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Antioxidant properties of methanolic extracts from *Antrodia camphorata* with various doses of γ-irradiation

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Abstract

The mycelia of *Antrodia camphorata* (Chang & Chou) Wu, Ryvarden & Chang were irradiated with gamma rays at doses of 2.5, 5, 10, 15 and 20 kGy and the antioxidant properties of the methanolic extracts were studied. At 2.5 mg/ml, antioxidant activities of methanolic extracts from 10 to 20 kGy-irradiated mycelia were significantly higher than those of the non-irradiated control. Reducing powers of methanolic extracts from unirradiated and 0.5–7.5 kGy-irradiated mycelia were comparable except for the 20 kGy-irradiated mycelia. At 2.5 mg/ml, all methanolic extracts showed excellent scavenging abilities of 92.3–103% on DPPH radicals. Scavenging abilities of methanolic extracts from 2.5 to 20 kGy irradiated mycelia were better than that of the unirradiated control at 10 mg/ml. With irradiation at 5–20 kGy, mycelia possessed higher chelating ability on ferrous ions than did the unirradiated control. The EC_{50} values were below 15 mg/ml, except for values of scavenging ability of the unirradiated control on hydroxyl radicals. Total phenols were the major naturally occurring antioxidant components found in the range of 13.0–15.5 mg/g. In summary, γ-irradiation not only maintained the antioxidant properties of mycelia but also enhanced the antioxidant properties to some extent.
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Keywords: *Antrodia camphorata*; Mycelia; Antioxidant activity; Scavenging ability; Chelating ability; Antioxidant components; γ-Irradiation

1. Introduction

Antrodia camphorata (Chang & Chou) Wu, Ryvarden & Chang (Chang-chih or niu-chang-ku) in the Polyporaceae (Aphyllophorales) causes brown heart rot of the endemic evergreen, *Cinnamomum kanehirai* Hay (Lauraceae), in Taiwan (Wu, Ryvarden, & Chang, 1997). "Niu-chang" is the Chinese common name for *C. kanehirai*, which is one of the endangered species in Taiwan; "ku" in Chinese means mushroom and "chih" means *Ganoderma*-like fungus. The red to light cinnamon, resupinate, effused-reflexed to pileate fruit bodies of *A. camphorata* are very bitter in taste and have a mild camphor oil odour like the host woods (Chang & Chou, 1995). The mycelia isolated from the fruit body forms orange red, orange brown to light cinnamon colonies (Chang & Chou, 1995).

Chang-chih, specifically referred to as the fruit body of *A. camphorata*, is well known in Taiwan as an expensive medicinal material, and is commonly used as an antidote, anticancer, antiitching and hepatoprotective drug. Fruit bodies of *A. camphorata* are expensive and scarce, partially due to their rareness and difficulty of cultivation. Thus, *A. camphorata* is mainly prepared from submerged culture in the form of mycelium for use in the formulation of nutraceuticals and functional foods.

During processing, storage and marketing, the harvested *A. camphorata* mycelium is prone to microbial contamination and insect infestation resulting in quality deterioration and economic loss. Although, several methods for preventing the contamination have been developed, exposure to ionizing radiation, such as γ-rays, is one of the currently practiced methods. Irradiation to an overall average dose

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of 10 kGy has proved to present no toxicological hazards and to introduce no specific microbial and nutritional problems [International Atomic Energy Agency (IAEA, 1992].

Several studies on spices γ-irradiation at doses up to 10–15 kGy have shown that no substantial changes occurred in volatile oils, favour profiles, spicing power (IAEA, 1992) or antioxidant properties (Kuruppu, Schmidt, Langerak, Van Duren, & Farkas, 1985). Nevertheless, many studies had shown that γ-irradiation really affected the antioxidant properties of truffles (Adamo et al., 2004), ground beef (Ahn & Nam, 2004), herbs and spices (Calucci et al., 2003), green tea leaf extracts (Jo, Son, Lee, & Byun, 2003), lupin seed products (Lampart-Szczapa, Korczak, Nogala-Kalucka, & Zawirska-Wojtasiak, 2003) and soybean (Variyar, Limaye, & Sharma, 2004). However, the effect of γ-irradiation on *A. camphorata* mycelia has not been investigated.

Our objective was to study and compare the antioxidant properties of methanolic extracts from *A. camphorata* mycelia with various doses of γ-irradiation. Antioxidant properties were assayed in terms of antioxidant activity by the conjugated diene method, reducing power, scavenging abilities on 1,1-diphenyl-2-picrylhydrazyl (DPPH·) and hydroxyl radicals and chelating ability on ferrous ions. The contents of potential antioxidant components of methanolic extracts were also determined.

2. Materials and methods

2.1. Mycelia

Freeze-dried red mycelia of *A. camphorata* were originally obtained from the Biotechnology Center, Grape King Inc., Chungli, Taiwan. Mycelia were ground into a fine powder (60 mesh) by using a mill (Retsch ultracentrifugal mill and sieving machine, Haan, Germany); a subsample (20 g) was packaged in polyethylene packaging. Packaged mycelia were then transported to China Biotech Corp., Taichung City, Taiwan. Mycelia were either unirradiated (control) or γ-irradiated with doses of 2.5, 5, 10, 15 or 20 (±10%) kGy of ^{60}Co (600,000 Ci, 6.78×10^2 kGy/h) radiation at ambient temperature. For each unirradiated or irradiated sample, three packages of mycelia were randomly selected for analyses. A subsample (2 g) was extracted by vortexing with 10 ml of methanol at 25 °C for 2 min and filtering through Whatman No. 4 filter paper. The residue was then extracted with two additional 5 ml portions of methanol, as described above. The combined methanolic extracts were then rotary evaporated at ambient temperature to dryness. The dried extract obtained was used directly for analyzing of antioxidant components or dissolved in methanol to a concentration of 30 mg/ml and stored at 4 °C for further use.

2.2. Antioxidant activity

The antioxidant activity was determined by the conjugated diene method (Lingnert, Vallentin, & Eriksson, 1979). Each extract (2.5–30 mg/ml) in methanol (100 μl) was mixed with 2 ml of 10 mM linoleic acid (Sigma Chemical Co., St. Louis, MO) emulsion in 200 mM sodium phosphate buffer (pH 6.5, Wako Pure Chemical Co., Osaka, Japan) in test tubes and placed in darkness at 37 °C to accelerate oxidation. After incubation for 15 h, 6 ml of 60% methanol (Mallinckrodt Baker, Paris, KY) in deionized water were added, and the absorbance of the mixture was measured at 234 nm against a blank in a Hitachi U-2001 spectrophotometer. The antioxidant activity was calculated as follows: antioxidant activity (%) = [(ΔA_{234} of control − ΔA_{234} of sample)/ΔA_{234} of control] × 100. A control consisted of methanol and the reagent solution without methanolic extracts added and the procedure was carried out as described above. A value of 100% indicated the strongest antioxidant activity. EC_{50} value (mg extract/ml) is the effective concentration at which the antioxidant activity was 50% and was obtained by interpolation from linear regression analysis. Ascorbic acid (Sigma), butylated hydroxyanisole (BHA, Sigma) and α-tocopherol (Sigma) were used for comparison.

2.3. Reducing power

The reducing power was determined according to the method of Oyaizu (1986). Each extract (0.5–10 mg/ml) in methanol (2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6, Wako) and 2.5 ml of 1% potassium ferricyanide (Sigma), and the mixture was incubated at 50 °C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid (w/v, Wako) were added, the mixture was centrifuged at 200*g* for 10 min. The upper layer (5 ml) was mixed with 5 ml of deionized water and 1 ml of 0.1% ferric chloride (Wako), and the absorbance was measured at 700 nm against a blank. A higher absorbance indicated a higher reducing power. EC_{50} value (mg extract/ml) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation from linear regression analysis. Ascorbic acid, BHA and α-tocopherol were used for comparison.

2.4. Scavenging ability on 1,1-diphenyl-2-picrylhydrazyl radicals

Each extract (0.5–10 mg/ml) in methanol (4 ml) was mixed with 1 ml of methanolic solution containing DPPH (Sigma) radicals, resulting in a final concentration of 0.2 mM DPPH·. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm against a blank (Shimada, Fujikawa, Yahara, & Nakamura, 1992). The scavenging ability was calculated as follows: scavenging ability (%) = [(ΔA_{517} of control − ΔA_{517} of sample)/ΔA_{517} of control] × 100. EC_{50} value (mg extract/ml) is the effective concentration at which DPPH radicals were scavenged by 50% and was obtained by interpolation from linear regression

analysis. Ascorbic acid, BHA and α-tocopherol were used for comparison.

2.5. Scavenging ability on hydroxyl radicals

The hydroxyl radicals reacted with the nitrone spin trap 5,5-dimethyl pyrroline-*N*-oxide (DMPO, Sigma) and the resultant DMPO-OH adducts were detected with an electron paramagnetic resonance (EPR) spectrometer. The EPR spectrum was recorded 2.5 min after mixing each extract (5.0 and 10 mg/ml) in methanol (200 μl) with 200 μl of 10 mM H_2O_2 (Merck, Darmstadt, Germany), 200 μl of 10 mM Fe^{2+} (Sigma) and 200 μl of 10 mM DMPO, using a Bruker EMX-10 EPR spectrometer at the following settings: 3480-G magnetic field, 1.0 G modulation amplitude, 0.5 s time constant, and 200 s scan period (Shi, Dalal, & Jain, 1991). The scavenging ability was calculated by subtracting the relative EPR signal intensity from 100. The relative EPR signal intensity was calculated as follows: relative EPR signal intensity (%) = [$h\Delta H^2$ (sample)/$h\Delta H^2$ (control)] × 100; h is the width of the peak; ΔH is the length of the peak. α-Tocopherol, at 10 mg/ml, was used for comparison.

2.6. Chelating ability on ferrous ions

Chelating ability was determined according to the method of Shimada et al. (1992). Each extract (0.5–10 mg/ml) in methanol (2 ml) was mixed with 200 μl of 1 mM tetramethyl murexide (TMM, Sigma) and 2 ml of the mixture reagent consisting of 30 mM hexamine (Wako), 30 mM potassium chloride (Sigma) and 9 mM ferrous sulfate (Union Chemical Works, Hsinchu, Taiwan). After 3 min at room temperature, the absorbance of the mixture was determined at 485 nm against a blank. A lower absorbance indicated a higher chelating power. EC_{50} value (mg extract/ml) is the effective concentration at which ferrous ions were chelated by 50% and was obtained by interpolation from linear regression analysis. Ethylenediaminetetraacetic acid (EDTA, Sigma) was used for comparison.

2.7. Determination of antioxidant components

Ascorbic acid was determined according to the method of Klein and Perry (1982). Each methanolic extract (20 mg) was extracted with 10 ml of 1% metaphosphoric acid (Union) for 45 min at room temperature and filtered through Whatman No. 4 filter paper. The filtrate (1 ml) was mixed with 9 ml of 2,6-dichloroindophenol (Sigma) and the absorbance was measured within 15 s at 515 nm against a blank. Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid.

β-Carotene was extracted and analysed as described by Rundhaug, Pung, Read, and Bertram (1988). Each methanolic extract (20 mg) was extracted with a solution of 1% pyrogallol (Wako) in 10 ml of methanol/dichloromethane (1:1, v/v) for 45 min at room temperature, filtered through Whatman No. 4 filter paper and adjusted by volume to 10 ml using the same solution. The filtrate was then passed through a filter unit (13 mm, Lida Corp., Kenosha, WI) and filtered using a 0.45 μm CA filter paper prior to injection onto a high performance liquid chromatograph (HPLC).

The HPLC system consisted of a Shimadzu LC-10AT VP pump, a Rheodyne 7725i injector, a 20 μl sample loop, SPD-10A VP UV–VIS detector, and a Li Chrospher 100 RP-18 column (4.6 × 250 mm, 5 μm, Merck, Darmstadt, Germany). The mobile phase was acetone/methanol/acetonitrile, 1:2:2 (v/v/v), at a flow rate of 0.7 ml/min and UV detection was at 470 nm. Content of β-carotene was calculated on the basis of the calibration curve of authentic β-carotene (Sigma).

Tocopherols were extracted and analyzed according to the method of Carpenter (1979). Each methanolic extract (50 mg) was suspended in 6 ml of pyrogallol (6% in 95% ethanol) and 4 ml of 60% aqueous potassium hydroxide solution, and the resulting mixture was saponified at 70 °C for 20 min. Deionized water (15 ml) was added and the mixture was extracted with 15 ml of *n*-hexane. The organic layer was washed with deionized water to neutralize, dried over anhydrous sodium sulfate, and rotary evaporated to dryness. The residue was redissolved in 5 ml of *n*-hexane and filtered prior to HPLC injection in the same manner as in the β-carotene assay.

The HPLC system was the same as for the β-carotene assay. The mobile phase was acetonitrile/methanol, 85:15 (v/v), at a flow rate of 1.0 ml/min and UV detection was at 295 nm. Content of each tocopherol was calculated on the basis of the calibration curve of each authentic tocopherol (Sigma).

Total phenols were determined according to the method of Taga, Miller, and Pratt (1984). Each methanolic extract (20 mg) was dissolved in a solution of 5 ml of 1.3% HCl in methanol/deionized water (60:40, v/v) and the resulting mixture (100 μl) was added to 2 ml of 2% aqueous sodium carbonate solution. After 3 min, 100 μl of 50% Folin–Ciocalteau phenol reagent (Sigma) were added to the mixture. After 30 min standing, the absorbance was measured at 750 nm against a blank. The content of total phenols was calculated on the basis of the calibration curve of gallic acid (Sigma).

2.8. Statistical analysis

For each methanolic extract from *A. camphorata* mycelia with various doses of γ-irradiation, three samples were prepared for assays of every antioxidant attribute and component. The experimental data were subjected to an analysis of variance for a completely random design to determine the least significant difference at the level of 0.05.

3. Results and discussion

3.1. Extraction yield

Extraction with methanol showed that 15 and 20 kGy-irradiated *A. camphorata* mycelia had higher yields (53.1 and 51.8%, respectively) than had non-irradiated control and mycelia irradiated with lower doses (Table 1). Higher doses of irradiation, such as 15 and 20 kGy could increase the extraction yields by 11.6 and 8.8%, respectively. It seems that 15–20 kGy, of irradiation might induce chemical reaction in components of *A. camphorata* mycelia, which might degrade or decompose large molecules into small molecules readily soluble in methanol, and then result in the production of more methanol-soluble substances.

Similarly, Kim, Yook, & Byun (2000) also found the total extraction yields in 15 kinds of Korean medicinal herbs, using various solvents, increased by 5–25% with a dose of 10 kGy of γ-irradiation. Apparently, 15 or 20 kGy irradiation might be an effective method for higher methanolic extraction yield of *A. camphorata* mycelia. Using the same extractant, the extraction yields of *A. camphorata* red mycelia, with or without γ-irradiation (44.6–53.1%) were higher than those of *A. camphorata* white mycelia (31.1%) and fresh and air-dried fruit bodies (41.6 and 33.9%; Huang, 2000). In addition, Mau, Huang, Huang, & Chen (2003) found that the methanolic yields constantly decreased from 32.56% for submerged mycelia harvested at day 7 to 21.8% for mycelia at day 13, and retained the level of 20.1–21.8% for mycelia at days 13–18. Mau, Huang, Huang, & Chen (2004) found that methanolic yields of *A. camphorata* red and white mycelia were 37.8 and 27.5%, respectively. However, the discrepancies in yields might be due to difference in strains used and extraction solvents, extraction methods and times employed.

3.2. Antioxidant activity

Using the conjugated diene method, the antioxidant activities increased as concentrations increased from 2.5 to 30 mg/ml for methanolic extracts of *A. camphorata* mycelia, with or without γ-irradiation (0–20 kGy) (Fig. 1). At 2.5 mg/ml, the antioxidant activities of all methanolic extracts were above 50%, indicating that *A. camphorata* mycelia had good antioxidant activity. At 2.5 mg/ml, the antioxidant activities of methanolic extracts from 10 to 20 kGy irradiated mycelia were significantly higher than those of the non-irradiated control.

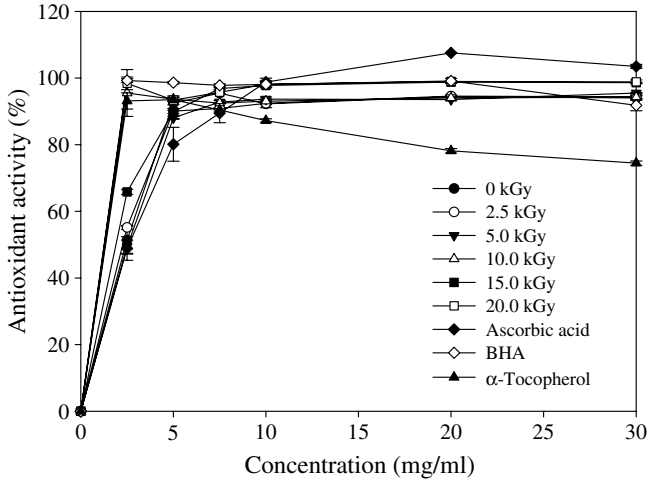

Fig. 1. Antioxidant activity of methanolic extracts from *Antrodia camphorata* mycelia with various doses of γ-irradiation (conjugated diene method). Each value is expressed as mean ± standard deviation ($n = 3$).

Surprisingly, the methanolic extracts from 10 to 20 kGy-irradiated *A. camphorata* mycelia exhibited excellent antioxidant activities of 92.5–95.5% and 93.4–99.0% at 2.5–30 mg/ml, respectively. In addition, the methanolic extracts from 15 to 20 kGy-irradiated *A. camphorata* mycelia showed slightly higher antioxidant activities (97.8–99.0%) than did that of non-irradiated control (92.3–94.0%) at 10–30 mg/ml. However, the antioxidant activities were 98.8% at 10 mg/ml for ascorbic acid, 99.3% at 2.5 mg/ml for BHA and 93.1% at 2.5 mg/ml for α-tocopherol.

Huang (2000) found that methanolic extracts, from white mycelia, fresh and air-dried fruit bodies, showed good antioxidant activities of 87.7, 93.0 and 91.2% at concentrations as low as 1 mg/ml, respectively. Song & Yen (2002) found that methanolic extracts from red mycelia inhibited lipid peroxidation by 44% at 0.2 mg/ml. Mau et al. (2003) found that, at 10 mg/ml, antioxidant activities of methanolic extracts were low for submerged mycelia at day 7 (42.2%), moderate for mycelia at day 10 (77.5%) and high for mycelia at days 13 and 18 (94.4–100%). At 5 mg/ml, Mau et al. (2004) found the antioxidant activities were 40.6% and 69.3% for red and white mycelia, respectively.

Evidently, 10–20 kGy of γ-irradiation could slightly enhance antioxidant activities of methanolic extracts from *A. camphorata* mycelia. Similarly, Ahn, Kim, Jo, Kim, and Byun (2004) reported that phytic acid, irradiated at 20 kGy, showed higher antioxidant activity, as evidenced by significantly reduced 2-thiobarbituric acid-reactive substances (TBARS) values in the lipids. Nevertheless, Byun, Yook, Kim, and Chung (1999) found that γ-irradiation

Table 1
Extraction yield of methanolic extracts from *Antrodia camphorata* mycelia with various doses of γ-irradiation

Dose (kGy)	Extraction % (w/w)
0 (Control)	47.6 ± 2.08 B[a]
2.5	44.6 ± 1.83 B
5	45.2 ± 1.41 B
10	46.3 ± 0.14 B
15	53.1 ± 1.72 A
20	51.8 ± 2.54 A

[a] Each value is expressed as mean ± standard deviation ($n = 3$). Means with different letters within a column are significantly different ($P < 0.05$).

of Korean medicinal herbs at 10 kGy had no effect on their antioxidative capacity.

Using thiobarbituric acid and peroxide values as indicators of the antioxidant activity of turmeric extracts, Chatterjee, Desai, & Thomas (1999) also found that 10 kGy of γ-irradiation did not show any effect on their antioxidant activity. In addition, using the β-carotene/linoleic acid system, Kitazuru, Moreira, Mancini-Filho, Delincée, & Villavicencio (2004) found that 5–25 kGy of γ-irradiation did not show any effect on the antioxidant potential of cinnamon compounds. On the other hand, Lampart-Szczapa et al. (2003) reported that increased doses of irradiation decreased antioxidant effects of most lupin extracts. Evidently, γ-irradiation would enhance the antioxidant activity in some foods whereas in others the antioxidant activity was reduced. So far, no general pattern of γ-irradiation on antioxidant activity has been found.

3.3. Reducing power

Reducing powers (expressed as absorbance at 700 nm) of all methanolic extracts from mycelia increased rapidly from 0.20–0.30 to 2.14–2.34 at low concentrations (from 0.5 to 5.0 mg/ml), except for 20 kGy-irradiated mycelia (Fig. 2). Reducing powers of methanolic extracts from unirradiated and 2.5–15 kGy irradiated mycelia were comparable, whereas reducing powers of the methanolic extract from 20 kGy-irradiated mycelia increased slowly from 0.20 to 1.10 at 0.5 to 10 mg/ml. However, reducing powers of ascorbic acid, BHA and α-tocopherol were 2.24, 2.25 and 2.06 at 0.5 mg/ml, respectively.

Using the ferric reducing/antioxidant power method, Ahn et al. (2004) reported that the reducing power of phytic acid was significantly increased by irradiation. In addition, using the same method, Ahn et al. (2005) found that 0.5 kGy of irradiation increased or maintained the antioxidant activity of the minimally processed Chinese cabbage. However, Byun, Yook, Kwon, and Kang (1997) applied γ-irradiation to Korean red ginseng powder and found that its hydrogen donating activity was not significantly changed by up to 10 kGy of γ-irradiation.

Huang, Huang, Chen, and Mau (1999) found that methanolic extracts from fresh and air-dried *A. camphorata* fruit bodies showed excellent reducing powers of 0.94 and 0.92 at 5 mg/ml, respectively, whereas the reducing power of methanolic extracts from white mycelia was 0.62. In addition, Mau et al. (2003) found that reducing powers of methanolic extracts from submerged mycelia at day 7, 10, 13, 16 and 18 were good (>0.64) at 2.5–10 mg/ml. Mau et al. (2004) also reported that reducing powers from *A. camphorata* white mycelia were higher than those from red mycelia at 2.5–7.5 mg/ml, but the reducing power from red mycelia increased to 1.24 and was higher than that from white mycelia at 10 mg/ml. The reducing power of *A. camphorata* mycelia might be due to its hydrogen-donating ability, as described by Shimada et al. (1992). Accordingly, *A. camphorata* mycelia might contain some reductones, which could react with free radicals to stabilize and terminate radical chain reactions. Apparently, the irradiation of *A. camphorata* mycelia might produce and release less reductones.

3.4. Scavenging ability on 1,1-diphenyl-2-picryhydrazyl (DPPH) radicals

Scavenging abilities of all methanolic extracts on DPPH radicals were 50.5–80.0% at 1 mg/ml, indicating that *A. camphorata* mycelia were also good in this scavenging ability (Fig. 3). At 2.5 mg/ml, all methanolic extracts showed excellent scavenging abilities of 92.3–103% on DPPH radicals. Obviously, scavenging abilities were comparable for all methanolic extracts from *A. camphorata* mycelia,

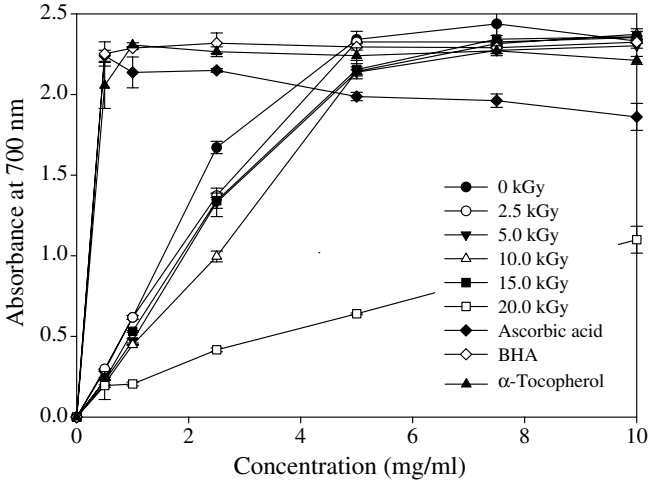

Fig. 2. Reducing power of methanolic extracts from *Antrodia camphorata* mycelia with various doses of γ-irradiation. Each value is expressed as mean ± standard deviation ($n = 3$).

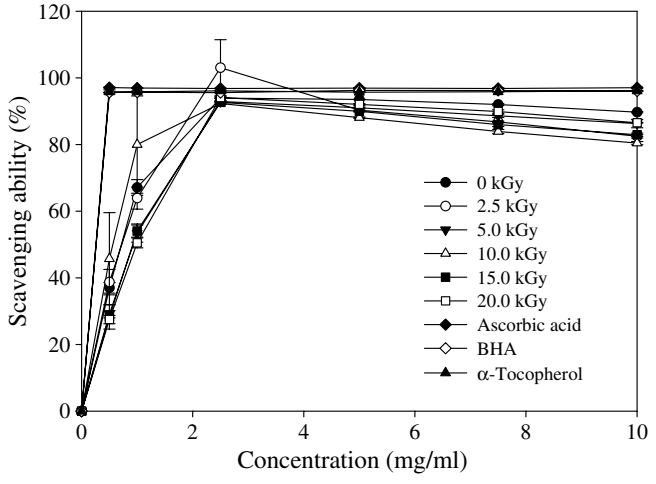

Fig. 3. Scavenging ability of methanolic extracts from *Antrodia camphorata* mycelia with various doses of γ-irradiation on 1,1-diphenyl-2-picrylhydrazyl radicals. Each value is expressed as mean ± standard deviation ($n = 3$).

regardless of γ-irradiation. However, at 0.5 mg/ml, the scavenging abilities of ascorbic acid, BHA and α-tocopherol were 97.1, 95.6 and 95.8%, respectively.

Huang et al. (1999) found that scavenging abilities of methanolic extracts from fresh and air-dried *A. camphorata* fruit bodies were 99.1 and 96.3% at 2.5 mg/ml, respectively, whereas that from white mycelia was 97.1% at 5.0 mg/ml. Scavenging abilities of methanolic extracts from most submerged mycelia on DPPH radicals were excellent (93.2–93.9%) at 0.5 mg/ml (Mau et al., 2003). In addition, at 5.0 mg/ml, scavenging abilities were 97.0 and 98.4% for red and white mycelia, respectively (Mau et al., 2004).

Variyar et al. (2004) found that the scavenging ability of soybean, with 0.5–5 kGy of γ-irradiation on DPPH radicals, increased with doses used. Ahn et al. (2004) reported that, after irradiation, scavenging ability of phytic acid on DPPH radicals was found. However, they pointed out that unirradiated phytic acid did not show scavenging ability. Green tea leaf extracts with 10 and 20 kGy of irradiation significantly increased the scavenging ability on DPPH radicals at 4 °C (Jo et al., 2003).

By contrast, Ahn et al. (2005) found that, immediately after irradiation, the scavenging ability of Chinese cabbage was reduced by 2 kGy of irradiation. No significant changes of the scavenging abilities were observed in unirradiated and 5, 10 and 20 kGy-irradiated *Chungkookjang* and *Doenjang* (Byun, Son, Yook, Lo, & Kim, 2002). However, electron donating activities of some Korean medicinal herbs were not influenced by 10 kGy of γ-irradiation at ambient temperature (Byun et al., 1999). Based on the results shown in Fig. 3, it seems that *A. camphorata* mycelium had good scavenging ability on DPPH radicals and any effect of irradiation on its ability was not noticeable.

3.5. Scavenging ability on hydroxyl radicals

Generally, scavenging abilities of methanolic extracts from most *A. camphorata* mycelia, with various doses of γ-irradiation, on hydroxyl radicals increased at 5–10 mg/ml (Table 2). Scavenging abilities of methanolic extracts from 2.5 to 20 kGy γ-irradiated mycelia were better than that of the unirradiated control at 10 mg/ml. However, the scavenging ability of α-tocopherol at 10 mg/ml was 54.5%.

Huang (2000) found that none to little scavenging effect on hydroxyl radicals occurred with methanolic extracts from fresh and air-dried *A. camphorata* fruit bodies, whereas the scavenging effect of that from white mycelia was low (1.46–23.1%) and not concentration-dependent. Scavenging abilities of methanolic extracts from *A. camphorata* mycelia, harvested at different days of incubation in submerged culture, on hydroxyl radicals showed different levels and these levels correlated well with increased incubation times (Mau et al., 2003). Mau et al. (2004) found none to little scavenging ability on hydroxyl radicals with methanolic extracts from white mycelia whereas those from red mycelia showed slight scavenging ability. However, these results were not higher than those shown in Table 2.

Since, the major constituent of living cells is water, exposure to high-energy radiation such as γ-rays, will result in hydroxyl radical production (von Sonntag, 1987). But, in this study, samples used were freeze-dried *A. camphorata* mycelia, in which insufficient water was available to react and generate hydroxyl radical. Therefore, the components that could scavenge hydroxyl radicals were not depleted. In addition, γ-irradiation might enhance the release of components which could scavenge hydroxyl radicals. Accordingly, irradiated *A. camphorata* mycelia possessed better scavenging ability at 10 mg/ml.

Table 2
Scavenging abilities of methanolic extracts from *Antrodia camphorata* mycelia with various doses of γ-irradiation on hydroxyl radicals

Sample	Scavenging ability(%)	
	5.0 mg/ml	10.0 mg/ml
0 kGy (Control)	11.5 ± 1.20 A[a]	14.0 ± 1.29 F
2.5 kGy	10.5 ± 2.87 A	38.0 ± 1.13 C
5.0 kGy	7.70 ± 1.46 A	38.7 ± 0.93 C
10.0 kGy	7.50 ± 2.41 A	29.0 ± 0.96 E
15.0 kGy	2.50 ± 0.95 B	42.4 ± 0.37 B
20.0 kGy	12.1 ± 2.69 A	31.4 ± 0.94 D
α-Tocopherol		54.5 ± 3.22 A

[a] Each value is expressed as mean ± standard deviation ($n = 3$). Means with different letters within a column are significantly different ($P < 0.05$).

3.6. Chelating ability on ferrous ions

Chelating abilities of all methanolic extracts on ferrous ions were 46.5–61.7% at 1 mg/ml, indicating that *A. camphorata* mycelia were also good in this chelating ability (Fig. 4). In addition, *A. camphorata* mycelia, with various doses of irradiation, showed medium (46.5–84.4%) and high (84.8–95.7%) chelating abilities on ferrous ions at

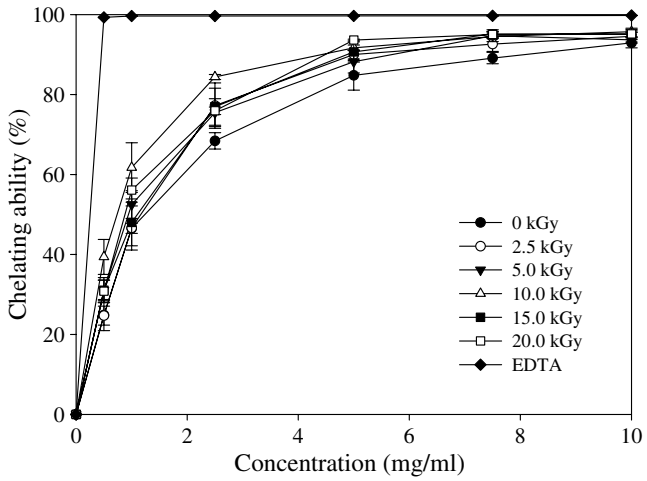

Fig. 4. Chelating ability of methanolic extracts from *Antrodia camphorata* mycelia with various doses of γ-irradiation on ferrous ions. Each value is expressed as mean ± standard deviation ($n = 3$).

1–2.5 and 5–10 mg/ml, respectively. At 7.5–10 mg/ml, the chelating ability reached a plateau of 89.1–95.7% for all samples. It seems that, with irradiation at 5–20 kGy, *A. camphorata* mycelia possessed higher chelating ability on ferrous ions than did the unirradiated control at the concentrations tested. However, EDTA showed an excellent chelating ability of 99.3% at 0.5 mg/ml.

Chelating abilities of methanolic extracts from white mycelia, fresh and air-dried *A. camphorata* fruit bodies were also concentration-dependent (Huang et al., 1999). At 10 mg/ml, the methanolic extracts from white mycelia, fresh and air-dried fruit bodies chelated ferrous ions by 89.0, 81.5 and 88.9%, respectively (Huang et al., 1999). Chelating abilities of methanolic extracts from submerged mycelia, harvested at day 7 and 10, on ferrous ions maintained the level of 83.4–92.3% at 5–10 mg/ml (Mau et al., 2003). In addition, at 10 mg/ml, chelating abilities declined significantly for mycelia at days 13–18 (Mau et al., 2003). Mau et al. (2004) found that chelating abilities of methanolic extracts from red and white mycelia, on ferrous ions increased with increasing concentration, and the chelating abilities reached a plateau of 95.7–98.7% for both mycelia at 7.5–10 mg/ml.

Since, ferrous ions are the most effective prooxidants in food systems (Yamaguchi, Tatsumi, Kato, & Yoshimitsu, 1988), the high chelating abilities of methanolic extracts from mycelia would be beneficial. Evidently, γ-irradiation did not diminish the high chelating ability of *A. camphorata* mycelia. On the contrary, γ-irradiation could slightly increase the chelating ability of *A. camphorata* mycelia.

3.7. EC_{50} in antioxidant properties

The antioxidant properties assayed herein are summarized in Table 3, and the results are normalized and expressed as EC_{50} values for comparison. The EC_{50} values were below 15 mg/ml, except values in scavenging ability of the unirradiated control on hydroxyl radicals, indicating that unirradiated and irradiated *A. camphorata* mycelia had good antioxidant properties. Based on EC_{50} values in antioxidant activity, 10–20 kGy-irradiated *A. camphorata* mycelia (1.27–1.90 mg/ml) were significantly lower ($P < 0.05$) than the unirradiated control (2.44 mg/ml). With regard to EC_{50} values in reducing powers of methanolic extracts, 5–20 kGy-irradiated mycelia (0.94–3.39 mg/ml) were higher than the unirradiated control (0.82 mg/ml), indicating that γ-irradiation might decrease their reducing powers.

The EC_{50} values in scavenging abilities on DPPH radicals were extremely low (0.59–0.99 mg/ml) for all samples. However, 10 kGy irradiated samples exhibited lower EC_{50} values than did the unirradiated control, whereas 5 and 20 kGy-irradiated samples had higher EC_{50} values. Apparently, EC_{50} values in the scavenging ability of methanolic extracts from irradiated mycelia on hydroxyl radicals were significantly lower (11.0–15.0 mg/ml) than the unirradiated control (82.0 mg/ml). EC_{50} values of 0.74–1.08 mg/ml, in chelating ability on ferrous ions, showed that the doses of 2.5–20 kGy did not change the excellent inherent chelating ability of *A. camphorata* mycelia.

The antioxidant properties of *A. camphorata* mycelia, with various doses of irradiation, studied herein, were assayed on their methanolic extracts. However, the extraction yields should be taken into consideration when the antioxidant properties are assessed, based on the dried samples instead of their methanolic extracts. Therefore, EC_{50} values, based on the dried extracts, were converted to EC_{50} values, based on the dried samples. For *A. camphorata* mycelia with 0, 2.5, 5, 10, 15 and 20 kGy of irradiation, EC_{50} values in antioxidant activity were 5.13, 5.09, 5.60, 2.83, 3.58 and 2.45 mg sample/ml; EC_{50} values in reducing power were 1.72, 1.82, 2.32, 2.46, 1.77 and 6.54 mg sample/ml; EC_{50} values in scavenging ability on DPPH radicals were 1.51, 1.61, 2.06, 1.14, 1.56 and 1.91 mg sample/ml; EC_{50} values in scavenging ability on hydroxyl radicals were 172.3, 27.3, 26.2, 32.4, 20.6 and 28.8 mg sample/ml; EC_{50} values in chelating ability on ferrous ions were 2.27, 2.44, 2.10, 1.60, 2.03 and 1.70 mg sample/ml, respectively.

Table 3
EC_{50} values of methanolic extracts from *Antrodia camphorata* mycelia, with various doses of γ-irradiation, in antioxidant properties

	EC_{50} value[a] (mg extract/ml)					
	0 kGy	2.5 kGy	5 kGy	10 kGy	15 kGy	20 kGy
Antioxidant activity (conjugated diene method)	2.44 ± 0.20 AB[b]	2.27 ± 0.02 B	2.53 ± 0.10 A	1.31 ± 0.09 D	1.90 ± 0.02 C	1.27 ± 0.02 D
Reducing power	0.82 ± 0.01 D	0.81 ± 0.01 D	1.05 ± 0.02 BC	1.14 ± 0.02 B	0.94 ± 0.01 C	3.39 ± 0.11 A
Scavenging ability on DPPH radicals	0.72 ± 0.01 C	0.72 ± 0.02 C	0.93 ± 0.05 AB	0.53 ± 0.15 D	0.83 ± 0.12 BC	0.99 ± 0.03 A
Scavenging ability on hydroxyl radicals	82.0 ± 1.76 A	12.2 ± 0.15 C[c]	11.8 ± 0.18 C[c]	15.0 ± 1.02 B[c]	11.0 ± 0.05 C[c]	14.9 ± 0.89 B[c]
Chelating ability on ferrous ions	1.08 ± 0.02 A	1.09 ± 0.15 A	0.95 ± 0.06 AB	0.74 ± 0.12 B	1.08 ± 0.18 A	0.88 ± 0.04 AB

[a] EC_{50} value: the effective concentration at which the antioxidant activity was 50%; the absorbance was 0.5 for reducing power; 1,1-diphenyl-2-picrylhydrazyl (DPPH·) or hydroxyl radicals were scavenged by 50%; ferrous ions were chelated by 50%. EC_{50} value was obtained by interpolation from linear regression analysis.

[b] Each value is expressed as mean ± standard deviation ($n = 3$). Means with different letters within a row at a specific EC_{50} are significantly different ($P < 0.05$).

[c] Obtained by extrapolation from linear regression analysis.

Table 4
Contents of ascorbic acid, β-carotene, tocopherols and total phenols of methanolic extracts from *Antrodia camphorata* mycelia with various doses of γ-irradiation

Compound	Content (mg/g)					
	0 kGy	2.5 kGy	5 kGy	10 kGy	15 kGy	20 kGy
Ascorbic acid	0.15 ± 0.11 A[a]	0.07 ± 0.07 A	0.10 ± 0.06 A	0.10 ± 0.03 A	0.20 ± 0.13 A	0.17 ± 0.14 A
β-Carotene	0.05 ± 0.00 C	0.06 ± 0.01 BC	0.11 ± 0.02 A	0.08 ± 0.02 B	0.08 ± 0.01 BC	0.06 ± 0.01 BC
α-Tocopherol	0.75 ± 0.24 A	0.47 ± 0.24 AB	0.39 ± 0.10 B	0.29 ± 0.10 B	0.46 ± 0.12 AB	0.47 ± 0.07 AB
γ-Tocopherol	0.25 ± 0.07 A	0.17 ± 0.15 AB	0.13 ± 0.05 AB	0.07 ± 0.02 B	0.21 ± 0.07 AB	0.16 ± 0.07 AB
δ-Tocopherol	0.08 ± 0.01 A	0.09 ± 0.01 A	0.07 ± 0.01 A	0.03 ± 0.01 B	0.07 ± 0.02 A	0.05 ± 0.01 AB
Total phenols	13.4 ± 2.89 A	14.6 ± 0.68 A	15.5 ± 0.38 A	14.8 ± 0.55 A	13.0 ± 0.97 A	14.5 ± 0.73 A

[a] Each value is expressed as mean ± standard deviation ($n = 3$). Means with different letters within a row are significantly different ($P < 0.05$).

3.8. Antioxidant components

Naturally occurring antioxidant components, including ascorbic acid, β-carotene, α-, γ- and δ-tocopherols and total phenols, were found in methanolic extracts from unirradiated and irradiated *A. camphorata* mycelia (Table 4). Total phenols were the major naturally occurring antioxidant components found in methanolic extracts and in the range 13.0–15.5 mg/g. Total antioxidant components varied among methanolic extracts and were 14.7, 15.5, 16.3, 15.4, 14.0 and 15.4 mg/g for *A. camphorata* mycelia with 0, 2.5, 5, 10, 15 and 20 kGy of irradiation, respectively.

Huang (2000) found that the contents of ascorbic acid, β-carotene, α-tocopherol and total phenols in methanolic extracts of *A. camphorata* white mycelia were 2.39, 2.15, 7.16 and 18.6 mg/g; for fresh fruit bodies, they were 0.16, 6.87, 25.3 and 21.2 mg/g; for air-dried fruit bodies, they were 0.02, 2.89, 24.3 and 23.3 mg/g, respectively. During incubation in submerged culture, Mau et al. (2003) found that contents of total antioxidant components were 28.0, 24.6, 18.6, 24.3 and 25.4 mg/g for mycelia at days 7, 10, 13, 16 and 18, respectively. However, Mau et al. (2004) found that tocopherols were the major naturally occurring antioxidant components found in methanolic extracts from *A. camphorata* red and white mycelia, and contents of total antioxidant components were 37.8 and 25.2 mg/g for red and white mycelia, respectively.

Calucci et al. (2003) reported that, after 10 kGy of γ-irradiation, significant losses of total ascorbate were found for black pepper, cinnamon, nutmeg, oregano and sage, whereas significant decrease of carotenoid contents were observed for cinnamon, oregano, parsley, bird pepper, and sage. In addition, the addition of ascorbic acid (0.1%) to ground beef might result in reducing lipid oxidation after 2.5 kGy of irradiation (Ahn & Nam, 2004). However, the amount of ascorbic acid in *A. camphorata* mycelia was small and the effect of irradiation on its content was also insignificant.

Ahn et al. (2005) found that γ-irradiation, at 1 kGy or more, significantly reduced the phenolic contents in cut Chinese cabbage and an increase of total phenols by irradiation at 0.5 kGy was observed in some samples. Adamo et al. (2004) found that, for truffles with ionizing radiation, a slight degradation of polyphenolic compounds occurred at 1.0–1.5 kGy, giving rise to an increase of soluble phenols as well as to an increase of other small metabolites. Interestingly, Variyar et al. (2004) found that, in soybean with 0.5–5 kGy of irradiation, the content of glycosidic conjugates decreased, and that of aglycones increased, with increased radiation dose. Accordingly, γ-irradiation might degrade antioxidant components or decompose some components into antioxidant components. This may change the content and composition of antioxidant components, thereby affecting the antioxidant properties. In addition, Adamo et al. (2004) proposed that the destructive processes of oxidation and γ-irradiation were capable of breaking the chemical bonds of polyphenols, thereby releasing soluble phenols of low molecular weight.

Phenols, such as BHT and gallate, are known to be effective antioxidants (Madhavi, Singhal, & Kulkarni, 1996). Yen, Duh, & Tsai (1993) found that the antioxidant activity of the methanolic extract from peanut hulls correlated with its content of total phenols. Therefore, the high content of total phenols in all methanolic extracts might explain their high antioxidant properties. It seemed that up to 20 kGy of irradiation did not markedly affect the amounts of total antioxidant components in *A. camphorata* mycelia, thereby, conserving its inherent good antioxidant properties. In addition, antioxidant activities and scavenging abilities of methanolic extracts from irradiated *A. camphorata* mycelia were better than those of methanolic extracts, from the unirradiated control. Furthermore, other antioxidant properties of all methanolic extracts, including reducing power, scavenging ability on DPPH radicals and chelating ability on ferrous ions were comparable and were not affected by the γ-irradiation applied. Change of total phenol composition as a result of irradiation, and the antioxidant mechanisms of phenolic components are prospective areas of future investigation.

4. Conclusions

Unirradiated and irradiated *A. camphorata* mycelia possessed excellent antioxidant properties, including antioxidant activity, reducing power, scavenging abilities on $DPPH^{\bullet}$ and hydroxyl radicals and chelating ability on

ferrous ions. Scavenging abilities of γ-irradiated *A. camphorata* mycelia on hydroxyl radicals were significantly higher than those of the unirradiated control. Summarily, γ-irradiation not only maintained the antioxidant properties of *A. camphorata* mycelia but also enhanced antioxidant properties to some extent. Taking the influence of γ-irradiation on antioxidant properties into account, γ-irradiation is a good method for preventing the contamination of *A. camphorata* mycelia. Further research on the effect of γ-irradiation on antioxidant properties is necessary.

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