Lipase-Catalyzed Hydrolysis of Palm Oil

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The hydrolysis of palm oil, palm olein and palm stearin, soybean oil, corn oil and peanut oil by the commercial lipase from *Candida rugosa* (formerly known as *C. cylindracea*) was studied. The optimal conditions for the hydrolysis of palm oil by the lipase were established. The lipase from *C. rugosa* exhibits an optimal activity at 37 C and at pH 7.5. The optimal oil to hexane ratio is 1 g of oil to 0.5 ml hexane. The rate of hydrolysis of palm oil by the lipase is linear on a logarithmic scale. Under the same conditions, palm oil and palm olein were hydrolyzed at the same rate, whereas palm stearin was hydrolyzed much more slowly.

Fatty acids are important raw materials for the chemical industry. Existing methods of fatty acid production are based on chemical and physical methods (1).

Lipases are enzymes that hydrolyze fats and oils to their basic components, fatty acids and glycerol. At present, a number of lipases are available commercially; the enzymology of these lipases is well documented (2,3). The use of lipase for the production of fatty acids from isolated triacylglycerols, olive oil, coconut oil, soybean oil, etc., has been reported (4-8). However, to date there has been no published report on how lipase would hydrolyze palm oil and palm oil fractions as compared to other oils. In this paper we describe the optimal conditions for the hydrolysis of palm oil by the lipase from *Candida rugosa* (formerly known as *Candida cylindracea*).

EXPERIMENTAL

Materials. Lipase from *Candida rugosa*, 2975 units activity per mg protein, wheat germ lipase, 7 units activity per mg protein and porcine pancreatic lipase, 38 units activity per mg protein, were purchased from Sigma Chemical Co. (St. Louis, Missouri). All lipase activities quoted above are according to the supplier.

Methods, lipase assay. Lipase activity was assayed as follows. 0.1 g of lipase solubilized in 10 ml of 0.1M Tris-HCl buffer, pH 7.5, was added to 1 g of palm oil or other oils dissolved in 0.5 ml of hexane. The flask was then incubated in a shaking water bath at 162 strokes per min at 37 C for 30 min or longer. At the end of the incubation, 5 ml of acetone-ethanol (1:1) were added to stop the reaction and to extract the free fatty acids and other lipids. The free fatty acids in the mixture were then estimated by direct titration with 0.5M NaOH using phenolphthalein as the indicator. A blank consisted essentially of the same components mentioned above, except a heat-inactivated lipase was used to correct for any background titer. Palmitic acid was used as the reference standard.

Separation and quantification of lipid classes. After the incubation, the lipids were extracted with chloroform-methanol (2:1) as described (9) and separated on TLC using hexane/diethyl ether/acetic acid (50:50:1, v/v/v) as the solvent system. The plate was then charred with 50% sulphuric acid and the lipid spots quantified by photodensitometry (10). Calibration curves were constructed with commercial lipid standards.

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RESULTS AND DISCUSSION

Selection of lipase. Linfield et al. (7) reported that lipases from *Candida rugosa* and *Aspergillus niger* performed similarly with olive oil, tallow and coconut oil as the substrates. In our study, lipase from *Candida rugosa* hydrolyzed palm oil much faster than did porcine pancreatic lipase and wheat germ lipase under the same conditions (Table 1). Hence, lipase from *Candida rugosa* was used in all further studies.

Linfield et al. (8) also reported that lipase from *Candida rugosa* hydrolyzed olive oil much faster than tallow and coconut oil under the same conditions. In our study, the same lipase hydrolyzed palm oil, soybean oil and corn oil at the same rate but hydrolyzed peanut oil at a much slower rate (Table 2). Recent results (unpublished data) in our laboratory show that the slow hydrolysis of peanut oil by the yeast lipase was due to the physical structure of the peanut triacylglycerols and not due to impurities in the oil as was encountered by Linfield et al. (8) in olive oil.

Hexane to oil ratio. Hexane is a common vehicle used to solubilize fats and oils in the laboratory. In this study, we solubilized palm oil in hexane because palm oil is semi-solid at room temperature. The optimal ratio of hexane to oil is 0.5 ml of hexane to 1 g of oil. Increasing or decreasing the hexane to oil ratio would result in decreased hydrolysis (Table 3). Linfield et al. (8), on the other hand, observed that hexane inhibited the hydrolysis of

TABLE 1

Lipolysis of Palm Oil by Different Lipases

Sources of lipase	FA released[a] (μmoles)	Specific activity (μmoles FFA/mg enzyme/min)
Candida rugosa	1023.5	0.34
Hog pancreas	213.9	0.07
Wheat germ	69.3	0.02

[a]FA = free fatty acids with reference to palmitic acid.

TABLE 2

Lipolysis of Different Oils by the Lipase from *Candida rugosa*

Types of oils	FA released (μmoles)	Specific activity (μmoles FFA/mg enzyme/min)
Soybean oil	1069.0	0.36
Corn oil	1069.0	0.36
Palm oil	1023.0	0.34
Peanut oil	697.6	0.23

tallow by lipase from *Candida rugosa.* The ratio of hexane to oil used by Linfield et al. (8) was 75% higher than ours. In a recent communication, Kim et al. (11) observed that the inhibition of lipase activity by several organic solvents was very pronounced with prolonged exposure of the lipase to the solvents. However, the inhibition of lipase activity by the organic solvents was minimal when the incubation time was 1 hr or less, especially when hexane was used.

Effect of temperature and pH. Temperature and pH are well known factors that affect enzyme activity. In our study the optimal temperature and pH for the lipase of *Candida rugosa* were 37 C and pH 7.5, respectively (Fig. 1a and 1b). Tomizuka et al. (4) reported that the optimal temperature and pH for the lipase isolated by them from *Candida rugosa* were 45 C and pH 7.2, respectively. Linfield et al. (8) showed that there was no appreciable change in the rate of lipolysis of olive oil between pH 4.8 and 7.2. The discrepancies in optimal temperature and pH for the same lipase could be due to different conditions used for the assay of the activity of the enzyme and the methods of analysis.

Effect of calcium ion. Calcium ions are considered to have a stimulatory effect on lipolysis by pancreatic lipase (2). However, calcium ions have different effects on lipolysis by other lipases. With the fungal lipase (12) calcium ions were observed to have no effect on the lipolysis. With the yeast lipase, Linfield et al. (3) observed that calcium ions have an inhibitory effect on the lipolysis of olive oil by the lipase from *Candida rugosa.* Our study with palm oil lipolysis by the same enzyme confirmed the above results. The inhibitory effect of calcium ions on lipolysis increased with increasing concentration of the calcium ions (Table 4).

Time course of lipolysis. Under the conditions outlined in the Methods section, complete hydrolysis of the substrate, palm oil, could be achieved in 5 hr (Fig. 2). The rate of hydrolysis is linear on a logarithmic scale. A similar observation was made by Linfield et al. (8) using olive oil as the substrate. However, the rate of lipolysis in our experiments was much faster than that observed by Linfield et al. (8). The differences in the rate of hydrolysis could be due to different experimental conditions and substrates. In our experiments, palm olein was

TABLE 3

Effect of Hexane on Palm Oil Hydrolysis

Hexane (ml)	FA released (μmoles)	Specific activity (μmoles FFA/mg enzyme/min)
0	558.1	0.18
0.5	1023.5	0.34
0.75	906.9	0.30
1.0	860.5	0.28
2.0	604.6	0.20

TABLE 4

Effect of Calcium Ions on the Lipolysis of Palm Oil by the Lipase from *Candida rugosa*

Calcium chloride (μmoles)	FA released (μmoles)	Specific activity (μmoles FFA/mg enzyme/min)
0	1023.5	0.34
1	976.7	0.32
2	651.1	0.22
4	558.1	0.18
6	325.6	0.11

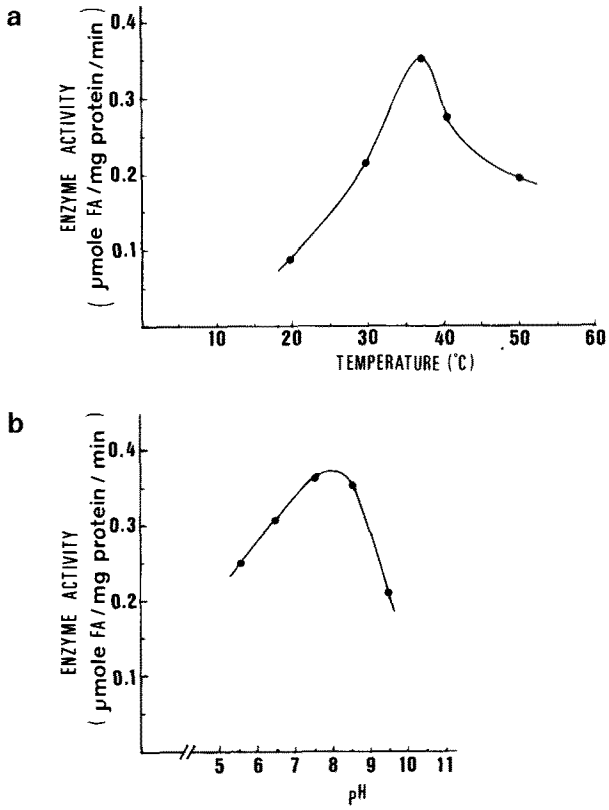

FIG. 1. Optimal temperature (a) and pH (b) for the hydrolysis of palm oil by the lipase from *Candida rugosa.*

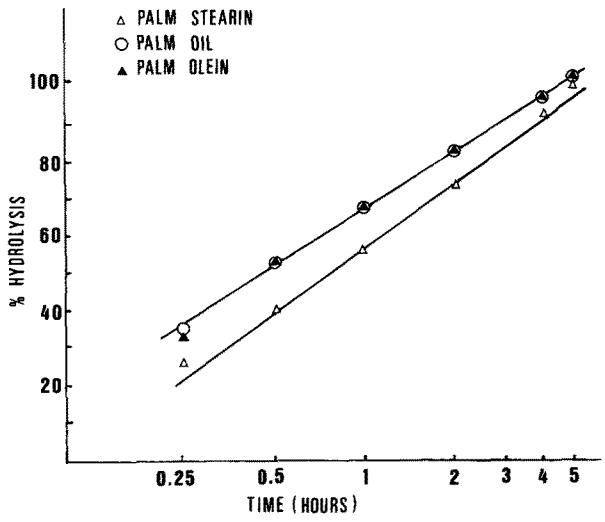

FIG. 2. Rate of hydrolysis of palm oil, palm olein and palm stearin by the lipase from *Candida rugosa.*

hydrolyzed at the same rate as palm oil, whereas palm stearin was hydrolyzed much more slowly (Fig. 2).

Effect of enzyme concentration of lipolysis. The effect of enzyme concentration of lipolysis was studied at 30, 60 and 90 min. A linear logarithmic relationship between enzyme concentration and rate of lipolysis was observed at enzyme concentrations up to 100 mg enzyme per 10 ml incubation medium. Above that enzyme concentration, the rate of lipolysis was no longer linear (Fig. 3). Complete hydrolysis of 1 g of palm oil could be achieved in 90 min if the enzyme concentration was increased to 200 mg enzyme per 10 ml incubation medium.

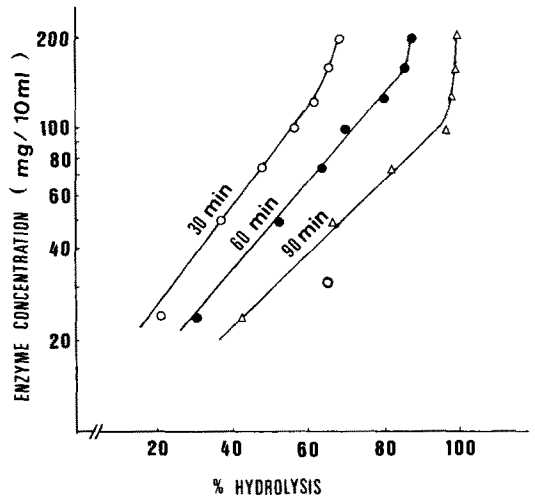

FIG. 3. Effect of enzyme concentration on the rate of hydrolysis of palm oil by the lipase from *Candida rugosa*.

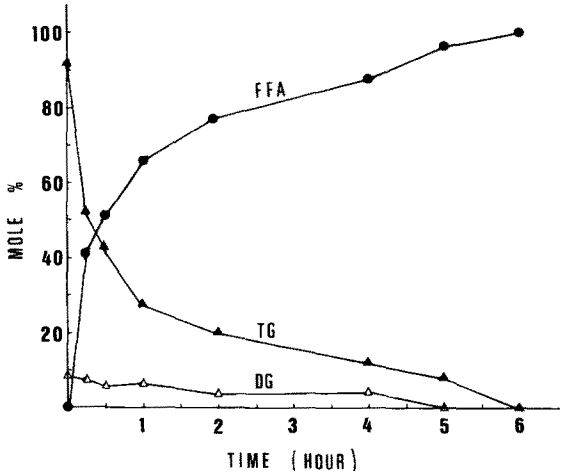

FIG. 4. Changes in major neutral lipid classes during hydrolysis of palm oil by the lipase from *Candida rugosa*.

Lipid classes at different stages of hydrolysis. TLC separation followed by photodensitometric quantification (10) of the lipid classes at different stages after hydrolysis showed that monoacylglycerol was present only at trace levels. TG was rapidly hydrolyzed to fatty acids and glycerol. Diacylglycerols serve only as a transient intermediate in the hydrolytic process (Fig. 4). After 6 hr, essentially all TGs are converted to fatty acids and glycerol.

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