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Original Article

Effects of Particle Sizes on Total Catching Content and Antioxidant Activity of Musa Paradisiacal Inflorescence using Supercritical Carbon Dioxide (SFE-CO2) Extraction

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Abstract: The inflorescence of Musa paradisiacal or Banana Nipah primarily serves as a staple food crop in Malaysia. It contains various polyphenols, including catching, and is renowned for its excellent ant oxidative properties. Supercritical carbon dioxide (SFE-CO2) is a green technology that preserves bioactive components while facilitating extraction. This study employed SFE-CO2 to investigate the catching extraction from different size ranges of M. paradisiacal inflorescences at different extraction parameters. The plant matrices (450 - 600 µm, 600 - 850 µm, and 850 µm - 1600µm) were extracted under different temperatures (40°C and 60°C) and pressures (25MPa and 45MPa), with a constant supercritical CO2 fluid and 50% (v/v) ethanol co-solvent flowed at 4 mL/min. Data were analysed using ANOVA in Minitab Software. The results indicated that the smallest particles (450 - 600 µm) exhibited the highest total oil extraction yield (29.40 ± 7.95%) at 60°C; 45 M Pa, while 850 µm - 1600µm particles had the least (9.54 ± 1.60%) at 40°C; 45MPa. Extraction efficacy of SFE-CO2 at 45MPa; 60°C demonstrated a comparable effect ($p > 0.05$) to the 10-hour Sox let extraction. Smaller plant matrices exhibited higher catching content at 40°C and 45 M Pa, particularly 81.51 ± 1.11 mg (450 - 600 µm)—increased total catching content correlated with higher DPPH radical scavenging activity. Kinetic modelling revealed that 93% to 95% of the catching extraction from the plant matrix followed a first-order kinetic model. In conclusion, the smallest plant matrix (450 - 600 µm) exhibited the highest catching yield and ant oxidative activity when extracted at 45MPa and 40°C.

Keywords: Musa paradisiacal inflorescence; Antioxidant activity; SFE-CO2; Catching; Particle sizes

1. Introduction

Musa paradisiacal is the first Linnaean name given to a banana. M. paradisiacal is a herbaceous, perennial, and monocotyledonous plant that belongs to the genus Musa from the Musa caeca family and primarily grows in the wet tropical biome (Diazgranados et al., 2022). M. paradisiacal also known as

Banana Nipah is a hybrid between (Ma et al., 2017), which are indigenous to Malaysia and Indonesia and have been distributed to other countries, including northern India, China, Philippines, Cambodia, Maldives, Kenya, etc. Each M. paradisiacal tree produces a single-dropping spike-shaped inflorescence. The inflorescence is a large (15 to 20 cm), scarlet-red, leathery texture bracts with opening in succession (Imam & Akter, 2011). The aerial stem of inflorescence emerges from the middle of overlapping, spirally arranged leaf sheaths (pseudo stem) (Kirchoff, 2017). The fruiting stem holds the yellow flowers, also known as racemes, enclosed by the inflorescence bracts. M. paradisiacal not only served as a dietary but also a traditional treatment for centuries until the present due to its rich source of multiverses nutrients and phytochemicals, including phenolic glycosides, tannins, and flavonoids (Imam & Akter, 2011). M. paradisiacal inflorescence consists of hemitrope node, glycoside, rose side, benzyl alcohol glycoside, Gallic acid, and catechol (Arun et al., 2017); (Le Quéré et al., 2018). It has been used as an indigenous treatment of dysentery menorrhagia and type 2 Diabetes in Malaysia Southern India, Taiwan, and Sri Lanka (Arun et al., 2017).

Catching is a monomeric flavan-3-ol, a secondary metabolite that can be harvested from many dietary foods, including herbs, fruits, vegetables, tea, and red wines. Catching is composed of two benzene rings and a dihydropyran heterocyclic with a hydroxyl group on carbon 3 where a chiral is positioned at the centers of Carbon 2 and 3 of the dihydropyran heterocyclic (Namal Senanayake, 2013) From a structural perspective, catching has four diastereoisomers trans configuration, also known as catching; cist configuration, which Esterifies with gal late groups to generate epicatechins, epigallocatechin, and epicatechin-3-gallate (Fan et al., 2017). catechist' antioxidant mechanisms work in two ways: first, scavenging and chelating reactive oxygen species (ROS), free radicals, and metal ions; second, catching will up regulate the production of antioxidant enzymes, blocking pro-oxidant enzymes, and stimulate the development of phase 2 detoxifying enzymes (Youn et al., 2006).

Supercritical carbon dioxide (SFE-CO2) is a fluid state of carbon dioxide where it exists at or above its critical pressure (73atm) and critical temperature (31.1°C). CO2 is a non-polar compound whose density can be modified by temperature and pressure (Cormier et al., 2014). Chemically, SFE-CO2 is non-toxic to the environment, inert, and not combustible because it has undergone a fully oxidised state (Karmee et al., 2009). In addition, the extraction process is conducted under a carbon dioxide atmosphere without oxygen; this indicates that SFE-CO2 can be the appropriate extraction method for those bioactive components that are easily oxidised. Since the critical temperature of carbon dioxide is only 31.1°C, the extraction can be conducted at room temperature. The extraction process's low critical temperature and pressure minimise thermal degradation's effect on bioactive compounds (Cabeza et al., 2017). SFE-CO2 is effective in dissolving lipid soluble and low-polar compounds. CO2 is the most commonly used supercritical fluid in various industries, yet it is only efficient in extracting non-polar compounds. Concerning this limitation, co-solvents such as ethanol, methanol, water, and acetic acid are added to improve the solvation power of SFE-CO2 for the non-polar solutes from plants, such as alkaloids, phenolic, and glycoside compounds (Herrero et al., 2010). In SFE, co-solvents can be implemented in three different ways: as a mixed fluid in a pumping system; the solvent was partially soaked in the co-solvent before extraction; added as a cylinder tank of pre-modified CO2 (Pourmortazavi & Hajimirsadeghi, 2007).

Sox let extraction, which was first used to extract analyses from solid materials in 1879, has endured as one of the most popular and reliable methods. It continues to be a mainstay in analytical labs and frequently acts as a standard by which the effectiveness of contemporary extraction procedures is evaluated. Due to its ease of use and efficiency in extracting various samples, including soils, sediments, and animal and plant tissues, this approach continues to be widely used. Furthermore, Sox let extraction enables various applications of an array of solvents, including dichloromethane (DCM), which can be used either in its pure form or when combined with acetone, hexane, or both. This adaptability makes the extraction process more practical for a wider range of applications by enabling analysts to customise it to the distinctive features of the sample and target chemicals (Reyes-Garcés et al., 2018). Meanwhile, one notable limitation of Sox let extraction is its time-consuming nature. A standard Sox let extraction process requires approximately 8 hours to complete a single extraction cycle (Subramaniam, 2017). On account of this time-intensive nature, Sox let extraction requires continuous supervision and upkeep as it involves cycles of solvent heating and condensation. Solvent consumption is another issue when conducting Sox let extraction. Not only raising concerns about environmental sustainability, solvent consumption also increases the overall cost of experiments (Kapadia et al., 2022).

The rising demand for high-quality foods has spurred an increased interest in functional foods and nutraceutical supplements, particularly in the exploration of edible flowers like M. paradisiacal inflorescence in Southeast Asia. Despite their potential as sources of beneficial phytochemicals, these

flowers are underutilised due to blooming seasons and concerns about adverse effects. The relationship between particle size and bioactive chemical extraction is critical for optimising operations and enhancing product quality in various sectors. Modern non-thermal processing technologies, such as SFE-CO2, promise to preserve the functionality of phytochemicals extracted from these flowers. The objectives of this study were to investigate The effect of particle size on the extraction oil yield of M. paradisiacal inflorescence using SFE-CO2 and to assess both the catching contents and antioxidant activity of the resulting extract.

2. Materials and Methods

2.1. Materials

The matured M. paradisiacal inflorescence with a violet leathery appearance was bought from banana suppliers from Johor (The Planet Farm, Johor). The banana inflorescences were dried in an oven at 60 °C for 4 hours to achieve a water activity 0.53. The dried banana inflorescence was ground into fine particles using a steel grinder (Moongiantgo 800g, Foodsense.). The fine particles of inflorescences are then classified into a range of 450 µm – 650 µm, 600 µm – 850 µm, and 800 µm – 1600 µm with the sieving process. The dried inflorescence powders were stored in the chiller (3°C) until ready to be used. Catching was purchased from Macklin Biochemical Co., Ltd (Hu & Chen, 2019). Absolute ethanol 99.86%, 2,2 - diphenyl-1-picrylhydrazyl (DPPH) reagent, pure n-hexane, hydrochloric acid (HC l), and vanillin powder were purchased from Thermo Fisher Scientific Chemicals (Waltham, MA).

2.2. Supercritical fluid-carbon dioxide extraction (SFE-CO2)

A10.0 grams of sieved M. paradisiacal inflorescence powders were placed inside the extraction cell of the SFE extraction system. The CO2 tank supplied the CO2 needed for the extraction process. The CO2 pumps (Lab Alliance, Series II Pump) chilled the gas into the supercritical state and started pumping the supercritical fluid to the extraction cell with a 10 mL/min solvent flow rate. The pump regulates the supercritical fluid and co-solvent (50% (v/v) ethanol); meanwhile, the data logger controls the temperature, pressure, and flow rates of supercritical fluid and co-solvent. The pump was connected to an extraction cell placed inside an oven and a back pressure regulator with a restrictor valve (JASCO, Model BP-2080). The oven controlled the set extraction temperature while the back pressure regulator governed the extraction pressure (Rizkiyah et al., 2023). The extraction parameters were categorised into independent, fixed, and dependent variables. The independent variable was the particle sizes of M. paradisiacal inflorescence powders ranging from 450 µm – 600 µm, 650 µm - 850 µm, 850 µm -1600 µ m; pressure level of 25 MP a, and 45 MP a; and temperature at 40°C, and 60°C. The independent fixed variables included 50% (v/v) ethanol and its flow rate at 1.0 mL/min. Lastly, the dependent variables were total extraction yield, total catching content (TCC) and antioxidant activity (%). The extracted oil was then collected in the collection vial attached to the restrictor valve every 40 minutes, 80 minutes, 120 minutes, and 160 minutes for kinetic modeling purposes. The extracted oil collected at different times will undergo spectrophotometry analysis to determine the amount of catching (mg / 2 mg extracted sample oil) and antioxidant activity (%). The extraction yield (%) of *M. paradisiacal* inflorescence was calculated as the equation described by (Abbas et al., 2021):

$$\text{Percentage of extraction yield (\%)} = \frac{\text{Weight of extracted oil (g)}}{\text{Weight of sample(g)}} \times 100\%, \quad (1)$$

2.3 Soxh let extraction

100 mL of 50% (v/v) ethanol was added to 10.0 g of M. paradisiacal inflorescence powder. The mixture was placed in a thimble inside the Sox let apparatus, and extraction was performed at 50°C for 10 hours to ensure the maximal extraction of M. paradisiacal inflorescence powder. During the extraction, cotton wool was plugged into the mouth entrance of the timber to avoid the transfer of sample powder to the distillation flask (Ntalikwa, 2021). Subsequently, a rotary evaporator was implemented to evaporate the ethanol residue from the extracted crude sample oil at 40°C and 121 M Pa. The antioxidant activity and the catching content of Sox let extracted M. paradisiacal inflorescence were investigated. The oil extraction yield (%) of M. paradisiacal inflorescence by Sox let extraction was calculated using (1) (Abbas et al., 2021).

2.4. Spectrophotometry analysis of catechin content in M. paradisiaca inflorescence

Two grams of sample oil extracted from M. paradisiacal inflorescence using SFE were dissolved in 2.0 mL of n-hexane. The mixture was thoroughly mixed using a vortex mixer for about 30 seconds, after the liquid-liquid extraction by adding 4.0 mL of 80% (v/v) ethanol to the mixture for the polar fraction (Sun et al., 2022). The final mixture was then centrifuged at 3500 revolutions per minute (rpm) for 10 seconds. The supernatant was collected into a sanitised centrifuge tube. The same mixture tube was subjected to a second and third extraction by adding 4.0 mL of 80% (v/v) ethanol only. The following steps were repeated as mentioned previously. The isolated supernatant yielded from the oil of M. paradisiacal inflorescence was stored in a refrigerator at -15°C until analysis. One millimeter of supernatant was added to 3.0 mL of vanillin-ethanol, followed by the top-off with 4.0% H C l - ethanol to a total volume of 6.0 mL mixture (He et al., 2009). The reactive mixture was then incubated for 30 minutes at 30°C. In the dark, and subsequently measured at 505 nm against the blank of ethanol using a UV-VIS micro plate reader a pure catching hydrate ((+)-Cyanidol-3) (98%) was used as a positive control.

2.5. Antioxidant activity by DPPH radical scavenging assay

A solution of 0.004% DPPH in methanol was prepared by dissolving 2.0 mg of DPPH powder in 12.74 mL of ethanol. One millimeter of the DPPH methanol solution was added to the 1.0 mL supernatant and vortexes; subsequently, the mixture reaction was incubated in the dark at room temperature (25°C) for 30 minutes. The absorbance of the reaction mixture was measured at 517 nm against blank using a UV-Vis spectrophotometer. 1mL of query cetin was used as the positive control while ethanol was the negative control (Baliyan et al., 2022). The scavenging activity of the extracted oil on DPPH radicals will be calculated based on the equation stated below:

$$\text{DPPH radical scavenging activity} = \frac{(\text{Absorbance control} - \text{Absorbance sample})}{\text{Absorbance control}} \times 100\%, \quad (2)$$

2.6. Kinetic modeling

In this experiment, the extraction kinetics of M. paradisiacal inflorescence matrix by supercritical fluid extraction was performed using first- and second-order kinetic modelling.

2.6.1. First-order kinetic modeling

The first-order kinetic modeling was used to describe the extraction behaviour that follows the first-order kinetic. In this case, the extraction rate of catching over time was analysed by applying the data to the first- order equation (Alara & Abdurahman, 2019). The first-order equation could be differentiated as the equation below;

$$\frac{dCt}{dt} = k_1(Cs - Ct), \quad (3)$$

Where Ct was the extraction capacity of M. paradisiacal inflorescence at different extraction times (t), while Cs denoted the concentration of the target polar compound extracted from the plant matrix at saturation, and k1 was the extraction rate coefficient per minute (min-1). Equation 3 was further integrated into Equation 4 and 5 as stated below with the boundary conditions of Ct = 0 and Ct = Ct.

$$In\left(\frac{Cs}{Cs-Ct}\right) = k_1(t), \quad (4)$$

$$log10(Cs - Ct) = \log(Cs) - \frac{k_1}{2.303}t, \quad (5)$$

The first-order kinetic model was expressed using exponential decay when taking the natural logarithm of the equation. Thus, in [Cs – Ct] was plotted against the extraction time to obtain slope and interception.

2.6.2. Second-order kinetic modeling

A second-order kinetic model was implemented to describe the two mechanisms during the extraction of M. paradisiacal inflorescence. The rate of dissolution has been expressed in Equation 6 as described by (Alara & Abdurahman, 2019).

$$\frac{dCt}{dt} = k_2\,(Cs - Ct)^2, \tag{6}$$

Where K_2 is denoted as the extraction rate coefficient of the second-order kinetic model in millimeters per milligram per minute (mL $mg^{-1}min^{-1}$) With the boundary conditions of $Ct = 0$ when $t = 0$; $Ct = Ct$ when $t = t$; Equation 6 had been further integrated into Equation 7.

$$Ct = \frac{Cs^2 k_2 t}{1 + Cs k_2 t}, \tag{7}$$

Equation 7 was then rearranged into Equation 8 and multiplied by $t = t$ to obtain a linearised Equation 9.

$$\frac{1}{Ct} = \frac{1}{k_2 Cs^2 t} + \frac{1}{Cs} \tag{8}$$

$$\frac{t}{Ct} = \frac{1}{k_2 Cs^2} + \frac{t}{Cs}\,, \tag{9}$$

Inversion of Equation 9 into Equation 10 and the initial extraction rate coefficient (h) were stated as Equation 11 mentioned below;

$$\frac{Ct}{t} = \frac{1}{\left[\frac{1}{k_2 Cs^2} + \frac{t}{Cs}\right]}\,, \tag{10}$$

$$h = k^2 Cs^2, \tag{11}$$

Thus, the substitution of Equation 11 into Equation 9 generated Equation 12 to identify the extraction rate coefficient (k_2), initial extraction rate coefficient (h), slope, and interception of the second-order kinetic model by plotting $1/(Cs) - 1/(Ct)$ against time.

$$\frac{t}{Ct} = \frac{1}{h} + \frac{t}{Cs}, \tag{12}$$

2.7. Statistical analysis

All data were expressed in mean ± standard deviation and interpreted using ANOVA analysis and kinetic modeling in Minitab Software and Microsoft Excel 2019, respectively.

3. Results

3.1. Total extraction oil yield (%)

Table 1 demonstrates the total extraction yield of the different-sized M. paradisiacal inflorescence matrix after 160 minutes of SFE-CO2 and 10 hours - Sechelt extraction. Notably, M. paradisiacal inflorescence matrix with a smaller particle size yielded a consistently higher total oil extraction than a larger particle size, as observed in both supercritical extraction and Sechelt extraction. Plant matrices with the smallest particle size (450 µm – 600 µm) exhibited the highest total oil extraction at 25MPa, 40°C; 45MPa, 40°C; and 45MPa, 60°C. At various extraction parameters, the total oil yield from plant matrices sized 450 µm – 600 µm and 600 µm – 850 µm exhibited no statistically significant difference ($p > 0.05$). Meanwhile, plant matrix with particle size in the range of 850 µm –1600 µm showed a significant difference ($p < 0.05$) when contrasted with both the particle size ranges of 450 µm – 600 µm and 600 µm – 850 µm. This significant effect emphasised the prominence of particle size in shaping the outcome of the

extraction process under these specific conditions. In Sechelt extraction, M. paradisiacal inflorescence with the size of 600 µm – 850 µm yielded the highest extracted oil (17.10 ± 0.99%) followed by 450 µm – 600 µm (16.15 ± 0.49%), and lastly 850 µm –1600 µm (14.25 ± 0.92%).

Table 1. Effect of particle size of M. paradisiaca inflorescence matrix on total extraction yield (%) in Soxhlet and supercritical fluid extraction

Particle size	Soxhlet extraction	Supercritical fluid extraction			
		25 MPa, 40°C	25 MPa, 60°C	45 MPa, 40°C	45 MPa, 60°C
450 µm – 600 µm	16.15 ± 0.49	16.71 ± 1.55^{bc}*	16.21 ± 2.39^{c}	17.16 ± 3.38^{c}	29.40 ± 7.95
600 µm – 850 µm	17.10 ± 0.99	15.60 ± 2.46^{a}	17.63 ± 3.20^{c}	16.95 ± 3.14^{c}	20.16 ± 3.99
850 µm –1600 µm	14.25 ± 0.92	16.32 ± 2.31^{a}	10.85 ± 1.57^{ab}*	9.54 ± 1.60^{ab}*	14.81 ± 2.62

The data was expressed in mean ± standard deviation. The superscript 'a', 'b', and 'c' indicates there is a significant difference ($p < 0.05$) as compared to 450 µm – 600 µm, 600 µm – 850 µm, and 850 µm-1.60mm, respectively. Data denoted with the symbol * indicates a significant difference ($p < 0.05$) between the Sechelt and supercritical fluid extraction. Meanwhile, data without a common superscript indicates no significant difference between the groups ($p > 0.05$).

3.2. Total catechin content (mg / 2g of extracted sample oil)

The increasing particle size of the M. paradisiacal inflorescence matrix decreased the total catching content. The results shown in Table 2 are derived from the intra-group comparison within the supercritical extraction groups revealing that the plant matrices with sizes ranging from 600 µm – 850 µm yielded the highest total catching content, followed by 450 µm – 600 µm, and 850 µm – 1600 µ m. The total catching content of the plant matrix with a particle size of 450 µm – 600 µm exhibited significant differences ($p < 0.05$) compared to those with the particle sizes of 600 µm – 850 µm and 850 µm – 1600 µ m across all four extraction parameters. This signifies that the impact of particle size strongly influenced the catching yield under different supercritical extraction parameters. Furthermore, the most substantial amount of catching content (89.52 ± 1.83 mg) was extracted from the M. paradisiacal inflorescence matrix with particle sizes ranging from 450 µm to 600 µm using the Sechelt extraction, which was also statistically comparable ($p > 0,05$) to that obtained from 450 µm – 600 µm plant matrix at 45 MP a, 40°C (81.51 ± 1.11 mg) using supercritical extraction. Overall, the significant difference ($p < 0.05$) between the Sechelt extraction and supercritical extraction groups highlighted their distinct effects on catching extraction, driven by their differing operating principles.

Table 2. Effect of particle size of M. paradisiaca inflorescence matrix on total catechin content (mg / 2g of extracted sample oil) in Soxhlet and supercritical fluid extraction

Particle size	Soxhlet extraction	Supercritical fluid extraction			
		25 MPa, 40°C	25 MPa, 60°C	45 MPa, 40°C	45 MPa, 60°C
450 µm – 600 µm	89.52 ± 1.83*	62.60 ± 0.65^{bc}*	32.53 ± 0.49^{b}*	81.51 ± 1.11^{bc}	21.03 ± 1.42^{bc}*
600 µm – 850 µm	39.52 ± 1.82*	55.64 ± 0.39^{ac}*	34.55 ± 0.27^{a}*	50.77 ± 0.73^{a}*	55.58 ± 0.42^{a}*
850 µm – 1600 µm	28.75 ± 1.31*	72.60 ± 0.17^{ab}*	35.38 ± 0.78*	50.99 ± 0.31^{a}*	55.58 ± 0.31^{a}*

The data was expressed in mean ± standard deviation. The superscript 'a', 'b', and 'c' indicates there is a significant difference ($p < 0.05$) as compared to 450 µm – 600 µm, 600 µm – 850 µm, and 850 µm-1.60mm, respectively. Data denoted with the symbol * indicates a significant difference ($p < 0.05$)

between the Sechelt and supercritical fluid extraction. Meanwhile, data without a common superscript indicates no significant difference between the groups ($p > 0.05$).

3.3. DPPH radical scavenging activity

A DPPH radicals scavenging experiment was conducted to further manifest the amount of catching isolated from M. paradisiacal inflorescence matrix of different sizes. Based on the data shown in Table 3, the DPPH scavenging activity of the plant extract decreased with the particle size increase. Across the four supercritical extraction parameters, the DPPH scavenging activity of the plant matrix with the smallest particle size range of 450 µm – 600 µm was approximately the highest, followed by 600 µm – 850 µm, and lastly 850 µm – 1600µm. This may relate to the total catching content shown in Table 2. The higher the catching content extracted, the greater the DPPH radical scavenging activity, in comparison to the supercritical extraction groups, the Sox let extracted M. paradisiacal inflorescence matrix exhibited a significant ($p < 0.05$) stronger radical scavenging effect.

Table 3. Effect of particle size of M. paradisiaca inflorescence matrix on DPPH scavenging activity (%) using Soxhlet and supercritical fluid extraction

Particle size	Soxhlet extraction	Supercritical fluid extraction			
		25 MPa, 40°C	25 MPa, 60°C	45 MPa, 40°C	45 MPa, 60°C
450 µm – 600 µm	67.59 ± 1.23	26.99 ± 0.49^{c}*	25.94 ± 1.20^{c}*	26.53 ± 0.72^{c}*	25.74 ± 1.70*
600 µm – 850 µm	59.24 ± 0.63	25.58 ± 0.87^{c}*	25.98 ± 1.81^{c}*	25.90 ± 0.40^{c}*	21.62 ± 1.15*
850 µm – 1600 µm	50.15 ± 0.63	22.32 ± 1.74^{ab}*	21.59 ± 1.07^{ab}*	23.95 ± 2.68^{ab}*	23.42 ± 2.23*

The data was expressed in mean ± standard deviation. The superscript ‘a’, ‘b’, and ‘c’ indicates there is a significant difference ($p < 0.05$) as compared to 450 µm – 600 µm, 600 µm – 850 µm, and 850 µm-1.60mm, respectively. Data denoted with the symbol * indicates there is a significant difference ($p < 0.05$) between the Sox let and supercritical fluid extraction. Meanwhile, data without a common superscript indicates there is no significant difference between the groups ($p > 0.05$).

3.4. Kinetic modeling

The experimental data of the catching content from M. paradisiacal inflorescence matrix by supercritical fluid were expressed and plotted as first-order and second-order kinetic models. The specific kinetic parameters, including rate constant (k), interception, and concentration of water-soluble compounds of M. paradisiacal inflorescence at saturation (Cs), coefficient determination (R2), and initial extraction rate (h) were identified based on the mathematical regression equations for each particle size and extraction parameters. In Table 4, the plant matrix's extraction kinetics (k1 values) with a particle size of 450 µm – 600 µm ranged from 0.02 to 0.04 min^{-1}. Meanwhile, the M. paradisiacal inflorescence matrix extraction demonstrated the lower k_2 values, ranging from 0.01 to 0.04 mL $mg^{-1}min^{-1}$, in the second-order kinetic model. The Cs value of water-soluble compounds in M. paradisiacal inflorescence was between 19.86 - 81.90 mL/mg and 2.00 - 5.26 mL/mg for the first- and second-order kinetic model, respectively. Moreover, a higher coefficient of determination (R^2) was observed in the first-order kinetic model, indicating a 95% fit for the extraction of M. paradisiacal inflorescence matrix with a particle size of 450 µm – 600 µm to the first-order modeling.

Table 4. First- and second-order kinetic model of M. paradisiaca inflorescence matrix with particle size range of 450 µm – 600 µm

Extraction parameters	k_1 (min^{-1})	k_2 (mL mg^{-1} min^{-1})	Initial extraction rate	Interception	Cs (mL/mg)	R^2

				(h)						
Pressure (MPa)	Temperature (°C)				First-order	Second-order	First-order	Second-order	First-order	Second-order
25	40	0.03	0.01	0.32	5.26	-0.66	63.10	5.26	0.9289	0.6860
25	60	0.02	0.01	0.13	2.58	-0.25	19.86	2.00	0.9526	0.9897
45	40	0.04	0.04	0.33	-1.38	-1.38	81.90	5.12	0.6270	0.6270
45	60	0.03	0.01	0.16	3.54	-0.55	21.89	3.95	0.9549	0.9293

The data above was obtained from Solver of Microsoft Excel®.

The M. paradisiacal inflorescence matrix with a particle size range of 600 µm – 850 µm demonstrated higher k1 values (0.031 - 0.039 min^{-1}) than the k_2 values (0.01 - 0.03 mL $mg^{-1}min^{-1}$) (Table 5). The Cs value for the plant matrix at the higher temperature of 60°C was lower than that extracted at 40°C in both kinetic models. In addition, a higher coefficient of determination (R^2) was observed in the first-order kinetic model (0.9735 - 0.9995) compared to the second-order model (0.7942 - 0.9198). This suggests that the extraction of M. paradisiacal inflorescence matrix with a particle size of 600 µm – 850 µm was more likely to be fitted to the first-order modeling.

Table 5. First- and second-order kinetic model of M. paradisiaca inflorescence matrix with particle size range of 600 µm – 850 µm

Extraction parameters		k_1 (min^{-1})	k_2 (mL mg^{-1} min^{-1})	Initial extraction rate (h)	Interception		Cs (mL/mg)		R^2	
Pressure (MPa)	Temperature (°C)				First-order	Second-order	First-order	Second-order	First-order	Second-order
25	40	0.039	0.03	0.76	4.92	-0.67	51.26	7.89	0.9995	0.7985
25	60	0.030	0.01	0.53	4.32	-0.58	34.55	4.54	0.9774	0.9198
45	40	0.036	0.02	0.60	5.23	-0.97	49.94	6.80	0.9735	0.8580
45	60	0.031	0.03	0.53	5.27	-1.49	43.99	5.92	0.9822	0.7942

The data above was obtained from Solver of Microsoft Excel®.

The extraction kinetic modeling of the M. paradisiacal inflorescence matrix with the particle size range of 850 µm – 1600 µ m was stated in Table 6 below. The k_1 values of the extraction vary between 0.025 and 0.039 min^{-1}, whereas, the k_2 values range from 0.01 to 0.02 mL mg^{-1} min^{-1}. Higher k values manifested that faster extraction kinetics was observed at the extraction parameters of 25MPa and 40°C. The saturation concentration of water coble compounds in the plant matrix was ranged 39.034 to 72.596 mL/mg for the first-order model and from 4.75 to 13.13 mL/mg for the second-order model. A higher R2 values signify a better fit to the kinetic models. Similar to the data in Table 4 and 5, lower R2 values were observed in the second-order kinetic model, suggesting that the first-order model provided a better fit to the data.

Table 6. First- and second-order kinetic model of M. paradisiaca inflorescence matrix with a particle size range of 850 µm – 1600 µ m

Extraction parameters	k_1 (min^{-1})	k_2 (mL mg^{-1} min^{-1})	Initial extraction rate (h)	Interception		Cs (mL/mg)		R^2	
Pressure (MPa)	Temper-			1st-order	2nd-order	1st-order	2nd-order	1st-order	2nd-order

	ature (°C)									
25	40	0.039	0.02	0.91	5.09	-0.27	72.596	13.13	1.00	0.8704
25	60	0.025	0.01	0.28	4.46	-0.62	39.034	4.94	0.97	0.8703
45	40	0.029	0.01	0.89	5.16	-1.20	70.201	6.50	0.99	0.7962
45	60	0.029	0.01	0.13	4.70	-0.30	63.236	4.75	0.93	0.8272

The data above was obtained from Solver of Microsoft Excel®.

4. Discussion

M. paradisiacal inflorescence matrix with a smaller particle size yielded a higher total oil extraction than a larger particle size. Under the supercritical extraction parameters of 25MPa, 40°C; 25MPa, 60°C; and 45MPa, 40°C, the influence of particle size in the range of 850 µm – 1600µm exhibited a statistically significant difference ($p < 0.05$) when contrasted with both the particle size ranges of 450 µm – 600 µm and 600 µm – 850 µm. This significant effect emphasised particle size's prominence in shaping the extraction process's outcome under these specific conditions. An investigation conducted by Hu & Chen, (2019) indicated that smaller particle sizes maximised the total surface area of the green tea powder, which in turn improved the mass transfer of oil from the tea matrix to the supercritical fluid. Nevertheless, a higher extraction yield was observed at 600 µm – 850 µm in both Sox let and supercritical fluid extraction. Specifically, M. paradisiacal inflorescence with the size of 600 µm – 850 µm yielded the highest extracted oil (17.10 ± 0.99%) followed by 450 µm – 600 µm (16.15 ± 0.49%), and lastly 850 µm – 1.60mm (14.25 ± 0.92%) (Table 1) Theoretically, finer particles enhance the extraction yield of plant oil, however, smaller particle size was susceptible to agglomerating or clumping together due to the presence of electrostatic or Van der Waals interactions between the particles, which in turn reduced the surface area in contact with the solvent (Yeop et al., 2017).

Agglomeration of fine particles would result in the elevation of surface area to mass ratio, limitation of mass transfer rate, uneven distribution of the solvent, and hindering the diffusion of M. paradisiacal inflorescence oil into the solvent (Zhang et al., 2018) (Rodríguez De Luna et al., 2020). Additionally, there was no significant difference between each group ($p > 0.05$) at 45MPa and 60°C. This indicated that the M. paradisiacal inflorescence matrix particle sizes did not demonstrate a significant impact ($0 > 0.05$) on the extraction efficiency compared to the Sox let method and other extraction parameters. In Sox let extraction, the desired chemicals are mostly accomplished through the diffusion of solvents into the solid-phase material (Zhang et al., 2018). The total extraction oil yield of the supercritical extracted plant matrix with a particle size range of 450 µm – 600 µm was not significantly different ($p > 0.05$) compared to the 10-hour Sox let extraction. It could be demonstrated that the effectiveness of both extraction techniques was at par ($p > 0.05$) in terms of extracting the plant matrix, which may be due to two possible factors, including the pore structure of the plant matrix at different particle sizes and the operational parameters of both extraction methods (Punín Crespo & Lage Yusty, 2005). Smaller particles have a higher surface area with smaller internal pores, which increase the particles porosity and pore volume, allowing more surface contact with the solvent during the extraction process.

Furthermore, the elevation of temperature and pressure would also enhance the extraction yield by increasing the pore volume of the plant matrix. The statement could shed light on the outcomes tabulated in Table 1, where the data revealed that supercritical extraction conducted at 40°C emitted a lower yield than the Sox let extraction, whereas, supercritical extraction operated at 60°C produced a greater extraction yield than Sox let extraction. However, temperature elevation will also cause degradation or alteration of the pore network, potentially reducing the pore volume and the extraction yield (Schenker et al., 2000). High pressure has an influence on pore volume in the same way that temperature does. An optimum pressure level will promote solvent adsorption within the pores of particles, but extremely high pressure also leads to pore deformation (Krause et al., 2020).

The total catching content was decreased with the increased particle size of M. paradisiacal inflorescence matrix (Table 2). The overall outcomes implied that the particle size did not exhibit a significant ($p > 0.05$) impact on the effectiveness of catching extraction. However, a significant distinction ($p < 0.05$) was observed between the plant matrices with particle sizes of 450 µm – 600 µm and 600 µm – 850 µm under supercritical fluid extraction conditions of 25MPa, 60°C as well as 45MPa, 60°C. Besides, a larger plant matrix (600 µm – 850 µm) yielded a greater amount of catching than the smaller size (450 µm – 600 µm) under the aforementioned extraction parameters. These scenarios could be due to the plant matrix's rapid oxidation of bioactive compounds. The smaller the particle size, the larger the surface area, which

could lead to increased exposure of catching molecules to oxygen in the surrounding environment (Fotsing Yannick Stéphane et al., 2022). The oxidation of catching involves the conversion of ortho - dihydroxy (catching) and ortho – trihydroxy - phenyl 'B' rings into reactive ortho quinones (Abudureheman et al., 2022). Consequently, this phenomenon could potentially influence the overall yield of catching extracted from the M. paradisiacal inflorescence matrix.

Under elevated temperature conditions, catching molecules gained greater kinetic energy, increasing the frequency of molecular collisions. Subsequently, as the catching molecules accumulate sufficient energy to overcome the activation energy barrier, the degradation process will be accelerated and lead to a faster degradation rate (Abudureheman et al., 2022). Higher pressure would also promote rapid degradation of target compounds. Additionally, there was no significant difference ($p > 0.05$) has been observed in the catching content between the plant matrices with sizes ranging from 850 µm – 1600µm and 600 µm – 850 µm across all four extraction parameters. This augmented the similarity in catching contents attributed to the comparable accessibility of the supercritical fluid to the solubility of catching molecules. Smaller particles exhibited a larger surface area for interaction that facilitated stronger intermolecular interaction between the catching and solvent (Nabil et al., 2020). Thus, solubility and diffusion of catching into the solvent were improved as substantiated by the observed significant catching content obtained from the plant matrix with particle sizes ranging from 450 µm – 600 µm.

Even though smaller particle sizes could yield more catching from the plant matrix, the implementation of larger particles characterised by a smaller surface area to volume ratio mitigated the oxidative reaction and degradation of catching (Yaneva & Ivanova, 2020). Moreover, the larger particle size had the potential to delay the onset of saturation points, wherein the supercritical fluid might become more saturated with the catching (Wang et al., 2014). Thus, a reduction of the propensity for saturation offered a hindrance to further extraction. Overall, the most substantial catching content (89.52 ± 1.83 mg) was extracted from the M. paradisiacal inflorescence matrix with particle sizes ranging from 450 µm to 600 µm using the Sox let extraction. The significant difference ($p < 0.05$) between the Sox let extraction and supercritical extraction groups highlighted their distinct effects on catching extraction, driven by their differing operating principles (Shi et al., 2022). Sox let extraction is a solid-solvent extraction technique that involves the continuous diffusion of the solvent into the plant matrix to extract the target compounds (Zygler et al., 2012).

Meanwhile, under precise pressure and Tempe rapture parameters, supercritical fluid extraction utilises carbon dioxide as a supercritical solvent. The pressurised supercritical fluid effectively penetrates solid matrices, dissolving their target compounds (Fraguela-Meissimilly et al., 2023). In contrast, the efficiency of catching extraction from the plant matrix was comparable ($p > 0.05$) using the supercritical extraction method at 45MPa and 40°C (81.51 ± 1.11 mg), achieving an indistinguishable outcome as a 10-hour extraction using the Sox let method ($p > 0.05$). This result suggested that the supercritical extraction method offered advantageous properties in reducing extraction time and lowering solvent usage (Cid-Ortega & Monroy-Rivera, 2018).

A DPPH radicals scavenging experiment was conducted to manifest further the amount of catching isolated from M. paradisiacal inflorescence matrix of different sizes. Based on the data in Table 3, the DPPH scavenging activity of the plant extract decreased with the particle size increase. At the extraction parameter of 45MPa and 40°C, the DPPH scavenging activity of the plant matrix with the smallest particle size range of 450 µm – 600 µm was 26.53 ± 0.72%, followed by 25.90 ± 0.40% (600 µm – 850 µm), and 23.95 ± 2.68% (850 µm – 1600µm). This may relate to the total extraction and catching content shown in Table 2. Generally, the higher the total extraction and catching content, the stronger the DPPH scavenging activity (Loganayaki et al., 2010) In the process of scavenging, catching's hydroxyl groups donate hydrogen atoms to DPPH radicals, concurrently engaging in the self-oxidation of catching as part of the radical neutralisation mechanism (He et al., 2009). Furthermore, the DPPH scavenging activity of M. paradisiacal inflorescence may also attributed to the presence of phenolic compounds other than catching, including rut in, Gallic acid, quercetin, and tannic acid (Amornlerdpison et al., 2020). A statistically significant difference ($p < 0.05$) in the DPPH scavenging activity of M. paradisiacal inflorescence matrix was observed among the extraction parameters based on particle size, except for the parameters at 45MPa and 60°C. At 45MPa and 60°C, there was no significant difference ($p > 0.05$) between the three particle sizes, which indicated that the effects of DPPH scavenging activity of the extracted catching were at par. Additionally, the scavenging activity of catching extracted from the plant matrix, with particle sizes ranging from 450 µm - 600 µm and 600 µm – 850 µm possessed no significant difference ($p > 0.05$), in which these outcomes corresponded to the total catching content that previously mentioned in Table 2. Thus, this could further explain that the DPPH scavenging activity of the plant matrix was highly dependent on the presence of catching (Anggraini et al., 2019).

In comparison to the extraction temperature at a specific pressure level, the reduction of DPPH scavenging activity of the plant matrix was observed at a higher temperature level of 60°C than 40°C. This may be due to the thermal degradation of catching and other phenolic compounds when exposed to higher temperatures. At elevated temperatures, the catching could undergo decarboxylation and result in the formation of a Quinone -like intermediate (Muzolf-Panek et al., 2008). Quinone-like intermediate is a byproduct of catching decarboxylation that was highly susceptible to further oxidation through reacting with the nucleophile molecules and led to the formation of covalent adducts between the intermediate and nucleophiles (Chen & Pignatello, 1997). Subsequently, the structural alteration of catching was facilitated by the intermediate nucleophile adducts, which potentially resulted in the degradation and loss of catching's biological activity (Chen & Pignatello, 1997). Moreover, the pressure of supercritical extraction appeared to have less or no impact on the DPPH scavenging activity of the plant matrix (Patras et al., 2009).

The DPPH scavenging activity of the plant matrix extracted using Sox let extraction exhibited a stronger effect on the DPPH radicals, and the results were consistent with the total catching content reported in Table 2. Further, a significant difference ($p < 0.05$) between the Sox let and supercritical extraction was observed. Greater amount of catching isolation and DPPH scavenging activity using Sox let extraction at 50°C with solvent of 50% (v/v) ethanol further demonstrated that the extraction temperature significantly influenced the molecular structure and antioxidant activity of the catechu, as lower extraction temperature prevented the degradation of catching from high temperature. The high scavenging activity of extracted Piece Abase Bark using 50:50 ethanol/water was investigated by Zeppetzauer et al., (2023) demonstrating the impacts of different ratio solvents on temperature, solvation power of solute, and solvent polarity.

Based on the theory of kinetic modeling, higher k values demonstrate a faster extraction rate (Alara & Abdurahman, 2019). In Table 4, the lowest k1 value of extraction was recorded at 25MPa, 60°C (0.02 min-1); while the highest k1 value was noted at 45MPa, 40°C (0.04 min-1). In the second-order kinetic model, extraction of M. paradisiacal inflorescence matrix at 45MPa and 40°C also acquired the highest k_2 value of 0.04 mg-1min-1. The k1 and K2 values of the extraction of catching from M. paradisiacal inflorescence matrix with particle size ranging from 600 µm – 850 µm were tabulated in Table 5. In both kinetic models, the highest k value of catching extraction was identified when the extraction process was conducted at 25 MP a and 40°C; whereas, catching content at 25MPa and 60°C obtained the lowest k1 values (0.030 min-1 and 0.01 mL mg-1min-1, respectively). The initial extraction rate (h) of catching content from the plant matrix with a particle size of 600 µm – 850 µm varied between 0.53 to 0.76 The kinetic parameters from the catching content from the largest particle size of the experimental M. paradisiacal inflorescence matrix (850 µm – 1600µm) are stated in Table 6. Similar to previous scenarios, catching extraction at 25 MP, 40°C and 25MPa, 60°C acquired the highest and the lowest k1 and k2 values, respectively. Nevertheless, the k values at 45MPa and 40°C were considerably low, exhibiting k values that were approximately on par with those obtained during extractions at 45MPa and 60°C. Therefore, the previously hypothesised favorable extraction condition of 25MPa and 45MPa at 40°C aforementioned was justified as the higher pressure promoted a better extraction rate, the more rapid the isolation of catching from the plant matrix, and the faster the extraction occurs (Chuang et al., 2015).

Cs or concentration of target compound of the plant matrix at saturation was related to the concept of solubility of the polar compound being extracted (Agu et al., 2021). The solubility of the catching in supercritical fluid was highly dependent on its polarity. The addition of 50% (v/v) ethanol a as co-solvent to increase the solvent power of supercritical CO2 fluid could by developing a hydrogen bridge between the molecules of supercritical CO2 and catching (Huaman-Castilla et al., 2019). The solubility of the catching could also be enhanced by elevating the extraction temperature and pressure. The extraction parameters of 25MPa, 40°C for the extraction of catching from the plant matrix with size ranging from 450 µm - 600 µm was recorded with the highest extraction capacity in both kinetic models, while 25MPa, 60°C got the lowest value in both kinetic models are shown in Table 4. The higher the Cs value, the greater the solubility of the target compound was demonstrated as evidenced by the highest and lowest achievement of catching content observed in the extraction conducted at 45MPa, 40°C and 25MPa, 60°C, respectively (Al-Hamimi et al., 2016). A greater temperature also boosts the solubility of the catching by enhancing the polar compound's molecular mobility in the non-polar supercritical fluid, which then increases the rate at which the plant matrix is diffused into the extraction phase (Atwi-Ghaddar et al., 2023).

However, the polar compounds may tend to degrade at higher temperatures, resulting in a lower yield of the extracted oil and catching content. The increased particle sizes range of M. paradisiacal inflorescence matrix (600 µm - 850 µm and 850 µm – 1600µm) influenced the extraction capacity by affecting the surface area available in contact with the supercritical fluid, the rate of diffusion, and the accessibility of the catching within the matrix. When the particle size of M. paradisiacal inflorescence matrix had increased to

850 µm – 1.60 mm, the extraction capacity of catching had slightly increased as shown in Table 6. In general, the smaller particle size would lead to greater surface area and enhance the extraction process. However, a decrease in extraction capacity was observed when the particle size of M. paradisiacal inflorescence matrix increased. The outcomes could be attributed to several factors, including the rapid oxidation of smaller particle size of the plant matrix resulted in the reduction in the available catching content, agglomeration of the smaller particle size created a barrier that impeded solvent penetration and hindered the diffusion between the supercritical fluid and catching (Sodeifian & Usefi, 2023); (Koina et al., 2023).

Based on the data shown in Table 4, the first-order kinetic model of catching content from the plant matrix ranged from 450 µm – 600 µm had the lowest R2 value of 0.63 when extracted at the parameters of 45MPa and 40°C. Generally, the R2 value ranging from 0.3 to 0.5, 0.5 to 0.7, and 0.7 to 0.9 are considered weak, moderate, and strong effect sizes, respectively (Moore, 1996). Thus, it could be suggested that the catching content was moderately fitted to the first-order kinetic model with an approximate 63% variation presented in the catching content; in other words, the prediction of the model was 63% close to the actual catching content (Akossou & Palm, 2013). Whereas, the R2 values of catching content obtained by different extracting conditions were around 0.93 and 0.95, indicating that the actual catching content was 93% to 95% fitted to the first-order kinetic model. In addition, a good relationship between the first-order predictor and catching content stipulated that the rate of catching extraction was more likely to be directly proportional to the amount of catching contented from the plant matrix (Wang et al., 2014), which the amount of catching had been decreased over the extraction time. Meanwhile, the R2 values scored by the predictive secondary-order model was relatively lower than the first-order model, except the R2 value of catching content isolated at 60°C under the pressure level of 25MPa and 45MPa (0.9897 and 0.9293, respectively). Thus, the higher R2 value indicated that the catching extraction process at these particular conditions was highly possibly influenced by the higher temperature of 60°C, which also meant that the extraction process may involve two mechanisms.

Higher pressure facilitated the extraction of compounds that dissolve from the plant matrix in supercritical extraction. The isolated compounds subsequently separated in the solvent via structural breakdown at high temperatures (Castellanos-Gallo et al., 2022). In the first order of catching content from the plant matrix with a sized of 600 µm – 850 µm (Table 5), the R2 values ranged from 0.9735 to 0.9995 across the extraction parameters, which could indicate that the actual yield of catching from the particular size of plant matrix was around 97% to 99%. In the second-order kinetic models, the R2 values were lower than the first-order kinetic models. Yet, a higher R2 value of 0.9198 was observed when catching extraction was processed at 25MPa and 60°C, which was about 92% fitted to the kinetic model, the mechanism of the extraction process could be influenced by both pressure and temperature simultaneously as discussed previously. Concerning the larger particle size of M. paradisiacal inflorescence (850 µm – 1600µm), the actual catching content was 93% to 100% fitted to the first-order kinetic model; meanwhile, the R2 values ranged between 0.7962 to 0.8704 (about 80% to 87% fitted) in the second-order model. It could be stipulated that the mechanism of the catching extraction was considerably to be the single-step mechanism. A single-step reaction primarily prevailed by the solubility and diffusion of the catching from the plant matrix oil in the supercritical CO2 fluid (Sovová et al., 2016). The increase in pressure and temperature could enhance the solubility of the catching and thus elevate the extraction yield.

5. Conclusions

The supercritical extraction of catching content from M. paradisiacal inflorescence involved a complex interplay of particle size, pressure, and pressure. This study determined the optimal conditions at 40°C with pressure levels of 25MPa and 45MPa, enhancing the solubility and total extraction yield. Particle size, particularly in 450 µm – 600 µm, exhibited a higher total oil yield and catching content than the other particle size ranges. The extraction kinetics were assessed through first- and second-order models, which revealed a single-step mechanism predominantly influenced by solubility and diffusion. Further studies may employ advanced analytical methods like NMR and HPLC for in-depth catching characteristics. Implementing statistical design experiments and response surface methodology is recommended to refine various extraction variables and optimise efficacy.

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