Studies on the Enzymatic Synthesis of Lipophilic Derivatives of Natural Antioxidants

H. Stamatis, V. Sereti, and F.N. Kolisis*

Chemical Engineering Department, Division IV, National Technical University of Athens, Zografou Campus, 15700 Athens, Greece

ABSTRACT: The esterification of some natural antioxidants such as cinnamic acid derivatives and ascorbic acid in nonaqueous media, catalyzed by immobilized lipases from *Candida antarctica* and *Rhizomucor miehei,* was investigated. The alcohol chain length affected the rate of esterification of cinnamic acids by both lipases. Higher reaction rates were observed when the esterification was carried out with medium- or long-chain alcohols. The rate also depended on aromatic acid structure. The reactivity of the carboxylic function of the cinnamic acids was affected by electron-donating substituents in the aromatic ring. Higher yields were observed for the esterification of *p*-hydroxyphenylacetic acid (97%) catalyzed by *C. antarctica* lipase and for the esterification of cinnamic acid (59%) catalyzed by *R. miehei* lipase. *Candida antarctica* lipase was more suitable for producing ascorbic acid fatty esters, catalyzing with a relatively high yield (up to 65% within 24 h) the regioselective esterification of ascorbic acid with various fatty acids in 2-methyl-2-propanol. The reaction rate and yield depended on the fatty acid chain length and on the molar ratio of reactants. All ascorbic acid fatty esters produced by this procedure exhibited a significant antioxidant activity in a micellar substrate composed of linoleic acid.

Paper no. J9139 in *JAOCS 76,* 1505–1510 (December 1999).

KEY WORDS: Ascorbic acid, cinnamic acids, esterification, lipase, natural antioxidants, phenolic acids.

Food antioxidants retard oxidative rancidity caused by atmospheric oxidation and thus protect oils, fats, and fat-soluble components, such as vitamins or carotenoids. Many compounds have been used as food antioxidants, including mainly artificial phenolic substances that terminate free radical reaction chains in lipid oxidation (1,2). Some of the most widely used synthetic phenolic antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and esters of gallic acid. Since some synthetic antioxidants, such as BHA and BHT, may promote carcinogenesis (3), there is great interest in naturally occurring antioxidants, which are presumed safe (4). Two such naturally occurring substances with a high reducing ability that are widely used in the food and cosmetic industries as oxygen scavengers are ascorbic acid (vitamin C) and erythorbic acid. These react with oxygen and can thus remove it in a closed system (5,6).

*To whom correspondence should be addressed.
E-mail: Kolisis@orfeas.chemeng.ntua.gr

On the other hand, natural phenolic compounds are numerous and widely distributed within the plant kingdom. A major portion of the antioxidant activity of oilseeds and oilseed flours and concentrates is attributed to phenolic acids (3). Phenolic acids, including hydroxylated (*p*-coumaric acid, caffeic acid) or methoxylated (ferulic acid) derivatives of cinnamic acids, are present in soybeans, cottonseeds, and peanuts in free forms or as carbohydrate esters (3,7). Owing to their phenolic nucleus and an extended side-chain conjugation, these acids can readily form resonance-stabilized phenoxy radicals, which account for their antioxidant performance in lipid-aqueous systems (8–11). Moreover, some phenolic acids such as ferulic and caffeic acids may serve an important antioxidant function in preserving the physiologic integrity of cells exposed to both air and impinging ultraviolet (UV) radiation (8,12).

Generally, the solubility of natural antioxidants, such as ascorbic acid or phenolic acids, in water is good. The hydrophilic character of these antioxidants reduces their effectiveness in stabilizing fats and oils and has been reported as a serious disadvantage if an aqueous phase is also present (5,13). Therefore, the modification of these compounds *via* esterification with aliphatic molecules (fatty acids or alcohols) can be used as a tool to alter solubility in oil-based formulae and emulsions. Thus, for example, ascorbic acid was converted into oil-soluble fatty acid esters, such as ascorbyl palmitate, which are used for the stabilization of fats, oils, and fatty products (14). These lipophilic derivatives are generally prepared by reacting ascorbic acid with strongly corrosive acids such as sulfuric acid or hydrogen fluoride followed by reesterification with fatty acids (5). On the other hand, the chemical synthesis of benzoic and phenolic acid esters is usually carried out with basic or acidic catalysts under reflux (15), but this procedure does not meet the requirements necessary for food applications. To overcome the disadvantages of the conventional processes, the use of enzymes in nonaqueous media has opened new avenues for producing many valuable products under mild conditions (16,17). Recently, the enzymatic esterification of several phenolic acids with aliphatic alcohols catalyzed by *Candida antarctica* lipase has been reported (18,19). In addition, the enzymatic acylation of ascorbic acid with palmitic acid using lipase Amano P or lipase from *C. antarctica* in methyl ethyl ketone, pyridine, dioxane, tetrahydrofuran, or 2-methyl-2-butanol has been investigated (20–22). As an extension of these works, we report

the use of two commercial lipases, from *C. antarctica* (Novozyme; Novo Nordisk, Bagsvaerd, Denmark) and *Rhizomucor miehei* (Lipozyme™; Novo Nordisk), in various solvent systems for the biotransformation of ascorbic acid, as well as various cinnamic acid derivatives, *via* esterification with fatty acids or fatty alcohols, respectively (Fig. 1). Solvents used in this work are nontoxic and can solubilize relatively large amounts of ascorbic and cinnamic acid derivatives. Our objective was to investigate the role of various physicochemical parameters—such as the structural characteristics of substrates, the concentration of reactants, and the nature of the organic medium used—which affect lipase catalytic behavior, in an attempt to optimize the esterification procedure.

MATERIALS AND METHODS

Materials. Lipase from *R. miehei* (Lipozyme™) immobilized onto a macroporous anion exchange resin was kindly provided by Novo Nordisk. The enzyme activity was 25 BIU/g (BIU = batch interesterification unit, defined as millimoles of palmitic acid incorporated into triolein per minute at 40°C). Lipase from *C. antarctica* (Novozyme 435) immobilized on a macroporous acrylic resin was also kindly offered by Novo Nordisk. Its activity was 7 PLU/mg (the enzyme activity refers to the millimoles of propyl laurate synthesized per minute at 60°C). Various phenolic acids, ascorbic acid, ascorbyl palmitate, aliphatic alcohols, fatty acids, and organic solvents were purchased from Aldrich (Milwaukee, WI), Merck (Darmstadt, Germany), or Sigma (St. Louis, MO), and were of the highest available purity.

Enzymatic esterification.The esterification of various cinnamic acids was carried out in sealed stirred flasks. In typical experiments, the reaction mixture consisted of various amounts of cinnamic acid derivatives (50–500 mg) in 5 mL of solvent (aliphatic alcohols or acetone) and 150 mg of immobilized lipases. The flasks were incubated in an orbital shaker at 200 rpm with the temperature fixed at 50°C. Control experiments were conducted without enzyme. Alcohols and acetone were dehydrated before use with 4 Å molecular sieves. To evaluate the rate of esterification, a solution sample (10 µL) was withdrawn and subjected to high-performance liquid chromatography (HPLC) analysis once every hour.

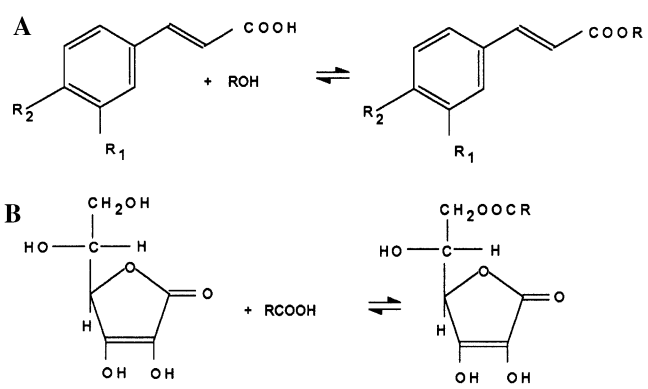


FIG. 1. Esterification of (A) cinnamic acids with aliphatic alcohols, and (B) ascorbic acid with long-chain fatty acids catalyzed by lipases from *Candida antarctica* and *Rhizomucor miehei*. $R_1 = R_2 = H$, cinnamic acid; $R_1 = R_2 = OH$, caffeic acid; $R_1 = OCH_3$ and $R_2 = OH$, ferulic acid; $R_1 = H$ and $R_2 = OH$, *p*-coumaric acid.

In a typical enzymatic synthesis of various ascorbyl esters, 0.15 mmol of ascorbic acid, 0.90 mmol of fatty acids, and 150 mg of immobilized lipases were incubated in 5 mL of organic solvent (acetone or 2-methyl-2-propanol). The reaction mixture was then incubated in an orbital shaker at 200 rpm at 45°C. Organic solvents were dehydrated before use with 4 Å molecular sieves. Control experiments were conducted without enzyme. Samples were withdrawn at various times to determine the concentration of substrate and product by HPLC. Syntheses on larger scales were carried out in sealed stirred flasks with the same proportion of reagents. All reactions were carried out in the presence of 4 Å molecular sieves (20 mg/mL of organic solvent).

Analytical methods. Qualitative analysis of reaction mixtures was made by thin-layer chromatography (TLC) on silica gel 60 plates (Merck) using a solvent mixture of $CHCl_3/CH_3OH/CH_3COOH$ (96:3:1, vol/vol/vol) or $CHCl_3/CH_3OH/CH_3COOH/H_2O$ (80:10:8:2, by vol). Lipids were visualized by spraying the plates with a 5% (vol/vol) ethanolic solution of H_2SO_4 and heating for 10 min at 150°C or visualized under a UV lamp.

Quantitative analysis of samples was done by HPLC on a C_{18} Nucleosil column. Particle size was 10 µm; length, 300 mm; diameter 3.9 mm. Detection was achieved at 280 nm based on calibration curves prepared using standard cinnamic acids, ascorbic acid, and ascorbyl esters solutions in methanol. Samples were first filtered to remove the enzyme and molecular sieves. The resulting clear solution (50 µL), was diluted with methanol (950 µL) before analysis. Elution was conducted with methanol or methanol/water (90:10, vol/vol) at a flow rate 1 mL/min. Yields for the synthesis of various phenolic esters were calculated from the amount of cinnamic acids reacted compared to the initial quantity of cinnamic acids.

In the figures and tables, the yields were calculated with respect to the limiting reagent (cinnamic acids or ascorbic acid). Reaction rates were calculated from the slope of the linear portion of plots of degrees of conversion vs. time, and expressed as millimoles per hour. All experiments were carried out in duplicate.

Ester purification. Esters of various cinnamic acid derivatives were obtained by extraction with diethyl ether followed by washing with saturated $NaHCO_3$ solution as described elsewhere (23). Various esters of ascorbic acid were isolated by a procedure similar to that described by Humeau *et al.* (22). The reaction mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure. Ascorbic acid was eliminated by water washing while ascorbic acid esters were obtained by recrystallization in hexane. The chemical structures

of the ascorbic esters were determined by ^{13}C nuclear magnetic resonance (NMR) in dimethylsulfoxide-d_6 by using tetramethylsilane as the internal reference on a Varian 300 MHz spectrometer (Palo Alto, CA).

Measurement of antioxidation effect of ascorbic acid and its esters. The autoxidation rate of linoleic acid was measured according to the methods of Chimi *et al.* (12) and Nishimura *et al.* (24). Linoleic acid (2.5×10^{-3} M) was dispersed with 0.5% Tween 20 in 50 mM phosphate buffer at pH 6.9 containing ascorbic acid or its fatty acid esters (10^{-2} M). All the samples were left in the dark and under air at 50°C. Samples without ascorbic acid and its esters, as well as controls without linoleic acid, were placed under the same conditions. The autoxidation rate of linoleic acid was estimated by the increase of conjugated diene levels in the sample which exhibited absorption at 234 nm. Samples were diluted 20 times with phosphate buffer before measuring the absorption.

RESULTS AND DISCUSSION

Enzymatic esterification of cinnamic acid derivatives in various organic solvents. The esterification of cinnamic acid with octanol has been evaluated as a model reaction with immobilized lipases from *C. antarctica* and *R. miehei* in various nontoxic organic solvents such as acetone, 1-octanol, 2-methyl-2-propanol, and 2-methyl-2-butanol. These solvents can solubilize relatively large amounts of cinnamic acid and other cinnamic acid derivatives. The two tertiary alcohols used cannot act as lipase substrates since they are too sterically hindered. Table 1 shows the effect of an organic solvent on the esterification of cinnamic acid with 1-octanol by both lipases during 12 d of reaction at 50°C. Reaction yields were dependent on the solvent and lipase used. When *C. antarctica* lipase was used, the octyl ester of cinnamic acid was formed in relatively good yields (up to 82%), whereas lipase from *R. miehei* showed moderate to low reaction yields depending on the nature of the solvent. For both lipases, higher yields were observed when the reaction was carried out without added solvent (solvent-free system). In this case, cinnamic acid was solubilized by the excess of alcoholic substrate, e.g., 1-octanol. Recently, Buisman *et al.* (19) reported that the esterification yield of cinnamic acid with 1-butanol in a reaction catalyzed by *C. antarctica* lipase in hydrocarbons such as *n*-pentane or cyclohexane was high (up to 85%), but the solubility of various cinnamic acids in these apolar solvents is very low.

TABLE 1
Effect of Organic Solvent on the Esterification of Cinnamic Acid with 1-Octanol Catalyzed by *Candida antarctica* and *Rhizomucor miehei* Lipases at 50°C[a]

	C. antarctica	*R. miehei*
Solvent	Conversion after 12 d (%)	
Acetone	38	Traces
1-Octanol	82	59
2-Methyl-2-propanol	52	12
2-Methyl-2-butanol	57	14

[a]Esterification with 0.5 mmol cinnamic acid, 5 mL 1-octanol, and 30 mg/mL *C. antarctica and R. miehei.*

The esterification of cinnamic acid in a solvent-free system was further studied using various primary aliphatic alcohols with chain lengths varying between 4 and 10 carbon atoms. As can be seen from Figure 2, the alcohol chain length affects the activity of both lipases. Notably, lipase from *R. miehei* showed 3–4 times higher reaction rates when the esterification was carried out with long-chain alcohols (1-nonanol and 1-decanol), rather than with short-chain ones such as 1-butanol. However, lipase from *C. antarctica* catalyzed the esterification of cinnamic acid with medium or high chain lengths with higher rates. It must be noted that the conversion yield of the reaction is about 80–85% after 12 d of reaction for both lipases used. However, the conversion yield for the esterification of cinnamic acid with 1-butanol and 1-hexanol catalyzed by *R. miehei* lipase is less than 45%. This behavior is probably due to the high concentrations of short-chain alcohols such as butanol, which cause deactivation of *R. miehei* lipase (25).

Effect of phenolic acids structure. Various hydroxylated or methoxylated derivatives of cinnamic acid as well as hydroxylated benzoic and phenylacetic acid were esterified with 1-octanol using lipases from *C. antarctica* and *R. miehei* under the experimental conditions described above. As can be seen from Table 2, both reaction rates and conversion yields depend on the structural characteristics of the acid substrate. Figure 3 depicts the esterification of various cinnamic acids, as well as *p*-hydroxyphenylacetic acid catalyzed by *C. antarctica* lipase in 1-octanol. Lipase from *C. antarctica* was able to catalyze the esterification of *p*-hydroxyphenylacetic and

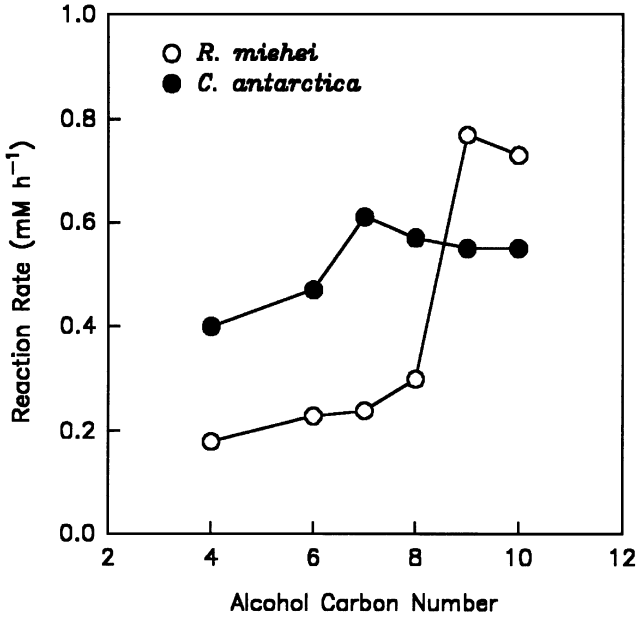


FIG. 2. Effect of the alcohol chain length on the reaction rate of esterification of 0.5 mmol cinnamic acid with 5 mL 1-octanol catalyzed by 30 mg/mL *Candida antarctica* and *Rhizomucor miehei* lipases at 50°C.

TABLE 2
Effect of Phenolic Acid Structure on the Reaction Rates and Conversion Yields for Esterification in 1-Octanol Catalyzed by *C. antarctica* and *R. miehei* Lipases[a]

	C. antarctica		*R. miehei*	
Acid	Reaction rate (mM h^{-1})	Conversion after 12 d (%)	Reaction rate (mM h^{-1})	Conversion after 12 d (%)
Cinnamic	0.57	82	0.32	59
o-Coumaric	0.18	21	0.20	18
m-Coumaric	0.27	38	0.25	32
p-Coumaric	0.18	25	0.18	22
Caffeic	ND[b]	Traces	ND[b]	Traces
Ferulic	ND[b]	11	0.10	30
p-Hydroxybenzoic	ND[b]	Traces	ND[b]	6
p-Hydroxyphenylacetic	8.5	97	0.10	32

[a]Reaction conditions described in Table 1; for abbreviations see Table 1.
[b]ND, not determined.

cinnamic acid with a high reaction rate and yield, while the hydroxy-substituted cinnamic and benzoic acid derivatives (especially *ortho*- and *para*-isomers) inhibited the catalytic action of lipase. When the side chain on the aromatic ring was saturated (*p*-hydroxyphenylacetic acid), *para*-hydroxylation had no effect on lipase activity. This inhibitory effect of electron-donating substituents in cinnamic or benzoic acid derivatives has been reported by other researchers (18,19) and could be attributed to an electronic and/or steric effect. It has been proposed that the electronic-donating effects deactivate the electrophilic carbon center of the carboxylic group for nucleophilic attack of the alcohol (19). A similar inhibitory effect by hydroxylated derivatives of cinnamic acid was also observed for *R. miehei* lipase (Table 2). However, this lipase seems to catalyze the esterification of methoxylated derivatives of cinnamic acid, such as ferulic acid, with higher rates and yield (30% conversion after 12 d; see Table 2) than *C. antarctica* lipase.

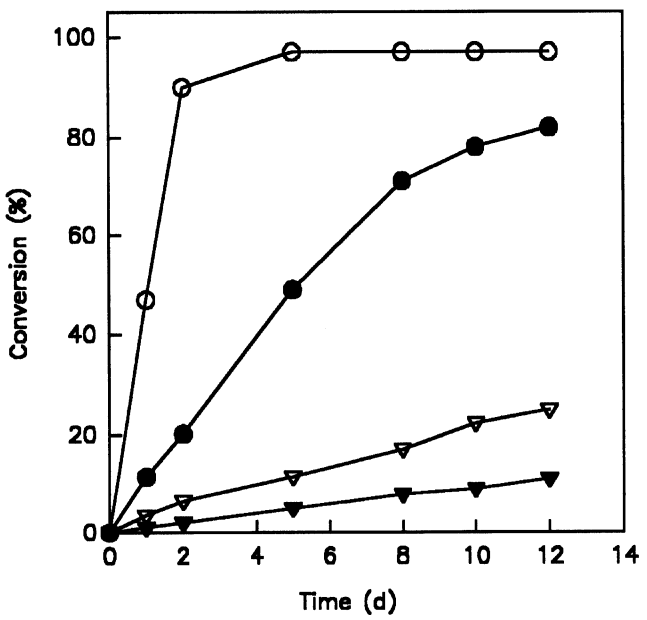
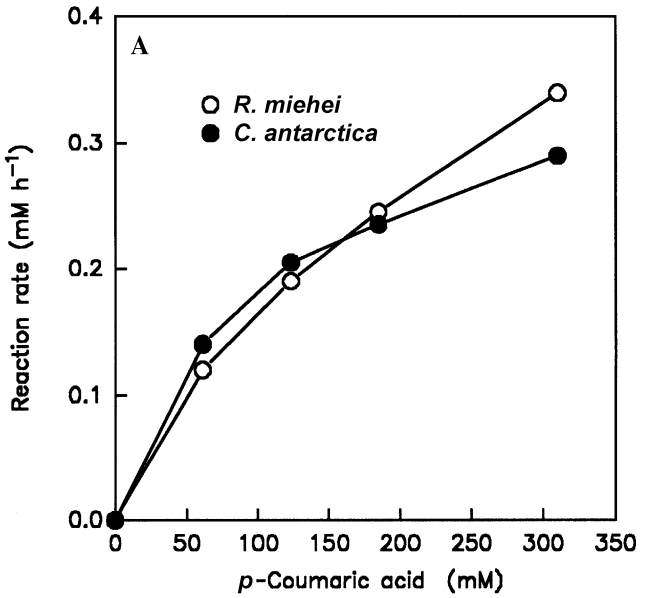


FIG. 3. Enzymatic esterification of *p*-hydroxyphenylacetic (○), cinnamic (●), *p*-coumaric (▽), and ferulic (▼) acids with 1-octanol catalyzed by *C. antarctica*. Reaction conditions as described in Figure 2; for abbreviation see Figure 2.

Effect of concentration of cinnamic acid derivatives. The effect of the concentration of cinnamic acid derivatives on lipase activity was studied. Figures 4A and B show the effect of the concentration of *p*-coumaric acid and cinnamic acid, respectively, on the rate of their lipase-catalyzed esterification with 1-octanol. An increase in cinnamic acid or *p*-coumaric acid concentration increased the reaction rate of both lipases. *Candida antarctica* lipase esterified cinnamic acid with 1-octanol two to three times faster than *R. miehei* lipase over all substrate concentrations tested (Fig. 4B). On the other hand, both lipases esterified *p*-coumaric acid with 1-octanol at similar rates (see Fig. 4A). It is interesting to note that the rate of esterification of *p*-coumaric acid by *C. antarctica* lipase was three to six times lower than the rate of esterification of cinnamic acid in a similar concentration range. This indicates that the lipase-inhibiting effect of hydroxylated derivatives of cinnamic acid (such as *p*-coumaric acid) is strong. Similar inhibition by various benzoic and cinnamic acids was also observed for the pancreatic lipase (26). Unfortunately, the relatively low reaction rates and the solubility limitation of cinnamic acids in 1-octanol make difficult an extensive kinetic study of the above esterification reaction.

Enzymatic esterification of ascorbic acid. Two nontoxic solvents, acetone and 2-methyl-2-propanol (*t*-butanol), have been tested in the enzymatic esterification of ascorbic acid with long-chain fatty acids (lauric, myristic, palmitic, and stearic acids), catalyzed by immobilized lipases from *C. antarctica* and *R. miehei,* respectively, since these solvents can solubilize relatively large amounts of the substrates. It was found that *C. antarctica* lipase catalyzed the acylation of ascorbic acid at a rate five times higher than *R. miehei* lipase in *t*-butanol. However, *R. miehei* lipase was unable to catalyze the same reaction in anhydrous acetone. Figure 5 shows a typical reaction profile for the acylation of ascorbic acid with the above-mentioned fatty acids catalyzed by *C. antarctica* lipase in *t*-butanol. Both reaction rate and yield after 24 h of reac-



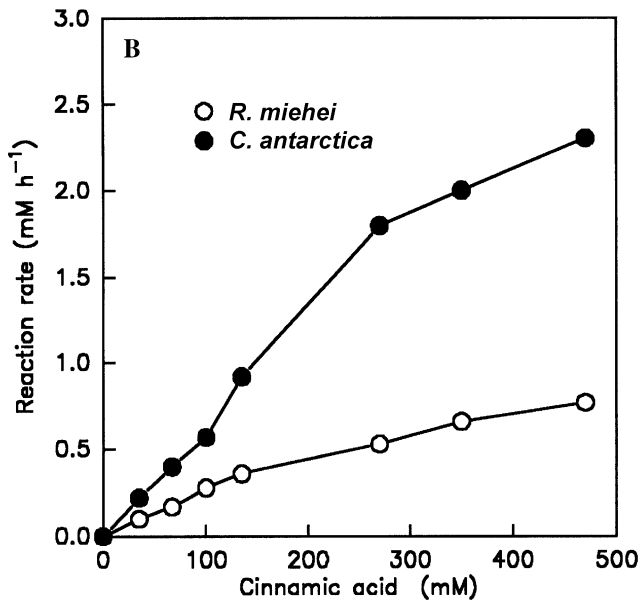


FIG. 4. Effect of the concentration of (A) *p*-coumaric acid and (B) cinnamic acid on the reaction rate of their esterification with 1-octanol catalyzed by lipases from *C. antarctica* and *R. miehei*. Reaction conditions as described in Figure 2; see that figure for abbreviations.

tion depended on the fatty acid chain length. An increase in the carbon number of fatty acids from 12 to 18 decreased the yield from 60 to 40%, while the esterification rate was decreased from 6.8 to 2.0 mM/h, respectively. The yield for the esterification of ascorbic acid with fatty acids was 10–15% lower in acetone than in *t*-butanol (data not shown). It must be noted that only one product was identified in all of the cases studied (using TLC and HPLC analysis), which indicates that this lipase-catalyzed esterification is regioselective. Various enzymatically synthesized fatty acid esters of ascorbic acid were purified and characterized by ^{13}C NMR analysis. By comparison with a commercial sample (6-*O*-palmitoyl L-ascorbic acid), it was determined that *C. antarctica* lipase acylated the ascorbic acid at the 6-OH group. ^{13}C NMR data for the dodecanoyl moiety in lauroyl ascorbate: δ 173.1 (C=O), 33.6 $CH_2(CH_2)_9CH_3$, 33.4 $CH_2CH_2CH_3$, 28.5–31.3 6 × CH_2, 24.3 $CH_2(CH_2)_8CH_3$, 22.1 CH_2CH_3, and 13.9 CH_3. In the ^{13}C NMR spectrum of lauroyl ascorbate, six carbon signals were observed for ascorbyl moiety: 174.8, 152.4, 118.4, 75.1, 65.6, and 64.5 ppm. The signal at 64.5 ppm was assigned to the C-6 of the ascorbyl moiety. This signal exhibited a downfield shift of 2.4 ppm in comparison with that of ascorbic acid (δ 62.1). These results suggest the presence of an ester bond on the C-6 of the ascorbyl moiety. Similar regioselective esterification of ascorbic acid catalyzed by the above lipase was also observed recently in 2-methyl-2-butanol by Humeau *et al.* (22).

Effect of molar ratio of reactants. Figure 6 shows the effect of molar ratio of myristic acid to ascorbic acid on the synthesis of myristoyl ascorbate catalyzed by *C. antarctica* lipase in *t*-butanol. The molar ratio of ascorbic acid to myristic acid was varied from 1:1 to 1:15 at fixed lipase concentration. As shown in the figure, the conversion yield of the reaction increased from 30 to 65% as the molar ratio increased. This behavior is similar to that observed when acetone was used as solvent (data not shown), as well as for the enzymatic esterification of other polar substrates such as monosaccharides (glucose or fructose) in hexane or supercritical carbon dioxide (26,27).

Antioxidation effect of ascorbic acid and its esters. The antioxidative effects of various ascorbic acid fatty esters toward linoleic acid were compared with those of ascorbic acid. The autoxidation of linoleic acid was accompanied by a rapid increase of conjugated diene level, reaching a maximal value at 5 d. Autoxidation of linoleic acid was inhibited by ascorbic

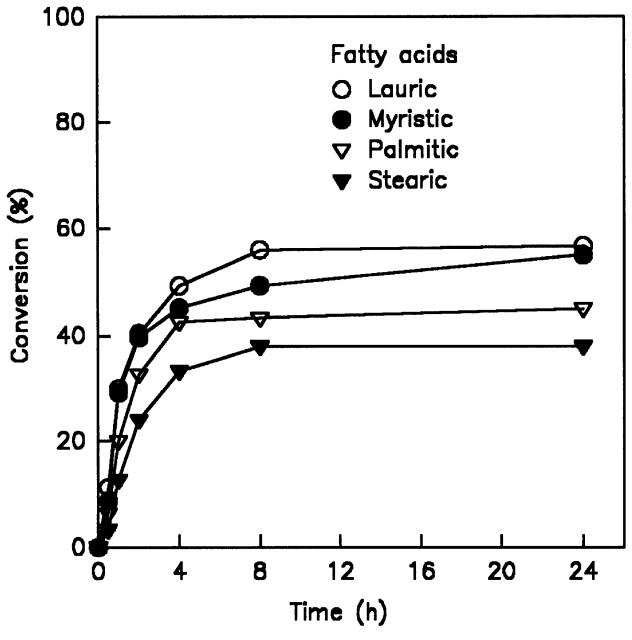


FIG. 5. Typical reaction profile for the acylation of ascorbic acid (0.15 mmol) with various fatty acids (0.90 mmol) catalyzed by *C. antarctica* lipase (30 mg/mL) in 2-methyl-2-propanol (5 mL) at 45°C. For abbreviation see Figure 2.

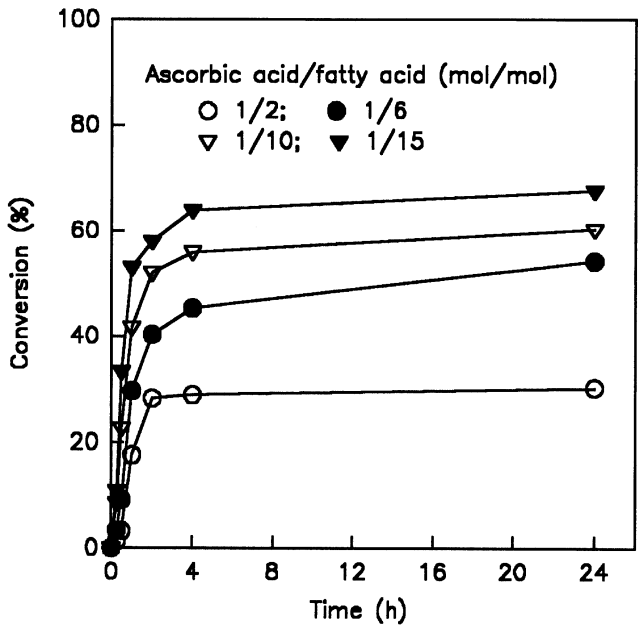


FIG. 6. Effect of varying myristic acid concentration on the synthesis of myristoyl ascorbate catalyzed by *C. antarctica* lipase in 2-methyl-2-propanol. Reaction conditions as described in Figure 5. For abbreviation see Figure 2.

acid and its fatty esters at a concentration of 10^{-2} M. On the fifth day of incubation, the absorption at 234 nm of the samples was 2–3 times lower compared to that of the control (without ascorbic acid or its esters), confirming the antioxidant activity of the tested ascorbic acid fatty esters (data not shown).

ACKNOWLEDGMENTS

The authors would like to thank Novo-Nordisk A/S Denmark, for the generous gifts of *C. antarctica* and *M. miehei* lipases. H. Stamatis thanks the Greek Foundation of Fellowships (I.K.Y.) for a grant.

REFERENCES

1. Sherwin, E.R., Antioxidants for Food Fats and Oils, *J. Am. Oil. Chem. Soc. 49*:468–472 (1972).
2. Kochhar, S.P., and J.B. Rossell, Detection, Estimation and Evaluation of Antioxidants in Food Systems, in *Food Antioxidants*, edited by B.J.F. Hudson, Elsevier Science Publishers, London, 1990, pp. 19–22.
3. Pratt, D.E., and B.J.F Hudson, Natural Antioxidants Not Exploited Commercially, in *Food Antioxidants*, edited by B.J.F. Hudson, Elsevier Science Publishers, London, 1990, p. 171.
4. Shahidi, F., P.K. Janitha, and P.D. Wanasundara, Phenolic Antioxidants, *Crit. Rev. Food Sci. Nutr. 32*:67–75 (1992).
5. Schuler, P., Natural Antioxidants Exploited Commercially, in *Food Antioxidants*, edited by B.J.F. Hudson, Elsevier Science Publishers, London, 1990, pp. 113–127.
6. Wang, X.Y., P.A. Seib, and K.S. Ra, L-Ascorbic Acid and Its 2-Phosphorylated Derivatives in Selected Foods: Vitamin C Fortification and Antioxidants Properties, *J. Food Sci. 60*:1296–1299 (1992).
7. Larson, R.A., The Antioxidants of Higher Plants, *Phytochemistry 27*:969–978 (1988).
8. Graf, E., Antioxidant Potential of Ferulic Acid, *Free Radicals Biol. 13*:435–448 (1992).
9. Yagi, K., and N. Ohishi, Action of Ferulic Acid and Its Derivatives as Antioxidants, *J. Nutr. Sci. Vitaminol. 25*:127–130 (1979).
10. Liu, X.-Y., F.-L. Guo, L.-M. Wu, and Z.-L. Liu, Remarkable Enhancement of Antioxidant Activity of Vitamin C in an Artificial Bilayer by Making It Lipo-Soluble, *Chem. Phys. Lipids 83*:39–43 (1996).
11. Marinova, E.M., and N.V. Yanishlieva, Effect of Lipid Unsaturation on the Antioxidative Activity of Some Phenolic Acids, *J. Am. Oil Chem. Soc. 71*:427–434 (1994).
12. Chimi, H., J. Cillard, P. Cillard, and M. Rahmani, Peroxyl and Hydroxyl Radical Scavenging Activity of Some Natural Phenolic Antioxidants, *Ibid. 68*:307–312 (1991).
13. Han, D., O.S. Yi, and H.K. Shin, Antioxidative Effect of Ascorbic Acid Solubilized in Oils *via* Reversed Micelles, *J. Food Sci. 55*:247–249 (1990).
14. Ito, S., C. Kobayashi, and S. Ikeda, *Jpn. Kokai Tokkyo Koho JP* 09065864 (1997).
15. Gutman, A.L., E. Shkolnik, and M. Shapira, A Convenient Method for Enzymatic Benzyl-Alkyl Transesterification Under Mild Neutral Conditions, *Tetrahedron 48*:8775–8780 (1992).
16. Ballesteros, A., U. Bornscheuer, A. Capewell, D. Combes, J.-S. Condoret, K. Koening, F.N. Kolisis, A. Marty, U. Menge, T. Scheper, H. Stamatis, and A. Xenakis, Enzymes in Non-Conventional Phases. *Biocatal. Biotransform. 13*:1–42 (1995).
17. John, V.T., and G. Abraham, Lipase Catalysis and Its Applications, in *Biocatalysis for Industry,* edited by J.S. Dodrick, Plenum Press, New York, 1991, pp. 193–218.
18. Guyot, B., B. Bosquette, M. Pina, and J. Graille, Esterification of Phenolic Acids from Green Coffee with an Immobilized Lipase from *Candida antarctica* in Solvent-Free Medium, *Biotechnol. Lett. 19*:529–532 (1997).
19. Buisman, G.J.H., C.T.W. van Helteren, G.F.H Kramer, J.W. Veldsink, J.T.P. Derksen, and F.P. Cuperus, Enzymatic Esterification of Functionalized Phenols for the Synthesis of Lipophilic Derivatives, *Ibid. 20*:131–136 (1998).
20. Enomoto, K., T. Miyamori, A. Sakimae, and R. Numazawa, Eur. Pat. Appl. EP, 401,704 (1990).
21. Sakashita, K., S. Miyamoto, and A. Sakimae, Eur. Pat. Appl. EP, 514,694 (1992).
22. Humeau, C., M. Girardin, D. Coulon, and A. Miclo, Synthesis of 6-*O*-Palmitoyl L-Ascorbic Acid Catalyzed by *Candida antarctica* Lipase, *Biotechnol. Lett. 17*:1091–1094 (1998).
23. Borneman, W.S., R.D. Hartley, W.H. Morrison, D.E. Akin, and L.G. Ljungdahl, Feruloyl and *p*-Coumaroyl Esterase from Anaerobic Fungi in Relation to Plant Cell Wall Degradation, *Appl. Microbiol. Biotechnol. 33*:345–351 (1990).
24. Nishimura, T., T. Kometani, H. Takii, Y. Terada, and S. Okada, Glucosylation of Caffeic Acid with *Bacillus subtilis* X-23 *a*-Amylase and a Description of the Glucosides, *J. Ferment. Bioeng. 80*:18–23 (1995).
25. Gandhi, N.N., S.B. Sawant, J.B. Joshi, and D. Mukesh, Lipozyme Deactivation by Butanol and Temperature, *Enzyme Microb. Technol. 17*:373–380 (1995).
26. Karamajc, M., and R. Amarowicz, Inhibition of Pancreatic Lipase by Phenolic Acids—Examination *in vitro*, *Z. Naturforsch. C. Biochem. Biophys. Biol. Virol. 51*:903–905 (1996).
27. Stamatis, H., V. Sereti, and F.N. Kolisis, Studies on the Enzymatic Synthesis of Sugar Esters in Organic Medium and Supercritical Carbon Dioxide, *Chem. Biochem. Eng. Q. 12*:151–156 (1998).
28. Tsitsimpikou, C., H. Stamatis, V. Sereti, H. Daflos, and F.N. Kolisis, Acylation of Glucose Catalysed by Lipases in Supercritical Carbon Dioxide, *J. Chem. Technol. Biotechnol.*