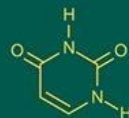


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 IJABR 2024; SP-8(8): 162-166
www.biochemjournal.com
 Received: 02-06-2024
 Accepted: 05-07-2024

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Study on extract yield of papaya leaf powder using a supercritical fluid extraction technique

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DOI: <https://doi.org/10.33545/26174693.2024.v8.i8Sc.1756>

Abstract

To obtain standardized parameters from the supercritical fluid extraction system for yield extraction, various factors, such as temperatures of 40, 55, and 70 °C and pressures of 100 bar, 175 bar, and 250 bar, respectively, were selected. During experimentation, the extraction times were 30, 75, and 120 minutes, and the particle sizes of the materials were varied from 0.50 mm to 2.0 mm. After analysis, the optimized conditions for the highest yield were a temperature of 40 °C, a pressure of 250 bar, a particle size of 0.50 mm and a 120 min dynamic time. The pressure and time are more important than the temperature in terms of obtaining the most supercritical fluid extraction.

Keywords: Papaya, supercritical, extraction, yield

Introduction

The superfluid extraction method is often used for extraction. Owing to the low density, high diffusivity and viscosity of supercritical carbon dioxide gas, the supercritical extraction method has become an alternative to conventional extraction methods such as the Soxhlet extraction method. Moreover, carbon dioxide has no side effects on the environment because it is nonflammable and is a “green” gas, and the influence of pressure on the solubility of desired compounds may increase the extraction efficiency. When the pressure and temperature are optimized, carbon dioxide may be preferable. There is no chance of mixing carbon dioxide in the final product received from supercritical sources, as carbon dioxide can be removed by depressurizing during the collection of extracts (Katarzyna, *et al.*, 2018) [3].

When a fluid (most often carbon dioxide) is subjected to a desirable combination of pressure and temperature, it can possess critical properties for the selective extraction of fat. Under controlled conditions of time, temperature, and pressure, the sample is exposed to supercritical fluid to allow dissolution of the fat from the sample in a supercritical fluid extraction system. The dissolved fat is then separated from the supercritical solvent by a significant drop in solution pressure (Sanaha, *et al.*, 2009) [8].

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 analytical supercritical extraction systems are used for the extraction of fat or compounds, with carbon dioxide having several benefits. Carbon dioxide is relatively inexpensive, highly pure, nontoxic and nonflammable. Carbon dioxide can be removed easily from the extract, which is best for lipophilic compounds. It has a relatively low critical pressure and temperature. (Pourmortazavi and Hajimirsadeghi, 2007) [6]

Materials & Methods

A sample of papaya leaves was prepared. This powder was obtained from papaya leaves that had been tray-dried at 50 °C. The objective of the experiment was to achieve a high yield of extraction using the supercritical fluid extraction system.

Supercritical fluid extraction technique

The supercritical fluid extraction assembly (Make: Applied Separations, USA) (Plate 3.9) has five components: a reciprocating compressor, a CO₂ cylinder, a CO₂ pump, an oven module and a chiller.

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Compressed air is necessary to drive the CO₂ pump, for which a reciprocating compressor (make: Anest Iwata, Noida) was used. It was provided with a 3-phase squirrel cage induction motor of 4 HPs with a rpm of 1424. The capacity of the reciprocating compressor is 180 L. The CO₂ cylinder (46.7 liter capacity) was connected to a CO₂ pump. This pump is used to pressurize CO₂ at the required pressure with a maximum limit of 690 bar. From the pump, pressurized CO₂ enters the oven module. The SFE oven module (Plate 3.21) consists of a rectangular chamber in which the sample holding vessel is fixed at the required temperature. In the oven, two sample holding vessels can be fixed at a time. The oven temperature, micrometering valve temperature, vessel temperature and set temperature are displayed in the bottom panel of the oven module. The CO₂ inlet valve and vent valve are located at the left sidewall of the oven, and the CO₂ outlet valve and micrometering valve are located at the right sidewall of the oven. The inlet valves are shut-off valves that supply supercritical CO₂ from the pump module to the extractor vessels, whereas the outlet valves are shut-off valves that supply supercritical CO₂ from the extraction vessel to the metering valves. The vent valves are used to vent the supercritical CO₂ from the extractor vessels to the atmosphere. The micrometer valves are flow control valves used to release supercritical CO₂ to the atmosphere. A thermocouple is placed inside the oven module to measure the temperature of the vessel. The extract is collected in a glass vial, which is fixed below the micrometering valve. A chiller is used for the recirculating coolant and is connected to the pump module. A recirculating coolant is required by the pump module for CO₂ pressurization. The recirculating coolant should be maintained at a controlled temperature of 0 °C or less. A mixture of ethylene glycol and water is used as a cooling medium.

$$\text{Extract yield (\%)} = \frac{\text{weight of extract}}{\text{weight of feed}} \times 100 \dots\dots\dots (3.22)$$

Results & discussion

Supercritical extraction

Effect of supercritical fluid extraction parameters on extract yield

Experiments on the supercritical fluid extraction of papaya leaf powder were conducted. The extraction yield was analyzed from the extracted yield of the papaya leaves.

The optimized combination of temperature, pressure, dynamic time and particle size was selected on the basis of the maximum yield recovery. The yield content was influenced by temperature, pressure, dynamic time and particle size.

The yield of the papaya leaf powder (%) varied from 0.12 to 6.27%. The maximum yield was obtained at 40 °C and 250 bar pressure, and the minimum yield was obtained at 70 °C and 100 bar pressure.

An analysis of variance was conducted on the experimental data, and the effects of temperature, pressure, dynamic time and particle size, as well as their interactions with yield, phenol, flavonoid and antioxidant activity, were estimated. The quadratic model was fitted to the experimental data, and the statistical significance of the linear, quadratic and interaction effects was calculated for each response (Table 1).

The Model F value of 15.42 implies that the model is significant in the case of yield. There is only a 0.01%

chance that an F value that is this large could occur due to noise. P values less than 0.0500 indicate that the model terms are significant. In this case, A, B, C, AD, BC, A², and B² are significant model terms. Values greater than 0.1000 indicate that 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 3.75 implies that there is a 7.86% chance that a lack of fit F value could occur due to noise.

Table 1: ANOVA for the quadratic model of yield

Source	df	Yield		
		F value	p value	
Model (Significant)	14	15.42	< 0.0001	significant
A-Temperature	1	7.22	0.0169	
B-Pressure	1	62.71	< 0.0001	
C-Time	1	74.19	< 0.0001	
D-Particle size	1	0.1584	0.6962	
AB	1	3.95	0.0655	
AC	1	0.2937	0.5958	
AD	1	7.06	0.0179	
BC	1	27.66	< 0.0001	
BD	1	2.79	0.1155	
CD	1	1.04	0.3235	
A ²	1	25.30	0.0001	
B ²	1	9.04	0.0088	
C ²	1	2.78	0.1161	
D ²	1	0.2106	0.6529	
Residual	15			
Lack of Fit	10	3.75	0.0786	not significant

The predicted R² of 0.7155 is in reasonable agreement with the adjusted R² of 0.8744; i.e., the difference is less than 0.2. Adeq Precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. In the present study, a ratio of 14.232 indicates an adequate signal. This model can be used to navigate the design space.

Table 2: Fit statistics (yield)

Std. Dev.	0.6158	R²	0.9350
Mean	2.40	Adjusted R ²	0.8744
C.V.%	25.71	Predicted R ²	0.7155
		Adeq Precision	14.2322

The response surface equation developed to predict the change in yield (%) with varying levels of processing parameters is as follows:

$$\text{Biochemical extract yield (\%)} = + 2.37 - 0.3900 * \text{Temperature} + 1.15 * \text{Pressure} + 1.25 * \text{Dynamic time} - 0.0578 * \text{Particle size} \dots\dots (4.1)$$

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 greater 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. A ratio of 10.911 indicates an adequate signal. This model can be used to navigate the design space.

Effects of temperature and pressure on yield

The effects of temperature and pressure on the extract yield are shown in Fig. 2. The influence of pressure was more prominent than that of temperature. When the pressure increased, the extraction yield increased. At a constant temperature, an increase in pressure increases the density of

SC-CO₂. The solvent strength of SC-CO₂ increases with increasing density of CO₂. As the density increases, the distance between the molecules decreases; therefore, the interaction between the analyte and CO₂ increases, leading to greater solubility of the analyte in CO₂ (De Castro *et al.*, 1994) [2]. An increase in pressure also accelerates the mass transfer of analytes and solvents in supercritical extractor vessels and improves the extraction yield (De Castro *et al.*, 1994) [2].

At higher pressures of 175 and 250 bar and lower temperatures of 40 and 55 °C, the extract obtained was viscous, waxy, dark green and yellow in color. Reverchon (1992) [7] reported that it is not simple to investigate extraction from herbaceous substrates because they contain many families of lipophilic solutes that have a high affinity for supercritical CO₂. In addition, other extractable compounds, such as fatty acids, vegetable wax, coloring matter, and resinoids, are also present. Therefore, it can be presumed that the waxy extract obtained was due to the presence of hydrocarbon compounds. When the pressure was reduced and the temperature increased, the extract was less viscous, and the color was yellow. The graph clearly shows that as the pressure increased, the yield of the extract also increased with increasing temperature. Minimum extract of 0.12% was obtained at 70 °C, 100 bar for a dynamic time of 30 min and a 0.5 mm particle size.

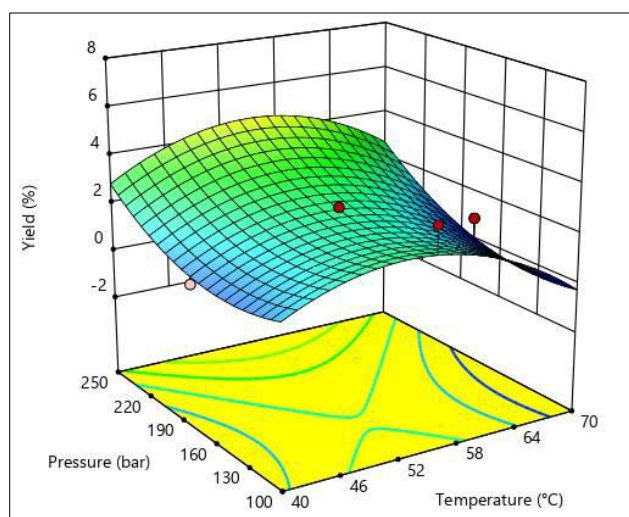


Fig 2: Effects of temperature and pressure on yield

A maximum oil yield of 6.27% was obtained at 40 °C, 250 bar for 120 min and a 0.5 mm particle size. The data clearly show that at the same dynamic time (120 min), pressure had a strong effect on the yield %. The same trend was observed by Bermejo *et al.* (2015) [1] and Katarzyna *et al.* (2018) [3].

Effects of temperature and time on yield

The influences of temperature and dynamic time on yield are shown in the figure. The RSM graph reveals that with increasing time, the yield (%) increased. The graph also indicates that at different temperatures, the yield varies. There was no specific trend observed for the temperature. Compared with relatively high temperatures, moderate temperatures resulted in greater extract yields. The temperature at constant pressure and the same particle size had no major effect on the yield. An extract of 1.68% was obtained at 70 °C, 250 bar for a dynamic time of 30 min and a 0.5 mm particle size, whereas an extract yield of 5.35%

was obtained at 70 °C, 250 bar for 120 min and a 0.5 particle size. This finding revealed that at the same temperature and different times, the yield was different. These results are also in accordance with those of Sardar *et al.* (2018) [9], who worked on supercritical fluid extraction from ajwain seeds.

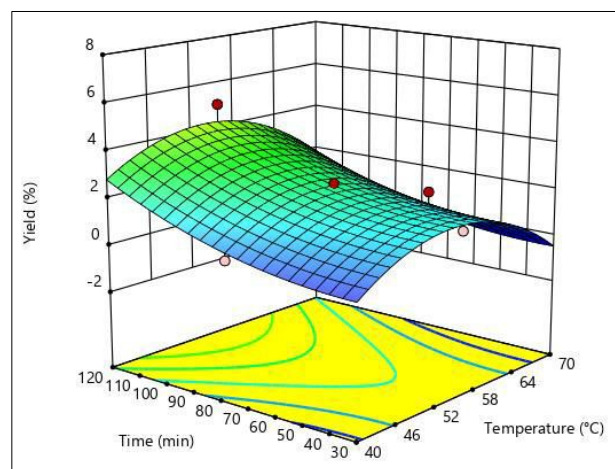


Fig 3: Effects of temperature and time on yield

Effects of temperature and particle size on yield

The effects of temperature and time on the yield content were studied, and the results are shown in Figure 4.27(a). From this figure, it can be concluded that when the yield was changed at different temperatures, a particular particle size was used. For example, at 40 °C, with a 0.5 mm particle size at constant pressure and time, the yield was 2.12%, whereas it was 0.112% at 70 °C and 0.5 mm in size. At a particular temperature, the yield also changed with different particle sizes at the same pressure and time, i.e., at 55 °C, the yield varied from 2.75 and 1.71% at 0.5 and 2 mm, respectively. The temperature index was controlled at approximately 55 °C for a relatively high yield. The findings of this study are similar to those of Nehul (2015) [5].

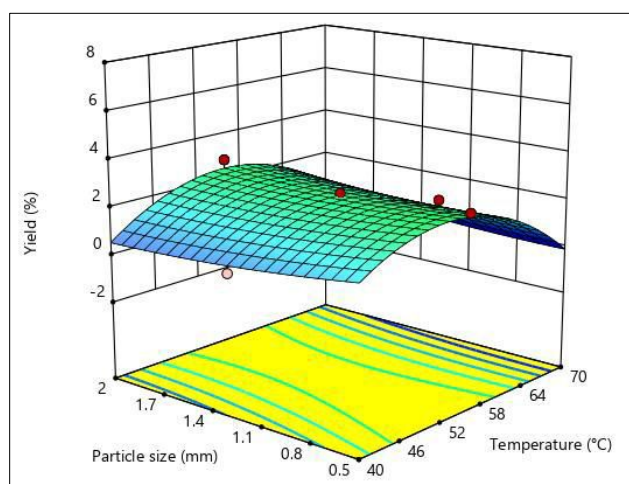


Fig 4: Effects of temperature and particle size on yield

Effect of pressure and time on yield

The effects of pressure and drying time on the extraction yield of papaya leaves are shown in Fig. 4.28 (a). An increase in extract yield with increasing pressure and dynamic time was observed; hence, an increase in the extraction yield of papaya leaves is directly proportional to

pressure and dynamic extraction time. The RSM graph revealed that the maximum extraction yield of the papaya leaves was 6.27 at a pressure of 250 bar and a dynamic time of 120 min. The RSM graph also revealed that the extraction yields of the papaya leaves obtained at dynamic times ranging from 120 min to 30 min were significantly different. The observations of Liza *et al.* (2010)^[4] are quite similar to the present observations.

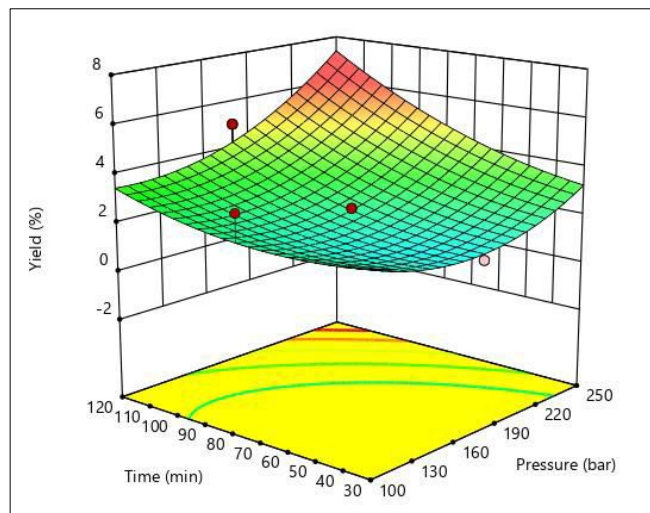


Fig 5: Effects of pressure and time size on yield

Effects of pressure and particle size on yield

An increase in the extraction yield of papaya leaves was recorded with increasing pressure, whereas there was no significant decrease in the extraction yield of papaya leaves with increasing particle size. This finding cannot be attributed to the fact that, owing to an increase in particle size, less surface area is available for extraction; therefore, the yield was greater, but the effect remained almost the same. Pressure is the main factor affecting the extraction of papaya leaf extracts. The effects of pressure and particle size on the extraction yield of papaya leaves are shown in Fig. 6. Pressure had a dominant effect on the extraction yield, as reported by Liza *et al.* (2010)^[4].

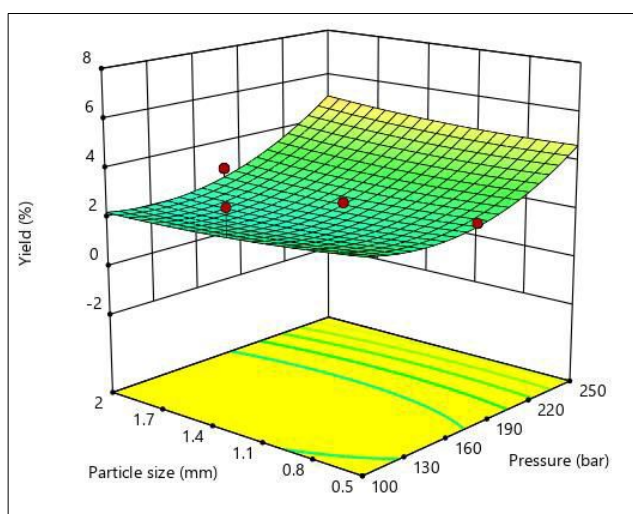


Fig 6: Effects of pressure and particle size on yield

Effect of time and particle size on yield

The effects of dynamic time and particle size on the percent extraction yield of papaya leaves are shown in Figure 7. The

extraction yield increased with increasing dynamic time. However, the extraction yield had no effect on the particle size. Similar trends in the yields of the extracts of papaya leaves were found for different particle sizes.

The effects of dynamic time and particle size on the percent extraction yield of papaya leaves are shown in Figure 7. The extraction yield increased with increasing dynamic time. However, the extraction yield had no effect on the particle size. Similar trends in the yields of the extracts of papaya leaves were found for different particle sizes.

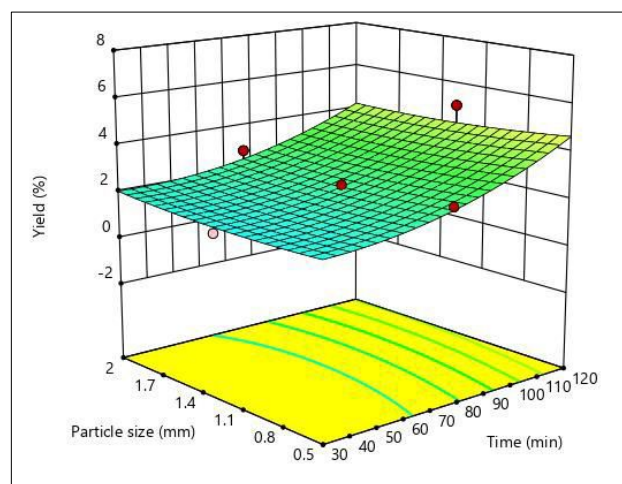


Fig 7: Effect of time and particle size on yield

Conclusion

The Supercritical fluid extraction yield from the papaya leaf powder was optimized by carefully adjusting several factors to achieve the best possible results. The extraction process was carried out at 40 °C, which represents an ideal balance between efficiency and preservation of the extract's quality. The pressure was maintained at 250 bar, which is a critical parameter that significantly influences the solubility and extraction efficiency of the target compounds. A dynamic time of 120 minutes is utilized to ensure thorough extraction while maximizing yield. The particle size of the papaya leaf powder was optimized at 0.50 mm, increasing the surface area available for extraction without causing excessive clogging. Under these optimized conditions, the extraction yield is determined to be 6.19%, demonstrating an efficient extraction process that effectively balances all the critical variables to achieve a high-quality output.

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