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Enzymatic synthesis of 1,3-dicaproyglycerol by esterification of glycerol with capric acid in an organic solvent system

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ABSTRACT

In this work, the esterification of glycerol with capric acid catalyzed by an immobilized form of a 1,3-positionally selective lipase (*Rhizomucor miehei*) showed to be effective for the synthesis of 1,3-dicaprin in *n*-heptane as the reaction medium.

The effects of the reaction parameters were studied using an experimental factorial design of three factors and three levels with two central points. The selected experimental variables were amount of glycerol adsorbed on silica gel (*G*), biocatalyst load (*E*) and reaction temperature (*T*), and the response variables were total conversion of capric acid, acylglycerol fractions, selectivity and yield of dicaprin, and acyl migration reaction. The range of each parameter was selected as follows: $G = 50–250$ mg, $E = 20–40$ mg and $T = 40–60\,^{\circ}C$. At optimum conditions 73% capric acid conversion was achieved, with 76% dicaprin selectivity, and selectivity to the specific 1,3-dicaprin of 70% of total products. An adequate selection of the reaction conditions is necessary not only to maximize the conversion of capric acid, but also to minimize the acyl migration reaction and the generation of undesired products. Evidence of kinetically controlled enzymatic acyl migration from sn-3/sn-1 to sn-2 is presented.

1. Introduction

In the last decades, there has been an increasing interest in the nutritional effects of food. New habits have caused the appearance of new products on the market such as those enriched with omega-3 and omega-6, foods fortified with vitamins and minerals (mainly iron, magnesium, zinc, calcium and vitamins B6 and B12), probiotic-enriched dairy products, low calorie foods and others for lowering cholesterol [1].

The increased public interest in fitness and healthy dietary habits has led to much research on the synthesis of "healthy lipids" through the modification of fats and oils. Modified lipids that have been restructured in terms of their native composition and/or distribution of fatty acids (FA) in the glycerol backbone are known as structured lipids (SL) [2]. The aim of the SL synthesis is to produce a lipid that could be used for nutritional purposes or for treating certain health problems, as well as to improve the physical and chemical properties of fats and oils.

In order to obtain lipids with specific properties, a wide range of fatty acids are incorporated in specific positions of the glycerol molecule [3–6]. They include short-chain fatty acids (SCFA or S), medium-chain fatty acids (MCFA or M), and long-chain fatty acids (LCFA or L). Structured lipids of the so-called MLM type contain medium-chain fatty acids at the sn-1 and sn-3 positions of glycerol and a long-chain fatty acid at the sn-2 position, and they are probably the structured lipids that have received the most important attention of science [5].

In this report the synthesis of 1,3-dicaproylglycerol was studied as a previous stage to the synthesis of MLM-type triglyceride. However, the synthesis of diacylglycerols (DAG) is of great importance itself. They are known to be used as additives or carriers in medicine, food and cosmetic industry, and so on [7]. The latest reports on the nutritional benefits of DAG have renewed the interest on this research topic. DAG, particularly the 1,3-isoform, has been confirmed as having certain nutritional benefits such as the ability to reduce serum triacylglycerol (TAG) concentration, body-weight and visceral fat [8–13]. There is no significant difference in energy value and absorption coefficient between DAG and TAG [14]. Numerous studies on the safety aspects of DAG on humans [14–17] and animals [18–20] demonstrated no adverse effects. With the goal of obtaining SL, the synthesis has been generally explored through acidolysis of vegetable oils or interesterification of vegetable oils. Palm oil acidolysis with capric or caprylic acid has become an option to obtain MLM SL. However, the complexity of the reaction substrates and products requires careful post-reaction

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separation steps and tedious analytical work. Undesired products are generated by acidolysis or interesterification of vegetable oils in higher relative amounts than in the case of direct esterification of glycerol with fatty acids [21].

In the present work 1,3-dicaproylglycerol was synthesized by direct esterification of glycerol with capric acid in an organic solvent using a commercial immobilized form of lipase from *Rhizomucor miehei* (Lipozyme RM IM) as catalyst. Being glycerol a low-cost by-product of the biodiesel industry, using it as a substrate is highly efficient in economic terms. Besides, as this is an esterification reaction with simple substrates such as glycerol and capric acid, the complexity of the reaction medium and analytical aspects were greatly reduced. In this paper we studied the effect of selected parameters (glycerol feed, enzyme dosage and reaction temperature) on the acid conversion, selectivity and diglyceride yield, as well as on the acyl migration process. The study was performed with a screening factorial design of three factors and three levels.

A number of studies in the literature focus on the synthesis of 1,3-DAG by esterification catalyzed by lipase, but no careful analysis of the impact of the silica (used as glycerol supplier) as fatty acid and monoglyceride adsorbent was included in those works, nor was the impact of the sampling procedure on the results considered or discussed. This impact may be huge in the case of solvent-free systems or a system with volatile substrates. No report on the subject, involving enzymes, has described the acyl migration mechanism with the level of detail presented in this manuscript. Watanabe et al. [22] studied the production of 1,3-DAG from a mixture of FA using the immobilized lipase from *R. miehei* in a solvent-free system. The kinetics of 1,3-DAG production from FA and glycerol was investigated. However, no detailed study of the acyl migration from sn-1 or sn-3 to sn-2 was included. The authors emphasized that the enzyme is regioselective for 1,3 positions, but they also reported that long reaction times, high temperatures and high concentrations of immobilized enzyme increased the acyl migration, triglyceride production and purity reduction of the 1,3-DAG. Purity was reduced from 94% to 68% when the immobilized lipase content was varied from 2.5% to 20%, but these results were not discussed. No explanation was provided, being the key question if the acyl migration responsible for the generation of triglycerides was induced by immobilized lipase support or by lipase. Other authors [23,24] studied the reaction conditions for the esterification mediated by Lipozyme RM IM. Reduced yields of the specific diglyceride were found due to acyl migration caused by the amount of biocatalyst, but no discussion of the causes of this reaction was included.

The present manuscript considers (a) the problems of the use of silica and the mistakes that may be present without careful sampling (errors as high as 20% or more in fatty acid conversion due to fatty acid adsorption on silica may be found, a phenomenon not taken into account or at least properly reported in the published literature), (b) a new explanation of the acyl migration mechanism (never presented before, to the best of our knowledge), and (c) a careful analysis of the 1,3-DAG isomeric distribution with simple gas chromatography.

2. Experimental

2.1. Materials

Lipozyme RM IM, which is a commercial form of the 1,3-specific lipase from *R. miehei* immobilized by adsorption on a macroporous anion exchange phenolic resin Duolite A-568, was kindly provided by Novo Nordisk A/S (Brazil). Glycerol, *n*-heptane, isopropyl ether and silica gel were supplied by Cicarelli Laboratorios. Capric acid, 1,2,4-butanetriol and silylation reagents were obtained from Fluka. Monocaprin, dipalmitin, trilaurin and tricaprin were supplied by Sigma–Aldrich. Absolute ethanol and ethyl ether were supplied by Dorwil, and phenolphthalein and pyridine were provided by Anedra S.A. All products were of analytical grade.

2.2. Adsorption of glycerol on silica gel

Glycerol was adsorbed as follows: 1 g of glycerol and 2 g of silica gel were mechanically mixed until total adsorption on the solid.

2.3. Lipase-catalyzed esterification

Esterification of glycerol was performed in 10 mL flasks, which were kept in a thermostatic bath with temperature control and magnetic stirring. The reaction time was 6 h. The reaction was carried out as follows: 110 mg of capric acid were dissolved in 3 mL *n*-heptane, then the amount of glycerol adsorbed onto silica fixed to each reaction under study was added. When the reactant mixture reached the selected temperature, the reaction was started by adding 50% of the total amount of enzyme to be added (time 0). The remaining 50% of the biocatalyst was added after 3 h of reaction. The values of glycerol, immobilized lipase dosage and reaction temperature were established according to the experimental design explained below. Highly hydrophilic polyols cause loss of enzymatic activity. This may be due to two factors: (1) in a hydrophobic reaction medium, polyols adhere to the support of the lipase impeding access of the acid to the active site, or (2) the hydroxyl groups of the polyol strongly interact with the active site of the enzyme.

Although silica gel behaves as a “polar substrate reservoir” and plays a protective role for the immobilized enzyme avoiding its blockage due to glycerol, the addition of the immobilized lipase in two steps minimizes the deactivation of the enzyme and maximizes the production of the desired product.

2.4. Experimental factorial design

A three-level-three-factor factorial design with two central points and a total of 10 experiments (Table 1) was applied in this study. The variables and their levels were: adsorbed glycerol on silica gel (50–250 mg), reaction temperature (40–60 °C) and immobilized lipase loading (20–40 mg). The studied responses were fatty acid conversion (mol%), enzymatic activity (μmol acid converted/mg immobilized lipase), monocaprin, dicaprin and tricaprin production (molar % of the total products), dicaprin selectivity and yield (mol%) and 1–2 dicaprin, 1–3 dicaprin and 2–3 dicaprin formation percentage (relative to total moles of produced dicaprin). All the experimental factorial design and the statistical analysis were performed using the STATGRAPHICS Centurion version XV.2 software. The factors and levels used and the obtained experimental responses are presented in Table 1. The order of the experiments was fully randomized to provide protection against the effects of lurking variables.

2.5. Statistical analysis

The complete statistical analysis was performed using the STATGRAPHICS Centurion software. The responses were adjusted by multiple regression, and the generated models were used to evaluate the effect of the selected experimental factors. The goodness of fit was assessed using the coefficient of determination (R^2). The statistically significant effect of the variables was tested using ANOVA statistical test. Non-significant coefficients were eliminated (p-value > 0.05) and the models were refined in order to consider