



Optimization of Process Parameters Using Response Surface Methodology for Essential Oil Extraction from *Canarium odontophyllum* Kernels by Subcritical Water Treatment

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Abstract

The physicochemical properties of dabai kernel oil as well as the morphology of dabai samples are affected by the optimal extraction method and process configurations. Subcritical water treatment prior to dabai kernel oil extraction was optimized through the utilization of response surface methodology based on central composite design. The subcritical water treatment was carried out within the following temperature ranges of 100 to 150 °C, 10- to 20-min reaction times, and 10:1 to 20:1 solvent to solid ratios. Results showed that temperature of 100 °C, reaction time of 10 min, and solvent to solid ratio of 10:1 were optimal parameters for dabai kernel oil by subcritical water treatment prior to Soxhlet extraction. Dabai kernel oil yields via subcritical water treatment were 96.53% on average, compared to 42.85% for Soxhlet extraction sample. Gas chromatography mass spectrometry analysis found for subcritical water treatment samples revealed value-added compounds, including oxalic acid and 9-octadecenoic acid, which can be utilized for cleaning and pharmaceutical applications. Scanning electron microscope images provided evidence that rapid extraction was driven by the degradation and aggregation of dabai kernel powder structure. The Brunauer–Emmett–Teller analysis indicated that the dabai kernel of subcritical water treatment displayed a greater surface area (14.813 m²/g) than the raw (2.804 m²/g) or Soxhlet extraction (13.452 m²/g) dabai kernel. Subcritical water treatment could be considered a promising method in combination with Soxhlet extraction in order to improve the oil yield from dabai kernels.

Keywords Dabai · Essential oil · Extraction · Subcritical water · Response surface methodology

Introduction

Dabai (*Canarium odontophyllum*) is unique to the Borneo Islands, specifically Sabah and Sarawak. It is a seasonal fruit in Malaysia, and Sarawak is the only commercial dabai producer state. The fruit looks like an olive; when it is fully ripe, the skin will turn dark purple, and the kernel will be creamy yellow (Chew et al. 2011). This fruit is also sensitive to hot temperatures. The fruit has a very fatty flavor and is eaten after soaking it in warm water to soften the pulps. Unlike the palm kernel shell, the dabai seed has a strong, woody endocarp that protects the edible cotyledon. This nutshell's physical properties are similar to those of a palm kernel shell (Anthonette et al. 2022). A dabai seed contains three chambers and a cross-section similar to a sub-triangle, with the

largest chamber containing the oil-rich kernel (Ideris et al. 2021). Dabai is typically consumed fresh, with the seeds being discarded after consumption.

Each dabai fruit has only 43–56% pulp; the remaining portion, which comprises the kernel (6–9%) and peel (7–16%), might be regarded as waste. In general, 100 g of fresh dabai kernel contains 7.3 to 11.6 g of protein and 23.6 to 33.1 g of lipid (Chua et al. 2015). The dabai kernel fat is highly saturated, and it has been used as a substitute for palm kernel fat and cocoa butter in manufacturing chocolate (Azlan et al. 2020). Additionally, it has been biologically shown that dabai fat from kernel improves the lipid profile of experimental New Zealand white rabbits (Shakirin et al. 2012a). Dabai kernels provide up to 22% dietary fiber, which lowers the risk of type 2 diabetes and heart disease (Chua et al. 2015). Anthocyanin, the main phenolic ingredient in dabai fruit, is also abundant in the kernel, which has strong antioxidant qualities (Salleh et al. 2022). Another study

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examined the possible health benefits of hexane-extracted dabai kernel oil, which decreased the plasma low-density lipoprotein levels of New Zealand white rabbits, hence exerting a hypercholesterolemic impact (Shakirin et al. 2012b). Dabai kernels can also yield oleoresin, a natural substance composed mostly of essential oils and resins.

The growing acknowledgement of the value of dabai kernel oils and their health advantages has sparked economic interest in their ability to be turned into a variety of agricultural and industrial products. Future research on oleoresin or essential oil extraction from dabai kernels should be undertaken given their high vitamin E content (Abdul Kadir et al. 2021a). However, the physical, chemical, and biological properties of dabai kernel oil may vary based on the process configurations. A considerable amount of literature has been published on dabai kernel oil extraction. Researchers have found that the utilization of solvent extraction for dabai kernel oil to study the fatty acids, vitamin E, and other physicochemical properties (Azlan et al. 2010) had the protective effect on blood lipids, lipid peroxidation, and antioxidant status in healthy rabbits (Shakirin et al. 2012a); antiatherosclerotic effect in rabbits fed high cholesterol diet (Shakirin et al. 2012b); fatty acid profile and antioxidant capacity (Salleh et al. 2022); and fatty acids, triacylglycerols, thermal properties, morphology, and antioxidant activity (Norazlina et al. 2023). Moreover, ultrasound-assisted oil extraction was optimized to extract dabai kernel oil as a novel biodiesel feedstock (Ideris et al. 2021).

To date, no evidence has been associated with the application of subcritical water (SCW) concept or treatment to extract dabai kernel oil. The method of SCW treatment before Soxhlet extraction (SE) was selected because it demonstrated the possibility of extracting essential oil, given the variety of promising technologies available to develop an extraction process. SCW can broadly be defined as a new sustainable extraction method compared to other conventional methods which is safe, economical, and environmentally friendly (Nastić et al. 2018). By using SCW, water is applied to agricultural materials at pressures of up to 218 atm and temperatures ranging from 100 to 374 °C to keep it liquid (Lisbona et al. 2023). Additionally, SCW is a better choice for separations involving slightly polar and polar compounds than supercritical extraction, which is typically used to recover more nonpolar analytes. However, the limitation of highly polar water for non-polar essential oil extraction has been successfully documented in the literature (Abdul Halim et al. 2021; Nuttawan et al. 2012). Overall, supercritical extraction, due to its low polarity, makes it difficult to extract polar analytes and has high investment costs (Hamzah et al. 2022). The toxicity of the solvent, the economy of the extraction process, and the selectivity of the manufacturing process must also be taken into account (Abdul Kadir et al. 2021b). As part of the process of this study,

SE was used as a conventional method to recover essential oil from the plant matrix. SE is the most used method for phenolic compound extraction because it has several advantages, including low processing costs, ease of operation, high performance, favorability for extract recovery, and less time and solvent usage (Alara et al. 2018).

The purpose of this study is to evaluate the potential of SCW treatment to produce high quantity and quality dabai kernel oil. The study specifically attempts to attain the following objectives: (1) to determine the optimal parameters of SCW including temperature, reaction time, and solvent to solid ratio by response surface methodology (RSM) and (2) to compare the dabai kernel oil yield and physical, chemical, and morphological properties of dabai kernel oil and kernel after SCW treatment and SE.

Material and Methods

Material Preparation

A local fruit supplier from Song, Sarawak, Malaysia, provided the Song variety dabai fruit. The dabai fruits were placed in an ice box and flown to Universiti Putra Malaysia on the same day. The fruit was kept in the freezer (SIA291583, Thematic A35900, Malaysia) at 4 °C immediately after it arrived. The dabai fruits were processed to separate the pulp and seeds (shell). Next, the pulp was separated from the seed using the dabai pulp separator machine (WJ200 Inverter, Hitachi, Malaysia). After that, the dabai seed was broken by using a hammer to get the kernel. The kernel was placed on a tray and put into a box dryer (WS 30, Japan) at 60 °C for 3 days. The temperature and time of dabai kernel drying were determined based on preliminary test. When the dabai kernels were dried in an oven, temperatures below 60 °C took longer to dry and resulted in fungal development on the kernel; temperatures above 60 °C could lead to overheating and the loss of the kernel's bioactive components (Bhat et al. 2023) with maximum moisture content of 8% (Jian et al. 2019). The dabai kernels were shredded manually into small pieces after the drying process. Figure 1 summarizes the steps taken to maximize dabai kernel oil extraction in this study.

Experimental Set-Up of Subcritical Water Treatment

The SCW treatment was carried out in a static-mode batch fluid extraction system consisting of a manufactured thermal bath equipped with a temperature controller, a stirring, and rocking bed mechanism (OB-30, Protech, Malaysia). The experimental set-up for SCW treatment prior to SE is shown in Fig. 2. The 20 runs of the treatment set were designed by RSM based on selected operational parameters. The desired

Fig. 1 Flow chart of the methodology for dabai kernel oil extraction

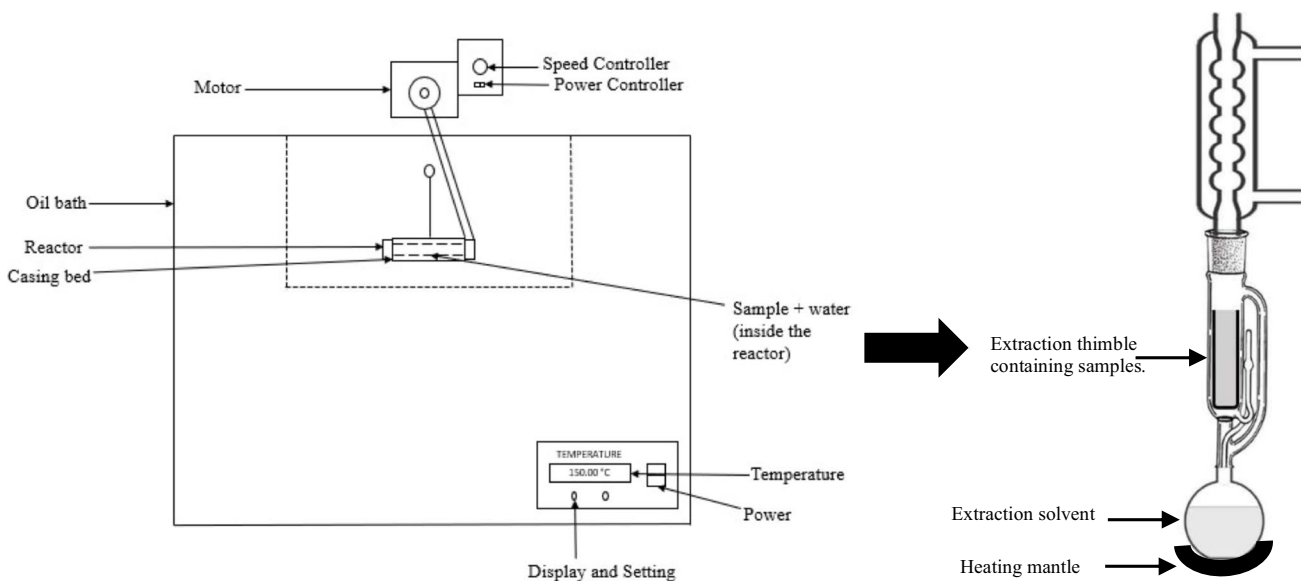
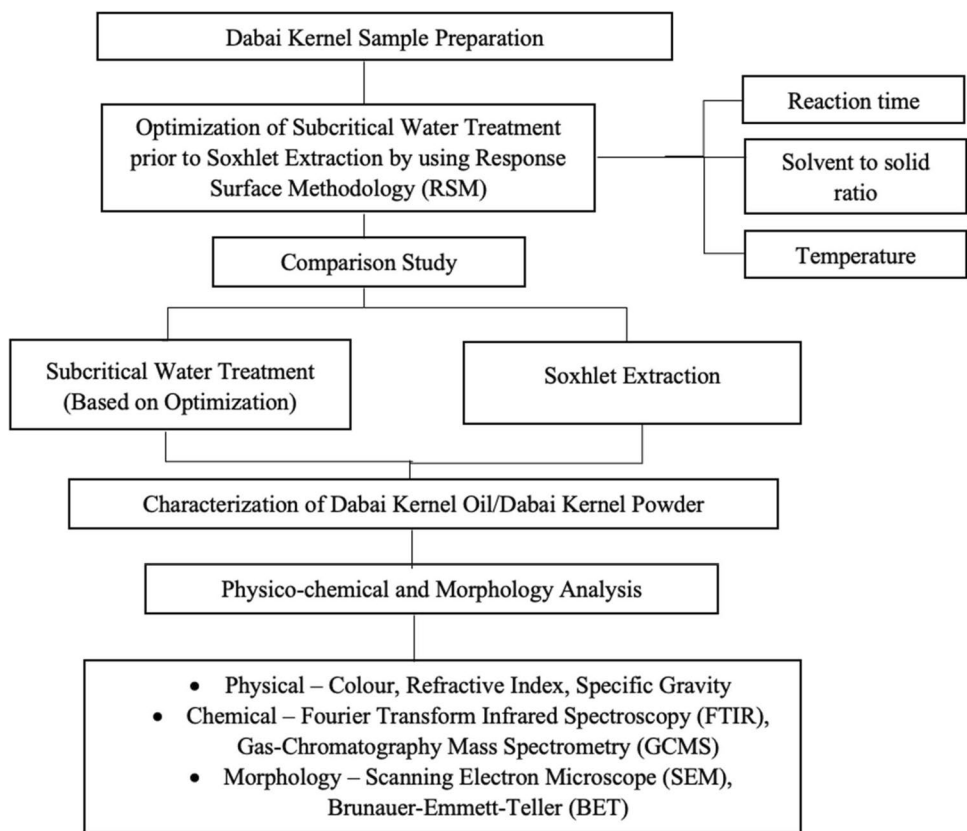


Fig. 2 Experimental set-up for SCW treatment and SE extraction

solvent to solid was prepared and poured into the 200-mm-long SS316L hydrothermal steel reactor tubes with removable caps for high pressure reactions between 100 (0.1 MPa) and 150 °C (0.48 MPa) (Swagelok Company, Japan) to make the final working volume of 30 mL. A controller (SRS11A,

SHIMADEN) increased the set point temperature to the set temperature and kept it stable during the reaction time. The oil bath was allowed to warm up to the desired temperature before each treatment. The reactor was heated with silicon oil for the treatment and placed in the casing bed to attach

to the oil bath, and the motor was switched on. Following the designated reaction time, the reactor was taken out and submerged in ice water to quickly cool down. The samples were allowed to cool for an hour in the reactor before being removed to reduce the pressure inside. Upon being taken out, the dabai kernels were placed in a laboratory oven (PF 200 Carbolite, Portugal) at 60 °C for the drying process before proceeding to SE.

Soxhlet Extraction (SE)

The SE apparatus consists of a distillation flask, cellulose extraction thimble, siphon, condenser, and heating mantle. About 4 g of the dried dabai kernel (SCW-treated and SE samples alone) was weighed using an electronic balance (SHIMADZU Electronic Balance TX223, Japan) and placed into a cellulose extraction thimble (CT30100, Aqualab Supplies, Spain). 200 mL of n-hexane was added to the empty round bottom flask. The extraction process was performed for 3 h in the SE apparatus. The dabai kernel oil was then separated from the flask using a rotary evaporator (Heidolph Laborota 4000, Germany) at 60 °C. Oil was extracted and weighed in a receiving flask, and its quantity was quantified using Eq. 1 (Xiao et al. 2022). In this study, the dabai kernel oil yield from SCW treatment before SE was compared to that of SE's samples alone using Eq. 1.

$$\text{Dabai kernel oil yield (\%)} = \frac{\text{Weight of oil recovered (g)}}{\text{Weight of dabai kernel (g)}} \times 100\% \quad (1)$$

Optimization of Dabai Kernel Oil Extraction by Response Surface Methodology (RSM)

The Design-Expert® v.11.1.2 (Stat-Ease Inc., Minneapolis, USA) software was used to optimize the effect of extraction parameters on the dabai kernel oil yield extraction. The interaction results and optimum parameters such as temperature, reaction time, and the solvent to solid ratio of SCW treatment were obtained. Table 1 shows the range and level of the parameters studied. The process parameters' actual levels and ranges were expressed as -1 (lowest), 0 (center), +1 (maximum), +, + α , and - α . The SCW treatment was performed at temperatures ranging from 100 to 150 °C, reaction times of 10 to 20 min, and solvent to solid ratios of 10:1 to 20:1. A three-level (three factors $n=3$, $\pm\alpha=1.682$) central

composite design (CCD) was used to obtain optimization of the design. This run was basically a complete 2^3 factorial plan expanded by six axial sets coded to $\pm\alpha$ and six duplications of central set points, amounting to 20 experimental data points. Analysis of variance (ANOVA) was used to perform a statistical analysis of the experiment. Table 2 presents 20 runs of experiments that were carried out to optimize oil yield. Each run was performed in triplicate.

Physicochemical and Morphology of Dabai Kernel Oil and Powder

Physical Analysis

Color The color of dabai kernel oil extracted using both methods was observed using a color reader (FRU Precise Color Reader, WR-18, China). The CIELab three-dimensional captures L (lightness), a (red/green value), and b (blue/yellow value) of the oil (Timar and Beldean 2022).

Refractive Index A drop of dabai kernel oil was placed with a pipette on the flat section of a glass prism. The refractive index of dabai kernel oil was then measured using a refractometer (ATAGO Pocket Refractometer, Japan) at 20 °C. The refractive index of the dabai kernel oil was examined between the sample from SE alone and the sample treated by SCW.

Specific Gravity The specific gravity of the dabai kernel oil extracted using both methods was determined using the method reported by Ong et al. (2021). The specific gravity of the samples was calculated according to Eq. 2.

$$\text{Specific gravity} = \frac{\text{Mass of oil sample (g)/ Volume of oil sample (mL)}}{\text{Density of water } \left(\frac{\text{g}}{\text{mL}}\right)} \quad (2)$$

Chemical Analysis

Fourier Transform Infrared Spectroscopy (FTIR) Chemical analysis of samples was performed using FTIR (Perkin Elmer FTIR-Spectrum 100, United States). A resolution of 4 cm^{-1} and 32 scans per sample was used to measure the infrared spectra at a wavelength spectrum of $650\text{--}4000 \text{ cm}^{-1}$. Dabai kernel (raw powder, SCW-treated, and

Table 1 Experiment data values of independent variables and their encoded levels for the RSM model

Symbol	Independent variables	Coded levels				
		- α	-1	0	1	+ α
A	Temperature (°C)	83	100	125	150	167
B	Reaction time (min)	6.6	10	15	20	23.4
C	Solvent to solid ratio (:)	7:1	10:1	15:1	20:1	23:1

Table 2 The independent variables and dabai kernel oil yield (experimental and predicted)

Run	Independent variables			Dabai kernel oil yield (%)	
	Factor 1	Factor 2	Factor 3	Experimental	Predicted
	A: temperature (°C)	B: reaction time (min)	C: solvent to solid ratio (:)		
1	125	15	7:1	80.94 ± 1.30	90.05
2	83	15	15:1	69.21 ± 0.74	72.60
3	100	10	10:1	98.29 ± 0.23	94.32
4	100	20	10:1	98.94 ± 0.91	93.52
5	100	10	20:1	71.04 ± 1.15	72.78
6	125	15	23:1	84.13 ± 0.26	80.22
7	150	10	20:1	98.28 ± 1.08	100.38
8	125	15	15:1	67.09 ± 0.65	66.87
9	150	10	10:1	75.63 ± 0.15	73.03
10	150	20	10:1	85.10 ± 0.63	80.03
11	100	20	20:1	54.61 ± 0.64	53.88
12	125	23.4	15:1	68.53 ± 1.05	73.22
13	125	15	15:1	67.09 ± 0.65	66.87
14	150	20	20:1	88.63 ± 0.44	89.28
15	125	15	15:1	67.09 ± 0.65	66.87
16	125	15	15:1	67.09 ± 0.65	66.87
17	125	15	15:1	67.09 ± 0.65	66.87
18	167	15	15:1	83.13 ± 0.54	84.45
19	125	15	15:1	67.09 ± 0.65	66.87
20	125	6.6	15:1	83.19 ± 0.52	83.21

SE method alone) and dabai kernel oil (SCW-treated and SE method alone) were all subjected to chemical analysis.

Gas Chromatography Mass Spectrometry (GCMS) Chemical analysis of samples was compared using a gas chromatography mass spectrometry (GCMS) (Perkin Elmer Clarus SQ 8, USA). Twenty microliters of dabai kernel oil was diluted with n-hexane. One milliliter of diluted dabai kernel oil was then injected with helium carrier gas. A 30 m × 0.25 mm column was used. The running time was set for 76.667 min, and the maximum temperature applied was 300 °C. The analysis involved two stages; temperature was increased from 3 °C per min to 230 °C and then 7 °C per min to 300 °C. After injections, the oven temperature was raised to 250 °C for 2 min, and a flow rate of helium carrier gas was set at 20 mL/min (Samadi et al. 2020a).

Morphological Analysis

Scanning Electron Microscope (SEM) Morphological analysis was carried out using a scanning electron microscope (SEM) (S-3400N Hitachi, Japan), with images taken at 1000 × magnification. Each sample was coated with a layer of platinum for 120 s using a coater (Quorumtech EMscope SC500 Sputter Coater, United Kingdom). The four dabai kernel samples observed were raw powder, SCW-treated

sample before SE, SCW-treated sample after SE, and SE alone.

Brunauer–Emmett–Teller (BET) The surface area and pore analysis of raw powder, SCW-treated, and SE's dabai kernel was performed using the Brunauer–Emmett–Teller (BET) by A3 Flex Surface Characterization (Micromeritics, United States). The sample was subjected to a pressure of 729.0293 mmHg. The degassing process of sample preparation includes stage 1, which was ramped up to 90 °C at 10 °C/min and held for 60 min, and stage 2, which was ramped up to 150 °C at 10 °C/min and held for 480 min.

Results and Discussion

Optimization of Subcritical Water Treatment for Dabai Kernel Oil Extraction by Response Surface Methodology

Table 2 presents the actual and predicted dabai kernel oil yields (%) for three SCW treatment parameters: solvent to solid ratio, temperature, and reaction time. RSM was shown to be a dependable and accurate way of providing reliable results when the experimental values of actual oil yield were compared to the projected values estimated by Design-Expert® 11.1.2 software.

Overall, the optimum parameters for obtaining 98.29% of the dabai kernel oil yield by SCW treatment before SE were 100 °C, 10 min of reaction time, and a solvent to solid ratio of 10:1. Time and temperature had a major impact on the yield of dabai kernel oil because temperature changes affect water's polarity, dielectric constant, and viscosity (Gil-Martín et al. 2022). As indicated in Table 2, the temperature of 100 °C (98.94%) provides a higher dabai kernel oil yield, as compared to the temperature of 125 °C, which produced a low output of dabai kernel oil yield (67.09%). Additionally, the dabai kernel oil yield is higher when the solvent to solid ratio is 10:1 (98.94%) than when it is 20:1, which resulted in a low dabai kernel oil yield (54.61%). Besides that, at 125 °C and 6.6 min, greater dabai kernel oil yield (83.19%) was achieved, but the longest time, 23.4 min, produced low oil yield (68.53%).

Interaction Between Independent Variables on Dabai Kernel Oil Yield

Three-dimensional (3D) surface plots and two-dimensional (2D) contour plots were created to determine the optimal value for each variable of dabai kernel oil extraction. Figure 3a–c illustrates the effects of manipulated variables on extraction yield. According to Fig. 3a, temperature and reaction time interacted with the percentage dabai kernel oil yield when the solvent to solid ratio is maintained at the optimal value of 15:1. High temperatures and short reaction times, as seen in the response plot, are associated with slightly increased oil production. Furthermore, when reaction times are kept at their optimal value of 15 min, the 3D surface response plot and contour plot in Fig. 3b showed mutual interactions between temperature and solvent to solid ratio on oil yield percentage. It was found that a rise in temperature and a higher solvent to solid ratio led to greater oil output. These findings showed that temperatures appear to significantly impact oil production more than the solvent to solid ratio. A graphical representation of the influence of reaction times and solvent to solid ratio on the percentage of dabai kernel oil yield is shown in Fig. 3c, at a constant temperature of 125 °C. From the results, it showed that the dabai kernel oil yield can be increased by combining a rapid reaction time with a greater solvent to solid ratio.

Based on the experimental work conducted at run 3, the dabai kernel oil yield was 98.29%. In accordance with the achieved results of the actual yield (94.32%), they were significantly different, a decrease of 3.97% from those investigated by the experimental work. Despite this, it remains unclear whether these optimized parameters were reliable. Therefore, the model should be validated and further supported by a test experiment performed under optimal parameters in the future, as predicted by RSM, to support its validity further (Table 3). Based on the validation test,

the average dabai kernel oil yield based on the chosen solution was $96.53 \pm 0.65\%$ which is higher than the expected (94.32%). The percentage of error is considered low which is 2.35% as the desirability to achieve the criteria was 1. The quadratic model was used to determine the best correlation between dabai kernel oil yield and parameters such as temperature, reaction time, and solvent to solid ratio.

SCW extraction was influenced significantly by temperature. High temperatures have various negative implications, including compound degradation, solubilization of undesired ingredients, and lower yield and selectivity of essential oils (Putra et al. 2023). The effect of temperature on the dabai kernel was investigated by changing the temperature from 83 to 167 °C. The optimum temperature of 100 °C produces the highest oil yield. It has been found that when the temperature reaches 100 °C, the dabai kernel cell walls are expected to be easily broken, hence easier extraction of essential oil (Mohamad et al. 2019). Despite this, water performed better as a solvent because its polarity and dielectric constant tend to decrease with temperature and is able to act like an organic solvent such as methanol and acetone (Samadi et al. 2020b).

Figure 3 depicts a 3D graph and contour plot of dabai kernel oil extraction, demonstrating that the oil yield increases as the reaction time increases. Despite this, incomplete extraction may lead to low yield at low temperatures and short extraction times because it takes time and energy to fully break down the cellulose, hemicellulose, and cell wall (Kumar and Sharma 2017). A study on mango seed kernel oil found that high temperatures and long extraction periods can cause charring, oxidation, and thermal degradation of targeted components, resulting in a low yield of essential oil (Balacuit et al. 2021). Therefore, a longer reaction time up to a certain limit provides more opportunities for the SCW to interact with the dabai kernel. As a result of this prolonged exposure, the oil-containing structures are more thoroughly broken down and a greater yield of oil is obtained.

The SCW process depends on the solvent to solid ratio because it determines the appropriate water content and sample weight. Oil output increased as the solvent to solid ratio decreased, which could be attributed to improved mass transfer (Patil et al. 2018). A larger concentration gradient between the solid and the solvent can be achieved by using an appropriate solvent-to-solid ratio, which could reduce solvent consumption. However, it has been discovered that increasing the solvent-to-solid ratio in SCW extraction is often preferable to get better oil yield (Zaini et al. 2022). Solvent to solid ratio contributes to more effective oil extraction by providing better solubility, improved mass transfer, and enhanced thermal effects. In another study, as the temperature increases, organic compounds, including oils, become more soluble in SCW (Mottahedin et al. 2017). Besides, using less solvent will allow the temperature to rise more rapidly and reach the desired extraction temperature quickly.

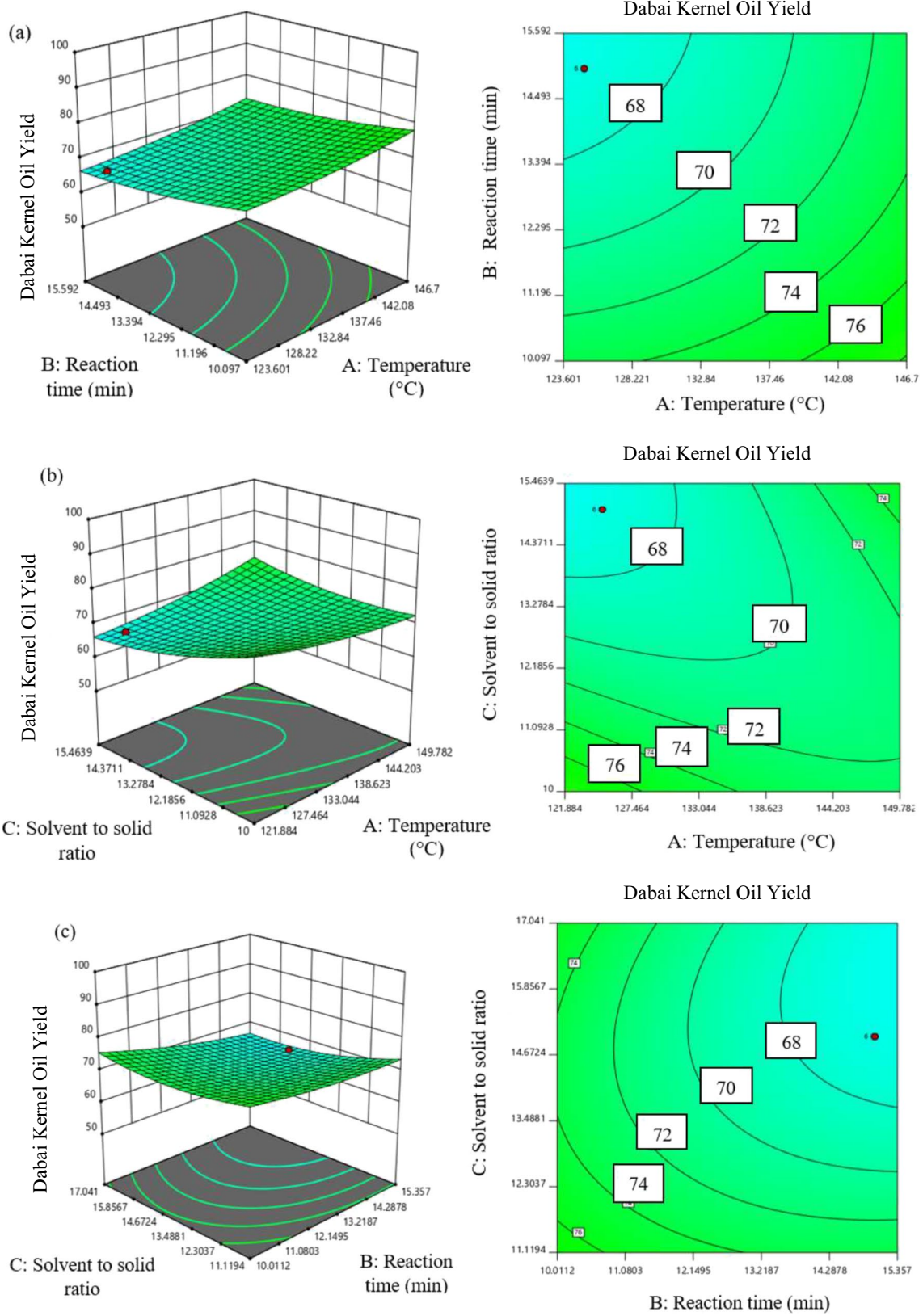


Fig. 3 The 3D graph and contour plot of dabai kernel oil extraction. Interaction between **a** temperature and reaction time on dabai kernel oil yield, **b** temperature and solvent to solid ratio on dabai kernel oil yield, and **c** reaction time and solvent to solid ratio on dabai kernel oil yield

Table 3 Numerical optimization solution generated by RSM and comparison between predicted and corresponding experimental dabai kernel oil yield based on optimal parameters

Numerical optimization solution generated by RSM					
Temperature (°C)	Reaction time (min)	Solvent to solid ratio (:)	Dabai kernel oil yield (%)	Desirability	
100	10	10:1	94.32	1.000	Selected
Comparison between predicted and corresponding experimental dabai kernel oil yield based on optimal parameters (validation)					
Predicted dabai kernel oil yield (%)			94.32		
Experimental dabai kernel oil yield (%)			96.53 ± 0.65		
Percentage of error (%)			2.35		

Optimization and Desirability

A numerical optimization approach was employed for this study. The optimization criteria were established to minimize all the treatment parameters. The solution provided by RSM yielded optimum result, and the optimal set with the highest desirability was chosen (Abdul Halim et al. 2021). The condition that had the maximum desirability of 1 was chosen as the optimal parameter among 35 solutions produced by the optimization. The optimal SCW treatment temperature was 100 °C for 10 min at a solvent to solid ratio of 10:1 to produce 94.32% oil yield (Table 3). The optimal parameters for the SCW were validated and verified through experiments in triplicate, and the results were compared with the values predicted by the model as discussed in the previous section. The obtained experimental findings (actual response) exhibited a high degree of consistency with the predicted responses, thereby confirming the validity of the optimized parameters (Ideris et al. 2021). For this optimal configuration, the desirability functions of response are 1 for a combined desirability of 1 (Fig. 4a). Desirability is measured on a scale of 0 to 1, with 1 being the most desirable (Durante et al. 2020). As a result, a value closer to 1 is seen to be best for the responses (Aili Hamzah et al. 2022).

Statistical Analysis

Multiple regression analysis showed a correlation between the response variable and the test variables based on the following second-order polynomial equation. RSM generates a regression model equation that is used to determine dabai kernel oil yield based on temperature (A), reaction time (B), and solvent-to-solid ratio (C). The second-order polynomial equation used to express the percentage of dabai kernel oil yield as a function of the coded parameters is as follows:

$$\text{Yield of dabai kernel oil (\%)} \quad (3)$$

$$= 442.81042 - 3.21110(A) - 4.65366(B) - 18.67950(C) + 0.015600(AB) + 0.097760(AC) - 0.181000(BC) + 0.006607(A^2) + 0.160791(B^2) + 0.285341(C^2)$$

where A is the temperature; B is the reaction time; C is the solvent to solid ratio; AB, BC, and AC are the interaction factors; and A^2 , B^2 , and C^2 are the second-order effects of the major terms.

The equation in terms of coded parameters can be used to anticipate the response for specific levels of each parameter. By default, the high levels of factors are coded as +1 and the low levels are coded as -1. The equation in coded form is useful for determining the relative impact of the parameters by comparing the parameter coefficients, whereas the equation with respect to the actual parameters can be used to anticipate the reaction to actual amounts of each parameter (Ezemagu et al. 2021). The analysis of variance (ANOVA) is used to determine whether the process parameters are statistically significant, as shown in Table 4. Model analysis, coefficient of determination (R^2) analysis, and the lack-of-fit test were also used to evaluate the models' suitability. The model's F -value of 14.01 with a low p -value of 0.0001 indicated that the regression model is statistically significant. Significant model terms have a p -value of less than 0.05 (Mushtaq et al. 2015). Criteria for statistical significance are defined as $\text{Prob} > F < 0.05$ ($\alpha = 0.05$) which is significant if $\text{Prob} > F > 0.1$ (Toor et al. 2021). The following was the order of significance for dabai kernel oil yield with regard to the F -value: temperature (7.73) > solvent to solid ratio (5.63) > reaction time (5.63). The lack of fit (LOF) of 219.75 also indicates no significance in relation to the pure error, implying that this experimental design model is suitable.

The R^2 value was 0.9265, indicating that the model could explain up to 92.65% of the variability in essential oil extraction. Nevertheless, there is a discrepancy of more than 0.2 between the adjusted R^2 of 0.8604 and the predicted R^2 (0.4639), which is not as close as one might typically expect. This could point to a significant block effect or a potential data issue. Therefore, the following things should

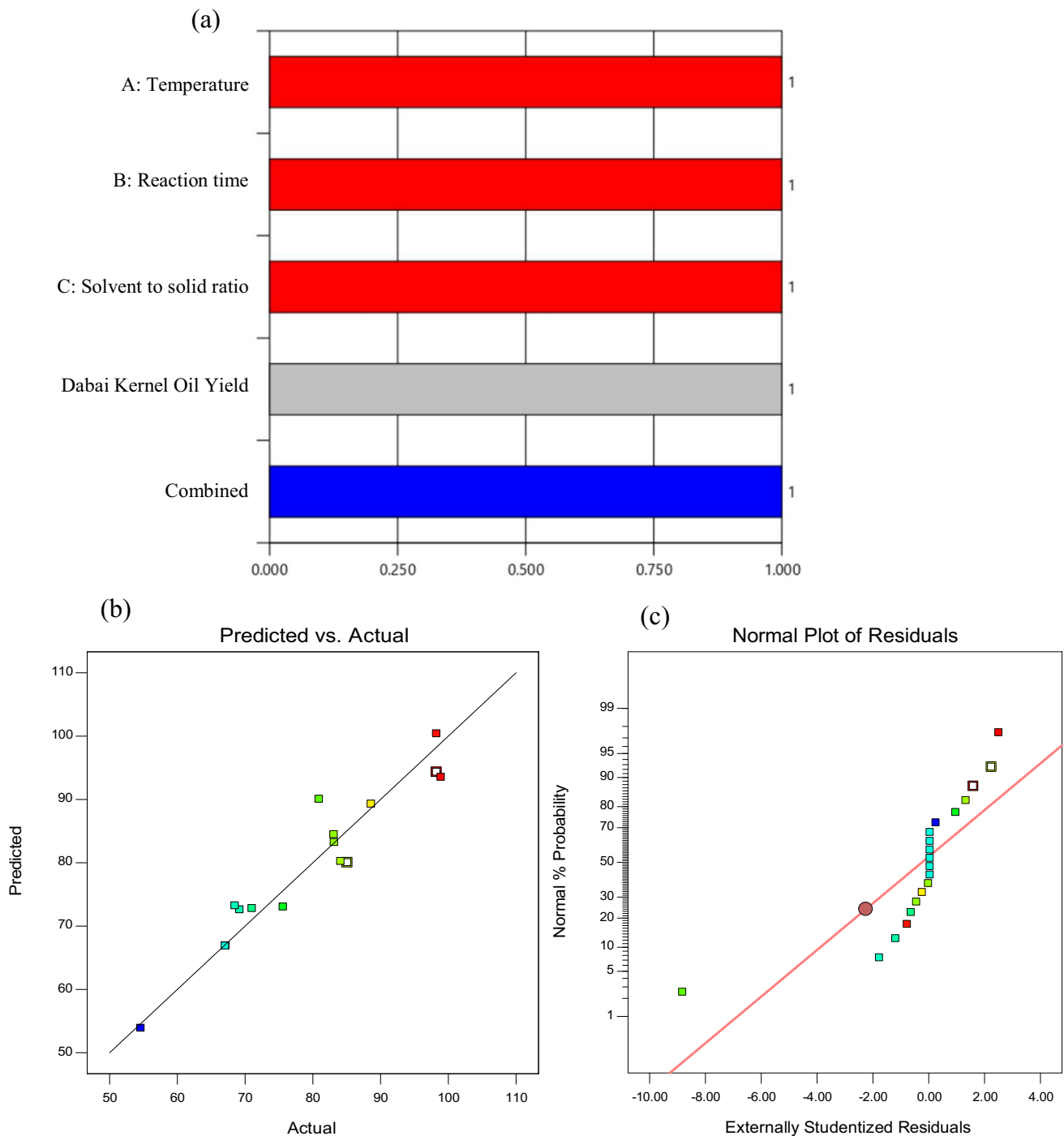


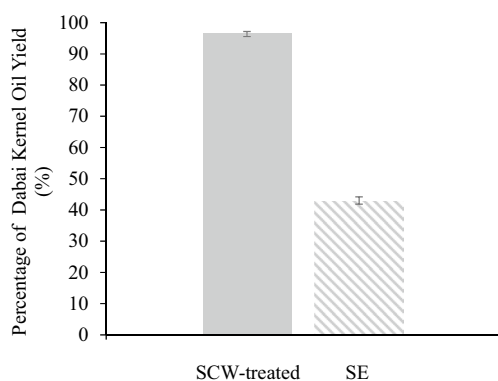
Fig. 4 a Pareto chart of desirability. b Predicted vs actual plot. c Normal plot of residuals

be considered: reduction of the model, response transformation, and outliers. Furthermore, the signal-to-noise ratio was measured with an acceptable precision of 14.027, which is significantly higher than the desired value of 4 (Ong et al. 2021). This indicates that the model is capable of exploring the design space.

The scatter plot of the regression graph within the RSM is displayed in Fig. 4. The observed and predicted response values are distributed at random along and near a straight line in Fig. 4b, indicating that the developed model is appropriate for optimizing essential oil yield. One of the fundamental

Table 4 ANOVA table for quadratic model

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	2770.98	9	307.89	14.01	0.0001	Significant
A-temperature	169.88	1	169.88	7.73	0.0194	
B-reaction time	120.74	1	120.74	5.49	0.0411	
C-solvent to solid ratio	123.76	1	123.76	5.63	0.0391	
AB	30.42	1	30.42	1.38	0.2666	
AC	1194.63	1	1194.63	54.36	<0.0001	
BC	163.81	1	163.81	7.45	0.0212	
A ²	245.90	1	245.90	11.19	0.0074	
B ²	233.00	1	233.00	10.60	0.0086	
C ²	629.20	1	629.20	28.63	0.0003	
Residual	219.75	10	21.98			
Lack of fit	219.75	5	43.95			
Pure error	0.0000	5	0.0000			
Cor total	2990.73	19				
Std. Dev	4.69		R ²	0.9265		
Mean	77.11		Adjusted R ²	0.8604		
C. V. %	6.08		Predicted R ²	0.4639		
			Adeq precision	14.0270		

**Fig. 5** Comparison of dabai kernel oil yield for SCW-treated and SE

conditions for an analysis of variance's validity is that the residuals follow a linear distribution, as shown by the normal probability plot in Fig. 4c. Furthermore, in comparison to the normal residual plot, the result is still considered acceptable because it remains close to the fitted line.

Comparison of Dabai Kernel Oil Yield and Physicochemical and Morphological Analysis Between Subcritical Water-Treated and Soxhlet Extraction

Dabai Kernel Oil Yield

The extracted oil of dabai kernel samples that were SCW-treated and SE is shown in Fig. 5. Using optimized parameters, the average oil yield for SCW-treated before SE

was $96.53 \pm 0.65\%$, nearly twice as high as from SE alone ($42.85 \pm 1.34\%$). According to Mottahedin et al. (2017), SCW had the highest concentration of oxygenated compounds (alcohols, aldehydes, ketones, acids, phenols, oxides, lactones, ethers, and esters) (Mottahedin et al. 2017). The extraction time was very short and required less energy. Furthermore, water temperature is an important factor in SCW because it affects the solubility of essential oils. Higher temperatures within certain reaction times cause the dielectric constant to decrease and the hydrogen bond between water molecules to weaken, facilitating the solubilization of more non-polar essential oils (Abdul Halim et al. 2021). Thus, the oil yield significantly increased by using SCW treatment. The next section presents a detailed comparison of the physical, chemical, and morphological analysis of the optimized dabai kernel oil yield or powder (SCW-treated) in comparison to SE alone.

Physical Analysis

Table 5 compares the physical properties (color, refractive index, and specific gravity) of dabai kernel oil for SCW-treated and SE. The appearance and color of the oil are important quality parameters. Therefore, three-dimensional coordinates L (lightness), a (red/green value), and b (blue/yellow value) were used to represent any color within the visible range (X-Rite 2016). According to Table 5, both oil samples had nearly identical L , a , and b values. Therefore, the oil sample appeared light yellowish based on the CIELab Color Chart (Paravina 2018).

Table 5 Physical properties of dabai kernel oil

Physical properties		SCW-treated	SE
Color	<i>L</i>	55.24 ± 0.38	54.04 ± 0.12
	<i>a</i>	1.17 ± 0.15	1.00 ± 0.04
	<i>b</i>	22.50 ± 0.02	20.82 ± 0.42
Refractive index		0.673 ± 0.01	0.658 ± 0.02
Specific gravity		1.03 ± 0.013	1.05 ± 0.005

In terms of refractive index, it describes how much light bends when passing through samples (Chinedu et al. 2017). From this study, the value of refractive index for the SCW-treated oil sample (0.673) is higher than for the SE oil sample (0.658). The density or weight of a liquid in comparison to the density of equal volumes of water is referred to as specific gravity (Eric et al. 2008). Based on Table 5, the oil extracted from the SE has a higher specific gravity (1.05) compared to the SCW-treated (1.03) due to the chemical compositions being affected by the nature of the procedure. There has been no previous research on the physical quality of oil extracted from dabai that can retain the quality of the fruits, which remains to be determined. Therefore, researchers seek to comprehend and determine the effect of SCW treatment on the quality of dabai extracted oil under a variety of reaction time–temperature–solvent to solid ratio configurations. This information will be useful in building future standards for essential oils from dabai fruit.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

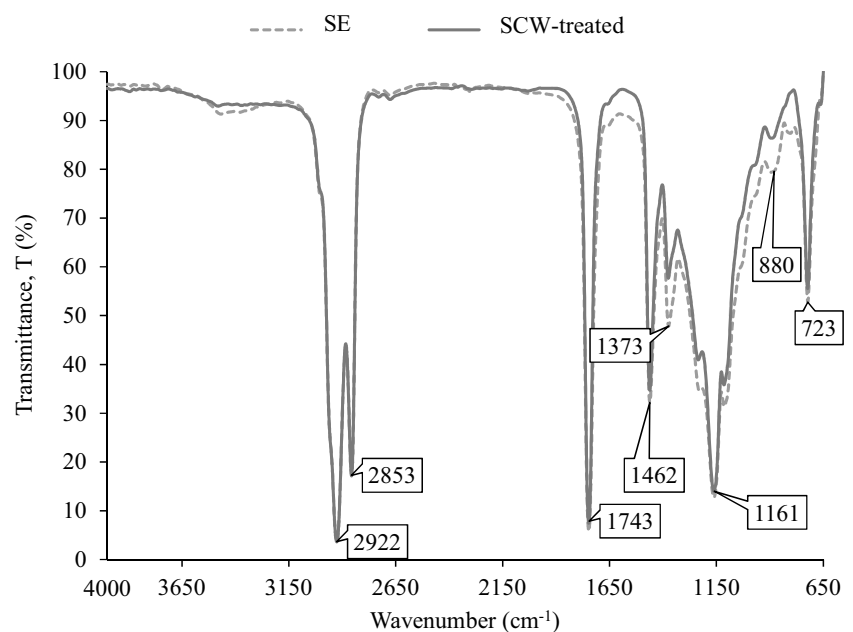
Dabai Kernel Oil Figure 6 presents the FTIR spectra of SE and SCW-treated dabai kernel oil. There were two distinct

spectral regions in the FTIR spectrum of dabai kernel oil: a region at 1600 to 4000 cm^{-1} and a fingerprint region at 1500 to 650 cm^{-1} .

The sharp peaks at 2922 cm^{-1} and 2853 cm^{-1} indicated that the stretching of C-H corresponds to an alkyl-saturated aliphatic group in the compound (Boughendjioua et al. 2020). When the lipid peroxy radical interacts with hydrogen absorbed from other lipid molecules, hydroperoxide and a new lipid alkyl radical are formed (Choe and Min 2006). The lipid alkyl radical and reactive oxidants are expected to accelerate oil oxidation. On the other hand, a C=O bond in the oil is indicated by the absorption peak at 1743 cm^{-1} , indicating the presence of ester (Ideris et al. 2021). The FTIR spectrum for the SCW-treated sample shows most of the absorption peaks that are also found in the spectrum for the SE sample, but they are slightly displaced from absorption peaks representing methyl groups (C-H) and ketone, aldehyde, or carboxyl (C=O).

The peaks 1462–1161 cm^{-1} indicated the bending vibrations of the methyl, methylene hydrocarbon, and unsaturated C=C bonds (Ideris et al. 2021). The rocking bending vibration of the methylene group and the C=O shear vibration is responsible for the peak absorption at 880 cm^{-1} (Qiu et al. 2019). Therefore, there is a greater peak intensity at 880 cm^{-1} in the SE sample than in the SCW-treated sample. The final vibration occurs as a superposition of the out-of-plane vibration of -cis disubstituted olefins (CH=CH) and the rocking vibration of methylene groups (-CH₂) at a peak of 723 cm^{-1} (Zahir et al. 2017). Consequently, during the SCW process, the absorbance bands of the treated samples either decreased or disappeared completely, indicating that the components had significantly separated during the extraction process (Jiao et al. 2013).

Fig. 6 FTIR spectra of SE and SCW-treated dabai kernel oil



Dabai Kernel Powder Figure 7 depicts the intermolecular interactions and structural alterations for raw, SE, and SCW-treated dabai kernel powder. The analysis of the spectra revealed distinctive bands within the range of 4000 to 650 cm^{-1} in each of the three different samples, indicating that the spectra differed among them, especially for raw samples.

At the infrared wavelength of 3283 cm^{-1} , the O–H stretching vibration is observed, as well as the presence of alcohol and phenol (Theivandran et al. 2015). According to the results, raw dabai kernel powder has a lower peak intensity than the SCW-treated and SE due to the modification of the chemical constituents during the extraction process. The sharp peak at 2922 cm^{-1} and 2853 cm^{-1} indicates that the compound consists of an alkyl-saturated aliphatic group (Boughendjioua et al. 2020). The absorption band at peak 1743 cm^{-1} suggests that there is a C=O bond present in the sample, indicating the presence of an ester (Ideris et al. 2021).

Amide I and amide II of proteins were found to have stretching vibrations corresponding to bands at 1643 cm^{-1} and 1541 cm^{-1} , respectively (Movasaghi et al. 2008). A weak intensity is observed for the raw dabai kernel powder compared to those from SCW-treated and SE. The amide I band is caused by carbonyl stretching vibrations, whereas the amide II band is caused by N–H bending vibrations (Gallagher 2009). The bending vibrations of methyl, methylene hydrocarbons, and unsaturated C=C bonds are responsible for the absorption peaks of 1400 cm^{-1} (Zaib et al. 2021). A peak of 1100 cm^{-1} may be attributed to vibrations of O–H bonds in the SE dabai kernel powder, which has a stronger intensity than raw and SCW-treated ones (Manaila et al. 2016). A prominent peak at 723 cm^{-1} arises, which indicates the superposition of the rocking vibrations of methylene groups ($-\text{CH}_2$) with those of -cis disubstituted olefins (Zahir et al. 2017).

The SCW-treated dabai kernel powder was generally shown to be substantially more remarkable than the SE, based on the peaks. FTIR analysis reveals a significant number of carbonyl and hydroxyl groups, as well as a mixture of carboxyl groups, which may serve as potential active sites for oil extraction and other applications such as bio-based materials, pharmaceuticals, and polymers (Rahmat et al. 2016; Riaz et al. 2018; Ricci et al. 2015).

Gas Chromatography Mass Spectrometry (GCMS) Analysis

The chemical compositions of dabai oil kernel for SCW-treated and SE are shown in Table 6. Recent research has suggested that the SCW process can selectively extract chemical compounds such as bioactive (Ko et al. 2020) and phenolic compounds (Machmudah et al. 2017). The results of GCMS analysis of dabai kernel oil showed that phthalic acid is the major component in both SCW-treated (49.29%) and SE (27.34%). Phthalic acid esters (PAEs) or phthalates are a class of synthetic chemicals used as plasticizers in various industries. Evidence suggests that oils inevitably come into contact with plastic containers and machinery during processing or storage may be the cause of the phthalates found in dabai kernel oil (Nanni et al. 2011). PAEs are susceptible to moving from packaging into foods or beverages because PAEs are merely physically bound to the plastic polymer macromolecules via hydrogen bonding or van der Waals forces instead of intimate chemical bonding (Qi et al. 2023). PAEs have been shown in studies on chronic rodents to cause liver injury, liver cancer, teratogenicity, and testicular damage (Čtveráčková et al. 2020). Furthermore, phthalates pose a high risk of impairing animal reproduction systems and preventing the development of young mammals (Estill et al. 2019). Due to this, PAEs directly pose a threat to human health. Hence, it is crucial to have a reliable and

Fig. 7 FTIR spectra of raw, SE, and SCW-treated of dabai kernel powder

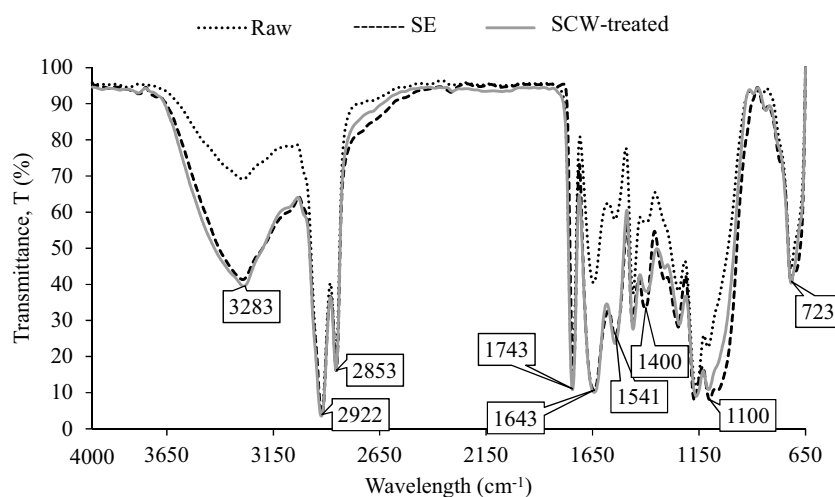


Table 6 Chemical compositions of dabai kernel oil

Chemical	Percentage (%)	
	SCW-treated	SE
Sabinene	0.69	14.39
D-Limonene	0.92	11.11
Dodecahydropyrido[1,2-b]isoquinolin-6-one	1.72	0.66
Nonanal	2.01	4.91
2-Decenal	10.28	2.97
Cyclohexane	1.12	2.03
Hexahydropyridine	1.04	0.58
Phthalic acid	49.29	27.34
Hexadecanoic acid	8.93	7.64
Hexanoic acid	n.d.	1.33
Octanal	n.d.	1.32
o-Cymene	n.d.	2.15
Nonanoic acid	n.d.	2.24
Nonane	1.20	n.d.
Oxalic acid	1.44	n.d.
9-Octadecenoic acid	12.93	n.d.

not detected (n.d.)

precise detection method for PAEs in order to guarantee food safety.

On the other hand, the chemical compound of hexadecanoic acid also appeared in the SCW-treated (8.93%) and SE (7.64%) dabai kernel oil. Numerous plants and animals naturally contain hexadecanoic acid, commonly referred to as palmitic acid. Hexadecanoic acid is found in some plant oils, such as coconut or palm oil, and it may be transferred to essential oils when used as diluents or carriers (Sundram et al. 2003). Hexadecanoic acid has no aroma and little waxy odor. Previous research has established that hexadecanoic acid has considerable natural antioxidant and anticancer properties, which could lead to the creation of effective treatments for various types of cancer in the future (Bharath et al. 2021). In another application, authors have highlighted that hexadecanoic acid is an excellent option for the creation of environmentally friendly anti-fouling paint and anti-biofilm chemicals (Bakar et al. 2017).

The compound, 2-decenal, was also detected higher in SCW-treated (10.28%) dabai kernel oil than SE (2.97%), which could be attributed to the structural modification that occurred throughout the treatment process. It is an organic compound with a mechanical and soapy flavor and exhibits antimicrobial properties, which can contribute to the preservation of essential oils (González-Aguilar et al. 2013). This substance may aid in the prevention of growth of certain microbes, such as bacteria and fungi, thus reducing the chance that the oil will spoil or become contaminated. Hong et al. (2022) also provided an in-depth investigation of the

2-decenal composition from *Alpinia coriandriodora* essential oil, demonstrating its potential as a new source of anti-cancer drugs in the pharmaceutical field (Hong et al. 2022).

Sabinene is an aromatic bicyclic monoterpene with a woody scent and a pleasant odor that has been isolated from essential oils extracted from a variety of plant species. Sabinene is an extremely weak acid and practically insoluble in water. Sabinene is used in the pharmaceutical, fragrance, and flavor industries because of its pleasant smell, anti-fungal, and anti-inflammatory properties (Menon and Padmakumari 2005; Valente et al. 2013). Specifically, sabinene acts as a promising agent with therapeutic potential for treating or preventing skeletal muscle atrophy (Ryu et al. 2019), cariogenic activity in the development of oral healthcare products (Park et al. 2019), and mediating SARS-CoV-2 (COVID-19) viral entry into cells (İstifli et al. 2020). d-Limonene, a monocyclic monoterpene of lemon-like odor, is a common component of many citrus oils, such as those from lemon, orange, lime, mandarin, and grapefruit. It is widely used in food and beverages, cosmetics, pharmaceuticals, and household cleaners (Kim et al. 2013; Ravichandran et al. 2018). In a comprehensive research of the neuroprotective potential of limonene and limonene-containing natural products, the authors reported that the compound reduces age-related neurodegeneration in different diseases, including Alzheimer, Parkinson, multiple sclerosis, and stroke (Eddin et al. 2021). Nonanal, a clear brown liquid which is insoluble in water, has a rose-orange odor. It is present in at least 20 essential oils, such as rose and citrus, and several types of pine oils (Nissen et al. 2022). Nonanal indicated the potential utility for addressing hair loss caused by various physiological conditions (Park et al. 2020). Nonanal from *Artemisia ludoviciana* also has demonstrated efficacy in symptomatic relief of induced diarrhea (Zavala-Sánchez et al. 2002).

For SE dabai kernel oil, several new chemical components were found such as hexanoic acid, octanal, o-cymene, and nonanoic acid. Hexanoic acid is a carboxylic acid produced from hexane, with a sour, cheesy, greasy, and waxy odor. Hexanoic acid possesses a wide range of applications including organic synthesis, perfume, medicine, lubricating grease, rubber, and dye production (Cheon et al. 2014). According to previous research, hexanoic acid is an effective supplement that increases the commercial yield of microbial oil while selectively increasing oleic acid content for microbial oil production (Choi et al. 2023). Furthermore, hexanoic acid may be a helpful nutritional approach to treat liver steatosis and hepatic insulin resistance (Rial et al. 2018). o-Cymene is a volatile component of woody-scented essential oil with antimicrobial properties (Thakre et al. 2016). This view is supported by Chelouati et al. (2023) who reported that this terpenoid from *Juniperus phoenicea* seed essential oil shows importance as a natural antioxidant and antibacterial medication against pathogenic strains that

are clinically relevant (Chelouati et al. 2023). Nonanoic acid is a clear, yellowish-oily liquid with an unpleasant smell that dissolves in ether, alcohol, and other organic solvents but is insoluble in water. The chemical has been reported to be used as thermal stabilizer in lubricating oils (Sahin et al. 2006). Another study also reported that nonanoic acid was powerful in reducing hilar neuronal death while having a reduced effect on seizure activity (Chang et al. 2013).

In addition, many chemical components including nonane, oxalic acid, and 9-octadecenoic acid were recovered from SCW-treated dabai kernel oil. Nonane which is colorless liquid with a gasoline-like odor can cause irritation to the eyes and skin and breathing difficulties (Ahmed et al. 2021). These effects were comparable to those described for nonane, which contributed to hematological and neurotoxicity implications from simulated inhalation exposures to volatile organic compounds generated by oil and gas operations (Holder et al. 2019). Furthermore, oxalic acid, also known as oxalate, a colorless powder or granular solid with no odor and non-volatile, can be found in many types of plants, including leafy greens, vegetables, fruits, cocoa, nuts, and seeds (García-Fernández et al. 2014; Mitchell et al. 2019). This is one of the strongest organic acids that can act as a catalyst in the SCW treatment technique (Li et al. 2021). Oxalate has remained a concern for human health due to its antinutritional effects and potential nephrotoxicity. For example, too much oxalate can cause stone formation, tubular cell injury, inflammation, tubular atrophy, or interstitial fibrosis, all of which are harmful to kidney function (Bargagli et al. 2020). In addition, 9-octadecenoic acid, which has a mild odor, is used commercially in the production of oleates and lotions as well as in pharmaceutical applications (Dinesha et al. 2018). It has previously been established that 9-octadecenoic acid has antioxidant and antimicrobial properties against many fungi and bacteria (Ghavam et al. 2021; Pinto et al. 2017). Considering all of this evidence, it appears that dabai kernel oil may find application in pharmacological, food, and commercial purposes.

Morphological Analysis

Scanning Electron Microscope (SEM) Analysis Figure 8a shows the morphological analysis of raw dabai kernel powder prior to SE. The rough and uneven pattern of the surfaces is clearly visible. However, the surface damage on the standard SE-processed sample is negligible (Dahmoune et al. 2013). In addition, no significant disruption of the sample's external structure was observed prior to extraction. Figure 8b illustrates the exterior structure of the SCW-treated dabai kernel powder prior to SE; the surface of the dabai kernel appeared to be flatter and smoother compared to the SE process due to swelling in the SCW process. It has been suggested that SCW may be more effective due to

its extreme extraction parameters of high temperature and pressure, which result in more effective plant cell breakage and compound desorption (Samadi et al. 2020a). A similar report by Md Sarip et al. (2016) found that severe cell wall ruptures were intensified with higher SCW treatment temperatures (120 to 180 °C) following the analysis of SEM images. When maintained at a suitable pressure, the water's properties can be altered between 100 °C and its critical temperature of 374 °C, as water's dielectric constant decreases with increasing temperature during the extraction process (Gallina et al. 2022). A decrease in viscosity also allows for better penetration into the matrices, while a prominent increase in the ionic product is associated with the depolymerization of complex structures.

Figure 8c reveals the image of the SCW-treated dabai kernel powder after SE with aggregation. The sample's cell structure was ruptured and destroyed, thus releasing the oil, as a result of a combination of the hydrothermal effects produced by SCW and the thermal expansion effects brought on by SE (Samadi et al. 2020b). In SCW, water becomes more efficient when the cell walls are disrupted, as a barrier to its flow is removed. SCW's mechanism is based on two important principles, namely, the enhancement of solubility and the effects of mass transfer resulting from the alteration of water's properties at higher temperatures and pressures (Ong et al. 2006). This results in a greater ability to solubilize analytes with less disturbance of surface equilibrium. The SEM morphology of the dabai kernel after the SE process is shown in Fig. 8d, which shows visible deformation with less-ruptured surface due to the loss of cell structure and the beginning of aggregate formation. The SEM results confirmed that SCW improves oil extraction by (i) rupturing the cell wall and (ii) changing the properties of water as a solvent, resulting in improved oil extraction. Nonetheless, due to the high temperature and pressure, SCW reduces the polarity of water, producing behavior similar to organic solvents (Islam et al. 2013).

Brunauer-Emmett-Teller (BET) Analysis Table 7 tabulates the BET analysis of raw, SE, and SCW-treated dabai kernel powder. The surface area of the SCW-treated dabai kernel is higher (14.813 m²/g) compared to the raw (2.804 m²/g) and SE dabai kernel (13.452 m²/g). However, the raw dabai kernel (219.645 nm) has a greater pore size than the SCW-treated (131.664 nm) and SE dabai kernel (139.700 nm). This is the result of SCW's pressure and temperature that caused the sample's small pores to grow larger and new pores were formed. This result emphasizes the significance of surface modification of raw agricultural waste prior to chemical processing (Haque et al. 2022; Yu et al. 2020). Increasing an adsorbent's specific surface area will result in a greater number of accessible active sites and increased adsorption capacity in the case of adsorbent studies (Elkhaleefa et al. 2021; Wulan et al. 2022). In this study,

Fig. 8 SEM images of **a** raw dabai kernel, **b** SCW-treated dabai kernel powder prior to SE, **c** SCW-treated dabai kernel powder after SE, and **d** dabai kernel powder after SE

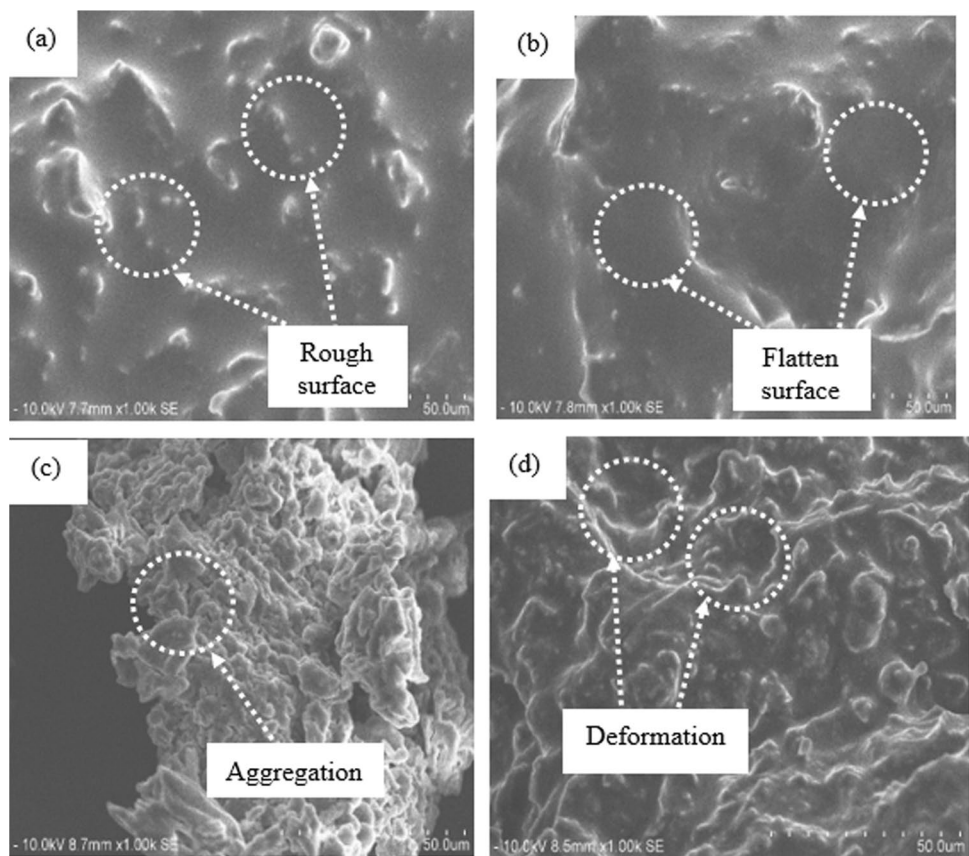


Table 7 BET analysis of raw, SE, and SCW-treated dabai kernel

Adsorbent	Pore volume (cm ³ /g)	Pore size (nm)	Surface area (m ² /g)
Raw dabai kernel	0.308	219.645	2.804
SE dabai kernel	0.886	139.700	13.452
SCW-treated dabai kernel	1.035	131.664	14.813

BET analysis showed small pore size and large surface area which were crucial to enhancing convective mass transport and extraction of desired compounds (Acquah et al. 2016). Therefore, these findings seem to be related to the greater yield of dabai kernel oil from the SCW-treated sample in comparison to other samples.

Conclusion

The finding from RSM showed that the SCW treatment achieved 98.29% of the dabai kernel oil yield when the temperature was 100 °C, reaction time was 10 min, and

the solvent to solid ratio was 10:1. In general, temperature has a greater impact on dabai kernel oil yield than the solvent to solid ratio. SCW treatment resulted in nearly two times (96.53%) the dabai kernel oil yield of the SE sample (42.85%). In terms of color, both SCW-treated and SE dabai kernel oil exhibited light yellowish color. As for the refractive index, SCW-treated dabai kernel oil (0.673) was higher than that of the SE (0.658), and the dabai kernel oil extracted from SE had a higher specific gravity (1.05) than the SCW-treated (1.03). The FTIR analysis showed that C=O and C-H functional groups were present in both the dabai kernel oil and powder samples. Meanwhile, the GC-MS data showed a notable improvement in the quality of the SCW-treated dabai kernel oil. BET analysis confirmed the SEM images, showing that the SCW-treated dabai kernel powder had a larger surface area than raw and SE. In conclusion, new products containing dabai oil as an ingredient such as functional foods, nutraceuticals, or natural cosmetics could be introduced. In addition to product safety risk analysis, sensory assessment is a significant practical consequence of the various goods provided. It is typically investigated to assess the acceptable and quality interpretation of products among consumers. Another research area that can be investigated in the future is exploring possible extraction methods or investigating the bioactivity of dabai kernel oil extracts.

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Data Availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethics Approval and Consent to Participate This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest Muhammad Hazwan Hamzah declares that he has no conflict of interest. Nurbaqlis Zulkefli declares that she has no conflict of interest. Adila Fazliyana Aili Hamzah declares that she has no conflict of interest. Rosnah Shamsudin declares that she has no conflict of interest. Hasfalina Che Man declares that she has no conflict of interest. Abd Halim Md Ali declares that he has no conflict of interest. Maimunah Mohd Ali declares that she has no conflict of interest. Bernard Maringgal declares that he has no conflict of interest. Mohd Hafizz Wondi declares that he has no conflict of interest.

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