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Optimization of process variables using response surface methodology for tocopherol extraction from Roselle seed oil by supercritical carbon dioxide

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ABSTRACT

Hibiscus sabdariffa L. (Roselle) seeds are rich in proteins, carbohydrates and unsaturated fatty acids, and are a good source of minerals and antioxidants. Supercritical carbon dioxide (SC-CO_2) extraction was applied for extraction of oil from Roselle seed at temperatures of 40 °C to 80 °C and pressures of 20 MPa to 30 MPa. The effects of temperature and pressure on the extraction yield and solubility of oil were determined. Process optimization was carried out using response surface methodology (RSM). A particle size of 300 µm, SC-CO_2 flow rate of 5 mL/min and extraction time of 180 min were held constant throughout this study. The overall oil yield increased as pressure and temperature was increased, while a reverse effect was observed at higher temperatures. The optimum extraction conditions for Roselle seed oil corresponded to a pressure of 30 MPa and temperature of 40 °C. According to the analysis of variance (ANOVA), the coefficient of determination R^2 for oil yield and gamma tocopherol concentration were 0.9723 and 0.9754, respectively, indicating a good correlation and agreement between the experimental and predicted values.

1. Introduction

Oil seed crops are an important source of natural oils for human nutrition, pharmaceutical and industrial applications. The global demand for seed oil is increasing every year due to the growing world population and the use of these materials for industrial purpose (USDA, 2017). Depending on the fatty acid composition, seed oils have different characteristics which determine their application. Seed oils such as palm oil, coconut oil, soybean oil, castor oil, rapeseed oil and sunflower seed oil are popular and are used for many commercial purposes, especially in manufacturing of soaps, detergents, lubricants, solvents, paints, inks, surfactants, cosmetics etc. (Patel et al., 2016). Seed oils are rich in proteins, fatty acids, minerals, fibers and vitamins that are important for human health. Presently, scientists are exploring new oil seeds to meet the growing demand for nutritional oils, especially seed oils containing fatty acids of oleic (Omega-9), linoleic (Omega-6) and linolenic (Omega-3) acids. These are the essential fatty acids (EFA) which humans are not able to synthesize, but have to obtain from external sources. EFA is important for fortifying the body and it has been shown that many common illness and conditions like hypercholesterolemia, low immune system, low metabolism rate are related to imbalances or deficiencies of these EFA (Sarwar et al., 2013; Bradberry et al., 2013). In addition, the Omegas are the building blocks of healthy cell membranes. The polyunsaturated fatty acids (PUFA) are skin's natural oil barrier and critical components to keep skin hydrated (Lin et al., 2018). Seed oils are widely used in cosmetic and skin care products, as the Omega-3 and Omega-6 fatty acids are reported to keep skin moisturized, reduce transepidermal water loss, as well as aid in healing of acne and sunburns (Zielinska et al., 2014).

Seed oil is also known to be highly rich in tocopherols content. Vitamin E is an important dietary nutrient for humans, and which is mainly synthesized from plant extract and seed oil extraction. Tocopherol mainly contains the alpha, beta, delta and gamma isomers. They are all chain breaking antioxidants with scavenging activities

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towards the free radicals of unsaturated lipids. Alpha-tocopherol is the most efficient bioactive isomer which has been reported as the first defence to prevent lipids from peroxidation in living cell membranes. Gamma tocopherol is another isomer found abundantly in seed oil which exhibits a superior antioxidant level compared to alpha-tocopherols. Some studies reported that it protects the cardiovascular system and associates plasma gamma-tocopherol concentration with lower risk of coronary disease (Marchese et al., 2014; Przygoda and Wejnerowska, 2015).

As the awareness of using healthy oils in both diet and personal care products increases, the demand for seed oils in natural products industries increases drastically. According to Abdul Afiq et al (2013), date seed oil was extracted to serve as a dietary seed oil alternative. In South Africa, Baobab (*Adansonia digitata L.*) seed oil generated great interest for use in the cosmetic industry (Komane et al., 2017). Researchers in West Africa explored the potential uses of oil extracted from *L. kerstingii* seeds, which is usually used as traditional medicine, to increase utilization in local communities (Judicael et al., 2017).

Hibiscus sabdariffa L. (Roselle) is a native plant of Malaysia, with high potential for seed oil extraction. It is also popular and grown worldwide in India, Mexico, Sudan, Saudi Arabia, Indonesia, Thailand, Philippines, Vietnam, Africa, Taiwan and Egypt. The plant takes only three to four months to reach the commercial stage of maturity where the calyces are harvested. The red calyces of Roselle have drawn the attention of academics, researchers in the food industry, as well as pharmaceuticals in past decades. The extract has an attractive red color and the good flavour makes Roselle calyces a valuable product in food processing for the preparation of jam, jellies and beverages. Moreover, it is used as a traditional medicine to treat hypertension, cancer, cough, fever and scurvy (Ologundudu et al., 2010). The antioxidant can be used as a component in cosmetics.

Beside the calyces, the other parts of Roselle remain unexplored. Roselle seed is usually discarded after mature calyces are harvested. They have been reported high in nutritional value, especially with regards to the oleic-linoleic group of fatty acids and the tocopherols content. Some studies reported that Roselle seed oil has higher protein, dietary fiber, and minerals content compared to other seeds like passion fruit and black seeds (Ismail et al., 2008). The tocopherol concentration in Roselle seed oil is four times more than Safflower and 20 fold higher than grapeseed oil, containing 25% of alpha tocopherols, 5% delta tocopherols and 70% of gamma-tocopherols of the total tocopherol content (Mohamed et al., 2007). Some previous investigations established the potential of Roselle seed oil utilization in food, pharmaceutical and cosmetic industries (Rimamcwe et al., 2017; Sahar et al., 2017). So far the reported extraction of Roselle seed oil has only been through the conventional solvent extraction route which is the most common method that has the benefit of more sample mass extracted than using other methods. However, there are some reports showing the disadvantages, as potential toxic solvents contaminate the sample extract during the process and large amount of solvent is wasted (Han et al., 2013). Besides, the extraction process is not suitable for heat-sensitive samples and the process usually takes 6–9 h to complete a cycle of extraction (Danlami et al., 2014).

In this study, a green technology is proposed for Roselle seed oil extraction. The supercritical carbon dioxide extraction method produces no hazardous waste since carbon dioxide is used as the solvent. This gas is inexpensive and can be recycled. The special fractionation capabilities allow specific compounds to be extracted by optimizing extraction parameters such as the temperature, pressure and solvent flow rate. Therefore in this study, tocopherol enriched Roselle seed oil was extracted using supercritical carbon dioxide extraction and optimization of the extraction parameters, viz. the particle size, extraction time, flow rates, temperature, and pressure was carried out using response surface methodology (RSM), in order to obtain the optimum conditions to extract the highest yield of tocopherol rich Roselle seed oil.

2. Materials and methods

2.1. Chemicals

Mature *Hibiscus sabdariffal L* (Roselle) calyces were collected from a Roselle planting field in Setiu Terengganu. A large amount of the calyces were collected (approximately 10 kg) to ensure the profiling for samples used throughout the experiments remained the same. The chemicals used in this study were light petroleum ether for Soxhlet extraction, methanol and acetonitrile for HPLC analysis.

2.2. Sample preparation and determination of seed moisture content

The pod of seeds in each calyces was pushed out of the calyces with a small hollow metal tube. The Roselle seeds were collected from each velvety capsule. The seeds were oven dried at 60 °C for a period of 12 h to minimize the water content. The dried seed were ground into a powder using a miller grinder (Werke, Germany). The samples were stored in an air tight zipper bad inside a freezer at a temperature of about -20 °C to maintain the freshness of the seeds.

The moisture content of sample was calculated using Eq. (1):

$$M_n = ((W_w - W_d)/W_w) \times 100\% \quad (1)$$

Where,

M_n = Moisture content of sample n
W_w = Sample weight
W_d = Weight of sample after drying

2.3. Optimum extraction parameters

This preliminary study was carried out to determine the constant parameters including the extraction time, seed particle size and supercritical carbon dioxide flow rate in extraction. These parameters were determined to obtain the optimum extraction yield.

2.3.1. Determination of optimum extraction time

In order to determine the optimum extraction time as a constant parameter throughout the experiment, experiments were carried out at various pressures of 20 MPa, 25 MPa and 30 MPa at a constant temperature of 40 °C and also at various temperatures of 40 °C, 60 °C and 80 °C at constant pressure of 30 MPa. The extracted sample was collected at intervals of 30 min over 240 min of extraction time. The best extraction time was determined by plotting the graph of extraction yield versus extraction time.

2.3.2. Determination of optimum particle size

The dried Roselle seeds were ground and sieved using laboratory test sieves (USA STD Test Sieve, Endecott Ltd, England). Five particle sizes (212 μm, 300 μm, 425 μm, 600 μm and 710 μm) of Roselle seeds powder were collected. The optimum seed particle size for extraction was determined by plotting the graph of extraction yield versus extraction time and the total oil collected from extraction. The oil yield was collected at intervals of 30 min over 210 min of extraction time. The experiments were carried out at a temperature of 40 °C and pressure of 30 MPa.

2.3.3. Determination of optimum flow rate

In order to determine the best flow rate for Roselle seeds extraction and set it as a constant parameter throughout the experimental campaign, experiments at various flow rates (3 mL/min, 4mlL/min and 5 mL/min) and pressures (20 MPa, 25 MPa and 30 MPa) at a constant temperature of 40 °C were carried out. The extracted sample was collected at intervals of 30 min over 240 min of extraction time. The best flow rate was determined by plotting the graph of extraction yield versus extraction time.

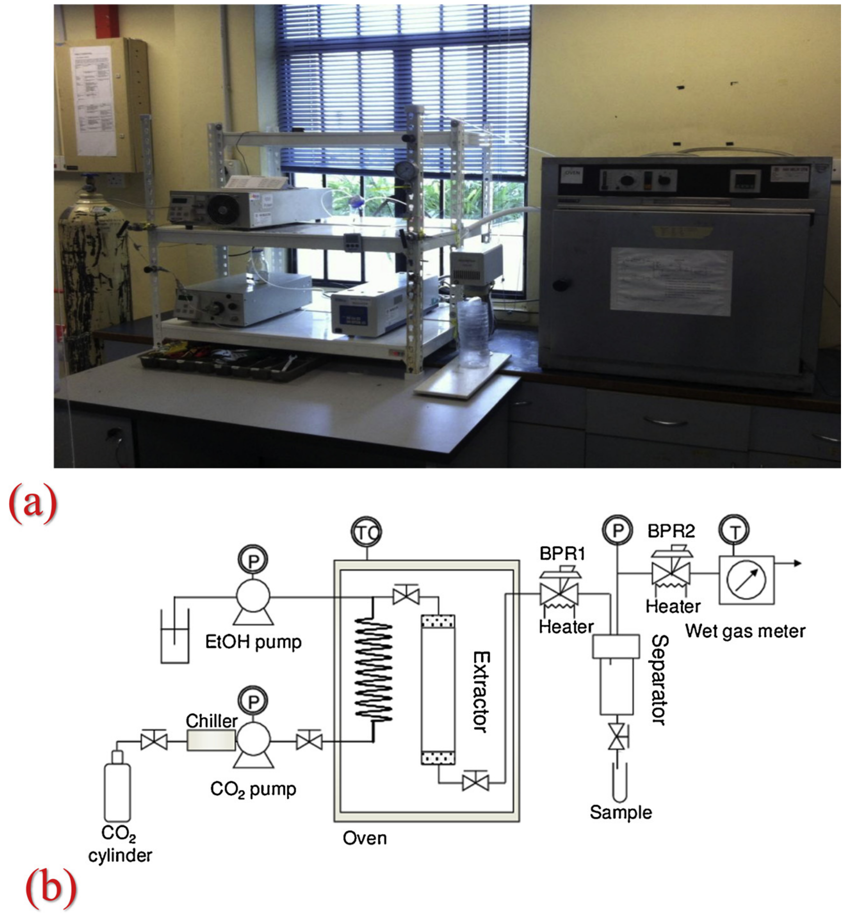

Fig. 1. a) Supercritical Carbon Dioxide (SC-CO_2) extraction system b) A schematic diagram of process flow of supercritical carbon dioxide extraction.

Table 1
Parameters applied in supercritical carbon dioxide extraction.

Parameters	Unit	Range/Value	Increment
Pressure	MPa	20 – 30	5.00
Temperature	°C	40 – 80	20.00
Flowrate	mL/min	3 – 5	–
Extraction Time	min	180	–
Mass of Sample	g	5.00	–
Particle Size	µm	300	–

2.4. Method of extraction

In this study, supercritical carbon dioxide extraction (SC-CO_2) and Soxhlet solvent extraction method were used to obtain *Hibiscus sabdariffa L.* (Roselle) seed oil.

2.4.1. Supercritical carbon dioxide extraction (SC-CO_2)

The Roselle seeds oil extraction was performed using a lab-scale Supercritical 24 fluid extractor (SSI, State College Pennsylvania, US) at the Centre of Lipids Engineering and Applied Research (CLEAR), Universiti Teknologi Malaysia, which consists of a constant flow pump, oven (Memmert, Germany), 10 mL stainless steel extraction vessel, pressure gauge (Swagelock, Germany) a carbon dioxide gas cylinder, programmable back pressure regulator (Model BP-2080, JASCO, Japan) and restrictor valve. Fig. 1a shows the experimental set up for the supercritical CO_2 extraction system and schematic a diagram of the lab-scale supercritical CO_2 extraction instrument is shown in Fig. 1b.

The Roselle seed samples were taken out of the freezer and kept at room temperature before weight measurement and extraction. Approximately 5 g of ground Roselle seeds were used for each extraction cycle. Roselle seeds samples were put into the extraction vessel and sealed tightly. The extraction was performed at a range of temperatures, namely 40 °C, 60 °C and 80 °C. The temperature was maintained in the oven. The pressures selected were 20 MPa to 30 MPa due to the limitation of the instruments. The extraction was processed under dynamic condition in order to determine oil solubility. During the experiment, carbon dioxide at a constant flow rate of 5 mL/min was allowed to flow dynamically for the desired extraction time of 180 min. The extract was collected in the vial that was attached to the restriction valve at the end of the extraction apparatus. The sample was collected in fractions at intervals of 30 min until 180 min.

The parameters and constant parameters used in this extraction process are presented in Table 1. Pressure and temperature were varied, while the responses were extracted oil yield and gamma tocopherol content. The collected extract was weighed and the total yield was calculated based on cumulative mass of extract.

2.4.2. Soxhlet extraction

In the soxhlet extraction process, 15 g of the ground Roselle seed were placed in the thimble and 150 mL of light petroleum ether was poured into the round bottom flask. The solvent was heated between 40 °C to 60 °C for 8 h using a heating mantle and the oil was recovered from the solvent by evaporation using a rotary vacuum evaporator (Model Heidolph) at 35 °C under reduced pressure and stored in a refrigerator at 4 °C for future use. The diagram for preparation of the soxhlet extraction is shown in Fig. 1b. The entire extraction process was repeated three times in order to determine the average results.

2.5. Calculation for extracted oil yield

The Roselle seeds oil yield was measured directly after the supercritical carbon dioxide extraction. It was determined as the mass difference between the sample before and after the extraction process at the desired temperature and pressure. The extracted oil yield was determined using Eq. (2).

$$W_{oil} = W_f - W_i \tag{2}$$

Where,
W_{oil} = weight of extracted oil, g
W_f = weight of sample bottle + extracted oil, g
W_i = weight of empty sample bottle, g

2.6. Overall extracted oil yield in percentage (Y, %)

To determine the total extraction of oil yield, Y(%), the mass of the extracted Roselle seed oil is divided by the mass of sample used and multiplied by 100%. The equation of the total extraction is expressed as Eq. (3).

$$Y\% = W_{oil}/W_s \text{ x } 100\% \tag{3}$$

Where,
W_{oil} = weight of extracted oil, g
W_s = weight of sample used, g
For the degree of extraction relating the extracted yield using $SC-CO_2$ and Soxhlet extraction, the relationship is expressed in Eq. (4).

$$Y\% = \frac{Y\,oil\,(SC-CO2)}{Y\,oil\,(Soxhelt)} \times 100\% \tag{4}$$

2.7. Solubility measurement

The solubility of Roselle seed oil in SC-CO_2 was determined by the dynamic method. The solvent flow rate is kept constant at 5.0 mL/min corresponding to a temperature range from 40°C to 80°C and at pressures from 20 MPa to 30 MPa. The solubility is measured by flowing the solvent CO_2 through an extraction vessel that is filled with the sample which has a large amount of free-oil, and then would be allowed to saturate the CO_2 leaving the extraction vessel. The solubility can be calculated from the slopes of the linear part of each extraction curve.

2.8. Quantification of tocopherol concentration by HPLC analysis

The extraction yield obtained from the SC-CO_2 extraction process was analyzed using high performance liquid chromatography (HPLC) analysis. Gamma-Tocopherols standards (99%) were procured from Sigma-Aldrich. HPLC grade of methanol and acetonitrile were purchased from Merck. The analysis procedure was followed according to Gomas et al., 2006, with modification. The tocopherol analysis was carried out using Waters HPLC system (Milford, MA, USA) consisting of a pump, system controller (Model Waters e2695) and photo-diode array detector (Model 2998). A LiChrosorb Si 60 column (5 µm, 4.6 × 250 mm) was used for compound separation. The mobile phase consisted of methanol (60%) and acetonitrile (40%). The injection volume of the sample was 20 µL and all samples were filtered with 0.45 µm nylon filters prior to injection. The detection was monitored at 295 nm and data were integrated by Empower 3 software (Waters) (Milford, MA, USA).

2.9. Design of experiments

The extraction of Roselle seed oil was modeled using Response Surface Methodology (RSM) which is a technique to describe the behavior of a set of data. Three-level factorial design was employed to find the relation between the dependent and independent parameters which took part in the extraction. In this study, Design-expert 6.4 software was used in order to determine the optimum conditions for the extraction method. The number of experiments is given by Eq. (5).

Table 2
Independent variables for factorial design.

Independent Variables	Coded Levels		
	−1.0	0	+1.0
Temperature (°C)	40	60	80
Pressure (MPa)	20	25	30

$$N = 3^k \tag{5}$$

Where,
N = number of experiment
k = number of factor
Temperature and pressure were studied in order to optimize the Roselle seed oil yield, solubility of oil in CO_2 and tocopherol concentration using the factorial design. A three level factorial is suitable for a second-order polynomial model of two factors. The coded values of the independent variables in the factorial design are given in Table 2.

3. Results and discussion

3.1. Determination of moisture content

In this study, the moisture content in Roselle seed is calculated using Eq. (1). From the ontained result, moisture content in Roselle seed is 8.03%. The oil extracted from the seed is clear, as shown in Fig. 2. Pre-

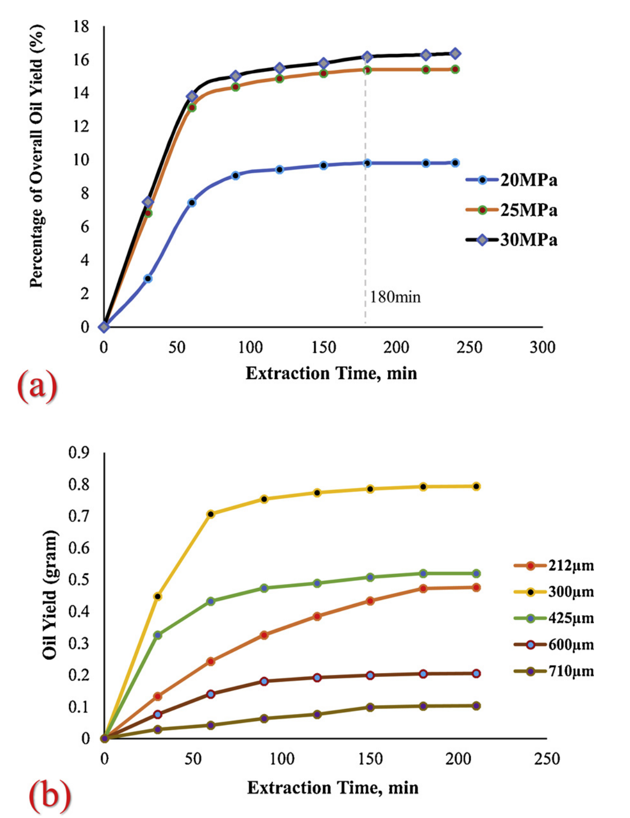

Fig. 2. a) The effect of extraction time on extraction yield at pressure 20, 25, 30Mpa at the temperature of 40 °C, 5 ml/min flow rate of carbon dioxide **b)** The effect of particle size on extraction yield at temperature 40 °C, pressure of 30Mpa and 5 ml/min flow rate of carbon dioxide.

treatment of sample in supercritical carbon dioxide extraction is very crucial as it may influence the extraction rate and kinetics to obtain the highest yield. The study by Asep et al. (2013) showed the significant effect of moisture content of cocoa nibs on the butter yield. As the moisture content increases, the yield increases and the highest yield is obtained at the moisture content of 9.79%, but a lower yield is collected in the sample with higher moisture content. High moisture samples inhibit the flow of the SC-CO_2 fluid by changing the surface tension and contact angles as a result of phase interaction between the three components – water, sample matrix and SC-CO_2 fluid (John and Sophia, 2016).

3.2. Determination of optimum extraction time

In this study, the best extraction time of Roselle seed was determined by performing the dynamic extraction method with the conditions of pressure 20, 25, 30 MPa at a temperature of 40 °C, 5 mL/min flow rate of carbon dioxide and; temperature 40 °C, 60 °C, 80 °C at a pressure of 25 MPa 5 mL/min flow rate of carbon dioxide. The extract was collected every 30 min over the 240 min of extraction time. The results of the best extraction time are presented in Fig. 2a and b.

The figure presents the oil yield in weight versus the extraction time in minutes. Temperature and pressure are the important factors influencing the mass transfer of solute. They were set in range in order to determine the optimum extraction time for best yield and compound extracted. Both of the results showed that the oil yield increased as the extraction time extended until 150 min, where the oil yield then approached a limit and achieved an asymptotic yield at nearly 180 min. Time is one of the indices for extraction efficiency evaluation which influences yield and the economic viability of the process. Moreover, the optimum extraction time should be obtained for extraction of the target compound with optimum concentration. Too short of extraction time might lead to incomplete extraction of the sample whilst when the extraction time is too long, extracted compounds might degrade or convert to other compounds. The literature shows that the concentration of monoterpenes increases as extraction time is increased due to the enhanced leaching of heavier compounds (Al-Asheh et al., 2012). Hence, 180 min of extraction time is determined appropriate for Roselle seed oil SC-CO_2 extraction.

3.3. Determination of optimum extraction particle size

Fig. 3a and b demonstrate the Roselle seed oil yield for different particle sizes in SC-CO_2 extraction at a constant pressure of 30 MPa and temperature of 40 °C. Results show that the mean particle size of 300 µm gave the highest Roselle seed oil yield which is 15.85% compared with other mean sizes. A higher yield of extract, 9.45% to 10.38% can be obtained from the particle size ranged from 212 µm to 425 µm but not in larger particle sizes of 600 µm and 710 µm, as these result in a lower yield of extract. The particle size controls the mass transfer kinetics and the access of CO_2 to the sample matrix. In this study, *Hibiscus sabdariffa* seed extraction behaviour of higher yield obtained from smaller particle size is similar to the results obtained from *Momordica charantia* extraction, for which a particle size of 300 µm gave the highest yield, and the lowest yield was achieved at a particle size of 700 µm. This may be due to the diffusion factor, where smaller particle size gives a larger surface area, SC-CO_2 molecules diffuse easier into and out of the smaller particles and thus shorten the diffusion path for the solute to dissolve itself in SC-CO_2 solvent in comparison with larger particle sizes (Aiysah et al., 2018), while large particle could lead to long and slow diffusion rate which may affect the extraction kinetics.

Similar findings to this study were presented by del-Valle and Uquiche (2002), the extraction of *Rosa aff. Rubiginosa* (Rosehip) seed oil, where the highest yield of oil was obtained from the seed milled into particle size range 150 µm to 425 µm compared to the particle size range 425 µm to 850 µm. The study claimed that the milling process not

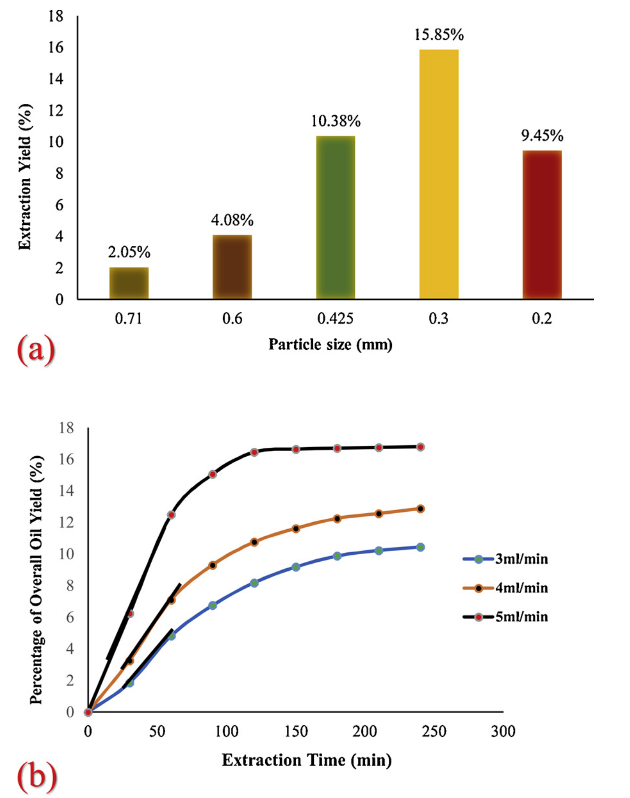

Fig. 3. a) The percentage of yield varied using different particle size of Roselle seed **b)** The effect of SC-CO_2 flow rate on extraction yield at temperature of 80 °C and pressure 30 MPa.

only increased the interfacial area to contact with SC-CO_2, but increased the release of oil from ruptured cells resulting in more free solutes released onto the surface and dissolved in the solvent. Studies have shown that oil cannot be extracted from intact seed, even using high pressure SC-CO_2, due to very low external mass transport for the whole or poorly ground seeds (Fiori et al., 2009). This phenomena is not only found in supercritical extraction but in conventional extraction as well (Lazaro et al., 2014). Apparently, the optimum particle size for Roselle seed oil extraction using SC-CO_2 is 300 µm in order to achieve the highest oil yield. This particle size of Roselle seed is used throughout the experiments.

3.4. Determination of optimum extraction flow rate

The extraction efficiency is strongly related to the SC-CO_2 flow rate in the extraction vessel. An optimum flow rate can be studied and its synergistic effect also studied with respect to temperature and pressure in order to achieve the maximum yield. In this study, the optimum SC-CO_2 flow rate was investigated by performing the extraction at constant temperature (80 °C) and pressure (30Mpa) with different flow rates of 3 mL/min, 4 mL/min and 5 mL/min. The extraction yield increases as the SC-CO_2 flow rate increases from 2 mL/min to 5 mL/min. The oil recovery at a flow rate of 5 mL/min showed the highest value at 16.7% compared to others which were 12.62% and 9.88% at the flow rate of 4 mL/min and 3 mL/min, respectively.

It can be observed that the increasing of SC-CO_2 flow rate significantly influences the diffusion rate of solute and shortens the extraction time. The higher flow rate could produce higher mass transfer of solute and the number of CO_2 molecules in contact with the solute molecules increase. More contact thus increases the intramolecular interaction between CO_2 and the solute, increasing the dissolution of solute in the fluid phase (Hostettmann, 2014; Salleha et al., 2014). Similar results can be seen in the study of S. Mahagoni for seed extraction

(Norodin et al., 2017), flaxseed extraction (Bozan and Temeli, 2002) and Kalahari melon seeds extraction (Nyam et al., 2011) in all of which the seed oil recovery increased as the SC-CO_2 flow rate increased. The higher SC-CO_2 flow rate decreases the external mass transfer resistance, leading to higher seed oil yield. Another study by Asep et al. (2013) on the SC-CO_2 extraction of cocoa butter showed that the yield is better at higher CO_2 flow rate, with an increase of temperature and pressure. The synergetic effect not only provides higher recovery but also maximizes the dissolution of selected solute as well. The extraction of *Pistacia terebinthus* gave a maximum yield for oleic acid (51.55%) at a higher flow rate of 17 g/min by raising the temperature to 54 °C and 24 MPa (Senyay et al., 2011).

3.5. Supercritical carbon dioxide extraction of roselle seeds oil

In this study, the optimum extraction time, particle size and SC-CO_2 flow rate were set constant at 180 min, 300 µm and 5 mL/min for the Roselle seed oil extraction. The effects of temperature and pressure on the Roselle seed oil yield were studied.

3.5.1. Effect of pressure on the overall oil yield

The effect of pressure on the overall extracted Roselle seed oil yield was investigated at temperatures of 40 °C, 60 °C and 80 °C. The results are shown in Fig. 4a, b and c. As the pressure increased, the SC-CO_2 density was elevated and the solubility of lipid compounds were influenced by the solvent solvation power and hence enhanced solubility

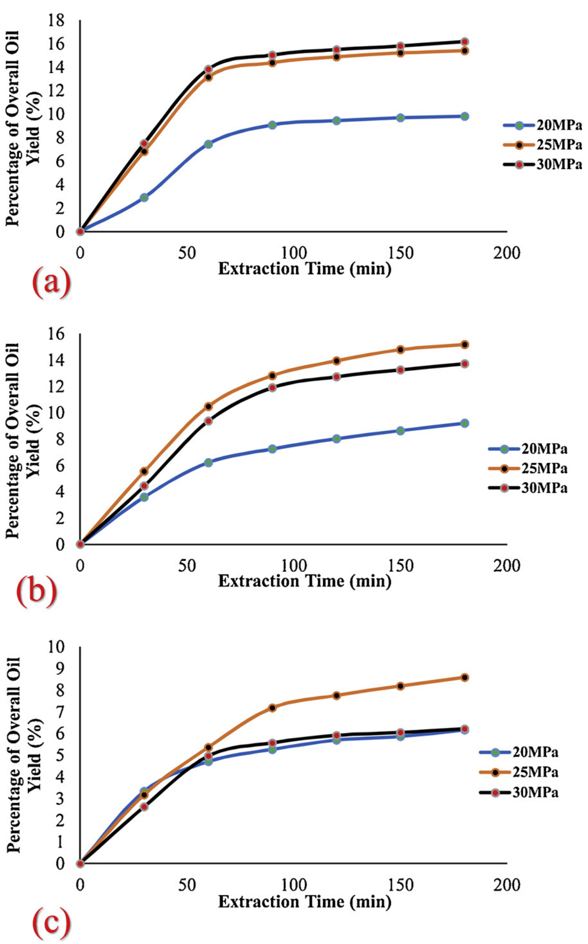

Fig. 4. a) The percentage of oil yield as a function of extraction time at 40 °C with 5 ml/min flow rate under different pressures **b)** The percentage of oil yield as a function of extraction time at 60 °C with 5 ml/min flow rate under different pressures **c)** The percentage of oil yield as a function of extraction time at 80 °C with 5 ml/min flow rate under different pressures.

of the solute. The extraction curves in Fig. 4a are plotted at the condition of constant temperature 40 °C and an increasing pressure fom 20 MPa to 30Mpa. The curves show that the constant extraction rate period is highly controlled by the solubility of oil in SC-CO_2 at the early stages of the extraction. The vapour pressure, which was elevated at high extraction pressure, facilitated the travel of oil onto the surface of seeds. The percentage of Roselle seed oil yield increased by 6.362% with the increase in extraction pressure. The highest Roslle seed oil yield was 16.168% obtained at lower temperature with higher pressure.

The same trend was observed in the extraction of soybean oil using SC-CO_2, in which the yield increases with increasing pressure from 200 bar to 300 bar at a constant temperature of 40 °C (Jokic et al., 2010, 2012). Another similar trend was found in the extraction of tomato seed oil in which the extraction rate was enhanced by nearly 30% when the pressure was raised from 20 MPa to 30 MPa at 40 °C (Shen and Xu, 2005). Grapeseed oil extraction using SC-CO_2 have been investigated by Diofanor et al. (2018) at 20Mpa and 30Mpa when the temperature, CO_2 flow rate and particle size were kept constant at 40 °C, 5 mL/min and 400 µm respectively. It was found that extraction yield increased when pressure was raised. The yield of bottle gourd seed oil at different pressure levels have been found to increase as the pressure increased from 20 MPa to 50 MPa. It was found that as the density of the solvent increases, the distance between molecules of solvent and solute decreases. Therefore, the interaction between oil and CO_2 increases which improves the solubility of oil in CO_2 (Said et al., 2014).

The effect of pressure on the overall Roselle seed oil yield at constant temperature of 60 °C and 80 °C have been investigated in this study. The oil yield increases when the pressure is raised from 20 to 25 MPa, while further increase in the pressure to 30 MPa caused the oil yield to decrease. The results indicate the increasing of solvent density enhances the solvent solvation power which eases the dissolution of oil in solvent (and increases the yield until the optimum yield is obtained at the pressure of 25 MPa. However, a slight drop in yield at a temperature of 60 °C and a more drastic reduction in yield at 80 °C was observed when the pressure was raised from 25 to 30Mpa. The results can be seen in Fig. 4a and b, and this may be due to the negative quadratic effect of pressure on the oil yield. Increasing pressure to a certain point reduces the diffusivity of SC-CO_2, hence reducing the contact of solvent with pores in the sample matrix, thereby reducing the solvation power (Belwal et al., 2016).

This is supported by the study on Kalahari melon seed oil extraction, in which the oil recovery increased slightly from low to moderate level of pressure (200–300 bar) and then decreased slightly at a higher level of pressure (300–400 bar). This may be due to the increasing repulsive solute-solvent interactions resulting from the highly compressed CO_2 at high-pressure levels. In some cases, increases in pressure causes the solid matrix to become compacted which results in negative extractive performance (Paula et al., 2013). On the other hand, the extraction of *Ziziphora tenuior* oil also exhibited an increased yield at the first stage of extraction with lower pressure of 160 to 175 bar where, the mass transfer coefficient in the fluid phase decreases with pressure increase due to the solvent viscosity. Meanwhile, the pressure had a negative effect in the second stage, when a pressure increase from 175 to 190 bar caused the extraction yield to reduce to 45%. They found the diffusivity causes a decrease in the interaction between the SC − CO_2 and the solute within the sample matrix (Darbandi et al., 2013).

3.5.2. Effect of temperature on the overall oil yield

The effect of temperature on the overall extracted Roselle seed oil yield was conducted at pressures of 20 MPa, 25 MPa and 30 MPa. The results are shown in Fig. 5a, b and c. The overall results show that the yield increases as temperatures decreases. At lower pressure of 20 MPa, the difference of overall oil yield is 3.65% when temperature decreases from 80 °C to 40 °C. At a pressure 25 MPa, the yield at 40 °C and 60 °C was almost the same at 15.39% and 15.18% respectively, while a drastic drop to 8.58% is observed when temperature increases to 80 °C.

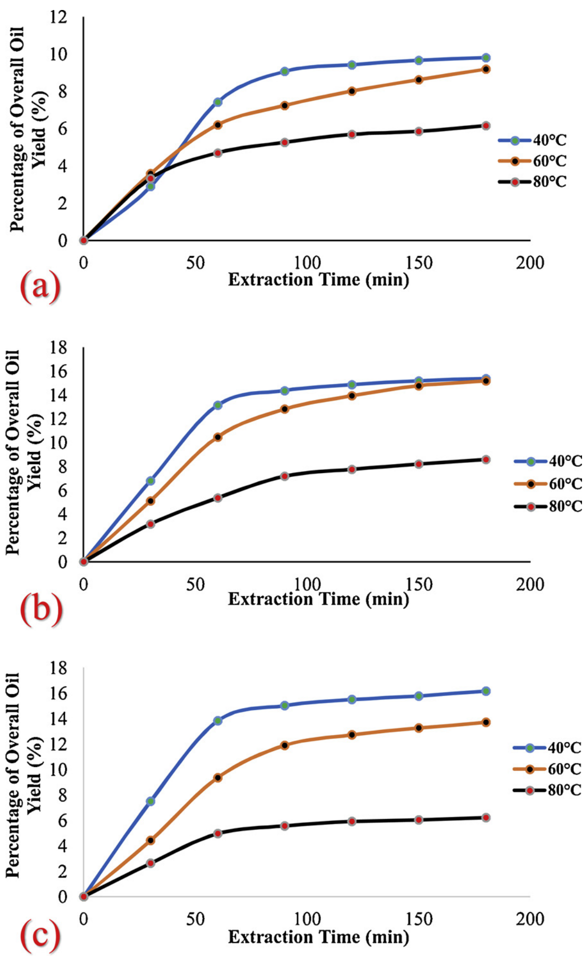

Fig. 5. a) The percentage of oil yield as a function of extraction time at 20 MPa with 5 ml/min flow rate under different temperatures **b)** The percentage of oil yield as a function of extraction time at 25 MPa with 5 ml/min flow rate under different temperatures **c)** The percentage of oil yield as a function of extraction time at 30 MPa with 5 ml/min flow rate under different temperatures.

At the higher pressure of 30 MPa, the highest yield of Roselle seed oil, 16.19% is achieved at 40 °C. Only 6.2% of oil yield was obtained at 80 °C with a pressure of 30 MPa but the yield is higher compared to the extraction at a pressure 20 MPa.

Higher temperature might lead to a saturated vapor pressure condition, oil volatility and mass transfer elevation, which allows the solute to easily dissolve in $SC-CO_2$. However, the increase in temperature at a constant pressure can cause the $SC-CO_2$ density to decrease (Zhao et al., 2014; Rivero et al., 2015). Hence the solubility of solute could have a positive or negative effect dependant on whether the solute vapor pressure or the solvent density is predominant. The trend of the observations in this study can be explained by the latter, where the reduction of SC-CO_2 density overcomes the vapor pressure and further decreased the interaction of CO_2 with seed matrix and hence reduces the dissolution of solute in the solvent. The similar tendency of extraction was observed in the study by Zermane et al. (2012), in which the recovery of Algerian Rosemary oil using SC-CO_2 was observed to decrease significantly with increasing temperature at pressures of 10–22 MPa. From Fig. 5a, the yield is higher at the very early stage at the temperature of 40 °C. However the yield increases rapidly as the extraction time progresses. This phenomenon is called "crossover effect" where higher temperature produces low yield while lower temperature can produce high yield (Khaw et al., 2017). This effect has been observed in the extraction of ginger oil where the increase of solute vapor pressure and reduction of solvent density were competing to become the main element in the extraction process (Bhupesh et al., 2006).

Table 3
The experimental solubility data of Roselle seed oil in $SC-CO_2$.

Temperature (°C)	Pressure (MPa)	SC-CO_2 Density (kg/m^3)	Solubility (g/kg CO_2)
40	20	839.9	0.65
	25	879.6	0.97
	30	910	0.99
60	20	723.8	0.80
	25	786.8	1.07
	30	830	0.92
80	20	594.1	0.58
	25	686.6	0.69
	30	746	0.59

3.5.3. Effect of extraction conditions on solubility of Roselle seeds oil

The solubility of Roselle seed oil in SC-CO_2 was measured at pressures of 20 MPa to 30 MPa and temperatures of 40–80 °C at a flow rate of 5 mL/min. Dynamic extraction was carried out in this study in order to obtain the solubility data. The solubility data is important to determine the conditions where the SC-CO_2 fluid works best and to optimize the operating temperatue and pressure. The measurement of solubility data were obtained from the slope of the linear portion of the overall extraction curve. The solubility data of Roselle seed oil are summarized in Table 3. From the results, it can be seen that the solubility of Roselle seed oil is low at higher temperature of 80 °C. The high temperature has limited the Roselle seed oil solubility. This might be due to the decreased SC-CO_2 density at higher temperature which decreases the solvation power of the solvent. At lower temperature of 40 °C and 60 °C, solubility of Roselle seed oil increased with the increase of pressure from 20 MPa to 25 MPa. The SC-CO_2 density at 20 MPa to 25 MPa is lower at the temperature of 60 °C compared to the SC-CO_2 at 40 °C. However the solubility at 60 °C is higher than that of 40 °C. It is mainly due to the oil vapor pressure effect being greater than solvent solvation power. The maximum solubility for Roselle seed oil was observed at 25 MPa and the temperature at around 60 °C.

The increasing of temperature from 40 °C to 60 °C at higher pressure lowers the solubility. This effect created a solubility cross over region which occured at the pressure of about 28 MPa and temperature of 40 °C to 60 °C where the competing effect of SC-CO_2 density with the vapor pressure occurred. Below the cross over zone, the high solubility of the oil achieved is mainly due to the solute vapour pressure dominating over the SC-CO_2 density resulting in a solubility increase as the temperature increases meanwhile above the cross over region, the solubility of Roselle seed oil increased as the SC-CO_2 density increased, hence increasing the solubility of oil at higher pressure.

3.6. HPLC analysis of gamma-tocopherol content in SC-CO_2 extracted Roselle seed oil

The SC-CO_2 extracted Roselle seed oil was analysed for gamma-tocopherol content. HPLC was used for quantification and the actual content was calculated according to the calibration curve generated. The content of gamma-tocopherol in Roselle seed oil using SC-CO_2 extraction is compared to coventional extraction.

3.6.1. Calibration curves of gamma-tocopherol standard

The gamma-tocopherol standard was diluted into five concentrations, 1 mg/g, 0.5 mg/g, 0.1 mg/g, 0.05 mg/g and 0.025 mg/g. The calibration curve was plotted with peak area as a function of injected gamma tocopherol concentration. The linear regression equation obtained was $y = 491.58x + 38.659$ with the R^2 equal to 0.9988 showing the high correlation.

Table 4
The Roselle seed oil yield and gamma tocopherol content in oil extracted under different conditions.

Pressure (MPa)	Temperature (°C)	The peak area (mAU)	Gamma tocopherol content (mg/100 g)
20	40	66.19	5.6
	60	60.29	2.7
	80	61.76	1.6
25	40	51.93	4.4
	60	47.02	1.7
	80	50.95	2.1
30	40	46.52	4.7
	60	48.98	2.5
	80	49.97	2.3

3.6.2. Effect of $SC\text{-}CO_2$ extraction conditions on the gamma-tocopherol content

Each of the Roselle seed oil samples extracted at diffent extraction conditions were analysed for their gamma tocopherol content. The chromatograms of oil samples were compared to the standards. The peak area of each oil samples were determined and the quantification of gamma tocopherol concentration was obtained from the calibration curve. The peak eluted at the retention time of 5.961 min. Other HPLC chromatograms were attached in Appendix A. The gamma tocopherol content in Roselle seed oil extracted under different extraction conditions were identified and presented in Table 4.

The concentration of gamma tocopherol in Roselle seed oil were in range of 1.7 mg/100 g to 5.6 mg/100 g. The higher gamma tocopherol content was found at lower temperature of 40 °C. The highest value was found at a temperature 40 °C with pressure 20 MPa although the oil yield is not very high in this case. A similar trend was observed for the extraction of grapeseeds oil at low pressure of 20 MPa, the extract yield is lower but the content of vitamin E extracted is higher (Gustinelli et al., 2018). This may be due to the solubility of Vitamin E being highly associated with its solubility in oil and availability on the seed surface. The oil extraction rate is higher in the initial $SC\text{-}CO_2$ extraction phase compared to the latter stage of the process. Although the lowest concentration of gamma tocopherol was found at a temperature of 60 °C with pressure 25 MPa, the increasing of pressure to 30 MPa increased the tocopherol content. This indicates the extraction pressure plays an important role in solubilizing tocopherols. As Yang et al. (2011) reported, the vitamin E solubility is dependant on pressure, especially in long extraction processes. The pressure increases the $SC\text{-}CO_2$ solvent interaction within the seed particles which leads to the increasing diffusion rate of tocopherol.

3.7. Comparison between soxhlet extraction and $SC\text{-}CO_2$ extraction

The extraction of Roselle seed oil using supercritical carbon dioxide ($SC\text{-}CO_2$) was compared with the conventional Soxhlet method in terms of the extraction oil yield and gamma tocopherol content. Table 5 compares the conditions and results for both method of extraction.

Table 5
Comparison of extraction performance between $SC\text{-}CO_2$ and Soxlet method.

Extraction method	$SC\text{-}CO_2$ Extraction	Soxhlet Extraction
Solvent used for extraction	Carbon dioxide	Light petroleum ether
Weight of seed used for extraction	5g	15 g
Extraction time	180 min (3 h)	480 min (8 h)
Particle size of seed used for extraction	300 µm	300 µm
Solvent flow rate	5 mL/min	–
Temperature	40 °C	60 °C
Pressure	30 MPa	0.1
Extraction Yield	16.17%	23.8%
Gamma tocopherol content	4.74 mg/100 g oil	1.32 mg/100 g oil

From the results, the extraction oil yield using Soxhlet is higher compared to the $SC\text{-}CO_2$ extraction while the gamma tocopherol content is found to be lower in Soxhlet extraction of Roselle seed oil. The results is similar to the studies of wheat germ oil extraction where the oil yield was found higher by using solvent extraction, especially using methanol. However, the concentration of alpha tocopherol extracted using $SC\text{-}CO_2$ at a temperature of 40 °C with pressures of 20 MPa to 30 MPa were found higher compared to the oil extracted using conventional solvents, methanol and hexane (Sirisetti et al., 2017).

3.8. Optimization of supercritical carbon dioxide extraction

Optimization of Roselle seed oil extraction using supercritical carbon dioxide extraction ($SC\text{-}CO_2$) was determined using response surface methodology (RSM). The interaction between temperature and pressure on the variables, extraction yield and gamma tocopherol content were studied in order to improve the operating conditions and achieve high extraction efficiency.

3.8.1. Fitting the response surface model

Design of experiment for Roselle seed oil was based on the three level factorial with 13 set of experiments with four repetitions at the middle point. The experimental data for the yield of Roselle seed oil and gamma tocopherol are presented in Table 6. The selection of the model for the experimental data was based on the R^2 analysis and Fisher *F*-test. R^2 is necessary to develop and study the suitability and adequacy of the equations and model for the true response surface (Daneshvand et al., 2012). An R^2 value more than 0.75 is considered accurate in developing a statistical model for optimization. In this study, the R^2 values for Roselle seed oil yield and gamma tocopherol concentration were 0.9723 and 0.9754 respectively, demonstrating good correlation between the experimental data to the predicted data. Fig. 6a and b show the experimantal data and predicted values by the model. Table 7 is the ANOVA table showing the Roselle seed oil yield and gamma tocopherol content are well fitted in the second-order polynomial model.

The F-value is calculated in order to determine the adequacy of the second order polynomial model. The calculated F-value for Roselle seed oil yield and gamma tocopherol concentration are 49.13 and 55.6 respectively. These values are greater than the tabulated F (5,7,0.05) which is 3.97, indicating the significance of the relationship between the variables with the responses at the 95% confidence level. The R^2 and F-value indicated the second-order polynomial model is suitable to depict the relationship between Roselle seed oil extraction yield and gamma tocopherol content.

Eqs. (6) and (7) are the second order polynomial models showing the relationship between independent variables, pressure (X_1) and temperature (X_2) with dependant variables, Roselle seed oil yield (Y_1)

Table 6
The Experimental data of Roselle seed oil yield and gamma tocopherol content.

Standard Order	Pressure X_1 (MPa) (Coded level)	Temperature X_2 (°C) (Coded level)	Roselle seed oil yield Y_1 (mg/g)	Gamma tocopherol content Y_2 (mg/g)
1	20 (-1)	40 (-1)	98.06	0.056
2	20 (-1)	60 (0)	91.92	0.027
3	20 (-1)	80 (+1)	61.64	0.016
4	25 (0)	40 (-1)	153.9	0.044
5	25 (0)	60 (0)	139.92	0.017
6	25 (0)	80 (+1)	85.84	0.021
7	30 (+1)	40 (-1)	161.68	0.047
8	30 (+1)	60 (0)	137.02	0.025
9	30 (+1)	80 (+1)	62.2	0.023
10	25 (0)	60 (0)	151.76	0.016
11	25 (0)	60 (0)	137.65	0.018
12	25 (0)	60 (0)	125.768	0.017
13	25 (0)	60 (0)	141.56	0.017

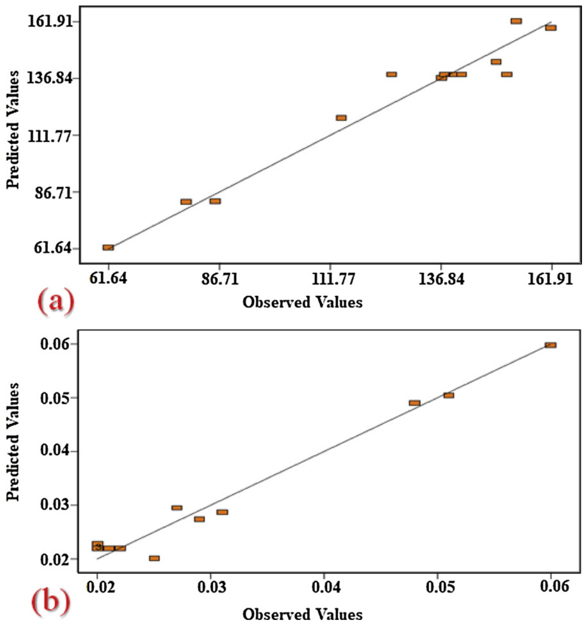

Fig. 6. a) The observed values versus predicted values for Roselle seeds oil yield **b)** The observed values versus predicted values for gamma tocopherol concentration.

Table 7
ANOVA for the response surface second-order polynomial model for Roselle seen oil yield.

Source	Sum of Square	Degree of Freedom	Mean Square	F-value	R^2
Roselle Seed oil yield					
Model	14161.74	5	2832.35	49.13	0.9723
Residual	403.54	7	70.68		
Total	12031.54	12	–		
Concentration of gamma tocopherol					
Regression	2.151×10^{-3}	5	4.302×10^{-4}	55.6	0.9754
Residual	5.416×10^{-5}	7	7.737×10^{-6}		
Total	2.205×10^{-3}	12	–		

and gamma tocopherol concentration (Y_2).

$$\mathbf{Y_1} = +139.21 - 33.99\,X_1 + 18.21\,X_2 - 19.03\,X_1^{\,2} - 24.43\,X_2^{\,2} - 15.76\,X_1X_2 \quad (6)$$

$$\mathbf{Y_2} = +\,0.018\,-0.015\,X_1\,-6.667 \times 10^{-4}\,X_2 + 0.013\,X_1^{\,2} + 6.103 \times 10^{-3}\,X_2^{\,2} + 4 \times 10^{-3}\,X_1\,X_2 \quad (7)$$

The coefficients of the second order polynomial quadratic equations for predicting the optimum point was obtained according to the three-level factorial design. The multiple regression coefficients of oil yield and gamma tocopherol were determined and are summarized in Table 8. The linear coefficient, X_1, X_2 the quadratic coefficient X_1^2, X_2^2 and interaction coefficient X_1X_2 for the extraction oil yield are very significant at $p < 0.01$ denoting that the temperature and pressure are very crucial for the Roselle seed oil yield. For the recovery of gamma tocopherol content, linear coefficient X_1 is very significant at $p < 0.01$, the temperature plays an important role while the linear coefficient X_2 for pressure is not significant with $p > 0.05$, which means the presusure does not affect the gamma tocopherol concentration in Roselle seed oil extraction. However, the quadratic coefficient X_2^2 and interaction coefficient X_1X_2 were significant, which shows the crucial effect of both pressure and temperature interaction.

Table 8
The regression coefficients for the Roselle seed oil yield and gamma tocopherol.

Variables	Extraction Oil Yield		Gamma Tocopherol Concentration	
	Regression coefficient	*p*-value	Regression coefficient	*p*-value
Intercept	139.21	< 0.0001	0.018	< 0.0001
Temperature (X_1)	−33.99	< 0.0001	−0.015	< 0.0001
Pressure (X_2)	18.21	0.0006	-6.667×10^{-4}	0.5756
X_1^2	−19.03	0.0042	0.013	0.0001
X_2^2	−24.43	0.0011	6.103×10^{-3}	0.0082
X_1X_2	−15.76	0.0043	4.000×10^{-3}	0.0238

3.8.2. Analysis of response surface model

The response surfaces can be ilustrated as 3-dimensional plots as shown in Fig. 7a and b which showing the Roselle seed oil yield and gamma tocopherol in relation to the extracting pressure and temperature. It was found that the temparature and pressure were both affecting on the extraction yield while temperatue was more prominent on gamma tocopherol concentration as compared to the pressure. From Fig. 7a, when the temperature was low, the extraction yield increases as the pressure is increased. The yield achieved the highest when the temperature is low with a high pressure. When the temperature is increased further, the extraction yield increases as the pressure increases until a maximum point then the yield begins dropping as the pressure is increasing. Fig. 7a shows the extraction yield was low when the extraction was carried out at high temperature with low pressure or high temperature with high pressure. From Fig. 7b, the highest gamma tocopherol concentrations were obtained at the lowest temperature of this study.

Optimum conditions of temperature and pressure were attained using the fitted model in order to predict the optimum oil yield and gamma tocopherol content. By using the desirability function which was proposed by Derringer and Suich in 1980, multiple responses can be optimised by inputting a set of parameters, adjusting the requirements and importance of responses, thereby achieving overall process improvement (Hu et al., 2008; Shi et al., 2010). According to Aggarwal et al. (2008), desirability function is more appropriate for readability, acceptability and visualisation as compared to other optimization techniques.

The goal for extraction parameters in Roselle seed oil SC-CO_2 extraction was set in the appropriate range, the extracted oil yield and gamma tocopherol concentrations were set as maximum. An importance value of three was chosen for all the parameters and responses in the study (Tables 4 and 6). Numerical optimization was chosen, as it locates a point that maximizes the desirability function. The desirability function transforms the estimated response into a scale free value indexed from zero, which is the least value, to unity, the most desirable value (Manohar et al., 2013). In this study, the optimum extraction conditions for Roselle seed oil SC-CO_2 extraction obtained by the application of response surface methodology (RSM) were found to be temperature of 40 °C and pressure of 30 MPa where the desirability value is 0.871.

4. Conclusion

The extraction of *Hibiscus sabdariffa L.* (Roselle) seed oil using supercritical carbon dioxide (SC-CO_2) was performed within the pressure range of 20 MPa to 30 MPa and temperature range of 40 °C–80 °C. The Roselle seed used for extraction contained approximately 8.03% moisture. The 300 μm particle size was found to have the highest yield compared to 212 μm, 425 μm, 600 μm and 710 μm. The optimum extraction time is 180 min for complete oil extraction, and 5 mL/min of SC−CO_2 flow rate was determined to give the highest extraction oil yield. The extraction of Roselle seed oil using SC-CO_2 was carried out,

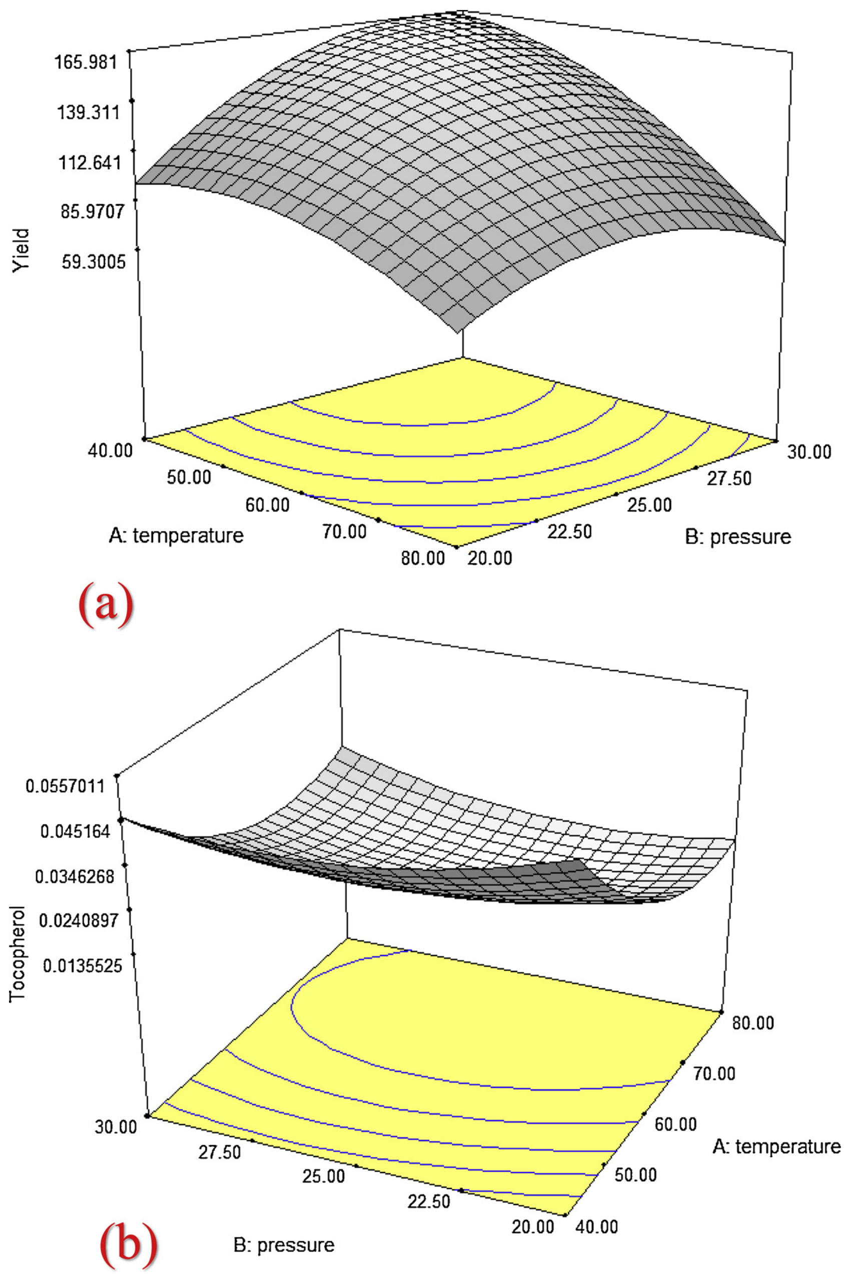

Fig. 7. **a)** Response surface of the oil yield expressed as the function of pressure and temperature **b)** Response surface of the gamma tocopherol concentration expressed as the function of pressure and temperature.

and it was shown that the highest extraction oil yield obtained was at 30 MPa and 40 °C while the lowest was at 30 MPa and 80 °C. The obtained oil yields were in the range 6.22–16.17%. The overall extraction oil yield using SC-CO_2 increased as the pressure increased at lower temperature. The gamma tocopherol content obtained were in the range of 1.6–5.6 mg per 100 g of oil. The gamma tocopherol concentration is much higher in oil extracted using SC-CO_2 extraction (4.7%) compared to the Roselle seed oil extracted using Soxhlet method (1.32%). This result has proven the selective nature of SC-CO_2 extraction and that it is a good choice for extraction of a high nutritive value plant. The experimental data of SC-CO_2 extraction obtained were fitted to a second-order polynomial model. The coefficient of determination values for Roselle seed oil yield and gamma tocopherol concentration were 0.9723 and 0.9754, respectively. In addition the F-value calculated using data was higher than the tabulated F-value which shows the experimental data were well fitted against the model.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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