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ARTICLE

Influence of roasting conditions on the chemical properties and antioxidant activity of perilla oils

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Abstract Chemical properties of perilla oils under a variety of roasting conditions were evaluated. Roasting was performed in a hot-air roaster, with inlet air temperatures of 150–240 °C. The major fatty acid contents in perilla oil were α-linolenic, oleic, and linoleic acid. Trans-fatty acid content was 0.10–0.31 % from perilla oil under different roasting conditions. Maillard reaction products increased significantly from perilla oils exposed to higher temperatures for longer times. In particular, 2-methylpyrazine and 2,5-dimethylpyrazine were highest in the P210/30 (roasting temp. 210 °C, roasting time 30 min) and P210/30 treatments, respectively. Total benzo[a]pyrene of 0.21 μg/kg was detected in perilla oil under different roasting conditions. Evaluation of radical scavenging activity revealed that antioxidant function of perilla oil was promoted by increasing the roasting temperature of perilla seeds. As roasting temperature and time increased, the oxidative stability of perilla oils increased. The average iodine value of perilla oil under different roasting conditions was 154.23. Oxidation stability increased in the P240/20 group.

Keywords Antioxidant activity · Maillard reaction products · Perilla oils · Roasting

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Introduction

Many systematic studies have investigated natural plant components (Kim et al. 2000). It has been revealed that specific components from natural plants have direct or indirect effects on various systems in the human body such as the nervous, circulatory, endocrine, and immune systems. The Korean diet, which is mainly organized around vegetables, has attracted global attention. In particular, Korean traditional edible oils are notable because of their functional components. Perilla has been grown and consumed as a food in Asian countries for a long time. Perilla seeds have high omega-3 fatty acid content such as α-linolenic, docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA). Furthermore, they contain unsaturated fatty acids such as oleic acid (18:1) and linoleic acid (18:3). Eating marine products that contain high levels of EPA and DHA reduces the incidence of stroke and coronary heart disease (Kremer 1996). Perilla seed oils are prepared by roasting, physical pressing, and refining the raw perilla seeds (Park et al. 2011b). The oil extracted from roasted seed has many benefits such as color and flavor than those of oil extracted from unroasted seeds (Kim et al. 2000). Perilla oil roasted from seed contains many aromatic compounds and should be produced under heat treatment. The flavor profiles of perilla oils change under different roasting temperatures and times (Kim et al. 1995). In particular, the volatility of perilla oils is one of the most important factors that affect consumer acceptability. The Maillard reaction is an important reaction during which the volatile components appear. Perilla oils have a distinctive odor and volatile substances from the process of roasting. Kim et al. (2000) obtained more than 80 volatiles from perilla oil treated under different roasting conditions. Perilla seeds roasted at <170 °C develop relatively high

contents of aldehydes such as 2-methyl butanal, pyrazine, and furans, whereas Maillard reaction products (MRPs) are detected in perilla oil when the perilla seeds are roasted at temperatures >170 °C. Perilla oils from roasted seeds develop higher acidification than other vegetable oils due to their high unsaturated fatty acids.

Oxidative stress can increase the level of reactive oxygen species (ROS) production and induces cellular damage linked with numerous human diseases (Valko et al. 2007). Several studies reported that MRPs from food products are closely related to high oxidative stability (Abou-Gharbia et al. 1997; Abou-Gharbia et al. 2000; Lee et al. 2007; Shahidi and Naczk 2004) and possess antioxidant activity that attenuates cellular oxidative damages (Vhangani and Wyk 2013; Jung et al. 2014; Wu et al. 2014). Perilla oil has been also regarded, which possesses biological activities against atherosclerosis (Sadi et al. 1996), chemically induced cancers (Onogi et al. 1996), and asthma in vivo and in vitro (Deng et al. 2007) due to its oxidative stability and bioactive component content (Kim et al. 2000; Lee et al. 2004; Yoshida et al. 1995). Although most of these diseases related to with cellular damages induced by ROS, correlation between bioactive contents and antioxidant activity of perilla oil has not been elucidated. In addition, no studies have investigated both roasting temperature and time or reported the physical and chemical characteristics of perilla oils during storage. To examine whether traditional edible oils can attenuate these cell damage, we evaluated antioxidant activity of perilla oil in different roasting conditions against oxidative stress.

The objectives of the present study were to determine the changes in chemical properties including fatty acids, MRPs, and polycyclic aromatic hydrocarbons (PAHs) in perilla seeds under different roasting conditions. Antioxidant properties of perilla oil prepared by various roasting temperature were evaluated at the cellular level. Furthermore, we observed the oxidation characteristics, including the acid value (AV), peroxide value (POV), iodine value (IV), and color during storage of perilla oils.

Materials and methods

Materials

Perilla (*Perilla frutescens* var. *japonica* HARA) seeds were purchased from Gyeongbuk Andong, Korea. All solvents were high-performance liquid chromatography grade and obtained from Burdick & Jackson (Muskegon, MI, USA). Fatty acid methyl ester (FAME) and MRP standards including 2-methylpyrazine, 2.5-dimethylpyrazine, furfuryl alcohol, guaiacol, and 2-phenylpyridine were purchased from Sigma Chemical Co. (St Louis, MO, USA). Solid-phase macroextraction (SPME) fiber (65 μm polydimethylsiloxane divinylbenzene (PDMS-DVB)) was purchased from Supelco (cat. no. 57310-U; Belafonte, PA, USA), Headspace vials (20 mL) with screw caps, and a PETT septum was purchased from Supelco (cat. no. SU860097). A DB-23 column (60 m × 0.250 mm i.d. × 0.25 μm) and a DB-WAX capillary column (30 m × 0.32 mm i.d. × 0.25 μm) were purchased from J & W Scientific (Folsom, CA, USA).

Sample preparation

Roasting conditions to obtain the perilla oil from seed were obtained from previous studies (Kim et al. 2000; Lee et al. 2010; Park et al. 2011b). The perilla seeds were washed in tap water to remove impurities and then dried under sunshine to remove the remaining water. The washed perilla seeds were put in the drum of a hot-air coffee roaster (Gene cafe CBR-101, Seoul, Korea) and roasted at temperatures of 150, 180, 210, or 240 °C for 10, 20, or 30 min. The perilla oil from the roasted seeds was extracted using a small expeller (National Eng., NEH-404 K, Tokyo, Japan). The control was perilla oil from unroasted seed. Perilla oil that was roasted from seed at 150 °C for 10 min was designated as P150/10, and the control was designated as P0/0. All samples were prepared in triplicate.

Analysis of fatty acid content

Fatty acid composition of perilla oil was analyzed using an AOAC method with an Agilent 7890A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector (FID) after esterification with 14 % BF_3 in methanol (AOAC 2006). The injector and detector temperatures were 270 and 280 °C, respectively. The column was held at 50 °C for 1 min and programmed to rise to 175 °C at a rate of 25 °C/min and to 230 °C at a rate of 4 °C/min. It was held at 230 °C for 5 min. The carrier gas was nitrogen, with a flow rate of 1 ml/min and a split ratio of 20:1. Each FAME in the chromatogram was identified by comparing the retention times of a standard FAME mixture (37 component FAME mix, Supelco, Belafonte, PA, USA).

Analysis of MRPs by HS-SPME

The HS-SPME analytic conditions for MRPs were modified from previous studies (Park et al. 2011a, b). Two grams of perilla oil was placed in a 20 mL headspace vial and sealed with a Teflon-coated rubber septum and an aluminum cap. The sample bottles were placed in a water bath for 5 min at 50 °C, and MRPs in the perilla oil were isolated with 65-μm PDMS-DVB SPME fiber at 50 °C for 20 min. The isolated

MRP components were determined by gas chromatography (GC)-FID (Agilent Technologies 7890A gas chromatograph, Santa Clara, CA, USA) equipped with a DB-23 column (60.0 m × 0.25 mm × i.d., 0.25 μm). Injection time of SPME was 5 min at 250 °C. The carrier gas was nitrogen, with a flow rate of 1 mL/min in splitless mode. The column was held at 50 °C for 3 min and programmed to rise to 220 °C at a rate of 5 °C/min. Each MRP in the chromatogram was identified by comparing the retention times of MRP standards.

Analysis of polycyclic aromatic hydrocarbons (PAHs)

A 10 g sample was weighed and deposited in a separatory funnel, spiked with 1 mL of 100 μg/kg benzo[a]pyrene-d12 as internal standard. Then, 50 mL of N,N-DMF:H_2O (9:1, v/v) and n-hexane were added, and the mixture was shaken. The n-hexane layer was added to another separatory funnel and extracted twice with 25 mL of N,N-DMF:H_2O (9:1, v/v) followed by adding 50 mL n-hexane. The n-hexane layer was transferred to another separatory funnel. A 35 mL aliquot of n-hexane was added, and the mixture was washed twice with 40 mL deionized-H_2O. The resulting solution was dehydrated with anhydrous sodium sulfate (15 g) and concentrated under reduced pressure on a rotary evaporator at 35 °C to a final volume of 2 mL. The cleaned-up samples were preconditioned with 10 mL of dichloromethane, and 20 mL of n-hexane was used to activate a solid-phase extraction cartridge. The sample was eluted with 5 mL of n-hexane and 15 mL of a n-hexane-dichloromethane (3:1) mixture. The solution was concentrated to near dryness under a gentle stream of nitrogen gas at 37 °C. The dry residue was re-dissolved in 1 mL of dichloromethane and passed through a 0.45 μm PTFE membrane filter. An aliquot of 20 μL of this solution was injected into the GC/MS system (Agilent Technologies 6890 N/5975 MSD GC–MS).

Analysis of oxidation characteristics

The degree of oil oxidation was evaluated by measuring the AV, POV, and IV. Each perilla oil sample was kept in an incubator at 25, 50, or 70 °C for 18 days, and the oxidation values were measured by AOCS methods (AOCS 1997a, b, c).

Chromaticity

The change in color during storage of the perilla oil was determined by measuring the L^*, a^*, and b^* color scales using a Hunter Lab colorimeter (NE 4000; Nippon Denshoku Co., Tokyo, Japan): L^*, lightness from 0 (dark) to 100 (light); b^*, yellowness from a positive value (yellow) to a negative value (blue); and a^*, redness from a positive value (red) to a negative value (green).

Cell culture

Mouse epithelial fibroblast (MEF) cell line was purchased from the American type culture collection (ATCC, VA, USA). The cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) with 10 % Fetal Bovine Serum (FBS), 1 % penicillin streptomycin and grown in an atmosphere containing 5 % CO_2 in air at 37 °C.

Cell toxicity assay

Cell viability was measured by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. MEF cells (5×10^3cells/well) were seeded in 96-well plates (SPL Inc., Pocheon, Korea) with various concentrations of perilla oil samples dissolved in DMSO for 24 h. After 24 h incubation, the cells were washed with PBS, and then 10 μL MTT (5 mg/mL) in 100 μL medium was added to each well and incubated at 37 °C for 3 h. After incubation, MTT solutions were removed, and dimethyl sulfoxide (DMSO) was added to dissolve formazan crystals. And the absorbance was measured using enzyme-linked immunosorbent assay (ELISA, Molecular Devices, CA, USA) at a wavelength of 570 nm, and the percentage of cell viability was calculated.

Analysis of free radical scavenging activity

DPPH (Sigma-Aldrich) was dissolved in 80 %(v/v) ethanol to 200 μM sonicated for 5 min to obtain the stable free radical DPPH. Different concentrations of various samples prepared from perilla oil were thoroughly mixed in a 96-well plate (SPL Inc., Pocheon, Korea) with 100 μL of freshly prepared 200 μM DPPH ethanolic solutions. The plate was incubated at the room temperature in the dark for 30 min. The absorbance of the mixture was measured by enzyme-linked immunosorbent assay (ELISA, Molecular Devices, Sunnyvale, CA, USA) at wavelength of 517 nm, and the percentage (%) of free radical scavenging activity was calculated according to the following formula:

$$\begin{aligned}\text{Scavenging activity } (\%) = [1 - 1] \\ \times \{(\text{A517nm, sample} - \text{A517 nm, blank} \\ -\text{A517 nm, standard})/\text{A517 nm, control}\} \times 100\end{aligned}$$

Measurement of intracellular ROS

The formation of intracellular ROS was evaluated by fluorescence marker 2′,7′-dichlorofluorescein diacetate (DCF-DA). DCFH-DA is diffused into cells and is deacetylated by cellular esterase to non-fluorescent 2′, 7′-

dichlorodihydrofluorescein (DCFH), which is rapidly oxidized to highly fluorescent 2′, 7′-dichlorodihydrofluorescein (DCF) by ROS. MEF cells (5×10^3cells/well) were seeded in 96-well plates and incubated with various perilla oil samples for 6 h, and then cells were stimulated with H_2O_2 for 18 h. After incubation, the cells were washed with DPBS and then incubation with 10 μM DCF-DA in DPBS for 30 min. Stained cells were examined under fluorescence microplate reader (Tecan Group Ltd., Männedorf, Switzerland) at 485 nm/535 nm. Intracellular ROS generation was measured by the DCF fluorescence. The percentage of DCF fluorescence was compared with control. DCF fluorescence intensity, which represents the amount of intracellular ROS, was observed using fluorescence microscopy (Leica Microsystem, Wetzlar, Germany).

Statistical analysis

The results were analyzed with Sigma Stat 2.0 (Jandel Corp., San Rafael, CA, USA). A one-way analysis of variance (ANOVA) was performed. Appropriate comparisons were made using the Student–Newman–Keuls test for the one-way ANOVA analysis. A $P < 0.05$ was considered significant.

Results and discussion

Fatty acid analysis

Table 1 shows the fatty acid chemical characteristics of the perilla oil extracted and roasted under different conditions. The order of fatty acid contents under all conditions was the same: α-linolenic acid (C18:3, $n-3$) > oleic acid (C18:1n9c) > linoleic acid (C18:2n6c). Trans-fatty acid was detected at 0.10–0.31 %, and these results did not exceed standard specifications. These chemical characteristics were within the ranges reported by other studies (Kashima et al. 1991; Shin and Kim 1994). In particular, α-linolenic acid had the highest fatty acid content in perilla oil. Other studies have shown that perilla oil retains α-linolenic acid at 59–65 % (Longvah and Deosthale 1912; Shin and Kim 1994). α-Linolenic acid is widely known as a EPA and DHA precursor (Narisawa et al. 1991; Sauer et al. 2000).

MRP analysis

Changes in total 2-methylpyrazine, 2.5-dimethylpyrazine, furfuryl alcohol, guaiacol, and 2-phenylpyridine (MRPs) in perilla oil under different roasting conditions are shown in Table 2. Total MRPs increased significantly compared to those in the control P0/0 ($P < 0.05$) as perilla seeds were roasted for longer times and at higher temperatures. Total MRP content in the P210/30 group was higher than that in the other groups, and all MRPs increased sharply following roasting at 210 °C. The more roasting temperature increased, and the more MRPs increased. But the MRPs decreased slightly after roasting at 240 °C/10 min. These results were similar to those observed in previous studies (Park et al. 2011a, b).

The MRP contents in perilla oils exposed to different roasting conditions and the SPME-GC-FID results are shown in Table 2. No MRP compounds were detected in the P0/0 sample (control). 2-Methylpyrazine and 2,5-

Table 1 Fatty acid compositions of perilla oil under different roasting conditions

Roasting temperature (°C)	Roasting time (min)	Fatty acid (%)[a,b]					
		16:0	18:0	18:1	18:2	18:3*n*3	Trans fat
	Untoasted	6.55 ± 0.01a	3.08 ± 0.00ab	15.83 ± 0.01ab	13.21 ± 0.01a	61.19 ± 0.02b	0.09 ± 0.01b
	10	6.55 ± 0.02a	3.08 ± 0.02ab	15.61 ± 0.01a	13.22 ± 0.01a	61.40 ± 0.01b	0.10 ± 0.00a
150	20	6.55 ± 0.01a	3.07 ± 0.01a	15.61 ± 0.01a	13.25 ± 0.02ab	61.38 ± 0.01b	0.10 ± 0.00a
	30	6.68 ± 0.09a	3.14 ± 0.05ab	15.81 ± 0.14ab	13.42 ± 0.10c	60.81 ± 0.38b	0.10 ± 0.00a
	10	6.58 ± 0.01a	3.08 ± 0.02ab	15.61 ± 0.02a	13.26 ± 0.02ab	61.31 ± 0.04b	0.10 ± 0.00a
180	20	6.58 ± 0.02a	3.08 ± 0.01ab	15.61 ± 0.00a	13.25 ± 0.01ab	61.34 ± 0.01b	0.10 ± 0.00a
	30	6.60 ± 0.01a	3.09 ± 0.01ab	15.60 ± 0.01a	13.27 ± 0.01abc	61.29 ± 0.02b	0.10 ± 0.00a
	10	6.63 ± 0.01a	3.11 ± 0.02ab	15.62 ± 0.03a	13.27 ± 0.01abc	61.21 ± 0.02b	0.11 ± 0.00a
210	20	6.65 ± 0.01a	3.13 ± 0.03ab	15.67 ± 0.02a	13.31 ± 0.00abc	61.07 ± 0.01b	0.11 ± 0.00a
	30	6.72 ± 0.01a	3.15 ± 0.02ab	15.72 ± 0.03ab	13.31 ± 0.01abc	60.92 ± 0.01b	0.12 ± 0.00a
	10	6.68 ± 0.01a	3.12 ± 0.01ab	15.65 ± 0.01a	13.29 ± 0.01abc	61.06 ± 0.04b	0.13 ± 0.02a
240	20	7.01 ± 0.24b	3.28 ± 0.11b	15.98 ± 0.18b	13.37 ± 0.01abc	59.90 ± 0.71a	0.31 ± 0.02a
	30	6.88 ± 0.01ab	3.22 ± 0.02ab	16.00 ± 0.01b	13.40 ± 0.02bc	60.17 ± 0.02ab	0.23 ± 0.01c

[a] Mean values ± standard deviations for triplicate samples

[b] Values within a column with different are significantly different ($P < 0.05$)

Table 2 Maillard reaction products of perilla oil under different roasting conditions

Roasting temperature (°C)	Roasting time (min)	Maillard reaction products (MRPs; mg/kg)[a,b]				
		2-methylpyrazine	2.5-dimethylpyrazine	Furfurly alcohol	Guaiacol	2-phenylpyridine
0	Untoasted	ND	ND	ND	ND	ND
	10	1.10 ± 1.06a	0.35 ± 0.21a	ND	ND	ND
150	20	1.90 ± 0.98a	0.67 ± 0.01a	ND	ND	ND
	30	2.82 ± 1.22a	0.86 ± 0.32a	ND	ND	ND
	10	10.51 ± 2.64ab	2.27 ± 0.31a	0.30 ± 0.02a	ND	ND
180	20	21.68 ± 5.21bc	4.63 ± 0.45a	0.54 ± 0.31a	0.05 ± 0.13a	0.05 ± 0.02a
	30	22.30 ± 3.21bc	19.43 ± 0.32bcd	1.92 ± 0.34a	0.33 ± 0.32	0.33 ± 0.12a
	10	31.10 ± 4.69cd	27.54 ± 2.1d	0.16 ± 0.03a	2.10 ± 0.45b	0.40 ± 1.36b
210	20	38.52 ± 8.16cd	23.42 ± 3.23cd	1.10 ± 0.98a	1.48 ± 0.31ab	0.95 ± 0.87ab
	30	44.86 ± 8.32d	20.56 ± 5.26bcd	2.49 ± 1.57a	1.03 ± 0.21ab	1.23 ± 0.65ab
	10	42.96 ± 2.32cd	22.20 ± 2.16bcd	1.03 ± 0.13a	1.33 ± 0.98ab	1.53 ± 0.74ab
240	20	30.40 ± 5.26cd	14.15 ± 2.13bc	7.38 ± 2.35b	5.25 ± 0.12c	6.26 ± 0.33c
	30	29.00 ± 3.13cd	12.22 ± 3.66b	4.84 ± 1.36ab	13.05 ± 0.00d	15.30 ± 2.36d

[a] Mean values ± standard deviations of triplicate samples

[b] Value within a column with different letters is significantly different ($P < 0.05$)

ND not detected

Table 3 Polycyclic aromatic hydrocarbons (PAHs) in perilla oil under different roasting conditions

Sample	PAHs (μg/kg)[a,b]								
	B[a]A	CRY	B[b]F	B[k]F	B[a]P	D[ah]A	B[ghi]P	I[cd]P	Total PAHs
P0/0	0.71a	0.45a	0.17a	ND	0.13a	ND	ND	ND	1.46
P180/20[c]	0.89a	0.53a	0.30a	ND	0.16a	ND	ND	ND	1.88
P210/20	1.24b	0.47a	0.29a	ND	0.21a	ND	ND	ND	2.21
P240/20	1.34b	0.52a	0.54b	ND	0.22a	ND	ND	ND	2.62
Total	4.18	1.97	1.3	ND	0.72	ND	ND	ND	8.17

[a] Mean values ± standard deviations of triplicate samples

[b] Value within a column with different letters is significantly different ($P < 0.05$)

ND not detected

[c] P180/20 indicates perilla oil prepared at a 180 °C roasting temperature and a 20-min roasting time

dimethylpyrazine, furfuryl alcohol, and guaiacol with 2-phenylpyridine were first detected in perilla oil from the P150/10, P180/10, and P180/20 treatments, respectively. 2-Methylpyrazine and 2,5-dimethylpyrazine were detected more often than furfuryl alcohol, guaiacol, and 2-phenylpyridine, which were similar to a previous report (Kim et al. 2000). 2-Methylpyrazine and 2,5-dimethylpyrazine increased significantly following exposure to 210 °C/30 min and 210 °C/10 min but decreased under the 240 °C/10 min and 210 °C/20 min conditions, respectively. Furfuryl alcohol, guaiacol, and 2-phenylpyridine increased significantly as the perilla seeds were roasted for longer times and at higher temperatures. Furfuryl alcohol, one of the most abundant furan compounds, was most highly detected in the P240/20, and guaiacol and 2-phenylpyridine were most highly detected in the P240/30 treatments.

A previous study identified pyrazines from roasted sesame oil. Pyrazines in sesame oil represent 60.56 % of total volatiles from roasted seeds at 247 °C for 28 min (Park et al. 2011a). Pyrazines are detected in roasted coffee and soybean (Jung et al. 1997). According to that report, pyrazines of 3.07, 9.46, and 19.7 mg/100 g were extracted from roasted soybean at 130, 150, and 170 °C, respectively. The Maillard reaction has been hailed as an important mechanism for the formation of pyrazines and pyridine (Lojzova et al. 2009). During the roasting process of perilla seeds, the Maillard reaction is a very important chemical reaction that can be confirmed by the formation of pyrazines, furans, guaiacol, and 2-furanmethanol.

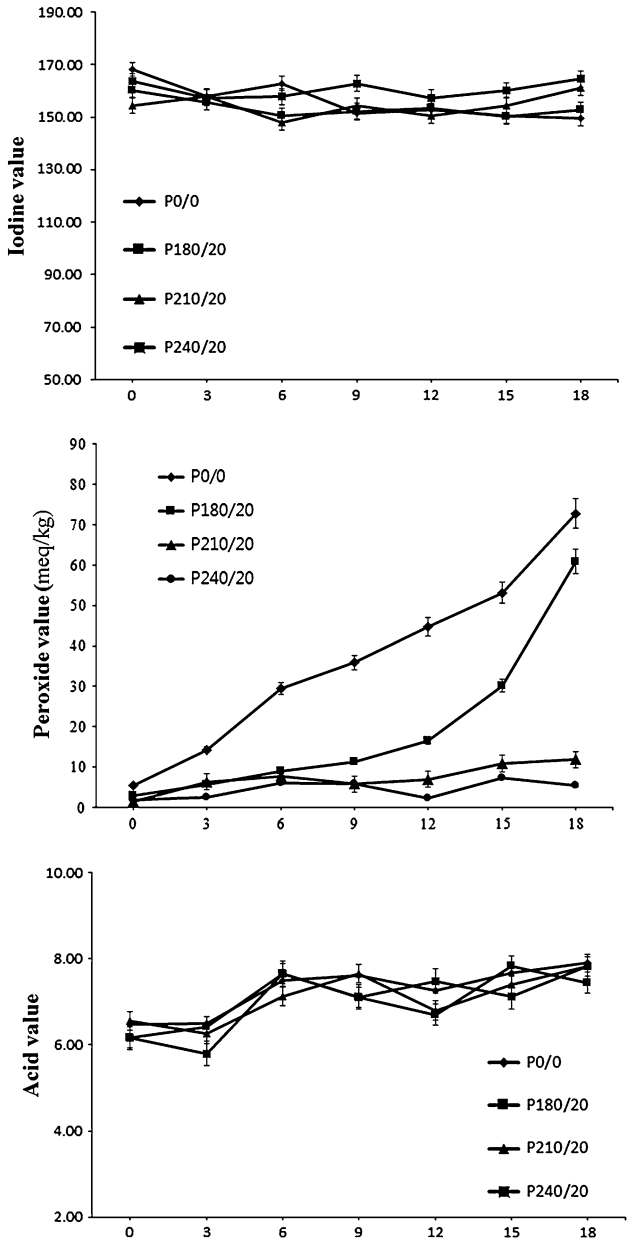

Fig. 1 Changes in oxidation characteristics of perilla oils under different roasting conditions during storage at 50°. Acid and peroxide values increased gradually, whereas the iodine value decreased during storage. Values are mean of three determinations

Analysis of PAHs

As perilla seeds were roasted for longer times and at higher temperatures, total PAHs of 8.17 μg/kg were detected (Table 3). Under each roasting condition, total PAH contents of the P0/0, P180/20, P210/20, and P240/20 were 1.46, 1.88, 2.21, and 2.62 μg/kg, respectively. Total benzo[a]pyrene of 0.21 μg/kg was detected in perilla oil, which was less than the 2.0 μg/kg detection limit offset by the European Food Safety Authority (EFSA).

Acid value analysis

Figure 1 shows the oxidation characteristics of perilla oil such as AV, POV, and IV under different roasting conditions. The AV and POV values increased gradually, whereas IV decreased during storage. Similar results were also observed in a previous report (Gökhan et al. 2006). AVs of stored perilla oil at 25, 50, and 70 °C are increased by an average of 2.0 under all roasting conditions. The amount of free fatty acids was represented by the AV in the edible oils, as AV increased by oxidation. The AV of edible oil is associated with lipase activity originating from microorganisms. A low AV is in edible oil <4.0 and shows that this limit was exceeded after the initial storage period.

Peroxide value analysis

The changes in POV of perilla oils during storage in an oven at 50 °C are shown in Fig. 1. The POV test, which is an index of the initial oxidation step, is used to evaluate the extent of lipid oxidation. The POVs of perilla oil stored at 50 and 70 °C increased significantly. As roasting temperature and time increased, the oxidative stability of the perilla oil under different roasting conditions increased. The POV of the P0/0 treatment was lower than the others following high roasting temperatures and time. The greater antioxidative stability of perilla oils prepared from seeds roasted at higher temperature and time probably occurred because of caramelization products that formed during roasting. The oxidative stability of sesame seed oil increases with increasing roasting temperature. Phosphorus and tocopherols have also been identified in perilla oil (Jung et al. 2012; Lee et al. 2004; Zhao et al. 2012). Higher roasting temperatures lead to greater formation of caramelization reaction products. MRPs are formed through the interaction of reducing sugars and proteins. Perilla oil shows strong antioxidant activities (Beckel and Waller 1983; Elizalde et al. 1992; Jung et al. 1999; Lee 1992).

Iodine value analysis

Oil with a high of IV contains a greater number of double bonds than one with a lower IV. The changes in the IV of perilla oils extracted under different roasting conditions are shown in Fig. 1. The perilla oils containing a large amount of $n-3$ PUFAs are more stable than other edible oils (Lee et al. 2008). The IVs of perilla oil under different roasting condition were not significantly different based on storage period. The average IV of perilla oil was 154.23. These values were considered acceptable.

Table 4 *L*, *a*, and *b* color changes in perilla oils under different roasting conditions

Storage temperature (°C)	Roasting temperature (°C)	Time (day)	Color		
			L^c	a^*	b^*
50 °C	Raw	0	75.43 ± 0.29j	−5.89 ± 0.96a	27.98 ± 0.69de
		3	75.46 ± 0.54j	−5.76 ± 0.17a	27.29 ± 0.33d
		6	64.86 ± 0.44hi	−3.81 ± 0.06abc	27.31 ± 0.97d
		9	64.70 ± 0.64hi	−3.69 ± 0.89abc	25.65 ± 0.46 cd
		12	67.64 ± 1.75i	−3.76 ± 0.04abc	24.68 ± 0.54bcd
		15	57.99 ± 1.80ef	−1.81 ± 0.31bcd	22.30 ± 1.23b
		18	59.60 ± 0.70fg	−1.67 ± 0.90bcd	19.86 ± 0.43a
	180 °C	0	62.66 ± 0.41gh	−4.61 ± 0.51ab	31.06 ± 0.92ef
		3	66.24 ± 0.27hi	−4.25 ± 0.55abc	30.95 ± 0.54ef
		6	51.96 ± 0.53cde	−2.11 ± 0.52bcd	28.28 ± 0.28de
		9	50.16 ± 1.88cd	−2.62 ± 0.69abc	28.06 ± 0.38de
		12	60.19 ± 1.49fg	−2.72 ± 0.84abc	27.13 ± 1.22d
		15	49.39 ± 0.39efg	−1.10 ± 1.08bcd	25.06 ± 0.82bcd
		18	44.49 ± 0.16d	−0.67 ± 0.05cd	22.62 ± 1.41bc
	210 °C	0	51.97 ± 0.46cde	−0.59 ± 1.88cd	40.01 ± 0.10h
		3	51.89 ± 0.42cde	−0.56 ± 1.63cd	39.22 ± 0.81h
		6	56.18 ± 0.36de	−1.30 ± 0.58bcd	39.59 ± 0.62h
		9	54.23 ± 1.92fgh	−2.02 ± 0.28bcd	37.97 ± 0.67gh
		12	54.04 ± 1.49fgh	−0.96 ± 0.73cd	36.59 ± 0.21g
		15	54.21 ± 0.59fgh	−0.71 ± 0.58cd	35.85 ± 0.41g
		18	37.26 ± 0.69c	1.42 ± 0.50d	30.74 ± 0.91ef
	240 °C	0	16.67 ± 0.82a	7.10 ± 0.41e	25.98 ± 0.45d
		3	16.50 ± 1.73a	7.05 ± 0.36e	25.29 ± 0.05bcd
		6	16.70 ± 1.39a	7.18 ± 0.17e	25.76 ± 1.08cd
		9	23.03 ± 0.64b	6.69 ± 0.89e	32.99 ± 0.46f
		12	22.62 ± 0.60b	6.65 ± 0.92e	32.68 ± 0.33f
		15	22.49 ± 0.58b	7.61 ± 0.61e	32.07 ± 1.07f
		18	23.24 ± 0.85b	6.36 ± 0.08e	32.42 ± 1.20f

[a] Mean values ± standard deviations of triplicate samples

[b] Value within a column with different letters is significantly different ($P < 0.05$)

[c] *L*, a^*, and b^* values are whiteness, redness, and yellowness, respectively

L *L* = 0 yields black and *L* = 100 indicates diffuse white, a^* Negative values indicate green while positive values indicate magenta, b^* Negative values indicate blue and positive values indicate yellow

Chromaticity analysis

Color changes in the perilla oils under different roasting condition using are shown in Table 4. As more thermal energy was introduced into the perilla seeds from roasting, the *L* values tended to decrease, whereas the *a* and *b* values appeared to increase. These values are commonly used to show changes in food color (Venkatesh Murthy et al. 2008). A dark brown color and a low *L* value were observed for perilla oils that had been exposed to higher roasting temperatures. The dark color was caused by chemical reactions including the Maillard reaction (Park et al. 2011b). The *L* value of perilla oil did not change significantly during storage. The *a* value increased slightly, whereas the *b* value decreased. The *L* value increased with increasing temperature, and the *b* value increased rapidly at 240 °C after 9 days.

DPPH free radical scavenging activity

The radical scavenging activity of perilla oil prepared under different roasting temperature and concentration was determined by using the chemical DPPH. The DPPH radical scavenging activity assay is a simple and accurate method in the evaluation of antioxidant activity in vitro. This stable radical is reduced to the corresponding hydrazine by changing their color once the free radical meets hydrogen donors (Molyneux 2004). The result showed that

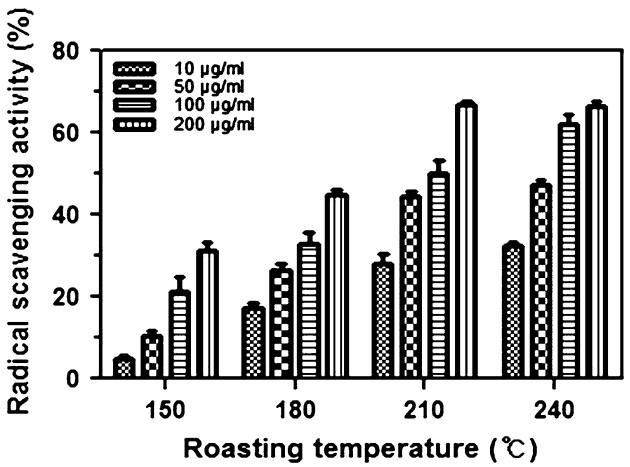

Fig. 2 DPPH radical scavenging activities of perilla oil under different roasting conditions. Values are mean of three determinations

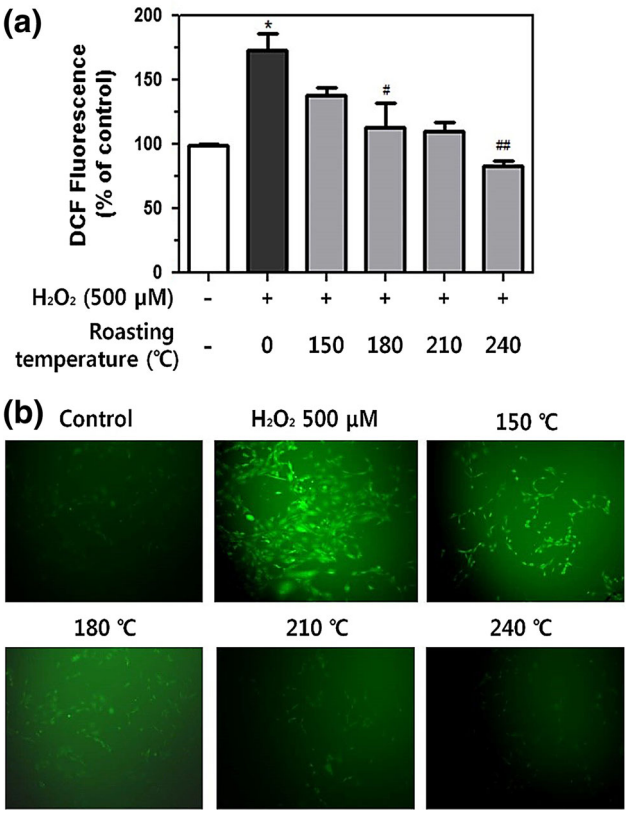

Fig. 3 The level of intracellular ROS on perilla oil under different roasting temperature in MEF cells (**a**). The level of ROS accumulation was determined using fluorescence microscope (×40) (**b**). Values are mean of three determinations. $^{*}P < 0.05$ was considered significant for control cells. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ was considered significant difference relative to H_2O_2-treated group

the removal of DPPH free radical was significantly increased by the perilla oil samples in roasting temperature and concentration-dependent manner (Fig. 2). Elevation on

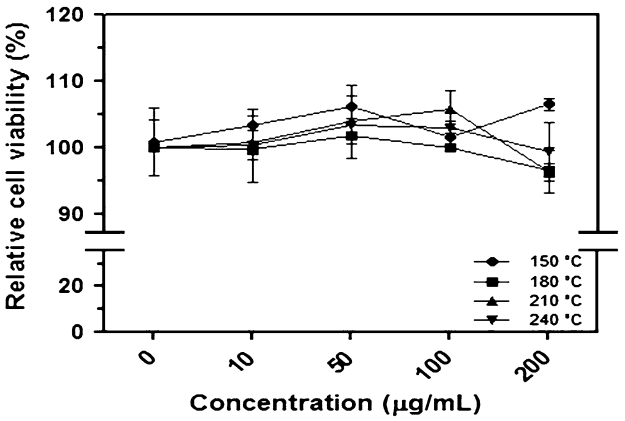

Fig. 4 Cytotoxicity of perilla oil prepared under different roasting conditions. Values are mean of three determinations

radical scavenging activity of P150/20, P180/20, P210/20, and P240/20 coincided with the increase in MRPs in the samples prepared at higher roasting temperature (Table 2). Hydroxyl radical scavenging activity of MRPs derived from fructose-lysine and ribose-lysine systems has been reported previously (Vhangani and Wyk 2013). Accordingly, our result suggests that the MRPs from perilla oils may act as an effective antioxidant due to their radical scavenging properties.

Measurement of intracellular ROS

ROS such as hydroxyl radicals, superoxide anions, and H_2O_2 are known for the main cause of cellular damages and linked with numerous human diseases (Valko et al. 2007). Therefore, one of the key roles to be an effective antioxidant is to remove excessive ROS from the cells. DCF diacetate was used to identify intracellular ROS generation. To examine the effect of roasting temperature in perilla oils preparation on the intracellular ROS content, MEF cells were treated with 100 μg/ml perilla oil of P150/20, P180/20, P210/20, and P240/20 and then exposed to H_2O_2. The amount of intracellular ROS, indicated by the fluorescence intensity, was increased by ~60 % by exposure MEF cells to H_2O_2, as compared to untreated control. However, treatment of the perilla oil resulted in the reduction of intracellular ROS (Fig. 3a). Higher the roasting temperature in perilla oil extraction, the effect of perilla oil on intracellular ROS removal was improved. Microscopic analysis also showed that less bright DCF green fluorescence was observed in the cells co-treated with perilla oil samples compared to the cells treated with H_2O_2 alone (Fig. 3b). These results suggest that the protective effect against oxidative stress by perilla oil was increased in a roasting temperature-dependent manner by attenuating intracellular ROS formation.

Cytotoxicity analysis

To examine whether the increased roasting temperature of perilla oil preparation affect the cell viability, we evaluated cytotoxicity of P150/20, P180/20, P210/20, and P240/20 on MEF cells by MTT assay. Cells were incubated with perilla oil prepared in different roasting conditions for 24 h. As shown in Fig. 4, more than 90 % of cells were survived with treatment all perilla oil samples, and no apparent cytotoxicity was observed compared to the control (Fig. 4). It suggested that increased bioactive compounds including PAHs and MRPs in the perilla oil obtained by raising the roasting temperature were not toxic to the cells .

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