Hydrolysis of Soybean Oil by a Combined Lipase System

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The hydrolysis of soybean oil by different lipases was compared, and the results demonstrated that lipases D, N and G hydrolyzed the oil to 44, 42 and 7.2%, respectively, after 10 hr reaction, which represents incomplete hydrolysis. But the combined enzyme systems (lipases (G+N and lipases G+D) hydrolyzed the oil to an extent of 95-98% after 10 hr reaction. Plotting the percentage hydrolysis of soybean oil by combined enzyme systems against the logarithm of the reaction time resulted in essentially straight lines.

It is known that there are two types of processes for the production of fatty acids, physicochemical and enzymatic processes. The present industrial process is a physicochemical process, involving pressures of ca. 700 psi and a temperature of 250 C or higher for a period of 2 hr in order to achieve 96-98% hydrolysis of fats and oils (1). The resulting fatty acids are unusable as obtained and need to be redistilled to remove color and byproducts. Furthermore, the process is energy-consuming and gives rise to a variety of undesirable side reactions such as the polymerization of highly unsaturated fatty acids and the production of ketones and hydrocarbons. Alternatively, the enzymatic process promises to conserve energy and minimize thermal degradation and could provide a useful method for generating fatty acids from unstable oils containing conjugated or highly unsaturated fatty acid residues (2,3).

Enzymatic hydrolysis of polyunsaturated lipids by the lipase from castor beans was used to a limited extent during the past year (4). It has the disadvantage of incomplete hydrolysis. A number of experiments have demonstrated that pancreatic lipase and lipase, from *Aspergillus niger, Rhizopus delemar* and *Rhizopus arrhizus* (5,6) exhibit specificity toward the 1,3-acyl esters of triglycerides. Therefore these enzymes are not able to provide complete hydrolysis of triglycerides. On the other hand, lipases from *Candida rugosa* (formerly *C. cylindracea*), *Penicillium cyclopium* and *Geotrichum candidum* are able to hydrolyze all the ester bonds of the triglycerides, producing fatty acids and glycerol (6,7).

Linfield et al. reported that the lipase from *C. rugosa* catalyzed 95-98% hydrolysis of lipids in 72 hr, and that the fatty acids were formed as an almost linear function of the logarithms of the reaction time and of the enzyme concentration, whereas the lipase from *R. arrhizus* gives a slow hydrolysis rate due to its specificity for certain acyl groups (3,8). Khor et al. also reported that the lipase from *C. rugosa* leads to complete hydrolysis of palm oil in 5 hr, and that the rate of hydrolysis is a linear function of the logarithm of the reaction time (9).

Recently, it was reported that the lipase from *C. rugosa* is being used to hydrolyze oils for the production of soaps and that the enzymatic method yielded products with a better odor and color and was a cheaper overall process than the conventional uncatalyzed splitting method, which is called the Colgate-Emery Process (10).

The objective of this article is to demonstrate the hydrolysis of soybean, corn and olive oils by a combined lipase system that consisted of both specific and non-specific lipases, and to determine the optimal enzyme concentrations for efficient hydrolysis.

MATERIALS AND METHODS

Materials. Lipase D from *R. delemar*, Lipase G from *Penicillium sp.* and Lipase N from *R. niveus* were purchased from Amano International Enzyme Co., Inc., Troy, Virginia.

Soybean and corn oils (FFA 1.3 and 1.4% respectively) were donated by the Minasa Oil Company, Campinas, Brazil, and olive oil (FFA 1.4%) was purchased from a commercial source.

Hydrolysis of oils. Three grams of the respective oils were placed in 125-ml Erlenmeyer flasks containing 15 pieces of glass bead and 5 ml of acetate buffer, pH 5.6, 0.05 M mixed with lipase. The flasks were placed in a water bath at 40 C, with shaking (130 oscillations min in a 4-cm amplitude). After the enzymatic reaction, a 20-ml portion of acetone-ethanol (1:1) containing phenolphthalein was added to the flask, which was shaken thoroughly to inactivate the enzyme. The mixture as then titrated with 0.1 N KOH aqueous solution to determine the total fatty acid generated.

Assay of lipase activity. Lipase activities were determined by incubating a reaction mixture containing 5 ml 0.05 M acetate buffer, pH 5.6; 1 g olive oil, and 1 ml enzyme solution at 30 C for 60 min, with shaking. After incubation, the reaction was stopped by the addition of 20 ml acetone-ethanol (1:1), and the fatty acids titrated with 0.1 N KOH in the presence of phenolphthalein as indicator. One unit of lipase activity is defined as that amount of enzyme which can release one μmol of fatty acid/min under the above conditions.

Analysis of lipid fractions. After the enzyme reaction, the products of hydrolysis were extracted with petroleum ether and separated on a thin layer chromatography plate (boric acid-impregnated silica gel) using a solvent system of petroleum ether/ethyl ether/acetic acid (85:15:1, vol/vol/vol). The spots of hydrolysis products were visualized by spraying with 50% sulfuric acid and heating at 150 C for 20 min.

RESULTS AND DISCUSSION

The hydrolysis of soybean oil by the three lipases are compared in Figure 1; the results demonstrated that the lipases D, N and G hydrolyzed the oil to 44, 42 and 7.2%, respectively, after 10 hr enzymatic reaction, which represents incomplete hydrolysis. The products of hydrolysis by lipases D and N (from *R. delemar*

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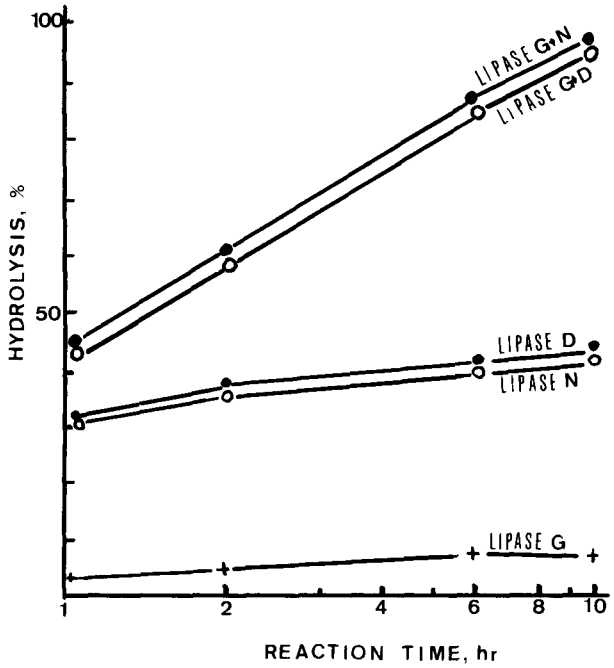

FIG. 1. Hydrolysis of soybean oil by single and combined lipase systems. Quantity of enzymes applied for 3 g of oil: D=3.6 units; N=5.4 units; G=3.0 units; G+N=3 units + 5.4 units; and G+D=3.0 units + 3.6 units. A mixture of 3 g soybean oil and 4 ml acetate buffer pH 5.6, 0.05 M containing respective enzyme was incubated at 40 C for 1-10 hr.

and *R. niveus*) on TLC plate indicated an accumulation of free acids, mono- and diglycerides. Therefore, the incomplete hydrolysis appears to be due to the specificity of the lipases. In fact, the lipases from *R. delemar* and *R. niveus* catalyze the release of fatty acids specifically from the outer 1- and 3-positions of glycerides, leaving 1,2(3)-diglycerides and 2-monoglycerides as reaction products (6,11). The lipase G from *Penicillium sp.* hydrolyzed slight amounts of triglyceride, and the TLC plate demonstrated only a small fatty acid spot, indicating that the triglycerides are an inadequate substrate in this case, although the lipase is able to hydrolyze all the ester bonds of the triglycerides, regardless of their positions. However, the combined enzyme systems demonstrated efficient hydrolysis as shown in Figure 1. The combined system (lipase G+N) hydrolyzed soybean oil to 98% after 10 hr of reaction, whereas lipase G+D hydrolyzed 95%. It was impossible to reach 100% hydrolysis of the substrate by various repetitions. Trace amounts of mono- and diglycerides were always detected on TLC plate. Plotting the percentage hydrolysis of soybean oil by combined enzyme systems against the logarithm of the reaction time, resulted in essentially straight lines. However, with single enzyme systems, straight lines were not observed. Linfield et al. and Khor et al. also observed that the rate of hydrolysis was linear on a logarithmic scale of reaction time when the lipase from *C. rugosa* hydrolyzed olive and palm oils (3,9).

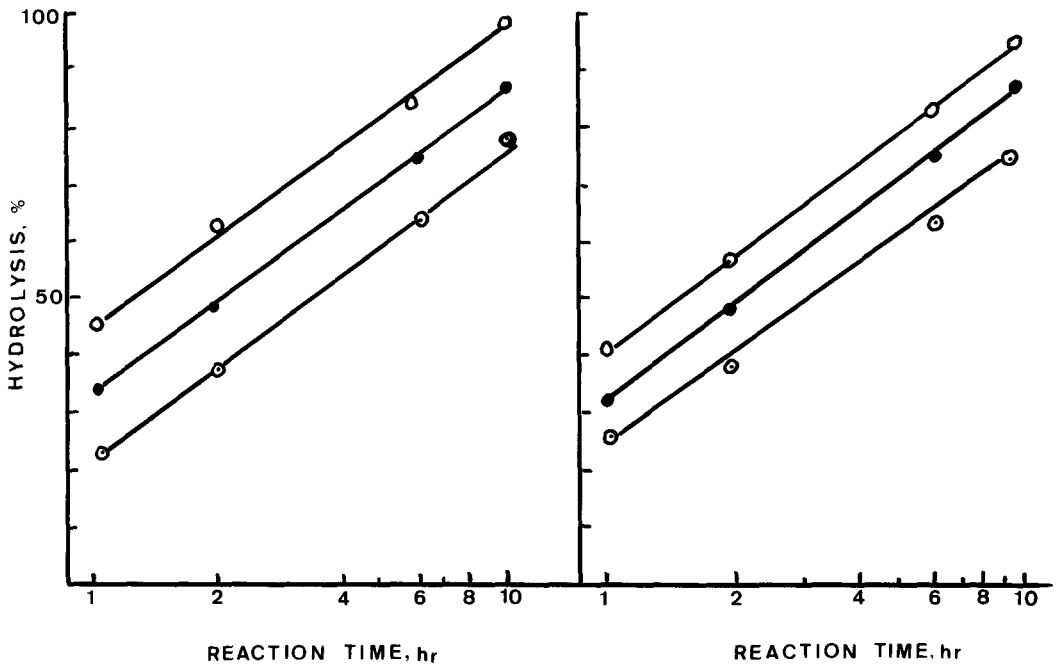

FIG. 2. Relationship between hydrolysis of soybean oil and combined lipase concentrations. A mixture of 3 g soybean oil and 5 ml acetate buffer pH 5.6, 0.05 M containing respective combined enzyme was incubated at 40 C for 1-10 hr. —○— G=3 units + N=5.4 units, G=3 units + D=3.6 units; —●— G=2.4 units + N=3.6 units, G=2.4 units + D=2.4 units; —⊙— G=1.8 units + N=1.8 units, —⊙— G=1.8 units + D=1.2 units.

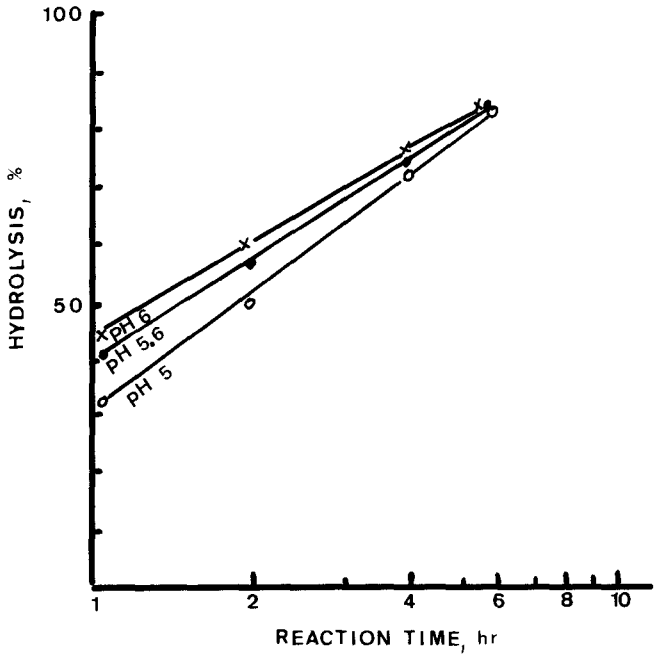

FIG. 3. Effect of pH on rate of hydrolysis. A mixture of 3 g soybean oil and 5 ml buffer solutions, (pH 5.0, 5.6 and 6.0, respectively) containing combined enzyme system (G = 3 units + N = 5.4 units) was incubated at 40 C for 1-6 hr. pH 5.0 and 5.6 were acetate buffer, 0.05 M, and pH 6.0 was phosphate buffer, 0.05 M.

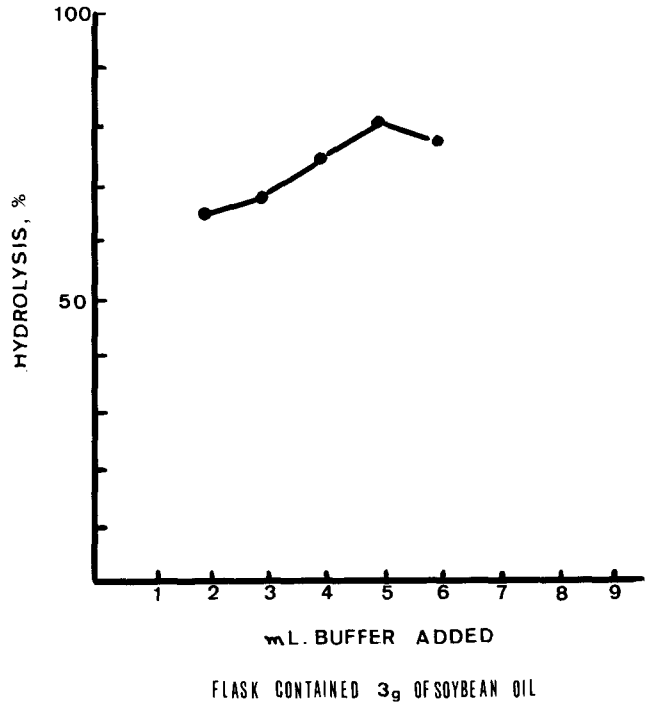

FIG. 4. Effect of quantity of buffer solution. A mixture of 3 g soybean oil and acetate buffer pH 5.6, 0.05 M (2-6 ml) containing combined enzyme system (G = 3 units + N = 5.4 units), was incubated at 40 C for 5 hr.

The relationship between the hydrolysis of soybean oil and the combined lipase concentrations is shown in Figure 2. The reaction kinetics of three different concentrations of lipases G+N and lipases G+D illus trated that the rates of hydrolysis of the substrate vs the logarithm of the reaction time were linear. Combined lipase systems (G = 3 units + D = 3.6 units and G = 3 units + N = 5.4 units) hydrolyzed 95-98% of the oil after 10 hr reaction. Corn and olive oils were also examined for hydrolysis with the same combined enzyme systems under the conditions described above and resulted in the same rate of hydrolysis as soybean oil. The plot shown in Figure 2 can be extrapolated to determine the reaction time required to reach 95-98% hydrolysis of soybean oil at lower enzyme concentrations. However, complete hydrolysis of the oil by lipase in a short period is desired, and higher concentrations of enzyme prevent possible microbial contamination.

The effect of pH on the rate of hydrolysis was examined by using the combined lipase system (G = 3 units + N= 5.4 units) and hydrolyzing soybean oil at pH 5, 5.6 and 6.0 under the conditions described above. pH 5 and 5.6 were acetate buffer, 0.05 M, and pH 6.0 was phosphate buffer, 0.05 M. Figure 3 shows that the 3 pH values gave the same results after 6 hr reaction.

The effect of the quantity of buffer solution in relations to the 3 g of soybean oil was also examined after 5 hr incubation, and the results are shown in Figure 4. A ratio of 5 ml of buffer solution to 3 g of substrate resulted in a slightly more efficient hydrolysis than other combinations.

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