Germinating Rapeseed as Biocatalyst: Hydrolysis of Oils Containing Common and Unusual Fatty Acids†

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Seedlings of low-erucic rape (*Brassica napus*) at day 3–5 of germination have been homogenized in Tris-HCl buffer and the homogenate used—as such or after defatting with pentane—as a biocatalyst for the hydrolysis of fats and oils containing common and unusual fatty acids. The rape seedlings were found to be highly active in the hydrolysis of triacylglycerols of linseed oil, castor oil, borage oil, coriander oil, *Hydnocarpus wightiana* oil, and hake (*Merluccius hubbsi*) liver oil but were unable to hydrolyze the wax esters contained in jojoba oil and orange roughy (*Hoplostethus atlanticus*) oil to any appreciable extent. In the hydrolysis of borage oil, coriander oil, *H. wightiana* oil, and hake liver oil the rape seedlings were found to discriminate against γ-linolenoyl, petroselinoyl, gorlioyl and n-3 docosahexaenoyl moieties, respectively. These findings show the potentials of germinating rape seedlings as a cheap and easy-to-obtain biocatalyst for complete hydrolysis of oils or for their partial selective hydrolysis for the enrichment of definite fatty acids from mixtures.

Keywords: *Germinating rapeseed; lipase from rapeseed; biocatalyst; Brassica napus*

INTRODUCTION

Lipase from germinating seedlings of oilseed rape (*Brassica napus* L.) has been used as a biocatalyst after partial purification and immobilization (Hills et al., 1989, 1990a,b, 1991; Hills and Mukherjee, 1990; Nćube et al., 1993).

We have described in an accompanying paper the use of germinating rapeseed homogenates—without any isolation or partial purification of the lipase—as biocatalysts for the hydrolysis of endogenous and exogenous triacylglycerols (Jachmanián et al., 1995). Here we report the substrate selectivity of germinating rapeseed in the hydrolysis of various seed oils and marine oils containing common and unusual fatty acids.

MATERIALS AND METHODS

Materials. Seeds of low-erucic rape (*B. napus*) cultivar Ceres were provided by Norddeutsche Pflanzenzucht, Hohenlieth, Germany. Seeds of high-erucic oriental mustard (*Brassica juncea*) were obtained from Chinese Cereals and Oilseeds Institute, Beijing, People's Republic China. Coriander (*Coriandrum sativum*) seed oil (Henkel, Düsseldorf, Germany), jojoba (*Simmondsia chinensis*) oil (Worleé, Hamburg, Germany), and borage (*Borago officinalis*) seed oil (International Food Science Centre, Lystrup, Denmark) were kind gifts from various industries. Castor (*Ricinus communis*) oil and linseed (*Linum usitatissimum*) oil were kindly provided by Dr. M. Rüsch gen Klaas, Münster, Germany. Seed oils of high-erucic oriental mustard, *Hydnocarpus wightiana*, and hake (*Merluccius hubbsi*) liver oil were obtained by extraction. Oil of orange roughy (*Hoplostethus atlanticus*) was provided by A. S. Johan, C. Martens, Bergen, Norway. Free fatty acid contents of the oils were as follows: oriental mustard, 0.8%; coriander, 0.6%; jojoba, 0.2%; borage, 2.2%; castor, 1.9%; linseed, 0.4%; hake, 0.2%; *H. wightiana*, 0.5%; orange roughy, 0.6%.

All chemicals of analytical grade and adsorbents were purchased from E. Merck, Darmstadt, Germany. Distilled solvents were used throughout.

Preparation of Biocatalyst. Germination of low-erucic rape and preparation of the biocatalyst by homogenization of the seedlings were carried out as described in the accompanying paper (Jachmanián et al., 1995). Alternatively, the homogenate was delipidated by repeated extraction with pentane at ambient temperature, followed by removal of residual pentane from the homogenate by a stream of nitrogen.

Hydrolysis Reactions. Routinely, the homogenates of 3 g of rape seedlings at day 5 of germination in 3 mL of Tris-HCl buffer (50 mM, pH 8.0) were incubated with 1.2 g of different oils for various periods at 30 °C by magnetic stirring. In one experiment the delipidated homogenate of low-erucic rape seedlings was incubated under similar conditions with hake liver oil.

Lipid Extraction and Analysis. Lipids were extracted from the seedlings and products of hydrolysis by extraction according to the method of Bligh and Dyer (1959). Subsequently, the residual lipids were recovered from the residue by repeated extractions with a mixture of hexane/diethyl ether (1:1 v/v). The lipid extracts were combined, the organic solvents were evaporated in a stream of nitrogen, and the residual lipids were weighed.

In most cases the proportion of fatty acids formed by hydrolysis of triacylglycerols was determined titrimetrically (IUPAC, 1987). All data presented are percent free fatty acids in the extracted oil. Preparative separation of lipid classes was carried out by thin-layer chromatography on silica gel H using hexane/diethyl ether/acetic acid (70:30:1 by vol) as developing solvent. The lipid fractions were made visible by exposing the edges of the chromatoplates to iodine vapor. The fractions containing fatty acids and acylglycerols (i.e. monoplus di- plus triacylglycerols) were scraped, eluted with water-saturated diethyl ether, and converted to methyl esters (Schulte, 1993), which were analyzed by gas chromatography as described in the accompanying paper (Jachmanián et al., 1995). The analysis of methyl ester samples containing methyl petroselinate was carried out in a Varian 3700 gas chromatograph on a 40 m × 0.18 mm i.d. fused silica DB-23 capillary

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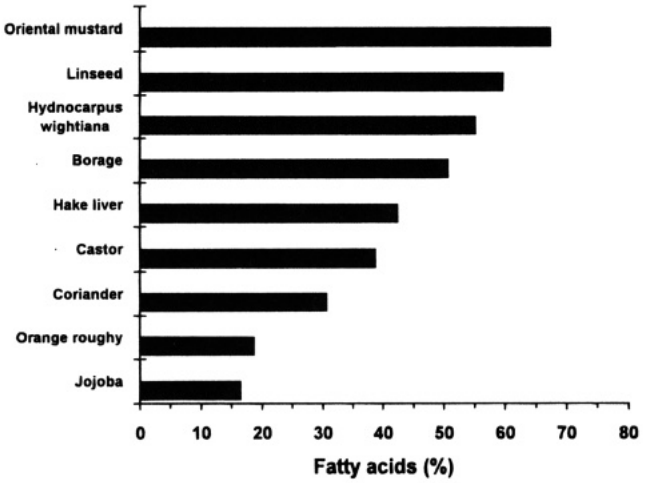

Figure 1. Relative rates of hydrolysis of oils containing common and unusual constituent fatty acids by lipolysis catalyzed by homogenized rape seedlings measured as percent fatty acids formed after incubation for 3 days.

column of 0.2 μm film thicknes (methyl/50% cyanopropyl silicone, J&W, Fisons Instruments, Mainz, Germany). Hydrogen was used as carrier gas at 20 cm/s linear velocity at a split ratio of 1:10. Temperature was programmed from 170 °C (17 min isothermal) at 7 °C/min to 225 °C (10 min isothermal).

Qualitative separation of lipid classes by thin-layer chromatography was done essentially as described above, and the lipid fractions were made visible by charring after the chromatoplates were sprayed with 50% sulfuric acid and heated.

In the case of lipolysis products formed from castor oil, the fatty acids were separated from acylglycerols by converting them to sodium salts followed by solvent partitioning (Rahmatullah et al., 1994). Subsequently, the two fractions were converted to methyl esters and analyzed by gas chromatography as described above.

RESULTS AND DISCUSSION

Homogenates of rape seedlings at day 5 of germination were incubated with different seed oils and marine oils containing common and unusual fatty acids as constituents of triacylglycerols or wax esters at a level of about 20% endogenous triacylglycerols in total lipids contained in the reaction mixture. Incubation under identical conditions led to different extents of hydrolysis, as shown by the various proportions of fatty acids formed (Figure 1). Among all of the oils examined in which the triacylglycerols are the major constituents, the highest rate of hydrolysis was obtained with the high-erucic oriental mustard oil, followed by linolenic acid-rich linseed oil, and the lowest rate was found with coriander oil in which petroselinic (*cis*-6-octadecenoic) acid is the major constituent. Intermediate rates of hydrolysis were observed for the hake liver oil containing polyunsaturated C_{20} and C_{22} fatty acids and oils of *H. wightiana*, borage, and castor, which contain the unusual fatty acids gorlic [13-(cyclopent-2-en-1-yl)tridec-6-enoic], γ-linolenic (*all-cis*-6,9,12-octadecatrienoic), and ricinoleic (12-hydroxy-*cis*-9-octadecenoic) acid, respectively (Figure 1).

The highest rate of hydrolysis of the high-erucic oriental mustard oil by the homogenate from low-erucic germinating rapeseed (Figure 1) agrees well with an earlier report on closely related substrate specificities of lipases from low-erucic and high-erucic rapeseed varieties, e.g. preference for erucic acid containing triacylglycerols (Huang, 1987). Since the low-erucic

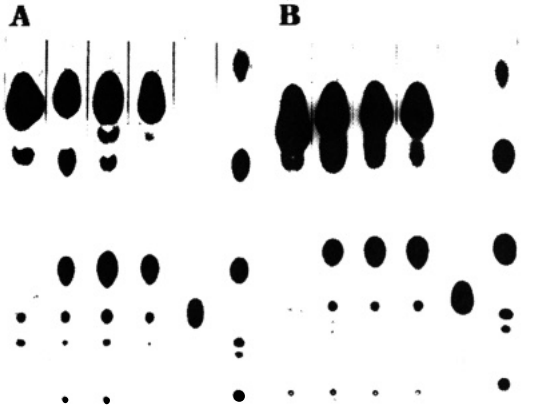

Figure 2. Thin-layer chromatogram showing the distribution of lipolysis products formed during incubation of orange roughy oil (A) and jojoba oil (B) with homogenized rape seedlings. Lanes (left to right): untreated orange roughy oil and jojoba oil, respectively; lipolysis products after 24 h; lipolysis products after 48 h; lipolysis products after 72 h; oleoyl alcohol; reference standards [with increasing R_f values: monoacylglycerols (origin), 1,2-(2,3-)diacylglycerols, 1,3-diacylglycerols, unesterified fatty acids, triacylglycerols and methyl esters of fatty acids.

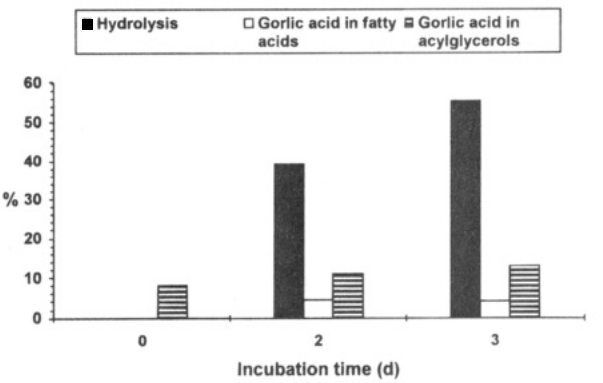

Figure 3. Distribution of gorlic acid in fatty acids and acylglycerols during lipolysis of *H. wightiana* oil catalyzed by homogenized rape seedlings.

varieties were obtained by breeding through the alteration of a few genes involved in the biosynthesis of erucic acid, it has been suggested that the lipase gene was not altered (Huang, 1987). Our data showed no selective cleavage of erucic acid from the high-erucic oriental mustard oil by the homogenate from germinating low-erucic rapeseed. Thus, after 3 days of incubation of the high-erucic oriental mustard oil that had resulted in the formation of 67.4% fatty acids, the levels of erucic acid in fatty acids and acylglycerols (mono- plus di- plus triacylglycerols) were 36.0 and 37.9%, respectively.

The data given in Figure 1 also show the lowest rate of hydrolysis of orange roughy oil and jojoba oil in which not the triacylglycerols but the wax esters are the most predominant constituents. The products of lipolysis of both orange roughy oil and jojoba oil were examined by thin-layer chromatography. The thin-layer chromatograms (Figure 2) show for both oils a progressive increase with time in the relative proportion of fatty acids and a concomitant decrease in the proportion of triacylglycerols, which originate mostly from the endogenous oil of the seedlings. Figure 2 shows furthermore that the relative proportions of wax esters (migrating ahead of the triacylglycerols) and long-chain alcohols barely change during the incubations. These findings, taken together with the data presented in Figure 1,

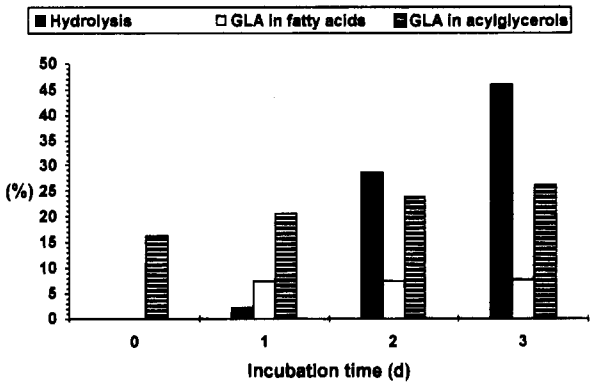

Figure 4. Distribution of γ-linolenic acid (GLA) in fatty acids and acylglycerols during lipolysis of borage oil catalyzed by homogenized rape seedlings.

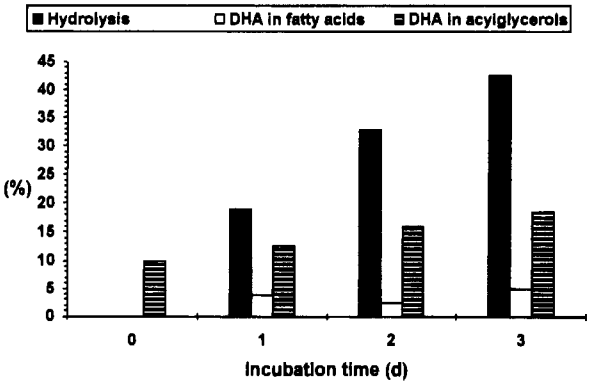

Figure 5. Distribution of docosahexaenoic acid (DHA) in fatty acids and acylglycerols during lipolysis of hake liver oil catalyzed by homogenized rape seedlings.

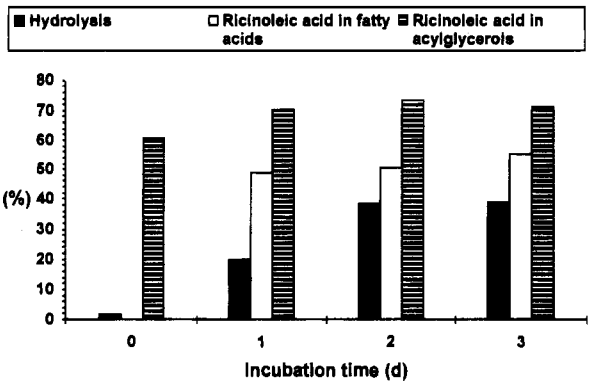

Figure 6. Distribution of ricinoleic acid in fatty acids and acylglycerols during lipolysis of castor oil catalyzed by homogenized rape seedlings.

show that homogenates of germinating rapeseed do not accept wax esters as substrates for hydrolysis.

Products of lipolysis of *H. wightiana* oil, borage oil, hake liver oil, and castor oil were fractionated into fatty acids and acylglycerols (mono- plus di- plus triacylglycerols), and the fatty acid composition of these two lipid fractions was determined to examine the substrate specificity of the rape seedling homogenate in the hydrolysis reaction. The results presented in Figures 3–7 show during the course of lipolysis an enrichment of gorlic acid, γ-linolenic acid, docosahexaenoic acid, ricinoleic acid, and petroselinic acid in the acylglycerol fraction, whereas the relative proportion of each of these fatty acids is concomitantly reduced in the fatty acid fractions. Obviously, triacylglycerols containing gorlic acid, γ-linolenic acid, docosahexaenoic acid, ricinoleic

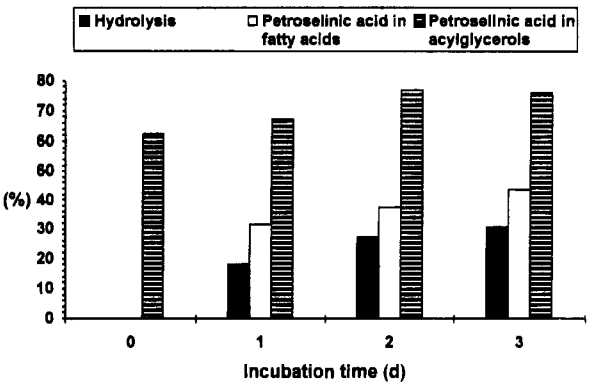

Figure 7. Distribution of petroselinic acid in fatty acids and acylglycerols during lipolysis of coriander oil catalyzed by homogenized rape seedlings.

acid, and petroselinic acid are discriminated against as substrates during hydrolysis catalyzed by the homogenates of germinating rape seedlings. Some of these substrate selectivities agree with the substrate specificities reported so far for purified rapeseed lipase (Hills et al., 1990b) and microbial lipases (Mukherjee et al., 1993), which have been found to discriminate against fatty acids/acyl moieties having a *cis*-4, *cis*-6, and *cis*-8 double bond as the first olefinic bond next to the carboxyl group as substrates; gorlic acid, γ-linolenic acid, and petroselinic acid are fatty acids having a *cis*-6 double bond and docosahexaenoic acid is a fatty acid having a *cis*-4 double bond as the first olefinic bond next to the carboxyl group. It should be possible to utilize such substrate selectivities for the enrichment of definite fatty acids from naturally occurring oils by selective hydrolysis, i.e. via kinetic resolution.

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Received for review April 4, 1995. Revised manuscript received August 8, 1995. Accepted August 28, 1995.[⊗]

JF950199C

[⊗] Abstract published in *Advance ACS Abstracts,* October 15, 1995.