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Evaluation of the composition of *Carica papaya* L. seed oil extracted with supercritical CO_2

Pedro T.W. Barroso[a], Pedro P. de Carvalho[b], Thiago B. Rocha[b], Fernando L.P. Pessoa[a], Debora A. Azevedo[c], Marisa F. Mendes[b,*]

[a] Universidade Federal do Rio de Janeiro, Escola de Química, Chemical Engineering Department, Brazil
[b] Chemical Engineering Department, Technology Institute, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro 23897-000, Brazil
[c] Universidade Federal do Rio de Janeiro, Instituto de Química, Ilha do Fundão, Rio de Janeiro, RJ 21941-909, Brazil

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ABSTRACT

Among the most important tropical fruit grown in the world today and in Brazil, papaya occupies a prominent place. Native to tropical America, papaya has spread to several regions of the world, and Brazil accounts for 12.74% of the world production, followed by Mexico, Nigeria and India. The culture reached a harvested area of 441,042 ha and production of 12,420,585 t worldwide. The largest interest in this fruit relies on its main constituent compounds, like vitamins A, B and C, alkaloids (carpaine and pseudocarpaine), proteolytic enzymes (papain and quimiopapain) and benzyl isothiocyanate, more known as BITC, which has anthelmintic activity. Because of that, the present work has as objective the evaluation of the efficiency and composition of the oil extracted from *Carica papaya* L. seeds with supercritical carbon dioxide. The experiments were performed in a unit containing mainly a high-pressure pump and a stainless steel extractor with 42 mL of volume. The sampling was performed at each 20 min until the saturation of the process. About 6.5 g of sample were fed for each experiment done at 40, 60 and 80 °C under the pressures of 100, 150 and 200 bar. Samples of the *Carica papaya* L. fruit were acquired in a popular market and free for personal use intended for the study. After collection, the seeds were crushed with the help of a pestle, and dried at 60 °C for 60 min. For each operational condition, the extraction curves were constructed relating cumulative mass of oil extracted in function of the operational time. The better efficiencies were found at 40 °C and 200 bar (1.33%) followed by 80 °C and 200 bar (2.56%). Gas chromatography and NMR analysis could identify an insecticide component (BITC) that enables new applications of this residue in pharmaceutical and chemical industries.

1. Introduction

Among the most important tropical fruit grown in the world nowadays and in the country, papaya (*Carica papaya* L.) occupies a prominent place [1]. Native to tropical America, the papaya has spread to several regions of the world, and its largest producers are Brazil, Mexico, Nigeria and India. Brazil is responsible for 12.74% of world production. The world production has a harvested area of 441,042 ha and 12,420,585 tons worldwide in the year of 2013. Nowadays, Brazil is the second largest fruit producer, producing over 1,517,696 tons/year, only behind India [1,2].

The papaya tree is typically tropical, semi-herbaceous stem, hollow, cylindrical and simple. It grows fast and can reach 8 feet tall. This is a fleshy fruit, large, indehiscent, with pulp soft, dense, aromatic and has different colors that vary from yellow to red. The bark is smooth and thin, green when harvested and becomes gradually yellow or orange during ripening. The internal cavity of the fruit contains numerous black seeds, edible, spicy flavor, coated with a mucilaginous substance [3].

This fruit is preferably consumed fresh, while offering many products and by-products through industrialization. Its pulp has organoleptic properties (texture, color and aroma), chemical (total soluble solids, acidity and good balance of sugars and organic acids) and digestive, making it an ideal food for healthy people of all ages [4].

The fruit of *Carica papaya* L. (papaya), rich in vitamins A, B and C, has also as constituents carbohydrates, proteins, alkaloids (carpaine and pseudocarpaine), proteolytic enzymes (papain and quimiopapain), and benzyl isothiocyanate, more known as BITC, which has anthelmintic activity [5].

* Corresponding author.
E-mail addresses: marisamf@ufrrj.br, marisamendes@globo.com (M.F. Mendes).

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BITC is a bioactive substance present in the papaya seed that has been studied among different areas due to its wide applications. BITC applications ranged from vascular relaxation [6] to inhibition of cancer proliferation [7].

The anthelmintic activity of this compound is the most studied but the occurrence of this activity depends on how the seeds are correctly treated. The seeds do not naturally contain BITC. The BITC production occurs when the benzyl glucosinolate, present in the interior of the seeds, contacts the myrosinase enzyme, present in the seed's surface. The enzyme catalyses the BITC production [8], as it is shown in Fig. 1.

This finding is interesting because it demonstrates an extremely effective defense mechanism in evolution. The seed to be broken by an animal generates a substance that has toxic effects to some organisms. This data also reveals the importance of the act of breaking the seed hull before eating it [5].

Due to the large production and consumption of this fruit in Brazil, the aim of this work is to recover high-value products such as BITC, present in the seed. For this purpose, it was carried out the extraction of oil present in papaya seeds using supercritical carbon dioxide, in order to verify the technical feasibility of the process.

This is considered a green process due to the absence of organic solvent utilization, using carbon dioxide in a supercritical state as solvent. Because of the carbon dioxide supercritical fluid densities, the extracted products have a high purity and are free of solvent. Moreover, an advantage of supercritical fluid extraction is the possibility of easy solvent recovery after the supercritical extraction process, only adjusting the pressure and temperature, and it can be continuously recycled [9].

Due to the absence in the literature of this type of raw material extraction using organic and supercritical fluid, it was used carbon dioxide as supercritical fluid, since it has low critical temperature (Tc = 31.04 °C), allowing the extraction of compounds without altering the thermo-sensitive properties of the extracts and critical pressure of 73.8 bar, easily achieved in industrial production units, in addition to the fact that it is inert, it does not present a risk of secondary reactions, it is harmless, non-explosive, non-toxic and promotes the effective separation from the extract by depressurization.

Thus, different conditions of temperature and pressure were applied in order to evaluate the solvency power of the solvent, and the presence of BITC in the samples.

2. Materials and methods

2.1. Materials

The samples from *Carica papaya* fruit L. were purchased in open-air market for their own use and seeds for the study. After collection, seeds were crushed with the help of a pestle, and stored in the refrigerator. Carbon dioxide (minimum purity of 99.99%) used in supercritical extraction was from White Martins (Rio de Janeiro, Brazil).

All the other reactants were purchased from Vetec Chemical Industry (Rio de Janeiro, Brazil).

2.2. Experimental procedure

The oil extraction of *Carica papaya* L. seeds with supercritical carbon dioxide was performed in Applied Thermodynamics and Biofuel Laboratory (Department of Chemical Engineering/UFRRJ). The experimental apparatus (Fig. 2) consists of a stainless steel 316S extractor with 42 mL of capacity. The extractor contains two canvas of 260 mesh to prevent the entrainment of material. A high-pressure pump (Palm model G100), specific for pumping CO_2 was responsible for feeding the solvent into the extractor. A thermostatic bath (Haake K15 model) was coupled in the extractor to control the temperature and a manometer was installed on line for control pressure.

It was fed into the extractor about 6.5 g of seeds and then the thermostatic bath was turned on, reaching the desired temperature.

The experiments were performed under the operational conditions of 100, 150 and 200 bar for pressure and 40, 60 and 80 °C for temperature. The maximum time of extraction was 180 min, when it was observed the saturation in the extraction curve. Sampling occurred at a maximum flow of 16.45 mL/min, controlled by a rotameter, and was performed at each 10 min using the technique of decompression through a micrometer valve. The reduction of the pressure facilitates the recovery of the samples in a polypropylene tube. The experiments were done in triplicate.

2.3. Sample treatment

Some extracts from the supercritical extraction had yet a little water quantity together with the oil. So, it was done a liquid-liquid extraction to remove the water from the sample to do a more precise analysis. The sample was diluted in dichloromethane and two phases were formed: an oily phase that contains the *Carica papaya* oil and the water still remained in the upper phase. Anhydrous sodium sulfate was added to the sample to adsorb water and then the solvent was separated from decantation. The dichloromethane was removed using a rotary evaporator.

2.4. BITC analysis

The BITC presence in the extracted oils were determined through the hydrogen nuclear magnetic resonance analysis (NMR 1H). The NMR spectra were obtained, at 200 MHz, in the Varian Gemini 200 equipment, using the tetra-methyl-silane (TMS) as an internal standard, at ambient temperature. The chemical shift values (d) were reported in parts per million (ppm) relative to TMS

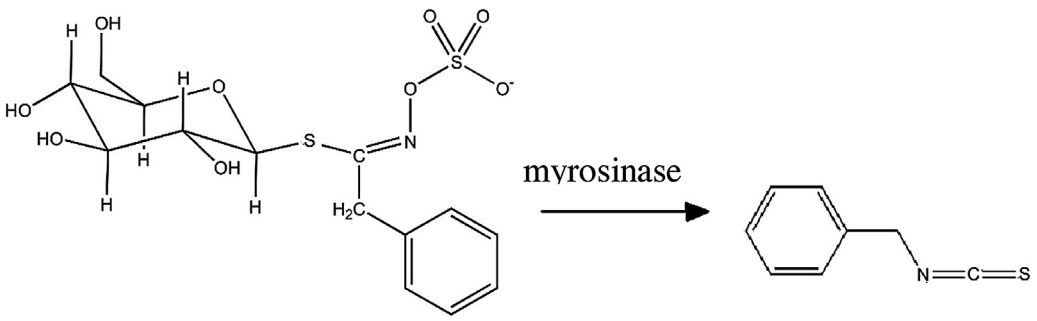

Fig. 1. BITC production process through the myrosinase enzyme action [8].

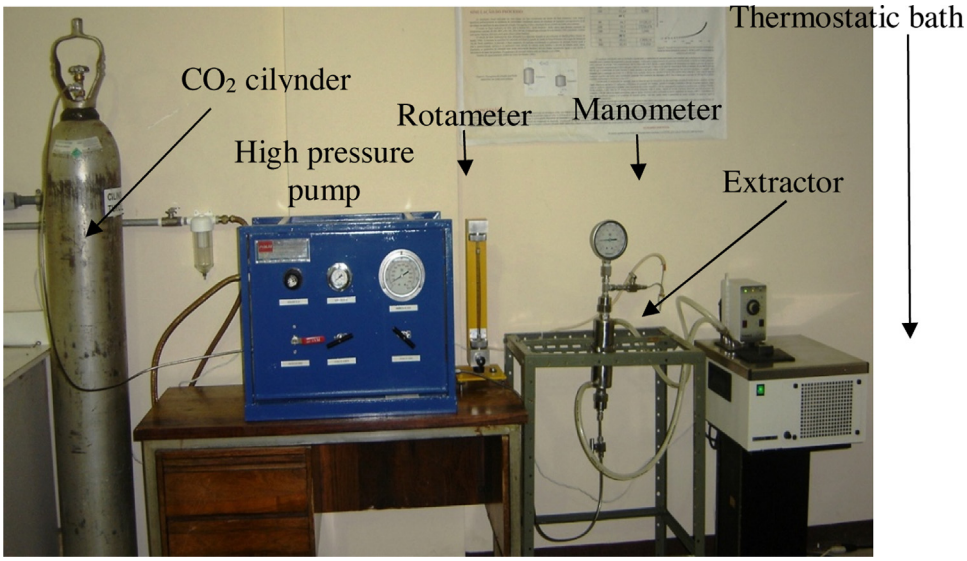

Fig. 2. Experimental apparatus of the supercritical extraction process.

and the values of the coupling constants (J) are reported in Hertz (Hz). The solvent used in this analysis was the deuterochloroform.

2.5. Sample analysis

The analysis of extract content, including fatty acids and other components, was performed using comprehensive two-dimensional gas chromatography coupled with time of flight mass spectrometry (GC × GC-TOFMS).

The two-dimensional system consists in two columns with different stationary phases which are connected in tandem and all compounds that elute from the first column are injected in the second one. In GC × GC the compounds are submitted to two separation mechanisms which distributes the compounds in an orthogonal plane, increasing the information about the samples. It is available in the literature review articles which explain the theoretical and practical aspects about the technique [10,11].

GC × GC can be coupled to several kinds of detectors [12], but the most powerful combination is GC × GC coupled to time of flight mass spectrometry (GC × GC-TOFMS) because of its high acquisition rate (until 500 spectra s^{-1}) [13,14].

Analysis was performed in a Pegasus 4D (Leco, St. Joseph, MI, USA) GC × GC-TOFMS, composed of an Agilent 6890 GC (Palo Alto, CA, USA) equipped with a secondary oven and a non-moving quad-jet dual-stage modulator and a Pegasus III (Leco, St. Joseph, MI, USA) time of flight mass spectrometer. A DB-5 column (Agilent, Palo Alto, CA, USA), 5%-phenyl-95%-methylsiloxane (30 m 0.25 mm i.d., 0.25 μm d_f) was used as the first dimension column (1D). A BPX-50 column (SGE, Ringwood, VIC, Australia), 50%-phenyl-50%-methylsiloxane (1.5 m 0.1 mm i.d., 0.1 μm d_f) was used as the second-dimension column (2D). The 2D column was transferred to the TOFMS by means of a 0.5 m 0.25 mm i.d. empty deactivated fused silica capillary, which was connected via SGE mini-unions and SiltiteTM 76 metal ferrules 0.1–0.25 mm i.d. (Ringwood, VIC, Australia) [14].

GC conditions: sample volume was 1 μL at 310 °C in split mode with split ratio of 5:1. Helium (99.9999% purity from White Martins) was used as carrier gas at a constant flow rate of 1.0 mL/min. The primary oven temperature program was set at 100 °C for 2 min, ramped at 10 °C/min to 150 °C, then ramped at 3 °C/min to 300 °C. The secondary oven temperature program was 10 °C higher than the primary. The modulation period was 6 s with 1.5 s hot pulse duration and the modulator temperature was 45 °C higher than the primary oven temperature. The total analysis time was 57 min.

Chemstation™ software was used for data acquisition and the identification of the compounds was performed using a NIST Mass Spectral Library software (NIST 08, version 2.0) for correct matching.

3. Results and discussion

With respect to the analysis, all extracts were homogeneous in composition, especially with regard to the presence of various fatty acids [oleic (OA), palmitic (PA) and stearic (SA) acids], among others. The presence of BITC was detected in all samples extracted having the highest concentration at 40 °C and 150 bar and 80 °C and 200 bar, with 11 and 7%, respectively [15]. The compositions of the majority components (components that are present in higher quantities) present in the extract are presented in Table 1, and they were calculated from peak areas.

2,2-Dithio diethanol is widely used as monomer for the synthesis of copolymers of polyurethanes. De Paz et al. [16] made use of this reagent to synthesize a polyurethane biodegradable derivative of L-arabinitol; the degradability of this polymer is mediated by glutathione. It was concluded that the ratio of units of dithio-diethanol in the final polymer has a fundamental role for the biodegradability of this product.

In the extraction performed using sohxlet by Viana [17], major triglycerides have been found as oleic acid (74.06%), followed by palmitic acid (21.21%) and stearic acid (4.73%), in the oil of the *Carica papaya* seeds. For the samples extracted with supercritical

Table 1
Composition (%) of the majority components present in the extracted oils at different operational conditions.

T (°C)	P (bar)	BITC	DDDTD [*1]	OA	PA	SA	DD [*2]
40	100	2%	13%	6%	3%	1%	5%
	150	11%	26%	6%	4%	1%	7%
	200	3%	7%	11%	6%	2%	7%
60	100	2%	12%	10%	6%	2%	0%
	200	2%	28%	18%	7%	2%	8%
80	100	1%	36%	8%	5%	1%	12%
	200	7%	12%	29%	8%	3%	3%

[*1]2,2-Dithio ethanol.
[*2]Diethanol disulfite.

Table 2
Extraction efficiency (%) of different compounds for each operational condition, calculated based on the seed mass.

T (°C)	P (bar)	BITC	DDDTD [*1]	OA	PA	SA	DD [*2]
40	100	0.37	2.41	1.11	0.56	0.19	0.93
	150	3.40	8.05	1.86	1.24	0.31	2.17
	200	1.38	3.22	5.07	2.76	0.92	3.22
60	100	0.52	3.15	2.62	1.57	0.52	0.00
	200	0.69	9.70	6.24	2.43	0.69	2.77
80	100	0.56	19.98	4.44	2.78	0.56	6.66
	200	6.72	11.51	27.83	7.68	2.88	2.88

[*1]2,2-Dithio ethanol.
[*2]Diethanol disulfite.

fluid, the same fatty acids were extracted, while other acids appeared in very small concentrations.

The efficiency of extraction and concentration of compound in the oil was calculated and these results are presented in Table 2. It can be seen more real values of the extraction efficiency, through Table 2, because although the extracted oil, at 40 °C and 150 bar, has the highest concentration of BITC in absolute terms, the condition of 80 °C and 200 bar extracted larger amounts of BITC.

Fig. 3 shows the two-dimensional chromatogram, as an example, of the oil sample extracted at 80 °C and 200 bar, showing the presence of BITC.

To evaluate the BITC presence in the supercritical fluid extracts, a NMR analysis was done due to the fact that BITC has two characteristic peaks. The major peaks are the related connection CH_2 neighboring the nitrogen present in the region of 4.6 ppm and the CH bonds of the aromatic rings in the region between 7.2 and 7.4 ppm. In Figs. 4–6, it can be seen the spectra carried out for three different conditions of 100, 150 and 200 bar.

As can be seen in the spectra shown in Figs. 5–7, the presence of BITC in the oil samples extracted using supercritical fluid was very small, being present in a concentration slightly higher in the samples of higher pressure. The presence of BITC is confirmed by the signals of aromatic hydrogens in the range 7.2–7.4 ppm, and also the signals for the benzyl hydrogen adjacent to the nitrogen at 4.7 ppm. The extraction of BITC as well as the total yield is directly dependent on the pressure of the system, being in a higher concentration in the sample of 200 bar.

According to the process operation, yields obtained are shown in Table 3. As the experiments were done in triplicate, the results presented are the mean values. Experimental yield was calculated according to equation 6.

$$e\% = \frac{\text{Extract mass}}{\text{Free solute feed mass}} \times 100 \quad (6)$$

with extract mass as the mass of extracted oil and the load-free mass as the solute free oil mass. The calculation of solute free of oil mass requires the default percentage of oil extracted applying a conventional method. From this experiment, it was obtained the value of 2.8% of oil extracted by Soxhlet extraction using pentane as solvent.

In general, it is known that, at constant temperature, the yield increases with increasing pressure. It can be observed at 40 and 80 °C, while at 60 °C all the efficiencies decreased with the pressure increase. This behavior is expected because when the pressure increases, the density of carbon dioxide also increases, at constant temperature.

At constant pressure, the increase of temperature causes an opposite behavior, affecting a decrease in the efficiency, in a general manner. In this case, this behavior only is observed between the efficiencies calculated at 40 and 60 °C. At 80 °C, the efficiencies increase again, because the vapor pressures of the components also increase, at high conditions of temperature. This behavior can be explained by the competition between carbon dioxide density (decreases with the temperature increase at constant pressure) and components vapor pressures (increase with temperature increase).

The accumulated yield in function of the operational time for the oil extraction from the seeds of *Carica papaya* fruit can be seen in Figs. 7–9 , respectively, according to the pressures applied. The extraction curves vary depending on the solubility of components in the supercritical carbon dioxide. The representation of the cumulative yield versus time is shown as a function of each mathematical model study.

The extraction curves with the experimental efficiencies are shown in Figs. 7–9 at temperatures of 40, 60 and 80 °C, respectively.

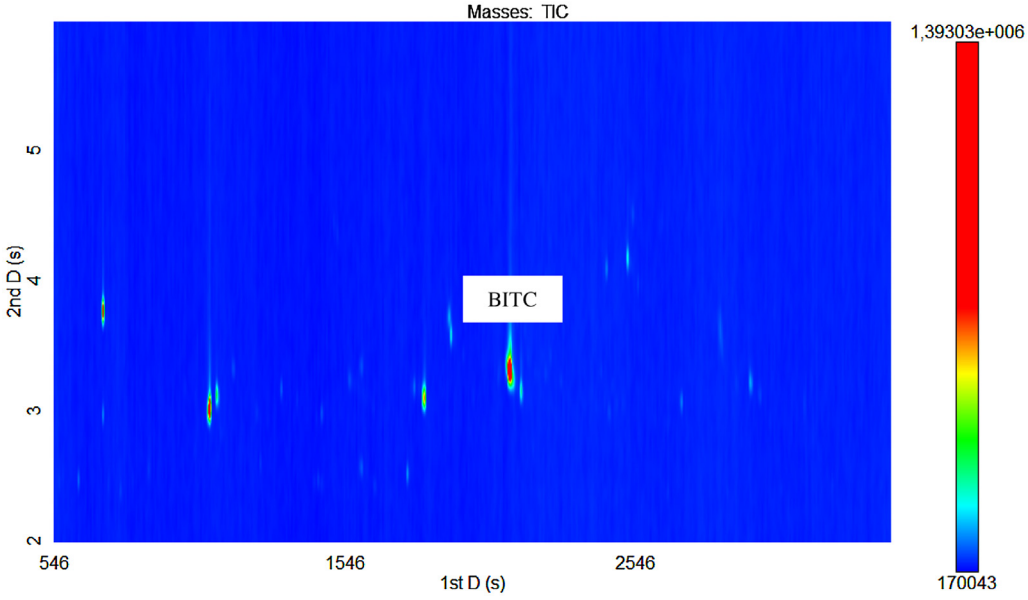

Fig. 3. Two-dimensional chromatogram of the oil sample extracted at 80 °C and 200 bar.

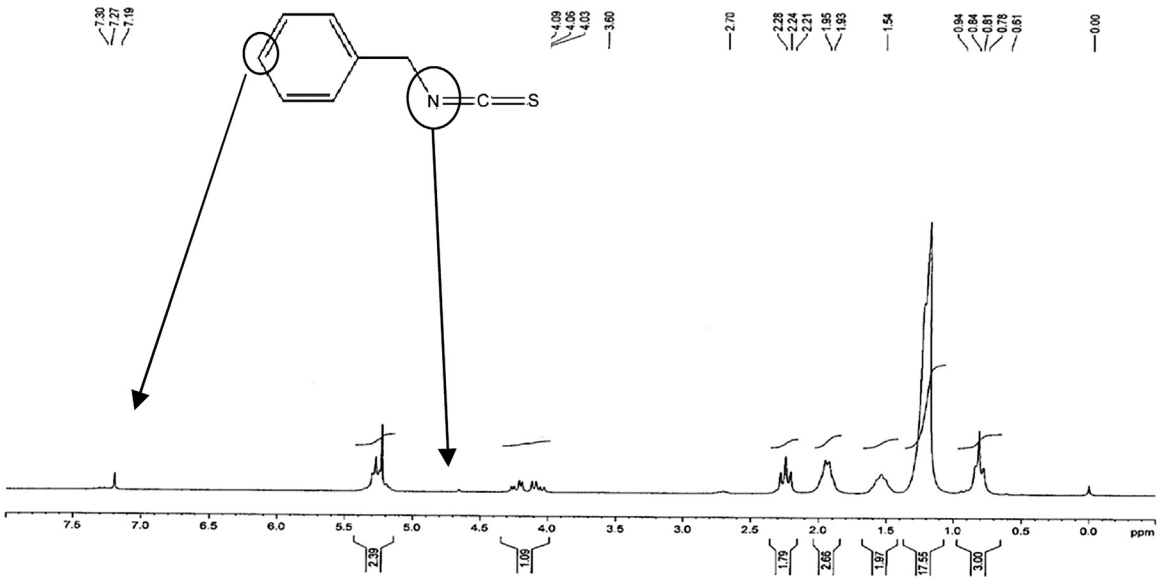

Fig. 4. NMR spectra for the oil sample extracted at 80 °C and 100 bar.

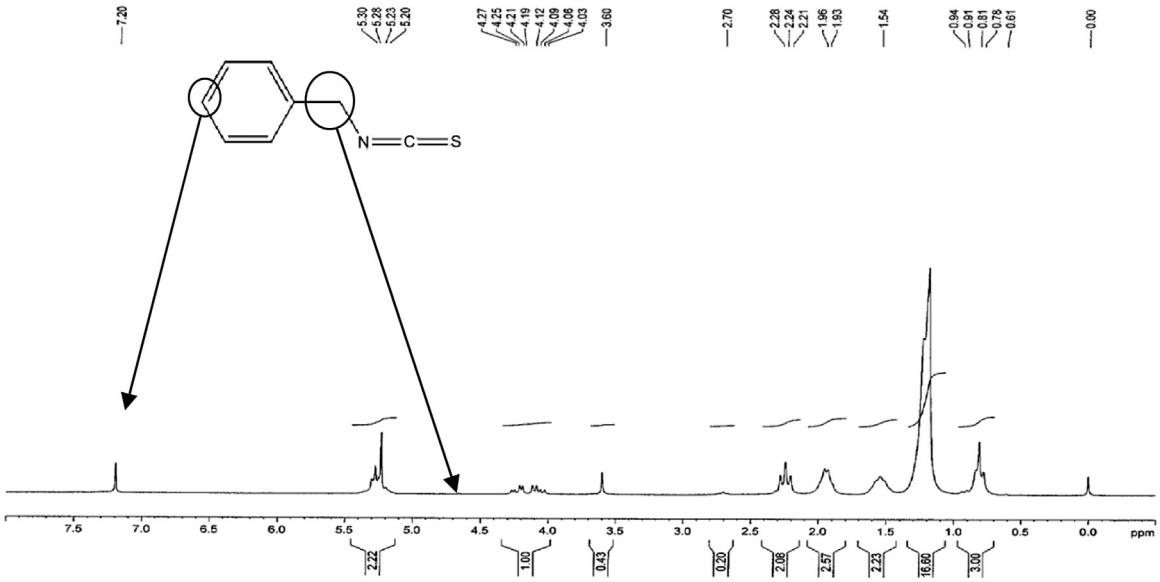

Fig. 5. NMR spectra for the oil sample extracted at 40 °C and 150 bar.

At 80 °C, it can be observed at 100 and 150 bar, a crossover behavior indicating that above 130 min, approximately, it is better to conduct the extraction at 150 bar and higher values of efficiency were obtained at 100 bar below 130 min of extraction.

4. Larvicide test

This test has a protocol well established by WHO [1]. The test consists in groups of 20 live larvae of *Aedes Aegypt* in stage L3 mixed with 249 mL of water. Then it is done an inoculation of 1 mL of a diluted solution of the oil in acetone, and the solution remained at rest for 24 h. The acetone is chosen to do the test because there is not larvicide activity in solutions with this organic solvent. After 24 h, the number of the dead larvae is counted. It was considered dead larvae those one that do not have movement, even with the pipet stimulus, or those who have limited movement.

It was done preliminary tests of the larvicide effect of the extracts, considering the quantitative data characterizing the presence of BITC in the samples.

From the extract obtained at 60 °C and 200 bar, it was prepared a concentration of 53.8 ppm; from the extract of 40 °C and 150 bar, it was prepared a solution of 40.8 ppm of concentration; for the sample obtained at 80 °C and 100 bar, it was prepared a solution of 30.4 ppm, and with the extract from 40 °C and 200 bar, it was prepared a solution with concentration of 32.6 ppm. All of the samples used for the tests were prepared with higher concentrations (30–50 ppm) to know the potential of the samples. Then, there were done dilutions from the original solutions to find the minimum values that have positive effect on the larvicide activity.

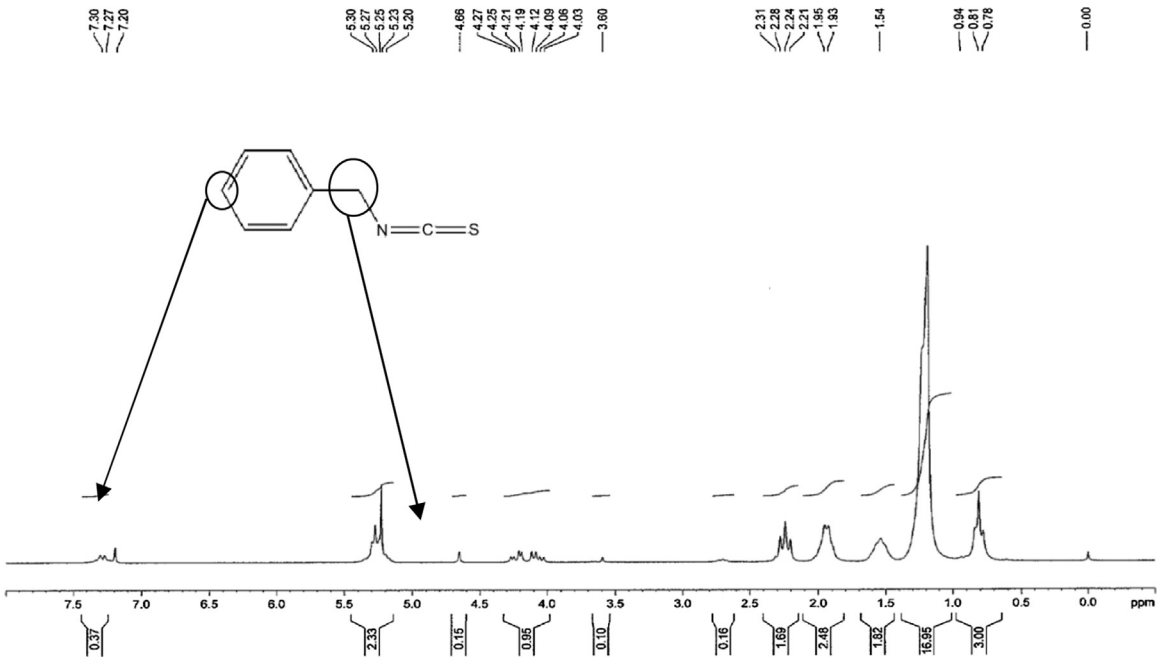

Fig. 6. NMR spectra for the oil sample extracted at 40 °C and 200 bar.

Table 3
Yield behavior in function of the operational conditions of temperature and pressure.

40 °C			
Pressure (bar)	100	150	200
Efficiency (e)	0.44	0.89	1.33
60 °C			
Pressure (bar)	100	150	200
Efficiency (e)	1.11	0.76	0.94
80 °C			
Pressure (bar)	100	150	200
Efficiency (e)	1.59	1.58	2.56

The concentrations investigated (from 30 to 50 ppm) were considered so high if the final objective is to use this product in a large scale. Even with high concentrations, none of the samples were capable to have activity against the *Aedes Aegypt* larvae.

Due to that, the larvicide action of the *Carica papaya* seed oil extracted using supercritical fluid was discarded. The BITC concentrations of the extracts from soxhlet and supercritical fluid techniques were similar (Table 4); however the supercritical fluid extracts did not have the expected activity, probably due to the low quantity of the bioactive component.

5. Conclusions

The supercritical carbon dioxide showed technical viability in the extraction of *Carica papaya* oil from seeds. The better yield results were obtained at 80 °C and 200 bar with 2.5% of oil extracted. It was observed that the efficiency increased with the pressure increase, at constant temperature. The temperature increase causes the decrease in the process efficiency, except at 80 °C. BITC was presented in all the extracts, but it was more

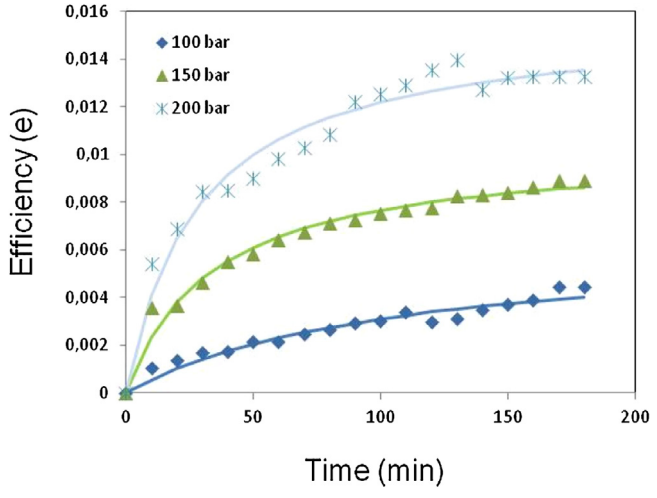

Fig. 7. Extraction curves of *Carica papaya* seed oil, at 40 °C, for the pressures of 100, 150 and 200 bar.

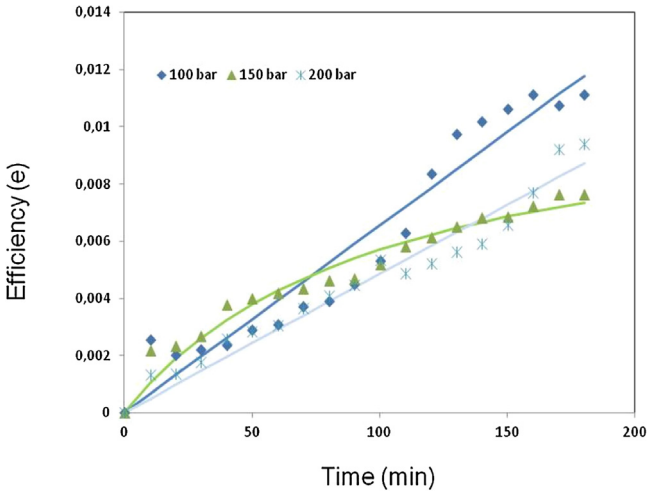

Fig. 8. Extraction curves of *Carica papaya* seed oil, at 60 °C, for the pressures of 100, 150 and 200 bar.

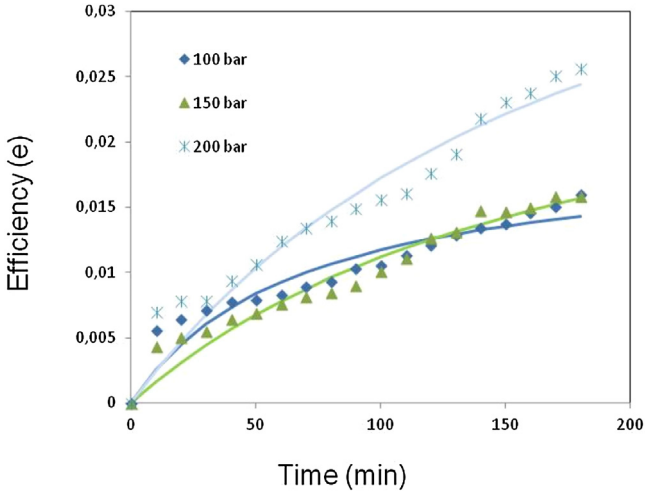

Fig. 9. Extraction curves of *Carica papaya* seed oil, at 80 °C, for the pressures of 100, 150 and 200 bar.

Table 4
Concentration of BITC encountered in the extracts obtained by soxhlet, hydro distillation and supercritical fluid.

		Aedes aegypti	
	BITC concentration (w/w)	CL50	CL99
Soxhlet	4.7–16%	12–13 ppm	31–32 ppm
Hydrodistillation	91–94%	1.65 ppm	5.64 ppm
Supercritical fluid	1–11%	>30 ppm	>>30 ppm

concentrated at 80 °C and 200 bar. The presence of this insecticide revealed a new application of this residue, being able to be used in pharmaceutical and chemical industries, although they did not have the expected activity against *Aedes Aegypt* larvae. Probably, higher conditions of temperature and pressure will be better to extract more quantity of BITC to promote activity against the mosquito.

Conflict of interest

None.

Acknowledgments

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