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Antiradical Capacities of Perilla, Sesame and Sunflower Oil

Sunhee Hong[1], Mijin Kim[1], Chanho Oh[2], Sukhoo Yoon[3], and Yeongok Song[1†]

[1]*Department of Food Science and Nutrition, Kimchi Research Institute, Pusan National University, Busan 609-735, Korea*
[2]*Department of Food & Biotechnology, Woosuk University, Jeonbuk 565-701, Korea*
[3]*Korea Food Research Institute, Gyeonggi 463-746, Korea*

Abstract

The aim of this study is to examine the radical scavenging activity of perilla and sesame oil that Koreans traditionally consume. For DPPH radical scavenging activity, oil and its hexane/70% methanol extracts (ME) are used and for superoxide and hydroxyl radical scavenging activities, ME are used. Unrefined perilla oil, sesame oil, and refined sunflower oil are used. The yields for ME of perilla, sesame and sunflower oil are 0.57, 0.61, and 0.30%, respectively, and the amounts of phenolic compounds in ME of corresponding oil are 18.77, 88.64 and 0.05 μg tannic acid/mg, respectively. IC_{50} for DPPH scavenging activity of perilla, sesame and sunflower oil are 2.12, 1.91, and 3.35 mg/mL, respectively and those for ME of corresponding oils are 0.42, 0.07, and 43.11 mg/mL, respectively. In DPPH assay, the solvent used for oil sample is iso-octane and that for ME is methanol. Superoxide anion scavenging activity of ME of perilla, sesame and sunflower oil tested at 1 mg/mL concentration are 21.10, 13.25, and 3.14%, respectively. Hydroxyl radical scavenging activities of those samples tested at 1 mg/mL concentration are 86.08, 93.30, and 93.17%, respectively. In summary, the refining process seems to remove the phenolic compound during oil processing. Antiradical substances in perilla and sesame oils responsible for scavenging DPPH radicals are present in the methanol fraction, while the antiradical substances in the sunflower oil are in the lipid fraction. DPPH scavenging activity of ME of sesame oil is significantly higher than that of perilla oil ($p<0.05$). However, superoxide anion scavenging capacity of ME of perilla oils was found to be greater than that of both sesame and sunflower oils ($p<0.05$).

Key words: perilla oil, sesame oil, sunflower oil, DPPH radical, superoxide anion

INTRODUCTION

Diets rich in lipids are not preferred in this day and age, because they are high in calories, as well as lipid radicals when metabolize in the biological system. Subsequently, plant oils composed of unsaturated fatty acid have been considered unhealthy due to their susceptibility to oxidation. However, there is a consensus of the health benefits of the Mediterranean-style diet in which olive oil is the principle fat source (1). This health promoting effect of olive oil on humans is explained by the presence of antioxidants, mainly phenolic compounds, in it (2). The amount of phenolic compounds in olive oil varies with the state of the oil, not by the location of production (3). Virgin olive oils are known to have greater health benefits than other olive oils that are consumed unrefined (4). Phenolic compounds in oils are believed to be removed during the various stages of refining (2). Therefore, plant oils consumed in the natural unrefined state might also have health benefits like those of virgin olive oil. Sesame oil and perilla oil are some of the oils consumed unrefined in Korea.

The physiological importance of long chain n-3 fatty acids on health benefits has been well documented in terms of prevention and treatment of cardiovascular diseases, inflammatory diseases and cancer. These effects might be due to either n-3 fatty acids themselves, as structural components of membrane phospholipids, or to their products, modulating biosynthesis of potential cellular mediators, eicosanoids. Eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and alpa-linolenic acid (ALA) belong to n-3 fatty acids and fish oils are rich dietary source for EPA and DHA. But fish oil is not easy to consume because of its smell, thus the majority of the population are interested in the alternative dietary source of long-chain n-3 fatty acids, ALA (5). ALA is found in many foods including perilla oil, flaxseed oil, and borage oil, as well as walnuts and leafy vegetables. Whether dietary ALA, as a precursor of EPA and DHA, has health benefits similar to EPA or DHA from long-term dietary intake, is being investigated. In Korea, perilla oil is commonly used in cooking with ses-

†Corresponding author. E-mail: yosong@pusan.ac.kr
Phone: +82-51-510-2847, Fax: +82-51-583-3648

ame oil, both of which are consumed in the natural, unrefined state. The major fatty acid in perilla oil is ALA and in sesame oil is linoleic acid.

The reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical, hydrogen peroxide, and singlet oxygen and reactive nitrogen species (RNS), such as nitric oxide and peroxynitrite, are responsible for oxidative stress that, once elevated, consequently leads to oxidative damage (6). The prevention and removal of ROS can be achieved enzymatically or non-enzymatically. Living cells have biological defense mechanisms that combat the effects of ROS generated during normal metabolic processes. These natural processes are called 'antioxidative enzyme systems'. Nonenzymatic reactions are also effective for scavenging ROS and RNS (4). The nonenzymatic antioxidants vitamin E and C, as well as many phenolic phytochemicals that possesses scavenging activity have received considerable attention in recent years because they function as chemopreventive agents against oxidative damage (7,8). In fact, foods having antioxidant properties or containing antioxidants are receiving much attention due to their ability to prevent oxidative damage to human body. The main dietary antioxidants are polyphenols followed by vitamins and carotenoids, quantitatively.

In this study, the antiradical activities of perilla oil, which usually consumed unrefined like olive oil, were studied. DPPH radical scavenging activities of the oil and methanol fractions of oil samples were analyzed and superoxide anion and hydroxyl radical scavenging ability were examined with the methanol fraction of oil, where the phenolic compounds were present. These activities of perilla oil are compared with those of sesame oil, which is also usually consumed unrefined. The antiradical effects of unrefined oil were compared with those of refined oil, sunflower oil and its methanol fraction.

MATERIALS AND METHODS

Oils

Perilla and sesame oils, extracted by cold pressure, were purchased at a local market (Busan, Korea). And the sunflower oil was kindly provided by L Company (Seoul, Korea). The sunflower oil was refined, but did not have antioxidants added.

Preparation of methanol extracts of oils

For the preparation of methanol extracts of oil, 100 g of oil was dissolved in 500 mL hexane followed by successive extractions three times with 200 mL of 70% aqueous methanol. The mixture was stirred each time for 1 hr. The extracts were combined together and then brought to dryness in a vacuum rotary evaporator at 40°C. The residue (methanol extracts) was dissolved in methanol and stored at -20°C until it was used (9).

Total polyphenols

The content of total phenols in methanol extracts (ME) of oil was determined according to the Folin-Ciocalteu colorimetric method using tannic acid as a standard (10). The procedure consisted of added 0.2 mL extract diluted to suitable concentration to 2.8 mL water, and addition of 0.2 mL Folin-ciocalteu reagent. After 7 min, 2 mL of 7% Na_2CO_3 solution was added. The extinction was measured after 90 min at 750 nm. Tannic acid served as a standard for preparing the calibration curve ranging 1～300 μg/mL assay solution.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging activity of oil and ME of oil was determined using a Microplate Reader described by Hatano et al. (11). For oil samples, iso-octane was used as a solvent (12). Briefly, 100 μL of oil diluted with iso-octane was mixed with 100 μL of 60 μM DPPH in 95% iso-octane. The reaction mixture was left to stand in the dark for 30 min, then the optical density was determined at 540 nm using a Microplate Reader (model 680, Bio-Rad Laboratories Ltd, UK). For ME of oil sample, the same assay procedure described above for the oil sample was used, except methanol was used as the solvent. Reaction mixture was prepared with 100 μL of ME of oil and 100 μL of 60 μM DPPH in 95% methanol. The scavenging activity of oil or methanol extracts of oil against DPPH radical was expressed as IC_{50}.

Superoxide radical scavenging activity

Superoxide anions (O_2^-) generated in an enzymatic system (xanthine-xanthine oxidase) were reduced by nitroblue tetrazolium (NBT) (13). The reaction mixture was prepared with 400 μL methanol extract, 100 μM xanhine, 60 μM NBT, 0.05 U/mL xanthine oxidase, and 0.1 M phosphate buffer (pH 7.4) to be a total volume 2.0 mL. This mixture was incubated at 37°C for 10 min and then measured the absorbance at 540 nm. The control was run without xanthine oxidsase.

Hydroxyl radical scavenging activity

In the presence of hydroxyl radical, 2-deoxyribose is oxidized and degraded to malondialdehyde (MDA) (14). The reaction mixture was prepared with 0.2 mL of 10 mM $FeSO_4 \cdot 7H_2O$ with 10 mM ethylenediaminetetraacetic acid (EDTA) in it, 10 mM 2-deoxyribose solution

(0.2 mL), sample solution (1.4 mL) or 0.2 M phosphate buffer (1.4 mL, pH 7.4). The reaction was initiated by addition of 10 mM H_2O_2 (0.2 mL) followed by incubation at 37°C for 4 hr. To stop the reaction, 1 mL each of 2.8% trichloroacetic acid (TCA) and 1.0% thiobarbituric acid (TBA) were added to the incubation medium immediately and then boiled for 10 min. The mixture was immediately cooled in the ice water. The absorbance of the mixture was measured at 540 nm. The -OH scavenging activity was calculated as the inhibition of 2-deoxyribose oxidation by comparing with the control run with the phosphate buffer.

Statistical analysis

Analysis of one-way variance (ANOVA) was followed by Duncan's multiple rage test in order to determine the statistical significance of measurements between groups, using the SAS software ($p<0.05$).

RESULTS

Yields and total phenol contents of methanol extracts of oils

Yields for ME of perilla, sesame and sunflower oils were 0.57, 0.61 and 0.30%, respectively (Table 1). The differences in extraction yields for three oils might be due to the oil state. Perilla and sesame oils were unrefined ones, obtained by cold pressure of the corresponding paste but the sunflower oil provided by company was refined oil produced by solvent extraction. The concentrations of phenolic compounds in ME of perilla, sesame and sunflower oils were 18.77, 88.64 and 0.05 μg tannic acid/mg, respectively (Table 1). Total phenol content in ME of sesame oil was the highest among three oils at 4.72, and 1772.8 folds greater than that for perilla and sunflower oil, respectively. In this study, the yields for ME and the concentration of phenolic compounds in ME of sesame oil are greater than those for perilla oil, which are in good agreement with results by Kim et al. (15) who used the same extraction procedure and the same method for total phenol determination as us.

Table 1. Yield for methanol extract of oils and the concentration of phenolic compounds

Oil	Yields (%)	Phenolic compounds (μg tannic acid/mg)
Perilla	0.57	18.77
Sesame	0.61	88.64
Sunflower[1)]	0.30	0.05

[1)]Sunflower oil was kindly provided by L Company (Seoul, Korea) without adding antioxidant in it but refined.

Table 2. IC_{50} for DPPH radical scavenging activity by the oil and its corresponding methanol extract (mg/mL)

	Oil[1)]	Methanol extract[2)]
Perilla	2.12[b]	0.42[b]
Sesame	1.91[c]	0.07[c]
Sunflower	3.35[a]	43.11[a]

[1)]Oil and DPPH were dissolved in iso-octane.
[2)]Methanol extract and DPPH were dissolved in methanol.
[a-c]Data with different letters are significantly different with ANOVA followed by Duncan's multiple range test at $p<0.05$.

Scavenging effect of oil and methanol extracts of oil on DPPH radical

As shown in Table 2, IC_{50} for DPPH radical of perilla, sesame, and sunflower oils were 2.12, 1.91, and 3.35 mg/mL, respectively and for ME of corresponding oils were 0.42, 0.07, and 43.11 mg/mL, respectively. The DPPH scavenging activity in ME of sesame oil was the highest and is recognizable compared with other samples ($p<0.05$) (16,17). According to these results, antiradical capacity in methanol extracts of sesame and perilla oil is superior to sunflower oil. However, antiradical activity in ME of sunflower oil is negligible. These results can be interpreted that antiradical substances in sesame and perilla oil are present in the methanol fraction while that for sunflower oil exist in the lipid fraction. These results are in good agreement with Espin et al. (16) who worked with 57 edible oils from different sources. They demonstrated that the highest antiradical capacity was in the methanol fraction of sesame and safflower oil, while the antiradical activity in the methanol fraction of sunflower oil was negligible. Dose dependent inhibition of DPPH radicals by oils (Fig. 1A) and its MEs (Fig. 1B) were observed. Linear inhibition curves for oil samples as well as ME samples were obtained. These results demonstrate that iso-octane can be used in the DPPH assay when oil sample is applied (12). According to the IC_{50} for DPPH of oil and the ME samples, the ME samples demonstrated greater DPPH radical scavenging activities than those of corresponding oils. These results indicate that antiradical capacity of phenolic compounds in the ME is greater than other antioxidants, such as tocopherols, in the lipid fraction of oil.

Free radical scavenging activity of methanol extracts of perilla, sesame and sunflower oil against reactive oxygen species

To evaluate the physiological activity of edible oils in terms of preventing oxidative damage, the free radical scavenging activities of oils were studied with methanol extracts in which phenolic compounds are present. As shown in Fig. 2A, superoxide anion scavenging activity

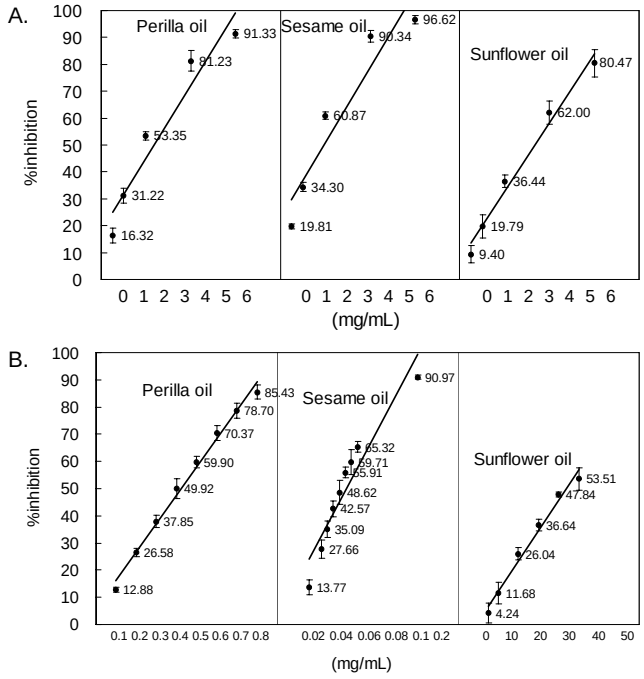

Fig. 1. Scavenging effect of oil and methanol extracts of oil on DPPH radical. A: Dose dependent inhibition on DPPH radicals by oils, B: Dose dependent inhibition on DPPH radicals by methanol extract of oils. Iso-octane was used for oil sample and methanol was used for methanol extracts of oil.

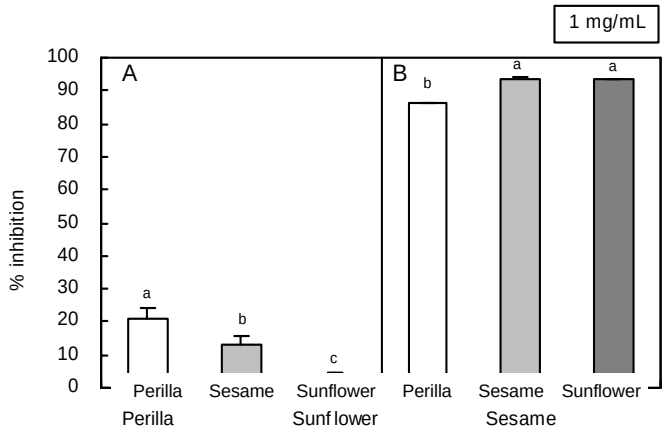

Fig. 2. Free radical scavenging activity of methanol extracts of perilla, sesame, and sunflower oils against reactive oxygen species. A: Superoxide anion scavenging activity of methanol extract of oils, B: Hydroxyl radical scavenging activity of methanol extract of oils. [a-c]Data with different letters are significantly different with ANOVA followed by Duncan's multiple range test at $p<0.05$.

of the perilla oil ME was the highest, followed by sesame and sunflower oil. The inhibition rate for the perilla oil ME tested at 1 mg/mL was 21.10±2.83% which is 1.6 and 6.7 folds greater than that for sesame (13.25± 2.60%) and sunflower oil (3.14±1.21%) MEs, respectively. Superoxide anion scavenging activity of perilla oil ME is higher than that for sesame oil, which is in line with the results reported by Kim et al. (15). Also, ME of sunflower oil showed antiradical activity against superoxide anion, which is in line with the result from DPPH radical assay; however, hydroxyl radical scavenging activities of those MEs observed in this study are much higher than for superoxide scavenging activity. Over 90% inhibition was observed from all three samples. The hydroxyl radical scavenging activity of sesame and sunflower oil MEs are significantly higher than the perilla oil ME.

In summary, antiradical capacity in the methanol fraction of edible oils was compared; for DPPH radicals, sesame oil showed greater activity than perilla oil. But for superoxide anion scavenging activity, perilla oil revealed superior activity to sesame oil. The methanol fraction of sunflower oil did not reveal any antiradical capacity to DPPH radical and superoxide anion.

DISCUSSION

The antiradical capacity of plant oils are widely studied using DPPH radical (16,18) with olive oils, and especially with virgin olive oils (4,9,19). The substance responsible for these antiradical activities is phenolic compounds, which are rich in unrefined olive oils, especially virgin olive oil (4). In this study we examined the antiradical capacity of perilla and sesame oils, which are consumed unrefined like virgin olive oil, and which Koreans favor in their cooking. Refined sunflower oil, whose major fatty acid is linoleic acid, was also examined. The yields for the ME of perilla and sesame oil were 2 fold higher than that for sunflower oil (refined oil), as expected. However, the amounts of phenolic compounds in ME from perilla and sesame oil are significantly different, although methanol extracts yields for both oils are not different. Kim et al. (15), who used same extraction process and assay method for phenol as we did, also reported higher phenol content in the methanol fraction of sesame oil compared to perilla oil. However, in our study, approximately 4.7 fold greater phenolic compounds in the ME of sesame oil than the ME of perilla oil were observed. For sunflower oil, very small amount of phenolic compounds were present in the ME. The refining process might remove the phenolic compounds (2). DPPH radical scavenging capacities are observed from both oil and methanol extracts of perilla and sesame oils. The antiradical activity in the methanol fractions are significantly more effective than the lipid fractions of these oils, indicating that the antiradical activity of phenolic compounds in ME are superior to the antioxidants present in lipid fraction. However, DPPH radical scavenging activity of sunflower oil is found be greater in the lipid fraction than the methanol fraction.

Antiradical capacity in methanol fraction of sunflower oil is negligible; IC_{50} for DPPH radical is 43.11 mg/mL. According to these results, antiradical substances seem to be present in the methanol fraction of perilla and sesame oils, but in the lipid fraction for sunflower oil. Soybean oil and corn oil are other oils that also have antiradical substance in the lipid fraction (16). These observations were confirmed in the present study when the antiradical effects of methanol extracts of the three oils were studied with superoxide anion. The MEs of perilla and sesame oil demonstrated antiradical capacity on superoxide anion while almost no effect was observed from ME of sunflower oil. When antiradical activity in methanol fraction of sesame and perilla oil is compared, sesame oil showed greater DPPH radical scavenging activity than perilla oil, but for superoxide anion, perilla oil demonstrated higher activity than sesame oil. These results are in good agreement with the results of Kim et al. (15). The differences in antiradical capacity against different radicals from identical oil samples might be due to the different types of antioxidants present in the corresponding oils. Both oxidant and antioxidants may, in some cases, act by multiple mechanisms depending on the reaction system. Furthermore, antioxidants may respond in a different manner to different radical or oxidant sources (20). A representative phenolic compound in sesame oil is sesamole derivatives. Among these, sesaminol is known to have the strongest antioxidant activity (21,22). And for perilla oil, several phenolic compounds such as rosemarinic acid, luteolin, chrysoeriol, quercetin, catcehin and apigenin have been identified (23-25). Besides these phenolic compounds in perilla oil, flavanoids and anthocyanins are also reported to have antioxidant properties. Not much information regarding the antioxidants in sunflower oil has been reported.

The antiradical effects of plant oil have been recently investigated, because of the low incidence of cardiovascular diseases in the populations that consume the Mediterranean-style diet in which the primary fat source is olive oil (1). These antiradical effects of olive oils have studied extensively with phenolic compounds primarily present in the methanol fraction of oil (4,16,19). These phenolic compounds have been shown to have good *in vitro* antioxidant and chemoprotective properties, which may have some biological functions *in vivo*. However, the extent of *in vivo* antioxidant and protective effects of phenolic constitutes depends on their bioavailability. Indeed, the apparent absorption of many phenolic acids after olive oil consumption has been reported in both human and animals. One important finding in this study regarding the DPPH assay is that iso-octane (12) can be used in the study using Microplate Reader for optical density measurement. The DPPH assay using microplate is introduced for mass sampling. In our study, ethyl acetate originally introduced in the DPPH assay for oil sample by Espin et al. (16) does not work properly when the microplate is used. DPPH radical dissolved in ethyl acetate demonstrated the stability and molar absorption same as in methanol solution (16). In our study, iso-octane worked on DPPH radicals properly (Fig. 1A) similarly to methanol (Fig. 1B). Linear inhibitions on DPPH radicals by various oil samples were observed to be concentration dependent, such as the ME of oil, in which iso-octane and methanol were used as solvent. Based on the results from this study, we can suggest a new method using iso-octane for the oil and lipid fraction in DPPH assay. If data can be collected from different fractions of the sample, such as total oil, methanol fraction and lipid fraction, then the data will be very informative for locating and identifying the antiradical substances present in the samples.

Oils composed of long chain unsaturated fatty acids are believed not to be healthy due to their susceptibility to oxidation. Lipid radicals produced during oxidation are very critical to the body because they readily participate in the chain radical reactions, which subsequently elevate the oxidative stress in the body. Oxidative damage by uncontrolled oxidative stress is well recognized as a cause of degenerative diseases, such as cancer, heart disease, atherosclerosis, rheumatoid arthritis and also for the normal aging process. Thus, it might be important to consume oils with high ROS scavenging activity for health concerns. In this study, the ME of the perilla oil showed the highest superoxide anion scavenging activity among three oils, and also demonstrated a competitive activity against DPPH and hydroxyl radicals to sesame oil.

In conclusion, the methanol fraction of cooking oils demonstrated antiradical activities against DPPH radical and ROS. The antiradical substances in methanol fraction of perilla and sesame oils, phenolic compounds, are more potent for scavenging DPPH radicals than the ones in the lipid fraction. Thus, we could conclude that unrefined oils contain greater antiradical activity in their methanol fractions than refined oils, and are recommended for daily consumption.

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