MAKING VALUE-ADDED PRODUCTS FROM PALM OIL BY 1-3 REGIOSELECTIVITY ENZYMATIC INTERESTERIFICATION

Keywords: Enzymatic interesterification; 1-3 Regioselectivity; Molten medium; Continuous reactor; Palm oil; Making value-added products

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Work was undertaken with a view to enhancing the commercial and nutritional quality of palm oil and its solid fraction by enzymatic interesterification, catalyzed by 1-3 regioselective lipases in a molten medium. The main aims are to develop base materials for table or confectionery margarine; and to produce liquid oils for salad-dressing and frying, possibly enriched with essential fatty acids, to meet technological and nutritional requirements.

The biological processing of palm oil and its solid fraction by interesterification in a tubular reactor with a fixed catalyst bed containing Lipozyme offers the advantage of continuous enzymatic transformation which makes for greater flexibility in obtaining products with specific properties. A study of reaction kinetics showed that transformation was complete after 5 h in the reactor, which corresponded to a flow rate of 3.5 ml per hour. For a given pair of oils in a defined oil/counter-oil ratio, establishment of kinetics showed that it is possible to manufacture products which all have rheological properties closely linked to the time spent in the reactor. Thus, 1-3 RI of the palm stearin/palm kernel oil (30:70) mixture for 30 min and 3h 30 min led to materials resembling a firm margarine in the first case and a soft margarine in the second case. Interesterification with fluid oils gives either plastic fats or oils virtually fluid at 20°C which could be used as salad oils in hot countries.

INTRODUCTION

Work was undertaken with a view to enhancing the commmercial and nutritional quality of palm oil and its solid fraction by enzymatic interesterification, catalyzed by 1-3 regioselective lipases in a molten medium. There were two main aims :

- development of base materials for table or confectionery margarine
- producing liquid oils for salad-dressing and frying, possibly enriched with essential fatty acids, to meet technological and nutritional requirements.

Interesterification is a reaction whereby, in an appropriate medium and at a suitable temperature, esters react by exchanging their acyl chains. The result is a significant modification of the natural distribution of fatty acid chains and consequently of the physical properties of oils and fats. This reaction makes it possible, by careful combination, to obtain products with predefined fatty acid compositions and rheological properties.

There are three major types of interesterification:

- stochastic chemical
- directed chemical
- regioselective enzymatic.

We shall consider only 1-3 regioselective enzymatic interesterification.

1-3 regioselective interesterification (1-3 RI), biocatalyzed by a 1-3 specific lipase affects only triglyceride positions 1 and 3, consequently limiting random distribution to these two positions, which leads to a smaller number of triglycerides. 1-3 RI obviously has many advantages over chemical interesterification :

1) The fatty acid at triglyceride position 2 remains unchanged, thus ensuring better bio-availability of the essential unsaturated fatty acids which frequently occupy this position in vegetable oils.

2) Enzymatic reactions are slow, which means that the interesterification reaction is easier to control from a kinetic point of view, hence making it possible to produce a wide range of products with distinct characteristics.

3) The formation of high melting point triglyceride generally observed in interesterification reactions is prevented or significantly limited.

4) Conventional interesterification necessitates working with partially refined, anhydrous substrates. This is no longer the case with 1-3 RI.

5) 1-3 RI calls for relatively low temperatures, between 30° C and 60° C ; quality should therefore be improved.

6) Last but not least, the possibility of working on raw or only lightly refined substrates at relatively low temperatures enables substantial savings of energy.

From a biotechnological point of view, the choice of the most appropriate enzyme is made essentially on the basis of specificity and ease of use.

Except for fractionation, which in any case cannot entirely solve the problem, most attempts to improve palm oil have not as yet given the industry entire satisfaction. Such attempts include chemical dehydrogenation, agricultural methods, the preparation of eutectic blends, *etc* ... ; 1-3 RI appears of particular interest as regards to palm oil when we consider that in its glyceride structure, around 90% of saturated fatty acids occupy the external glycerol positions.

Accordingly, and with the stated aims in view, 1-3 RI was studied in a molten medium, using palm oil and/or its solid fraction (palm stearin) as the basic oil; the counter-oils used included coconut and palm kernel oil from the lauric group, rapeseed, sunflower, soya bean and ricebran oils, representing all the types of liquid oils, and lastly two specialty oils, borage oil, a γ linolenic oil, and *Myrianthus arboreus* oil in which linoleic acid accounts for more than 90% of the fatty acids.

Developing the use of 1-3 RI regioselective lipases in various forms (in natural form or fixed) necessitated:

- a study of the fatty acid composition and distribution of the triglycerides in the oils used, which is essential for forecasting fatty acid rearrangement on the triglycerides.

Also necessary, using a simple coconut oil/methyl stearate model, were:

- the development of a test for determining the specific transesterification action of the various biocatalysts, with a view to comparing them significantly and reliably; it was found that for a given lipase the activity increases in the following way:

TABLE 1. KINETICS OF INTERESTERIFICATION BETWEEN PALM OIL AND *Myrianthus* OIL (60:40). THE RESULTS FOR THE MAIN TRIGLYCERIDES ARE EXPRESSED AS PERCENTAGES BY WEIGHT.

Triglycerides	Palm oil	*Myrianthus* oil	Palm oil/*Myrianthus* oil (60:40) 0 min	26 min	lh 4min	1.5 h	3 h	5 h
LLL		79.9	31.1	28.3	26.0	23.8	21.8	21.0
OLL		12.8	4.8	5.4	5.9	6.3	7.8	7.8
PLL	1.0	2.7	1.7	3.0	5.6	7.4	8.9	9.0
POL	7.9	0.4	5.4	7.0	8.6	9.8	11.1	11.2
PPL	7.2		4.5	5.1	5.0	5.0	5.3	5.2
OOO	3.5		2.2	2.5	2.4	2.2	2.0	2.0
POO	25.1		14.9	14.7	14.4	13.7	11.7	11.7
POP	35.2		20.7	19.1	17.6	17.2	15.6	15.9
PPP	9.5		5.5	4.7	3.8	4.5	4.0	4.0
P.CtO	5.5		3.4	3.1	3.5	3.0	3.0	2.8

TABLE 2. RETENTION OF 1-3 REGIOSELECTIVITY BY LIPOZYME DURING 1-3 RI IN THE REACTOR ON THE PALM OIL/*Myrianthus* OIL MIXTURE. THE RESULTS FOR THE MAIN TRIGLYCERIDES ARE EXPRESSED IN MOLAR PERCENTAGES OF THE TOTAL QUANTITY OF FATTY ACID IN THE MIXTURE.

Fatty acid	Triglyceride position	0 min		26 min	lh 4min	1.5 h	3 h	5 h
		2	1 + 3	2	2	2	2	2
16:0	28.8	3.6	25.2	3.5	3.6	3.6	3.6	3.6
18:0	3.1	0.4	2.7	0.5	0.4	0.4	0.4	0.4
18:1	24.9	13.5	11.4	13.5	13.6	13.6	13.6	13.5
18:2	41.7	15.6	26.1	15.6	15.6	15.6	15.5	15.6

TABLE 3. SOLID CONTENT (%) IN RELATION TO TEMPERATURE OF THE PALM OIL/COCONUT OIL MIXTURE (70:30) INTERESTERIFIED IN A REACTOR FOR BETWEEN 30 MIN AND 4 HOURS.

Temperature (°C)	Palm oil	Coconut oil	Palm oil/Coconut oil (70:30)				
			0 min	30 min	1 h	2 h	4 h
0	54.5	79.6	53.4	45.6	38.6	35.7	33.9
5	49.6	68.2	42.6	32.8	27.9	42.7	22.2
20	15.4	31.0	17.3	11.1	6.0	5.6	4.7
29	8.9	0.2	5.2	3.8	2.6	2.2	1.9
37	3.7	—	1.8	0.7	—	—	—
42	2.2	—	1.3	0.3	—	—	—
48	—	—	—	—	—	—	—

TABLE 4. SOLID CONTENT (%) IN RELATION TO TEMPERATURE OF THE PALM STEARIN/ PALM KERNEL MIXTURE (30:70) INTERESTERIFIED IN A REACTOR FOR BETWEEN 35 MIN AND 4 HOURS 37 MINUTES.

Temperature (°C)	Palm stearin	Palm kernel oil	Palm stearin/Palm kernel oil (70:30)				
			0 min	35 min	lh 3min	2 h	4h 37min
0	66.0	70.5	64.7	63.0	60.3	58.1	56.5
5	62.7	66.7	54.7	52.4	50.3	49.4	46.1
20	33.3	40.7	23.3	21.5	18.6	17.2	15.8
29	23.6	3.3	8.5	6.4	4.9	3.6	2.8
37	14.0	—	3.0	2.0	1.0	0.9	0.4
42	8.3	—	2.4	0.5	0.1	—	—
48	2.7	—	—	—	—	—	—

TABLE 5. SOLID CONTENT (%) OF THE REFERENCE PRODUCTS AND OF THE INTERESTERIFIED MIXTURES OIL PALM STEARIN/PALM KERNEL OIL (30:70) AND PALM OIL/ COCONUT OIL (70:30).

Temperature (° C)	Firm margarine	Soft margarine	Palm stearin/Palm kernel oil		Palm oil/coconut oil
			35 min	4h 37min	30 min
20	18.6	13.2	19.4	15.8	11.1
30	5.0	2.3	5.3	2.7	2.7
37	—	—	2.0	0.4	0.7

LIPASE IN ITS NATURAL FORM < SUPPORTED LIPASE (celite) < DEVITALIZED CELLS < LIPASE FIXED BY IONIC BONDING.

• a study of transesterification conversion rates in relation to the activity of water, making it possible to define the relevant hydration conditions in which the biocatalysts are most effective and by-product formation is kept to a minimum.

In fact, even in a molten medium, the minimum hydration required to preserve the activity of the lipase inevitably causes slight hydrolysis, releasing partial glycerides and FFAs. In all cases, the best results are obtained with a water activity of between 0.25 and 0.5 which corresponds to hydration rates in the reaction medium of around 0.5% to 1 %, including catalyst hydration. For water activities under 0.25, the activity of the biocatalyst is too low. With water activity exceeding 0.45, hydrolysis begins to take over from 1-3 RI.

• checking the stability of biocatalyst 1-3 regioselectivity within the time limits of industrial reactions. This check is made on the simplified coconut oil/methyl stearate model by monitoring stearic acid incorporation at position 2 of the triglycerides during the reaction process up to the stage of equilibrium.

After this preliminary study, the Lipozyme, *Mucor miehei* lipase fixed on to a macroporous anion exchange resin, was chosen as the reaction biocatalyst, basically for the following reasons :

The steps described above to determine operating procedure showed that Lipozyme was the most active enzyme complex of all the biocatalysts tested; we also checked that 1-3 regioselectivity was maintained for at least 12 hours, *i.e.* long enough to be compatible with the demands of industry and sufficient to ensure virtually complete fat transformation (conversion rate of 95 % before stearic acid is detected in position 2).

A batch study revealed the kinetics of the reaction and enabled us to determine the time taken for the reaction to reach equilibrium. Interesterification was carried out with palm oil or palm stearin and the counter-oil at ratios between 20:80 and 80:20.

A reaction at 60° C for six hours with 6 g of the mixture of basic oil and counter-oil and 0.3 g of Lipozyme showed that interesterification was complete. It was then possible to plot the curve of solid content against temperature for each oil/counter-oil ratio. These curves were compared with those of the commercial margarines used for reference.

The 1-3 RI conducted in batches between palm oil or palm stearin and lauric oils showed that several mixtures (70:30 palm oil/coconut oil, 70:30 palm oil/palm kernel oil and 30:70 palm stearin/palm kernel oil) when totally interesterified, showed certain similarities with the solid content of the firm control margarine.

Other mixtures (80:20 palm oil/coconut oil, 70:30 palm oil/palm kernel oil, and 20:80 and 30:70 palm stearin/palm kernel oil) once totally interesterified were characterized by solid contents similar in certain temperature ranges to those of soft sunflower margarine.

Likewise, total interesterification between palm oil or palm stearin and the fluid oils made it possible to obtain fluid oils at 20° C-25° C that are very suitable for hot countries. This was the case with mixtures of palm oil/rapeseed oil, palm oil/rice bran oil, palm oil/soya bean oil, palm oil/borage oil in the ratio of 40:60 and palm stearin/rapeseed oil, palm stearin/soya bean oil, palm oil/*Myrianthus* oil and palm oil/borage oil mixtures in the ratio of 30:70.

RESULTS AND DISCUSSION

Studies in a Continous Reactor

In the study conducted in a continuous reactor described below, only the oil/counter-oil ratios that gave products with noteworthy rheological properties were used. The solid content in relation to temperature was systematically determined for each period spent in the reactor.

Reaction conditions

The fixed catalytic bed reactor consisted of a double-jacketed column with an internal diameter of 1 cm, temperature-regulated at 60 °C. The catalytic bed consisted of 9 g of Lipozyme (25 cm^3). The mixture to be interesterified, kept at 60 °C ±1 °C in a tank was fed into the bottom of the reactor via a peristaltic pump. The time spent in the reactor could vary from 30 min to 5 h corresponding to flow rates of 35 ml/h^{-1} to 3.5 ml/h^{-1}.

Given that the reaction performances in batch operation and in a continuous reactor are not identical, we thought it wise to check the reaction kinetics, to ascertain the contact time required for the reaction to reach equilibrium. It was also useful to check whether the biocatalyst retained its 1-3 regioselectivity as regards the substrate under these conditions, *i.e.* whether there was a risk of acyl migration at the end of lengthy reactions.

Reactions kinetics and determination of time taken to reach equilibrium

As in a non-continuous reactor, the mixture used in this study was palm oil/*Myrianthus* oil in a ratio of 60:40, since these oils are characterized by 26 % POO and 35 % POP for palm oil and 80 % LLL for *Myrianthus* oil. These tracer triglycerides help in monitoring the reaction as it proceeds.

Five gram samples of the interesterified mixture were taken at given times for periods spent in the reactor varying from 30 min to five hours. Interesterification was checked by HPLC according to triglyceride partition numbers. The values given in *Table 1* show the changes in concentration (weighted %) for the different triglycerides, depending on the amount of time spent in the reactor ; these data make it possible to keep track of interesterification kinetics, taking into account the following main triglycerides: LLL, PLL ,POL, POP and PPP. The concentration of LLL, POP and PPP gradually fell during the reaction, whereas that of POL and PLL increased.

Interesterification would appear to be complete in 5 h, the time taken for the concentration of the different glycerides to stop changing. The concentration of LLL and POP dropped from 31.1 % to 21.0% and from 20.7% to 15.9% respectively ; POL and PLL increased from 5.4% to 11.2% and from 1.7% to 9% respectively.

Consequently, interesterification in the reaction was then studied by establishing kinetics for times spent in the reactor varying from about 30 min to five hours.

Study of the stability of the biocatalyst's 1-3 regioselectivity

The palm oil/*Myrianthus* oil (60:40) mixture was interesterified for times spent in the reactor varying from 26 min to five hours. Two gram effluent samples were taken during the reaction from the reactor outlet and chromatographed on silica gel to separate the pure triglycerides.

Internal/external fatty acid distribution was then determined. The results obtained are given in *Table* 2 and call for the following comments :

A comparison of the mean molar composition of the fatty acids in the internal position of the unprocessed mixture with that of the mixtures interesterified for between 26 min and 5 h showed that these compositions were virtually the same ; the mean molar composition of the fatty acids in the internal position of the triglycerides thus remained constant throughout the reaction. Lipozyme 1-3 regioselectivity was retained and 1-3 RI took place satisfactorily since no acyl migration was seen during the reaction for any period up to five hours.

1-3 RI with lauric oils

The oil mixtures used for this biological transformation were as follows : 70:30 palm oil/coconut oil and 30:70 palm stearin/palm kernel oil. Interesterification was carried out for reaction times varying from 30 min to 4 h and from 35 min to 4h 37 min respectively. The effluent was checked by low resolution pulse NMR.

The values for solid content in relation to the temperature and the time spent on the reactor are given in *Table 3* for the palm oil/coconut oil mixture (70:30), and in *Table 4* for the palm stearin/palm kernel oil mixture (30:70); they can be interpreted as follows :

- The interesterified mixtures are characterized by a distinctly lower solid content than that of the respective unprocessed mixtures;

- The solid content of the interesterified mixtures varies with the time spent in the reactor, falling gradually during the interesterification reaction;

- At 37 °C, the solid content of the interesterified mixtures fluctuated between 0% and 2%; in particular, it was nil for the interesterified 70:30 palm oil/coconut oil mixture for at least one hour; it reached 2% for the intresterified 30:70 palm stearin/palm kernel oil mixture after 35 min and 0.4 % after 4h 37 min for the same mixture.

The solid contents of the interesterified mixtures were compared with those of the reference products, firm margarine and soft sunflower margarine (*Table 5*). Taking into account the values for solid content at 20°C, 30°C and 37°C only, it appeared, under these conditions, that :

• The palm oil/coconut oil mixture (70:30) and the palm stearin/palm kernel oil mixture (30:70), interesterified for 30 min and 4h 37 min respectively, were comparable to soft sunflower margarine, with solid contents at 30°C of 2.7 % and 2.3% respectively. At 37°C, the solid content of these mixtures was negligible : 0.7% and 0.4% respectively. These are consequently base materials suitable for use as ingredients in the production of table margarine.

• The palm stearin/palm kernel oil mixture (30:70), interesterified for 35 min, resembled the firm control margarine, with solid contents of 5.3% for the product and 5.0% for the firm margarine at 30°C. However, this mixture was characterized by a not inconsiderable solid rate of 2.0% at 37°C. It is therefore a base material suitable for use in cooking or confectionery margarines.

Interesterification with fluid oils

A study in a non-continuous reactor enabled us to opt for the following oil mixtures : 40:60 palm oil/ rapeseed oil, 30:70 palm stearin/soy bean oil and 60:40 palm oil/*Myrianthus* oil.

a) *1-3 RI with rapeseed oil*

The palm oil/rapeseed oil mixture (40:60) was interesterified in the reactor for periods varying from 35 min to four hours.

The solid contents in relation to the temperature and the time spent in the reactor are shown in *Table 6*. It was thus possible to monitor the change in solid content in the interesterified mixture in relation of the time spent in the reactor.

The results call for the following comments :

• The interesterified mixtures has lower solid contents than the unprocessed mixtures.

• The solid contents of the processed mixtures varied with the time spent in the reactor, falling gradually during the reaction and becoming nil at 37°C.

• The palm oil/rapeseed oil mixture (40:60), interesterified for 2 to 4 h was virtually fluid at 20°C - 25°C ; the solid contents at 22°C were 0.9 % after 2 h and 0.4 % after four hours in the reactor.

b) *1-3 RI with soya bean and* Myrianthus *oil*

The palm stearin/soya bean oil (30:70) and palm oil/*Myrianthus* oil (60:40) mixtures were interesterified for between 35 min and 5 h and between 26 min and 5 h respectively. The figures for solid content in relation to the temperature and the time spent in the reactor are given in *Table 7* for the palm stearin/soya bean oil (30:70) mixture and in *Table* 8 for the palm oil/*Myrianthus* oil (60:40) mixture.

The results can be interpreted as follows :

• Interesterification lowered the solid contents of simple mixtures.

• The solid content of interesterified mixtures varied with the contact time, as in the previous cases ; it fell during the reaction.

• The palm stearin/soya bean oil (30:70) mixture, interesterified for between 75 min and 5 h, was characterized by a nil solid content at 37 °C, as was the palm oil/*Myrianthus* oil (60:40) mixture interesterified for between 64 min and five hours.

• The palm stearin/soya bean oil (30:70) mixture, interesterified for between 2h 45 min and 5 h was a plastic fat at room temperature, with a solid content at 22°C of 1.8% (after 2h 45 min) and 1.2 % after 5 hours in the reactor. It could be used as an ingredient in the production of shortening. It was also completely fluid at 37 °C after a reaction lasting 2h 45 min.

• The palm oil/*Myrianthus* oil (60:40) mixture as virtually fluid at room temperature after 5 h in the reactor ; the solid content at 22°C was 0.7 per cent.

TABLE 6. SOLID CONTENT (%) IN RELATION TO TEMPERATURE OF THE PALM OIL/RAPESEED OIL MIXTURE (40:60) INTERESTERIFIED IN A REACTOR FOR BETWEEN 35 MIN AND 4 HOURS.

Temperature (° C)	Palm oil	Rapeseed oil	Palm oil/rapeseed oil (40:60)				
			0 min	35 min	lh 9min	2h 5min	4 h
0	54.5	0.4	14.7	12.1	10.5	8.8	7.5
5	49.6	—	9.4	7.8	6.7	5.4	4.6
10	34.7	—	5.5	4.7	3.3	3.0	2.0
19	25.3	—	3.5	3.3	2.1	1.8	1.2
22	15.4	—	2.3	2.1	1.0	0.9	0.4
29	8.9	—	2.0	1.6	0.3	0.1	—

TABLE 7. SOLID CONTENT (%) IN RELATION TO TEMPERATURE OF THE PALM STEARIN/SOYA BEAN OIL MIXTURE (30:70) INTERESTERIFIED IN A REACTOR FOR BETWEEN 35 MIN AND 5 HOURS 5 MINUTES.

Temperature (° C)	Palm stearin	Soya bean oil	Palm stearin/Soya bean oil (30:70)				
			0 min	35 min	lhl5min	2h45min	5h5min
0	66.0	0.3	16.1	14.3	12.3	10.3	8.0
5	62.7	—	11.3	9.9	8.0	7.1	5.6
10	53.3	—	9.0	7.3	5.7	4.7	3.1
15	45.0	—	7.3	5.7	4.1	3.2	2.1
22	33.3	—	5.4	4.3	2.3	1.8	1.2
29	23.6	—	4.0	2.6	1.0	0.9	0.3
37	14.0	—	1.3	0.7	0.2	—	—
42	8.3	—	1.0	0.3	—	—	—
48	2.7	—	—	—	—	—	—

TABLE 8. SOLID CONTENT (%) IN RELATION TO TEMPERATURE OF THE PALM OIL/ *Myrianthus* OIL MIXTURE (60:40) INTERESTERIFIED IN A REACTOR FOR BETWEEN 26 MIN AND 5 HOURS.

Temperature (°C)	Palm oil	*Myrianthus* oil	Palm oil/*Myrianthus* oil (60:40)					
			0 min	26 min	lh 4min	1.5 h	3 h	5 h
0	54.5	—	25.3	23.8	20.1	18.8	15.7	14.3
5	49.6	—	19.4	17.7	15.7	12.9	10.9	8.6
10	34.6	—	13.0	11.4	9.3	8.3	6.3	5.0
15	25.3	—	9.4	7.4	5.7	5.3	4.0	3.0
22	15.4	—	5.7	4.3	3.0	2.4	1.3	0.7
29	8.9	—	2.3	1.3	0.3	0.1	—	—
37	3.7	—	1.0	0.3	—	—	—	—
42	2.2	—	—	—	—	—	—	—

CONCLUSION

The biological processing of palm oil and its solid fraction by interesterification in a tubular reactor with a fixed catalyst bed containing Lipozyme offers the advantage of continuous enzymatic transformation (by comparison with a batch reaction with the same catalyst) which makes for greater flexibility in obtaining products with specific properties. A study of reaction kinetics showed that transformation was complete after 5 h in the reactor, which corresponded to a flow rate of 3.5 ml per hour. For a given pair of oils in a defined oil/ counter-oil ratio, establishment of kinetics showed that it is possible to manufacture products which all have rheological properties closely linked to the time spent in the reactor.

Thus, 1-3 RI of the palm stearin/palm kernel oil (30:70) mixture for 30 min and 3h 30 min led to materials resembling a firm margarine in the first case and a soft margarine in the second case. Interesterification with fluid oils gives either plastic fats or oils virtually fluid at 20°C which could be used as salad oils in hot countries. Interesterification between palm oil or palm stearin and rapeseed oil would be worth studying in greater detail in a tubular reactor. This process would make it possible to increase the value of both oils; the interesterified mixture could be a fluid oil at room temperature or a more stable plastic fat with a low α-linoleic acid content of around 2-3 per cent.

The advantage offered by this process as compared to hydrogenation would be that it does not cause any isomerization of double bonds whether geometric or in terms of position. We have thus been able to devise a polyunsaturate-enriched base material for margarine, with the same rheological properties as sunflower margarine. Since interesterification is 1-3 regioselective, the base materials and the fluid oils obtained have better essential fatty acid bioavailability than products obtained through chemical interesterification, since the essential fatty acids mainly occupy the internal position of the triglycerides. These products therefore satisfy both technological and nutritional requirements.

Making base materials for table or confectionery margarines through 1-3 RI between oils is therefore a process well worth investigating. Economically speaking, the biocatalyst used, Lipozyme, is very expensive. The use of whole cells seems promising as a solution, in that from a technical and economic point of view, they are easier to produce than actual lipases, since the preparation of the latter involves precipitation and purification stages. Moreover, whole cells provide a naturally fixed lipase; it is easy to confine the cells, either by simply mixing them with an inert mineral medium, or in an appropriate polymer reticulum. A reactor with a fixed bed comprising confined cells would be cheaper at present than a reactor with a bed consisting of a fixed lipase.

Be that as it may, in conclusion, it seems that we

are on the verge of an industrial development than can be expected to express its full potential by the end of the 20th century.

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