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Antioxidant activity of brown pigment and extracts from black sesame seed (*Sesamum indicum* L.)

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Abstract

The antioxidant activities of brown pigment, extract of *n*-hexane and extract of supercritical carbon dioxide extraction of black sesame seeds were investigated in this study. Kinetics of anti-radical activity showed that the reaction between DPPH· and brown pigment of sesame seed was rapid and reached the steady state in 10 min. Extracts from supercritical carbon dioxide extraction and *n*-hexane extraction reacted with DPPH· slowly and the absorbance became stable after 35 min. However, α-tocopherol and trolox were rapid while the kinetic behaviour of BHA was intermediate. The brown pigment of sesame seed also showed a lower EC_{50} (13.5 $\mu g\,ml^{-1}$) than the other two extracts and α-tocopherol, which was about 7–10 fold of the antioxidant activity of the supercritical carbon dioxide extract and the *n*-hexane extract. The ferric thiocyanate (FTC) method also showed that the brown pigment of sesame seed provided higher inhibition activity against lipid peroxidation at 200 $\mu g\,ml^{-1}$ than did the supercritical carbon dioxide extract an *n*-hexane extract at 1 $mg\,ml^{-1}$. However, the activities of the supercritical carbon dioxide extract and *n*-hexane extracts were significantly higher than that of α-tocopherol. In the linoleic acid system, the brown pigment of black sesame seed showed an equal antioxidant activity to BHA and higher than trolox and α-tocopherol. The results indicated that the brown pigment of sesame seed possessed excellent antioxidant activity.

Keywords: Antioxidant activity; Brown pigment of black sesame seed; Extracts

1. Introduction

Sesame (*Sesamum indicum* L.) is one of the most important oilseed crops (because of its high content of lipid) in the world (Shyu & Hwang, 2002). It is not only a source of edible oil, but also widely used in baked goods and confectionery products (Namiki, 1995). It is also consumed as a nutritious food, beneficial to health in oriental countries. Many studies have been conducted to investigate the health-promoting effect of sesame (Shyu & Hwang, 2002). Budowski (1964) noted that sesame oil was highly stable to oxidation compared with other plant oils. The main sesame lignans, namely sesamin and sesamolin, which are found in sesame oil, possess no antioxidative activity (Kamal-Eldin & Appelqvist, 1994). During sesame oil manufacturing, however, sesamilin can be converted to other lignans, such as sesamol, sesaminol and sesamol dimer (Fukada, Nagata, Osawa, & Namiki, 1986). These components are believed to play an important role in the oxidative stability of sesame oil (Shyu & Hwang, 2002). Sesamol, sesaminol and α-tocopherol could not be responsible for the activity of crude methanol extracts of either unroasted or roasted sesame since they were not found in these extracts (Shyu & Hwang, 2002). It is certain that there

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are other unknown antioxidants present in sesame (Shyu & Hwang, 2002). Lignan glycosides existing mainly in the defatted sesame, are regarded as hydrophilic antioxidants. However, the most abundant lignan glycosides of Burma black sesame, namely sesaminol triglucoside and sesaminol diglycoside showed poor antioxidative activity (DPPH free-radical-scavenging and inhibition of LDL oxidation) (Shyu & Hwang, 2002). It was also reported that water extract (black material) of black sesame seed coat possessed strong antioxidant activity (Nagashima, Fukuda, & Ito, 1999) and that the brown material isolated from the crude lignan glycosides extract showed excellent antioxidant activity, and the brown material is different from the water extract (black material) of black sesame seed coat because of the difference in their retention times by preparative HPLC (Chang, Yen, Huang, & Duh, 2002; Namiki, 1990; Shyu & Hwang, 2002). It is of interest to further investigate the antioxidant activity of brown pigment present in sesame seeds.

The objective of the present research was to illustrate the contribution of brown pigment, *n*-hexane extracts and extracts from supercritical carbon dioxide extraction of black sesame seeds responsible for antioxidant activities assayed by the 2,2-Diphenyl-1-picrylhydrazyl hydrate free radical and inhibiting peroxidation of linoleic acid.

2. Materials and methods

2.1. Materials and reagents

Black sesame (*Sesamum indium* L.) seeds were purchased from Nanjing Weigang Supermarket (Nanjing, China).

Linoleic acid (99%) was purchased from Wako Chemical Pure Chemical Industries, Ltd. (Osaka, Japan). 6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (trolox), α-tocopherol, α,α-diphenyl-β-picrylhydrazyl (DPPH·) and butylated hydroxyanisole (BHA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ammonium thiocyanate and other reagents were of analytic reagent grade.

2.2. Preparation of brown pigment of black sesame seeds

Black sesame seeds (200 g) were thrice extracted for 24 h with 800 ml of 75% (v/v) ethanol at room temperature. After being filtered, the combined filtrates were evaporated under vacuum below 40 °C using a rotary evaporator to a final volume of 5 ml and defatted with three volumes of chloroform. Then the extract was lyophilized into a dry black material (5.935 g), and kept in a freezer for further use.

2.3. Preparation of n-hexane extract of black sesame seed

The black sesame seed (20 g) was extracted three times by *n*-hexane (at fivefold 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 rotary evaporator.

2.4. Preparation of extracts of black sesame seed by supercritical carbon dioxide (SC–CO_2) extraction

Extractions were conducted in one litre stainless steel vessels with a Hua'an (Hua'an Co., Nantong, China) supercritical fluid extractor. Black sesame seed (200 g) were ground into powder and filled into the extractor vessel and extracted at 35 °C, 20 MPa and the CO_2 flow rate of 2.5 ml/min for 3 h. The extracts were collected for determination of antioxidant activity.

2.5. Preparation of antioxidant solutions

Brown pigment, BHA, α-tocopherol and trolox were used at the final concentration of 200 $\mu g\,ml^{-1}$. SC–CO_2 extract and *n*-hexane extract were used at 1 $mg\,ml^{-1}$.

2.6. Determination of antioxidant activity by DPPH· radical-scavenging

The antioxidant activities of brown pigment, *n*-hexane extracts and extracts of supercritical carbon dioxide extraction of black sesame seeds, BHA, α-tocopherol and trolox were determined by using DPPH· (Saint-Cricq de Gaulejac, Provost, & Vivas, 1999; Sānchez-Moreno, Larrauri, & Saura-Calixto, 1999); 0.1 ml of the above sesame seed extracts, BHA, α-tocopherol or trolox were added to 3.9 ml of 2×10^{-4} M 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 steady state. The EC_{50} value, defined as the amount of antioxidant necessary to decrease the initial DPPH· concentration by 50%, was calculated from the results.

2.7. Determination of antioxidant activity by inhibition of peroxidation of linoleic acid method

Two millilitre of brown pigment, *n*-hexane extracts or extract of supercritical carbon dioxide extraction of black sesame seeds, 2 ml of 2.51% (w/v) linoleic acid in ethanol, 4 ml of 0.05 M phosphate buffer (pH 7.0) and 2 ml of distilled water were mixed in a 10 ml vial with a screw cap and then kept in a 40 °C water bath in the dark; 0.1 ml 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 M 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 one week. The ferric thiocyanate (FTC) method was described in detail by Kikuzaki and Nakatani (1993).

2.8. Statistical 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 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).

3. Results and discussion

3.1. Antioxidant activity of brown pigment of ethanolic extracts, n-hexane extracts and extracts from supercritical carbon dioxide extraction of black sesame seeds assessed by DPPH radical-scavenging

The DPPH· radical is considered to be a model of a stable lipophilic radical. A chain reaction in lipophilic radicals was initiated by the lipid autoxidation. Antioxidants react with DPPH·, reducing a number of DPPH· molecules equal to the number of their available hydroxyl groups. Therefore, the absorption at 517 nm was proportional to the amount of residual DPPH·. The kinetic classification, according to the time at the steady state, has been reported as follows: Rapid <5 min, intermediate 5–30 min and slow >30 min (Sānchez-Moreno, Larrauri, & Saura-Calixto, 1998).

Fig. 1 illustrates the kinetic behaviour of various black sesame extract and antioxidants as radical scavengers toward DPPH·, and different kinetics were observed. In our study, all the sesame extracts exhibited significant antiradical activity. The absorbance of SC–CO_2 extract and *n*-hexane extract reached a plateau in 35 min and the reactions were slow. However, reaction of brown pigment of black sesame seed was intermediate according to the evaluation and the reaction reached a steady state in 10 min (Sānchez-Moreno et al., 1998). Three antioxidants reacted rapidly with the DPPH radical and α-tocopherol and trolox reached the steady state in five minute while the absorbance of BHA was stable till 15 min (Fig. 1). This result was in agreement with data reported by Sānchez-Moreno, showing that the kinetic classification of BHA was slow and α-tocopherol was intermediate (Sānchez-Moreno et al., 1998).

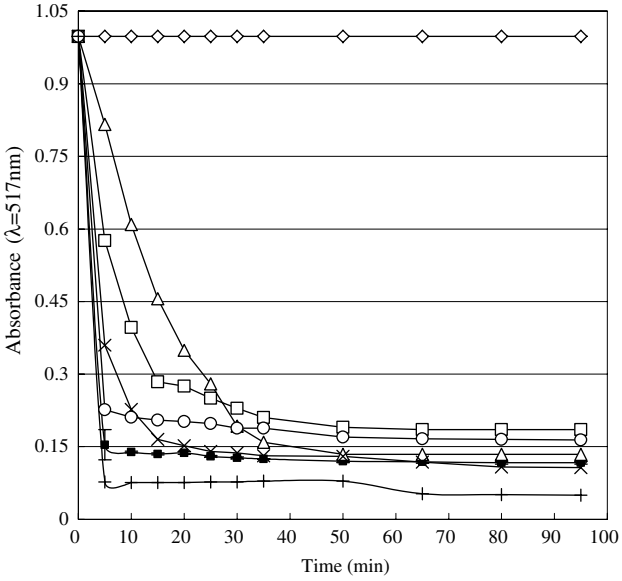

Fig. 1. Antioxidant activities of different black sesame extracts as assessed by DPPH· method compared to BHA, α-tocopherol and trolox. —◇—, Blank; —■—, Brown pigment of black sesame seed; —□—, SC-CO_2 extract; —△—, *n*-hexane extract; —×—, BHA; —○—, α-tocopherol; —+— trolox.

The EC_{50}, meaning the concentration of antioxidant needed to decrease (by 50%) the initial substrate concentration, is a parameter widely used to measure the antiradical efficiency (Kanner, Frankel, Granit, German, & Kinsella, 1994; Vinson, Jang, Dabbagh, Serry, & Cai, 1995). The lower the EC_{50}, the higher is the antioxidant power. The values of various sesame extracts, BHA, α-tocopherol and trolox are compared and shown in Table 1. Based on Table 1, the brown pigment of black sesame seed was the most efficient by the lowest EC_{50} values of 13.3 $\mu g ml^{-1}$ among all the extracts, which is about 7–10 fold that of the SC–CO_2 extract and *n*-hexane extracts. The activity decreased in the order: Ethanolic extract > SC–CO_2 extract > *n*-hexane extract. Compared to reference antioxidants, the brown pigment of black sesame seed provided a lower EC_{50} of 13.3 $\mu g ml^{-1}$ than α-tocopherol (19.7 $\mu g ml^{-1}$) and higher EC_{50} than BHA and trolox. Trolox has the lowest EC_{50} of 5.30 $\mu g ml^{-1}$ among the three antioxidants and BHA was second. This result was in agreement with the previous report that BHA and α-tocopherol had EC_{50} values of 93 g antioxidant kg^{-1} DPPH· and 201 g antioxidant kg^{-1} DPPH·, respectively (Sānchez-Moreno et al., 1998). Shimada reported that the activities of antioxidants correspond to the number of hydrogens available for donation by hydroxyl groups (Shimada, Fujikawa, Yahara, & Nakamura, 1992). It is well known that monophenols are less efficient than polyphenols, but in BHA the methoxy substitution substantially increases the antioxidant power of monophenols.

Table 1
Radical-scavenging activity of black sesame extracts expressed by EC_{50}

Samples	EC_{50} ($\mu g mL^{-1}$)
Brown pigment of black sesame seed	13.3 ± 0.69a*
SC–CO_2 extract	114 ± 3.45b
n-Hexane extract	78.3 ± 4.77c
BHA	12.0 ± 1.01a
α-Tocopherol	19.7 ± 0.99d
Trolox	5.30 ± 0.48e

* Within the same column, means followed by different letters are significantly different at $P < 0.05$.

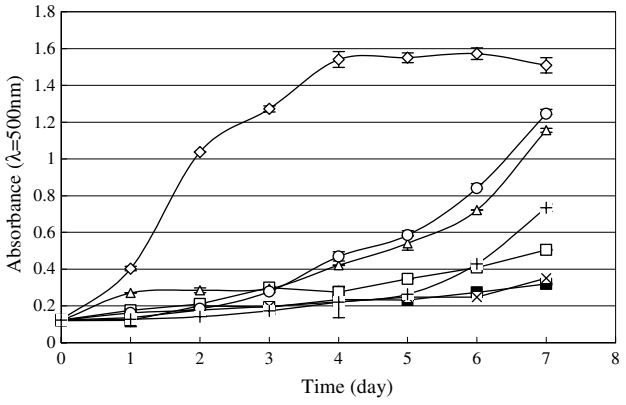

Fig. 2. Antioxidant activities of different black sesame extracts as assessed by linoleic acid compared to BHA, α-tocopherol and trolox. —◇—, Blank; —■—, Brown pigment of black sesame seed; —□—, SC-CO_2 extract; —△—, *n*-Hexane extract; —×—, BHA; —○—, α-tocopherol; —+— trolox.

3.2. Antioxidant activity of brown pigment of ethanolic extracts, n-hexane extracts and extracts from supercritical carbon dioxide extraction of black sesame seeds assessed by inhibition peroxidation of linoleic acid

Fig. 2 displays the inhibitive activity of black sesame extracts and reference antioxidants against linoleic acid peroxidation. All the black sesame extracts showed significantly higher inhibition activities than the control and the antioxidant activity followed the increasing order: α-Tocopherol < *n*-hexane extract < trolox < SC–CO_2 extract < brown pigment = BHA. However, in our study the concentrations of SC–CO_2 extract and *n*-hexane extract were 1 $mg ml^{-1}$, ten fold those of brown pigment and antioxidants. The antioxidant activity of brown pigment of black sesame seed is still higher than those of the other two extracts obtained by SC–CO_2 or *n*-hexane extraction. This result was similar to data from the DPPH radical-scavenging method. However, due to their unequal concentrations to sesame seed extracts and brown pigment, trolox and α-tocopherol exhibited lower inhibition activities than the SC–CO_2 extract.

The black sesame seeds were extracted with 75% ethanol and defatted with chloroform to obtain the brown pigment. Based on the above results, brown pigment of black sesame seed provided about a 7–10 fold higher antioxidant activity than those obtained by SC–CO_2 or *n*-hexane extraction. It seems that the higher antioxidant activity of black sesame may be attributed to brown pigment, which is responsible for its various physiological functions.

In conclusion, the white and black sesame seeds are widely consumed. However, the consumer accepts black sesame seed better than white sesame seed. The brown pigment of ethanolic extracts provided a stronger antioxidant activity than *n*-hexane extracts or extracts of supercritical carbon dioxide extraction from black sesame seeds, assayed by 2,2-diphenyl-1-picrylhydrazyl hydrate free radical and inhibition of peroxidation of linoleic acid. The brown pigment in ethanolic extract plays a prominent role in the antioxidant activity of black sesame seed.

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