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Research Report

Chemical Composition and Antioxidant Properties of Cultivated Button Mushrooms (*Agaricus bisporus*)

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Abstract. Mushrooms contain a variety of secondary metabolites, including various phenolic compounds and ergothioneine, which have been shown to act as excellent antioxidants. The present study reports a comparison of the antioxidant properties, total phenolic, ergothioneine and mineral contents of the most-consumed strains of *Agaricus bisporus* (white or brown colors). The samples were fruiting body, mycelia produced at farm (old mycelium) and in vitro (young mycelium). The antioxidant activity was measured by DPPH, FRAP and ABTS assays. The analysis of phenolic compounds was performed by Folin-Ciocalteau method. The mushroom strain with the highest ergothioneine, phenolic compounds and antioxidant potential was the strain A-SPA15 (brown color). Generally, fruiting body samples revealed higher ergothioneine, phenolic compounds and antioxidant properties than mycelia obtained from farm and in vitro culture. There were variations in the mineral content of *A. bisporus* harvested from the fruiting body, old mycelium, and young mycelium. Furthermore, the lowest heavy-metal concentrations (Ni, Pb, Cd, and Cr) were detected in mycelium samples in each strain. Results demonstrated that mushrooms contained high amounts of copper, zinc, iron and manganese could be used in well-balanced diets. This study contributes to the data relative to *A. bisporus* consumed as fresh mushrooms and the possibility of in vitro production as a source of bioactive compounds.

***Additional key words*:** ABTS, *Agaricus bisporus*, DPPH, ergothioneine, FRAP, mineral content

Introduction

For centuries, mushrooms have been consumed by humans in many cultures, not only as a part of the normal diet, but also as a delicacy because they have a desirable taste and aroma (Mattila et al., 2001). Edible mushrooms are rich in protein, essential amino acids, fiber, are very low in fat, and also provide vitamins and minerals (Mattila et al., 2001). Besides their nutritional properties, mushrooms have proven antioxidant, antibacterial, antifungal, antitumor, immunomodulatory, anti-inflammatory, and antiviral properties (Barros et al., 2007; Dore et al., 2007; Faccin et al., 2007; García-Lafuente et al., 2010).

In recent times, consumption of mushrooms has risen greatly due to continuous development in cultivation, harvest, post-harvest, processing, and storage treatments that facilitate consumption throughout the year. More than 2,000 edible species of mushroom are known but only 25 are commercially cultivated. In Iran, the main species cultivated are *Agaricus bisporus* and *Pleorotus spp*. which, both mushroom play a significant economic role in the global market (Bernas et al., 2006).

Agaricus bisporus is the most widely consumed mushroom in the world, with a high nutritional value and many bioactive compounds, including L-ergothioneine (2-mercaptohistidine trimethylbetaine, ERGO), polysaccharides, amino acids, phenolics, dietary fiber, ergosterol, vitamins, and minerals (Lindequist et al., 2005; Mattila et al., 2002). Phytochemicals play an important role in preventing oxidative stress, which contributes to cataracts, cardiovascular disease, atherosclerosis, chronic in flammation, and neurodegenerative diseases such as Alzheimer's and Parkinson's disease (Ghahremani-Majd et al., 2012). Fungi are an important source of antioxidant and other medicinal compounds. Recently, research is being conducted on the use of mushrooms to reduce damage caused by oxidizing agents (Ozturk et al., 2011).

Current research suggests that one bioactive compound responsible for the antioxidant activity of mushroom extract is L-ergothioneine or ERGO. In animals, ERGO provides several physiological benefits, such as enhancement of met-

abolic energy, protection against formation of cataracts, and molecular regulation of anti-inflammatory mechanisms in the lungs, etc. (Rahman et al., 2003). Synthesis of ERGO in nature is restricted to fungi and mycobacterium. ERGO synthesized by these organisms is taken up by plants from the soil and then passed to animals and humans, where it accumulates at different concentrations in tissue and blood (Ey et al., 2007). Nutritional information is increasingly important both for health care professionals and for consumers; however, little is known about the nutritional value of the edible mushrooms cultivated in Iran. The main focus of this work was to compare the nutritional value and chemical composition of commercial strains *A. bisporus* (fruiting bodies and mycelia) produced in laboratory and farm conditions.

Material and Methods

Chemicals

The DPPH (2,2′-Diphenyl-1-picrylhydrazyl) and BHT (butylatedhydroxytoluene) were purchased from Alfa Aesar (Lancaster, UK). The ABTS [(2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonate), diammonium salt)], potassium persulfate ($K_2S_2O_8$), Trolox C (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), plus a water-soluble analogue of vitamin E, iron (III) chloride, 2,4,6-tripyridyl-S-triazine (TPTZ), acetate buffer, and $FeSO_4 \cdot 7H_2O$ were obtained from Sigma Chemical (St. Louis, MO, USA). Organic solvents (methanol, ethanol, chloroform, and dimethyl sulphoxide) were purchased from Rathburn Chemicals Ltd. (Walkerburn, Scotland). The HNO_3, H_2SO_4, and H_2O_2 were obtained from Merck (Darmstadt, Germany). Ergothioneine standard, ethanol, acetonitrile, acetic acid, diethiothreitol (DTT), betaine, 2-mercapto-1-methyl imidazole (MMI), sodium dodecylsulfate (SDS), sodium phosphate, and triethylamine were purchased from Sigma Chemical (St. Louis, MO, USA).

Mushroom cultivation

Various mushroom spawn were donated by Balot Co. (Tehran, Iran). Information about the studied species is provided in Table 1. *Agaricus bisporus* was grown on a pasteurized composted substrate consisting of wheat straw, bedded poultry manure, and gypsum. Mushroom spawn was evenly distributed on compost beds; after 14 to 21 days of spawning, mushroom mycelia were covered with a uniform moist layer of casing (pasteurized peat moss) to trigger the mushroom mycelia into a reproductive fruiting button stage. The old mycelium and mushrooms were harvested before casing and 12 to 15 days after casing. After harvest, two samples of mushrooms from each strain were selected for in vitro isolation and culture. An internal section of the mushroom mycelium was excised and isolated in a Petri dish containing synthetic potato dextrose agar (PDA). The cultures were incubated for seven days at 25°C, stored at 4°C and sub-cultured every two months. Cultivation in liquid media was carried out in 250-mL Erlenmeyer flasks containing 100 mL of 200 $g \cdot L^{-1}$ potato, 20 $g \cdot L^{-1}$ glucose, 3 $g \cdot L^{-1}$ yeast extract, 2 $g \cdot L^{-1}$ KH_2PO_4, and 1 $g \cdot L^{-1}$ $MgSO_4 \cdot 7H_2O$, with natural pH. Flasks were inoculated with a 0.5 cm mycelia block of *A. bisporus* from the solid media, incubated at 25°C for 24 h without shaking, and then shaken on a rotary shaker at 140 rpm for 25 days.

Table 1. Mushroom strains used in this study.

Sample	Type	Strain no.
S1	Brown	A-SPA15
S2	White	S2200
S3	Brown	DAF-A15
S4	White	IM008

Extraction procedures

The mushroom samples, including young mycelia, old mycelia, and fruiting body, were freeze-dried and finely milled. Mushroom powder was placed in a Soxhlet apparatus with methanol (MeOH) at 60°C for 4 h. The extract was filtered using a Whatman No. 1 filter paper and concentrated in a vacuum rotary evaporator at 40°C. These methanolic extracts were used for the determination of total phenolics, radical scavenging activity, and reducing capacity.

DPPH radical scavenging activity

The DPPH free radical scavenging assay was performed according to the Bozin et al. (2007) method with some modification. Various concentrations of each extract were added to 1 mL of 90 μM DPPH solution and made up with methanol (95% v/v) to a final volume of 3 mL. The mixture was shaken immediately after adding DPPH solution, and was allowed to stand for 1 hour at room temperature in darkness. Absorbance was read at 517 nm against the blank (the same solution with no added extract). Three replicates were recorded for each sample. Synthetic antioxidant BHT (butylated-hydroxytoluene) was used as a positive control. The radical scavenging capacity (RSC) was calculated using the following equation:

$$\text{Radical scavenging activity\%} = \left(A_{blank} - A_{sample}\right) / A_{blank} \times 100$$

The IC50, which expresses the concentration of the extract that causes 50% inhibition, was obtained from the plot of RSC versus extract concentrations.

Ferric reducing antioxidant power (FRAP) assay

The ability to reduce ferric ions was measured using a

modified version of the Benzie and Strain (1996) method. To prepare fresh FRAP reagent, 100 mL acetate buffer (300 mM, pH 3.6) was added to 10 mL 2,4,6-tripyridyl-S-triazine (TPTZ) (10 mM in 40 mM/HCl) and 10 mL $FeCl3·6H_2O$ (20 mM) in a ratio of 10:1:1 at 37°C. The absorbance of test tubes containing 2 mL reagent and 200 µL sample extracts was read at 593 nm after 5 min using FRAP solution as blank. Trolox was used for calibration. The range of absorbance should be within 0.2. Antioxidant capacity based on the ability to reduce ferric ions of the sample was calculated from the linear calibration curve and expressed as µmol Trolox equivalents per gram of tissue dry weight.

ABTS radical cation scavenging assay

Standard TEAC (Trolox equivalent antioxidant capacity) assay was performed according to the method of Gao et al. (2007), with slight modification. ABTS was prepared by mixing 7 mM aqueous ABTS with potassium persulfate (2.4 mM) in the dark at room temperature for 16 h. For the evaluation of antioxidant activity, the solution was diluted with ethanol to reach an absorbance of 0.70 ± 0.02 at 734 nm. Different concentrations of the extracts were mixed with ABTS solution. The final absorbance was read at 734 nm after 6 min with 1 min intervals. The results were obtained using a calibration curve of Trolox as standard and were expressed as µmol Trolox equivalents per gram of plant dry weight. All experiments were performed in three replications.

Total phenolic content

Total phenolic content of the extracts was measured using the Folin-Ciocalteau (Slinkard and Singleton, 1997) method with some modifications. A volume of 20 µL from each extract was mixed with 100 µL Folin-Ciocalteau reagent and 1.6 mL distilled water. After 3 min, 300 µL of 7% sodium carbonate solution was added to the mixture. The final solution was shaken for 2 h and then the absorbance was read at 765 nm. According to the calibration curve of gallic acid (ranging 0-1000 ppm), results were obtained and expressed as mg gallic acid equivalents per gram of dry weight.

Determination of metal contents

Fruiting bodies and the mycelia were oven dried at 40°C for 48h. Dried samples were homogenized using an agate homogenizer, and stored in pre-cleaned polyethylene bottles until analysis. One gram of sample was placed in a porcelain crucible and ashed at 450°C for 18 to 20 hours. The ash was dissolved in 1 mL concentrated HNO_3 (Merck, Darmstadt, Germany), evaporated to dryness, heated again at 450°C for 4 h, treated with 1 mL concentrated H_2SO_4, 1 mL HNO_3 and 1 mL H_2O_2, and then diluted with double deionized water up to a volume of 10 mL. Three blank samples were treated in the same way. For the element analyses, AAS (A Perkin-Elmer Analyst 700 model atomic absorption spectrometer, Waltham, MA, USA) was used. The Pb and Cd levels in the mushroom samples were determined by HGA graphite furnace, using argon as an inert gas. Determinations of other heavy metal contents were carried out in an air/acetylene flame. All the experimental results were means ± SD of three parallel measurements.

Ergothioneine (ERGO) analysis

The method used to quantify ERGO in the mushrooms is outlined in detail by Dubost et al. (2007). In short, analysis was carried out using an HPLC with separation carried out on two Econosphere C18 columns (Alltech Associates, Deer field, IL, USA) with each column being 250 mm × 4.6 mm, 5 µm particle size connected in tandem. The isocratic mobile phase was 50 mM sodium phosphate in water with 3.0% acetonitrile and 0.1% triethylamine adjusted to a pH of 7.3 with a flow rate of 1 mL per minute. A UV-VIS detector equipped with a wavelength of 254 nm was employed. The injection volume was 10 µL, with the column temperature being ambient. ERGO was quantified by monitoring absorbance at 254 nm and comparing the peak area of the sample to peak areas obtained from different concentrations of the authentic standard. All data was expressed as milligrams of ERGO per gram of dry weight (mg $ERGO·g^{-1}$ DW). Triplicate extractions were performed and used for ERGO analysis from one crop for each of mushroom strain tested.

Results

Antioxidant activity DPPH radical scavenging

There were significant differences among mushroom strains by the DPPH assay (Table 2). All extracts possessed free-radical scavenging activity, but at different levels. The fruit body of S1 strain had the greatest antioxidant activity (with an IC50 at 1.16 ± 0.12 $mg·mL^{-1}$), followed by the fruit body of S3 strain (with an IC50 at 1.18 ± 0.09 $mg·mL^{-1}$) and fruit body of S2 strain (with an IC50 at 1.39 ± 0.11 $mg·mL^{-1}$). The lowest antioxidant activity was detected in the mycelium of S4 strain (with an IC50 at 3.38 ± 0.26 $mg·mL^{-1}$).

ABTS radical cation scavenging activity

The ABTS radical assay is a rapid and reliable method widely used to assess the total amount of radicals that can be scavenged by an antioxidant. The ABTS assay in this study showed similar results to those obtained through DPPH reaction (Table 2). Statistical Analysis Showed that the fruit body of S1 strain extract had the highest level (466.8 ± 16.1 µmol $Trolox·g^{-1}$), while young mycelium of the S4 strain extract had the lowest activity (212.6 ± 7.3 µmol $Trolox·g^{-1}$).

Table 2. Antioxidant activity of various extracts of the *A. bisporus* strain by the DPPH, ABTS, FRAP, and total phenolic assays[z].

Strain	Sample	DPPH assay IC50 ($mg \cdot mL^{-1}$)	ABTS assay (μmol Trolox$\cdot g^{-1}$)	FRAP assay (μmol Trolox$\cdot g^{-1}$)
S1	Fruit body	1.1 ± 0.12 e	466.8 ± 16.1 a	489.7 ± 15.8 a
	Old mycelium	2.6 ± 0.21 b	353.2 ± 12.1 c	392.7 ± 12.6 b
	Young mycelium	3.1 ± 0.24 a	313.5 ± 10.8 d	319.6 ± 10.3 c
S2	Fruit body	1.3 ± 0.11 e	410.0 ± 14.1 b	470.4 ± 15.1 a
	Old mycelium	2.0 ± 0.16 c	310.1 ± 10.6 d	376.9 ± 12.1 b
	Young mycelium	2.7 ± 0.21 b	275.3 ± 9.4 e	306.6 ± 9.8 c
S3	Fruit body	1.1 ± 0.09 e	358.4 ± 12.3 c	396.6 ± 12.7 b
	Old mycelium	1.4 ± 0.11 de	270.9 ± 9.3 e	316.5 ± 10.2 c
	Young mycelium	2.3 ± 0.18 c	240.5 ± 8.2 f	257.2 ± 8.3 d
S4	Fruit body	1.7 ± 0.13 d	317.1 ± 10.9 d	371.1 ± 11.9 b
	Old mycelium	3.0 ± 0.24 a	239.5 ± 8.2 f	295.7 ± 9.5 c
	Young mycelium	3.3 ± 0.26 a	212.6 ± 7.3 g	240.2 ± 7.7 d

[z]IC50 values represent the means ± standard deviation of three parallel measurements.

FRAP assay

The principle of the FRAP method is based on the reduction of a ferric tripyridyltriazine complex to its ferrous colored form in the presence of antioxidants. This property indicates that the antioxidant compounds are electron donors and can reduce the oxidized intermediates of the lipid-peroxidation process, so that they can act as primary and secondary antioxidants (Yen and Chen, 1995). The reducing powers of the different strains are shown in Table 2. In the present study, this method showed similar results to those obtained by ABTS assay.

All strains demonstrated reducing power, but they showed considerably different values of FRAP. Again, the highest activity was found in the fruit body of S1 strain (489.7 ± 15.8 μmol Trolox$\cdot g^{-1}$) and the lowest in young mycelium of the S4 strain (240.2 ± 7.7 μmol Trolox$\cdot g^{-1}$).

Total phenolic content

Phenolic contents of mushroom strain extracts were measured using the Folin-Ciocalteu reagent (Fig. 1). The highest phenolic content was found in the fruit body of S1 strain (11.9 ± 0.2 mg GAE$\cdot g^{-1}$), followed by the Fruit body of S3 (10.8 ± 0.2 mg GAE$\cdot g^{-1}$). Also the lowest phenolic contents were detected in the young mycelium of the S4 strain (6.0 ± 0.1 mg GAE$\cdot g^{-1}$). Hence, a high phenolic content was found to be a factor in determining the antioxidant activity of medicinal mushrooms.

Determination of metal contents

Four elements (Cu, Mn, Zn and Fe) and four heavy metals (Ni, Pb, Cd, and Cr) were present in four *A. bisporus* strains. Element concentrations of the various mushroom strains are presented in Table 3. The most abundant elements were iron and copper, followed by zinc. Manganese was the scarcest element presented in this table (between 10.3 and 36.2 $\mu g \cdot g^{-1}$, DW); the most abundant heavy metal was nickel (Table 3). The amount of nickel ranged between 2.2 and 10.4 $\mu g \cdot g^{-1}$, DW. When compared with nickel, the amount chromium was much lower, ranging between 1.9 and 8.8 $\mu g \cdot g^{-1}$, DW among the mushroom strains studied. The Cd was present in the lowest concentrations, ranging from 0.46 to 2.4 $\mu g \cdot g^{-1}$, DW.

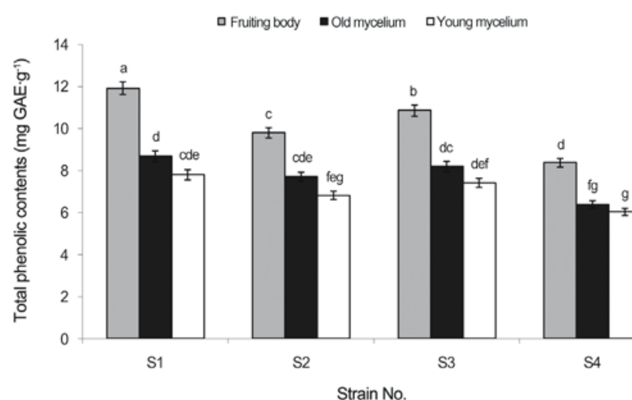

Fig. 1. Concentration of total phenolic contents in different mushroom strains.

Ergothioneine content

The biological role of ERGO is currently under investigation for its impact on the inflammatory process and certain diseases. In this study, ERGO concentration of the mushrooms ranged from 0.46 to 1.79 mg$\cdot g^{-1}$ DW (Fig. 2). Statistical Analysis Showed that among the mushroom strains, the fruit body of S1 strain contained the highest ERGO and young mycelium of the S4 strain contained the least.

Table 3. Concentrations of Cu, Zn, Mn, Fe, Cr, Ni, Cd, and Pb of the mushroom samples analyzed.

Strain	Sample	Cu	Zn	Mn	Fe	Cr	Ni	Cd	Pb
		($\mu g \cdot g^{-1}$ dry weight)							
S1	Fruit body	152.3 ± 7.2^{b}	72.9 ± 4.0^{b}	23.5 ± 1.3^{bcde}	322 ± 13^{d}	7.0 ± 0.5^{cd}	8.7 ± 0.5^{c}	2.2 ± 0.2^{b}	3.04 ± 0.1^{b}
	Old mycelium	123.2 ± 5.8^{cd}	61.7 ± 3.2^{def}	19.9 ± 1.1^{cdef}	272.8 ± 2^{f}	5.9 ± 0.4^{e}	7.5 ± 0.4^{de}	1.8 ± 0.1^{de}	2.5 ± 0.1^{d}
	Young mycelium	91.8 ± 3.9^{f}	39.2 ± 2.1^{hi}	11.8 ± 0.7^{fg}	136 ± 7^{i}	1.9 ± 0.1^{f}	2.4 ± 0.1^{f}	0.57 ± 0.0^{g}	0.67 ± 0.0^{fg}
S2	Fruit body	137.3 ± 6.5^{cb}	69.6 ± 3.8^{bcd}	36.2 ± 2.0^{a}	369 ± 15^{b}	8.8 ± 0.6^{a}	8.1 ± 0.5^{cd}	1.8 ± 0.1^{de}	2.5 ± 0.1^{d}
	Old mycelium	111.1 ± 5.2^{de}	58.9 ± 3.1^{ef}	30.7 ± 1.7^{ab}	312 ± 12^{d}	7.5 ± 0.5^{bc}	7.0 ± 0.4^{e}	1.7 ± 0.1^{e}	2.0 ± 0.1^{e}
	Young mycelium	62.6 ± 2.7^{g}	37.5 ± 2.0^{hi}	14.6 ± 0.9^{efg}	156 ± 8^{gh}	2.1 ± 0.1^{f}	2.2 ± 0.1^{f}	0.54 ± 0.0^{g}	0.55 ± 0.0^{fg}
S3	Fruit body	119.6 ± 5.7^{cd}	62.6 ± 3.4^{cde}	29.1 ± 1.6^{abc}	403 ± 17^{a}	8.2 ± 0.6^{ab}	10.4 ± 0.6^{a}	2.1 ± 0.1^{bc}	3.3 ± 0.21^{a}
	Old mycelium	96.8 ± 4.6^{ef}	71.0 ± 3.7^{bc}	24.6 ± 1.4^{bcd}	341 ± 13^{c}	6.9 ± 0.5^{cd}	9.0 ± 0.5^{bc}	1.4 ± 0.1^{f}	2.7 ± 0.1^{c}
	Young mycelium	72.1 ± 3.1^{g}	45.2 ± 2.5^{gh}	10.3 ± 0.6^{g}	171 ± 8^{g}	2.2 ± 0.1^{f}	2.8 ± 0.1^{f}	0.46 ± 0.04^{g}	0.72 ± 0.05^{f}
S4	Fruit body	164.5 ± 7.8^{a}	83.9 ± 4.6^{a}	20.5 ± 1.1^{cdef}	345 ± 14^{c}	7.7 ± 0.5^{bc}	9.7 ± 0.6^{ab}	2.4 ± 0.2^{a}	2.4 ± 0.1^{d}
	Old mycelium	133.1 ± 6.3^{c}	53.0 ± 2.7^{fg}	17.4 ± 1.0^{defg}	291 ± 12^{e}	6.5 ± 0.5^{de}	8.4 ± 0.5^{c}	1.9 ± 0.1^{cd}	1.9 ± 0.1^{e}
	Young mycelium	99.2 ± 4.3^{ef}	33.7 ± 1.8^{i}	18.1 ± 1.1^{defg}	146 ± 8^{hi}	2.4 ± 0.1^{f}	2.7 ± 0.1^{f}	0.62 ± 0.0^{g}	0.53 ± 0.0^{g}

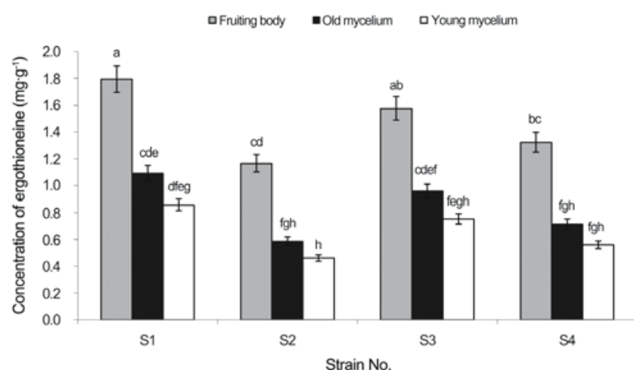

Fig. 2. Concentration of ergothioneine in different mushroom strains.

Discussion

In recent years, antioxidant properties of mushroom products have been of interest to the food industry and in pharmacology. Although current research mainly focuses on the fruiting body of A. *bisporus*, cultured mycelia can be considered as a source of bioactive substances. Currently, no effort has been made to compare the antioxidant bioactivity of fruiting bodies and mycelia of *A. bisporus*. It was demonstrated that different culture conditions have an effect on the production of antioxidant molecules in submerged cultivation (Anne Elise et al., 2012). It is probable that different culture conditions, such as carbon and nitrogen sources, temperature, and other factors, are related to production of antioxidant compounds by *A. bisporus*.

The present study was the first investigation of the antioxidant activities of Iranian mushroom strains through comprehensive in vitro methods. The results showed that extracts of all *A. bisporus* had antioxidant activity. Mushrooms contain an array of different classes and types of antioxidants; therefore, various antioxidant assays can be used to determine the total antioxidant capacity.

Mushrooms contain a variety of secondary metabolites, including various phenolic compounds, which have been shown to act as antioxidants (Mau et al., 2002). The identification and evaluation of phenolic compounds in mushrooms is of importance both in their nutritional and functional characterization. Phenolics are secondary metabolites commonly found in plants, and fungi have been reported to exert multiple biological effects including antioxidant activity (Dimitrios, 2006; Kim et al., 2008).

Statistical analysis displayed a significant linear correlation between the antioxidant activities and phenolic content (Table 4). Higher phenolic content was positively correlated with higher total antioxidant activity. Phenolic compounds have the capacity of reducing oxidative cellular damage caused

Table 4. Correlation (r) between total phenolics (TF), DPPH, FRAP, and ABTS in mushroom strain's.

	DPPH	ABTS	FRAP	TF
DPPH	1.0			
ABTS	0.86**	1.0		
FRAP	0.93**	0.96**	1.0	
TF	0.91**	0.91**	0.87**	1.0

**Correlation is significant at $p < 0.01$ level.

by free radicals (Dimitrios, 2006). DPPH had a strong correlation with phenolics (r = 0.91). ABTS and FRAP also correlated with phenolics, with r = 0.91 and r = 0.87, respectively. These indicate that higher total phenolic content resulted in higher total antioxidant activity. According to Dubost et al. (2007), the linear correlation between antioxidant activity and polyphenol content underlie the fact that phenolic compounds of mushrooms contribute to their antioxidative effects. Researchers study showed that edible mushrooms, including *A. bisporus*, have been found to possess significant in vitro antioxidant activity, which was well correlated with their total phenolic content. Various concentrations of methanolic extracts from mushrooms exhibited scavenging activity of free radicals (Cheung et al., 2003; Mau et al., 2002; Yang et al., 2002).

Our results suggest that different sample of mushrooms have different antioxidant activities. Observed biological activity of mushrooms could be due to various chemical compounds, such as polyphenolic compounds like flavonoids, and also ergothioneine. Differences in extract activities could be attributed to the combined influences of both genetic factors and growing conditions. Differences in antioxidant capacity reflect the variability of strains. Rajesh and Nageswara (2013) showed variation in the amount of electrochemical behavior and antioxidant properties of cultivated commercial mushroom extracts. Dubost et al. (2007) reported a variation between polyphenols and ergothioneine contents in cultivated mushrooms. Our results suggest that button mushrooms could be consumed as a source of natural antioxidants, and also used in pharmaceutical and food industries.

Four mushroom strains contained high amounts of copper, zinc, iron and manganese. This is in agreement with the report of analysis of cultivated mushrooms (Mattila et al., 2001). Minerals in the diet are required for metabolic reactions, transmission of nerve impulses, rigid bone formation and regulation of water and salt balance (Genccelep et al., 2009). The levels of essential elements in mushroom species were higher than those of toxic elements. Mushrooms are a good source of iron, copper, manganese, zinc and calcium (Table 3). Manganese is essential for most physiological functions such as bone and cartilage formation; amino acid, cholesterol and glucose metabolism, and manganese-mediated antioxidant enzymes (Koyyalamudi et al., 2013). The recommended dietary allowances (RDA) for adults are 0.90 mg copper/day (European Commission, 2003). In general, copper levels in mushrooms are higher than other vegetables, and could be considered a source of this element (Isiloglu et al., 2001). Present concentrations of copper in mushrooms are not considered a health risk. Zinc is one of the most important trace metals for normal growth and development of humans. Zinc deficiency can result from inadequate dietary intake, impaired absorption, excessive excretion, or inherited defects in zinc metabolism. Mushrooms are known as zinc accumulators and sporophores. The results obtained for trace elements in analyzed mushroom species seem acceptable for human consumption at nutritional levels.

Mineral contents of two different cultures (farm and mycelia) showed variant mineral content in mushrooms. This is indicative of the difference in mineral composition of compost and seed culture. The variation of mineral content in mushrooms between farm and mycelia culture appeared to reflect differences in mineral composition of compost and seed culture, although significant variations between the two cultures were observed for most mineral elements, especially higher contributing elements such as Cr, Ni, Cd, and Pb. Taken together, the presence of poultry manure in the bed suggests an accumulation of these toxic trace elements in the mushrooms during cultivation.

The content of ergothioneine in fruiting bodies was higher than that in mycelia. Among the fruiting bodies, S1 contained the highest amount of ergothioneine ($1.79\ mg{\cdot}g^{-1}$). This result is consistent with the fact that ergothioneine is mainly synthesized in fungi. In the present study, the brown mushroom contained a considerably higher amount of ergothioneine. Dubost et al. (2007) also found the ergothioneine content of brown mushrooms to be the highest. Ergothioneine is present in human tissues at concentrations up to 1-2 mM (Hartman, 1990) and has been detected in the liver, kidney, seminal vesicles, bone marrow, optic lens, and central nervous system (Chen et al., 2012). Cells lacking ergothioneineare susceptible to oxidative stress, resulting in increased mitochondrial DNA damage, protein oxidation, and lipid per-oxidation (Paul and Snyder, 2010). The biological role of ergothioneine is still under investigation for its impact on the inflammatory process and certain diseases.

The ramifications of this study could provide valuable new opportunities for mushroom growers, since mushrooms can serve as an excellent source for ergothioneine and provide yet another reason to incorporate mushrooms into the human diet. With this methodology, researchers can begin to quantify this antioxidant in food sources and ultimately in the average consumer's diet. Ergothioneine may be a new vitamin with physiologic roles in antioxidant cytoprotection (Chen et al., 2012; Paul and Snyder, 2010). Overall, the results of this study suggest that differences in strain, along with growing conditions, can affect the level of ergothioneine produced in the mushrooms.

Conclusions

We have shown that various strains of button mushrooms could be a viable and economical source of antioxidants in the diet. Also, the results of this study indicate that *A. bisporus* (specifically S1) mushrooms have significantly higher anti-

oxidant potential relative to the other mushrooms tested. In addition, the TP content in the mushrooms is of value. *A. bisporus* and its extracts maybe used for the development of safe food and pharmaceutical additives. However, further studies, especially in vivo activity tests on extracts and isolated constituents, are needed.

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