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Article

Recovery of *Moringa oleifera* Oil from Seed Cake by Supercritical Carbon Dioxide Extraction

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Abstract. Commercial *Moringa oleifera* seed oil has been currently produced via mechanical extraction using a screw press machine. Approximately 30 %wt. of the residue oil remains in the seed cake. Thus, this study aims to examine the effects of pressure and solvent-to-feed (S/F) ratio on the supercritical CO_2 extraction of seed cake obtained from mechanical extraction. The antioxidant activities of extracted oil were determined using the standard in *vitro* biological assays. The supercritical CO_2 extraction duration of seed cake is shortened because the mechanical extraction reduces oil content before performing supercritical CO_2 extraction. At 50 °C and 30.0 MPa, S/F ratios of 0.4 /min and 1.0 /min have corresponded to an extraction duration of 300 min and 180 min, respectively. Up to 90 %wt. of the oil content in the seed cake can be recovered at 30.0 MPa, 50 °C, and after 200 min of extraction duration. Regardless of the extraction pressure, the antioxidant activities of *M. oleifera* seed oil were maximized at 60 °C.

Keywords: Supercritical carbon dioxide extraction, extraction recovery, moringa oleifera, oil seed, screw press.

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1. Introduction

Moringa oleifera is identified as a softwood tree that is indigenous to regions on the equator. The seed oil of *M. oleifera* has been reported to contain high oleic acid of up to 70%. The sterol and tocopherol contents in Moringa spp. were reported in the range of 41.37 mg/kg oil to 56.91 mg kg/oil and 34.00 mg/kg oil to 373.31 mg kg/oil, respectively [1]. *M. oleifera* seed oil has many biological activities, including its anti-inflammatory, antidiabetic, antimicrobial, and antioxidant properties [2]. Therefore, *M. oleifera* seed oil is more expensive than common seed oil. For instance, in Thailand, *M. oleifera* seed oil costs €100/L to €500/L, depending on the quality and source of the raw material. *M. oleifera* oil seed can be directly used as a skin and hair moisturizer to relieve muscle pains and aches, or it can be processed to other skincare and haircare products. Stohs and Hartman (2015) intensively reviewed the safety and efficacy of using *M. oleifera* [3].

The oil content in *M. oleifera* seed is 25 %wt. to 40 %wt. of that in a dry seed; therefore, commercial seed oil is currently produced via mechanical extraction using a screw press machine. To prevent the thermal degradation of active compounds during compression, the temperature in the pressing barrel is maintained below 70 °C; this process is traditionally called the cold-pressed method [4]. The friction force between the seed, screw, and barrel has been determined to generate a significant amount of heat. If extraction conditions are not carefully monitored and controlled, the pressing temperature can increase up to 120 °C. The pressing temperature can be controlled by controlling various parameters such as feed rate, barrel and screw gap, and screw rotating speed. The maximum amount of extracted oil from *M. oleifera* seed by a screw press machine is ~70 % of its oil content [5, 6]; in other words, ~30 %wt. of the residue oil remains in the seed cake. The residue cake can be used as an adsorbent or a coagulant for wastewater treatment [7], lamb feed [8], and feed for Bocourti's catfish (*Pangasius bocourti*) [9].

Table 1. Experimental parameters and oil yield for $SCCO_2$ extraction of *M. oleifera* kernel, as reported in the literature.

Parameters								Maximum yield obtained condition	Oil yield (%)	Ref.
P (MPa)	T (°C)	CO_2 flowrate (kg/h)	Particle size (mm)	%EtOH	Time (min)	Sample wt. (g)	Extractor volume (L)			
20 to 40	60 to 100	0.30 to 0.90	0.50 to 1.00	0 to10	250	50	1.0	40 MPa, 80°C, 0.60 kg/h, 0.75 mm, 250 min, 5% EtOH	N/R	[24]
20 to 60	40 to 120	0.11 to 0.55	<1.00	-	30 to 150	10	0.05	50 MPa, 100°C, 0.44 kg/h, 120 min	37.12	[11]
15 to 30	35 to 60	0.45	0.16 to 1.12	10	420	N/R	0.5	29 MPa, 44 °C, 0.54 mm, 420 min	37.84	[9]
15 to 35	25 to 35	20	<1.00	-	300	850	2.0	35 MPa, 30°C, 20 kg/h, 300 min	28.87	[10, 13]

Because supercritical carbon dioxide ($SCCO_2$) has been identified as a suitable solvent for seed oil extraction, many studies have used $SCCO_2$ extraction of *M. oleifera* kernel without pre-extraction, as summarized in Table 1. A small amount of ethanol was added into the system as co-solvent or enhancer to improve the extraction efficiency. However, the study on $SCCO_2$ extraction of *M. oleifera* seed cake is presently scarce.

For all studies in Table 1, the seed samples were dehulled, milled, and sieved before extraction by $SCCO_2$. Optimal extraction time is the time required for the oil yield to become constant. For example, the extraction duration of 140 min for $SCCO_2$ extraction is observed at 100 °C, 50 MPa, a CO_2 flow rate of 7.36 g/min, and a solvent-to-feed (S/F) ratio of 0.74/min [10]. Increasing the temperature has been reported to reduce the viscosity of *M. oleifera* oil, enhancing the extraction efficiency. At high temperatures, the density of $SCCO_2$ decreases; thus, high pressure is required to maintain the $SCCO_2$ extraction efficiency. In contrast, the optimal extraction duration prolonged to 420 min and 300 min when $SCCO_2$ extraction was performed at 40 °C and 44.2 °C, respectively [11–13]. The extended extraction durations reported at low temperature were attributed to the high viscosity of *M. oleifera* seed oil and the high oil content of *M. oleifera* kernel.

Ultrahigh-pressure $SCCO_2$ extraction of *M. oleifera* kernel can dramatically shorten the extraction duration without pre-extraction [14]. It is well-known that increasing the pressure improves the extraction efficiency because of increasing $SCCO_2$ density. However, ultrahigh-pressure $SCCO_2$ extraction is considered practical for analytical-scale (5 mL) because the fabrication cost of the extractor exponentially increases with the operating pressure, especially for the large extractor in the industrial scale (<1000 mL). Nowadays, the commercial $SCCO_2$ extractor has the maximum working pressure of 40.0 MPa to 45.0 MPa. The extractor volumes are 1 L to 100 L. The economic analysis of basil seed oil extraction by $SCCO_2$ was reported in our previous work [15].

This study first aims to examine the mechanical extraction of the whole seed and kernel of *M. oleifera* by a single-screw press machine. The report on mechanical pressing of *M. oleifera* seed has been identified to be presently scarce. The pre-extraction of *M. oleifera* seed by mechanical pressing could reduce the extraction duration of $SCCO_2$ extraction. The second aim is to recover the residue oil in *M. oleifera* seed cake using the $SCCO_2$ extraction. The antioxidant activities were determined by following the in vitro methods to check the quality of extracted oil.

2. Materials and Methods

2.1. Chemicals and Raw Materials

Petroleum ether (Certified ACS Reagent, CAS 8032-32-4) was obtained from Fisher Scientific Co, Ltd. All chemicals used to analyze the fatty acid profile, including 14 % BF_3 in methanol (CAS 373-57-9), *n*-heptane (CAS 142-82-5), and NaOH (CAS 1310-73-2), were purchased from SAC Sci-Eng, Co, Ltd., Thailand. The carbon dioxide (ALPHAGAZ SFC; purity CO_2 >99.995 %; impurity H_2O <5 ppm, O_2 <2 ppm, and N_2 <50 ppm) was purchased from Air Liquide Co., Ltd. (France).

Dried *M. oleifera* seeds were purchased from agriculturists coming from the Phitsanulok province, Thailand. The naturally dried *M. oleifera* pods were manually collected, and the seed samples were separated into whole seed and kernel, as shown in Fig. 1. Whole seed and kernel were priced at €6.50/kg and €9.50/kg, respectively. As reported from the agriculturists, the whole seed consist of 10%wt. to 15%wt. of shell. The samples were preserved in a cold room at 4 °C before extraction using a single-screw press machine.

2.2. Mechanical Extraction

The 4.0 kg of whole seed and kernel were separately extracted using a single-screw press machine (Model YZYX70-ZWY), which was obtained from Mianyang Guang Xin Machinery Factory Co, Ltd. The mechanical extractor was located at The Institute of Biotechnology and Genetic Engineering, Chulalongkorn University, Thailand. Both whole seeds and kernel were mechanically extracted at a constant screw rotation speed of 25 rpm. Because of the different physical characteristics of the seed, the feed rates for whole seed and kernel were 3.6 kg/hr and 1.2 kg/hr, respectively. Seed samples were compressed twice. The oil and cake samples for each compression step were separately collected, and the weights of oil and seed cake were then recorded. To examine the actual oil yield, the solid impurities were removed from the extracted oil via centrifugation and vacuum filtration. The amount of residue oil remaining in the seed cake was determined via Soxhlet extraction using petroleum ether as the solvent.

Fig. 1. Images of *M. oleifera* (a) whole seed, (b) kernel, and (c) whole *M. oleifera* seed cake (2nd compression).

2.2. Solvent and $SCCO_2$ Extractions

Solvent extraction was performed in a 500 mL 24/40 glass Soxhlet extractor. The sample was filled in a cellulose timber. The glass condenser was cooled by the circulating water bath. The sample size and petroleum ether (36 °C to 60 °C) were 10 g and 250 mL, respectively, and the solvent was heated using an electrical heating mantle. The extraction was performed at 60 °C for 8 hours.

The $SCCO_2$ extractions were performed in 50 mL extractor under extraction conditions of 40 °C to 70 °C and 15.0 MPa to 60.0 MPa. Flow rates were varied within 10 g/min to 30 g/min. To normalize the effects of particle size, the seed cake was milled and sieved until the particle size was 1.00 mm to 1.18 mm in order to normalize the effects of particle size. The particle distribution was then measured by sieve analysis. The $SCCO_2$ extractor was located at Laboratoire de Génie Chimique (LGC), Toulouse, France [16]; its dimensions are 2.0 cm inner diameter and 18.0 cm, and its maximum working pressure is 110.0 MPa. The schematic diagram of the extraction experiment is shown in Fig. 2.

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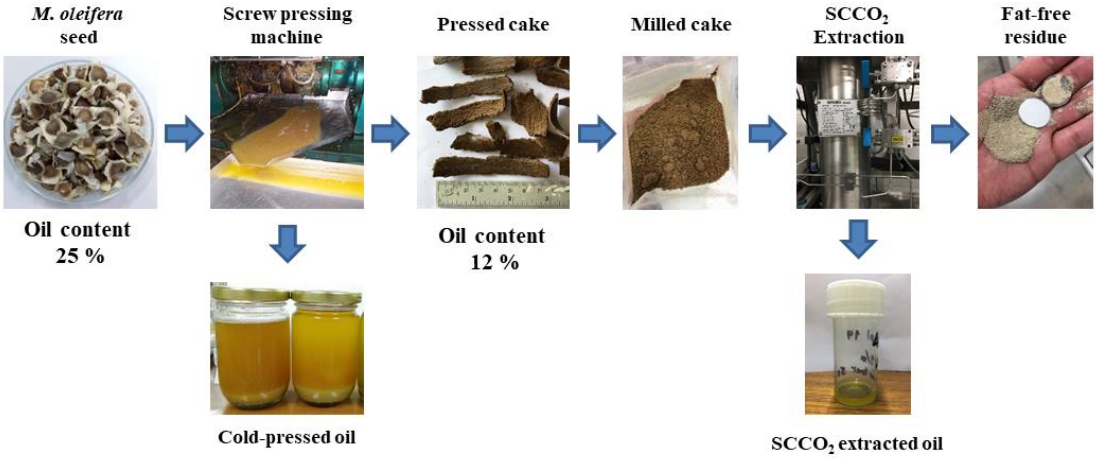

Fig. 2. Schematic of the overall experiment.

2.4. Analytical Methods

The extracted oil yield is determined as follows:

$$\%\text{Oil yield} = \frac{W_1}{W_2} \times 100 \quad (1)$$

where W_1 and W_2 are the weights of the extracted oil and individual samples, respectively. The samples are whole seed, kernel, whole seed cake, and kernel cake.

The physicochemical characteristics, including moisture (Ca 2d-25) and lipid (Am 5-04) contents, and fatty acid profile (Ce 1k-09 and Ce 1h-05) of the extracted oil were analyzed using standardized AOCS methods [18, 19]. The total protein was estimated by the total nitrogen obtained from Kjeldahl's method. The antioxidant activities were determined by following the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide (NO) *in vitro* methods [20, 21]. Finally, the α-tocopherol content was analyzed by HPLC using a published in-house method [22]. Significant differences and mean values were analyzed using Duncan's multiple range test, and all statistical analyses were performed using IBM SPSS Statistics 22.

3. Results and Discussion

3.1. Solvent and Mechanical Extractions of Whole Seed and Kernel

The oil contents obtained via the 8-h Soxhlet extraction using petroleum ether as the solvent were 25.39 %wt. and 37.92 %wt. on a dry basis for whole seed and kernel, respectively. The oil content in the kernel is almost similar to that reported previously [11, 13, 23–26]. The material balance for the mechanical extraction process is listed in Table 2.

The mechanical extraction of kernel was deemed unsuccessful by using. At the beginning of extraction, the kernels were cracked, but no oil content was observed. When a higher pressure was applied, the kernels turned into the doughy paste and percolated through the barrel of the single-screw press machine. The large amount of weight loss of 44.75 % is shown in Table 2. Thus, the mechanical extraction of kernel is determined impracticable. Consequently, we did not undertake $SCCO_2$ extraction of kernel cake in this study.

Table 2. Material balance for the mechanical extraction process of whole seed and kernel.

Property	Whole seed	Kernel
Total feed weight (kg)	4.000	4.000
Cake weight (1st compression, kg)	3.420	1.940
Raw oil weight (1st compression, kg)	0.275	0.270
Weight loss (1st compression, kg) [a]	0.305	1.790
Cake weight (2nd compression, kg)	2.875	N/R
Raw oil weight (2nd compression, kg)	0.290	N/R
Weight loss (2nd compression, kg) [b]	0.255	N/R
Total raw oil weight (kg)	0.565	N/R
Total weight loss (kg) [c]	0.560	N/R
%Raw oil yield	14.13	N/R
Purified oil weight (kg) [d]	0.500	N/R
%Purified oil yield	12.50	N/R

N/R: not reported

[a] Weight loss (1st compression, kg) = total feed weight (kg) − cake weight (1st comp., kg) − raw oil weight (1st comp., kg)

[b] Weight loss (2nd compression, kg) = cake weight (1st comp., kg) − cake weight (2nd comp., kg) − raw oil weight (2nd comp., kg)

[c] Total weight loss = total feed weight (kg) − total cake weight (kg) − total raw oil weight (kg)

[d] Oil weight after centrifugation and filtration

For the mechanical extraction of oil seed, the seed shell has been identified to play an important role. The hardness of the shell provides the suitable friction force for the screw press process;4 the results are similar to those reported for linseed oil. After mechanical extraction, the whole seed turns into a fragile flake that can be easily milled. The size and shape of the flake were sporadic, as shown in Fig. 1(c). The average flake measured approximately 10 × 1.5 cm^2. Thus, a raw flake could not be directly placed into the lab-scale extractors. These raw flakes were milled and sieved to a particle size in the range of 1.00 mm to 1.18 mm before $SCCO_2$ was conducted as mentioned in Section 2.3.

Table 3 shows the moisture and lipid contents of whole seed, kernel, and whole seed cake. Whole seed contains slightly more moisture than kernel. After removing the seed shell, the oil content in the kernel increases to ~10 %wt. For the whole seed cake, the oil contents in the 1st and the 2nd compressions do not significantly differ. The mechanical press can extract 10 %wt. to 12 %wt. of the oil from the *M. oleifera* whole seed. The seed cake thus retains high oil content that could be extracted by $SCCO_2$ in a future study.

Table 3. Moisture and lipid contents of *M. oleifera* whole seed, kernel, and whole seed cake.

Property	Whole seed	Kernel	Whole seed cake	
			1st comp.	2nd comp.
Moisture (%wt.)	7.08	4.76	6.33	6.30
Lipid (%wt.)	25.39	36.12	15.62	13.14

3.3. $SCCO_2$ Extraction of *M. oleifera* Seed Cake

Effect of temperature on the extraction yield has successfully investigated in our previous works [29-30]. The increasing temperature slightly improves the extraction yield, but the exceeding temperature reduces the oil yield. It was concluded that the optimal temperature for $SCCO_2$ extraction of *M. oleifera* oil at 30.0 MPa is 50 °C. Thus, the experiments were performed at higher pressure to explore the effect of pressure on the $SCCO_2$ extraction of *M. oleifera* seed cake.

Effect of pressure on the extraction yield has been shown in Fig. 3. The results show that an increase in pressure considerably enhances the oil yield. At 60.0 MPa, the extraction curve reaches the diffusion-controlled region within an hour. In contrast, the extraction curve is linear at 30.0 MPa.

To evaluate the solubility of *M. oleifera* oil in $SCCO_2$, the solvent to feed (S/F) is defined as the CO_2 flow rate (g/min) divided by the weight of the sample (g). The S/F ratio is one of the most important parameters for scale-up the extraction process. The solubility of vegetable oils in $SCCO_2$ can be estimated from the plot of percent oil yield versus CO_2 consumption per feed weight [28].

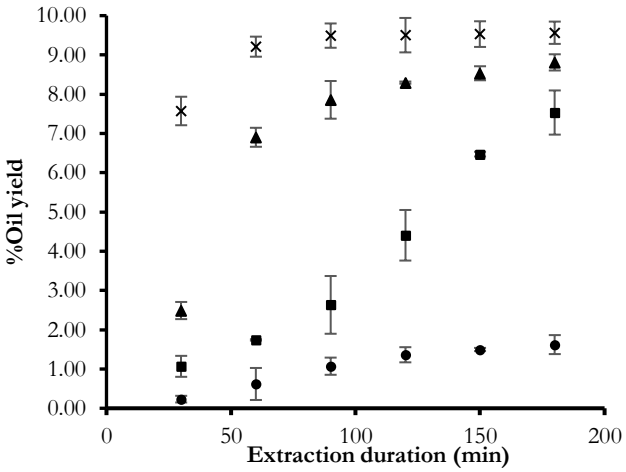

Fig. 3. Effects of pressure on %oil yield of $SCCO_2$ extraction at 50 °C, 15 MPa (●), 30.0 MPa (■), 40.0 MPa (▲), and 60.0 MPa (×).

Figure 4 shows the results of the experiments performed under 50 °C and 30 MPa in the various S/F ratios. The x- and y-axes are the percent oil yield and the CO_2 consumption (g) divided by the sample mass (g), respectively. To estimate the solubility of *M. oleifera* oil in $SCCO_2$ at 50 °C and 30.0 MPa, the sample weight was fixed at 25 g and the CO_2 flow rate was varied to obtain S/F ratios between 0.4 /min and 1.0 /min. The extraction curve divides into external mass transfer- and diffusion-controlled regimes due to the oil concentration in the sample.

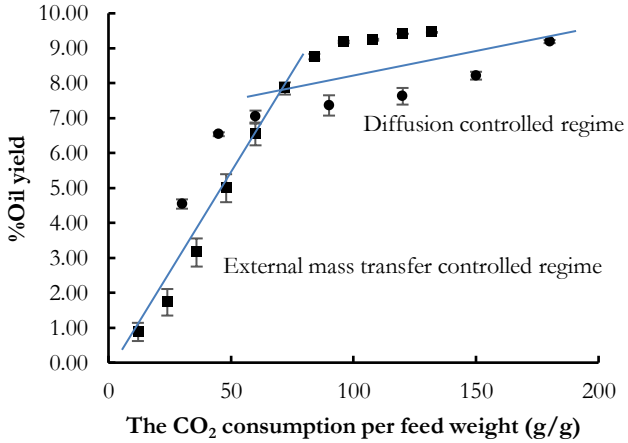

Fig. 4. Percent oil yield as a function of CO_2 consumption divided by sample weight of $SCCO_2$ extraction at 50 °C and 30.0 MPa, (■) S/F = 0.4/min and (●) S/F = 1.0/min.

The oil yield linearly increases with S/F and then becomes constant around 8 % because the solubility of *M. oleifera* has been found to be unique under the same conditions. From the slope of the linear part in Fig. 4, the solubility of *M. oleifera* oil is 4.67 g oil/kg CO_2 at 50 °C and 30.0 MPa. It was reported that the solubility of *M. oleifera* oil in $SCCO_2$ is 3.68 ± 0.03 g oil/kg CO_2 at 60 °C and 30.0 MPa [10]. The solubilities of *M. oleifera* are slightly difference because of many parameters such as the particle size and the sample matrix.

As per Table 1, the %oil yield obtained from this work is determined to be threefold lower than that of the other works because the oil content in *M. oleifera* seed is observed to be reduced by the screw press machine. The extraction duration of this work was lowered as well. The aim to reduce mass transfer limitation during the $SCCO_2$ extraction process was attempted successfully. Increasing the pressure to 60.0 MPa was determined to improve the efficiency of $SCCO_2$ extraction process compared to the $SCCO_2$ extraction at 30.0 MPa. Further study on the economic analysis of the $SCCO_2$ extraction at 30.0 MPa and 60.0 MPa should be conducted to evaluate the viability of production process.

3.4. Antioxidant activity of *M. oleifera* seed oil

DPPH and NO activities, and α-tocopherol content of *M. oleifera* seed oil obtained from Soxhlet and $SCCO_2$ extractors are listed in Table 4.

Table 4. Percent inhibition and α-tocopherol content of *M. oleifera* oil obtained from seed cake under various conditions.

Temperature (°C)	Pressure (MPa)	%Inhibition		α-tocopherol (mg/kg oil)	%Oil yield
		DPPH	NO		
Soxhlet extraction		90.84 ± 0.09 [a]	37.22 ± 0.29 [c]	190.11 ± 8.45 [e]	13.14 ± 0.84 [h]
50	30	80.47 ± 0.09 [b]	35.87 ± 0.11 [d]	148.88 ± 2.91 [f]	9.45 ± 0.44 [i]
50	40	89.25 ± 0.17 [a]	32.22 ± 0.14 [e]	169.97 ± 9.79 [g]	9.81 ± 1.22 [i]
50	60	88.94 ± 0.09 [a]	35.11 ±1.11 [d]	171.88 ± 6.03 [g]	9.56 ± 0.54 [i]

Significant difference between the values with different alphabets at the 5 % confidence level

The DPPH and NO activities of *M. oleifera* seed oil obtained from Soxhlet and mechanical extractions were higher than that of $SCCO_2$ extraction because petroleum ether could extract more compounds than $SCCO_2$ did. However, the trace amount of petroleum ether could contaminate in the extracted oil and interfere the activity testing. Although the %oil yield of Soxhlet extraction was significantly higher than that of $SCCO_2$ extraction, the extracted oil was not appropriate for food and pharmaceutical industries.

For the $SCCO_2$ extraction, the sample obtained from 30.0 MPa had the lowest DPPH activity that conformed with the α-tocopherol content. The samples extracted at 40.0 MPa and 60.0 MPa had insignificantly different in terms of DPPH activity, α-tocopherol content, and %oil yield. Nonetheless, the %oil yield of the $SCCO_2$ extractions at 40.0 MPa and 60.0 MPa were 9.49% and 7.89%, respectively (Fig. 3).

The antioxidant activities are reportedly related to multiple bioactive compounds in the extracted oil, and not only to α-tocopherol. It was reported that other forms of tocopherol such as δ- and γ-tocopherol were found in cold-pressed and solvent-extracted *M. oleifera* seed oils [2]. The δ- and γ-tocopherol content in *Moringa* spp. reported in the literature was in the range of 3.55 mg/kg oil to 108.01 mg/kg oil and 50.5 mg/kg oil to 161.30 mg/kg oil, respectively [1].

Table 5. Fatty acid profiles of *M. oleifera* seed oil obtained under various conditions.

Press. (MPa)		Fatty acid (%w/w)						
		C16:0	C16:1	C18:0	C18:1	C20:0	C20:1	C22:0
Soxhlet		6.01±0.71	0.93±0.07	2.01±0.37	81.59±0.43	1.87±1.07	3.52±0.47	4.06±0.13
30	50 °C	6.78±0.46	0.98±0.84	1.99±0.49	82.92±1.79	1.18±0.62	1.86±0.60	3.91±0.08
40	50 °C	7.24±0.25	1.58±0.46	2.11±0.36	81.88±1.72	2.54±0.11	2.49±0.30	2.55±0.56
60	50 °C	7.66±1.09	1.96±0.31	2.27±0.95	82.71±1.07	2.11±0.29	3.57±0.93	3.17±0.07
Overall mean		7.18±0.15	1.62±0.16	2.09±0.16	81.83±0.29	1.97±0.17	1.74±0.15	3.75±0.18

3.5. Fatty Acid Profiles of *M. oleifera* Seed Oil

Table 5 shows the fatty acid profiles of extracted oil seed. The major fatty acid, >80 %wt. was oleic acid (C18:1), was similar to the previous literatures [1, 2, 12, 13]. In this study, extraction pressure and temperature have somewhat affected the fatty acid profiles of *M. oleifera* seed oil. The fatty acid compositions were not significantly different from the overall mean and the averaged value of all experiments at 95 % confidence interval. However, increasing the pressure enhanced the extraction of unsaturated fatty acids [12, 13]. The fatty acid profiles of *M. oleifera* seed oil obtained from Soxhlet and $SCCO_2$ extractions were similar.

4. Conclusion

$SCCO_2$ extraction of *M. oleifera* seed cake was successfully performed herein. Approximately 90 %wt. of oil content in the seed cake could be removed at 30.0 MPa and 50 °C, and after an extraction time of 200 min. When the extraction was performed at 60.0 MPa and 50 °C, the extraction time was reduced to 100 min. The oil-free seed has been determined to have high protein content up to 25%wt. that would be useful as feedstock to produce protein hydrolysate.

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