Review Article

The evolution of enzymatic interesterification in the oils and fats industry

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Immobilised lipases have now become accepted as a mainstream technology for fat modification. This paper presents the development of this technology in particular for the production of *trans*-free fats. One of the factors influencing this development has been concern for health, which has made *trans* fats a major issue for food manufacturers and consumers. Enzymatic interesterification is a relatively new method for producing *trans*-free alternatives for fats used in conventional margarines and shortenings. The development of this technology is examined from the perspective of an eventual industrial application rather than operation only at the laboratory scale. This paper also covers the practical means of operating immobilised enzyme columns and gives examples of how formulations can be adapted to match existing specifications. There are environmental benefits when choosing enzyme technology in comparison with chemical-based routes. Life cycle assessments have been used to quantify the differences in environmental impact of this new technology. The final process is both capable of providing fats with the correct melting properties but without *trans* fats and of reducing the environmental impact of fat processing. Finally, the future developments that are anticipated in the applications of this technology are considered.

Keywords: Enzymatic interesterification / Fat modification / Immobilised lipases / Speciality oils / *Trans* fats

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1 Introduction

Modification of fat melting properties is the keystone for the production of margarine and baking fats. Simple blending of two or more fats is often not enough to provide the right melting and/or crystallisation characteristics and hence the techniques of fractionation, hydrogenation and chemical interesterification (CIE) have been used for many years [1]. Hydrogenation has been in use for more than 100 years, whereas CIE is a more recent technology [2].

By the 1960s, the convenience of margarines and lower costs compared to butter had resulted in a large swing from dairy fats and there were also claims that they were more healthy. In many cases the fats used in these margarines were partially hydrogenated to obtain the correct melting properties and consequently contained high *trans* fat levels. The introduction of softer margarines resulted in a lowering of *trans* fat content but this was to a large extent negated by the increased consumption of these fats *via* bakery products. In conjunction with this, studies in the 1960s concluded that the cholesterol raising effect of hydrogenated fats was slightly lower than that of saturated fats, supporting to some extent the early health claims. However, in 1990, it was observed that, although *trans* fatty acids increase LDL cholesterol to a similar degree to saturated fat, they decrease HDL cholesterol relative to both *cis*-unsaturated and saturated fats. These initial studies have been followed up by a wide range of investigations where the overall conclusion remains that *trans* fatty acid consumption substantially increases the risk of coronary heart disease [3].

In the USA, figures from 2003 revealed that the main sources of *trans* fatty acids in the diet are baked goods (40%), animal products (21%), and margarine (17%). The other sources are snacks, candies, fried potatoes and household shortenings [4]. By contrast in France, 18% is derived from baked goods and 53% from dairy products [5], possibly reflecting different dietary habits in the two countries. Reducing the level of *trans* fats in food products would appear to have positive health consequences and the food industry has

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Abbreviations: **CIE**, chemical interesterification; **EIE**, enzymatic interesterification; **FFA**, free fatty acid; **LCA**, Life cycle assessment; **SFC**, solid fat content; **PV**, peroxide values

been actively investigating and applying options for this, particularly in light of current and forthcoming legislation in this area. The new *trans* fat labelling introduced in the USA in January 2006 created a wave of interest in enzymatic interesterification (EIE) for the production of *trans*-free margarine fats for the US market. Since then, a number of other countries have followed the lead of Denmark and the USA and have introduced similar regulations covering labelling of foods and control over *trans* fat content of foods. Food manufacturers have also made a number of changes in food composition and announcements on reducing *trans* fat contents. However, the options open to them were basically blending of fractionated fats or CIE, both of which have drawbacks and hence research was started into a third process, enzymatic interesterification to fill this gap.

2 Historical context

Enzymes have a long history of application within the food industry but their cost-effective application for bulk fat modification is a recent development. The normal mode of operation for enzymes within the food industry is to carry out hydrolysis of substrates to produce simpler molecules, *e.g.* starch to glucose, lactose to glucose and galactose and high molecular weight pectins to pectic acid. These are all operations within an aqueous environment and it was assumed that enzyme proteins could not function in the absence of water and/or in organic solvents. However, this assumption was found to be incorrect and dry enzyme powder products were found to be both active and stable in non-aqueous environments under certain conditions [6]. These and similar observations indicated the possibility that fats could be modified by lipase enzymes.

Research into lipase applications commenced in the early 1980s both at Unilever and Novozymes and a number of other companies including Fuji oil were also active in this segment. The application that received the most attention was the production of a cocoa butter equivalent (CBE) utilising the sn-1.3 specificity of certain fungal lipases. Macrae [7] describes the use of a lipase product absorbed onto a kiselguhr matrix to convert a mixture of palm mid fraction and stearic acid into CBE-like product containing increased levels of the desired triglycerides, 1(3)-palmitoyl-3(1)-stearoyl-2-monooleine (POSt) and 1,3-distearoyl-2-monooleine (StOSt). The immobilised lipase was prepared by preparing a slurry of the enzyme with kiselguhr and then adding a solvent such as acetone or an alcohol (ethanol or methanol) to precipitate the enzyme onto the inorganic particulate material. The immobilised enzyme was then separated by filtration, dried and then stored until required for use.

In use these catalysts had to be reactivated by the addition of 10% water to obtain high enzyme activity and, if stearic acid was used, it was presented to the enzyme dissolved in petroleum ether. A further complication of the process was that the water initially added to the enzyme would be gradually lost through hydrolysis as part of the triglyceride was converted into diacyl and monoacyl glycerol. Additional water addition was required to maintain enzyme activity and there were mixing problems when this was scaled up from the laboratory and pilot scale.

The type of immobilised enzyme made according to the above type of procedure would not be very suitable for operation in a packed bed column reactor needed for an industrial application. This is because the small particle size would have resulted in a high pressure drop if the height to diameter ratio of the column exceeded 0.5:1.0. An alternative mode of operation could have been in a fluidised bed reactor, but there, particle attrition could result in loss of enzyme from the carrier. As a final observation, the use of inflammable solvents complicates operations in a factory environment. For all these reasons, the kiselguhr-immobilised enzyme was difficult to use in practice and could only be tolerated for products with a much higher value than margarine fats. The overall result was that this promising application of a new enzyme technology languished and efforts were focused in other areas.

3 Alternative immobilisation systems

There are a number of alternative immobilisation systems which have been developed for lipase and other enzymes, including encapsulation, covalent linkage to carriers, adsorption onto polymer-based carriers or cross-linking using, for example, glutaraldehyde. The main reason to immobilise an enzyme is to separate it from the reaction products and allow for its reuse. Christensen *et al.* [8] list the issues that need to be addressed when choosing or developing an immobilization technique.

- The immobilisation should increase the thermal stability and working life of the enzyme
- The enzyme should maintain its activity throughout the immobilisation process
- The process of immobilisation should be robust and reproducible
- It should be cost efficient and not occupy too many production resources
- The materials and production equipment should be suitable for the production of food-grade enzymes
- From an application standpoint the enzyme should be physically robust.

The weaknesses in the immobilisation system as outlined by Macrae [7] were to a large extent addressed in the second generation enzymes where an ion exchange resin carrier was used instead of an inorganic absorbent. One of these products Novozyme 435 has become one of the most widely cited products used in a wide range of synthesis reactions. This *Candida antartica* B lipase is probably the most widely known lipase with over 23 000 listings in Google by May 2008 [9]. This form of immobilisation produces a lipase capable of

working in the presence or absence of organic solvents. However, the carrier material for this enzyme is expensive and the immobilisation procedure is not simple, resulting in a high cost product with its application restricted to high-value segments such as synthesis of cosmetic esters or production of omega 3-boosted fish oils. This area was extensively covered by a review by Hayes in 2004 [10].

A further weakness of the kiselguhr-based systems was the requirement to continually add water. The ion exchange resin-based enzyme products do not suffer to the same extent from this problem. Zhang *et al.* [11] describe the use of a *Rhizomucor miehei* lipase immobilised onto an ion exchange resin for the production of margarine fats. They used the enzyme for 10 batch reactions to interesterify a blend of palm stearine and coconut oil and observed that the enzyme was able to retain enough water within the granulate to carry out sequential reactions without the need to add further amounts of water.

Despite all these advances the resin-based enzymes were still too expensive for the production of bulk fats such as those used in margarines and shortenings, and further research was initiated to find alternative immobilisation systems that combined the low cost of the kiselguhr precipitates with the larger particle size and water-retaining efficiency of the resin-based products.

3.1 Production of a cost-effective immobilised lipase

The process that was developed and which is described by Christensen *et al.* [8] combines the use of an inorganic support material and an organic binder to produce a final enzyme particle. Typical particle sizes are in the range of 300–1000 μm, with a mean of 500–600 μm. The lipase is distributed throughout the particle in comparison to the resin-based enzymes, where the lipase is mainly found close to the particle surface.

In addition to this, by immobilising the enzyme in a carrier that could itself bind water, the small amount of water needed for the reaction to proceed can be carried and maintained within the enzyme product itself. Finally, as the enzyme is immobilised in a carrier, it remains there and will not be found in the final modified fat product. The lipase used was derived from *Thermomyces lanuginosus* with the transferred gene for the production expressed in *Aspergillus* sp. It was the combination of these two technologies that enables the production of a robust enzyme particle containing a cost-effective lipase. Prior to this, as indicated above, either the enzyme or the support material was too costly and resulted in a product that was not able to be used economically in the production of margarine fats.

Initial studies made on interesterification reactions focussed on the porosity of the particles and the particle size distribution. In use, the interesterification reaction produces fats with similar melting properties to those derived from CIE (Figure 1).

Zhang *et al.* [12] carried out a number of batch reactions using a 75:25 blend of palm stearine and coconut oil. They observed that in their batch system the enzyme was stable over an operating range of 55–85°C and that drying the enzyme from an initial moisture content of 6% to 3% had no negative impact on stability but did reduce the generation of free fatty acids (FFA) when the enzyme became dehydrated. They also commented that, using either a 1-kg or a 300-kg batch reactor,

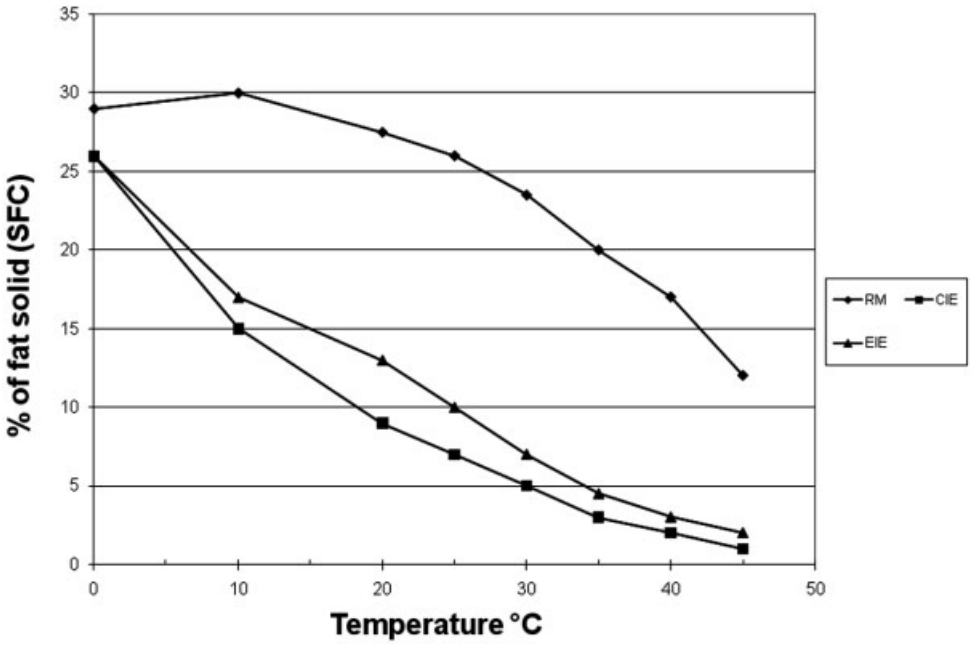

Figure 1. Solid fat content (SFC) of a 75% soybean: 25% fully hardened soybean oil before (RM) and after chemical (CIE) or enzymatic interesterification (EIE).

the enzyme appeared to be physically stable, managing 11 and 9 re-uses, respectively, without change in the particle size distribution. Also they were able to confirm the observation that water addition was not required, which dramatically simplifies the operation of an immobilised lipase process. This review concentrates on the application of immobilised enzymes in the production of margarine fats. However, the application of silica-immobilised *T. lanuginosus* enzyme (Lipozyme TL IM) for other interesterification processes has been reviewed by Yang *et al.* [13].

4 Scale up of the EIE process

For large-scale use, the batch mode of operation of the enzyme was not thought to be the most optimum as many cycles of use would be required and attrition of the enzyme particle would become a factor. Also there would be loss of enzyme through the batch system during filling and emptying of reactors, so a plug flow column system was developed to address these issues. In September 2001, through collaboration with Novozymes, Karlshamns (now AAK) in Sweden became the first company in the world to use the new immobilised enzyme Lipozyme® TL IM in the production of margarine fats. To simplify the installation process, the enzyme was supplied in a purpose-built reactor, which could be connected to the existing equipment in the factory (Fig. 2).

The 1-m^3 plug-in reactor was installed in September 2001 and went on operating for more than a year. This was the first "Plug&Play" reactor and it demonstrated that high enzyme productivity was possible when operating with incoming oil of very high quality. The results obtained from these large-scale production trials in Sweden were a major stepping stone for continued development work. Even though the development work faced a lot of hurdles and there were question marks about the critical influence of oil quality on enzyme productivity, the trials in Sweden showed without a doubt that a very acceptable production economy for EIE was possible.

In July 2002, ADM (Archer Daniels Midland Company) built the first commercial units in the USA for EIE using Lipozyme TL IM from Novozymes. They were the US pioneers in the production of *trans*-free margarines and shortenings.

The single reactor concept used for the first large-scale trials proved the concept of EIE, but the practical operation did not fit well into a continuously operating industrial setting. When in use the enzyme activity within a reactor gradually reduces as a function of the amount of oil passing down the column [14]. A typical inactivation curve for a single column operating at 70°C and with a 70:30 blend of palm stearine and coconut oil is shown in Fig. 3. To operate in this mode in a production environment would result in fluctuating output from the reactor, which is not desirable, and the logical solution is to have several reactors in series. This is the mode of operation that was introduced in 2002 and has remained the model followed.

By operating several columns in series, it is no longer necessary to have full conversion in the first column, as the load can be spread over several reactors. This results in a more even flow and output from the reactor series. In addition, as the first column in the series can now be operated down to effectively zero activity, the overall economy is improved. When the enzyme in the first column is exhausted, it can easily be replaced without disrupting production, as the remaining columns can still be utilised. A fresh charge of enzyme then replaces the exhausted material and this column is then connected to the end of the conversion line through the pipe work system. Spent enzyme catalyst is easily disposed of and the most convenient option is *via* the same route as used for bleaching earth.

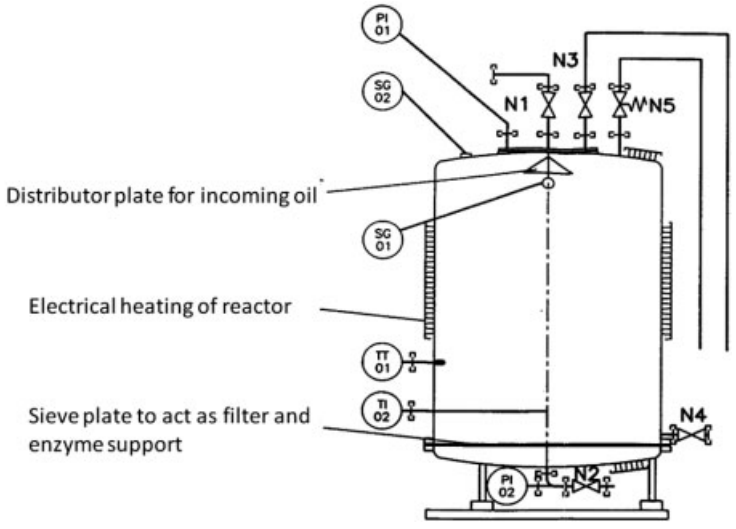

Figure 2. First industrial scale reactor containing 400 kg of immobilised lipase.

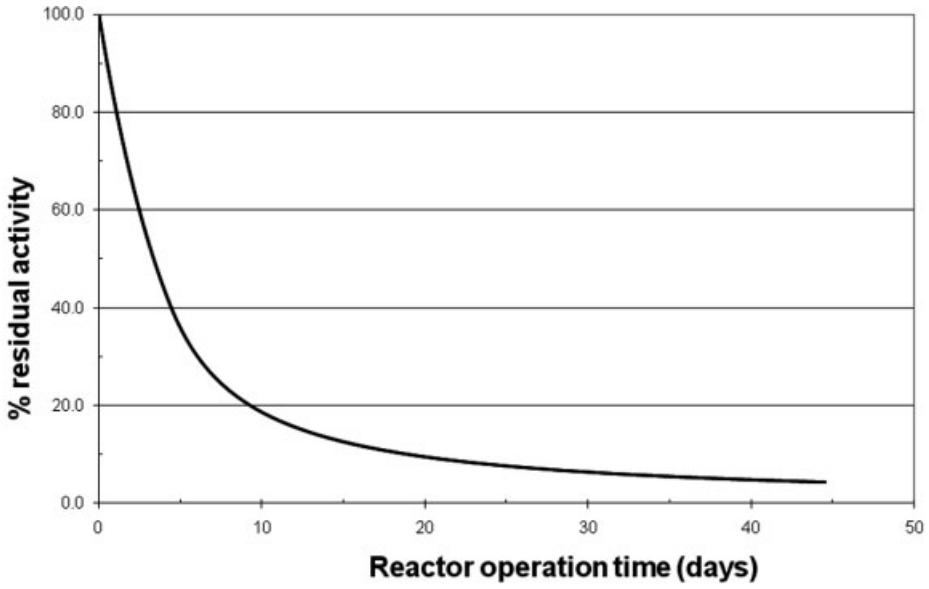

Figure 3. Activity decay *versus* time at 70°C for Lipozyme TL IM using a 70:30 palm stearine-coconut blend as feed stock for interesterification.

5 Controlling the quality of bulk fats produced by EIE

The aim of EIE is to produce a fat with the correct melting properties compared to that obtained by mainly CIE or hydrogenation. Although, the melting properties of fats produced by the two interesterification methods are similar, they are never 100% the same. The observed differences between the end result of interesterification of a fat blend by either method is partly due to the higher level of diglycerides produced in CIE and partly because the enzymatic process does not produce full randomisation.

When operating EIE, this can be overcome by fine-tuning of the fat blend to be used. In Fig. 4, the solid fat content (SFC) curve for three blends of palm stearine and sunflower oil are compared to a product obtained from CIE of a 30:70 blend of the same fats. The curve for the same proportion blend made by EIE is slightly below that of the chemical process. By adjusting the blend used for EIE in the direction of a 40:60 proportion, an exact match can be achieved.

Similarly, if the fats to be replaced are partly hydrogenated, then a similar strategy can be applied, but a wider range of fat blend compositions may be required to fully match the properties of the originally hydrogenated fat. For example, partially hydrogenated soya bean oil can be substituted by interesterification of fully hydrogenated soya bean oil with liquid soya bean oil. This produces the desired physical properties, does not include *trans* fats and does not increase overall the level of saturated fats.

In another example, the desired SFC profile to be matched was 75–85% solids at 10°C, 50–60% at 20°C, 26–34% at 30°C and 8–13% at 40°C. In this example, the fats available for inclusion were rapeseed oil, palm mid fraction, palm olein and palm stearine. Laboratory studies using a batch interesterification process were carried out. First of all, different blends of palm stearine and palm olein were interesterified and their SFC and melting profiles determined and compared to the desired result. Table 1 shows the results from these laboratory tests.

To match the desired properties more closely, the interesterified blend was then mixed with different amounts of palm mid fraction, and the properties determined (Table 2).

As results from these simple laboratory experiments can be directly converted to parameters to run the continuous interesterification, designing fat blends to reach a desired SFC profile is both simple and rapid if the correct raw materials are available. However, there are a considerable number of patents in this area and care should be taken when considering a particular blend.

5.1 Properties of margarines and shortenings made with EIE

EIE does not modify the oxidative stability of the component oils as there is no influence on the degree of saturation. In studies of the interesterification of palm stearine and sunflower oil, the residual tocopherol level was 30% of the starting level following chemical and 65% following EIE. In some cases, therefore, it might be expected that oxidative stability was improved or the amount of additional anti-oxidants required could be reduced.

Zang *et al.* [15] reported studies of the stability of four margarines produced by combination of a hardstock with sunflower oil. The initial composition of the hardstock was the same in all cases; however, one was enzymatically inter-

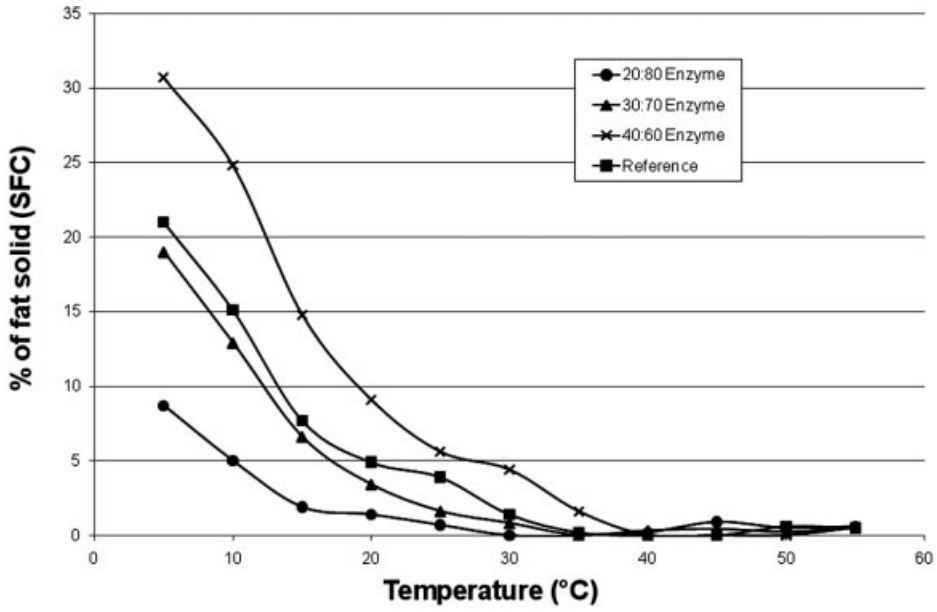

Figure 4. Comparison of EIE and CIE of palm stearine and sunflower oil blends.

Table 1 Achieved SFC profile for the final modified fat using different starting blends (EIE, enzymatic interesterification; PS, palm stearine; PL, palm olein).

Blend	(50% PS, 50% PL 60)		(60% PS, 40% PL 60)		(70% PS, 30% PL 60)		Specification
Process	Feed	EIE	Feed	EIE	Feed	EIE	
Slip point, °C	48.0	46.7	51.5	48.4	52.3	49.6	(42 – 45)
SFC							
10°C	61.0	69.4	66.4	73.9	71.1	77.8	(75 – 85)%
20°C	40.4	46.8	46.9	51.7	52.8	56.9	(50 – 60)%
30°C	22.7	24.5	27.8	28.2	32.9	32.6	(26 – 34)%
40°C	12.3	10.5	15.9	13.1	19.2	15.9	(8 – 13)%
45°C	7.6	6.1	10.5	9.0	13.4	10.8	
50°C	1.5	0.0	4.0	1.8	6.1	3.3	

Blend	(50% PS, 50% PL 60)		(60% PS, 40% PL 60)		(70% PS, 30% PL 60)		Specification
Process	Feed	EIE	Feed	EIE	Feed	EIE	
Slip point, °C	48.0	46.7	51.5	48.4	52.3	49.6	(42 – 45)
SFC							
10°C	61.0	69.4	66.4	73.9	71.1	77.8	(75 – 85)%
20°C	40.4	46.8	46.9	51.7	52.8	56.9	(50 – 60)%
30°C	22.7	24.5	27.8	28.2	32.9	32.6	(26 – 34)%
40°C	12.3	10.5	15.9	13.1	19.2	15.9	(8 – 13)%
45°C	7.6	6.1	10.5	9.0	13.4	10.8	
50°C	1.5	0.0	4.0	1.8	6.1	3.3	

Table 2. Properties of final fats obtained from blending of the interesterified material with palm mid fraction (PMF).

Blend	(70% PS, 30% PL)		Blending EIE with			Specifications
Process	Feed	EIE*	10% PMF	20% PMF	30% PMF	
% FFA		1.14				
Slip point, °C	52.3	49.6	49.3	47.6	*44.8*	(42 – 45)
SFC						
10°C	71.1	77.8	77.1	75.8	*73.9*	(75 – 85)%
20°C	52.8	56.9	55.6	54.1	*51.9*	(50 – 60)%
30°C	32.9	32.6	30.1	27.6	*24.6*	(26 – 34)%
40°C	19.2	15.9	14.6	12.7	*10.1*	(8 – 13)%
45°C		10.8	9.4	7.6	*5.4*	
50°C		3.3	2.2	0.9	*0*	

esterified to 80% and a second to 100%. A third hardstock was produced by CIE and the fourth by blending of the components. A number of physical parameters of the resulting margarines were studied on storage, including hardness, crystal form and dropping point as well as sensory evaluation. They concluded that stability increased at higher conversion degrees and that colour in the margarines derived from enzymatically produced hardstock was closer to that of the simple blend. Margarines produced from the enzymatically produced hardstock had lower peroxide values (PV) to that from the chemically interesterified hardstock but no differences in tocopherol content or stability were observed.

As well as margarine, shortenings and baking margarines are another major potential source of *trans* fat in the diet. To successfully replace partially hydrogenated fats in these products, baking performance has to be at least equivalent to the standard material.

Studies on baking performance of margarines produced from enzymatically interesterified hardstock were reported by Kirkeby [16]. When compared in baking tests to produce puff pastry, the margarine from the enzymatically interesterified hardstock gave superior results to that from CIE.

Additional studies made by Cowan *et al.* [17] have demonstrated that shortenings of good quality can be produced from enzymatically interesterified hardstocks using tropical oils as a base. Baking tests with some of these blends have been carried out with good results compared to the commercial shortening. Siew *et al.* [18] studied the interesterification of hard palm stearine with canola oil with the aim of producing a range of different margarine and fat products. They observed that by varying the proportions between the two fats they could produce modified fats with physical properties suitable for the production of stick margarine, shortenings, puff pastry margarine and vanaspati.

While the number of studies carried out are insufficient to conclude that margarines and shortenings made from hardstocks containing enzymatically interesterified fats are superior in performance to those made by CIE, they are at least equal. As tocopherol retention in these hardstocks is superior to those from CIE, the addition of anti-oxidants may possibly be reduced. This topic is currently being further investigated at a number of locations.

6 Factors influencing enzyme activity

Enzyme stability in aqueous media is influenced primarily by temperature and pH and to some extent by the presence of the substrate. Heavy metals and oxidising chemicals will also have a negative impact on enzyme stability. In non-aqueous environments, enzyme stability has already been shown to be different and part of the development of this technology was the elucidation of the roles that traditional factors for enzyme inactivation would play. Although lipases were known to be more thermostable in the absence of water, many of these determinations were made over short time periods and did not involve continuous operation. Initially enzyme stability was determined by incubating samples of the immobilised enzyme product in oil at different temperatures for a fixed time period. Following the incubation, residual interesterification activity was determined. These results demonstrated that at temperatures up 70°C the enzyme was stable but would start to lose activity when incubated at >80°C (Fig. 5).

Since that first observation, further research has demonstrated that 70°C is the optimum temperature for EIE, combining long working life with the ability to be above the melting point of all the fat blends likely to be used. Short periods of time at 80°C are also possible without serious loss of activity but at this elevated temperature, other oil quality factors also exert an elevated effect.

Fat blends for CIE have to meet various quality specifications to be used and the criteria applied here were investigated for their relevance to enzyme stability. Levels of some components such as FFA were not found to have a negative impact on enzyme stability but do indicate something about the processing history of the oil blend. In contrast for CIE, high levels of FFA cause increased catalyst consumption and hence raised losses through soap production, which always includes some neutral oil. Components such as phosphorus (incomplete degumming) and nickel soaps are capable of causing column blocking as the enzyme will act as a filter bed and must be kept at low levels.

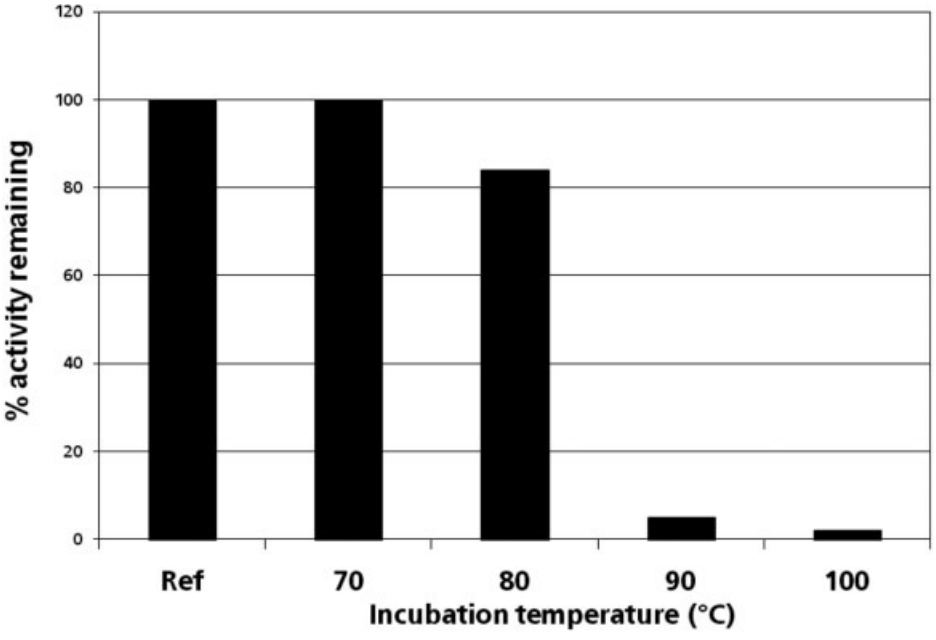

Figure 5. Residual enzyme activity following incubation of Lipozyme TL IM in oil for 168 h at different temperatures.

Two groups of compounds that have specific relevance for enzyme working life have been identified and are residual inorganic acids from degumming or bleaching earth together with citric acid from deodorization and oxidation compounds, specifically those measured as the PV. Oils coming from tropical crops, *e.g.* palm, have often been subjected to phosphoric acid degumming and or bleaching with acid-activated bleaching earth. Acid residues from these processes together with citric acid, transfer from the oil to the enzyme granulate, reducing the internal pH and thus the half-life of the enzyme. The lipase used has an alkaline pH optimum and it is at this pH that it is the most temperature stable. Reducing the pH within the micro-environment of the enzyme will thus have an impact on both enzyme activity and stability. Although the pH of oil is impossible to determine, the pH of a water extract of the oil can be made and the presence of inorganic and water soluble organic acids determined by measuring the pH of this extract. Studies have been made of enzyme half-life (the amount of oil passing over the enzyme that reduces the activity to 50% of the starting value) and the pH of a water extract of the oil. The effect that pH of the water extract on half-life (V1/2) is shown in Fig. 6.

The presence of oxidation compounds as determined by the PV also has a negative effect on enzyme stability. Comparison of oil PV with enzyme half life (V1/2) indicates that high PV are associated with reduced enzyme operating life (Fig. 7).

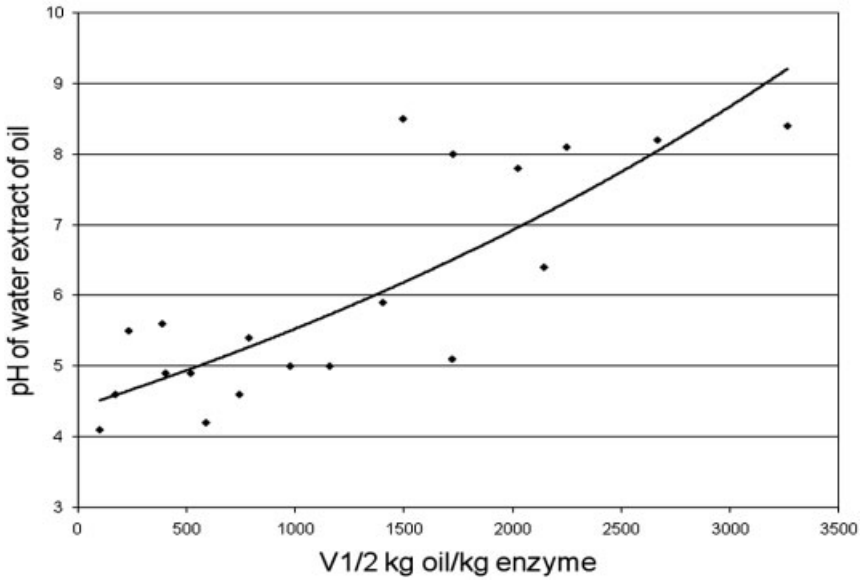

Figure 6. Influence of water extract pH on enzyme half-life.

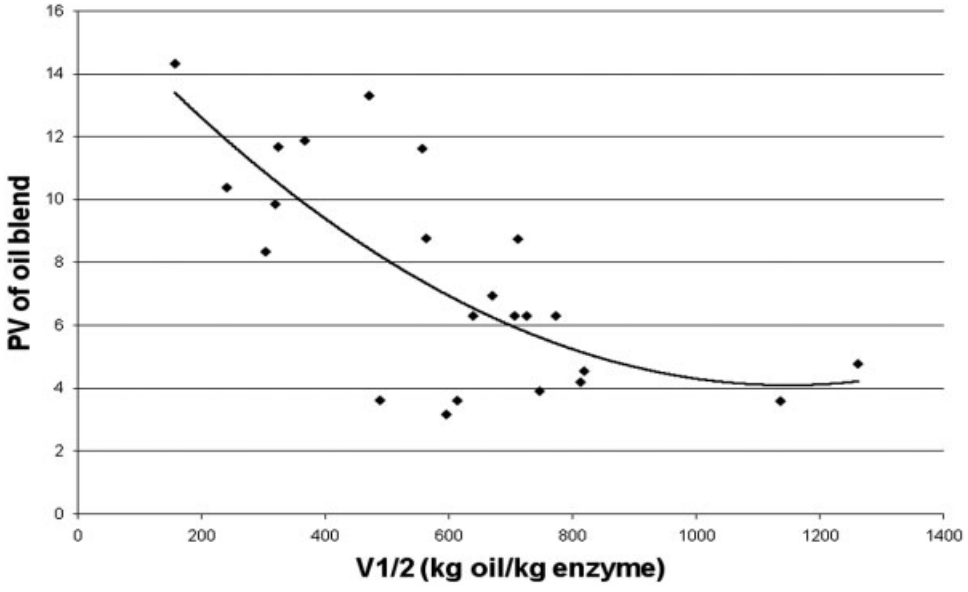

Figure 7. Influence of oxidation (PV) on enzyme operating life.

Within these two data sets there is also an interrelationship and further work is necessary to determine how the two factors are connected. However, it is clear that, for optimum operating life, residual acid and high PV must be avoided. This was also observed by Osório *et al.* [19] who commented that oil blends with a high oxidation risk gave lower enzyme productivity than blends where oxidation was absent.

Several strategies are possible to provide oils of the correct quality and to assess their likely effect on enzyme stability. Cowan *et al.* [20] used a multiple batch assay where one aliquot of enzyme is sequentially brought into contact with separate amounts of the oil blend to be tested and the SFC change monitored for each separate oil batch. From the derived SFC change, the V1/2 can be calculated. Bertram *et al.* [21] used a microtitre plate method where a lipase was incubated together with the oil and a colorimetric pH indicator. Inhibition of enzyme activity due to compounds present in the oil was revealed by a decrease in hydrolytic activity and reduced colour formation. They compared the results of this assay with performance on the immobilised enzyme in a column performance test and were able to rank oil blends with the best correlation being obtained for blends with the worst performance in the column test. However, in these studies they did not identify the causative compounds responsible for the activity loss.

While the values for PV and acid extract pH have been shown to be critical, they cannot always be controlled if the refining is carried out at another location. In some cases it may be required to re-process oils to ensure that the correct quality is achieved. Sodium carbonate has been applied (Cowan [20]) to remove acidity but this is more difficult to apply in practice. Lee *et al.* [22] proposed the use of a number of different pre-treatment materials to be used in a column including activated carbon, spent enzyme keiselguhr, and silica. They reported that for a soya bean oil-based blend, the flow rate required for full conversion was 10% of the starting level after 40 days for the standard blend. For the reactor in which the oil had received silica pre-treatment, the flow was 18% of the starting level, indicating almost double the residual enzyme activity at this time. Based on the type of materials being used, it would seem that they were primarily removing oxidation compounds. Ibrahim *et al.* [23] also focussed on oxidation compounds and proposed the use of spent enzyme catalyst as a means of removing enzyme inactivating species. Using molecular sieves, activated carbon and deactivated but unused enzyme they increased productivity (kg oil converted/kg enzyme) by a factor of 3.1-, 7.4- and 4.1-fold, respectively. From this they concluded that spent enzyme might be an effective purifying material but this assumes that the sites of absorption for poisons have not all been occupied during the normal use of the enzyme product. Overall, the best method for ensuring good enzyme productivity remains to supply the reaction with oil of the appropriate quality, low in oxidation compounds (<2 meq/g) and residual acids.

7 Environmental benefits of EIE

The environmental benefits of enzyme processing can be compared by a side by side examination. As hydrogenation is being used less frequently due to *trans* fat generation, we have compared the two interesterification processes. They are outlined in Fig. 8, which lists the individual steps and their relative energy consumption. CIE requires more processing steps, uses higher temperatures and therefore more energy. The product becomes discoloured and therefore requires bleach-

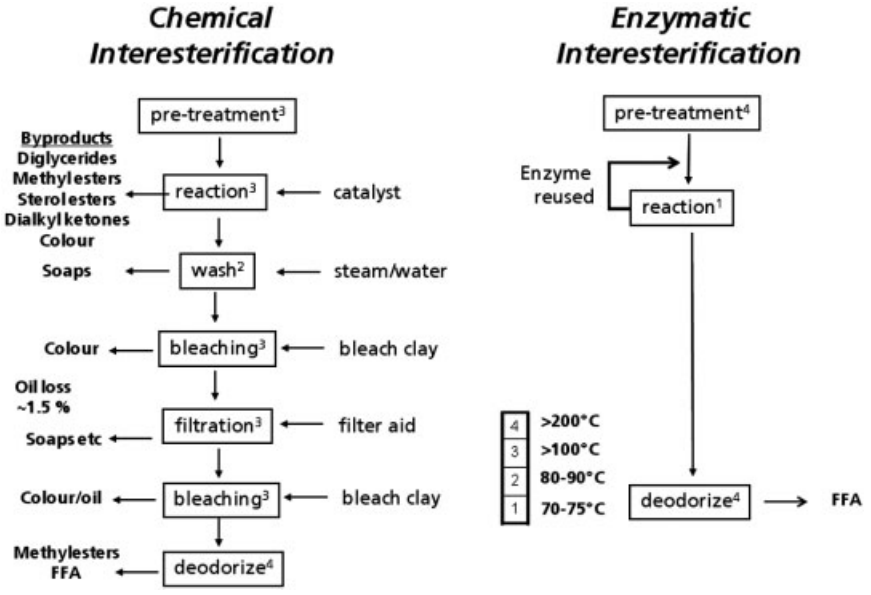

Figure 8. Comparison of process steps and temperatures for CIE and EIE.

ing plus some other purification steps. The enzymatic process is much simpler and takes place in a fixed-bed reactor under mild conditions. The end-product is ready to deodorise without purification.

As a result of the non-specific nature of the catalyst, by-product formation is greater, associated with increased yield loss, post process purification costs and waste generation, when compared to EIE. Colour formation is reduced and retention of tocopherol is increased in EIE due to the milder processing conditions of EIE.

Enzymatic processing of oils and fats should therefore be a more sustainable way of processing but the potential benefit needs to be quantified. Life cycle assessment (LCA) is a method that enables us to quantify the environmental impacts of new technology. It can examine all processes in the product chain – from raw material extraction through production and use to final disposal. LCA is used to compare environmental impacts of two or more alternative processes providing the same benefit to the user. LCA has been applied to three processes (degumming, interesterification and ester synthesis) used within the industry to examine the environmental impact of the conventional and enzymatic alternative. In each case, inputs and outputs have been quantified and the potential savings have been calculated in terms of energy, global warming contribution, acidification, *etc.*

For EIE, comparison was made between the CIE and EIE of soybean oil and fully hydrogenated soybean oil to produce a hardstock for margarine production. The analysis compared the parts of the life cycle, which differ between the two processes, therefore as they use the same raw materials, inputs relating to soybean farming, oil extraction/refining and hydrogenation are not included in the study. However, the pre-use purification required for both processes is included in the analysis. Catalyst production (sodium methoxide or enzyme) is included, as well as catalyst and by-product disposal. In the case of the spent enzyme catalyst the assumption used is that it is used as component of animal feed due to its protein and energy content.

We carried out the Life cycle assessment according to the procedures of Wenzel *et al.* [24] and the assessments are in agreement with ISO 14040 and are based on the principles described by Hauschild *et al.* [25], with the modelling carried out in SimaPro 6.0 software. Characterisations of environmental impacts are based on Eco-indicator 95 v2. The assessment covers four environmental indicators: global warming, acidification, nutrient enrichment and photochemical ozone formation. The conclusions of this study are shown in Fig. 9.

In this case, the major savings area is that of energy as the process runs at a lower temperature. A smaller benefit is that due to reduced losses, vegetable oil consumption per ton of produced margarine is lower. However, the values for disposal of the enzyme catalyst have been deliberately set on the conservative side and we can anticipate further savings when these are more accurately quantified. A second LCA analysis of the EIE process based on a margarine blend of palm stearine and palm kernel oil has been completed and it shows similar savings to those presented above. The study is undergoing external validation and the results will be presented in more detail at a later date.

8 Current and future developments

EIE has become an established technology within the oils and fats processing industry and is one of the few successful applications of immobilised enzymes. However, as evidenced by several review papers, particularly those of Xu [26] and

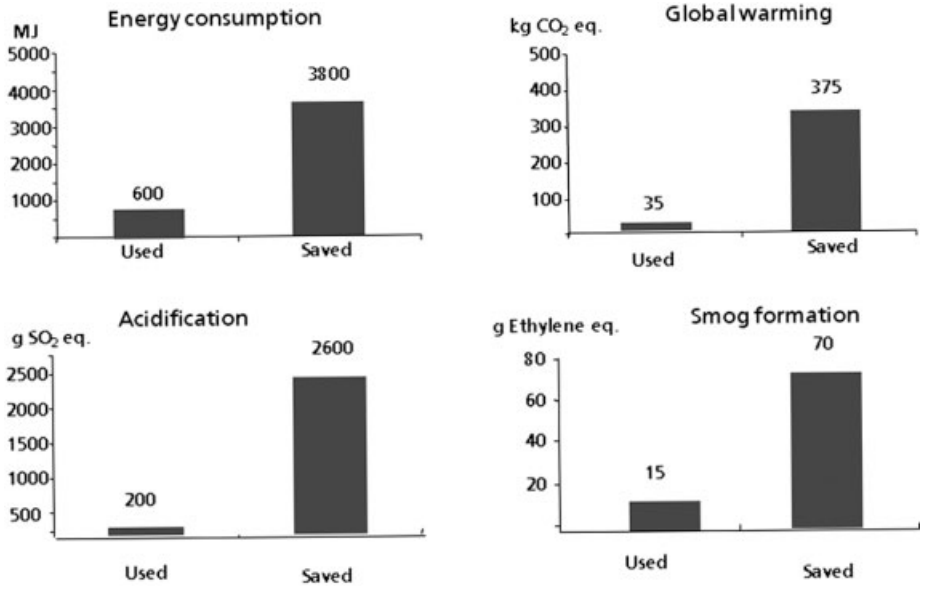

Figure 9. Comparison of the environmental impact of EIE and CIE.

Hayes [10], the number of potential enzymatic processes is much larger than that currently applied. The problems that had to be overcome in the development of this application and the new discoveries that had to be made concerning factors regulating enzyme activity and in producing cost-effective immobilised enzyme catalysts do go some way to explaining this observation.

However, a number of other potential applications are under consideration by different research groups including *trans*-esterification for biodiesel, production of speciality food fats and the use of water-based, non-immobilised enzymes in an oil-based medium and use of several lipases in one reaction.

8.1 Lipase-based *trans*-esterification for biodiesel

Current biodiesel production processes are largely based on an alkaline-catalysed production of FAME with glycerol as the main by product. In almost all cases oils of food quality are used due to the interference of FFA in the alkali-catalysed reaction. Nielsen and Holm [27] commented that, while there were more than 40 references to lipase-catalysed biodiesel, the overall conclusion was that the enzymatic process was not yet cost effective. One observed problem has been the methanol toxicity for the enzyme when the entire methanol is added at the start of the reaction. Watanabe *et al.* [28] demonstrated that sequential methanol addition could overcome this toxicity to some extent and this has become the method of choice.

One problem observed with second quality or used oils is the presence of oxidation compounds known from studies with EIE to have a detrimental effect on enzyme stability and activity. In addition, these oils often contain high FFA levels, which makes use of a conventional catalyst or acid pre-treatment too costly. Li *et al.* [29] have described a three-part system where tertiary butanol is used as a solvent for both methanol and these impurities to separate them from the immobilised lipase. Under these conditions, they were able to drastically extend the operational life of a pilot enzyme reactor. Obtaining the correct mixture between the oil, alcohol and enzyme phases is also critical in obtaining good results and this has been demonstrated by Jachmanián *et al.* [30]. The use of ethanol rather than methanol in a lipase catalysed *trans* esterification combined with the recycling of part of the produced FAEE, resulted in a superior efficiency of conversion. By focussing on oils of lower quality and using the insensitivity of lipases to the presence of FFA, it is anticipated that a cost effective biodiesel process can be developed. Current developments are focussed upon adapting the immobilisation technology used for EIE to other lipases, which are more efficient at producing ethyl esters from oils containing high levels of FFA. Substituting methanol by ethanol or other alcohols could improve the enzymatic conversion and provide biofuels of superior cold flow and/or lubrication properties.

8.2 Speciality fats

As described above the first application of immobilised lipase was in the field of CBE production using the inherent sn-1.3 specificity of the *Rhizomucor miehei* lipase. A subsequent development in this area was the synthesis of the 1,3-diacylglycerol products as described by Watanabe *et al.* [31]. One drawback of these forms of immobilised enzymes has been the support material, which is expensive but it may be possible to use less expensive supports of the type developed for the EIE process. Preliminary results with a silica-immobilised enzyme compared to the standard immobilised product on Duolite are shown in Table 3

These results, which need further and wider investigations, may offer the possibility to extend the range of speciality fat products by making it possible to produce structured fats for other applications where the cost of conversion is currently prohibitive.

Table 3. Acidolysis of a 25:75 blend of palm stearine and oleic acid using two forms of *R. miehei* lipase at 65°C for 48 h.

TAG type	Palm stearine	After acidolysis with standard enzyme	After acidolysis with enzyme on new carrier
PPP	62.7%	1.8%	4.5%
POP	13.2%	17.2%	29.1%
POO/OPO	4.4%	37.8%	36.3%
OOO	0.6%	25.0%	11.3%

8.3 Application of water-based enzymes in oils

Water-based enzyme products would not normally be expected to function in an oil-based environment and this is one of the justifications for using immobilised enzymes in interesterification. However, water-based enzymes have been successfully used for a number of years in enzymatic degumming where high speed mixing is used to form micro droplets of water, distributed throughout the oil mass and with the enzyme functioning at the oil-water interface.

The success of this application has prompted research into carrying out similar reactions in oils and with the enzyme finely dispersed into the oil mass. One model system used to investigate the possibility of using liquid enzymes in oil is the removal of FFA from an oil by reaction with glycerol or mono- or diglycerides. The reaction takes place under reduced pressure to remove the generated water and with initial dispersion of the enzyme throughout the reactants with the help of a high shear mixer. Distillate obtained from palm oil refining was mixed in a stochiometric ratio with glycerol and liquid *C. antartica* B lipase added at 50, 100 or 175 lipase units (LU)/g glycerol. Following high speed mixing with an Ultra turrax, the reaction was allowed to proceed with stirring under vacuum at 65°C. Samples were withdrawn at intervals for determination of the FFA level.

Despite relatively high water levels coming from the released water and the portion introduced together with the enzyme itself, FFA was quickly removed from the reaction (Fig. 10). Removal of the remaining FFA was not complete

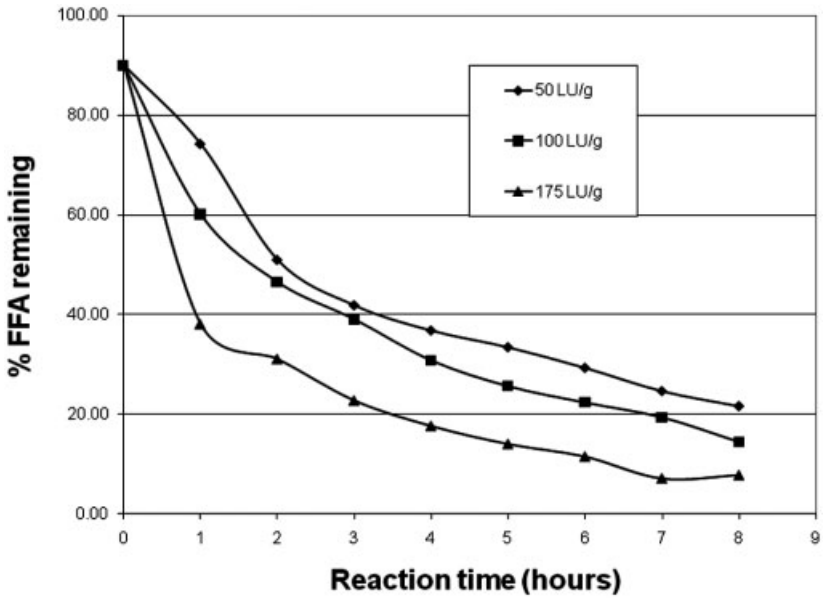

Figure 10. Removal of FFA by reaction with glycerol in the presence of a liquid lipase (Lipozyme CalB L).

when the reaction was terminated and this will be studied in future experiments. However, the possibility to use water-based enzymes in a non-aqueous environment has been demonstrated and this opens up the possibility of carrying out many different types of lipase modification without the need for an immobilised enzyme preparation.

8.4 Simultaneous lipase application

The development of robust enzyme immobilisation processes opens up the possibility of extending the range of enzyme types available. From there it is also feasible to consider the use of two or possibly more lipases in a single reaction. This is an area currently being researched but some preliminary ideas of how this could be beneficial were demonstrated by Ibrahim *et al.* [32]. They observed that, when a second lipase (either Novozyme 435 or Lipozyme RM IM) was added to an interesterification reaction with Lipozyme TL IM, there was on overall enhancement of the enzymatic reaction. This synergistic effect appeared to be mediated by the carrier, as the effect was more pronounced with the hydrophilic carrier of the Lipozyme RM IM. When the additional enzyme was presented in a non-immobilised form, synergism was also observed, suggesting that the immobilised enzyme had also acted as a carrier for the other lipase.

Conclusions

The negative health consequences of *trans* fats have driven a search for alternative processing methods for the production of fats with the correct melting properties. EIE is one of the best tools available to the food industry to produce modified fats without *trans* fatty acids.

However, there is not only a desire to eliminate *trans* fats in food but also to reduce and, as far as possible, replace chemical processing of foods around the world. The use of immobilised lipase, eliminates the need for chemical additions both in the interesterification process itself and in downstream processing. EIE has the lowest environmental impact of all the processing methods. This is a result of reduced generation of by-products and a corresponding reduced need for their removal, the lower operating temperatures and the reduced consumption of water during the process.

The margarines and shortenings produced by this method have physical properties at least equivalent to those produced by the alternative technologies and the process is simple to operate and reliable in large-scale production.

The research carried out in the development of this process will have a general beneficial effect on the industry because it will increase the range of applications by allowing for the development of more flexible and less costly enzyme product.

Conflict of interest statement

The authors have declared no conflict of interest.

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