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Discrimination against diacylglycerol ethers in lipase-catalysed ethanolysis of shark liver oil

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ABSTRACT

Lipase-catalysed ethanolysis of squalene-free shark liver oil was investigated. The mentioned shark liver oil was comprised mainly of diacylglycerol ether and triacylglycerols. In order to test discrimination against diacylglycerol ether, up to 10 different lipases were compared. The ratio of oil to ethanol and lipase stability were also evaluated. Surprisingly, lipase from *Pseudomonas stutzeri* was the fastest biocatalyst among all assayed, although poor discrimination against diacylglycerol ether was observed. The best results in terms of selectivity and stability were obtained with immobilised lipase from *Candida antarctica* (Novozym 435). Ethanolysis reaction after 24 h in the presence of Novozym 435 produced total disappearance of triacylglycerol and a final reaction mixture comprised mainly of diacylglycerol ethers (10.6%), monoacylglycerol ethers (32.9%) and fatty acid ethyl esters (46.0%). In addition, when an excess of ethanol was used, diacylglycerol ethers completely disappeared after 15 h, giving a final product mainly composed of monoacylglycerol ethers (36.6%) and fatty acid ethyl esters (46.4%).

1. Introduction

Glycerolipids are molecules that contain a glycerol backbone linked to different alkyl chains by different bond types. Depending on the number of chains linked to the glycerol molecule, glycerolipids can be classified into three categories: triradyl-, diradyl- and monoradyl-glycerols. Fig. 1 shows the structural differences of the main subclasses of triradyl-, diradyl- and monoradyl-glycerols.

Alkylglycerols (AKG), monoacylglycerol ethers (MAGE), diacylglycerol ethers (DAGE), alkylglycerophospholipids and their derivatives, commonly known as ether lipids, have been the subject of much attention, due to their special health-promoting effects in humans (Magnusson & Haraldsson, 2011; Pugliese, Jordan, Cederberg, & Brohult, 1998). Ether lipids have been used in the therapy of cancer (Andreesen, 1988), since they are potent antineoplastic agents which inhibit growth, show antimetastatic activity and induce differentiation and apoptosis in cancer cells (Berdel, 1991; Diomede et al., 1993). Immune stimulators properties have also been attributed to dietary ingestion of these substances (Palmblad, Samuelsson, & Brohult, 1990). In addition, recent studies indicate that these compounds could improve the bioavailability of other lipid molecules, such as butyric or omega-3 fatty acids (Martín, Morán-Valero, Señoráns, Reglero, & Torres, 2011; Torres, Vázquez, Señoráns, & Reglero, 2009). Hence, extent and type of esterification can play a significant role in their biological activity.

Ether lipids are widely distributed in marine and terrestrial animals, but they are not or rarely found in plant sources. Non-polar glyceryl ether lipids of the 1-*O*-alkyl-2,3-diacyl-*sn*-glycerol type or DAGE are major constituents in the liver oils of various species of elasmobranch fish, such as dogfish and shark (Haraldsson & Kristinsson, 1998). However, due to the current need to avoid shark overfishing, alternative sources of ether lipids have been identified. Hence, high contents of these compounds can be found in some species of pteropoda (*Clione limacina*) (Böer et al., 2005), liver of deep-sea squids (*Berryteuthis magister*) (Hayashi & Kishimura, 2002), marine invertebrates (sponge *Niphates digitalis*) (Meimetis et al., 2011) and species of coral (*Gersemia rubiformis*) (Imbs, Demina, & Demidkova, 2006).

Ether lipids are commonly found in shark liver oil together with high amounts of squalene, triacylglycerols (TAG) and omega-3 fatty acids. Since these compounds may provide similar bioactive effects in the human body, methods for purification of ether lipids from natural sources, to identify with accuracy their specific physiological role, are highly demanded. Isolation and purification of ether lipids from natural sources are difficult, mainly because of the presence of TAG, which have analogous structure, molecular weight, polarity and volatility.

Some common procedures used at large-scale to concentrate specific lipids from different raw materials are urea complexation (Senanayake & Shahidi, 2000), low temperature crystallisation (winterisation) (Vázquez & Akoh, 2011), supercritical fluid extraction (SFE) (Vázquez, Fornari, Señoráns, Reglero, & Torres, 2008) or short-path distillation (Vázquez & Akoh, 2010). These methods are

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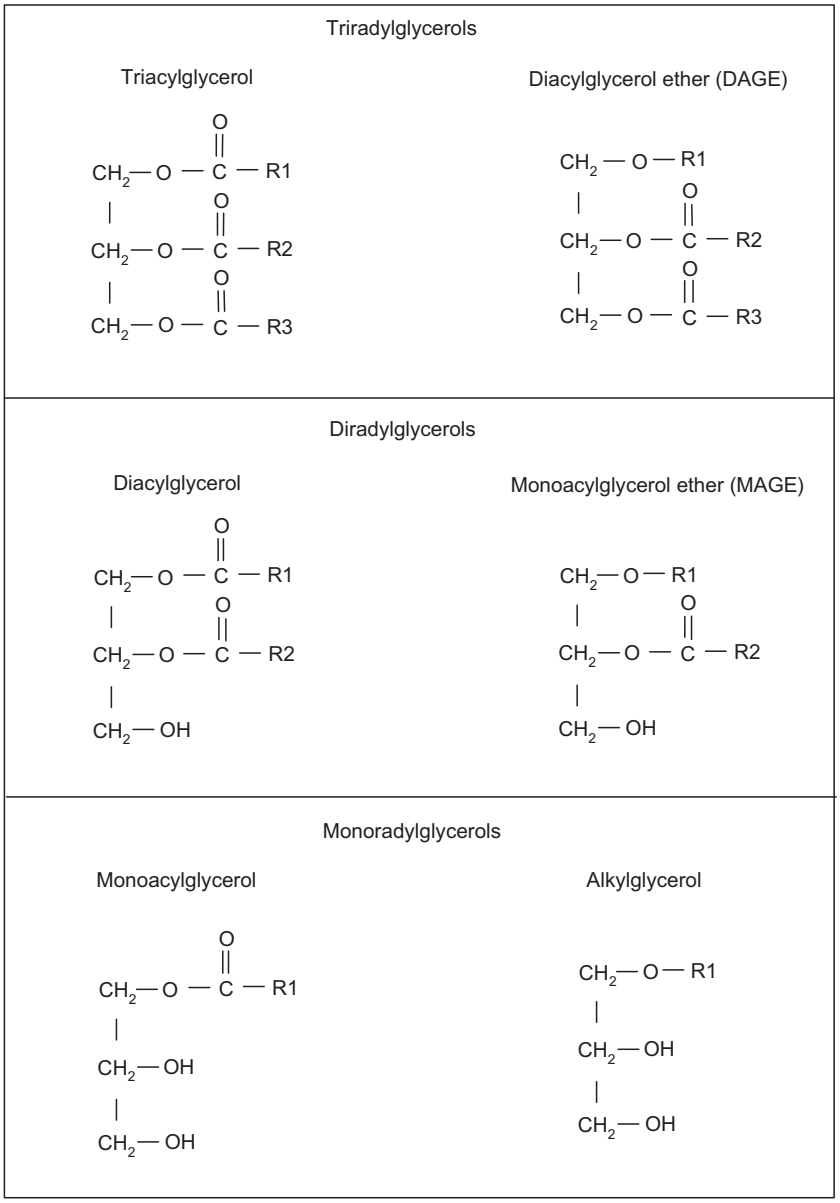

Fig. 1. Chemical structure of the main subclasses of triradyl-, diradyl- and monoradyl-glycerols. Positional isomers are not included in the figure.

commonly based on differences in the molecular weight, degree of unsaturation, volatility and solubility in CO_2 of the compounds. In these terms, ether lipids and TAG are similar molecules and their separation by using the aforementioned technologies can be unfeasible since these compounds would have similar behaviour.

Recently, our research group has been able to concentrate AKG by a two-step methodology based on the transesterification by ethanolysis of shark liver oil and subsequent SFE to purify the products (Vázquez et al., 2008). The transesterification reaction converts triacylglycerols and DAGE into the corresponding fatty acid ethyl esters (FAEE), plus AKG together with minor amounts of MAGE and lower glycerides. Saponification has also been used to modify the original shark liver oil (Torres, Vázquez, Señoráns, & Reglero, 2007). As stated previously, saponification or ethanolysis reactions are necessary because of the presence of TAG that could interfere in the separation. However, no discrimination between DAGE and TAG were observed during these chemical transformations. Besides, significant amounts of unidentified material that may come in part, from undesired oxidation and/or polymerisation reactions, were generated in these processes (Tenllado, Reglero, & Torres, 2011).

In order to modify only the TAG present in the shark liver oil avoiding the generation of undesired byproducts, enzymatic technology has shown very interesting capabilities. Several advantages have been attributed to lipase-catalysed reactions, such as milder reaction conditions, non-toxic reactants and catalysts, and high specificity (Irimescu, Furihata, Hata, Iwasaki, & Yamane, 2001). The mildness that they offer in terms of temperature, pH and pressure most certainly protects them from partial destruction of their natural all-*cis* framework by oxidation, *cis–trans* isomerisation or double-bond migrations (Halldorsson, Kristinsson, & Haraldsson, 2004). Numerous studies have demonstrated the efficiency of several lipases to effectively discriminate between lipids with slight differences in their structure (Shimada, Sugihara, & Tominaga, 2001). These processes are based on the specificity of different lipases that discriminate or act very weakly on the desired molecule. The different sources of lipases bring about different substrate specificities, which are determined by the differences in their