Antioxidant Capacity, Phenolic Content, and Polysaccharide Content of *Lentinus edodes* Grown in Whey Permeate-Based Submerged Culture

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ABSTRACT: Total antioxidant capacity (TAC), phenolic content, and crude water-soluble polysaccharide (WSP) were determined for *Lentinus edodes* mycelia grown on both whey permeate (WP)-based medium with lactose content of 4.5% and controlled medium, and harvested after 5, 10, 15, or 20 d of fermentation at 25 °C. Both methanol extracts and water extracts of *L. edodes* in this study were found to exhibit high free radical scavenging capacity. The harvesting time was found to contribute to most of the variability in the free radical scavenging capacity. High levels of antioxidant capacities (0.28 ± 0.03 and 0.29 ± 0.06 mmol TAE/g dry weight for methanol and water extracts, respectively) were observed in mycelia grown on whey permeate and harvested on day 10. Harvesting time and the type of media can interact to alter the chemical content of mycelia. Mycelia grown in whey permeate had greater ($P < 0.05$) WSP than mycelia grown in the synthetic media. High levels of WSP ($4.1 \times 10^2 \pm 71$ mg polysaccharide/g dried mycelia) were found in mycelia grown in whey permeate and harvested on day 10. Whey permeate grown mycelia had phenolic compounds ranging from 4.2 ± 0.1 to 8.0 ± 0.8 mg GAE/g dried mycelia. The overall means of total phenolic contents of mycelia grown in whey permeate were 5.9 ± 0.5 and 6.2 ± 0.6 mg GAE/g dried mycelia for methanol and water extracts, respectively.

Keywords: antioxidant capacity, *Lentinus edodes*, mycelia, phenolic compound, polysaccharides, whey permeate

Introduction

Free radicals released during oxidative stress pose the major endogenous damage in the biological system (Cheung and others 2003). This type of damage is often associated with various degenerative diseases and disorders such as cancer, cardiovascular disease, immunofunction decline, and aging (Kaur and Kapoor 2001; Cheung and others 2003). Free radicals are highly reactive molecules having unpaired electrons (Kaur and Kapoor 2001). They can be produced by radiation or as by-products of the metabolic process (Cheung and others 2003; Kang and others 2003). To gain stability, free radicals capture electrons quickly from other compounds. The attacked compound becomes a free radical itself, which continues to attack other compounds and leads to a chain reaction. This results in the disintegration of cell membranes and cell compounds, including lipid, protein, and nucleic acids (Kaur and Kapoor 2001). Besides damage to living cells, free radicals are the major cause of food deterioration through lipid oxidation, which ultimately affects the organoleptic properties and edibility of foods (Cheung and others 2003). Hence, intervention of an antioxidant may provide therapeutic functions (Jose and others 2002) and maintain the freshness of food products (Kaur and Kapoor 2001).

Recent research suggested that synthetic antioxidants could promote tumor formation as well as provide anticarcinogenic properties (Cheung and others 2003). Due to these contradictory properties, the application and exploration of natural antioxidants has received more attention. The consumption of fruits and vegetables has been proven to provide protection against various degenerative diseases (Kaur and Kapoor 2001; Cheung and others 2003). Dietary antioxidants from fruits and vegetables have been shown to increase plasma antioxidant capacities (Kaur and Kapoor 2001). Other research has also shown that the consumption of plants is associated with lower incidences of cancer (Kaur and Kapoor 2001). A great number of naturally occurring substances have been recognized to have antioxidant abilities. Flavonoids and other phenolic compounds are among the major contributors to such activity (Kang and others 2003). Other active compounds include fiber, conjugated isomers of linoleic acid, some vitamins, calcium, uric acid, glutathinone, and protease inhibitors. These compounds may work independently or synergistically (Kaur and Kapoor 2001).

Mushrooms have been recognized as a nutritious food and an important source of bioactive compounds. Studies in antioxidant capacity of mushrooms have begun to take the spotlight in recent research (Mattila and others 2001; Mau and others 2002, 2004; Badalyan 2003a, 2003b; Cheung and others 2003). Antioxidant activities have been discovered in some mushrooms at different growth stages (Badalyan 2003a, 2003b). However, data from most studies often focus on certain antioxidant capacity of fruiting bodies. Studies concerning antioxidant capacity of mushroom mycelia using radical scavenging capacity as measurement are rare. There have been no data on the antioxidant activity for mycelia grown in whey permeate culture and its changes occurring during growth of mycelia.

Our previous research (Wu and Hansen 2006) suggests that whey permeate can be utilized as an alternative medium for the growth of *Lentinus edodes*. However, the interaction between the type of media and harvesting time changes the proximate composition of the mycelia.

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The objective of this study was to evaluate the effects of whey permeate-based medium and harvesting time on the antioxidant capacity measured by radical scavenging capacity, phenolic content, and crude water-soluble polysaccharide (WSP) of *L. edodes* mycelia.

Materials and Methods

Culture, subculture, and storage condition

L. edodes culture was obtained from the American Type Culture Collection (ATCC, Manassas, Va., U.S.A.) and maintained in a potato dextrose agar (PDA) (Becton Dickinson, Sparks, MD., U.S.A.) slant at 4 °C. The seed culture was transferred to a Petri dish containing PDA medium and incubated at 25 °C for 8 d. Microorganisms were subcultured at regular intervals (50 d) to maintain viability (Mukhopadhyay and others 1999).

Media preparation

The fungi were grown on both whey permeate and controlled medium (synthetic medium). The whey permeate was supplied by Gooding Cheese and Whey Plant (Glanbia Inc., Gooding, Idaho, U.S.A.). The lactose concentration of the whey permeate-based medium was adjusted to 4.5% (Mukhopadhyay and others 2002). The pH of whey permeate was adjusted to pH 5.5. Sterilization of the whey permeate media was achieved by filtration through a 0.2-μm membrane filter (Fisherbrand, Pittsburgh, Pa., U.S.A.). The control medium consisted of 10 g/L yeast extract (Difco Laboratories, Detroit, Mich., U.S.A.), 10 g/L glucose (Mallinckrodt Chemicals, Phillipsburg, N.J., U.S.A.) and 10 g/L peptone (Becton Dickinson,) (Song and others 1987). The pH of the control medium was also adjusted to pH 5.5 prior to autoclaving.

Inoculation and fermentation condition

Mycelial agar discs from the margin of 8-d-old colonies were isolated from the PDA culture by using inoculating loops. The mycelia agar pellets were transferred to potato dextrose broth (PDB) (Becton Dickinson,) enrichment media (5 discs/100 mL media) and cultivated at 25 ± 1 °C, pH 5.5 for 8 d.

Fermentation was carried out in 2000 mL baffled flasks. Each flask contained 600 mL media. About 60 mL of the same PDB culture were inoculated in each 600 mL media. The flasks were incubated at 25 ± 1 °C on a rotary shaker (New Brunswick Scientific Co. Inc., Series 25 Incubator Shaker, Edison, N.J., U.S.A.) at 120 rpm until the assigned harvesting days (Mukhopadhyay and others 1999; Wasser and others 2003; Wu and Hansen 2006).

Harvesting

The fermentation was terminated at 4 different intervals (5, 10, 15, 20 d). At the end of each fermentation process, the mycelia were harvested and separated from the media by filtration through Whatman Nr 1 filter paper. About 20 mL of distilled water were used to wash the filtered mycelia 3 times (Elisashvili and others 2004). The growth of mycelia was measured as dried mycelial mass. The filtered mycelia were dried by lyophilization for 40 h. Dried mycelia were kept at 4 °C until use (Mukhopadhyay and others 1999).

Preparation of mycelial extracts

The freeze-dried mycelia (about 0.02 g) were extracted with 80% (v/v) methanol (1 mL) or distilled water (1 mL) at 80 °C for 30 min. The extracts were centrifuged at 6000 rpm for 5 min, and the supernatant was collected. Extracts from 80% methanol were vaporized under vacuum at 40 °C for 3 h. All extracts were stored at −20 °C. The powders of methanol extracts were reconstituted with 1 mL assay buffer from the antioxidant assay kit (Cayman Chemical Co., Ann Harbor, Mich., U.S.A.) prior to analyses.

Trolox equivalent antioxidant capacity (TEAC) assay

The total antioxidant capacity (TAC) was measured as described by Miller and others (1993, 1995) and Miller and Rice-Evans (1997). The antioxidant assay kit was purchased from Cayman Chemical Co. Absorbance was monitored at 750 nm for 5 min. Changes in absorbance were calculated and plotted with respect to concentrations of the standards and samples. The final TEAC value was expressed as millimoles of Trolox antioxidant equivalent (TAE) per gram of dried mycelia. Higher TEAC values suggested a stronger free radical scavenging capacity.

Crude WSP and protein contents

The procedure used in determining the total protein content was adapted from the AOAC Official Method of Analysis (1990). The Kjeldahl method was used to determine the total nitrogen. The factor of 6.25 was used to calculate the crude protein content (Wang and others 2001).

The crude water WSP were measured by the phenol-sulfuric acid method as described by Dubois and others (1956). The dried mycelia were mixed with distilled water at a ratio of 1:4. Mixture was boiled in a water bath at 100 °C for 12 h. The extracts were then centrifuged (Beckman Coulter™, Allegra™ X-22 Centrifuge, Germany) at 9000 rpm for 15 min. The supernatant was collected for the assay with the phenol-sulfuric acid (Basbitskaya and others 2003). Absorbance was measured at 490 nm with standard glucose solutions from 0 to 100 mg/L spectrophotometrically (Perkin Elmer, Lambda 3B, Norwalk, Conn., U.S.A.).

Determination of total phenolic content

The total phenolic content was determined using the Folin–Ciocalteau method (Singleton and Rossi 1965). About 100 μL of the extracts, 625 μL of Folin–Ciocalteau reagent, 4 mL distilled water, and 4 mL of 10% sodium carbonate were added and mixed completely. After 2-h incubation in a 35 °C water bath, the absorbance of the solution at 765 nm was measured with a spectrophotometer (Perkin Elmer). Quantification was based on the standard curve for gallic acid (0 to 0.5 mg/mL). The results were expressed in milligrams of gallic acid equivalents (GAE) per gram of dried mycelia.

Statistical analysis

The results were expressed as means ± the standard error of means (SEM) of the triplicate experimental results. Statistical analysis was performed using SAS version 9.1 (SAS Inst. Inc., Cary, N.C., U.S.A.). Data sets were evaluated by analysis of variance (ANOVA). Statistically significant difference was identified at the 95% confidence level. Post-hoc means comparison were made based on P value ($\alpha = 0.05$) using the Ryan–Einot–Gabriel–Welsh method. Correlation coefficient (r) was used to evaluate the relationship of mycelia composition and TEAC values.

A 2-way factorial model with triplicates was used for analyzing the chemical results. The type of media (whey permeate, control media) and harvesting times (5, 10, 15, 20 d) were set as fixed factors. Dependent variables were analyzed using a natural log transformation to satisfy the assumption of normality and homoscedasticity of the data. Untransformed data were used in tables and figures for the ease of interpretation.

Results and Discussion

Water-soluble polysaccharides

Data in Figure 1 show that whey permeate always resulted in a higher concentration of WSPs in the mycelia, as compared to the controlled media, at each level of harvesting time. The interaction term between harvesting time and type of media was found to be statistically significant ($P = 0.0258$, Table 1). The factor of harvesting time was nonsignificant ($P = 0.0916$, Table 1). The type of media was found to contribute to most of the variability. Highly statistically significant differences ($P < 0.0001$) were found between the pooled means of mycelia grown on whey permeate ($2.6 \times 10^2 \pm 36$ mg polysaccharide/g dried mycelia) and the pooled means of mycelia grown on the controlled media (72 ± 4 mg polysaccharide/g dried mycelia). This indicated that the production of polysaccharide greatly depended on the compound presented in the medium. As shown in our previous study (Wu and Hansen 2006), whey permeate was more favorable than the controlled media in the production of WSPs for *L. edodes*.

The highest mean value of WSP was found in whey-permeate grown mycelia harvested on day 10 ($4.1 \times 10^2 \pm 71$ mg polysaccharide/g dried mycelia; Figure 1). The WSP/g of mycelia increased from day 5 to day 10. However, a reduction in the WSP/g of mycelia occurred from day 10 to day 20. By day 20, WSP/g of mycelia was statistically significantly lower than the mycelia harvested on day 10.

Total phenolic content

Cheung and others (2003) said that most soluble components in mushroom fruiting bodies have high polarity. The antioxidant activities of mushrooms seemed to rely highly on the polarity of the solvent used in extraction. Extracts using polar solvents such as methanol and water had higher total phenolic content (TPC) than those extracted with hexane (Cheung and others 2003; Kang and others 2003). Total phenolic content varied in mycelia harvested at different times and grown with different media. Whey permeate grown mycelia had less phenolic compounds, ranging from 4.2 ± 0.1 to 8.0 ± 0.8 mg GAE/g dried mycelia, while mycelia grown on controlled media were composed of phenolic compounds ranging from 9 ± 1 to 14 ± 1 mg GAE/g dried mycelia (Figure 2 and 3).

Methanol extracts. There was no statistically significant interaction between the harvesting time and the type of media on the variability of total phenolic content in mycelia, if they were extracted with 80% methanol. Harvesting time and type of media were found to contribute to most of the variability independently ($P = 0.0002$ or 0.0001, respectively; Table 1). As shown in Figure 2, mycelia harvested on day 5 or day 15 consisted of significantly higher phenolic content than mycelia harvested on day 10 or day 20, regardless of the type of media used for growth. When grown in whey permeate, mycelia had a lower overall mean value of total phenolic (5.9 ± 0.5 mg GAE/g dried mycelia), in comparison to mycelia grown in controlled media (11 ± 1 mg GAE/g dried mycelia). However, in our study, in the methanol extracts, mycelia

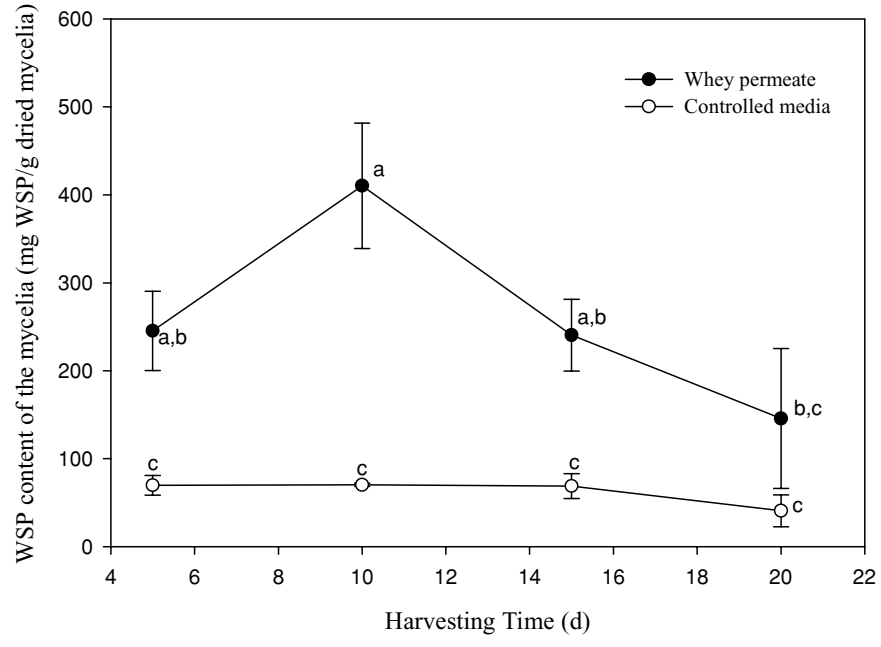

Figure 1 — Water-soluble polysaccharides content of the mycelia due to the interaction of the types of media and the harvesting time. Means sharing letter are not different at $P < 0.05$.

Table 1 — Summary of significance as determined by the ANOVA.

	WSP[a]	PRO[b]	PCME[c]	PCWE[d]	TEAC-ME[e]	TEAC-WE[f]
Media	**	**	**	**	NS	NS
Harvesting time	NS	*	**	*	**	**
Media × harvesting time	*	**	NS	**	NS	*

* = significant at $P < 0.05$; ** = significant at $P < 0.01$; NS = not significant at $P < 0.05$.
[a]Water-soluble polysaccharides (WSP).
[b]Protein content (PRO).
[c]Phenol content in methanol extracts (PCME).
[d]Phenol content in water extracts (PCWE).
[e]TEAC in methanol extracts (PCME).
[f]TEAC in water extracts (PCWE).

grown in whey permeate was found to produce phenolic compounds with a value close to or even higher than the value of *L. edodes* fruiting bodies reported by Cheung and others (2003), which was about 4.79 ± 1.2 mg GAE/g dried fruiting bodies weight.

Water extracts. There was a statistically significant interaction ($P < 0.05$) between the harvesting time and the type of media on the variability of total phenolic content in mycelia (Table 1), if they were extracted with water. Water extracts from mycelia grown on whey permeate exhibited peak values of total phenolic content at day 5, while extracts from mycelia grown on the controlled media exhibited peak values at day 10 (Figure 3). Phenolic content of mycelia in water extracts evaluated in this study was much higher (Figure 3) than phenolic content of the fruiting bodies of *L. edodes* (1.33 ± 0.04 mg GAE/g dry weight) reported by Cheung and others (2003). When grown in whey permeate, mycelia had a lower overall mean value of total phenolic (6.2 ± 0.6 mg GAE/g dried mycelia), in comparison to mycelia grown in controlled media (17 ± 1 mg GAE/g dried mycelia).

Water extracts showed different patterns in phenolic content if different media were used for cultivation. When controlled media was used, the highest phenolic content was observed on day 10; and then a reduction of phenolic content was seen (Figure 3). When whey permeate was used, the pattern of phenolic content in water extract was similar to the pattern for methanol extracts. Mycelia

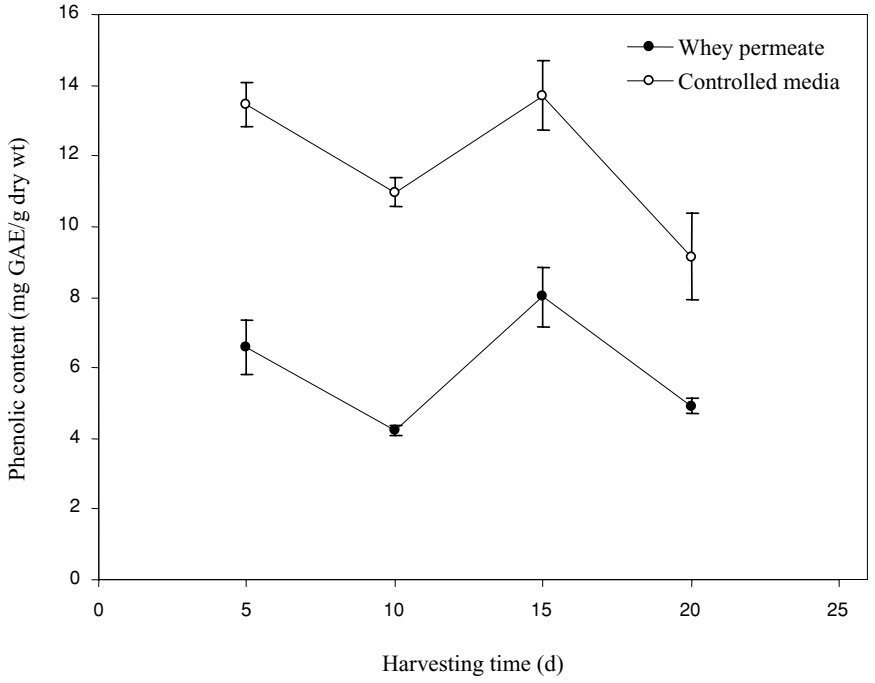

Figure 2—Total phenolic content of *Lentinus edodes* grown on whey permeate and controlled media extracted by 80% (v/v) methanol.

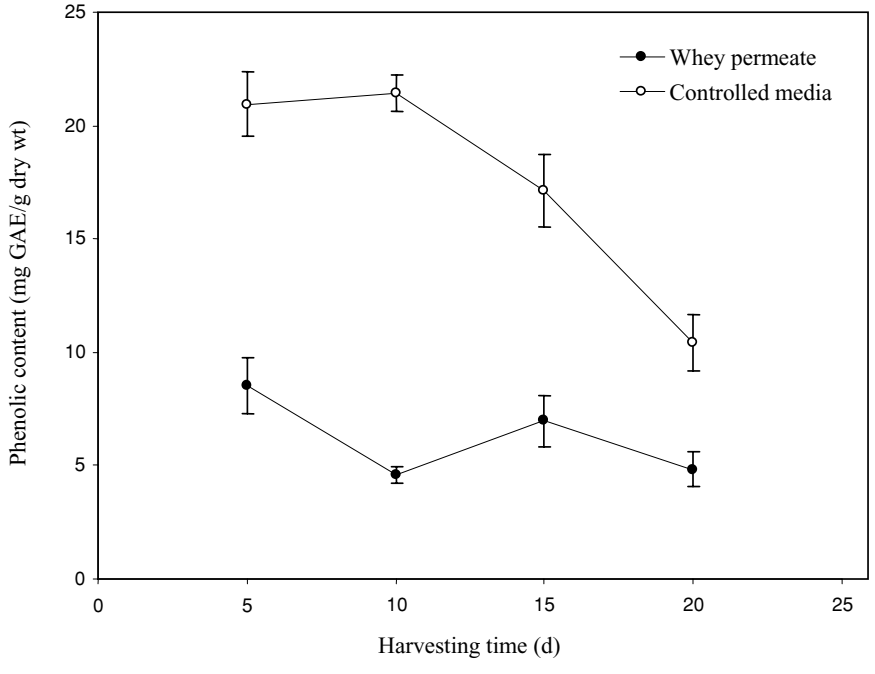

Figure 3—Total phenolic content of *Lentinus edodes* grown on whey permeate and controlled media extracted by water.

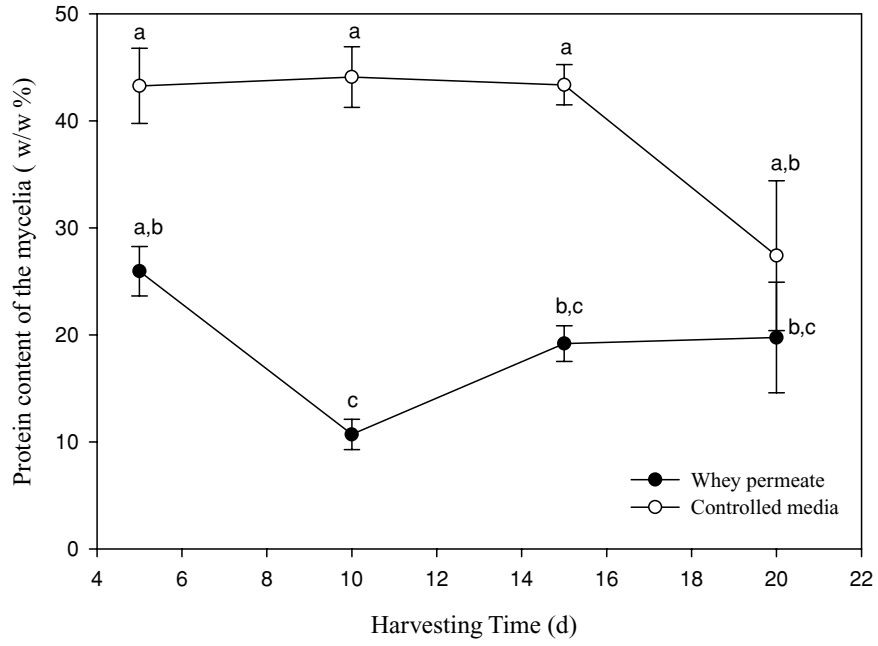

Figure 4—Protein content of the mycelia due to the interaction of the types of media and the harvesting time. Means sharing letter are not different at $P < 0.05$.

harvested on day 5 or day 15 consisted of higher phenolic content than mycelia harvested on day 10 or day 20. A slightly higher phenolic content was found in water extracts than in methanol extracts. This suggested that the increase of polarity could contribute to more effective extraction of phenolic compound in the mycelia of *L. edodes*.

Protein content

The crude protein content of mycelia was found to be statistically significantly different due to the effect of the type of media (Figure 4). Mycelia grown on the whey permeate media had an overall mean value of 19 ± 2% in crude protein content, which was about 50% of the overall mean of mycelia grown on controlled media (40 ± 3%).

Total antioxidant capacity

The antioxidant activities of mycelia extracts were evaluated by means of Trolox equivalent antioxidant capacity (TEAC) assay. Miller and others (1993) introduced the TEAC assay by the measurement of the color in the solution with $ABTS^{+}$ radical cation. In the presence of antioxidative substances, the colorization process can be delayed due to the radical scavenging ability of antioxidants. Miller and others (1995) in their later study further demonstrated that the assay could be applied to pure solutions as well as mixtures of substances. It provided reproducible and practical ways to estimate antioxidant activity, especially for food matrixes.

Both methanol extracts and water extracts of *L. edodes* were found to exhibit free radical scavenging capacity (Table 2).

Methanol extracts. No interaction between harvesting time and type of media was found in the ANOVA for the TEAC of the methanol extracts. The harvesting time was found to contribute to most of the variability ($P < 0.0001$) (Table 1). There was no significant difference between methanol extracts from mycelia grown on either medium. This indicated that the antioxidative ability of methanol extracts from mycelia was not affected by substituting controlled medium with whey permeate.

For methanol extracts, highly statistically significant differences were found among the TEAC of mycelia harvested at different time intervals. Methanol extracts of mycelia harvested on day 10 showed

Table 2—Antioxidant capacity (AOA) of the methanol and water extracts of *L. edodes* mycelia grown on whey permeat (WP) or controlled media (CM) expressed as millimoles of Trolox antioxidant equivalent (TAE) per gram of dried mycelia (mmol Trolox/g dry weight).

Harvest time (d)	Methanol extract		Water extract	
	WP grown	CM grown	WP grown	CM grown
5	0.06 ± 0.02^{b}	0.11 ± 0.02^{b}	0.12 ± 0.03^{b}	0.13 ± 0.03^{b}
10	0.28 ± 0.03^{a}	0.29 ± 0.06^{a}	0.29 ± 0.03^{a}	$0.16 \pm 0.04^{a,b}$
15	0.11 ± 0.02^{b}	0.12 ± 0.01^{b}	0.07 ± 0.01^{b}	0.10 ± 0.02^{b}
20	0.06 ± 0.02^{b}	0.12 ± 0.03^{b}	0.22 ± 0.02^{a}	0.20 ± 0.02^{a}

Means from the same type of extract columns and sharing the same letter are not significantly different ($P < 0.05$).

significantly higher ability in inhibiting free radicals. When expressed as millimoles of Trolox antioxidant equivalent per gram of dried mycelia (mmol TAE/g dry weight), TEAC of mycelia grown on whey permeate and controlled media were 0.28 ± 0.03 and 0.29 ± 0.06, respectively for day 10 harvest. These TEAC values were at least 2 times higher than extracts from the mycelia harvested on other intervals. As mycelia grew, the TEAC increased until it reached a peak value at day 10; and this was followed by a reduction of the TEAC value (Table 2).

Badalyan's study (2003b) suggested that the strength of antioxidant activities in certain types of mushroom could depend on the length of its growth time. In their study, studying another medicinal mushroom *Flammulina velutips*, they found no notable antioxidant activity until the growth at week 3. This suggested that the timing of mycelia harvesting could contribute to the variability of their nutriceutical properties.

Water extracts. The interaction term between the type of media and harvesting time was found to be statistically significant ($P = 0.045$), which suggested a contribution of that interaction to variability in the TEAC of harvested mycelia (Table 1). Unlike methanol extracts, water extracts of the mycelia exhibited peak values of TEAC at both days 10 and 20 (Table 2). The TEAC of water extracts from mycelia grown on whey permeate was slightly higher than one grown on controlled media, even though no significant difference was found between the types of media. Harvesting time

was found to contribute to most of the variability in TEAC ($P < 0.001$), as shown in Table 1. Water extracts of mycelia harvested on the 10th and 20th day showed significantly higher ability in the inhibition of free radicals. The TEAC values of mycelia grown on whey permeate and controlled media were 0.29 ± 0.03 and 0.16 ± 0.04 mmol TAE/g dry weight, respectively, if the harvesting was conducted on day 10.

Pellegrini and others (2003) provided detailed data on the antioxidant activity of vegetables and fruits by the TEAC assay. Spinach and pepper in the vegetable category were found to have the highest antioxidant capacity. Among the 30 different types of fruits, high antioxidant capacity was found in berries, such as blackberry, strawberry, and raspberry. Compared to the data provided by Pellegrini and others (2003) (Table 3), the TEAC values of mycelia grown on whey permeate and harvested on day 10 in our study were much higher, regardless of the type of extract solvent used.

Correlation of mycelia composition and TEAC value

High TEAC values and crude WSP content were detected in mushroom mycelia grown in whey permeate and harvested on day 10. In spite of a lower level of phenolic content in the whey permeate grown mycelia when compared to mycelia from controlled media, similar or higher levels of total antioxidant capacity were observed on day 10. However, neither total phenolic content nor crude WSP content in this study was found to be significantly correlated with the TEAC values of either methanol or water extracts directly. Rather than a single type of component, it is possible that TAC of mushroom extracts is the result of a sum of various antioxidative components in extracts, including WSP and various phenolic compounds. Mau and others (2005) reported in their research on *Ganoderma tsugae*, a different type of mushroom, that total phenol content in methanol extracts did not correlate with the discrepancy in antioxidant activities.

In our study, when water was used as solvent for extraction, the TEAC value of the extract was found to be loosely but positively correlated to the ratio of WSP to protein ($r = 0.46$, $P = 0.023$) as well as the ratio of WSP to total phenolic in water extracts ($r = 0.45$, $P = 0.026$). The low value of correlation coefficient suggested that in spite of a significant correlation between the TEAC value and the 2 ratio values, the relationship was nonlinear. Liu and others (1997) suggested in their research that the free radical scavenging activity of polysaccharide could depend on the ratio of WSP to protein. More specifically, the ratio of bound protein in the polysaccharide–protein complexes was considered to be more essential to the scavenging activity.

A loose correlation was also found between the TEAC values of methanol extracts and water extracts ($r = 0.60$, $P = 0.039$). The types of media were found to affect the correlations of various components in mycelia with TAC of mushroom. When mycelia grew in the controlled media, no significant correlation was found between the chemical parameters and the TEAC values. However, when whey permeate was used to grow *L. edodes*, the ratio of WSP to total phenolic in extracts was loosely but positively correlated to the TEAC values of mushrooms (in water extracts: $r = 0.58$, $P = 0.048$; in methanol extracts: $r = 0.64$, $P = 0.026$). Again, the low values of correlation coefficient suggested that in spite of a significant correlation between the TEAC value and the 2 ratio values, the relationship was nonlinear. For methanol extracts from WP grown mycelia, there was a close to linear relationship between the TEAC value and the ratio of WSP to protein ($r = 0.82$, $P = 0.011$). The TEAC value increased along with the increase of the ratio value. The TEAC values of the methanol extracts were also found to be loosely but negatively correlated to total protein content of mycelia ($r = -0.67$, $P = 0.017$).

Due to the concern of potential toxic and carcinogenic effects in some synthetic antioxidants, studies in the effectiveness of natural antioxidants have gained increased interest since the 1990s (Kaur and Kapoor 2001). A number of plant extracts and plant byproducts have been identified with antioxidative properties. Broccoli, cabbage, cauliflower, spinach, and beet have been reported to have high levels of antioxidant capacity. The active components being studied include fiber, polyphenol, flavonoids, vitamins, and other phytochemicals (Kaur and Kapoor 2001). It is believed that the antioxidant properties of phenolics are a result of their ability to act as reducing agents, hydrogen donors, and free radical quenchers (Rice-Evans and others 1997; Kaur and Kapoor 2001). Depending on the structure of the molecules, phenolics can also act as metal chelators which prevent the catalytic function of metal in the process of initiating radicals (Salah and others 1995; Kaur and Kapoor 2001).

There are few reports on the antioxidant capacity of polysaccharides. The mechanism of polysaccharides as antioxidative reagents is still not fully understood (Liu and others 1997; Jiang and others 2005). Chen and others (2005) found that in low-grade green tea, which contained low levels of phenolic content,

Table 3—Comparison of TEAC values of various vegetables, fruits, and *L. edodes* mycelia grown on whey permeate.

		Extract solvent	Growth length (d)	TEAC (mmol/g dry weight)
Mycelia[a]	*L. edodes* from WP	Methanol	10	0.28 ± 0.03
		Water	10	0.29 ± 0.03
		Moisture[b] (%)	**TEAC[c] (mmol/kg FW)**	**TEAC[d] (mmol/g dry weight)**
Vegetable	Red pepper	92.21	8.40	0.108
	Spinach	91.40	8.49	0.099
	Asparagus	93.22	3.92	0.058
	Zucchini	94.64	2.86	0.053
	Radish	95.27	2.22	0.047
	Beet	87.58	5.21	0.042
Fruit	Blackberry	85	20.24	0.135
	Strawberry	90	10.94	0.109
	Raspberry	84	16.79	0.105
	Pineapple	85	9.91	0.066
	Orange	86	8.74	0.062

[a]Data of mycelia were obtained from this study.
[b]Moisture contents of vegetables and fruits were obtained from USDA (1981, 2006), respectively.
[c]TEAC values, based on fresh weight, were initially reported by Pellegrini and others (2003).
[d]For the ease of comparison, TEAC values of fresh weight were converted to values based on dry weight, according to data from USDA.

polysaccharides contributed to the antioxidant capacity. Polysaccharides from green tea exerted inhibitory effects on both hydroxyl and superoxide radicals. Jiang and others (2005) reported that WSPs extracted from *Isaria farinose* mycelia had Fe^{2+} chelating activity. This minimizes the amount of catalytic metal available for the Fenton Reaction, which ultimately inhibits the generation of free radicals (Jiang and others 2005).

When mycelia were grown in whey permeate and harvested on day 10 in our study, crude WSP made up almost 40% of the dried weight. It was found that glucan and its derivatives, which were identified to be the major components of the water soluble fraction of polysaccharides in mushroom fruiting bodies (Mizuno 1995), could exhibit various degrees of free radical scavenging activities in Tsiapali and others' study (2001). The antioxidant activities appeared to be partially due to the monosaccharide constituents. And the polymeric structure in polysaccharides seems to enhance the free radical scavenging capacity, when compared to monomeric units, such as dextrose (Patchen and others 1987; Tsiapali and others 2001). In Patchen and others' study (1987), the ability to scavenge radiation induced free radicals was compared between *D*-glucose and its polymer glucan. The glucan concentration required for exhibiting the scavenging ability was 10 times lower than the glucose concentration. Tsiapali and others (2001) proposed that this enhancement may be due to the ease of abstraction of an anomeric hydrogen from one of the monosaccharide units on the backbone rather than from the reducing ends.

Conclusions

This study provided data on total antioxidant capacity (TAC), phenolic content, and crude WSP of mycelia grown on WP-based media at different growth stages in comparison with the control media. In this study, both methanol extracts and water extracts of *L. edodes* were found to exhibit free radical scavenging capacity, which was used to measure the total antioxidant capacity. Harvesting time played an important role in the variability of the free radical scavenging capacity. Methanol and water extracts of mycelia harvested on day 10 showed significantly higher ability to inhibit free radicals. In methanol extracts, the antioxidative ability of mycelia was not affected by the substitution of controlled medium with whey permeate. In water extracts, the antioxidative ability of mycelia was affected by the interaction between the types of media and harvesting time. The proportion of different chemicals in the mycelia changed due to the impact of the type of media and harvesting time. When grown in whey permeate and extracted with methanol, mycelia had a lower overall mean value of total phenolic (5.9 ± 0.5 mg GAE/g dried mycelia) in comparison to mycelia grown in controlled media (11 ± 1 mg GAE/g dried mycelia). Similar to methanol extracts, water extract of whey permeate grown mycelia had a lower overall mean value of total phenolic (6.2 ± 0.6 mg GAE/g dried mycelia) in comparison to mycelia grown in controlled media (17 ± 1 mg GAE/g dried mycelia). But compared to other reported phenolic contents of the fruiting bodies of *L. edodes*, phenolic content of mycelia evaluated in our study was much higher. Growing mycelia in whey permeate was more favorable for the production of WSP in this study in comparison to the synthetic medium. The maximum production of WSP ($4.1 \times 10^2 \pm 71$ mg polysaccharide/g dried mycelia) in this study was found in the whey-permeate grown mycelia harvested on the 10th day. It is possible that TAC of mushroom extracts can be the result of a sum of various antioxidative components such as WSP and phenolic compounds.

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