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Enzymatic Synthesis DE sn2-Monoacylglycerols: A Mini-Review

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ABSTRACT

Sn2-monoacylglycerols (2-MAG) are very relevant in food chemistry. They are excellent emulsifiers and they may have excellent functional properties. Their synthesis via classical chemistry means that it is complex because the reactivity of the secondary hydroxyl of glycerol is much lower than the one of the primary hydroxyls. Because of the regioselectivity of certain lipase derivatives and the very mild reaction conditions, the different enzymatic routes may become very promising. In this mini-review, different enzymatic routes will be discussed: regioselective hydrolysis of oils, regioselective ethanolysis of oils, glycerolysis of oils and glycerolysis of pure esters of fatty acids. “Pros” and “cons” of each strategy will be discussed. In addition, some problems related to the acyl migration of 2-MAG and to the relevance of biocatalyst engineering in activity, stability and regioselectivity of the immobilized lipase will be also commented.

Keywords: Food chemistry, Regioselectivity of lipases, Different lipase derivatives in different reaction media, Regioselectivity of lipases towards glycerol

INTRODUCTION

Sn2-monoacylglycerols (2-MAG) are non-ionic surfactants which have excellent properties as emulsifiers in food industry [1]. They are also useful in pharmaceutical and cosmetic industry [2]. Moreover, 2-MAG are intermediates in the synthesis of structured lipids, MLM. For example, these lipids may contain medium-size fatty acids (M) in the 1,3-positions of glycerol and polyunsaturated fatty acids (PUFA) in the position 2. These lipids have important benefits in the immune function [3] and they exhibit a better physiological adsorption than lipids containing PUFA in 1,3-positions or in random positions [4,5]. Structured lipids containing docosahexaenoic acid (DHA) in position 2 have an excellent bio-availability and they are related to brain development in early ages [6-8]. 2-monoolein is other MAG with also an excellent emulsifier and it has relevant antioxidant function [9-11]. The 2-MAG of arachidonic acid is also very useful for treatment of pain, anxiety and depression [12,13].

Chemical synthesis of 2-MAG is very complex because of the low reactivity of the secondary hydroxyl of glycerol. Chemical means that it produces undesirable by-products and they are not very suitable for food industry. Selective enzymatic synthesis under very mild conditions seems to be the most suitable strategy [14-19]. In this review, different enzymatic routes of 2-MAG catalyzed by selective lipases will be commented:

Regioselective Hydrolysis of Oils

It is considered the simplest protocol but it has been hardly reported because of relevant migration problems, from 2-MAG to 1-MAG, in aqueous medium at moderate temperatures and neutral pH values. Unmodified oils may be hydrolyzed, by a 1,3-regioselective lipase, to a 33% of 2-MAG and a 66% of fatty acids. Nieto et al. described the preparation of sn-2 eicosapentaenoyl glycerol (2-EPA) and sn-2 docosahexaenoyl glycerol by hydrolysis of fish oils catalyzed by a commercial derivative of a 1,3-regioselective lipase from *Rhizomucor miehei*(Lipozyme IM-20) [20]. At the beginning of hydrolysis a high amount of 2-MAG is produced but after long-term reactions, a high amount of 1-MAG is produced because of migration processes.

Regioselective ethanolysis of Oils

It is the most widely reported route in literature. Unmodified

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oil and ethanol are the substrates and 1,3 regioselective lipase derivatives are the biocatalysts. Again, a 33% of a mixture of 2-MAG is obtained [3]. The by-products of the reaction are ethyl esters of fatty acids present in 1,3 position of the oil. The ideal reaction systems would be a mixture of oil and ethanol with no solvent (solvent-free systems). Nevertheless, lipase derivatives are unstable under these conditions and these processes have to be highly optimized. Ethanolysis in the presence of solvents, such as hexane, have to be carefully designed. The solvent, the biocatalyst, the excess of ethanol, the temperature, etc. may greatly modulate the activity, stability and selectivity of the biocatalyst and even the rate of migration of the product, 2-MAG. Piyatheerawong et al. [21] have observed that the polarity of the solvent strongly modulates the selectivity of some lipase derivatives (Novozym 435 andLipozyme 435).Next, the preparation of different MAGs by enzymatic ethanolysisis reported.

Table 1. Some examples of the preparation of sn-2 monoacylglycerols (2-MAG)

Oil Source	Biocatalyst	Product	Reference
Algal oil	Lipozyme 435	2-DHA	11
High oleic sunflower oil	*Thermomyces lanuginosus* adsorbed on hydrophobic supports	2-monoolein	22
Pacific oyster oil	Lipozyme TL IM	2-EPA and 2-DHA	23
Refined olive pomace oil	*Candida antarctica* (immobilized on a macroporous acrylic resin)	2-monoolein	24
Arachidonic acid-rich oil	Novozym 435	2-Arachidonoyl glycerol	25
Castor oil	Novozym 435	2-Monoricinoleoylglycerol	26
Cod liver oil and Tuna oil	Novozym 435	2-MAG	19
Trioleoin	Novozym 435	2-monoolein	27

Regioselective glycerolysis of Oils

Glycerolysis is the alcoholysis of oils by using glycerol as acceptor alcohol. When using an excess of glycerol, a very high yield in monoacylglycerols (MAG) can be obtained [28,29]. Conversion of triglycerides in more than 90% of MAG have been reported [30,31]. When immobilized lipases have a regio-preference towards the 2-position of the glycerol as acceptor, a very high yield in 2-MAG may be obtained. A complex mixture of 2-MAG had to be separated into pure 2-MAG of a given fatty acid. Purification has to be very carefully designed in order to avoid acyl migrations.

Regioselective glycerolysis of Ethyl Esters of Pure Fatty Acids

The use of pure esters and a regioselective lipase (preference for the 2-position of glycerol as acceptor) may be useful to obtain a very high yield of pure 2-MAG. By using immobilized RML (immobilized by interfacial adsorption of C-18 supports) more than 95% of the 2-MAG of docosahexaenoic acid (2-DHA) was obtained. The process was carried out in a solvent-free system under very mild experimental conditions [32,33]. The obtained pure 2-MAG does not require complex purification.

Acyl migration of 2-MAG

Acyl migration is a relevant problem during synthesis and handling of 2-MAG. The synthesis of structured lipids (MLM or SLS) avoids this problem. In organic solvents, the hydrophobicity of the solvent and the excess of ethanol may modulate the migration [34-36]. Furthermore, the support used for lipase immobilization is another key factor. For example, hydrophobic supports do not promote acyl migration but polar alumina and cationic Amberlyst 15 greatly increase the migration rates [37].

In aqueous medium (for example oil hydrolysis) the temperature and pH can play a relevant role in acyl migration [20]. Moderately high temperatures and neutral or alkaline pH values may promote very intense migrations. Perhaps, the migration can be strongly reduced by working at low temperature (4°C) and acidic pH as it has been observed for the regioselective de-acetylation of per-acetylated sugars catalyzed by immobilized derivatives of lipases [38].

Biocatalyst Engineering

The immobilization of a given lipase on different supports strongly modulates its functional properties (activity, stability and regio-selectivity [39,40]. This is particularly the case of *Thermomyces lanuginosus* lipase (TLL). On one hand, when immobilized by interfacial adsorption on octadecyl supports it is not regioselective for ethanolysis of oils in solvent-free systems or in the presence of solvents [22]. In this way, a complete ethanolysis of oils can be performed. On the other hand, the same enzyme, also immobilized by interfacial adsorption but on divinyl benezene supports, exhibits a clear 1,3-regioselectivity and it promotes the synthesis of a 33% of 2-MAG. (**Table 2).** Additionally the pore sizes of supports seem to play a critical role in lipase activity in solvent-free systems containing very hydrophobic substrates (oils, fatty acid ethyl esters and so on) [33].

Table 2. Selectivity of TLL immobilized by interfacial adsorption on hydrophobic supports

Supports for immobilization of TLL	Oil source	Product
Sepabeads C18	High-oleic sunflower oil	99% of ethyl ester
Purolite C18	High-oleic sunflower oil	99 % of ethyl ester
Lewatit DVB	High-oleic sunflower oil	33% of 2-monolein and 66% of ethyl ester
Purolite DVB	High-oleic sunflower oil	33% of 2-monolein and 66% of ethyl ester

CONCLUSIONS

There are a number of enzymatic strategies to produce sn2-monoacylglycerols. All of them require a complex design of the biocatalyst and the reaction conditions. In addition to that acyl migration should be prevented. We propose an interesting strategy:

a- Complete ethanolysis of oil by non regioselective selective lipase derivatives (for example Tll adsorbed on C-18 supports). Production of more than 95% of ethyl esters.

b- The purification of the most interesting esters

c- Glycerolysis of a given pure ester with RML adsorbed on C18 supports (selective for the position 2 of glycerol as acceptor). Production of more than 95% of 2-MAG

d- Synthesis of structured lipids (MLM or SLS) in order to prevent acyl migration under physiological conditions.

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