

Effect of supercritical parameters on phenol content

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. Maximum phenol amount 1868.25 mg/100gm was obtained at the at the 70 °C and 250 bar pressure and minimum phenol 508.20 mg/100gm was found at 70 °C with 100 bar pressure in the supercritical fluid extraction method.

Key word : *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 thousand metric tonne in 2019-20. Total area of crops in Gujarat during 2020-2021 was estimated 18.189 thousand hectares and the production was about 1107.880 thousand metric tonnes. (Anonymous, 2021).

Phenolic compounds is having biological properties like antidiabetic, antioxidant, gastroprotective, anti-inflammatory, spasmolytic, antimicrobial, anticarcinogenic (Pavic,*etal.*,2017 and Veggi, *et al.*, 2014). Antiseptic, disinfectant (Magdalena,*etal.*, 2016), hepato-protective, hypotensive and cardio protective (Rodriquez,*et al.*,2016). It has influence on chronic and degenerative diseases and different types of cancer (Abbsi :, 2008 and Liu,*et al.*, 2007). 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 (Katarzyna, 2018).

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 (Saneha,*et al.*,2009)

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. (Pourmortazavi and Hajimirsadeghi, 2007)

2. Materials and methods:

Fresh papaya leaves 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.

Cleaned papaya leaves were weighed in digital balance (Atom scale make, Capacity: 1 Kg, least count: 0.01 g). 100 gram samples were prepared and placed in perforated aluminum tray in the hot air tray dryer .

Drying experiments were performed in a cabinet type laboratory hot air tray dryer manufactured by Navrang Scientific Works Pvt. Ltd., New Delhi. Air velocity was set at 1.0 m/s.

Extraction of bioactive compound from papaya leaves powder was experimentally done using supercritical fluid extraction assembly. Surface response method was used to get the combination of temperature , pressure, time and particle size.

Total phenol from the papaya leaves sample was estimated by the method as described by Sadasivam and Manickam, 1992 .

3. Result 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/100gm (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.

Table 1: Effect of SFE parameters on bioactive compounds

Run	Temperature (°C)	Pressure (bar)	Time (min)	Particle Size (mm)	Phenol mg/100g
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

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, A², B² 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 2 : ANOVA for Quadratic model of yield and phenol

Source	df	Phenol		
		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

The Predicted R² of 0.1682 is not as close to the Adjusted R² 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.

Table 3 :Table Fit Statistics (Phenol)

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

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

$$\text{Phenol (mg/100g)} = + 1419.30 - 69.78 * \text{Temperature} + 205.13 * \text{Pressure} + 218.11 * \text{Dynamic time} - 8.47 * \text{Particle size} \dots 1$$

Effect of Temperature and Pressure on Phenol

The effect of temperature and pressure on the phenol content was studied which is shown in the figure 1. 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.

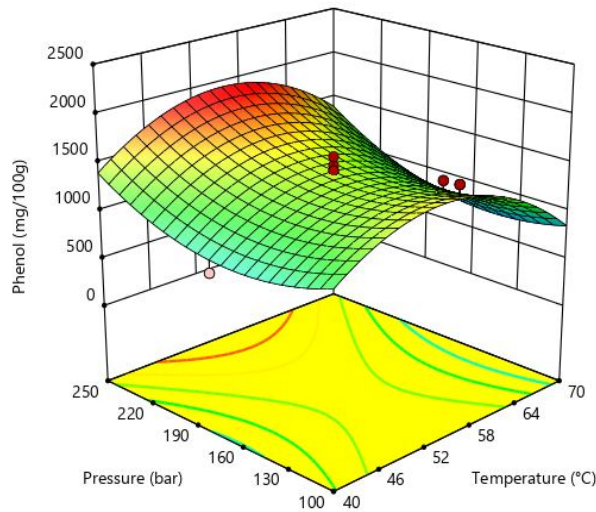


Figure.1: Effect of temperature and pressure phenol

Effect of Temperature and Time on Phenol

The effect of temperature and time on the phenol content was studied which is shown in the figure 2. 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 particular time, at 40, 55 and 70 °C, the phenol content was 810.13, 1570.18 and 929.88 mg/100g.

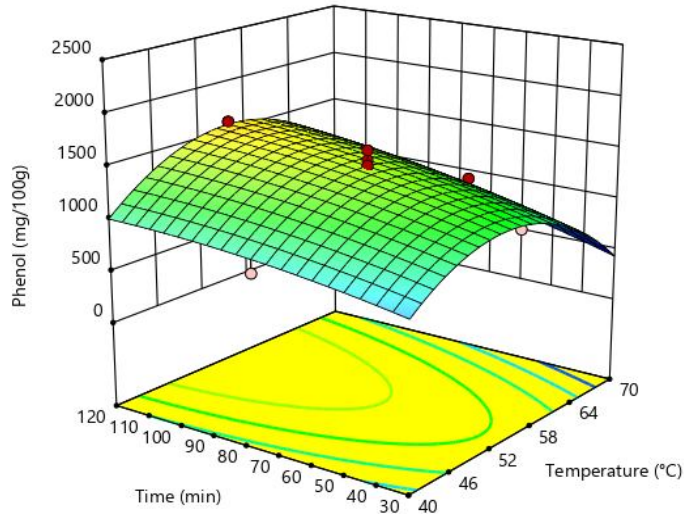


Fig.2 : Effect of Temperature and Time on Phenol

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 figure 3. 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/100g 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/100g and 1401.12 mg/100g 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.

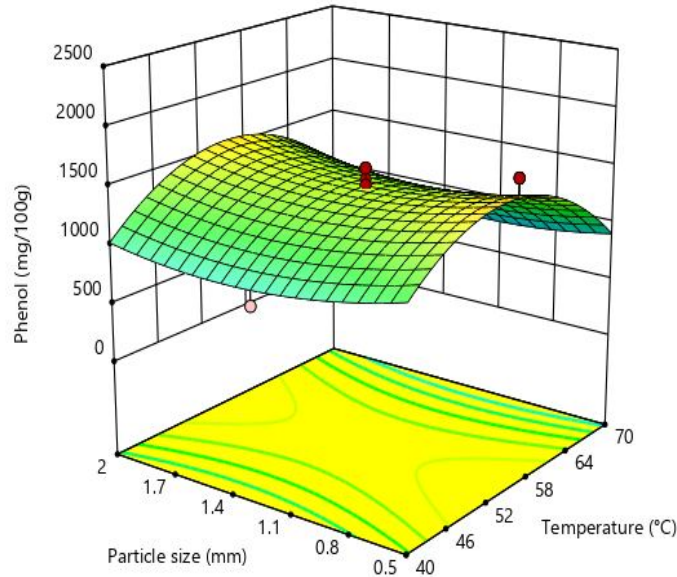


Fig.3: Effect of Temperature and Particle Size on Phenol

Effect of Pressure and Time on Phenol

Effect of pressure and dynamic time on phenol content of extract yield is shown in Fig. 4. Increase phenol with increasing pressure and dynamic time was observed and hence increase in 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.

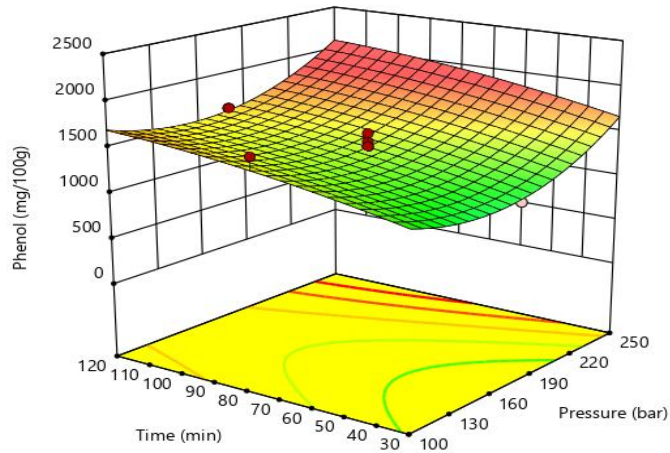


Fig.4: Effect of Pressure and Particle Size on Phenol

Effect of pressure and time on Phenol

It can be seen that increase in 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.5.

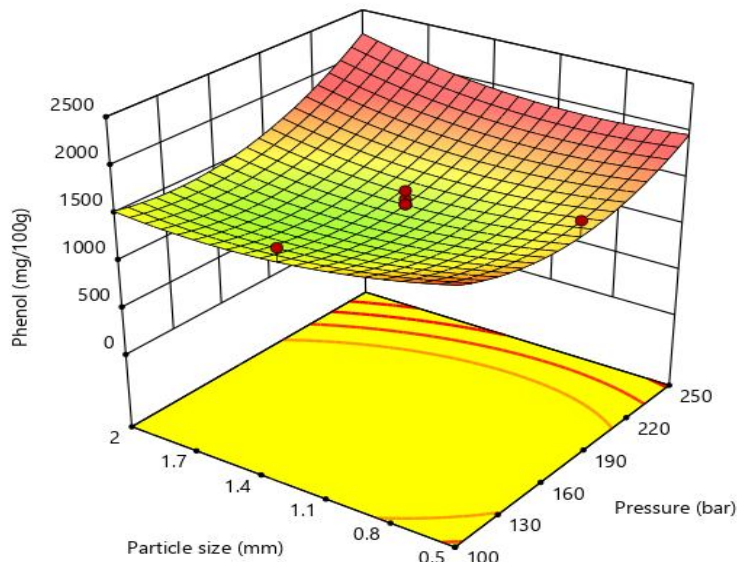


Fig.5 : Effect of Pressure and particle size on Phenol

Effect of Particle size and Time on Phenol

It can be seen that increase in 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. 6.

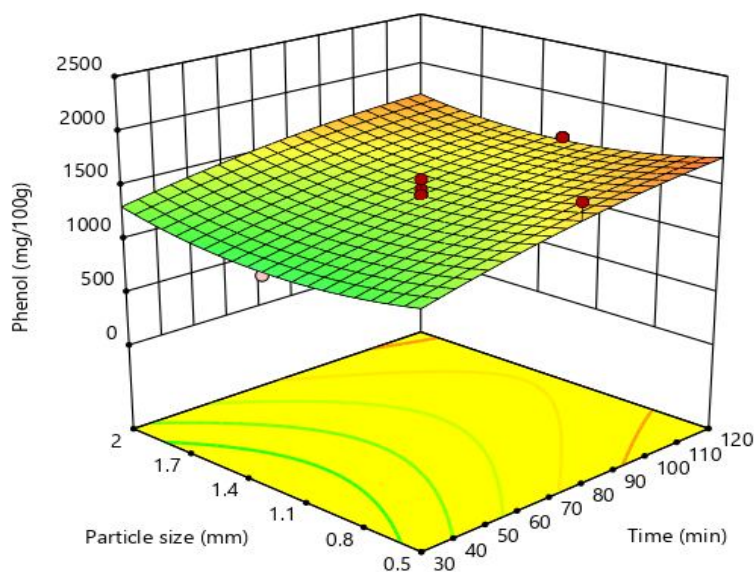


Fig.6: Effect of time and particle size on phenol

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. References

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