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Enzyme-catalysed modification of oils and fats

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Various processes are used industrially to modify the composition and hence the physical properties of edible oils and fats. One of these processes is interesterification, in which a chemical catalyst such as sodium methoxide is used to promote interchange of fatty acid groups between the component triglycerides of a mixture of fats.

Microbial lipases are enzymes that in nature catalyse the hydrolysis of glycerides. This reaction is reversible, and as a result of concurrent hydrolysis and resynthesis of triglycerides, interesterification occurs when fats are treated with lipases under conditions of limited water availability. By exploiting the specificity of microbial lipases it is possible to produce, by enzyme-catalysed interesterification reactions, useful triglyceride mixtures that cannot be obtained by conventional chemical processing.

Introduction

The main components of oils and fats are triglycerides, which are triesters of glycerol and fatty acids. The physical properties of oils and fats are to a large extent determined by the structure of the fatty acid groups and their distribution within the triglycerides. The application of a particular triglyceride mixture depends upon its physical properties, and a number of processing techniques are used industrially to modify and manipulate the physical properties of naturally occurring oils and fats. These processes include hydrogenation, which reduces the unsaturated fatty-acid content of triglycerides, thereby raising their melting point; fractional crystallization, which separates triglycerides according to melting point; and interesterification. In this last process a chemical catalyst such as sodium methoxide is used to promote migration of fatty acid groups between glyceride molecules, so that the product consists of triglycerides in which the fatty acid groups are randomly distributed amongst the glyceride molecules (Sreenivasan 1978). The change in the distribution of the fatty acid groups amongst the triglycerides alters the physical properties of a fat mixture.

Developments in plant breeding techniques may enable the oils and fats industry to obtain raw materials that are more closely matched to the compositions required for use in food and other products, but there will still be a requirement for processing techniques to manipulate the functional properties of available oils and fats. Accordingly there is an interest in the exploitation of biocatalysis for the modification of oils and fats, either to replace existing processes with milder, more natural, techniques or to provide novel products that cannot be produced readily by existing methods (Werdelmann & Schmid 1982).

In this paper, enzyme-catalysed interesterification processes for the modification of oils and fats are discussed. By exploiting the specificity of the lipase catalysts it is possible to produce useful triglyceride mixtures which cannot be obtained by conventional chemical interesterification processes.

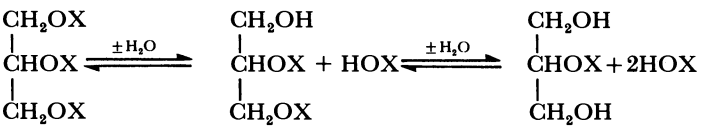
Compared with most other industrial enzyme processes the interesterification process has two unusual features. First, it uses a two-liquid-phase reaction system, in which the reactants are

dissolved in a water-immiscible solvent and the enzyme is located in a small volume of aqueous phase. Secondly the ability of a hydrolytic enzyme to catalyse the reverse of its natural reaction is exploited.

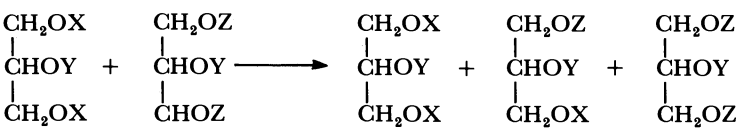
Reactions catalysed by microbial lipases

The enzymes used as catalysts for interesterification are regiospecific extracellular microbial lipases (glycerol ester hydrolases E.C. 3.1.1.3). These enzymes are excreted by fungi such as *Aspergillus niger* and various *Rhizopus* and *Mucor* species into their growth medium, to assist in the digestion of lipids (Macrae 1983*a*). They catalyse the hydrolysis of oils and fats releasing fatty acid specifically from the outer 1- and 3- positions of triglycerides to give free fatty acid, 1,2- and 2,3-diglycerides and 2-monoglycerides (scheme 1). The reaction is reversible and the

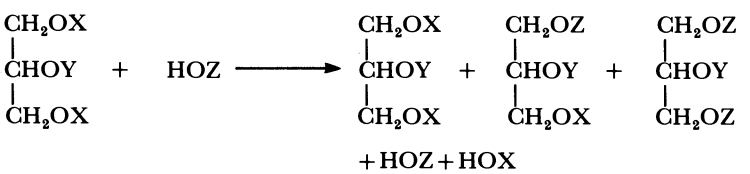
hydrolysis:



interesterification of triglycerides:



interesterification of triglyceride plus free fatty acid



Scheme 1. Reactions catalysed by regiospecific lipases. X, Y and Z are fatty acyl groups. (*a*) Hydrolysis; (*b*) interesterification of triglycerides; (*c*) interesterification of triglyceride plus free fatty acid.

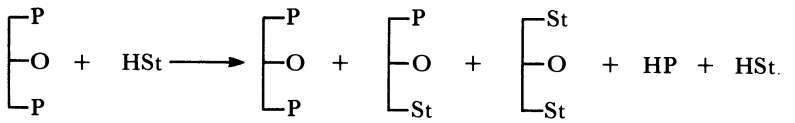
lipases can be shown to catalyse the formation of triglycerides from partial glycerides and free fatty acid (Tsujisaka *et al.* 1977). Because of this reversibility, hydrolysis and resynthesis of triglycerides occur when lipases are reacted with oils and fats, causing interchange of fatty acid groups between the 1- and 3-positions of triglyceride molecules and giving selectively interesterified products (scheme 1). Under conditions in which the amount of water in the reaction system is restricted to less than 1% of the reactants, hydrolysis of the fat can be minimized so that interesterification becomes the dominant reaction. Mixtures of triglycerides and free fatty acids can also be used as reactants for interesterification. In these cases, free fatty acid exchanges with fatty acid groups of the triglyceride to produce a new triglyceride enriched selectively in the 1- and 3- positions with the added fatty acid.

The ability to produce novel triglyceride mixtures by using regiospecific lipases is of interest

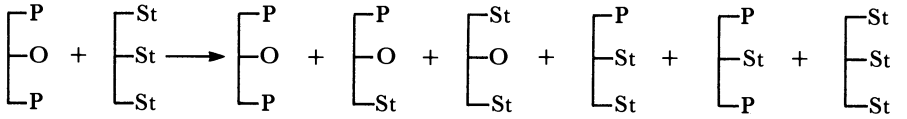
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

to the oils and fats industry because some of these mixtures have compositions and properties which makes them valuable. This is illustrated by reactions of use for the production of confectionery fats. Interesterification of 1,3-dipalmitoyl-2-monoolein (POP; this is the major triglyceride of the mid-fraction of palm oil) with either stearic acid or tristearin gives products enriched in the triglycerides 1(3)-palmitoyl-3(1)-stearoyl-2-monoolein (POSt) and 1,3-distearoyl-2-monoolein (StOSt) (scheme 2). 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 cheap starting materials. StOSt can also be generated from industrially available fat fractions containing a high proportion of 1,2(2,3)-dioleoyl-3(1)-monostearin (StOO). Interesterification of this triglyceride gives a mixture containing StOSt, StOO and triolein (scheme 2).

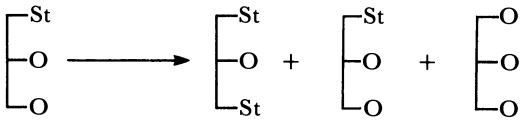
(*a*) palm mid-fraction + stearic acid

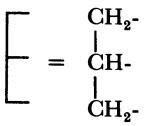


(*b*) palm mid-fraction + tristearin



(*c*) dioleoylmonostearin





SCHEME 2. Interesterification reactions for production of confectionery fats. (*a*) Palm mid-fraction + stearic acid; (*b*) palm mid-fraction + tristearin; (*c*) dioleoylmonostearin. P = palmitate; St = stearate; O = oleate.

INTERESTERIFICATION REACTION SYSTEMS

Because lipases work at lipid–water interfaces (Brockerhoff & Jensen 1974), rapid rates of interesterification are obtained only if the reaction system has a large interface area between the reactant phase and the small volume of aqueous enzyme phase. This can be achieved by using macroporous particles coated with hydrated lipase as catalysts for the interesterification reactions. Materials suitable as supports for lipases include kieselguhr, hydroxylapatite and

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

alumina. The catalysts can be prepared by addition of a solvent such as acetone to a slurry of the particles in buffered lipase solution. The solvent-precipitated enzyme coats the particles, and the lipase-coated particles are collected by filtration, dried and stored (Coleman & Macrae 1980).

An alternative method of preparing interesterification catalysts is to thoroughly mix microbial lipase solution with an excess of inorganic particles and then to dry the resulting powder under controlled conditions (Matsuo *et al.* 1983). Fully dried catalyst particles are essentially inactive as interesterification catalysts, and to obtain reasonable reaction rates it is necessary to activate them by hydration with up to 10% water. The activated catalyst particles are then contacted with the water-insoluble reactants. Thus the interesterification reaction mixture consists of a water-immiscible reactant phase and a hydrated enzyme phase located and immobilized on porous particles.

Liquid oils and fatty acids can be interesterified without solvent. However in most cases it is necessary to add a water-immiscible solvent to ensure that the reactants and products remain dissolved in the organic phase. Various solvents can be used, but hexane is preferred for commercial operation because this solvent is already used industrially for the extraction and processing of oils and fats.

TABLE 1. TRIGLYCERIDES FORMED BY INTERESTERIFICATION OF A MIXTURE OF PALM MID-FRACTION (1.0 PARTS) AND STEARIC ACID (0.5 PARTS) WITH *A. NIGER* LIPASE AS CATALYST

	amount in triglyceride	
fatty acid	palm mid-fraction (percentage)	interesterified product (percentage)
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[a]		
SSS	5	13
POP	58	19
POSt	13	32
StOSt	2	13
SSO	7	2
SLnS	9	7
SOO	4	7
Others	2	3

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

Interesterification reactions can be performed either batchwise in stirred-tank reactors or continuously by using packed-bed reactors. In a typical batch reaction a mixture of palm mid-fraction and stearic acid dissolved in petroleum ether was stirred for 16 h at 40 °C with hydrated catalyst prepared from *A. niger* lipase and kieselguhr. Analysis of the product showed that as a result of the interesterification reaction stearate groups were incorporated selectively into the 1- and 3- positions of the triglyceride, with the production of the valuable POSt and StOSt triglycerides (table 1). Diglycerides and additional free fatty acid were formed as by-products, a proportion of the water required to activate the catalyst being consumed in this hydrolysis reaction.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

On completion of the reaction the catalyst particles were removed by filtration and a cocoa butter equivalent was isolated from the filtrate by conventional fat fractionation techniques such as distillation, liquid–liquid extraction and crystallization from solvents. The catalyst has good stability under the conditions used in the stirred-tank reactor and use of the same catalyst particles in many successive batch reactions is possible.

For operation of packed-bed reactors the reactants are treated by conventional refining techniques to remove particulate materials and enzyme catalyst poisons, and dissolved in hexane or petroleum ether. The solution of the reactants is then partly saturated with water and pumped through a bed of hydrated catalyst particles. Water must be added to the feedstream to prevent dehydration and loss of activity of the catalyst bed, and it is essential that this added water is completely dissolved in the feedstream and not present as a second liquid phase. Second-phase water in the feedstream collects on the catalyst bed, and if an appreciable amount accumulates the lipase, which is water-soluble, washes off the kieselguhr particles.

The performance of a reactor containing a bed of hydrated catalyst prepared from *Rhizopus niveus* lipase and kieselguhr was studied by using a feedstream consisting of palm mid-fraction and myristic acid dissolved in petroleum ether. The temperature of the reactor was maintained at 40 °C and the mean residence time of the feed in the reactor was approximately 30 min. Essentially complete interesterification as determined by the myristate content of the triglycerides was obtained throughout 400 h of continuous operation. The products from the packed-bed reactor were collected and compared with those generated from the same feedstock in a stirred-tank reactor (table 2). Because of the short residence time of the fats in the packed-bed reactor and the ability to restrict water addition in this system, less by-product formation occurred in the packed reactor than in the stirred-tank reactor. The overall content of potentially valuable SOS triglycerides in the packed-bed reactor product was 41 % compared with 32 % for the stirred-tank product. This clearly demonstrates the advantage of using the continuous packed-bed reactor system in preference to the batch stirred-tank method.

The interesterification catalysts are reasonably stable under the conditions prevalent in packed-bed reactors. This is illustrated by the performance of a reactor containing hydrated

TABLE 2. COMPARISON OF PRODUCTS FORMED BY INTERESTERIFICATION OF A MIXTURE OF PALM MID-FRACTION (2.5 PARTS) AND MYRISTIC ACID (1.0 PARTS) WITH THE USE OF A BATCH STIRRED-TANK REACTOR OR A CONTINUOUS PACKED-BED REACTOR

	reactant composition (percentage)	product composition stirred-tank reactor (percentage)	product composition packed-bed reactor (percentage)
triglyceride	70	52	60
fatty acid	29	36	33
diglyceride	1	12	7
triglyceride fraction			
SSS	—	11	10
SOS	—	62	68
SSO	—	1	1
SLnS	—	9	7
others	—	17	14
total amount of SOS	—	32	41

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

catalyst prepared from *Mucor miehei* lipase and kieselguhr and operated with a feedstock consisting of a fraction from palm oil and stearic acid dissolved in petroleum ether. The activity of the catalyst decayed exponentially with a half-life of approximately 30 d, showing that packed-bed reactors can be operated continuously for long periods with an acceptable loss of catalytic activity. The product from this reactor was collected and fractionated to give a fat that closely resembled cocoa butter in its physical properties and chemical composition (table 3).

TABLE 3. COMPOSITION OF A CONFECTIONERY FAT ISOLATED FROM THE PRODUCTS OF INTERESTERIFICATION OF A MIXTURE OF PALM OIL MID-FRACTION (1 PART) AND STEARIC ACID (0.5 PARTS) WITH THE USE OF A PACKED-BED REACTOR

triglyceride	amount in enzymically produced confectionery fat (percentage)	amount in cocoa butter (percentage)
SSS	3.0	1.0
POP	16.2	16.3
POSt	38.8	40.8
StOSt	28.5	27.4
SLnS	8.0	7.5
SOO	4.0	6.0
others	1.5	1.0

CONCLUSIONS

The use of regiospecific microbial lipases as interesterification catalysts enables the oils and fats industry to produce triglyceride mixtures which cannot be obtained by conventional chemical interesterification methods. The usefulness of the enzyme technique has been illustrated by the development of processes for the production of high-value confectionery fat components; however the technique can also be expected to have applications in the production of novel triglyceride mixtures with valuable functional properties in other edible fat products such as margarines.

Regiospecificity is only one of three types of specificity known to be exhibited by lipases. Fatty-acid-specific lipases, such as that produced by the fungus *Geotrichum candidum*, release a particular type of fatty acid from glycerides (Jensen 1974), and stereospecific lipases from some animal tissues catalyse reaction preferentially at either the 1- or 3- position of glycerol (Jensen *et al.* 1983). Development of interesterification processes using these enzymes as catalysts may eventually allow production of a different range of novel triglyceride mixtures to those produced with the use of regiospecific lipases. Some of these alternative mixtures may also have properties of value to the edible oils and fats industry.

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