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Quantification of polyphenols and ergothioneine in cultivated mushrooms and correlation to total antioxidant capacity

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Abstract

Agaricus bisporus, *Lentinula edodes*, *Pleurotus ostreatus*, *Pleurotus eryngii* and *Grifola frondosa* were analyzed for antioxidant capacity, as measured by oxygen radical absorbance capacity (lipophilic and hydrophilic) ($ORAC_{total}$), hydroxyl radical averting capacity (HORAC), peroxynitrite radical averting capacity (NORAC), superoxide radical averting capacity (SORAC) assays, Folin-Ciocalteu reagent and ergothioneine (ERG) content. $ORAC_{total}$values ranged from 39 to 138 μmol of Trolox equivalents (TE)/g dry weight (dw). HORAC values ranged from 3.0 to 13.6 μmol of caffeic acid equivalents/g dw. NORAC values ranged from 2.0 to 9.0 μmol TE/g dw. SORAC values ranged from 0.37 to 2.6 kunit superoxide dismutate equivalents/g dw. Polyphenols ranged from 4.2 to 10.6 mg gallic acid equivalents/g dw. *A. bisporus* mushrooms, especially portabellas, had higher antioxidant capacity relative to the specialty mushrooms tested. ERG ranged from 0.21–2.6 mg/g dw with *L. edodes*, *P. ostreatus*, *G. frondosa* containing a statistically significant greater amount compared to *A. bisporus*. A good correlation was found between $ORAC_{total}$ and polyphenols ($R^2 = 0.86$).

Keywords: Mushrooms; Ergothioneine; Phenolics; ORAC; HORAC; NORAC; SORAC

1. Introduction

Oxidative stress can be defined as the state in which the balance between prooxidants and antioxidants is disturbed resulting in an increased rate of oxidation. This imbalance can ultimately result in damage to molecules, including proteins, lipids, carbohydrates and DNA. The most common harmful reactive oxygen or nitrogen species existing in the body are peroxyl ($ROO^{\bullet}$), hydroxyl ($HO^{\bullet}$), hydrogen peroxide (H_2O_2), superoxide (O_2^-), singlet oxygen ($^1O_2$) and peroxynitrite ($ONOO^-$) (Halliwell, 1990). Free radicals or reactive oxygen/nitrogen species may ultimately play a major factor in many diseases, such as cancer (Ames, Gold, & Willet, 1995), Alzheimer's (Christen, 2000), atherosclerosis (Diaz, Frei, & Keaney, 1997), and the entire aging process (Yu, 1996). Epidemiological studies have demonstrated an inverse relationship between the intake of fruits and vegetables, rich in antioxidants and the occurrence of these diseases (Willet, 2001). Commonly researched antioxidants are vitamin E, vitamin C, carotenoids, and more recently, phenolic compounds (Katz, 2003; Lee, Koo, & Min, 2004; Schmidl & Labuza, 2000).

Phenolic compounds are one of the most widely distributed plant secondary products. The ability of these compounds to act as antioxidants have been well established (Decker, 1995; Rice-Evans, Miller, & Paganga, 1996). Polyphenols are multifunctional antioxidants by acting as reducing agents, hydrogen donating antioxidants and singlet oxygen quenchers (Rice-Evans et al., 1996). The dietary intake of these polyphenols has been estimated to be from 20 mg to 1 g per day (Hertog, Feskends, Hollman, Katan, & Kromhout, 1993). A well-established method for determining total polyphenols relies on color development due to the reduction of the Folin-Ciocalteu reagent

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(FCR) by the reductant (polyphenol). This particular method utilizes a reference standard, such as gallic acid, with absorbance readings taken with a spectrophotometer (Singleton & Rossi, 1965).

Mushrooms contain a variety of secondary metabolites, including various phenolic compounds, which have been shown to act as excellent antioxidants (Ishikawa, Morimoto, & Hamasaki, 1984; Mau, Lin, & Song, 2002). One specific antioxidant, ergothioneine (ERG) has been identified and quantified in various genera of mushrooms utilizing HPLC-MS (Dubost, Beelman, Peterson, & Royse, 2007). It has been documented that ERG is an antioxidant *in vivo* (Hartman, 1990; Akanmu, Cecchini, Aruoma, & Halliwell, 1991) and a cellular protector against oxidative damage (Aruoma, Spencer, & Mahmood, 1999; Chaudiere & Ferrari-Iliou, 1999).

There is a growing interest to measure the antioxidant capacity in foods. The antioxidant capacity refers to a full spectrum of antioxidant activity against various reactive oxygen/nitrogen radicals. Cao, Alessio, and Cutler (1993) developed an assay called the oxygen radical absorbance capacity (ORAC) to quantify the antioxidant capacity of a number of products including fruits and vegetables (Cao, Sofic, & Prior, 1996; Prior & Cao, 2000; Wang, Cao, & Prior, 1996; Wu et al., 2005). Currently, various employed assays are focused on the antioxidant activity against the peroxyl, hydroxyl, superoxide and peroxynitrite radicals (Huang, Ou, & Prior, 2005).

In this study we selected the most commonly consumed mushrooms in the United States, *Agaricus bisporus* (white, crimini and portabella), and the specialty strains, *Lentinula edodes* (shiitake), *Pleurotus osteratus* (oyster) and *Grifola frondosa* (maitake) to determine the antioxidant capacity (radical scavenging and chelating ability) using multiple assays, including the oxygen radical absorbance capacity ($ORAC_{ROO}$, both hydrophilic and lipophilic), hydroxyl radical averting capacity (HORAC), peroxynitrite radical averting capacity (NORAC), and superoxide radical averting capacity (SORAC). ERG was quantified in each of these mushrooms in order to correlate this data with the antioxidant capacity assays as an indicator of whether ERG is contributing to the antioxidant capacity of the mushrooms tested. In addition, FCR was used to quantify the total phenolics (TP) of the same mushrooms in order to evaluate their contribution to antioxidant capacity. The results of this study provide a comprehensive set of data on the antioxidant capacity of the selected mushrooms.

2. Materials and methods

2.1. Chemicals and apparatus

Ergothioneine standard, ethanol (HPLC grade), acetonitrile (HPLC grade), diethiothreitol (DTT), betaine, 2-mercapto-1-methyl imidazole (MMI), sodium dodecylsulfate (SDS), sodium phosphate, triethylamine, Folin-Ciocalteu reagent (2.0 M), hydrogen peroxide, gallic acid, caffeic acid, ethanol, xanthine oxidase, xanthine, superoxide dismutase from bovine erythrocytes, dihydrorhodamine (DHR-123), 3-morpholinosydnonimine hydrochloride (SIN-1) and sodium carbonate were purchased from Sigma Chemical Co. (St. Louis, MO). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA (Richmond, VA). 6-Hydroxy-2,5,7,8-tetramethylchroman2-carboxylic acid (Trolox), Cobalt(II) fluoride tetrahydrate, picolinic acid (PA) and fluorescein disodium (Fl) were obtained from from Aldrich (Milwaukee, WI). Randomly methylated β-cyclodextrin (RMCD) was obtained from Cyclodextrin Technologies Development Inc. (High Springs, FL). Hydroethidine fluorescent stain was purchased from Polysciences, Inc. (Warrington, PA). ORAC, HORAC, SOAC and NORAC analysis was performed using a Precision 2000 eight channel liquid handling system and Synergy HT microplate UV–VIS and fluorescence reader (Bio-tek. Inc., Winooski, VT). Total phenolics (TP) were analyzed on an UV–VIS spetrophotometer at a wavelength of 765 nm (Thermo Spectronic, Rochester, NY). Quantification of ERG was completed by an HPLC 600E system controller (Waters Corp., Milford, CT) and a UV–VIS 490E detector (Waters Corp., Milford, CT).

2.2. Samples analyzed

Samples of the various mushrooms were collected from The Pennsylvania State University Mushroom Test Demonstration Facility (MTDF), The Pennsylvania State University Mushroom Research Center (MRC), and Modern Mushroom Farm Inc. (Toughkennamon, PA). A list of the fungi analyzed is shown in Table 1. The *A. bisporus* mushrooms were grown using the standard tray system (Hartman, 1998) under standard, controlled conditions that are representative of growth conditions at mushroom farms across the country. The mushrooms are harvested in optimum maturation stage with the caps closed and 2.0–2.5 in. in diameter. Mushrooms from the second break of

Table 1
List of fungi analyzed for antioxidant capacity (ORAC, HORAC, SORAC, and NORAC assays), total polyphenols (Folin-Colcalteu assay) and ergothioneine

Content		
Mushrooms species	Sample type	Source
Agaricus bisporus	White button (all crops)	Penn State University
	Brown mushroom (crimini)	Penn State University
	Portabella	Penn State University
Lentinula edodes	Basidioma	Modern Mushroom Farm Inc.
Grifola frondosa	Basidioma	Modern Mushroom Farm Inc.
		Penn State University
Pleurotus osteratus	Basidioma	Modern Mushroom Farm Inc.
Pleurotus eryngii	Basidioma	Modern Mushroom Farm Inc.

the *A. bisporus* crops were tested. Samples from the *A. bisporus* included in this survey were brown (crimini) mushrooms, portabella mushrooms (mature, brown mushrooms with exposed hymenia), and the common white button mushrooms. The growing conditions of the specialty mushrooms were those currently employed at mushroom farms across the country; thus, the mushrooms analyzed would be comparable to those normally available to consumers. Mushrooms for analysis were harvested on the peak production day.

2.3. Preparation of samples

Harvested mushrooms were expediently transferred by vehicle from the growing facilities to the laboratory. Immediately following this and after random sampling from each genera of the mushroom crop, the mushrooms were cleaned, sliced and stored in a walk-in cooler at 0 °C for 24 h. The mushrooms were then freeze-dried (Model 15 SRC-X; Virtis Genesis Co, Inc., Gardiner, NY), ground to a fine powder and sieved through a size 16 mesh screen. The mushroom powder was collected in sterile sample bags (Fisher Scientific, Pittsburgh, PA) and stored in the dark at room temperature in desiccators over $CaSO_4$ prior to analysis.

2.4. Determination of total phenolics

TP concentrations were measured using FCR. The ethanolic extracts were used for analyzing their phenolic compounds following a modified method of Fu, Shieh, and Ho (2002). Five grams of the freeze-dried mushroom powder was added to 60 ml of 80% ethanol and heated to 60 °C for one hour using a water bath (Precision Scientific Co., Chicago, IL). The sample was filtered after one hour and the procedure was repeated two additional times. After a total of three hours, the extract was filtered, combined with the previous extracts and diluted with 80% ethanol to a final volume of 200 ml, which was then well blended (Model LB10 with 250 ml stainless steel container: Waring Laboratory, Torrington, CT) for 30 s. One milliliter of the ethanolic extract was added to four mls of the FCR, which was diluted with distilled water (1:10). After 3 min, 5 ml of a 7.5% aqueous sodium carbonate solution was added and then after 30 min, absorbance was measured by an UV–VIS spetrophotometer at a wavelength of 765 nm (Thermo Spectronic, Rochester, NY). Gallic acid was used as the standard in order to create a calibration curve by plotting absorbance versus concentration. TP content was standardized against gallic acid and the data was expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight (mg GAE/g dw). The linearity range for this assay was determined as 0.025–0.3 mg/ml GAE ($R^2 = 0.9964$), yielding an average absorbance range of 0.15–2.7 AU. Each sample was extracted in triplicate with from one crop for each genera of mushroom analyzed.

2.5. ERG analysis

The method used to quantify ERG 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, Deerfield, IL) 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% acetonitrile and 0.1% triethylamine adjusted to a pH of 7.3 with a flow rate of 1 ml per minute. An UV–VIS detector equipped with a wavelength of 254 nm was employed. The injection volume was 10 μl; with the columns temperature being ambient. ERG 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 ERG per gram of dry weight (mg ERG/g dw). Triplicate extractions were performed and used for ERG analysis from one crop for each of mushroom genera tested.

2.6. Lipophilic and hydrophilic $ORAC_{FL}$ assay

Approximately 15 g of ground mushrooms were weighed into a 50 ml polyethylene centrifuge tube with 20 ml mixture of 50%H_2O–50%Aceton. The sample was extracted for 1 h at room temperature on an orbital shaker operated at 400 rpm then centrifuged at 5900 rpm and the supernatant was immediately analyzed, the same extract was used for HORAC, NORAC and SORAC described below. The lipophilic $ORAC_{FL}$ assay ($ORAC_{lipo}$) was based on a previous reported method (Huang, Ou, Woodill, Flanagan, & Prior, 2002). Hydrophilic $ORAC_{FL}$ assay ($ORAC_{hydro}$) was based on a previous reported method (Ou et al., 2002). All data was expressed as micromoles of Trolox equivalents (TE) per gram of dry weight (μmol TE/g dw). The freeze-dried forms of the mushrooms were used for testing and the data was reported as dry weight. When reporting amount per serving, the data was converted to a fresh weight basis according to the dry weight of the fresh mushroom samples. For each analysis, triplicate extractions were performed from one crop and used for analysis. $ORAC_{total}$ was determined by calculating the sum of $ORAC_{lipo}$ and $ORAC_{hydro}$ results.

2.7. HORAC assay

The HORAC assay was based on a previously reported method (Ou et al., 2002) with modified for the FL600 microplate fluorescence reader (Bio-Tek Instruments, Inc., Winooski, VT). The FL600 microplate fluorescence reader was used with fluorescence filters for an excitation wavelength of 485 ± 20 nm and an emission wavelength of 530 ± 25 nm. The plate reader was controlled by software KC4 3.0. Caffeic acid (CA) was used as the standard due to CA providing a wider linear range as compared to

gallic acid. All data was expressed as micromoles of caffeic acid equivalents (CAE) equivalents per gram of dry weight (μmol CAE/g dw). The freeze-dried forms of the mushrooms were used for testing and the data was reported as dry weight. When reporting amount per serving, the data was converted to a fresh weight basis according to the dry weight of the fresh mushroom samples. For each analysis, triplicate extractions were performed from one crop and used for analysis.

2.8. NORAC assay

The NORAC assay was modified on a previously reported method (Chung, Choi, Park, Choi, & Choi, 2001). Briefly, a stock solution of DHR 123 (5 mM) in dimethylformamide was purged with nitrogen and stored at −80 °C. A working solution with DHR 123 (final concentration, f.c., 5 M) diluted from the stock solution was placed on ice in the dark immediately prior to the study. The buffer of 90 mM sodium chloride, 50 mM sodium phosphate (pH 7.4), and 5 mM potassium chloride with 100 M (f.c.) diethylenetriaminepentaacetic acid (DTPA) was purged with nitrogen and placed on ice before use. $ONOO^-$ scavenging by the oxidation of DHR 123 was measured with a microplate fluorescence reader FL 600 with excitation and emission wavelengths of 485 and 530 nm, respectively, at room temperature. The background and final fluorescent intensities were measured 5 min after treatment with or without SIN-1 (f.c. 10 M) or authentic $ONOO^-$ (f.c. 10 M) in 0.3 N sodium hydroxide. Oxidation of DHR 123 by decomposition of SIN-1 gradually increased, whereas authentic $ONOO^-$ rapidly oxidized DHR 123 with its final fluorescent intensity being stable over time.

All data was expressed as micromoles of Trolox equivalents (TE) equivalents per gram of dry weight (μmol of TE/g dw). The freeze-dried forms of the mushrooms were used for testing and then the data was converted to a FW basis according to the dry weight of the fresh mushroom samples, which were obtained from one crop. For each analysis, triplicate extractions were performed and used for analysis.

2.9. SORAC assay

SORAC assays based on the previously described method by Zhao et al. (1999). Simply, hydroethidine was used as a probe in measuring $O_2^{-\cdot}$ scavenging capacity. Non-fluorescent hydroethidine was oxidized by $O_2^{-\cdot}$ generated by the mixture of xanthine and xanthine oxidase to form a species of unknown structure that exhibit strong fluorescence signal at 586 nm. Addition of SOD inhibits the hydroethidine oxidation. All data was expressed as superoxide equivalents per gram of dry weight (kunitSOD eq/g dw). The freeze-dried forms of the mushrooms were used for testing and the data was reported as dry weight. When reporting amount per serving, the data was converted to a fresh weight basis according to the dry weight of the fresh mushroom samples. For each analysis, triplicate extractions from one crop were performed and used for analysis.

2.10. Statistical analysis

All statistical analysis was performed using MiniTab Inc. (State College, PA). Means ± standard deviations (SD) were calculated for all mushrooms tested. Tukey's procedure was used to determine if significant differences existed between mean scores of mushrooms tested ($\alpha = 0.05$). Regression analysis was completed to obtain a coefficient of determination (R^2) between the antioxidant assays ($ORAC_{total}$, HORAC, NORAC, SORAC) and ERG content of the mushrooms and also between the antioxidant assays and TP (FCR).

3. Results and discussion

Plant foods contain an array of classes and types of antioxidants; therefore, various antioxidant assays can be used to determine the total antioxidant capacity. Using multiple assays, such as ORAC, NORAC, SORAC and HORAC, along with the quantification of TP provides evidence of the antioxidant capacity within the food material able to scavenge various biological significant free radicals. In order to determine total antioxidant capacity of a variety of mushrooms, numerous *in vitro* assays were used. However one must remember that the assays used in this study do not reflect *in vivo* antioxidant capacity. Absorption, metabolism and secondary metabolites should also be considered to determine biological impact.

3.1. Ergothioneine content

ERG is present in human tissues at concentrations up to 1–2 mM (Melville, 1958; Brummel, 1985; Hartman, 1990). The biological role of ERG is currently being explored and is under investigation for its impact on the inflammatory process and certain diseases. Dubost et al. (2007) developed a method to quantify the ERG in various genera of edible mushrooms. In this current study ERG concentration of the mushrooms ranged from 0.21 to 2.6 mg/g dw (Table 2). There was no significant difference found within *A. bisporus* mushrooms; however a trend was evident where the common white button mushroom contained the least ERG and portabellas contained the highest value. The specialty strains tested all contained greater amounts of ERG as compared to *A. bisporus*. A significant difference was found between all of the specialty mushrooms. Among the specialty mushrooms, maitake contained the least ERG and oyster contained the highest value. Based on the amount of ERG found in these mushrooms and on a fresh weight basis, a serving of these mushrooms (85 g) would provide between 1 and 26 mg of ERG (Table 2). Mushrooms, particularly the specialty strains, can serve as an excellent source for ERG.

Table 2
Concentration of ergothioneine in mushrooms

Sample type	mg/g dw of ergothioneine[a]	Moisture (%)	mg ERG/serving[b]
White button	0.21 ± 0.01^{A}	92	1.4
Crimini	0.40 ± 0.03^{A}	91	3.1
Portabella	0.45 ± 0.03^{A}	93	2.7
Maitake	1.13 ± 0.05^{B}	83	16.3
Shiitake	1.98 ± 0.11^{C}	88	20.2
Oyster	2.59 ± 0.18^{D}	88	26.4

[a] Mean mg/g dry weight (dw) ± standard deviation for three samples tested from one crop followed by different capital letters differ significantly ($p = 0.05$, Tukey's method).
[b] Serving size from USDA National Nutrient Database for standard reference for mushrooms (85 g).

3.2. Lipophilic and hydrophilic $ORAC_{FL}$

$ORAC_{hydro}$, $ORAC_{lipo}$ and $ORAC_{total}$ of the mushrooms are presented in Table 3. The $ORAC_{hydro}$ values of the different mushrooms ranged from 33 to 131 μmol TE/g dw, while $ORAC_{lipo}$ values had a narrow range between 5 and 7 μmol TE/g dw. $ORAC_{total}$ values, calculated by adding the $ORAC_{hydro}$ and $ORAC_{lipo}$ values, ranged between 39 and 138 μmol TE/g dw.

A. bisporus were found to have the highest values among the mushrooms tested with a range between 80 and 131 μmol TE/g dw. The specialty strains provided a range between 33 and 49 μmol TE/g dw. The $ORAC_{hydro}$ values translate to approximately 5–9 μmol TE/g (fw). Wu et al. (2005) analyzed a wide variety of vegetables using the $ORAC_{hydro}$ assay with many of the vegetables tested having values less than 15 μmol TE/g fw.

In order to obtain a complete picture of antioxidant capacity of mushrooms, both hydrophilic and lipophilic components should be tested for ORAC analysis. There was no major difference found among the mushrooms in $ORAC_{lipo}$ values. Crimini, maitake and oyster mushrooms had identical values of 5.67 μmol TE/g dw. Both portabella and shiitake mushrooms had the highest $ORAC_{lipo}$ values at 7.00 μmol TE/g dw. The $ORAC_{lipo}$ values translate to approximately 0.48–0.96 μmol TE/g fw. Mushrooms are low in fat with linoleic acid being the most predominate fatty acid (Breene, 1990); therefore, there may be not be a large concentration of lipophilic antioxidant(s) present to produce a high $ORAC_{lipo}$ value.

Wu et al. (2005) analyzed a wide variety of vegetables using the $ORAC_{lipo}$ assay and found a range between 0.09 and 4.20 μmol TE/g fw with many of the vegetables tested provided values below 1.0 μmol TE/g fw (Wu et al., 2005). When considering the $ORAC_{total}$ values there was a significant difference found within the different *A. bisporus* mushrooms; portabellas was highest and the common white button the lowest value. However, all of the *A. bisporus* mushrooms were significantly higher than the specialty mushrooms. Among the specialty mushrooms, maitake contained the lowest $ORAC_{total}$ value with shiitake containing the highest. A significant difference was found between shiitake and maitake, however no significant difference was found between oyster and maiitake and also between oyster and shiitake. It is interesting to note the difference in antioxidant potential among the various genera of mushrooms. Genetics (species or strain), growing conditions, and environmental conditions can affect the amount of secondary metabolites produced by the plant. Since the serving size for mushrooms is equivalent to 85 g (USDA National Nutrient Database for Standard Reference), $ORAC_{total}$ values would range between 564 and 823 μmol TE/serving.

In addition to testing mushroom samples for ORAC values, ERG was also tested using the $ORAC_{hydro}$ assay and was found to have a value of 6440 μmol TE/g indicating that it is a powerful scavenger of $ROO^{\cdot}$ radicals. The results of this study concur with other previous studies that have indicated ERG is a scavenger of $ROO^{\cdot}$ radicals (Asmus, Vensasson, Bernier, Houssin, & Lands, 1996).

To our knowledge this is the first published $ORAC_{total}$ analysis of commonly consumed mushrooms in the United States. Most consumers believe that mushrooms provide little nutritional value or contain few antioxidants; however, the results of this study indicate otherwise. The hydrophilic components of the mushrooms are predominately providing the antioxidant potential indicating that the water-soluble components of the mushrooms have a higher antioxidant capacity against peroxyl radicals as compared to the lipophilic components.

Table 3
Lipophilic ($ORAC_{lipo}$), hydrophilic ($ORAC_{hydro}$), and $ORAC_{total}$ values for mushrooms[a]

Sample type	$ORAC_{lipo}$[b] (μmol of TE/g)	$ORAC_{hydro}$ (μmol of TE/g)	$ORAC_{total}$[c,d] (μmol of TE/g)	$ORAC_{total}$/serving[e] (μmol of TE)
White button	6.33 ± 0.58	80.00 ± 1.7	86.33^{A}	587
Crimini	5.67 ± 0.58	100.00 ± 9.6	105.67^{B}	808
Portabella	7.00 ± 0.00	131.33 ± 10.0	138.33^{C}	823
Maitake	5.67 ± 0.58	33.67 ± 1.5	39.33^{D}	568
Shiitake	7.00 ± 0.00	55.67 ± 4.5	62.67^{E}	639
Oyster	5.67 ± 0.00	49.67 ± 3.8	55.34^{DE}	564

[a] Data expressed as dry weight basis and presented as mean ± standard deviation for three samples tested from one crop.
[b] ORAC data expressed as micromoles of trolox equivalents per gram (μmol of TE/g).
[c] $ORAC_{Total} = ORAC_{lipo} + ORAC_{hydro}$.
[d] $ORAC_{total}$ values followed by different capital letters differ significantly ($p = 0.05$, Tukey's method).
[e] Serving size from USDA National nutrient database for standard reference for mushrooms (85 g).

3.3. HORAC

$HO^{\cdot}$ radicals are highly reactive and can be generated via the Fenton reaction. Due to the fact that $HO^{\cdot}$ radicals are short lived with a high rate constant, it is unlikely that

antioxidants present at biological concentrations will be able to scavenge the HO· radical. However antioxidants, which are able to act as metal chelators, may be able to prevent the formation of the HO· radical, thus acting as a preventative antioxidant (Ou et al., 2002). The HORAC assays measures the ability of the antioxidant present to chelate Co(II) prior to the Fenton reaction occurring (Huang et al., 2005; Ou et al., 2002). HORAC values of the mushrooms are presented in Table 4. HORAC values ranged between 3.0 and 13.6 μmol CAE/g dw. There was a significant difference found within the different *A. bisporus* mushrooms. Portabellas contained the highest value with the common white button containing the lowest value. However, all of the *A. bisporus* mushrooms were significantly higher than two of the specialty mushrooms (oyster and maitake) with shiitake mushrooms not being significantly different than the white button mushrooms. Among the specialty mushrooms, maitake contained the least HORAC value with shiitake containing the highest. However no significant difference was found among the specialty mushrooms. HORAC values provided a range between 30 and 81 μmol CAE/85 g serving size. Generally compounds that can chelate metals, such as Co(II) provide high HORAC values (Ou et al., 2002).

Studies have shown that ERG is a powerful scavenger of HO at high rates (Asmus et al., 1996). Unlike other scavengers, ERG is able to inhibit iron and copper-ion dependent generation of HO (Akanmu et al., 1991). Through its sulfur atom, ERG has the ability to complex with divalent metal ions; such as copper, cadmium and mercury (Motohashi, Mori, & Sugiura, 1976). The HORAC value of ERG was 231 μmol CAE/g. This value is quite high relative to other common natural antioxidants that were previously tested (Ou et al., 2002).

Table 4
HORAC and NORAC values for mushrooms

Sample type	HORAC[a]	μmol CAE/serving[b]	NORAC[c]	μmol TE/serving[b]
White button	5.33 ± 0.58^{A}	36.2	6.33 ± 0.58^{A}	43.0
Crimini	7.67 ± 0.58^{B}	58.7	6.33 ± 0.58^{A}	43.0
Portabella	13.67 ± 0.58^{C}	81.3	9.00 ± 0.00^{B}	53.6
Maitake	2.67 ± 0.58^{D}	38.6	2.00 ± 0.00^{C}	28.9
Shiitake	4.00 ± 0.00^{AD}	40.8	5.00 ± 0.00^{D}	51.0
Oyster	3.00 ± 1.00^{D}	30.6	2.33 ± 0.58^{C}	23.8

[a] Data expressed as dry weight basis and presented as μmol of caffeic acid equivalents per gram (μmol of CAE/g ± standard deviation) for three samples tested from one crop followed by different capital letters differ significantly ($p = 0.05$, Tukey's method).

[b] Serving size from USDA National Nutrient Database for Standard Reference for Mushrooms (85 g).

[c] Data expressed as dry weight basis and presented as μmol of trolox equivalents per gram (μmol TE/g ± standard deviation) for three samples tested from one crop followed by different capital letters differ significantly ($p = 0.05$, Tukey's method).

3.4. NORAC

Under physiological conditions, the formation of $ONOO^-$ can easily result due to the cellular production and interaction of nitric oxide and superoxide radicals. Damage to protein, especially aromatic compounds can result from $ONOO^-$ (Saran, Micel, & Bors, 1990). A limited number of papers have been published regarding the antioxidant capacity against $ONOO^-$. The NORAC assay is a relatively new assay. SIN-1 is used in the NORAC assay, which when heated decomposes to produce $ONOO^-$. DHR-123 (non-fluorescent) is used as the probe, which becomes oxidized by the presence of $ONOO^-$ to become rhodamine 123 (fluoresces). If antioxidants present in the sample are able to scavenge $ONOO^-$, a delay in fluorescence will occur due to the protection of the probe offered by the antioxidant. Generally, compounds that can scavenge $ONOO^-$ will provide high NORAC values. The reaction mechanism for both the ORAC and NORAC assays are very similar since both assays are measuring the capacity of chain-breaking antioxidants.

NORAC values obtained with the mushrooms are presented in Table 4 and they ranged between 2.0 and 9.0 μmol TE/g dw. There was a significant difference found within the different *A. bisporus* mushrooms. All of the NORAC values for the *A. bisporus* mushrooms were significantly higher than the specialty mushrooms and portabellas contained the highest value with the common white button and crimini providing the same values. Among the specialty mushrooms, shiitake had the highest value and was significantly higher than the other specialty mushrooms. NORACvalues provided a range between 23 and 53 μmol TE/85 g serving size.

Studies have shown that ERG is a powerful scavenger of $ONOO^-$ and is able to provide protection from nitration to biological compounds, such as tyrosine (Aruoma, Whiteman, England, & Halliwell, 1997). The NORAC value of ERG was 407 μmol TE/g, which appears to be quite high.

3.5. SORAC

Another biological relevant free radical is O_2^- which is less reactive than HO, however O_2^- does react very quickly with certain radicals, such as nitric oxide (Halliwell & Whiteman, 2004). The SORAC assay is a newly developed assay. Hydroethidine (non-fluorescent) is used as the probe, which is oxidized (fluoresces) in the presence of O_2^- that is generated from a xanthine and xanthine oxidase mixture. If antioxidants present in the sample are able to scavenge O_2^-, a delay in fluorescence will occur due to the protection of the probe offered by the antioxidant (Huang et al., 2005). Generally, compounds that can scavenge O_2^- will provide high SORAC values. The reaction mechanism for the ORAC, NORAC and SORAC assays are very similar since the assays are measuring the capacity of chain-breaking antioxidants.

SORAC values of the mushrooms are presented in Table 5 and they ranged between 0.37 and 2.6 kunit SODeq/g dw. Among the *A. bisporus* mushrooms, portabellas contained the highest value and the common white button contained the lowest value. Among the specialty mushrooms, shiitake had the highest value. Maitake and oyster contained lower values, which were not significantly different from each other. NORACvalues provided a range between 3.9 and 16 kunit SODeq/85 g serving size.

To our knowledge, studies have not shown that ERG is a powerful scavenger of O_2^-. In fact, the NORAC value of ERG was non-detectable.

3.6. Total phenolics

TP in all mushroom samples were analyzed using the FCR and the amount in the mushrooms ranged from 4.2 to 10.6 mg GAE/g dw (Table 5). The specialty mushrooms all contained amounts ranging from 4.1to 4.3 GAE/gdw. All of the *A. bisporus* mushrooms contained significantly greater TP and ranged between 8.0 and 10.7 mg GAE/g dw. Portabellas and crimini contained significantly higher TP than white button.

Based on the amount of TP found in these mushrooms and on a fresh weight basis, a serving of these mushrooms (85 g) would provide 43–75 mg of TP (GAE) (Table 5). A higher free radical scavenging activity has been shown in mushrooms that contain higher TP (Fu et al., 2002). Cheung, Cheung, and Ooi (2003) analyzed shiitake mushrooms and found a positive correlation between total phenolic content in the mushroom extracts (4.79 mg GAE/g dw) and antioxidant capacity. Yang, Lin, and Mau (2002) analyzed shiitake and oyster mushrooms for TP and found between 6 and 15 mg/g dw of TP depending on the species of mushroom chosen. The authors concluded that TP were the major occurring antioxidant component found in the mushrooms and contributed significantly to the antioxidant capacity.

The principal soluble phenolic compound found in the skin of mushrooms is γ-L-glutaminyl-4-hydroxybenzene (GHB). The precursors of GHB include chorismic acid, prephenic acid, tyrosine, 4-aminobenzoic acid and 4-amino-phenol. Polymerization of these phenols ultimately results in melanin production, which produces a brown discoloration of mushrooms (Beaulieu, Beliveau, D'Aprano, & Lacroix, 1999). Choi and Sapers (1994) found *A. bisporus* to have 5.4 mg/g dw of soluble phenols, as measured by FCR. They analyzed *A. bisporus* for phenols and found significant amounts of GHB in every part of the fruiting body at a higher concentration than other phenolic acids. Rajarathnam, Shashirekha, and Rashmi (2003) investigated the phenolic compounds present in button and oyster mushrooms and found the phenolic content about three times higher in button mushrooms. Based on the ability of phenol oxidase to oxidize various phenolic compounds, the predominate phenolics identified in the button mushrooms included tyrosine, catechol, and the phenolic acids, *p*-hydroxy-benzoic acid, *p*-coumaric acid and vanillic acid. The oyster mushrooms contained the phenolic compounds, syringaldehyde, guaiacol and catechol with no detection of tyrosine. Maitake mushrooms were analyzed for phenolic acids and were found to be high in *p*-hydroxy-benzoic acid (66.4 μg/100 g fw) relative to the other phenolic acids identified, tr-cinnamic acid (13.4 μg/100 g fw) and caffeic acid (4.2 μg/100 g fw). The predominate phenolic acids identified in *A. bisporus* was tr-cinnamic acid (20.7 μg/100 g fw) for the white button and *p*-hydroxy-benzoic acid (50.3 μg/100 g fw) for crimini (Mattila et al., 2001). The discolouration of button mushrooms is more extensive as compared to the specialty strains, which is believed to be due to the differences found in the total amount of phenolic compounds and also the diverse functional groups present.

Quantification of TP using FCR is based on electron-transfer reactions (Huang et al., 2005), therefore one must consider non-phenolic compounds present in the sample that could contribute to the transfer of electrons, such as vitamin C. *A. bisporus*, *L. edodes*, *P. ostreatus*, and *G. frondosa* have been analyzed for vitamin C content yielding a range between 17 and 60 mg/100 g dw, with *A. bisporus* containing the lowest amount (Liu, Ooi, & Chang, 1997; Mattila et al., 2001). Due to the fact that *A. bisporus* was found to have the highest level of TP and along with the fact that this genera of mushrooms do not provide significant amounts of vitamin C, it is unlikely that vitamin C contributes significantly to the TP value, however other non-phenolic compounds may be present that are contributing to the TP value.

Table 5
SORAC and total phenolic (TP) values for mushrooms

Sample type	SORAC[a]	kunit SODeq/serving[b]	TP (mg GAE/g)[a]	mg GAE/serving[b]
White button	0.90 ± 0.10^{A}	6.1	8.00 ± 0.48^{A}	59.4
Crimini	1.61 ± 0.11^{B}	12.3	9.89 ± 0.43^{B}	75.7
Portabella	2.69 ± 0.11^{C}	16.0	10.65 ± 0.61^{B}	63.4
Maitake	0.37 ± 0.04^{D}	5.3	4.17 ± 0.06^{C}	60.3
Shiitake	0.77 ± 0.02^{A}	7.9	4.32 ± 0.27^{C}	44.1
Oyster	0.38 ± 0.02^{D}	3.9	4.27 ± 0.69^{C}	43.6

[a] Data expressed as dry weight basis and presented as milligrams of gallic acid equivalents per gram (mg of GAE/g ± standard deviation) for three samples tested from one crop followed by different capital letters differ significantly ($p = 0.05$, Tukey's method).

[b] Serving size from USDA National Nutrient Database for Standard Reference for Mushrooms (85 g).

3.7. Correlation between ERG and antioxidant assays

The ERGO content of the various mushrooms was correlated to the antioxidant potential as measured by the $ORAC_{total}$, HORAC, NORAC and SORAC assays. No significant correlation was found between ERGO and $ORAC_{total}$ ($R^2 = 0.50$), ERGO and HORAC ($R^2 = 0.36$), ERGO and SORAC ($R^2 = 0.37$), and ERGO and NORAC ($R^2 = 0.50$). Based on these results, there appears to be no

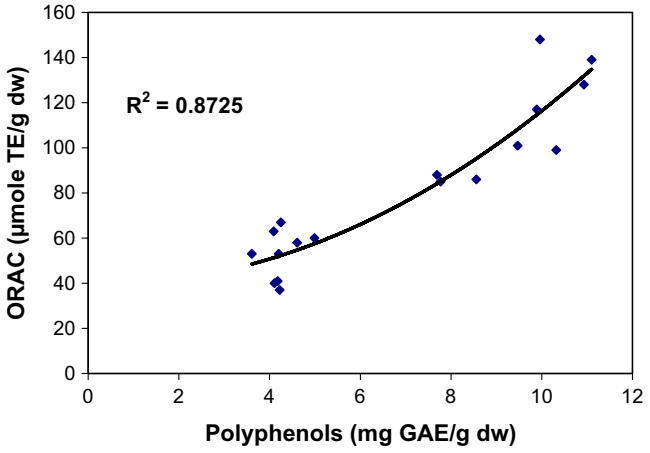

Fig. 1. Relationship ($R^2 = 0.87$) between oxygen radical absorbance capacity ($ORAC_{total}$) expressed as micromoles trolox equivalents per gram dry weight (µmol TE/g dw) and polyphenols expressed as milligrams gallic acid equivalents per gram dry weight (mg GAE/g dw) in cultivated mushrooms.

relationship between these parameters in the six cultivated mushrooms measured. However, this does not provide evidence that there will be less biological activity from ERGO.

3.8. Correlation between TP and antioxidant assays

TP may account for the antioxidant capacity found among fruits and vegetables. Previous studies have shown a linear correlation between polyphenols and antioxidant capacity (ORAC) (Adamson et al., 1999); however, not all types of foods demonstrate a good correlation (Wu et al., 2005). Generally, a relationship is observed within a food or similar foods most likely due to the similar profiles of the antioxidant phytochemicals.

As mentioned earlier, previous studies conducted with mushrooms have shown a positive correlation between the polyphenol content and antioxidant capacity. Within this study, TP content of the various mushrooms was correlated to the antioxidant potential as measured by the $ORAC_{total}$, HORAC, NORAC and SORAC assays. Plotting TP content versus $ORAC_{total}$ values (Fig. 1) yields a nonlinear relationship with a good correlation ($R^2 = 0.87$) between these two parameters, indicating that $ROO^{\cdot}$ radicals are scavenged at a greater rate as the TP content of the mushroom increases. One possible mechanism by which phenolic compounds act as antioxidants is through hydrogen donation. The chemistry of the ORAC assay has been shown to proceed through hydrogen atom transfer (HAT) mechanism (Huang et al., 2005). Thus, the polyphenols present in the mushrooms are able to donate a hydrogen to the $ROO^{\cdot}$ radicals present.

A similar trend was observed for all of the other antioxidant assays data and TP content. A nonlinear relationship was found between SORAC and TP ($R^2 = 0.90$) and HORAC and TP ($R^2 = 0.85$). A linear relationship was found between NORAC and TP ($R^2 = 0.76$). Overall a positive correlation can be seen between the TP content and antioxidant capacity. As the TP content increases, the antioxidant capacity of the mushrooms also increases, indicating the TP in the mushrooms are able to scavenge peroxynitrite, superoxide and peroxyl radicals and in addition able to chelate metals, such as CO(II).

4. Summary

The biological role of ERG and polyphenols has been explored and is still under investigation for its impact on the inflammatory process and certain diseases. We have shown that various genera of edible mushrooms could be a viable and economical source of antioxidants in the diet. Also, results of this study indicate that *A. bisporus*, specifically portabella and crimini, mushrooms have significantly higher antioxidant potential relative to the other mushrooms tested. In addition, TP content in the mushrooms is significantly correlated to $ORAC_{total}$ values. The ramifications of this study could provide valuable new opportunities for mushroom growers, since mushrooms can serve as an excellent source of antioxidants, specifically ERG and TP and provide yet another reason to incorporate mushrooms into the human diet.

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