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Antioxidant Activity of Extracts of Black Sesame Seed (*Sesamum indicum* L.) by Supercritical Carbon Dioxide Extraction

QIUHUI HU,[†] JUAN XU,[†,‡] SHUBING CHEN,[†] AND FANGMEI YANG*,[†]

Key Laboratory of Food Processing and Quality Control, College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, People's Republic of China, and Department of Biology, Changshu College of Science and Technology, Changshu 215500, People's Republic of China

Antioxidant activities of extracts derived from sesame seed by supercritical carbon dioxide (SC-CO_2) extraction and by *n*-hexane were determined using α,α-diphenyl-β-picylhydrazyl (DPPH) radical scavenging and linoleic acid system methods. The highest extracted yield was given at 35 °C, 40 MPa, and a CO_2 flow rate of 2.5 mL min^{-1} by an orthogonal experiment. The yields of extracts increased with increasing pressure, and yields at 40 and 30 MPa were higher than that by solvent extraction at 46.50%. Results from the linoleic acid system showed that the antioxidant activity follows the order: extract at 35 °C, 20 MPa > BHT > extract at 55 °C, 40 MPa > extract at 55 °C, 30 MPa > Trolox > solvent extraction > α-tocopherol. The SC-CO_2 extracts exhibited significantly higher antioxidant activities comparable to that by *n*-hexane extraction. The extracts at 30 MPa presented the highest antioxidant activities assessed in the DPPH method. At 20 MPa, the EC_{50} increased with temperature, which indicated that the antioxidant activity was decreased in a temperature-dependent manner. The significant differences of antioxidant activities were found between the extracts by SC-CO_2 extraction and *n*-hexane. However, no significant differences were exhibited among the extracts by SC-CO_2 extraction. The vitamin E concentrations were also significantly higher in SC-CO_2 extracts than in *n*-hexane extracts, and its concentrations in extracts corresponded with the antioxidant activity of extracts.

KEYWORDS: Antioxidant activity; sesame; seed; black extracts

INTRODUCTION

Black sesame seed (*Sesamum indicum* L.) is one of the most important oil seed crops. Besides being a source of edible oil, the seed itself serves as a nutritious food for humans in China and other East Asian countries (*1*). A number of lipid souble antioxidants have been isolated from sesame seeds, including sesamin, sesaminol, and sesamolinol (*2*). These compounds present in sesame oil are responsible for many of its unique chemical and physiological effects, such as antioxidant and antimutagenic activities. Lignans are found to reduce the incidence rates of breast and prostate cancer and reduce serum cholesterol levels (*3*, *4*). Sesamol and sesamolin exhibited powerful inhibitory effects on lipid peroxidation of liposomes in rat liver and kidney (*5*). Sesamol was shown to exhibit strong antimutagenic activity in the Ames tester strains TA100 and TA102 (*6*). However, the oil prepared from roasted sesame seeds has a distinctive flavor but has a lower quality (*7*). Microwaving and steaming were used to extract oil from sesame seed (*8*). It was reported that the components, such as Se, Zn, Fe, Mg, sesamin, and vitamin E, were different between the white and the black coat sesame and the mice lipid peroxidation was significantly decreased by black sesame seed rather than white seed (*9*−*11*). Ethanol extracts of black-coated sesame seed provided suppression of growth of cultured malignant cells (*12*). The crude extract of lignan glycosides obtained form unroasted defatted black sesame seeds showed better antioxidant activity (*13*). It is of great interest to explore the active components in black-coated sesame seed including oil, lignan, and other active components.

Supercritical fluid extraction (SFE) is an attractive alternative to conventional liquid extraction due to its use of supercritical fluid without being nontoxic, nonexplosive, and easily removable from products. Furthermore, SFE has been proposed for antioxidants from rosemary leaves, sage, and herbaceous matrixes (*14*−*16*). The antioxidant activity of extracts by SFE was significantly higher than that of extracts by conventional means (*17*, *18*). However, little is reported to prepare natural antioxidant from sesame seed by supercritical carbon dioxide (SC-CO_2). The objective of this study was to investigate the influences of extracting pressure, temperature, and flow rate on

* To whom correspondence should be addressed. Fax: 86-25-4396431. E-mail: qiuhuihu@njau.edu.cn.
[†] Nanjing Agricultural University.
[‡] Changshu College of Science and Technology.

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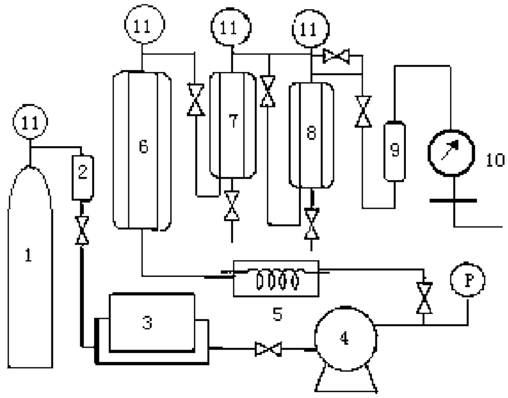

Figure 1. Scheme of supercritical CO_2 extraction: 1, gas cylinder; 2, filter; 3, cooler; 4, compressor; 5, preheater; 6, extractor; 7 and 8, separator; 9, flow meter; 10, pressure cumulative flow meter; and 11, pressure indicator.

the yield of sesame seed extract and the antioxidant activity of extracts by SF-CO_2 and solvent from black sesame seed as compared to α-tocopherol, Trolox, and butylated hydroxytoluene (BHT).

MATERIAL AND METHODS

Reagents and Materials. The chemicals used were as follows: linoleic acid (ca. 99%) (Wako Chemical Industries Ltd., Osaka, Japan); α,α-diphenyl-*β*-picrylhydrazyl (DPPH) (Aldrich Co., Milwaukee, WI); α-tocopherol (Sigma Chemicals Co., St. Louis, MO); and BHT, ammonium thiocyanate, and ascorbic acid (Nanjing Chemical Industry, Nanjing, China). For analytical SFE, CO_2 was 99.99% pure (Taixing, China). *n*-Hexane and other reagents were of analytic grade produced in Nanjing. Black sesame (*Sesamum indicum* L.) seeds were obtained from a local supermarket.

Sample Preparation and Analysis. Extractions were conducted in 1 L stainless steel vessels with a Hua'an (Hua'an Corp., Nantong, China) supercritical fluid extractor. The schematic flow diagram of SFE is shown in **Figure 1**. The major components of the apparatus include a high-pressure extraction vessel and two separator flasks. Flow rates and accumulated gas volumes were controlled with micrometering valves. Pressure was monitored by semiautomatic back pressure valve with an accuracy of ±0.3 MPa in the extractor and ±0.1 MPa in the separators, and the temperature was adjusted by thermostats with an accuracy of ±1 °C. Liquid CO_2 was supplied from a gas cylinder and passed into the inner storage vessel. It was then compressed to a desired pressure by means of the pump and heated to a specified extraction temperature to be brought into supercritical state before it passed into the extraction vessel filled with samples. The oil and other lipophilic substances were extracted. The solution left the extractor and through the pressure reducing valve flowed into the first separator. The nonpolarity product settled to the bottom and was collected and weighed. The remaining solution was passed into the second separator, where the CO_2 was evaporated and the product was recovered. Extracts obtained from two separators were combined together for further antioxidant assay.

Before the extraction, samples of black sesame seed (200 g) with the moisture of 3.15 ± 0.25% were ground into powder and weighed accurately before filled into the extractor vessel. An orthogonal experimental design covering four factors and three levels was employed as in **Table 1**. The extracts were collected for 180 min under the desired conditions and weighed. The extracted yields of crude products and vitamin E were expressed as the ratio of the amounts of SC-CO_2 extracts to the amount of sesame seed powder in the extractor. Vitamin E concentrations in extracts were also determined by the colorimetric method (*19*).

Solvent Extraction (SE) of Sesame Seed. The black sesame seed was ground into a powder by a mortar grinder, and 20 g of sesame powder was extracted three times by *n*-hexane at 5-fold volume of sample in an ultrasonic bath for 60 min at 30 °C. The samples were stirred every 10 min to ensure a well-mixed extraction. The extracts were obtained by removing *n*-hexane in vacuo by a rotator evaporator.

Table 1. Orthogonal Design of SC-CO_2 Extraction L_9 (3^3) and Extracted Yield and Vitamin E Concentration of Black Sesame Extracts

experiment	temp (°C)	pressure (MPa)	CO_2 flow rate (L h^{-1})	extracted yield (%)	vitamin E (μg mL^{-1})
1	35	20	20	44.56	41.11
2	45	20	15	41.73	34.44
3	55	20	25	50.41	28.89
4	35	30	15	51.56	28.44
5	45	30	25	45.88	36.22
6	55	30	20	48.13	33.11
7	35	40	25	51.83	20.44
8	45	40	20	48.88	30.00
9	55	40	15	47.30	46.67
solvent extract				33.31	24.67

Preparations of SFE Extract Standards. Stock solutions of 10 extracts for antioxidant activity were prepared by dissolving measured quantities of extracts in methanol given to the final concentration of 0.01 g extract per mL methanol. BHT, Trolox, and α-tocopherol were used as reference antioxidants at the concentration of 200 μg mL^{-1}.

Assay of DPPH Radical Scavenging Activity. The antioxidant activities of extracts of sesame seed, BHT, α-tocopherol, and Trolox were determined using the DPPH radical method (*20*, *21*). A 0.1 mL amount of above sesame seed extracts, BHT, and α-tocopherol was added to 3.9 mL of 2×10^{-4} mol L^{-1} ethanol solution of DPPH. Absorbance measurements commenced immediately. The decrease in absorbance was determined at 515 nm and continuously at 5 min intervals with a spectrophotometer until the reaction reached a steady state. The percentage of DPPH remaining at the steady state was calculated as a function of the molar ratio of antioxidant to DPPH. The EC_{50} value defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50% was calculated from the results.

Determination of Antioxidant Activity with the Ferric Thiocyanate (FTC) Method. Two milliliters of 0.1 g mL^{-1} extract, 2 mL of 2.51% (w/v) linoleic acid in ethanol, 4 mL of 0.05 mol L^{-1} of phosphate buffer (pH 7.0), and 2 mL of distilled water were mixed in a vial of 10 mL with a screw cap and then kept at 40 °C water bath in the dark. A 0.1 mL amount of the above mixture was added to 9.7 mL of 75% (v/v) ethanol and 0.1 mL of 30% (w/v) ammonium thiocyanate. After 5 min, 0.1 mL of 0.02 mol L^{-1} ferrous chloride in 3.5% (v/v) hydrochloric acid was added to the above mixture and then mixed. The absorbance of mixture was measured at 500 nm every 24 h for 1 week. The FTC method was described in detail by Kikuzaki (*22*).

Statistics Analysis. The data were presented as means ± standard deviations of three determinations. Statistical analyses were performed using Student's *t*-test and one way analysis of variance. Multiple comparisons of means were done by the LSD (least significant difference) test. A probability value of <0.05 was considered significant. All computations were made by employing the statistical software (SPSS, version 11.0).

RESULTS AND DISCUSSIONS

Effect of Extracting Conditions on Extracted Yield of Black Sesame. An orthogonal design [L_9 (3^3)] was performed to optimize the extract temperature, pressure, and CO_2 flow rates. The highest extracted yield was given at 35 °C, 40 MPa, and a CO_2 flow rate of 2.5 mL min^{-1}. The extracting temperature was found to exhibit the principle effect on extracted yield as compared to pressure. The CO_2 flow rate provided ignorable influence on the yield of sesame seed extracts (**Table 1**). The extractions were evaluated in terms of their extract yields and compared with results from *n*-hexane extraction. All of the SFE extractions possessed much higher yields than *n*-hexane

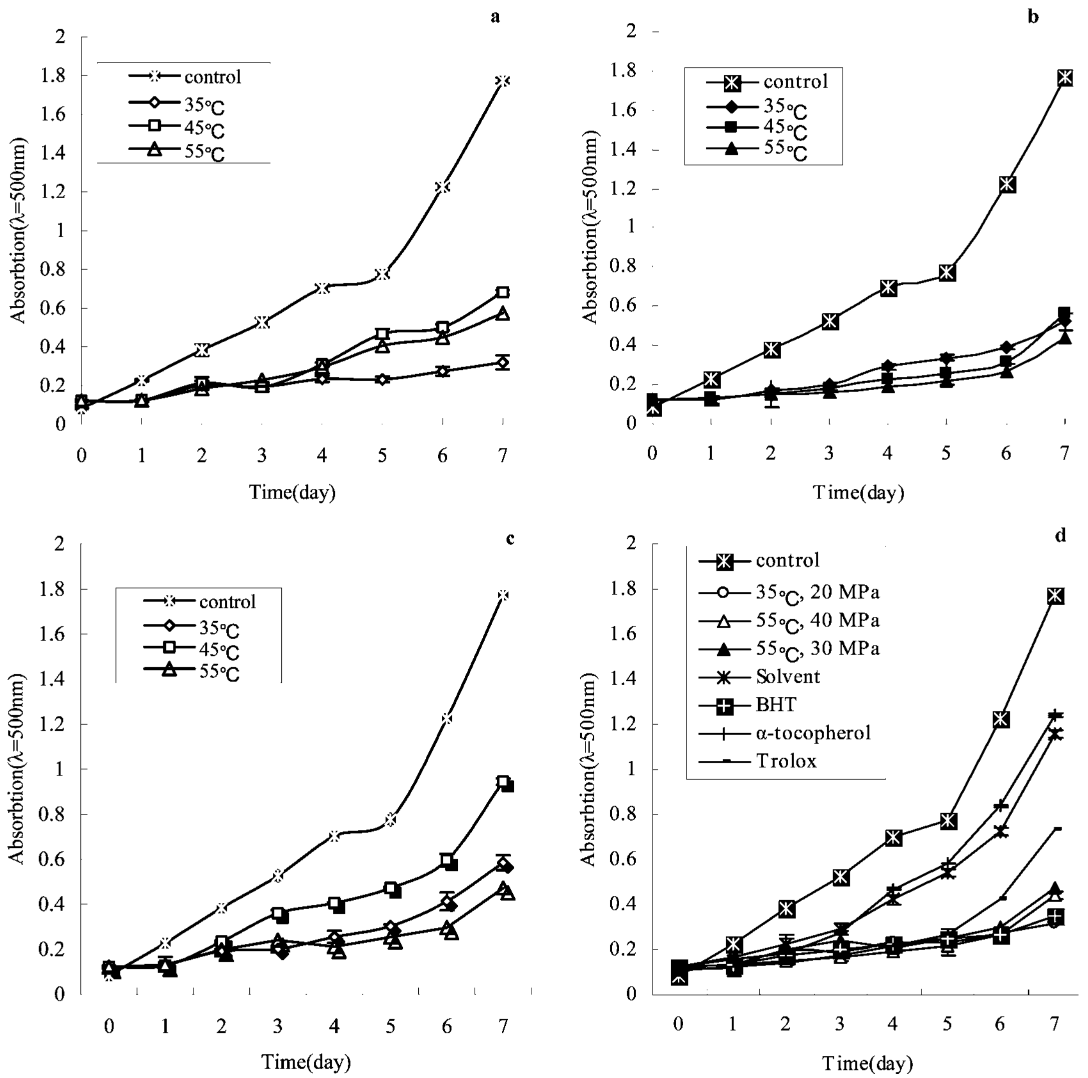

Figure 2. Effect of extraction temperature and pressure on the antioxidant activities of the black sesame extracts with supercritical CO_2 extraction by linoleic acid method and comparisons of the antioxidant activities of black sesame extracts, BHT, Trolox, and α-tocopherol: (**a**) 20, (**b**) 30, and (**c**) 40 MPa.

extract with a yield of 33.31% (**Table 1**). Generally, the yields increased with the increasing pressure (*23*). At 40 MPa, the extraction has a higher average yield than that at 30 or 20 MPa, which indicated that higher pressures led to a higher efficiency in SC-CO_2 extraction due to the enhancement of CO_2 density. However, an increase of temperature at higher pressures led to a lower extraction yield.

This result was in good agreement with the reports that the supercritical carbon dioxide extraction presented a higher yield than Soxhlet extraction and conventional crushing extracts. Cheung showed that SC-CO_2 provided the highest lipid yield and higher concentrations of total and individual ω-3 fatty acids from brown seaweed as compared to that of the Soxhlet method (*24*). The same result was reported in *Forsythia suspensa* that SC-CO_2 gave a higher yield, a shortened extraction time, and the same composition of the extracting components (*25*). However, the SC-CO_2 extraction had an opposite effect on ginseng root hair oil (*26*).

Antioxidant Activity of Extracts by Supercritical Carbon Dioxide Extraction Assessed by Linoleic Acid System. The extract at 35 °C possessed the highest activity against lipid peroxidation while that at 45 °C had the lowest antioxidant activity among three extracts ($P < 0.05$) at 20 MPa (**Figure 2a**). The antioxidant activities of extracts increased with the temperature at 30 MPa. However, no significant differences were found between the antioxidant activity of three extracts ($P < 0.05$) (**Figure 2b**). At 40 MPa, the extract at 55 °C showed the highest antioxidant capacity while the extract at 45 °C gave the weakest lipid inhibition activity. There were significant differences between the antioxidant activities of three extracts ($P < 0.05$) (**Figure 2c**). The extract with the highest antioxidant activity at each pressure was chosen to compare to the reference antioxidants including BHT, Trolox, and α-tocopherol. The antioxidant activity follows the order: extract at 35 °C, 20 MPa > BHT > extract at 55 °C, 40 MPa > extract at 55 °C, 30 MPa > Trolox >SE > α-tocopherol. There was no significant difference of antioxidant activity among extracts at 55 °C, 40 MPa; 55 °C, 30 MPa; and 35 °C, 20 MPa ($P < 0.05$) (**Figure 2d**). The SC-CO_2 extracts showed a significantly higher antioxidant activity than that by SE ($P < 0.05$). SC-CO_2 extracts also presented a significantly higher antioxidant activity than α-tocopherol and Trolox ($P < 0.05$) in the linoleic acid system.

Determination of Antioxidant Activity of SC-CO_2 Extracts by DPPH Radical Scavenging Activity. The radical scavenging activity was expressed with EC_{50}. The lower the value, the stronger the antioxidant activity is. Generally, the EC_{50} of extract at 30 MPa is lower than those at 40 and 20 MPa, showing that extracts at 30 MPa possess the higher antioxidant activity than

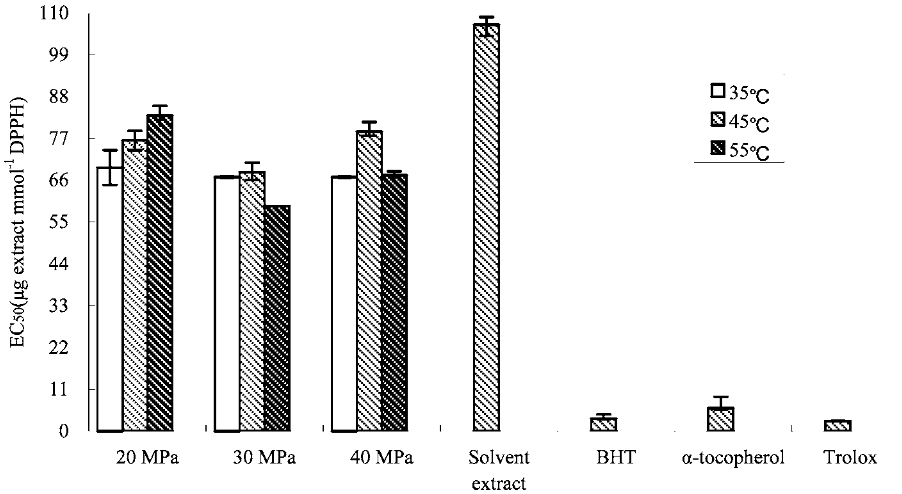

Figure 3. Antioxidant activity of black sesame extracts with supercritical CO_2 extraction as assessed by the DPPH method at 0.01 g mL^{-1} as compared to BHT, α-tocopherol, and Trolox at 200 μg mL^{-1}.

those at 40 and 20 MPa. At 20 MPa, the EC_{50} increased with temperature, which indicated that the antioxidant activity decreased in a temperature-dependent manner (**Figure 3**). The EC_{50} of extract at 35 °C was 69.35 μg mmol $^{-1}$ DPPH, which was lower than that at 45 and 55 °C. However, no marked differences were found between them ($P < 0.05$).

A similar result of antioxidant activity determined by the linoleic acid system was found on the DPPH radical scavenging method at 30 MPa. The extract at 55 °C possessed a lower EC_{50} than that at other two temperatures. At 40 MPa, 35 °C had a lower EC_{50}, which indicated that the extract at 35 °C had a higher radical scavenging activity (**Figure 3**). However, no significant differences were observed among all of the SC-CO_2 extracts ($P < 0.05$). As compared to the extracts by SC-CO_2 extractions, *n*-hexane extract presented significantly lower antioxidant activity ($P < 0.05$). This result was similar to the report that extracts of rosemary leaves by SC-CO_2 exhibited a higher antioxidant activity than that by SE (*27*). The same results were also found on the higher antioxidant activity of *Eucalyptus camaldulensis* var. brevirostris leaf oils extracted by SC-CO_2 than by hydrodistillation (*28*). At low temperature, pressure displayed no apparent effect on the extracts. The temperature exerted the same pattern on antioxidant activity at 45 and 55 °C; SC-CO_2 extracts possessed the highest activities in both cases.

Comparisons were also made between extracts and reference antioxidants. Trolox had significantly higher radical scavenging activities than BHT and α-tocopherol ($P < 0.05$), about 20-fold antioxidant effect than SC-CO_2 extracts and 30-fold than *n*-hexane extract (**Figure 3**). However, in the linoleic acid system, BHT showed a different antioxidant effect from the DPPH method, due to the unequal concentrations of antioxidants to SC-CO_2 extracts. These data indicated that it is more liable to adopt the EC_{50} value as the assessment of the efficiency of antioxidants. Therefore, it is promising to obtain natural antioxidants from black sesame seed.

The concentrations and compositions of antioxidants in the products extracted by different methods or conditions lead to the difference in their antioxidants (*29*). At lower pressure, more saturated fatty acids were extracted whereas when pressure increases, the concentrations of unsaturated fatty acid increase, which lead to higher antioxidant activity (*24*). In addition, SC-CO_2 produces higher concentrations of phenolic compound, such as sesamol, sesamolin, sesaminol, and sesamolinol, which exhibited powerful inhibitory effects on lipid peroxidation of liposomes (*10*, *29*). Vitamin E is also a potent fat soluble antioxidant that inhibits lipid peroxidation in biological membranes, especially α-tocopherol. In this study, vitamin E was also determined to explore the relationships between the concentrations of vitamin E in extracts and their antioxidant activities. The vitamin E concentrations were significantly higher in SC-CO_2 extracts than that in *n*-hexane extract (**Table 1**). At 20 MPa, the vitamin E concentration decreased with the temperature and the extract at 55 °C contained less vitamin E than that at 35 and at 45 °C. At high pressure, the vitamin E concentration increase with the temperature and the extract at 55 °C provided the highest vitamin E concentration. It is of great interest that the concentrations of vitamin E in extracts corresponded with the antioxidant activity of extracts.

Therefore, further studies are needed to clarify the difference in the components of SC-CO_2 extracts and *n*-hexane extract and their contribution to the difference in the antioxidant activity of extracts under different conditions and obtained by different methods.

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