INFORMACIÓN

Biotechnology of lipids: The use of lipases for the structural modification of fats and oils.

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RESUMEN

Biotecnología de lípidos: Uso de lipasas para la modificación estructural de grasas y aceites.

La industria de alimentos utiliza procedimientos de modificación química y enzimática de las grasas y aceites con el propósito de mejorar sus características organolépticas y/o nutricionales. Debido a que los procedimientos químicos presentan muchas limitaciones e inconvenientes, la aplicación de tecnologías que utilizan enzimas aparece como muy prometedora para el desarrollo de nuevos tipos de grasas y aceites. Las reacciones de interesterificación catalizadas por lipasas permiten la modificación estructural de los lípidos al cambiar en forma selectiva la composición de los triacilgliceroles bajo condiciones de reacción muy suaves y controladas. Este trabajo revisa las principales características de las lipasas y sus reacciones, el uso de lipasas para la realización de reacciones de interesterificación, y las ventajas y limitaciones del uso de lipasas inmovilizadas para la modificación de grasas y aceites. Se discute, además, la aplicación de lipasas de diferente procedencia para la hidrólisis de grasas y el uso potencial de lipasas estereoespecíficas para la producción de lípidos estructurados.

PALABRAS-CLAVE: Biotecnología — Información (artículo) — Lipasa — Lípido — Modificación estructural.

SUMMARY

Biotechnology of lipids: The use of lipases for the structural modification of fats and oils.

The food industry has introduced chemical and enzymatic procedures for the modification of fats and oils to allow the improvement of their organoleptic and/or nutritional properties. As chemical procedures have many drawbacks, the application of enzyme technology shows as a promising approach for the development of new fats and oil-derived products. Lipase-catalyzed interesterification reactions allow the structural modification of lipids by selectively changing the composition of a triacylglycerol under very mild and controlled conditions. This work reviews the main characteristics of lipase reactions, the use of lipases for interesterification procedures, and the advantages and limitations of lipase immobilization for the enzymatic modification of fats and oils. The use of lipases from different sources for fat splitting and the potential application of stereospecific lipases for the production of structured or designer lipids is also discussed.

KEY-WORDS: Biotechnology — Information (paper) — Lipase — Lipid — Structural modification.

1. INTRODUCTION

The Food Industry is now facing the important challenge to produce more and improved nutritional products for a consumer population which is becoming increasingly health conscious (Fitch-Haumann, 1993). The oleochemical industry has not remained indifferent to this challenge, its interest being now centered around the chemical modification of natural fats and oils triacylglycerols from plant or animal sources to obtain products having a wide range of applications (Kinsella, 1988). The term "designed" or "structured" lipids has been introduced by the fats and oils chemists to name those triacylglycerols obtained from the structural modification of natural fats or oils by chemical and/or enzymatic procedures (Kennedy, 1991). Sabatier and co-workers laid the foundations of the structural modification of lipids by chemical methods when they introduced the hydrogenation of oils by catalysis. From Sabatier´s procedures to our days a number of different technologies have been developed for the industrial modification of lipids. Chemical modification of fats and oils are at present the procedure mainly used by the oleochemical industry to obtain the myriad of different products which are now commercially offered. However, in spite of their extensive application chemical procedures have many drawbacks, being necessary the expenditure of energy (heat and/or vacuum or pressure), the maintenance of costly installations and equipments, and having relatively low efficiency in terms of product yielding. At present the interest of industry is centered in the development of manufacturing procedures involving low energy consumption, more simple equipments, and the highest yields.

Concerning these requeriments, biotechnology shows as an approach which is beginning to be exploited by the oleochemical business promising to provide new procedures for the chemical modification of fats and oils. Biotechnology encompasses the use of living systems including plants, animals, microbes, or any part of these organisms, for the production of useful products. We have been using biotechnology for centuries. In the food sector, cheese and bread manufacture could be classified as biotechnological processes because they are fermentations that rely on living organisms —bacteria and yeast— for commercial production (Harlander, 1989). Three biotechnologies have, at present, major potential application to the fats and oils industries (Krishnamurthy, 1992): crop biotechnology, microbial biotechnology and enzymatic biotechnology. Crop biotechnology requires longer to achieve the same results than the other two approaches. Microbial biotechnology, the use of live organisms to modify base materials, can results in the manufacture of products having new and different characteristics. However, the increased use of microbial biotechnology will depend on the identification of low-cost

substrates, the development of genetically modified microbes and effective downstream processes. Enzymatic biotechnology involves the isolation of a required enzyme, generally a lipase, from its natural source —of animal, vegetal or microbial origen— and its application to produce a desired end product. From these three biotechnologies, the last one, i.e. enzymatic biotechnology, appears at present as the most serious alternative for the structural modification of fats and oils. Although enzymes have been used for centuries to modify the structure and composition of foods, they have only recently become available on a large scale for application in modern industrial processes (Neidleman, 1991). One of the more recent development in this area is the use of lipases to modify the structure and composition of fats and oils (Haraldsson, 1992a).

The use of lipases for the modification of fats and oils is not recent. Since Claude Bernard discovered that pancreatic extracts were capable of hydrolyzing neutral fats in 1849, a number of lipases obtained from different sources have been described for their catalytic activity, chemical specificity and operation conditions. The enzymes which have the largest number of potential application in the oleochemical industry are extracellular microbial lipases (Casey & Macrae, 1992). In the past, because of low fermentation yield, microbial lipases have been expensive in comparison with other extracellular enzymes such as proteases and carbohydrases. However, recently the successful application of recombinant DNA technology has enabled enzyme manufacturers to produce microbial lipases in high yields (Wasserman et al., 1988). Several types of microbial lipases are now commercially offered under different trade mark names. This review is focussed on the application of lipases for the modification of fats and oils, mainly for food manufacture, and the developments which have led to full scale implementation of this technology.

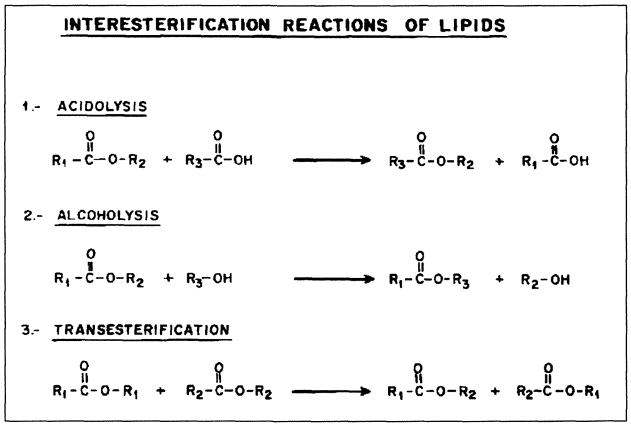
2. GENERAL CHARACTERISTICS OF LIPASES.

The literature distinguishes between esterases and lipases. According to Huang & Dooley (1976), an esterase is an enzyme which catalyses the hydrolysis of a carboxyester bond, whereas a lipase is an enzyme which catalyses the hydrolysis of a specific type of ester bond, i.e. mono, di or triacylglycerols (Zaks & Klibanov, 1985; Tsujita et al., 1990). Lipases are often named triacylglycerol acylhydrolases (EC 3.1.1.3) and have many biochemical features in common with other enzymes, such as proteases. However, their characteristic property is the capability of acting on lipids at the lipid/water interface. Lipases are the only enzymes that have their fastest turn-over rate in the presence of a substrate-water interface, and also display activity in an isotropic medium of small molecular aggregates (Leger & Charles, 1980), and show weak activity towards monomeric carboxilic esters (Semeriva & Desnuelle, 1979). The natural and most specific substrates for lipases are long-chain fatty acid esters of glycerol. Short-chain fatty acid esters of glycerol are hydrolyzed more rapidly than long-chain esters, but are less-specific substrates for lipases. The rate of hydrolysis is fastest for triacylglycerols, followed by di- and monoacylglycerols, the latter being split very slowly (Brockerhoff, 1968). Triacylglycerols without branch points near the ester bond are hydrolyzed regardless of the state of hydration, but double bonds near the ester bond impede hydrolysis. Electrophilic substituents on the alcohol side of the ester bond enhance the rate of hydrolysis (Semeriva & Desnuelle, 1979).

Lipase activity is not dependent on the amount of substrate, but instead depends on both the concentration of the substrate interface (expressed as surface area, e.g. m^2/L emulsion) and the quality of the interface between the insoluble substrate and the aqueous phase. It is at this interface that lipase acts to hydrolyze its substrate (Tietz & Shuey, 1993). Lipases may catalyze several different reactions with rather wide substrate specificity (Huang & Dooley, 1976). In terms of their substrate selectivity, lipases usually can be classified in three main groups. They can be sn-1, 3-regiospecific or non-regiospecific toward triacylglycerols, and they can possess selectivity toward particular types of fatty acids. Additional lipases are known that show specificity for partial glycerols and unsaturated fatty acids (Hills et al., 1990). Although there have been reports in the literature of an effective lipase with specificity for the sn-2 position, such a lipase has not yet become available commercially (Semeriva & Desnuelle, 1979).

Porcine pancreatic lipase and lipases obtained from some yeasts and fungus are examples of sn-1, 3-regiospecific enzymes (Haraldsson, 1992a). These lipases hydrolyse triacylglycerols to yield free fatty acids, 1,2 and 2,3-diacylglycerols and 2-monoacylglycerols. The remaining 2-monoacylglycerols, probably because of steric hindrance cannot be hydrolyzed by lipase, but can spontaneously isomerize to 3-monoacylglycerol on which the enzyme can act. Lipases obtained from the yeast *Candida rugosa* are examples of non-specific lipases. They can catalyze the complete splitting of triacylglycerols to free fatty acids and glycerol. On the other side, the lipase obtained from the yeast *Geotrichum candidum* is an example of a lipase possesing fatty acid specificity toward long-chain fatty acids containing a cis-double bond in the 9-position (Jensen et al., 1983). Moreover, most lipases possess enantioselectivity towards various ester, alcohol and acid substrates. This characteristic has been utilized for laboratory and industrial resolution of racemic mixtures of substrates, being highly important in organic synthesis (Jones, 1986).

The natural function of lipases is to catalyze the hydrolysis of acylglycerols and other fatty acid esters, but this reaction may be easily reversible. If the water content of the reaction medium is reduced, some lipases will continue to catalyze reactions where interesterification begins to dominate over hydrolysis (Sonnet & Gazzillo, 1991). Consequently, lipases may be also efficient catalysts for a wide range of interesterification reactions (Sonnet, 1988). These reactions, shown in Fig. 1, may be classified in three main types: acidolysis, alcoholysis and transesterification (Quinlan & Moore, 1993). Although these overall schemes show only the changing of acylglycerol composition, for these reactions to proceed at appreciable rates water is still necessary, albeit at very low concentration. According to several authors the optimal moisture content control is the key factor for success with the various interesterification processes catalyzed by lipases in organic media (Klibanov, 1986; Goderis et al., 1987).



Acidolysis involves the reaction of a fatty ester with an acid, usually a fatty acid. This reaction can produce an equilibrium mixture of reactants and reaction products, or it can be driven to completion by physically removing one of the reaction products. For example, coconut oil and stearic acid can be reacted so as partially to supplant the short-chain acids of the coconut oil with the higher melting stearic acid. The reaction can be carried out under temperature and vacuum conditions such that C6 to C10 acids volatilize as fast as they are freed from the glycerol and are thereby removed from the reaction batch.

Alcoholysis is the reaction of a fat (or oil) with an alcohol. A triacylglycerol can be reacted with a methyl or ethyl alcohol to produce the corresponding methyl or ethyl ester and free glycerol in virtually stoicheometric yields. Again the reaction can be driven to completion by removal of the more volatile reaction products. The production of mono- and diacylglycerols from fat is actually an alcoholysis reaction, with glycerol as the alcohol (the reaction is named glycerolysis). Sorbitol and sucrose esters are also made by alcoholysis as well as by direct esterification with fatty acids. A mutual solvent for fat and alcohol is frecuently required for such reactions.

Transesterification (or ester interchange), the final class of interesterification reactions, involves the exchange of acid radicals from one ester to another. In other words, the fatty acids in a fat are mixed up or rearranged. When it pertains with triacylglycerols, transesterification can be of two types, random or directed. In random rearrangement the fatty acid radicals freely move from one position to another in a simple triacylglycerol or from one triacylglycerol to another. As the fatty acids rearrange, they reach an equilibrium which is based on the composition of the starting material and is predictable from the laws of probability. Directed transesterification refers to the modification of randomized triacylglycerols by up-setting the equilibrium mixture, usually by cristalization of one or more of the higher melting components. Obviously transesterifications employing random lipases offers little advantage over standard chemical techniques. The use, however, of sn-1, 3-specific lipases in acidolysis allows a limited range of triacylglycerols to be produced which then can be separated using physical methods. Figure 1 summarizes the main interesterification reactions.

3. CHEMISTRY OF LIPASE ACTIVITY

Lipases are belived to act as catalysts by a mechanism in which a serine hydroxyl group of the enzyme is acylated by either free fatty acid or ester to give a fatty acyl-enzyme complex. Under non-aqueous conditions this complex can be attaked by various nucleophiles including alcohols, thiols, amines and hydrogen peroxide. As to specificity, lipases which preferentially cleave primary HO-group esters are distinguished from those enzymes which indiscriminantly hydrolyze all three ester bonds of acylglycerols. The lipase secreted by the human pancreas, which is very similar to that secreted by the swine pancreas, is the most studied. It is a glycoprotein containing 420 amino acid residues with a molecular weight of 48 kdal (Vandermeers et al., 1974). Serine 152 is identified as the nucleophilic residue essential for catalysis. It is located in the larger N-terminal domain at the C-terminal edge of a doubly wound parallel beta-sheet and is part of an Asp-His-Ser triad. The hydrolytic site is covered by a surface loop and is thus inaccessible to solvent (Winkler et al., 1990). The enzyme cleaves the following types of acylglycerols, with a decreasing rate of hydrolysis: triacyl-> diacyl->> monoacylglycerols. The third acyl residue of a triacylglycerol is cleaved only after acyl migration, which requires a longer incubation time. The smaller the size of the oil droplet, the larger the oil/water interface and, therefore, the higher the lipase activity. This relationship should not be ignored when substrate emulsions are prepared for the assay of lipase activities.

A model for pancreatic lipase has been suggested to account for the enzyme property to be active on the oil/water interface. The lipase "hydrophobic head" is bound to the oil droplet by hydrophobic interactions, while the enzyme active site alings with and binds to the substrate molecule. The splitting of the ester bond occurs with the involvement of serine, histidine and aspartate residues on the enzyme by a mechanism analogous to that of the proteolytic enzyme chymotrypsin. Because the aspartate residue is located in hydrophobic surroundings, it can polarize the functional groups in close proximity to it. Thus, histidine acts as a strong general base and abstracts a proton from the OH-group of the neighboring serine residue. The oxygen remaining on the serine residue thus becomes a strong nucleophile and attacks the carbon of the carbonyl group of the ester bond of the triacylglycerol. At this stage a free fatty acid (the first product) is released and the transient covalently-bound acyl enzyme is formed. A deacylation step follows. The previous position of the fatty acid is occupied by a water molecule. Again the histidine residue, through support from the aspartate residue, serves as a general base, abstracting the proton from water. This is followed by nucleophilic attack of the resultant OH- ion on the carbon of the carbonyl group of the acyl enzyme, resulting in free enzyme and the second product of the enzymic convertion (the fatty acid-free acylglycerol). Although active site of lipase resembles that of serine proteinase, both enzymes show some dissimilarities. Lipases have also a leucine residue within this site in order to stablish hydrophobic contact with the lipid substrate and to align it with the active center. Figure 2 shows a hypotetical model of pancreatic lipase fixation on an oil/water interface.

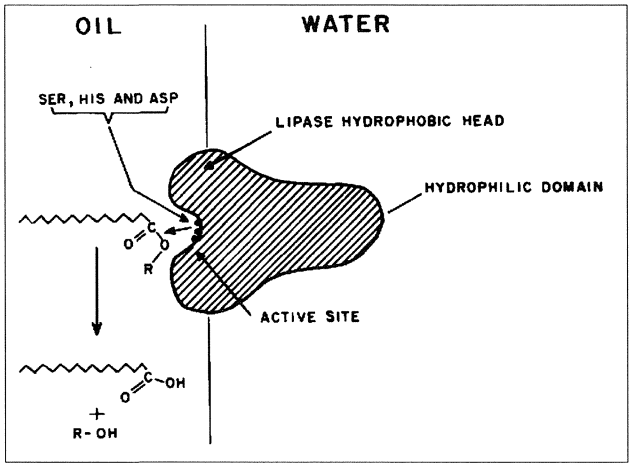


Figure 2
Hypotetical model of pancreatic lipase fixation on an oil/water interface (modified from Brockenhoff, 1968).

4. LIPASE FAT AND OIL SPLITTING

The use of lipases for the hydrolysis of fats and oils is today an alternative approach to the conventional fat splitting by chemical procedures, i.e. hydrolysis by high-pressure steam splitting and alkaline hydrolysis (Mukherjee, 1990). These methods offer high degree of hydrolysis and short reaction times (2 to 3 hours), however both methods need high capital investment and energy cost. In addition, the product obtained by the steam-splitting method is usually dark colored and must be distilled. Also, the resulting soap from the alkaline saponification process need acidification to release free fatty acids. These drawbacks favours the potential application of lipases for fat splitting. Lipase fat-hydrolysis needs much milder conditions (30°C to 50°C at atmospheric pressure) therefore provides a suitable procedure for the obtention of free fatty acids from high polyunsaturated oils, such as marine oils, which are extremedly sensitive to temperature-dependend oxidation (Nieto et al., 1993).

Several lipases are now commercially available from different sources, the microbial lipase obtained from the yeast *Candida rugosa* being the most suitable for fat splitting (Haraldsson, 1992a). This lipase is now successfully used by some Japanese factories to perform the hydrolysis of linseed oil, and also for the production of soap powder. *Candida rugosa* lipase has been used by Linfield and co-workers (Linfield et al., 1984) for the hydrolysis of olive oil, coconut oil and tallow. Khor and co-workers (Khor et al., 1986) have studied the kinetic and the effect of different solvents on the hydrolysis of palm oil, palm olein and stearin, soybean oil, corn oil, and penaut oil catalized by *Candida rugosa* lipase.

An ideal lipase for use in fat splitting should possess several kinetics and molecular characteristics. It should be non-specific toward all positions in the triacylglycerols; otherwise, the non-enzymatic acyl migration would become the rate-determining step in the hydrolysis rather than the enzymatic process, as occurs with the 1,3-specific lipases where 2,1-migration must be avoided to preserve the enzyme specificity. Lipases should not display substrate specificity, and should also hydrolyse a variety of fats and oils with high degree of efficacy. It should be thermostable for enabling the hydrolysis of solid fats, such as lard and tallow, which need temperatures above 50°C to reach the liquid state, allowing the lipase action. The commercial price of the lipase is a crucial factor when the feasibility of an enzymatic fat splitting procedure is compared with a conventional one.

5. APPLICATION OF LIPASES FOR INTERESTERIFICATIONS OF FATS AND OILS

Ester interchange reactions can be catalyzed chemically using sodium, sodium-potassium alloy, or sodium alkoxide. These procedures, which are widely used by the oleochemical industry, result in random acyl migrations and exchanges between the different positions in the triacylglycerol being difficult the evaluation of the process in terms of product yielding. Lipases due substrate and position specificity, and mainly as immobilized enzymes, appear like ideal tools for the production of new types of triacylglycerols. However, a number of problems are associated with the use of lipases in nonaqueous media. The first of these is "solubilizing" the enzyme in the oils. Second, for kinetic reasons, lipases show generally lower rates in interesterification than in hydrolysis. Commercially, therefore, at the levels required to give acceptable reactions rates, the lipase must be recorverable for reuse. Although techniques are available to solubilize free enzymes and recover them, for practical reasons immobilized enzymes systems are preferred.

A variety of supports have been used to immovilize lipases. Membranes have been employed where there is an advantage if substrate and product species are separated. Generally, however, porous particulate materials providing high surface areas per unit volume have been used. Typical examples would be ion exchange resins, silicas, macroporous polymers, clays, etc. To function effectively, any support must satisfy a number of criteria (Quinlan & Moore, 1993): a) the lipase must be absorb irreversibly in a suitable conformation to function; b) the pore sizes should not be so small so as to unduly restrict reactions rates; c) it must be robust within the intended process; d) it must not contaminate the product and; e) ideally it should be inexpensive. In addition to the advantages mentioned above, immobilized systems can show improved enzyme properties both in intrinsic activity and, of particular importance in term of fat processing, improved thermal stability. The immobilization of enzymes allows higher stabilization, continuous processes, the possibility of re-use and lowers the cost, being these factors decisive for the economical evaluation of the application of immobilized enzymes for industrialization processes (Roig et al., 1990).

Different immobilized lipases have been experimentaly used for triacylglycerol interesterification, however Novo Nordisk (Denmark) offers a commercial inmobilized 1,3-specific microbial lipase obtained from the fungus *Mucor miehei* immobilized on a macroporous anionic exchange resin carrier (Lipozyme IM-20). Lipases are proteins and as such will begin to denature when temperature begin to exceed 40°C in aqueous media. Lipozyme IM-20 show good thermal stability at temperatures required to process

most oils and fats and therefore most suitable for biomodification of triacylglycerols. Several examples for the application of immobilized lipases are described by the literature. Macrae (1983) describes the use of a sn-1,3-specific *Aspergillus niger* lipase immobilized on kieselguhr to produce a cocoa butter-equivalent product from dipalmitoyl-2-monoolein, obtained from palm oil, reacting with stearic acid (acidolysis) or tristearin (transesterification) to yield 1(3)-palmitoyl-(1)-stearoyl-2-monoolein and 1,3-distearoyl-2-monoolein, which are the main components of cocoa butter. Neidleman and Geigert (1984) have reported the random transesterification of coconut and olive oil (1:1) by an immobilized non-specific *Candida rugosa* lipase impregnated with 10% water. Goderis et al. (1987) have reported the acidolysis of triolein with palmitic acid by a 1,3-specific *Rhizopus arrhizus* lipase immobilized on Celite. Hansen and Eigtved (1986) showed that Lipozyme IM-20 allow the solvent-free transesterification of 40% soy bean oil and 60% beef tallow at 60°C both batchwise and in a packed-bed reactor. Goh and co-workers (1993) have examined the effect of different solvents in the transesterification of cocoa butter by immobilized 1,3 specific fungal lipases. Mutua and Akoh (1993) have also studied the effect of immobilized lipases in the structural modification of biosurfactant phospholipids by incorporation of n-3 polyunsaturated fatty acids such as eicosapentaenoic acid (C20:5, EPA) and docosahexaenoic acid (C22:6, DHA). Table I shows examples for lipase specificity.

***Table I.* EXAMPLES FOR LIPASE SPECIFICITY**

SITE OF HYDROLYSIS AT TRIACYLGLYCEROL	LIPASE SOURCE
acyl residues in positions 1 and 3	human and porcine pancreas, *mucor miehei, pseudomona fragi, rhizopus arrhizus*
acyl residues in positions 1, 2 and 3	*candida rugosa aspergillus flavus*
oleic and linoleic acid in positions 1, 2 and 3	*geotrichum candidum*

6. LIPASES FOR THE PRODUCTION OF NUTRITIONALLY IMPROVED FATS

Immobilized lipases can be used to impart new and improved physical and nutritional properties to fats and oils. In relation to physical properties, enzymic interesterification can improve structuring and/or melting behaviour of fats for application in products such as spreads, nondairy creams, and confectioneries. The use of such triacylglycerols in food products can be applied to improve organoleptic properties (for example, to reduce waxiness or to impart cool melting), to reduce the total saturated fat content of a product, and in some cases can provide the technical edge necessary to develop a new product. Specific functional triacylglycerols also can be made which at low levels can improve the processing or storage properties of the product, for example reducing fat recrystallization in confectionery products. Of increasing interest is the application of enzymic interesterification to improve the nutritional properties of dietary triacylglycerols. This stems from the growing realization that both the site of esterification of fatty acid in the triacylglycerol molecule and the molecular weight distribution of component triacylglycerols can affect their digestion and metabolism (Borum, 1992). Enzymic interesterification is particularly suited to the production of such triacylglycerols and already has been used to generate a number of novel products with commercial potential. Such is the case of the named "structured" or "designed" lipids obtained from lipase fats and oils modification, from which some examples are summarized bellow.

Saturated fatty acids of chain length C16 or greater are less well absorbed than their unsaturated counterparts, particularly when the former are esterified on the sn-1,3 position of the dietary triacylglycerol (Mascioli et al., 1988). Saturated fatty acids in the sn-1,3 position are released by pancreatic lipase during the digestive process and have a tendency to form insoluble, poorly absorbed soaps with calcium. The same fatty acid esterified in the sn-2 position of the dietary triacylglicerol is efficiently absorbed as the sn-2 monoacylglycerol, in part because soap formation is avoided. This is a major reason why human milk fat, which contains a large proportion of the major saturated fatty acids in the sn-2 position, is so well absorbed by infants in comparison to vegetable fat sources of similar fatty acid composition where the saturated fatty acids are mainly esterified to the sn-1,3 positions (Borum, 1992). Researchs of the Colworth Laboratory (Unilever) (Quinlan and Moore, 1993) have used this principle to develop a novel triacylglycerol based on vegetable fats which closely mimics the fatty acid distribution of human milk fat. This triacylglycerol can only be made by the use of a 1,3 specific lipase, by reacting tripalmitin with unsaturated fatty acids. The product obtained from such procedure is currently under commercial development for infant nutrition under the trade name "Betapol".

By the same principle, triacylglycerols containing long-chain polyunsaturated fatty acids (C18:3; C20:5 or C22) in the sn-2 position and medium-chain fatty acids (mainly C8:0 and C10:0) in the sn-1,3 position have successfully obtained by our group (Valenzuela et al., 1993a). These triacylglycerols are formed by reacting sn-2 monoacylglycerols, obtained from vegetal or fish oil triacylglycerols treated with a sn-1,3 specific lipase (Lipozyme IM-20), with C8:0 and C10:0 fatty acid ethyl esters obtained from distilled fractions of coconut oil. The reaction between the fatty esters and the monoacylglycerols was carry out by the same lipase but in a non-aqueous media. This procedure allows the obtention of "structured" triacylglycerols with potential application in clinical nutrition (Heird et al., 1986). Medium-chain fatty acids provide rapid delivery of energy because they are easily absorbed by the intestine cells (enterocytes), secreted directly to the portal vein, and rapidly oxidized by the liver. Instead, long-chain polyunsaturated fatty acids esterifiying the sn-2 position of the structured triacylglycerol are reesterified at the intestinal cells an secreted as triacylglycerol-rich quilomicrons allowing the supply of essential fatty acids to the periferic extrahepatic tissues (Schmidl et al., 1988).

Therefore, triacylglycerols containing mixed fatty acids have potential applications in the development of new clinical products for the parenteral or enteral nutrition of surgical, polytraumatized, burned or septic patients. Figure 3 shows a schematic procedure for the obtention of structured triacylglycerols. Figure 4 shows the metabolic fate of medium-chain fatty acid triacylglycerols, long-chain fatty acid triacylglycerols, and of a structured mixed medium and long-chain fatty acid triacylglycerol.

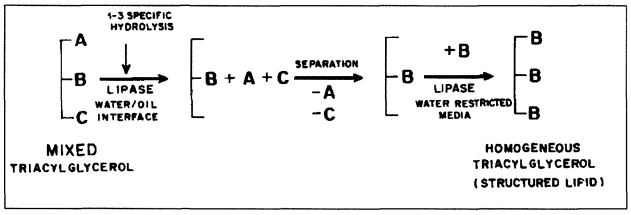


Figure 3
Schematic procedure for the obtention of stuctured triacylglycerols by specific lipases.

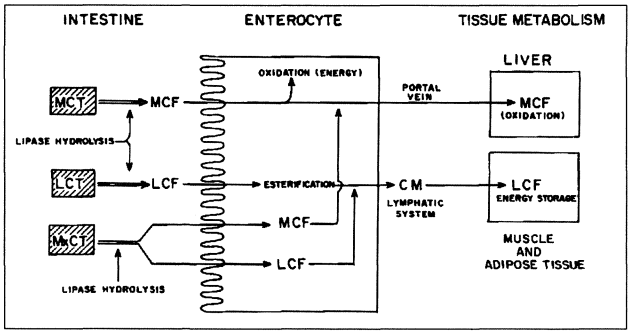


Figure 4
Metabolic fate of medium-chain fatty acid triacylglycerols (MCT), long-chain fatty acid triacylglycerols (LCT), and of a stuctured mixed medium and long-chain fatty acid triacylglycerol (MxCT). MCF: medium-chain fatty acid. LCF: long-chain fatty acid. CM: chylomicron.

Another potential and practical application for lipase-catalyzed interesterification reaction is in the obtention of polyunsaturated fatty acid-rich triacylglycerols (Haraldsson, 1992b) and for the development of new food emulsifiers and surfactants (Kawahara, 1993). Nutritionally important polyunsaturated fatty acids, particularly those containing more than two double bonds, are found in natural fats and oils but at relatively low concentrations. In nutritional aplications where it is desirable to maximize the intake of a particular polyunsaturated fatty acid, a large quantity of undesirable fatty acids are consumed. For this reason there is growing interest in the obtention of polyunsaturated fatty acid-rich triacylglycerols for specific nutritional interventions. A unique property of lipases relevant to this area is their ability to discriminate between fatty acids on the basis of the position of double bonds and fatty acid chain length. This property of lipases has been used in the selective hydrolysis of polyunsaturated-containing oils, and a resultant concentration of some particular fatty acids such as alpha or gamma linolenic acid (C18:3, n-3 or n-6, respectively), EPA or DHA in the remaining monoacylglycerol (Valenzuela et al., 1993b). The polyunsaturated fatty acid-rich triacylglycerol may be obtained by reacting monoacylglycerols, obtained after lipase treatment, with ethyl or methyl esters of the same polyunsaturated fatty acid (Uauy & Valenzuela, 1992). This last reaction may be catalyzed by the same sn-1,3 specific lipase but in non-aqueous media as applied for the obtention of structured triacylglycerols described above.

7. FUTURE TRENDS FOR LIPASE APPLICATIONS

Lipase interesterification technology has become now a commercial reality and it can be anticipate that this technology will be an important tool for the oleochemical industry. Currently applications are only confined to products with high added value due to the cost of the process. However, the development of less expensive and novel processes will bring this technology into major consumer products. Enzymic interesterification technology is now being used as a research tool by food technologists anf food ingeniers to explore the relationship between the structure and function of triacylglycerols, and for the development of nutritionally and healthy improved novel food products. The improvement of the chemical and organoleptic characteristics of natural fats and oils, or the development of enterely new nutritional or industrial products, is a fascinating challenge for food scientists and technologists and for the oleochemical business.

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