Hydroesterification of crambe oil (*Crambe abyssinica* H.) under pressurized conditions

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ABSTRACT

In this study the continuous production of esters from the hydroesterification (hydrolysis followed by esterification in two-step) of crambe oil was performed under pressurized conditions without a catalyst. For this purpose the effects of experimental variables were evaluated aiming to maximize the formation of free fatty acids (FFA) in the hydrolysis and the ester content in the esterification, keeping the pressure fixed at 15 MPa. Transesterification was also performed for comparative purposes. The results indicate that the combination of high temperature and the addition of a cosolvent (*n*-hexane), for a low oil to water mass ratio, favors the FFA formation. The kinetics reaction is fast and optimal conditions for the hydrolysis were identified as: temperature of 320 °C, water to oil mass ratio of 1:1, 75% of cosolvent and reaction time of 14 min. In the esterification step, increasing the temperature and molar ratio has a considerable effect and favors the FFA conversion and esters content for a residence time of $\leq$20 min, after which the thermodynamic equilibrium of the reaction was reached. The hydroesterification provided a higher esters content (~98%) compared to transesterification (~89%).

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1. Introduction

In the search for alternative raw materials the technological aspects of biodiesel production should be considered along with agronomic factors, such as the oil content, yield, production system and crop cycle (Rogério et al., 2013). Since the raw material represents around 75% of the total production cost, the selection of an oilseed with lower added value is vital to ensure a competitive cost for the biofuel (Atabani and César, 2014). According to the Brazilian National Petroleum Agency (ANP, 2015), most of the biodiesel produced in Brazil is obtained from soybeans and, since this is a crop mainly grown for human consumption, research on the exploitation of other oilseed crops with the potential to provide biodiesel has been identified.

Crambe oil (*Crambe abyssinica* Hochst) is an attractive option for biodiesel production because it is drought tolerant, has a cycle of approximately 90 days and the total seed yield is around 1000–1500 kg per hectare (Falasca et al., 2010; Viana et al., 2013). The oil content of crambe seed is 48–60% (Silva et al., 2013; Santos et al., 2015), and it has a high content of erucic acid (50–60%), which is toxic, causing heart disease through increased cholesterol levels and lipidosis in the heart tissues, and thus the oil is unsuitable for human consumption (No et al., 2013). Crambe oil can therefore only be used for industrial purposes such as biodiesel production.

Crambe oil is obtained by extraction through pressing and is not passed through a refining process. Thus, the free fatty acids (FFA) content is above that suitable for processing by the conventional biodiesel production method, which can be applied to raw materials containing up to 1% of FFA and 0.06% of water (Dermibas, 2009). For the processing of vegetable oils with high acidity, hydroesterification can be applied, since this process permits the use of low quality raw materials, which are completely converted into esters. Therefore, instead of decreasing the acidity of the feedstock through a refining process, in the hydrolysis step the acidity is increased.

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Subsequently, the hydrolyzate is esterified and fatty acid esters are obtained with water as a byproduct.

The processes generally employed in hydrolysis are catalytic, via chemical or enzymatic routes. The chemical process involves acid catalysis, generally conducted in the presence of sulfuric acid (Ngaosuwan et al., 2009), and alkaline catalysis using sodium and potassium hydroxide (Asai et al., 1997). The use of such catalysts requires long reaction times (20–48 h), besides causing oxidation and dehydration of the products obtained, resulting in a dark colored product, which requires purification to remove the color and excess catalyst (Castro et al., 2004). Enzymatic catalysis has recently been used in hydrolysis reactions. However, it presents several technical difficulties, including long reaction times, loss of enzyme activity with increasing temperature and contact with certain solvents, in addition to the high cost of the catalyst (Hama and Kondo, 2013).

The esterification of the hydrolyzate, which is rich in free fatty acids, is usually carried out via a catalytic route using homogeneous acid catalysts (Brinks et al., 2013). However, the use of this type of catalysts is associated with slower reaction rates and difficulties related to the separation and purification of the final product (Christopher et al., 2014). Enzymatic catalysis can also be applied in the esterification reaction, however, as mentioned above, the high cost of the enzymes and long reaction times are significant disadvantages. Moreover, studies have shown that the enzymes are easily deactivated by the alcohol used (Hama and Kondo, 2013).

To minimize the problems associated with the use of catalysts in reactions for biodiesel production, the recent literature reports the application of technology involving fluids under sub- or supercritical conditions, without the addition of a catalyst in the process (Minami and Saka, 2006; Silva and Oliveira, 2014). The properties of a fluid under these conditions are intermediate between a gas and a liquid and one of the advantages is that many of the physical properties, such as density, dielectric constant and solubility, can be easily adjusted through slight variations in the temperature and pressure (Wen et al., 2009).

Water in the subcritical state (10–20 MPa and 270–350 °C) has recently been used in hydrolysis reactions. Since the water temperature is high there is a reduction in the polarity, which is due to a decrease in the dielectric constant. In addition, the kinetic energy of water also increases, resulting in a greater distance between the molecules. In the subcritical region, water has properties (density and dielectric constant) similar to an organic solvent at room temperature. This allows an increase in the reaction rate without using a catalyst (Carr et al., 2011). Subcritical water is a technically feasible and environmentally acceptable alternative in comparison with water in the supercritical state, which is associated with serious hazards since it is toxic, flammable, explosive and corrosive (Shin et al., 2012; Kansedo and Lee, 2014).

In the esterification step, the alcohol used under pressurized conditions shows an autocatalytic mechanism due to dissociation of the free fatty acids in the reaction medium under these conditions (Akgün et al., 2010). Thus, catalysts are not used in the process, eliminating their cost and the need to remove them from the final product (Pinnarat and Savage, 2010). The use of elevated temperatures and pressures in the method leads to a higher reaction rate compared to the conventional alkaline method due to decreased mass transfer limitations resulting from the high miscibility between the substrates (Alenezi et al., 2010a; Abdala et al., 2014a).

In this context, the aim of this study was to evaluate the continuous production of esters from the hydroesterification of crambe oil under pressurized conditions. The effects of the experimental variables were investigated in the hydrolysis step in order to maximize the production of free fatty acids, and the residence time was also studied. In the esterification step the effects of the temperature, and

ethanol to FFA molar ratio and residence time on the FFA conversion and esters content were investigated. Transesterification was also performed for comparison purposes. The results of this study contribute to research on the production of crambe biodiesel and the use of hydroesterification under pressurized conditions, for which there are very few reports available in the literature.

2. Materials and methods

2.1. Materials

Crambe oil, donated by the MS Foundation, and water, from a Millipore (ZICW300UK) deionization system, were used as substrates in the hydrolysis reactions. Ethanol (JT Baker, 99.8%) was used in the esterification and transesterification reactions. n-Hexane (Anidrol) was used as a cosolvent in the hydrolysis and transesterification reactions. In the determination of the FFA content and composition and the glycerol and ethyl esters content as well as the qualitative analysis by thin layer chromatography the following reagents were used: sodium hydroxide (Anidrol, 97%), ethyl ether (Anidrol), ethanol (Anidrol, 95%), phenolphthalein (Nuclear), the derivatizing agent BF_3-methanol (Sigma-Aldrich), sodium periodate (Vetec), ethylene glycol (Vetec, 99.5%), sulfuric acid (Anidrol, 98%), bromothymol blue (Synth), heptane (Anidrol), n-hexane (Anidrol), acetic acid (Nuclear), silica-coated plates (Polygram Sil G), solid iodine (Synth, 99.8%), 3 Å molecular sieves (Sigma-Aldrich) and methyl heptadecanoate (Sigma-Aldrich, 99.9%).

2.2. Oil characterization

The crambe oil used was characterized in terms of the free fatty acids content, fatty acids composition and water content, using the official methods recommended by the AOCS (1990): Ca 5a-40, Ce 2-66 and 984.20, respectively. After derivatization, the fatty acids composition was determined using the method described by Trentini et al. (2016).

2.3. Experimental procedure

The reactions were carried out in a tubular reactor with a packed bed, the specifications for which are given in detail by Abdala et al. (2014b). The reactor had a void volume of 60.7 mL and was made of stainless steel tubing (6 m, 316 L 1/4 in outer diameter (OD) and inner diameter (ID) 3.2 mm HIP) and stainless steel tubing (0.15m, 304 L 30.5 mm OD and ID 13 mm HIP). Fig. 1 shows the schematic diagram of the experimental apparatus used. The substrates were kept under constant stirring using a mechanical stirrer (IKA® RW 20 D) and pumped through an isocratic pump (Waters, 515 HPLC) at a given volumetric flow rate until the reactor was filled. The reaction temperature was monitored using three thermocouples (Salvi Casagrande) coupled to the reactor and the system pressure was controlled by a pressure control valve (Swagelok) and monitored by a pressure indicator (Record). The residence time was computed by dividing the void volume of the reactor (mL) by the flow rate of the substrates (mL min^{-1}). After passing through the reactor the reaction mixture was sent to a cooling system maintained at 10 °C by a thermostatic bath with circulation (Tecnal, TE-184).

At the end of the hydrolysis reaction, samples were placed in a centrifuge (Quimis, Q222E) and submitted to centrifugation for 15 min at 3500 rpm, to remove the glycerol. The oil phase was again transferred to a sample bottle and left in an oven with air circulation (Marconi, MA035) at 80 °C until constant weight. For the samples taken during the esterification and transesterification steps and at the end of the reaction, unreacted ethanol was removed in an oven with circulation (Marconi, MA035). Next, 2 mL
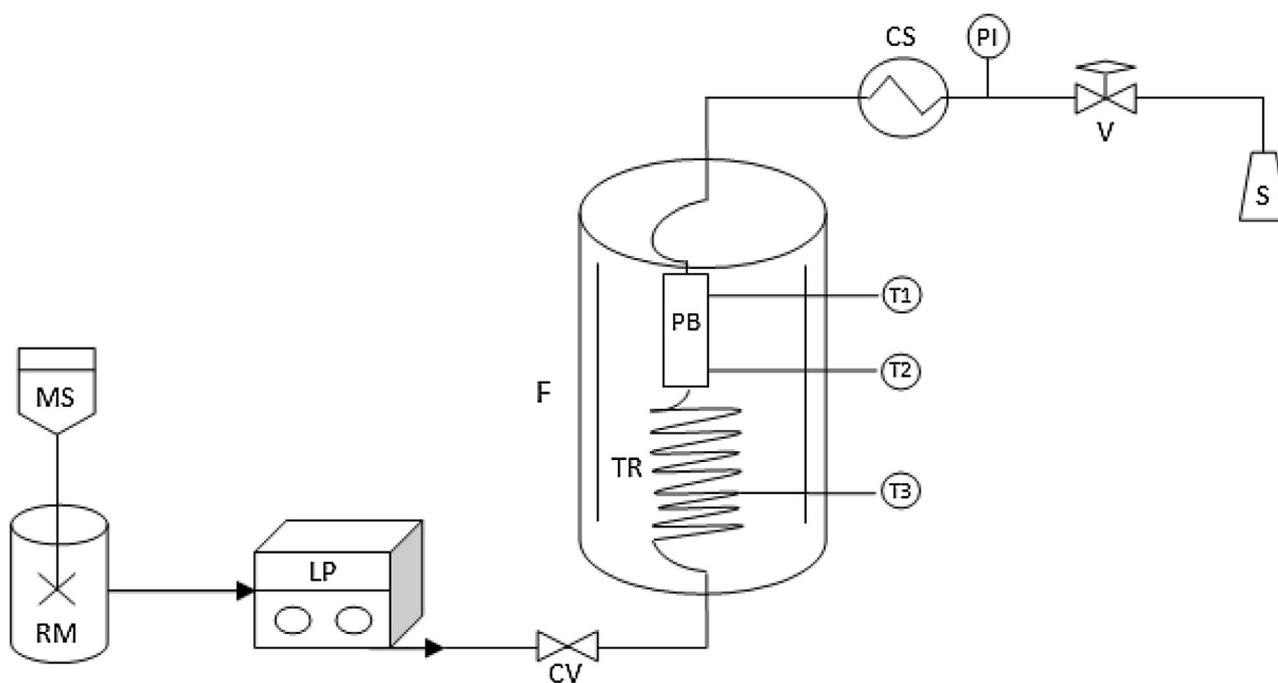
Fig. 1. Schematic diagram of the experimental apparatus: (RM) reaction mixture, (MS) mechanical stirring device, (LP) high pressure liquid pump, (F) furnace, (TR) tubular reactor, (PB) packed bed, (T1, T2 and T3) temperature indicators, (CS) cooling system, (CV) check-valve, (V) pressure control valve, (PI) pressure indicator, (S) sampling.

Table 1
Factors and levels for the Box-Behnken design for the hydrolysis of crambe oil with subcritical water.

Factors	Levels		
	Low (-1)	Central (0)	High (1)
Temperature (°C) − T	275	290	305
Water to Oil Mass Ratio − R	1:1	2:1	3:1
Cosolvent (wt%) − CS	25	50	75

of n-hexane and 2 mL of distilled water were added to the samples which were transferred to centrifuge tubes and subjected to centrifugation (3500 rpm for 15 min). The supernatant was transferred to sample bottles and again left in the oven until constant weight.

To determine the optimum levels for the operating variables for the hydrolysis reaction, in order to maximize the FFA content, a Box-Behnken design was generated using the software Statistica® 8.0. Analysis of variance (ANOVA) was used to evaluate the effect of the independent variables on the responses, using a 95% confidence interval, and experimental data were fitted to a second-order polynomial model.

In the experimental runs, the pressure was kept fixed at 15 MPa and the time at 14 min. Hexane was used as the cosolvent and its concentration was calculated based on the mass of oil used. The levels used for the independent variables (temperature, water to oil mass ratio and cosolvent concentration), along with the real and coded data, are shown in Table 1. The effect of the residence time was evaluated under the optimum conditions of the experimental design.

The reactions with the crambe oil hydrolyzate were conducted keeping the pressure fixed at 15 MPa and the reaction conditions were selected based on the work of Abdala et al. (2014a). The application of temperatures of 275 and 300 °C, residence times of 10, 20 and 30 min, and ethanol to FFA molar ratios of 6–15 was evaluated.

The transesterification reactions were conducted according to the conditions reported by Silva et al. (2014). The pressure was fixed at 15 MPa, oil to ethanol mass ratio at 1:1 and n-hexane as the

cosolvent at 20% (relative to oil mass), while temperatures of 300 and 325 °C and different residence times were applied.

Data obtained for esterification and transesterification reactions were subjected to ANOVA using Excel® 2010 software and Tukey tests (95% confidence interval) to evaluate differences between the media.

2.4. Analytical methods

The method Ca 5a-40 (AOCS, 1990), based on acid-base titration, was used to determine the FFA content of the hydrolysis and esterification reactions according to Eq. (1):

$$FFA(\%) = \frac{C \times MM \times v}{(10 \times m)} \tag{1}$$

where C is the concentration of sodium hydroxide (mol L^{-1}) used as titrant, MM corresponds to the molar mass of the predominant fatty acids in the sample, v is the volume required for the titration (ml) and m is the mass of sample (g).

The conversion of the esterification reaction was determined according to Eq. (2):

$$Conversion(\%) = \frac{FFA_i\text{-}FFA_f}{FFA_i} \times 100 \tag{2}$$

where FFA_i is the initial FFA content in the hydrolyzate and FFA_f is the FFA content in the final sample of the reaction medium.

The thin layer chromatography technique was used for the qualitative determination of the classes of lipids present in the hydrolyzate (Achmanian et al., 2002). The glycerol content was determined by titration, using the sodium periodate method described by Cocks and Van Rede (1996).

The samples obtained from the esterification and transesterification were analyzed by gas chromatography with mass spectrometry to determine the ethyl esters content. Samples were prepared (after evaporating the unreacted ethanol) by placing ~300 mg of sample in sample bottles and then adding 150 mg of 3 Å molecular sieves, previously dried in a muffle furnace at 270 °C. In the next step, 150 mg of the sample from the sample
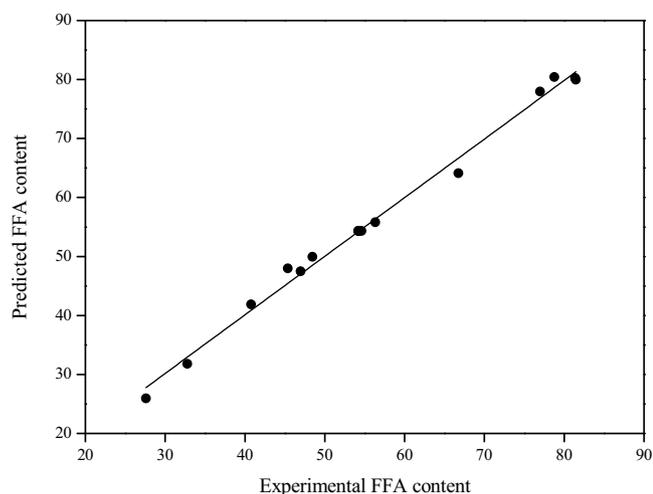
Fig. 2. Correlation between the experimental and predicted FFA contents (according to second-order polynomial model – Eq. (4)) for hydrolysis of crambe oil in subcritical water.

Table 2
Fatty acids composition of crambe oil.

Fatty acid	Content (%)
Palmitic (C16:0)	2.24 ± 0.05
Palmitoleic (C16:1)	0.13 ± 0.01
Stearic (C18:0)	1.14 ± 0.04
Oleic (C18:1)	20.58 ± 0.63
Linoleic (C18:3)	4.71 ± 0.05
Linolenic (C18:3)	1.07 ± 0.10
Arachidonic (C20:0)	1.17 ± 0.02
Gadoleic (C20:1)	4.21 ± 0.02
Eicosadienoic (C20:2)	0.74 ± 0.01
Behenic (C22:0)	2.20 ± 0.01
Erucic (C22:1)	61.82 ± 0.76

Table 3
Matrix of experimental design (coded values) with responses in terms of FFA content (residence time fixed at 14 min). .

Run	T[a]	R[a]	CS[a]	FFA (%)
1	−1	−1	0	32.79
2	1	−1	0	81.36
3	−1	1	0	40.76
4	1	1	0	76.97
5	−1	0	−1	48.45
6	1	0	−1	78.78
7	−1	0	1	27.59
8	1	0	1	81.47
9	0	−1	−1	56.33
10	0	1	−1	66.74
11	0	−1	1	45.37
12	0	1	1	46.98
13	0	0	0	54.22
14	0	0	0	54.22
15	0	0	0	54.60
16	0	0	0	54.24

[a] See Table 1.

Table 4
ANOVA for the factors evaluated in the subcritical hydrolysis of crambe oil.

	Sum of squares	Degrees of freedom	Mean square	F	p[a]
T (L)	3569.70	1	3569.70	102186.1	<0.001
T (Q)	78.63	1	78.63	2250.9	<0.001
R (L)	30.42	1	30.42	870.8	<0.001
R (Q)	2.46	1	2.46	70.3	0.003
CS (L)	298.78	1	298.78	8552.8	<0.01
CS (Q)	0.41	1	0.41	11.6	0.04
T*R	38.19	1	38.19	1093.3	<0.01
T*CS	138.65	1	138.65	3969.0	<0.01
R*CS	19.36	1	19.36	554.2	<0.01
Lack of fit	28.63	3	9.54	273.2	<0.01
Pure Error	0.10	3	0.03		
Total	4205.34	15			

[a] Statistical significance $p < 0.05$; L — linear effect and Q — quadratic effect.

3. Results and discussion

3.1. Oil characterization

Table 2 shows the composition of fatty acids present in the crambe oil. The FFA and water content were determined as $6.11 \pm 0.34\%$ and $0.121 \pm 0.01\%$, respectively.

The predominant fatty acid found in crambe oil was erucic acid (61.82%), followed by oleic acids (20.58%) and linoleic and linolenic acids (4.71 and 4.21%, respectively). These fatty acids are classified as unsaturated and they represent ∼92% of the total composition of crambe oil. The concentrations obtained in this study are consistent with values found in the work of Santos et al. (2015), who reported ∼94% of unsaturated fatty acids and 59.4 and 20.17% of erucic and oleic acids, respectively.
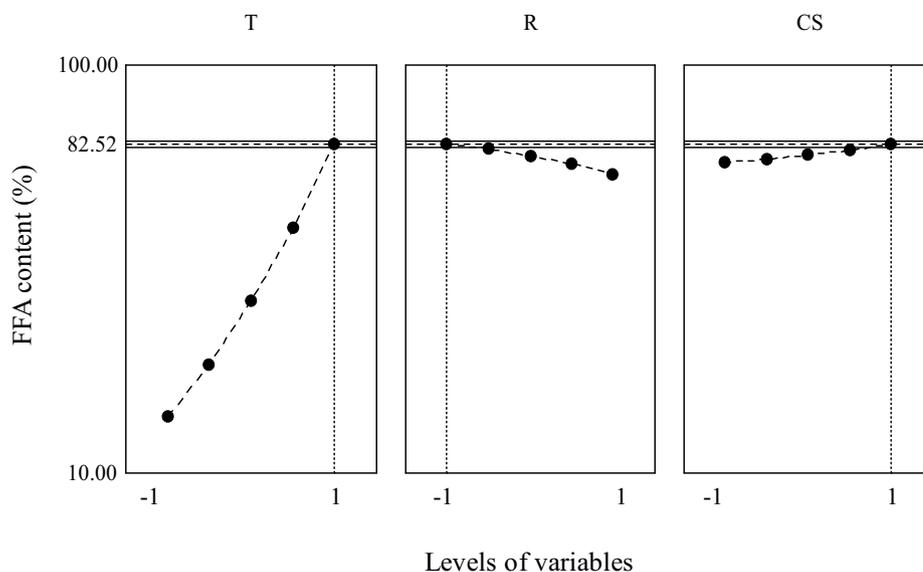
3.2. Hydrolysis of Crambe oil

3.2.1. Effect of operational variables

Table 3 shows the experimental conditions for the Box-Behnken design, as well as the results for the FFA contents obtained under the conditions evaluated.

The fitting of the model to the experimental data for the free fatty acids content according to the independent variables is shown in Eq. (4).

$$FFA(\%) = 56.96 + 21.12T + 1.95R - 6.11CS - 2.21T^2 + 0.39R^2 - 0.16CS^2$$
$$- 3.09T * R + 5.88T * CS - 2.20R * CS \quad (4)$$

The analysis of variance (ANOVA) of the regression model fitted to the experimental values is shown in Table 4 and demonstrates

bottle was transferred to a 5 mL volumetric flask and the volume was completed with heptane. An aliquot of this solution was then transferred to a 1 mL volumetric flask in order to obtain a concentration of $1000 \, mg \, L^{-1}$ and then added to the internal standard (methyl heptadecanoate) at a concentration of $150 \, mg \, L^{-1}$. This solution was injected, in duplicate, into a gas chromatograph (Agilent 7890 B) equipped with an Agilent HP-5MS capillary column ($30 \, m \times 0.250 \, mm \times 0.25 \, \mu m$) and the chromatographic conditions were those described by Visioli et al. (2016). The carrier gas (helium) flow was set at $1 \, mL \, min^{-1}$. The temperatures of the transfer line, ion source and quadrupole were 250, 230 and 150 °C, respectively. The compounds were identified by comparison of their mass spectra with the NIST 11.0 library spectra and quantified upon analysis using methyl heptadecanoate as internal standard. Eq. (3) was used to calculate the ethyl esters content:

$$\text{Esters content} \; (\%) = \left(\frac{\sum A\text{-}AP}{AP} \frac{CP}{CA} \right) \times 100 \quad (3)$$

where ΣA is the sum of the areas of the peaks corresponding to the esters and the internal standard, AP is the area for the internal standard, CP is the concentration of the internal standard and CA is the concentration of the injected sample.

Fig. 3. Optimization of operational variables, obtained from Eq. (4), in subcritical hydrolysis of crambe oil: T – temperature; R: water to oil mass ratio; CS: cosolvent.

that the model results were statistically significant ($p < 0.05$) for the linear and quadratic effects of each variable and the effects of interactions between them. From the ANOVA it was observed that the proposed mathematical model (Eq. (4)) was valid for the representation of the experimental data (F_{calc} (83.97) > F_{tab} (4.10) and coefficient of determination of 0.99). Fig. 2 shows the strong correlation between the experimental data and the values predicted by the model.

The temperature was found to be of greater significance than the other variables, and an increase in temperature favors the formation of FFA. It can be observed for experiments 1 and 2 in Table 3 that on increasing the temperature from 275 to 305 °C, while keeping the mass ratio and percentage of co-solvent fixed, the FFA content obtained was considerably higher, and a difference of ~48% can be observed from the above experiments.

An increase in temperature allows a better solubility of the triglycerides and fatty acids in subcritical water and it increases the rate of the hydrolysis reaction, which can be completed in a relatively short time (Satyarthi et al., 2011; Shin et al., 2012). These effects are also related to the carboxylic group of the fatty acid molecules which act both as a donor and a receiver hydrogen at elevated temperatures, contributing to improved dissolution in water (Huang et al., 2013). Ravber et al. (2015) showed that with an increase in temperature there is a decrease in the polarity and viscosity of water, resulting in better contact between the substrates in the reaction medium. The effects of increased temperature on the hydrolysis of vegetable oils and lipid raw materials have been studied in detail by several researchers. Baig et al. (2013) reported FFA contents of ~20% and 70% at 270 and 310 °C, respectively, for the hydrolysis of sunflower oil at 20 MPa for 25 min. Ilham and Saka (2010) demonstrated the effect of temperature in the subcritical hydrolysis of *Jatropha* oil, observing FFA contents of 80% at 225 °C and ~99% at 300 °C, using a pressure of 29 MPa for 12 min.

An increase in the cosolvent concentration in the reaction medium decreases the formation of FFA. Klein et al. (1992) explained that the addition of solvent increases the reaction rate in the first minutes, when the density of water is still relatively high. However, once water diffusion between the liquid and gaseous state begins, the effect of the solvent is not observed. Ngaosuwan et al. (2010) noted that when *n*-hexane is added to the hydrolysis reaction the induction period becomes relatively low. However, the solubility improves in the initial minutes, increasing the formation

of free fatty acids, which act as emulsifiers, inhibiting the reactivity of the solvent.

For the interaction between the variables temperature and addition of cosolvent, it was noted that a simultaneous increase in the respective variables favors the obtainment of higher levels of FFA. It is well known that increasing the temperature causes the dielectric constant of water to decrease until it reaches a value close to 0.1 (Pollet et al., 2014). This also occurs when a solvent is subjected to pressurized conditions under which its dielectric constant is reduced and hence the miscibility between the substrates increases (Townsend et al., 1987).

The variable water to oil mass ratio showed a relatively weak influence ($p < 0.05$) on the response compared with the other variables, but the addition of water to the reaction medium increased the FFA content. When larger amounts of water are used the equilibrium of the hydrolysis reaction was shifted towards the products, with an increase in glycerol formation (Pinto and Lanças, 2006). The glycerol produced in the oil phase of the reaction is then diffused in the aqueous phase due to its hydrophilic properties. Thus, the free fatty acids remain in the oil phase and the diffusion of water to the reaction phase favors the formation of FFA (Kansedo and Lee, 2014; Micic et al., 2015).

Kusdiana and Saka (2004) studied the effect of the water to oil mass ratio (0.2:1.0 to 3.8:1.0) on rapeseed oil hydrolysis and the best FFA yields were achieved at a ratio of 0.9:1 (at 270 °C with a reaction time of 20 min). The authors observed no significant effect on increasing of the amount of water in the reaction, where a thermodynamic equilibrium can be noted for mass ratio of 0.9:1 (water to oil). Kansedo and Lee (2014) observed, in the non-catalytic hydrolysis of *Cerbera odollam* oil, that increasing the water to oil mass ratio from 0.7:1.0 to 1.9:1.0 favored the production of FFA, providing an increase from ~70 to ~82%.

The use of high temperatures and quantities of water in the reaction medium did not favor the reaction ($p < 0.05$). One possible reason for this result, as proposed by Ryu et al. (2014), is that regardless of the variations in the proportions of water to oil, the temperature favors the formation of FFA until equilibrium is reached.

The use of a high water to oil mass ratio and percentage of cosolvent led to a decrease in the formation of FFA, this effect being possibly caused by the high dilution of the reaction medium, since
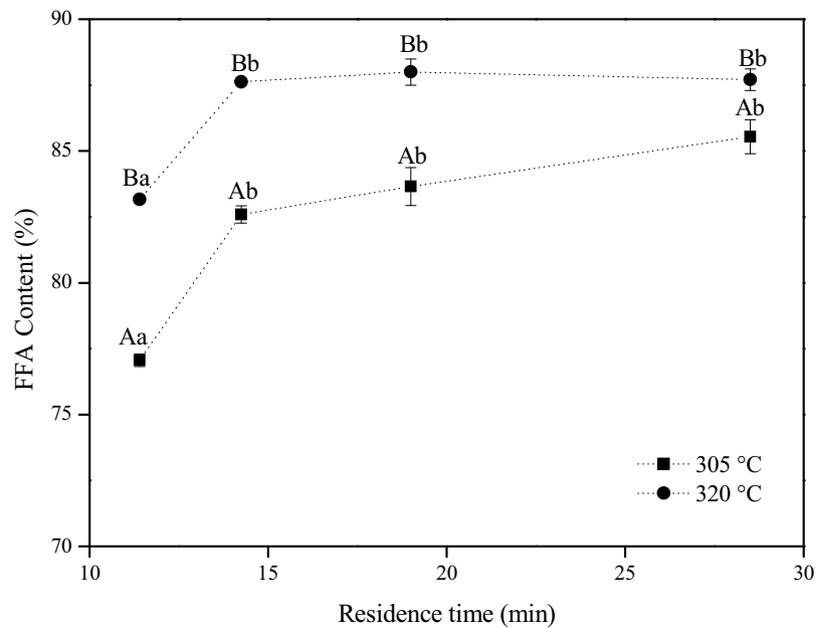
Fig. 4. FFA content as a function of residence time for subcritical hydrolysis of crambe oil. Means followed by same lowercase letters (for the same temperature) and uppercase letters (for the same residence time) did not differ statistically ($p > 0.05$).
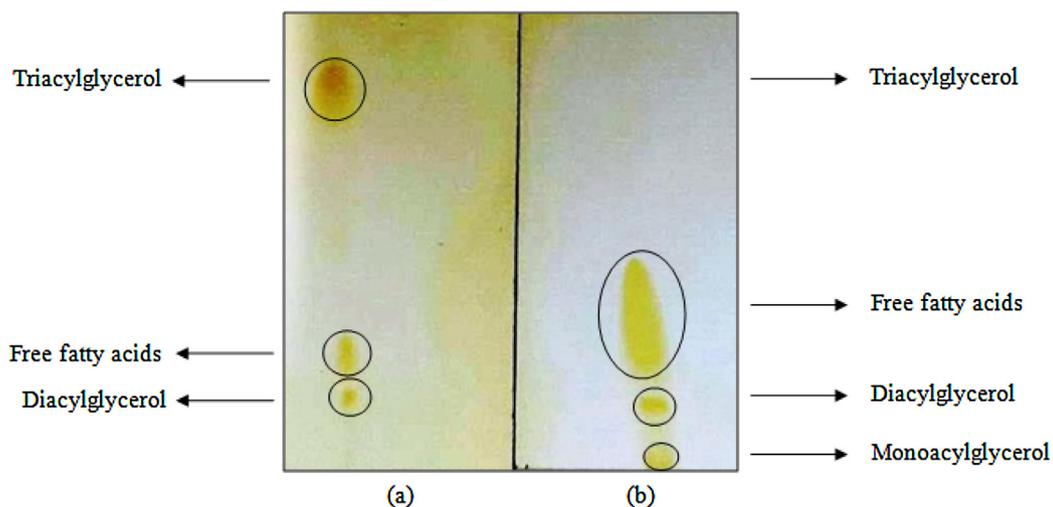
Fig. 5. Identification of lipid classes by thin layer chromatography: (a) crambe oil and (b) crambe oil hydrolyzate.

the FFA formation rate is dependent on the triacylglyceride concentration in the reaction medium.

3.2.2. Optimization

From the predictive equation, Eq. (4), the optimal values for the experimental variables, i.e., those which provide the maximum FFA content, were obtained for the experimental range evaluated and shown in Fig. 3. As shown in this figure, the maximum FFA content was 82.52% under the following conditions: temperature of 305 °C, water to oil mass ratio of 1:1 and the addition of 75% of cosolvent to the reaction medium. To confirm the predictive ability of the equation, experiments were performed in triplicate under the optimum conditions and $82.47 \pm 0.4\%$ of FFA was obtained. The Student test was applied, with a 95% confidence level, and it was observed that the content obtained experimentally did not differ significantly from that predicted by the model.

3.2.3. Effect of residence time

Fig. 4 reports the results for the effect of the residence time on the crambe oil hydrolysis reaction in subcritical water, which was determined considering the optimum values obtained in the experimental design (75% of cosolvent, and oil to water mass ratio of 1:1 at temperatures of 305 and 320 °C).

As shown in Fig. 4, a significant increase ($p < 0.05$) in the FFA content can be observed when the reaction time increased from 11.5 to 14 min, for both temperatures tested, and the use of longer residence times had no significant effect ($p > 0.05$), possibly because the reaction had reached equilibrium. The FFA content gradually increased with increasing temperature from 305 to 320 °C for all time intervals assessed, and ~88% of FFA was obtained at 320 °C with a reaction time of 14 min.

King et al. (1999) reported increased soybean oil miscibility in water on increasing the temperature from 300 to 325 °C at ~13 MPa and consequently a higher FFA content was obtained. The effect of temperature on the hydrolysis of rapeseed oil was studied by

Minami and Saka (2006), who reported FFA yields of 30 and ~90% for 270 and 300 °C respectively, with a reaction time of 20 min. The use of longer residence times did not affect the yield for the reactions conducted at 300 and 320 °C.

The best FFA content reported herein (~88%) was compared with the results of other studies available in the literature. In the hydrolysis of soybean oil in subcritical water, Milliren et al. (2013) obtained ~80% of FFA using a batch reactor, 30 min of reaction, 300 °C, 3.3 MPa and an oil to water mass ratio of 1:4. On evaluating the continuous hydrolysis of canola oil in subcritical water, Wang et al. (2012) achieved ~76% of FFA at 260 °C at a pressure of 5.5 MPa with a relatively long residence time (210 min). These results were lower than the one obtained in the present study due mainly to the low pressure used, in addition the authors report the use of larger reaction times. Alenezi et al. (2010b) studied the hydrolysis of sunflower oil with subcritical water under continuous flow and obtained ~90% of FFA at 300 °C, with ~15 min of reaction and a water to oil ratio of 1:1 (by volume) at 20 MPa, result very close to that obtained in this study due to the similarity of the operational conditions applied.

3.2.4. Hydrolyzate characterization

The crambe oil hydrolyzate collected under optimum conditions showed a free fatty acids content of $88.42 \pm 0.4\%$, water content of $0.865 \pm 0.07\%$, and glycerol content of $0.22 \pm 0.001\%$. This product was obtained considering the results of the experimental design for the evaluation of the effect of residence time, which were 320 °C, oil to water mass ratio of 1:1, 75% of cosolvent, 15 MPa and 14 min of reaction. The hydrolyzate and crambe oil were subjected to qualitative analysis by thin layer chromatography to determine the lipid classes, and the results can be seen in Fig. 5.

As seen in Fig. 5, column (a), in the crambe oil the triglycerides were present in higher quantity. Diglycerides were also present in the oil, but in lesser amounts. Since crambe oil has a low acidity, a lower intensity is observed for the FFA. In column (b), the presence of small quantities of monoacylglicerides and diacylglicerides in the hydrolyzate can be verified as well as greater intensity for the free fatty acids. No triglycerides were observed in the sample due to their conversion into fatty acids, which also indicates that the hydrolysis reaction efficiency was retained in the presence of subcritical water.

3.3. Reaction carried out with Crambe oil hydrolyzate

The crambe oil hydrolyzate was reacted with ethanol in the same experimental apparatus used in subcritical hydrolysis. Fig. 6 shows the results for the conversion of the FFA in the crambe oil hydrolyzate applying different temperatures and ethanol/FFA molar ratios as a function of the residence times. Based on the data given in this figure, it appears that for the reactions conducted at 275 °C an increase in the ethanol/FFA molar ratio from 6 to 12 favored the conversion at 10 min ($p < 0.05$), with an increase in the conversion from 88.68 to 95.39%. For 20 and 30 min of residence time, only an increase in the ratio from 6 to 9 influenced the reaction. For all reaction times evaluated the use of a molar ratio of 15 did not result in a significant increase in the FFA conversion ($p > 0.05$). At 300 °C, the use of a molar ratio of ≥ 9 had no influence on the conversion ($p > 0.05$) for all residence times evaluated.

In the non-catalytic reactions, an increased alcohol content in the reaction medium should provide greater contact between the substrates, improving the conversion reaction (Abdala et al., 2014a). An increase in the proportion of ethanol causes the reaction equilibrium to shift towards the formation of products and the conversion of the free fatty acids into esters increases proportionally (Jin et al., 2015) until reaching equilibrium.

Pinnarat and Savage (2010) investigated the esterification of oleic acid in pressurized ethanol and found that an increase in the molar ratio (ethanol/oleic acid) of 7–10 provided an increase in the conversion from 70 to ~80%. Similarly, Abdala et al. (2014a) reported that increasing the ethanol/oleic acid molar ratio from 6 to 9 resulted in better FFA conversions which increased from ~87% to ~92%.

Regarding the temperature, the greatest effects of this variable on the FFA conversion were observed at ethanol/FFA molar ratios of 6 and 9, where it appears that an increase in the system temperature results in a faster reaction rate. Accordingly, the time required for the reaction to approach equilibrium decreases as the temperature increases. For ratios of 12 and 15, the temperature has no effect on the formation of free fatty acids and the equilibrium of the reaction was observed for all residence times considered.

In general, the data in Fig. 6 demonstrate that high conversion rates can be obtained within a short reaction time, indicating fast reaction kinetics. This has been observed in other studies on FFA esterification with an alcohol under pressurized conditions. Alenezi et al. (2010a) reported a fast reaction rate at temperatures of ≥ 300 °C. These authors observed that the conversion rapidly approached equilibrium within 10 min of reaction for experiments conducted at 270 and 290 °C. Similarly, Visioli et al. (2016) observed equilibrium of the esterification reaction of a soybean oil deodorizer distillate under pressurized ethanol within only 10 min of residence time for reactions carried out at 275–300 °C using a molar ratio (ethanol/FFA) of 7.

Taking into consideration the fact that molar ratios of 12 and 15 (ethanol/FFA) achieved equilibrium within all residence times assessed, the ethyl esters content in the samples was determined by gas chromatography, and are reported in Fig. 7. An increase in the temperature and molar ratio (ethanol/FFA) favored the production of esters ($p < 0.05$), especially in the initial phase of the reaction (10 min). For a molar ratio (ethanol/FFA) of 12, 81% of esters was obtained at 275 °C and 87% at 300 °C. At 300 °C, the esters content obtained increased from 87% to 97% with an increase in the molar ratio (ethanol/FFA) from 12 to 15. For the other residence times evaluated (20 and 30 min), changes in the other variables had little influence on the esters content obtained. For 12 and 20 min of reaction, the residence time has a greater influence than the molar ratio, after the equilibrium has been reached.

Warabi et al. (2004) explained that fatty acids are more soluble in alcohol than the triglycerides and faster reaction kinetics can
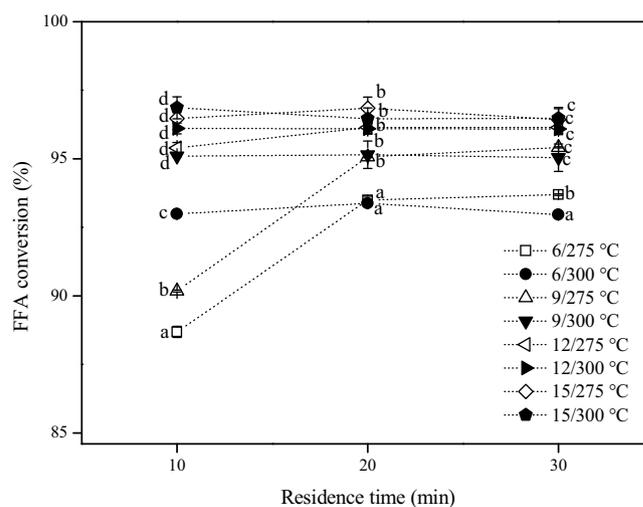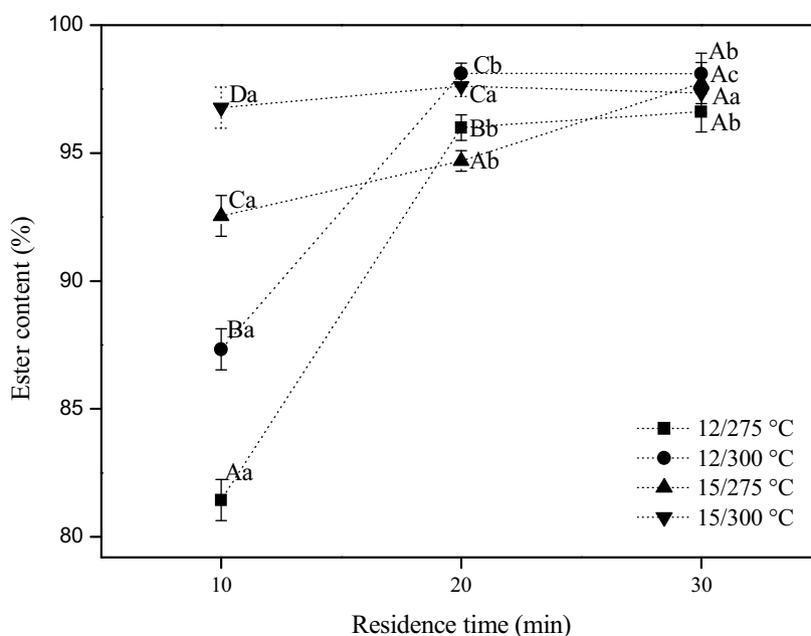

Fig. 6. Effect of molar ratio (ethanol/FFA) and temperature on the FFA conversion of crambe oil hydrolyzate at 15 MPa. Means followed by same letters (for the same residence time) did not differ statistically ($p > 0.05$).
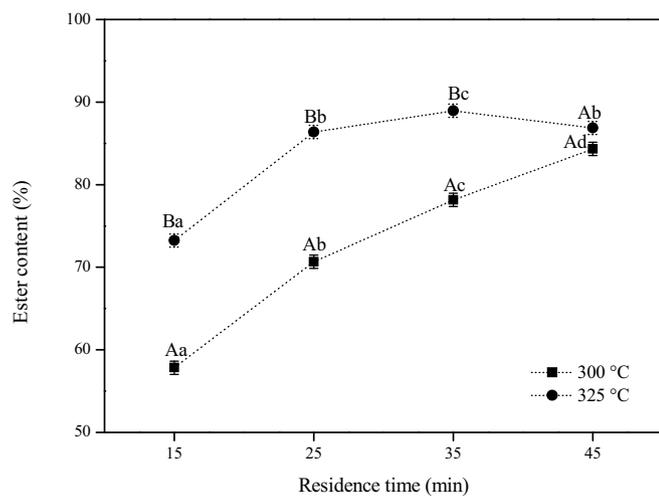
Fig. 7. Effect of molar ratio (ethanol/FFA) and temperature on the esters content obtained for crambe oil hydrolyzate at 15 MPa. Means followed by same lowercase letters (under the same experimental conditions) and uppercase letters (for the same time) did not differ statistically (p > 0.05).

be observed (Silva et al., 2014). In addition, the activation energy of esterification is low compared to that of transesterification, as reported by Ong et al. (2013). Thus, in this study, samples with ~98% of esters were obtained in reactions conducted at 300 °C, with a molar ratio (ethanol/FFA) of 12 and residence time of 20 min.

3.4. Transesterification of crambe oil

Fig. 8 shows the results obtained for the transesterification of crambe oil. It can be seen from the data in Fig. 8 that the esters content is favored (p < 0.05) at higher temperatures and the esters content increased from 80% at 300 °C to 88.9% at 325 °C with a residence time of 35 min.

On evaluating the results obtained at different residence times (Fig. 8) it was observed that at 300 °C the esters content increased significantly (p<0.05) with the residence time for the times eval-

uated (15–45 min). At 325 °C, a similar effect was observed for residence times of 15–35 min, however, a decrease (p>0.05) was observed at 45 min. This effect was probably due to the thermal decomposition of the esters produced during the reaction, which is more severe at temperatures above 300 °C and for long residence times (Silva and Oliveira, 2014; Ortiz-Martínez et al., 2016).

Fatty acid decomposition was identified by several authors using different raw materials. For the non-catalytic supercritical transesterification of used frying oil, Abdala et al. (2014b) reported ~85% of ethyl esters and ~12% of fatty acid decomposition at 300 °C, with 20 MPa and a residence time of 70 min. Silva et al. (2014) obtained ~90% of esters and ~7% of fatty acids decomposition at 325 °C, with 20 MPa and a residence time of 52 min for the transesterification of *Jatropha curcas* L. with supercritical ethanol. Ortiz-Martínez et al. (2016) reported obtaining ~80% of esters in the transesterification reaction of *Pongamia pinnata* with supercritical methanol at 325 °C and 35 MPa with a residence time of 60 min, and under these conditions the authors obtained ~10% decomposition of fatty acids.

In the transesterification of crambe oil it was possible to obtain ~89% of esters at 325 °C with a reaction time of 35 min. For the hydroesterification, high amounts of ethyl esters (~98%) were obtained at 320 and 300 °C for the first and second step, respectively, with 34 min of residence time (sum of the combined steps applied). Both processes were conducted at 15 MPa.

The main advantage of hydroesterification is that a high esters content can be achieved, since the hydrolysis step provides a hydrolyzate rich in FFA, allowing the rapid production of esters. Furthermore, in this step a decrease in the esters content with increasing residence time is not observed, since the removal of glycerol, after the hydrolysis step, prevents the consumption of esters and ethanol in side reactions (Minami and Saka, 2006; Aimaretti et al., 2009; Lee et al., 2012).

Aimaretti et al. (2009) reported that glycerol alcoholysis consumes the alcohol in the reaction medium and the glycerol is transformed into products of smaller molecular size along with water. Lee et al. (2012) observed the reaction of glycerol with ethanol at 270 °C and 10 MPa, and identified oxygenated compounds and ethers as products of this reaction

Fig. 8. Transesterification of crambe oil in ethanol at 15 MPa, oil to ethanol mass ratio of 1:1 and 20% of cosolvent. Means followed by same lowercase letters (for the same temperature) and uppercase letters (for the same time) did not differ statistically (p > 0.05).

4. Conclusions

In this study, the hydroesterification of crambe oil under pressurized conditions was investigated. In the hydrolysis step the use of a high temperature and a solvent in the reaction medium favored the production of FFA at a low water to oil mass ratio. Water in the subcritical state is an effective alternative for the hydrolysis of crambe oil, since high levels of free fatty acids can be obtained without the use of catalysts in the reaction and within relatively short residence times. A reaction temperature of 320 °C, mass ratio (water:oil) of 1:1, 75% of the cosolvent (*n*-hexane) and a residence time of 14 min were identified as the optimal conditions, providing 82.52% of FFA content. In the esterification step, the kinetics showed that the reaction rate is fast and the thermodynamic equilibrium was rapidly reached. Thus, the effect of temperature and the ethanol/FFA molar ratio can be observed only for short residence times, where it was found that increases in these variables favor the FFA conversion and ester formation. In the evaluation of transesterification, increased temperature and residence time favored the obtainment of esters, however, a decrease in the esters content was observed at high temperatures and with long exposure times. A comparison between the transesterification and hydroesterification reactions indicated better results in the latter case, demonstrating that the process carried out in two steps can be considered an efficient method for the production of biodiesel under pressurized conditions. This method should be explored and developed on a pilot scale in order to address the demand for biofuels in the Brazilian energy scenario. This research provides an important contribution to the literature due to the lack of reports on the use of hydroesterification under pressurized conditions to obtain esters.

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