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The potential use of lipases in the production of fatty acid derivatives for the food and nutraceutical industries

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Abstract

The potential of lipases (E.C.3.1.1.3.) as biocatalysts for the production of fatty acid derivatives for the food and nutraceutical industries, such as flavouring esters, fatty acid esters of antioxidants and structured lipids, is enormous, mainly due to their high regio- and stereo-selectivities, in addition to the other well-known advantages of enzymatic processes. The replacement of chemical catalysts by lipases presents great benefits in terms of the nutritional properties of the obtained products and environmental care. The reactions performed for the production of these compounds, as well as the best operation conditions, the biocatalysts used, reactor types and operation mode, are addressed in this review.

Keywords: fatty acids derivatives, lipases, nutraceutical industries.

INTRODUCTION

Lipases

In the food industry, biocatalysis offers a clean and ecological way to perform biochemical reactions under mild conditions and with high specificity. Additionally, enzymes are accepted as process aids in most food legislations. Lipases (triacylglycerol acyl-hydrolase, E.C. 3.1.1.3) are one of the most useful enzymes for the food industry (Jaeger and Eggert, 2002). Indeed, they have broad substrate acceptance, stability in many organic solvents, do not need cofactors, robust lipases are commercially available and many tools for its optimization and production are available (Sandoval, 2012). Reactions catalyzed by lipases are presented in Figure 1. The natural substrates of lipases are triacylglycerols (TAGs) and in their natural media (aqueous) the products of hydrolysis of TAGs are free fatty acids (FFAs) and glycerol (Figure 1a), or *sn*-2 monoacylglycerol and glycerol, respectively when non regioselective or *sn*-1,3 regioselective lipases are used. In non-aqueous media, lipases can perform synthesis reactions. They catalyze synthetic biotransformations that involve the carboxyl group, such as esterification, transesterification and aminolysis in which the natural nucleophile (water) is replaced by an alcohol or an amine (Figure 1b-g), (Naik et al. 2010; Sandoval, 2012).

Due to the importance and great variety of applications, lipases from many different sources have been purified (Table 1) and sequenced to obtain their three-dimensional structure, which allowed a better understanding of their unique structure-function relationships in various hydrolytic and synthetic reactions (Casas-Godoy et al. 2012).

Regarding its specificity (Figure 2), lipases could be: i) chemoselective (for instance they can selectively perform aminolysis or acylation of a bifunctional molecule (Figure 2a)); ii) regioselective (Figure 2b), the 1,3 *sn*-selectivity towards the external positions of triglycerides of many lipases has been successfully applied to the synthesis of "structured lipids" (Ferreira-Dias, 2010; Jala et al. 2012); and iii) stereoselective (Figure 2c). Indeed, more than a thousand racemic compounds have been resolved by lipases (Bornscheuer et al. 2002). Regarding the selectivity of lipases towards different chain-length acyl groups, there is some controversy about the definition of a "true lipase", term usually referred to lipases that are able to hydrolyze long chain TAGs, while esterases usually prefer short chain acyl groups. However, lipase B from *Candida antarctica,* one of the most applied lipases in food industries and in organic synthesis, do not fulfill this definition. To avoid such controversy, a new classification of the carboxylester hydrolases group has been proposed, based on various criteria of physico-chemical, chemical, anatomical or cellular nature (Ali et al. 2012). Therefore, due to their selectivity and stability and the biotechnological tools available, lipases are used in many different industrial areas such as food, nutraceuticals, cleaning, fine chemicals, agrochemicals, energy and others.

The development of novel recombinant DNA technologies such as metagenomics (Ferrer et al. 2007) and molecular directed evolution (Khurana et al. 2011; García-Ruíz et al. 2012), together with the implementation of high-throughput platforms, are favouring the expression and production of large amounts of recombinant lipases, with new- or tailored catalytic activities.

Lipase applications in food industries

Lipases can be used as additives or as biocatalysts to manufacture food ingredients. As additives, these enzymes have been used to modify flavour by synthesizing esters of short chain fatty acids and alcohols, which are known flavour and fragrance compounds. Lipases are extensively used in the dairy industry for the hydrolysis of milk fat. Common applications include the flavour enhancement of cheeses, the acceleration of cheese ripening, the manufacturing of cheese-like products, and the lipolysis of butterfat. Thus, the addition of lipases that begin releasing short chain fatty acids (principally C4 and C6) lead to the development of a sharp, tangy flavour, whereas the release of medium chain (C12, C14) fatty acids tend to confer a soapy taste to the product (Gupta et al. 2003; Kilara, 2011).

Lipases have been also used in the improvement of flavour in coffee whiteners to produce the creamy flavour, and buttery texture of toffees and caramel (Hasan et al. 2006). Blue cheese flavour development is due to enzymes from *Penicillium roqueforti.* Lipases are also used to extend the freshness of baked products (Bárcenas et al. 2003) and as additives in animal feed (Houde et al. 2004).

As biocatalysts, lipases are important in the lipid industry because they can be exploited for the retailoring of vegetable and animal oils. Tailored vegetable oils with nutritionally important structured triacylglycerols and modified physicochemical properties have a big potential for future marketing. Cheap oils could also be upgraded to synthesize nutritionally important structured triacylglycerols like cocoa butter substitutes, low calorie triacylglycerols, human milk fat substitutes and oils enriched with specific fatty acids such as oleic, stearidonic, gamma-linolenic (GLA), conjugated linoleic (CLA) or omega-3 polyunsaturated (ω3 PUFA) fatty acids (c.f. 4.).

Bioactives, nutraceuticals and functional foods

In recent years, consumers have been increasingly confronted with the so-called functional foods and nutraceuticals, first introduced in Japan, which are claimed to promote health and well-being beyond their nutritive properties (Inglett, 1999; Espin et al. 2007; Ozen et al. 2012; Shahidi, 2012). Similarly, "bioactives" are nutritive substances that have favourable impact on human health (Biesalski et al. 2009; Bernhoft, 2010). The term "nutraceutical" is also used as synonym of bioactive compound. Nutraceuticals market will reach US$243 billion in 2015 (GIA, 2010), but the most rapidly growing market is for the "functional foods".

Definitions of “functional foods” could vary from place to place. In USA, the Institute of Food Technologist (IFT) defined functional foods as foods and food components that provide a health benefit beyond basic nutrition for the intended population (IFT, 2005). In Europe, the European Commission Concerted Action on Functional Food Science in Europe (FUFOSE) published a consensus concept that states that a food is “functional” if it is satisfactorily demonstrated to beneficially affect one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health, well-being and or reduction of disease risk (FUFOSE, 1999). Later on, the European Food Safety Authority (EFSA) adopted regulations about health claims which state, suggest or imply that a relationship exists between a food category, a food or one of its constituents (EFSA, 2011). In Japan, the term “Food with Health Claims” (FHC) is used instead and refers to foods that comply with the specifications and standards established by the Ministry of Health, Labor and Welfare (MHLW) and are labeled with certain nutritional or health functions (MHLW, 2012).

Lipases as biocatalysts for the production of nutraceuticals

To be formulated in functional foods, nutraceutical supplements or in “cosmeceuticals” (Draelos, 2008), bioactive compounds must have some characteristics as solubility, stability, bioavailability, etc., that in many cases the original bioactive molecule does not have. Therefore, there is growing interest to perform synthesis and modification of bioactive compounds at mild conditions compatible with the requirements of food and pharmaceutical industries and current legislation. In this context, as seen before, the characteristics of lipases convert them into ideal biocatalysts to perform these bioconversions. Some examples of nutraceuticals production trough biocatalysis using lipases are presented in Table 2, including mainly hydrolysis and acylation reactions. Specific examples of lipase application to obtain improved structured lipids, flavours, antioxidants and emulsifiers in a practical, ecological and often economical way, are presented in the next sections.

PRODUCTION OF FLAVOURS

Flavours and fragrances

A wide range of chemicals are flavour or fragrance compounds, ranging from terpenes to phenols and from aldehydes to esters. The demand for fragrance and flavour esters for food, cosmetics and pharmaceutical industries represent a quarter of the world market of food additives (Ahmed et al. 2010). Other important fact is that many of these compounds are chiral (Heinsman et al. 2002). A database for organoleptic compounds can be consulted in www.leffingwell.com/chirality/chirality.htm.

The production of these compounds is obtained by the direct extraction from fruits, or by chemical synthesis based on direct chemical esterification in the presence of inorganic catalysts at high temperatures (Heinsman et al. 2002). Direct extraction entails a high cost due to the expensive treatment of the fruit, the downstream processes and the shortage of some natural materials, but the products obtained could be labeled as “natural” (Guillén et al. 2012). Chemical esterification has associated problems of non-selectivity, undesirable side reactions, low yields, and high energy consumption, and the final products cannot be labeled as “natural” reducing the final price of the product (Abbas and Comeau, 2003; Ozyilmaz and Gezer, 2010).

The use of enzymes in flavour synthesis is an important alternative for these synthesizing processes, because the obtained products can be labeled as “natural” and they have better smell and colour than products obtained from chemical esterification (Longo and Sanromán, 2006, Ahmed et al. 2010, Ozyilmaz and Gezer, 2010). In addition, natural flavouring products can also be obtained by fermentation or bioconversion using microorganisms as *Geotrichum*, *Kluyveromyces*, and others *(*Longo and Sanromán, 2006, Mdaini et al. 2006).

Lipases have been successfully applied in the production of flavours and fragrances, in non-aqueous media, under appropriate working conditions (Park et al. 2009, Ozyilmaz and Gezer, 2010). Pancreatic lipases obtained from pig and human pancreas have been the most often investigated lipolytic enzymes (Ferrer et al. 2001). However, due to that practically all microorganisms are potential lipase producers, they are nowadays, the main source of these enzymes. More than 40 microbial commercial lipases are available (White and White, 1997). Among them *Candida antarctica, Candida rugosa, Penicillium* sp*., Mucor miehei, Rhizopus oryzae, Aspergillus niger, Thermomyces lanuginosa,*

Staphylococcus xylosus, Bacillus sp.*,* and *Acinetobacter* are the main lipase producing microorganisms.

Also cutinase, a small carboxyilic ester hydrolase, has shown a great selectivity toward the production of short-chain carboxylic acid esters (De Barros et al. 2012). *Fusarium solani* (De Barros et al. 2010) and *Burkholderia cepacia* (Dutta and Dasu, 2011) are examples of microorganism producing cutinases.

Initially, microbial lipases were obtained from fermentation with natural microorganisms and subsequent downstream processing. Nowadays, with the boom of molecular biology and protein engineering, the majority of lipases are recombinant proteins, produced in host microorganisms, in many cases with a modified sequence to obtain a more efficient biocatalyst.

There are many applications of alkyl esters as flavors. Ethyl acetate is used as artificial fruit essence and aroma enhancer, butyl butyrate contributes to the fragrance of pineapple, apple, banana, apricot and butter. Also, ethyl hexanoate, hexyl acetate or hexanoate are related to similar fragrances including pear, green notes flavor, citrus aromas, while ethyl valerate is the typical fragrance of green apple (Horchani et al. 2012). Octyl esters are responsible of apple, pear and strawberry flavours (De Barros et al. 2012). Butyl acetate and isoamyl esters, as isoamyl acetate (banana flavour) or valerate, are fruity flavours (Rodrigues and Fernández-Lafuente, 2010).

Terpene esters of fatty acids are essential oils that find several applications in the food, cosmetic and pharmaceutical industries. Among them, the acyclic terpene alcohols geraniol and citronellol are commercially the most important (Serri et al. 2010). Free and immobilized *Rhizopus* lipases were used in the synthesis of citronellyl butyrate and valerate, with better results when the immobilized enzyme was used (Melo et al. 2005). Several citronellyl esters (acetate, propionate, butyrate, caprate and laurate) have been synthesized using immobilized *Candida antarctica* lipase B (Lozano et al. 2007). The same biocatalyst has also been selected for the synthesis of citronellyl laureate in organic solvents and supercritical carbon dioxide (Habulin et al. 2008) and immobilized *Candida rugosa* lipase has been used in the same bioconversion (Abdullah et al. 2009).

Other important product is the enantiomeric pure alcohol *l*-menthol, which has a refreshing flavour, whereas *d*-menthol has an undesirable taste. It has been obtained by stereoselective hydrolysis of the corresponding racemic esters or by esterification from the mixture of the racemic alcohol (Wang et al. 2002).

The classical approach for producing flavours in biocatalysis processes using lipases are synthesis reactions involving the use of organic solvents (Ben Salah et al. 2007). Hydrophobic solvents, such as n-hexane, n-heptane, cyclohexane and isooctane are routinely used due to the difficulty to penetrate the water layer around the enzyme surface. However, a process using organic solvents cannot be considered green so the use of solvent-free systems is a preferred option despite the problems of mass transfer limitations and low reaction rates (Melo et al. 2005; Longo and Sanromán, 2006).

One of the alternatives to the use of organic solvents is the use of non conventional media. Ionic liquids (ILs) are an attractive option, being environmentally friendly solvents compared to volatile organic solvents (De los Ríos et al. 2008). Other approach is the use of supercritical fluids, especially supercritical carbon dioxide (Sc-CO_2) for the synthesis of natural compounds with similar conversions to those obtained in the presence of organic solvents (Kumar et al. 2005; Rodrigues and Fernández-Lafuente, 2010). For instance, citronellyl esters synthesis catalyzed by immobilized *Rhizopus oryzae* lipase on a film was reported under Sc-CO_2 conditions (Dhake et al. 2011).

One of the main drawbacks for the scale-up of bioprocesses for the synthesis of flavours to industrial scale is the relatively high cost of enzymes. Thus, process optimization is necessary to produce biocatalysts at low cost. On the other hand, due to the characteristics of the bioconversion (ester synthesis in non-aqueous medium using lipases as catalysts for esterification or transesterification reactions in organic medium or solvent-free medium), free lipases are used only for preliminary studies for characterization and performance evaluation of the biocatalyst, but not for industrial application.

Production of flavours and fragrances requires the use of immobilized lipases to be a real alternative to a chemical process. Immobilization may improve the stability of the biocatalyst, offering the possibility

of a better control of the reaction and the design of operational strategies (batch, fed-batch or continuous) using different bioreactor configurations (classical, packed bed, fluidized bed) and biocatalyst reuse (Ozyilmaz and Gezer, 2010). Thus, the optimization of the support to improve enzyme stability and the selection of the bioreactor and the operational conditions (Pires-Cabral et al. 2010) are key points for the feasibility of these bioprocesses.

The methods usually applied to immobilize lipases are based on adsorption or covalent attachment (Guillén et al. 2011). Different kind of supports has been used: polypropylene powder, Eupergit C, Octadecyl Sepabeads (Guillén et al. 2011), microemulsion based organogels (Ahmed et al. 2010), Amberlite IRC 50 (Abbas and Comeau, 2003), calcium alginate gel (Ozyilmaz and Gezer, 2010), multipoint covalent attachment using different supports (Rodrigues and Fernández-Lafuente, 2010), hydrophilic polyurethanes (Dias et al. 1991; Pires-Cabral et al. 2005a; Pires-Cabral et al. 2005b; Pires-Cabral et al. 2007; Pires-Cabral et al. 2009; Pires-Cabral et al. 2010) and others (Table 3).

Once the best support has been selected, it is important to determine the best conditions to maximize flavour compounds synthesis, such as the nature of the biocatalyst, the temperature (directly related with the reaction kinetics and stability of the biocatalyst), the enzyme loading and the solvent used.

The effect of water content is essential in reactions involving organic solvents or substrates. Water is essential for the formation of hydrogen bonds which are fundamental in the interactions for maintaining the conformation of the enzymes.

Substrates concentrations and molar ratio are important factors to avoid inhibitory effects by alcohol or acid excess, and will affect the selection of the operational strategy together with variables directly associated to enzyme reactor design, such as agitation speed.

Several studies have been conducted with the objective of testing the efficiency of novel or recombinant lipases in the synthesis of flavour compounds or to optimize the parameters involved in batch reactors of reduced volume (millilitre scale). The design of novel bioreactor configurations, operation modes and reaction conditions are the basic requirements for scaling up the production of flavours to industrial scale since they would affect the operational stability of the biocatalysts used. Thus, a comprehensive screen of immobilized supports and reaction conditions, bioreactor design and operating regime is crucial to obtain highly productive processes (Saponjic et al. 2010).

PBR has been tested in the production of ethyl butyrate using *Candida rugosa* lipase immobilized in polyurethane foams: high operational stability was obtained when reaction media with low substrate concentrations were used. However, at high substrate concentrations, a fast deactivation of the biocatalyst was observed, due to the accumulation of the substrate (ethanol) inside the hydrophilic support provoking an inhibitory effect of the substrate. The performance of PBR was also compared with a strategy using repeated batch reactors (Pires-Cabral et al. 2010).

The continuous esterification of malonic acid with citronellol using *Candida rugosa* lipase immobilized on Amberlite MB-1 in a PBR has been reported. Bioreactor configuration was a two-stage PBR, the first column containing the immobilized lipase and the second one containing molecular sieve particles. The effect of different substrate concentrations and feed-flow rates were analyzed. Full conversion was obtained at high citronellol concentration (50 mmol/L) reaching steady state after 180 min of reaction (Serri et al. 2010).

Fluidized bed reactor (FBR) operating by cycles has been used in the amyl caprylate synthesis using *Candida rugosa* lipase immobilized on Sepabeads. Flow pattern behaviour, substrate flow rate effect and long-term stability were studied. Conversions around 90.2% after 14 hrs were obtained and the system was stable for up to 70 hrs (Saponjic et al. 2010).

FBR configuration using *Candida rugosa* lipase immobilized on Sepabeads was also applied to the synthesis of geranyl butyrate. Although at optimal conditions, lower molar conversion was achieved in FBR compared to batch system, where the volumetric productivity was more than fivefold higher (Damnjanovic et al. 2012).

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In recent years, successful applications of enzymatic microreactors using lipases as catalysts for the hydrolysis of TAGs or umbelliferone acetate have been reported (Urban et al. 2006). In flavour synthesis, *Candida antarctica* lipase B has been successfully used in the synthesis of isoamyl acetate in a continuously operated pressure-driven microreactor (Znidarsic-Plazl and Plazl, 2009). A summary of different bioreactor configuration for the production of flavours and fragrances is presented in Table 4.

One of the drawbacks in the application of immobilized lipases in commercial processes is their high cost. To circumvent this problem, an enzyme-displaying yeast cell as whole-cell biocatalyst has been used. The synthesis of 2-phenyl acetate has been made in the presence of *Yarrowia lipolytica* biomass (Bialecka-Florjanczyk et al. 2012). *Candida antarctica* lipase B has also been displayed on yeast cell surface using the α-agglutinin and Flo1p (FS) anchor systems in *Pichia pastoris* as biocatalyst for the production of ethyl hexanoate (Su et al. 2010). Recently, a comparison in the production of 12 flavour esters using CALB-displaying yeast whole cells with other studies has been reported. The results show that this approach is promising for further large-scale production of flavour esters in nonaqueous media (Jin et al. 2012).

PRODUCTION OF FATTY ACID ESTERS OF ANTIOXIDANTS

Antioxidants in food nutraceuticals

Heating or long-term storage of fats, oils and lipid-based foods cause deterioration through several degradation reactions (Pokorny, 2001). The main deterioration processes are oxidation reactions that result in development of rancidity and formation of off-flavour compounds thus decreasing the nutritional value and sensory quality of food products. The spontaneous reaction of atmospheric oxygen with lipids is known as autooxidation (Gordon, 2001). The autooxidation of lipids starts with an initiation reaction during which free radicals are formed, followed by a series of propagation reactions of such radicals, and finally a termination reaction that involves the combination of two radicals, with the formation of stable products.

The prevention of oxygen access, the use of low temperatures, the inactivation of certain redox enzymes, and a suitable packaging contribute to minimize oxidative damage. Oxidation may be also inhibited by the use of specific additives (antioxidants) that may vary in their chemical structure and mode of action. Antioxidants, which were first used for food preservation before World War II, are substances that when present at low concentration −compared with the oxidizable substrate− delay or prevent its oxidation (Yeo et al. 2011). Antioxidant molecules prevent unsaturated oil products from becoming rancid during storage, thus extending its shelf life (Safari et al. 2006). This is particularly important for polyunsaturated fatty acids (ω-3, ω-6) which are rather prone to oxidation. The most common food antioxidants (primary antioxidants) interfere with lipid autoxidation by rapid donation of hydrogen atoms to lipid radicals. Natural antioxidants such as vitamins C and E are the best accepted for food applications. For example, alpha-tocopherol (vitamin E) suppresses the propagation of radical chain reactions at the stage of the peroxy radical. Synthetic phenolic antioxidants, like t-butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate are not so well accepted by consumers as dietary components as there has been growing concern about their possible side-effects. In this context, natural phenolic molecules such as flavonoids are much better accepted (Landete, 2012).

Free radicals and other reactive species are produced in the body, primarily as a result of aerobic metabolism, causing the so-called oxidative stress. Scientific evidence exists that the excessive production of free radicals in the organism, and the imbalance between their concentration and the antioxidant defenses, may be related to processes such as aging (Calabrese and Maines, 2006) and several diseases such as cancer, atherosclerosis, stroke, rheumatoid arthritis, neurodegeneration, inflammatory disorders or diabetes (Geronikaki and Gavalas, 2006; Siekmeier et al. 2007). Oxidative inhibitors (*e.g.* glutathione, arginine, taurine, creatine, selenium, zinc, vitamin E, vitamin C, vitamin A, and tea polyphenols) and several enzymes (*e.g.* superoxide dismutase and catalase) are excellent antioxidants that exert synergistic actions in reducing free-radical lipid oxidation and protect living cells against the effects of harmful free radicals (Zhou and Liu, 2005). The role these antioxidants play in protecting living systems against lipid peroxidation and other anomalous molecular modifications is of great interest.

Over the past three decades, there has been growing evidence showing that malnutrition or excess of certain nutrients give rise to the oxidation of biomolecules and cell injury. Most antioxidants are common food components, and have been used in the diet for thousands of years. They are classified into two groups: (i) those whose ingestion is essential in human feed -nutrients- comprising vitamins C and E and vitamin precursors (carotenoids); (ii) plant-derived compounds of low molecular weight, basically polyphenols (*e.g.* flavonoids), which have not been demonstrated to be essential for health.

Enzymatic modifications of antioxidants

The modification of natural antioxidants can pursue different objectives: (i) to improve their chemical, oxidative or heat stability, especially in lipid-containing systems; (ii) to alter their hydrophile-lipophile balance (HLB) and, as a consequence, their miscibility with lipids; (iii) to improve their bioavailability in nutraceutical formulations. In this context, various semisynthetic antioxidants (*e.g.* L-ascorbyl stearate, tocopheryl acetate) are being commercialized (Wüstenberg et al. 2011). These derivatives may have impact not only as food preservatives but also as components of functional foods and nutraceuticals.

The acylation of an antioxidant by esterification or transesterification may impart novel properties to the molecule, both in terms of physical and biological properties. The esters of antioxidants are generally prepared using strongly corrosive acids such as sulfuric acid or hydrogen fluoride followed by a re-esterification step (Stamatis et al. 2001). For example, the chemical synthesis of benzoic and phenolic esters is commonly performed with acid or basic catalysts under reflux (Netscher et al. 2003), but these procedures do not fulfill the requirements needed for food applications. To overcome the shortcomings of conventional processes, new approaches based on the so-called “green chemistry” are being developed. Biocatalytic processes fully participate in the “green chemistry” conceptthat was introduced in the 90’s and its impact on sustainability is now well established (Alcalde et al. 2006). Biocatalysts (either enzymes or whole-cells) constitute a greener alternative to traditional stochiometric methods, offering appropriate tools for the industrial transformation of natural and synthetic materials under mild reaction conditions and with low energy requirements. In addition, the undesirable isomerization and rearrangement side reactions are minimized (Azerad, 2001; Sabally et al. 2007).

In the case of antioxidants, mild reaction conditions are particularly desirable to avoid irreversible degradation of such labile compounds. Lipases and carboxyl-esterases represent the most important groups of enzymes that catalyze the formation or transfer of ester bonds (Plou et al. 2003), and have been successfully applied to the acylation of antioxidants. In these synthetic applications, the immobilization of the enzyme usually facilitates the substrates to reach the catalytic site, minimizing protein-protein contacts that are present when using enzymes suspended in organic solvents (Torres-Salas et al. 2011).

The enzymatic methodologies described to acylate the two major vitamin antioxidants (C and E) as well as different phenolic antioxidants of natural origin, will be discussed in the next section.

Lipase-catalyzed production of L-ascorbic acid fatty acid esters

L-ascorbic acid (vitamin C) belongs to the group of vitamin-based antioxidants and is the major water-soluble natural antioxidant. Acting as a free radical scavenger, L-ascorbic acid and its derivatives react with oxygen, thus removing it in a closed system. The combination of L-ascorbic acid and primary antioxidants like α-tocopherol makes a synergistic effect that results in the “vitamin E recycling system” (Noguchi et al. 2000). This mixture of vitamins is usually added to cookies, pastes, meat products and other fatty products to maintain their quality and extend their shelf-life (Kochhar, 2000). However, due to the low miscibility of L-ascorbic acid with α-tocopherol or with oily products, it is more convenient to use ascorbyl fatty acid derivatives instead of vitamin C.

Ascorbic acid has only one primary alcohol, capable of reacting with an acyl donor, yielding the corresponding 6-*O*-ascorbyl ester (Figure 3). It has been demonstrated that the addition of one or more hydrocarbon chains to the ascorbic acid ring, preferably in the 6-position, retains or even enhances the physiological and antioxidant activity performed by the vitamin C (Lonostro et al. 2000; Song et al. 2004). Thus, esters of L-ascorbic acid and long-chain fatty acids (usually palmitic or stearic) are employed as additives (E-304) for the stabilization of fats, oils and fatty products, as they retard the autooxidation of unsaturated fatty acids. Although ascorbyl esters of saturated fatty acids are more soluble in fats than L -ascorbic acid itself, their only moderate miscibility with edible fats and oils limits

their use. Ascorbyl esters of unsaturated fatty acids, such as oleic (Figure 3) or linoleic, exhibit improved properties, in particular their miscibility with α-tocopherol, oils and lipid-containing products.

Ascorbyl palmitate and stearate are currently produced by reacting ascorbic acid with sulphuric acid followed by re-esterification with the corresponding fatty acid, and subsequently purified by re-crystallization. This chemical process has some disadvantages such as the use of strong acids, the low yields due to non-regioselective reactions and the need of tedious product isolation.

As an alternative, lipases have been successfully used to catalyze the synthesis of ascorbyl esters in different solvents (Song and Wei, 2002), employing saturated and unsaturated free fatty acids, alkyl and vinyl esters as acyl donors. In order to reduce the cost of the process, the use of TAGs and oils as acyl donors has been successfully explored (Figure 3) (Burham et al. 2009; Reyes-Duarte et al. 2011). Table 5 summarizes the experimental conditions and yields described in the literature for the acylation of vitamin C. Tertiary alcohols and acetone are commonly employed as reaction solvents. The latter is especially appropriate as it is inexpensive, volatile and permitted in most countries for use in the manufacture of food products. The use of ionic liquids as alternative media for the acylation of L-ascorbic acid has been also described (Park et al. 2003). The enzyme-catalyzed synthesis of acyl L-ascorbates presents several advantages compared with the classical processes, in particular its high regioselectivity and the moderate reaction conditions required (Yan et al. 1999). The main disadvantage is the price of the immobilized lipases compared with the chemical reagents, although prices are lowering fast due to the advances in recombinant DNA technologies and downstream processing.

The yield and conversion reported in the acylation of L-ascorbic acid, especially with the lipase B from *Candida antarctica*, are quite good (Table 5); however, these processes have not been adopted by the industry yet. Except from the works from Kuwabara et al. (2003a) and Watanabe et al. (2003), in which continuous PBR were used, the rest of processes employed BR.

Lipase-catalyzed reactions on tocopherols (vitamin E)

Among the natural antioxidants, the term vitamin E describes the beneficial biological activity on humans of a group of structurally related compounds, in particular α, β, γ and δ-tocopherol, and α, β, γ and δ-tocotrienol (Cerecetto and López, 2007). Vitamin E enhances the resistance to oxidation of the organisms, owing to its ability to protect polyunsaturated fatty acids from peroxidation and to scavenge free radicals. It is a primary antioxidant as it terminates the free radical chains in lipid oxidation. Vitamin E has an important presence in the animal nutrition market, where high doses of this antioxidant are applied to improve the quality and shelf-life of meat (Valentin and Qi, 2005).

Tocopherols have three different domains: the functional one, responsible of the antioxidant activity; the signaling one, regulator of the protein kinase C; and the hydrophobic one, which interacts with membranes and lipids. The tocopherols are characterized by the 6-chromanol ring structure methylated to varying degrees at the 5, 7 and 8 positions (Figure 4). For example, the α-tocopherol is trimethylated. At the position 2, there is a C16 saturated side that has no effect on its antioxidant activity but serves to insert and hold the chemically reactive “head” in biomembranes (Chen et al. 2006). Tocopherols have three chiral centers at carbons 2, 4’- and 8’-, and the naturally occurring isomers have the RRR-configuration (Weiser et al. 1986). It is generally accepted that the RRR-α-tocopherol is the most bioactive compound as it is specifically recognized by membranes (Cerecetto and López, 2007). For example, the isomer with inverted stereochemistry at position 2 has only 30% of the biological activity of the RRR isomer. Regarding the effect of methylation degree of the chromanol ring on the antioxidant activity, it follows the order of δ>ɣ>β>α.

Commercially available RRR-α-tocopherol is derived from deodorizer distillate, a by-product of soybean oil refining process. The synthetic vitamin E (α-tocopherol) is obtained by reaction of trimethylhydroquinone with isophytol (Bonrath et al. 2002b), without any control of stereochemistry. The obtained product, consisting of eight stereoisomers in equal proportions, is designated all racemic (all-*rac*)-α-tocopherol. Despite being less biologically active than the natural RRR stereoisomer, its production is higher than 1000 t per annum (Bonrath and Netscher, 2005). However, the derivative tocopheryl acetate (Figure 4) is the major sales form of vitamin E, both in the human (Winklhofer-Roob et al. 1996) and animal (Özkan et al. 2007) nutrition markets.

Acetylation of vitamin E increases its stability in the presence of light and oxygen. In fact, vitamin E is generally administered as a prodrug in the form of all-*rac*-α-tocopheryl acetate (vitamin E acetate) or all-*rac*-α-tocopheryl succinate (vitamin E succinate). These derivatives carry an acetyl or succinyl moiety at the C-6 phenolic group that blocks the antioxidant properties (Schneider, 2005). However, unspecific esterases rapidly cleave *in vivo* the ester bond and release the active α-tocopherol.

The vitamin E acetate is synthesized by chemical acylation all-*rac*-α-tocopherol with acetic acid or acetic anhydride as acyl donors using different acid or base catalysts such as sulphuric acid or pyridine (Bonrath and Giraudi, 2004; Bonrath et al. 2007). Several continuous or discontinuous processes have been described (Bonrath and Netscher, 2005).

Two different routes have been described for the enzymatic preparation of tocopheryl acetate: the direct process (Figure 4a) and the strategy based on hydroquinone-1-monoacetate (Figure 4b). The direct enzymatic acetylacion of vitamin E is a difficult reaction due to the large molecular size of the acceptor (tocopherol) and its steric hindrance with the active site of the enzyme. Torres et al. (2008) described for the first time the enzymatic acetylation of vitamin E using vinyl acetate as acylating agent. Out of 15 lipases, esterases and proteases screened, only the lipase B from *C. antarctica* was an effective biocatalyst for this reaction. To improve biocatalyst performance *C. antarctica* lipase B has been immobilized in several carriers; the reaction was faster with the enzyme immobilized in polypropylene, which was correlated with the higher porosity of the support. A hexane/2-methyl-2-butanol 90:10 (v/v) mixture was found to be the optimum medium, as it represents a compromise between substrates solubility and biocatalyst efficiency. The acetylation of δ-tocopherol was faster than that of α-tocopherol, probably due to its lower methylation degree. This methodology was further applied to the enzymatic synthesis of vitamin E succinate (Yin et al. 2011) and ferulate (Xin et al. 2011).

By computational conformation studies, it has been demonstrated that the acceptor binding site of lipase B from *C. antarctica* is deep (compared with other lipases, *e.g.* that from *Thermomyces lanuginosus)* (Pleiss et al. 1998), which partly explains the broader specificity of *C. antarctica* lipase B (Uppenberg et al. 1995; Fuentes et al. 2004).

An alternative route to *all-rac*-α-tocopheryl acetate is the reaction of trimethyl-hydroquinone-1-monoacetate with isophytol (Figure 4b) (Bonrath et al. 2002a). The diacetate is easy to obtain in large amounts from cheap α-isophorone; however, the selective hydrolysis to 1-monoacetate is difficult to achieve by standard chemical methods. As an alternative, the regioselective lipase-catalyzed hydrolysis has been reported (Bonrath et al. 2002a). Lipase from *T. lanuginosus* (formerly *Humicola lanuginosa*) hydrolyzed regioselectively the diacetate in water-saturated *t*-butyl methyl ether. This lipase is inexpensive as it is used in detergent formulations. The authors demonstrate that the immobilization on polypropylene enhanced activity without affecting regioselectivity. The 4-monoacetate isomer and the free hydroquinone (the product of total hydrolysis) were not detected in measurable amounts, making this process superior to chemical deacetylation. The 1-monoacetate can further react with isophytol to yield the corresponding tocopheryl acetate.

Lipase acylation of phenolic antioxidants

In the last years, the natural phenolic compounds found in vegetables and fruits have gained interest both as alternative antioxidants in foods (Medina et al. 2010) and as functional ingredients (Shahidi, 2012). In general, phenolic antioxidants (Figure 5) are hydrophobic scaffolds exhibiting poor absorption, resulting in a very low concentration in the circulatory system (Biasutto et al. 2009). The modification of their physicochemical properties such as solubility and partition coefficient by acylation or glycosylation, may exert a positive influence on the entry of polyphenols into enterocytes and thus in their bioavailability (Ratnam et al. 2006). For example, glucoside and ester derivatives of quercetin, a flavonoid widely distributed in nature, (Arts et al. 2004; Biasutto et al. 2007) or resveratrol, a phytoalexin found in seeds and skin of grape berries and other plants (Biasutto et al. 2009), exhibited improved bioavailability. Table 6 contains examples of phenolic antioxidants whose lipase-catalyzed acylation has been reported. The antioxidants in Table 6 are grouped into families based on their hydrocarbon skeleton.

One of the approaches to increase bioavailability of resveratrol is to protect its 3-OH phenolic group. Regioselective acylation of resveratrol at 3-OH was achieved by transesterification with vinyl acetate

catalyzed by immobilized lipase from *Alcaligenes* sp. (Torres et al. 2010). The maximum yield of 3-*O*-acetylresveratrol was approximately 75%, as the lipase also catalyzes its further acetylation yielding the diester 3,4'-di-*O*-acetylresveratrol and finally the peracetylated derivative (Figure 6). In contrast, the lipase B from *Candida antarctica* catalyzes the acylation of the phenolic group 4'-OH with negligible formation of higher esters (Cardile et al. 2005).

Hydroxytyrosol is recognized as the main antioxidant and protective principle in olive oil (Azaizeh et al. 2012). The increase of lipophilic character of hydroxytyrosol by acylation may favour its use as additive for preserving foods from oxidative processes or as bioactive in functional foods and nutraceuticals for the prevention and treatment of pathologies associated with free radicals (Baldioli et al. 1996). Lipase B from *Candida antarctica* is particularly effective to obtain hydroxytyrosol fatty acid esters in good yields and short reaction times using the corresponding ethyl esters (Buisman et al. 1998), including those of polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) acids. The latter derivatives may combine the antioxidant properties of PUFAs with those of the cathecol moiety of hydroxytyrosol (Torres De Pinedo et al. 2005).

PRODUCTION OF STRUCTURED LIPIDS

Definition and principal types of structured lipids

Natural fats present a wide variety of functional, nutritional and organoleptic properties which depend on (i) their composition in saturated and polyunsaturated fatty acids, (ii) fatty acid chain length and (iii) on the distribution of the different fatty acids in TAGs (positions *sn*-1, *sn*-3 or *sn*-2). Thus, novel TAGs can be obtained (i) by the incorporation of new fatty acids (FAs), (ii) by changing the positions of FAs or the FA profile, from the natural state, or (iii) by the synthesis of completely new TAGs (Xu, 2000). These modified fats, known as structured lipids (SLs) or "taylor-made fats", can be produced either chemically or enzymatically. The cheap natural oils and fats can be used as raw materials for the synthesis of high added-value products*, i.e.* the SLs. They present important medical and functional properties for food applications as described in several reviews (Willis et al. 1998; Gunstone, 1999; Osborn and Akoh, 2002; Gupta et al. 2003; Sakurai and Pokorný, 2003; Ferreira-Dias, 2010).

In the field of edible oils and fats, the interest on the lipase-catalyzed production of structured lipids (SL) presenting specific functional properties has greatly increased due to the benefits of the enzymatic route relatively to chemical processes. Also, when *sn*-1,3 regioselective lipases are used, the original FA at the *sn*-2 position in the TAG is preserved, and the obtained SLs present a novel structure and properties not attainable by chemical catalysis. However, even using *sn*-1,3 specific lipases, the undesirable and unavoidable side reaction of acyl migration will occur to some extent in the overall process of SLs production, with the formation of undesirable TAG products. Acyl migration depends on several parameters, namely reaction temperature, lipase load, immobilization supports, water content and water activity, solvent type and reaction system (Xu, 2000; Xu, 2003). Thus, in the optimization of lipase-catalyzed processes for the production of SLs, acyl migration must be minimized.

Lipase-catalyzed synthesis of different types of SLs, either in solvent-free media or in the presence of an organic solvent, in bioreactors operating batchwise or in continuous mode, has been widely described in the literature (Table 7 and Table 8). In most of these studies, high-cost *sn*-1,3 regioselective immobilized commercial microbial lipases have been used. However, the high cost of commercial lipases and the low operational stability of some of them have been recognized as the main constraints to the use of lipase-catalyzed processes for SLs production in the food industry. Therefore, the search for novel biocatalysts, presenting both high activity and operational stability, is a way to make enzymatic processes competitive. Lipases extracted from plants show some advantages over the microbial counterparts, namely the lower cost and the ready availability combined with wide versatility and stability in organic media (Paques and Macedo, 2006). Among them, *Carica papaya* lipase has emerged as a promising biocatalyst for oil and fats restructuring (Villeneuve, 2003; Domínguez de María et al. 2006; Tecelão et al. 2012a).

Lipase-catalyzed production of triacylglycerols modified in their fatty acid composition

The production of TAGs modified in the fatty acid composition has been usually carried out by (i) acidolysis of an ester (a single TAG, oil or fat) with a free fatty acid or (ii) by interesterification of a

single TAG, oil or fat with ethyl or methyl esters, catalyzed by *sn*-1,3 specific lipases. The term "acidolysis" is classically used to describe a reaction where a fatty acyl group is exchanged through a combination of hydrolysis of a donor ester and esterification of the released alcohol moiety with another free fatty acid.

Examples of SLs, obtained by lipase-catalyzed modification of the original FA composition in TAGs, are: (i) low calorie TAGs, (ii) human milk fat substitutes, (iii) cocoa butter substitutes and (iv) TAGs rich in omega-3 PUFA or in other specific fatty acids.

Low calorie and dietetic triacylglycerols. The most usual types of low caloric and dietetic TAGs consist of (i) TAGs with short-chain fatty acids, S, at the positions *sn*-1 and *sn*-3, and the long chain fatty acid, L, in the internal position (SLS), (ii) TAGs with medium chain fatty acids, M, in the positions *sn*-1 and *sn*-3, and L at position *sn*-2 (MLM) and (iii) MMM type SLs. These SLs present lower caloric value (21 to 29 kJ/g) than conventional fats and oils (38 kJ/g) because of the lower caloric content of S and M, compared to their L counterparts (Smith et al. 1994). During digestion, M are metabolized as rapidly as glucose. Since they are not readily re-esterified into new TAGs, they have little tendency to accumulate in the body as stored fat, with weight control benefits (Osborn and Akoh, 2002). MLM are also important as food source for persons with fat malabsorption and metabolism problems and can also be used in cosmetics formulations (Low et al. 2007).

Lipase-catalyzed acidolysis, in which M (caprylic, C8:0, or capric acids, C10:0) are used as acyl donors and vegetable or fish oils are used as the source of glycerol backbone and L, is one of the most commonly used methods to produce MLM. The presence of unsaturated L at *sn*-2 position is beneficial in terms of their absorption as *sn*-2 monoacylglycerols.

Among vegetable oils, virgin olive oil is an interesting substrate for the production of MLM because of its high oleic acid (55.0-83.0 wt-%) and moderate linoleic acid contents (3.5-21.0 wt-%) mainly at *sn*-2-position in its TAGs. The structured TAGs, obtained by acidolysis of virgin olive oil with M catalyzed by *sn*-1,3 specific lipases, will present oleic acid at the *sn*-2 position, with absorption benefits, while M will be mainly esterified at the external positions in the TAG molecules (Nunes et al. 2011b; Nunes et al. 2011c; Nunes et al. 2012a; Nunes et al. 2012b) Dietary ingestion of virgin olive oil has been reported to have physiological benefits such as lowering serum cholesterol levels, suppressing certain types of cancer, enhancing liver function, and reducing the effects of aging and heart disease (Covas, 2007).

Table 7 presents some examples on the enzymatic production of low calorie and dietetic TAGs, of the types SLS, LSL, MLM and MMM, as well as the reaction conditions followed to attain the best results. In most of these experiments, commercial immobilized *sn*-1,3 specific lipases were used in loads varying from 4% (Shimada et al. 1996) to 20% (w/w) of the substrate amounts used (Jennings and Akoh, 2000; Jennings and Akoh, 2001; Turan et al. 2006). To lower biocatalyst cost, the search for cheaper immobilized lipase preparations, with high activity and operational stability has been reported (Nunes et al. 2011c; Nunes et al. 2012a; Nunes et al. 2012b).

Human milk fat substitutes. Human milk fat (HMF) contains L, namely oleic (30-35%), palmitic (20-30%), linoleic (7-14%) and stearic acids (5.7-8%). Unlike in vegetable oils and in cow's milk fat, in HMF, palmitic acid, the major saturated fatty acid, is mostly esterified at the *sn*-2 position of the TAGs, while unsaturated fatty acids are at the external positions. The fatty acid profile of HMF has a crucial effect on its digestibility and intestinal absorption in infants. In fact, the presence of palmitic acid at the *sn*-2 position provides a more efficient absorption of palmitic acid as *sn*-2 monoacylpalmitate (López-López et al. 2001). The use of vegetable oils and cow's milk fat as a substitute of HMF in infant formulas may cause a deficient calcium and fatty acid absorption, due to the formation of insoluble calcium soaps with saturated fatty acids released by the action of the *sn*-1,3 specific pancreatic lipase (Sahín et al. 2005a). Therefore, the production of SLs resembling human milk fat has been a challenge for the food industry.

Human milk fat substitutes (HMFS) have been obtained by *sn*-1,3 lipase-catalyzed (i) acidolysis of tripalmitin, butterfat, palm oil, palm stearin or lard (rich in palmitic acid in *sn*-2 position) with free fatty acids (FFA) from different sources (Yang et al. 2003; Sahín et al. 2005a; Sahín et al. 2005b; Sahín et al. 2006; Sorensen et al. 2010; Tecelão et al. 2010; Ilyasoglu et al. 2011; Qin et al. 2011; Zou et al. 2011; Tecelão et al. 2012a; Tecelão et al. 2012b; Zou et al. 2012), (ii) interesterification of tripalmitin or lard with vegetable oils (Maduko et al. 2007a; Maduko et al. 2007b; Silva et al. 2009) and (iii) synthesis

of 1,3-dioleoyl-2-palmitoylglycerol (OPO), an important structured TAG in infant formulas, by multistep reactions (Schmid et al. 1999; Chen et al. 2004; Qin et al. 2011).

Nowadays, "Betapol™" and "InFat™" are examples of commercial HMFS manufactured by biocatalytic processes. "Betapol™" is produced by IOI Loders Croklaan (http://www.betapol.com/ January 2013) and "InFat™", by Advanced Lipids (http://www.aak.com/en/productsapplications/food-ingredients/infant-nutrition/infat/, January 2013). Both HMFS are synthesized by acidolysis of fats containing palmitic acid predominantly esterified at the *sn*-2 position (*e.g.*, fractionated palm stearin or tripalmitin) with blends of free fatty acids rich in oleic acid, catalyzed by *sn*-1,3 selective lipases such as *Rhizomucor miehei* lipase and *Rhizopus oryzae* lipase (Meiri-Bendek et al. 2011).

The heterologous *Rhizopus oryzae* lipase (r-ROL) immobilized in different supports (Accurel MP 1000, Eupergit C and Lewatit VP OC 1600) was successfully used for the acidolysis between tripalmitin and oleic acid, batchwise, at 60ºC, in solvent-free media, to produce OPO (Tecelão et al. 2012b). Both activity (30 mol-% of oleic acid incorporation after 6 hrs reaction time) and batch operational stability (half life time of 202 hrs) of r-ROL in Lewatit VP OC 1600, were comparable or even better than those obtained with commercial immobilized lipases working in similar reaction systems (Tecelão et al. 2010). Also, *Carica papaya* latex showed to be a promising low-cost biocatalyst for the production of HMFS in solvent free media, presenting similar activity and batch operational stability to the values observed for commercial immobilized lipases (Tecelão et al. 2012a).

Some interesting examples of works on enzyme-catalyzed production of HMFS are shown in Table 9.

Cocoa butter substitutes. Cocoa butter substitutes are very important SLs for the food industry. Cocoa butter is mainly formed by TAGs with saturated fatty acids (palmitic and stearic acids), in the external positions, and a monounsaturated fatty acid (oleic acid) at position *sn*-2. This particular structure is responsible for the unique rheological and sensory properties of cocoa butter. The enzymatic process was successfully developed by Unilever and Fuji Oil to produce cocoa butter substitutes at a large scale via lipase-catalyzed reactions (Coleman and Macrae, 1977; Matsuo et al. 1981). Products similar to cocoa butter were also obtained by lipase-catalyzed (i) interesterification of tea seed oil with methyl palmitate and methyl stearate (Wang et al. 2006) and (ii) acidolysis of olive pomace oil with a mixture of palmitic and stearic acids in *n*-hexane, in a batch PBR with product recirculation (Ciftci et al. 2009).

Triacylglycerols rich in specific long-chain fatty acids. SL containing conjugated linoleic acid (CLA), gamma-linolenic acid (GLA), omega-3 PUFA and/or other special fatty acids can also be obtained by *sn*-1,3-specific lipase-catalyzed acidolysis or interesterification with fatty acid ethyl esters, either in organic or in solvent-free media, as reported in several studies (Senanayake and Shahidi, 2002a; Torres et al. 2005; Villeneuve et al. 2007; Alim et al. 2008).

Lipase-catalyzed production of triacylglycerols modified in the original position of the fatty acids

The physical properties of the natural oils and fats (*e.g.*, crystallization pattern and melting properties) can be modified by interesterification (ester interchange) without modifying their fatty acid composition and without *trans*-fatty acid formation. This operation is very important for the food, pharmaceutical and cosmetics industries Healthy margarines, dressing oils and shortenings can be obtained by mixing interesterified blends, in different proportions, with natural liquid oils and fats (Moustafa, 1995).

In the food industry, the interesterification reaction of fat blends is currently carried out at high temperature (up to 270ºC), under reduced pressure, for less than 2 hrs, using metal alkylates or alkali metals as catalyst. The interchange of acyl groups proceeds at random, final products may remain contaminated by residual catalyst and the formation of considerable amounts of side products, with a subsequent decrease in yield, may occur (Erickson, 1995).

The replacement of inorganic catalysts by lipases, for the interesterification of fat blends, has been attempted also due to the benefits of the enzymatic route relatively to chemical processes. Lipase-catalyzed interesterification of oils and fats is frequently carried out in batch and continuous reactors, either in the presence of organic solvent or in solvent-free media (Table 8).

The use of thermostable lipases is very important when these reactions occur in solvent-free media, where at least one of the substrates has a high melting point (m.p.), such as palm stearin (m.p. = 47-54ºC). To carry out these reactions at near-room temperature, an organic solvent to dissolve the solid fats is needed. This will increase the complexity of the system, as well as the costs related with solvent and downstream processing. In the last decade, these facts, together with the search for green processes, have drawn special attention to the search for lipases produced by thermophilic microorganisms.

In the margarine industry, conventional fat blend formulations contain (i) palm stearin or fully hydrogenated oils to provide the spreadability of the margarines and (ii) palm kernel or coconut oils (lauric oils) to increase the diversity of fatty acid profile, contributing to the presence of the desirable β' crystals in the margarine structure (Kim et al. 2008). The addition of polyunsaturated oils to these blends will decrease the atherogenicity, mainly ascribed to the presence of saturated fatty acids (lauric, myristic and palmitic acids) in palm stearin, palm kernel and coconut oils, considered the primary risk factors for coronary heart disease (Shin et al. 2009). Besides mixtures of vegetable fats currently used for margarine production (*e.g.*, palm stearin, palm, coconut, palm kernel, canola, soybean and sunflower oils), commercial concentrates of triacylglycerols rich in EPA and DHA have been also used in order to obtain interesterified fat blends rich in omega-3 PUFA (Osório et al. 2001; Nascimento et al. 2004; Nascimento et al. 2006; Osório et al. 2006; Osório et al. 2008; Osório et al. 2009a; Osório et al. 2009b). In these studies, results obtained were very promising, since for reaction times not longer than 2 hrs, the obtained SLs presented rheological properties similar to that of the chemically interesterified fat blends and, thus, fulfilled the technological requirements for the production of margarines.

However, during lipase-catalyzed interesterification, the accumulation of FFA in reaction media occurs even when using the biocatalysts at low initial a_w values (Ghazali et al. 1995; Osório et al. 2001; Nascimento et al. 2004; Rasera et al. 2012). This results from the mechanism of lipase-catalyzed interesterification which involves the hydrolysis of ester bonds in TAGs followed by esterification. Thus, the optimization of interesterification reactions results from a balance between the rates of hydrolytic and esterification reactions, which is not always easy to achieve.

When the enzymatic interesterification of palm stearin with soybean oil was performed in solvent-free media, in continuous PBR and FBR, the FFA of the interesterified fat blend rapidly decreased to values between 0.8 and 3%, remaining approximately constant during the running period, which are considerably lower than that of the interesterified fats obtained batchwise (Osório et al. 2005; Osório et al. 2006; López-Hernández et al. 2007; Osório et al. 2009b). A similar trend was observed during batch reutilization of Lipozyme RM IM (Posorske et al. 1988; Zhang et al. 2000) and Lipozyme TL IM (Zhang et al. 2001).

A high operational stability of the biocatalysts is a key-factor to make the use of lipases as catalysts for the production of commodity fats economically feasible. Promising results were obtained for (i) Novozyme 435, used at 70ºC as catalyst for the interesterification of palm stearin with soybean oil, in a continuous FBR, with a half-life of 17 days (Osório et al. 2005) and (ii) Lipozyme TL IM in a continuous PBR, for the interesterification of ternary blends of palm stearin, palm kernel oil and sunflower oil (half life of 135 hrs) or palm stearin, palm kernel oil and a concentrate of TAGs rich in omega-3 PUFA, with a half life of 77 hrs (Osório et al. 2006).

As an alternative to high cost commercial immobilized lipases, the performance of *Candida parapsilosis* lipase/acyltransferase immobilized on Accurel MP 1000 as catalyst for interesterification was studied. This enzyme, which preferentially catalyzes alcoholysis over hydrolysis, when in aqueous or in biphasic aqueous/organicmedia (Briand et al. 1995a; Briand et al. 1995b) was able to catalyze the interesterification of fat blends of palm stearin, palm kernel oil and TAGs rich in omega-3 PUFA, in solvent-free media, batchwise and in a continuous FBR (Osório et al. 2009a; Osório et al. 2009b). However, the operational stability of this biocatalyst (half-life of 10 hrs and 18 hrs in batch reactor, without or with rehydration between batches; 9 hrs in fluidized-bed reactor) was lower than that observed with commercial immobilized lipases in the presence of similar fat blends (Osório et al. 2006).The operational stability depends, not only on the biocatalyst used but also on the refining degree of the fat blends used.

The enzymatically interesterified fat blends have been used for the manufacture of margarines by several authors. As an example, table margarines were prepared at (i) lab-scale from interesterified fat

blends of milk fat stearins and oleins with high- and low-melting point natural fats (De et al. 2007) and blends of canola oil, palm stearin and palm kernel oil (Kim et al. 2008), and (ii) pilot-scale from interesterified blends of palm stearin with palm kernel olein, (Laia et al. 2000), and palm stearin with soybean oil, obtained in a FBR (Fonseca et al. 2001; Osório et al. 2003; Osório et al. 2005). The texture of "enzymatic" margarines was similar to that of the "chemical" margarines, prepared from chemically interesterified fat blends, using the same margarine formulation (Osório et al. 2003).

CONCLUDING REMARKS

This review showed the potential of lipases as biocatalysts for the production of fatty acid derivatives for the food and nutraceutical industries, namely the production of flavouring esters, fatty acid esters of antioxidants and structured lipids.

Nowadays, the main constrains for the industrial implementation of these enzymatic processes refer to economical aspects related with the high cost of most of the biocatalysts used, together with a low activity and operational stability of some of them. Also, the use of organic solvents must be avoided in order to decrease costs of solvent, product recovery and purification, and to obtain a green process, adequate for the food and nutraceutical industries. However, the search for stable and active low-cost lipases and the optimization of the enzymatic processes will allow in the near future that many of these processes will be catalyzed by lipases instead of inorganic catalysts, with great benefits in terms of nutritional and environmental aspects.

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Tables

Table 1. Examples of lipase sources and their applications.

Type	Lipases	Industry	Action	Product or application
Fungal	*Candida rugosa*	Dairy	Hydrolysis of milk fat; Lipolysis; and modification of butterfat and cream	Fragrance agents in cheese, milk, and butter
	Candida antarctica A/B	Detergents	Hydrolysis	Fats removal (decomposition of lipids), soap production, dish washing, dry cleaning solvents
	Thermomyces lanuginosus	Baked products and confectionery	Flavor improvement; control non-enzymatic browning; quality improvement	Extend shelf-life, increase loaf volume, Improve crumb, structure, Mayonnaise and dressings emulsifiers
	Yarrowia lipolytica	Waste treatment		Degradation of organic debris, clearing of drains, sewage treatment plants, water reconditioning
	Aspergillus niger and *oryzae*	Food industry	Cheese flavoring and ripening	Flavoring agents (acetoacetate, beta-keto acids, methyl ketones, flavor esters, and lactones)
	Pseudomonas sp.	Pulp and paper industry	Hydrolysis	Wastepaper deinking, increase paper whiteness, reduce waste water pollution
	Achromobacter sp.	Fine chemicals	Enantioselectivity	Resolution of racemic mixtures
	Acinetobacter calcoaceticus	Bioremediation	Hydrolysis	Remove fat layer
Animal	Pig pancreatic	Fats and oils	Transesterification, hydrolysis	Butter substitutes (cocoa butter), Glycerides for butter, and margarine

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Table 2. Examples of lipase-catalyzed production of nutraceuticals.

Compound	Reaction	Biological activity	Lipase used	Reference
L-menthol	Enantio-resolution	Local anestesic, anti-irritant	*C. rugosa*	Vorlová et al. 2002
Carnosine	Acylation	Antioxidant, neuroprotector	Novozyme 435	Husson et al. 2011
Phytosterols	Acylation	Hipocholesterolemic, antiinflamatory, neuroprotector	Novozyme 435	Hernández Soto et al. 2008
Saponins	Hydrolysis	Antiinflamatory cicatrizing agent	Novozyme 435	Monti et al. 2005

Table 3. Enzymatic production of different flavours.

Flavour	Lipase from	Reaction	System	Support	Reference
Ethyl butyrate	*Candida rugosa*	Esterification	n-hexane	Hydrophilic polyurethanes	Dias et al. 1991 Pires-Cabral et al. 2005a Pires-Cabral et al. 2005b Pires-Cabral et al. 2007 Pires-Cabral et al. 2009 Pires-Cabral et al. 2010
Ethyl valerate	*Candida rugosa*	Esterification	n-hexane solvent free	aminated polyproplilene membranes	Bayramoglu et al. 2011
	Candida rugosa	Esterification	cyclohexane	organogels	Raghavendra et al. 2010
Butyl acetate	r-*Staphylococcus xylosus*	Esterification			Mosbah et al. 2010
	Candida antarctica	Esterification	n-hexane	Novozyme 435	Martins et al. 2011
	r-*Rhizopus oryzae*	Esterification	n-hexane	Octadecyl Sepabeads	Guillén et al. 2012
	Rhizopus oryzae		n-hexane,n-heptane solvent free	Celite 545	Ben Salah et al. 2007
Butyl butyrate	*Rhizomucor miehei*	Esterification	n-hexane	Lipozyme RM-IM	Lorenzoni et al. 2012
isoamyl acetate	*Geotrichum* sp. *Rhizopus* sp.	Esterification	solvent free	no	Macedo et al. 2004
	Bacillus licheniformis S-86	Esterification	n-hexane	DEAE Sepharose	Torres et al. 2009
L-menthol	r-*Candida rugosa* LIP1	Hydrolysis	aqueous	no	Vorlová et al. 2002
L-menthol	*B. subtilis* esterase	Hydrolysis	cosolvent	no	Zheng et al. 2009
Citronellyl butyrate	*Rhizopus* sp.	Esterification	solvent free	Celite	Melo et al. 2005
Cis-3-hexen-1-yl-acetate	*Rhizomucor miehei*	Transesterification	n-hexane	Lipozyme IM-77	Chiang et al. 2003
n-amyl isobutyrate	*Candida rugosa*	Esterification	n-hexane	hydrogels	Milasinovic et al. 2012
2-phenylethyl acetate	*Candida antarctica*	Transesterification	n-hexane	Novozyme 435	Kuo et al. 2012
Cinnamyl acetate	*Candida antarctica*	Transesterification	Solvent free	Novozyme 435	Geng et al. 2012
Cinnamyl acetate	*Candida antarctica*	Transesterification	Toluene	Novozyme 435	Yadav and Davendran, 2012
Ethyl caprylate	*Acinetobacter EH28*	Esterification	Isooctane and others	Non AOT based organogels	Ahmed et al. 2010

Table 4. Examples of different bioreactor configurations for the production of flavours and fragrances.

Flavour	Lipase origin	Support	Bioreactor	Reference
Ethyl butyrate	*Candida rugosa*	Polyurethane foams	PBR Repeated batch reactors	Pires-Cabral et al. 2010
Citronellyl malonate	*Candida rugosa*	Amberlite	PBR	Serri et al. 2010
Amyl caprylate	*Candida rugosa*	Sepabeads	FBR Batch reactor	Saponjic et al. 2010
Geranyl butyrate	*Candida rugosa*	Sepabeads	FBR Batch reactor	Damnjanovic et al. 2012
Isomayl acetate	*Candida antarctica*	no	microreactor	Znidarsic-Plazl and Plazl 2009

Table 5. Enzymatic synthesis of fatty acid esters of L-ascorbic acid.

Type of acylating agent	Acyl donor	Solvent	Biocatalyst	Reaction conditions	Conversion (%)	Reference
Alkyl esters	Methyl palmitate	2M2B [a]	Novozym 435[b]	1.36 mmol ascorbic acid, 6.8 mmol methyl palmitate; 200 mg biocatalyst; 20 ml solvent; 55ºC	68	Humeau et al. 1995
	Methyl oleate	Acetone	Chirazyme L-2[b]	0.15 mmol ascorbic acid, 0.60 mmol methyl oleate; 50 mg biocatalyst; 5 ml solvent; 45ºC	60	Adamczak et al. 2005
	EPA ethyl ester, DHA ethyl ester[c]	2M2B	Novozym 435	12 g/l ascorbic acid, 112 g/l EPA ethyl ester or 121 g/l DHA ethyl ester; 16 mg/ml biocatalyst; 55ºC	≤ 40	Humeau et al. 1998
	Palm oil methyl ester	Acetone	Novozym 435	1 mmol ascorbic acid, 4 mmol acyl donor; 300 mg biocatalyst; 10 ml solvent; 50ºC; 24 hrs	62	Hsieh et al. 2005
	Methyl L-lactate	2M2B	Novozym 435	0.5 mmol ascorbic acid, 0.5 mmol acyl donor; 100 mg biocatalyst; 5 ml solvent; 250 mg molecular sieves; 55ºC; 8 hrs	80	Maugard et al. 2000
Fatty acids	Lauric acid, Myristic acid, Palmitic acid, Stearic acid	2M2P [d]	Novozym 435 Lipozyme IM[e]	0.15 mmol ascorbic acid, 0.90 mmol fatty acid; 150 mg biocatalyst; 5 ml solvent; 45ºC; 24 hrs	≤ 65	Stamatis et al. 1999
	Lauric acid	Acetonitrile	Novozym 435	Ascorbic acid to lauric acid molar ratio 1:4.3; 34.5% w/w biocatalyst referred to ascorbic acid; 30.6ºC; 6.7 hrs	93	Chang et al. 2009
	Palmitic acid	Hexane	Lipase from *B. stearothermophilus*	50 mmol ascorbic acid, 250 mmol palmitic acid, 1litre solvent, 5 g biocatalyst, 60ºC, 2 hrs	97	Bradoo et al. 1999
	Palmitic acid, Oleic acid	2M2B	Novozym 435	16 mmol ascorbic acid, 32 mmol oleic acid, 184 ml solvent, 600 mg biocatalyst, 2.5 g molecular sieves, 65°C, 48 hrs	71-86	Viklund et al. 2003
	Capric acid, Lauric acid, Myristic acid	Acetone	Chirazyme L-2	Packed-bed reactor; 200 mM fatty acid, ascorbic acid at the top of column, 50ºC	[60 g/ L reactor·day]	Watanabe et al. 2003
	Capric acid, Lauric acid, Myristic acid, Oleic acid, Linoleic acid, Arachidonic acid	Acetone	Chirazyme L-2	Packed-bed reactor; 200 mM fatty acid, powdered ascorbic acid in column, 50ºC	[1.6-1.9 kg/ L reactor·day]	Kuwabara et al. 2003b
	Palmitic acid, Oleic acid	Ionic liquids	Novozym 435	0.1 mmol ascorbic acid, 0.12 mmol fatty acid; 20 mg biocatalyst; 0.5 ml solvent;	≤ 61	Park et al. 2003

				50 mg molecular sieves, 60°C, 24 hrs		
	Oleic acid, Linoleic acid	2M2B	Novozym 435	4 mmol ascorbic acid, 4 mmol fatty acid; 300 mg biocatalyst; 10 ml solvent; 500 mg molecular sieves, 12 h	38-44	Song et al. 2004
	Oleic acid	Ionic liquids	Chirazyme L-2	0.12 mmol ascorbic acid, 0.1 mmol oleic acid; 20 mg lipase; 0.5 ml ionic liquid; 60°C; 24 h	72	Adamczak and Bornscheuer, 2009
	Oleic acid	2M2B	Lipase from *Candida* sp.	2 mmol ascorbic acid, 1.5 mmol oleic acid; 300 mg lipase; 10 ml solvent; 55°C; 24 hrs	n.r.	Song and Wei, 2002
	Linoleic acid	Acetone	Chirazyme L-2	0.125 mmol ascorbic acid, 1.25 mmol linoleic acid; 50 mg biocatalyst; 2.5 ml solvent; 50ºC; 48 hrs	70	Watanabe et al. 2008
Vinyl esters	Vinyl octanoate, Vinyl decanoate, Vinyl laurate, Vinyl palmitate	*t*-butanol	Chirazyme L-2	1 mmol ascorbic acid, 3 mmol vinyl ester; 50 mg biocatalyst; 1 ml solvent; 200 mg molecular sieves; 40ºC; 48 hrs	≤ 91	Yan et al. 1999
Triglycerides	Palm oil	2M2B	Novozym 435	1.5 mmol ascorbic acid, 12 mmol palm oil; 0.5 ml solvent; 40ºC; 16 hrs	70-75	Burham et al. 2009
	Olive oil	2M2B	Lipozyme TL IM[f] Novozym 435[a]	0.5 mmol ascorbic acid, 0.5 mmol olive oil; 5 ml solvent; 125 mg biocatalyst; 40ºC; 168 hrs	27-33	Reyes-Duarte et al. 2011

Table 6. Examples of phenolic antioxidants whose lipase-catalyzed acylation has been reported.

Family	Structure	Example	Enzymatic acylation
Simple phenols and derivatives	C6	Resorcinol	(Miyazawa et al. 2008)
		Hydroxytyrosol	(Torres De Pinedo et al. 2005)
		Tyrosol	(Fernández et al. 2012)
Phenolic acids	C6-CX (X= 1-4)	Dihydrocaffeic acid	(Sabally et al. 2007)
		Ferulic acid	(Safari et al. 2006)
Xanthonoids	C6-C1-C6	Mangiferin	(Wang et al. 2010b)
Stilbenoids	C6-C2-C6	Resveratrol	(Torres et al. 2010)
Flavonoids	C6-C2-C6	Quercetin	(Christelle et al. 2011)
		Hesperetin	(Chebil et al. 2007)
		Rutin	(Ardhaoui et al. 2004)
		Naringin	(Kontogianni et al. 2001)

Table 7 Examples of lipase-catalyzed production of low caloric and dietetic TAGs.

Product	Biocatalyst	Substrates	Reaction System	Operation mode	Best reaction conditions	Best results	Reference
MLM LLL rich in DHA	*Rhizopus delemar* lipase in a ceramic carrier	Tuna oil + C8:0 Tuna oil + DHA	Solvent-free	Batch	[Biocatalyst] = 4wt-% MR = 1:12 T = 30ºC t = 48 hrs	65 mol-% C8:0	Shimada et al. 1996
MLM	Lipozyme RM IM	Rapeseed oil + C10:0 Safflower oil +C8:0	Solvent-free	Continuous PBR	MR = 1:5 T= 60ºC Residence time = 90min	MLM 65 mol-% C10:0	Xu et al. 1998
MLM	Lipozyme RM IM	Rice bran oil + C10:0	Solvent-free *n*-hexane	Batch	MR = 1:8 T = 55ºC Solvent-free: [Biocatalyst] = 20 wt-% Hexane: [Biocatalyst] = 10 wt-%	Solvent-free: 43.2 mol-% C10:0 Hexane: 53.1 mol-% C10:0	Jennings and Akoh, 2000
MLM	Lipozyme RM IM	Menhaden fish oil + C10:0	Solvent-free *n*-hexane	Batch	MR = 1:8 T = 55ºC t = 72 hrs Solvent-free: [Biocatalyst] = 20 wt-% Hexane: [Biocatalyst] = 15 wt-%	Solvent-free: 36.7 mol-% C10:0 Hexane: 50.7 mol-% C10:0	Jennings and Akoh, 2001
LSL	Chirazyme L-2	Triacetin + C18:0	Solvent-free	Batch - open reactor -closed reactor -vacuum reactor	[Biocatalyst] = 4 wt-% MR = 1:1.73 T = 80ºC - open reactor: t = 6 hrs - vacuum reactor: P = 700 mm Hg t = 4 hrs	- open reactor: 85.5% C18:0 - vacuum reactor: 83.3% C18:0	Yang et al. 2001
MLM	Lipozyme RM IM	Rapeseed oil +C6:0	Solvent-free	Batch	[Biocatalyst] = 14wt-% MR = 1:5 T = 65ºC t = 17 hrs	55 mol-% C6:0	Zhou et al. 2001
MLM	Lipozyme RM IM	Sea blubber oil + C10:0	Several solvents Solvent-free	Batch	[Biocatalyst] = 10wt-% MR = 1:3 T= 45ºC t = 24 hrs	In hexane: 27 mol-% C10:0 Solvent-free:	Senanayake and Shaidi, 2002b

					[molecular sieve] = 1%	24 mol-% C10:0.	
MLM	Lipozyme RM IM	Olive oil + C8:0	Solvent-free	Continuous PBR	MR = 1:5 T = 60ºC Residence time = 2.7 hrs	43 mol-% C8:0	Fomuso and Akoh, 2002
MLM	Lipozyme RM IM	Palm olein + C8:0	Solvent-free	Continuous PBR	MR = 1:5 T = 60ºC Flow rate = 1 mL/min	30.5 mol-% C8:0	Lai et al. (2005)
MLM	Lipozyme RM IM	Roasted sesame oil + C8:0	Solvent-free	Continuous PBR	MR = 1:6 T = 45ºC Flow rate = 1.15 mL/min Residence time = 7.8 hrs	42.5 mol-% C8:0	Kim and Akoh, 2006
MLM	Lipozyme TL IM	Soybean oil + C8:0	Solvent-free	Batch	[Biocatalyst] = 20 wt-% MR = 1: 1 T = 45ºC t = 6 hrs	13 mol-% C8:0	Turan et al. 2006
MLM	Several lipases	Lard + C10:0	Several solvents	Batch	[Lipozyme TL IM] = 10% hexane or isooctane MR = 1:2 T = 50-55ºC t = 24 hrs	50.14 mol-% C10:0	Zhao et al. 2007
MMM	Lipozyme RM IM Lipozyme TL IM Novozym 435	Glycerol+ FFA (Palm kernel oil distillate)	Solvent-free	Batch	[Novozym 435] = 2 wt% MR: Gly:FA = 1:4 T = 90ºC t = 10 hrs	73.3%	Low et al. 2007
MLM	Lipozyme RM IM	Fish oil + C10:0 methyl ester Fish oil + Medium-chain TAG	Solvent-free	Batch	[Biocatalyst] = 5 wt-% T = 60ºC t = 6 hrs P = 56 mm Hg	Rich in EPA, DHA and C10:0	Feltes et al. 2009
MLM & LLL	Lipase from *M. miehei*	Terebinth fruit oil + C8:0+C18:0	*n*-hexane	Batch	[Biocatalyst] = 30 wt-% MR = 1:1:3 T = 50ºC t = 18 hrs	19 mol-% C8:0 + 14 mol-% C18:0	Koçak et al.2011
MLM	Lipozyme RM IM Lipozyme TL IM Novozym 435	Olive oil + C8:0 Olive oil + C10:0	Solvent-free *n*-hexane	Batch	[Biocatalyst] = 5wt-% MR = 1:2 T = 45ºC t = 24 hrs	Solvent-free 25 mol-% C8:0 27-30 mol-% C10:0	Nunes et al. 2011b
MLM	*Rhizopus oryzae* recombinant lipase in	Olive oil + C8:0	Solvent-free	Batch	[Biocatalyst] = 5wt-% T = 40ºC	21.6 mol-% C8:0 34.8 mol-% C10:0	Nunes et al. 2011c

	Eupergit C	Olive oil + C10:0	*n*-hexane		MR = 1:2 t = 24 hrs		
MLM	*Rhizopus oryzae* recombinant lipase in Eupergit C or Lewatit VP OC 1600	Olive oil + C8:0 Olive oil + C10:0	Solvent-free	Batch	[Biocatalyst] = 5 wt-% T = 40ºC MR = 1:2 t = 24 hrs	r-ROLin Lewatit with rehydration ($t_{1/2}$ = 234 hrs; C10:0)	Nunes et al. 2012
MLM	several commercial lipases	Canola oil + C8:0	Solvent-free	Batch	[Lipozyme RM IM] = 10 wt-% MR = 1:4 T= 50-60ºC t = 15 hrs	44% C8:0	Wang et al. 2012
MLM	Lipozyme RM IM	Modified pine nut oil + C10:0	Solvent-free	Continuous PBR	MR = 1:5 [H_2O] = 0.04 % T = 60ºC	MLM with pinolenic acid	Choi et al. 2012
MLM	Lipozyme RM IM	Palm olein +C8:0+C10:0	hexane	Batch	t = 24 hrs [Biocatalyst] = 10 wt-%	38% C8:0 43% C10:0	Chnadhapuram and Sunkireddy, 2012
LML	Lipozyme RM IM	Tricaprin + *n*-3 PUFA Tricaprin + *n*-3 PUFA ethyl esters	Solvent-free	Batch	*n*-3 PUFA EE [Biocatalyst] = 6 wt-% T = 60°C; MR = 1:6 t = 6 hrs [H_2O] = 3 %	49.6% *n*-3 PUFA	Chen et al. 2012

t = reaction time; DHA= docosahexaenoic acid; FA= fatty acid; L= long-chain fatty acid; M= medium-chain fatty acid, MR= molar ratio TAG:FA; P= pressure; PBR= packed-bed reactor; S= short-chain fatty acid; T= temperature; TAG= triacylglycerol; Acids: C6:0= caproic acid; C8:0= caprylic acid; C10:0= capric acid; C12:0= lauric acid; C18:0= stearic acid; [Biocatalyst] = biocatalyst concentration. Commercial biocatalysts: Chirazyme L-2 = immobilized lipase B from *Candida antarctica*; Lipozyme TL IM= immobilized lipase from *Thermomyces lanuginosa*; Lipozyme RM IM= immobilized lipase from *Rhizomucor miehei*; Novozym 435= immobilized lipase from *Candida antarctica.*

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Table 8. Examples of lipase-catalyzed production of *trans*-free and nutritionally improved SLs for commodity fats for the margarine industry and specialty fats.

Biocatalyst	Substrates	System type	Operation mode	Best reaction conditions	Reference
Novozym 435	PS + soybean oil + "EPAX 2050TG"	Solvent-free	Batch	[Biocatalyst] = 5 wt-% 50% PS+32% soybean oil+18% EPAX T = 60ºC	Osório et al. 2001
Novozym 435	PS + soybean oil	Solvent-free	Continuous FBR	T = 70ºC Residence time = 19 min	Osório et al. 2005
Lipozyme TL IM	Blend A: PS + PK + sunflower oil Blend B: PS + PK + "EPAX 4510TG"	Solvent-free	Continuous PBR	T = 70ºC Residence time= 15 min Blend A = 55:25:20 Blend B = 55:35:10	Osório et al. 2006
Lipozyme TL IM	Sesame oil + FHSO	Solvent-free	Batch Continuous PBR	T = 70ºC 4-6 hrs Residence time = 15 min- 2 hrs	López-Hernández et al. 2007
Lipozyme RM IM LIP1	Canola oil + C18:0 Canola oil + ethyl stearate	*n*-hexane	Batch	Lipozyme RM IM (interesterification): [Biocatalyst] = 10 wt-% T = 50ºC t = 12 hrs	Lumor et al. 2007
Novozym 435	Olive oil + FHPO	Solvent-free	Batch	[Biocatalyst] = 10 wt-% T = 75ºC t = 3 hrs	Criado et al. 2008
Lipozyme TL IM	PS + PK + canola oil	Solvent-free	Batch	[Biocatalyst]= 5 wt-% T = 60ºC t = 18 hrs	Kim et al. 2008
Lipozyme TL IM	PS + PK + "EPAX 4510TG"	Solvent-free	Batch	[Biocatalyst]= 6 wt-% T = 60ºC t = 1 hr P = 0.1-150 MPa	Osório et al. 2008
Lipozyme TL IM	Rice bran oil + Palm stearin	Solvent-free	Batch	[Biocatalyst] = 3 wt-% T = 60ºC	Reshma et al. 2008
Lipase/acyltransferase on Accurel MP 1000	PS + PK + "EPAX 4510TG"	Solvent-free	Batch	[Biocatalyst] = 5 wt-% a_w = 0.97 t = 120 min T = 55-65ºC	Osório et al. 2009a
Lipozyme RM IM	Butterfat+ PS+ flaxseed oil (source of C18:3)	Solvent-free	Continuous PBR	T = 60ºC Residence time = 140 min	Shin et al. 2009
Lipozyme TL IM	PS + SBO	Solvent-free	Batch Continuous PBR (2 columns)	[Biocatalyst] = 4 wt-% T= 70ºC PS/SBO = 70/30	Costales-Rodríguez et al. 2009

				t = 4 hrs Residence time = 27 min	
Lipozyme TL IM	Beef tallow + High oleic sunflower oil (HOSFO)	Solvent-free	Batch	[Biocatalyst] = 10 wt-%; T = 60ºC t = 24 hrs	Segura et al. 2011
Aspergillus niger lipase immobilized on SiO_2-PVA composite	Milkfat+Soybean oil	Solvent-free	Batch	T = 45ºC t = 72 hrs MF:SBO = 67:33	Nunes et al. 2011a
Fermented solids by *Rhizopus oryzae* and *Rhizopus microsporus*	PS + PK + "EPAX 4510TG"	Solvent-free	Batch	fermented solids by *R. oryzae* 38 wt-% PS + 47% PK + 15%. EPAX T = 65ºC t = 24 hrs	Rasera et al. 2012
Lipozyme TL IM Novozym 435	Stearidonic acid SBO + high stearate SBO	Solvent-free	Batch	T_{opt} = 50ºC t = 18 hrs St SBO: SSBO = 2:1 T_{opt} = 58ºC t = 14 hrs St SBO:SSBO = 2:1	Pande and Akoh, 2012

Legend: a_w= water activity; C18:3= linolenic acid; "EPAX 2050TG"= concentrate of TAG rich in omega-3 PUFA (c.c. 20% EPA+ 50% DHA); EPAX 4510TG"= concentrate of TAG rich in omega-3 PUFA (c.c. 45% EPA+ 10% DHA); FBR= fluidized bed reactor; FHPO= fully hydrogenated palm oil; FHSO= fully hydrogenated soybean oil; P= pressure; PK= palm kernel oil; PS= palm stearin
Biocatalysts: LIP1- free lipase isoform 1 from *Candida rugosa.* See legend of Table 7 and Table 9.

Table 9. Examples of lipase-catalyzed production of human milk fat substitutes (HMFS).

Biocatalyst	Substrates	System type	Operation mode	Best reaction conditions	Best results	Reference
Carica papaya latex Lipozyme RM IM	PPP + low erucic rapeseed oil FA	Solvent-free	Batch	[Biocatalyst] = 9.9 wt-% MR = 1:1 T = 60ºC t = 6 hrs	HMFS with essential PUFA	Mukherjee and Kiewitt, 1998
Lipozyme RM IM	Lard+soybean oil FA	Solvent-free	Batch	[Biocatalyst] = 13.7 wt-% MR = 1:2.4 T = 61ºC t = 1 hr	HMFS similar to Chinese mothers' milk fat	Yang et al. 2003
Lipozyme RM IM	PPP + hazelnut FA + stearic acid (C18:0)	*n*-hexane	Batch	[Biocatalyst] = 10 wt-% MR = 1:12:1.5 T = 65ºC t = 24 hrs	HMFS 47% of C18:1	Sahín et al. 2005a
Lipozyme RM IM Lipozyme TL IM	PPP + [hazelnut FA + GLA]	*n*-hexane	Batch	T = 55ºC t =24 hrs [Lipozyme RM IM] = 10% MR =1:14.8 [Lipozyme TL IM]= 6% MR =1:14	HMFS 10% GLA; 45% C18:1	Sahín et al. 2005b
Lipozyme RM IM	Lard+soybean oil FA	Solvent-free	Continuous PBR	MR = 1:3 T = 65ºC Residence time = 1.5 hrs	HMFS similar to Betapol	Nielsen et al. 2006
Lipozyme RM IM	PPP + [hazelnut FA+ + omega-3 PUFA]	*n*-hexane	Batch	[Biocatalyst] = 10 wt-% MR = 1:12.4 T = 55ºC t = 24 hrs	HMFS with omega-3 PUFA	Sahín et al. 2006
LIP1 Lipozyme RM IM	PPP + C18:1 PPP + Methyl oleate	*n*-hexane	Batch	[Lipozyme RM IM] = 10% PPP + Methyl oleate MR = 1:3 T = 65ºC t = 24 hrs	HMFS 49.4% C18:1	Srivastava et al. 2006
Lipozyme RM IM	PPP + [coconut + safflower+ soybean] oils	*n*-hexane	Batch	[Biocatalyst] = 10 wt-% MR = 1:1 (PPP:vegetable oils) T = 55ºC t = 12 hrs	HMFS	Maduko et al. 2007a
Lipozyme RM IM	PPP + [safflower+ soybean] oils	*n*-hexane	Batch	[Biocatalyst] = 10 wt-% MR = 1:3 (PPP vegetable oils) T = 55ºC t = 14.4 hrs	HMFS 40% C16:0 at *sn*-2 position	Maduko et al. 2007b

Lipozyme TL IM	Lard + soybean oil	Solvent-free	Batch	[Biocatalyst] = 5 wt-% T = 60ºC t = 14.4 hrs	HMFS	Silva et al. 2009
Lipozyme RM IM-	Butter fat + Rapeseed oil and soybean oil fatty acids	Solvent-free	Continuous pilot-scale PBR	T = 65ºC MR = 1:2 [H_2O]= 0.07 wt%, 500 mL/h	HMFS	Sorensen et al. 2010
Lipozyme RM IM	Lard + FA from PK, tea seed and soybean oils	Solvent-free	Batch	[Biocatalyst] = 7 wt-% T = 60ºC MR = 1:2 t = 1 hr	HMFS of Guangzhou mothers	Wang et al. 2010a
Lipozyme TL IM Lipozyme RM IM Novozym 435 *Candida parapsilosis* Lipase/Acyltransferase	PPP+ C18:1 PPP + omega-3 PUFA	Solvent-free	Batch	T = 60ºC t = 24 hrs MR = 1:2 (TAG:FFA)	27 mol-% C18º:1 (commercial lipases); 20-22 mol% omega-3 PUFA (LIpozyme RM IM; Novozyme 435)	Tecelão et al. 2010
Carica papaya latex	PPP+ C18:1 PPP + omega-3 PUFA	Solvent-free	Batch	PPP + C18:1 T = 60ºC t = 24 hrs MR = 1:2 (TAG:FFA)	22.1 mol-% C18º:1	Tecelão et al. 2012a
Novozym 435 Lipozyme TL IM	PPP+ stearidonic acid enriched soybean oil	Solvent-free	Batch	[Biocatalyst] = 10.7 wt-% T = 50ºC MR = 1:2 t = 15.6 hrs (Novozym 435) t = 18 hrs (Lipozyme TL IM)		Teichert and Akoh, 2011
Lipozyme RM IM.	Chemically interesterified PS + (rapeseed oil + sunflower oil)FA + C14:0 + C18:0	solvent free	Batch	[Biocatalyst] = 10.7 wt-% MR = 1:14.6 T = 57ºC t = 14.4 hrs		Zou et al. 2011
Lipozyme TL IM	PPP+ hazelnut FA + *Echium* oil FA	*n*-hexane	Batch	Topt = 60ºC t = 8 hrs MR = 1:4	HMFS with 2.0 mol-% stearidonic acid (C18:4n-3) + 22.9 mol-% C18:1	Yüksel and Yesilcubuk, 2012

C18:1 = oleic acid; PPP = Tripalmitin. LIP1- free lipase isoform 1 from *Candida rugosa.* See legend of Table 7.

Figures

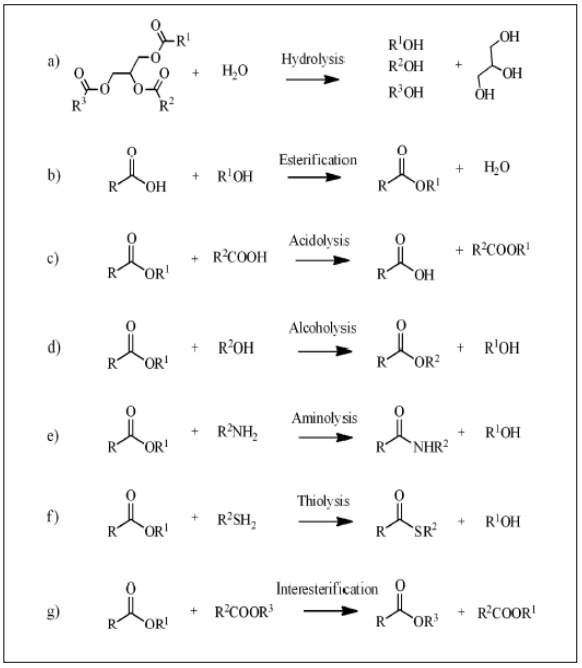

Fig. 1 Scheme of lipase-catalyzed reactions.

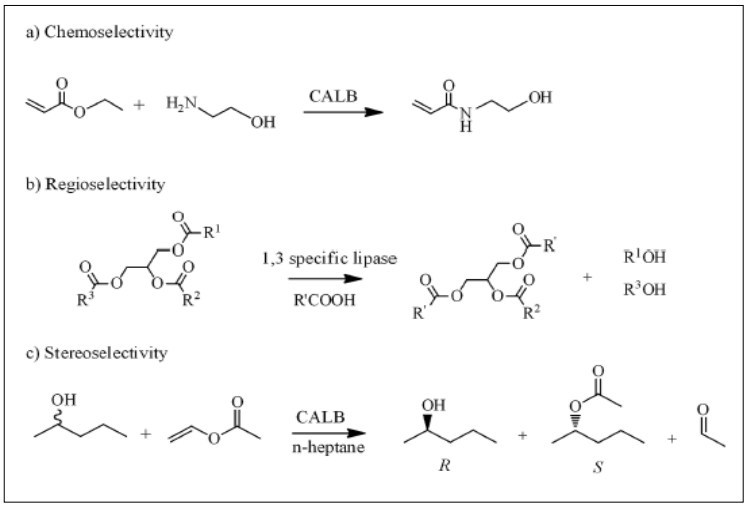

Fig. 2 Examples of lipase selectivity. CALB: *Candida antactica* B lipase.

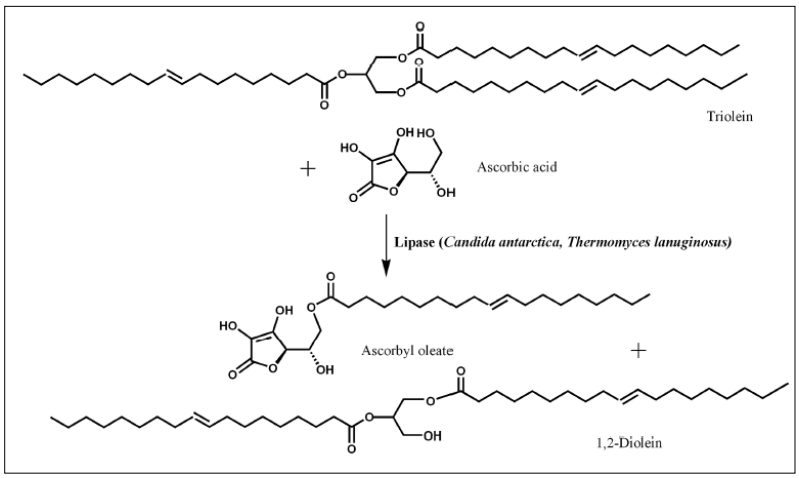

Fig. 3 Scheme of the lipase-catalyzed transesterification between L-ascorbic acid and triolein.

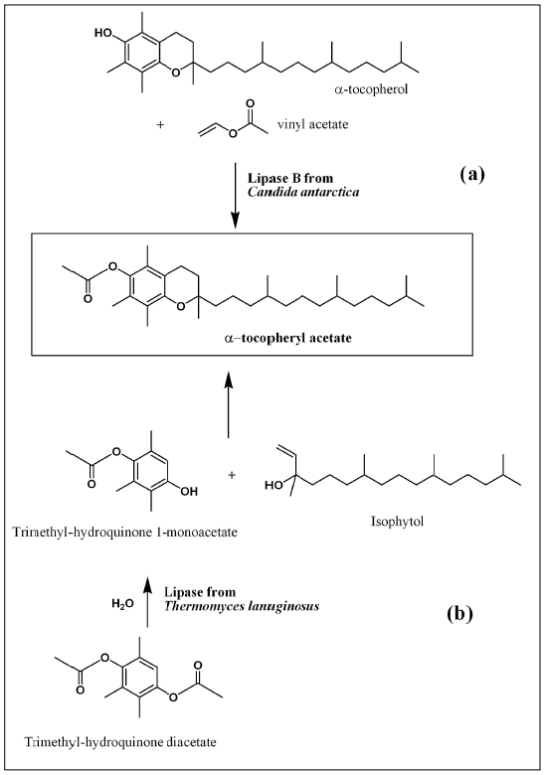

Fig. 4 Routes for the synthesis of vitamin E acetate. (a) Lipase-catalyzed direct acetylation of α-tocopherol. (b) Chemoenzymatic process based on the regioselective hydrolysis of trimethyl-hydroquinone diacetate followed by condensation with isophytol.

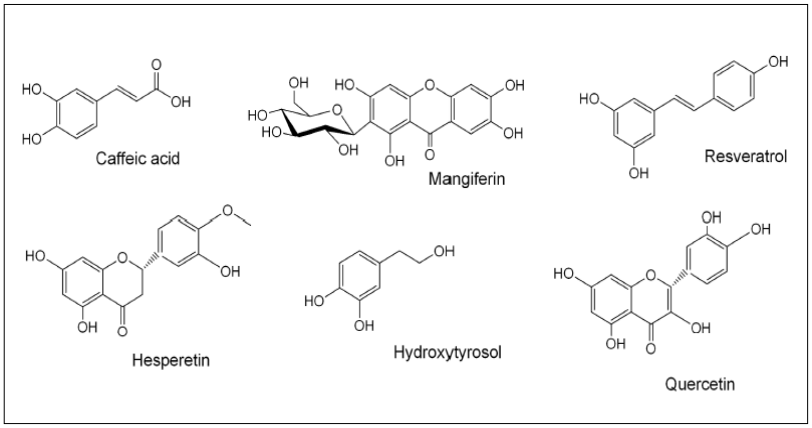

Fig. 5 Structure of some phenolic antioxidants.

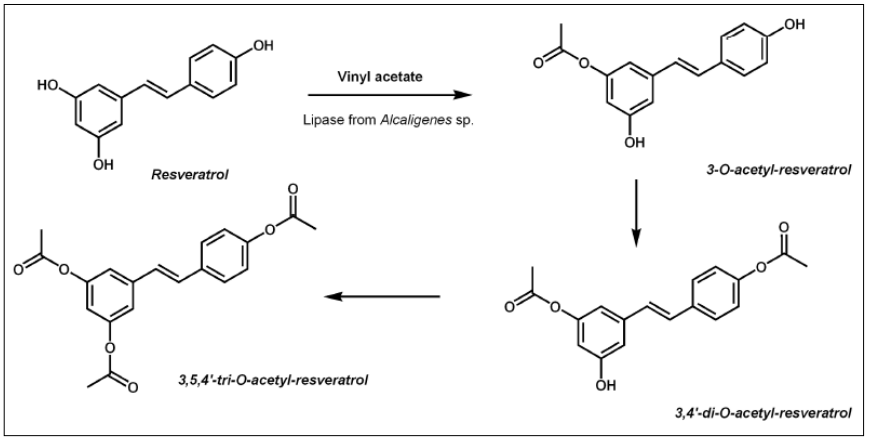

Fig. 6 Scheme of the acetylation of resveratrol catalyzed by lipase from *Alcaligenes* sp.