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Antioxidant activity of white and black sesame seeds and their hull fractions

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Abstract

The total phenolic content (TPC), total antioxidant status (TAS), free radical scavenging capacity, inhibition of low density lipoprotein (LDL) cholesterol and metal chelating capacity of extracts of whole black and whole white sesame seeds and their hull fractions in 80% aqueous ethanol were investigated. The TPC of whole black sesame seeds and hull extracts were 29.9 ± 0.6 and 146.6 ± 0.6 mg catechin equivalents/g crude ethanolic extract, respectively. The corresponding values for white sesame were 10.6 ± 1.6 and 29.7 ± 0.9 mg catechin equivalents/g crude ethanolic extract. The TAS as determined by Trolox equivalent antioxidant capacity assay and expressed as Trolox equivalents was highest for black sesame hulls (65.9 ± 1.7) while white seeds showed the lowest (4.4 ± 0.6). Free radical scavenging capacity of sesame extracts (5–40 μg/mL) was measured using 2,2-diphenyl-1-picrylhydrazyl radical. The highest scavenging capacity was obtained at 40 μg/mL and was 94.9 ± 0.8, 25.1 ± 0.4, 14.4 ± 0.9 and 2.5 ± 0.4 for black sesame hulls, black sesame seeds, white sesame hulls and white sesame seeds, respectively. Inhibition of LDL oxidation at 100 ppm level was highest for black sesame hulls (96.7%) followed by those for white sesame hulls (84.6%), black sesame (78.4%) and white sesame seeds (57.3%). Sesame products displayed good ferrous ion chelating capacities, which ranged from 12% to 46% and 17% to 62% at 50 and 100 ppm levels, respectively. Results demonstrated considerable antioxidant activity of sesame products tested especially black sesame hulls.

Keywords: Antioxidant activity; *Sesamum indicum*; Phenolics; Free radical scavenging; LDL oxidation

1. Introduction

Sesame (*Sesamum indicum* L.) is an important oilseed crop in the world and provides a good source of edible gourmet oil (Namiki, 1995). Several studies have reported the health-promoting properties of sesame (Kita et al., 1995; Sugano et al., 1990; Yamashita, Lizuka, Imai, & Namiki, 1995). Sesame serves as a nutritious food for humans and is used widely in bakery and confectionery products (Abou-Gharbia, Shahidi, Shehata, & Youssef, 1997). Sesame is cultivated on a worldwide basis for both oil and protein and the seed is composed of 55% lipid and 20% protein (Abou-Gharbia et al., 1997). The defatted sesame meal contains nearly 50% protein (Sen & Bhattacharyya, 2001) and seed hulls contain large quantities of oxalic acid and fiber (Abou-Gharbia et al., 1997). For certain applications, dehulling of sesame seeds may be necessary (Sen & Bhattacharyya, 2001). Chang, Yen, Huang, and Duh (2002) have shown that sesame hulls possess considerable antioxidant activity, partly due to their high level of phenolic compounds.

Epidemiological studies have shown that consumption of plant foods is beneficial to health and contributes to the prevention of degenerative processes, hence lowering the incidence and mortality rate from cancer and cardio- and cerebro-vascular diseases (Halliwell, 1997). Such protection has been attributed to various antioxidants and phytonutrients contained in these plant foods (Rapisarda

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et al., 1999). It has been shown that free radicals cause oxidative deterioration of biomolecules such as membrane lipids, proteins and nucleic acids. Therefore, antioxidants that quench free radicals may play a significant role in the prevention of numerous degenerative diseases (Rice-Evans & Diplock, 1993). In addition to free radical chain breaking, chelation of metal ions and quenching of singlet oxygen are the major characteristics of antioxidant activity (Hall & Cuppett, 1997). Currently, there is much interest in replacing synthetic antioxidants with natural alternatives due to safety concerns. Many naturally occurring phytochemicals have received much attention as safe antioxidants (Namiki, 1990). In fact, natural antioxidants have captured the interest of consumers and scientists of the medical and pharmaceutical industries due to their antitumor, antimutagenic as well as anticarcinogenic properties (Shahidi, Wanasudara, & Hong, 1992). Therefore, it is important to focus on antioxidants from natural sources and to develop food products with maximum retention of endogenous antioxidants both for food stabilization and for nutritional purposes (Bryngelsson, Dimberg, & Kamal-Eldin, 2002).

Both edible and non-edible plants and plant-derived products are known to contain a complex mixture of phenolic compounds that possess multiple biological effects including antioxidant activity. Shahidi and Naczk (2004) have reported the antioxidant activity of naturally occurring phenolics in different model systems. The antioxidant activity of phenolic compounds has been attributed to their redox properties and may function as free radical scavengers, reducing agents, potential chelators of prooxidant metals and quenchers of singlet oxygen (Watanabe, 1998). Phenolic compounds may also play a significant role in preventing undesirable changes in flavor and nutritional quality of foods by acting as antioxidants (Zielinski & Kozlowska, 2000). Antioxidant phytochemicals may also play an important role in human health via scavenging reactive oxygen and nitrogen species (Bravo, 1998). In this study, an attempt was made to determine the antioxidant activity of white and black sesame and their hull fractions.

2. Materials and methods

2.1. Materials

The samples of white and black sesame as such and their hull fractions were obtained from a supermarket in Singapore. The chemicals used were obtained from Sigma Chemical Co. (St. Louis, MO) or Aldrich Chemical Co. (Milwaukee, WI). Solvents used in this study were ACS-grade or better quality and obtained from Fisher Scientific Co. (Nepean, ON).

2.2. Sample preparation

Sesame seeds were ground in an electric coffee grinder (Black and Decker Canada Inc., Brockville, ON) for 10 min. Ground sesame was then defatted by blending with hexane (1:5 w/v, 5 min × 3) in a Waring blender (Model 33BL73, Dynamics Corp. of America, New Hartford, CT) at ambient temperature. The resulting slurry was filtered under suction and the residue was air dried for 12 h. The dried defatted meal was stored in vacuum packaged polyethylene pouches at −20 °C prior to analysis. Sesame hulls were also defatted in the same manner prior to analysis.

2.3. Preparation of crude extracts of sesame

Phenolic constituents of both sesame meal and hull samples (6 g) were extracted into 80% aqueous ethanol (100 mL) at 70 °C for 30 min. The resulting slurries were centrifuged for 5 min at 4000*g* (ICE Centra M5, International Equipment Co., Needham Heights, MA). The supernatants were collected and the residue was reextracted under the same conditions. The solvent from the combined supernatants was removed under vacuum at 40 °C and the resulting concentrated solutions were lyophilized for 72 h at −49 °C and 46×10^{-3} mbar (Freezone 6, Model 77530, Labconco Co., Kansas City, MO).

2.4. Determination of total phenolic content

The content of total phenolics was determined according to a modified version of the procedure described by Singleton and Rossi (1965). Extracts were dissolved in methanol to obtain a 1 mg/mL solution. Folin and Ciocalteu's reagent (0.5 mL) was added to centrifuge tubes containing 0.5 mL of the extracts. Contents were mixed and 1 mL of a saturated solution of sodium carbonate was added to each tube, followed by adjusting the volume to 10 mL with distilled water. The contents in the tubes were thoroughly mixed by vortexing. Tubes were allowed to stand at ambient temperature for 45 min until the characteristic blue color developed and then centrifuged for 5 min at 4000*g* (ICE Centra M5, International Equipment Co., Needham Heights, MA). Absorbance of the supernatants was read at 725 nm using a Hewlett–Packard spectrophotometer (Agilent, Palo Alto, CA). The content of total phenolics in each extract was determined and results were expressed as milligram catechin equivalents per gram of extract.

2.5. Measurement of total antioxidant status (TAS) by Trolox equivalent antioxidant capacity (TEAC) assay

Total antioxidant status was determined according to the procedure described by van den Berg, Haenen, van den Berg, and Bast (1999) with some modifications. The extracts and reagents were prepared in a 0.1 M phosphate buffer (pH 7.4) solution containing 0.15 M sodium chloride (PBS buffer solution). A solution of 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonate) radical anion ($ABTS^{\bullet-}$) was prepared by mixing 2.5 mM 2,2′-azobis-(2-methylpropionamidine)-dihydrochloride (AAPH) with 2.0 mM $ABTS^{2-}$ in a 1:1 (v/v) ratio, and heating at 60 °C for 20 min. The radical solution

was stored at room temperature and in the dark. A standard curve was prepared using different concentrations of Trolox. The reduction in absorbance of the $ABTS^{\bullet-}$ solution (1960 μL) at different concentrations of Trolox (40 μL) over a 6 min period was measured and plotted. TEAC values of the extracts (1 mg/mL) were determined in the same way and expressed as Trolox equivalents.

2.6. Quenching of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical by sesame extracts

The method described by Kitts, Wijewickreme, and Hu (2000) was used to assess the DPPH radical scavenging capacity of sesame extracts. A 0.1 mM DPPH solution in ethanol was mixed with various amounts of sesame extracts (5, 10, 20, 40 μg/mL) and vortexed thoroughly. The mixtures were allowed to stand at ambient temperature for 30 min. The absorbance was measured at 519 nm using a Hewlett–Packard spectrophotometer (Agilent, Palo Alto, CA). The scavenging percentage was calculated according to the following equation:

$$\%\text{scavenging} = \{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})/\text{Abs}_{\text{control}}\} \times 100$$

2.7. Determination of the effects of sesame extracts on oxidation of human low density lipoprotein (LDL) cholesterol

The procedure described by Hu and Kitts (2001) was employed in this study. Human LDL cholesterol was dialyzed in 10 mM PBS (pH 7.4) at 4 °C in the dark for 24 h. LDL (0.2 mg protein/mL) was mixed with different amounts of sesame extracts (25–100 ppm phenolics). Catechin was used as the reference antioxidant compound. Reaction was initiated by adding a solution of $CuSO_4$ (10 μM); samples were then incubated at 37 °C for 22 h. The formation of conjugated dienes was measured at 234 nm using a Hewlett–Packard spectrophotometer (Agilent, Palo Alto, ON).

2.8. Measurement of iron (II) chelating capacity

Solutions of ferrous sulphate (400 μM), extracts/standard were prepared in a 10 mM hexamine–HCl buffer containing 10 mM KCl (pH 5.0). One millilitre of ferrous sulphate was mixed with 1 mL of extracts/standard followed by the addition of 0.1 mL of a 1 mM solution of tetramethylmurexide prepared in the same buffer. The final concentration of extracts and standards in the assay medium was 50 or 100 ppm based on catechin equivalents. Absorbance of the reaction mixture was recorded at 460 and 530 nm and the ratio of A_{460}–A_{530} calculated. A standard curve of absorbance ratio vs. free iron (II) concentration was prepared. The difference between the total iron (II) and the free iron (II) indicates the concentration of chelated iron (II) (Terasawa, Murata, & Homma, 1991). Iron (II) chelating capacities of extracts/standards were calculated using the following equation:

$$\text{Iron(II)chelating capacity, \%} = \{\text{concentration of chelated iron(II)/concentration of total iron(II)}\} \times 100$$

2.9. Statistical analysis

All experiments were carried out in triplicate and the significance of differences among means were determined at $p < 0.05$ using one way ANOVA followed by Tukey's multiple range test. The type of relationship between variables was determined by simple regression analysis.

3. Results and discussion

The content of total phenolics in whole white and black sesame seeds and their hull fractions is given in Table 1. The level of total phenolics as determined by the Folin–Ciocalteu method was highest in black sesame hulls (146.6 ± 4.1 mg catechin equivalents/g crude extract) and lowest in white sesame seeds (10.6 ± 1.6 mg catechin equivalents/g crude extract). The total phenolic content was significantly ($p < 0.05$) higher (≈5 times) in the black than that in the white sesame seed hulls.

Phenolic compounds are widely distributed in the plant kingdom. These compounds serve as important antioxidants because of their ability to donate a hydrogen atom or an electron in order to form stable radical intermediates. Hence, they prevent the oxidation of various biological molecules (Cuvelier, Richard, & Berset, 1992). In fact, several oilseeds and their byproducts have been investigated for phenolic compounds in search for safe sources of natural antioxidants (Wettasinghe, Shahidi, & Amarowicz, 2002).

In the case of cereal grains it has been shown that their outer layers such as husk, pericarp, testa and aleurone cells contain the highest concentration of total phenolics while their concentration is considerably lower in the endosperm layers (Kahkonen et al., 1999). Naczk and Shahidi (1998) have shown that canola hulls also contain a high

Table 1
Content of total phenolics (milligram catechin equivalents per gram ethanolic extract) and total antioxidant status (TAS; Trolox equivalents) of whole sesame seeds and hulls[a]

Component	Total phenolic content	Total antioxidant status
Black sesame hull	146.6 ± 4.1	65.9 ± 1.7
Whole black sesame	29.9 ± 0.6	18.8 ± 0.5
White sesame hull	29.7 ± 0.9	13.1 ± 0.7
Whole white sesame	10.6 ± 1.6	4.4 ± 0.6

Values are means of three determinations ± standard deviation.
[a] Two milligrams of crude extract were dissolved in 1 mL solution of PBS for TAS determinations.

proportion of phenolic compounds. The same observation was made in sesame; both white and black sesame showed a higher phenolic content in their hull fractions as compared to their whole seed counterparts. With respect to whole sesame, both black and white seeds had a low content of phenolics due to a dilution effect as the endosperms contain only a very low amount of phenolics compared to those of the hulls.

Total antioxidant status (TAS) of different sesame components, expressed as Trolox equivalents, is shown in Table 1. The concentration of the extracts used in the determination of TAS was 1 mg/mL. The total antioxidant activity of the samples tested was in the decreasing order of black sesame hulls > black sesame > white sesame hulls > white sesame. The TAS procedure measures the relative activity of antioxidant substances to scavenge DPPH radical anion compared to the reference Trolox which is a water-soluble vitamin E analogue. The TAS trend obtained corresponded directly with the content of total phenolics in the extracts with a correlation coefficient of $r = 0.995$. Thus, it is apparent that there is a strong relation between total phenolics content of different sesame fractions and their respective total antioxidant activity.

The scavenging activity of sesame crude phenolics compared to catechin for DPPH radical is shown in Table 2. The results indicate a concentration-dependent scavenging activity of the DPPH radical. At 20 and 40 μg/mL concentrations scavenging activity of phenolics from black sesame hulls did not show any significant difference from that of catechin, but at 5 and 10 μg/mL it did. Scavenging activity of extracts of black sesame seeds, white sesame hulls and white sesame seeds were significantly lower than that of black sesame hulls, thus phenolics of black sesame hulls exhibited the strongest DPPH scavenging potency.

Numerous methods have been proposed to evaluate the antioxidant activity of a test compound based on free radical chain breaking, metal chelating and singlet oxygen quenching activities, among others (Amarowicz, Naczk, & Shahidi, 2000). One of the mechanisms involved in antioxidant activity is the ability of a molecule to donate a hydrogen atom to a radical and the propensity of hydrogen donation is the critical factor that involves free radical scavenging (Hu, Zhang, & Kitts, 2000), including that for DPPH as proposed by Blois (1958). Hence, DPPH radical scavenging activity of sesame extracts may primarily be related to their hydrogen donation ability. The values obtained for DPPH radical scavenging capacity corresponded well with those of the total antioxidant activity and total phenolics. Thus, sesame extracts may also possess a strong antimutagenic activity which is attributed to the ability of the molecules involved to scavenge free radicals (Hochstein & Atallah, 1998). Catechin demonstrated similar DPPH radical scavenging activity despite the existing concentration differences. This may be due to a saturation effect or due to some other yet unexplained factors.

The antioxidant effects of sesame extracts were investigated individually at four different levels of phenolics against inhibition of Cu^{2+}-induced oxidation of human LDL in vitro by monitoring conjugated diene formation. Table 3 summarizes the results for the effects of sesame extracts on the Cu^{2+}-induced oxidation of human LDL as compared with those of catechin. In this study, sesame extracts, especially black sesame hulls, demonstrated a considerable inhibitory effect against oxidation of human LDL in a concentration-dependent manner. Hence, a higher concentration of phenolics in the assay medium may lead to greater inhibitory effects on the Cu^{2+}-induced LDL oxidation.

In general, in vitro LDL oxidation has been used effectively in several investigations to characterize antioxidant activity of a number of phytochemicals from different plants (Auroma et al., 1998; Hu & Kitts, 2001; Hu et al., 2000). Sesame extracts exerted considerable antioxidant potency toward Cu^{2+}-induced human LDL oxidation in vitro with black sesame hulls being the most potent among samples/fractions tested. In fact, the inhibitory effect of black sesame hulls was comparable to that of catechin against Cu^{2+}-induced LDL oxidation. The variability in inhibition of LDL oxidation between different extracts may be explained by the existing differences in their total phenolic content. Further, the differences in antioxidant activities can be attributed to variations in the chemical structures of different phenolics present in different sesame fractions. Hence, further studies on evaluating such structural features are required. Within the concentration range tested no prooxidant activity toward LDL by sesame extracts was detected. Metal chelation may have also been involved in the protective mechanisms against transition

Table 2
Scavenging capacity of DPPH radical by extracts of sesame seeds and hulls

Component	DPPH scavenging capacity (%)			
	5 μg/mL	10 μg/mL	20 μg/mL	40 μg/mL
Black sesame hull	28.9 ± 0.6	55.3 ± 1.3	93.3 ± 0.6	94.9 ± 0.8
Whole black sesame	2.1 ± 0.2	5.1 ± 0.4	12.7 ± 0.6	25.1 ± 0.4
White sesame hull	3.2 ± 0.6	4.3 ± 0.1	6.4 ± 0.3	14.4 ± 0.9
Whole white sesame	0.1 ± 0.05	1.0 ± 0.4	1.3 ± 0.4	2.5 ± 0.4
Catechin	93.6 ± 0.2	93.6 ± 0.1	93.8 ± 0.2	93.9 ± 0.2

Values are means of three determinations ± standard deviation.

Table 3
Inhibition of Cu^{2+}-induced human LDL cholesterol oxidation by extracts of sesame seeds and hulls

Component	Concentration of phenolics (ppm)			
	25	50	75	100
Black sesame hull	57.3	85.4	94.2	96.7
Whole black sesame	31.2	55.4	61.7	78.4
White sesame hull	42.2	61.3	75.4	84.6
Whole white sesame	24.4	40.4	44.8	57.3
Catechin	59.3	88.7	94.4	99.0

Values are means of three determinations ± standard deviation.

Table 4
Chelation capacity of ferrous ions by extracts of sesame seeds and hulls

Component	50 ppm	100 ppm
Black sesame hull	46.0 ± 1.6	62.0 ± 0.9
Whole black sesame	39.0 ± 1.9	50.0 ± 2.9
White sesame hull	20.0 ± 0.9	25.8 ± 2.3
Whole white sesame	12.0 ± 0.9	17.1 ± 2.1
Catechin	92.0 ± 2.4	99.0 ± 1.0

Values are means of three determinations ± standard deviation.

metal ion-induced LDL oxidation (Hu et al., 2000). Hence, the mechanism of activity of sesame extracts to inhibit Cu^{2+}-induced LDL oxidation could be attributed to phenolic compounds that are known to act as good transition metal ion chelators (Rice-Evans, Miller, Bolwell, Bramley, & Pridham, 1995).

Low density lipoprotein cholesterol is the major cholesterol carrier in blood and an elevated plasma level of LDL is correlated with an increased risk of atherosclerosis and cardiovascular disease (Ross, 1993). It is well established that LDL does not form atherosclerotic plaques in its native form. However, oxidatively modified LDL is responsible for the pathogenesis of atherosclerosis that may lead to the build up of plaque in the arteries and consequently the occurrence of coronary heart disease (Esterbauer, Gebiski, Puhl, & Jurgens, 1992; Steinberg, 1997). Therefore, dietary antioxidants that inhibit LDL oxidation may be of paramount importance in protection against these diseases (Esterbauer et al., 1992).

Table 4 shows the chelating effect of sesame extracts on ferrous ions. The greatest chelating activity was detected in black sesame hulls both at 50 (46%) and 100 ppm (65%) levels of phenolics, followed by black sesame seeds, white sesame seed hulls and white sesame. Different sesame fractions differed significantly in their chelating activities at both concentration levels tested. The total phenolic content and chelating activity with both 50 ($r = 0.771$) and 100 ppm ($r = 0.805$) levels were moderately strong. The reference compound, catechin, exhibited a strong iron (II) chelation capacity at both levels tested. Transition metal ions such as those of iron and copper are major primary catalysts that initiate oxidation in vivo and in vitro (Tichivangana & Morrissey, 1985). Metal ions play an important role in the acceleration of oxidation of important biological molecules; for instance they may catalyze the formation of first few radicals that may lead to propagation of the radical chain reaction in lipid peroxidation (Nawar, 1996). Chelating agents, on the other hand, are known to stabilize pro-oxidative transition metal ions by complexing them, where unshared pairs of electrons in the molecular structure of chelators promote the complexation (Dziezak, 1986). Citric acid and its salts, phosphates and salts of ethylenediaminetetraacetic acid (EDTA) are among the most commonly used chelators. In addition, plant phenolic compounds have also been found to be good metal ion chelators (van Acker et al., 1996). Thus, certain phenolic compounds possessing *ortho* dihydroxyl groups or a properly located carbonyl and hydroxyl groups.

In conclusion, defatted sesame extracts and their hulls possess good antioxidant activity. This activity was higher for black sesame and concentrated mainly in the hull fraction. Both free radical scavenging and chelation mechanisms were found to be involved in the overall efficacy of the extracts.

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