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Industrial use of lipases to produce fatty acid esters

A wide range of fatty acid esters can be synthesised by esterification and transesterification reactions catalysed by lipases in non-aqueous systems. As well as modifications to the natural substrate triacylglycerol, many other commercially important substances can be produced using this enzyme technology, as lipases have been shown to have broad substrate specificities and can thus catalyse conversions with many other non-glycerol based compounds. In mild reaction conditions, emollient esters for use in the personal care industry can be made with excellent quality.

Development of economical processes was necessary to commercialise this technology for the manufacture of cosmetic ingredients. Enzymatic production methods had to be established which could compete against standard established chemical synthetic methods. Now relatively simple esters are being produced enzymatically. Extensive purification procedures to clean up products after high temperature reaction steps are not required. Product quality and specifications, such as the acid and hydroxy values of commercial ester products, could be improved. Examples of products manufactured with better purity and yields in economical and ecologically friendly processes are decyl oleate, cetyl ricinoleate, myristyl myristate and decyl cocoate.

Keywords: Lipase, fatty acid ester, production, skin care, emollient.

1 Introduction

Lipases are well known to catalyse hydrolysis, esterification and transesterification reactions on a wide range of substrates [1–3]. As well as hydrolysing their natural substrate triglycerides, this group of enzymes are very versatile in a variety of bioconversions on natural and synthetic substances, ranging from regio- and stereoselective hydrolyses to syntheses in organic, non-aqueous media. Many alcohols other than glycerol can be substrates for lipases, including simple *n*-alcohols and polyhydroxy compounds such as carbohydrates. Reactions can be carried out on the complete range of carboxylic acids from acetic acid to greater than C₂₀ fatty acids.

The mechanism of lipase catalysis is common to all variants of this enzyme class. The formation of an acyl-enzyme intermediate on a serine residue in the active site is the key step. A triad of amino acids is responsible for the *sn*-2 type reaction mechanism. Lipases act preferably at oil/water interfaces, where they attach themselves to emulsified oil droplets. They contain a hydrophobic region on the protein surface near the active site and often have a special loop called a lid which can open and close upon attachment to the water insoluble substrate. The 3D structures of some lipases have been determined in the last few years.

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The enzymatic hydrolysis is an equilibrium reaction performed in an excess of water to split the ester bonds. The “reverse hydrolysis” reaction, as it is sometimes named, is simply brought about by changing the equilibrium conditions. In media containing no or little water, alcohols act as nucleophiles and form esters instead of free fatty acids, when water breaks the acyl-enzyme intermediate. It is thought that the “interface” of organic/aqueous phase lies directly on the insoluble enzyme in this case and an alcohol is usually in excess of water.

The potential of this relatively easy to perform bioconversion for industrial purposes seems to be enormous, but there are only few examples of successful production processes in practice [4]. There are some important reasons for this.

Development of the enzymes into active and stable (bio)catalysts, usually by appropriate immobilisation techniques on support materials suitable for large scale production processes on a multi-tonne basis is very important. At the moment there are only a few off-the-shelf purpose made biocatalysts commercially available which are suitable for industrial production. The use of dried enzyme powders, although often reported in laboratory scale experiments, is generally unsuitable for large scale processing in non-aqueous media.

Process economics, which involves enzyme or biocatalyst costs related to productivity (kg product/g biocata-

lyst) need to be favourable and at least comparable with current chemical manufacturing practices. Enzymes and biocatalysts are (at the moment) more expensive than chemical catalysts, for example sulphuric acid or its derivatives which are commonly used as esterification catalysts. For relatively low priced bulk and semi-bulk products this necessitates recycling of the biocatalyst to be competitive. If enzymatically manufactured products are to compete with current market products, the price of products made in this new way should not be higher, otherwise they will not be considered as a real alternative. Also, new products must fall into the same price range of standard products with similar application properties. Many products can be synthesised using lipases, but value added by enzyme-catalysed production must be demonstrated, for example by improved effectivity in special applications.

Finding the right products to introduce on the market which fit into a marketing strategy which expands an existing product portfolio must also be considered. New products for cosmetics generally take a long time-to-market due to intensive application testing and can involve other costs (e.g. registration of new substances) which must be taken into account.

Another hindrance is the lack of commitment of chemical companies to establish a new biocatalysis-based production to complement or broaden their manufacturing base, although this situation seems to be gradually changing for the better.

The business unit Care Specialties of *Degussa* (formerly *Goldschmidt*) has developed economical enzymatic processes for the manufacture of fatty acid esters for use in cosmetic skin care products on a multi-tonne scale. Here it was possible to show the benefits of a low temperature biocatalysed production process to manufacture finer products for personal care use.

2 Development of industrial scale enzymatic ester production for the cosmetic industry

Fatty acid esters are important components in many cosmetic formulations. The range of applications is broad – pure oils for skin care (emollients), re-fattening agents, emulsifiers, thickening agents, solubilisers and (multi)functional additives are some examples [5].

Emollient esters are the second main component after water in cosmetic emulsions. They function to protect the skin against drying and to keep it smooth, soft and elastic. Therefore the synonymous term “emollient” is often used which literally means “softener”. The chemical composition of emollient esters can be widely varied to suit a variety of properties, which makes this class of compounds

very versatile. They are mostly made from straight or branched chain, saturated, unsaturated or functionalised alcohols and fatty acids. Their properties can be differentiated into basic properties – viscosity, surface tension, spreadability, polarity, pour point and cloud point – which have a strong impact on both the colloid chemistry (e.g. viscosity, stability) and the sensory properties (skin feel) of emulsion preparations [6]. Obviously for personal care applications high quality oils are needed. They must be clean in terms of light colour and good odour.

In the design and development of a biocatalysed process using an immobilised lipase in a non-aqueous environment, engineering principles involving heterogenous catalysts can be applied, but with some new restraints introduced for biocatalysts. Enzymes are known for their ability to catalyse reactions in neutral and mild conditions, but are unstable in harsh environments, such as extremes of pH and some organic solvents. An upper temperature limit must not be exceeded at any point or time in a production process, as the thermostability of enzymes places a restriction on the process. As in chemical reactions increasing the temperature raises the enzyme-catalysed reaction rate, but with enzymes only to a certain limit.

Considering these factors, several constraints in the development of an economical enzyme-catalysed manufacturing procedure need to be taken into account. However, the low temperature enzymatic synthesis shows considerable advantages over standard production methods. By fine tuning of an enzymatic production process, many steps in chemical catalysed esterification processes could be eliminated.

In an enzymatic ester synthesis, the reaction can be performed so that complete quantitative conversion is achieved and standard product specifications met, so that no cost intensive product purification is necessary. Many ester products for the cosmetic industry are defined with specific, low acid and hydroxy values, which are important for their application for example as cosmetic oils and waxes. In a new production method, these must be adhered to, otherwise the marketing and customer acceptance of products replacing standard products will be more difficult, as they will have to be declared as new products and accordingly tested and approved, even though they are the same chemically, before market introduction.

Many reported lipase catalysed syntheses are carried out in organic solvents. However, residues of organic solvents in products is undesired and many solvents which could be used are even toxic and are not allowed in processing procedures to make products for personal care applications. As well as that, removal of organic solvent traces in products requires extra expense and increases manufacturing costs. Solvent-free processes are thus desired.

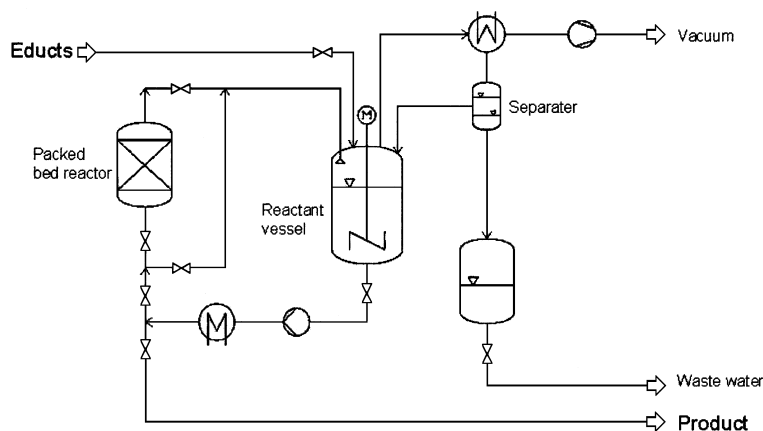


Fig. 1. Flowsheet of the packed bed reactor.

Ester synthesis conditions can be found in the literature [7, 8] and these have been further optimised for large scale production. The most efficient and economical way to commercially manufacture fatty acid esters for cosmetics is using an enzyme packed bed reactor connected in a loop to a vessel and recycling the reaction mixture which consists of only the reactants in an optimised composition [9]. Here maximum productivity is achieved at the highest possible concentration of substrates and products, which lies in the range 1.5–3.5 M for the emollient esters described in this paper. The desired conversion is achieved under vacuum to remove reaction water in esterifications, or alcohol in transesterifications if fatty acid esters are used as the raw material. In this way the reaction equilibrium could be successfully shifted so that at the end of the reaction, products meeting given specifications could be made without the need for any further purification steps. Reaction times of 4–8 h have been achieved by optimising the amount of biocatalyst. This also depends on the substrates, which react with different velocities, and efficiency of water removal, especially towards the end of the reaction. In the enzymatic

production, the reaction time makes up the bulk of total production time, which in general are shorter than in chemical processes due to avoidance of purification procedures. As the biocatalyst is contained in the packed bed, the biocatalyst is separated from products and reuse is easy (Fig. 1).

It was even possible in some cases to make improvements over the old specifications of ester products. The low temperature enzymatic production is more controllable as co-distillation of reaction components with the water is less problematic. For example, many raw materials for cosmetic fatty acid esters are blends of fatty acids extracted from plant oils with specified compositions. A partial fractionation due to inefficient distillation conditions could alter the fatty acid distribution in the final ester product, resulting in batches which do not meet product specifications.

The effect on process economics by reuse of immobilised enzyme biocatalyst, i.e. the cost reduction of biocatalyst per kilogram product, a very important factor when considering production of low and medium cost bulk or semi-bulk commodities, is clearly evident in Fig. 2. As long as

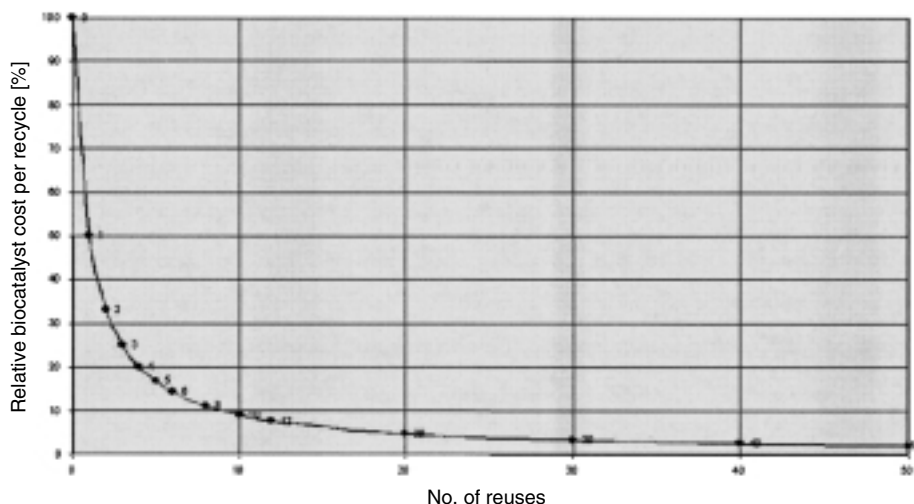


Fig. 2. Effect of recycling on biocatalyst cost.

the biocatalyst remains stable and active in the process conditions, biocatalyst costs can be minimised to an acceptable fraction of product cost. This development has now enabled economical production of substances not previously commercially available. Several hundred tonnes per year of a variety of products are currently being manufactured, and the market and product range is expanding.

3 Advantages of enzymatic production over chemical manufacturing methods

The low temperature biocatalysed esterification process has many advantages over conventional chemical methods. These employ strong acid, alkaline or metal-based (Lewis acid) chemical catalysts which function only at high temperatures and result in unwanted side reactions causing darkening and formation of odours. Chemically catalysed esterifications are usually conducted at temperatures above 160 °C. They are unselective, so when more than one alcohol group is present there will always

be a mixture of esters in the products. Functionalised components, *e.g.* containing double bonds, are especially susceptible to side reactions under such conditions. In the cosmetic industry oleic acid esters are very important products widely used in many formulations for skin care. The presence of by-products resulting from reactions other than esterification is thus highly undesired. By-products and catalyst residues in a chemical esterification must be removed in extensive post-reaction purification procedures to produce clean, high quality emollient esters fit for use in cosmetic products. Neutralisation, steam treatment, deodourising and bleaching stages must be performed to produce acceptable, light coloured products. Even so, remains of odours may be detectable in products. Bleaching can also be considered as a compendium of chemical reactions to remove discolourations. Commonly used are bleaching earths and hydrogen peroxide which, although fulfilling their function, generate other impurities. Bleaching reactions are generally undefined, thus it is not known what type of contaminants could remain in products, even after extensive clean-up

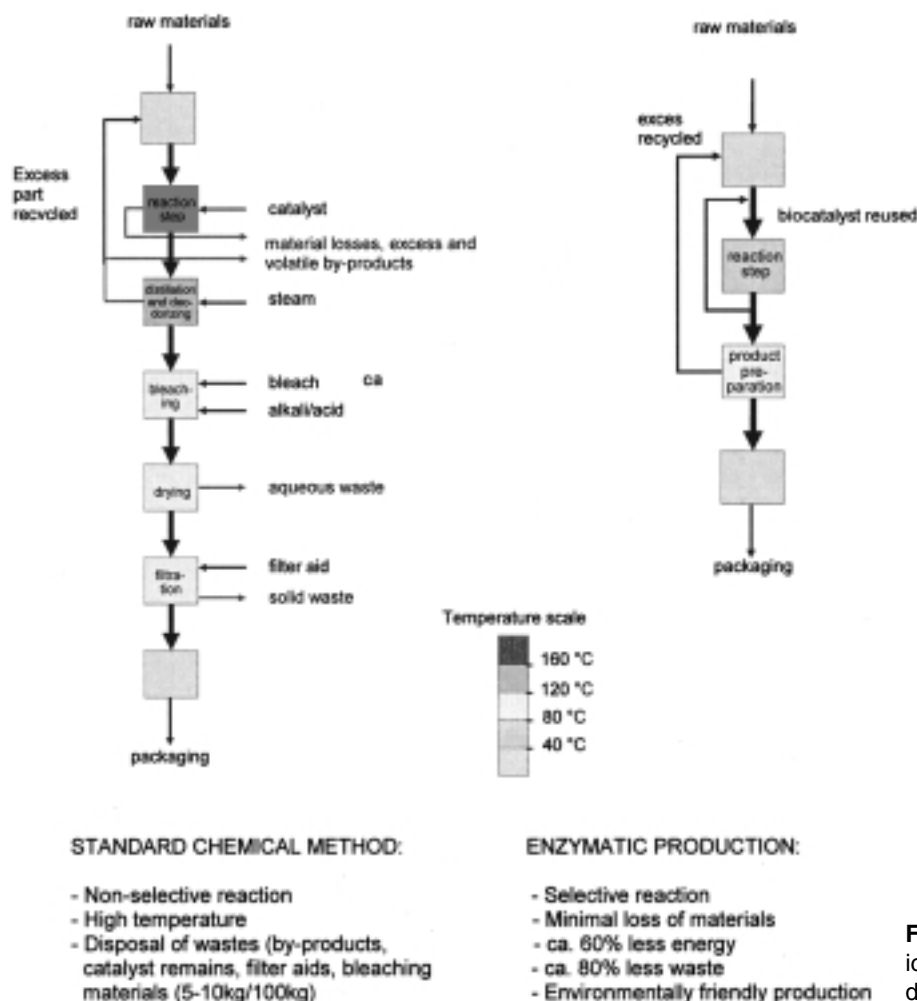


Fig. 3. Schematic diagram of chemical and enzymatic production procedures.

procedures. All in all, post reaction purification stages can lead to a loss of up to five percent of product yield and generate extra wastes which have to be disposed of.

All these steps can be avoided by a selective enzymatic ester production, where only esterification is catalysed. The avoidance of the formation of by-products in the low temperature synthesis means that purification procedures are no longer necessary. In particular, as there is no bleaching, no bleaching residues or remains of bleaching agents contaminate the products. Thus cleaner products with better colour and odour, which are important criteria for cosmetic products, and with narrower specifications due to the more efficient synthesis can be obtained.

4 Products for the cosmetic industry

The superior quality achieved by enzymatic production over standard products, such as better (lighter) colour, less (more natural) odour, fewer by-products, narrower specifications achievable and higher content of actives due to better selectivities are given in more detail in the following examples. The differences between conventional chemical and enzymatic syntheses are exceptionally shown with decyl oleate and cetyl ricinoleate.

Decyl oleate is widely used in many cosmetic products, as it has a composition similar to that of natural skin lipids. It is an emollient also with special properties of re-fattening and good solvency for lipophilic active ingredients.

Here the low temperature production and thus minimisation of side reactions and formation of by-products, especially detrimental dark colouring, eliminating the need for bleaching, is particularly noteworthy. The possible side reactions in a chemical esterification process due to the presence of the double bond in oleic acid are numerous: oxidative and reductive cleavage, cis-trans isomerisation, double bond rearrangements, dimerisation, metathesis, peroxidation and breakdown of peroxides are some of the possible reactions which reduce the purity and quality of

oleic acid based products. After cleaning-up procedures to produce a light coloured product, complete removal of all reaction by-products is unlikely.

Technical grade oleic acid (70–80% oleic acid content only) used contains considerable amounts of polyunsaturates, namely linoleic and linolenic acids, which are even more susceptible to undesired degradative side-reactions.

In gas chromatography – mass spectrometry (GC/MS) analyses of chemically synthesised decyl oleate in laboratory experiments using p-toluene sulphuric acid and tin oxalate, some by-products found were azelaaldehyde decyl ester, formed from splitting of the oleic acid at the $\Delta 9$ double bond, and unidentifiable olefinic and aldehydic compounds. In the product made by esterification using p-toluene sulphuric acid, decyl esters of the acid chemical catalyst were positively identified. Didecyl ether was also shown to be present as a by-product, which demonstrates the non-selectivity of the chemical reaction. Using catalysts based on organo-tin derivatives, formation of tin salts of oleic acid is possible, which then have to be removed. This is obviously not a comprehensive summary of possible contaminants, but just examples of some by-products which are formed, and further investigations are being carried out.

Cetyl ricinoleate is also currently available on the market for cosmetic applications. Ricinoleic acid is a natural, vegetable based unsaturated hydroxy fatty acid. Its cetyl ester is a wax ester which melts at skin temperature, giving a silky but non-oily feel to the skin. It is mostly applied in cosmetic formulations for dry and sensitive skin and lipsticks. However, due to the hydroxy group on the fatty acid high temperature synthesis of this product is also accompanied with a competing esterification reaction. As well as other side reactions, such as rearrangements and dehydration, considerable amounts of dimers of ricinoleic acid are formed, as can be seen in the gas chromatography analysis (Fig. 4). Enzymatic synthesis is regioselective, resulting in a product with a high content of the target

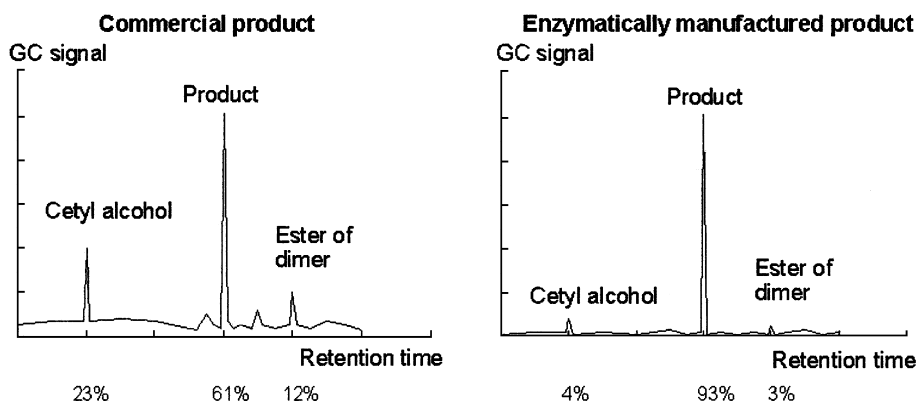


Fig. 4. Gas chromatograms of a typical commercially available cetyl ricinoleate compared with the enzymatically manufactured product.

Tab. 1. Comparison of purity of myristyl myristate obtained by enzymatic synthesis with chemically synthesised commercial products.

Property	Enzymatic production Average of 3 batches	Commercial product 1	Commercial product 2	Commercial product 3	Commercial product 4
Acid value [mg KOH/g]	0.5	0.2	0.8	0.2	0.6
OH-value [mg KOH/g]	6.0	9.6	8.7	14.0	13.0
Saponification value [mg KOH/g]	130.0	129.0	134.0	132.0	134.0
Peroxide value [mVal O ₂ /kg]	<0.1	0.1	1.7	<0.1	<0.1
Melting point [°C]	41	40	40	40	39
Colour [Hazen at 50 °C]	28	59	32	cloudy at 50 °C	cloudy at 50 °C
GC: MM-content [%]	96.4	90.7	88.6	94.5	80.1
GC: C ₁₄ OH-content	1.4	4.2	0.8	4.7	4.1
GC: non-identifiable peaks	0.4	1.4	2.2	0.4	4.2

molecule and, perhaps more importantly for its use in cosmetics, a low cetyl alcohol content. Other commercially available cetyl ricinoleate products can contain anything between 10 and 30% cetyl alcohol. Cetyl alcohol is used in cosmetic formulations as a viscosity enhancer for cremes, so undefined amounts of this alcohol in this ester product causes difficulties in formulating skin care products. The new enzymatically manufactured product is now specified with a defined low cetyl alcohol content, which will allow development of new types of formulations with this product as the rheology effects of cetyl alcohol in cosmetic emulsions can now be controlled.

Myristyl myristate is a simple ester in terms of structure and is widely used in a broad range of cosmetic products, but is also shown to be synthesised enzymatically with a better quality than standard products on the market. The table of specifications and compositions of some commercially available products which were analysed by gas chromatography shows clearly the enzymatically made product with an actives content of myristyl myristate higher than commercial products (Tab. 1). Also noteworthy to mention about product purity is that the commercial products have considerably higher hydroxy values due to the myristyl alcohol content and which contain traces of unidentified compounds too. Even more unexplainable is the finding that two commercial products were not clear liquids when molten at 50 °C.

Decyl cocoate is a new emollient ester being introduced on the market made exclusively by enzymatic esterification. It is, of course, as its name implies, a completely natural product based on coconut oil fatty acids. This natural blend is composed of 50% lauric acid, 20% myristic acid, ca. 20% other saturated fatty acids and ca. 10% unsaturates, mainly oleic acid. It has the special property of low spreadability

which makes it suitable for topical applications. As it spreads only slowly over the skin, decyl cocoate is designed for inclusion in formulations targeted for special uses where localised application of functional and active compounds is desired. One example of this is around the eyes, where diffusion of a cosmetic product could cause unpleasantness if it spreads onto sensitive tissues. Tab. 2 shows a model formulation developed by the applications group of the business unit Care Specialties.

Decyl cocoate can also be made enzymatically with conservation of the coconut aroma present in the raw fatty acid starting material, demonstrating the exceptional mildness of the enzymatic synthesis. Coconut oil fatty acids produced from the triglyceride oil contains the natural odour components of the coconut. These aroma components are (un)naturally destroyed in a high temperature chemical esterification. However, due to the problems associated with perfuming of cosmetic formulations, the oil with natural aroma did not yet find any commercial success.

5 Conclusions

The advantages of enzymatic syntheses, even of relatively simple esters, is here clearly to be seen. For personal care applications, where product purity is very important, biocatalysis can play an important role in future developments. Improvements in the manufacture of standard products on the market and new products with special properties which could not previously be made have been clearly demonstrated. Besides the development of economical processes, the environmental friendly nature of enzymatic production methods should also not be neglected. Reduction in energy usage and disposal of waste materials generated are points to note in establishing ecologically favourable “green” sustainable technology.

Tab. 2. Model cosmetic formulation containing decyl cocoate.

O/W Eye Care Cream		%
A		
TEGO® Care 450	Polyglycerol-3 Methylglucose Distearate	3.0
TEGIN® M	Glyceryl Stearate	2.0
TEGO® Alkanol 18	Stearyl Alcohol	2.0
TEGOSOFT® CT	Caprylic/Capric Triglyceride	7.5
TEGOSOFT® DC	Decyl Cocoate	9.5
Avocado (Persea Gratissima) Oil		
B		
Glycerin		3.0
Water		69.3
C		
LACTIL	Sodium Lactate; Sodium PCA; Glycine; Fructose; Urea; Niacinamide; Inositol; Sodium Benzoate; Lactic Acid	1.0
Panthenol		0.2
Tocopheryl Acetate		0.5
Z		
Preservative, Parfum		q.s.

In getting products in a form for presentation on the market and selling to customers many other factors necessary also need to be mentioned. For the successful commercialisation of enzyme technology in industry, the support of management in all areas of research, engineering, production and marketing is essential. To many, enzyme technology is a new and unknown concept, and the cooperation within chemical companies to understand and learn new (bio-)chemistry principles internally along with the commitment to developing products made in this way are often barriers to overcome. Also, in this particular case the parallel development of applications of new products is just as important. Emollient esters are a part of a cosmetic product and the benefits of the improved manufacturing method needed to be specifically demonstrated. The work of the applications groups in the business unit Care Specialties of *Degussa* in developing new formulations was also essential to the success. Once this has been achieved, there is another aspect to be considered which is the acceptance of products on the market place by consumers. Here the virtues of enzymatic production for commercial products for the personal care industry are extremely well demonstrated.

In the future, further examples of the selectivity of low temperature enzyme-catalysed reactions to make cleaner and more effective products in more efficient production processes are certain to arise. Polyfunctional products with regioselectively synthesised ester groups giving unique properties over non-selectively made products

may be an area where enzyme technology can show real benefits in the manufacturing industry. New types of formulations and applications for skin care using nature-based products made in a natural way using biocatalysts can be envisaged.

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[Received: June 12, 2003; accepted: August 22, 2003]