High-Yield Preparation of Wax Esters via Lipase-Catalyzed Esterification Using Fatty Acids and Alcohols from Crambe and Camelina Oils

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Fatty acids obtained from seed oils of crambe (*Crambe abyssinica*) and camelina (*Camelina sativa*) via alkaline saponification or steam splitting were esterified using lipases as biocatalysts with oleyl alcohol and the alcohols derived from crambe and camelina oils via hydrogenolysis of their methyl esters. Long-chain wax esters were thus obtained in high yields when Novozym 435 (immobilized lipase B from *Candida antarctica*) and papaya (*Carica papaya*) latex lipase were used as biocatalysts and vacuum was applied to remove the water formed. The highest conversions to wax esters were obtained with Novozym 435 (≥95%) after 4−6 h of reaction, whereas with papaya latex lipase such a high degree of conversion was attained after 24 h. Products obtained from stoichiometric amounts of substrates were almost exclusively (>95%) composed of wax esters having compositions approaching that of jojoba (*Simmondsia chinensis*) oil, especially when crambe fatty acids in combination with camelina alcohols or camelina fatty acids in combination with crambe alcohols were used as substrates.

Keywords: *Camelina (Camelina sativa) oil; Candida antarctica lipase B; crambe (Crambe abyssinica) oil; esterification; jojoba (Simmondsia chinensis) oil; Novozym; papaya (Carica papaya) lipase; wax ester*

INTRODUCTION

Lipase-catalyzed reactions offer great potentials for the preparation of a wide variety of wax esters under mild reaction conditions (*1*). Thus, wax esters have been obtained in high yield by esterification of fatty acids with long-chain alcohols (*2*−*11*) and in moderate to good yields by interesterification (alcoholysis) of triacylglycerols or natural fats and oils with long-chain alcohols (*5*, *7*, *9*, *12*−*14*). Moreover, lipase-catalyzed alcoholysis of alkyl esters of fatty acids with long-chain alcohols has been carried out successfully to prepare wax esters (*10*, *15*, *16*).

We have shown recently that alcoholysis of triacylglycerols from seed oils of crambe (*Crambe abyssinica*) and camelina (*Camelina sativa*) with long-chain and very long-chain alcohols, catalyzed by Novozym 435 (immobilized lipase B from *Candida antarctica*), Lipozyme IM (immobilized lipase from *Rhizomucor miehei*), and papaya (*Carica papaya*) latex lipase, yields wax esters in moderate to high yields (*17*). Here, we report the lipase-catalyzed synthesis of wax esters by esterification of long-chain and very long-chain fatty acids with the corresponding alcohols, both derived from seed oils of crambe and camelina. Novozym 435 and papaya latex lipase were used as biocatalysts, and the water formed during esterification was removed under vacuum with the objective to obtain wax esters in high yields. Seed oils of crambe and camelina were chosen as starting materials because these plants have great potentials as crops for the future (*18*, *19*).

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MATERIALS AND METHODS

Materials. Refined crambe and camelina oils were supplied by the Institut für Pflanzenbau der Landesforschungsanstalt für Landwirtschaft und Fischerei Mecklenburg-Vorpommern, Gülzow, Germany. Novozym 435 (NOV) (*Candida antarctica* lipase B immobilized on polyacrylic resin) with an activity of 10.500 propyl laurate units (PLU)·g^{-1} was a kind gift from Novo GmbH, Mainz, Germany. *Carica papaya* latex (CPL) was obtained as a crude powder from Sigma-Aldrich-Fluka, Deisenhofen, Germany. The granular latex preparation was ground in a mortar with a pestle to a fine powder and sieved to 0.8 mm mesh size. All distilled solvents and reagents of analytical grade were obtained from Merck, Darmstadt, Germany. Reference lipid standards were from Sigma-Aldrich-Fluka and Nu-Chek-Prep, Elysian, MN.

Fatty acids from crambe and camelina oils were prepared via saponification with potassium hydroxide followed by treatment with hydrochloric acid (*20*). Fatty acids, prepared by steam splitting of the oils at 250 °C and 50 bar, followed by distillation under 1 mbar vacuum at 275 °C (crambe) or 240 °C (camelina), were provided by the Deutsche Hydrierwerke (DHW), Rodleben, Germany. Compositions of the fatty acid mixtures used are given in Table 1. The oils were transesterified with methanol using sodium methoxide as catalyst at 65−70 °C, and the methyl esters formed were recovered by distillation at 235 °C and 1 mbar vacuum. The methyl esters of crambe and camelina oils were converted to alcohols via hydrogenolysis at 240 bar hydrogen pressure and 300 °C using ZnO/Cr_2O_3 as catalyst, and the alcohols were recovered by distillation at 250 °C and 1 mbar vacuum at a pilot plant of DHW. Compositions of the alcohols are given in Table 2. Oleyl alcohol (technical grade, containing 4% stearyl alcohol, 90% oleyl alcohol, 5% linoleyl alcohol, and 1% eicosenyl alcohol) was purchased from Sigma-Aldrich-Fluka and percolated prior to use over alumina under argon.

Lipase-Catalyzed Esterification. Equimolar amounts of the fatty acids and fatty alcohols (total amount of substrates

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Table 1. Composition of Fatty Acids Obtained via Alkaline Saponification and High-Pressure Steam Splitting of Crambe Oil and Camelina Oil

	composition (% w/w)			
	crambe		camelina	
fatty acid[a]	saponification	steam splitting	saponification	steam splitting
16:0	1.6	1.9	5.0	6.1
18:0	0.7	0.8	2.6	2.9
18:1	14.8	14.8	14.4	17.6
18:2	7.7	7.7	16.3	18.3
18:3	6.1	4.1	35.7	25.4
20:0	0.9	1.0	1.4	1.4
20:1	3.8	3.9	15.8	16.4
20:2	0.2	0.2	2.1	2.0
20:3	tr[b]	tr	1.7	0.7
22:0	2.1	2.1	0.3	0.4
22:1	57.7	59.3	3.3	4.1
22:2	0.6	0.6	0.1	tr
22:3	0.3	tr	0.5	0.2
24:0	0.8	0.7	0.2	0.2
24:1	1.5	1.3	0.8	0.6

[a] Fatty acids are designated by number of carbon atoms:number of double bonds. [b] Traces.

Table 2. Composition of Alcohols Obtained from Methyl Esters of Crambe Oil and Camelina Oil via Hydrogenolysis

	composition (% w/w)	
alcohol[a]	crambe	camelina
16:0	23.0	10.0
18:0	2.0	5.0
18:1	21.0	30.0
18:2	1.0	10.0
18:3	2.0	3.0
20:0	2.0	6.0
20:1	4.0	27.0
22:0	5.0	tr[b]
22:1	56.0	9.0
22:2	0.6	tr
24:0	2.0	tr
24:1	2.0	tr

[a] Alcohols are designated by number of carbon atoms:number of double bonds. [b] Traces.

0.6 or 60 g) were reacted in the presence of the lipase preparation (5% w/w of the substrates) by magnetic stirring in a screw-capped tube under vacuum (20 mbar) at 60 °C for various periods with water trapping in the gas phase using potassium hydroxide pellets. Samples of the reaction products were withdrawn at various intervals, taken up in isohexane, and centrifuged to separate the biocatalyst. An aliquot of the supernatant was analyzed as described below. Similarly, the final product at the end of the reaction was worked up and analyzed.

Lipid Analysis. All analyses were carried out by gas chromatography in a Hewlett-Packard 5890 series 2 instrument (Hewlett-Packard GmbH, Waldbronn, Germany) equipped with flame ionization detectors. Peaks in gas chromatograms were assigned by comparison of their retention times with those of known standards. Peak areas and percentages were calculated using Hewlett-Packard ChemStation software.

The fatty acids used as starting materials were converted to methyl esters by treatment with diazomethane and the methyl esters analyzed in a DB-23 (methyl/50% cyanopropyl silicone) fused silica capillary column, 40 m × 0.18 mm i.d., 0.2 m film (J&W, ASS-Chem, Bad Homburg, Germany). Methyl esters were separated using hydrogen as carrier gas (linear velocity = 27 cm·s^{-1}), initially at 170 °C for 17 min, followed by linear programming from 170 to 225 °C at 7 °C·min^{-1}. The final temperature was kept constant for 10 min.

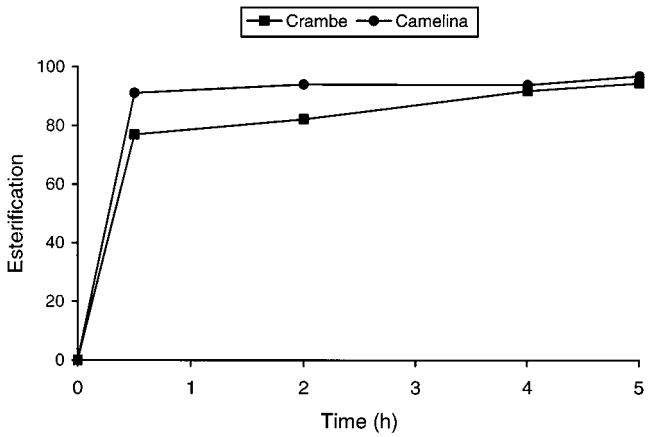

Figure 1. Time course of lipase-catalyzed esterification of fatty acids of crambe oil and camelina oil with oleyl alcohol (substrate mixture = 60 g; lipase = Novozym 435, 5% w/w of substrate mixture; molar ratio of fatty acids/oleyl alcohol = 1:1).

The split ratio was 1:10, and the injector as well as the flame ionization detector temperature was 270 °C.

Alcohols were analyzed by gas chromatography on an HP-1 column (25 m × 0.32 mm i.d. × 0.52 μm film) (Hewlett-Packard) with nitrogen as carrier gas (linear velocity = 44 cm·s^{-1}). Temparature was programmed from 180 °C (2 min isothermal) to 260 °C at 6 °C·min^{-1} (5 min isothermal).

The reaction products formed by lipase-catalyzed esterification were treated with diazomethane to convert the unesterified fatty acids to methyl esters, and the resulting mixture consisting of methyl esters, wax esters, and alcohols was analyzed by gas chromatography on a Quadrex 400-5HT column (25 m × 0.25 mm i.d. × 0.1 μm film) (Quadrex Corp., New Haven, CT) with hydrogen as carrier gas (linear velocity = 31 cm·s^{-1}). Temperature was programmed from 160 °C (2 min isothermal) to 310 °C at 10 °C·min^{-1} and then to 420 °C at 5 °C·min^{-1} (5 min isothermal).

RESULTS AND DISCUSSION

Lipase-Catalyzed Esterification of Fatty Acids 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 esterification with crambe and camelina fatty acids. The results, given in Figure 1, show that with both fatty acid mixtures the conversions to wax esters using Novozym 435 are well above 90% after a reaction period of only 4−5 h. With papaya latex lipase as biocatalyst, however, a high degree of conversion (∼90%) to wax esters is reached after a reaction period of 24 h (Figure 2).

The compositions of the wax esters formed by esterification of the fatty acids of crambe and camelina oils with oleyl alcohol are as expected from the composition of the oils, given in Table 1. Figure 3 shows, for example, in the case of the esterification catalyzed by papaya latex lipase that esterification of crambe fatty acids with oleyl alcohol leads mainly to wax esters with the chain lengths C36 and C40, whereas the corresponding products from camelina fatty acids are predominated (>70%) by C36 chain length. It can be seen from Figure 3 that the composition of the wax esters obtained is distinctly different from that of jojoba oil. Essentially identical results were also obtained when Novozym 435 was used as biocatalyst for the preparation of wax esters by esterification of crambe and camelina fatty acids with oleyl alcohol (data not shown).

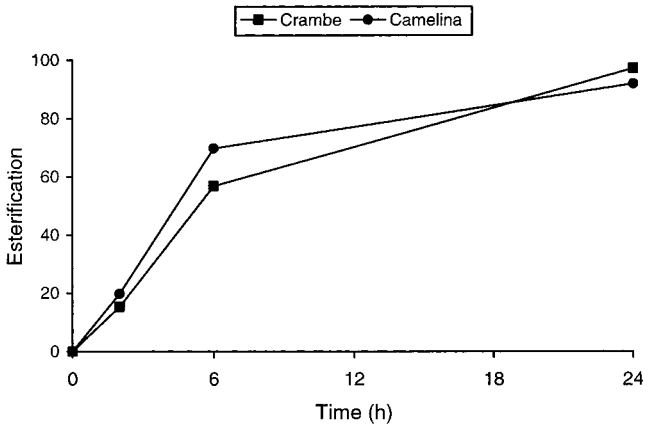

Figure 2. Time course of lipase-catalyzed esterification of fatty acids of crambe oil and camelina oil with oleyl alcohol (substrate mixture = 0.6 g; lipase = papaya latex, 5% w/w of substrate mixture; molar ratio of fatty acids/oleyl alcohol = 1:1).

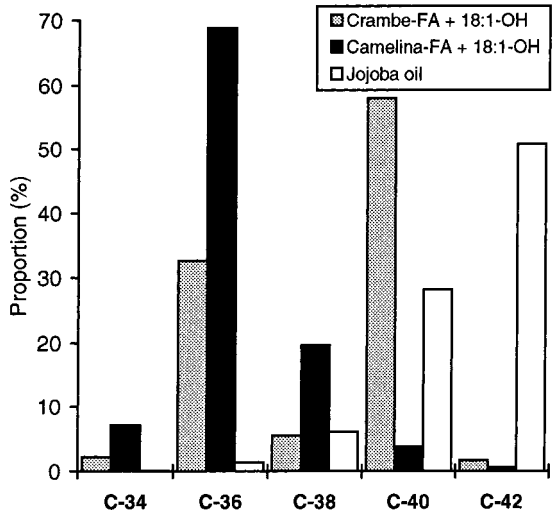

Figure 3. Composition of wax esters of various carbon numbers formed by lipase-catalyzed esterification of fatty acids from crambe oil (crambe-FA) and camelina oil (camelina-FA) with oleyl alcohol (18:1-OH) in comparison with wax esters of jojoba oil (substrate mixture = 0.6 g; lipase = papaya latex, 5% w/w of substrate mixture; molar ratio of fatty acids/oleyl alcohol = 1:1).

Table 3. Time Course of Esterification of Crambe Fatty Acids and Camelina Fatty Acids with Crambe Alcohols and Camelina Alcohols (Substrate Mixture = 0.6 g) Catalyzed by Papaya Lipase

substrate		esterification (%) at reaction time of	
fatty acids	alcohols	6 h	24 h
crambe	crambe	42.6	92.1
crambe	camelina	16.1	98.6
camelina	camelina	81.3	99.9
camelina	crambe	57.6	96.1

Lipase-Catalyzed Esterification of Fatty Acids from Crambe and Camelina Oils with Alcohols from Crambe and Camelina. Table 3 shows the time course in the esterification of crambe fatty acids (crambe-FA) and camelina fatty acids (camelina-FA), respectively, with crambe alcohols (crambe-ALC) and camelina alcohols (camelina-ALC) using papaya latex lipase as biocatalyst. Both fatty acids in combination with each of the two alcohols lead to >90% conversion to wax esters after a reaction period of 24 h (Table 3). Novozym 435 as biocatalyst gives such high conversions within a reaction period of 4−5 h, as will be seen later.

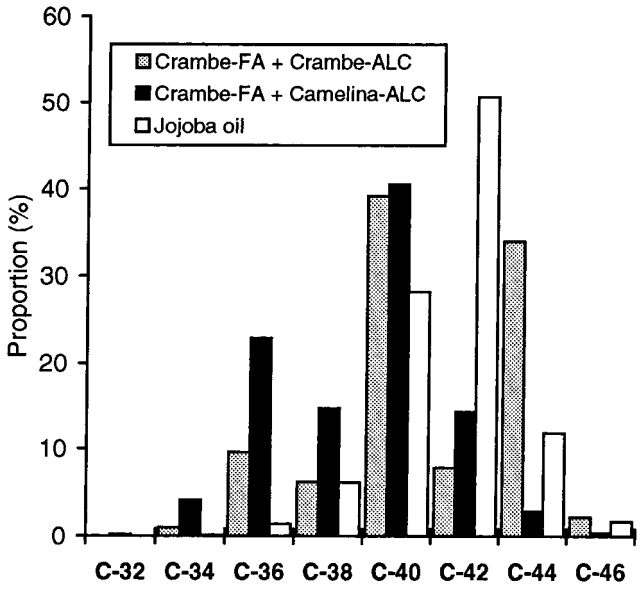

Figure 4. Composition of wax esters of various carbon numbers formed by lipase-catalyzed esterification of crambe fatty acids (crambe-FA) with crambe alcohols (crambe-ALC) and camelina alcohols (camelina-ALC) in comparison with wax esters of jojoba oil (substrate mixture = 0.6 g; lipase = papaya latex, 5% w/w of substrate mixture; molar ratio of fatty acids/alcohol = 1:1).

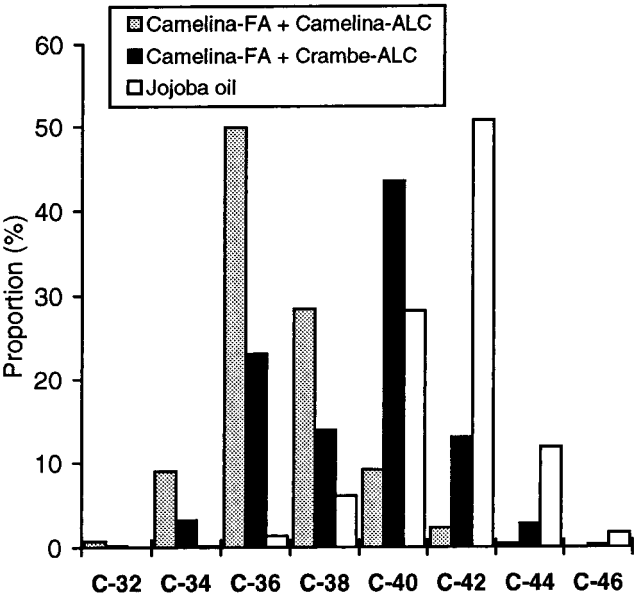

Figure 5. Composition of wax esters of various carbon numbers formed by lipase-catalyzed esterification of camelina fatty acids (camelina-FA) with crambe alcohols (crambe-ALC) and camelina alcohols (camelina-ALC) in comparison with wax esters of jojoba oil (substrate mixture = 0.6 g; lipase = papaya latex, 5% w/w of substrate mixture; molar ratio of fatty acids/alcohol = 1:1).

Figures 4 and 5 show the composition of the wax esters formed by esterification of crambe fatty acids and camelina fatty acids, respectively, with crambe alcohols and camelina alcohols using papaya latex lipase as biocatalyst. The composition of jojoba oil is given in Figures 4 and 5 for comparison. The main wax ester constituents of joboba oil are those with chain lengths C42 (≈50%), C40 (≈30%), and C44 (≈10%). Using papaya latex lipase as biocatalyst, the wax esters

Table 4. Composition of Products Obtained in Preparative Scale (60 g) by Novozym 435-Catalyzed Esterification of Fatty Acids from Crambe Oil and Camelina Oil with Oleyl Alcohol

		composition (% w/w)		
fatty acid substrate	reaction time (h)	wax ester	alcohol	unesterified fatty acid
crambe (via alkaline saponification)	3	97.2	1.0	1.8
crambe (via steam splitting)	5.5	97.8	1.0	1.1
camelina (via alkaline saponification)	2	95.0	4.6	0.4
camelina (via steam splitting)	5	98.3	0.9	0.9

Table 5. Composition of Products Obtained in Preparative Scale (60 g) by Novozym 435-Catalyzed Esterification of Fatty Acids from Crambe Oil and Camelina Oil with Alcohols Derived from These Oils

			composition (% w/w)		
fatty acid substrate[a]	alcohol substrate	reaction time (h)	wax ester	alcohol	fatty acid
crambe	crambe	5	95.0	1.9	3.1
crambe	camelina	5	97.4	0.3	2.3
camelina	crambe	4.5	95.1	2.3	2.5
camelina	camelina	4.5	98.3	0.7	0.9

[a] Obtained by steam splitting. [b] Obtained by hydrogenolysis of fatty acid methyl esters.

obtained by esterification of crambe fatty acids with crambe alcohols constitute a product of mainly C40 (≈40%), C44 (≈35%), and C36 as well as C42 (both ≈10%) chain lengths (Figure 4). The corresponding esters of camelina fatty acids with camelina alcohols contain C36 (≈50%), C38 (≈30%), and C34 as well as C40 (both ≈10%) (Figure 5). If crambe fatty acids are esterified with camelina alcohols (Figure 4) or camelina fatty acids with crambe alcohols (Figure 5), the wax esters in the products consist of C40 (≈40%), C36 (≈25%), and C38 as well as C42 (both ≈15%) in both cases. Thus, the two last mentioned products show compositions that are closest to the wax ester composition of jojoba oil. Results essentially identical to those above were obtained when Novozym 435 was used as biocatalyst (data not shown).

Table 4 shows the composition of lipid classes in the reaction products formed by preparative scale (60 g total substrates) esterification of crambe and camelina fatty acids with oleyl alcohol, catalyzed by Novozym 435. It is evident that the reaction products are almost exclusively composed of wax esters, obviously due to high conversion within ~5 h and the use of a stoichiometric mixture of substrates as starting materials. The chain length distribution of the wax esters is essentially identical (data not shown) to that of the corresponding products obtained by esterification catalyzed by papaya latex lipase (Figure 3).

Table 5 shows the composition of lipid classes in the reaction products formed by preparative scale esterification of crambe and camelina fatty acids with crambe and camelina alcohols, catalyzed by Novozym 435. Again, it is evident that the reaction products are almost exclusively composed of wax esters, obviously due to high conversion within 5 h and the use of a stoichiometric mixture of substrates as starting materials. The chain length distribution of the wax esters is essentially identical (data not shown) to that of the corresponding products obtained by esterification catalyzed by papaya latex lipase (Figures 4 and 5).

Our studies show that lipase-catalyzed esterification, under vacuum, of stoichiometric mixtures of long-chain and very long-chain fatty acids with the corresponding mixtures of alcohols, both derived from crambe and camelina oils, gives nearly quantitative yields of mixtures of long-chain and very long-chain wax esters which should be potentially suitable for applications in cosmetics, drugs, and special lubricants. Of the two lipases tested as biocatalyst for esterification, Novozym 435 was distinctly more active than papaya lipase, yet the latter should be interesting for commercial application because it is an inexpensive plant enzyme preparation occurring in crude papaya latex containing papain that has been used in the food and beverage industries for a long time. Of the two processes for the lipase-catalyzed preparation of esters, that is, alcoholysis as reported recently (*17*) and esterification as reported in the present work, the products of lipase-catalyzed esterification under vacuum contain waxes in >95% purity due to high conversion into wax esters starting from stoichiometric mixtures of substrates. The lipase-catalyzed alcoholysis of triacylglycerols with alcohols, however, is a direct one-step process, although the products of alcoholysis contain distinctly fewer wax esters compared to those obtained by esterification.

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