

Enzymatic synthesis of designer lipids

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Oils and fats play a very important role in human diet by providing calories along with essential fatty acids and bioactive components like vitamins, antioxidants, etc. Nature has made available to the consuming public a large variety of dietary fats. However, attempts are made to reduce consumption of oils and fats in these days, in order to reduce the cholesterol-related health problems such as obesity, diabetes, heart attack and other diseases. Thus, there is an increased awareness among the people to reduce the intake of calories derived from fats. The designer oils produced by lipid modifications enhance the role of fats and oils in food, nutrition, and health applications. As the health marketing becomes a major tool in developing market growth, "designer oils" with health attributes will increasingly find their way into everyday foods for health related reasons in the future. There has been a list of review publications in food science and food safety with varied range of information about the production, medical, nutritional and functional food applications of designer lipids in the last few years [1-3].

Lipid modification strategies for the production of functional or designer fats and oils include chemical- or lipase-catalyzed (inter)esterification reactions and genetic engineering of oilseed crops. Nowadays enzymatic (inter)esterification is more popular in comparison to chemical (inter)esterification because chemical methods result in a more-random rearranging of fatty acids or non-specific locations to spe-

Abstract: Even though natural oils and fats play an important role in human nutrition, its excessive intake became major cause for so many health related problems and hence designer lipids came into focus. Designed or structured lipids are nothing but tailor-made oils and fats with improved physical and organoleptic properties to enhance the role of fats and oils in food, nutrition, and health applications. These designer lipids can be produced by chemical- or enzymatic (inter)esterification reactions and genetic engineering of oilseed crops. This review gives a general idea about the enzymatic modifications of natural lipids and their derivatives for the preparation of designer lipids. The commercialization outlook, food, nutritional and pharmaceutical applications of designer lipids are also briefly discussed.

Key words: designer lipids, interesterification, enzymatic modifications, nutritional and pharmaceutical applications

cific positions, whereas enzymatic (inter)esterification is more precise and controlled. The catalysts in enzymatic (inter)esterification offer both substrate and stereo-specificity, require simple and cheap refining and purification techniques with additional potential benefits like high catalytic activity, eco-friendly processes and environmental biodegradability. Enzymatic (inter)esterification has gained lot of importance from nutritional and functional standpoints because of the possibility to produce trans free margarines, cocoa butter substitutes, and reduced calorie foods; to improve functional and physical properties of foods; and to improve the nutritional quality of fats and oils.

In this paper, we give a general idea about enzymatic modifications of natural lipids and their derivatives for the preparation of different designer lipids with nutritional benefits. We also present the current state-of-the-art of some of the commercially available structured triacylglycerols, diacylglycerols, monoacylglycerols, phospholipids, spingolipids, and bioactive compounds like flavonoids.

Designer triacylglycerols

All animal and vegetable oils and fats are triacylglycerols (TAG), composed of glycerol chemically combined with fatty acids. The fatty acids can be varied both for saturated degrees i.e. saturated (SFA) or unsaturated fatty acid (UFA), and carbon chain length including short

chain (<C₆), medium chain (C₆-C₁₀), and long chain (>C₁₂) fatty acids. The composition of TAG and the position of FA are related to their physical and chemical properties as well as the nutritional values. The so called "Designer triacylglycerols" or "structured lipids" are tailor-made fats and oils derived from natural oils and fats, but with their molecules rearranged in such a way to give modified structure with improved nutritional or physical properties. Designed TAGs can be produced by hydrogenation, fractionation, blending, interesterification including enzymatic and chemical methods, esterification, and lipids from gene modified plants, such as low erucic rapeseed and laurate Canola or microbial sources, such as single cell oils. From the production of designed TAG, enzymatic modification can rearrange fatty acids at the specific positions on the glycerol backbone to obtain their special functionality at a mild reaction condition. This is especially favourable for products involved in polyunsaturated fatty acids (PUFA) in the reactions. Work on structured lipids with desired fatty acids were designed to provide simultaneous delivery of beneficial long chain fatty acids (LCFAs) at a slower rate and medium chain fatty acids (MCFAs) at a quicker rate [4, 5]. The concern of nutritive and therapeutic performance is also reported in literature [6-9]. For the most recent advances one can refer the review on modification of oils and fats to produce structured lipids by Trivedi and Singh [10].

Table 1 shows typical commercialized or pilot-scale produced designed TAGs in different groups of TAGs. The reason for producing regio-designed TAGs is the degradation process of lipids in human body is regio-specific and ideally results in the formation of sn-2 monoacylglycerols (MAGs) and free fatty acids (FFA). If FFAs are the short or medium chain FA, they are more easily to be liberated and produce lower calories. Therefore, it can be used for the body weight reduction and the treatment of lipid malabsorption, such as Salatrim, MCT. However, MCT does not supply essential FA. Therefore, sn-2 MAG contained essential fatty acids, such as EPA/DHA, which has effect on visual and auditory performance, brain, and liver, is desired. In the commercial products, marine oil is normally added to increase PUFA, such as products produced from Novartis Nutrition and Nestlé Nutrition. It can also be produced by using sn-1,3 specific lipases through acidolysis or interesterification between marine oil with medium chain FA or MCT. For special group of people, such as infants, a specially designed lipid is required (table 1) i.e. Betapol, in which unsaturated fatty acids are located at sn-1 or -3 positions, and palmitic acid located at the sn-2 position to supply required energy for infants with respect to bone growth and body development. For more diversified designer TAGs, chemoenzymatic approaches can offer new possibilities. It

can not only make symmetric products but also possible for asymmetric products where in some cases it is hard to make it possible with only enzyme approaches [9].

Designer partial acylglycerols

Partial acylglycerols, or in a more common term expressed as mono-and di-glycerides, are commercially produced emulsifiers emerging from oleo chemistry. Today, they are widely used in the food, cosmetic and pharmaceutical industries as well as in the textile, fiber and plastic industries. The partial acylglycerols are estimated to account for as much as approximately 75% of the world wide emulsifier production, corresponding to approximately 250,000 metric tons per year. The popularity of partial acylglycerols as emulsifiers, especially pure monoacylglycerols, is due to their dietary safety together with their molecular structure, which combines a hydrophilic and hydrophobic portion. This gives the capability to aid the formation of a stable and homogenous emulsion in all kinds of products where water-soluble and water-nonsoluble compounds are included [11-14].

Partial acylglycerols are chemically characterized by one (mono) or two (di) fatty acyl chains esterified to a glycerol backbone as illustrated in figure 1. The fatty acid residues (marked as R in figure 1) can in principle obtain many different

chemical profiles. Typically, R can contain 12 to 18 chain with zero (saturated profile) or one or more double bonds (mono- or polyunsaturated profile, respectively).

Traditionally, partial acylglycerols on the global market has been dominated by saturated fatty acid profiles. This is partially due to the unmanageable damage of the heat-sensitive unsaturated fatty acid structures under the current chemical process performed at 220-260 °C. In contrast, the recent progress in enzyme technology has made possible for more gentle processing methods. Thus, damage of the fats and oils can be avoided due to the much lower temperature required (below 80 °C), so that the processing of more heat-sensitive partial acylglycerols with designed unsaturated fatty acid profile has become feasible. Among interesting designer partial acylglycerols are the ones containing polyunsaturated fatty acid (PUFA) residues such as C18 n-3 PUFAs. The PUFA are of great interest because a number of them are essential micronutrients or have been ascribed particular health benefits. Therefore, MAGs containing PUFAs are expected to have plenty application possibilities like incorporation into functional foods and cosmetics, as dietary supplements and as ingredients in pharmaceuticals.

Today, it is possible to apply enzyme technology to produce healthful and functional partial acylglycerols in laboratory. Different reaction

Table 1. Commercially or pilot scale produced designed triacylglycerols.

Brand	Manufactured method	Application	Type	Company
Caprenin	A synthetic fat formulated from glycerol and behenic, capric, and caprylic acids and designed for lowering the caloric content of food.	Chewing gum Cocoa butter substitute	MCT	Procter & Gamble
Betapol	Enzymatic interesterified product. 66-76% palmitic acid at sn-2 position	Infant formula	P at sn-2 / UFA at 1,3 positions	Lipid Nutrition, Loders Croklaan
Salatrim /benefat (Cronym for short- and long-chain TAG)	Salatrim is prepared by interesterification of triacetin, tripropionin, or tributyrin, or their mixtures with hydrogenated canola, soybean, cottonseed, or sunflower oil. TAGs with three short-chain fatty acids are removed in the process. 30-67 mol-% short-chain fatty acids (SCFA) and 33-70 mol-% long-chain fatty acids (LCFA); Stearic acid is the predominant LCFA.	Cooking, baked and dairy products	SLS/ SSL/ LLS/ LSL	Danisco A/S
Captex	It is known as medium chain TAGs. They are manufactured by esterification of fractionated coconut oil or palm kernel oil fatty acids (mainly, caprylic and capric) and glycerine.	Clinical applications	MCT	Abitec Crop.
Neobee				Stepan company
Impact	Protein: 22% , Carbohydrate: 53% , Fat: 25% (Palm Kernel Oil, Sunflower Oil, Menhaden Oil)	Clinical applications	n-6/n-3 1.4:1, EPA/DHA 1.7 g/L	Novartis Nutrition
Crucial	50% fat source as MCT + Marine oil and soy oil	Clinical applications	MCT + n6/n3 1.5/1	Nestlé Nutrition
Peptamne junior	60% fat source as MCT	For children (ages 1-10)	n6/n3 4.8/1	
Peptamme	70% fat source as MCT	For adults	n6/n3 7.4/1	
MLM-type oils	Lipozyme RM IM-catalyzed acidolysis of fish oils or vegetable oil with caprylic or capric acid; oleic acid with MCT oil.	Functional applications	MLM or LML	Pilot plant (X. Xu for more information)

*Abbreviations: MCT, medium chain triacylglycerols; S, M and L, short chain, medium chain, and long chain fatty acid; UFA, unsaturated fatty acid; EPA/DHA, eicosapentaenoic acid/docosahexaenoic acid; P, palmitic acid; O, oleic acid; St, stearic acid.

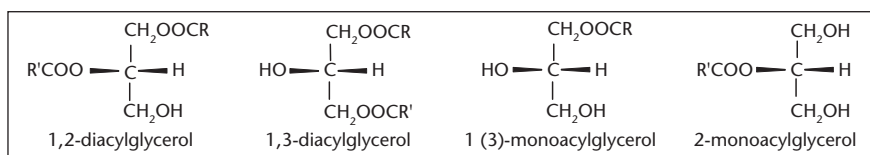


Figure 1. Structure of partial acylglycerols ($R, R' = \text{fatty acid}$).

routes have been applied including glycerolysis, hydrolysis, esterification, and transesterification reactions like acidolysis and ethanolysis. The enzymatic glycerolysis seems to be a very promising approach that converts glycerol and vegetable oils into partial acylglycerols containing PUFAs in a simple and relatively cheap way. However, due to the low temperature used, mixing of the water soluble glycerol with lipids is proved very difficult. Therefore, one of the challenges is to improve the contact between the reactants. Adding solvents to the glycerolysis reaction has proven very promising for an efficient MAG production system from unsaturated oils. Furthermore, the solvent helps facilitate continuous reactor processes by forming more homogenous and less viscous reactant mixtures [12, 13].

Traditional cooking oils consist mostly of triacylglycerols with a small amount of diacylglycerols. The latest structured lipids to hit the headlines as designer oils with health benefits is a diacylglycerol (DAG) oil. Xu et al. summarized the recent progresses in the enzymatic modification of natural oils into DAG oils [15]. Enova oil with 80% DAG is available in market from ADM prepared from soya and canola oils through an enzymatic esterification process developed by Kao. Because of the changed molecular structure, the DAG oil is digested differently from conventional oils and fats, so that it is absorbed in the small intestine without resynthesizing into a neutral triacylglycerol. As a result, it is claimed to reduce the level of body fat that helps consumers maintain, not gain, weight.

These recent results imply a high feasibility of enzymatic MAG and DAG production in practical applications. Accordingly, it is likely that the future will bring the enzyme-catalyzed glycerolysis into industrial plants as a supplementary processing method of nutritional high-valued mono- and di-acylglycerols carrying important PUFAs.

Designer glycerophospholipids

Glycerophospholipids are major and the most abundant class of natural phospholipids (PL). Structurally, glycerophospholipids contain a glycerol backbone, covalently bound two acyl groups and one phosphate moiety. The nature

of the acyl and the type of the end group (X) of phosphate moiety decide the classification, property, and also biological functions of the PLs. When the end-group is substituted by choline, ethanolamine, etc., the relevant individual phospholipid species are given the name of phosphatidylcholine (PC), phosphatidylethanolamine (PE), etc. Those only having one acyl group at 1- or 2-position of the glycerol backbone are their corresponding lyso species (monoacylglycerophospholipids). As integral components of biomembrane, glycerophospholipids carry important biological functions and involve in many metabolism-related and neurological diseases as well as regulate basic biological processes as signalling compounds. Many nutritional and pharmaceutical experiments have led to the renovation of the concepts with regards to the nutritional value of PLs. The importance to human health as well as the market demands spurs the PLs product development, especially for those with specific structure and high purity to meet the particularly nutritional and pharmaceutical requirements. Designer glycerophospholipids are accordingly termed to integrate the work concerning any functional glycerophospholipid preparations from a natural phospholipid species or a synthesized product.

If designer glycerophospholipids are referred as to the glycerophospholipids with defined structure that plays a kind of biological or nutritional function or developed for a particular application, all efforts to this goal should be taken into account. Guo *et al.* summarized the publications and patents concerning recent progresses in physical modification of naturally sourced phospholipids to enrich certain PL species and the advances in chemical derivation of natural phospholipid for different industrial applications (hydrolysis, hydroxylation, acetylation, and hydrogenation) and semi- or *de novo* synthesis of a specific glycerophospholipids [16]. The readers could read more details from the thorough review paper. Obviously, the strategy making for modification of glycerophospholipids is strongly dependent on the structure characteristics of starting materials and enzyme specificities and activity. Phosphatidylcholine (PC) is naturally occurring and representative phospholipid species. Many phospholipases show good activity to PC, therefore, PC constitutes a good starting sub-

strate for enzymatic modifications (figure 2). The chemical bonds in PC molecules could be principally classified into aliphatic ester and phosphate ester bond. The enzymes involved in bond cleavage of esters include lipases and phospholipase A1 and A2, phospholipase B and lysophospholipase. Phospholipase A1 (phosphatidylcholine 1-acylhydrolase, EC 3.1.1.32) and A2 (phosphatidylcholine 2-acylhydrolase, EC 3.1.1.4) belong to acyl hydrolase, which specifically hydrolyze 1- and 2-acyl ester bond of phospholipids, respectively. The phospholipase that can hydrolyze both positional acyl ester bonds is called phospholipase B (EC 3.1.1.5). Lysophospholipase (EC 3.1.1.5) refers to the enzyme preferable to catalyze monoacylphospholipids to glycerol phospholipids. Phospholipase C (phosphatidylcholine cholinephosphohydrolase, EC 3.1.4.3) and D (phosphatidylcholine phosphatidohydrolase, EC 3.1.4.4) show similar activity to phosphodiesterases to cleave the phosphorus-oxygen bond between glycerol and phosphate, and phosphate and headgroup, respectively. Figure 2 gives a diagrammatic representation of enzymatic transformation of glycerophospholipids in terms of enzyme, reaction and destination products.

Figure 2 depicted that lysophospholipids could be produced by lipase- or phospholipase-catalyzed selective hydrolysis or alcoholysis from PLs, or acylmigration from Sn-2 to Sn-1 lysoPC, or esterified from glycerophosphorylcholine. Modified PC could be prepared by selective insertion of defined acyl chains through lipase or phospholipase specific recognition or esterification from glycerophosphorylcholine. Besides enzyme specificity, solvent and water activity are found to play important roles [16]. PLC is capable to catalyze synthesis of some organic phosphate. PLD-catalyzed modification of polar head group by transphosphatidyltransfer to those acceptors with reactive hydroxyl groups has found very broad applications, including increasing the content of particular PL species, such as PC, PS, by starting from naturally abundant lecithin sources, or to converting one phospholipid species to another one; transformation of currently known drugs such as genipin, ascorbic acid, arbutin, etc. to alter their physicochemical properties; or prepare novel derivatives with potentially pharmaceutical values, such as alkylphosphate ester and plasmalogen [17]. The reaction rate and product yield depend largely on the activity and specificity of PLD, structure of acceptor alcohol and property of the medium [18].

Designer sphingolipids

Since sphingolipids have many biological functions, such as the regulation or mediation of

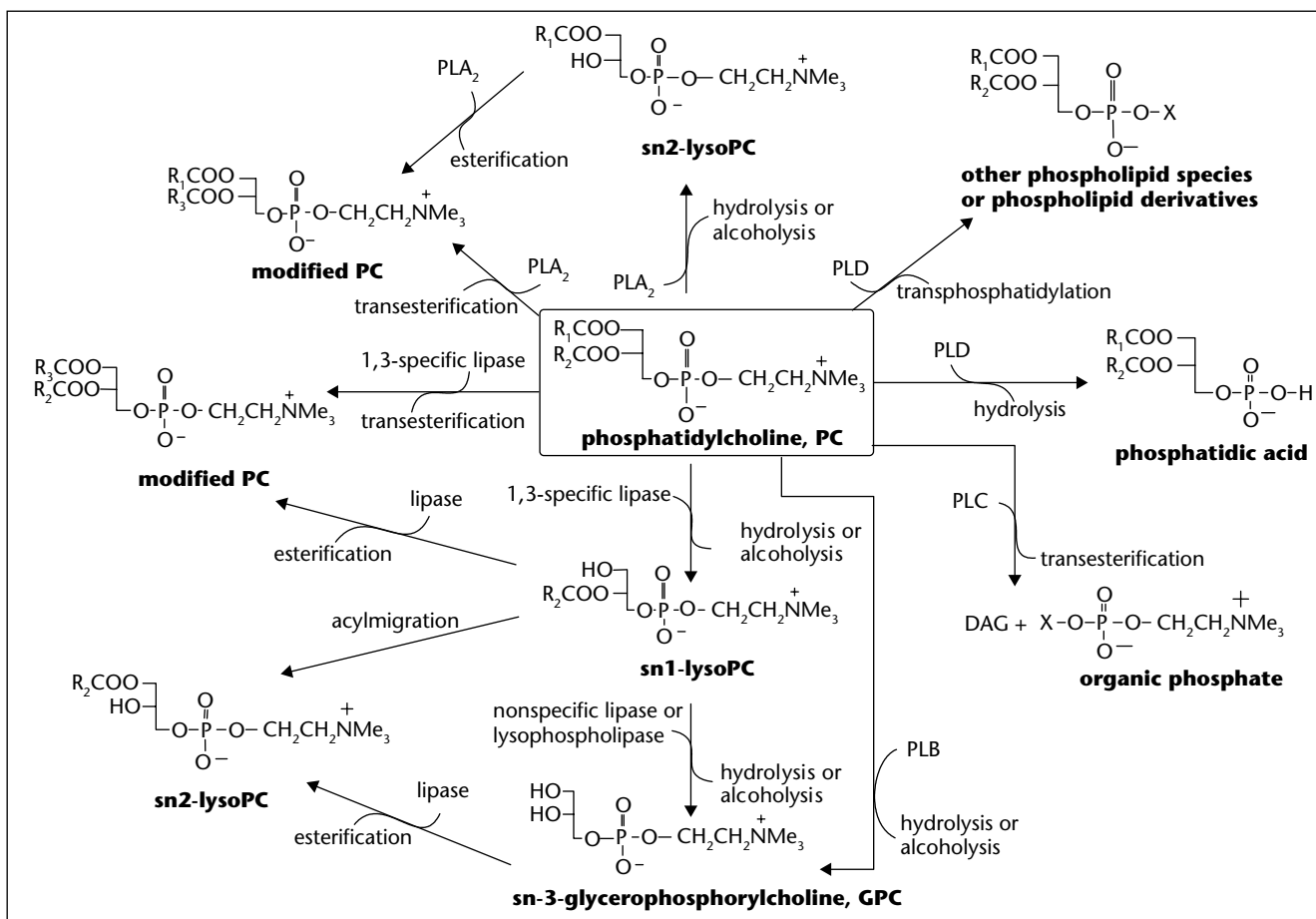


Figure 2. Phospholipase/lipase-catalyzed transformation of glycerophospholipids (Redrawn from [16]).

cell differentiation, transformation, proliferation and apoptosis, a large number of physiological studies have been conducted. The achievements from these studies have led to the discovery of industrial applications of sphingolipids and their derivatives. Furthermore, many biologically active sphingolipids and relevant enzymes have become commercially available. Therefore, the situation for the technical and engineering study of sphingolipid modifications is changing rapidly. The modification sites and relevant enzymes are shown in figure 3. Sphingolipids can be labelled through the replacement of the original fatty acyl chain with radioisotope or fluorescent labelled one [19]. The new labelled sphingolipids can be used to detect the activity of sphingolipid-degradation enzymes, like ceramidase and sphingomyelinase, from biological samples. Since the fatty acyl composition of the ceramide moiety in glycosphingolipids is related to cell-cell interactions and the formation of microdomains on the plasma membranes of vertebrates, remodelling the fatty acyl composition could attract interest in sphingolipid design. Lyso-sphingolipids, which is produced from the *N*-deacylation of sphingolipids

(figure 3), are involved in a signal transduction cascade and useful in many physiological studies [20]. Ceramides have great commercial potential in cosmetics and pharmaceuticals due to their major role in maintaining the water-retaining properties of the epidermis. They have been broadly used as a moisture-retaining ingredient for human skincare products. However, chemical synthesis of ceramides is a costly and time-consuming process. An alternative production method has been developed through enzy-

matic hydrolysis of sphingomyelins, which are ubiquitous component of animal cell membranes and are rich in dairy products or by-products. The modification of the polar group in sphingomyelins can be catalyzed by phospholipase D, where the choline moiety in sphingomyelins is replaced by serine, with the product valuable in the therapy of many diseases of the central nervous system [16]. Lyso-sphingolipids production and the modification of the fatty acyl composition in sphingolipids can be achieved by one enzyme. Sph-

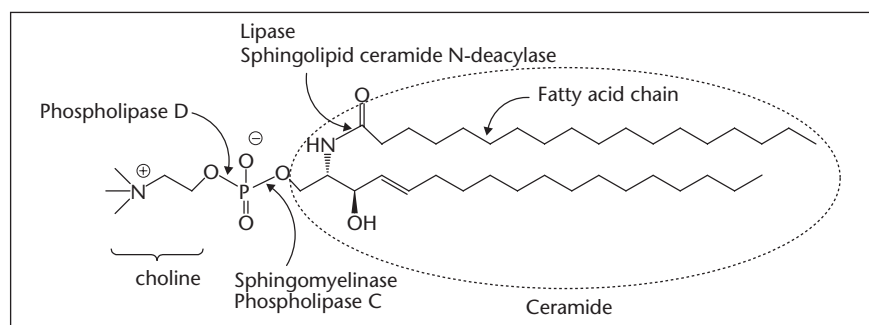


Figure 3. Structural moieties of sphingomyelin, specific enzymes and their acting-sites for modification.

ingolipid ceramide *N*-deacylase produced from culture fluid of *Pseudomonas sp.* TK4 can cleave the *N*-acyl linkage of ceramide moieties in sphingolipids to yield their lyso forms and fatty acids. Moreover, this enzyme is also capable of catalyzing the acylation of lyso-sphingolipids, which is the reverse of above deacylation reactions. With the same enzyme, sphingolipid deacylation proceeds more efficiently at acidic pH in a high detergent concentration, whereas the preferred conditions for the reverse reaction are at neutral pH with a low detergent concentration. Therefore, the direction of the catalytic reaction can be controlled through the manipulation of reaction conditions and substrate concentrations. Consequently, remodelling the fatty acyl composition in sphingolipids can be achieved in two steps, the deacylation of natural sphingolipids and the acylation of the reaction intermediate (lyso-sphingolipids) with new fatty acid, using the same enzyme in different conditions. Enzymes such as phospholipase C and sphingomyelinase (EC 3.1.4.12), can break the bond between the primary hydroxyl group of ceramides and choline phosphate ester in sphingomyelins to generate ceramides. The hydrolysis of sphingomyelins for ceramide production has been improved through evaluation and the optimization of several important factors, and phospholipase C from *Clostridium perfringens* shows high catalytic activity towards the hydrolysis reaction [21]. The reaction is more

efficient in two-phase (water: organic solvent) system than in one-phase (water-saturated organic solvent) system. The reusability of phospholipase C has been enhanced by immobilizing the enzyme on a carrier.

Designer bioactive compounds

There is a growing awareness that certain types of foods are particularly good for our health, including a wide range of fruits and vegetables, wine, tea, oilseeds and even cocoa. In actual fact, these foods contain bioactive compounds such as phenolic acids, flavonoids and tocopherols which contribute to the maintenance and improvement of human health, and have even been linked to the prevention of cardiovascular disease [22]. To date, investigations have already identified levels of individual bioactive compounds in many foodstuffs, such as quercetin in red wine and catechins in green tea. A key attribute of the bioactive compounds detailed above is their strong antioxidant activity, the potency of which is of course linked to their particular structures. While specific *in vivo* mechanisms are still being investigated, it is clear that the antioxidative properties of these compounds confer a protective effect on the body. Moreover, the popularity of these compounds as natural antioxidants has grown considerably over time due to an increased demand from consumers; however, their use in

the food, pharmaceutical and even cosmetics industries has been limited due to practical issues such as their comparatively low solubility & miscibility in hydrophobic environments. For this reason, the addition of acyl groups to these compounds expands potential applications through adjustment of the physico-chemical properties of the product while still maintaining desirable antioxidative properties. Changes in product partitioning as well as improved emulsification properties also expand the applications of these products. To date, designer bioactive compounds have already been successfully synthesized [23-27] using a host of bioactive structures and acyl groups of varying chain lengths and unsaturation through enzymatic modification as shown in figure 4.

Lipases were used to catalyze both the esterification and transesterification reactions of bioactive compounds with acyl groups. While lipases from many sources have been employed to date, the immobilized lipase Novozym 435 from *C. antarctica* is extremely robust and among the most effective and commonly used. With regards to the reaction system, synthesis of bioactive compounds has been carried out in organic solvent [23], solvent-free systems [24] as well as in novel media, such as room temperature ionic liquids (RTILs) [28]. In all of these systems, the major challenge lies in the bringing together of substrates (i.e. hydrophilic flavonoid and hydro-

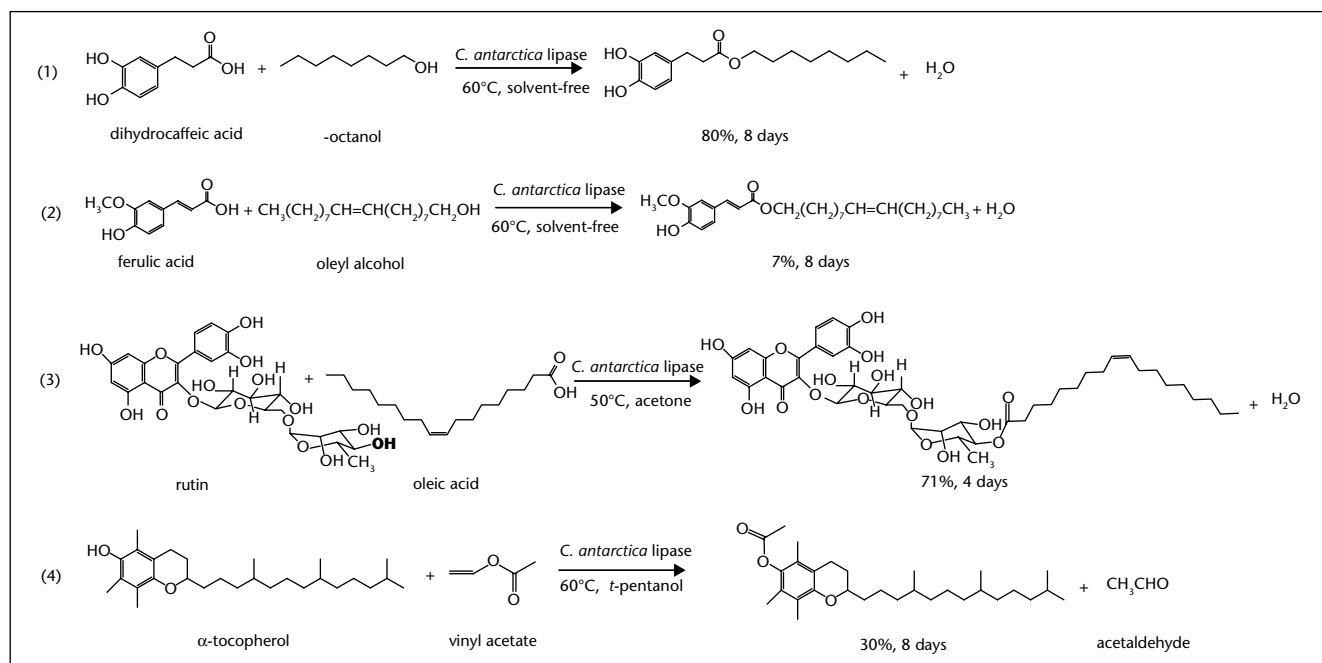


Figure 4. Biosynthesis of designer bioactive compounds (1) reaction of dihydrocaffeic acid with octanol in a solvent free system yields 80% product after 8 days; (2) reaction of ferulic acid with oleyl alcohol in a solvent free system yields 7% product after 8 days; (3) reaction of rutin with oleic acid in acetone yields 71% product after 4 days; (4) reaction of α -tocopherol with vinyl acetate in 2-methyl-2-butanol yields 30% product after 8 days.

phobic long chain fatty acid) with widely differing polarities. Most often, good contact between the substrates and lipase requires at least some compromise during solvent selection: *tert*-butanol, acetone and even co-solvent systems such as octane/2-butanone have been shown to work. Solvent-free systems seem most effective when the substrates in question were fluid and mass transfer limitations reduced. Other systems that have shown promising results include RTILs such as 1-butyl-3-methylimidazolium hexafluorophosphate (BMIM PF₆) and trioctylmethyl-ammonium bis-(trifluoro-methylsulfonyl)imide (TOMA TF₂N), both of which possess higher substrate solubilization capacities than many organic solvents [28]. Some other industrially important factors to consider include reaction temperature, substrate ratio, enzyme load, pH and water content of the system as well as the possibility of recovering/recycling the lipase. Proper adjustment of the above-mentioned parameters can help to push the reaction equilibrium towards the production of these bioactive compounds and result in a more cost effective reaction set-up. *Figure 4*, details the reactions of several bioactive compounds, including dihydrocaffeic acid [23], ferulic acid [24], rutin [25] and α -tocopherol [26, 27] to yield some designer bioactive compounds. It may be noted from this figure that things like the placement of substituent on the phenolic ring and the presence of a double bond on the side chain conjugated with the ring structure affect bioconversion yield. Moreover, the presence of increasingly bulky substrates and/or longer acyl chains may also contribute to the need for longer reaction times. Despite this, careful consideration of reaction parameters often allows for sufficiently high bioconversion yields within a reasonable frame of time, as seen in *figure 4*.

Remarks

This writing is intended to document the concept of designer lipids in a simple way. Designer lipids are in many ways depicted as functional lipids in terms of biological, physical and chemical properties. With the advanced understanding of lipids in different applying systems either *in vivo* or *in vitro*, we are now not quite satisfied with the lipids created from nature. This certainly offers possibilities to tailor-make the lipids structures to meet the needs of what we want.

Lipids tailor-making can be conducted by chemical approaches for certain reactions. However, enzymatic approach has proved to be able to offer more obvious or potential merits as indicated in the introduction. More importantly, enzymes can be tailor-made

themselves to meet the needs of specificity, stability, system efficiency, etc. through modern genetic engineering. This in many ways can relieve the critical concern of economical considerations. There have been a number of commercial products already in the market made by enzymatic approaches such as Betapol for infant formula, Econa diacylglycerol oil for frying, cocoa butter equivalents for confectionery products, etc. This certainly from one way demonstrates that products from enzymatic approaches can be economically favored. It is more potential if you consider the endless improvement of enzymes as well as processes. This writing has a focus on structure re-designing with particular nutritional functions. However, physical functions have been recently also a target concerning new fat design for margarine uses using enzyme technology to replace the conventional chemical interesterification. The work has been moved to a number of industrial companies. This again demonstrates the economical potentiality for the use of enzymes in lipid re-structuring since margarine fats are normally cheap products in the market.

REFERENCES

- OSBORN HT, AKOH CC. Structured lipids-novel fats with medical, nutraceutical and food applications. *Comprehensive reviews in food science and food safety* 2002; 1: 93-103.
- LAVERS B. Technical Focus - Oils and fats: The growth of "designer oils". *Food Ingr Health Nutr* 2002; 12: 12-5.
- LAVERS B. Designer oils revisited. *Food Ingr Anal Int* 2003; 25(5): 10-2.
- BABAYAN VK. Medium chain triglycerides and structured lipids. *Lipids* 1987; 22(6): 417-20.
- AKOH CC. Structured lipids. In: Min DB, ed. *Food Lipids: Chemistry, Nutrition, and Biotechnology*. New York: Marcel Dekker Inc., 1998: 699-727.
- LEE KT, AKOH CC. Structured lipids: synthesis and applications. *Food Rev Int* 1998; 14(1): 17-34.
- GUNSTONE FD. Enzymes as biocatalysts in the modification of natural lipids. *J Sc Food Agr* 1999; 79: 1535-49.
- XU X. Production of specific-structured triacylglycerols by lipase-catalyzed reactions: a review. *Eur J Lip Sc Technol* 2000; 102: 287-303.
- IWASAKI Y, YAMANE T. Enzymatic synthesis of structured lipids. *J Mol Catalyl* 2000; 10: 129-40.
- TRIVEDI R, SINGH RP. Modification of oils and fats to produce structured lipids. *J Oleo Sc* 2005; 54(8): 423-30.
- KROG N. Food Emulsifiers. In: Gunstone FD, ed. *Lipid Technologies and Applications*. New York: Marcel Dekker Inc., 1997: 521-34.
- YANG T, REBSDORF M, ENGELRUD U, XU X. Enzymatic production of monoacylglycerols containing polyunsaturated fatty acids through an efficient glycerolysis system. *J Agr Food Chem* 2005; 53: 1475-81.
- KAETHONG W, SIRISANSANEYAKUL S, PRASERTSAN P, H-Kittikun A. Continuous production of monoacylglycerols by glycerolysis of palm olein with immobilized lipase. *Proc Biochem* 2005; 40: 1525-30.
- ELFMAN-BORJESSON I, HARROD M. Synthesis of monoglycerides by glycerolysis of rapeseed oil using immobilized lipase. *J Am Oil Chem Soc* 1999; 76(6): 701-7.
- XU X, JANNI BK, HONG Z. Production of structured lipids with fictional health benefits. In: Rastall R, ed. *Novel enzyme technology for food applications*. New York: CRC Press, 2000: 270-84.
- GUO Z, VIKBJERG AF, XU X. Enzymatic modification of phospholipids for functional applications and human nutrition. *Biotechnol Adv* 2005; 23: 203-59.
- SERVI S. Phospholipases as synthetic catalysts. *Top Cur Chem* 1999; 200: 127-58.
- ULBRICH-HOFMANN R, LERCHNER A, OBLOZINSKY M, BEZAKOVA L. Phospholipase D and its application in biocatalysis. *Biotechnol Let* 2005; 27: 535-43.
- ITO M, MITSUTAKE S, TANI M, KITA K. Enzymatic synthesis of [C-14] ceramide, [C-14] glycosphingolipids, and omega-aminoceramide. *Met Enzymol* 2000; 311: 682-9.
- KITA K, KURITA T, ITO M. Characterization of the reversible nature of the reaction catalyzed by sphingolipid ceramide N-deacylase. A novel form of reverse hydrolysis reaction. *Eur J Biochem* 2001; 268: 592-602.
- ZHANG L, HELLGREN LI, XU X. Enzymatic production of ceramide from sphingomyelin. *J Biotechnol* 2006; 123: 93-105.
- JUAN CE, MARIA T-C, FRANCISCO A-B. Nutraceuticals: Facts and fiction. *Phytochemistry* 2008; 68: 2986-3008.
- LOPEZ-GIRALDO LJ, LAGUERRE M, LECOMTE J, FIGUEROA-ESPINOZA MC, PINA M, VILLENEUVE P. Quality and food safety - Lipophilisation de composés phénoliques par voie enzymatique et propriétés antioxydantes des molécules lipophiles. *OCL* 2007; 4(1): 51-60.
- CHEBIL L, HUMEAU C, FALCIMAIGNE A, ENGASSER JM, GHOUL M. Enzymatic acylation of flavonoids. *Proc Biochem* 2006; 41(11): 2237-51.
- GUYOT B, BOSQUETTE M, PINA M, GRAILLE J. Esterification of phenolic acids from green coffee with an immobilized lipase from *Candida antarctica* in solvent-free medium. *Biotechnol Let* 1997; 19(6): 529-32.

26. MELLOU F, LOUVRARI H, STAMATIS H, ROUSOS C, KOLISIS FN. Enzymatic esterification of flavonoids with unsaturated fatty acids: Effect of the novel esters on vascular endothelial growth factor release from K562 cells. *Proc Biochem* 2006; 41(9): 2029-34.
27. TORRES P, REYES-DUARTE D, LÓPEZ-CORTÉS N, FERRER M, BALLESTEROS A, PLOU FJ. Acetylation of vitamin E by *Candida antarctica* lipase B immobilized on different carriers. *Proc Biochem* 2008; 43(2): 145-53.
28. KATSOURA MH, POLYDERA AC, KATAPODIS P, KOLISIS FN, STAMATIS H. Effect of different reaction parameters on the lipase-catalyzed selective acylation of polyhydroxylated natural compounds in ionic liquids. *Process Biochem* 2007; 42: 1326-34.