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THE APPLICATION OF LIPASES IN MODIFYING THE COMPOSITION, STRUCTURE AND PROPERTIES OF LIPIDS – A REVIEW

Marek Adamczak

Chair of Food Biotechnology, University of Warmia and Mazury in Olsztyn, Olsztyn

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Structured triacylglycerols (sTAG), not so long ago called "lipids of the future", have become functional food available on the market. In the nearest future, some fatty products designed for infant and children nutrition in the USA will contain increased concentrations of arachidonic (AA) and docosahexaenoic (DHA) acids. Many formulae and dietetic products containing triacylglycerols with modified chemical structure and composition – structured triacylglycerols (sTAG) – are available as supplements to human nutrition. This paper reports on the directions and possibilities of lipid modification catalyzed by lipases. Two basic methods for the synthesis and modification of triacylglycerols to obtain sTAG are characterized. The main properties of sTAG are also discussed.

INTRODUCTION

Fats and oils are the major food components and the main energy source of the human body. The use of the following terms: "fats", "oils" or "lipids" can be often misleading. In this paper, these terms will be interchangeable and will refer to triacylglycerols. To quote Christie [http://www.lipid.co.uk/infores/lipids.html], "Lipids are fatty acids and their derivatives, and substances related biosynthetically or functionally to these compounds". Fats and oils are used in human nutrition directly as natural products or, more often, after appropriate modification. Naturally-occurring fats and oils do not always meet all nutritional recommendations or possess desirable physicochemical properties. The modification of fatty acid composition as well as the regio- and stereo-chemical structure of triacylglycerols improves their nutritive value and changes their physicochemical properties.

An excessive intake of lipids and consumption of the so-called "bad" fat may produce health problems, *i.e.* heart diseases, arteriosclerosis or neoplasms. The signifi-cance of risk factors of heart diseases, including those disturbing the qualitative proportion between cholesterol present in LDL and HDL, may be suppressed or eliminated by changing the source, the amount and the chemical structure of the fats and oils consumed. Some fatty acids are precursors of substances able to regulate physiological processes [Kritchevsky, 1998], and *n*-3 and *n*-6 polyenoic fatty acids are essential to the synthesis of prostaglandin and other eicosanoids [Gill & Valivety, 1997a; b; Shahidi & Wanasundara, 1998].

Recent years have witnessed an extraordinary interest in the enzymatic modification and synthesis of lipids with desirable nutritional properties, including the so-called "structured triacylglycerols" (sTAG) [Bornscheuer *et al.*, 2003; Xu, 2000]. The term "structured triacylglycerol" was introduced by Babayan [1987] to describe fats and oils obtained upon modification, which aimed at changing the composition of fatty acids and the structure of triacylglycerols after hydration, interesterification, position-specific esterification and the use of genetic engineering techniques, *etc*. [Bednarski & Adamczak, 2003]. Still, the "true" sTAG should be defined as triacylglycerols with a precisely specified composition and position of fatty acids esterified with glycerol. Only oils and fats containing such a defined sTAG are able to meet recommendations for functional foods. The synthesis of such sTAG is difficult and possible only by means of lipases. The objective of this paper is to present the directions and possibilities of using lipases to modify the composition and properties of triacylglycerols.

Enzymatic synthesis of structured triacylglycerols (sTAG)

The application of selective lipases can produce pure sTAG (Figure 1A) and it makes them advantageous to chemical catalysts and chemical synthesis, whose products are a mixture of TAG (Figure 1B). Evidently, the enzymatic modification of lipids is also beneficial due to, for example, mild conditions of the reaction or the possibility of monitoring the enzymatic activity.

Requirements for modified TAG and characteristics of available sTAG

The modification of the composition, structure and properties of triacylglycerols aims at: reducing the consumption of saturated fatty acids and their *trans* isomers; increasing the content of polyenoic fatty acids, including those of *n*-3 and *n*-6 group, in triacylglycerols; limiting the

Author's address for correspondence: Marek Adamczak, Chair of Food Biotechnology, University of Warmia and Mazury in Olsztyn, J. Heweliusza St. 1, 10-718 Olsztyn; tel./fax.: (48 89) 523 38 38; e-mail: Marek.Adamczak@uwm.edu.pl

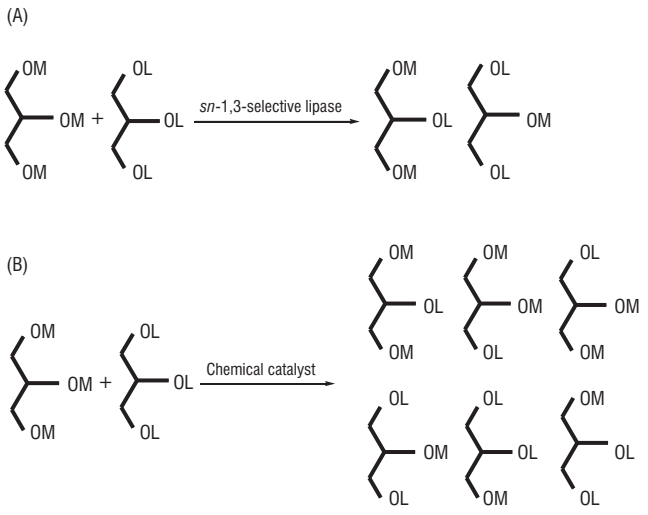

FIGURE 1. Synthesis of sTAG catalyzed by lipase (A) and a chemical catalyst (B). M – medium-chain fatty acid, L – long-chain fatty acid. Figure presents only the main reaction products.

caloric value of fats and oils; improving the physicochemical properties of fats and oils, *e.g*. oxidative stability, freezing and melting point; synthesis of fats and oils useful in different industry branches, *e.g*. bakery, confectionery fats, and frying oils; production of fats and oils characterized by health-promoting properties, *e.g*. synthesis of triacylglycerols with a higher concentration of conjugated linoleic acid (CLA); production of fat and oil substitutes characterized by desirable physico-chemical (substitutes of cocoa butter and jojoba oil) and nutritional (substitutes of human milk fat) properties, *etc*.

The modification of naturally-occurring TAG involves obtaining especially attractive sTAG of the MLM type which contain polyenoic acids (L) in the *sn*-2 position and medium-chain fatty acids (M) in the *sn*-1,3 positions [Adamczak & Bednarski, 2003]. Such sTAG are beneficial to the human immune system, readily transported from the cardiovascular system, and what is most important – useful for patients with pancreopathy. The consumption of this type of sTAG is also beneficial to brain and liver functions [Gill & Valivety, 1997a; b]. It is also important for MLM type sTAG to contain the following fatty acids: γ-linolenic (GLA; 18:3) and arachidonic (AA; 20:3), essential for the proper functioning of the human body.

Triacylglycerols containing palmitic acid in the *sn*-2 position and short-chain fatty acids in the *sn*-1,3 position are low-calorie sTAG (short-chain fatty acids demonstrate a lower caloric value than long-chain fatty acids and stearic acid is used by the human body only to a limited extent and provides few calories) [Babayan & Rosenau, 1991; Fitch Haumann, 1997; Kennedy, 1991; Megremis, 1991]. A drawback of such low-calorie triacylglycerols is the fact that they do not constitute a source of polyenoic fatty acids essential to the human body. Alternatives to such triacylglycerols are fats or oils made of sTAG of MLM type, which are more rapidly assimilated by the human body compared to free polyenoic fatty acids.

Triacylglycerols containing medium-chain fatty acids (MCT; C8-C12) are a very attractive nutrient to our organism. High amounts of medium-chain fatty acids have been observed in coconut oil, palm seed oil, lactic fat, *etc*. Despite containing saturated fatty acids, MCT are liquid at room temperature and do not cause arteriosclerosis. The MCT are utilized by the human body more readily than triacylglycerols containing other fatty acids. Their digestion process omits the lymphatic system and they enrich the cardiovascular system without hydrolysis or re-esterification. Therefore, MCT do not accumulate in the fatty tissue and do not form a reserve fat and, unlike other triacylglycerols, they have lower caloric values [Leman, 1993; Megremis, 1991; Ziemlański & Budzyńska-Topolowa, 1991a]. Thus, MCT are used as a source of easily available energy and a low-calorie product. The sTAG, containing medium-chain fatty acids, are applied in the nutrition of infants as well as in the clinical nutrition of patients with digestion or nutrient absorption disorders, since their digestion requires negligible amounts of bile salts and pancreatic lipase. Those triacylglycerols also exhibit desirable physico-chemical properties and oxidative stability, thus are successfully applied in confectionery production as solvents and carriers of dyes, vitamins, *etc*. The market offers a wide variety of MCT-containing products, including those produced in Poland [Ziemlański & Budzyńska-Topolowa, 1991b].

The conjugated linoleic acid is a mixture of geometrical and positional isomers of linoleic acid. CLA has been identified in fried meat, cheeses, pasteurized milk, and human milk as well [Ha *et al.*, 1987; Lin *et al.*, 1995; McGuire *et al.*, 1997]. Studies performed mainly on animals have shown that the CLA is able to reduce the risk of neoplasm incidence and beneficially affects arteriosclerosis and the immune system [Pariza, 1991; Park *et al.*, 1997]. Recently, CLA has been used for the synthesis of sTAG with MCT [Kim *et al.*, 2001a; b] or with tristearin [Torres *et al.*, 2002]. Watanabe *et al.* [2002] also suggested a methodology for the production of monoacylglycerols with an increased content of CLA, which can be further used for sTAG synthesis. Products rich in CLA, *e.g*. Clarinol™ (Loders Croklaan) or CLA 750 (AST Research), are available on the market.

Polyenoic acids forming sTAG are more rapidly adsorbed by the human body and exert a more beneficial effect on brain and liver than the same polyenoic acids occurring as free fatty acids [Gill & Valivety, 1997a; b]. Commercial concentrates of those acids are available, *e.g*. GLA – Gammanol™ (Loders Croklaan), DHA, EPA – EPA-DHA Complex™ (Metagenics™), and Marinol™ (Loders Croklaan).

The major TAG component of human milk fat is palmitic acid found in acylglycerols, mainly in the *sn*-2 position. However, its occurrence in the *sn*-1 and *sn*-3 positions, leads – after its release by pancreatic lipase – to the formation of barely-soluble calcium soaps and a loss of free calcium. The Loders Crooklan company (Unilever) produces an sTAG preparation under a commercial name "Betapol" as a substitute of human milk fat with a low concentration of palmitic acid in the *sn*-1,3 position.

Salatrim and Caprenin are widely known as the low-calorie fats [Auerbach *et al.*, 1998]. Salatrim is a mixture of short- (2–4) and long-chain (16–22) fatty acids forming TAG [Smith *et al.*, 1994], produced as a result of interesterification followed by vacuum distillation, which in turn aims at removal of the remaining free short-chain fatty acids [Klemann *et al.*, 1994].

The enzymatic synthesis can also produce products similar to Salatrim. Investigations have been carried out into the use of *e.g*. lipases from *Rhizomucor miehei* or lipase from *Carica papaya* for the transesterification of triacetate with hydrogenated soybean oil or stearic acid. The yield of that process has been reported to reach 85% [Fomuso & Akoh, 1997; Mangos *et al.*, 1999; Yang *et al.*, 2001]. Caprenin – a low-calorie fat containing caprylic (8:0), caproic (10:0) and behenic (22:0) acids, is synthesized with both chemical [Kleusener *et al.*, 1992] and enzymatic methods [Akoh & Yee, 1997; McNeill & Sonnet, 1995; Yankah & Akoh, 2000b]. In one of the first methods of Caprenin enzymatic synthesis, esterification catalyzed by *Geotrichum candidum* lipase at a temperature of 50°C reached a yield of 75% [McNeill & Sonnet, 1995].

Lipases and conditions of lipid modification

Lipases are used for the modification of fats and oils mainly due to their selectivity, *i.e*. [Diks & Bosley, 2000; Stadler *et al.*, 1995]: regioselectivity: *sn*-1,3-regioselectivity or a lack of selectivity; fatty acid selectivity: long-chain polyenoic fatty acids, saturated fatty acids, *cis n*-9 unsaturated fatty acids or short-chain fatty acids; acylglycerol selectivity: mono-, di- or triacylglycerols.

The selectivity of lipases is determined by the parameters of the reaction medium, *i.e*. the solvent's polarity or log P (hydrophobic-hydrophilic coefficient), water activity (a_w), the immobilization carrier, *etc*. [Catoni *et al.*, 1996]. The properties of lipases can be affected by techniques of reaction medium engineering, by their chemical modification or by covering the enzymes with surfactants or lipids [Villeneuve *et al.*, 2000].

Table 1 compiles some information on the selectivity of lipases significant to sTAG synthesis.

Lipases are also capable of discriminating lipid enantiomers. Chandler *et al.* [1998] used lipases from *Rhizomucor miehei* and *Rhizopus delemar* for the synthesis of chiral triacylglycerols. A ^{13}C-NMR analysis of the stereoselectivity of *Rhizomucor miehei* lipase immobilized on ion-exchange resin showed the lipase to be very strongly stereoselective to the *sn*-1 position, compared to the *sn*-3 position of triacylglycerols. Under optimal conditions (in hexane medium, at a temperature of 60°C and a_w=0.11), over 80% of synthesized stereoisomers were identified as 1-stearynoyl-2--oleinoyl-3-palmitynoyl-*sn*-glycerol. Under the same conditions, but with the use of lipase from *Rhizopus delemar*, only a racemic mixture of triacylglycerols was obtained.

Generally, the enzymatic synthesis of sTAG may be performed with one- or two-step methods. The one-step reaction consists in the interesterification of two triacylglycerols containing essential fatty acids with the use of *sn*-1,3-regioselective lipase or in triacylglycerol acidolysis with two equivalents of fatty acids (or their esters) (Figure 2). The yield of sTAG synthesis in the interesterification reaction is similar to that of chemical interesterification. The main problem is that the sTAG obtained are difficult to separate from the remaining triacylglycerols. The acidolysis of triacylglycerols with fatty acids results in obtaining sTAG with a higher yield, due to the reduced amount of by-products.

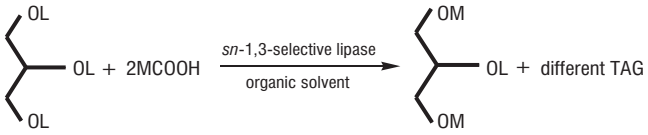

FIGURE 2. A one-step method of sTAG synthesis (acidolysis), L – long-chain fatty acid, M – medium-chain fatty acid.

In the two-step method, pure triacylglycerols or natural fats/oils are first subjected to alcoholysis with *sn*-1,3-regioselective lipase to obtain pure 2-monoacyl-*sn*-glycerols (2-MAG). Pure 2-MAG should be isolated from the reaction mixture as simply and rapidly as possible, *e.g*. by the crystallization. The 2-MAG obtained, together with fatty acids, are used in the second step of esterification aimed at obtaining the desired sTAG (Figure 3). The key to obtaining a high yield of sTAG synthesis is an appropriate performance of alcoholysis and avoiddance of acyl group

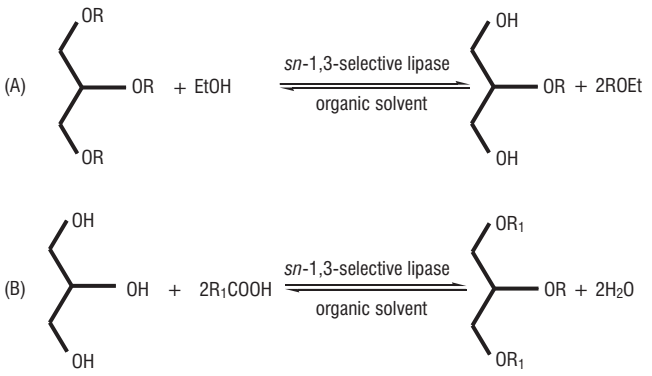

FIGURE 3. A two-step method of sTAG synthesis: (A) alcoholysis, (B) esterification.

TABLE 1. The selectivity of lipases used for the synthesis of sTAG [Xu, 2000].

Lipase	Selectivity with reference to fatty acids	Regio-selectivity	Reference
Rhizopus delemar	M,L>>S	1,3>>2	[Shimada *et al.*, 1997a]
Rhizomucor miehei	S>M,L	1>3>>2	[Chandler *et al.*, 1998]
Porcine pancreas	S>M,L	1,3	[Berger & Schneider, 1991]
Humicola lanuginosa	S,M,L	1,3>>2	[Berger & Schneider, 1991]
Pseudomonas sp.	S,M,L	1,3>2	[Shimada *et al.*, 1997b]
Candida rugosa	Non-selective	Non-selective	[Benjamin & Pandey, 1998]
Penicillium camembertii	Non-selective	2	[Watanabe, *et al.*, 2002]
Candida antarctica	M,L>S	2	[Irimescu *et al*, 2002]

TABLE 2. Examples of one-step synthesis of sTAG.

Lipase	Reaction	Reference
Candida antarctica	Transesterification of triolein with ethyl caprinate	[Huang & Akoh, 1996b]
Rhizopus delemar	Acidolysis of safflower oil or linseed oil with caprylic acid	[Shimada *et al.*, 1996]
Rhizomucor miehei	Acidolysis of rapeseed oil and caprylic acid	[Xu *et al.*, 1998a]
Rhizomucor miehei		
Candida antarctica	Transesterification of tricaproin and trilinolein	[Fomuso & Akoh, 1998]
Rhizomucor miehei	Acidolysis of triolein and lauric acid	[Miura *et al.*, 1999]
Rhizomucor miehei	Transesterification of tricaprylin with EPA ethyl ester	[Han *et al.*, 1999]
Carica papaya	Acidolysis of chicken fat with caprylic acid	[Lee & Foglia, 2000a]
Rhizopus delemar	Acidolysis of tripalmitin with arachidonic acid	[Shimada *et al.*, 2000]
Rhizopus javanicus	Transesterification of LCT and MCT	[Mogi *et al.*, 2000]
Rhizopus japonicus	Acidolysis of tripalmitin with stearic acid	[Basheer *et al.*, 1995]
Rhizomucor miehei	Transesterification of EPAT and caprylic acid ethyl ester	[Irimescu *et al.*, 2000]

LCT-triacylglycerol containing long-chain fatty acids, MCT-triacylglycerols containing medium-chain fatty acids, EPAT-triacylglycerols containing eicosapentaenoic acid; * – molar yield of fatty acid incorporation or sTAG synthesis

migration from the *sn*-2 position to the *sn*-1 or *sn*-3 positions. A lipase immobilization carrier, organic solvent, reaction conditions, *etc*. may effect the migration of acyl groups.

One-step synthesis of sTAG

Despite the fact that the yield of sTAG synthesis in a one-step reaction is usually quite low, this method is commonly used especially due to its simplicity. Table 2 presents the substrates, reaction conditions, and lipases used for sTAG synthesis through interesterification. The main role of substrates to be used for acidolysis or transesterification reactions is played by those which contain long-, medium-chain or polyenoic- fatty acids [Xu, 2000].

The most common example of sTAG one-step synthesis is the production of a cocoa-butter substitute (CBE) [Quinlan & Moore, 1993]. The cocoa-butter composition is predominated by triacylglycerols constituted from saturated fatty acids esterified in the *sn*-1,3 position and containing oleic acid in the *sn*-2 position. The content of palmitic, stearic and oleic acids in cocoa-butter exceeds 95% of the total fatty acids. The melting point of cocoa-butter reaches 25–35°C, which determines its beneficial organoleptic properties.

Unilever [Coleman & Macrae, 1977] and Fuji Oil [Matsuo *et al.*, 1981] companies were the first to be granted patents in the enzymatic synthesis of cocoa-butter substitutes. Currently, both companies use *sn*-1,3-selective lipases for replacing palmitic acid with stearic acid in the *sn*-1 and sn-3 position of triacylglycerols. This process proceeds through transesterification or acidolysis of cheap oils and tristearin or stearic acid as a donor of acyl groups. Apart from palm oil, CBE synthesis is performed with sunflower, rapeseed [Adlercreutz, 1994], or olive oils [Chang *et al.*, 1990].

Issues connected with the optimization of synthesis conditions for other sTAG have also been the subject of studies into protein engineering and reaction medium engineering.

Of the numerous lipases assessed in terms of their suitability for the incorporation of medium-chain fatty acids into sTAG, the best results were reported for lipases from: *Rhizomucor miehei* (Lipozyme RM IM), *Rhizopus delemar* (RDL), and *Candida antarctica* B (CAL-B) [Huang & Akoh, 1996a; b]. The maximum degree of fatty acid incorporation into sTAG did not exceed 40–65 mol%. The fatty acids were incorporated into triacylglycerols through acidolysis with free fatty acids and an interesterification reaction with ethyl esters of fatty acids. The ethyl esters seem to be a better substrate than the free fatty acids, since they are highly soluble and do not affect the changes in the acidity of the reaction medium or suppress the stability of lipase activity.

Apart from pure triacylglycerols, sTAG synthesis may be performed with natural vegetable oils (*e.g.* cotton, safflower or rapeseed oil), fish oils and poultry fat. Despite optimization of the conditions of sTAG synthesis with a one-step method, with special attention paid to the molar ratio of substrates, water activity or content, amount of enzyme or reaction temperature, no conditions were elucidated for obtaining pure sTAG. It seems that the only possibility for synthesis of pure sTAG by one-step reaction is their isolation and purification with column chromatography, which was suggested by Miura *et al.* [1999]. The application of this technique for the industrial production of modified lipids will be, however, difficult due to the use of organic solvents and high cost of the processes.

Shifting the reaction equilibrium into sTAG synthesis often requires the reaction to be run in a medium with a reduced pressure, enabling removal of the water produced in the course of reaction. Using this method, Han and Yamane [1999] carried out transesterification of tricaprylin with eicosapentaenoic acid (EPA) ethyl ester and obtained sTAG with a yield reaching 87% after 24 h.

The synthesis of sTAG by a one-step method was performed by Xu *et al.* [1998a; 2000b; 2000a] in a continuous process, in a semi-technical scale with the use of a bed reactor and a flat membrane reactor. The reaction conditions of these continuous systems were found to inhibit the migration of acyl groups in triacylglycerol molecules [Xu *et al.*, 1998b].

Natural vegetable oils and triacylglycerols containing medium-chain fatty acids have been used for the synthesis of sTAG through interesterification [Fomuso & Akoh, 1998; Soumanou *et al.*, 1997]. After a 24-h reaction with the use of lipases from *Rhizopus delemar* or

Lipozyme RM IM, 35% of sTAG were obtained and the efficiency of lipase from *Candida rugosa* was higher in the interesterification process than in acidolysis [Soumanou, 1997]. Generally, the relatively low yield of sTAG synthesis in such reactions may be explained by the fact that they should be mediated by lipases with high activity to triacylglycerols containing long- and medium-chain fatty acids. The activity of the lipases can be modified by coating the lipases with surfactants, *e.g*. lipase from *Rhizopus javanicus* coated with a surfactant, in a medium with water activity of a_w=0.33, enhancing the yield of sTAG synthesis up to 74% [Mogi *et al.*, 2000].

In Poland, studies into the enzymatic synthesis of sTAG by a one-step method are being carried out at the Department of Fat Technology and Chemistry, Technical University of Gdańsk [Ledóchowska, 1999].

Two-step synthesis of sTAG

The production of pure sTAG should be possible upon running a one-step acidolysis and incorporation of appropriate fatty acids into the *sn*-1,3 position of triacylglycerols with no interruption of the fatty acid located in the *sn*-2 position. In practice, however, migration of acyl groups in an aqueous medium results in the production of many by-products.

The basis for elaborating a two-step method was the observation by Adlercreutz and co-workers (University of Lund, Sweden) that pure 2-monoacyl-*sn*-glycerols (2-MAG) can be obtained by the triacylglycerol ethanolysis in the medium of methyl-tert-butyl ether (MTBE), with a controlled a_w [Millqvist *et al.*, 1996a; b; Millqvist Fureby *et al.*, 1994]. The main advantage of a two-step synthesis of sTAG is the fact that it can obtain high-purity products [Adamczak *et al.*, 2003b; c]. The first reports on the application of ethanolysis as the first step of a two-step method were made by Soumanou *et al.* [1998a]. The best results of ethanolysis were obtained upon the application of immobilized lipase from *Rhizopus delemar*, in a medium at a_w=0.11 [Soumanou *et al.*, 1999].

The two-step method of sTAG synthesis was also used for the synthesis of 1,3-dioleinoyl-2-palmitoyl-*sn*-glycerol (OPO), which may be applied for the nutrition of infants. At present, this sTAG is produced by the acidolysis of tripalmitin and oleic acid with the use of lipase from *Rhizomucor miehei* (Lipozyme RM IM), and is sold under the commercial name "Betapol". The obtained product contains, however, as little as 65% of palmitic acid in the *sn*-2 position.

To compare, the alcoholysis of tripalmitin with ethanol carried out with *Rhizopus delemar* lipase immobilized on a polypropylene carrier (EP-100) disabled obtaining 95% of monopalmitin (with a purity over 95% after crystallization). After a few hours of 2-MAG esterification with oleic acid in *n*-hexane, the yield of OPO synthesis reached 70%. The product contained over 92% of palmitic acid in the *sn*-2 position [Schmid *et al.*, 1999].

Recently, studies have focused on determining the conditions of a two-step process on a semi-technical scale. The synthesis of OPO is now possible in a continuous reaction, which can be performed for few weeks with no considerable decrease in the yield, product purity or enzyme activity. In this case pervaporation, instead of crystallization, was used to isolate 2-MAG.

The two-step method is also used to synthesize sTAG containing medium-chain fatty acids in the *sn*-1 and *sn*-3 position and polyenic long-chain fatty acids in the *sn*-2 position [Soumanou *et al.*, 1998a; b]. This method was used for the synthesis of symmetric sTAG containing docosahexaenoic acid (DHA) in the *sn*-2 position and caprylic acid in the *sn*-1 and *sn*-3 positions [Irimescu *et al.*, 2001]. Ethanolysis was performed with the oil of mackerel-related fish (bonito), and the reaction was catalyzed by *Candida antarctica* lipase B. After 2 h of the reaction, 92.5% of the 2-MAG contained 43.5% DHA. After removal of *Candida antarctica* lipase B, Lipozyme RM IM was added (without purification of the 2-MAG), and the yield of sTAG synthesis reached 51% (w/w).

The two-step synthesis of sTAG is a very attractive method of obtaining high-purity sTAG. Both reactions proceed quite rapidly: 4–8 h in the case of the first step, 1–3 h in the case of the second step. This method, however, requires substitution of MTBE with another solvent, as it is banned in food production.

Another type of two-step sTAG synthesis was suggested by Lee and Foglia [2000a; b]. In the first step, they fractionated chicken fat with organic solvents to obtain TAG fractions with higher concentrations of monoenoic fatty acids. The synthesis of sTAG was performed by acidolysis of the obtained triacylglycerols and caprylic acid.

A chemoenzymatic two-step procedure for the synthesis of sTAG with high contents of EPA and DHA was proposed by a research group from Island [Halldorsson *et al.*, 2001a; b; Haraldsson *et al.*, 2000]. The first step involved an enzymatic reaction using *sn*-1,3 selective lipase to obtain 1,3-diacyl-*sn*-glycerols. The second step was a chemical esterification of diacylglycerols and polyenoic fatty acids.

Recently, an original two-step method for sTAG synthesis was presented [Wongsakul *et al.*, 2003]. It consists of enzymatic synthesis of 1,3-diacyl-*sn*-glycerols and selective esterification of a fatty acid into the *sn*-2 position. The method seems very promising, although it still needs determination of the optimal reaction conditions and screening of selective lipases, because the maximum yield of sTAG synthesis reached *ca*. 50%.

Stability of sTAG composition and properties

The synthesis of sTAG containing polyenoic fatty acids may be limited due to their susceptibility to oxidative changes, disadvantageous taste-flavour changes as well as the accumulation of toxic compounds, often carcinogenic. The simplest method to inhibit oxidative changes is the addition of antioxidants: butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) or esters of gallic acid. These substances are, however, assumed to be the promoters of neoplasmatic changes. The use of natural and safe antioxidants, *e.g*. ascorbic acid, polyphenols, tocopherols or nordihydro-guaiaretic acid (NDGA), is therefore more desirable. Generally, natural antioxidants are hydrophilic substances, which decrease the efficiency of their stabilizing activity against fats and oils. The solubility of natural antioxidants in fats and oils as well as the efficiency of their activity can be modified by the esterification of antioxidants with aliphatic compounds.

Lipases have been successfully used for the synthesis of acyl esters of ascorbic acid in the solid phase [Yan

et al., 1999] or in the medium of organic solvents [Adamczak *et al.*, 2003a; Humeau *et al.*, 1998; Stamatis *et al.*, 1999; Watanabe *et al.*, 2000; 2001]. A high synthesis yield of lipophylic derivatives of natural antioxidants was obtained with the application of lipases from *Candida antarctica* (97%) or *Rhizomucor miehei* (59%) [Stamatis *et al.*, 1999].

The efficiency of eicosapentaenoic acid in the inhibition of oxidation was determined by Watanabe *et al.* [2000]. It was found that 80% of 6-*O*-eicosapentaenoyl ascorbate did not undergo oxidation after being kept at 65°C and neutral air humidity, whereas under the same conditions, a pure eicosapentaenoic acid was completely oxidized even after 3 h. Watanabe *et al.* [2001] also found that the auto--oxidation of linolenic acid is successfully suppressed upon the addition of ascorbic acid or linoleinoyl ascorbate in a molar-ratio to linolenic acid equal or higher than 0.2.

Moussata and Akoh [1998] reported the improvement of the oxidative stability of triacylglycerols obtained after interesterification of melon seed oil with sunflower oil with a higher concentration of oleic acid. On the other hand, a decrease in the oxidative stability of sTAG was observed after: acidolysis of menhaden oil and/or canola oil with caprylic acid [Akoh & Moussata, 2001]; interesterification of palmitoyl stearate and palm oil [Chu *et al.*, 2000]; acidolysis of algae oil with caprylic, oleic or stearic acid [Yankah & Akoh, 2000a]. The oxidative instability of some sTAG results obviously from an increased concentration of polyenoic fatty acids in triacylglycerols, but may be also caused by the loss of natural antioxidants present in fats and oils upon purification and isolation of sTAG.

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ZASTOSOWANIE LIPAZ W MODYFIKACJI SKŁADU, STRUKTURY ORAZ WŁAŚCIWOŚCI LIPIDÓW

Marek Adamczak

Katedra Biotechnologii Żywności, Uniwersytet Warmińsko-Mazurski w Olsztynie, Olsztyn

Strukturyzowane triacyloglicerole (sTAG) nazywane jeszcze niedawno lipidami przyszłości stały się dostępną na rynku żywnością funkcjonalną. W najbliższym czasie niektóre produkty tłuszczowe przeznaczone dla żywienia niemowląt i dzieci w USA będą zawierały zwiększoną ilość kwasu arachidonowego (AA) i dokozaheksaenowego (DHA). Dostępnych jest wiele odżywek, produktów dietetycznych, wspomagających żywienie człowieka zawierających triacyloglicerole o zmodyfikowanej budowie chemicznej i składzie – strukturyzowanych triacylogliceroli (sTAG). W pracy przedstawiono kierunki i możliwości modyfikacji lipidów z wykorzystaniem jako biokatalizatora lipaz. Scharakteryzowano dwie podstawowe metody syntezy i modyfikacji triacylogliceroli celem uzyskania sTAG. Omówiono także podstawowe właściwości otrzymywanych produktów.