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Enzymatic hydrolysis of soybean and waste cooking oils under ultrasound system

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ABSTRACT

This work reports the enzymatic hydrolysis of soybean and waste cooking oils in ultrasound system towards producing free fatty acids hence allowing faster esters production. For this purpose, the following experimental conditions were investigated: temperature (40–60 °C), molar ratio of oil to water (1:9; 1:10; 1:20, 1:40 and 1:46), enzyme (Lipozyme TL IM) concentration (1–10 wt%, based on the total mass of substrates), keeping the reaction time fixed at 2 h, 300 rpm stirring rate and ultrasonic output power of 132 W. Results showed a content of 60 wt% FFA for soybean oil while 61 wt% was reached for waste cooking oil. After the experimental design, a kinetic study was performed at the best temperature, molar ratio (oil:water), enzyme concentration varying reaction times (until 24 h) with and without ultrasound. The use of ultrasound showed to be effective, increasing the initial reaction, providing very satisfactory reaction yields at short reaction times with no damage to protein structure thus allowing enzyme reuse.

1. Introduction

In recent years there has been a remarkable increase in the production and modification of oils and fats from both animal and plant sources (Clauss, 2013; Lima and Nassu, 1996). One of the first steps for obtaining chemical compounds derived from vegetable and animal oils may be via hydrolysis. In the hydrolysis of oils and fats a triglyceride molecule is transformed in the presence of a catalyst (chemical or enzymatic) and water, to form fatty acids and glycerol (Gioielli et al., 1995).

The free fatty acids produced by hydrolysis can be esterified by a short chain alcohol (methanol or etanol), giving as products esters and water. This process is called by hydroesterification and represents a new alternative for the production of esters, since permits the use of any grease raw material (plant oils, industrial greasy residues, waste cooking oils, byproducts of refining oils and fats), independent from the free fatty acids and water contents (Cardoso, 2008; Encarnação, 2008).

The traditional oil hydrolysis is carried out using a chemical catalyst at high temperature and pressure (250 °C and 70 bar) (Murty et al., 2002). Under these conditions, undesirable reactions such as oxidation, dehydration of free fatty acids, or interesterification of triglycerides may occur (Murty et al., 2002; Rooney and Weatherley, 2001). On the other hand, hydrolysis reactions catalyzed by lipases can be carried out at lower temperatures so as to save energy, and exhibits a high selectivity which leads to high-quality products with less reaction side compounds (Murty et al., 2002; Castro et al., 2004; Hasan et al., 2006; Sharma et al., 2009).

In recent decades, lipases gained increased attention worldwide because of its wide range of applications, including large-scale industry (Neena, 1997; Yadav and Borkar, 2009; Yadav et al., 2008; Yadav and Devi, 2004) and commercially employed for breakage the glycerides binding, in the presence of a sufficient amount of water, leading to the formation of monoglycerides, diglycerides, free fatty acids and glycerol (Villeneuve et al., 2000; Pilarek and Szewczyk, 2007; Hasan et al., 2009). Lipases are interfacial enzymes and remain active in the oil–water interface, due to the lid open conformation of lipases in the biphasic system interface (Yan et al., 2011; Maruyama et al., 2000). It is known that lipases act through oil–water interface and for this reason the total free interfacial area is a great requirement to increase the yield of different reactions (Sharma et al., 2012). Tools may be used such as mechanical stirring at low speed and more recently ultrasound. The ultrasound technology has an interesting advantage for accelerate the initial rates of many chemical reactions (Huang et al., 2010). In enzymatic reactions, the use of ultrasound has shown improvements in enzymatic synthesis with an increase of stability and catalytic activity (Batistella et al., 2012; Lerin et al., 2014), and consequently, allow-

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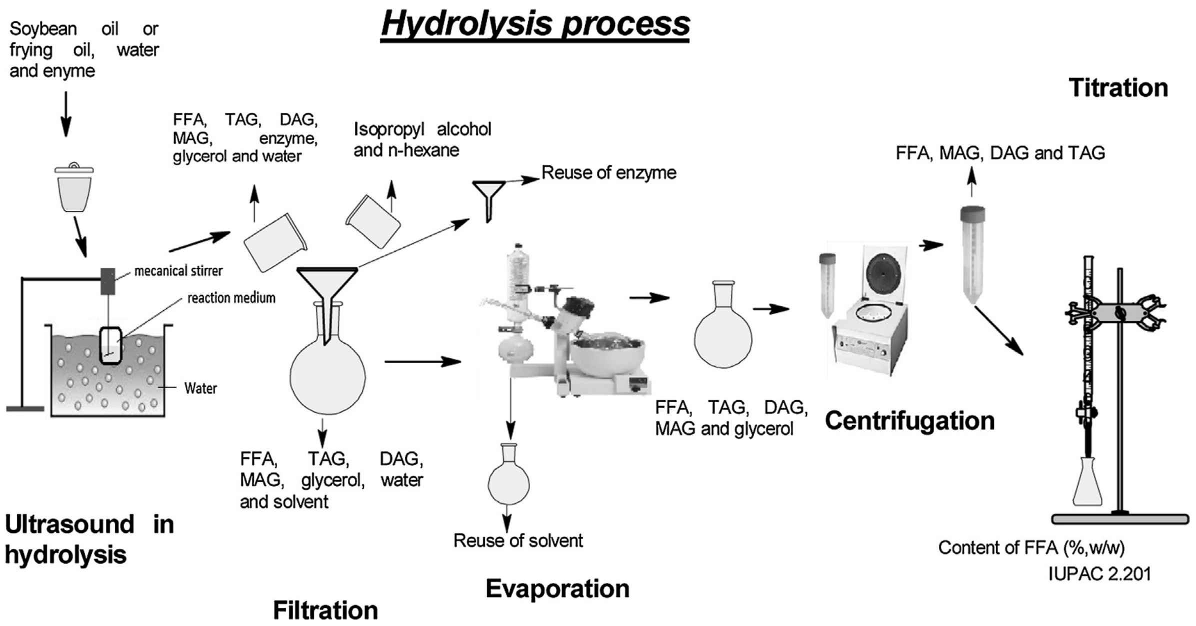

Fig. 1. Experimental apparatus used in the enzymatic hydrolysis.

ing a stabilization of the biocatalyst in the presence of an organic solvent, leading to higher reaction yields compared to the traditional methods.

This nonconventional method can comprise an important tool seeking for environmentally sustainable technologies, especially related to the concepts called "green chemistry" due to its high efficiency, low instrumentation requirements, significant reduction in processing time compared with other conventional techniques and toward an economically viable performance (Mason et al., 2015). Some studies report that the use of ultrasound facilitates the formation of emulsions even without the presence of surfactants due to the occurrence of huge agitation of the reaction medium, thus promoting effective phase dispersion in heterogeneous systems.

In this context, this study aims to investigate the potential of ultrasound to produce fatty acids from soybean oil and frying oil through enzymatic hydrolysis reaction in organic solvent-free system with subsequent recover and reuse of both lipase and solvent.

2. Experimental

2.1. Materials

Soybean oil (Soya, Brazil) was used as a model system and (acid number 0.29 mg KOH/g) purchased from a local market and used without any further treatment. A commercially immobilized lipase was kindly supplied by Novozymes® (Araucária, PR, Brazil): Lipozyme TL IM, from *Thermomyces lanuginosus* (TL), specific for position 1-3 triglyceride bond, immobilized on silica gel (0.15 U/g and 4 wt% water) and used as catalyst. Bi-distilled and deionized water was used to accomplish the hydrolysis reactions. For filtration and washing of the enzyme, *n*-hexane (Vetec) and isopropyl alcohol (Vetec) were used. The waste cooking oil was kindly donated by local restaurants and presented na acid number of 1.26 mgKOH/g.

2.1.1. Apparatus and experimental procedure

Enzymatic ultrasound-assisted hydrolysis reactions were carried out using an ultrasonic bath (Unique apparatus—temperature accuracy of ±0.5 °C) in which Erlenmeyer flasks (50 mL) were immersed in water with the content mechanically stirred (IKA-RW 20 digital stirrer) at constant stirring rate of 300 rpm for all experimental conditions. The experimental setup consists of an ultrasonic bath equipped with a transducer having longitudinal vibrations. The ultrasonic unit has an operating frequency of 40 kHz and a maximum electrical power rated output of 132 W. The ultrasonic transducer (surface area of 282.2 cm^2) is fitted at the bottom of the bath, horizontally along the length of it, which has been considered a suitable ultrasonic system. Fig. 1 shows the schematic diagram of the experimental apparatus used in this work.

The evaluated variables for hydrolysis were temperature (40–60 °C), oil to water molar ratio (1:9; 1:10; 1:20; 1:40 and 1:46), enzyme concentration C_E (1–10 wt%, based on the total amount of substrates), fixing 2 h of reaction time, 300 rpm of stirring rate (naval ship propeller type with axial flow) and an ultrasonic electric power of 132 W. These initial conditions were established based on preliminary tests (data not shown) performed in our lab. After the reaction two different solvents were tested, *n*-hexane and isopropyl alcohol, for washing the enzyme and removing the product by vacuum filtration to identify which solvent would be the best to drag the product and not damage the enzyme. At the best temperature, oil to water molar ratio, enzyme concentration and best solvent, destructive kinetic assays were performed (0.25; 0.50; 1, 2, 4, 6, 8, 10, 12; 16, 18, 20 and 24 h), and after each reaction time the enzyme recovered was applied in new kinetics. A reaction kinetics without ultrasound was also accomplished.

After each experimental reaction time, the samples passed through filtration to separate the enzyme, then the filtrate was concentrated in a rotary evaporator to remove the remaining solvent and centrifuged to separate the glycerol and water that did not react, providing only fatty acids, monoglycerides, diglycerides and unreacted triglycerides. The FFA content (wt%), monoglycerides, diglycerides and unreacted triglycerides was determined. In addition, it was measured the hydrolytic activity of the recovered enzyme, after both reactions with and without ultrasound for its subsequent reutilization. The solvent was removed using a rotary evaporator and used in other reactions. After reaching an optimum condition of the soybean oil hydrolysis, a test with frying oil (soybean cooking oil, supplied from a local restaurant) was also carried out.