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Ethanolysis of a waste material from olive oil distillation catalyzed by three different commercial lipases: A kinetic study

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Abstract

Ethanolysis of a raffinate product-obtained after distillation of olive oil-catalyzed by three commercial lipases (from *Candida antarctica*, *Rhizomucor miehei* and *Thermomyces lanuginosa*) was studied. Uni-response model derived from a generalized Michaelis–Menten mechanism was utilized to describe the rates of formation of ethyl esters from the fatty acids present in the precursor oil. The rate constants for the ethanolysis of triacylglycerols and steryl esters under the reaction conditions were compared. Moreover, inactivation of three lipases was evaluated. Because the half-life of the enzyme is comparable to or even shorter than the half-life of the reaction, the intrinsic reaction rate and enzyme deactivation must both be considered in modeling the kinetics.

Keywords: Deodorizer distillate; Kinetic parameters; Lipase; Modeling; Steryl esters; Transesterification

1. Introduction

The cholesterol lowering effect of sterols has been extensively studied [1,2]. Both sterols and steryl esters show the same physiological effect [3]. This physiological activity has led to the development of several functional foods, such as salad oils, dressings, margarines, etc., containing sterols and/or steryl esters. However, the solubility of steryl esters in edible oils is much higher than that of free sterols. Hence, steryl esters are preferred and considerable attention has been particularly addressed to their addition to oil based foods.

At present, free sterols are obtained as a by-product of the tocopherols purification process from vegetable oil deodorizer distillates (VODD). However, the isolation of the sterol fraction requires multiple steps such as short-path distillation, ethanol fractionation, crystallization, etc. Additionally, process yields are not high, and sterol compounds contained in VODD are wasted in spite of being used as functional food materials. An attractive alternative is to find raw material sources that contain steryl esters, i.e. sterols in their esterified form. One of these materials is the raffinate product-obtained after distillation of olive VODD, also known as olive oil pitch (OOP). This waste material contains mainly acylglycerols, steryl esters and hydrocarbons. The separation of steryl esters from acylglycerols results difficult due to the similarities in the physical properties of these compounds. The isolation of steryl esters from the OOP waste can be greatly facilitated if the acylglycerol compounds are selectively converted to fatty acid alkyl esters. Numerous studies of lipase catalyzed reactions have shown very high efficiency and selectivity for enrichment or purification of useful components in oils [4,5]. For example, sterols can be converted to their fatty acid esters by lipase catalyzed esterification or transesterification [6,7].

The present study describes the ethanolysis of an OOP waste, obtained after distillation of olive VODD, in the presence of three different commercial lipases: *Candida antarctica*, *Rhizomucor miehei* and *Thermomyces lanuginosa*. The same reaction conditions were employed for the three lipases assayed and hence, the lipase selectivity to discriminate against the steryl esters in ethanolysis reactions was studied and compared. Additionally, the activity and stability of the three lipases used have been determined and compared. Hirota et al. [8] have been previously carried out similar studies via lipase catalyzed hydrolysis reactions.

Abbreviations: B, ethanol; E, active form of the enzyme; E_d, inactive form of the enzyme; FAEEs, fatty acid ethyl esters; G, triacylglycerols; MSS, mean sum of squares; OOP, olive oil pitch; P, lower glycerides; Q_1, fatty acid ethyl esters involved in the ethanolysis of triacylglycerols; Q_2, fatty acid ethyl esters involved in the ethanolysis of steryl esters; RSS, relative sum of squares; S, sterols; SE, steryl esters; VOOD, vegetable oil deodorizer distillates

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Nomenclature

$a\{t\}$	enzyme activity at time (t)
a_0	enzyme activity at time (t_0)
k_d	first order enzyme deactivation rate constant
t	time
t_0	starting time of the first reaction cycle experiment
t_1	starting time of a particular reaction cycle experiment
t^*	pseudo reaction time

A model for the ethanolysis reaction is proposed and is formally equivalent to the Michaelis–Menten mechanism, which takes into account both reaction reversibility and product inhibition. A first order enzyme deactivation term [9] was incorporated in the kinetic model to describe the loss of enzyme activity with time. The kinetic model developed provides mechanistic information concerning the enzymatic ethanolysis of acylglycerol + acylsterol mixtures, and permits estimating the differences in the rate of release of the fatty acid residues present in the acylglycerol molecules and those present in the steryl ester molecules. This information, together with lipase deactivation, is of great importance for process analysis and design.

2. Materials and methods

2.1. Materials

OOP waste was obtained from Grupo SOS (Madrid, Spain). Immobilized lipases from *T. lanuginosa*, *R. miehei*, and *C. antarctica* were kindly donated by Novozymes (Bagsvaerd, Denmark). All solvents used were HPLC grade from Lab-Scan (Dublin, Ireland).

2.2. Methods

2.2.1. Reaction mixtures

OOP waste (10 g) and ethanol (0.85 g) were added to a 50 ml flask containing 0.7 g dodecane (used as an internal standard) and mixed by swirling. Then the immobilized lipase was added. The flasks were stoppered and placed in an orbital shaker (200 rpm) at 30 °C. Samples (50 μl) were withdrawn periodically. The reaction was allowed to proceed for 48 h.

2.2.2. Lipase recovery

In order to study the stability of the three lipases assayed, two charges of reactants according to the procedure described above were carried out with the same initial mixture of OOP waste and ethanol. For each charge, the lipase was used to catalyze the reaction for 48 h and then recovered at room temperature using the following washing procedure. First, the lipase was submerged in a fresh reaction mixture for 5 min (to allow the liquid to wet the sample) and dried at room temperature under vacuum for 10 min. Then, two washings with acetone were effected to eliminate the reaction mixture from the biocatalyst. After each washing the lipase was again dried under vacuum for 15 min. Finally, the lipase was weighed to ensure that no traces of either reaction mixture or acetone were present, and reused in the next charge to the batch reactor. The recovery process took approximately 0.75 h.

2.2.3. HPLC analysis

The analyses were effected on a Kromasil silica 60 column (250 mm × 4.6 mm, Análisis Vínicos, Tomelloso, España) coupled to a CTO 10A VP 2 oven, a LC-10AD VP pump, a gradient module FCV-10AL VP, a DGU-14A degasser, and an evaporative light scattering detector ELSD-LT from Shimadzu (IZASA, España). The column temperature was maintained at 35 °C. The mobile phase utilized has been previously reported by Torres et al. [10].

2.2.4. Gas chromatography

A Perkin-Elmer Autosystem XL gas chromatography (Perkin-Elmer, Norwalk CT, USA) equipped with a programmed split/splitless injector (PSS) and a flame ionization detector (FID) was used to analyze fatty acid ethyl esters (FAEEs). The system was coupled to a Perkin-Elmer chromatography software system (Turbochrom). A 30 m × 0.25 mm i.d. BTR-Carbowax capillary column (Quadrex Corporation, New Haven, USA) coated with a 0.25 μm layer of polyetilene glycol–oxygen resistant was employed. The temperature program was as follows: starting at 100 °C and then heating to 180 °C at 20 °C/min; followed by heating from 180 to 220 °C at 15 °C/min. The final temperature (220 °C) was held for 35 min.

Helium was used as a carrier gas with a flow rate of 1.2 ml/min. The split ratio was 20:1. Sample injection volume was 1 μl for all the extracts.

The retention times of the diverse FAEEs were determined injecting a solution of PUFA no. 1 Marine Source, Supelco (4–7033) (Bellefonte, PA).

3. Mathematical modeling: dual response model

A modified version of the Michaelis–Menten generalized rate expressions, proposed by Malcata et al. [11] and by Torres et al. [12] for lipase catalyzed lipolysis reactions, was employed. The proposed reaction mechanism is shown in Fig. 1 In the ethanolysis kinetic modeling, the step involving the ester bond rupture is assumed to be the rate-determining step. The total rate of disappearance of triacylglycerols (G) and steryl esters (SE) can be written for the rate-limiting steps as:

$$-\frac{d[SE]}{dt} = k_t[FS][B] - k_{-t}[EQ_2][S] \quad (1)$$

$$-\frac{d[G]}{dt} = k_p[FG][B] - k_{-p}[EQ_1][P] \quad (2)$$

where Eqs. (1) and (2) refer, respectively, to the net rate at which fatty acid residues in steryl esters and triacylglycerols are consumed due to the ethanolysis reaction. Additionally, B, P, S and Q represent, respectively, the concentrations of ethanol, lower glycerides, sterols and released fatty acid ethyl esters. The reader