Free Radical Scavenging Capability of Various Defatted Sesame Seed Cakes and Hulls Using EPR Compared with In Vitro Testing and HPLC Analysis

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Abstract: The free radical scavenging activities of black and white sesame seed hulls and the powder of black and white sesame seed cakes were investigated using noninvasive continuous wave electron paramagnetic resonance (EPR) and antioxidant assays. With black sesame seed hulls and the powder of black sesame seed cakes, EPR detected the very strong single-line signal intensities that correspond to the stable organic radicals, while the spectrum of the white sesame seed hulls and the white sesame seed cakes showed no signal. The in vitro antioxidant activities of black and white sesame seed cake extract were evaluated by DPPH free radical scavenging activity, hydrogen peroxide scavenging activity, and ferric reducing antioxidant power (FRAP) assay. The results indicated that the extract from black sesame seed cake possessed a greater DPPH radical inhibitory activity and hydrogen peroxide inhibitory activity than white sesame seed cake extract, with IC₅₀ values of 0.847 ± 0.011 mg/mL and 0.338 ± 0.007 mg/mL, respectively. Black sesame seed cake extract also showed a strong reducing power with a FRAP value of 1.307 ± 0.037 mM Fe (II)/g of extract weight and an EC₁₀ value of 0.683 ± 0.002 mg/mL. The main compounds from the black and white sesame seed cake extracts were analysed using high-performance liquid chromatography (HPLC). The results revealed that the main compounds in black and white sesame seed cake extracts were in a group of water-soluble lignans, mainly sesaminol triglucoside and sesaminol diglucoside. However, sesaminol diglucoside was found in large amounts in the black sesame seed cake extract, while it was found in a very small amount in the white sesame seed cake extract. Therefore, these results demonstrated considerable antioxidant capacity of the sesame seed, especially in the black strain.

Key words: sesame hull, sesame seed cake, free radicals scavenging, EPR, HPLC

1 Introduction
Sesame (Sesamum indicum L.) is a valuable oilseed plant that has been used to enhance energy and is widely believed to have anti-ageing properties¹,². Sesame seed contains 45 to 50% of the oil used in various traditional cuisines all over the world. Moreover, sesame oil has been used in the manufacture of pharmaceutical products, cosmetics and toiletries³⁻⁴. Sesame oil consists of triacylglycerols, diacylglycerols and free fatty acids, and they present a rich source of furofuran lignans with a wide range of potential biological activities. The two main groups of lignans in sesame seed are oil-soluble lignans and water-soluble lignans. Oil-soluble lignans, including sesamin, sesamolin, sesaminol and sesamolinol, are the main lignan in sesame oil, while water-soluble (glycosylated) lignans have been found in defatted sesame seed cake⁵. Lignan glycosides isolated from defatted sesame seed cake include sesaminol glucosides, pinoresinol glycosides, and sesaminol glycosides⁶⁻⁷. Defatted sesame seed cake, a by-product of the sesame oil industry, is uninteresting and is commonly

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used as cattle feed in several countries\(^3\). However, numerous groups of antioxidants and bioactive compounds have been found in sesame seed cake, including phenolics, phytates and short-chain peptides\(^1,6,7\). A recent study presented that polar-soluble crude extracts from defatted white and gold sesame seed flour exhibited good antioxidant capacity, which was evaluated by DPPH free radical scavenging activity and oxygen radical absorbing capacity (ORAC)\(^1\).

According to traditional beliefs, black sesame seed is a more valuable health food than brown or white sesame seed. Shahidi \textit{et al.} (2006) examined the total phenolics content (TPC) and the antioxidant activity of extracts from black and white sesame seeds and their hulls. This study found that the extracts from whole black sesame seeds and hulls possessed a higher TPC than the extracts from whole white sesame seeds and hulls. Furthermore, the total antioxidant status, free radical scavenging capacity, and metal chelating capacity were correlated with the TPC\(^1\). Fukuda \textit{et al.} (1991) investigated the antioxidant activities of the extracts from black seeds compared with the extracts from white seeds. All the extracts from black seeds showed stronger antioxidant activity than the extracts from white strains. The black pigment in the hulls was more soluble in distilled water than in ethanol or chloroform\(^6\). However, various antioxidant compounds in the hull have not yet been investigated.

Electron paramagnetic resonance (EPR), also known as electron spin resonance (ESR), is a highly specific and sensitive technique for direct detection of free radicals and other paramagnetic species\(^10\). EPR also identifies a type of radical in the sample that is presented in g-values. The g-values are the constant of proportionality between the frequency and the magnetic field at resonance expressed, and they present a specific characteristic of each type of paramagnetic species\(^11\). The EPR method is useful for applications in medicine, biology, pharmacology, cosmetology, and biotechnology\(^15\). Furthermore, the applications of EPR in nutraceutical and food analysis have enormously increased, because this method can be used to investigate the antioxidant capacity, quality, stability and shelf life of foods\(^14\). Because the unpaired electrons in the structure of antioxidants form a paramagnetic centre, then the antioxidant compounds can be analysed by EPR. Many researchers have used EPR for this purpose\(^7,18\). Several previous studies have used EPR spectroscopy to demonstrate a strong and stable radical signal in foods and beverages (e.g. the antioxidant properties of rice, herbs, cereals, tea, coffee, wine and beer)\(^13,20\).

The aim of the present study is to investigate the EPR spectral characteristics of stable organic radical between defatted black and white sesame seed cake and hull, and compare with \textit{in vitro} antioxidant capacities. The antioxidant capacities were compared with standards. Moreover, the antioxidants compounds present in the black and white defatted sesame seed cake extracted were also characterized using HPLC.

2 Experimental Procedures

2.1 Chemical materials

2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tri[2-pyridyl]-s-triazine (TPTZ), sesamin, sesaminol diglucoside, sesaminol triglucoside, sodium acetate \((\text{C}_2\text{H}_3\text{NaO}_2)\) and trifluoroacetic acid were purchased from Sigma-Aldrich (Schnelldorf, Germany). Ferrous sulphate (heptahydrate) 99\% \((\text{FeSO}_4\cdot7\text{H}_2\text{O})\) and sodium acetate (trihydrate) 99.5\% were purchased from LOBA Chemie (Mumbai, India). Acetonitrile, dimethyl sulphoxide (DMSO), ethanol, hydrogen peroxide \((\text{H}_2\text{O}_2)\) 30\%, iron (III) chloride hexahydrate \((\text{FeCl}_3\cdot6\text{H}_2\text{O})\) and methanol were purchased from Merck (Darmstadt, Germany).

2.2 Plant materials

The seeds of \textit{Sesamum indicum} \(L\). (black sesame) were collected during September to October from the Mae Tang district in Chiang Mai, Thailand. The seeds of \textit{Sesamum indicum} \(L\). (white sesame) were purchased from a local market in Chiang Mai, Thailand. Black and white sesame seed hulls were obtained by removing the endosperm of the sesame seeds and were kept in a moisture-protected place before use. The black and white sesame seed cakes were obtained from cold-press oil processing and were dried in a hot-air oven for 24 hrs at a temperature of 45\(^\circ\)C. The seed cake was ground into a fine powder and was kept in a moisture-protected place before use.

2.3 Plant extraction

The black and white sesame seed cakes were macerated with a 1:1 mixture of ethanol and water 3 times 24 hrs. The solution of extracts was concentrated using a rotary evaporator to remove the organic solvent, then the water was eliminated by spray drying. The concentrated extracts were stored at \(-4\)\(^\circ\)C until use.

2.4 EPR measurements

All the EPR measurements were performed on the black and white sesame seed hulls and the powder of the black and white sesame seed cakes without any further treatment. Each sample, including each seed hull and 0.5 g of each defatted seed cake, was inserted into an EPR tube \((\text{outer diameter (OD)}, 5.0 \text{ mm, inner diameter (ID)} 4.0 \text{ mm})\), Wilmad LabGlass, Vineland, NJ] where each sample was directly measured by microwave radiation. For the EPR measurements, a Bruker EMX plus EPR spectrometer at X-band was used. The system was operated at 9.845 GHz using a 100.0 kHz modulation frequency. All continuous
wave (CW) EPR spectra were obtained in a single scan. The typical CW EPR settings were as follows: microwave power, 2.0 mW; time constant, 0.1 s; sweep time, 2.6 min; magnetic field modulation, 0.32 mT; and magnetic field sweep width, 5–300 mT. All measurements were performed at ambient temperature\textsuperscript{21, 22}.

2.5 Antioxidant activity assays

2.5.1 Determination of antioxidant activity with 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

Five different concentrations of the black and white sesame seed cake extracts were prepared in DMSO (from 0.625 \(\mu\)g/mL to 20.0 \(\mu\)g/mL) and mixed with 167 \(\mu\)M DPPH in methanol. Then, the mixtures of samples were incubated for 30 min. At the endpoint of reaction, the mixtures were measured at 520 nm using a spectrophotometer microplate reader. The percentage of inhibition was calculated by the equation

\[
\% \text{ Inhibition} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100,
\]

Whereas; \(A_{\text{control}}\) is the absorbance of the control

\(A_{\text{sample}}\) is the absorbance of the test sample

The IC\textsubscript{50} (concentration providing 50\% inhibition) was calculated on a calibration curve by plotting the sample concentration and the \% Inhibition.

2.5.2 Determination of antioxidant activity with hydrogen peroxide inhibition assay

For this experiment, a solution of hydrogen peroxide (2.0 mmol/L) was mixed with various concentrations of the black and white sesame seed cake extracts while using a control consisting of the extract solution. After 10 min, the absorbance was determined at 230 nm using a spectrophotometer. The percentage of hydrogen peroxide inhibition was calculated by the equation

\[
\% \text{ Inhibition} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100,
\]

Whereas; \(A_{\text{control}}\) is the absorbance of the control

\(A_{\text{sample}}\) is the absorbance of the test sample

The IC\textsubscript{50} (concentration providing 50\% inhibition) was calculated on a calibration curve by plotting the sample concentration and the \% Inhibition.

2.5.3 Determination of antioxidant activity with ferric reducing antioxidant power (FRAP) assay

FRAP values for the extraction samples of the black and white sesame seed cakes were determined using a calibration curve for standard ferrous sulphate. Briefly, the extracts and the standard were dissolved in 20% Tween 20 in deionised (DI) water and mixed with freshly prepared FRAP reagent. The FRAP reagent was a mixture of acetate buffer, TPTZ solution, FeCl\textsubscript{3} solution and DI water. Then, the mixtures were incubated at room temperature for 5 min. At the endpoint of reaction, absorbance at 593 nm was measured by spectrophotometer. The regression equation from the standard curve was used to calculate the FRAP value of each sample.

2.6 HPLC analysis

The extraction samples of the black and white sesame seed cakes were tested by an analytical HPLC equipped with an UltiMate 3000 Photodiode Array Detector\textsuperscript{23}. This method was modified from Müller et al. (2017). A reversed phase Kinetex\textsuperscript{24} 5 \(\mu\)m C18 100 Å, 150 × 4.6 mm column was used in the experiments and eluted with a mobile phase consisting of 5\% acetonitrile in DI water with 0.1\% trifluoroacetic acid as solvent A and 0.1\% trifluoroacetic acid in acetonitrile as solvent B. The elution conditions were 0–7 min (5\% B), 13 min (18\% B), 31 min (35\% B), 41 min (40\% B), 51 min (80\% B), and 54–55 min (70\% B) at a flow rate of 0.8 mL/min.

2.7 Statistical analysis

All the experiments were performed in triplicate, and the data were presented as mean ± standard deviation (SD). The data were subjected to analysis of variance using ANOVA at a 95\% confidence level (\(p<0.05\)) by SPSS version 17.0.

3 Results and Discussion

3.1 EPR of black and white sesame seed hulls and the powder of black and white sesame seed cakes

The stable radicals of the black and white sesame seed hulls and the powder of black and white sesame seed cakes were evaluated using CW-EPR. Figure 1 exhibits the EPR spectra of (A) black sesame seed hulls and (B) white sesame seed hulls. The spectrum of the black sesame seed hulls (A) presents very strong single-line signal intensities that correspond to the stable organic radicals, while the spectrum of the white sesame seed hulls shows no signals. According to the EPR signal of both sesame seed hulls (as shown in Fig. 1), the robust signal of black sesame seed hulls (A) had a g-value at ≈ 2.00, corresponding with the g-value of the free radical organic compounds found in antioxidant substances\textsuperscript{11, 14, 18, 23}. Sesame seed hull, botanically termed the testa, is the part responsible for the colour of the sesame. It is associated with the biochemical and antioxidant properties because it contains a high proportion of crude fibre, polyphenolic compounds, and oxalic acid. Moreover, Nakagawa and Maeda (2017)\textsuperscript{24} found that the stable organic radicals in each seed were primarily located in the pigmented region of the seed coat, while small amounts of organic radicals were found in the seed cotyledon.

Figure 2 illustrates the EPR spectra of (A) black sesame seed cake and (B) white sesame seed cake. Both black and
white sesame seed cake obtained from cold-press oil processing were inserted into an EPR tube (≈ 0.50 g) for each measurement. The spectra of the black sesame seed cake and the white sesame seed cake corresponded with the EPR spectra of the black and white sesame seed hulls, respectively. The spectrum of black sesame seed cake revealed the strong single line with the g-value at ≈ 2.00, while the spectrum of white sesame seed cake showed an extremely weak signal. The g-value of black sesame seed cake was nearly the same as the g-value of black sesame seed hull, and it was similar to the g-value of free radical organic compounds including carbon-centred radicals such as hydrocarbons with hydroxyl groups, flavo-semiquinone compounds and benzo-semiquinone compounds. In this study, the EPR spectra of the black and white sesame seed hulls and the powder of black and white sesame seed cakes correspond to the CW EPR spectra of various sesame seeds from the previous report by Nakagawa and Hara (2015). Moreover, these EPR signals correlate with those of Shahidi et al. (2006), who determined that defatted sesame extracts and their hulls possess good antioxidant activity. This activity was higher for the black sesame, especially in the hull fraction.

**3.2 Determination of antioxidant activity**

The extracts from the black and white sesame seed cake, and the standards—including ascorbic acid and butylated hydroxytoluene (BHT)—were evaluated for antioxidant activity by the DPPH radical scavenging assay, the hydrogen peroxide (H₂O₂) scavenging assay and the FRAP assay, as shown in Table 1. The IC₅₀ value of black sesame seed cake extract (BS) was 0.847 ± 0.011 mg/mL while the white sesame seed cake extract (WS) exhibited an IC₅₀ value of 2.150 ± 0.130 mg/mL. Among the standard antioxidants, trolox produced the lowest IC₅₀ value of 0.012 ± 0.004 mg/mL, followed by ascorbic acid. In accordance with antioxidant capacity, the DPPH assay is based on both the single electron transfer mechanism (SET), in which the antioxidant compound donates an electron to the free radical and then becomes a radical cation, and the hydrogen atom transfer (HAT) reactions, in which the free radical molecule removes one hydrogen atom from the antioxidant compound and then the antioxidant becomes a radical. The radical scavenging ability of the tested compounds is measured as a relative decrease in the amount of DPPH. The decrease in the DPPH absorption is related to the concentration of radicals that are being scavenged. The antiradical...
activity of each sample was expressed in terms of the IC50 value, which indicates the effectiveness of the compounds in inhibiting 50% of the radical, as shown in Table 1. The scavenging ability of BS was significantly greater than that of WS (p < 0.05). This result was in accordance with various studies of the antioxidant activity of sesame seed extract related to the hydrogen donating ability of its compounds.

Table 1 Free radical scavenging activity of the extracts from black sesame seed cake (BS) and white sesame seed cake (WS) and standards.

| Samples     | DPPH scavenging activity IC50 (mg/mL) | H2O2 scavenging activity IC50 (mg/mL) | FRAP value (mM Fe (II)/g of extract weight) | Reducing power EC1 (mg/mL) |
|-------------|---------------------------------------|---------------------------------------|--------------------------------------------|----------------------------|
| BS          | 0.847 ± 0.011a                         | 0.338 ± 0.007a                         | 1.307 ± 0.037a                             | 0.683 ± 0.002a             |
| WS          | 2.150 ± 0.130b                         | 0.412 ± 0.009b                         | 0.629 ± 0.001b                             | 1.659 ± 0.003b             |
| Gallic acid | 0.012 ± 0.001c                         | ND                                    | 1.340 ± 0.010c                             | 0.081 ± 0.001c             |
| Trolox      | 0.012 ± 0.004d                         | ND                                    | 1.380 ± 0.001d                             | 0.061 ± 0.001d             |

Mean ± standard deviation was used to present the mean results of three determinations. Different letters represent significant differences at the 0.05 level (p < 0.05).

Furthermore, in a biological system, the potent reactive species are the hydroxyl radical (·OH) and hydrogen peroxide (H2O2), which react with polyunsaturated fatty acid moieties of the cell membranes and cause cell damage. H2O2 is not a free radical, but it is considered as a ROS because it can be transformed into other free radicals such as the hydroxyl radical. The ability of plant extracts to inhibit the generation of hydroxyl radicals is related to the electron donor in the antioxidant structure. The antioxidant properties of the sesame seed cake extracts (BS and WS) determined by different mechanisms were correlated with the EPR spectra of black and white sesame seed hulls and the powder of black and white sesame seed cakes as described above.

3.3 HPLC analyses of BS and WS

The major components of BS and WS were evaluated using HPLC with a photodiode array detector. The maximum absorption wavelength of the samples was 290 nm, which usually corresponds to a lignan compound. Figure 3 illustrates the HPLC of BS and WS. The highest peak in both chromatograms was attributed to sesaminol triglucoside, which was identified by comparing the specific retention time and absorption spectrum with those of the authentic standard. The sesaminol triglucoside was quantified by determining the peak areas in the HPLC chromatograms. The amounts of sesaminol triglucoside found in BS and WS were 3482.9 mg/100 g dry weight and 3269.8 mg/100 g dry weight, respectively. The amount of sesaminol triglucoside found in BS was significantly higher than in WS (p < 0.05). The HPLC chromatogram of BS also revealed another major compound that was identified as sesaminol diglucoside. BS contained sesaminol diglucoside at a level of 1928.6 mg/100 g dry weight, while WS possessed...
only 156.04 mg/100 g dry weight. Moreover, BS possessed a small amount of sesamin, while the sesamin peak did not appear in the WS chromatogram. Various studies have suggested that the antioxidant properties of sesame are related to lignan compounds, such as sesamin, sesamolin, sesamol and sesaminol glucoside, that occur in their seeds. However, some studies have suggested that the antioxidant effects of sesame seeds may not correlate only with lignan contents but also with the pigment in their hulls.

According to the HPLC results, the major components found in BS were sesaminol triglucoside, sesaminol diglucoside and sesamin, which are potent antioxidant compounds of both the lignans and glycosylated lignans. WS possessed sesaminol triglucoside and a small amount of sesaminol diglucoside, and sesamin found in BS compared to WS were responsible for its greater radical scavenging ability. They could be identified as stable organic radicals (carbon-centred radicals with a g-value at ~2.00). In the EPR study, BS also demonstrated a stronger signal than WS. Various studies have presented the antioxidant properties of sesame seeds that relate to lignan components, particularly sesamin, sesamol, sesaminol triglucoside and sesaminol diglucoside. These compounds exhibited strong hydrogen-donating capacity, metal-chelating ability and hydroxyl radical scavenging.

Furthermore, Nakagawa et al. (2015) also studied the distribution of stable radicals in sesame seeds which were not treated by any chemicals. They found that a high radical concentration was observed in the hilum region and the black sesame seed coat, whereas they were not found inside the seed. Moreover, the white sesame seed presented a very low-intensity image although they irradiated the white sesame seeds. Thus, the pigmented compounds in the black sesame seed coats could be associated with the stable paramagnetic species. Nevertheless, none of the previous research has exhibited a stable paramagnetic species in the pigments of the black sesame seed coat.

4 Conclusion
In this study, the stable organic radicals related to the antioxidant activity of the black and white sesame seed hulls and the powder of black and white sesame seed cakes which were the waste that obtained from cold-press oil processing were evaluated using the noninvasive EPR technique. The EPR spectra of all the samples were correlated with the antioxidant properties that were investigated in the in vitro study. The extract from the black sesame seed cake presented greater DPPH radical scavenging and hydrogen peroxide inhibition than that of the extract from white sesame seed cake. Furthermore, the black sesame seed cake extract exhibited reducing power comparable with that of the standard gallic acid. The HPLC analysis demonstrated the peak of the sesaminol triglucoside, sesaminol diglucoside and sesamin of the black sesame seed cake extract that was related to its antioxidant ability. The black sesame seed cake extract could be a precious natural source of antioxidants that will be further developed into nutraceutical products for health benefits.

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References
1) Bedigian, D. *Sesame: the genus Sesamum*. CRC
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Press, Boca Raton, United States (2010).

2) Lim, T.K. Sesamum indicum. in Edible medicinal and non-medicinal plants (Lim, T.K. ed.) Vol. 4. Springer, Dordrecht, Netherlands, pp. 187-213 (2012).

3) Othman, S.B.; Katsuno, N.; Kanamuru, Y.; Yabe, T. Water-soluble extracts from defatted sesame seed flour show antioxidant activity in vitro. Food Chem. 175, 306-314 (2015).

4) Xu, J.; Chen, S.; Hu, Q. Antioxidant activity of brown pigment and extracts from black sesame seed (Sesamum indicum L.). Food chem. 91, 79-83 (2005).

5) Sarkis, J.R.; Michel, I.; Tessaro, I.C.; Marczak, L.D.F. Optimization of phenolics extraction from sesame seed cake. Sep. Purif. Technol. 122, 506-514 (2014).

6) Suja, K.P.; Jayalekshmy, A.; Arumughan, C. Antioxidant activity of sesame cake extract. Food Chem. 91, 213-219 (2005).

7) Dar, A.A.; Arumugam, N. Lignans of sesame: purification methods, biological activities and biosynthesis—a review. Bioorg. Chem. 50, 1-10 (2013).

8) Shahidi, F.; Liyana-Pathirana, C.M.; Wall, D.S. Antioxidant activity of white and black sesame seeds and their hull fractions. Food Chem. 99, 478-483 (2006).

9) Fukuda, Y.; Osawa, T.; Kawakishi, S.; Namiki, M. Antioxidative activities of fractions of components of black sesame seeds. Nippon Shokuhin Kogyo Gakkaishi 38, 915-919 (1991).

10) Drouza, C.; Spanou, S.; Keramidas, A.D. EPR Methods Applied on Food Analysis. in Topics From EPR Research (Maghraby, A.M. ed.) IntechOpen, London, United Kingdom, pp. 46-56 (2018).

11) Morsy, M.A.; Khaled, M.M. Novel EPR characterization of the antioxidant activity of tea leaves. Spectrochim. Acta A 58, 1271-1277 (2002).

12) Nakagawa, K. Free Radicals in Nonirradiated and Irradiated Foods Investigated by ESR and 9 GHz ESR Imaging. in Electron Spin Resonance in Food Science (Shukla, K. ed.) Academic Press, London, United Kingdom, pp. 123-136 (2017).

13) Suzen, S.; Gurer-Orhan, H.; Saso, L. Detection of reactive oxygen and nitrogen species by electron paramagnetic resonance (EPR) technique. Molecules 22, E181 (2017).

14) Schaich, K.M. EPR methods for studying free radicals in foods. in Free Radicals in Food Chemistry, Nutrition, and Health Effects (Morello, M.J., Shahidi, F., Ho, C.T. eds.) American Chemical Society, Washington DC, United States, pp 12-34 (2002).

15) Zdybel, M.; Pilawa, B. Application of electron paramagnetic resonance spectroscopy in ophthalmology. in Ophthalmology-Current Clinical and Research Up-