratio on the alcoholysis of crambe oil and camelina oil with oleyl alcohol. In the alcoholysis of crambe oil (Fig. 3A) and camelina oil (Fig. 3B) with oleyl alcohol using Novozym 435 as biocatalyst, the conversions (80–95%) are very little affected by the molar ratio of the substrates. It is obvious that the very high rate of conversion achieved with Novozym 435 at stoichiometric molar ratio of oil to alcohol (1:3) was further increased very little by increasing the molar excess of alcohol up to 10:1. In the case of Lipozyme IM, however, the conversions are much higher (≥90%) if the reaction takes place at a molar ratio of 1:10 of oil to alcohol compared to a ratio of 1:3 of oil to alcohol (50–60 %) as shown in Figure 3.

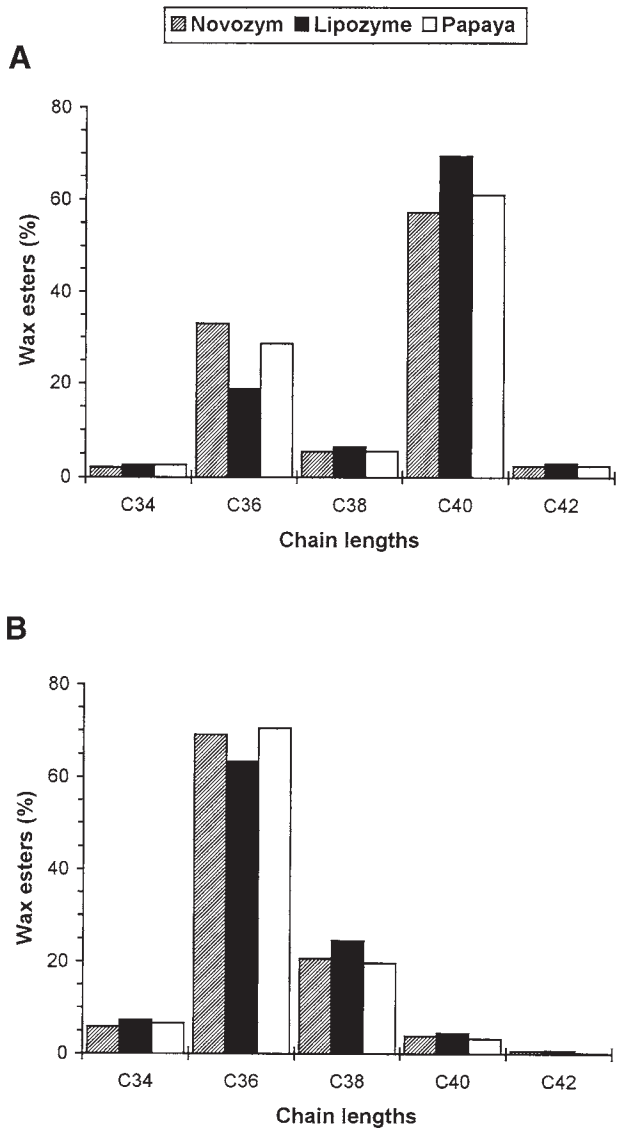

FIG. 2. Composition of the wax esters formed by lipase-catalyzed alcoholysis of (A) crambe oil and (B) camelina oil with oleyl alcohol (molar ratio of triacylglycerols/alcohol = 1:3, time of reaction = 24 h). For enzyme sources see Figure 1.

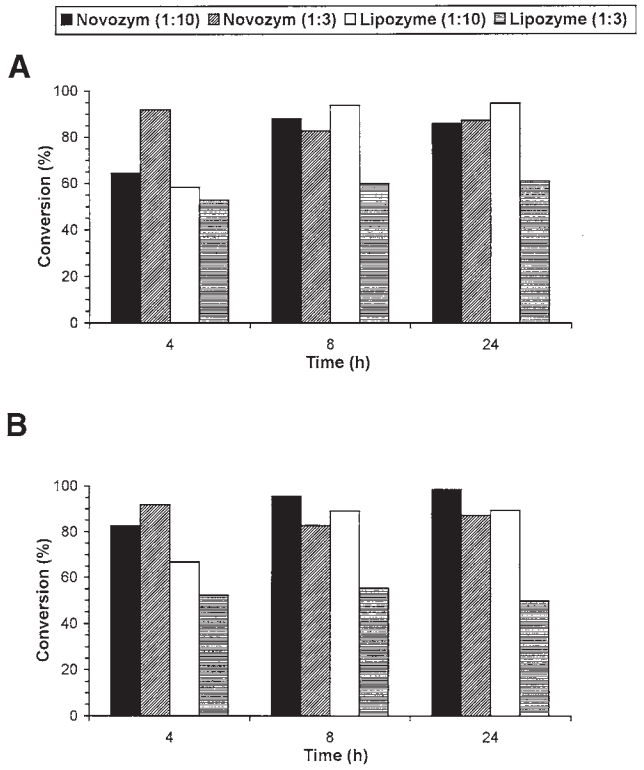

FIG. 3. Time course of lipase-catalyzed alcoholysis of (A) crambe oil and (B) camelina oil with oleyl alcohol at different molar ratios of triacylglycerols/alcohol. For enzyme sources see Figure 1.

Alcoholysis of crambe oil and camelina oil with crambe alcohols and camelina alcohols. Figure 4A shows the time course in the alcoholysis of crambe oil with crambe alcohols and camelina alcohols. Novozym 435 as biocatalyst gives conversions of about 70% with crambe alcohols and about 65% with camelina alcohols (Fig. 4A). The reaction with Novozym 435 levels off after 4 h. In the alcoholysis of crambe oil, Lipozyme IM leads to conversions of approximately 50% with crambe alcohols and *ca.* 40% with camelina alcohols; similarly, papaya latex lipase gives conversions of 45–50% with crambe alcohols and 40% with camelina alcohols (Fig. 4A). With Lipozyme IM and papaya lipase the conversions increase slightly for a reaction time up to 24 h.

The results of alcoholysis of camelina oil with crambe alcohols and camelina alcohols are shown in Figure 4B. The conversions are very similar to those for crambe oil (Fig. 4A), but camelina alcohols give higher conversions than crambe alcohols.

In the alcoholysis of crambe oil and camelina oil with crambe alcohols and camelina alcohols, the conversions are about 20% lower (Fig. 4) than the corresponding reactions with oleyl alcohol (Fig. 1).

Figure 5 shows the composition of the wax esters formed by Novozym 435-catalyzed alcoholysis of crambe oil and camelina oil with crambe alcohols and camelina alcohols as compared to the wax ester composition of jojoba oil. The main wax ester constituents of joboba oil are those with chain lengths C42 (≈50%), C40 (≈30%), and C44 (≈10%). The wax esters obtained by Novozym 435-catalyzed alcoholysis of crambe oil with crambe alcohols constitute a product of mainly C40 (≈40%), C44 (≈35%), and C36 as well as C42 (both ≈10%) chain lengths. The corresponding esters of camelina oil with camelina alcohols contain C36 (≈50%), C38 (≈30%), and C34 as well as C40 (both ≈10%). If crambe oil is transesterified with camelina alcohols or camelina oil with crambe alcohols, respectively, the wax esters in the products consist of C40 (≈40%), C36 (≈20%), and C38 as well as C42 (both ≈15%) in both cases (Fig. 5). Thus, these last two products show compositions that are closest to the wax ester composition of jojoba oil.

Alcoholysis of crambe oil and camelina oil with n*-octanol.* When *n*-octanol was used as alcohol substrate in the lipase-catalyzed alcoholysis of crambe oil and camelina oil, the conversions were lower (Fig. 6) than with the long-chain alcohols

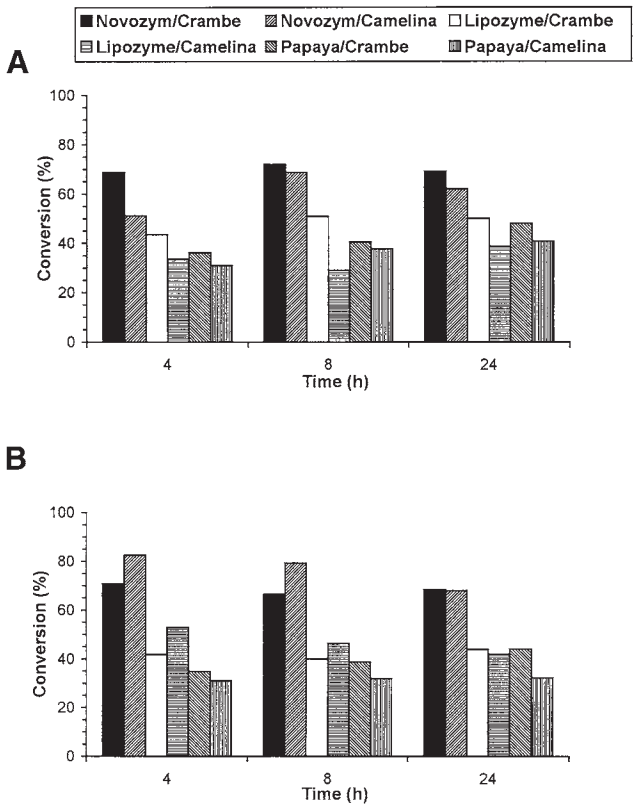

FIG. 4. Time course of lipase-catalyzed alcoholysis of (A) crambe oil and (B) camelina oil with crambe alcohols and camelina alcohols (molar ratio of triacylglycerols/alcohol = 1:3). For enzyme sources see Figure 1.

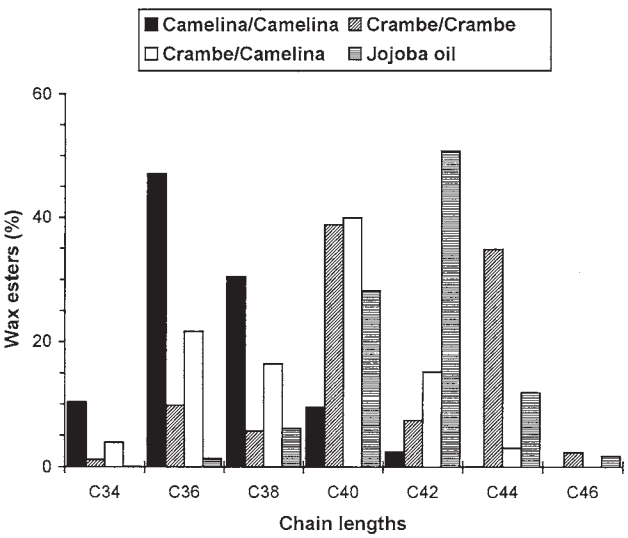

FIG. 5. Wax ester composition of the products obtained by Novozym 435-catalyzed alcoholysis of crambe oil and camelina oil with crambe alcohols and camelina alcohols in comparison to wax esters of jojoba oil (molar ratio of triacylglycerols/alcohol = 1:3, time of reaction = 24 h). For enzyme source see Figure 1.

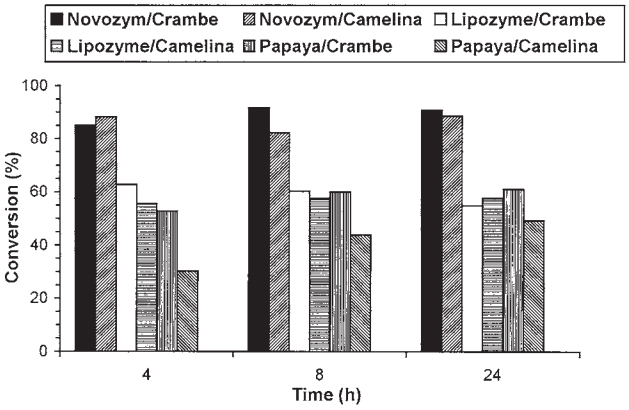

FIG. 6. Time course of lipase-catalyzed alcoholysis of crambe oil and camelina oil with *n*-octanol (molar ratio of triacylglycerols/alcohol = 1:3). For enzyme sources see Figure 1.

(Figs. 1, 3, 4). Similar to the experiments described above (Figs. 1, 3, 4), Novozym 435 gave the highest conversions (80–90%) of the three biocatalysts, whereas Lipozyme IM and papaya latex gave conversions of 45–60% (Fig. 6).

Figure 7 shows the composition of the *n*-octyl esters formed by Novozym 435-catalyzed alcoholysis of crambe oil and camelina oil with *n*-octanol. It is evident that alcoholysis of crambe oil leads mainly to *n*-octyl esters with the chain lengths of C26 and C30, while the corresponding products from camelina oil are predominated (70%) by C26 chain length (Fig. 7).

Alcoholysis of crambe oil and camelina oil with isopropanol. As reported in the accompanying paper (16) no alcoholysis of crambe oil and camelina oil with isopropanol could be achieved using potassium hydroxide as catalyst. In contrast enzymatic alcoholysis of crambe oil and camelina oil with isopropanol using Novozym 435 and Lipozyme IM as biocatalyst led to isopropyl esters (Fig. 8). The conversions were moderate (about 40%) with Novozym 435 and low (<10%) with

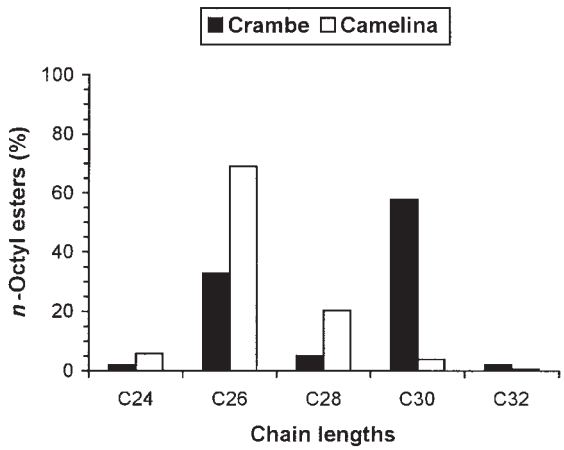

FIG. 7. *n*-Octyl ester composition of the products obtained by Novozym 435-catalyzed alcoholysis of crambe oil and camelina oil with *n*-octanol (molar ratio of triacylglycerols/alcohol = 1:3, time of reaction = 24 h). For enzyme source see Figure 1.

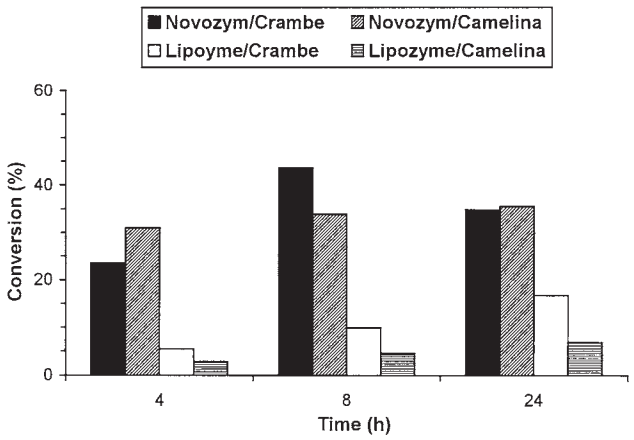

FIG. 8. Time course of lipase-catalyzed alcoholysis of crambe oil and camelina oil with isopropanol (molar ratio of triacylglycerols/alcohol = 1:3). For enzyme sources see Figure 1.

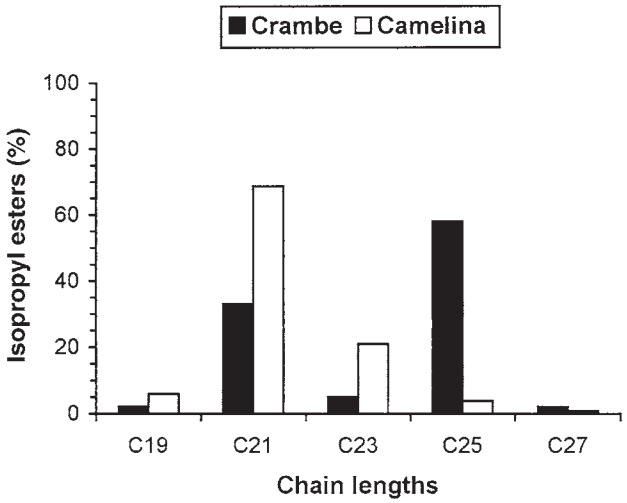

FIG. 9. Isopropyl ester composition of the products obtained by Novozym 435-catalyzed alcoholysis of crambe oil and camelina oil with isopropanol (molar ratio of triacylglycerols/alcohol = 1:3, time of reaction = 24 h). For enzyme source see Figure 1.

Lipozyme IM (Fig. 8). Papaya latex lipase was unable to catalyze the alcoholysis of the oils with isopropanol under the reaction conditions used, which is attributed to the strong regiospecificity of papaya latex lipase for the primary hydroxy groups (23–25).

Figure 9 shows the composition of isopropyl esters formed by Novozym 435-catalyzed alcoholysis of crambe oil and camelina oil with isopropanol. It can be seen that crambe oil yields mainly isopropyl esters with chain lengths of C21 and C25, whereas the corresponding products from camelina oil contain predominantly esters of C21 chain length (Fig. 9).

In conclusion, the lipase-catalyzed alcoholysis of crambe oil and camelina oil with long-chain alcohols such as oleyl alcohol or alcohols derived from these oils is a successful way to obtain wax esters for cosmetics and lubricants. Novozym 435 is an outstanding biocatalyst for the preparation of alkyl esters by alcoholysis of crambe oil and camelina oil with oleyl alcohol, crambe alcohols, camelina alcohols, *n*-octanol, and isopropanol. With Novozym 435 as catalyst the conversions with long-chain alcohols and *n*-octanol are high (around 80–95%), whereas isopropyl esters are only formed to an extent of about 40% conversion. Lipozyme IM and papaya latex lipase do not give as high conversions as Novozym 435. With Lipozyme IM and papaya latex lipase the conversions can be enhanced by increasing the molar excess of the alcohol in the reaction mixture. All three biocatalysts show a dependence on the chain length of the alcohol employed. The conversions are highest with the long-chain fatty alcohols (oleyl alcohol, crambe alcohols, and camelina alcohols) followed by the medium-chain alcohol (*n*-octanol), and finally the short-chain secondary alcohol (isopropanol). Composition of the alkyl esters formed by lipase-catalyzed alcoholysis of crambe oil and camelina oil with long-chain, medium-chain, or short-chain alcohols was very similar for each set of substrates, irrespective of the biocatalyst used, and it was also similar to the composition of the corresponding products formed by alcoholysis catalyzed by potassium hydroxide (16).

A comparison of the results of the lipase-catalyzed alcoholysis of crambe and camelina oils with long-chain, medium-chain, and short-chain alcohols with those of the potassium hydroxide-catalyzed alcoholysis, described in an accompanying paper (16), shows that the lipase-catalyzed alcoholysis led to higher conversions. Treatment of the reaction products was simpler in the lipase-catalyzed alcoholysis because no potassium soaps were formed. Furthermore under the conditions used, the isopropyl esters were formed only by lipase catalysis. The reaction periods in the lipase-catalyzed alcoholysis of crambe and camelina oils were, however, somewhat longer than those of the alkali-catalyzed alcoholysis. Since the lipases are more expensive than potassium hydroxide, reuse properties of the biocatalysts will be decisive for the economy of such a process.

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