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BIOCATALYTIC MODIFICATION OF FOOD LIPIDS: REACTIONS AND APPLICATIONS

MODIFICACIÓN BIOCATALÍTICA DE LÍPIDOS ALIMENTARIOS: REACCIONES Y APLICACIONES

R. Baeza-Jiménez[1], L.X. López-Martínez[2] and H.S. García[3*]

[1]*Centro Conjunto de Investigación en Química Sustentable UAEM-UNAM. Carr. Toluca-Atlacomulco Km 14.5, Unidad San Cayetano. 50200. Toluca, Estado de México, México.*
[2]*Facultad de Química, Universidad Autónoma del Estado de México. Paseos Colón y Tollocan. 50000. Toluca, Estado de México, México.*
[3]*UNIDA, Instituto Tecnológico de Veracruz. M.A. de Quevedo 2779, Col. Formando Hogar. Veracruz, Veracruz 91897, México*

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Abstract

The acylglycerol structure exemplifies the major lipid building block and therefore is an interesting structure to modify. Such modification is driven by: (1) consumers who have become more concerned about the relationship between diet and wellness, and (2) new and novel functional compounds can be prepared when the original structure of a lipid is modified. This trend has led to the design of functional foods or nutraceuticals, namely, fortified, enriched, modified and enhanced foods. Advances in the biochemistry and engineering of enzymatic reactions and reactors have improved the knowledge and understanding of such reaction systems and thus, make available a generation of structured lipids. In the present work, we detail several efforts carried out to prepare novel compounds, as well as industrial applications and possible future enzymatic procedures to obtain new food products.

Keywords: enzymes, lipids, modification.

Resumen

La estructura de un acilglicerol representa al mayor eje lipídico de construcción y por tanto es susceptible de modificación. Dicha modificación resulta de: (1) que los consumidores han tomado conciencia de la relación entre la dieta y su bienestar, y (2) nuevos y novedosos compuestos funcionales pueden prepararse cuando la estructura original de un lípido es modificada. Esta tendencia ha llevado al diseño de alimentos funcionales o nutracéuticos, tales como alimentos fortificados, enriquecidos, modificados y mejorados. Los avances en bioquímica e ingeniería de reacciones enzimáticas, así como del diseño de reactores, han mejorado el conocimiento y entendimiento de dichos sistemas de reacción y de esta forma hacen disponibles esta generación de lípidos estructurados. En el presente trabajo, se detallan varios trabajos realizados para preparar novedosos compuestos, así como sus aplicaciones industriales y posibles procesos enzimáticos para obtener nuevos productos alimentarios.

Palabras clave: enzimas, lípidos, modificación.

[*]*Corresponding author. E-mail*: hsgarcia@itver.edu.mx

1 Introduction

Biological lipids are a chemically diverse group of compounds; the common and defining feature of which is their insolubility in water. The biological functions of lipids are as diverse as their chemistry. Fats and oils are the main forms of stored energy in many organisms. Phospholipids (PL) and sterols are major structural components of biological membranes. Other lipids, although present in relatively small quantities, play crucial roles as enzyme cofactors, electron carriers, light-absorbing pigments, hydrophobic anchors for proteins, "chaperones" to aid in folding of membrane proteins, emulsifying agents in the digestive tract, hormones, and intracellular messengers (Nelson and Cox, 2004).

In addition to their essential physiological and biochemical roles, research on lipids has also focused on the preparation of new foods by the modification of the original fatty acid composition. Fatty acids (FA) are the basic building blocks of most of the compounds that we term 'lipids'. Indeed, some consider them the defining lipid constituents. Polyunsaturated fatty acids (PUFA) from the essential omega-6 (n-6) and omega-3 (n-3) families are the biosynthetic precursors of prostaglandins and other eicosanoids, including leukotrienes, thromboxanes, lipoxins and others (AOCS Lipid Library). The aim of these modifications is to supply the body with beneficial components like partial glycerides, PUFA and PL.

To meet different industrial application requirements, chemical and enzymatic approaches have been performed. Compared to chemical methods, enzymatic modification of lipids has some advantages. Selectivity of enzymes makes the modification simple and straightforward. Enzymatic reactions are often conducted under mild conditions, and this biocatalytic approach performed in microaqueous reaction schemes greatly reduces the use of toxic, undesirable solvents (Guo *et al*., 2005).

The first group of enzymes that could be used for the modification/synthesis of lipids is lipases, which represent a wide variety of ubiquitous enzymes extensively employed in the processing of fats and oils, detergents and degreasing formulations, food processing, synthesis of fine chemicals and pharmaceuticals, paper manufacture, and production of cosmetics. Lipases can also be used to accelerate the degradation of fatty wastes and polyurethane (Sharma *et al*., 2001). The second group of enzymes corresponds to the phospholipases. These enzymes play crucial roles in cellular regulation, metabolism, biosynthesis and selective modification of lipids (D'Arrigo and Servi, 1997).

The purpose of this review is to offer a current and detailed assessment of the different processes developed for the modification of food lipids. We first describe the main groups of lipids, then different strategies for their modifications, some industrial applications and finally possible future enzymatic procedures to obtain new food products are described.

2 Lipids

Lipids comprise a diverse range of compounds for which no agreed definition exists. They include a diverse range of compounds, like acylglycerols, PL, FA and their derivatives, carotenoids, terpenes, steroids and bile acids. Fahy *et al*. (2005, 2009) developed a classification system for lipids as follows: lipids are hydrophobic or amphipathic small molecules that may originate entirely or in part by carbanion-based condensations of thioesters and/or by carbocation-based condensations of isoprene units. The comprehensive classification system organized by the LIPID MAPS database includes: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids and polyketides (Fahy *et al*., 2009).

The most common lipid classes in nature consist of FA linked by an ester bond to the trihydric alcohol - glycerol, or to other alcohols such as cholesterol, or by amide bonds to sphingoid bases, or on occasion to other amines. In addition, they may contain alkyl moieties other than FA, phosphoric acid, organic bases, carbohydrates and many more components, which can be released by various hydrolytic procedures.

2.1 Fatty acids (FA)

Carboxylic acids occur in many molecular forms. The majority of the FA found in lipids is in the form of monocarboxylic acids; some of them are dicarboxylic and constitute important metabolic or oxidation products of the previous ones. To describe precisely the structure of a FA molecule, one must give the length of the carbon chain (number of carbons), the number of double bonds and also the exact position of these double bonds. This will define the biological reactivity of the fatty acid molecule and even of the lipid containing the FA studied.

FA's are compounds synthesized in nature via

condensation of malonyl coenzyme A units by a fatty acid synthase complex. They usually contain even numbers of carbon atoms in straight chains (commonly C_{14} to C_{24}), and may be saturated or unsaturated. The latter have a great importance and many reports in the technical literature refer their benefits. For the number of unsaturations, FA can be divided in mono- and polyunsaturated fatty acids (PUFA, Cyberlipid Center).

PUFA can be divided into two main subcategories, the n-3 and the n-6 FA. Both of them are considered "essential" because they cannot be synthesized by humans and thus must be obtained through diet or supplementation. α-Linolenic acid (ALA, 18:3 n-3) is found in certain plant oils, seeds, green leafy vegetables, beans, and nuts. Linoleic acid (LA, 18:2 n-6) is found in grains, meats, and the seeds of most plants. Through an enzymatic process of desaturation (see Fig. 1), ALA evolves into eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3). EPA and DHA are precursors to a group of eicosanoids (prostaglandins, thromboxanes, and leukotrienes) that have anti-inflammatory, antithrombotic, antiarrhythmic, and vasodilatory properties. Arachidonic acid (ARA, 20:4 n-6) is a derivative of LA and a precursor to another group of eicosanoids responsible for pro-inflammatory and pro-thrombic effects. ALA and LA use and compete for the same enzymes in the production of EPA and ARA. The ingestion of fish and fish oils provides both EPA and DHA directly, thereby avoiding the competition for enzymes for the conversion of ALA to EPA (Baeza-Jiménez and Garc, 2013).

2.2 Glycerolipids

The glycerolipids essentially encompass all glycerol-containing lipids. These compounds are important as membrane constituents, metabolic fuels, and signalling molecules. The glycerolipid category is dominated by the mono-, di- and trisubstituted glycerols, the most well known being the fatty acid esters of glycerol: acylglycerols (Figure 2C, AOCS Lipid Library). Additional subclasses are represented by the glycerolglycans, which are characterized by the presence of one or more sugar residues attached to glycerol via a glycosidic linkage. Macrocyclic ether lipids also occur as glycerolipids in the membranes of archaebacteria (Fahy *et al*., 2005). The glycerolipids category was reorganized to include two new main classes (glycosyldiradylglycerols and glycosylmonoradylglycerols) that contain key plant structural lipids, such as the sulfoquinovosyldiacylglycerols found in chloroplasts. The existing glycerolglycoside subclases were removed (Fahy *et al*., 2009).

Acylglycerols, namely, monoacylglycerols (MAG), diacylglycerols (DAG) and triacylglycerols (TAG), are the main components of oils and fats and have many commercial applications. In the food industry, TAG are the primarily components of oils. It has been reported that oils rich in certain saturated fatty acids cause coronary heart disease and premature development of atherosclerosis in obese subjects (Prentice and Poppitt, 1996; Hopkins, 2003; Kolovou *et al*., 2004). On the other hand, DAG are natural components of various edible oils and recent studies have indicated that DAG beneficially affect lipid metabolism by increasing fat oxidation and decreasing storage of TAG in adipose tissue (Nagao *et al*., 2000; Kamphuis *et al*., 2003), weight loss, lower liver fat content and lower abdominal fat content after intake of DAG oil as compared to ordinary TAG oil (Murase *et al*., 2002; Yuan *et al*., 2010). Moreover, in different degrees of purity, DAG are used as additives or carriers in the food, medicinal and cosmetic industries (Fureby *et al*., 1997). Furthermore, mixtures of DAG combined with MAG are good emulsifiers for preparing food and cosmetics emulsions, and pharmaceutical applications.

DAG can be manufactured either chemically or enzymatically. Enzymatically, DAG has been produced by direct esterification, glycerolysis, interesterification, partial hydrolysis, or a combination of partial hydrolysis and esterification (Arcos *et al*., 2000; Torres *et al*., 2001; Martínez *et al*., 2005; Miranda *et al*., 2012). Typically, chemical glycerolysis for production of DAG is carried out at 220-260^{o}C with sodium and potassium hydroxides but the purity and yield are very low. However, lipase-catalysed glycerolysis is a potential alternative to the high temperature chemical process usually applied at an industrial scale for MAG and DAG production, not only for being conducted at mild conditions, but also for the high yield and specificity of the enzyme. On the other hand, there are also some reports related to the production of enriched TAG (Huesca-Toral *et al*., 2005; Choi *et al*., 2012, Hong *et al*., 2012).

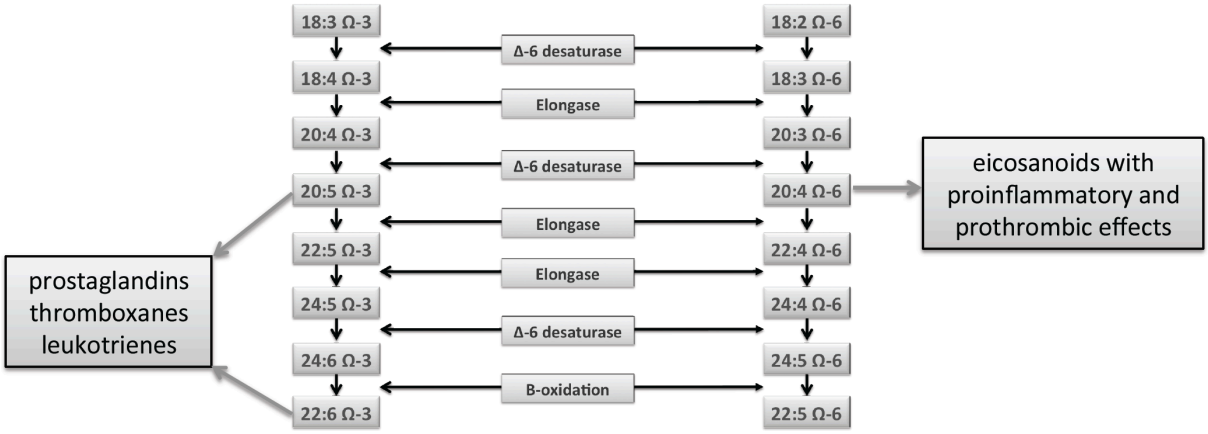

Fig. 1. Enzymatic process of desaturation to synthesize n-3 and n-6 fatty acids.

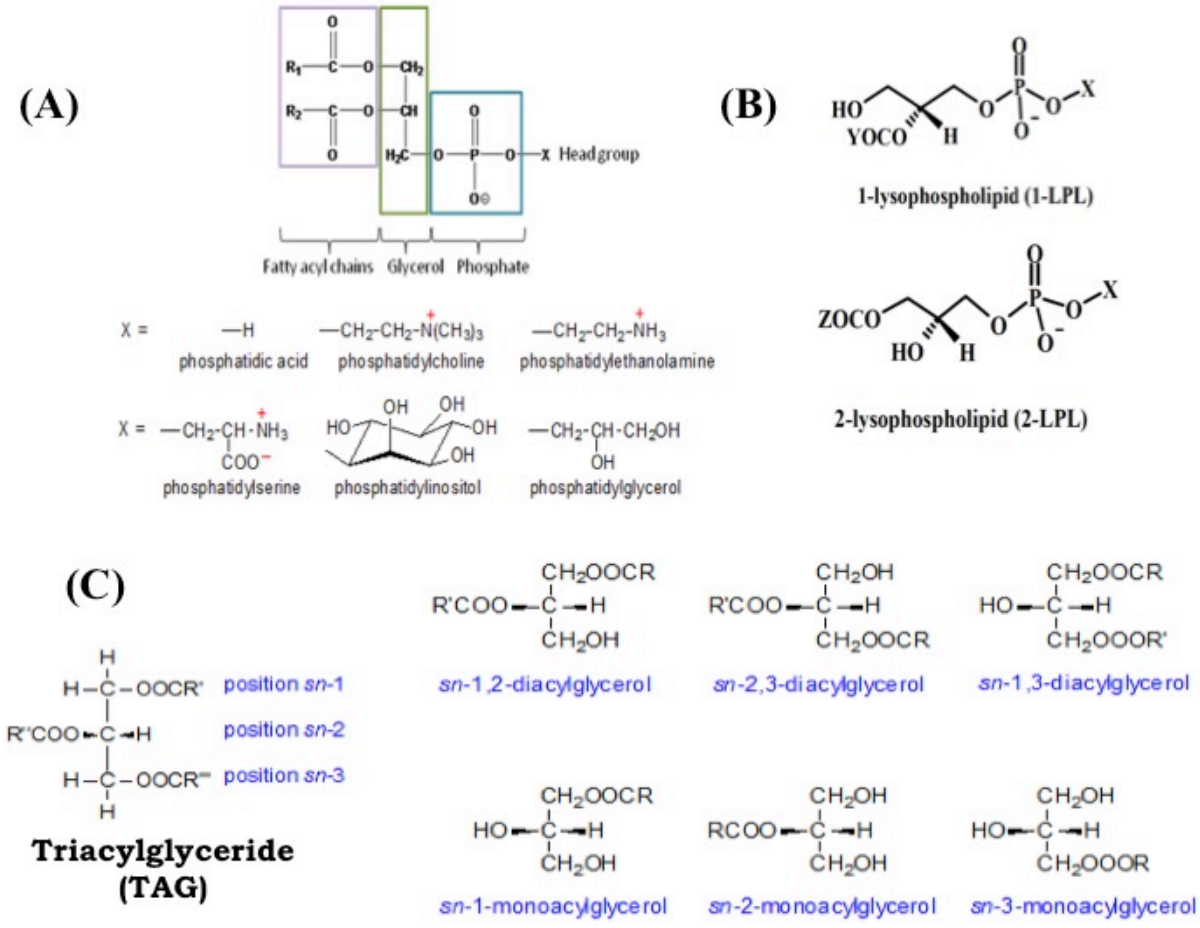

Fig. 2. Structure of the major classes of lipids: (A) phospholipids, (B) lysophospholipids and (C) acylglycerols.

2.3 Glycerophospholipids

PL are a class of lipids that consist of two fatty acyl molecules esterified at the *sn*-1 and *sn*-2 positions of glycerol, and contain a head group linked by a phosphate residue at the *sn*-3 position (Figure 2A, AOCS Lipid Library).

The head group forms a hydrophilic region and determines the type of PL. The fatty acyl side chains are hydrophobic; this amphipathic property of PL provides the basis for the compartmentalization of cells. PL are the main constituent of biological membranes. The size, shape, charge, and chemical composition of different phospholipid classes play a role in the formation and maintenance of the plasma membrane bilayer of cells, as well as membranes surrounding subcellular organelles and vesicles. An asymmetric distribution of PL types within the membrane imparts different functional characteristics between the inner and outer leaflets. PL are involved in stabilizing proteins within the membrane, facilitating the active conformational structure of proteins, and as cofactors in enzymatic reactions. PL are essential for the absorption, transport and storage of lipids. PL are secreted into the bile to aid in the digestion and absorption of dietary fats. They form the monolayer on the surface of lipoproteins, which function is to transport neutral lipids throughout the body. Lastly, PL serve as a reservoir for signalling molecules, such as ARA, phosphatidate, DAG and inositol triphosphate (AOCS Lipid Library).

For the glycerophospholipids category, the subclass 1-alkyl glycerophosphocholines has been replaced by the more generic monoalkylglycerophosphocholines, because 1-acyl-2-alkyl-glycerophosphocholines exist in nature. Examples are the ladderane phospholipids in anammox bacteria. Similar updates have been made for the other glycerophospholipid headgroups. The glycerophosphoglucose lipids class has been replaced by the glycosylglycerophospholipids class to allow coverage of glycerolipids with sugar groups other than glucose (Fahy *et al*., 2009).

For the above-mentioned physical properties, biocompatibility, and nutritional functions, PL are useful in industrial fields such as food, cosmetics, and pharmaceuticals. PL can be used as emulsifiers, components of cosmetics, medical formulations, and for liposome preparation.

2.4 Sphingolipids

Sphingolipids are a complex family of compounds that share a common structural feature, a sphingoid base backbone that is synthesized de novo from Serine and a long-chain fatty acyl-CoA, then converted into ceramides, phosphosphingolipids, glycosphingolipids, and other species, including protein adducts. A number of organisms also produce sphingoid base analogues that have many of the same features as sphingolipids (such as long-chain alkyl and vicinal amino and hydroxyl groups) but differ in other features. These have been included in this category because some are known to function as inhibitors or antagonists of sphingolipids, and in some organisms, these types of compounds may serve as surrogates for sphingolipids (Fahy *et al*., 2005).

Sphingolipids can be divided into several major classes: the sphingoid bases and their simple derivatives (such as the 1-phosphate), the sphingoid bases with an amide-linked fatty acid (e.g., ceramides), and more complex sphingolipids with head groups that are attached via phosphodiester linkages (the phosphosphingolipids), via glycosidic bonds (the simple and complex glycosphingolipids such as cerebrosides and gangliosides), and other groups (such as phosphono- and arsenosphingolipids).

2.5 Sterol lipids

The sterol category is subdivided primarily on the basis of their biological function. The sterols, of which cholesterol and its derivatives are the most widely studied in mammalian systems, constitute an important component of membrane lipids, along with the glycerophospholipids and sphingomyelins. There are many examples of unique sterols from plant, fungal, and marine sources that are designated as distinct subclasses in this schema.

The steroids, which also contain the same fused four-ring core structure, have different biological roles as hormones and signalling molecules. These are subdivided on the basis of the number of carbons in the core skeleton. The C18 steroids include the estrogen family, whereas the C19 steroids comprise the androgens such as testosterone and androsterone. The C21 subclass, containing a two-carbon side chain at the C17 position, includes the progestrogens as well as the glucocorticoids and mineralocorticoids. The secosteroids, comprising various forms of vitamin D, are characterized by cleavage of the B ring of the core structure, hence the "seco" prefix. Additional classes

within the sterols category are the bile acids, which in mammals are primarily derivatives of cholan-24-oic acid synthesized from cholesterol in the liver and their conjugates (Fahy *et al*., 2005).

2.6 Prenol lipids

Prenols are synthesized from the five-carbon precursors isopentenyl diphosphate and dimethylallyl diphosphate that are produced mainly via the mevalonic acid pathway. In some bacteria (e.g., *Escherichia coli*) and plants, isoprenoid precursors are produced by the methylerythritol phosphate pathway. Because the simple isoprenoids (linear alcohols, diphosphates, etc.) are formed by the successive addition of C5 units, it is convenient to classify them in this manner, with a polyterpene subclass for those structures containing more than 40 carbons. Note that vitamin A and its derivatives and phytanic acid and its oxidation product pristanic acid are grouped under C20 isoprenoids (Fahy *et al*., 2005).

Carotenoids are important simple isoprenoids that function as antioxidants and as precursors of vitamin A. Another biologically important class of molecules is exemplified by the quinones and hydroquinones, which contain an isoprenoid tail attached to a quinonoid core of nonisoprenoid origin. Vitamins E and K as well as the ubiquinones are examples of this class. Polyprenols and their phosphorylated derivatives play important roles in the transport of oligosaccharides across membranes. Polyprenol phosphate sugars and polyprenol diphosphate sugars function in extracytoplasmic glycosylation reactions, in extracellular polysaccharide biosynthesis (for instance, peptidoglycan polymerization in bacteria), and in eukaryotic protein N-glycosylation. The biosynthesis and function of polyprenol phosphate sugars differ significantly from those of the polyprenol diphosphate sugars; therefore, we have placed them in separate subclasses. Bacteria synthesize polyprenols (called bactoprenols) in which the terminal isoprenoid unit attached to oxygen remains unsaturated, whereas in animal polyprenols (dolichols) the terminal isoprenoid is reduced. Bacterial polyprenols are typically 10 to 12 units long, whereas dolichols usually consist of 18 to 22 isoprene units. In the phytoprenols of plants, the three distal units are reduced (Fahy *et al*., 2005).

3 Chemical modification of food lipids

Chemical and physical properties of lipids depend on their molecular structure. Such structure can be modified by chemical and enzymatic catalysis with the aim to prepare new and novel species with physiological and technological properties that the original ones do not possess and develop low-cost and procedures that could be easily scaled-up for the potentially applicable products. The chemical processes reported include hydrolysis, hydroxylation, acetylation, and hydrogenation, among others.

There are three major routes currently used for the hydrolysis of fats and oils in the production of FA: high pressure steam splitting, alkaline and enzymatic hydrolysis. The high temperatures (typically 250°C) and pressures (7×10^6 Pa) needed for steam splitting make this process unsuitable for processing sensitive TAG. There are also difficulties associated with alkaline hydrolysis, namely high-energy costs and the need to acidify the soaps formed (Xu, 2003).

The hydrolysis process is also conducted to prepare lysophospholipids (LPL), which are known to have better surfactant properties than PL. Hydroxylation involves insertion of two hydroxyl groups at the carbon-carbon double bonds of unsaturated fatty acids in PL under acidic environments, provided by lactic acid, etc. The purpose of acylation is to improve the oil/water emulsifying properties of PL. On the other hand, hydrogenation is performed to convert unsaturated fatty acids existing in phosphatides to the saturated forms to increase product stability against oxidation.

As we mentioned above, the chemical modification processes imply high temperatures and pressures, toxic catalysts, low specificity and selectivity, lack of food grade status and high-energy consumption. On the other hand, the different raw materials employed as well as the products prepared can be affected by the associated elevated temperatures. Therefore, the enzymatic approach emerges as a better alternative for modification of lipids.

4 Enzymatic modification of phospholipids

The progress in biochemistry and molecular biosciences has led to the understanding of the

enzyme 3D structure, the interfacial activation, improvement of immobilization and the development of more efficient enzyme processes. Hence, enzymatic reactions are more specific, require less severe reaction conditions and produce less waste. The main enzyme groups widely explored for modification of lipids are phospholipases and lipases.

4.1 Phospholipases

Phospholipases are enzymes, which hydrolyse PL. On the basis of the ester bond that is cleaved within a PL molecule, phospholipases are grouped into four families, namely A, B, C and D. Phospholipase A cleaves the acyl ester bond at either the *sn*-1 (phospholipase A_1, PLA_1) or *sn*-2 (phospholipase A_2, PLA_2) position (Fig. 3, AOCS Lipid Library). Phospholipase B hydrolyses acyl ester bonds at both *sn*-1 and *sn*-2 positions. Enzymes grouped under phospholipase C cleave the glycerophosphate bond, while phospholipase D enzymes remove the polar head group (Fig. 3, AOCS Lipid Library).

Phospholipases exist in almost every type of cell analysed for their presence, and most cells contain a variety of these enzymes. For a given PL ester bond, there can be a variety of subtypes of a phospholipase that are specific for it, that can either exist as secreted, membrane associated, or in cytoplasmic form. They may also require cofactors for activity, depending on the isoforms. The functions of phospholipases, where known, are as varied as their cellular and tissue localization and properties.

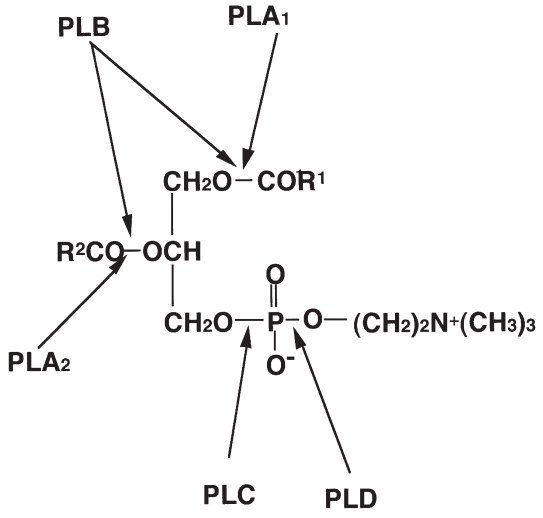

Fig. 3. Phospholipid structure and the site of action of phospholipases on PC.

Nonetheless, three general functions can be ascribed to the physiological relevance of phospholipases: (1) they can serve as digestive enzymes, e.g., PLA are ubiquitous in snake and vespid venoms; (2) they can play an important role in membrane maintenance and remodelling, *i.e.*, FA chains of PL can be cleaved and exchanged by an acyl hydrolase and an acyltransferase, respectively; and (3) they can regulate important cellular mechanisms, e.g., creation of bioactive lipid molecules used in signal transduction (Richmond and Smith, 2011).

PLA_1 (EC 3.1.1.32) has been found in many cells and tissues from various organisms. This enzyme is also a member of the triacylglycerol lipase family and shows a considerable sequence similarity to human hepatic and pancreatic lipases and the guinea pig pancreatic lipase-related protein 2 (GPLRP2). Two molecular characteristics of these PLA_1 emerge from these sequence/structure comparisons: the presence of a very short lid and the deletion of a loop (the β9 loop) compared to the pancreatic lipases. These differences were suggested to be the key to the PLA_1 activity. All these enzymes share the typical Ser-His-Asp catalytic triad (Aloulou *et al*., 2012). We have conducted several PL modifications with this phospholipase.

4.2 Lipases

Lipases (triacylglycerol hydrolases; E.C.3.1.1.3) are the other widely explored group that belong to the family of Serine hydrolases and can be found in animals, plants, fungi and bacteria. Their active site consists of a Ser-His-Asp/Glu catalytic triad; that is similar to that observed in Serine proteases, and therefore catalysis by lipases is thought to proceed along a similar mechanism as in Serine proteases.

Three types of lipases can be identified according to their coordination-substrate site: (a) Lipases corresponding to the *Rhizomucor* family including *Thermomyces* (formerly *Humicola*) *lanuginosa*, which have active sites and lids on the surface of the enzymes; (b) Lipases corresponding to the *Pseudomonas* and *Candida antarctica* family, which have active sites and funnel-like lids. *Candida antarctica* lipase B has a very small lid and a funnel-like binding site; and (c) Lipases corresponding to the *Candida rugosa* family, characterized by the presence of active sites at the end of tunnels containing the lids in their external segments (Jaeger *et al*., 1999; Zarevúcka and Zdeněk, 2008).

Regarding to enzyme-catalysed modification of lipids, it is worth mentioning that it is mainly based

on the positional and selective recognition of PLA_1, PLA_2 and 1,3-lipases. Two main mechanisms are involved in this process. The first one consists of two steps: hydrolysis followed by re-esterification. The second mechanism occurs in only one step of interesterification between PL and acyl donors.

5 Strategies performed for the enzymatic modification of lipids

Several enzyme-catalysed processes have been conducted in order to modify the original structure of natural lipid using lipases (Yagi *et al*., 1990; Svensson *et al*., 1992; Mutua and Akoh, 1993; Haraldsson and Thorarensen, 1999; Reddy *et al*., 2005; Du *et al*., 2010;), PLA_1 (Vijeeta *et al*., 2004; Garcia *et al*., 2008; Kim *et al*., 2010; Baeza *et al*., 2012; Baeza *et al*., 2012b), PLA_2 (Lilja-Hallberg and Härröd, 1995; Madoery *et al*., 1995; Kim *et al*., 2001; Yamamoto *et al*., 2006; Vikbjerg *et al*., 2007) or combination of both types of enzymes (Mustranta *et al*., 1994; Aura *et al*., 1995; Hossen and Hernandez, 2005; Baeza *et al*., 2013).

The major enzymatic reactions for the modification of lipids are mainly referring to acidolysis, alcoholysis, hydrolysis and esterification. Some details are depicted below for these reaction mechanisms.

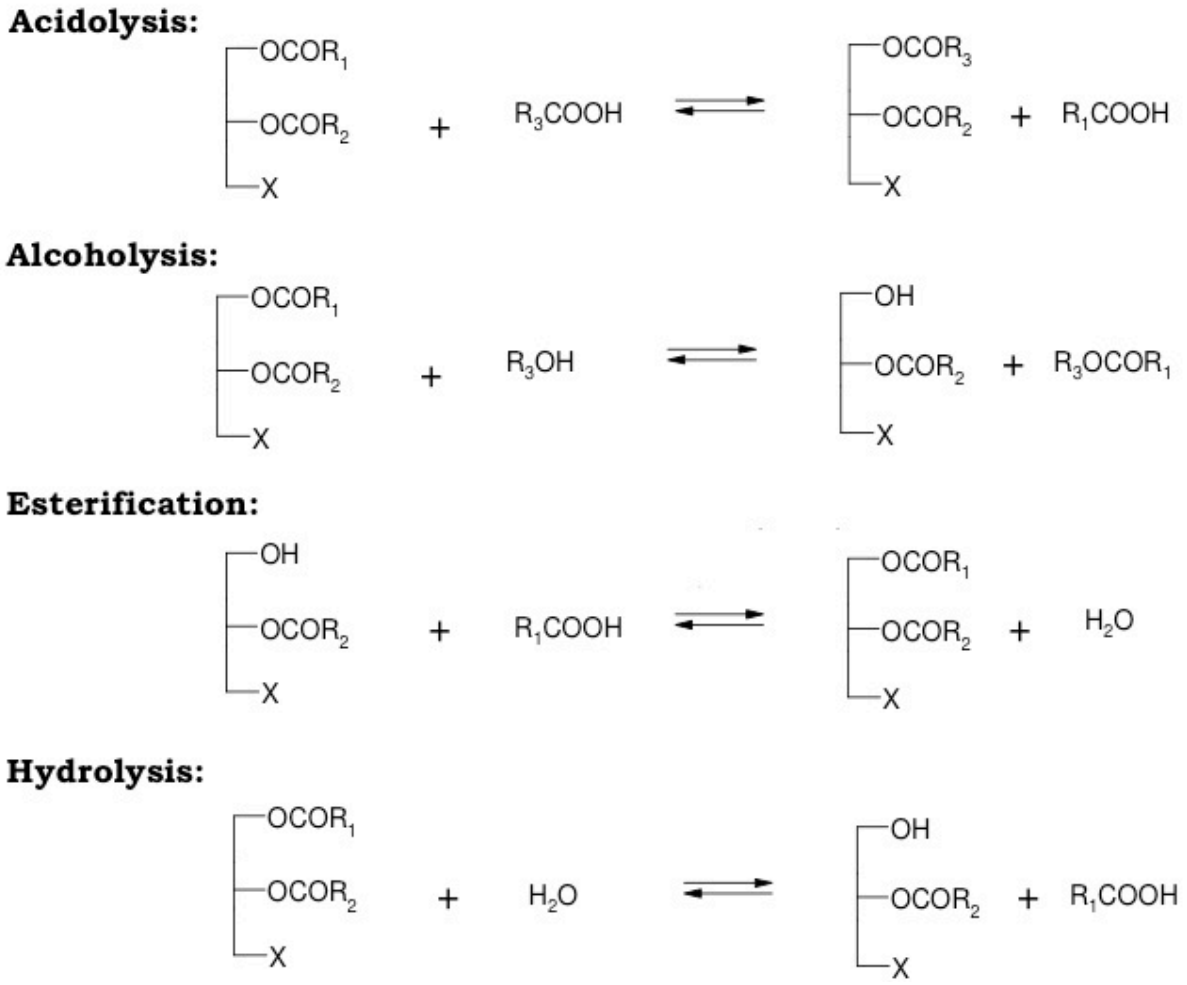

Fig. 4. Enzyme-catalysed modification of lipids.

Table 1. Enzyme-catalyzed interesterifications with different fatty for production of structured phosphatidylcholine

Reference	Incorporation	Substrates molar ratio	Enzyme load	Temperature
Baeza *et al*. (2012)	90% of CLA into PC	1:4 (PC/CLA)	15% immobilized PLA_1	50°C
Kim *et al*. (2010)	43% of n-3 PUFA into PC	1:8 (PC/n-3 PUFA)	15% immobilized PLA_1	50°C
Chojnacka *et al*. (2009)	28% ALA into the *sn*-1 position of egg-yolk PC	1:5.5 (PC/ALA)	20% Novozyme 435	52 - 55°C
	25% ALA into the *sn*-2	1:13 (PC/ALA)	60% excess PLA_2	25°C for 48 h and 40°C for next 48 h
Garcia *et al*. (2008)	35% of n-3 PUFA into PC	1:8 (PC/n-3 PUFA)	10% immobilized PLA_1	50°C
Kim *et al*. (2007)	28% of n-3 PUFA into PC	1:10 (PC/n-3 PUFA)	10% PLA_1	55°C
Vikbjerg *et al*. (2007)	25% of CA into PC	9 (CA/PC)	30% immobilized PLA_2	45°C
	30 and 20% of CLA and DHA into PC respectively	3 (FA/PL)		
Hossen and Hernandez (2005)	16% of CLA into soy PC	1:5 (PL/CLA)	20% Lipozyme TL IM	57°C
Vikbjerg *et al*. (2005)	49% of CA into PC	6 (CA/PC)	40% Lipozyme RM IM	55°C
Peng *et al*. (2002)	35% of CA into PC	5.5:1 (FA/PL)	20% Lipozyme TL IM	60°C
	ca. 30% of CLA into PC			
	18.9% of EPA and DHA into PC			

PUFA: polyunsaturated fatty acid, **PC**: phosphatidylcholine, **PLA_1**: phospholipase A_1, **ALA**: α-linolenic acid, **PLA_2**: phospholipase A_2, **CA**: caprylic acid, **CLA**: conjugated linoleic acid, **DHA**: docosahexaenoic acid, **FA**: fatty acid, **PL**: phospholipid, **EPA**: eicosapentaenoic acid

5.1 Acidolysis

This reaction occurs between an ester and an acid, resulting in an exchange of acyl groups (Fig. 4) and it has been used to incorporate free acid or ethyl ester forms of EPA and DHA, or other fatty acids with biological functions such as conjugated linoleic acid (CLA), as it is shown in Table 1. With this reaction system, the aim has not only been the enrichment of oils, as it has also been the synthesis of structured lipids.

Compared to the enzymatic process, chemical acidolysis has fewer applications due to the lack of positional specificity. Enzymatic acidolysis is a reversible reaction and is commonly considered as a two-step reaction: hydrolysis and esterification, yielding the incorporation of new FA into the original lipid.

Typically, the so-called structured lipids are those in which FA or other molecular components have been replaced or modified. By replacing the existing FA asymmetrically in an original lipid molecule with desired residues, new physical properties and special functionalities can also be achieved (Peng *et al*., 2002; Hossen and Hernandez, 2005; Vikbjerg *et al*., 2007). Acidolysis is also a common method for production of cocoa butter substitutes (Bloomer *et al*., 1990; Chong *et al*., 1992).

5.2 Alcoholysis

This reaction takes place between an ester and an alcohol, producing an ester with a different alkyl group (Figure 4) and it has been employed in the production of methyl esters from esterification of TAG and methanol with yields of up to 53%. During alcoholysis, hydrolysis of TAG to produce DAG and MAG can occur, in some cases reaching levels as high as 11%, although the presence of small amounts of alcohol can inhibit hydrolysis.

The main use of alcoholysis is in the implementation of glycerolysis reactions (García *et al*., 2000; Willis and Marangoni, 2002; Martínez *et al*., 2005; Miranda *et al*., 2012; Baeza *et al*., 2013b), with some particular applications (Xu, 2003): (1) *sn*-2 MAG, the intermediate for the synthesis of structured TAG, can be produced with *sn*-1,3 specific lipases; (2) MAG containing PUFA of higher quality can be produced, since milder conditions are applied than in chemical processes; and (3) easy recovery of glycerol and possible uses of waste oils and fats are a partial argument for the enzymatic production of biodiesels.

5.3 Hydrolysis

This reaction is conducted to prepare the partly hydrolysed form of PL, LPL. LPL are glycerophospholipids in which either the *sn*-1 position (1-LPL) or the *sn*-2 position (2-LPL) is not esterified with a FA residue (Figure 2B, AOCS Lipid Library). LPL play essential biological roles, mainly as signalling molecules. They are also key intermediates in PL remodelling, since once produced they can be recycled by specific acyltransferases leading to new PL. In addition, LPL have many applications in the food, cosmetic and pharmaceutical industries because of their emulsifying properties, considered being even greater than those of PL (Baeza *et al*., 2013).

The most widely used LPL is lysolecithin, which is obtained by the hydrolysis of one fatty acyl residue from naturally abundant sources of lecithin. LPL have been produced through enzyme-catalysed hydrolysis of PL involving PLA_1 (Mustranta *et al*., 1994; Kim *et al*., 1997; Baeza *et al*., 2013), PLA_2 (Mustranta *et al*., 1994; Kim *et al*., 2001; Adlercreutz and Wehtje, 2004) or lipases (Haas *et al*., 1994; Mustranta *et al*., 1994; Sarney *et al*., 1994; Kim and Kim, 2000; Virto and Adlercreutz, 2000; Baeza *et al*., 2013).

PLA_2 and 1,3-specific lipases have been used to modify phosphatidylcholine (PC) at the *sn*-2 and *sn*-1 positions, respectively. The most important reaction is the PLA_2-catalyzed hydrolysis of PC to produce 1-acyl lysophosphatidylcholine (LPC), an effective bioemulsifier and important intermediate in the synthesis of PC with defined FA composition. Remarkably, LPC has been produced from glycerol-3-phosphatidylcholine (GPC).

Conversely, enzymatic hydrolysis of oils and fats involves three steps from TAG to glycerol and free fatty acids (FFA). A batch-stirred tank reactor (STR) is often used for the reaction but packed bed reactors (PBR) are another type of reactors that can be used for the hydrolysis of oils and fats through lipase catalysis (Arcos *et al*., 2000; López-Hern *et al*., 2007). One of the interesting systems for the enzymatic hydrolysis of oils and fats is the application of a membrane reactor. In the typical setup, the lipase is immobilized on the surface of the membrane. The oil phase is circulated on one side and the water phase is circulated in the other side (Torres *et al*., 2001; Xu, 2003). An application of this type of reactor is the enrichment of PUFA from fish oil, widely reported in technical literature.

5.4 Esterification

It is the reverse reaction to hydrolysis and conducts to the production of an ester (and water) from an acid and alcohol (Fig. 4). This reaction is only possible and useful in a microaqueous reaction system where hydrolysis should be minimized and controlled by keeping limited amounts of catalytic and conformational water in the system. In high-moisture reaction systems, hydrolysis is the main reaction and it is difficult to make a viable esterification reaction.

Water is one of the direct products from the reaction and it has dramatic effects on the shifting of reaction equilibrium. It has to be continuously removed from the reaction system in order to minimize the reverse hydrolysis reaction. The methods for removing water from the reaction mixtures include the application of nitrogen, molecular sieves, saturated salt solutions and vacuum. However, a certain dynamic water environment should be maintained in order to preserve high enzyme activity.

The most explored products obtained by this reaction include production of biodiesel, MAG, DAG, sugar esters, PL, among others. In most of these applications, the reactions involve three phases in the simple reaction system in a batch STR: a hydrophobic phase, a hydrophilic phase, and a solid enzyme phase. Particular concern is paid to ensure an efficient reaction system including water removal, homogeneity, and system sustainability. In many cases, solvents have to be used as the medium to increase the homogeneity, depending on the particular application.

Given the difficulty to remove water from the reaction medium, a circulation system is often employed, in which a packed enzyme bed is the reaction unit. Vacuum is applied to the circulation tank to remove water. Membrane reactors have been widely used in the esterification system for different purposes. Lipase can be immobilized on the membrane surface and products formed will be either in the hydrophobic phase or the hydrophilic phase depending on the product property. Membrane devices have also been used for the removal of water through water pervaporation (Xu, 2003).

PUFA can be enriched in the FFA fraction by the esterification of FFA originating from PUFA-containing oil with an alcohol using a lipase that acts on PUFA very weakly. In general, because esterification proceeds efficiently in a reaction mixture without water, dehydrated substrates have been used and water generated by the reaction has been removed

by reducing the pressure in the reactor, or by adding molecular sieves into the reaction mixture.

A very important aspect in esterification reactions is the alcohol employed. Fatty alcohols are good substrates for lipases, and the esterification reaction proceeds efficiently even in the presence of a large amount of water, because the esterification products (fatty alcohol esters) are weakly hydrolysed by lipases. The catalytic mechanism occurring for the esterification reaction has been previously explained and reported (Zaidi *et al*., 1995, 2002).

6 Factors affecting the enzymatic modification of lipids

The replacement of acyl groups in the *sn*-1 and *sn*-2 positions has been carried between CLA, EPA, DPA, DHA, CA (caprylic acid) as acyl donors and PL, LPL and acylglycerols. With no regard to any specific reaction, the next parameters are involved in the modification of food lipids and their effects on the extent of the reaction are described as follows.

6.1 Reactivity of the substrates

The reactivity of different FA is influenced by lipase specificity and by some inhibition effects. Egger *et al*. (1997) mentioned that reaction rates seem to be the same for saturated FA of carbon number lengths of 6 - 12, but lower for C14 and C16. Highest reaction rates were found for C18:1 but higher degree of unsaturation resulted in lower reaction rates. This is based on enzyme selectivity.

Different FA may be applied as acyl donors for the acidolysis reaction. However, FA usually have different reactivities, because of FA specificity or by possible inhibition effects. Under the same conditions, different FA often result in different extents of incorporation into PL or different yields. The acyl donor for acidolysis reactions most widely employed are: CLA, EPA, DPA, DHA and CA.

As depicted in Table 1, Kim *et al*. (2007, 2010) and Garcia *et al*. (2008), reported the incorporation of n-3 PUFA (defined as the sum of EPA+DPA+DHA) into PC. These authors noted that EPA was the most reactive FA, followed by DPA and finally DHA. In a separate study, Vikbjerg *et al*. (2007) found CLA as the most reactive FA when it was compared with CA and DHA. The authors attained 30, 25.3 and 20.2% incorporations respectively. On the other hand, Peng *et al*. (2002) found CA as the most reactive FA when it was evaluated with CLA and the sum of EPA+DHA. They yielded incorporations of 35, 30 and 18.9%, respectively.

6.2 Substrates molar ratio

Increased yield for both esterification and transesterification reactions while increasing FFA concentration has been reported (Adlercreutz *et al*., 2002). However, changes in polarity or viscosity in the reaction medium while increasing FFA concentration have been documented (Egger *et al*., 1997).

In our experimental background, we were able to observe an increase in CLA incorporation into PC when substrates molar ratio (PC:CLA) increased from 1:2 to 1:4, when we studied the production of structured phosphatidylcholine (Baeza *et al*., 2012; Baeza *et al*., 2012b). This was clearly influenced by a greater enzyme load. From those reports, we were able to incorporate 90% of CLA for a 1:4 (PC:CLA) ratio. Vikbjerg *et al*. (2005) reached a 49% incorporation of CA into PC for a 1:6 (PC:CA) ratio. In the case of Kim *et al*. (2010) and Garcia *et al*. (2008), they attained 43% and 35% incorporation of n-3 PUFA into PC, respectively when the substrates molar ratio was increased to 1:8 (PC: n-3 PUFA).

6.3 Temperature

Given the nature of enzyme-catalysed reactions, temperature is a relevant variable to be considered for modification of lipids. In general, increasing temperature conducts to better yields during reactions but very high temperatures can reduce reaction rates due to irreversible denaturation of the enzyme.

In a solvent-free system, temperature must be high enough to keep the substrate in the liquid state. Temperatures do not need to be as high in systems containing organic solvents since they easily solubilize hydrophobic substrates. However, for food applications, where organic solvents are avoided, reaction temperatures are usually higher. Sometimes temperature has to be increased to as high as 60^{o}C to melt the substrate. Such high temperatures can seriously impair lipase stability, although immobilization is known to improve the stability of lipases under high temperature conditions. Immobilization fixes the enzyme in a conformation, which reduces the susceptibility of the enzyme to denaturation by heat. The optimal temperature for most immobilized lipases falls within the range of 30

- 62 °C, whereas it tends to be slightly lower for free lipases (Willis and Marangoni, 2002).

We observed an increment in CLA incorporation into PC when temperature increased from 20 to 50°C when we studied the production of structured PC (Baeza *et al*., 2012; Baeza *et al*., 2012b). Reports evaluating temperature, cited 50°C as the best for modifying lipids (Garcia *et al*., 2008; Kim *et al*., 2010). Other works evaluating the effect of temperature have been reported by Kim *et al*. (2007), who obtained an increment of n-3 PUFA into PC and LPC incorporation when temperature increased from 25 to 65°C. Vikbjerg *et al*. (2007), suggested 45°C as the temperature for reaching the maximum incorporation of CA into PC; when evaluating higher temperatures the acyl exchange was smaller. In a similar work Vikbjerg *et al*. (2005), also reported a decrease in the incorporation of CA into PC when temperature changed from 40 to 60°C but for LPC the behaviour was the opposite. In the experiments carried out by Peng *et al*. (2002), 55°C appeared to be the optimal temperature to attain the maximum incorporation of CA into PL when evaluating higher temperatures the incorporation was smaller.

6.4 Enzyme load

Enzyme load is the main variable in the enzyme-catalysed modification of lipids. When the effect of enzyme loading is evaluated, usually higher incorporations are attained, and this is an effect favoured by temperature. It has been reported that high enzyme loads are needed for effective incorporation of novel FA into PL by acidolysis in a solvent-free system (Aura *et al*., 1995; Haraldsson *et al*., 1999). However, the use of high enzyme loads produced problems with agitation and hindered mass transfer.

In our experimental background, we were able to observe an increment in CLA incorporation into PC when the enzyme load was increased to 15% (with respect to the total weight of substrates) for the production of structured PC (Baeza *et al*., 2012; Baeza *et al*., 2012b). Other reports in the technical literature also refer that an increase in enzyme load leads to better incorporation extents. Garcia *et al*. (2008) reported 35% incorporation of n-3 PUFA into PC for 1:8 substrates molar ratio when 10% enzyme load was employed. When 15% enzyme load was tested, Kim *et al*. (2010) obtained 43% esterification of n-3 PUFA into PC for 1:8 substrates molar ratio. Chojnacka *et al*. (2009) measured 28% incorporation of ALA into the *sn*-1 position of egg-yolk PC using 20% enzyme load. Hossen and Hernandez, (2005), obtained 16% incorporation of CLA into soy PC using a 5:1 substrate molar ratio and 20% enzyme load. Peng *et al*. (2002) reported 35% incorporation of CA into PC, ca. 30% of CLA into PC and 18.9% of EPA and DHA into PC employing 5.5 substrates molar ratio and 20% enzyme load.

6.5 pH

Lipases are only catalytically active at certain pH values, depending on their origin and the ionization state of residues in their active sites. While lipases contain basic, neutral, and acidic residues, the residues in the catalytic site are only active in one particular ionization state. The pH optima for most lipases lies between 7 and 9, although lipases can be active over a wide range of acid and alkaline pHs, from about pH 4 to pH 10.

The effect of immobilization on the pH optimum of lipases is dependent on the partitioning of protons between the bulk phase and the microenvironment around the support and the restriction of proton diffusion by the support. If the lipase is immobilized on a polyanionic matrix, the concentration of protons in the immediate vicinity of the support will be higher than in the bulk phase, thereby reducing the pH around the enzyme in comparison with the pH of the bulk phase. Since there is a difference in the perceived pH of the solution as measured by the pH of the bulk phase, the lipase would exhibit a shift in pH optimum toward a more basic pH. For instance, for a free lipase that has a pH optimum of 8.0, when immobilized on a polyanionic matrix, with the bulk solution at pH 8.0, the pH in the immediate vicinity of the lipase might be only 7.0. Therefore, while the reaction pH is 8.0, the lipase is operating at pH 7.0, which is below its optimum. The pH of the bulk solution would have to be increased to pH 9.0 to get the pH around the lipase to its optimum of 8.0. This phenomenon is only seen in solutions with ionized support and low ionic strength systems (Willis and Marangoni, 2002).

Enzymatic reactions are strongly pH dependent in aqueous solutions. Studies on the effect of pH on enzyme activity in organic solvents show that enzymes "remember" the pH of the last aqueous solution to which they were exposed. That is, the optimum pH of the enzyme in an organic solvent coincides with the pH optimum of the last aqueous solution to which it was exposed. This phenomenon is called pH memory. A favorable pH range depends on the nature of the enzyme, the substrate concentration, the stability of

the enzyme, the temperature, and the length of the reaction (Akoh, 2002).

6.6 Water content and water activity (a_w)

It is generally accepted that water is essential for enzymatic catalysis due to the role water plays in all non-covalent interactions. Water is responsible for maintaining the active conformation of proteins, facilitating reagent diffusion, and maintaining enzyme dynamics.

The activity of lipases at different water contents or a_w is dependent on the source of the enzyme. Lipases from molds seem to be more tolerant to low a_w than bacterial lipases. The optimal water content for interesterification by different lipases ranges from 0.04% to 11% (w/v), although most reactions require water contents of less than 1% for effective interesterification. Water content in a reaction system is the determining factor whether the reaction equilibrium would lean toward hydrolysis or ester synthesis. Lipases tend to retain the greatest degree of original activity when immobilized on hydrophobic supports. When the immobilized lipase reaches an oil-in-water emulsion, the oil phase tends to associate with and permeate the hydrophobic support, so that there is no aqueous shell surrounding the enzyme and support. It can be assumed that there is an ordered hydrophobic network of lipid molecules surrounding the support. Any water that reaches the enzyme for participation in hydrolysis and interesterification reactions must diffuse there from the bulk emulsion phase. Therefore, to avoid diffusional limitations, the oil phase must be well saturated with water. Too much water can inhibit interesterification, probably due to decreased access of hydrophobic substrates to the immobilized enzyme (Willis and Marangoni, 2002).

Due to the crucial role of water on the equilibrium of the reaction, several reports have referred the effect observed in the results obtained. When producing structured PC containing n-3 PUFA, Kim *et al*. (2010), observed a marked increase in the proportion of n-3 PUFA residues present in the total PC when a_w increased from 0.43 to 0.65. However, when aw was further increased from 0.65 to 0.95, the incremental raise in the percentage of n-3 PUFA residues was relatively small. Garcia *et al*. (2008) indicated that the greatest incorporation of n-3 FA is achieved at the lowest levels of added water. Vikbjerg *et al*. (2007), cited that water addition was the most significant factor on the PLA_2-catalysed acidolysis reactions in terms of incorporation and recovery. This is in contrast to a previous report by the same authors when evaluating lipase-catalysed acidolysis; water content had no effect on the incorporation in a solvent-free system (Vikbjerg *et al*., 2005).

Other approaches have been attempted to control and remove water present in the reaction medium. Abraham *et al*. (1998), found that in a solvent-free system, interesterification dominated hydrolysis up to a water-to-lipase ratio of 0.9, after which hydrolysis became the predominant reaction. During interesterification, the reaction equilibrium can be forced away from ester synthesis due to water accumulation, 1 mole of which is produced for every mole of ester synthesized during the reaction. Equilibrium can be pushed back toward ester synthesis by continuous removal of water produced during the reaction. Water activity can be kept constant by having a reaction vessel with a saturated salt solution in contact with the reaction mixture via the gas phase, in order to continuously remove the water produced in the course of interesterification. Another method of water activity control that has proven useful with interesterification reactions is the use of silicone tubing containing the salt solution, immersed in the reaction vessel. Water vapour can be transferred out of the reaction system across the tubing wall and into the salt solution (Svensson *et al*., 1994).

7 Applications

Enzymatic modification of lipids has been reviewed above. The different reactions employed have allowed us to prepare very interesting products with an improved functionality, based on simple processes and environmental considerations. This results from consumers concern about their health and the food they eat. The key issue when modifying food lipids is the amount of active compounds (PUFA, vitamins, etc.) that consumers should eat to receive a benefit from food intake. In order to achieve it, there are three main processes for designing functional foods: (1) to modify the composition of raw material, (2) to modify the technological process and (3) to modify the formulation of the recipes (Fogliano and Vitaglione, 2005).

Among the strategies implemented for the enzymatic modification of lipids described before, we can mention some particular efforts cited in technical literature by Willis and Marangoni (2002). The relationship between stereospecific FA location and lipid nutrition suggests that the process of

interesterification, or acidolysis, could be used to improve the nutrition profile of certain TAG. Manufacturers of specialty food ingredients for infant formulas and enteral supplements should design fats with saturated FA at the *sn*-2 position to provide increased caloric intake. Transesterification has been used to improve the textural properties of tallow and rapeseed oil mixtures as well as in the development of cocoa butter equivalents.

7.1 Infant formulas

Ideally, the fat component of infant formulas should contain the FA, such as medium-chain FA, LA, linolenic acid, and PUFA in the same position and amount as those found in human milk. The fat in most infant formulas is of vegetable origin and tends to have unsaturated fatty acids in the *sn*-2 position. In one study it was found that infants fed human milk had 26% palmitic acid in their plasma TAG, compared with 7.4% in infants fed vegetable oil based infant formula with the same total concentration of palmitic acid, but not at the *sn*-2 position. Therefore, high proportions of palmitic acid at the *sn*-2 position in a modified lipid would provide a fat with improved absorption capability in infants (Willis *et al*., 1998).

7.2 Reduced calorie fats

With increasing consumer awareness of the risks associated with high fat intake, a market for reduced calorie fats and fat replacers has opened up. Carbohydrate and protein-based fat replacers are currently available, but cannot be exposed to high temperatures. Therefore, lipid-based fat substitutes are the only option for use in cooking and deep-frying applications and for mimicking all the attributes of a natural fat.

This type of modified lipids are designed by taking advantage of either limited absorption of long-chain saturates or the low caloric value of short-chain FA. The majority of reduced calorie fats and fat substitutes available today contain FA that are not naturally present in edible oils and fats, but may match the chemistry and functions of natural fats (Osborn and Akoh, 2002).

7.3 Plastic fats for food applications

Margarine, modified butters, and shortenings are "plastic", that is, they have the appearance of solids in that they resist small stresses, but yield to a deforming stress above a certain minimal value (the yield stress) to flow like liquid. The relative proportion of solid to liquid crystals is the dominant controlling factor for hardness, followed by crystal size and polymorphic form (Johnson, 1998). Manufacturers demand fats with a steep solid fat content (SFC) curve for margarine production. They want their product to be solid in the refrigerator, but spread easily upon removal and melt quickly in the mouth.

Interesterification is useful for producing plastic fats and oils suitable for use in margarines and shortenings, because chemical properties of the original fat are relatively unaffected and the fat inherent properties are not changed. Additionally, unsaturation levels stay constant in the FA, and there is no cis-trans isomerization. When short or medium chain FA and long-chain FA are incorporated, they can produce TAs with good spreadability and temperature stability.

The most used oil for these food applications is palm oil. Different studies in the technical literature prove it. For example, Zhang *et al*. (2013) studied the effect of temperature on the crystalline form and fat crystal network of palm oil-based shortening. On the other hand, Nor Aini and Miskandar (2007) described several palm oil-prepared food products.

7.4 Cocoa butter alternatives

Cocoa butter (CB) is the fat of choice in the confectionery industry. Its polymorphism greatly affects the physical properties of chocolate products, such as gloss, snap, contraction, heat resistance, quick and sharp melting in the mouth, and bloom-resistance. CB is extracted from the seeds of the *Theobroma cacao* tree, a species that only can thrive in certain locations with a tropical climate. This fact and the sensitivity of the plant to pests make the cocoa supply somewhat uncertain and variable (Knapp, 2007). Furthermore, the rise in the price of CB in recent years due to its increasing demand has increased the interest in developing cheaper and more readily available alternatives to this fat particularly interesting.

For that reason, fat alternatives to CB have been developed and are classified into three groups: (1) cocoa butter substitutes (CBS), fats based on palm kernel oil or coconut oil, (2) cocoa butter replacers (CBR), non-polymorphic non-lauric fats based on partially hydrogenated oils, and (3) cocoa butter equivalents (CBE), polymorphic non-lauric fats that are defined as fat or fat blends with a similar melting

profile, composition and polymorphism as CB, which should be compatible with CB without exhibiting any eutectic behaviour (McGinley, 1991).

Attempts have been made to prepare cocoa butter-like fats by interesterification of hydrogenated cottonseed oil and olive oil and subsequent fractionation (Chang *et al*., 1990). Edible beef tallow also has been solvent-fractionated from acetone to produce cocoa butter-like fractions (Luddy *et al*., 1973). The preparation of CBS by means of lipase-catalyzed interesterification constitute an attractive research field (Abigor *et al*., 2003) providing the availability of lipases that catalyse the regioselective interchange of acyl groups at the *sn*-1 and *sn*-3 positions of the TAG structure. When using enzymatic processes for production of cocoa butter alternatives, several factors need to be taken into consideration. The melting behaviour has to be very similar to cocoa butter in order to achieve the same cooling effect in the mouth. An alternative fat that is to be used in conjunction with cocoa butter should not interfere with the correct crystallization of the cocoa butter during tempering.

The most common cocoa butter equivalents to date include palm oil, palm mid-fractions, illipe (*Shorea stenoptera*) fat, shea (*Butyrospermum parkii*) butter, sal (*Shorea robusta*) fat, and kokum (*Garcinia indica*) butter. When these natural fat sources are modified by incorporating either palmitic or stearic acid, using *sn*-1 and *sn*-3 selective lipases, it is possible to produce a cocoa butter-like fat, in which the FA composition closely resembles that of cocoa butter (Osborn and Akoh, 2002).

Conclusions

Research focused on the benefits of food and human health has been widely studied in the past decade, driven by consumers becoming more concerned about the relationship between diet and wellness. This has led to the development of novel functional lipids, mainly via modified fatty acid composition. To obtain such compounds several variables need to be controlled; namely, enzyme load, temperature, substrates molar ratio and water content. Once new products are produced, they could reach the market. The backbone of these modifications is to preserve the main sensory characteristics and physical properties of the original food in order to be accepted by consumers. The steps forward in functional foods development are headed to enhanced starting materials, new processes conducted under mild conditions, health benefits, better physical and chemical properties and intake of essential groups of lipids such PL, LPL and acylglycerols.

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