Effect of Lipid Oxidation Products on the Transesterification Activity of an Immobilized Lipase

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The effect of varying amounts of linoleic acid hydroperoxides on the activity of an immobilized lipase, Lipozyme, used for the transesterification of fats has been studied. The initial activity of the enzyme is not significantly reduced even by a peroxide value of 50 mequiv/kg, but the operational stability is reduced if the peroxide value of the oil is above 5 mequiv/kg, with the rate of enzyme inactivation increasing with peroxide value. A sample with initial peroxide value of 50 mequiv/kg caused a 50% loss of activity after eight batch treatments of 10 h per treatment. The loss of activity is due to generation of free radicals in the enzyme following hydroperoxide decomposition.

INTRODUCTION

Catalytic transesterification is commonly used in the oils and fats industry to modify the properties of edible fats. Chemical catalysts such as sodium alkoxide or sodium metal are used on an industrial scale to promote migration of fatty acyl groups among triglyceride molecules to form a product in which the fatty acyl groups are randomly distributed. In recent years there has been considerable research on catalysis by lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) that are capable of selective transesterification of oils and fats. In particular, lipases that selectively catalyze the transesterification of acyl groups at the 1,3-positions have potential for the economic synthesis of cocoa butter equivalents which cannot be obtained by conventional chemical transesterification. The development of immobilized lipases that may be recovered and reused or directly employed in continuous reactors has been important for potential industrial application of the process (Macrae, 1983; Kurashige et al., 1989; Wisdom et al., 1987; Posorske et al., 1988). To minimize the cost of the process, it is important to extend the lifetime of the enzyme as far as possible.

Biotechnologists have identified several microbial lipases with strong activity, good selectivity, and reasonable stability. A commercially available lipase, Lipozyme, which is an enzyme from *Mucor miehei* immobilized on an anion-exchange resin, showed the best transesterification activity and operational stability in the study by Bloomer et al. (1990).

Wills (1961) reported that lipid oxidation products have little effect on the activity of lipases in an aqueous medium, but recent research has demonstrated that lipases are inactivated by lipid oxidation products in nonaqueous media (Kurashige et al., 1989; Posorske et al., 1988; Ohta et al., 1989). Fat peroxides cause the inactivation of *Pseudomonas fluorescens* lipase during glycerolysis (Ohta et al., 1989). The activity of Lipozyme decreased more rapidly in soybean oil than in olive oil, and this was ascribed to the effect of lipid oxidation products in the more unsaturated soybean oil (Posorske et al., 1988). Previous studies have not involved the use of hydroperoxides synthesized under conditions designed to avoid hydroperoxide decomposition. It was the aim of the present study to investigate quantitatively the effect of linoleic acid hydroperoxides on the activity of Lipozyme during the transesterification of oils.

MATERIALS AND METHODS

Lipozyme IM 20 was donated by Novo Nordisk Bioindustries U.K. Ltd. Lipozyme IM 20 is a preparation of a *Mucor miehei* lipase immobilized on a macroporous anion-exchange resin (Eigtved, 1985). The enzyme activity was 28 BIU/g, and the moisture content was 12% (m/m). Soybean lipoxygenase (Sigma type I, 126 500 units/mg of solid), triolein (95%), lauric acid (99%), and linoleic acid (99%) were purchased from Sigma Chemical Co.

Linoleic acid hydroperoxides were prepared by lipoxygenase-catalyzed oxidation of linoleic acid according to the method of Kaplan et al. (1984). The concentration of conjugated lipids was determined spectrophotometrically at 234 nm and calculated from the molar absorption coefficient for hydroperoxides of 26 000 L/(mol·cm) (Matthew et al., 1977). The amount of this sample added to the triolein allowed the initial conjugated lipid value (CL) of the sample to be calculated. Chemical determination of PV was performed according to the IUPAC method on samples of 100–200 mg at the beginning and end of 10-h batch transesterification reactions as shown in Table I. Transesterification was performed with triolein (250 mg), lauric acid (500 mg), linoleic acid (30 mg, including hydroperoxides as required), water (8 mg), and Lipozyme (78 mg) in petroleum ether (5 mL, bp 100–120 °C). The mixture was stirred at 100 rpm in a 25-mL glass-stoppered flask at 65 ± 0.5 °C. Each batch reaction was conducted for 10 h. To determine the operational stability, the batch reaction product was removed with a syringe fitted with a filter, and the enzyme on the filter was twice washed back into the flask with hexane.

Sodium hydroxide (0.05 M) was added to the crude reaction product in a separating funnel to remove free fatty acids. The petroleum ether solution was separated and washed with water and saturated salt solution. The petroleum ether was evaporated. The acylglycerols were converted to fatty acid methyl esters by using sodium methoxide (0.5 M) in methanol. Analysis was performed in duplicate with a packed glass column containing 15% diethylene glycol succinate on Chromosorb W AW-DMCS (100–120 mesh) at 175 °C.

RESULTS AND DISCUSSION

Selection of Conditions. The concentration of lauric acid by mass in the acylglycerols was in excess of 50% after 10 h at 65 °C. This compares with the calculated equilibrium concentration of 50.5% (m/m) lauric acid in the acylglycerols, assuming exchange only at the 1,3-

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Table I. Peroxide Values (PV)[a] and Conjugated Lipids (CL)[b] by UV Absorption during Transesterification with Lipozyme

initial CL (calcd)	no. of 10-h batch transesterification reactions											
	0		2		4		6		8		10	
	PV	CL	PV	CL	PV	CL	PV	CL	PV	CL	PV	CL
0	0	4.9					0	5.1			0	5.1
10	8.0	14.1	2.6	12.9	4.0	11.2	4.5	13.2	3.6	10.7	3.7	11.7
30	22.1	31.0	10.3	25.2	11.4	25.2	13.6	25.8	13.4	29.3	13.6	26.0
50	36.9	46.8	20.4	36.1	24.7	34.4	26.7	40.3	25.4	35.3	28.7	34.1

[a] Units of mequiv/kg; determined by titration at the end of each transesterification batch reaction. [b] Units of mmol/kg; determined by UV absorption at the end of each transesterification batch reaction.

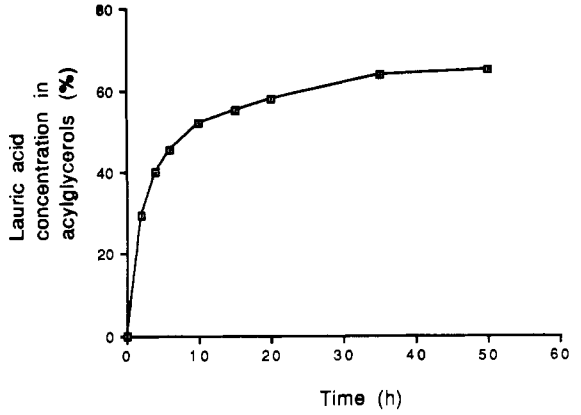

Figure 1. Incorporation of lauric acid into acylglycerols during transesterification with triolein.

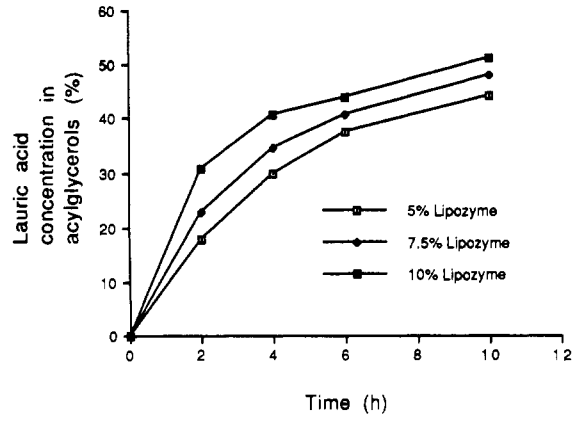

Figure 2. Effect of amount of Lipozyme on the rate of transesterification of triolein and lauric acid.

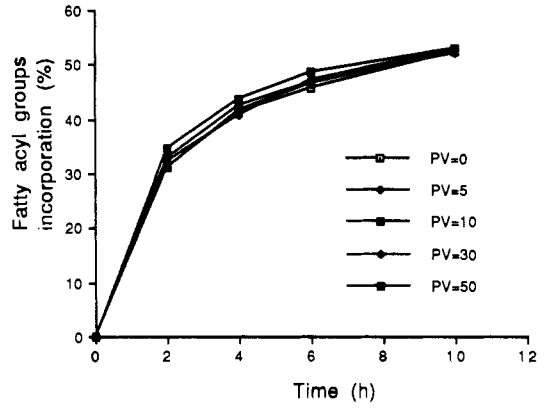

Figure 3. Effect of hydroperoxides on the transesterification of fatty acids with triolein.

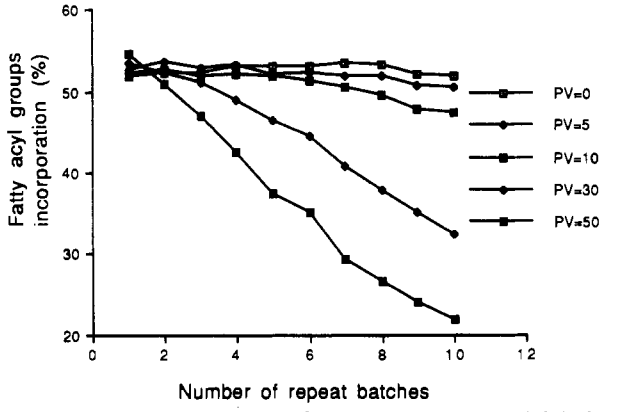

Figure 4. Changes in fatty acid incorporation into triolein in a 10-h batch transesterification with repeat usage of Lipozyme.

positions of the triacylglycerols and ignoring the presence of diacylglycerols. The increase in lauric acid incorporation after 10 h was slow (Figure 1), and it was therefore decided to use this period for batch reactions. Long transesterification periods, e.g., 50 h, caused the lauric acid concentration in the acylglycerols to increase to 60%, and this indicates that acyl migration in the acylglycerols occurred. The use of 10% (m/m) Lipozyme was preferred to smaller enzyme concentrations to allow this time period to represent a reasonable approach to equilibrium (Figure 2).

Effect of Hydroperoxides on the Initial Reaction Rate. The presence of hydroperoxides clearly did not cause any reduction in the initial rate of transesterification (Figure 3). No significant difference in the incorporation of lauric acid was detectable during the initial 10-h reaction time. This observation contrasts with that of Ohta et al. (1989), who found that samples with increased peroxide value reduced the initial rate of glycerolysis by *P. fluorescens* lipase.

The peroxide values quoted in Figure 3 are based on conjugated lipid determinations. These values were in general higher than the values determined chemically as is clear from Table I, indicating that hydroperoxide decomposition products contribute to the conjugated lipid values.

Operational Stability. The operational stability tests indicated that a substrate of PV less than 5 mequiv/kg did not cause significant inactivation of Lipozyme over 10 repeated batch reactions. Above this PV, inactivation increased with hydroperoxide content. Oil with a PV of 50 rapidly reduced the activity of Lipozyme with the enzyme activity falling to half in eight batches (Figure 4). The effect of PV on the time for the loss of 10% transesterification activity is shown in Figure 5. It is clear that samples with PV > 5 mequiv/kg would strongly reduce the useful life of the enzyme, but the use of good quality oils with PV < 5 mequiv/kg should allow the enzyme to be used over a long period of time.

It is clear that the decomposition of hydroperoxides is occurring during the reaction since the peroxide value determined chemically showed a consistent fall during the initial batches, while the UV absorption at 234 nm remained reasonably steady (Table I). The UV absorption is not reduced when hydroperoxides decompose to un-

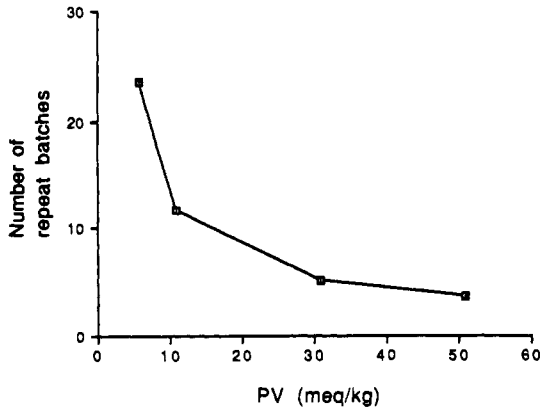

Figure 5. Effect of peroxide value (PV) on the time for the loss of 10% transesterification activity of Lipozyme. (Points at PV = 5 and 10 mequiv/kg obtained by extrapolation of data from 10 batches.)

saturated aldehydes and ketones, while the chemically determined peroxide value is a measure of the hydroperoxides. Since the hydroperoxides do not affect the initial rate of transesterification (Figure 3), it can be deduced that hydroperoxide decomposition products, probably free radicals, are causing inactivation of the enzyme which is not reversed by washing with hexane. Ohta et al. (1989) have shown that polymerization of lipase occurs in the presence of oils with a high PV, and this is consistent with lipid radicals formed from the hydroperoxides generating enzyme radicals that cause the enzyme to polymerize.

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LITERATURE CITED

Bloomer, S.; Adlercreutz, P.; Mattiasson, B. Triglyceride Interesterification by Lipases. I. Cocoa Butter Equivalents from a Fraction of Palm Oil. *J. Am. Oil Chem. Soc.* **1990**, *67*, 519–524.

Eigtved, P. Immobilised Lipase Preparation and its Use. Eur. Patent EP 140542, 1985.

Kaplan, E.; Ansari, K. Reduction of Polyunsaturated Fatty Acid Hydroperoxides by Human Brain Glutahione Peroxidase. *Lipids* **1984**, *19*, 784–789.

Kurashige, J.; Matsuzaki, N.; Makabe, K. Modification of Fats and Oils by Lipases. *J. Dispersion Sci. Technol.* **1988**, *10*, 531–559.

Macrae, A. R. Lipase-Catalysed Interesterification of Oils and Fats. *J. Am. Oil Chem. Soc.* **1983**, *60*, 291–294.

Matthew, J. A.; Chan, H. W.-S.; Galliard, T. A Simple Method for the Preparation of Pure 9-D-Hydroperoxide of Linoleic Acid and Methyl Linoleate Based on the Positional Specificity of Lipoxygenase in Tomato Fruit. *Lipids* **1977**, *12*, 324–326.

Ohta, Y.; Yasmane, T.; Shimizu, S. Inhibition and Inactivation of Lipase by Fat Peroxide in the Course of Batch and Continuous Glycerolyses of Fat by Lipase. *Agric. Biol. Chem.* **1989**, *53*, 1885–1890.

Posorske, L. H.; LeFebvre, G. K.; Miller, C. A.; Hansen, T. T.; Glenvig, B. L. Process Considerations of Continuous Fat Modification with an Immobilised Lipase. *J. Am. Oil Chem. Soc.* **1988**, *65*, 922–926.

Wills, E. D. *The Enzymes of Lipid Metabolism*; Pergamon Press: New York, 1961; pp 74–77.

Wisdom, R. A.; Dunnill, P.; Lilly, M. D. Enzymic Interesterification of Fats: Laboratory and Pilot-Scale Studies with Immobilised Lipase from *Rhizopus arrhizus*. *Biotechnol. Bioeng.* **1987**, *29*, 1081–1085.

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Registry No. Lipozyme IM 20, 135105-44-1; triolein, 122-32-7; lauric acid, 143-07-7; linoleic acid hydroperoxide, 25657-09-4.