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Volatile Compounds Fingerprints of Black Cumin (*Nigella sativa* L.) Seed Oil Extracted by Supercritical Carbon Dioxide †

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Abstract: Black cumin (*Nigella sativa* L.) seed oil consists of many volatile oils dissolved in the fixed-oil. In this work, the seed oil samples were obtained from supercritical CO_2 ($SCCO_2$) extraction under various pressures (20.0–30.0 MPa) and temperatures (40–60 °C). The volatile compounds fingerprints of $SCCO_2$ extracted oils were analyzed by the static headspace-gas chromatography (SH-GC-FID) without using any organic solvent. The comparison of volatile compounds fingerprints of $SCCO_2$ and *n*-hexane extracts was compared with direct analysis of milled seed.

Keywords: black cumin; *Nigella sativa* L.; supercritical carbon dioxide; volatile fingerprint; static-headspace gas chromatograph

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1. Introduction

Black cumin (*Nigella sativa* L.) is a small shrub that commonly cultivates in Eastern Europe, Asia, and Middle East. The black cumin seed has been used in food and medical applications for many centuries because it has a strong unique flavor and various herbal pharmacological activities [1]. In this work, we focus on the aroma profiles of the black cumin oil extracted by mechanical or solvent, including supercritical carbon dioxide ($SCCO_2$) extractions.

The black cumin oil consists of 2 parts: the fixed oil and the volatile oil. Indeed, the volatile oil is dissolved in the fixed oil. The hydrodistillation method can extract only the volatile oil from the black cumin seed [2]. The total oil content in black cumin seed was reported in range of 34.5–41.8%wt, while the volatile oil was in range of 0.4–2.8%wt [3]. However, the volatile fingerprint of black cumin seed oil was not clearly reported.

In this work, the static headspace-gas chromatography (SH-GC-FID) was applied to analyze the volatile components in black cumin seed oil from various extraction methods. The SH-GC-FID is capable to examine the volatile compound in the raw seed or the extracted oil without using any organic solvent. It should be noticed that using organic solvent as the dilutant interferes with the low-molecular weight compounds which located at a small retention time (0–3 min) in GC analysis. The effects of temperature and pressure

in $SCCO_2$ extraction on the volatile fingerprint were also investigated. The objective of this work is to discover the effects of extraction method on the volatile fingerprint of black cumin seed oil compared with the virgin oil in the raw black cumin seed.

2. Materials and Methods

2.1. Chemicals and Raw Materials

The solvent used in Soxhlet extraction, *n*-hexane (99.99%) purchased from RCI LABSCAN Co, LTD., Thailand. Carbon dioxide (HiQ CO_2; purity CO_2 >99.99%; impurity H_2O < 10 ppm, O_2 < 10 ppm, and N_2 < 50 ppm) was supplied by Linde Co, Ltd. (Thailand). The black cumin seed was imported from Guangxi Qinzhou province, The People's Republic of China as claimed by a local contributor. The black cumin seed was collected at room temperature in a desiccator before using in the experiments.

2.2. Extraction Methods

Soxhlet extraction was performed in a 500-mL 24/40 glass extractor assisting with the circulating water bath. The sample size and *n*-hexane were 20 g and 200 mL, respectively. The sample was milled by the household blender before it was filled in a cellulose timber and the solvent was heated using an electrical heating mantle. The extraction was conducted at 63 °C for 8 h. The extract phase was put in a rotary evaporator to remove *n*-hexane at 50 °C and 170 mbar. The solvent removal was performed until the drop of solvent did not observe at the condenser, approximately for 2 h.

The mechanical extraction was operated in a household single screw press machine (BEAUTISUN Model LBT01) at room temperature and constant rotating speed of 20 rpm. The seed feed rate was 1 kg/h to control the extraction temperature (less than 50 °C). The exceed feed rate generated the excess heat and led to the plug of the seed cake as well. The extracted oil was decanted overnight at room temperature in an airtight container and was filtered to remove remaining sediment.

The $SCCO_2$ extraction was performed in a 130-mL semi-continuous extractor. The 20 g of milled sample was filled into the extraction tube. The extraction temperature and pressure were in range of 40–60 °C and 200–300 bar, respectively. The carbon dioxide flow rate was kept constant at 10 g/min for all experiments. Further details on the $SCCO_2$ extraction apparatus were given in our previous work [4].

2.3. Static Headspace-Gas Chromatography (SH-GC-FID)

The gas chromatograph (Shimadzu, GC2030) equipped with the static-headspace autosampler (Shimadzu, HS-10) was employed to analyze all samples. Helium (99.995%) was used as carrier gas at constant linear velocity of 40 cm/s. The temperature of sample oven, sampling line, and transfer line were set at 100 °C, 120 °C, and 150 °C, respectively. The injection temperature was 200 °C The 20 mm aluminum crimp capped vial was equilibrated at 100 °C for 10 min and pressurized to 100 kPa for 1 min before injection. The injection volume was 1.00 mL by using a split ratio of 1:10 with 1.00 min of sampling time. The capillary column (DB-1, 0.25 mm ID × 0.25 μm × 30.0 m) was employed for all analysis. The column oven was held constant at 50 °C for 2 min, then heat up at 5 °C/min to 150 °C and held for 2 min. After complete analysis, the column oven was held at 150 °C for 5 min to purge the remaining high molecular weight molecule. The FID temperature was held constant at 280 °C. The flow rate of detector gases, H_2, Air zero, and make-up N_2 was set at 24, 32, and 200 mL/min, respectively. The total analysis time was 30 min per sample.

3. Results and Discussion

3.1. Volatile Figerprints of Raw Seed and Oils Extracted by Mechanical and n-Hexane Extractions

The GC chromatograms of (a) the extracted oil obtained from a screw press machine (Oil_{SM}) and (b) the milled raw seed (Oil_{RS}) are shown in Figure 1. The Oil_{SM} has a similar

composition to Oil_{RS}, whereas the Oil_{RS} has lower concentration of each component than that of Oil_{SM}. Therefore, the volatile components in black cumin seed were concentrated by a screw-press machine without damage to the mid-molecular weight components.

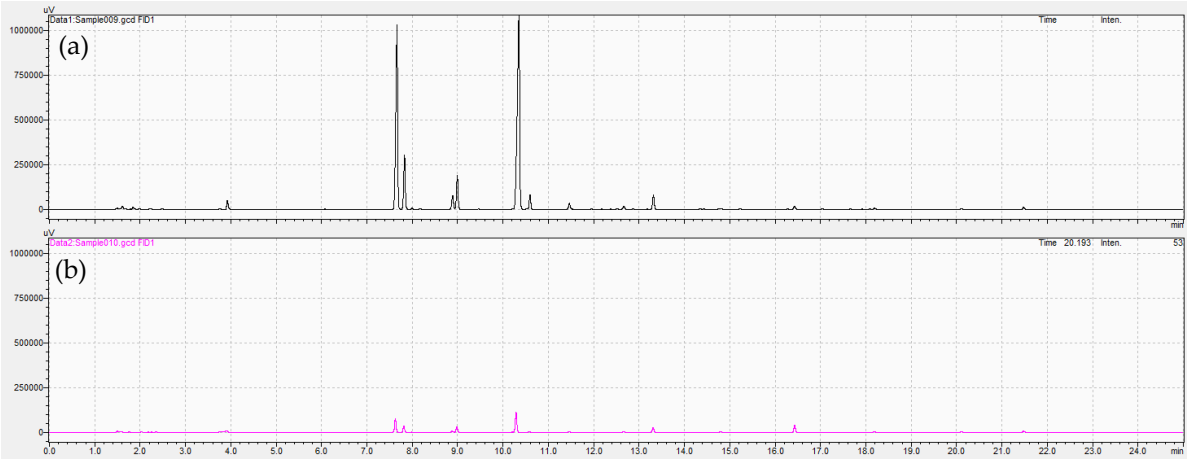

Figure 1. The GC chromatograms of (**a**) the extracted oil obtained from a screw-press machine (Oil_{SM}) and (**b**) the milled raw seed (Oil_{RS}). The y-axis is normalized to the similar maximum intensity of 10.25×10^4 μV.

Figure 2 depicts the chromatograms of (a) n-hexane, (b) Oil obtained from Soxhlet extraction of milled raw seed (Oil_{SE}), and (c) the extracted oil obtained from a screw-press machine (Oil_{SM}). The compositions of Oil_{SE} were considerably different from Oil_{SM}, especially at retention times between 1.00 min to 6.00 min. As illustrated in Figure 2a, the contaminates in the Oil_{SE} are the residual *n*-hexane (Retention time 3.00 min). However, the heavy contaminate in *n*-hexane (Retention time 7.00 min to 9.00 min) did not detect in Oil_{SE}. The major components in black cumin seed oil were detected at retention times of 7.60 min, 7.80 min, 8.90 min, 9.00 min, 10.40 min, 10.60 min, 11.50 min, and 13.25 min. It was reported that the major compositions of essential oil from black cumin seed are *p*-Cymene (60.2%, RI_{exp} = 1022), γ-Terpinene (12.9%, RI_{exp} = 1051), and *trans*-4-Methoxythujane (4.0%, RI_{exp} = 1110). RI_{exp} is experimental retention index given for CP-Sil5 column [5]. Moreover, the concentrations of major components in Oil_{SE} were lower than that in Oil_{SM}. It was hypothesized that the volatile compounds were thermal degraded by the extraction temperature and duration (63 °C and 8 h).

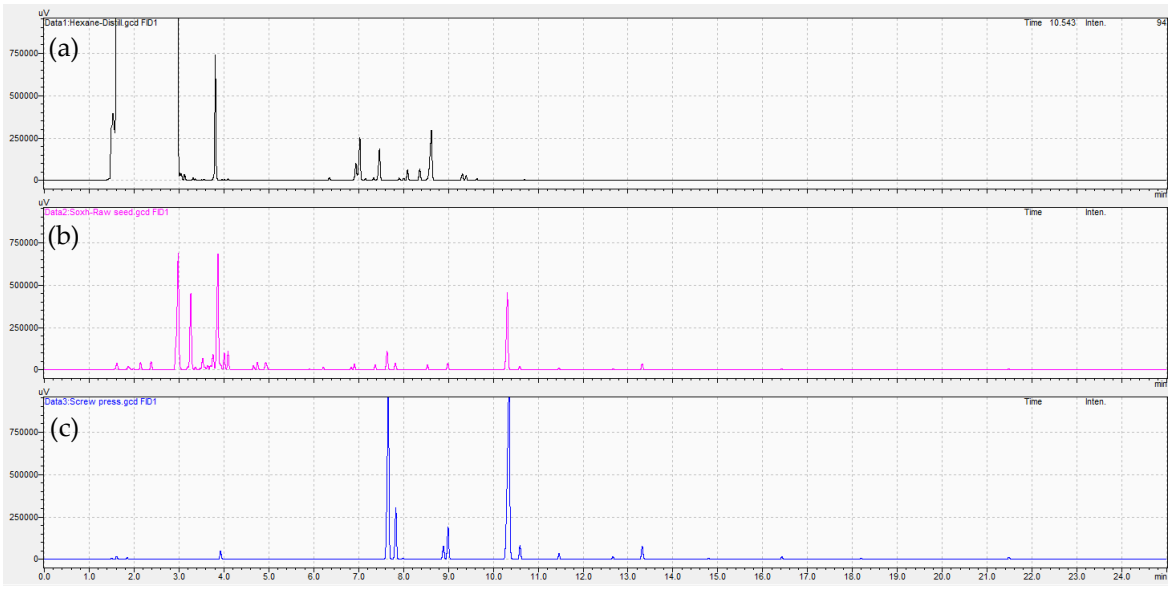

Figure 2. The GC chromatograms of (**a**) *n*-hexane, (**b**) Oil obtained from Soxhlet extraction of milled raw seed (Oil_{SE}), and (**c**) the extracted oil obtained from a screw-press machine (Oil_{SM}) The y-axis is normalized to the similar maximum intensity of 10.0×10^4 μV.

3.2. Volatile Figerprints of Black Cumin Seed Oil Obtained from $SCCO_2$ Extraction

The chromatograms of black cumin seed oil extracted by $SCCO_2$ at 20.0 MPa and 30.0 MPa are revealed in Figures 3 and 4, respectively.

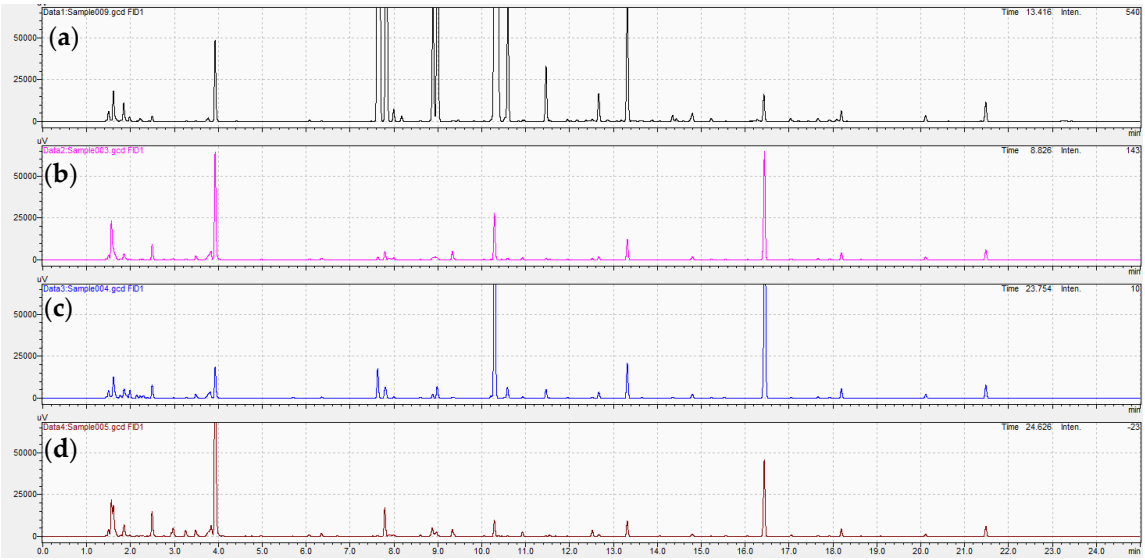

Figure 3. The GC chromatograms of black cumin seed oils obtained from (**a**) screw press machine and $SCCO_2$ extractions at 20.0 MPa (**b**) 40 °C, (**c**) 50 °C, and (**d**) 60 °C. The y-axis is normalized to the similar maximum intensity of 7.5×10^4 μV.

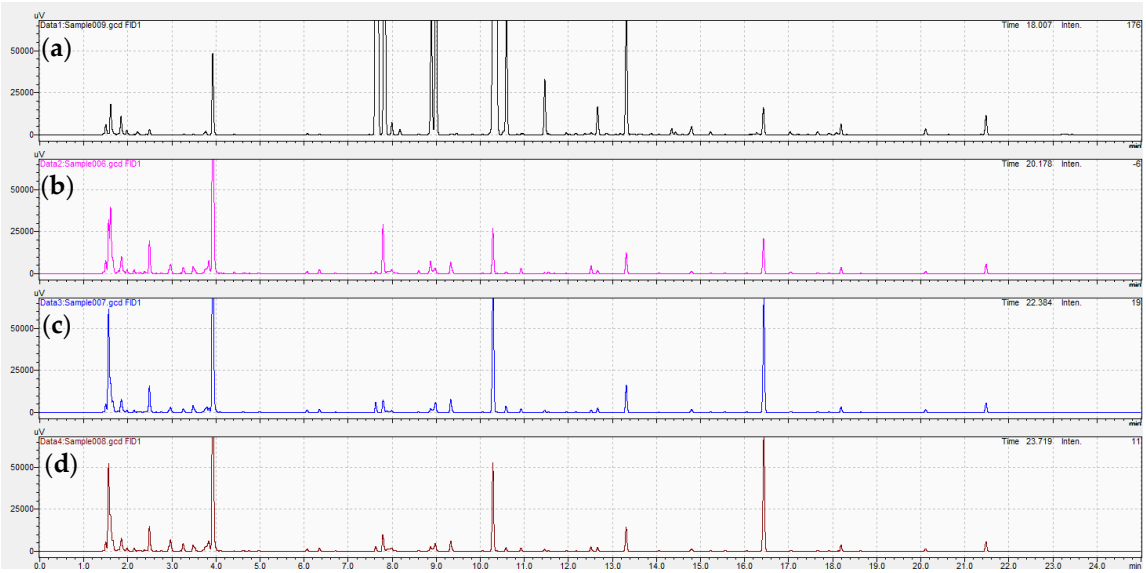

Figure 4. The GC chromatograms of black cumin seed oils obtained from (**a**) screw press machine and $SCCO_2$ extractions at $SCCO_2$ extractions at 30.0 MPa (**b**) 40 °C, (**c**) 50 °C, and (**d**) 60 °C. The y-axis is normalized to the similar maximum intensity of 7.5×10^4 μV.

According to Figures 3 and 4, the volatile compounds fingerprint of black cumin seed oil extracted by $SCCO_2$ depends on the extraction temperature and pressure. It is clear that the volatile fingerprints of $SCCO_2$ extracted oils are noticeably dissimilar to black cumin seed oils (Oil_{RS} and Oil_{SM}) as shown in Section 3.1. The major compounds in the $SCCO_2$ extracted oils were detected at retention times of 3.90 min, 10.40 min 13.25 min, and 16.50 min. It was reported that the major components in $SCCO_2$ extraction are o-Cymene (11.0–

7.6%, RI_{Exp} = 1027), Tymoquinone (86.2–77.2%, RI_{Exp} = 1252), Carvacrol (2.9–5.8%, RI_{Exp} = 1303), and Longifolene (1.9–2.4%, RI_{Exp} = 1402). RI_{exp} is experimental retention index given for HP-5 column [6]. The $SCCO_2$ extracted oil from 20.0 MPa has lower amount of low-boiling point compounds, retention time less than 5.00 min than that of the $SCCO_2$ extracted oil from 30.0 MPa. Those low-boiling point compounds are slightly observed in the Oil_{RS} (see Figure 1b) and Oil_{SM} (see Figure 3a). The compound at retention of 7.80 min was absent in the $SCCO_2$ extraction samples. Furthermore, the $SCCO_2$ extracts high amount of the component at retention time of 16.50 min.

4. Conclusions

The volatile compounds fingerprints of black cumin seed oil were preliminarily identified by SH-GC-FID. The highly volatile compounds that generally overlap with the *n*-hexane were detected at retention time below 3.00 min. The results show that the volatile compound concentration increases by the mechanical extraction. Regardless of the extraction method, the preferable temperature was 50 °C due to the degradation of volatile compounds. The screw press method was suitable to extract the mid-molecular weight compounds, while the $SCCO_2$ extraction was capable to extract the low- and high-molecular weight compounds. The $SCCO_2$ extraction revealed its selectivity on specific compound based on the extraction temperature and pressure. The unknown compounds will be identified by gas chromatograph-mass spectrometer equipped with the static-headspace autosampler (SH-GC-MS) in the further study.

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Conflicts of 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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