Lipase-Catalyzed Interesterification of Oils and Fats

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ABSTRACT

Extracellular microbial lipases can be used as catalysts for the interesterification of oils and fats. Use of specific lipases gives products which are unobtainable by chemical interesterification methods. Some of these products have properties of value to the oils and fats industry. The catalysts for enzymatic interesterification are prepared by coating inorganic support materials with the lipases. For batch interesterification reactions, the catalyst particles are activated by addition of a small amount of water and then stirred with a reactant mixture dissolved in petroleum ether. At the end of the reaction period, the catalyst particles are removed by filtration, and the interesterified triglycerides isolated by conventional fat fractionation techniques. The catalyst can be used in subsequent batch reactions. As an alternative to the batch reaction system, continuous enzymatic interesterification processes can be operated by pumping water containing feedstock through a packed bed of activated catalyst.

INTRODUCTION

Interesterification is a process which is used in the oils and fats industry to modify the properties of triglyceride mixtures. In this process, a chemical catalyst such as sodium metal or sodium alkoxide is used to promote acyl migration among glyceride molecules so that the products consist of glyceride mixtures in which the fatty acyl residues are randomly distributed amongst the glyceride molecules (1). In this paper, the use of microbial lipases as catalysts for interesterification is discussed. By exploitation of the specificity of the lipases, it is possible to produce useful glyceride mixtures which cannot be obtained by conventional chemical interesterification processes.

Substrate Specificity of Microbial Lipases

Extracellular microbial lipases (glycerol ester hydrolases EC 3.1.1.3) are excreted by microorganisms into their growth medium to assist in the digestion of lipid materials. They catalyze the hydrolysis of fats to give free fatty acid, partial glycerides and glycerol. The reaction is reversible and the enzymes can be shown to catalyze the formation of glycerides from glycerol and free fatty acid under certain conditions (2). The synthetic reaction is of no significance in the biosynthesis of oils and fats.

The naturally occurring triglycerides of long-chain fatty acids are water insoluble, and lipases are characterized by the ability to catalyze rapidly the hydrolysis of ester bonds at the interface between the insoluble substrate phase and the aqueous phase in which the enzyme is soluble. Thus the enzymes catalyze the hydrolysis of a wide range of insoluble fatty acid esters, although glycerides are normally the preferred substrates, while hydrolysis of water-soluble carboxylic acid esters by most lipases is very slow (3).

The microbial lipases can be placed in three groups according to their specificity (Fig. 1). The first group shows no marked specificity both as regards the position on the glycerol molecule which is attacked and the nature of the fatty acid released. These lipases catalyze the complete breakdown of triglycerides to free fatty acid and glycerol, but diglycerides and monoglycerides appear as intermediates in the reaction. Examples of enzymes of this type are the lipases from *Candida cylindracae* (4), *Corynebacterium acnes* (5) and *Staphylococcus aureus* (6).

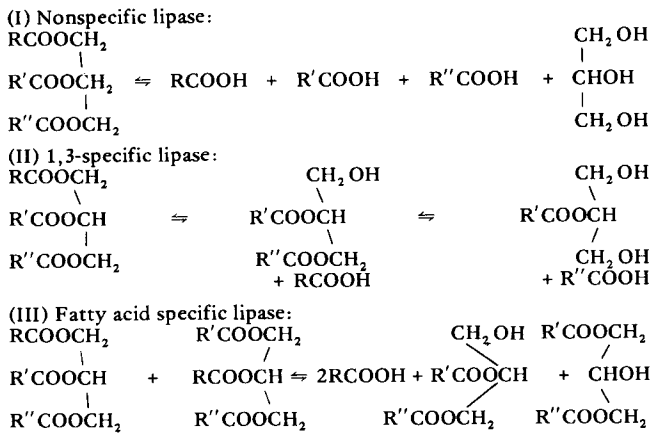

FIG. 1. Products formed by lipase-catalyzed hydrolysis of triglycerides

The second group of lipases catalyzes the release of fatty acids specifically from the outer 1- and 3-positions of glycerides. With these lipases, triglycerides are hydrolysed to give free fatty acids, 1,2(2,3)-diglycerides and 2-monoglycerides as reaction products. Because 1,2(2,3)-diglycerides and especially 2-monoglycerides are chemically unstable and undergo acyl migration to give 1,3-diglycerides and 1(3)-monoglycerides, respectively, prolonged incubation of a fat with a 1,3-specific lipase will give complete breakdown of some of the triglycerides with the formation of glycerol. 1,3-specificity is common amongst microbial lipases, and examples of enzymes from this group are the lipases from *Aspergillus niger, Mucor javanicus* and various Rhizopus species (7-9). The stereospecificity (i.e., relative catalytic activity at the *sn*-1 and *sn*-3 positions of the glycerol moiety) of *Rhizopus arrhizus* lipase has been investigated (10). It was shown that fatty acid is released by the enzyme at a similar rate from the *sn*-1 and *sn*-3 positions of enantiomeric forms of phosphatidylcholine, therefore *R. arrhizus* lipase and in all probability other microbial lipases show no stereospecificity. The positional specificity of the 1,3-specific lipases probably results from an inability of the sterically hindered esters of secondary alcohols, e.g., those of the 2-position of glycerol, to enter the active site of the enzyme.

The third group of lipases catalyses the specific release of a particular type of fatty acid from glyceride molecules. Most extracellular microbial lipases show little fatty acid specificity when incubated with natural oils and fats. However, the lipase produced by *Geotrichum candidium* has been shown to possess a very marked specificity for the hydrolysis of esters of a particular type of long-chain fatty acid. Thus the enzyme preferentially releases from triglycerides long-chain fatty acids containing a *cis* double bond in the 9-position. Saturated fatty acids and unsaturated fatty acids without a double bond in the 9-position are only slowly released from triglycerides (11).

Lipases as Interesterification Catalysts

Since lipase reactions are reversible hydrolysis and resynthesis of glycerides occur when lipases are incubated with oils and fats. This hydrolysis and resynthesis causes acyl migration between glyceride molecules and gives interesterified products. Under conditions in which the amount of water in the reaction system is restricted, hydrolysis of the fat can be minimized so that lipase catalyzed interesterification becomes the dominant reaction (12-14).

If a nonspecific lipase is used to catalyze the interesterification of a triglyceride mixture, the triglycerides produced are similar to those obtained by chemical interesterification. However, with a 1,3-specific lipase as catalyst, acyl migration is confined to the 1- and 3-positions, and a mixture of triglycerides which is unobtainable by chemical interesterification is produced (Fig. 2). Mixtures of triglycerides and free fatty acid can also be used as reactants for lipase-catalyzed reactions. In these cases, free fatty acid exchanges with the acyl groups of the triglycerides to produce new triglycerides enriched in the added fatty acid. With nonspecific lipases enrichment of all three glyceride positions occurs, but with 1,3-specific lipases the reaction is confined to the 1- and 3-positions of the glycerides. If a fatty acid specific lipase is used, a particular fatty acid from a mixture of fatty acids can be selectively introduced.

The similarity of the products obtained by the nonspecific lipase and chemically catalyzed methods is illustrated by examination of the triglycerides formed by interesterification of a 1:1 mixture of coconut oil and olive oil (Table I). The starting mixture contained predominately C_{52} and C_{54} triglycerides from the olive oil and medium chain fatty acid (C_{32} to C_{38}) triglycerides from the coconut oil. Interesterification using either *C. cylindracae* lipase or chemical catalysis gave closely similar mixtures in which the C_{42} to C_{48} triglycerides predominated.

The type of reaction obtained with a 1,3-specific lipase is demonstrated by analysis of the products formed by interesterification of a mixture of olive oil and stearic acid using *Rhizopus delemar* lipase as catalyst (Table II). Stearate residues were introduced into triglyceride, but no enrichment of the 2-position was observed and the stearate residues

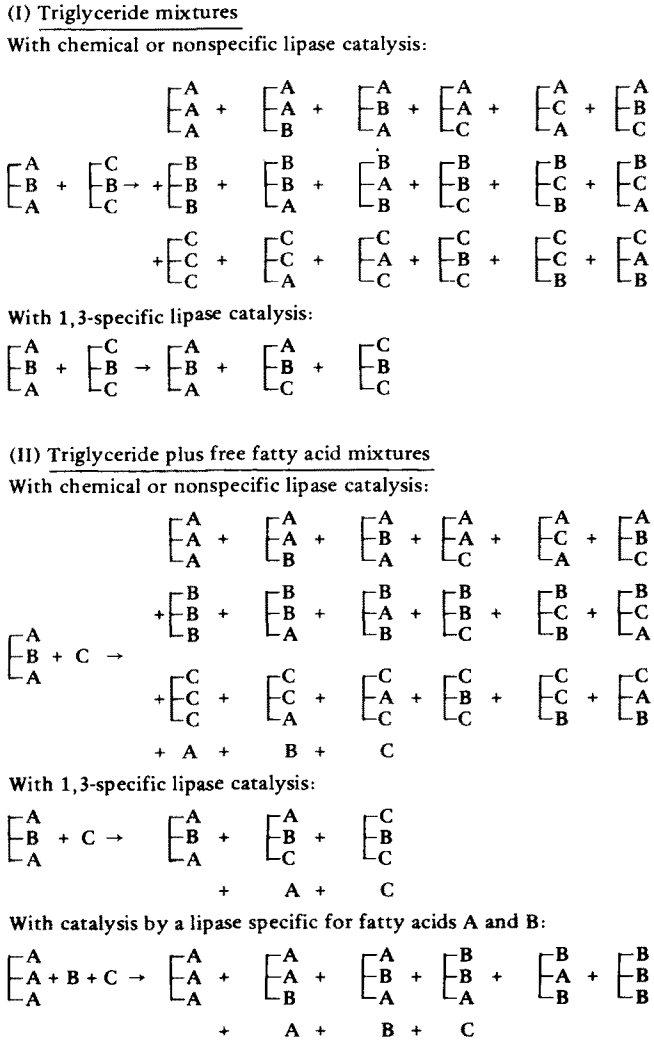

FIG. 2. Products formed by interesterification of mixtures of fats.

TABLE I

Product formed by Interesterification of a 1:1 Mixture of Coconut Oil and Olive Oil

Triglyceride carbon no. (excluding glycerol residue)	Composition of triglyceride		
		Interesterified oils	
	Starting mixture (wt %)	with alkali metal catalyst (wt %)	with *C. cylindracae*[a] lipase catalyst (wt %)
26	0.1	0.1	0.3
28	0.3	0.2	0.5
30	1.1	0.5	0.7
32	5.0	1.4	2.1
34	6.7 } 28.8	2.1 } 14.9	2.1 } 15.4
36	8.4	4.2	4.0
38	7.6	6.7	6.5
40	4.5	7.2	6.6
42	3.4	13.1	12.6
44	1.9 } 12.0	11.6 } 60.9	11.4 } 60.3
46	1.2	11.6	11.7
48	1.0	17.4	18.0
50	4.7	6.8	7.4
52	21.2	7.4	7.2
54	31.8 } 58.7	9.3 } 23.9	8.4 } 23.5
56	1.0	0.4	0.5

[a]A mixture of olive oil (25g) and coconut oil (25g) was stirred at 40 C for 66 hr with hydrated catalyst (3.2g) prepared from *C. cylindracae* lipase and kieselguhr.

TABLE II

Triglycerides Formed by Interesterification of a Mixture of Olive Oil (5 parts) and Stearic Acid (1 part) using *Rhizopus delemar* Lipase as Catalyst[a]

	Amount in olive oil			Amount in interesterified triglycerides		
Fatty acid	Total TG (%)	2-position (%)	1- and 3-positions (%)	Total TG (%)	2-position (%)	1- and 3-positions (%)
16:0	16.6	3.5	23.2	13.7	3.2	18.9
16:1	1.8	1.3	2.0	1.6	1.6	1.6
18:0	2.0	1.0	2.5	15.6	0.7	23.0
18:1	66.8	72.0	64.2	56.6	72.2	48.8
18:2	12.8	22.2	8.1	12.6	22.3	7.7

[a]A mixture of olive oil (2.5g) and stearic acid (0.5g) dissolved in 60-80 C petroleum ether (6.0g) was stirred at 40 C for 24 hr with hydrated catalyst (250 mg) prepared from *R. delemar* lipase and kieselguhr.

were selectively incorporated into the 1- and 3-positions.

Using the fatty acid specific lipase from *Geotrichum candidum*, Δ9 unsaturated fatty acids can be selectively exchanged with other Δ9 fatty acid residues in triglycerides. Interesterification of a mixture of olive oil, linoleic acid and stearic acid gave triglycerides which were enriched in linoleate at the expense of oleate, the saturated fatty acyl content of the triglycerides remaining substantially unchanged (Table III).

The ability to produce novel triglyceride mixtures using specific lipases is of interest to the oils and fats industry because some of these mixtures have properties which make them valuable. This is illustrated by the following example: 1,3-specific lipase-catalyzed interesterification of 1,3-dipalmitoyl-2-monooleine (POP), which is the major triglyceride of the midfraction of palm oil, with either stearic acid or tristearine gives products enriched in the valuable 1(3)-palmitoyl-3(1)-stearoyl-2-monooleine (POSt) and 1,3-distearoyl-2-monooleine (StOSt) (Fig. 3). POSt and StOSt are the main components of cocoa butter, and therefore it is possible by the interesterification reaction to produce a valuable cocoa butter equivalent from cheaper starting materials (12,13).

Interesterification Reaction Systems

The catalysts used for enzymatic interesterification are prepared by addition of a solvent such as acetone, ethanol or methanol to a slurry of an inorganic particulate material such as kieselguhr, hydroxylapatite or alumina in buffered lipase solution. The precipitated enzyme coats the inorganic particles, and the lipase-coated particles are collected by filtration, dried and stored. In the dried form the particles are almost inactive as interesterification catalysts, and to obtain high catalytic activity it is necessary to hydrate the particles by addition of up to 10% water prior to their use in the interesterification reaction systems.

In a typical batch stirred tank interesterification reaction, a mixture of palm midfraction (1.0 parts) and stearic acid (0.5 parts) dissolved in petroleum ether was stirred at 40 C for 16 hr with hydrated catalyst prepared from the 1,3-specific *A. niger* lipase and kieselguhr. The progress of the reaction is shown in Figure 4. Interesterification as measured by stearate incorporation into triglyceride proceded smoothly over the whole reaction period, but was accompanied by the formation of byproducts of diglycerides and addition free fatty acids. Most of the byproducts were formed in the first hour of the reaction. During this period, an equilibrium between triglyceride, water, 1,2-diglyceride and free fatty acid was established. The subsequent slow generation of more diglyceride and free fatty acid can be attributed to

TABLE III

Triglyceride Formed by Interesterification of a Mixture of Olive Oil (1.0 parts), Stearic Acid (0.15 parts) and Linoleic Acid (0.15 parts) using *Geotrichum candidum* Lipase as Catalyst[a]

Fatty acid	Amount in olive oil (%)	Amount in interesterified triglyceride (%)
16:0	11.6	11.5
16:1	0.8	0.5
18:0	3.6	4.5
18:1	72.8	64.8
18:2	10.6	18.3
20:1	0.6	0.4

[a]A mixture of olive oil (2.5g), stearic acid (0.375g) and linoleic acid (0.375g) dissolved in petroleum ether (4.0g) was stirred at 40 C for 3 days with hydrated catalyst prepared from *G. candidum* lipase and kieselguhr.

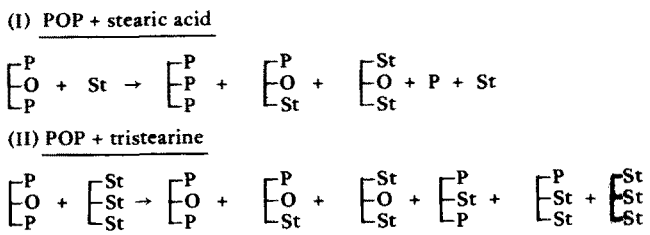

FIG. 3. Products formed by interesterification of 1,3-dipalmitoyl-2-monooleine (POP) with either stearic acid or tristearine using a 1,3-specific lipase as catalyst

the formation of 1,3-diglyceride from 1,2-diglyceride by a slow chemical isomerization reaction. Detailed analysis of the products obtained after 16 hr reaction shows that, as a result of the interesterification, stearate residues were incorporated specifically into the 1- and 3-positions of the triglyceride with the generation of the valuable POSt and StOSt triglycerides (Table IV). At the end of the reaction period, the catalyst was filtered off and a fraction containing the POSt and StOSt triglycerides isolated by conventional fat fractionation techniques such as countercurrent liquid-liquid extraction and crystallization from solvents. The catalyst was washed free of fatty materials, dried and

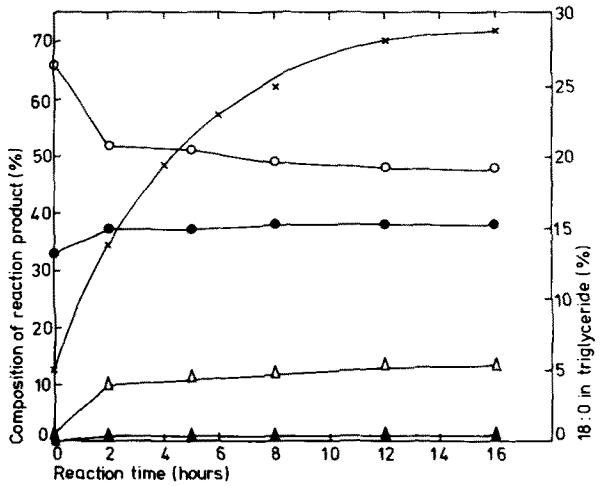

FIG. 4. Products formed during a stirred tank interesterification reaction. Palm midfraction (240g) and stearic acid (120g) dissolved in 60-80 C petroleum ether (550g) were stirred with hydrated catalyst prepared from *A. niger* lipase and kieselguhr (26g) at 40 C for 16 hr. Samples were taken periodically for analysis. ○: triglyceride; ●: free fatty acid; △: diglyceride; ▲: monoglyceride; X: 18:0 in triglyceride.

TABLE IV

Triglycerides Formed by Interesterification of a Mixture of Palm Midfraction (1.0 parts) and Stearic Acid (0.5 parts) using *A. niger* Lipase as Catalyst[a]

Fatty acid	Amount in triglyceride	
	Palm midfraction (%)	Interesterified product (%)
14:0	0.7	0.7
16:0	57.0	37.0
18:0	6.0	28.9
18:1	31.8	30.2
18:2	3.6	3.5
20:0	0.2	0.2
Triglyceride species[b]		
SSS	5	13
POP	58	19
POSt	13	32
StOSt	2	13
SSO	7	2
SLnS	9	7
SOO	4	11
Others	2	3

[a]The reaction conditions are given in Figure 4. The product obtained after 16-hr reaction was analyzed.

[b]S = saturated fatty acid group; P = palmitate; St = Stearate; O = Oleate; Ln = linoleate.

then reused in subsequent interesterification reactions. Use of the same catalyst particles in 10 successive batch interesterification reactions has proved to be possible.

Lipase-catalyzed interesterification reactions can also be performed continuously using packed bed reactors. For operation of the packed bed reactors, the feedstock mixture dissolved in petroleum ether is treated to remove particulate materials and enzyme catalyst inhibitors and poisons, and then partially saturated with water prior to being pumped through a bed of hydrated catalyst particles. The performance of a reactor containing a bed of catalyst prepared from *Rhizopus niveus* lipase and kieselguhr was studied using a feedstock consisting of a mixture of palm midfraction and myristic acid dissolved in 100-120 C petroleum ether (Fig. 5). Essentially complete interesterification as evidenced by myristate incorporation into triglyceride was obtained throughout the 400 hr of reactor operation. After an initial equilibration period, the diglyceride and free fatty acid content of the product stream remained constant at a level indicating that slight hydrolysis of triglyceride occurred in the reactor. During the equilibration period, which lasted for a few hours, water was removed from the hydrated catalyst particles with generation of large quantities of diglyceride and free fatty acid until steady-state conditions were attained. In the steady state, some of the water present in the feed to the reactors was consumed in the reactors with the generation of a small quantity of diglyceride and free fatty acid so as to set up an equilibrium involving triglyceride, water, free fatty acid and 1,2-diglyceride. The lipase catalysts are reasonably stable under the conditions prevalent in the packed bed reactors, and it is possible to operate these reactors continuously for up to 600 hr with an acceptable loss of catalytic activity.

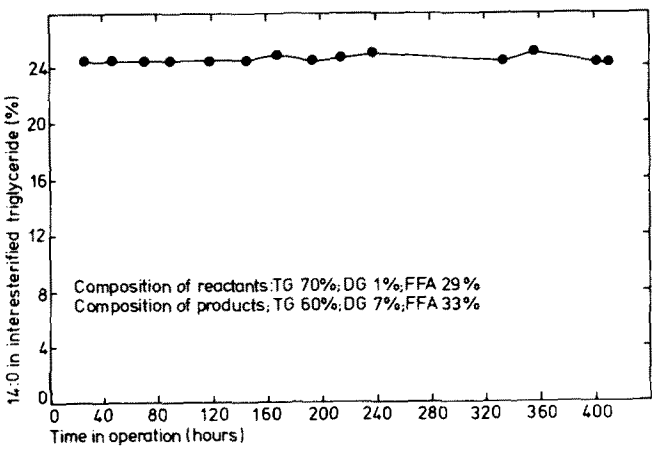

FIG. 5. Interesterification using a packed bed reactor. A mixture of palm midfraction (1 part) and myristic acid (0.4 parts) dissolved in 100-120 C petroleum ether (3.2 parts) was saturated with water and then continuously pumped at a flow rate of 22 mL/hr through a bed of hydrated catalyst (5.0g) prepared from *Rhizopus niveus* lipase and kieselguhr. The temperature of the reaction system was 40 C. Samples from the product stream were taken periodically for analysis.

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