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A two-step enzymatic strategy to produce ethyl esters using frying oil as substrate

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ABSTRACT

This work reports a strategy for enzymatic esters production through hydroesterification using soybean and frying oils by ultrasound bath-assisted, comprising two steps, enzymatic hydrolysis in batch mode followed by esterification in continuous mode. The hydrolysis reaction was conducted using 1:20 molar ratio (oil/water), ultrasound power 132 W, 40 °C, 300 rpm, 2 h of reaction time, and 10 wt% Lipozyme TL IM. For esterification reactions, fatty acids obtained in the previous hydrolysis step was employed at a molar ratio of 1:6 (fatty acid/ethanol), 132 W, 65 °C, substrate feed rate 2.5 mL/min, and 1 wt% Novozym 435. The experimental apparatus used for the esterification step consisted in a packed glass reactor, submerged in an ultrasound bath. Results showed a conversion to ethyl esters as high as 94% after 28 min for soybean oil and 79% in 9 min using frying oil in the esterification step. Finally, and based on our results, the procedure suggested here, enzymatic hydroesterification, is a good alternative to the continuous production of ethyl esters from low cost raw materials.

1. Introduction

In recent years, esters have attracted considerable worldwide attention being used as alternative biofuels (biodiesel) and additives in food, detergents, cosmetics and pharmaceutical industries (Dormo et al., 2004; Gryglewicz et al., 2013; Ganguly and NandI, 2015). The most common process for obtaining esters is the transesterification of triglycerides from vegetable oils and animal fats. Recently, the hydroesterification has increasing its application due to the possibility of using a variety of low-cost commercial lipases and a greater source of raw materials, including frying oils, high acidity oils, tallow and waste greases from processing oils, such as grease strap and brown grease (Kabbashi et al., 2015).

The use of enzyme technology is earning space, with great industry interest due to the high biotechnological potential, showing versatility to catalyze hydrolysis, transesterification and hydroesterification reactions (Brockman, 1984; Castro et al., 2004; Sánchez et al., 2016). In the biotechnological processes, enzymes are responsible to perform the reactions on water – oil interface, since the substrates are characteristically mutually insoluble (Navarro and Brace, 1997; Ren et al., 2011). The factors that influence the enzymatic reactions efficiency are: enzyme characteristics and concentration, time, temperature and pH of the reaction, molar ratio of substrate, type of alcohol, presence of impurities, and water content in the reaction medium (Pourzolfaghar et al., 2016).

Hydroesterification is a process that involves a hydrolysis step followed by an esterification one. The hydrolysis consists in a chemical reaction between fat or oil with water, thus producing glycerin and fatty acids (and, obviously, mono and diglycerides). After hydrolysis, glycerin is removed and fatty acids obtained are then esterified with an alcohol, which neutralizes the acidity present, producing esters with high purity. In the reaction, water is also obtained as by-product, which can return to hydrolysis process (Machado et al., 2015; Aguieira et al., 2014; Cavalcanti-Oliveira et al., 2011).

To date, triglycerides of edible vegetable oils are still the raw materials most used for esters production (Canakci and Sanli, 2008). However, these oils are relatively expensive, since the raw materials for biodiesel synthesis correspond to 50–85% of total production costs and therefore it is desirable to use low cost feedstocks to increase commercial biodiesel competitiveness (Kabbashi et al., 2015; Chen et al., 2009; Halim et al., 2009). Raw materials include low-value animal fats and residual oils, as frying oil used in this work. Ethanol was selected as the alcohol, due to its the low toxicity when compared with methanol and huge availability in Brazil.

In the search for new techniques that can improve the efficiency of industrial processes, ultrasound technology is proving to be a promising alternative. Some studies have reported the use of ultrasound towards emulsions formation even without surfactants presence, due the

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efficient phase dispersion promoted by the ultrasound irradiation in heterogeneous systems (Kwiatkowska et al., 2011; Chandralapa et al., 2012). Combined with ultrasound, packed bed reactors (PBR) can be considered as an economically viable process mainly when using enzymatic catalyst where this catalyst can be reused, reducing the process cost (Al-Zuhair et al., 2011).

Fixed bed reactors are traditionally employed in the most biocatalyst reactors in large scale, due to the high efficiency, low cost, ease construction, operation, scale-up, automatic control, and lower shear degree force that, consequently, reduce the biocatalyst deactivation (Halim et al., 2009; Baltaru et al., 2009; Chang et al., 2009; Chen and Lin, 2010; Wang et al., 2011).

Based on the above-mentioned facts and considering the possibility of process scaling, this work aims to investigate new alternatives for esters production, specifically the enzymatic hydroesterification of frying oil using ultrasound bath-assisted by two enzymatic steps: hydrolysis in batch mode followed by esterification of fatty acids in a continuous packed-bed reactor.

2. Materials and methods

2.1. Materials

The biocatalysts used in this work were the commercial immobilized lipases Lipozyme TL IM and Novozym® 435, kindly donated by Novozymes Brazil. The choice for the enzyme type considered the enzyme specificity in each reaction step. As substrates for enzymatic hydrolysis were used distilled water, refined soybean oil and frying oil (used as received, without any pre-treatment) from local restaurants. The substrates used in the esterification process were those obtained from the hydrolysis step. Ethanol (Merck, 99.9%) was used in the esterification step, and isopropanol (Vetec, 95%) was used in the hydrolyzed oil filtration to remove the enzyme.

For acid number and acidity determination, potassium hydroxide (Vetec), ethanol:diethyl ether (1:2 v/v) (95%, Vetec), and phenolphthalein (Nuclear) were used. All reactants were used as received without further purification.

2.2. Step 1: batch hydrolysis process in ultrasound-assisted system

The experimental apparatus used for the hydrolysis experiments consisted in an ultrasound bath (model USC 1800A, UNIQUE – frequency of 40 kHz and maximum power output of 132 W); inside ultrasound bath a 50 mL Erlenmeyer flask containing a mechanical stirrer (mechanical stirrer RW 20 model D, IKA) was used as reaction vessel. The flowchart of Fig. 1 shows the hydrolysis process used to obtain the hydrolysates for both soybean and frying oils.

For each experiment, 1:20 molar ratio oil to distilled water was used and the enzyme amount (10 wt%) Lipozyme TL IM was calculated on the total weight of substrate. The reaction mixture was kept under mechanical agitation (300 rpm) in ultrasound bath for 2 h of reaction time. The hydrolysis reactions were conducted at 100% of ultrasound power (132 W) and at 40 °C. The ultrasound bath parameters used in this step were fixed based on previous studies of our research group, where it was observed that ultrasound bath helped to increase the FFA content (Zenevicz et al., 2016). After reaction time, the sample was filtered for enzyme separation using isopropanol, then, isopropanol was removed by vacuum distillation, glycerol was separated by centrifugation and the acid number was then measured. The obtained hydrolysate (free fatty acids from soybean and frying oils) was used as substrate in the esterification reaction.

2.3. Step 2: continuous esterification in ultrasound-assisted system

The experimental apparatus (Fig. 2) used in this step consisted in a 29 mL glass reactor, (13 mm of internal diameter, 171 mm length), packed with immobilized lipase Novozym 435 (10 g), submerged in the ultrasound bath (Unique Ultra Sonic Cleaner model: USC-1800A, frequency of 40 kHz, 132 W irradiation power), fed with reaction mixture of substrates (free fatty acid plus glycerol, mono, di and triglycerides from hydrolysis of soybean and frying oils, in the previous step) and ethyl alcohol and kept at 65 °C. The residence time of incoming feed was estimated according to Dalla Rosa et al. (Dalla Rosa et al., 2009) Here, the influence of ultrasound on the esterification reaction was evaluated.

The substrates were previously homogenized under mechanical stirring (RW model 20 D IKA) during all reaction time, and volumetric flow rate of feed substrate (2.5 mL/min) was kept till complete reaction system filling using a peristaltic pump (MARLOW 323). After one residence time, samples were collected in sample bottles (previously weighed) and forwarded to quantification analysis.

2.4. Free fatty acids and ethyl esters quantification

The determination of free fatty acids (FFA) content was carried out by titration with KOH, according to IUPAC 2.201 and AOCS Cd 3d-63 method. Ethyl esters quantification was determined by gas chromatography in triplicate follow EN14103 (2001) by using a flame ionization detector (GC/FID Shimadzu model 2010) equipped with a nonpolar capillary column Model RT-WAX (polyethylene glycol containing stationary phase), 30 m length, 0.32 mm internal diameter with following chromatographic conditions: initial temperature of 150 °C column remained in this condition for 1 min, heating rate 10 °C/min with final temperature of 250 °C column remained in this condition for 1 min. The temperature of the injector and detector was set at 250 °C. Injected solution sample was 1 μL.

2.5. Monoacylglycerol (MAG), diacylglycerol (DAG), triacylglycerol (TAG) and glycerol quantification

MAG, DAG and TAG contents in the samples was performed by gas chromatography (Shimadzu 2010, autosampler on-column and flame ionization detector (FID)) equipped with a capillary column of 5% phenyl polydimethylsiloxane length of 10–15 m, internal diameter 0.32 mm and film 0,1 μm, gun (on-column), flame ionization detector and integrator (computer with appropriate software). The operating conditions were used according to European Standard No.14105, Committee for Standardization and ASTM D 6584. Glycerol content was determined by sodium periodate methodology (Dalla Rosa et al., 2009).

3. Results and discussion

3.1. Enzymatic hydroesterification of soybean oil

3.1.1. Enzymatic hydrolysis of soybean oil

Firstly, the enzymatic hydrolysis of soybean oil in ultrasound-bath mode was evaluated under reaction conditions of: 40 °C, molar ratio soybean oil to water 1:20, 10 wt% of Lipozyme TL IM (by weight of total reaction substrates), ultrasound power 132 W, 2 h of reaction time and 300 rpm. The hydrolyzed soybean oil under these conditions afforded an acid number of 120 mg KOH/g (60 wt% of FFA), 2.4 wt% of MAG content, 16 wt% of DAG content, 17.4 wt% of TAG and around 4.7 wt% of glycerol.

Although the presence of glycerol in the hydrolysate soybean oil may decrease the esterification reaction efficiency due to viscosity increase and glycerol adsorption on the catalyst surface, hence reducing mass diffusion between immobilized enzyme and substrates, it was decided to adopt an industrial practical approach and then not to remove the residual glycerol (Shimada et al., 2002; Huma et al., 2011; Xu et al., 2011).