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Simultaneous enzymatic synthesis of FAME and triacetyl glycerol from triglycerides and methyl acetate

E.M. Usai [b], E. Gualdi [c,d], V. Solinas [b], E. Battistel [a,*]

[a] Research Center for Non-Conventional Energies, Istituto Eni Donegani, via Fauser 4, 28100 Novara, Italy
[b] Department of Chemical Sciences, University of Cagliari, Cittadella Universitaria, S.S. 554 Bivio per Sestu, 09042 Monserrato CA, Italy
[c] Istituto G. Donegani, Novara, Italy
[d] Chemistry Department, University of Modena, Italy

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ABSTRACT

In the presence of methyl acetate triglycerides such as vegetable oils are transformed simultaneously into the corresponding fatty acid methyl esters and triacetyl glycerol (triacetin). The reaction, catalyzed by lipases, was studied as a function of some critical parameters, such as type of catalyst, enzyme hydration and immobilization support. The aim of the work was to achieve a conversion of the triglyceride as high as possible and to maximize the yield of the triacetin, the reaction end point. It was found that by using the immobilized lipase from *Candida antarctica* yields as high as 80% of both fatty acid esters and triacetin could be achieved. These results were obtained by carefully controlling the amount of water present in the reaction medium and the hydration level of the enzyme macromolecule.

1. Introduction

The production of biodiesel (fatty acid methyl esters, FAME) significantly increased in the last few years in Europe inducing a concurrent large increase of glycerol availability on the market. Glycerol is the main by-product (about 10% by weight) of FAME synthesis by transesterification of vegetable oils with methanol (Schuchardt et al., 1998). Although glycerol has many commercial uses, its demand has been stable for many years, making difficult for the market to absorb the glycerol surplus related to biodiesel production. As a consequence, the effort has been focused on the use of glycerol-based oxygenated compounds as a mean to reappraise the commercial value and to conveniently dispose of the abundance of this feedstock.

As an interesting possible alternative to the conventional industrial application is the use of glycerol derivatives as additives for fuels in the transportation sector (Zhou et al., 2008; Behr et al., 2008). Indeed these derivatives, including carbonates (Fabbri et al., 2007), acetals (García et al., 2008), ethers (Klepáčová et al., 2005) and esters (Melero et al., 2007), when blended with biodiesel and diesel–biodiesel mixtures, were found to improve some of the otherwise detrimental characteristics of biodiesel such as cold properties, fine particulate emission, viscosity and even flash point and oxidation stability.

Among the oxygenated derivates synthesized in the literature, glycerol esters showed promising properties. For instance, glycerine triacetate (triacetin), synthesized by acid catalyzed esterification of glycerol with acetic acid, can be used for biodiesel formulation, resulting in a final fuel having enhanced cold and viscosity properties (Melero et al., 2007). The addition of 10% (by weight) of triacetin to a biodiesel obtained from rapeseed oil, lowered the melting point of the mixture from −7 to −17 °C.

In a large scale industrial prospective, a possible improvement of the biofuel production could be a single-step process for the simultaneous synthesis of biodiesel (FAME) and glycerol derivatives to yield a blended mixture already qualified as fuel additive, without co-production of glycerol. More specifically, the reactions could be carried out by using a bifunctional group-donor agent for the simultaneous fatty acid esterification and glycerol derivatization, affording a mixture of FAME and the corresponding glycerol derivatives (Bianchi and Battistel, 2008). A suitable bi-functional agent is methyl acetate, which can be used as a source of both methanol (for the transesterification to yield FAME) and acetic acid (for acetylation of glycerol to triacetin), as shown in Fig. 1. In the case of rapeseed oil, for example, if the reaction is complete, a mixture of FAME (81% by weight) and triacetin (19%), without co-production of glycerol, can be obtained.

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* Corresponding author. Tel.: +39 0321 447444; fax: +39 0321 447241.
E-mail address: ezio.battistel@eni.it (E. Battistel).

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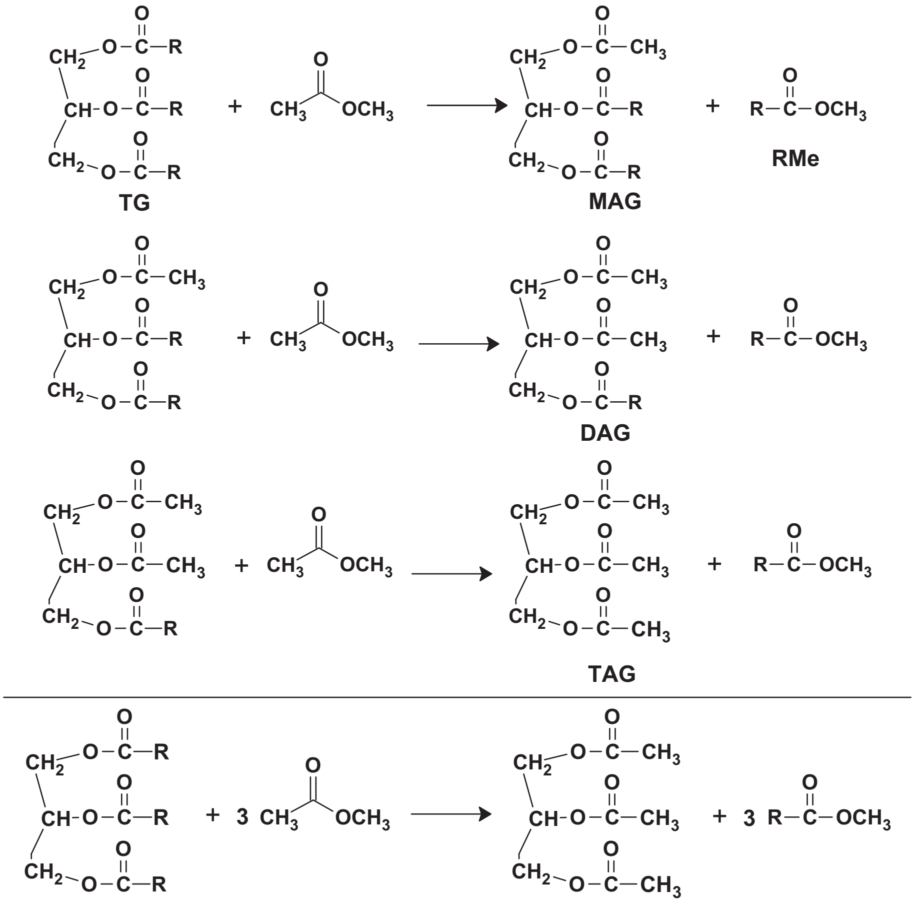

Fig. 1. Reaction between a triglyceride (TG) and methyl acetate to yield the corresponding fatty acid methyl ester (RMe) and triacetylglycerol (TAG). The consecutive steps are the progressive acetylation of a trygliceride (TG) to the corresponding monoacetyl-diacyl glycerol (MAG), di-acetyl-monoacyl glycerol (DAG).

The reaction between vegetable oils and methyl acetate is an interesterification or ester-exchange reaction and can be catalyzed by acids, bases (Bianchi and Battistel, 2008) and enzymes (Du et al., 2004) such as lipases. These enzymes also catalyze efficiently the transesterification reaction for the production of FAME from vegetable oils (Iso et al., 2001). The ability of methyl acetate or ethyl acetate as acyl acceptors has been already explored in the literature, on the attempt to overcome the major drawbacks associated with the classical transesterification reaction with methanol. These include enzyme inactivation (due to the excess of methanol) and glycerol accumulation on the immobilized enzyme on heterogeneous supports (due to preferential interaction between the protein molecule and/or absorption with consequent detrimental effects on the catalytic activity).

It was found that methyl acetate does not affect the long-term operational stability of lipase, even after enzyme immobilization on heterogeneous supports, as opposite to methanol in similar reaction conditions (Du et al., 2004). Moreover, large-scale inexpensive biodiesel production by using methyl acetate and immobilized lipase was studied with different vegetable oils with the aim to optimize FAME yields as a function of reaction time and recycling of the catalyst (Huang and Yaa, 2008; Modi et al., 2007). A detailed kinetic study indicated that the actual rate limiting step of the enzyme-catalyzed reaction between methyl acetate is the first acylation step of glycerol (Xu et al., 2005).

In all these studies, the use of methyl acetate in lipase mediated synthesis of biodiesel has been optimized for FAME production and, therefore, no many details of the overall reaction including the fate of triacetin and intermediates were discussed. Moreover, the sensitivity of the final yields on the amount of water present in the system was not explored in depth.

In this report, the reaction between triglycerides and methyl acetate to yield FAME and triacetin has been further explored including a complete analysis of the intermediates and end product as a function of various reaction parameters such as catalyst type, time and water content.

2. Methods

2.1. Materials

Lipase from porcine pancreas (LPP), from *Pseudomonas cepacea* (LPsC), *Candida cylindracea* (LCC), *Pseudomonas sp.* (LPs), *Mucor javanicus* (LMJ) and α-chymotrypsin were purchased from Sigma–Aldrich (Italy) and used without further treatment, unless otherwise specified. Lipase from *Candida antarctica* (LCA) immobilized on acrylic resin, a Novozyme (Denmark) product, was also purchased from Sigma–Aldrich. Lipase from *Candida rugosa OF* (LOF) was from Amano (Japan).

Lipase from *Pseudomonas fluorescens* "lipase AK" (Amano, Japan) immobilized on SBA-15 (mesoporous silica nanoparticles) and OMX (silica based organically modified xerogels) (Basso et al., 2003) were a generous gift of Department of Chemical Sciences (University of Cagliari, Italy) and they were used without further treatment.

Reagent grade methyl acetate, tributyrin, tetradecane, tricaprin, olive oil and Celite 545, a diatomaceous earth, were from Acros Organics (Italy). The chemical composition and fatty acids distribu-