EXTRACTION OF BASIL OIL (Ocimum basilicum L.) USING SUPERCRITICAL FLUID

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Abstract - Among the aromatic herbs, basil (Ocimum basilicum L) has economic importance in Brazil, and its consumption both in nature is used for industrial processing. The essential oil is valued in the international market and widely used in the industries of condiments, cosmetics and medicinal. The extract obtained can be using conventional methods (hydrodistillation, steam distillation, solvent extraction, employing Soxhlet) and unconventional one, using supercritical fluid extraction. The optimization of the operating conditions such as temperature and pressure, involves high rate of mass transfer, which added to increase the density of CO_2 is a good alternative of this technology in the extraction, so that it is highly selective in obtaining specific substances. After analysis it was concluded that the best performance was obtained by Soxhlet extraction (2.39) followed by the SC-CO_2 (0.43). The use of CCRD in the process of extraction of essential oil of basil was efficient because it showed that it is possible to optimize the extraction process. The technique of DPPH used to evaluate the antioxidant activity confirmed that the basil oil had 83 % of activity. It could be concluded that the use of different process conditions caused the antioxidant activity reduction in 56 % of the samples.

Keywords: optimization, carbon dioxide, experimental design, pressure.

1. Introduction

Among the aromatic herbs, basil (Ocimum basilicum L – Figure 1) has economic importance in Brazil, and its consumption occurs both in nature and added for industrial processing. The essential oil is valued in the international market and widely used in condiments, cosmetics and medicinal industries of [1].

Figure 1. Illustration of Ocimum basilicum L.

The Basil oil has high economic value due to the presence of specific substances such as estragol, lineol, linalool, eugenol, methyl cinamato, limonene and geraniol [2].

Studies of the oils from aromatic and medicinal plants is growing because these species are known specifically for possessing biological activity, such as antibacterial, antifungal and antioxidant properties [3].

The antioxidants can be classified as any substance, present in low concentration, compared with a oxidizable substrate, delays or inhibits oxidation of the substrate effectively [4].

Essential oils extracted from the plants are in a complex mixture of terpenes, sesquiterpenes, oxygenated derivatives and other aromatic compounds. These components are characteristic for basil aroma, which are precursors to the presence of 1,8-cineole, methyl cinnamate, methyl cavicol and linalool. In general, these substances are volatile and are present at low concentrations [5].

However, these molecules are extremely sensitive to heat and due to it, they are subject to chemical changes, and may occurs some losses of volatile compounds in higher or lower concentrations, depending on the method used in the extraction process, resulting in low yields due to the degradation of esters of unsaturated compounds coming hydrolytic or thermal effects [6].

Several methods can be used to essential oils extraction, like the conventional methods (hydrodistillation, steam distillation, solvent extraction, employing Soxhlet) and an unconventional one, using supercritical fluid extraction. However, the choice of each technique depends on the objective to be achieved by research. Among all processes that can be used in oil extraction, the supercritical fluid extraction method is selective and considered clean because it is possible to obtain pure extracts free of organic solvents [7]

The optimization of the operational conditions such as temperature and pressure, involves high rate of mass transfer to produce a selective extract with high yields. In most studies, low temperature are employed to preserve the thermo-sensitive compounds that influence the yield of extract [8, 9].

Therefore, the objective of this study was to compare the SFE efficiency in basil oil extraction with the conventional methods (hydrodistillation and soxhlet) using a central composite rotational design (CCRD), and study the influence of supercritical fluid on the antioxidant activity of the extracts.

2. Materials and Methods

2.1. Materials

The raw material used in this analysis was basil (Ocimum basilicum L), acquired in CEASA-RJ, in August 2012, coming from the mountain region of Teresopolis, Rio de Janeiro.

Carbon dioxide (CO_2) was from White Martins S.A. (Rio de Janeiro) with 99.995 of minimum purity.

Hexane from Vetec Química Fina Ltda. (Rio de Janeiro, Brazil) and distilled water were used in the conventional methods to extract the oil.

2.2. Experimental Procedure

Preparation of raw material. The leaves were dried in order to reduce the initial moisture content (90 % of humidity), in an oven with air renewal and circulation. The drying temperature was 50 °C and was kept constant for 5 hours until constant moisture.

After drying, the sheets were grinded in order to increase the contact surface reducing the resistance to oil extraction. The dried leaves were placed in sealed plastic bags, protected from light and moisture, and stored in a refrigerator with low humidity.

Soxhlet Extraction. The extraction process using soxhlet was conducted to determine the percentage of oil in the raw material used. The extractions were performed in a triplicate way. It was used, approximately 5 g of basil, with 200 mL of hexane. The extraction time was fixed in 4 hours, after reaching the boiling temperature around 69 °C**.**

Hydrodistillation Extraction. The extraction was done in a Clevenger apparatus, coupled to a bottom flask of 500 mL. It was added 30 g of crushed leaves of basil and 300 mL of water into the flask. The extraction time was fixed at 4 hours. The extract oil was diluted in hexane and filtered after separation. Then, it was dried using Na_2SO_4 to remove the water and after that, the solutions were separated by rotaevaporador.

Supercritical carbon dioxide extraction. For this study, it was used a supercritical extraction unit to determine the total yield and the kinetics of extraction. The unit consists of an stainless steel 316S extractor with 42 mL of capacity and 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 pressure measurement. The flowsheet of the experimental apparatus is shown in Figure 2.

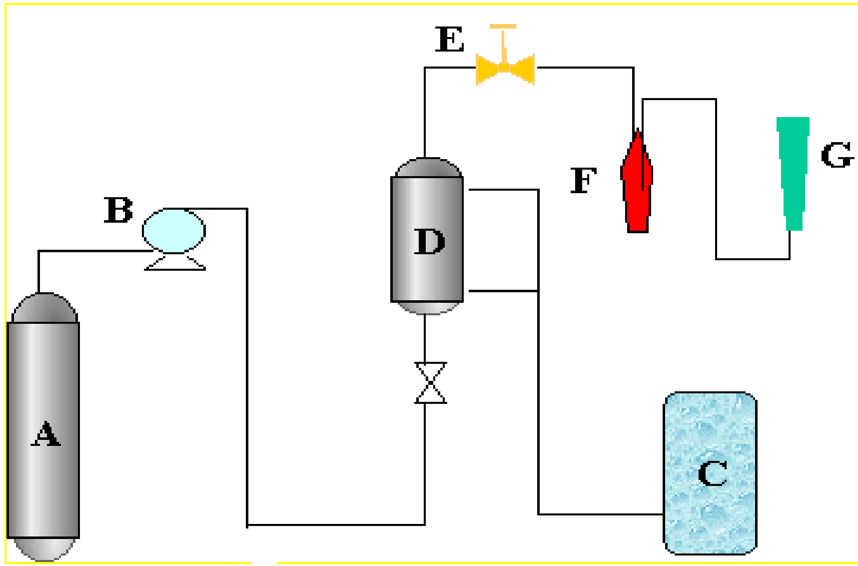

Figure 2. Flowsheet of the experimental apparatus [10,11,12,13], where A. Cylinder of CO_2; B. High-pressure pump; C. Healting bath; D. Extractor; E. Micrometric valve; F. Sample; G. Flowmeter.

The extractions were carried out with approximately 4.5 g of material. The CO_2 pumped into the extractor remained in contact with herbaceous matrix about 20 min before starting the extractions. The extracts were collected in test tubes by opening the micrometric valve located at the outlet of the extractor. The basil extraction fractions were collected in vials at intervals of 10 minutes and weighed; the values were used to calculate the mass of extract. The yield was calculated as the ratio of extracted oil and oil-free mass fed, using Equation 1:

$$Yield\ \% = 100\,\frac{extracted\ solute\ mass}{solute - free\ mass} \tag{1}$$

The solute-free mass means the difference between the sample mass and the extracted mass.
The process yield using soxhlet and hydrodistillation was determined using Equation 2,

$$Yield\ \% = \frac{kg\ of\ extract}{kg\ of\ initial\ oil\ content}\ x\,100 \tag{2}$$

The extractions were carried out at constant temperature and pressure for 4 h. The flow of carbon dioxide was kept constant at 16.4 mL/min for all experiments. The temperature range investigated was 30 to 50 °C and pressures between 100 and 300 bar.

2.3. Experimental Planning

The study of the extraction of basil essential oil was performed using a CCRD 2^2, with two variables, three central points, four axial points, totaling eleven essays, considering the significance level of 95 %. The selected variables were temperature and pressure, represented by X1 and X2 (dimensionless variables coded). Table 1 correlates coded and actual values of each variable. Three replicates at the central point of the design were performed to allow the estimation of pure error (Tests 9, 10 and 11).

Table 1. Levels of coded and actual variables.

Essays	Temperature		Pressure	
	Cod.	**Real (ºC)**	**Cod.**	**Real (bar)**
1	-1	(33)	-1	(129)
2	1	(47)	-1	(129)
3	-1	(33)	1	(271)
4	1	(47)	1	(271)
5	-1,41	(30)	0	(200)
6	+1,41	(50)	0	(200)
7	0	(40)	-1,41	(100)
8	0	(40)	+1,41	(300)
9	0	(40)	0	(200)
10	0	(40)	0	(200)
11	0	(40)	0	(200)

2.4. Analysis of the total antioxidant activity by DPPH capture free radical on essential oil of basil (Ocimum basilicum L)

This method was modified by [14] for measuring the kinetic parameters and adapted by [15]. The technique is based on electron transfer where, by the action of an antioxidant (HA) or a species radical, DPPH that has the purple color formed is reduced diphenyl-picryl-hydrazine, of yellow color, with consequent disappearance of absorption the same being monitored by the decrease in absorbance at 515 nm. From the results it was determined the percentage of antioxidant or free radical scavenging.

On preparation of the solution of DPPH was used 1 ml of DPPH, was added to the solution in a 10 ml flask and completed with methanol 1/10 ml. The solution was used to reading in the spectrophotometer and the remainder reserved.

It was used 1 mL of dichloromethane in the extracts obtained from supercritical fluid extraction. The solution was placed in an ultrasound apparatus while maintaining the sample under stirring for 10 min. Then this solution was added into a flask with 3 mL of dichloromethane. This procedure was used for 9 samples obtained under different conditions of extraction of essential oil of basil by supercritical CO_2.

From the extract obtained in the preceding item in the absence of light, it was transferred an aliquot of 0.15 mL of each dilution of the extract to the test tubes in duplicate with 2.85 mL of DPPH. The solution was homogenized into tubes stirrer for approximately 2 min.

A negative control was made by adding 0.15 mL of dichloromethane and 2.85 mL of DPPH. Dichloromethane was used to make the standard sample to calibrate the spectrophotometer. Samples were kept in dark, for 60 min after starting the procedure of reading the absorbance in a spectrophotometer at 515 nm. The ability to eliminate DPPH (antioxidant activity) was calculated using the following Equation 3

$$AA\,\% = \left(\left(\frac{\text{Abs sample}}{\text{Abs DPPH}} - Abs\ white\right) X100\right) \quad (3)$$

3. Results and discussion

3.1. Supercritical Carbon Dioxide Extraction

Table 2 shows the yields obtained from supercritical fluid extraction. Equation 1 was used to calulate the yield values of experimental performance.

The best results were observed at 33 º C and 271 bar, 50 º C and 200 bar, 40 º C and 200 bar equal to 0.43 and the yield was lower than 47 º C and 271 bar (0.32). Taking into account only the conditions employed, the maximum yield of essential oil of basil (Ocinum Selloi) was also obtained at 200 bar and 40 ° C, similar to operating condition found in this work [16].

Table 2. Experimental yields obtained in the basil oil extraction with supercritical fluid

Essays	Temperature (ºC)	Pressure (bar)	Yield (%)
1	33	129	0,34
2	47	129	0,38
3	33	271	0,43
4	47	271	0,32
5	30	200	0,41
6	50	200	0,43
7	40	100	0,38
8	40	300	0,37
9	40	200	0,43
10	40	200	0,43
11	40	200	0,38
standard deviation	-	-	0,038

Among the methods used to extract the basil oil, Table 3 shows the best performance obtained with each method applied. Comparing all the technologies studied, hydrodistillation had the lowest yield (0.26%), since the results with Soxhlet (hexane as a solvent), provided yields of around 2.39%, higher than found with SC-CO_2 (0.43%). This behavior can be explained by the CO_2 in supercritical state (the low polarity) be less effective for the extraction of more polar compounds from natural sources.

Table 3. Yield values of basil essential oil extracted by different processes.

Extraction	Temperature (°C)	Pressure (bar)	Yield (%)
SC-CO2	33	271	0.43
Soxhlet	69	-----	2.39
hydrodistillation	100	-----	0.26

The yield obtained in supercritical fluid extraction was higher than that found in hydrodistillation. This can be explained by the high temperature steam distillation, which can result in degradation volatiles, causing a decrease in throughput. Besides the volatile compounds at low efficiency can also be linked oil composition rich in apolar substances and high molecular weight, not being extracted by water.

The used solvent in extraction with Soxhlet can have result in the extraction the polar or no polar compounds, favoring the increase or decrease in income. In SC-CO_2 extraction, the operational conditions controlling could provide a lower yield, but a higher selectivity towards compounds extracted. This behavior was also observed by [16] in the extraction of *Artemisia annua* oil through the same extraction methods. It was obtained a lower yield in hydrodistillation (0.49%), 5.27% using SC-CO_2, and 7.28% using Soxhlet [17]. using SC-CO_2, in 1 hour extraction, found lower yield than found in this study, and 0.0044 g of extracted essential oil.

3.2. Results of experimental design

As described in Table 4, it can be observed that only the mean and the interaction of two variables (X1 and X2) were statistically significant at 95% of significance ($p<0.05$). Thus, it was validated by the values analysis shown in Table 5.

The variance analysis (ANOVA) done for the tests of the planning CCRD, show the non-statistically significant parameters (Table 5).

It was observed that the percentage of variation was explained by the model fit (R^2 = 69.1) and the calculated F factor for the regression was greater than tabled F factor, demonstrating that the model is representative and response surface can be obtained. According to the model, the response surface described in Figure 3 determines the regions of maximum extraction of essential oil of basil.

Table 4. Regression coefficient and standard deviations of supercritical extraction results.

Factors	Regression coefficient	standard deviation	t (5)	p-value
Média *	0,41	0,02	24,26	0,00
(X1)Temperature (L)	ns	0,01	-0,37	0,72
(X1)Temperature (Q)	ns	0,01	-0,40	0,71
(X2) Pressure (L)	ns	0,01	0,14	0,89
(X2) Pressure (Q)	-0,02	0,01	-2,04	0,09
Temp. X Pressure *	-0,04	0,01	-2,61	0,05

ns-not statistically significant to 95 % significance.
*Statistically significant factors for 95 % significance.

Table 5. Analysis of variance of CCRD (2 ²) for the yield of basil essential oil extraction

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	$F_{calculado}$	p-value
Regression	0,0061	1	0,0061		
Residue	0,0083	9	0,0009	6,8	0,0499
Total	0,0141	10			

Percentage of variance explained (R^2) 69,1% F_{tab} 0,05; 1; 9 5,12

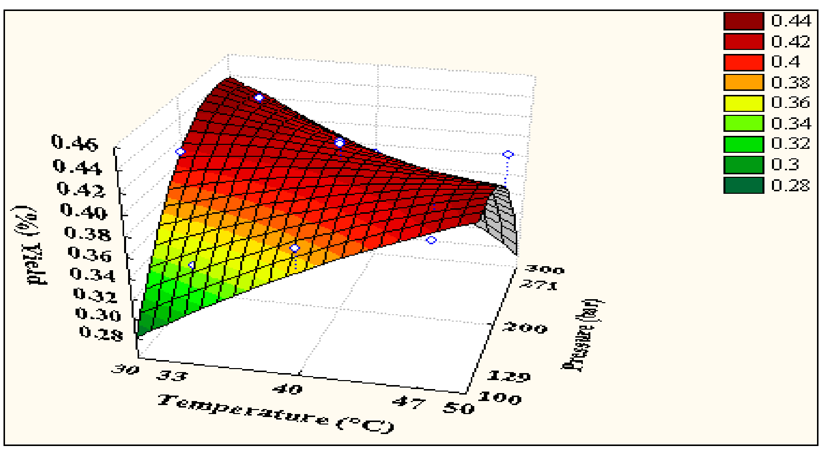

Figure 3. Chart on yield and response surface of basil essential oil.

It was verified by analyzing the response surface from Figure 3, two better regions for extraction; the first is in the temperature range of 30 to 33 ° C and pressure from 271 to 300 bar. This implies considering that, under conditions of low temperatures and high pressures achieved a good result in the extraction of essential oil of basil; addition, low temperatures preserve the presence of aromatic compounds in the extracts.

The second region is in the range of 45 to 50 ° C and 100-129 bar. For these thermal conditions, the density of the solvent that increases as the temperature rises, resulting in an increase in the solvating power of CO_2, and consequently in higher overall yield. [18] through extraction by supercritical CO_2 volatiles from spices (basil and oregano) has proved that this method is suitable for volatile components yields of volatiles were obtained at 40 º C and a pressure of 120 bar. It was found in this analysis that to optimize the extraction process and increase throughput, it is necessary to combine low temperatures at high pressures or high temperatures at low pressures.

It was mentioned by Menakera et al. [17] that the best conditions of temperature, using supercritical CO_2, varies from 40 to 50 °C, as evidenced in this study and corroborates the behavior observed.

3.3. Evaluation of results of antioxidant activity for DPPH.

The results of the analyzes using the DPPH method, showed that all the extracts obtained, with SC-CO_2, have antioxidant activity of an average of 83 % of activity.

Kwee et al. [19] evaluated the antioxidant activity of 15 species of Ocimum basilicum through the FRAP and DPPH methods, and found some differences in antioxidant capacity ranging from 0.28 to 11.5 g/mµg. Also, using the methodology of DPPH, it was found 0.49% of antioxidant activity by [20].

Table 6 presents the different conditions applied in supercritical extraction processes, and results of the averages found in the analysis using the program Sisvar 5.1 using the Skott-Knott test. It was evaluated whether operating conditions interfered the antioxidant activities of the extracts.

Table 6. Evaluation of antioxidant activity of extracts of basil as a function of operating conditions (temperature and pressure) through the DPPH assay.

Tratamentos	Condições	Médias
T6	47 ºC-129 bar	0.0570^{b}
T7	50 ºC-200 bar	0.0795^{b}
T4	30 ºC-200 bar	0.0810^{b}
T5	40 ºC-200 bar	0.0815^{b}
T8	33 ºC-271 bar	0.0875^{b}
T9	47 ºC-271 bar	0.1055^{a}
T2	33 ºC-129 bar	0.1205^{a}
T1	40 ºC-100 bar	0.1240^{a}
T3	40 ºC-300 bar	0.1465^{a}

Treatment means with different letters differ significantly at Skott-Knott test (p 0.05).

The result of this analysis showed that the use of different process conditions caused reduction of antioxidant activity in 56% of samples. There was no statistically difference between the percentage of antioxidant activity among treatments ranging from T6 to T8 for ranging from T9 to T3. It was noted that among the conditions for best activity antioxidants not occurred statistical difference between the values obtained in conditions of 40 °C and 100 bar, 40 °C and 300 bar and 33 °C and 129 bar. This information is of great importance to choose the best option for the extraction of essential oil of basil, in the case of raw materials with volatile substances and cost savings for operating conditions.

4. Conclusion

It can be concluded that the best performance was obtained using Soxhlet extraction (2.39%) followed by the SC-CO2 (0.43%). The use of DCCR in the process of basil essential oil extraction was efficient because it was shown that it is possible to optimize the extraction process according to the response shown in the chart surface. It is necessary to increase the pressure and decrease the temperature or decrease the pressure and temperature increase to obtain higher yields.

The technique of DPPH used to evaluate the antioxidant activity confirmed that the basil oil has 83% of activity. It was showed that the use of different process conditions, caused reduction of antioxidant activity in 56% of samples. There is no statistically difference between the values of antioxidant activity obtained at40 °C and 100 bar, 40 °C and 300 bar and 33 °C and 129 bar.

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