Fung. Sci. 18(3, 4): 59–71, 2003

Time course for antioxidants production by *Antrodia camphorata* in submerged culture

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(Accepted May 26, 2003)

ABSTRACT

The mycelia of *Antrodia camphorata* (Chang & Chou) Wu, Ryvarden & Chang were grown in a 50-ton fermentor for 18 days. The antioxidant properties and antioxidant components of methanolic extracts from mycelia at different days of incubation were studied. In the antioxidant activity by the conjugated diene method, EC_{50} value of mycelia at day 18 (1.46 mg/mL) was the best, followed by values of mycelia at days 10, 13 and 16. Reducing powers were good (> 0.64) at concentrations of methanolic extracts higher than 2.5 mg/mL and EC_{50} values were considerately low (0.40–0.53 mg/mL) for mycelia at days 7 to 16. At 0.5 mg/mL, the scavenging effect on 1,1-diphenyl-2-picrylhydrazyl radicals was excellent (93.2–93.9%) and EC_{50} values were extremely low (0.27 mg/mL) for mycelia at days 7 to 16. The chelating effects on ferrous ions were good (58.9–92.3%) at 2.5 to 10 mg/mL. Contents of naturally occurring antioxidant components were found in the order of total phenols > tocopherols > ascorbic acid > β-carotene. Methanolic extracts from *A. camphorata* red mycelia in submerged culture were good in the antioxidant properties tested, except for the scavenging effect on hydroxyl ions. More specifically, the antioxidant properties were good for mycelia at days 10 to 16.

Key words: antioxidant activity, antioxidant components, *Antrodia camphorata*, chelating effect, reducing power, scavenging effect.

Introduction

A new basidiomycete *Antrodia camphorata* (Chang & Chou) Wu, Ryvarden & Chang (Chang-chih or niu-chang-ku) in the Polyporaceae (Aphyllophorales), which causes brown heart rot of endemic evergreen *Cinnamomum kanehirai* Hay (Lauraceae) in Taiwan, was identified (Wu *et al.*, 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. Chang-chih is well known in Taiwan as an expensive medicinal material. Traditionally, it is commonly used as an antidote, anticancer, antiitching and hepatoprotective drug. 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 odor like its host woods (Chang and Chou, 1995). The mycelia isolated from the fruit body formed orange red,

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orange brown to light cinnamon colonies (Chang and Chou, 1995).

Chien *et al*. (1997) first examined solid and liquid mycelial cultures of *A. camphorata* and found new triterpenoids therefrom. In 1998, a submerged mycelial culture was established on a 50-ton scale in Grape King Inc. under standard fermentation procedure for mushrooms. Contrary to that observed on plates, the harvested mycelia were white in color. However, antioxidant properties of *A. camphorata* were evaluated using fruit bodies and white mycelia (Huang *et al.*, 1999; Huang, 2000). Also, the non-volatile taste components of *A. camphorata* white mycelia were determined (Chang *et al.*, 2001). Due to the unacceptable white color, a specific fermentation procedure was then established for *A. camphorata* in which citric acid was added to maintain the pH of culture at pH 4.5, the culture turned orange red in fermentor at days 8–10 and the mycelia thus harvested at days 13–16 were called red mycelia (Chen *et al.*, 2001).

Later, *A. camphorata* red mycelia was found to exhibit significant cytotoxicity against leukemia HL-60 cells but no against cultured human endothelial cells (Hseu *et al*., 2002). The fermented filtrate from submerged culture strongly inhibited lipid peroxidation (Song and Yen, 2002). Both the fermented filtrate and water extract from mycelia showed marked scavenging abilities on free radicals. Moreover, the fermented filtrate reduced the incidence of liver lesions, including neutrophil infiltration, hydropic swelling, and necrosis induced by carbon tetrachloride in rats (Song and Yen, 2003).

Fruit bodies of chang-chih are expensive and less available partially due to their rareness and difficulty in cultivation. Thus, *A. camphorata* is mainly prepared from submerged culture in the form of mycelia for use in the formulation of nutraceuticals and functional foods. To provide the information to optimize the harvest time point in addition to other parameters such as biomass, reducing sugar and polysaccharide contents, it is of great interest to examine other chemical or biological properties during the mycelial growth in submerged culture. Accordingly, our objective was to study and compare the antioxidant properties of methanolic extracts from *A. camphorata* mycelia during its growth in submerged culture. Antioxidant properties were assayed in terms of antioxidant activity by the conjugated diene method, reducing power, scavenging effects on radicals and chelating effect on ferrous ions. The contents of potential antioxidant components of mycelia harvested at different days of incubation in submerged culture were also determined.

Materials and Methods

Mushroom mycelia

Freeze-dried mycelia of *A. camphorata* were obtained from the Biotechnology Center, Grape King Inc., Chungli, Taiwan. Mycelia were grown in a 50-ton fermentor with 35-ton working volume. The medium consisted of 2.0% glucose, 1.0% cornstarch, 0.5% yeast extract, 0.5% peptone, 0.3% $(NH_4)_2SO_4$, 0.3% $MgSO_4 \cdot 7H_2O$, 0.3% KH_2PO_4, 0.05% citric acid and pH 4.5 (Chen *et al*., 2001). The working conditions were: temperature, 28°C; aeration rate, 0.5 vvm; agitation speed, 90 rpm; and the inoculum rate, 1% (Chen *et al*., 2001). The culture in the fermentor turned orange red at day 8. At different days of incubation, mycelia were sampled, filtered and freeze-dried.

For each mycelia harvested at different days of incubation, three samples (≈50 g each) were randomly selected and prepared for analyses. After a fine powder (20 mesh) was obtained using a mill (Restsch ultra centrifugal mill and sieving machine, Haan, Germany), a subsample (10 g) was extracted by stirring with 100 mL of methanol at 25°C at 20 × *g* for 24 h and filtering through Whatman No. 4 filter paper. The residue was then extracted with two additional 100 mL portions of methanol as described above. The combined methanolic extracts were then rotary evaporated at 40°C to dryness. The dried extract was used directly for analyses of antioxidant components or redissolved in methanol to a concentration of 50 mg/mL and stored at 4°C for further uses.

Antioxidant activity

The antioxidant activity was determined by the conjugated diene method (Lingnert *et al.*, 1979). Each mycelial extract (0.5–20 mg/mL) in methanol (100 μL) was mixed with 2 mL of 10 mM linoleic acid emulsion (pH 6.5) 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 blank in a Hitachi U-2001 spectrophotometer. The antioxidant activity (AOA) was calculated as follows: AOA (%) = [(ΔA_{234} of control − ΔA_{234} of sample)/ΔA_{234} of control] × 100%. An AOA value of 100% indicates the strongest antioxidant activity. EC_{50} value (mg methanolic extract per mL) is the effective concentration at which the antioxidant activity was 50% and was obtained by interpolation from linear regression analysis. Butylated hydroxyanisole (BHA) and α-tocopherol (vitamin E) were used as controls.

Reducing power

The reducing power was determined according to the method of Oyaizu (1986). Each mycelial 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 Pure Chemical Co., Osaka, Japan) and 2.5 mL of 1% potassium ferricyanide (Sigma Chemical Co., St. Louis, MO), and the mixture was incubated at 50 °C for 20 min. After 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 blank in a Hitachi U-2001 spectrophotometer. A higher absorbance indicates a higher reducing power. EC_{50} value (mg/mL) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation from linear regression analysis. BHA and α-tocopherol were used as controls.

Scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals

Each mycelial 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 blank (Shimada *et al*., 1992). EC_{50} value (mg/mL) is the effective concentration at which DPPH radicals were scavenged by 50% and was obtained by interpolation from linear regression

analysis. BHA and α-tocopherol were used as controls.

Scavenging effect on hydroxyl radicals

The hydroxyl radical reacted with the nitrone spin trap 5,5-dimethyl pyrroline-N-oxide (DMPO, Sigma) and the resultant DMPO-OH adduct was detected with an electron paramagnetic resonance (EPR) spectrometer. The EPR spectrum was recorded 2.5 min after mixing 200 μL of each mycelial extract (0.1–5 mg/mL) in methanol 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 *et al*., 1991). BHA was used as a control.

Chelating effects on ferrous ions

Chelating effect was determined according to the method of Shimada *et al*. (1992). To 2 mL of the mixture consisting of 30 mM hexamine (Wako), 30 mM potassium chloride (Sigma) and 9 mM ferrous sulfate (Union Chemical Works, Hsinchu, Taiwan) was added 2 mL of each mycelial extract (0.5–10 mg/mL) in methanol and 200 μL of 1 mM tetramethyl murexide (TMM, Sigma). After 3 min at room temperature, the absorbance of the mixture was determined at 485 nm against blank. A lower absorbance indicates a higher chelating power. EC_{50} value (mg/mL) is the effective concentration at which ferrousions were chelated by 50% and was obtained by interpolation from linear regression analysis. Ethylenediaminetetraacetic acid (EDTA) was used as a control.

Determination of antioxidant components

Ascorbic acid was determined according to the method of Klein and Perry (1982). Each dried 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 sec at 515 nm against blank. Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (Sigma).

β-Carotene was extracted and analyzed as described by Rundhaug *et al*. (1988). Each dried 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; the volume was adjusted 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 Hitachi D-6200 pump, a Hitachi L-5000 LC controller, a Rheodyne 7161 injector, a 20-μL-sample loop, a Hitachi D-2500 chromato-integrator, a Hitachi L-4000 UV detector, and a Prodigy 5 ODS-2 column (4.6 × 250 mm, 5 μm, Phenomenex Inc., Torrance, CA). 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 dried 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 neutral, 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 *et al*. (1984). Each dried 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 reagent (Sigma) were added to the mixture. After 30 min of standing, absorbance was measured at 750 nm against blank. The content of total phenols was calculated on the basis of the calibration curve of gallic acid (Sigma).

Statistical analysis

For each methanolic extract from mycelia harvested at different days of incubation, 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 as described by Steel *et al*. (1997) to determine the least significant difference at the level of 0.05.

Results and Discussion

The mycelia of *A. camphorata* were grown in a 50-ton fermentor for 18 days. The contents of residual sugar were 12.5, 10.0, 2.8, 0.7, and 0.2 mg/mL at days 7, 10, 13, 16, and 18, respectively. The contents of dry matter (mycelia) were 0.930, 1.263, 1.361, 1.335, and 1.306 mg/mL at days 7, 10, 13, 16, and 18, respectively (Table 1). The criterion for harvesting mycelia in Grape King Inc. under standard fermentation procedure is the residual sugar content of < 1.0 mg/mL. Under this criterion, the preferable harvest time point is near or at day 16. However, the mycelial contents were at the stationary phase of days 10 to 18. As a reference for the right harvest time point, the antioxidant properties during the mycelial growth in submerged culture were determined.

Using methanol as the extractant, the yields constantly decreased from 32.56% for mycelia

Table 1. The dry biomass of *Antrodia camphorata* mycelia and yield of methanolic extracts at different days of incubation in submerged culture

Mycelia	Dry biomass (g/L)	Extraction % (w/w)
Day 7	0.930	32.6 ± 8.4 [a]*
Day 10	1.263	26.0 ± 2.0 [b]
Day 13	1.361	21.8 ± 4.7 [c]
Day 16	1.335	21.2 ± 3.7 [c]
Day 18	1.306	20.1 ± 6.7 [c]

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

harvested at day 7 to 21.83% for mycelia at day 13, and retained the level of 20.11 to 21.83% for mycelia at days 13 to 18 (Table 1). It is apparent that yields of methanolic extracts were stable after 13 days of incubation. As compared to the yield of methanolic extracts from *A. camphorata* white mycelia (31.10%) in Huang (2000), only the yield from mycelia at day 7 was comparable. However, at day 7 the color of the culture in the fermentor has not turned orange red yet. On the contrary, the yields from red mycelia at day 10 to 18 were much lower than that from white mycelia observed in Huang (2000).

Antioxidant activity

Using the conjugated diene method, methanolic extracts showed three different patterns of antioxidant activities (Fig. 1). Generally, the antioxidant activities of methanolic extracts were low for mycelia at day 7, moderate for mycelia at day 10 and high for mycelia at days 13 to 18. Like the yields, good antioxidant activities were observed after 13 days of incubation. At 5 mg/mL, the antioxidant activities of methanolic extracts were 76.6–81.6% for mycelia at days 13 to 18, slightly lower than that of BHA and α-tocopherol (84.9 and 82.3%, respectively). At 10 mg/mL, antioxidant activities of methanolic extracts were 78.9–100% for mycelia at days 13 to 18, higher than that of BHA and α-tocopherol (87.7 and 88.3%, respectively).

Huang (2000) found that methanolic extracts from white mycelia, fresh and air-dried fruit bodies showed good antioxidant activities of 87.7, 87.7, 93.0, and 91.2% at the concentrations as low as 1 mg/mL, respectively. Song and Yen (2002) found that methanolic extracts from red mycelia inhibited lipid peroxidation by 44% at 0.2 mg/mL. However, the effective concentrations in this study seemed to be much higher than that found in Huang (2000) and Song and Yen (2002).

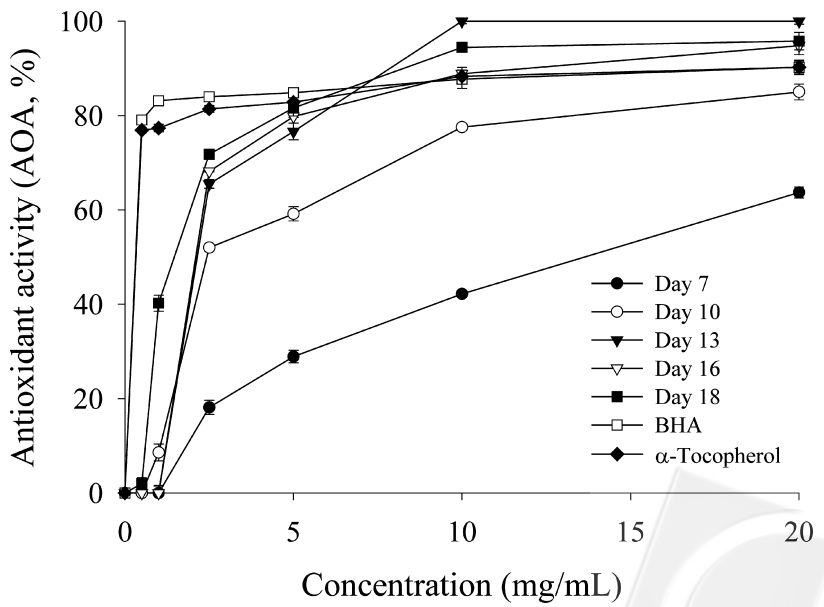

Figure 1. Antioxidant activity of methanolic extracts from *Antrodia camphorata* mycelia harvested at different days of incubation in submerged culture. Each value is expressed as mean ± standard deviation (n = 3).

Reducing power

Reducing powers of methanolic extracts increased rapidly at low concentrations from 0.5 to 2.5 mg/mL, after which all reducing powers became parallel plateaus with different levels (Fig. 2). Overall, reducing powers of all methanolic extracts were good (> 0.64) at concentrations higher than 2.5 mg/mL. However, the reducing powers of BHA and α-tocopherol maintained the levels of 0.95–1.02 and 0.70–0.78 at 0.50–10 mg/mL, respectively. At 5–10 mg/mL, reducing powers were in the steady state and in the descending order of mycelia at day 7 > mycelia at days 10 and 18 > BHA > mycelia at day 16 > mycelia at day 13 and α-tocopherol.

Huang *et al*. (1999) found that methanolic extracts from fresh and air-dried 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. At 2.5 mg/mL, those from white mycelia, fresh and air-dried fruit bodies showed low to moderate reducing powers of 0.30, 0.64 and 0.54, respectively (Huang *et al*., 1999). However, the reducing powers of methanolic extracts from red mycelia in this study were comparable to those from fruit bodies but higher than that from white mycelia.

Scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals

At 0.5 mg/mL, the scavenging effects of methanolic extracts from most mycelia on DPPH radicals were excellent (93.2–93.9%) and comparable to that of BHA and α-tocopherol (93.4–95.7%), except for that of methanolic extract from mycelia at day 18 (36.7%) (Fig. 3). The scavenging effects of methanolic extracts from fresh and air-dried 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 (Huang *et al.*, 1999). Song and Yen (2002) found that methanolic extracts from red mycelia scavenged DPPH radi-

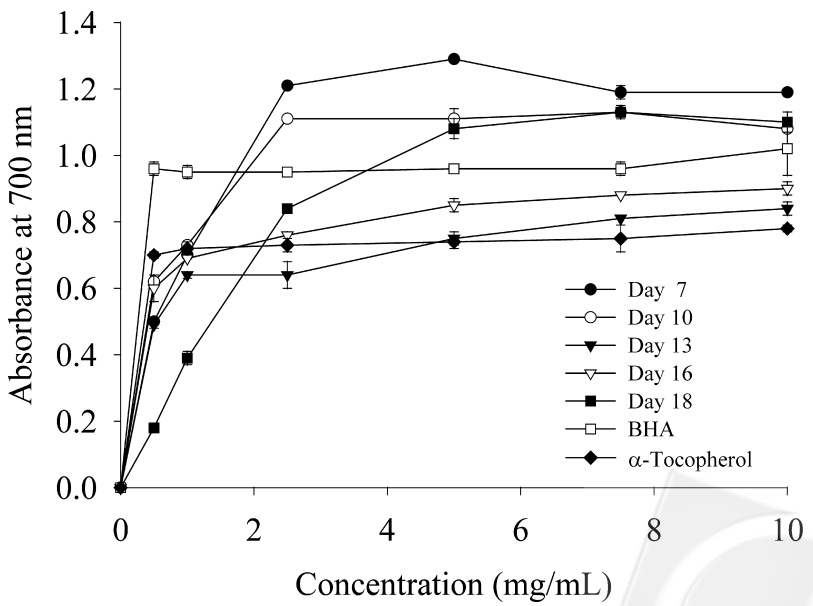

Figure 2. Reducing power of methanolic extracts from *Antrodia camphorata* mycelia harvested at different days of incubation in submerged culture. Each value is expressed as mean ± standard deviation (n = 3).

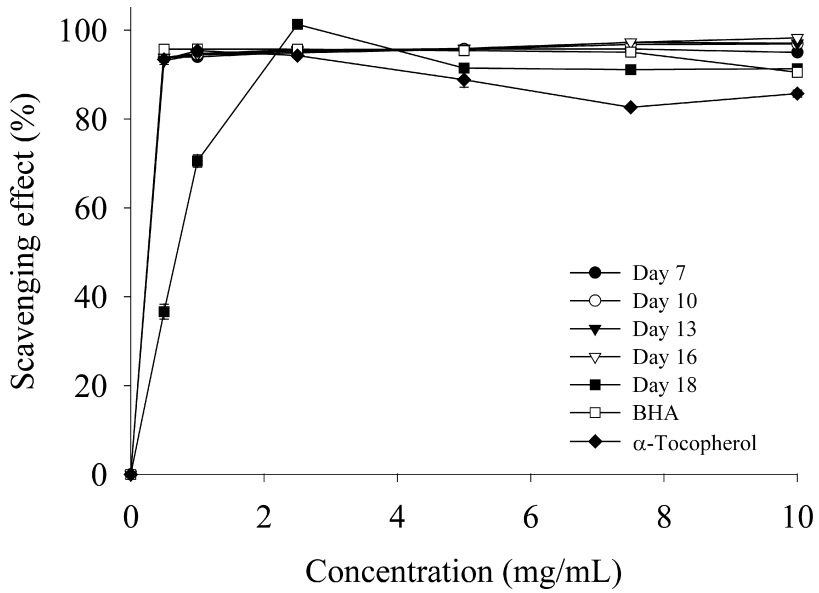

Figure 3. Scavenging effect of methanolic extracts from *Antrodia camphorata* mycelia harvested at different days of incubation in submerged culture on 1,1-diphenyl-2-picrylhydrazyl radicals. Each value is expressed as mean ± standard deviation (n = 3).

cals by 46% at 0.2 mg/mL. However, no results were shown at higher concentrations. Overall, white and red mycelia and fresh and air-dried fruit bodies were effective in scavenging DPPH radicals. More specifically, methanolic extracts from red mycelia in this study were more effective.

Scavenging effect on hydroxyl radicals

The scavenging effects of methanolic extracts on hydroxyl radicals showed different levels and these levels correlated well with the increased incubation times (Table 2). For mycelia at days 7 and 10, the scavenging effects on hydroxyl radicals were concentration-dependent. However, for mycelia at days 13 to 18, the scavenging effects were independent of concentration tested. It revealed that the components possessing the scavenging ability on hydroxyl radicals might be produced and accumulated at prolonged incubation time. However, the scavenging effect of BHA at 1 mg/mL was 12.2%. Huang (2000) found that no to little scavenging effects on hydroxyl radicals were observed with methanolic extracts from fresh and air-dried fruit bodies, whereas the scavenging effect of that from white mycelia was low (1.46–23.07%) and not concentration-dependent.

Chelating effects on ferrous ions

The chelating effects of methanolic extracts on ferrous ions were good (58.9–92.3%) at 2.5 to 10 mg/mL (Fig. 4). For mycelia at days 7 and 10, the chelating effects maintained the level of 82.4–92.3% at 5–10 mg/mL. At 10 mg/mL, the chelating effects declined significantly for mycelia at days 13 to 18. However, the chelating effect of EDTA at 1 mg/mL was 100%. Since ferrous ions are the most effective pro-oxidants in the food system (Yamaguchi *et al*., 1988), the high chelating effects of methanolic extracts from mycelia would be beneficial. The chelat-

Table 2. Scavenging effect of methanolic extracts from *Antrodia camphorata* mycelia harvested at different days of incubation in submerged culture on hydroxyl radicals

Amount (mg/mL)	Scavenging effect (%)				
	Day 7	Day 10	Day 13	Day 16	Day 18
0.1	4.74 ± 0.27 [d]*	6.95 ± 0.21 [c]	17.78 ± 0.25 [b]	18.09 ± 0.30 [b]	31.20 ± 0.47 [a]
0.5	6.52 ± 0.20 [e]	10.17 ± 0.14 [d]	19.36 ± 0.21 [c]	20.25 ± 0.27 [b]	27.39 ± 0.34 [a]
1.0	7.91 ± 0.19 [e]	12.76 ± 0.22 [d]	18.41 ± 0.30 [c]	20.25 ± 0.21 [b]	32.43 ± 0.52 [a]
5.0	9.23 ± 0.20 [d]	13.96 ± 0.28 [c]	14.39 ± 0.18 [c]	20.66 ± 0.36 [b]	39.42 ± 0.46 [a]

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

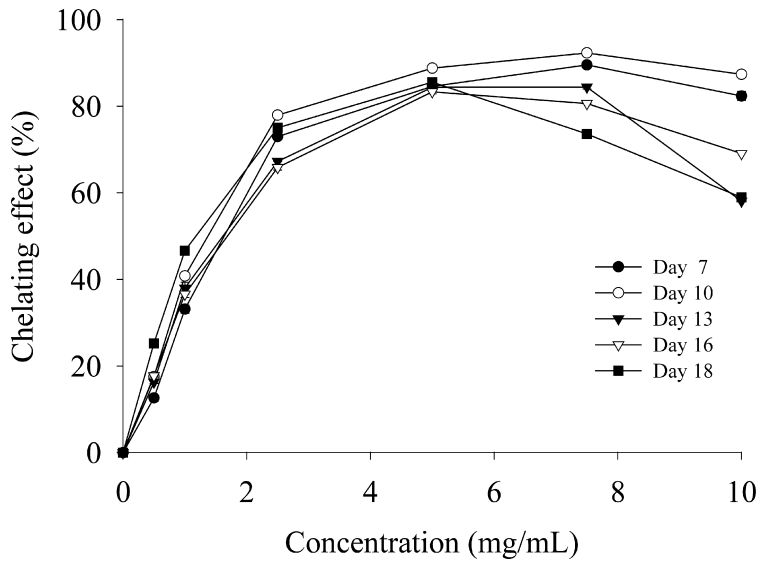

Figure 4. Chelating effect of methanolic extracts from *Antrodia camphorata* mycelia harvested at different days of incubation in submerged culture on ferrous ions. Each value is expressed as mean ± standard deviation (n = 3).

ing effect of methanolic extracts from white mycelia, fresh and air-dried fruit bodies increased with the increased concentrations and however, no decline was observed at 10 mg/mL (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).

EC_{50} values in antioxidant properties

The antioxidant properties assayed herein was summarized in Table 3 except for the scavenging effect on hydroxyl radicals, and the results were normalized and expressed as EC_{50} values (mg methanolic extract per mL) for comparison. With regard to EC_{50} values in the antioxidant activity of methanolic extracts by the conjugated diene method, the value of mycelia at day 18 (1.46 mg/mL) was the best, followed by values of mycelia at days 10, 13 and 16 (2.15–2.43 mg/mL). However, EC_{50} value of mycelia at day 7 (13.64 mg/mL) was significantly high. The EC_{50} values in the reducing powers of methanolic extracts were considerately low (0.40–0.53 mg/mL) for mycelia at days 7 to 16, whereas

Table 3. EC_{50} values of methanolic extracts from *Antrodia camphorata* mycelia harvested at different days of incubation in submerged culture in antioxidant properties

	EC_{50}* (mg/mL)				
	Day 7	Day 10	Day 13	Day16	Day18
Antioxidant activity	13.64 ± 0.05^{a}**	2.43 ± 0.03^{b}	2.15 ± 0.01^{c}	2.20 ± 0.01^{c}	1.46 ± 0.08^{d}
Reducing power	0.51 ± 0.01^{b}	0.40 ± 0.01^{c}	0.53 ± 0.04^{b}	0.42 ± 0.04^{c}	1.36 ± 0.07^{a}
Scavenging effect on DPPH radicals	0.27 ± 0.01^{b}	0.27 ± 0.01^{b}	0.27 ± 0.01^{b}	0.27 ± 0.01^{b}	0.70 ± 0.01^{a}
Chelating effect on ferrous ions	1.63 ± 0.03^{a}	1.37 ± 0.03^{b}	1.61 ± 0.06^{a}	1.68 ± 0.04^{a}	1.18 ± 0.02^{c}

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

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

EC_{50} value (1.36 mg/mL) was relatively high for mycelia at day 18. Similar to reducing powers, the EC_{50} values in the scavenging effect on DPPH radicals were extremely low (0.27 mg/mL) for mycelia at days 7 to 16, whereas EC_{50} value (0.70 mg/mL) was relatively high for mycelia at day 18. The EC_{50} values in the chelating effect on ferrous ions were also low and in the order of mycelia at days 7, 13 and 16 > mycelia at day 10 > mycelia at day 18.

Antioxidant components

Generally, contents of naturally occurring antioxidant components were found in the methanolic extracts of *A. camphorata* mycelia in the order of total phenols > tocopherols > ascorbic acid > β-carotene (Table 4). Ascorbic acid contents fluctuated between 1.89 to 2.69 mg/mL from days 10 to 18. β-Carotene contents were in the low range of 0.02 to 0.50 mg/mL. Total tocopherol contents decreased from mycelia at day 7 to 10, then reached a plateau and were 10.34, 6.56, 5.46, 5.46, and 5.91 mg/mL, for mycelia at days 7, 10, 13, 16, and 18, respectively. However, the total phenol contents were consistently high. Phenols such as BHT and gallate were known to be effective antioxidants (Madhavi *et al.*, 1996). Total antioxidant component contents were 28.03, 24.61, 18.55, 24.28, and 25.37 mg/mL, for mycelia at days 7, 10, 13, 16, and 18, respectively. It seems that contents of total antioxidant components were responsible for these good antioxidant properties observed. However, the profile of phenols and key components are needed to be study further.

Huang (2000) found the ascorbic acid content in white mycelia to be 2.39 mg/mL, similar to those found in Table 4, whereas the contents in fruit bodies (0.02–0.16 mg/mL) were much lower. As compared to red mycelia, β-carotene content (2.15 mg/mL) was high in white mycelia but contents of tocopherols and total phenols (7.61 and 18.56 mg/mL, respectively) were comparable (Huang, 2000). However, the contents of β-carotene (6.87 and 2.89 mg/mL), tocopherols (25.32 and 24.30 mg/mL) and total phenols (21.22 and 23.34mg/mL) were high in fresh and air-dried fruit bodies, respectively (Huang, 2000).

Table 4. Contents of ascorbic acid, β-carotene, tocopherols and total phenols of methanolic extracts from *Antrodia camphorata* mycelia harvested at different days of incubation in submerged culture

Compound	Content (mg/g)				
	Day 7	Day 10	Day 13	Day 16	Day 18
Ascorbic acid	2.40 ± 0.03^{c}*	2.15 ± 0.07^{d}	1.89 ± 0.01^{e}	2.52 ± 0.06^{b}	2.69 ± 0.02^{a}
β-Carotene	0.50 ± 0.01^{a}	0.02 ± 0.01^{c}	0.12 ± 0.14^{bc}	0.20 ± 0.01^{b}	0.15 ± 0.04^{bc}
α-Tocopherol	0.45 ± 0.11^{b}	0.56 ± 0.01^{b}	0.97 ± 0.08^{a}	0.16 ± 0.01^{c}	0.20 ± 0.01^{c}
γ-Tocopherol	3.75 ± 0.46^{a}	2.13 ± 0.07^{b}	2.03 ± 0.06^{b}	2.37 ± 0.37^{b}	1.99 ± 0.16^{b}
δ-Tocopherol	6.14 ± 1.27^{a}	3.87 ± 0.50^{b}	2.46 ± 0.46^{b}	2.93 ± 0.60^{b}	3.72 ± 0.66^{b}
Total phenols	14.79 ± 0.06^{b}	15.88 ± 0.38^{a}	11.08 ± 0.19^{c}	16.10 ± 0.16^{a}	16.62 ± 0.03^{a}

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

As shown in Table 3, methanolic extracts from *A. camphorata* red mycelia in submerged culture were good in the antioxidant properties tested, except for the scavenging effect on hydroxyl ions. More specifically, the antioxidant properties were good for mycelia at days 10 to 16. As shown in Table 4, the contents of total antioxidant components in methanolic extracts were low for mycelia at day 13 and high for mycelia at day 10. Upon the consideration of dry matter and residual sugar contents along with antioxidant properties, the preferable time point for harvesting mycelia should be at days 13 to 16. Nevertheless, other chemical and biological properties of mycelia and fermented filtrate at different days of incubation would be another area of investigation.

Acknowledgements

The study was supported by National Science Council, R.O.C., project no. NSC 90-2815-C005-021B.

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深層培養中樟芝抗氧化物產生之時程

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摘 要

樟芝 (*Antrodia camphorata* (Chang & Chou) Wu, Ryvarden & Chang) 菌絲體在 50 噸發酵槽中生長 18 天。在不同培養時間下，菌絲體甲醇萃取物的抗氧化性質與抗氧化成份經探討。在共軛雙烯法的抗氧化力中，第 18 天菌絲體的 EC_{50} 值 (1.46 毫克/毫升) 最佳，接著為第 10、13 和 16 天菌絲體者。還原力在甲醇萃取物濃度高於 2.5 毫克/毫升時良好 (> 0.64)，而第 7 至 16 天菌絲體的 EC_{50} 值則相當低 (0.40–0.53 毫克/毫升)。在 0.5 毫克/毫升時，對 1,1-二苯基-2-苦味基月井基團的清除作用極佳 (93.2–93.9%)，而第 7 至 16 天菌絲體的 EC_{50} 值極低 (0.27 毫克/毫升)。在 2.5–10 毫克/毫升時，對亞鐵離子的螯合作用良好 (58.9–92.3%)。天然來源的抗氧化成份含量經發現依以下的順序：總酚類 > 生育酚類 > 抗壞血酸 > β-胡蘿蔔素。深層培養的樟芝紅菌絲體甲醇萃取物在所測試的抗氧化性質上表現良好，除了對羥離子的清除作用。更特定地，第 10 至 16 天菌絲體有著良好的抗氧化性質。

關鍵詞：抗氧化力、抗氧化成份、清除作用、樟芝、螯合作用、還原力。