Industrial Crops and Products 97 (2017) 218–223

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Enzymatic interesterification of crambe oil assisted by ultrasound

Gilmar Roberto Tavares [a], José Eduardo Gonçalves [b], Wanderley Dantas dos Santos [c], Camila da Silva [d,*]

[a] Departamento de Ciências Agronômicas, Universidade Estadual de Maringá (UEM), Estrada da Paca s/n, Umuarama, PR, 87500-000, Brazil
[b] Programa de Mestrado em Tecnologias Limpas e Programa de Mestrado em Promoção da Saúde, Centro Universitário de Maringá, Av. Guedner 1610, Maringa, PR, 87050-900, Brazil
[c] Departamento de Bioquímica, Universidade Estadual de Maringá (UEM), Avenida Colombo 5790, Maringa, PR, 87020-900, Brazil
[d] Departamento de Tecnologia, Universidade Estadual de Maringá (UEM), Avenida Ângelo Moreira da Fonseca 180, Umuarama, PR, 87506-370, Brazil

ARTICLE INFO

Article history:
Received 23 July 2016
Received in revised form 5 October 2016
Accepted 18 December 2016

Keywords:
Methyl acetate
Biodiesel
Crambe abyssinica H.

ABSTRACT

In this work, the production of fatty acid methyl esters (FAME) from crambe oil by enzyme interesterification assisted by ultrasound was investigated. The experiments evaluated the effect of temperature, reaction time, methyl acetate (MA)/oil molar ratio, enzyme loading, catalyst reuse and influence of ultrasound. The best results were obtained at 60 °C, temperature at which the reaction reached equilibrium in 6 h. FAME yield was maximal at MA/oil molar ratio of 12 and the enzyme loading of 20 wt% (relative to oil mass) gave the best yields. The use of methyl acetate promoted the enzyme stability throughout successive cycles, with a decrease of only 10% and 8% in FAME yield and enzymatic activity. The results allow us to conclude that ultrasound reduced total reaction time and percentage of enzyme loading, when compared to the process without ultrasound.

1. Introduction

Biodiesel production is presented as sustainable alternative to petroleum diesel. For obtaining it should be prioritized non-food raw materials and with low cost. Crambe (*Crambe abyssinica* H.) is an interesting option, due to high oil content in its seeds (30–50%), short cycle (on average 90 days) and seed yield between 1000 and 1500 kg per hectare (Viana et al., 2013; Singh et al., 2014; Brandão et al., 2014; Prates et al., 2014).

The crambe oil is predominantly formed by erucic acid (56–66%). The consumption of the this acid increases the level of cholesterol and lipidosis in heart tissues (Goswami et al., 2012), which makes the crambe unfit for human consumption (Wazilewski et al., 2013; Maciel et al., 2014). On the other hand, the erucic acid is stable at high temperatures and low melting point. The crambe oil still present high content of oleic acid and antioxidants allowing excellent fuel production (Wazilewski et al., 2013; Nadaletti et al., 2014) with performance similar to that of mineral diesel and considerably lower emissions of polluting gases (Rosa et al., 2014). Despite their qualities, crambe oil is still little explored for the production of biodiesel, with a low number of papers on the subject in the literature.

In chemical routes conventionally used for the production of biodiesel, transesterification of triglycerides with short chain alcohols such as methanol and ethanol leads to the formation of glycerol as byproduct. Thus, by classical methods, the increasing production of biodiesel lead to increased glycerol generation. As biodiesel production generates approximately 10% glycerol by volume, with the replacement of petroleum diesel per biodiesel, glycerol can make an economic and environmental liability (Leoneti et al., 2012). To mitigate this effect, it has been developed alternative route to the transesterification. The idea is to replace the alcohols by methyl acetate (MA) as acyl acceptor, route known as interesterification. In this new route, instead of producing glycerol, the reaction yields triacetin as coproduct. Triacetin has wide industrial application (Casas et al., 2013) and has no adverse effects on the quality of fuel (Saka and Isayama, 2009), being allowed its addition to biodiesel up to 10% by weight (Casas et al., 2011; Jung et al., 2012; Go et al., 2013).

Among the catalysts used for interesterification of vegetable oils, immobilized enzymes show advantages, because are easily separated from the reaction medium resulting in greater purity of coproducts and reusability for several cycles without generating toxic waste (Ranganathan et al., 2008; Fjerbaek et al., 2009). The enzymes still allow the reactions are conducted at mild temperatures, which prevents the degradation of the products and reduces

* Corresponding author.
E-mail address: camiladasilva.eq@gmail.com (C. da Silva).

http://dx.doi.org/10.1016/j.indcrop.2016.12.022
0926-6690/

energy costs (Antczak et al., 2009). The use of methyl acetate have the additional advantage of not reduce the enzymatic activity in the early cycles, thus allowing more efficient re-use of enzymes (Huang and Yan, 2008; Ruzich and Bassi, 2010), which does not occur with the use of short chain alcohols (ethanol, methanol), because the glycerol can lead to inactivation of the enzyme, thereby decreasing its enzymatic activity (Jeong and Park, 2010).

The use of immobilized catalysts may limit the mass transfer, since the supports may hinder the access of substrate to the catalytic site (Fjerbaek et al., 2009). The contact between the two phases is usually promoted by mechanical agitation. However, it has recently been shown that the application of ultrasound may offer advantages (Yu et al., 2010), because cavitation (formation, rise and implosion of bubbles in the reaction medium) generated by ultrasound accelerate chemical reactions by increasing mass transfer between phases, and providing activation energy (Veljković et al., 2012; Lerin et al., 2014). Cavitation causes localized increase in temperature on the border of the phases and mechanical energy which enhance mixing. The collapse of cavitation bubbles disrupts the boundary between phases, and promotes emulsification by ultrasonic jet. These effects provide increased reaction rates and obtaining high yields (Thank et al., 2010), reducing the need for large amounts of catalysts and the power consumption compared to the process with mechanical stirring (Chand et al., 2010).

Based on the context described, in this work was investigated the enzymatic interesterification assisted by ultrasound of crambe oil, using methyl acetate as acyl acceptor. For this purpose we evaluated the effect of operational variables (temperature, time, enzyme loading and MA/oil molar ratio) in the FAME yield as well as reuse of the catalyst and the effect of ultrasound.

2. Material and methods

2.1. Materials

Crambe oil donated by MS Foundation (cultivate Bright FMS), methyl acetate (Sigma Adrich, 99% purity) and lipase Novozym® 435 were used in the experiments. In chromatographic analyzes were used standard chromatographic of methyl heptadecanoate (Sigma Adrich, >99% purity) and heptane as solvent (Anidrol). For the determination of enzyme activity and oil characterization were used: *n*-hexane (Panreac), lauric acid (Vetec), *n*-propyl alcohol (Panreac), acetone (Vetec), ethanol (Anidrol), sodium hydroxide (Anidrol), ethyl ether (Anidrol), phenolphthalein (Nuclear), methanol (BT Baker) and derivatising BF_3-methanol (Sigma Adrich).

2.2. Characterization of crambe oil

The oil used in the reactions were characterized in terms of free fatty acids and fatty acid composition, using official methods recommended by American Oil Chemists' Society (1990): 940.28 and Ce 2–66, respectively. After derivatization, the fatty acids composition was determined using the method described by Santos et al. (2015). The water content was determined using Karl Fischer titrator (Orion, AF8).

2.3. Experimental procedure

The reactions were conducted in ultrasound bath with indirect contact (Unique Q 5.9/25 A), 165 W power and 25 kHz frequency, using round bottom flask, with a volume of 50 mL, as reactor positioned in the center of the ultrasonic bath. The reactor was connected to a condenser with water recirculation provided by thermostated bath (Marconi, model MA 184). The reactions were carried out using the enzyme Novozym® 435, based on the works of Xu et al. (2003), Huang and Yan (2008) and Lei and Li (2015), who report higher yields with this enzyme. Prior to use, the enzyme catalyst was maintained at 40 °C for 1 h in an oven with air circulation (Marconi, MA035) for its activation. At the same time, ultrasound bath was fired at 165 W and the reaction temperature, and the temperature being kept constant by means of a thermostated bath with water recirculation (Marconi, model MA 184). To check the effect of ultrasound in the process, reaction was performed on an orbital shaker (Marconi, MA 839/A) at 40 rpm. In each reaction, 1 g of oil was added to the flask together with methyl acetate (MA) and the enzyme, in amounts set for each experimental condition. After weighing the substrates and enzymes, the reactor was placed in ultrasonic bath and connected to the condenser coupled to thermostatic bath at 10 °C.

After the reaction time, removal of the enzymes was performed by filtration in quality paper with a diameter of 15 cm and weight of 80 g m^{-1}, and the excess solvent in the filtrate was evaporated via the evaporator route (Marconi, MA120) to constant weight and stored under refrigeration for conducting further analysis.

2.4. Determination of FAME yield

To quantify the FAME content, samples were prepared according to the procedure reported by Silva et al. (2010). The analysis of samples was conducted in a gas chromatograph coupled to mass spectrum (Agilent) equipped with a capillary column Agilent HP-5MS (30 m × 0.250 mm × 0.25 μm), using the following conditions: injection 0.4 of μL in split mode 1:10, initial temperature of the column 120 °C, maintained at this temperature for 5 min, increasing to 180 °C at a rate of 15 °C min^{-1} and for 240 °C at a rate of 5 °C min^{-1}, staying for five minutes. The flow of carrier gas, helium, was 1 mL min^{-1}. The temperature of ionization and quadrupole source were 230 and 150 °C, respectively. Compounds were quantified upon analysis using methyl heptadecanoate as internal standard and FAME yield was then calculated based on the content of methyl esters in the analyzed sample and on the reaction stoichiometry (Tan et al., 2011; Maddikeri et al., 2013).

2.5. Reuse of biocatalyst and enzymatic activity

After use, the enzymes were washed with 10 mL of methyl acetate (twice) and dried in oven at 40 °C for 1 h. Recuperated enzyme was then kept in desiccator for 24 h prior to measurement of its activity and reutilization (Michelin et al., 2015). To determine the enzymatic activity was used the method described by Oliveira et al. (2006), wherein it consists in quantifying the lauric acid consumption in the esterification reaction between lauric acid and *n*-propyl alcohol, at 60 °C with 5 wt% enzyme (in relation to the mass of substrate) kept under stirring for 40 min.

To evaluate the reuse of the enzyme were adopted experimental conditions of MA/oil molar ratio of 12, 60 °C, 30 wt% of enzyme (relative to mass of oil) and reaction time of 6 h. A total of five cycles were conducted and after each cycle the FAME yield and enzyme activity were evaluated.

2.6. Analysis of data

The analyzes were performed in duplicate and data were subjected to ANOVA using Excel® 2010 software and Tukey tests (using a 95% confidence interval), to evaluate differences among the media.