Interesterification of Lipids Using an Immobilized *sn*-1,3-Specific Triacylglycerol Lipase[1]

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Triacylglycerols of ucuhuba (*Virola surinamensis*) that contain almost exclusively (90%) lauroyl and myristoyl moieties were interesterified with methyl esters of saturated fatty acids (C_{13}, C_{15}, C_{17}, C_{19}, C_{20}), unesterified fatty acid (heptadecanoic acid), triacylglycerols containing C_{18} acyl moieties (trioleoylglycerol, sunflower oil, corn oil), long-chain alcohol (octadecyl alcohol), and glycerol with an immobilized *sn*-1,3-specific lipase (Lipozyme) from *Mucor miehei*. Exchange (transfer) of acyl moieties occurred at the *sn*-1,3-positions of the triacylglycerols, and the rates of interesterification with various substances were of the order long-chain alcohol > fatty acid > triacylglycerol > methyl ester > glycerol. Rate of interesterification of methyl esters decreased with increasing chain length. Melting behavior of the triacylglycerols formed by interesterification reveals their potential use in food and dietetic products. These studies show the possible applications of immobilized lipases in the production of various acylglycerols and alkyl esters of fatty acids.

It is often desirable to alter the composition of acyl moieties of triacylglycerols naturally occurring in fats and oils in order to modify their physical and chemical properties. One of the processes commonly used to alter the physical properties of triacylglycerols by manipulating the composition of their acyl moieties is interesterification. The classical interesterification is characterized by a randomization in the distribution of acyl moieties in the triacylglycerol molecule by applying a chemical catalyst such as sodium alkoxide (Sreenivasan, 1978).

The use of lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) to catalyze interesterification reactions has received considerable attention lately, because of certain advantages over chemical catalysts. By using a *sn*-1,3-specific lipase, for example, the exchange of acyl moieties is confined to the *sn*-1- and *sn*-3-positions, giving rise to products with characteristics that cannot be obtained by chemical interesterification (Macrae, 1984). A few lipases immobilized on inorganic supports, such as Kieselguhr, have been studied in interesterification reactions (Yokozeki et al., 1982; Wisdom et al., 1984); such biocatalysts may be recovered and reused or may be directly employed in continuous reactors.

We report here the performance of an immobilized lipase (Lipozyme) from *Mucor miehei*, supported on a macroporous anion-exchange resin, in various interesterification reactions that were carried out in hexane. This lipase hydrolyzes specifically the acyl moieties from the *sn*-1- and *sn*-3-positions of triacylglycerols. Interesterification reactions were carried out using triacylglycerols of ucuhuba (*Virola surinamensis*) seed, which contains about 90% medium-chain acyl moieties (lauroyl, myristoyl). As reaction partners various classes of lipids were used, such as methyl esters of saturated fatty acids (C_{13}, C_{15}, C_{17}, C_{19}, C_{20}), unesterified fatty acid (heptadecanoic acid), triacylglycerols containing C_{18} acyl moieties (trioleoylglycerol, sunflower oil, corn oil), and long-chain alcohol (octadecyl alcohol), as well as glycerol.

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EXPERIMENTAL SECTION

Materials. Triacylglycerols were isolated from ucuhuba (*V. surinamensis*) seeds, which were provided by W. Gomes da Silva, University of Amazonas, Manaus, Brazil. The composition of the acyl moieties of these triacylglycerols was as follows: lauroyl (12:0), 14.3%; myristoyl (14:0), 71.5%; myristoleoyl (14:1), 2.2%; palmitoyl (16:0), 5.5%; stearoyl (18:0), 0.7%; oleoyl (18:1), 4.2%; linoleoyl (18:2), 0.8%. Refined sunflower oil (Tommy, Deutsche Tommy GmbH, 7500 Karlsruhe, Federal Republic of Germany) and corn oil (Mazola, Maizena Markenartikel GmbH, 7100 Heilbronn, Federal Republic of Germany) were purchased from local stores. Methyl esters of fatty acids, heptadecanoic acid, glycerol, silica gel H for thin-layer chromatography (TLC), and analytical-grade reagents were from E. Merck AG, 6100 Darmstadt, Federal Republic of Germany. Trioleoylglycerol, octadecyl alcohol, and pancreatic lipase were from Sigma Chemie GmbH, 8000 München, Federal Republic of Germany. Lipid standards and column packings for gas chromatography (GC) were from Applied Science Laboratories, State College, PA. Distilled solvents were used throughout. Immobilized lipase of *M. miehei* (Lipozyme) was a product of Novo Industrie GmbH, 6500 Mainz, Federal Republic of Germany. Activity of Lipozyme was 25 BIU (batch interesterification units)/g; 1 BIU corresponds to 1 μmol of palmitic acid incorporated into trioleoylglycerol/min from an equimolar mixture at 40 °C.

Interesterification Reactions. Triacylglycerols of ucuhuba seeds (0.5 mmol) and 0.5 mmol each of methyl esters of fatty acids, heptadecanoic acid, trioleoylglycerol, sunflower oil, corn oil, octadecyl alcohol, or glycerol were dissolved in 5 mL of hexane, and the solution was saturated with water at 45 °C. Lipozyme (10% of the total weight of the reactants) was added and the mixture stirred at 45 °C. Samples were withdrawn from the reaction mixture after 2, 4, and 8 h, and the enzyme was removed by centrifugation. Several interesterification reactions using triacylglycerols of ucuhuba and sunflower oil or corn oil were carried out with larger lots (25 mmol) as well.

Analytical Procedures. The products of interesterification of triacylglycerols of ucuhuba with each of the following reaction partners were analyzed as described below.

Methyl Esters of Fatty Acids. The products were fractionated by TLC with hexane/diethyl ether (90:10,

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v/v). The methyl ester fraction was eluted with diethyl ether, saturated with water, and analyzed by GC on glass columns (1.8 m × 4 mm) packed with 10% (w/w) Silar 5CP on Gas-Chrom Q (80–100 mesh) in a Perkin-Elmer F-22 instrument equipped with flame ionization detectors (Perkin-Elmer, GmbH, 7700 Überlingen, Federal Republic of Germany). Nitrogen (40 mL/min) was used as carrier gas, and the column temperature was programmed from 150 to 230 °C at 2 °C/min. The amount of methyl esters that was exchanged against the acyl moieties of the triacylglycerols of ucuhuba was calculated from the composition of the methyl ester fraction.

Heptadecanoic Acid. The products of interesterification were fractionated by TLC with hexane/diethyl ether/acetic acid (70:30:1, v/v). The unesterified fatty acid fraction was scraped off and transmethylated in situ (Chalvardjian, 1964). The methyl esters were analyzed by GC, as described above. The amount of heptadecanoic acid that was exchanged against the acyl moieties of the triacylglycerols of ucuhuba was calculated from the composition of the unesterified fatty acid fraction.

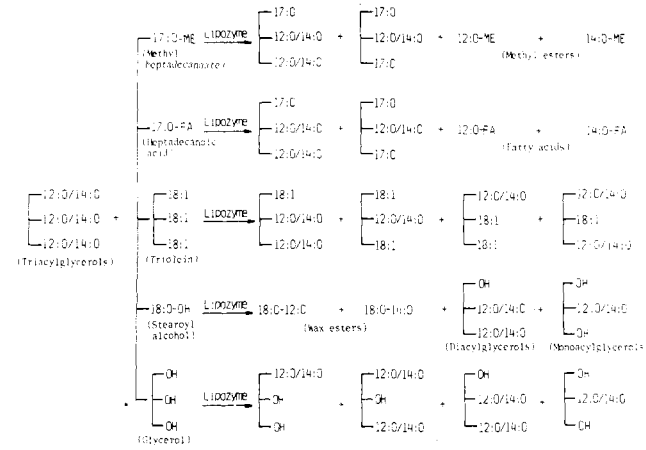
Trioleoylglycerol, Sunflower Oil, and Corn Oil. Reaction products were fractionated by TLC with hexane/diethyl ether/acetic acid (70:30:1, v/v). The triacylglycerol fraction was eluted and separated according to carbon number by GC on glass columns (50 cm × 4 mm) packed with 3% (w/w) OV-1 on Gas Chrom Q (100–120 mesh). Nitrogen (60 mL/min) was used as carrier gas, and the column temperature was programmed from 250 to 350 °C at 4 °C/min. The amount of trioleoylglycerol interesterified with the triacylglycerols of ucuhuba was calculated from the reduction in the relative proportion of trioleoylglycerol (carbon number 54). Similarly, the amount of sunflower oil or corn oil (each almost exclusively composed of C_{18} acyl moieties) interesterified with the triacylglycerols of ucuhuba was calculated from the reduction in the relative proportion of triacylglycerols having the carbon number 54.

Octadecyl Alcohol. An aliquot of the reaction product was mixed with a known amount of an internal standard, methyl heptadecanoate, and the mixture fractionated by TLC with hexane/diethyl ether/acetic acid (80:20:1, v/v). The fractions of wax esters and methyl esters were eluted together with diethyl ether, saturated with water, and separated according to carbon number by GC as described above but using a temperature program of 130–250 °C, 4 °C/min. The amount of octadecyl alcohol, to which the acyl moieties of triacylglycerols of ucuhuba were transferred, was calculated from the ratio of peak areas of wax esters and methyl heptadecanoate.

Glycerol. The products were fractionated by TLC as follows. The chromatograms were first developed twice with diethyl ether up to a height of 2 cm and then up to 19 cm with hexane/diethyl ether/acetic acid (70:30:1, v/v). Triacylglycerols, diacylglycerols, and monoacylglycerols were scraped off and transmethylated as described before. The relative proportions of these fractions were determined by GC of their methyl esters using methyl heptadecanoate as internal standard (Christie et al., 1970). The amount of glycerol, to which the acyl moieties of the triacylglycerols of ucuhuba were transferred, was calculated from the total proportion of monoacylglycerols and diacylglycerols formed.

Positional Specificity of Interesterification Reactions. In several experiments, the triacylglycerols were isolated from the reaction mixture by TLC and positional distribution of the acyl moieties was determined by hydrolysis with pancreatic lipase (Christie, 1982) and TLC on boric acid impregnated silica gel H (Thomas et al., 1965) in conjunction with GC, as described above.

Scheme I. Interesterification Reactions



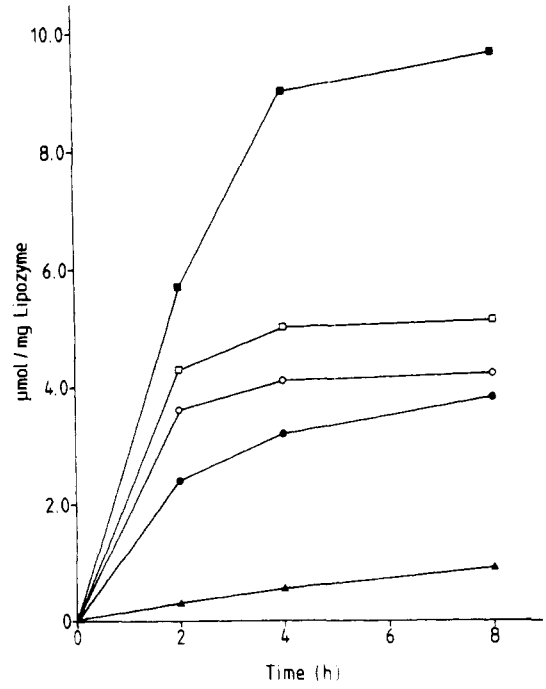


Figure 1. Rates of interesterification of the triacylglycerols of ucuhuba with methyl heptadecanoate (●), heptadecanoic acid (□), trioleoylglycerol (○), octadecyl alcohol (■), and glycerol (▲) using Lipozyme.

Solid Fat Content. Triacylglycerols of ucuhuba and the products of interesterification of these triacylglycerols with sunflower oil and corn oil for 0, 2, and 4 h were analyzed by pulse NMR (Arens and Kroll, 1986) in a Minispec p-20 instrument (Brucker Instruments, Inc., Analytische Messtechnik GmbH, 7512 Rheinstetten, Federal Republic of Germany).

RESULTS AND DISCUSSION

The triacylglycerols of ucuhuba seed that mainly contain lauroyl and myristoyl moieties were subjected to interesterification in hexane with various reaction partners using a *sn*-1,3-specific lipase from *M. miehei* supported on macroporous anion-exchange resin. The reactions investigated and the products expected are presented in Scheme I in a simplified manner.

The rates of interesterification given in Figure 1 show that with both methyl heptadecanoate and heptadecanoic acid an equilibrium is reached in about 4 h. Moreover, a higher rate of interesterification is observed with hepta-

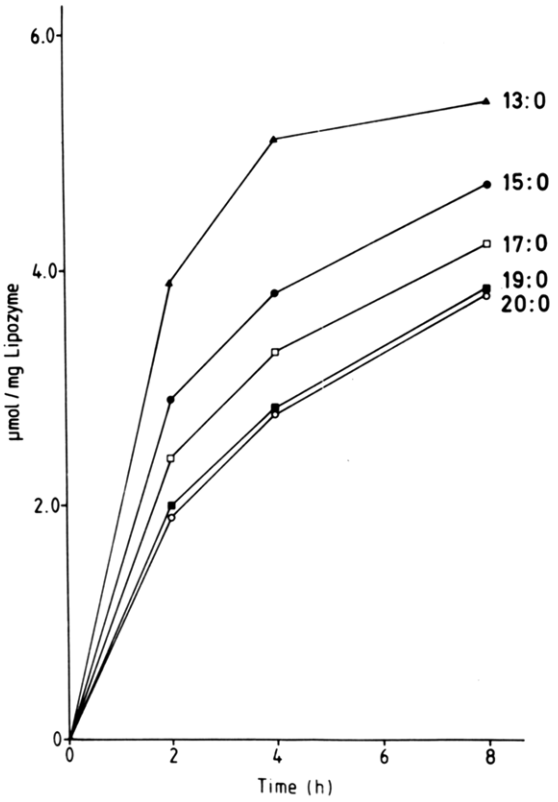


Figure 2. Rates of interesterification of the triacylglycerols of ucuhuba with methyl tridecanoate (▲), methyl pentadecanoate (●), methyl heptadecanoate (□), methyl nonadecanoate (■), and methyl arachidate (○) using Lipozyme.

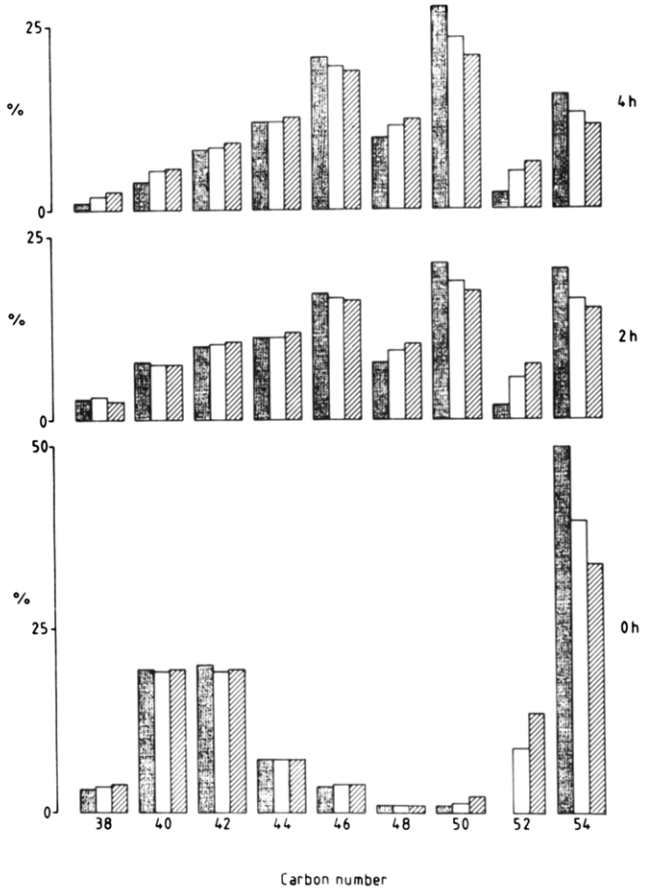


Figure 3. Composition (%) of triacylglycerols in the products formed by interesterification of the triacylglycerols of ucuhuba with trioleoylglycerol (▣), and the triacylglycerols of sunflower oil (□) and corn oil (▨) for different periods.

decanoic acid than with methyl heptadecanoate. In both interesterification reactions, hydrolysis of the triacylglycerols occurred to the extent of about 10%, and this was taken into account in calculating the reaction rates.

In order to assess the chain length specificity or selectivity of the lipase from *M. miehei*, the medium-chain triacylglycerols were interesterified with a known mixture of methyl esters of fatty acids of different chain lengths (13:0, 15:0, 17:0, 19:0, 20:0). The rates of interesterification of the individual components of the methyl ester mixture are found to increase distinctly with decreasing chain length (Figure 2).

The lipase-catalyzed interesterification of the medium-chain triacylglycerols with trioleoylglycerol or triacylglycerols containing C_{18} acyl moieties, such as those of sunflower oil or corn oil, results in an exchange of the C_{18} acyl moieties against the 12:0 and 14:0 acyl moieties at the *sn*-1- and *sn*-3-positions of the triacylglycerols (Scheme I). The rate of interesterification with trioleoylglycerol is found to be intermediate between those with methyl heptadecanoate and heptadecanoic acid (Figure 1) and similar to those with sunflower oil and corn oil (data not shown). In each case, an equilibrium is reached in about 4 h. In each of the interesterification reactions with triacylglycerols containing C_{18} acyl moieties, hydrolysis occurred to an extent of about 10%, and this was taken into account in calculating the reaction rates.

The formation of triacylglycerols differing in carbon number during the interesterifications of triacylglycerols of ucuhuba seed with trioleoylglycerol, sunflower oil, and corn oil is shown in Figure 3. The changes observed are marked by a reduction in the content of triacylglycerols with carbon numbers 38, 40, and 42 (corresponding to triacylglycerols from ucuhuba) and those with carbon number 54 (corresponding to trioleoylglycerol) and 52 and 54 (corresponding to triacylglycerols of sunflower oil and corn oil). Concomitantly, an increase in the content of triacylglycerols with carbon numbers from 44 to 50 is observed. The reaction with trioleoylglycerol shows the formation of triacylglycerols with carbon number 52 that are not present in the starting mixture.

The melting behavior of the products of lipase-catalyzed interesterification of triacylglycerols of ucuhuba seed with sunflower oil and corn oil for 0, 2, and 4 h was investigated by pulse NMR, and the data are presented in Figure 4. A decrease in the solid fat content is observed in both interesterification reactions. The melting behavior is similar for the products obtained after 2 and 4 h in the reactions with both sunflower oil and corn oil. Such products are of interest as fat components for special margarines with specific texture and consistency (Chrysam, 1985).

The lipase-catalyzed interesterification of the medium-chain triacylglycerols with octadecyl alcohol yields the octadecyl esters of the medium-chain fatty acids with concomitant formation of *sn*-1,2(2,3)-diacylglycerols and *sn*-2-monoacylglycerols, as outlined in Scheme I. The rate of this interesterification is found to be highest among all the reactions studied (Figure 1). The formation of wax esters and diacylglycerols and simultaneous reduction in the relative proportions of the reaction partners, i.e. triacylglycerols and octadecyl alcohol, are quite evident from the thin-layer chromatogram. Octadecyl laurate (ca. 20%) and octadecyl myristate (ca. 70%) are the principal constituents of the wax esters formed (Table I).

Interesterification of the medium-chain triacylglycerols with glycerol, catalyzed by the *sn*-1,3-specific lipase from *M. miehei*, yields a mixture of isomeric monoacylglycerols

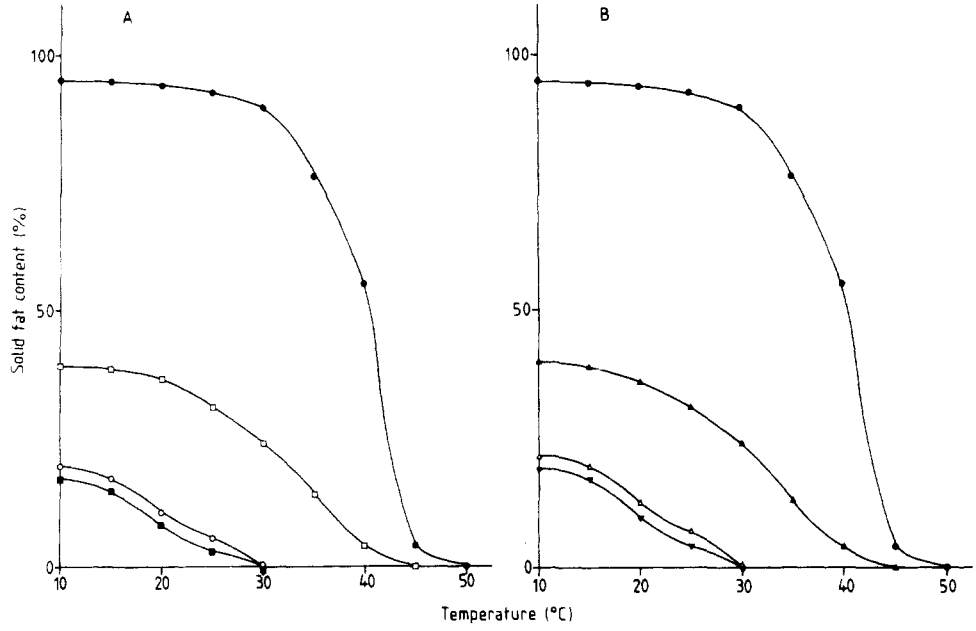


Figure 4. Solid fat content of triacylglycerols of ucuhuba (●) and of products obtained by interesterification of these triacylglycerols with those of sunflower oil (A) for 0 h (□), 2 h (○), and 4 h (■) and corn oil (B) for 0 h (▲), 2 h (△), and 4 h (▼).

Table I. Composition of Wax Esters Formed during the Interesterification of Triacylglycerols of Ucuhuba with Octadecyl Alcohol

	wax esters,[a] %			
reactn time, h	C_{30} 18:0–12:0	C_{32} 18:0–14:0	C_{34} 18:0–16:0	C_{36} 18:0–18:1, 18:0–18:2
2	23.4	68.9	4.7	2.9
4	20.4	71.9	4.5	3.2
8	21.8	70.0	4.9	3.3

[a] The wax esters are abbreviated by alkyl moiety–acyl moiety.

and diacylglycerols as outlined in Scheme I. The rate of this interesterification reaction is found to be the lowest of all the reactions investigated (Figure 1). This is most likely due to the lack of solubility of glycerol in the solution of the triacylglycerols in hexane.

The results reported in the present investigation demonstrate the potentials of lipase-catalyzed interesterification reactions for the preparation of a wide variety of acylglycerols and alkyl esters of fatty acids that are useful as food additives and agrochemicals. The use of an *sn*-1,3-specific lipase, as described here, ensures modification of the acyl composition of triacylglycerols exclusively at the *sn*-1- and *sn*-3-positions (Macrae, 1984), yielding products that cannot be obtained by conventional interesterification using chemical catalysts, i.e. randomization. It also appears quite feasible that triacylglycerols having unusual structures seldom occurring in nature can be prepared by interesterification of common fats and oils using *sn*-1,3-specific lipases. For example, triacylglycerols containing a high level of linoleoyl moieties at the *sn*-1,3-positions and almost exclusively medium-chain acyl moieties at the *sn*-2-position, which rarely occur in nature and are difficult to prepare by chemical synthesis, can be easily obtained by interesterification of medium-chain triacylglycerols, e.g. those of ucuhuba, with the triacylglycerols of corn oil or sunflower oil, as described in the present study. Such triacylglycerols could be of interest as dietetic products, since the linoleoyl moieties at the *sn*-1,3-positions would be rapidly released by the pancreatic lipases of most mammalian organisms and the linoleic acid would be readily available to the organism as an essential fatty acid. Future studies in this direction could be rewarding.

ACKNOWLEDGMENT

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Registry No. 17:0-Me, 1731-92-6; 17:0-FA, 506-12-7; 18:0-OH, 112-92-5; 13:0-Me, 1731-88-0; 15:0-Me, 7132-64-1; 19:0-Me, 1731-94-8; 20:0-Me, 1120-28-1; lipozyme, 9001-62-1; trioleoylglycerol, 122-32-7; glycerol, 56-81-5.

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