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Effect of Supercritical Process Parameters on Phenolic Compound

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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Original Research Article

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ABSTRACT

Carica papaya belonging to the Caricaceae family is an effective medicinal herb that is used as a folk medicine for the treatment of various diseases throughout the world. The present study was designed for evaluation of bioactive compound of dried papaya leaves extract received in supercritical fluid extraction method with special emphasis to the biochemical compound like phenol as well as the effect of various parameters like temperature, pressure, time and particle size on the phenol content. Maximunm phenol amount 1868.25 mg/100 gm was obtained at the at the 70°C and 250 bar pressure and minimum phenol 508.20 mg/100 gm was found at 70°C with 100 bar pressure in the supercritical fluid extraction method.

Keywords: Carica papaya; phenol; supercritical extraction method.

1. INTRODUCTION

The papaya (*Carica papaya Linn.*) is native of America and condition for its growth in tropical region. It is a large plant, like a tree, without branches it is a herbaceous plant because the stem does not have much wood and remains soft and green until its death. The single stem grows from 5 to 10 m tall with all the leaves on the top and large, deeply lobed, palmate, with long petiole; 50–70 cm wide. Papaya grows best in a well-drained, well aerated and rich organic matter soil, pH 5.5 – 6.7 while water logging of soils often results in the death of tress within 3-4 days. Papaya is grown extensively through India and in terms of area and production, Andhra Pradesh ranks first followed by Gujarat. Total area of India is 149 thousand hector and 6050

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thousand metric tonne in 2019-20. Total area of crops in Gujarat during 2020-2021 was estimated 18.189 thousand hectors and the production was about 1107.880 thousand metric tonnes.

Papaya leaf has a numberless of benefits for dengue fever, white blood cells and platelets can be increased by using the papaya leaf juice and that also helps normalizing the blood clotting, prevents the damage of cancer i.e. cancer cell growth inhibition. The production of key signalling molecules called Th1-type cytokines can be boosted through the papaya leaf juice and also helps to regulate the immune system, antimalarial and antiplasmodial activity. Papaya leaves are useful for malaria, and for digestion. Chemical compounds of carpain is available in the papaya leaves, the carpain and microorganisms which often interfere with the digestive function are killed using the carpain. Papaya leaves possess the additional benefits like an acne medicine, increase appetite, easemenstrual pain, meat tenderizer and relieve nausea [1].

Phenolic compounds is having biological properties like antidiabetic, antioxidant, gastroprotective, anti-inflammatory, spasmolytic, antimicrobial, anticarcinogenic [2,3]. Antiseptic, disinfectant [4], hepato-protective, hypotensive and cardio protective [5]. It has influence on chronical and degenerative diseases and different types of cancer [6,7]. The plant materials of the papaya are having the phenol contents, due to this compound in the plant material which determines their use in the pharmaceutical industries, in various areas of cosmetic and food. Different groups of polyphenols are present in the different sources of plant material [8].

Super fluid extraction method is often used for extraction of bioactive compound, due to low density, high diffusivity and viscosity of the super critical carbon dioxide gas, the super critical extraction method becomes the alternative to the conventional extraction methods such as soxhlet extraction method, Moreover, carbon dioxide has no side effect on the environment as it is non-flammable and it is “green” gas, The influence of the pressure on the solubility of desired compounds may increase the extraction efficiency. When the pressure and temperature are optimized, the carbon dioxide may be more preferable. There is no chance of mixing of carbon dioxide in the final product received from super critical as carbon dioxide can be removed by depressurizing during the collection of extracts [8].

When the fluid (most often carbon dioxide) is brought to the desirable combination of the pressure and temperature, which allows it to possess the super critical properties for the selective extraction of the fat. Under the controlled conditions of time, temperature, and pressure, the sample is exposed to the supercritical fluid for allowing the dissolution of the fat from the sample in super critical fluid extraction system. The dissolved fat will be then separated from the supercritical solvent by a significant drop in solution pressure [9].

Supercritical fluids have been used as solvents for a wide variety of applications such as essential oil extraction, metal cation extraction, polymer synthesis and particle nucleation. Most of the analytical supercritical extraction system is used for the extraction of fat or bioactive compound with the carbon dioxide having several benefits. Carbon dioxides is having relatively low cost, high purity, nontoxic and non-flammable properties. Carbon dioxide can be removed easily from the extract and is best for the lipophilic compound. It is having relatively low critical pressure and temperature [10].

2. MATERIALS AND METHODS

Fresh papaya leaves (Mandhubindu cv.) were taken from the plants grown in the Horticulture Farm of College of the Horticulture, Anand Agricultural University, Anand. Papaya leaves washed and thoroughly cleaned with tap water to remove soil and dust particles if any attached to it after hand picking. The soft stems were removed of papaya leaves and cut into pieces for further drying process.

Drying experiments were performed in a cabinet type laboratory hot air tray dryer manufactured by Navrang Scientific Works Pvt. Ltd., New Delhi and fitted with manually controlled digital thermostat, PT-100 thermocouple, a blower driven by 0.4 hp motor.. The dryer was adjusted to the selected temperature (50°C) for about half an hour before the start of experiment to achieve the steady state condition. Air velocity was set at 1.0 m/s and maintained by adjustable flap throughout drying time and measured by digital anemometer. Then 100 g of untreated samples of papaya leaves were uniformly spread in the tray.

The moisture loss from the papaya leaves was recorded at every 15 minute interval at 50°C temperature during drying using top pan digital weighing balance. The drying process was stopped when the final moisture content reached to about 4-6% (db). The product was then cooled for 10 minutes after drying and packed in Aluminium laminated pouch bags. All the experiments were conducted in triplicate for each air temperature and pre-treatment. The average values are reported.

Extraction of bioactive compound from papaya leaves powder was experimentally done using supercritical fluid extraction assembly. Carbon dioxide was pressurized with a high-pressure pump and then charged into the extraction vessel to desired pressure. The extraction vessel was packed with dried and sieved papaya leaves. Polypropylene wool and frits were used for proper packing and empty space was filled with glass beads. The extraction vessel was heated in the oven and its temperature was controlled by a thermocouple. The supercritical CO_2 with dissolved compounds passed through a heated micrometre valve, and was subsequently expanded at ambient pressure and temperature leaving the extract in a glass vial.

Surface response method was used to get the combition of temperature (40-70°C), pressure (100-250 bar), time (30-120 min) and particle size of dried papaya leaves (0.5-2.00) for the extraction.

2.1 Phenol Content

Total phenol from the papaya leaves extract was estimated by the method as described by Sadasivam and Manickam, [11] 1g of sample was homogenized in 80% methanol using mortar and pestle and the final volume was made to 10 mL. The content was refluxed for two hours on boiling water bath at 65° C. Supernatant was collected and the residue was re-extracted twice with 80% methanol. All supernatants were combined, and the final volume was made to 10 mL. The extract was used for the assay of total phenol. Aliquot 0.2 mL was taken and made the final volume 1.0 mL with distilled water. For standard separate test tubes. 0.2, 0.4, 0.6, 0.8, and 1 mL of the working standard solution were pipetted out into a series of test tubes and made the final volume 1.0 mL with distilled water. To this add 1 mL of Folin–Cio catechol reagent and after 3 min 2 mL of 20% Na_2CO_3 was added, and the tubes were incubated at room temperature for 30 min and made the total volume of 5 mL with distilled water. The absorbance was measured at 650 nm. Phenol content was calculated from the standard curve prepared from catechol as standard.

$$\text{Phenol (\%)} = \frac{\text{Reading x Graphfactor x Totalvolumex } 10^{-6}}{\text{TakenvolumexSampleweigt(g)}}$$

Fig. 1. Super critical fluid extractor

3. RESULTS AND DISCUSSION

Experiments on supercritical fluid extraction (SFE) of papaya leaves powder were conducted. Phenol was analysed from the extracted yield of papaya leaves.

The data obtained for all experiments are tabulated in Table 1. It was observed that phenol was greatly influenced by pressure, dynamic time and less with temperature, particle size.

The phenol of papaya leaves powder extract varied from 1868.25 to 508.20 mg/100 gm (Table 1). The maximum phenol was obtained at 70 °C and 250 bar pressure and minimum phenol was found at 70 °C with 100 bar pressure. Central Composite Design was employed to evaluate the combined effects of different parameters such as temperature, pressure, dynamic time and particle size.

The analysis of variances was conducted on experimental data and the significance of temperature, pressure, dynamic time and particle size as well as their interactions on phenol, was estimated. The quadratic model was fitted to the experimental data and statistical significance of linear, quadratic and interaction effects were calculated for each response.

The Model F-value of 7.19 implies the model is significant in the case of phenol. There is only a 0.02% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case B, C, BD, A^2, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms, model reduction may improve the model. The Lack of Fit F-value of 3.80 implies there is a 7.69% chance that a Lack of Fit F-value this large could occur due to noise.

Table 1. Effect of SFE parameters on phenol

Run	Temperature (℃)	Pressure (bar)	Time (min)	Particle Size(mm)	Phenol mg/100 g
1	40	100	120	2.00	1355.05
2	55	175	75	2.00	1401.12
3	55	175	75	0.50	1750.18
4	55	175	75	1.25	1250.13
5	40	100	30	0.50	1401.20
6	55	175	120	1.25	1601.71
7	55	175	75	1.25	1477.12
8	55	175	30	1.25	1108.03
9	55	175	75	1.25	1423.79
10	40	250	30	2.00	1501.36
11	70	100	30	2.00	508.20
12	70	250	120	0.50	1715.05
13	40	250	30	0.50	1290.98
14	55	175	75	1.25	1423.79
15	55	100	75	1.25	1680.15
16	40	100	30	2.00	801.15
17	55	175	75	1.25	1380.51
18	40	250	120	2.00	1648.15
19	55	250	75	1.25	1801.95
20	70	250	30	2.00	1650.15
21	40	175	75	1.25	810.13
22	70	250	120	2.00	1868.25
23	70	100	120	2.00	1088.81
24	55	175	75	1.25	1570.18
25	70	100	120	0.5	1350.88
26	70	100	30	0.50	703.20
27	70	175	75	1.25	929.88
28	70	250	30	0.50	750.36
29	40	250	120	0.50	1683.74
30	40	100	120	0.50	1329.05

Table 2. ANOVA for quadratic model of phenol

		Phenol		
Source	**df**	**F-value**	**p-value**	
Model (Significant)	14	7.19	0.0002	Significant
A-Temperature	1	2.72	0.1199	
B-Pressure	1	23.50	0.0002	
C-Time	1	26.56	0.0001	
D-Particle size	1	0.0400	0.8441	
AB	1	2.32	0.1482	
AC	1	3.74	0.0721	
AD	1	1.92	0.1861	
BC	1	0.0003	0.9866	
BD	1	9.89	0.0067	
CD	1	0.3645	0.5550	
A^2	1	24.11	0.0002	
B^2	1	8.40	0.0110	
C^2	1	0.3171	0.5817	
D^2	1	2.01	0.1771	
Residual	15			
Lack of fit	10	3.80	0.0769	Not significant

Table 3. Table fit statistics (Phenol)

Std. Dev.	179.54	R^2	0.8703
Mean	1341.81	Adjusted R^2	0.7492
C.V. %	13.38	Predicted R^2	0.1682
		Adeq Precision	10.9111

The Predicted R^2 of 0.1682 is not as close to the Adjusted R^2 of 0.7492 as one might normally expect; i.e. the difference is more than 0.2. This may indicate a large block effect or a possible problem with the model and/or data. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Ratio of 10.911 indicates an adequate signal. This model can be used to navigate the design space.

The response surface equation developed to predict the change in phenol (mg/100g) with varying levels of processing parameters is as under:

Phenol (mg/100g) = + 1419.30 – 69.78 * Temperature + 205.13 * Pressure + 218.11 *Dynamic time – 8.47 * Particle size.

3.1 Effect of Temperature and Pressure on Phenol

The effect of temperature and pressure on the phenol content was studied which is shown in the Fig. 2. From this figure it can be concluded that when the pressure increased, the phenol content slowly increased at the initial stage and afterwards that somewhat decreased and again that increased at the higher pressure. There was gradual increment of the phenol content from the 40 to 55°C and afterwards the phenol content slowly decreased from 55 to 70°C as the temperature increased i.e. at the 70°C. The minimum phenol content 508.20 mg/100g was found in the 70°C and 100 bar pressure. The maximum phenol content 1868.25 mg/100g was found at the 250 bar pressure. Thus the pressure had the more effect than the temperature in the case of phenol.

3.2 Effect of Temperature and Time on Phenol

The effect of temperature and time on the phenol content was studied which is shown in the Fig. 3. From this figure it can be concluded that when the time increased, the phenol content increased at the constant temperature. For example at 55°C constant temperature, the phenol content increased from 1108.03 at 30 minute to 1601.71 mg/100g at 120 minute. At the constant time, the phenol content initially increased to the 55°C and afterwards that decreased. At perticular time, at 40, 55 and 70°C, the phenol content was 810.13, 1570.18 and 929.88 mg/100g.

3.3 Effect of Temperature and Particle Size on Phenol

The effect of temperature and time on the phenol content was studied which is shown in the Fig. 4. From this figure it can be observed that the phenol was changed on different temperature at particular particle size. For example 40°C at 0.5 mm particle size at constant pressure and time, the phenol was 1401.20 mg/100 g and that was 703.20 mg/100 g at the 70°C and 0.5 mm at size. And at particular temperature, the phenol also changed on different particle size on same pressure and time, i.e. at the 55°C, the phenol was varied from 1750.18 mg/100 g and 1401.12 mg/100 g on the 0.5 and 2 mm respectively. It is observed that the temperature index to be controlled at about 55°C for higher phenol in major cases. There was also found that phenol content was lower at the middle level of particle size.

3.4 Effect of Pressure and Time on Phenol

Effect of pressure and dynamic time on phenol content of extract yield is shown in Fig. 5. Increment of phenol content was observed with increasing pressure and dynamic time and hence increment of phenol content of extract is directly proportional to pressure and dynamic extraction time. From the RSM graph it can be observed that phenol content of leaves extract obtained maximum was 1868.25 mg/100g at pressure of 250 bar and dynamic time of 120 min. From the RSM graph it also can be observed that phenol content of papaya leaves extract obtained at dynamic time from 120 min to 30 min (750.37 mg/100mg) is significantly different.

3.5 Effect of Pressure and Particle Size on Phenol

It can be seen that increment of bioactive phenol content of papaya leaves extract was recorded when there was increase in pressure whereas, there was no major changes in phenol content of papaya leaves extract with increase in particle size. Pressure is main factor for the extraction of phenol content. Effect of pressure and particle size on bioactive phenol content of papaya leaves extract is shown in Fig. 6.

3.6 Effect of Particle size and Time on Phenol

It can be seen that increment of bioactive phenol content of papaya leaves extract was recorded when there was increase in time whereas, there was no much decrement in phenol content of papaya leaves extract with increase in particle size. Time is dominant factor over the particle size for the extraction of phenol content. Effect of pressure and particle size on bioactive phenol content of papaya leaves extract is shown in Fig. 7.

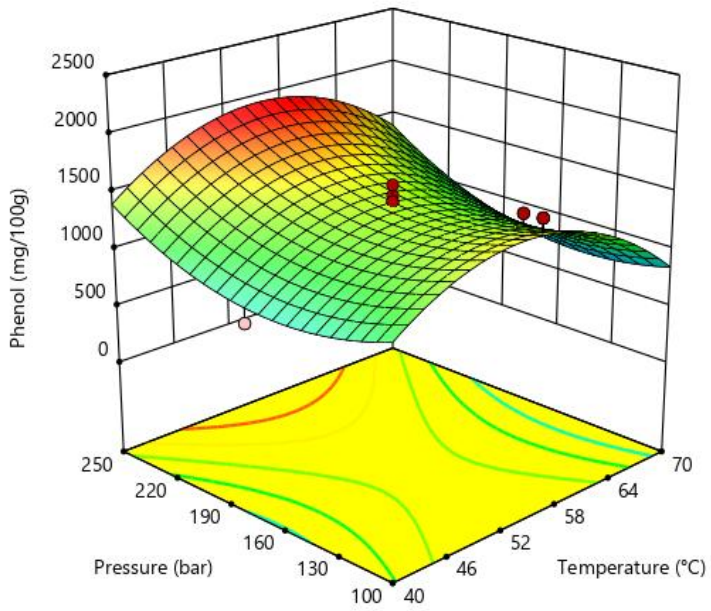

Fig. 2. Effect of temperature and pressure phenol

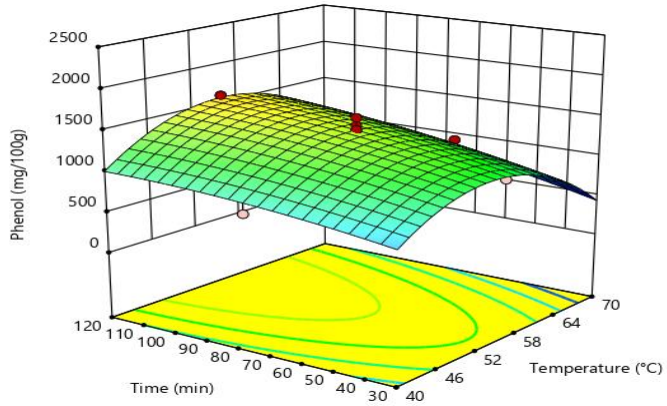

Fig. 3. Effect of temperature and time on phenol

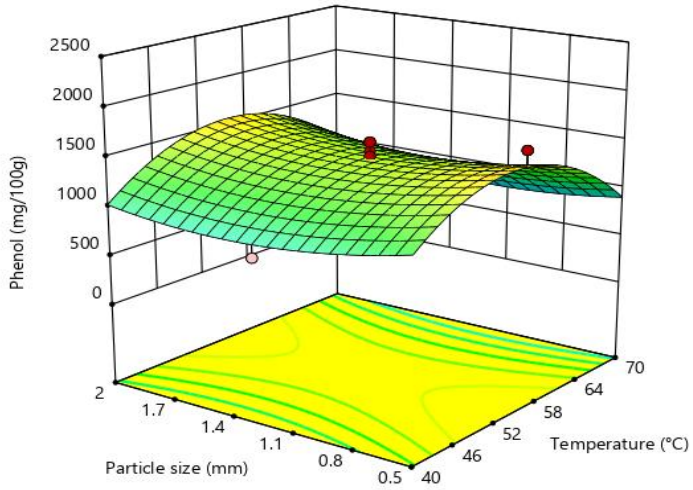

Fig. 4. Effect of temperature and particle size on phenol

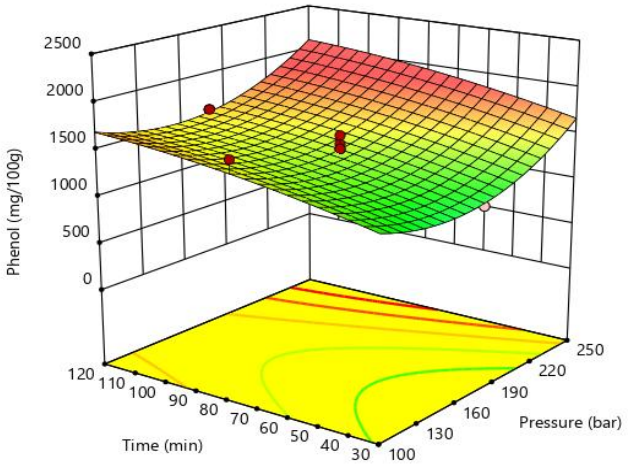

Fig. 5. Effect of pressure and time on phenol

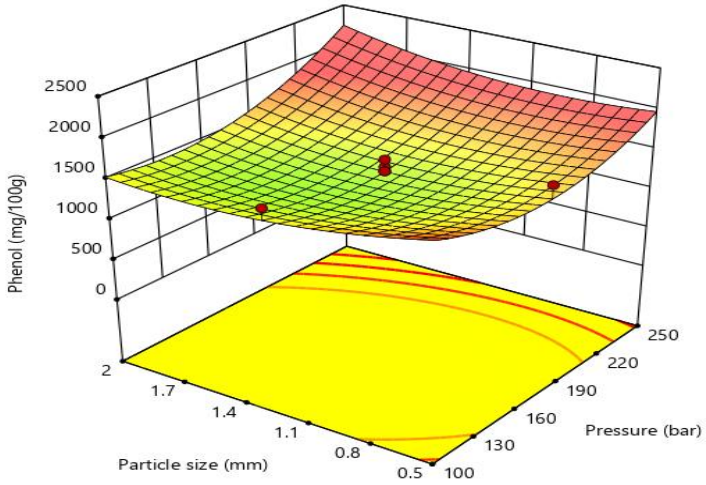

Fig. 6. Effect of pressure and particle size on phenol

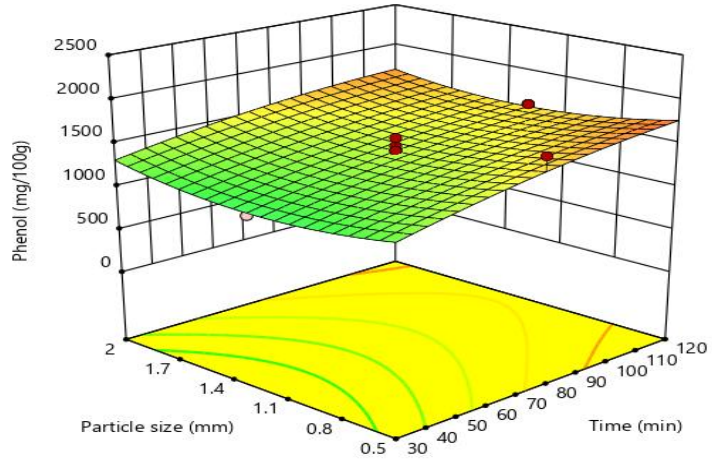

Fig. 7. Effect of time and particle size on phenol

4. CONCLUSION

On the basis of experimental results and data analysis the following conclusions are drawn given as under:

1. Pressure and time were more dominant factors than the temperature to phenol compound in super critical fluid extraction.
2. Particle size and temperature had less effect as compare to the pressure and time.

5. IMPORTANCE OF SUPERCRITICAL EXTRACTION METHODS

There are many methods available to extract the bioactive compound from the medicinal plants like water distillation, steam distillation, super critical extraction with solvents and super critical extraction with carbon dioxide. But among these super critical extraction with carbon dioxide as solvent has the certain advantages to chemical separation processes. Often used as supercritical fluid, Carbon Dioxide presents numerous advantages non-toxic, non-flammable, Inexpensive, Low chemical reactivity, Critical conditions easy to reach and High diffusivity. The special properties of supercritical fluids bring certain advantages to chemical separation processes. supercritical fliud extraction method is useful in a) Nutraceuticals b) Pharmaceuticals and Chemicals: c) Food and Flavouring-Extraction of natural spices d) Natural products.

Bioactive compounds are varied on location, soil, variety, climate and weather. So this experiment

was carried out to find out the extraction amount of bioactive compound at particular temperature, pressure, time and particle size.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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