



Proceeding Paper

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

Winatta Sakdasri ¹ , Buntita Sakulkittiyut ², Somkiat Ngamprasertsith ^{2,3} and Ruengwit Sawangkeaw ^{4,*}

¹ Program in Food Process Engineering, School of Food Industry, King Mongkut's Institute of Technology Ladkrabang, 1 Chalong Krung 1 Alley, Lad Krabang, Bangkok 10520, Thailand; winatta.sa@kmitl.ac.th

² Fuels Research Center, Department of Chemical Technology, Faculty of Science, Chulalongkorn University, 254 Phayathai Road, Pathumwan, Bangkok 10330, Thailand; planoiiz14180@gmail.com (B.S.); somkiat.n@chula.ac.th (S.N.)

³ Center of Excellence on Petrochemical and Materials Technology, Chulalongkorn University, 254 Phayathai Road, Pathumwan, Bangkok 10330, Thailand

⁴ Research Unit in Bioconversion/Bioseparation for Value-Added Chemical Production, Institute of Biotechnology and Genetic Engineering, Chulalongkorn University, 254 Phayathai Road, Pathumwan, Bangkok 10330, Thailand

* Correspondence: rueangwit.s@chula.ac.th; Tel.: +66-22-18-80-73

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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 CO₂ (SCCO₂) extraction under various pressures (20.0–30.0 MPa) and temperatures (40–60 °C). The volatile compound fingerprints of SCCO₂-extracted oils were analyzed by static headspace-gas chromatography (SH-GC-FID) without using any organic solvent. The comparison of volatile compound fingerprints of SCCO₂ and *n*-hexane extracts was compared with the 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 the 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 means or solvent, including supercritical carbon dioxide (SCCO₂) extractions.

Black cumin oil consists of two 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 to be in the range of 34.5–41.8%wt, while the volatile oil was in the range of 0.4–2.8%wt [3]. However, the volatile fingerprint of black cumin seed oil was not clearly reported.

In this work, static headspace-gas chromatography (SH-GC-FID) was applied to analyze the volatile components in black cumin seed oil from various extraction methods. SH-GC-FID is capable of examining 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 are located at a

small retention time (0–3 min) in GC analysis. The effects of temperature and pressure in SCCO₂ extraction on the volatile fingerprint were also investigated. The objective of this work is to discover the effects of the 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%), was purchased from RCI LABSCAN Co, LTD., Bangkok, Thailand. Carbon dioxide (HiQ CO₂; purity CO₂ > 99.99%; impurity H₂O < 10 ppm, O₂ < 10 ppm, and N₂ < 50 ppm) was supplied by Linde Co, Ltd. (Bangkok, 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 was not observed 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 SCCO₂ 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 SCCO₂ 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 heated 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, H₂, air zero, and make-up N₂ 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 Fingerprints 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.

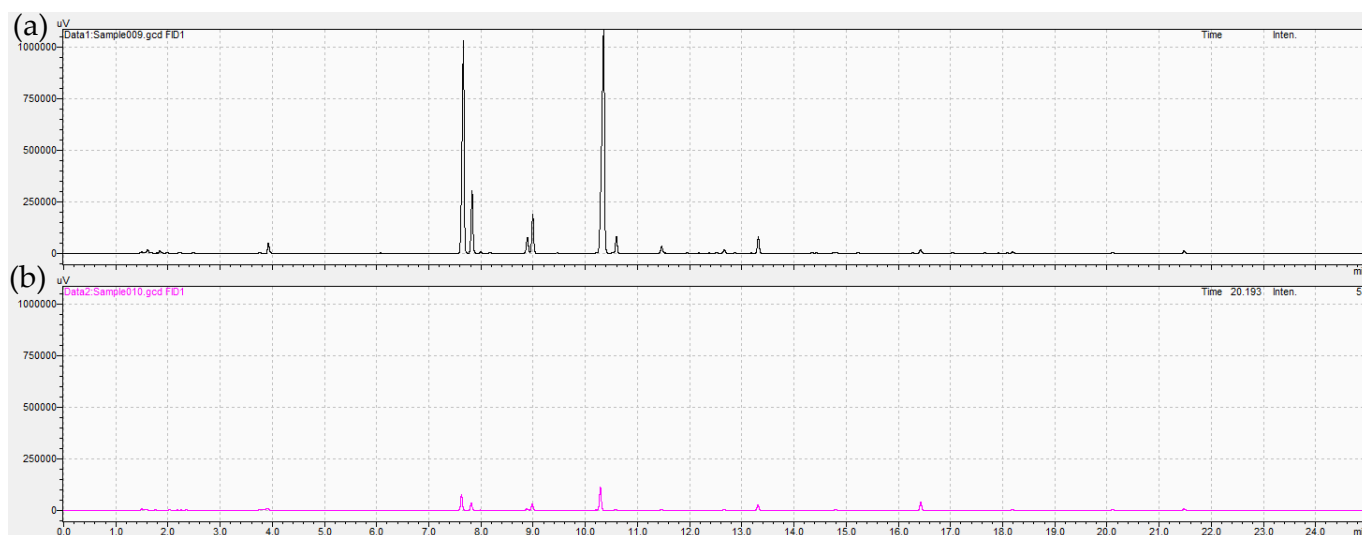


Figure 1. The GC chromatograms of (a) the extracted oil obtained from a screw press machine (Oil_{SM}) and (b) the milled raw seed (Oil_{RS}). The y-axis is normalized to the similar maximum intensity of $10.25 \times 10^4 \mu\text{V}$.

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 contaminants 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) were not detected 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 those in Oil_{SM}. It was hypothesized that the volatile compounds were thermal degraded by the extraction temperature and duration (63 °C and 8 h).

3.2. Volatile Fingerprints of Black Cumin Seed Oil Obtained from SCCO₂ Extraction

The chromatograms of black cumin seed oil extracted by SCCO₂ at 20.0 MPa and 30.0 MPa are revealed in Figures 3 and 4, respectively.

According to Figures 3 and 4, the volatile compound fingerprint of black cumin seed oil extracted by SCCO₂ depends on the extraction temperature and pressure. It is clear that the volatile fingerprints of SCCO₂-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 SCCO₂-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 SCCO₂ extraction are *o*-cymene (11.0–7.6%, RI_{Exp} = 1027), thymoquinone (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 the experimental retention index given for HP-5 column [6]. The SCCO₂-extracted oil from 20.0 MPa has a lower amount of low-boiling-point compounds and a retention time 5.00 min less than that of the SCCO₂-extracted oil from 30.0 MPa. Those low-boiling-point compounds are slightly observable 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 SCCO₂ extraction samples. Furthermore, the SCCO₂ extracted a high amount of the component at a retention time of 16.50 min.



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 $10.0 \times 10^4 \mu V$.

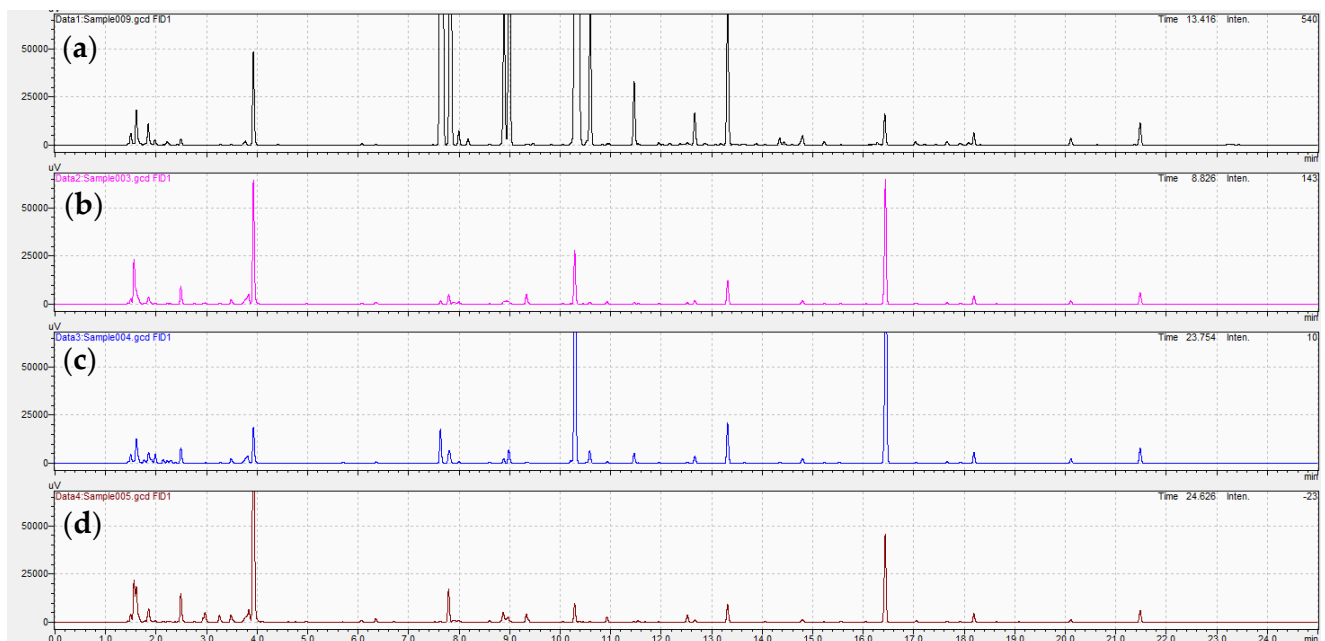


Figure 3. The GC chromatograms of black cumin seed oils obtained from (a) screw press machine and SCCO₂ extractions at 20.0 MPa, (b) 40 °C, (c) 50 °C, and (d) 60 °C. The y-axis is normalized to the similar maximum intensity of $7.5 \times 10^4 \mu V$.

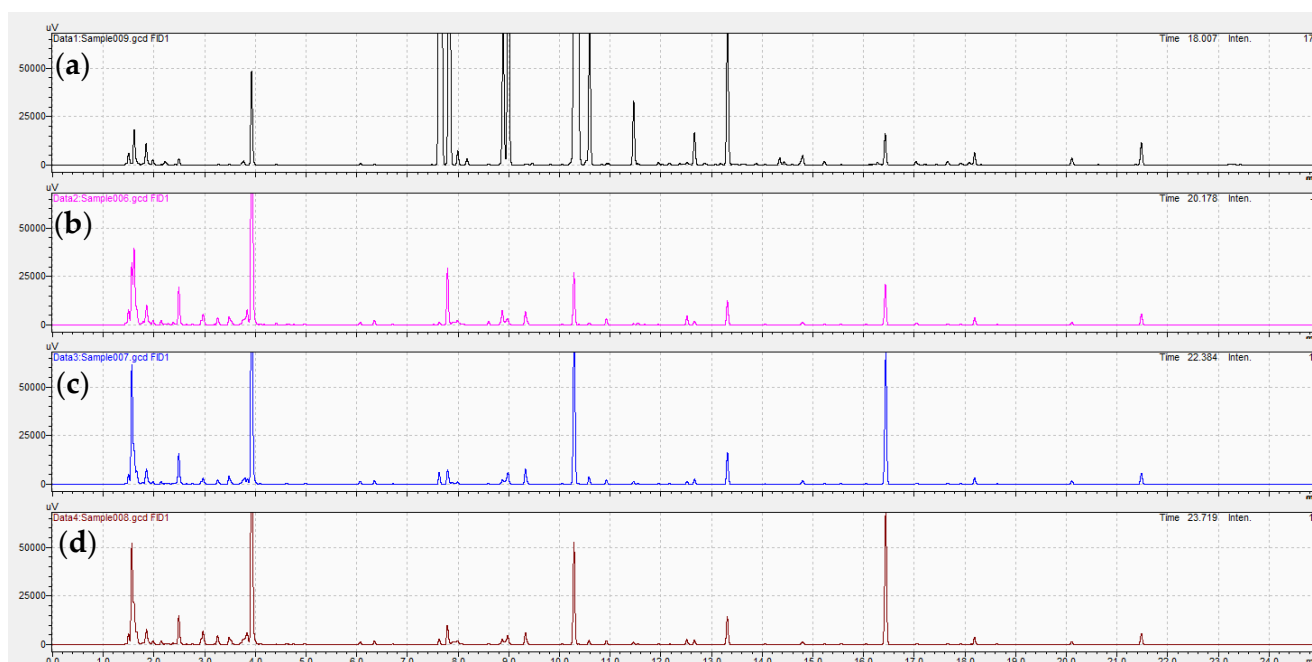


Figure 4. The GC chromatograms of black cumin seed oils obtained from (a) screw press machine and SCCO₂ extractions at 30.0 MPa (b) 40 °C, (c) 50 °C, and (d) 60 °C. The y-axis is normalized to the similar maximum intensity of $7.5 \times 10^4 \mu\text{V}$.

4. Conclusions

The volatile compound 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 a retention time below 3.00 min. The results show that the volatile compound concentration increases with mechanical extraction. Regardless of the extraction method, the preferable temperature was 50 °C due to the degradation of the volatile compounds. The screw press method was suitable to extract the mid-molecular-weight compounds, while the SCCO₂ extraction was capable of extracting the low- and high-molecular-weight compounds. The SCCO₂ extraction revealed its selectivity on specific compounds based on the extraction temperature and pressure. The unknown compounds will be identified by a gas chromatograph mass spectrometer equipped with the static headspace autosampler (SH-GC-MS) in further studies.

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Conflicts of Interest: The authors declare no conflict of interest.

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