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Effect of Roasting Condition on the Antioxidative Activity of the Methanolic Extract from Defatted Sesame Meal

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ABSTRACT

Sesame seeds are usually roasted prior to expelling oil. The defatted sesame meal contains lignan glycosides in its methanolic extract. The objective of this study was to investigate the antioxidative activity of methanolic extract from defatted black sesame meal roasted at three different temperatures (180, 200, 220°C) for different durations (5-30 min). The antioxidative activities were evaluated by the 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) radical scavenging ability and the inhibition of Cu^{2+} induced oxidation of human low-density lipoprotein (LDL). The effects of roasting condition on the browning index of sesame meal and the total phenolic content of the methanolic extract were also investigated. Results showed that as roasting temperature and time increased, the DPPH radical scavenging ability and the inhibition of Cu^{2+} induced oxidation of human LDL also increased. Meanwhile, the total phenolic content in the methanolic extract from defatted sesame meal and the browning level of sesame meal also increased with roasting temperature and time. These results suggested that the enhancement of antioxidative activity would probably be due to total phenolic compounds and Maillard reaction products formed during roasting process.

Key words: antioxidant, LDL-oxidation, lignan glycoside, sesame roasting

INTRODUCTION

Sesame seed is one of the important oil seed crops in the world. It is not only a good source of edible oil, but also widely used in baked goods and confectionery products[(1)]. Traditionally, sesame is considered as a healthy food in Oriental countries[(2)]. For example, it has been reported that sesame exhibits antioxidant, anti-hypertension and anti-cancer activities, and also possesses the ability to reduce cholesterol and to improve liver function[(3-5)]. Lignans and lignan glycosides present in sesame appear to be the important functional components. Lignans are found in sesame oil, while lignan glycosides exist mainly in the defatted sesame meal. There have been reports on the antioxidative activities and composition of lignans and lignan glycosides in sesame seed[(6-9)]. Although sesame seeds are often roasted to enhance the aroma and oxidative stability of sesame oil in addition to facilitate the pressing of the oil[(10)], little information is available on how roasting condition affecting the antioxidative activity of sesame meal. Thus, the main purpose of this study was to investigate the effect of roasting condition on the antioxidative activity of sesame meal. The results should provide information on how to improve the processing condition in order to increase the antioxidant activity of sesame meal, which, in turns, would enhance the utilization of sesame meal.

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MATERIALS AND METHODS

I. *Materials*

Burma black sesame seeds were obtained from Sweet Garden Agricultural Foods Co., (Taichung, Taiwan, R.O.C.). α-Tocopherol was purchased from Sigma Chemical Co. (St. Louis, MO, USA). 2,2-Diphenyl-1-picrylhydrazyl hydrate (DPPH) was purchased from Aldrich Co. (Milwaukee, WI, USA). Methanol (HPLC grade) was

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purchased from Tedia Co. (Fairfield, OH, USA). Cholesterol standard solution, copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), Folin-Ciocalteu's phenol reagent, sodium bromide and sodium chloride were purchased from Merck Co. (Darmstadt, German). 1-Butanol and sodium carbonate were purchased from Wako Co.(Osaka, Japan).

II. *Roasting of Sesame Seeds*

Sesame seeds (150 g) were roasted at three different temperature (180, 200, 220°C) and four different heating durations (5, 10, 20, 30 min) in an automatic roasting machine (Chung-Hou Co., Taipei, Taiwan, R.O.C.). The roasted seeds were ground with a commercial blender (Model 36BL23, Waring Co., New Harlford, CT, USA) and pressed at a pressure of 200 Kg/cm^2 to obtain sesame oil and sesame meal, the latter was used in the following experiments.

III. *Preparation of the Methanolic Extract from Sesame Meal* [9]

Sesame meal (50 g) obtained from sesame seed under different roasting condition was extracted with n-hexane (1:10, w/v) by stirring at room temperature for 24 hr. The insoluble residue of sesame meal (defatted sesame meal), after filtration, was extracted with 80% methanolic solution for 24 hr. The resultant extract was concentrated under reduced pressure to afford the methanolic extract from defatted sesame meal for this study.

IV. *HPLC Analysis of the Crude Extract of Lignan Glycosides*[11]

The methanolic extract (0.5 g) from defatted sesame meal was dissolved in 100 mL of methanol and the solution was filtered through a 0.45 μm filter. A 20 μL portion of the sample (5 mg/mL) was injected into HPLC column. The conditions of HPLC analysis were: column, Hypersil HS C18 (4.6 mm i.d. × 250 mm, 5 mm particle size); mobile phase, methanol/H_2O = 10:90 (v/v) initially and linear gradient to methanol/H_2O = 90:10 (v/v) in 60 min; flow rate, 0.8 mL/min; wavelength for detection, 280 nm.

V. *Determination of the Browning Level of Sesame Meal* [12]

Sesame meal (0.5 g) obtained from each roasting condition was added to 10 mL of distilled water. After vigorous shaking, the mixture was filtered through a Whatman No. 1 filter paper. The absorbance of the supernatant was measured at 420 and 550 nm. A browning index, A_{420}-A_{550}, was used to represent the browning level of sesame meal.

VI. *Determination of Total Phenolic Content* [13]

The methanolic extract (50 μL, 5 mg/mL) was added to 2 mL distilled water in a 10 mL tube. After adding 1 mL Folin-Ciocalteu's phenol reagent and 5 mL Na_2CO_3 solution (20%), the mixture was kept at room temperature for 20 min and then the absorbance at 735 nm was measured. The total phenolic content in the methanolic extract was determined by comparison with the absorbance of gallic acid.

VII. *Assay for DPPH Radical Scavenging Activity* [14]

Briefly, 0.2 mL of methanol and 0.3 mL of methanolic extract with various concentrations (5 μg/mL - 250 μg/mL) was mixed in a 10 mL test tube. Two point five mL of 75 μM DPPH in methanol was then added to achieve a final volume of 3 mL. The solution was kept at room temperature for 90 min and the absorbance at 517 nm was measured. The DPPH radical scavenging ability was calculated as follows: scavenging ability (%) = $[(A_0-(A-A_b))/A_0] \times 100\%$, where A_0: absorbance of DPPH without sample; A: absorbance of sample and DPPH; A_b: absorbance of sample without DPPH.

VIII. *Measurement of the Antioxidative Activity Toward Cu^{2+}- induced Oxidation of Human LDL* [15]

Human serum was obtained from a healthy male after overnight fasting. The LDL was isolated from serum by ultracentrifugation. The isolated LDL was dialyzed overnight at 4°C in the dark against phosphate buffer saline (PBS) (5 mM, pH 7.4, 125 mM NaCl). The integrity and the purity of lipoprotein preparations were checked by agarose gel electrophoresis. The cholesterol concentration of LDL was determined using the cholesterol enzymatic kit (Merck, Darmstadt, Germany).

The dialyzed LDL was diluted with PBS to a final concentration of 50 μg cholesterol/mL. For monitoring the formation of conjugated dienes, the absorbance of the mixture, containing 500 μL of LDL, 100 μL of test solution (20 μg/mL of the methanolic extract of defatted sesame meal dissolved in PBS), 200 μL of PBS and 200 μL of 25 μM $CuSO_4$ (the final concentration was 5 μM), was measured at 232 nm at 15 min intervals in an U-3210 spectrophotometer (Hitachi, Tokyo, Japan). The lag time of LDL oxidation, defined as the time interval (min) between the intercept of the curve slope in propagation phase with the initial absorbance axis, was measured.

IX. *Statistical Analysis*

Data in this paper were presented as the mean and standard deviation. One-way analysis of variance (one-way ANOVA) was conducted using a package (SAS Institute Inc., Cary, NC, USA). A significance level of 5% was adopted for all comparisons. Duncan's multiple ranges test was used to determine the significant difference between different treatments.

RESULTS AND DISCUSSION

Lignan glycosides, existing mainly in the defatted sesame meal, are regarded as hydrophilic antioxidants. However, the most abundant lignan glycosides of sesame, namely sesaminol triglucoside and sesaminol diglucoside, showed poor antioxidative activity[9]. It was also reported that the sesamin and sesamolin showed very low antioxidative properties[16,17]. The roasting process is the key step for producing sesame oil, since it not only provides special flavor but also increases the extractability of lipid. It is of interest to investigate the antioxidative activity of sesame meal after roasting.

I. *Effect of Roasting Condition on the Antioxidative Activities of the Methanolic Extract from Sesame Meal*

(I) *DPPH Radical Scavenging Ability*

The auto-oxidation of unsaturated fatty acids occurs autocatalytically in a chain reaction through free radical. Antioxidants can interrupt lipid autoxidation by scavenging the free radicals. DPPH is a stable free radical chemical that shows maximum absorption at 517 nm in methanolic solution. When DPPH free radical encounters an electron-donating substance, the radical would be scavenged and the absorbance at 517 nm is reduced. Based on this principle, the antioxidative activity of a test sample can be expressed as its ability in scavenging the DPPH free radical. The effect of roasting condition on the DPPH radical scavenging ability of the methanolic extract from sesame meal is shown in Figure 1. α−Tocopherol was used as the positive control in our study, with the highest DPPH radical scavenging ability (92.5%). The DPPH radical scavenging ability of the methanolic extract from roasted sesame meal was found to be significantly higher than that from unroasted sesame meal which exerted the lowest scavenging ability (16.0%) among all test samples. Moreover, the scavenging ability increases with increasing temperatures from 180 to 220°C.

It is therefore evident that roasting of sesame seed can not only produce desirable color and flavor of sesame oil[18,19] but also enhance the DPPH radical scavenging ability of the defatted sesame meal.

(II) *Antioxidative Activity Against Human LDL Oxidation*

It has been shown that oxidation of low density lipoprotein (LDL) leads to the pathogenesis of atherosclerosis. Studies on the antioxidants for inhibiting LDL oxidation have therefore received increasing attention[20]. However, no study has been conducted to investigate the antioxidative activity toward human LDL oxidation of sesame lignan glycosides. Figure 2 shows the effect of roasting condition on the ability of methanolic extract from sesame meal to inhibit Cu^{2+} induced human LDL oxidation. The lag time represents the resistance of LDL oxidation. Namely, the longer the lag time, the higher the resistance toward human LDL oxidation. The lag times of LDL oxidation of the control, unroasted, 180°C/10 min, 200°C/10 min, 220°C/10 min roasted sesame meal and Trolox (the positive control) are 84 ± 6, 148 ± 26, 191 ± 18, 268 ± 13, 268 ± 23 and 173 ± 9 min, respectively. Our data clearly show that the lag time of LDL oxidation

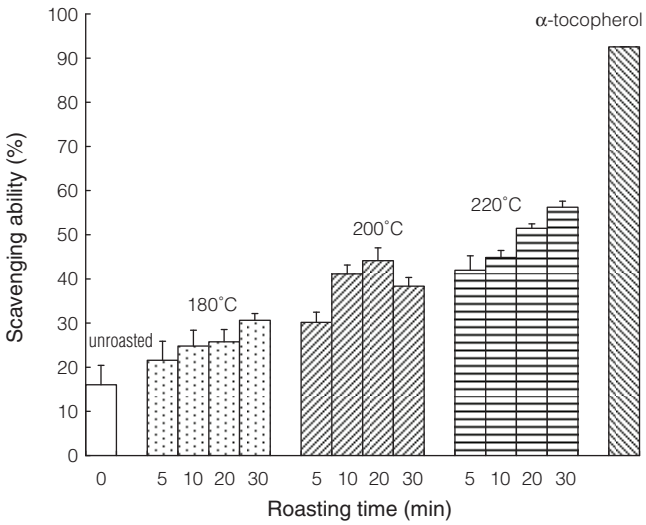

Figure 1. Effect of roasting condition on the DPPH scavanging ability of the methanolic extract from sesame meal. (sample concentration: 100 μg/mL).

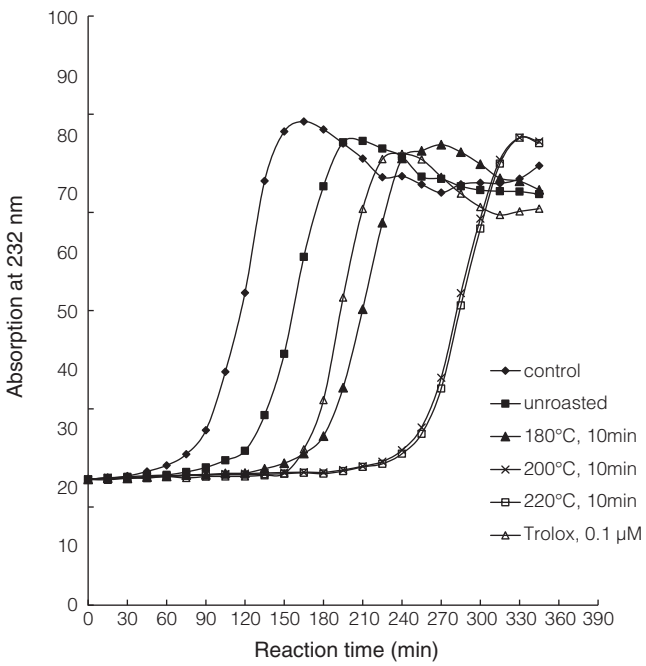

Figure 2. Effect of roasting condition on the inhibitory ability of the methanolic extract from sesame meal toward Cu^{+2} induced oxidation of human LDL.
((◆) control; (■) unroasted; (▲) 180°C, 10 min; (×) 200°C, 10 min; (□) 200°C, 10 min; (△) Trolox, 0.1μM). Sample concentration: 20 μg/mL.

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increases after roasting process. This is the first report on the antioxidative ability of the methanolic extract of the defatted roasted sesame meal towards human LDL oxidation. We are therefore curious to know the source of the antioxidant activity.

II. *Effect of Roasting Condition on Sesame Lignan Glycosides of the Methanolic Extract from Sesame Meal*

In attempting to search for the origin of the antioxidant activity of the methanolic extract of roasted sesame meal, the analysis of sesame lignan glycosides was conducted. Figure 3 shows the HPLC chromatograms of the methanolic extracts from unroasted and roasted sesame meals at three different roasting temperatures for 10 min. The retention time for sesaminol triglucoside (peak 1) and sesaminol diglucoside (peak 2) of the methanolic extract from unroasted sesame meal are 37.9 and 42.8 min, respectively. In addition, sesaminol triglucoside has much higher peak area than sesaminol diglucoside. We find that the chromatographic profiles of lignan glycosides (retention times of 35 to 45 min) are similar between unroasted and roasted sesame meals.

III. *Effect of Roasting Condition on the Browning Level of Sesame Meal*

Sesame oil is quite prevalent in the Oriental society. One of the reasons is its unique flavor, which comes from the Maillard reaction products during roasting process[21].

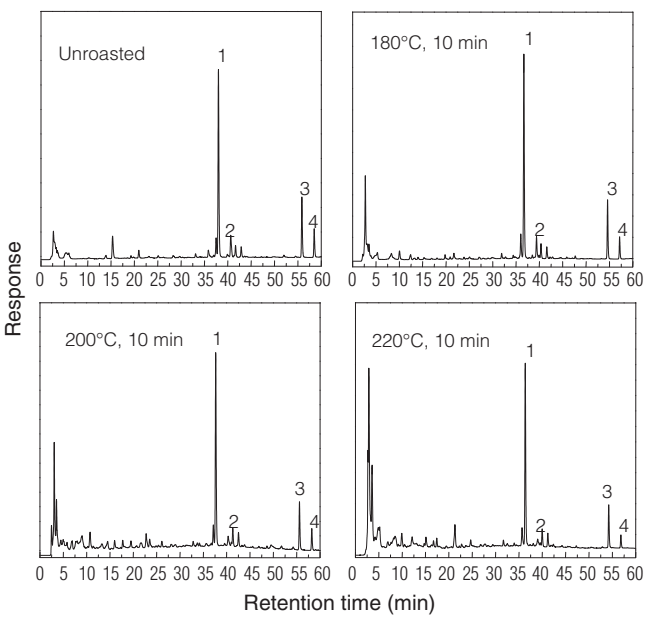

Figure 3. HPLC chromatograms of the methanolic extract of sesame meal roasted at different temperatures.
Peak 1: sesaminol triglucoside (t_R 37.9 min); Peak 2: sesaminol diglucoside (t_R 42.8 min); Peak 3: sesamin (t_R 55.8 min); Peak 4: sesamolin (t_R 58.5 min).

The effect of roasting condition on the browning level of sesame meal is shown in Figure 4. Our data show that the browning level increased significantly with roasting temperature. In general, the browning level also increased with the roasting time. But, the effect of time seems to be less important than temperature. Browning caused by Maillard reaction involves the interaction between the degradation products from proteins and carbohydrates. A

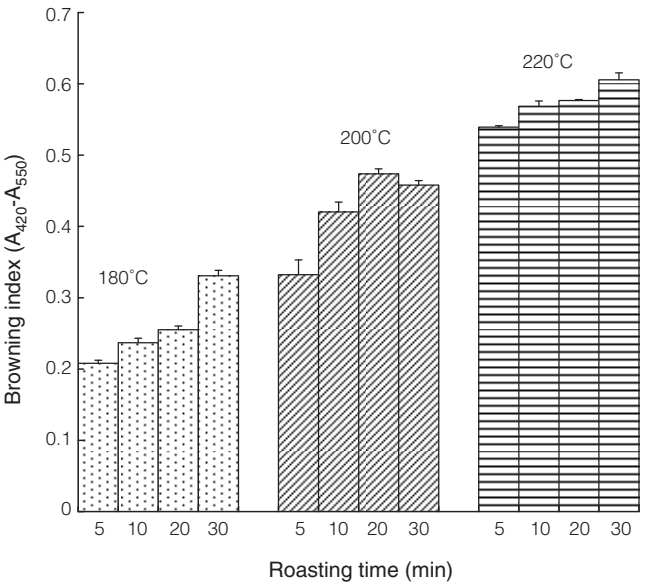

Figure 4. Effect of roasting condition on the browning level of sesame meal.

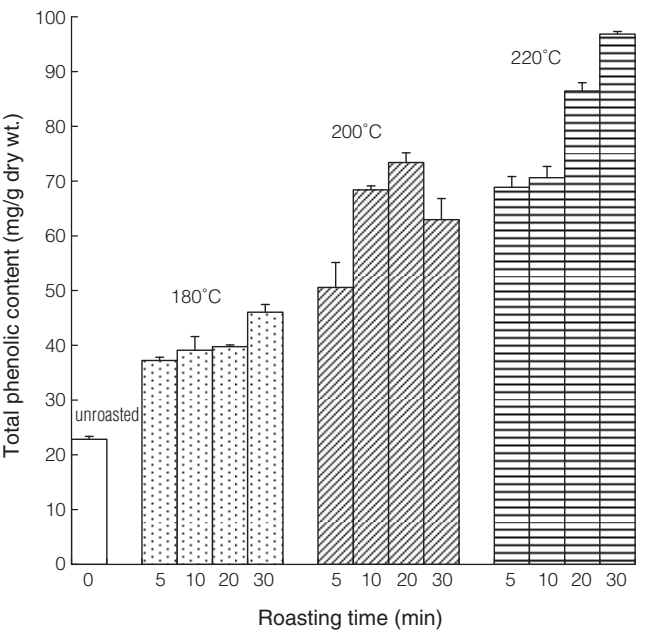

Figure 5. Effect of roasting condition on the total phenolic content of the methanolic extract from sesame meal.

positive contribution of roasting is the formation of Maillard reaction products, especially melanoidins, which are known to possess pronounced antioxidant properties[22,23]. Some reports have documented that the browning substances are generally very polar due to several active radicals in the compounds. Increase of the absorbance at 420 nm probably reflects color formation due to non-enzymic browning substances[24]. The increase in browning substances may be attributed to the increase of total lipids extracted from the roasted seeds because the sugar moiety of glycolipids could produce browning reaction products[25]. Our results suggest that raising the roasting temperature could increase the extent of Maillard reaction and thus increase the browning level in sesame meal.

IV. *Effect of Roasting Condition on the Total Phenolic Content of the Methanolic Extract from Sesame Meal*

Figure 5 shows the effect of roasting condition on the total phenolic content of the methanolic extract from sesame meal. The total phenolic content is 11.42 mg/g dry wt. in the methanolic extract from unroasted sesame meal. The total phenolic content increases dramatically with the roasting temperature. Compared to the total phenolic content of unroasted sesame meal, roasting at 220°C for 30 min resulted in about 4.2 fold increase in total phenolic content. Generally speaking, the total phenolic content also increased with the roasting time at the roasting temperature of 180-220°C. The increment of the total phenolic content might be caused by the degradation of lignan glycosides during the heating process[26]. Results of our HPLC analysis of the lignans and lignan glycosides (Table 1) indicate that the degradation of lignan glycosides was dependent upon the roasting temperatures. Thus, the temperature dependent degradation of lignan glycoside may be responsible for the increase of total phenolic content. More detailed examination of the chemical aspects is in progress.

Table 1. Effect of roasting condition on the lignans and lignan glycosides contents of the methanolic extract from sesame meal

Roasting condition	Sesaminol triglucoside (mg/g crude extract)	Sesaminol diglucoside (mg/g crude extract)	Sesamin (mg/g crude extract)	Sesamolin (mg/g crude extract)
Unroasted	82.77 ± 3.29^{a}	9.84 ± 0.37^{ab}	9.21 ± 0.78^{a}	4.44 ± 0.55^{a}
180°C, 10 min	78.36 ± 4.03^{a}	8.94 ± 0.36^{b}	5.41 ± 0.31^{b}	1.64 ± 0.28^{b}
200°C, 10 min	46.91 ± 1.61^{b}	3.52 ± 0.84^{c}	3.20 ± 0.08^{c}	0.84 ± 0.09^{c}
220°C, 10 min	38.97 ± 0.88^{c}	2.73 ± 0.62^{c}	2.50 ± 0.08^{c}	0.14 ± 0.03^{c}

The values are mean ± standard deviation.
$^{a\text{-}c}$The values in the same column followed by different superscripts were significantly different. (P<0.05).

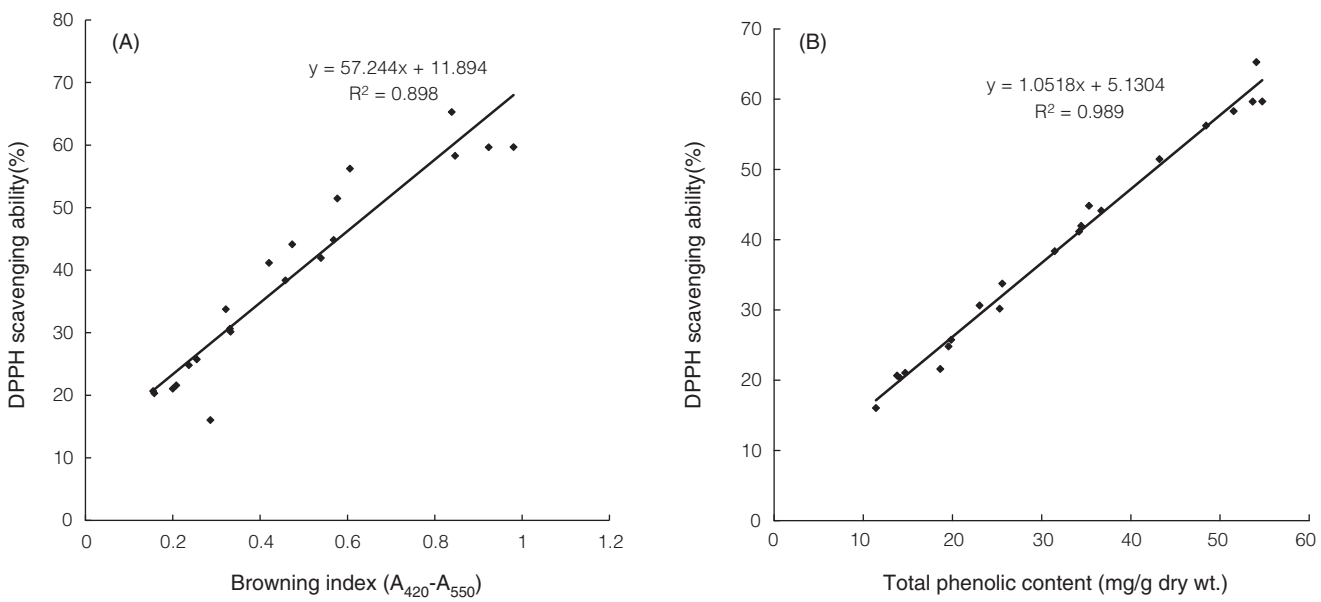

Figure 6. The correlation between DPPH scavenging ability and (A) browning index, (B) total phenolic content.

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V. *Correlation Between Antioxidant Activity and Total Phenol/Browning Level of Roasted Sesame Meal*

Figure 6 shows the relationship between DPPH scavenging ability and (A) browning index, (B) total phenolic content. The linear fit in Figure 6(A) is $y = 57.244x + 11.894$ with a correlation coefficient $R^2 = 0.898$ ($n = 21$), and the linear fit in Figure 6(B) is $y = 1.0518x + 5.1304$ with a correlation coefficient $R^2 = 0.989$ ($n = 21$). This implies that the DPPH free radical scavenging ability corresponds well with the browning level and the total phenolic content of the roasted sesame meal. The existence of such good correlation is not surprising, since our data indicate that higher roasting temperature significantly increase the browning level, the total phenolic content and the DPPH scavenging ability of the methanolic extract from sesame meal. Reports have shown that a remarkable increase in antioxidative activity of sesame with increasing roasting temperature and time. The various antioxidative mechanisms may be attributed to the combination of browning substances, γ-tocopherol, and degradation products of lignan glycosides (sesamol, sesaminol, and phenolic compounds)[16,26]. Fukuda[6] examined the antioxidative activity of methanolic extract from defatted white sesame meal and found that the antioxidative activity increased with roasting temperature. They suggested that the Maillard reaction products might play an important role in antioxidative activity. Our results also confirm this suggestion. However, in our study both the level of browning and the total phenolic content show good correlation with antioxidative activity and the correlation coefficient is higher for total phenolic content ($R^2 = 0.989$) than for browning index ($R^2 = 0.898$). Phenolic compounds have been demonstrated to exhibit a scavenging effect for free radicals and possess metal-chelating ability[27]. We, therefore, assume that both the total phenolic compounds and the products from Maillard browning reaction contributed to the antioxidative activity of defatted roasted sesame meal.

CONCLUSIONS

The methanolic extract of the defatted roasted sesame meal exhibits good DPPH radical scavenging ability and antioxidative activity against human LDL oxidation. Under the roasting temperatures from 180 to 220°C, the browning level, the total phenolic content and the antioxidative activities of the methanolic extract from sesame meal increased with the roasting temperature. The increment of antioxidative activity of the methanolic extract is probably due to the total phenolic compounds and Maillard reaction products formed during the roasting process.

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