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Kinetic modeling of lipase-catalyzed glycerolysis of olive oil

Fernando Voll[c], Roberta L. Krüger[b], Fernanda de Castilhos[a], Lúcio Cardozo Filho[c], Vladimir Cabral[c], Jorge Ninow[b], Marcos L. Corazza[a,*]

[a] Department of Chemical Engineering, Paraná Federal University (UFPR), Polytechnic Center (DTQ/ST/UFPR), Jardim das Américas, Curitiba, PR 82530-990, Brazil
[b] Department of Chemical and Food Engineering, UFSC – Campus de Florianópolis, Caixa Postal 476, 88040-900 SC, Brazil
[c] Department of Chemical Engineering, Maringá State University, (UEM), Av. Colombo 5790, Maringá, PR 87020-900, Brazil

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ABSTRACT

This work reports experimental data and the kinetic modeling of monoacylglycerols (MAG) and diacylglycerols (DAG) production from olive oil using a commercial immobilized lipase (Novozym 435) and tert-butanol as solvent. The kinetic modeling presented is based on the ordered-sequential Bi Bi mechanism considering glycerolysis and hydrolysis/esterification steps. Moreover, an empirical enzyme deactivation term, due to an excess of glycerol in the system, is proposed. The experiments were performed in batch mode evaluating the effects of temperature (40–70 °C), enzyme concentration (2.5–15 wt%) and glycerol to oil (G:O) molar ratio (0.5:1.5–6:1). Good conversions of MAG (~65 wt%) and DAG (~57 wt%) were obtained with relatively low enzyme concentrations (10 wt %) in relatively short reaction times (720 min) for different initial G:O molar ratios. A very satisfactory agreement between the experimental data and modeling results was obtained under various conditions of enzyme concentration, glycerol to olive oil molar ratio and temperature, thus allowing a better understanding of the reaction kinetics.

1. Introduction

Monoacylglycerols (MAG) and diacylglycerols (DAG) are non-ionic and amphiphilic molecules with excellent emulsifying properties [1]. For that reason, and because they have low odor and taste, MAG and DAG are widely used in the food, cosmetic and pharmaceutical industries [2]. Due to their excellent lubricant and plasticizing properties, MAG is also used in textile processing, production of plastics, and oil formulations for different types of machinery [3–6]. MAG and DAG are readily biodegradable and generally recognized as safe (GRAS) [7]. In addition, DAG oil has beneficial effects on the prevention and management of obesity compared with triacylglycerols (TAG), the main components of edible oils. Consumption of DAG has also been shown to reduce accumulation of visceral abdominal fat [8–10]. It is suggested that the beneficial health effects of DAG are due to differences in the digestion and absorption of TAG and DAG [11].

DAGs are naturally occurring minor constituents of edible fats and oils, which are mainly constituted by triacylglycerols (TAG). DAG has attracted much attention in recent years due to its several important beneficial properties in terms of human health [12]. Studies indicate that DAG, particularly sn-1,3-diacylglycerols, may have beneficial effects on obesity and lipemia prevention [13,14]. One should also note that mixtures of mono- and diacylglycerols are important emulsifiers widely used in industrially processed foods [15].

With the establishment of government biodiesel programs worldwide, huge amounts of glycerol surplus are expected to occur in the near future, which will represent an important driving force for the development of new technologies devoted to the transformation of this byproduct of industrial biodiesel processing.

In contrast to the conventional chemical glycerolysis technique, in which fats and oils are submitted to high temperatures (200–250 °C) in the presence of inorganic alkaline catalysts (e.g., KOH and NaOH), research efforts have been directed toward producing MAG and DAG using enzyme-catalyzed reactions. This is because the commercially used method leads to the formation of dark-colored, burnt-tasting products, and also due to the fact that the chemical catalysis process is energy intensive, provides low yields (30–40%) and there is the need for product post-purification by molecular distillation [16–18].

In order to overcome these drawbacks glycerolysis has been carried out with lipases, in organic media [3–6,19], in solvent-free systems [1,15,20], with free or immobilized enzymes [1,3–6,15,20], in ionic liquids [21] or using compressed fluids as the reaction media [12,22].

The use of a solvent improves the miscibility between substrates resulting in a more homogeneous system, with higher mass trans-

* Corresponding author. Tel.: +55 41 3361 3178; fax: +55 41 3361 3277.
E-mail address: corazza@ufpr.br (M.L. Corazza).

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Nomenclature

[*TAG*] triacylglycerol concentration (mmol/$g_{substrate}$)
[*DAG*] diacylglycerol concentration (mmol/$g_{substrate}$)
[*MAG*] monoacylglycerol concentration (mmol/$g_{substrate}$)
[*FA*] fatty acid concentration (mmol/$g_{substrate}$)
[*G*] glycerol concentration (mmol/$g_{substrate}$)
[*W*] water concentration (mmol/$g_{substrate}$)
[*E*] free enzyme concentration (mmol/$g_{substrate}$)
[E_T] total enzyme concentration (mmol/$g_{substrate}$)
[*ET*] total enzyme concentration (g_{enzyme}/$g_{substrate}$)
[E_{act}] total active enzyme concentration (g_{enzyme}/$g_{substrate}$)
[*TAG* × *E* × *G*] triacylglycerol × enzyme × glycerol complex concentration (mmol/$g_{substrate}$)
[*DAG* × *E* × *MAG*] diacylglycerol × enzyme × monoacylglycerol complex concentration (mmol/$g_{substrate}$)
[*DAG* × *E* × *G*] diacylglycerol × enzyme × glycerol complex concentration (mmol/$g_{substrate}$)
[*MAG* × *E* × *MAG*] monoacylglycerol × enzyme × monoacylglycerol complex concentration (mmol/$g_{substrate}$)
[*TAG* × *E* × *MAG*] triacylglycerol × enzyme × monoacylglycerol complex concentration (mmol/$g_{substrate}$)
[*DAG* × *E* × *DAG*] diacylglycerol × enzyme × diacylglycerol complex concentration (mmol/$g_{substrate}$)
[*TAG* × *E* × *W*] triacylglycerol × enzyme × water complex concentration (mmol/$g_{substrate}$)
[*DAG* × *E* × *FA*] diacylglycerol × enzyme × fatty acid complex concentration (mmol/$g_{substrate}$)
[*DAG* × *E* × *W*] diacylglycerol × enzyme × water complex concentration (mmol/$g_{substrate}$)
[*MAG* × *E* × *FA*] monoacylglycerol × enzyme × fatty acid complex concentration (mmol/$g_{substrate}$)
[*MAG* × *E* × *W*] monoacylglycerol × enzyme × water complex concentration (mmol/$g_{substrate}$)
[*G* × *E* × *FA*] glycerol × enzyme × fatty acid complex concentration (mmol/$g_{substrate}$)
a enzyme activity
A_i (i = 1, ..., 12) pre-exponential factors ($g_{substrate}^2$/(g_{enzyme} min mmol))
Ea_i (i = 1, ..., 12) energy parameters
k_i (i = 1, ..., 36) kinetic constants
K_i (i = 1, ..., 12) equilibrium constants ($g_{substrate}^2$/mmol^2)
kd deactivation constant ((min $mmol_{glycerol}$)$^{-1}$)
n deactivation order related to the activity
NOBS number of experimental observations (experimental data points)
NCOM number of components considered in the fitting procedure (FA, MAG, DAG and TAG)
q deactivation order related to the glycerol concentration
rmsd root-mean-square deviation
V_i (i = 1, ..., 12) apparent rate constants ($g_{substrate}^2$/(g_{enzyme} min mmol))
V^{max} (i = 1, ..., 12) maximum initial reaction rate (mmol/($g_{substrate}$ min))
X_{jk}^{exp} experimental FA, MAG, DAG and TAG concentrations free of solvent and glycerol (g/$g_{substrate}$)
X_{jk}^{calc} calculated FA, MAG, DAG and TAG concentrations free of solvent and glycerol (g/$g_{substrate}$)

fer and lower viscosity [4], and consequently with higher MAG and DAG formation. Solvents such as n-hexane, n-heptane, dioxane, acetonitrile, acetone, isooctane, tert-butanol, tert-pentanol, or mixtures of some of these compounds, are useful for different lipase-catalyzed interesterification reactions [19,23].

Modeling approaches of the enzymatic glycerolysis are scarce in the literature. Moquin et al. [12,24] carried out kinetic modeling of a noncatalytic glycerolysis reaction in supercritical medium using a sequence of reversible reactions including the glycerolysis and hydrolysis steps. In a more recent work, Valério et al. [25] utilized the same approach to model the lipase-catalyzed glycerolysis of olive oil in a solvent-free system and obtained a good agreement between the experimental data and the model. Also, Tan and Yin [26] presented a kinetic model for glycerolysis using a 1,3 position-specific lipase obtained from *Rhizopus arrhizus* considering that the enzyme had a specific affinity for this position in triacylglycerol. The kinetic model presented by Cheirsilp et al. [27] is based on the Ping-Pong Bi Bi mechanism for the glycerolysis of palm olein by immobilized lipase considering a simple scheme for the glycerolysis reaction based on hydrolysis and reesterification steps. Although the modeling results were reliable, this model can only be applied to high water content conditions, since it takes into account the hydrolysis as its first and most important step, and most of the glycerolysis reaction are conducted under lower water content conditions [4,15,20,25,28].

The main objective of this study is to report experimental data and a new kinetic model based on the ordered-sequential Bi Bi mechanism for a lipase-catalyzed glycerolysis in an organic solvent system based on glycerolysis and hydrolysis/esterification steps.

2. Materials and methods

2.1. Materials

The substrates used in this were commercial olive oil (Arisco, Brazil) and glycerol (Merck, 99.5%). The organic solvent used was tert-butanol (99%, Vetec). A commercial immobilized lipase from *Candida antarctica* (Novozym 435) was purchased from Novozymes (Araucária, PR, Brazil). n-Hexane solvent (Quimex, 99.5%) was used in the removal and washing of enzymes in the reaction medium at the end of reaction. n-Heptane, pyridine, MSTFA (derivatization grade) were used in the chromatographic analysis. The external standards used were: monoolein, diolein, triolein and oleic acid, all purchased from Sigma–Aldrich.

2.2. Analytical methods

Analyses were carried out in a Shimadzu 2010 gas chromatograph with an automatic and on-column injector and a flame ionization detector (FID). The following instrumentation and conditions were used: a DB-5 capillary column (30 m × 0.25 mm × 0.1 μm), column temperature: 50 °C/min, 15 °C/min up to 180 °C, 7 °C/min up to 230 °C, and 10 °C/min up to 380 °C, standing for 8 min. The detector temperature was 380 °C, carrier gas was H_2, pressure was 80kPa and injected volume was 1 μL. The quantification of reaction products was carried out using authentic standards of MAG, DAG, TAG and FA. Calibration curves were built with the following concentrations: 100, 200, 300, 500, 1000, 2000, 3100 and 6200 ppm for MAG; 50, 200, 530, 730, 890, 1000, 1400 and 2100 ppm for DAG; 50, 200, 300, 500, 3000, 5000, 7500 and 10,000 ppm for TAG; and 25, 50, 100, 300, 500, 750 and 1000 for FA. Derivatization grade MSTFA (100 μL) was added to each calibration solution, which was then stirred and kept at ambient temperature for 15 min for the derivatization of reactant. The content of the reaction products was expressed in terms of the