Effect of Roasting and Vacuum Microwave Treatments on Physicochemical and Antioxidant Properties of Oil Extracted from Black Sesame Seeds

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Abstract
Unroasted, roasted (at roasting temperatures of 100, 150 and 200°C and roasting times of 10, 20 and 30 min) and vacuum microwaved (at microwave watt powers of 800, 1440, 2400 and 3600 watts/kg black sesame seeds, for heating times of 10, 20 and 30 min) black sesame seeds were processed to extract oil using a single screw press at a constant pressing temperature of 50°C. The results revealed that different heat pre-treatments significantly affected yield and physiochemical and antioxidant properties of extracted oils. The extracted oil samples exhibited significantly different levels of total phenolic compounds, sesamin, sesamolin, and DPPH• and ABTS•+ scavenging activity. Additionally, it was found that these values of roasted and vacuum microwaved black sesame seed oils were significantly higher than those of unroasted oil. Sesamin, sesamolin, total content of phenolic compounds, and DPPH• and ABTS•+ scavenging activity of extracted black sesame oils increased when the roasting temperature and watt power increased. Black sesame oil obtained from unroasted, roasted and vacuum microwaved dried black sesame seeds contained linoleic and oleic acids as major fatty acids. Black sesame oil extracted from roasting and vacuum microwave treatments for 10 min at higher roasting temperature and microwave watt power had higher total phenolic content leading to a reduction of peroxide value and elevated stability of soybean oil when it was added during storage time at temperature of 65°C.

Keywords
Black Sesame Seeds; Roasting; Sesamin; Sesamolin; Vacuum Microwave.
Introduction

Black sesame seeds (*Sesamum indicum* L.) are an economically important oilseed crop and are also extensively planted in different parts of the world.\(^1,2\) Black sesame seeds are primarily found in China and other East Asian countries.\(^3\) Black sesame seeds contain more functional components than white and brown sesame seeds and are widely used for edible oil manufacturing and for the preparation of paste, cake or flour confectionary.\(^3,4\) Back sesame seeds may be extensively used because they have unique physiological and chemical properties; for example, antioxidant and antimutagenic activities, superior oxidative stability and savoury roasted flavour.\(^2,3,5\) Makinde and Akinoso\(^6\) reported that the black seeds contain fat (45.6%), crude protein (23.64%), carbohydrates (10.83%), crude fibre (7.15%), ash (7.34%) and moisture (5.41%). Moreover, the black sesame seeds contain calcium (521.88 mg/100 g), potassium (468.83 mg/100 g), phosphorus (482.82 mg/100 g), magnesium (380.60 mg/100 g), zinc (7.90 mg/100 g), manganese (6.22 mg/100 g) and iron (5.54 mg/100 g).\(^6\) Moreover, black sesame seeds contain unsaturated fatty acids, for example, oleic acid (56.88%), linoleic acid (50.73%), palmitic acid (21.97%), stearic acid (5.47%), arachidic acid (0.75%), α-linolenic acid (0.70%), palmitoleic acid (0.42%), and eicosenoic acid (0.38%).\(^7\) Black sesame seeds have a high excellent antioxidant activity. This is attributed the high oxidative stability of black sesame oil due to its natural antioxidants; for example, such as sesamin, sesamolin, sesamol and tocopherols.\(^8\)

Mostly, black sesame seeds and their oils are used for cooking to enhance the flavour of food products and beverages. Sesame oil has an important property in being resistant to oxidative degradation. Generally, the conventional method of sesame oil production consists of several procedures as follow: cleaning, roasting, cooking, grinding and pressing, without a refining procedure.\(^9,10\) Roasting results in a change in the chemical composition of sesame seeds and oil. Oil products obtained from roasted sesame seeds is mostly used as cooking oil due to a distinctive flavour and an extension of shelf life.\(^11,12\) Hama\(^10\) revealed that the traditional preparation method for tehina (sesame butter) involves the procedures of dehulling, roasting and grinding. The roasting condition is a significant factor which affects the quality of tehina such as colour, flavour, taste and composition.\(^1\) Additionally, the roasting temperature has affected oil yield, an oxidative stability of sesame oil, antioxidant activity and enzyme inactivation. Oil obtained from the roasted seeds had higher resistance to oxidation.\(^13,14,15\)

The microwave process is energy efficient and rapid method for reheating foods.\(^16,17\) Microwaves have a high power to penetrate into food products for cooking. Food products containing a high content of moisture and fat will be quickly and easily cooked or baked in microwave equipment. In recent years, the use of microwave processing for food preparation and food processing has received increasing interest because of its convenience and speed for preheating, including the heating and roasting of sesame seeds before the oil extraction process. Additionally, the microwave pretreatment before oil seed extraction by pressing could enhance chemical properties, antioxidant activity, polyphenol content and oxidative stability of the extracted oil.\(^18,19\)

After roasting or the microwave treatment process, black sesame oil is usually extracted by using a screw press machine that is widely used for sesame oil extraction. Despite the fact that the roasting and microwave treatments might have deleterious effects on oil quality of oilseeds reported by several researchers; these treatments before oil extraction could be used to increase phenolic contents, fatty acids, antioxidant activity and oxidative stability of the extracted oils. Therefore, the objective of this research was to determine the conditions of roasting and vacuum microwave treatments that affect oil yield and physicochemical and antioxidant properties of oil extracted from black sesame seeds, including the application of extracted black sesame oil as an alternative natural antioxidant for enhancing oxidative stability of soybean oil.

Materials and Methods

Samples

Black sesame seeds were purchased at the Royal Project Foundation, Muang District, Royal Park Rajapruek, Chiang Mai, Thailand. Unroasted black sesame seeds (control sample), containing 4.58% moisture, 18.83% protein, 51.54% fat, 16.09% carbohydrate, 4.50% fibre and 3.77% ash, were packed in vacuum sealed plastic bags and stored at temperature of 25°C until use. Soybean oil (Angoon...
Brand, Thai Vegetable Oil Public Co., Ltd., Thailand) obtained from local supermarket was used for comparison.

**Chemicals**

Sesamin, sesamolin, standard gallic acid, 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), boron-trifluoride and HPLC grade acetonitrile were obtained from Sigma-Aldrich Company (St. Louis, Missouri, USA). Trolox and Folin–Ciocalteu reagent were purchased from Merck, Germany. Ascorbic acid, anhydrous sodium carbonate and sodium acetate were obtained from Loba Chemie (India). Potassium chloride was purchased from Ajax Finechem Pty Ltd. (Australia). Chloroform, glacial acetic acid, 37% w/w hydrochloric acid, ethanol, methanol, petroleum ether, sodium chloride, phenolphthalein and Wijs solution were obtained from RCI Labscan Limited (Bangkok, Thailand). Potassium hydroxide, potassium iodide, soluble starch and sodium thiosulphate were purchased from Qrec, New Zealand. In the experiments, all chemicals and reagents used were analytical grade.

**Roasting and Vacuum Microwave Treatments of Black Sesame Seeds**

Approximately 500 g of black sesame seeds were roasted using an electric roaster (Barwell Model SCR-301, Shenzhen Darren Trade Co., Ltd., China) at roasting temperatures of 100, 150 and 200°C for the roasting times of 10, 15 and 20 min at each roasting temperature with constant stirring. For each vacuum microwave treatment, 500 g of black sesame seeds placed in petri dish was roasted in a vacuum microwave dryer at 800, 1440, 2400 and 3600 watts per kg black sesame seeds and vacuum microwave times of 10, 20 and 30 min at each vacuum microwave power level for roasting. Each roasting and vacuum microwave condition was prepared in triplicate.

After roasting and vacuum microwave treatments, black sesame seeds were immediately equilibrated at temperature of 25°C and mixed well prior to oil extraction. Roasted and vacuum microwaved black sesame seeds were kept in sealed polyethylene plastic bags and then stored at temperature of 25°C for oil extraction.

**Oil Extraction by a Single Screw Press Machine**

After roasting and vacuum microwave treatment, thoroughly black sesame oil was obtained by pressing approximately 500 g of unroasted, roasted and vacuum microwaved black sesame seeds with the Model FEA-101ss-M-H-Tc-2015 screw press developed by Friend Energy Limited Partnership, Chiang Mai, Thailand. This screw press has a 26 mm internal diameter of the press barrel and the screw rotation speed used was approximately 24.2 rpm. Each condition of screw press extraction was performed in triplicate. Sesame oil of each of the studied roasting and vacuum microwave conditions was extracted using a single screw press method at a constant pressing temperature of 50°C. The control sample was black sesame seed oil prepared from black sesame seeds without roasting and vacuum microwave treatments.

After the screw press extraction, each crude oil sample obtained was kept in dark bottle, subsequently wrapped with aluminium foil and stored in a chiller at the temperature of 4°C for 48 hr. After 48 hr, the foreign materials in the crude oil were completely settled down. Next, the crude oil samples obtained were centrifuged for 30 min at 5,500 rpm for separation of fine particles in the oil samples, purged with nitrogen gas and finally stored in a freezer (-18°C). The total mass of black sesame seeds was calculated using the total mass balance as follows:

\[
\text{Total mass balance} \% = \frac{\text{summation of mass products}}{\text{mass of black sesame seeds}} \times 100 \quad (1)
\]

where mass products include mass of crude oil and pressed black sesame cake.

The percentage of oil yield was calculated using the equation below.

\[
\text{The percentage of oil yield} \% = \frac{\text{weight of crude oil}}{\text{weight of black sesame seed sample}} \times 100 \quad (2)
\]

**Determination of Acid Value, Free Fatty Acids, Iodine Value, Saponification Value and Peroxide Value**

Acid value and free fatty acids were determined according to the AOAC official standard method 940.28. Iodine value, saponification value and
peroxide value of the crude oil samples were analysed according to the AOAC official methods 920.159, 920.160 and 965.33, respectively.20

Analysis of Fatty Acid Compositions in Extracted Oil

The fatty acid compositions of black sesame oil samples were investigated by gas chromatography according to method of Morrison and Smith21 and AOAC method 996.06.22 Briefly, black sesame oil sample (5 g) was added with sodium hydroxide (0.5 M) in methanol (5 ml). The mixture was added with tridecanoic acid solution (1 ml) used as an internal standard at its concentration of 10 mg/ml. After that, the mixture was refluxed at temperature of 110–120°C for 5 min, and then was cooled down at temperature of 25°C before adding boron trifluoride in methanol (20% w/v) (5 ml) and further refluxing the mixture for 5 min. Afterwards, the mixture was left to cool at temperature of 25°C and added to saturated sodium chloride solution (10 ml). Then, the fatty acid methyl esters (FAMEs) were extracted with hexane (1.5 ml). The mixture was vigorously shaken and then was left to separate into layers. The top hexane layer was filtered by using a 0.45-micron filter and injected into a gas chromatograph (Bruker, Scion 436-GC, Germany) using RT®-2560 column (biscyanopropyl polysiloxane; 100 m length \( \times 0.25 \) mm i.d., 0.2 µm film thickness) (Restek®, USA) and a flame ionisation detector (FID). The oven temperature was initially set at 100°C and maintained for 4 min, subsequently increased from 100 to 230°C at a heating rate of 3°C per min, and then maintained at a constant temperature of 230°C for 15 min. The injector and detector temperatures used were 225 and 250°C, respectively. Nitrogen was used as a carrier gas and maintained at a constant flow rate (1 ml/min). About 1 µl sample was injected at a split ratio of 100:1. The identification of FAMEs in the sample was performed by comparing with Supelco 37 component FAME mix as standard. The relative values of compositions were calculated based on the total peak level by using the device software. The amount of each fatty acid composition in the extracted oil samples was recomputed and shown as grams per 100 g of extracted oil sample.

Determination of Total Content of Phenolic Compounds

Total content of phenolic compounds of the crude oil samples were measured by modifying the methods of George et al.,23 and Houshia et al.24 The oil sample (about 1 g) was dissolved in hexane (1 ml). Then, the extraction of total phenolic compounds was performed using aqueous 80% methanol (2 ml). The mixture was subsequently vortexed for 15 min. After that, the solution was centrifuged for 10 min at a speed of 5,500 rpm, and the methanol layer (2 ml) obtained was pipetted and mixed with 10% Folin–Ciocalteu solution (5 ml) for 5 min, spiked with 20% sodium carbonate (1 ml), vigorously shaken and left in the dark for 1 hr at temperature of 25°C. After the reaction time of 1 hr, the prepared mixture samples were measured their absorption values at a wavelength of 725 nm. The total content of phenolic compounds of mixture samples was calculated by using the standard curve of gallic acid. Results of total content of phenolic compounds are shown as micrograms of gallic acid equivalents per gram of extracted oil sample (µg GAE/g oil).

Determination of Antioxidant Activity by DPPH and ABTS Methods

DPPH method

The extracted black sesame oil samples were evaluated for their DPPH• scavenging activity according to the method of Krzyczkowska and Kozlowska.25 Briefly, Trolox was used as a standard. The black sesame seed oil sample (100 mg) was extracted with methanol (1.5 ml). The oil-methanol mixture obtained was centrifuged for 10 min at 5,500 rpm. Subsequently, methanolic extract or standard (0.5 ml) was added to 1 mM DPPH (0.20 ml) in methanol. The mixture was kept in the dark for 30 min at temperature of 25°C. Next, the absorption value of the prepared mixture sample was measured at 515 nm by using a UV-vis spectrophotometer (Perkin Elmer, UV WINLAB, Germany). DPPH• scavenging activity was computed from Trolox standard curve (at Trolox concentration of 0, 0.78, 1.56, 3.12, 6.24, 12.5, 25, 50, 100, 200 and 400 µM in methanol) and shown as micromole of Trolox equivalents per gram of extracted oil.

ABTS Method

The ABTS radical scavenging activity was analysed according to the method of Chen.26 The ABTS•– solution was produced by reacting 7 mmol/l of ABTS stock solution with 2.45 mmol/l of potassium persulfate at the ratio of 1:1.95 and leaving the mixture to stand in the dark for 16 hr at temperature of 25°C. After 16 hr, the ABTS•– solution was diluted
with 80% methanol to obtain an absorbance of 0.7 ± 0.02 at 734 nm. Trolox was used as a standard. Approximately 100 mg of black sesame seed oil sample was diluted with 1.5 ml of methanol and vigorously shaken. The solution was centrifuged for 10 min at 5,500 rpm. Subsequently, 0.1 ml solution samples were pipetted, mixed with 0.7 ml of diluted ABTS\(^{•+}\) solution and left the solution to react for 6 min. Absorption was measured at 734 nm by using a UV-Vis spectrophotometer (Perkin Elmer, UV WINLAB, Germany). ABTS\(^{•+}\) scavenging activity was computed using Trolox standard curve (at Trolox concentration of 0, 0.78, 1.56, 3.12, 6.24, 12.5, 25, 50, 100, 200 and 400 µM in methanol) and shown as micromole of Trolox equivalents per gram of extracted oil.

**Determination of Sesamin and Sesamolin Content by HPLC**

Sesamin and sesamolin contents in extracted black sesame oil samples were determined by analysis according to the method of Schwertner and Rios.\(^27\)

About 100 mg of each extracted black sesame oil sample was accurately weighed. Then 4 ml of methanol was added to each oil sample. The mixture was vortexed and centrifuged at 4,800 rpm for 2 min. The obtained supernatant was put in a volumetric flask (10 ml) and diluted to volume with methanol. All extracted solutions were filtered by using Nylon filters (Chrom Tech, Apple Valley, MN, USA) having a pore size of 0.45 µm.

Sesamin and sesamolin in the extracted solutions were separated and isolated using a high-performance liquid chromatograph (HPLC) (Agilent 1260, Agilent technologies, USA) equipped with a diode array detector, a temperature-controlled column oven and a binary pump. In this study, a reversed-phase Poroshell 120 EC-C18, particle size 4 µm, HPLC column (250 mm length × 4.6 mm i.d.) was used as stationary phase. The mixture of methanol-deionised water in the ratio of 70:30 v/v was used as mobile phase. Approximately 20 µl of the mobile phase was injected into the column at flow rate of 0.8 ml/min. Sesamin and sesamolin in eluent were monitored at 290 nm and identified by comparison of the retention times with standard compound of sesamin and sesamolin. Standard curves of sesamin and sesamolin were prepared to quantify the peak of sesamin and sesamolin in HPLC. All samples were repeated in triplicate, and the obtained values were calculated as the average result of sesamin or sesamolin content.

**Determination of Oxidative Stability**

The extracted black sesame oil samples obtained were used to analyse their oxidative stability using the modified method of Fazel et al.,\(^28\) and Przybylski et al.,\(^29\) The oil samples were filled in dark brown bottles and kept in an oven at an accelerated storage temperature of 65°C. The extracted oil samples were taken on 0, 3, 7, 14, 21, 28 and 35 days to determine their peroxide values. Afterwards, the oxidative stability of each extracted oil sample was evaluated by its peroxide value.

**Determination of Soybean Oil Stability by Addition of Extracted Black Sesame Oil**

Extracted black sesame oil sample was added to soybean oil at a concentration of 20% w/w based on oil extract weight. BHA and BHT at a level of 85 mg/l were also applied for comparison. Each bottle was completely filled with an oil sample. The extracted black sesame oil samples were stored in an oven for 15 days under accelerated oxidation condition at temperature of 65°C. Samples were removed for analysis periodically every 0, 3, 6, 9, 12 and 15 days. After storage period, oil samples were withdrawn for triplicate analyses immediately.

**Statistical Analysis**

Experimental data are calculated and reported as the mean ± standard deviation for triplicate determinations. The experimental data were analysed using an analysis of variance (ANOVA). The differences in means at the 95% confidence level were compared using Duncan’s new multiple range test. SPSS statistics for Windows version 17.0 was used to analyse the experimental data.

**Results and Discussion**

**Roasting and Vacuum Microwave Treatments Effect on Crude Oil Yield**

Crude oil yield, pressed black sesame cake and total mass balance of oil extraction from unroasted, roasted and vacuum microwaved black sesame seeds using a single screw press at a constant pressing temperature of 50°C are presented in Table 1.
From Table 1, the highest crude oil yield (44.46%) was obtained from unroasted black sesame seeds (used as control sample), whereas the lowest oil yield (5.77%) was achieved at roasting temperature of 200°C for 30 min, and low oil yield (16.73 and 18.97%) was obtained at a wattage of vacuum microwave treatment of 1440 and 3600 watts/kg sample for 30 min and 10 min, respectively. For oil seeds, the roasting and vacuum microwave treatments could generally destroy the structure of the cell membrane in plant tissues, generating permanent pores, allowing oil to move through the permeable cell walls leading to higher content of crude oil.

<table>
<thead>
<tr>
<th>Roasting treatment</th>
<th>Crude oil yield (%)</th>
<th>Pressed cake (%)</th>
<th>Total mass balance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temperature (°C)</strong></td>
<td><strong>time (min)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>10</td>
<td>32.10±3.68bcd</td>
<td>70.30±3.68gh</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>29.64±2.77cde</td>
<td>72.97±2.77gh</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>29.89±2.27cde</td>
<td>73.78±3.09fg</td>
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<tr>
<td>150</td>
<td>10</td>
<td>35.86±2.53bcd</td>
<td>67.70±1.06h</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>30.79±3.36cde</td>
<td>72.64±3.36gh</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>32.99±4.73bcde</td>
<td>74.69±1.76fg</td>
</tr>
<tr>
<td>200</td>
<td>10</td>
<td>16.11±1.31gh</td>
<td>82.91±1.31b</td>
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<tr>
<td></td>
<td>20</td>
<td>13.61±0.91h</td>
<td>83.49±0.91b</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>5.77±1.21i</td>
<td>92.50±1.68a</td>
</tr>
<tr>
<td><strong>Vacuum microwave treatment</strong></td>
<td><strong>Watts/kg sample</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>10</td>
<td>42.30±4.04a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>30.14±1.55cde</td>
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<td></td>
<td>30</td>
<td>27.36±0.69def</td>
</tr>
<tr>
<td>1440</td>
<td>10</td>
<td>28.09±1.11cdef</td>
<td>69.35±3.77gh</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>25.15±1.68def</td>
<td>77.53±2.58cdef</td>
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<tr>
<td></td>
<td>30</td>
<td>16.73±1.14gh</td>
<td>78.89±1.27b</td>
</tr>
<tr>
<td>2400</td>
<td>10</td>
<td>24.24±1.13f</td>
<td>81.68±4.24bd</td>
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<td></td>
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<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>3600</td>
<td>10</td>
<td>18.97±1.69i</td>
<td>76.91±1.11cdef</td>
</tr>
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<td></td>
<td>20</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td><strong>Non-roasting treatment</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>44.46±1.50a</td>
<td>50.63±0.84i</td>
<td>95.09±0.67e</td>
</tr>
</tbody>
</table>

Data are means ± standard deviation. Means in the same column are significantly different at 95% confidential level. nd: not detected.

The vacuum microwave treatment which were evaporated water from the plant structure at low temperature and pressure causes material and membrane decomposition. The oil moved from the cell membrane, causing a higher efficiency of the oil extraction using a screw press. Conversely, it was observed that the crude oil yield decreased with a higher roasting temperature, higher wattage of vacuum microwave and longer roasting and vacuum microwave time. The higher roasting temperature, higher wattage of vacuum microwave and also longer time of roasting and vacuum microwave treatments may result in a creation of excess emulsion in a matrix of black sesame seed samples,
in which the oil cannot pass through the oil outlet, leading to a decrease of the oil yield. The cause of this phenomenon might be lower friction between the surface of black sesame seeds roasted and vacuum microwaved in the oil and black sesame seeds themselves. The occurrence of low friction between black sesame seeds and a screw surface could also lead to lower oil yield. Additionally, the efficiency of oil extraction was reduced when roasting temperature and wattage of the vacuum microwave increased excessively, probably due to a degraded internal structure of black sesame seeds and a closure of degraded seeds at oil outlet.

The results of this study were in accordance with the study of Akinoso et al.,30 and Orhevba et al.,31 who investigated oil extraction from sesame seeds and neem seed kernels, respectively. When the heating temperature was increased, the oil recovery decreased due to reduction of cake plasticity.32 The cake plasticity decreased due to water loss at the higher heating temperature. Kittipoom and Sutasinee33 observed that oil yield was significantly higher as increasing microwave pre-treatment time from 0 to 60 s. Nevertheless, at microwave power of 110 watts, increasing microwave pre-treatment time from 90 s to 150 s had no effect on a significant increase in extracted oil yield. The microwave pre-treatment time was increased to longer than 90 s at a microwave power of 330 watts and to longer than 30 s at a microwave power of 550 watts, but did not show significant differences in the extracted oil yield. In contrast, Azadmard-Damirchi et al.,34 and Uquiche et al.,35 found that increasing the microwave treatment time significantly increased the extracted oil yield obtained from rapeseed and Chilean hazelnut.

Using wattage of a vacuum microwave of 2400 and 3600 watts/kg sample for heating times of 20 and 30 min, The oil could not be extracted under these conditions (as shown in Table 1). This means that at very high wattage for a longer heating time, black sesame seeds and their structure were severely damaged, and also, their appearance was darker. The rise in the burning of oil that occurred subsequently led to a decreased amount of pressed crude oil. In addition, the decrease in crude oil yield might be due to thermal polymerization and carboxylation occurring at higher temperatures or longer times of pre-treatments.36 Therefore, the roasting and vacuum microwave period of 10 min was a suitable condition to produce a high oil yield. Thus, this study selected the extracted oil sample obtained from this period for the following experiments to elucidate their physicochemical properties (acid value, free fatty acids, iodine value, peroxide value and saponification value), which are shown in Table 2. The total content of phenolic compounds, sesamin and sesamolin of the selected oil samples and their DPPH• and ABTS•+ scavenging activity are presented in Table 3.

**Physicochemical Properties of Unroasted, Roasted and Vacuum Microwaved Black Sesame Oil**

Table 2 shows the acid value and free fatty acids of unroasted, roasted and vacuum microwaved black sesame oil samples. With the increase in roasting temperature, the acid value and free fatty acids of black sesame oil samples increased. However, the acid value of roasted black sesame oil was not significantly influenced by an increase in roasting temperature (p>0.05). This result is in accordance with previous works on sesame oils reported by Yoshida.37 The increase of acid value may be due to decomposition of some phospholipids and triacylglycerols to glycerol and free fatty acids. As seen in Table 2, it was noticed that the vacuum microwave treatment did not affect the acid value and free fatty acids. The acid value and free fatty acids of unroasted black sesame oil were higher than those of vacuum microwaved and roasted oils, respectively.

Generally, the iodine value can be used to measure the unsaturation level of oils and fats. During lipid oxidation, free radicals have attracted the double bonds of unsaturated fatty acids, causing the reduced amount of unsaturation fatty acids. The iodine value of extracted oil from unroasted black sesame seeds (control sample) was 97.92 g I₂/100 g of oil. As roasting temperature increased from 100°C to 150°C, the iodine values of the extracted oils were not different and slightly decreased at a roasting temperature of 200°C. The decreases in iodine value might be attributable to a reduction in amount of unsaturation oils due to oxidation, polymerisation or breakage of the long chain fatty acids. The lower iodine value indicates a lower degree of unsaturation of oils.1 From the obtained results as shown in Table 2, it could be also noted that the wattage of
a vacuum microwave had significantly affected the iodine value \((p<0.05)\). The iodine value of vacuum microwaved oils was increased as a result of the possibility of formation unsaturated compounds during increasing wattage of a microwave from 800 to 3600 watts/kg sample.

Table 2: Acid value, free fatty acids, iodine value, peroxide value and saponification value of extracted black sesame oil

<table>
<thead>
<tr>
<th>Roasting treatment</th>
<th>Temperature (^\circ)C</th>
<th>Acid value (mg KOH/g oil)</th>
<th>Free fatty acids (as % oleic acid)</th>
<th>Iodine value (g I(_2)/100 g oil)</th>
<th>Saponification value (mg KOH/g oil)</th>
<th>Peroxide value (meq O(_2)/kg oil)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>1.45±0.05(^c)</td>
<td>0.72±0.02(^c)</td>
<td>98.62±0.00(^abc)</td>
<td>186.14±1.94(^c)</td>
<td>1.46±0.02(^c)</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>1.52±0.10(^c)</td>
<td>0.76±0.05(^c)</td>
<td>98.74±0.16(^abc)</td>
<td>197.35±0.51(^bc)</td>
<td>1.45±0.00(^b)</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>1.60±0.07(^c)</td>
<td>0.80±0.03(^c)</td>
<td>97.84±0.08(^abc)</td>
<td>198.54±0.00(^bc)</td>
<td>2.96±0.01(^a)</td>
</tr>
<tr>
<td>Vacuum microwave treatment</td>
<td>Watts/kg sample</td>
<td>time (min)</td>
<td>Acid value (mg KOH/g oil)</td>
<td>Free fatty acids (as % oleic acid)</td>
<td>Iodine value (g I(_2)/100 g oil)</td>
<td>Saponification value (mg KOH/g oil)</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>10</td>
<td>1.99±0.13(^b)</td>
<td>0.99±0.06(^b)</td>
<td>95.51±0.48(^bc)</td>
<td>200.24±0.87(^a)</td>
</tr>
<tr>
<td></td>
<td>1440</td>
<td>10</td>
<td>2.00±0.00(^a)</td>
<td>1.00±0.00(^a)</td>
<td>99.07±1.12(^bc)</td>
<td>197.07±2.07(^a)</td>
</tr>
<tr>
<td></td>
<td>2400</td>
<td>10</td>
<td>1.99±0.00(^a)</td>
<td>0.99±0.09(^a)</td>
<td>98.85±2.42(^ab)</td>
<td>197.18±1.47(^ab)</td>
</tr>
<tr>
<td></td>
<td>3600</td>
<td>10</td>
<td>2.00±0.01(^a)</td>
<td>1.00±0.00(^a)</td>
<td>101.55±2.71(^a)</td>
<td>197.01±1.97(^ab)</td>
</tr>
<tr>
<td>Non-roasting treatment</td>
<td>2.87±0.06(^a)</td>
<td>1.43±0.03(^a)</td>
<td>97.92±0.57(^abc)</td>
<td>203.17±2.28(^a)</td>
<td>0.93±0.00(^c)</td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± standard deviation. Means in the same column are significantly different at 95\% confidential level.

As shown in Table 2, the saponification value of the unroasted oil sample was 203.17 mg KOH/g oil, while the saponification values of roasted and vacuum microwaved oil samples were in the ranges of 186.14–198.54 mg KOH/g oil and 197.01–200.24 mg KOH/g oil, respectively. It noticed that the saponification value of oil samples increased with increasing roasting temperature. Using higher roasting temperatures, the higher saponification values in oil samples obtained could be attributed to the higher decomposition of long-chain fatty acids to short-chain fatty acids. The results demonstrated that no significant differences \((p>0.05)\) in the saponification values were found among oil samples obtained at wattage of a vacuum microwave of 800, 1440, 2400 and 3600 watts/kg for 10 min. Also, the saponification values of vacuum microwaved oils did not differ significantly \((p>0.05)\) from that of the unroasted oil (control sample) and roasted oils obtained at roasting temperatures of 150 and 200\(^\circ\)C.

In the early step of lipid oxidation, the hydroperoxides are produced. Thus, the degree of lipid oxidation is determined using the peroxide value. In the roasting process, the results eventually confirmed that an increasing trend in the peroxide value was intensified with the roasting temperature. This might be the result of the greater amount of hydroperoxides and/or thermal oxidation of unsaturated fatty acids in black sesame seed oils. During roasting process at the high roasting temperature, the peroxide value was also increased. These results were consistent with those found by Akinoso et al.,\(^{30}\) who concluded that the peroxide value depends on several factors, for example, air exposure, extraction method and fatty acid compositions of oil. The heating temperature also had affected the peroxide value of sesame oil. The peroxide value increased when the heating temperature increased. Yoshida and Takagi\(^{38}\) found that the peroxide value of oil samples extracted from roasted sesame seeds using an electric oven increased when the roasting temperature and time increased. They concluded that the peroxide value of sesame oil samples slightly increased when the sesame seeds were roasted for 25 min. It was observed that the peroxide value increased, which might be the occurrence of lipid oxidation during roasting process. Gao et al.,\(^{39}\) found that when the
walnut kernels were roasted for 10 and 15 min at a roasting temperature of 180°C, the acid values and free fatty acids of walnut oils slightly changed while the peroxide values of oils were significantly increased.

Table 2 illustrates that the peroxide value of extracted oil samples obtained from the vacuum microwave process was 0.94 to 0.96 meq O₂/kg oil. The higher wattage of a vacuum microwave had no effect on the peroxide value of the oil samples. Moreover, the peroxide value of extracted oil samples obtained from the vacuum microwave process was lower than that from the roasting process but not different from the unroasted oil sample ($p \leq 0.05$).

Furthermore, in this study, the peroxide value of extracted oil samples obtained from roasted black sesame seeds at different roasting temperatures, was greater than that obtained from the vacuum microwaved and unroasted black sesame seeds. This result was consistent with that found Özdemir et al. $^{17}$ who reported that the peroxide value of tehina (sesame butter) oil obtained from sesame seeds, which were roasted by the conventional roasting treatment, was greater than that of the roasted seeds by microwave treatment. In this study, it was observed that the roasted black sesame seed oils had considerably higher peroxide value than that of vacuum microwaved and unroasted oils, indicating a higher content of hydroperoxides in roasted oils.

### Table 3: Content of total phenolic compounds, sesamin, sesamolin, and DPPH• and ABTS•+ scavenging activity of extracted black sesame oil

<table>
<thead>
<tr>
<th>Roasting treatment</th>
<th>Temperature (°C)</th>
<th>Roasting time (min)</th>
<th>Content of total phenolic compounds (µg gallic acid equivalent/g oil)</th>
<th>Sesamin (mg/g oil)</th>
<th>Sesamolin (mg/g oil)</th>
<th>DPPH• scavenging activity (µmol Trolox equivalent/g oil)</th>
<th>ABTS•+ scavenging activity (µmol Trolox equivalent/g oil)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>33.21±2.40$^e$</td>
<td>2.81±0.14$^{cd}$</td>
<td>2.38±0.15$^b$</td>
<td>0.26±0.02$^a$</td>
<td>0.04±0.01$^e$</td>
</tr>
<tr>
<td>Vacuum microwave</td>
<td>100</td>
<td>10</td>
<td>196.49±4.30$^c$</td>
<td>3.10±0.03$^{cd}$</td>
<td>2.06±0.06$^{cd}$</td>
<td>0.81±0.03$^c$</td>
<td>0.14±0.01$^h$</td>
</tr>
<tr>
<td>Watts/kg sample</td>
<td></td>
<td></td>
<td>482.73±8.11$^a$</td>
<td>5.49±0.21$^a$</td>
<td>1.34±0.09$^a$</td>
<td>1.16±0.02$^a$</td>
<td>0.27±0.00$^a$</td>
</tr>
<tr>
<td>time (min)</td>
<td>1440</td>
<td>10</td>
<td>35.10±0.97$^e$</td>
<td>3.21±0.17$^b$</td>
<td>2.26±0.13$^{cd}$</td>
<td>0.54±0.05$^e$</td>
<td>0.08±0.01$^e$</td>
</tr>
<tr>
<td></td>
<td>2400</td>
<td>10</td>
<td>35.49±3.70$^b$</td>
<td>4.16±0.07$^b$</td>
<td>2.87±0.00$^a$</td>
<td>0.39±0.03$^{cd}$</td>
<td>0.05±0.01$^d$</td>
</tr>
<tr>
<td></td>
<td>3600</td>
<td>10</td>
<td>52.17±1.60$^d$</td>
<td>2.89±0.28$^{cd}$</td>
<td>1.97±0.22$^d$</td>
<td>0.41±0.0$^d$</td>
<td>0.05±0.01$^d$</td>
</tr>
<tr>
<td>Non-roasting treatment</td>
<td>328.56±8.85$^b$</td>
<td>10</td>
<td>32.56±8.85$^b$</td>
<td>2.64±0.30$^{cd}$</td>
<td>0.85±0.08$^{b}$</td>
<td>1.28±0.06$^b$</td>
<td>0.25±0.00$^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>33.69±1.53$^a$</td>
<td>3.22±0.18$^b$</td>
<td>2.25±0.06$^{bc}$</td>
<td>0.48±0.06$^b$</td>
<td>0.02±0.00$^a$</td>
</tr>
</tbody>
</table>

Data are means ± standard deviation. Means in the same column are significantly different at 95% confidential level.

As illustrated in Table 3, the total phenolic content of roasted and vacuum microwaved black sesame oils was remarkably higher than unroasted black sesame oils. The content of total phenolics in oil samples increased when roasting temperature and wattage of vacuum microwave increased. The increase of phenolic compounds production during roasting and vacuum microwave treatments might be related to the increased formation of Maillard reaction products. $^{15, 40, 41, 42}$ Therefore, the roasting and vacuum microwave treatments could contribute to increase the content of total phenolics in the extracted black sesame oils.

Additionally, Jeong et al. $^{43}$ concluded that natural phenolic compounds in plants that are present in
bound form could be separated and released at a high roasting temperature. However, at a high roasting temperature might cause an adverse effect on the phenolic compound content, this was supported by Jannat et al., who reported that the total content of phenolic compounds, extracted from the roasted black sesame seeds using 50% methanol extraction for extraction of 2 hr, increased significantly when the roasting temperature and time increased until 200°C, and then decreased at roasting temperature of 220°C. The highest amount of total phenolic compounds was approximately 110.66 µmol/mL at roasting temperature of 200°C for 20 min.

The content of sesamin and sesamolin of black sesame seed oil samples obtained from non-roasting, roasting and vacuum microwave treatments was measured and also illustrated in Table 3. The sesamin content was slightly higher than that of sesamolin. Additionally, it was observed that sesamin was not damaged or changed during the roasting and vacuum microwave process. Meanwhile, sesamolin content slightly decreased due to thermal degradation during the roasting and vacuum microwave processes. As seen in Table 3, the results showed that the sesamin and sesamolin content of roasted and vacuum microwaved black sesame oil samples ranged from 2.64 to 5.49 mg/g and 0.85 to 2.87 mg/g oil, respectively. It seems that the sesamin content was higher than the sesamolin content (as shown in Table 3). Rangkadilok et al., reported that the two major lignans of sesame oil from black and white sesame seeds were sesamin and sesamolin. The content of sesamin and sesamolin in black and white sesame seeds was 0.30–0.74 g/kg oil and 0.93–2.89 g/kg, respectively. Additionally, the sesamin content found in both black and white sesame seeds was higher than sesamolin and other glucosides content, respectively.

The DPPH• and ABTS•+ scavenging activity of extracted oil are illustrated in Table 3. The extracted oil obtained from roasted and vacuum microwaved black sesame seeds had higher antioxidant activity than that from unroasted black sesame seeds. The antioxidant activity of extracted oil increased when the roasting temperature and watts of vacuum microwave increased. The high amount of released total phenolics into the extracted oil is a result of the heat damage to oilseed cell. When black sesame seeds received higher thermal energy during the roasting and vacuum microwave processes, sesamolin can be changed into sesamol due to the thermal decomposition. Increasing sesamol as a phenolic compound increased the content of total phenolic compounds and DPPH• and ABTS•+ scavenging activity. Therefore, the antioxidant activity is enhanced due to the increase in content of phenolic compounds. Jannat et al., suggested that the content of phenolic compounds found in Iranian sesame seeds increased significantly (p<0.05) when the roasting temperature and time increased from 180°C to 200°C for 10, 15 and 20 min. However, the total phenolic content decreased when the roasting temperature was higher than 220°C for a roasting time of 20 min. Moreover, Jannat et al., observed that the roasting treatment can increase in the content of γ-tocopherol and polyphenol compounds in the extracted sesame oil samples leading to increase their radical scavenging activities. The studies of Jeong et al., and Jannat et al., reported that the content of phenolic compounds and radical scavenging activity of sesame meal extract increased and also phenolic compounds were found in the sesame meal after roasting sesame seeds at high roasting temperature of 200°C for 60 min.

**Fatty Acid Compositions**

Fatty acid compositions of unroasted (control sample), roasted and vacuum microwaved black sesame seed oils are illustrated in Table 4. The extracted oils obtained from the different pre-treatments had similar fatty acid compositions. The major fatty acid components of the unroasted (control sample), roasted and vacuum microwaved black sesame seed oil samples were oleic and linoleic acids. The low content of palmitic, stearic, and arachidic acids in oil samples was detected. This study revealed that the roasting and vacuum microwave treatments did not lead to a significant difference of fatty acid compositions of black sesame oil resulting in the same fatty acid profile. It was noted, based on the results obtained, that the unroasted, roasted and vacuum microwaved black sesame seed oil samples composed of two major fatty acid components as oleic and linoleic acids, and these were detected in unroasted and roasted white sesame seed oil and brown sesame seed oil. Furthermore, the results showed similarities with those obtained by Ji et al., Yoshida and Yen.
who found no obvious difference in the fatty acid compositions of sesame oil when sesame seeds were treated by a roasting process. Moreover, the results of fatty acid compositions are in accordance with the results of Gharby et al., who reported that the sesame seed oil from Morocco, extracted by using screwless cold presses, contained high content of oleic acid (41.9%) and linoleic acid (42.1%) and the low content of arachidic, linolenic, palmitoleic and stearic acids.

Table 4: Fatty acid compositions of extracted black sesame oil

<table>
<thead>
<tr>
<th>Fatty acid composition (g / 100 g)</th>
<th>Non-roasting treatment/pressing 50°C</th>
<th>Roasting treatment 100°C, 10 min pressing 50°C</th>
<th>Roasting treatment 200°C, 10 min pressing 50°C</th>
<th>Vacuum microwave treatment 800 watts/kg, 10 min pressing 50°C</th>
<th>Vacuum microwave treatment 3600 watts/kg, 10 min pressing 50°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid (C16:0) must</td>
<td>9.03±0.13</td>
<td>9.15±0.03</td>
<td>9.34±0.08</td>
<td>9.22±0.25</td>
<td>9.15±0.16</td>
</tr>
<tr>
<td>Stearic acid (C18:0) m,c</td>
<td>5.19±0.09</td>
<td>5.10±0.04</td>
<td>5.23±0.06</td>
<td>5.17±0.37</td>
<td>5.26±0.07</td>
</tr>
<tr>
<td>Oleic acid (C18:1 n9c) m</td>
<td>41.34±0.68</td>
<td>42.06±0.01</td>
<td>41.94±0.29</td>
<td>41.16±0.24</td>
<td>41.80±0.80</td>
</tr>
<tr>
<td>Linoleic acid (C18:2 n6c) m</td>
<td>43.74±0.80</td>
<td>43.30±0.26</td>
<td>42.96±0.17</td>
<td>43.95±1.41</td>
<td>43.27±0.18</td>
</tr>
<tr>
<td>Arachidic acid (C20:0) m</td>
<td>0.51±0.01</td>
<td>0.49±0.02</td>
<td>0.53±0.07</td>
<td>0.50±0.00</td>
<td>0.52±0.01</td>
</tr>
</tbody>
</table>

Data are means ± standard deviation. Mean in the same row are not significantly different at 95% confidential level. ns: not significant.

It was found that black sesame oil contained valuable fatty acid compositions like in other types of sesame oil and vegetable oils. It also was valuable to note that the fatty acid compositions in sesame seeds could be affected by biochemical and physiological responses among various types of seed origin. Moreover, fatty acid composition data could be used as tool to classify adulteration of sesame oil.

Oxidative Stability of Unroasted, Roasted and Vacuum Microwaved Black Sesame Oil

The oxidative stability of the extracted oils obtained from unroasted (control sample), roasted and vacuum microwaved black sesame seeds are illustrated in Fig. 1.

The peroxide value was used to indicate the amount of primary oxidation products formed in oils. When the storage time increased, a gradual increase in peroxide value of all the samples was noticed (Fig. 1). At all stages, the highest peroxide value was observed for the vacuum microwaved (800 watts/kg sample for 10 min), roasted (200°C for 10 min), vacuum microwaved (3600 watts/kg sample for 10 min) and commercial sesame oil samples, respectively, during storage time. It was revealed that the temperature of roasting treatment and wattage of vacuum microwave treatment increased, the peroxide value of the extracted black sesame oil samples decreased. Although the high amount of heat obtained from roasting and vacuum microwave treatments accelerates the production of some harmful and unwanted compounds; for example oxidation products, unpleasant colour, during the roasting and vacuum microwave treatments of black sesame seeds, it could produce higher phenolics, sesamin and sesamolin, preventing oxidation occurrence which lead to decrease peroxide value. Moreover, the tocopherols and Maillard reaction products, formed during roasting and vacuum microwave treatments, might be used to inhibit lipid oxidation in oil samples due to their high antioxidant activity. Results of our study were found in accordance with Kim who reported that the unroasted sesame oil had lower storage stability than the roasted sesame oil. The highest storage
stability was obtained from roasting temperature of 200°C. Yen\(^47\) reported that the roasted sesame oil had a greater oxidative stability as a result of the formation of some antioxidant compounds such as tocopherols, sesamolin and sesamol.

Additionally, as shown in Fig.1, the decrease in peroxide value observed from 200°C roasting temperature and 3600 watts/kg sample treatments, compared to milder treatments, at 100°C roasting temperature and 3600 watts/kg sample, might be due to the decomposition of hydroperoxides into more stable compounds such as aldehydes and ketones. Under a mild condition of heating treatment, peroxidative oxidation could occur and subsequently continue to increase during storage time, while at more severe conditions, the formation of aldehydes and ketones, led to cause a constant value of peroxide value during storage time.\(^{52, 53, 54}\)

**Soybean Oil Stability by Addition of Extracted Black Sesame Oil**

Compounds produced from severe conditions at 200°C roasting temperature and 3600 watts/kg sample vacuum microwave treatments, as explained in Fig. 1, were considered to be useful as antioxidative compounds. Consequently, the oxidative stability of soybean oil with added roasted (200°C for 10 min) and vacuum microwaved (3600 watts/kg sample for 10 min) oils from black sesame seeds was further evaluated during 15 days of storage at an accelerated temperature of 65°C, as shown in Fig. 2.

As seen in Fig. 2, the peroxide value for all the samples increases in all storage periods. The soybean oil added with roasted and vacuum microwaved oil had a lower peroxide value than soybean oil without the addition of roasted and vacuum microwaved oil from black sesame seeds (control sample) during storage time. Roasted and vacuum microwaved sesame oil at a higher roasting temperature and higher wattage of a vacuum microwave decreased the peroxide value of the soybean oil, which concluded the excellent antioxidant ability in enhancing stability of the soybean oil during storage period.
Meanwhile, the peroxide value of the soybean oil added to the roasted and vacuum microwaved oil from black sesame seeds at mixture ratio of 4:1 w/w was higher when compared to BHT and BHA. However, the present results revealed that black sesame oil samples obtained from a roasting and vacuum microwave treatment at a higher roasting temperature and higher wattage of a vacuum microwave have better antioxidant activity and could be considered a natural antioxidant as a BHT and BHA alternative for stabilising the soybean oil during storage time. Results in this study were similar to that found by Jannat et al.,44 and Gharby et al.,48 who concluded that the sesame seed oil could be used to enhance oxidative resistance or to improve the oxidative stability of other vegetable oils.

Fig. 2: Oxidative stability of soybean oil added with roasted and vacuum microwaved oil from black sesame seeds during 15 days of storage at accelerated temperature of 65°C

Additionally, Gao et al.,39 explained that a roasting treatment for 5, 10 and 15 min at all roasting temperatures of 140, 160 and 180°C could enhance the yield of bioactive compounds (such as polyphenols, tocopherols, squalene and phytosterols), leading to an increase of the oxidative stability and free radical scavenging activity of screw-pressed walnut oils. The screw press method could be used to produce oil from roasted walnut kernels that contain a high amount of bioactive compounds. This was consistent with the results of Vaidya and Choe,50 Jannat et al.,46 and Belcadi-Haloui et al.,56 in their studying of the roasting of oilseeds. The results found that roasting could help to extract amount of antioxidant compounds, leading to a higher stability of vegetable oils.

Conclusions
The roasting and vacuum microwave treatments before using a screw press oil extraction had considerably affected oil yield. The high oil yield of 44.46%, 32.10–35.86% and 42.30% was obtained from the non-roasting, roasting and vacuum microwave treatments, respectively. Roasting and vacuum microwave time and temperature affected the acid value, free fatty acids, iodine value, saponification value and peroxide value of black sesame seed oils. The roasted and vacuum microwaved oil samples obtained from higher roasting temperature and wattage of a vacuum microwave showed higher DPPH and ABTS antioxidant activity due to a greater content of total phenolics, sesamin and sesamolin. This suggests
that the roasting and vacuum microwave treatments are the preferred processing method to achieve better quality of black sesame oil product.

The results indicate that roasting and vacuum microwave treatments can potentially promote oxidative stability of extracted black sesame seed oils. The addition of roasted and vacuum microwaved sesame oil could also improve thermo-oxidative stability of soybean oil over storage period for 15 days under the accelerated storage condition at temperature of 65°C. The extracted black sesame oil could be used as a natural resource of oleic acid (41.16–42.06%) and linoleic acid (42.96–43.95%). black sesame oil was found to be an important and abundant source of several essential nutrients positively affecting human health. The roasting and vacuum microwave processes made it possible to confirm that black sesame oil has a high potential to produce edible oils or as a natural antioxidant for human consumption.

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Conflicts of interest
No conflicts of interest to declare.

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