PHORBOL ESTERS EXTRACTION FROM *Jatropha curcas* SEED CAKE USING SUPERCRITICAL CARBON DIOXIDE

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Abstract. *Jatropha curcas* seeds contain 40–60% of oil, which can be converted to high quality biodiesel. However the seeds contain many toxic compounds with the most important ones known as phorbol esters (PEs). Phorbol esters are derivatives of the tigliane compound and they are considered toxic and maybe with carcinogenic activity. It is reported in the literature that it is possible to have four to six types of phorbol esters or its derivatives. Different chemical and physical methods have been employed for the removal or inactivation of phorbol esters present in different raw materials. This study has as aim the study of the technical feasibility in the use of supercritical fluid for the extraction of phorbol esters present in the *Jatropha* seed cake. The operational conditions investigated were 70ºC - 100Bar; 50ºC – 160Bar; 90ºC – 160Bar; 40ºC - 300Bar; 70ºC - 300Bar; 50ºC - 440Bar and 90ºC - 440Bar. Defatted *Jatropha curcas* seed cake was obtained by a screw press.

Keywords: Supercritical CO_2 extraction, Physic nut, Phorbol esters

1. Introduction

Jatropha curcas L. seeds, known as “pinhão-manso” in Brazil, is an important energy plant which has received great attention in recent years for its utilization in biodiesel production [1]. *Jatropha curcas* oil is usually extracted by screw presses and according to [2] for every thousand litres of *Jatropha* oil, around 2 tonnes of press cake is produced. The press cake remaining after oil extraction is rich in proteins but it contains toxic compounds [3]. The toxicity is caused by phorbol esters and this phorbol ester is the parameter that limits the utilization of the protein rich press cake for animal nutrition [4, 5]. Different chemical, physical and biologic methods have been employed for the removal or inactivation of phorbol esters present in different raw materials [3, 5, 6, 7, 8, 9, 10, 11, 12].

There are six types of phorbol ester present in the Jatropha [13] and many works have been studied in order to extract the phorbol esters to add value to this byproduct [2, 14]. According to [15], it was suggested that the aqueous/organic solvent extracts from Jatropha oil/seed are effective as insecticidal and antimicrobial agent in vitro and in majority of the studies, the activities are attributed to the presence of phorbol esther. Some studies report the antifungal, insecticidal the extract properties from J. curcas seed cake [15, 16, 17].

Development of green chemical process implies using the principles of green chemistry and engineering, from process inception in the research environment to process application on a commercial scale. [18]. The carbon dioxide in their supercritical states, is as solvents for green chemical processes and due to its characteristics like inertness, non-toxicity, no flammability, non-explosiveness, and availability with high purity at low cost [18,19]. This study has as aim the study of the technical feasibility in the use of supercritical fluid for the extraction of phorbol esters present in the *Jatropha* seed cake, but, until now, it was studied the kinectics of the extraction process applying a statistical experimental planning.

2. Materials and Methods

2.1. Preparation of *Jatropha* press cake

The *Jatropha* seeds were kindly provided by Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG) grown in the region of Janaúba city, located in the north of Minas Gerais state, in Brazil.

The *Jatropha* press cake was obtained using a tubular radial screw press with capacity of 50 kg/h (SCOTTECH). After pressing, the material was stored in a plastic bag in the refrigerator for later experiments.

The flowsheet of the experimental is shown in Figure 1.

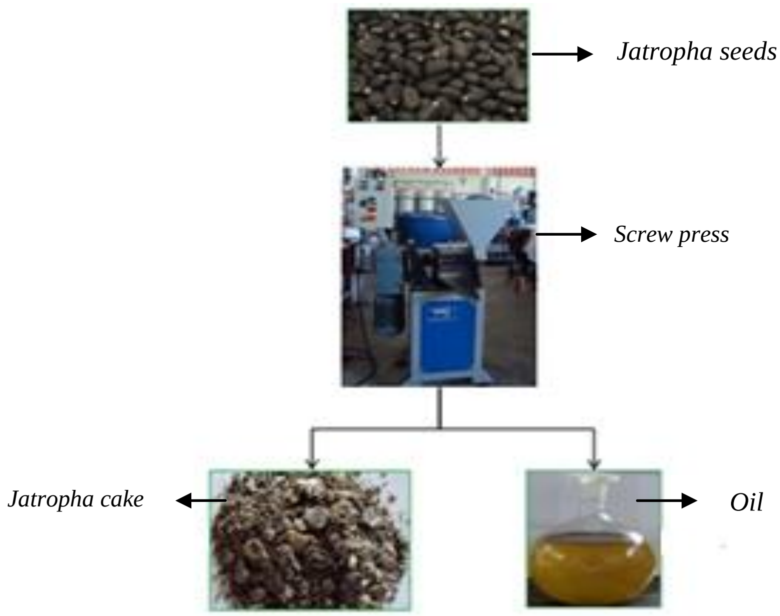

Figure 1. Flowsheet of the experimental apparatus for obtained *Jatropha* press cake [20]

2.2. Experimental design

The *Jatropha* cake press was submitted to the extraction process following a central composite rotational design (CCRD) 2^2 with two variables (pressure and temperature). Table 1 shows the values of the coded and real levels of the design.

Table 1. Central composite rotatable design matrix of the supercritical fluid extraction

Variables	**Code**	**-1.41**	**-1**	**0**	**+1**	**+1.41**
Temperature (ºC)	x1	40	50	70	90	100
Pressure (Bar)	x2	100	160	30	440	500

2.3. Supercritical carbon dioxide extraction

The experimental measurements of the extraction had been performed in an apparatus, built in the Applied Thermodynamics and Biofuels Laboratory at DEQ/UFRRJ, 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 (Fisatom) was coupled in the extractor to control the temperature and a manometer was installed on line for control pressure. Liquid CO_2 (99.9% pure) was from White Martins (Rio de Janeiro). The flowsheet of the experimental apparatus is shown in Figure 2.

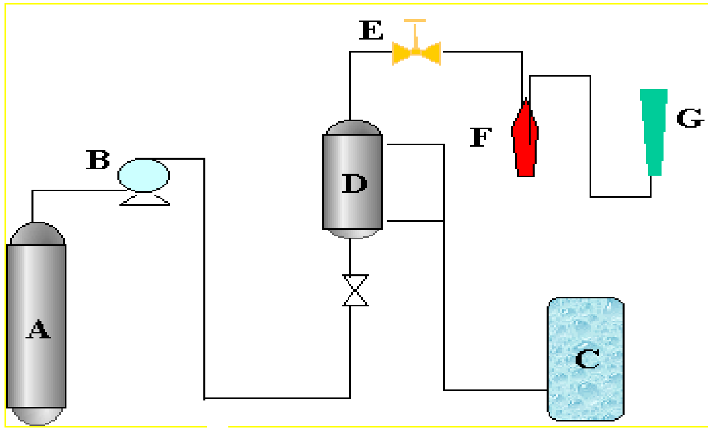

Figure 2. Flowsheet of the experimental apparatus;
A. Cylinder of CO2; B. High-pressure pump; C.Healting bath; D. Extractor;
E. Micrometric valve; F. Sample; G. Flowmeter

The same apparatus has been used in numerous studies done by the research group [21, 22, 23, 24].

The experimental procedure was carried out in a semi-batch way. Initially, the extractor was filled with the solid material, approximately 10g of Jatropha press cake. The sampling was done using the micrometric valve, reducing the pressure, and the oily extract was recovered in a previously weighed collector. Sampling occurred at each 10 minutes using the technique of decompression through the valve. With the pressure reduction, the sample is recovered in a polypropylene tube.

In this work the operational conditions investigated were 70ºC - 100Bar; 50ºC – 160Bar; 90ºC – 160Bar; 40ºC - 300Bar; 70ºC - 300Bar; 50ºC - 440Bar and 90ºC - 440Bar. For each operational condition, the extraction curves were constructed relating cumulative mass of extracted in function of the operational time. The maximum process time was 300 minutes.

3. Results and Discussion

The results of yield for extracts of Jatropha cake with supercritical carbon dioxide is shown in Table 2.

Table 2. Yields of the extracts of *Jatropha cake* with supercritical CO_2

Operational Conditions	70ºC 100Bar	50ºC 160Bar	90ºC 160Bar	40ºC 300Bar	70ºC 300Bar	50ºC 440Bar	90ºC 440Bar
Yield (%)	0.65	2.20	2.12	2.35	2.90	6.16	5.73

According to the results, the best yields were obtained at high pressures and the best extraction conditions were obtained at 90°C and pressure of 440 bar with the yield of 6,16%.

The accumulated yield in function of the extraction time of *Jatropha* cake can be seen in Figures 3 and 4. The extraction time was 300 minutes for all experiments.

It was observed that the yield increases with increasing pressure. Higher pressures (300 and 440 Bar) have better yields when compared to pressures (100-160 Bar). According to Figure 3, the increase in temperature, at constant pressure (300Bar), leads to an increase in yield. This may be related to the competitive effects of the vapor pressure of the solute and solvent density, The vapor pressure of the solutes increased, favoring the extraction process. At 160 Bar, it was observed a cross-over behavior between the curves of 50ºC and 90ºC, showing the effects of density and vapor pressure of the solute in the extraction efficiency.

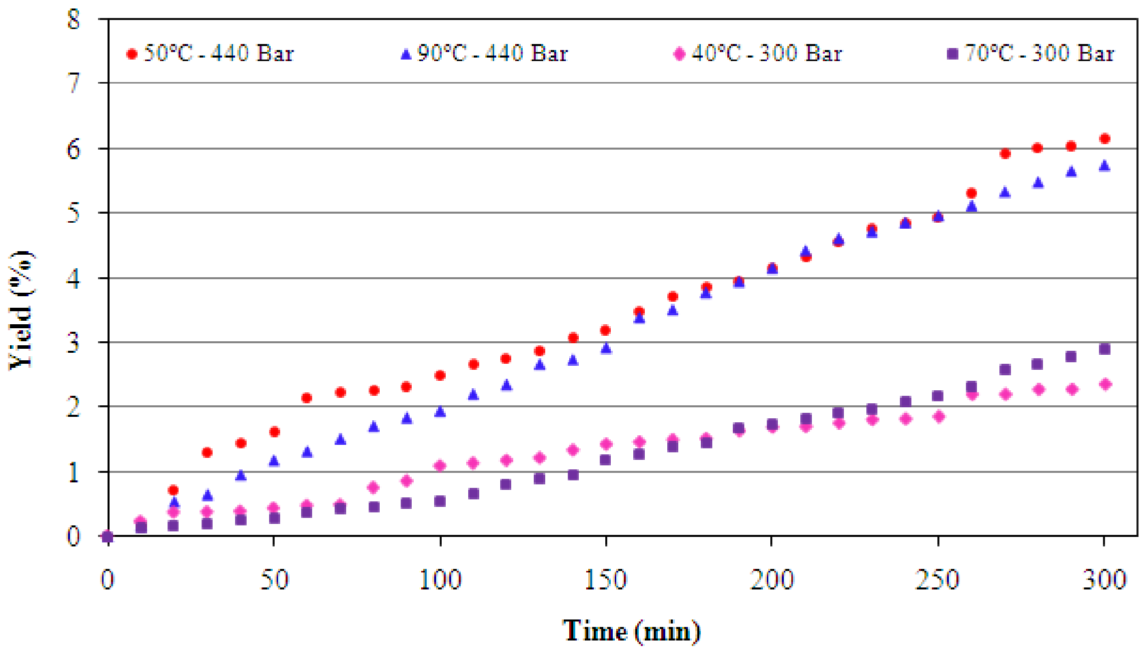

Figure 3. Extraction curve of *Jatropha* cake press with with supercritical CO2 at pressures 300 and 440 Bar.

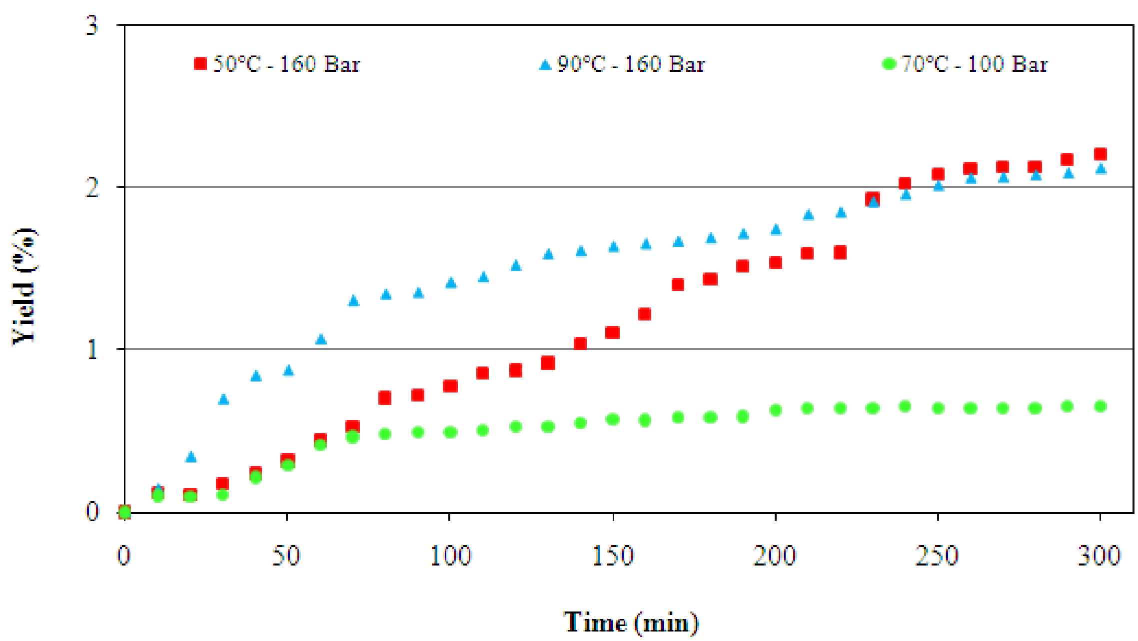

Figure 4. Extraction curve of *Jatropha* cake press with with supercritical CO2 at pressures 100 and 160 Bar.

4. Conclusion

In this work it is investigated the application of supercritical fluid extraction to obtain the phorbol esters from *Jatropha curcas* cake. The supercritical fluid extraction was very effective in the extraction of the *Jatropha* press cake. The study by the kinectics of the extraction process applying the statistical experimental planning showed the best results in the operational conditions of 50 °C and 440 bar with the yield of 6.16%. It was observed that the yield increases with increasing pressure. Higher pressures (300 and 440 Bar) have better yields when compared to pressures (100-160 Bar).

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