

Technical and Economic Evaluation of Phorbol Esters Extraction from *Jatropha curcas* Seed Cake using Supercritical Carbon Dioxide

Cristiane de Souza Siqueira Pereira^{1*}, Fernando Luiz Pelegrini Pessoa¹, Simone Mendonca², Jose Antonio de Aquino Ribeiro² and Marisa Fernandes Mendes³

¹Technology of Chemical and Biochemical Processes, Chemistry School, Federal University of Rio de Janeiro, Brazil

²Embrapa Agroenergy, Brasília, Brazil

³Chemical Engineering Department, Federal Rural University of Rio de Janeiro, Brazil

Abstract

Jatropha curcas plant shrub of *Euphorbiaceae* family is a plant whose seeds are rich in oil that can be used for biofuel production. However the seeds contain many toxic compounds which the most important ones are known as phorbol esters (PEs). This study has as aim the study of the technical and economic feasibility of the supercritical fluid for the PEs extraction present in the *Jatropha* seed cake. The effect of temperature (40-100°C) and pressure (100-500 bar) on the phorbol yield was investigated using a central composite design methodology to determine the significance and interactions of these parameters. PEs in the extracted samples were analyzed and quantified by HPLC. The supercritical fluid extraction was effective in the recovery of PE extracted from *Jatropha curcas* cake varying from 23.0%, at 70°C and 500 bar to 2.6% at 90°C and 160 bar. The results showed that pressure had the most significant enhancing effect on the phorbol ester yield. Simulations of phorbol ester extraction from *Jatropha curcas* cake were carried out using SuperPro Designer 9.0 (Intelligen, Inc) to evaluate production costs of an industrial process to treat the necessary quantity of cake. It was possible to conclude that the supercritical extraction is viable to be applied.

Keywords: Detoxification; Screw press; Design experiment; Scale-up

Introduction

Jatropha curcas L. seed, known as “pinhão-mansão” in Brazil, is an important oleaginous which has received great attention in recent years due to its utilization in biodiesel production [1]. *Jatropha curcas* oil is usually extracted by screw presses and for every thousand of liters of *Jatropha* oil, around 2 tonnes of press cake are produced [2].

The cake remaining after oil extraction is rich in proteins but it also contains toxic compounds [3]. The toxicity is due to the presence of high levels of toxic and anti-nutritional components as trypsin inhibitors, lectins, saponins, phytate and PEs. Although others compounds are present, PE is the main toxic component of *Jatropha curcas* and the concentration of them is the parameter that limits the utilization of the protein rich pressed cake for animal nutrition [4].

The phorbol esters molecules are tetracyclic diterpenoids with a tigliane skeletal structure [5]. These authors isolated six different types of PE of *Jatropha curcas* and all of these compounds possess the same diterpene core, namely, 12-deoxy-16-hydroxyphorbol. These compounds present in the seeds were designated as jatropha factors C1, C2, C3, epimers C4, C5 and C6, with the molecular formula C₄₄H₅₄O₈.

Different chemical, physical and biologic methods have been employed for the removal or inactivation of PEs from oil, kernel or pressed cakes in order to promote their use as a protein source for animal feed [6-10]. PEs are double-edged swords, having a lot of negative effects on human and livestock. They also possess some beneficial effects, because not all of the PEs are toxic and their activity and potency vary from one type of PE to another [11]. Many works have been studied in order to extract the PEs to add value to this by product [12-15]. The purified PEs could also be converted or transformed chemically into nontoxic compounds with beneficial activities such as the hydrolysis of 12-deoxy-16-hydroxyphorbol that results in the

synthesis of 12-deoxyphorbol-13-phenyl acetate, a compound that is considered as a promising adjuvant for antiviral therapy because of its anti-HIV properties [14].

There are several studies on the detoxification of *Jatropha curcas* cake using organic solvents and chemical treatments, for example, hexane, methanol, petroleum ether and potassium hydroxide [16,17]. However, authors reveal that these techniques are not economically feasible and the multiple steps involved in the processing of the biomass up to the detoxification stage are also costly and not environment friendly. Based on this fact, the supercritical extraction process has advantages compared to conventional methods with organic solvent. This technology implies in the use of 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 its supercritical state is a promising solvent due to its characteristics like inertness, non-toxicity, no flammability, non-explosiveness, and availability with high purity at low cost [19].

There are no reports in the literature concerning the extraction of PEs from *Jatropha curcas* cake with supercritical carbon dioxide.

***Corresponding author:** Cristiane de Souza Siqueira Pereira, Technology of Chemical and Biochemical Processes, Chemistry School, Federal University of Rio de Janeiro, Brazil, Tel: +5524981240208; E-mail: crispereirauss@gmail.com

Marisa Fernandes Mendes, Chemical Engineering Department, Federal Rural University of Rio de Janeiro, Brazil, Tel: +55-21 37873742; Fax: 55-37873750; E-mail: marisamf@ufrj.br

Received July 20, 2015; Accepted August 07, 2015; Published August 14, 2015

Citation: Pereira CSS, Pessoa FLP, Mendonca S, Ribeiro JAA, Mendes MF (2015) Technical and Economic Evaluation of Phorbol Esters Extraction from *Jatropha curcas* Seed Cake using Supercritical Carbon Dioxide. J Adv Chem Eng 5: 132. doi:10.4172/2090-4568.1000132

Copyright: © 2015 Pereira CSS, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Because of the scarce of data concerning PEs extraction with supercritical CO₂, this study has as aim the technical and economical evaluation of this type of extraction of PEs present in the *Jatropha curcas* seed cake.

Materials and Methods

Materials

Jatropha curcas 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 (Brazil). The *Jatropha curcas* press cake was obtained using a tubular radial screw press with capacity of 50 kg/h (Brand: SCOTTECH), which provided approximately 19.6% of oil. The press cake composition was detailed in Ref. [10]. After pressing, the material was stored in a plastic bag in the refrigerator for later experiments. Liquid CO₂ (99.9% pure) was from White Martins (Rio de Janeiro, Brazil).

Experimental design

Jatropha pressed cake was submitted to the extraction process following a central composite rotational design (CCRD) with two independent variables (pressure and temperature), commonly studied in a supercritical extraction process. Table 1 shows the coded and actual levels of variables and describes the 11 experiments that were carried out. The results were statistically analyzed using a statistical program.

Supercritical carbon dioxide extraction

The supercritical fluid extraction (SFE) experiments were performed in an apparatus, built in the Applied Thermodynamics and Biofuel Laboratory at Chemical Engineering Department/UFRRJ, consisting 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 CO₂ was responsible for the solvent feeding into the extractor. A thermostatic bath (Fisatom model) was coupled in the extractor to control the temperature and a manometer was on line installed for pressure measurement. The flowsheet of the experimental apparatus is shown in Figure 1. The same apparatus has been used in numerous studies done by the research group [20] and the experimental procedure was done in a semi-batch way. Initially, the extractor was filled with the solid material, approximately, 10 g of *Jatropha* pressed

cake. The sampling was done using a micrometric valve, reducing the pressure, and the oily extract was recovered in a previously weighed polypropylene tube. Sampling occurred at each 10 minutes with the depressurization of the system.

The extraction yield was estimated according to equation 1:

$$Yield (\%) = \frac{\text{mass of extract (g)}}{\text{mass of cake (g)}} \times 100 \quad (1)$$

with mass of extract as the oily fraction extracted at each 10 min and the mass of cake was 10 g, approximately, for all the experiments.

Phorbol ester analysis

The PEs from extracts of *Jatropha* cake with supercritical CO₂ and from cake pressed was analyzed in Embrapa Agroenergia (Brasília, Brazil).

Phorbol esters analysis from pressed cake

The methodology used in this work was adapted from Makkar et al. [4]. It was transferred, approximately, 4 g of *Jatropha curcas* cake to cells accelerated solvent extractor (ASE 350). The samples contained in the cells were extracted with methanol using the following conditions: temperature: 60°C; heat time: 5 min; static time: 2 min; number of cycles: 5; rinse volume: 150% and a purge time of 60 s. The extracts from the ASE tubes were evaporated under vacuum in a water bath at 60°C (rotaevaporator). It was added 2.5 mL of HPLC grade methanol to the ASE tubes and it was mixed for 20 to 30 seconds. The methanolic extracts were transferred to a test tube of 10 mL and centrifuged at 4000 rpm for 3 minutes. The clear supernatant was transferred to a volumetric flask of 5 mL with the aid of micropipette. This procedure was repeated with a further portion of 2.5 mL of HPLC grade methanol, bringing the mixture methanol plus residue at the same test tube. The methanol solution was filtered to vial (VertiPure PTFE Syringe, 13 mm, 0.2 µm) and 25 µL were injected into the chromatographic system.

HPLC analysis of PEs extracts from supercritical CO₂ process

It was added 3 mL of HPLC grade methanol to the falcon tube containing the extract obtained by supercritical fluid extraction. The tube was mixed for 20 to 30 seconds and centrifuged at 9000 rpm for 5 minutes. The clear supernatant was transferred to a 10 mL volumetric flask with the aid of micropipette. This procedure was repeated with two further portions of 3 mL of methanol, adding the methanolic extracts in the same 10 mL volumetric flask and completing the volume with methanol. The methanolic solution was filtered to vial (VertiPure PTFE Syringe, 13 mm, 0.2 µm) and 25 µL were injected into the chromatographic system. A gradient of (A) phosphoric acid 0.1% (V/V) and (B) acetonitrile was used as following described: start with 60% of B, increase B to 100% in the next 25 min, and keep 100% B for the next 3 min. Then the column was washed with 2-propanol in the next 5 min and equilibrated with the starting conditions (60% of B) for 10 min. The chromatographic conditions were also adapted from Makkar et al. [21]. The PEs were analyzed and quantified by HPLC (Agilent) on a reverse phase C18 SB-C18 250 × 4.6 mm (5 µm), maintained at 40°C.

Phorbol-12-myristate 13-acetate (PMA) was used as an external standard, which has a retention time around 23.5 min. PEs peaks were integrated at 280 nm, and the concentration was expressed as equivalent to PMA. The PEs peaks appeared between 17.5 and 21.5 min. The percentage of PEs present in the extracts were calculated as a ratio between the areas of the PEs analysed in the supercritical extracts and the areas of PEs presented in the original cake.

Run	Coded level of variables		Actual level of variables	
	Temperature (x1)	Pressure (x2)	Temperature (°C)	Pressure (Bar)
Factorial points				
1	-1	-1	50	160
2	+1	-1	90	160
3	-1	+1	50	440
4	+1	+1	90	440
Axial points				
5	-α (-1.41)	0	40	300
6	+α (+1.41)	0	98	300
7	0	-α (-1.41)	70	100
8	0	+α (+1.41)	70	500
Center points				
9	0	0	70	300
10	0	0	70	300
11	0	0	70	300

Table 1: Central composite rotatable design matrix applied for the supercritical fluid extraction.

Economic evaluation

According to Moraes, Zabet and Meireles [22], studies involving economic aspects are needed to transfer the knowledge acquired at laboratory/pilot scales to industrial scale. Many of them have simulated the manufacturing cost (MC) of extracts, mostly obtained by supercritical fluid extraction from vegetal raw materials and reported the financial viability of the process such as antioxidant extracts from *Myrciaria cauliflora* [23] and production of phenolic rich extracts and extraction of carotenoids from Brazilian plants [24,25].

Due to the fact that the technical feasibility of PEs extraction with supercritical carbon dioxide was assured and because of an absence of works using this type of raw material, an economic evaluation was done to predict the extraction behaviour of a process that will be conducted in a pilot scale of 42 L, using the better operational conditions of temperature (70°C) and pressure (500 bar) obtained in the experimental unit, until the cake was considered detoxified. The scale up criterion adopted consisted in maintaining solvent mass to feed mass ratio (S/F) constant. The overall extraction curves obtained from laboratory scale experiments were used as reference, so that S/F = 53.6.

The commercial simulator SuperPro Designer v9.0 (Intelligen, Inc) was used to simulate the extraction process and to estimate the production cost of phorbol esters extracted with supercritical fluid until the cake was detoxified. This simulator has been used by other authors to simulate supercritical fluid extraction processes using different raw materials [25,26]. The method utilized by the software to estimate the manufacturing cost [27,28] which uses the summation of the fixed cost of investment (FCI), the cost of utilities (CUT), the cost of labor (COL), the cost of raw material (CRM) and the cost of waste treatment (CWT) involved in the studied chemical process to compose the manufacturing cost.

The extraction process was simulated in a batch mode using a solid-liquid extractor present in the database of the simulator. The scale-up criterion adopted consisted of maintaining a constant solvent to feed ratio (S/F). The cost of solid-liquid extractor of 42 L (size 2) of capacity was expressed by equation 2, based on the value US\$ 500.000 for extractor 50 L (size 1) provided by Prado [29], considering an annual depreciation rate of 10%.

$$Cost\ 2 = Cost\ 1 \left(\frac{Size\ 2}{Size\ 1} \right)^{0.6} \quad (2)$$

The *Jatropha* cake press cost (US\$ 75/ton) was based on those reported by Sriram [30]. The cost of CO₂ was considered US\$ 19.76/Kg (White Martins) and it was adopted that the solvent can be recycled without further treatment. The utility cost was based from a simulation of the energy balance in SuperPro Designer: steam at US\$ 4.2/MT and the electricity at US\$ 0.092/kWh. The revenue of the plant may consist of phorbol ester. The cost of phorbol ester was considered based on the reference price of phorbol 12-myristate 13-acetate at US\$ 212.85/mg [31]. This consideration was necessary due to the absence of this price in the literature.

Results and Discussion

The total yields, presented in equation 1, for each operational condition were shown in Table 2.

According to the results, the best yields were obtained at high pressures and the best extraction conditions were obtained at 70°C and pressure of 500 bar with the yield of 9.33%. The accumulated yield in

function of the extraction time of *Jatropha* cake can be seen in Figure 2. The extraction time was different from one condition to another according to the saturation of the raw material: 200 minutes for 98°C at 300 bar, 300 minutes for 70°C at 100 bar, 90°C at 160 bar, 40°C at 300 bar, 70°C at 500 bar and 400 minutes for 50°C and 160 bar, 70°C at 300 bar, 50°C at 440 bar and 90°C at 440 bar.

It is important to note that Table 2 and Figure 3 show the total yield of the extraction, which not represents the PE yield for each operational condition. These behaviours cannot be represented because only the last fraction with the accumulated mass was analysed to know the quantity of PEs. It was observed that the yield increases with increasing pressure and higher pressures (300, 440 and 500 bar) had better yields when compared to lower ones (100 and 160 bar).

According to Figure 2, the increase in temperature, at constant pressure (300 bar), leads to an increase in yield. This may be related to the competitive effect of the vapor pressure of the solute and solvent density, due to the fact that when the vapor pressure of the solutes increased, the extraction process is favoured. At 160 bar, it was observed a cross-over behaviour between the curves of 50°C and 90°C, showing the competitive effects of density and vapor pressure of the solute in the extraction efficiency.

The extracts obtained with supercritical extraction in different conditions were analysed to better quantify the content of PEs by HPLC and shown in Table 3.

The PE content in untreated seed cake was 1.97 mg/g. This measure is the average of the HPLC analysis done in triplicate. Table 3 shows that the extraction at 70°C and 500 bar and 50°C and 440 bar were able to remove 22.19% and 23.03% of the PE present in the seed cake, respectively. The profile of the best condition of extraction (70°C and 500 bar) is shown in Figure 3 and indicates four peaks between retention times of 17 and 21.5 minutes, that represents the PE derivatives.

A central composite rotatable design (CCRD) was used to evaluate the PE yields extracted. The influence of the two variables (temperature and pressure) was statistically investigated at 95% of confidence level ($p \leq 0.05$). The linear and quadratic coefficients of the variables studied and their interactions, the standard error, the significance of each coefficient determined by the p-value and the values of the analysis of variance (ANOVA) are listed in Table 4. The p-values of the regression coefficients suggest that only pressure, as a linear factor, was significant ($p \leq 0.05$). This result corroborated the experimental results presented in Figure 3, in which the better operational conditions occurred at higher pressures. Moreover, the statistical analysis of the experimental data showed that the pressure had a significant effect on PE removal. A first order model was established (Equation 3) based on ANOVA which described the yield of PEs extracted as a function of the significant

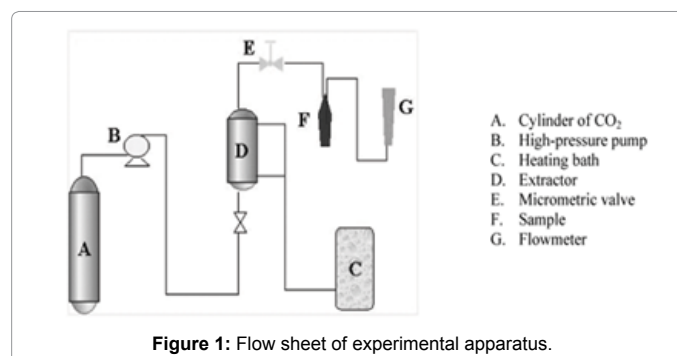
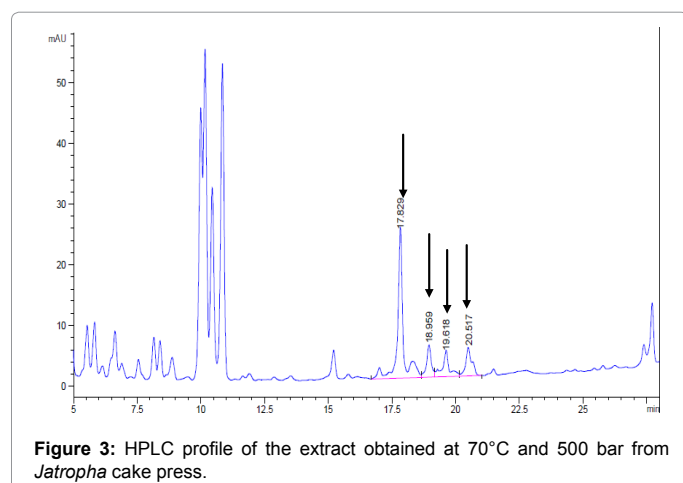
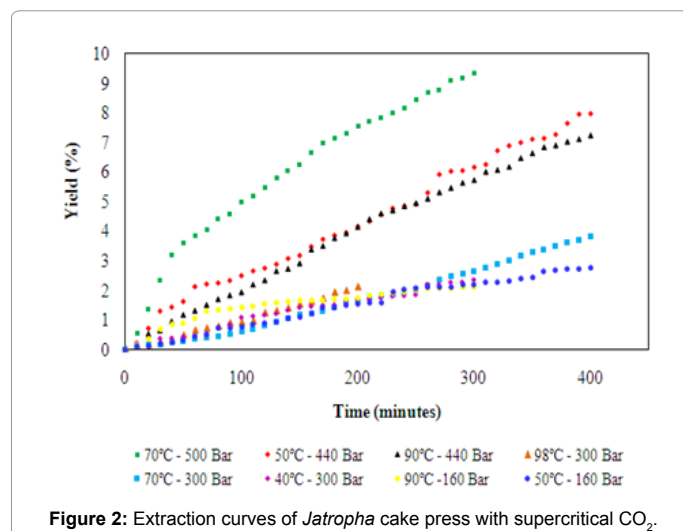


Figure 1: Flow sheet of experimental apparatus.



independent variables (pressure).

$$Y = 9.19 + 7.29 x_2 \quad (3)$$

where Y is the yield of PEs and x_2 is the pressure.

It was observed that the calculated F factor for the regression was greater than the tabulated one, demonstrating that the model is representative and, because of that, a response surface can be obtained.

Figure 4 shows the response surface illustrating the extraction yield as a function of temperature and pressure.

Analysing the response surface generated by the first order model, it can be observed an optimized region at higher pressures. The data indicated that, at any temperature, the yield of the extraction and consequently PEs concentration will increase with pressure increase.

The results of efficiency and temperature and pressure influences on the PEs extraction cannot be compared with another work, because until now, it was not found in the literature works concerning the extraction of PEs from *Jatropha curcas* cake with supercritical carbon dioxide to compare data.

As the phorbol ester content in untreated seed cake is 1.97 mg/g and the percentage extracted was 23%, it can be concluded that the best operational condition (500 bar and 70°C) was not able to remove the

phorbol esters to a tolerable level of 0.11 mg/g according to Makkar and Becker [32]. Due to that, it was simulated a supercritical extraction process involving more than one batch to assure the total extraction of PEs, based on the best operational conditions obtained in the experimental step.

Economic evaluation

The better condition of temperature and pressure (500 bar and 70°C) was used to simulate the application of supercritical carbon dioxide in the PEs extraction until the cake was detoxified. A preliminary simulation using only one extractor was not sufficient and because of that, this study considered the industrial SFE containing 24 extractors (Figure 5), with a total of 7866 h of operation per year, cycle time of 60 hours which represents a total of 132 batch/year. The experimental data corroborated the experimental data.

The annual throughput of phorbol ester was 2.47 kg/yr and a concentration in PE of 0.11 mg/g could be reached with the simulation. The seed cake can be considered non-toxic with this level, confirming the efficiency of the methodology studied in this work.

Table 5 provides a summary of the overall material balances and material consumption of this process.

The direct fixed capital (DFC) refers to the fixed assets of an investment, such as plant and equipment. It is calculated at the process section level as the sum of direct, indirect and miscellaneous costs that are associated with a plant capital investment. The direct costs include cost elements that are directly related to an investment, such as the cost of equipment, process piping, instrumentation, buildings, facilities, etc. The indirect costs include costs that are indirectly related to an investment, such as the costs of engineering and construction. Additional costs such as the contractor's fee and contingencies are included in miscellaneous costs. In this study, the total DFC is around US\$ 98 million approximately and the individual cost items that contributed to the DFC are shown in Table 6.

Figure 6 shows the breakdown of the operating cost. The facility dependent cost is the most important item, accounting for 90% of the overall operating cost. In cases of designs, where there is no prior experience on the use of equipments, this is typically calculated as the sum of the costs associated with equipment maintenance, depreciation of the fixed capital cost, costs such as insurance, local taxes and possibly other overhead-type of factory expenses. Raw materials account for around 5.7% of the overall cost. Carbon dioxide is the most expensive raw material, accounting for 99.99% of the raw materials cost, comparing to the *Jatropha* press cake. Labour costs were based on the sum of the labour requirements for each unit procedure multiplied by a fixed labour rate (which was based on a basic labour rate, plus adjustments for fringe benefits, administration, and others). Supercritical fluid extraction is an environmentally safe technology, thus the cost of waste treatment was considered null.

Table 7 shows the results of the economic evaluation. For a plant of this capacity (42 L), the total capital investment is US\$ 108,417,000. Assuming a selling price of US\$ 212.85/mg, the project yields an after-tax internal rate of return (IRR) of 132.58% and a net present value (NPV) around US\$ 2 billion (assuming a discount interest of 7%). The NPV is an indicator of how much value an investment adds to the industry. In this case, NPV is positive, what means that the investment would add value to the industry and therefore the project is economically viable and may be accepted and implemented. Based on these results, this project represents an attractive investment. It is

Operational Conditions	70°C 100 bar	50°C 160 bar	90°C 160 bar	40°C 300 bar	70°C 300 bar	98°C 300 bar	50°C 440 bar	90°C 440 bar	70°C 500 bar
Yield (%)	0.65	2.76	2.01	2.35	3.83	2.12	7.97	7.25	9.33

Table 2: Yields of the extracts of *Jatropha* cake with supercritical CO₂.

Run	Temperature (°C)	Pressure (bar)	PE (mg/g cake)	Yields PE (%)
1	50	160	0.1639	8.32
2	90	160	0.0508	2.58
3	50	440	0.4536	23.03
4	90	440	0.3812	19.35
5	40	300	0.1761	8.94
6	98	300	0.1057	5.36
7	70	100	0.0640	3.25
8	70	500	0.4370	22.19
9	70	300	0.2230	11.32
10	70	300	0.1355	6.88
11	70	300	0.1860	9.44

Table 3: PEs yields from *Jatropha* cake extracted with supercritical CO₂.

Factors	Regression coefficient	Standard deviation	p - value	
Constant	9.19	1.29	0.0190	
(x ₁) Temperature (L)	-1.81	0.79	0.1483	
Temperature (Q)	-0.18	0.94	0.8654	
(x ₂) Pressure (L)	7.29	0.79	0.0115	
Pressure (Q)	2.62	0.94	0.1085	
Temperature by Pressure	0.51	1.11	0.6894	
ANOVA				
	Sum of squares	Degrees of freedom	Mean sum of squares	F-value
Regression	429.73	1	429.73	37.32
Residue	103.62	9	11.51	
Total	533.35	10		

Table 4: Coefficients of regression of the CCRD and analysis of variance.

Bulk Material	Annual Amount (kg)	kg/batch	Unit Cost (US\$)	Annual Cost (US\$)
Carbon Dioxide	70,831.00	536.60	19.76	1,399,625.00
<i>Jatropha</i> Cake	1,320.00	10.00	0.075	99.00

Table 5: Overall Balance and Material Consumption.

A. Total Plant Direct Cost (TPDC)	
1. Equipment Purchase Cost	13,009,000.00
2. Installation	6,361,000.00
3. Process Piping	4,033,000.00
4. Instrumentation	1,691,000.00
5. Insulation	390,000.00
6. Electricals	3,773,000.00
7. Buildings	781,000.00
8. Yard Improvement	1,301,000.00
9. Auxiliary Facilities	7,155,000.00
TPDC	38,494,000.00
B. Total Plant Indirect Cost (TPIC)	
10. Engineering	12,318,000.00
11. Construction	13,088,000.00
TPIC	25,406,000.00
C. Total Plant Cost (TPC = TPDC + TPIC)	
TPC	63,899,000.00
D. Contractor's Fee and Contingency	
12. Contractor's Fee	11,502,000.00
13. Contingency	23,004,000.00
CFC	34,506,000.00
E. Direct Fixed Capital Cost (DFC = TPC + CFC)	
DFC	98,405,000.00

Table 6: Fixed capital estimate summary (2014 prices US\$).

Direct Fixed Cost	98,405,000.00
Total Investment (US\$)	108,417,000.00
Operating Cost (US\$) /yr	24,572,000.00
Total Revenues (US\$) /yr	525,425,000.00
Cost Basis Annual Rate mg ester/yr	37,197.00
Unit Production Cost US\$/mg ester	660.60
Gross Margin (%)	95.32
Return On Investment (%)	285.35
Payback Time (years)	0.35
IRR After Taxes%	132.58
NPV at (7.00%) US\$	2,094,841,000.00

Table 7: Key economic evaluation results.

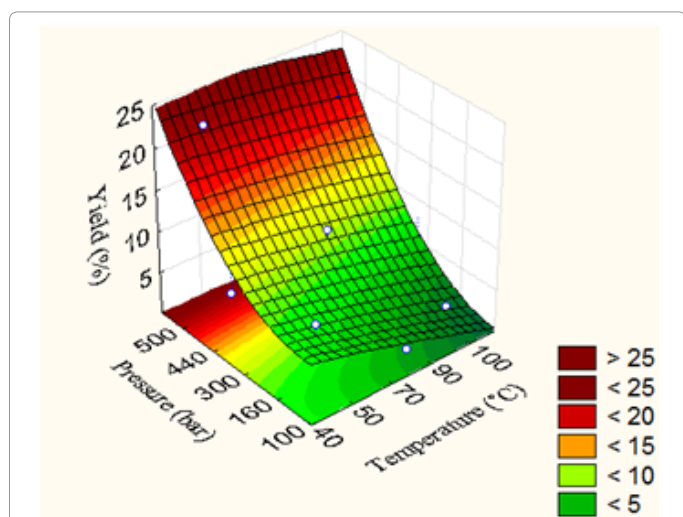


Figure 4: Response surface relating the PE concentration in function of temperature and pressure.

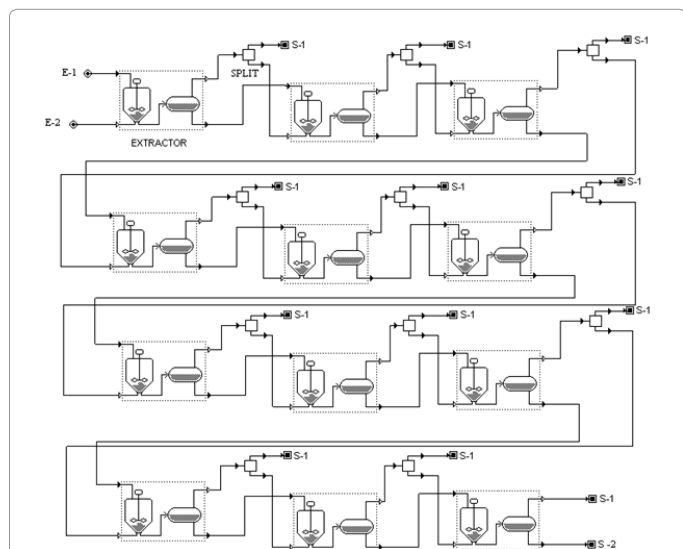


Figure 5: Process flowsheet built in Superpro Designer used for economic evaluation, E-1: *Jatropha* cake stream, E-2: CO₂ stream, S-1: extract stream, and S-2: extract stream.

important to note that this industrial plant can be used with other raw materials to extract different high aggregated components.

The analysis was based on the assumption that a new facility will be constructed for this process with a construction time of 30 months; start-up of 4 months and a 15 years project lifetime. The internal rate of return (IRR) is compared to the minimum attractive rate of return (MARR) or the cost of capital of the company. The decision criteria for accepting the project is to have an IRR greater than or equal to the minimum acceptable [20]. The criterion for evaluating the IRR in this study was that this is equal to or greater than 25% per year. A sensitivity study varying the selling price of phorbol ester in relation to total costs was conducted. The influences of the price in internal rate of return on total revenue and payback time are presented in Figure 7.

Figure 8 show the costs and profits in function of the rate of the production for phorbol ester. The break-even point was calculated in function of the direct fixed cost, variable costs and revenue. The break-even point was situated in the figure at, approximately, 20% of the rate of production. It means that if the plant is operated above this rate of production, the profits are higher than the total costs of production.

The Superpro Designer was also used to simulate the operating cost of the cake using chemical treatment described by Guedes et al. [10], to compare the process costs. The detoxification of seed cake was obtained with soxhlet apparatus, reducing the PE content in 97.30% (0.10 mg/g) using methanol, extraction time of 8 hr and solute/solvent ratio of 1:10 (w/v). Soxhlet simulation considered an industrial setup with three extractors of 42 L. The results showed that the Soxhlet extraction presented lowest operating cost US\$ 16,195,000.00/yr

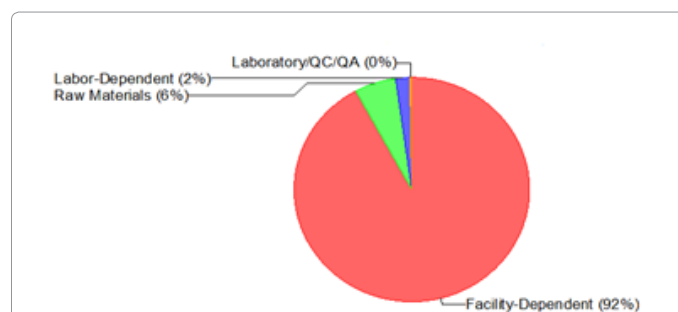


Figure 6: Breakdown of manufacturing cost for PEs extraction using supercritical CO₂.

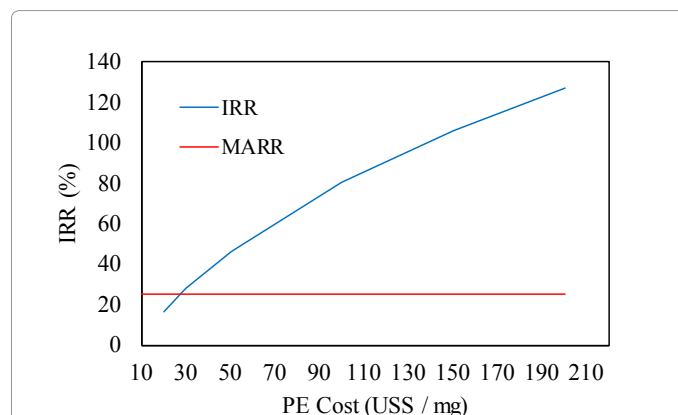


Figure 7: Influence of cost PE on the internal rate of return.

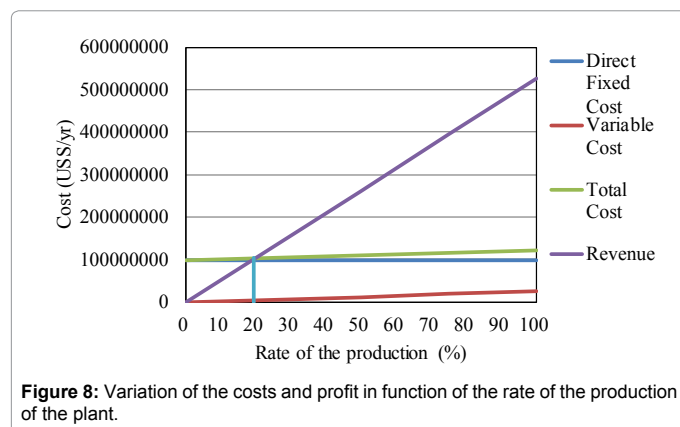


Figure 8: Variation of the costs and profit in function of the rate of the production of the plant.

compared with the operating cost of the supercritical fluid extraction (US\$ 24,572,000.00/yr). Probably, this behaviour is associated with the number of extractors used in the processes. On the other hand, the carbon dioxide in supercritical state is a promising solvent for green chemical processes due to its characteristics like non-toxicity, no flammability and non-explosiveness. Moreover, perhaps, it will be necessary more separation steps, using organic solvent, to eliminate the presence of solvent in the cake, able to be used in animal feed.

Conclusion

It was studied the technical evaluation of supercritical fluid to extract PEs from *Jatropha curcas* cake. The experiments were done applying a statistical experimental planning, showing that the best results occurred at 70°C and 500 bar with the yield of 9.33%. It was observed that the yield increases with increasing pressure and a statistical analysis corroborated this behavior. The supercritical fluid extraction was very effective in the recuperation of the PE from *Jatropha curcas* cake around 23.0%. The economic evaluation indicates that the process is economically viable and can be implemented to obtain detoxified cake and add value to phorbol ester.

Acknowledgement

The authors would like to thank Embrapa Agroenergy (Brasília, Brazil), for the analysis and FAPERJ for the financial support.

References

- Ceasar SA, Ignacimuthu S (2011) Applications of biotechnology and biochemical engineering for the development of *Jatropha* and biodiesel: a review. *Renew Sustain Energy Rev* 15: 5176-5185.
- Kootstra AMJ, Beertink HH, Scott ES, Sanders JPM (2011) Valorization of *Jatropha curcas*: solubilization of proteins and sugars from the NaOH extracted de-oiled press cake. *Industrial Crops and Products* 34: 972-978.
- Herrera JM, Martinez CJ, Ayala AM, Siciliano LG, Escobedo RM, et al. (2012) Evaluation of the Nutritional Quality of Nontoxic Kernel Flour From *Jatropha Curcas* in rats. *J Food Qual* 35: 152-158.
- Makkar HPS, Becker K, Sporer F, Wink M (1997) Studies of nutritive potential and toxic constituents of different provenances of *Jatropha curcas*. *J Agric Food Chem* 45: 3152-3157.
- Haas W, Sterk H, Mittelbach M (2002) Novel 12-deoxy-16-hydroxyphorbol diesters isolated from the seed oil of *Jatropha curcas*. *J Nat Prod* 65: 1434-1440.
- Devappa RK, Makkar HPS, Becker K (2010) Optimization of conditions for the extraction of phorbol esters from *Jatropha* oil. *Biomass Bioenergy* 34: 1125-1133.
- Joshi C, Mathur P, Khare SK (2011) Degradation of phorbol esters by *Pseudomonas aeruginosa* PseA during solid-state fermentation of deoiled *Jatropha curcas* seed cake. *Bioresour Technol* 102: 4815-4819.
- Xiao J, Zhang H, Niu L, Wang X, Lu X (2011) Evaluation of detoxification methods on toxic and antinutritional composition and nutritional quality of proteins in *Jatropha curcas* meal. *J Agric Food Chem* 59: 4040-4044.
- Phasukarratchai N, Tontayakom V, Tongcumpou C (2012) Reduction of phorbol esters in *Jatropha curcas* L. pressed meal by surfactant solutions extraction. *Biomass and Bioenergy* 45: 48-56.
- Guedes RE, Cruz FDA, Lima MCD, Sant'Ana LDO, Castro RN, et al. (2014) Detoxification of *Jatropha curcas* seed cake using chemical treatment: Analysis with a central composite rotatable design. *Industrial Crops and Products* 52: 537-543.
- Joshi C, Khare SK (2011) Utilization of deoiled *Jatropha curcas* seed cake for production of xylanase from thermophilic *Scytalidium thermophilum*. *Bioresour Technol* 102: 1722-1726.
- Devappa RK, Malakar CC, Makkar HP, Becker K (2013) Pharmaceutical potential of phorbol esters from *Jatropha curcas* oil. *Nat Prod Res* 27: 1459-1462.
- Roach JS, Devappa RK, Makkar HP, Becker K (2012) Isolation, stability and bioactivity of *Jatropha curcas* phorbol esters. *Fitoterapia* 83: 586-592.
- Wender PA, Kee JM, Warrington JM (2008) Practical synthesis of prostratin, DPP, and their analogs, adjuvant leads against latent HIV. *Science* 320: 649-652.
- Devappa RK, Angulo-Escalante MA, Makkar HPS, Becker K (2012) Potential of using phorbol esters as an insecticide against *Spodoptera frugiperda*. *Industrial Crops and Products* 38: 50-53.
- Aregheore EM, Becker K, Makkar HPS (2003) Detoxification of a toxic variety of *Jatropha curcas* using heat and chemical treatments, and preliminary nutritional evaluation with rats. *South Pac J Nat Sci* 21: 51-56.
- Martinez-Herrera J, Siddhuraju P, Francis G, Davila-Ortiz G, Becker K (2006) Chemical composition, toxic/antimetabolite constituents, and effects of different treatments on their levels, in four provenances of *Jatropha curcas* L from Mexico. *Food Chem* 96: 80-89.
- Machida H, Takesue M, Smith Jr RL (2011) Green chemical processes with supercritical fluids: Properties, materials, separations and energy. *The Journal of Supercritical Fluids* 60: 2-15.
- Brunner G (1994) Gas extraction: an introduction to fundamentals of supercritical fluids and the application to separation process.
- Mendes MF, Pessoa FLP, Uller AMC (2005) Optimization of the process of concentration of vitamin E from DDSO using supercritical CO₂. *Brazilian Journal of Chemical Engineering* 22: 83.
- Makkar H, Maes J, De Greyt W, Becker K (2009) Removal and degradation of phorbol esters during pre-treatment and transesterification of *Jatropha curcas* oil. *Journal of the American Oil Chemists' Society* 86: 173-181.
- Moraes MN, Zabot GL, Meireles MAA (2014) Applications of Supercritical Fluids in Latin America: Past, Present and Future Trends. *Food and Public Health* 4: 162-179.
- Cavalcanti VMS, Aznar M, Melo SAV, Cruz FG (2013) Economic evaluation based on an experimental study of extraction of alkylamides from *Spilanthes* genera using supercritical CO₂. In: 10th Conference on Supercritical Fluids and Their Applications 1: 476.
- Prado JM, Assis AR, Maróstica-Júnior MR, Meireles MAA (2010) Manufacturing cost of supercritical-extracted oils and carotenoids from amazonian plants. *J Food Process Eng* 33: 348-369.
- Prado JM, Dalmolin I, Carareto NDD, Basso RC, Meireles MAA, et al. (2012) Supercritical fluid extraction of grape seed: process scale-up, extract chemical composition and economic evaluation. *J Food Engineering* 109: 249-257.
- Delgado B, Pessoa FLP (2014) Simulation of butanol production by an integrated fermentation process using supercritical fluid extraction with carbon dioxide. *New Biotechnology*.
- Peters MS, Timmerhaus KD (1991) Plant design and economics for chemical engineers. McGraw-Hill, USA.
- Turton R, Bailie RC, Whiting WB, Shaeiwitz JA (2003) Analysis, Synthesis, and Design of Chemical Processes. Prentice Hall, USA.
- Prado IM, Albuquerque CLC, Cavalcanti RN, Meireles MAA (2009) Use of commercial process simulator to estimate the cost of manufacturing (COM) of carotenoids obtained via supercritical technology from palm and buriti trees. 9th International Symposium on Supercritical Fluids, Arcachon, France.
- Sriram S (2012) Potential of New *Jatropha* Varieties.
- Sigma Aldrich.
- Makkar HP, Becker K (1999) Nutritional studies on rats and fish (carp *Cyprinus carpio*) fed diets containing unheated and heated *Jatropha curcas* meal of a non-toxic provenance. *Plant Foods Hum Nutr* 53: 183-192.