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Antioxidant and Ergothioneine Assessment of Nutritionally Enriched *Agaricus bisporus* Powders

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Abstract

MBio produces a range of nutritionally enriched *Agaricus bisporus*- derived powder products, such as vitamin D enriched mushroom powders, selenium enriched mushroom powders, and vitamin B12 enriched mushroom powders that may have numerous health benefits. This study aimed to assess the antioxidant activity nutritionally enriched (Vitamin D, Selenium and Vitamin B12) mushroom powders and a whole mushroom powder. This study reports comparative antioxidant assessment of MBio's proprietary *Agaricus bisporus* derived powder products with five different commercially available mushroom powders. The antioxidant activity was evaluated through *in vitro* free radical scavenging activity (DPPH, nitric oxide, ABTS and hydroxyl radical), total polyphenols and ergothioneine content. The results confirmed that the vitamin B12 enriched mushroom powder exhibited higher DPPH activity (5.18 - 9.23 mg/g) and polyphenol content (5.66 - 5.94 mg/g) followed by the vitamin D enriched mushroom powder (DPPH 3.33 - 5.86 mg/g, polyphenols 4.86 - 5.73 mg/g), the selenium enriched mushroom powder (DPPH 2.57 - 5.05 mg/g, polyphenols 4.32 - 5.58 mg/g) and the whole mushroom powder (DPPH 3.36 - 6.72 mg/g, polyphenols 4.56 - 5.52 mg/g) compared to the commercial mushroom powders. Furthermore, the selenium enriched mushroom powder exhibited the highest nitric oxide scavenging activity (NOSA 23.18 - 25.73 mg/g) and the lowest IC_{50} (1.35 - 2.89 mg) for scavenging the ABTS radical indicating better antioxidant activity compared to vitamin B12, vitamin D, whole mushroom powder and five commercial powders. Additionally, the vitamin B12 enriched mushroom powder and the selenium enriched mushroom powder exhibited significantly higher hydroxyl radical scavenging activity (OHSA) compared to the commercial powders. Finally, ergothioneine analysis confirmed that the vitamin D enriched mushroom power (1303 mg/kg) and the selenium enriched mushroom powder (1290.2 mg/Kg) exhibited higher ergothioneine compared to the commercial mushroom powders. Conclusively, this antioxidant assessment confirmed that the nutritionally enriched (selenium, vitamin D and vitamin B12) mushroom powders exhibited significantly higher overall antioxidant activity compared to the commercially available mushroom powders. Therefore, this study confirmed that the nutritionally enriched *Agaricus bisporus* powder products can play a significant role as a natural, whole food source of antioxidants.

Keywords: *Antioxidant; Agaricus bisporus; Mushroom Powders; Nutritionally Enriched; Selenium; Ergothioneine*

Abbreviations

OS: Oxidative Stress; ROS: Reactive Oxygen Species; WMP: Whole Mushroom Powder; DPPH: 2,2-Diphenyl-1-Picrylhydrazyl Reagent; NOSA: Nitric Oxide Radical Scavenging Activity; OHSA: Hydroxyl Radical Scavenging Activity; HPLC: High-Performance Liquid Chromatography; LC-MS: Liquid Chromatography - Mass Spectrometer, CP: Commercial Powder; Se: Selenium, vit D: Vitamin D; vit B12: Vitamin B12

Introduction

Oxidative stress is defined as a significant imbalance between reactive oxygen species (ROS) production and antioxidant defences [1]. The ROS have adverse reactions with polyunsaturated fatty acids, proteins and nucleotides that could lead to lipid peroxidation, inactivated proteins and impaired DNA and RNA. If there is no adequate defence against ROS by enzymatic and non-enzymatic antioxidants, it can induce modifications in signal transduction pathways and can be damaging for cellular functions [2,3]. This results in potential tissue damage leading to a range of disorders such as aging, Alzheimer's disease, Parkinson's disease, immunological and many other neural disorders [4].

Among all free radical species, hydroxyl and superoxide radicals are found to be the main culprits in the damage that free radicals induce in biological systems. The hydroxyl ($^{\cdot}OH$) radical can cause lipid peroxidation, an oxidative modification of low-density lipoproteins (LDLs), which may play role in the development of arthrosclerosis and rheumatoid arthritis [5]. Under stress conditions, different cells of the brain, vascular endothelial cells and phagocytes converts L-arginine to nitric oxide radical. Nitric oxide (NO) radical has an unpaired electron, which reacts with certain types of proteins and other free radicals. The toxicity of NO becomes adverse when it reacts with superoxide radical, forming a highly reactive peroxynitrite anion ($ONOO^{-}$) [6]. Chronic exposure to NO radical is associated with various carcinomas and inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis, and ulcerative colitis [7].

In normal body conditions, the balance between free radicals is maintained by the body's antioxidant system. This is encompassed by enzymes, such as superoxide dismutase and catalase, and/or by compounds, such as ascorbic acid (vitamin C), tocopherols and glutathione. However, in certain situations, this mechanism of antioxidant protection becomes unbalanced and, therefore, antioxidants sourced through the diet play important role in reducing oxidative damage.

Mushrooms have been used for several thousand years, initially as a source of food but later for their medicinal properties [8]. Mushrooms are an excellent source of nutrients, including B vitamins and minerals such as selenium, copper and potassium. Mushrooms are also rich in dietary fiber, chitin and β-glucans, which are main constituents of their cell wall [9]. Many varieties of mushrooms are receiving scientific attention due to their proteins, minerals and different bioactive molecules [10], which have been associated with a reduced risk of many health- related disorders [11]. Indeed, mushroom bioactive proteins and peptides, including lectins have been reported to have anti-tumoural, anti-cancer, antibacterial, antiviral, antifungal, and immuno-modulatory activities [10,12]. It has also been demonstrated that mushrooms can be an abundant source of vitamin D2 when exposed to UV light. Of particular interest, mushrooms have been found to contain high levels of antioxidants, including phenolic compounds and, in particular, the sulphur-containing amino acid ergothioneine [13]. Additionally, compounds from a variety of mushrooms, including *A. bisporus*, such as terpenes, diterpenes, indoles and various phenolic compounds have been reported to have anti-inflammatory, neuroprotective, antioxidants, and antimicrobial effects [14].

Ergothioneine is mainly biosynthesized in fungi, some cyanobacteria and mycobacteria using histidine with cysteine and methionine, which provides sulphur and methyl groups, respectively [13]. In mammals, ergothioneine is mainly found in red blood cells, where it has exhibited an antioxidant role and was proposed as a possible therapeutic for prevention of erythrocytes disorders due to oxidative damage [15]. Additionally, it was proposed that ergothioneine has the ability to act with other antioxidants to protect against oxidative stress in the mitochondria and therefore, acts as a critical biological antioxidant [16]. Earlier research work demonstrated that ergothioneine is a naturally occurring dietary antioxidant [17-19] and present in higher levels in mushrooms compared to any other dietary source.

MBio produces a range of nutritionally enriched *Agaricus bisporus* derived powder products that have numerous health benefits (and associated health claims). This study aimed to assess the antioxidant activity as one of the health benefits of MBio's mushroom powders. Radical scavenging activities are very important to prevent the deleterious role of free radicals in different diseases, including cancer [20].

Aim of the Study

The current study was aimed to assess the mushroom powders using various important radical scavenging activities including DPPH, nitric oxide, hydroxyl and ABTS radical, total polyphenol and ergothioneine content.

Materials and Methods

Chemicals

DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent, methanol, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as a standard antioxidant, for polyphenols: Folin Ciocalteau reagent (2N), sodium bicarbonate ($NaHCO_3$), for NOSA: sodium nitroprusside (SNP), phosphate buffered saline (PBS pH 7.4), sulphanilamide, glacial acetic acid, naphthylethylene diamine hydrochloride (NED), for ABTS: ABTS reagent (Sigma), manganese dioxide (MnO_2), for OHSA: ascorbic acid, ferrous chloride ($FeCl_3$), H_2O_2, EDTA, 2deoxy ribose, thiobarbituric acid (TBA), trichloroacetic acid (TCA). Trolox and gallic acid were used as a standard antioxidant. Ergothioneine analysis was conducted at CampdenBRI group UK on contractual basis. All the materials and chemicals were obtained from Lennox Ireland and Sigma.

Preparation of mushroom extracts for antioxidant analysis

A number of MBio's proprietary mushroom powder products (Table 1) were obtained from MBio, Monaghan Mushrooms Ireland Unlimited Company. The random commercial market mushroom powder samples were used as commercial powders for antioxidant comparison. The details of the production of MBio's mushroom powder products, source and nutritional composition is described below. Each sample (0.1g) was extracted in 10 mL of the extracting solvent (Table 2) for 14h at room temperature in a shaking incubator. The supernatants were used for the assessment [21].

Mushroom Powders	Codes
Whole mushroom powder (WMP)	Batch 1
	Batch 2
	Batch 3
Vitamin D enriched mushroom powder (8,000 IU/g)	Batch 1
	Batch 2
	Batch 3
Vitamin B12 enriched mushroom powder (600 IU/g)	Batch 1
	Batch 2
	Batch 3
Selenium enriched mushroom powder (5 - 10 mg/kg)	Batch 1
	Batch 2
	Batch 3
Commercial Powder of Shitake mushrooms	CP1
Commercial Powder of Chaga mushrooms	CP2
Commercial Powder of Reishi mushrooms	CP3
Commercial Powder of Lion's Mane mushrooms	CP4
Commercial Powder of a mixture of different mushrooms	CP5

Table 1: *Mushroom powder products obtained from final commercial process.*

Assays	Extraction Solvents	Standards	Wavelengths (nm)
DPPH	Methanol	Trolox	515
Total poly-phenols	Water	Gallic acid	700
NOSA	Water	Gallic acid	540
ABTS	Methanol	Trolox	734
OHSA	Water	Gallic acid	520

Table 2: *Antioxidant assay extraction solvents and standards.*

Vitamin D enriched mushroom powder

The source of vitamin D2 mushroom powder is commercially cultivated, freshly harvested fruiting bodies and stalk component of *Agaricus bisporus* obtained from single batch, which is sourced from Monaghan Mushrooms Ireland Unlimited Company. The vitamin D2 enhancement is achieved post-harvest processing of the dried powder using UV light exposure. In addition, there were no significant differences in the nutritional information for fresh *Agaricus bisporus* and novel vitamin D enriched mushrooms bar a concentration effect from the removal of water. The vitamin D2 mushroom powder has been confirmed to provide more than 10% of the recommended daily intake for adults for copper, potassium, selenium and vitamin D. In addition, the vitamin D2 mushroom powder is also high in proteins, fibers and minerals.

Vitamin B12 enriched mushroom powder

The vitamin B12 enriched mushroom powder is derived from freshly harvested *Agaricus bisporus*, common button mushrooms naturally enriched with B12 during the cropping cycle, obtained from single batch, dried and milled under mild conditions to preserve its nutrient rich content. The powder is nutritionally rich and is high in protein, fiber, B vitamins and minerals. The powder feedstock is sustainably sourced, fully traceable and derived from a vegan source.

Selenium enriched mushroom powder

The selenium enriched mushroom powder is derived from freshly harvested *Agaricus bisporus*, common button mushrooms naturally enriched with selenium during the cropping cycle, obtained from single batch, dried and milled under mild conditions to preserve its nutrient rich content. The powder is nutritionally rich and is high in protein, fiber, B vitamins and minerals. The powder feedstock is sustainably sourced, fully traceable and derived from a vegan source.

Whole mushroom powders

The whole mushroom powder is derived from freshly harvested fruiting bodies and stalk component of *Agaricus bisporus* including white closed cup mushrooms obtained from single batch, dried and milled under mild conditions to preserve its nutrient rich content. The whole mushroom powder is 100% food grade mushroom powder. The powder feedstock is sustainably sourced, fully traceable and derived from a vegan source.

The powders were analysed for nutritional composition, which revealed that the mild drying process does not affect the nutritional status of the powders, bar a concentration effect from the removal of water. Furthermore, whole mushroom powder nutritional analysis confirmed that the mushroom powder is high in proteins, high in B vitamins, high in fiber, provides the recommended intake of vitamin D and also high in minerals, which further contributes to the nutritional value of the *Agaricus bisporus*.

Commercial mushroom powders

Randomly available marketed mushroom powders were obtained from online shops. The samples include different strains of mushroom powder such as: Shitakeii, Chaga, Reishi, Lion's Mane and commercial mixture of different mushroom powders. During the experiments, the marketed mushroom powders were coded as commercial powders starting from CP1, CP2, CP3, CP4 and CP5, respectively.

DPPH radical scavenging activity

DPPH is a well-known radical that scavenges other radicals, which is used as an indicator of the rate reduction of a chemical reaction. DPPH radical shows deep violet colour in solution, which upon neutralization, it changes to pale yellow or colourless. Therefore, the reaction monitors the number of radicals in presence of antioxidant samples [22] The DPPH radical scavenging activity was conducted by the method of Ariga and Hamano [23] with slight modifications; 1mL of DPPH reagent (75 µM in methanol) and 20 µL of test samples and 180 µL methanol were incubated at 37°C for 80 min. Trolox (0.1 mg/mL) was adopted as a positive control (5 - 30 µL) and the reduction of the absorbance at 515 nm was monitored and expressed as mg of trolox equivalent DPPH radical scavenging activity. The samples were analysed in triplicate.

Total polyphenol content

A polyphenol contains polyphenolic substructure, which acts as an antioxidant by scavenging the free radicals and up-regulates certain metal chelation reactions. The total polyphenol quantification is based on Folin-Ciocalteau method, which relies on the transfer of electrons in alkaline medium from phenolic compounds to form a blue chromophore [24]. Briefly, 0.2 mL of the sample was added to 0.6 mL of distilled water followed by 0.2 mL of Folin Ciocalteau reagent (1:1 diluted reagent with distilled water (D/W)) except sample blanks. After 5 mins, 1 mL of saturated solution of 8% $NaHCO_3$ in D/W was added and the volumes were made up to 3 mL with D/W. The samples were incubated at 37°C for 30 min and the absorbance was measured using a spectrophotometer at 765 nm against the reagent blank. Gallic acid was used as a standard antioxidant (0.2 mg/mL) in the range of 20 - 100 µL. The total phenolic concentration was calculated and the results were expressed as mg of gallic acid equivalents (GAE)/g of the sample. Analysis was completed in triplicate.

Nitric oxide scavenging activity (NOSA)

The procedure was based on the principle that sodium nitroprusside (SNP) in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent [25]. The reaction mixture contained 2 mL of 10 mM SNP, phosphate buffered saline (pH 7.4) and 200 µL of the test solution in a final volume of 3 mL. After incubation for 150 min at 37°C, 1mL sulphanilamide (0.33% in 20% glacial acetic acid) was added to 0.5 mL of the incubated solution and allowed to stand for 5 min. Then 1 mL of N-(1-Napthayl)ethylenediamine dihydrochloride (NED) (0.1% w/v) was added and the mixture was incubated for 30 min at 37°C. The pink chromophore generated by diazotization of nitrite ions with sulphanilamide and subsequent coupling with NED was measured spectrophotometrically at 540 nm against a blank sample. Gallic acid (0.1 mg/mL) was used as a standard. All tests were performed in triplicate.

ABTS radical scavenging activity

Free radicals are an outcome of various metabolic activities and their excess production leads to many diseases. According to Scopus citation, the 2,2 -azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical cation-based assays and the 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical-based assays are most widely used to predict the antioxidant activity. The trolox equivalent antioxidant activity (TEAC) was estimated using the $ABTS^{\bullet+}$ system according to the method of Zielińska., *et al* [26]. The radical scavenging capacity of samples was evaluated against $ABTS^{\bullet+}$ radical, which was generated by oxidizing a 5 mM of ABTS (2, 2'-azinobis 3-ethylbenzothiazoline-6-sulfonic

acid) diammonium salt, with manganese dioxide in PBS (pH 7.4) at ambient temperature for 14h in dark. The reaction mixture contained 2.0 mL of $ABTS^{\bullet+}$ with the test sample from 10 - 50 µL and the absorbance was recorded at 734 nm. A similar reaction was conducted for the standard trolox (0.1 mg/mL in methanol) as a positive control. The results were expressed as IC_{50} as mg of the powder required for 50% inhibition of $ABTS^{\bullet+}$ radical. Each sample was analysed in triplicate for IC_{50}. Lower the IC_{50} is considered as better antioxidant activity equivalent to Trolox.

Hydroxyl radical scavenging activity (OHSA)

The hydroxyl radical is one of the potent reactive oxygen species that reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell [2], modifies important biomolecules. These radicals reduce disulphide bonds in proteins, in particular fibrinogen, resulting in unfolding and abnormal spatial configurations. These reactions are the reason for many diseases including atherosclerosis, cancer and neurological disorders, and can be prevented by the action of non-reducing substances. OHS assay was conducted using ascorbic acid-iron-EDTA model of hydroxyl radical generating system. The total volume of the reaction mixture was 1 mL, which included 0.4 mL sodium phosphate buffer (20 mM, pH7.4), 0.1 mL of the sample extract, 0.1 mL of 2-deoxy ribose (60 nM/L), 0.1 mL ferric chloride (1mM/L), 0.1 mL EDTA (1.04 mM/L), 0.1 mL ascorbic acid (2 mM/L) and the reaction was initiated by adding 0.1 mL of H_2O_2 (10 mM/L). The standard gallic acid (0.1 mg/mL) was used ranging from 0.02 - 0.1 mL for the standard curve. The reaction mixture was incubated at 37°C for 1h. The reaction was stopped by adding 1 mL of trichloroacetic acid (TCA 17mM) and 1 mL of thiobarbituric acid (TBA 17mM). The tubes were placed in boiling water bath at 100°C for 15 mins and immediately cooled on ice. The pink colour was measured at 532 nm. The sample blanks were prepared by replacing TBA with 1 mL of water. The results were expressed as mg of gallic acid equivalent/g of sample. Analysis was conducted in triplicate.

Ergothioneine analysis

This analysis was conducted by CampdenBRI, UK on contractual basis using a modified method to quantify ergothioneine in the mushroom powders [17]. Briefly, 1g of the sample was extracted into 20 mL ethanol/water (50:50) and centrifuged prior to filtration through a 0.45 µm nylon filter. Ergothioneine stock solution was made in ethanol and a linear calibration curve was obtained from 10 mg/kg - 0.01 mg/kg. The separation was achieved on a hilic column with a water/acetonitrile mobile phase (0.1% formic acid). The LC/MS/MS was used for detection. The mass spectrophotometry was run in positive ionisation mode optimized for the detection of ergothioneine. One sample of each matrix type was analysed in duplicate with spike to check extraction efficiency. All samples were prepared for a standard addition analysis to accurately quantify the levels; the % recovery on the spiked samples was 85%. This analysis was performed twice to confirm the results. The results were expressed as mg of ergothioneine/Kg of powders.

Statistical analysis

The data was analysed using MS-EXCEL. One way Anova was used for statistical analysis for comparison across batches, different type of mushroom powders.

Results

DPPH radical scavenging activity

Whole mushroom powder: The DPPH scavenging activity was reported as equivalent to standard antioxidant Trolox. The DPPH scavenging activity of WMP ranged from 3.36 - 6.72 mg/g (Table 3). There was no significant ($p = 0.06$) difference in the DPPH activity of Batch 1 (4.72 mg/g) and Batch 2 (6.72 mg/g). Similarly, no significant difference ($p = 0.1$) was observed in Batch 1 and Batch 3 (3.36 mg/g) (Figure 1). Although, Batch 2 exhibited significantly higher ($p < 0.001$) DPPH scavenging activity compared to Batch 3, overall results confirmed that WMP exhibited a consistent retention of antioxidant activity across the production of different batches.

Mushroom powders		Average ± SD					
		DPPH (mg equivalent to trolox/g powder)	Total Polyphenols (mg equivalent to gallic acid/g powder)	NOSA (mg equivalent to gallic acid/g powder)	ABTS (IC50:mg of powder equivalent to trolox for 50% inhibition of ABTS)	OHSA (mg equivalent to gallic acid/g powder)	Ergothioneine (mg/Kg powder)
WMP	1	4.72 ± 0.15	5.52 ± 0.31	15.35 ± 3.82	2.81 ± 0.41	26.23 ± 2.81	547
	2	6.72 ± 1.70	4.82 ± 0.37	14.00 ± 1.32	1.87 ± 0.37	20.78 ± 1.28	1009
	3	3.36 ± 1.26	4.56 ± 0.18	14.54 ± 1.03	2.67 ± 0.42	29.96 ± 1.63	1009
Vit D	1	5.86 ± 1.53	4.86 ± 0.27	14.22 ± 2.17	1.93 ± 0.37	24.38 ± 2.08	978.5
	2	3.33 ± 1.28	5.05 ± 0.25	15.71 ± 1.41	2.99 ± 0.42	24.98 ± 5.24	1087
	3	5.03 ± 0.40	5.73 ± 1.07	11.63 ± 0.33	2.46 ± 0.17	10.87 ± 1.16	1843.5
Vit B12	1	9.23 ± 2.35	5.91 ± 0.49	14.32 ± 1.39	1.62 ± 0.32	38.37 ± 4.38	541.5
	2	5.18 ± 1.30	5.94 ± 0.19	21.88 ± 0.09	1.74 ± 0.19	17.39 ± 2.37	189.5
	3	5.31 ± 0.17	5.66 ± 0.18	14.75 ± 3.21	2.89 ± 0.62	24.98 ± 1.36	827
Selenium	1	5.05 ± 0.43	5.58 ± 0.08	25.73 ± 1.93	1.13 ± 0.16	25.59 ± 0.13	1266
	2	3.07 ± 1.08	4.59 ± 0.03	23.35 ± 0.66	1.35 ± 0.21	26.98 ± 0.99	1160.5
	3	2.57 ± 0.88	4.31 ± 0.06	23.18 ± 0.83	2.09 ± 0.18	23.98 ± 0.44	1444
Commercial powders	CP1	1.19 ± 0.21	3.43 ± 0.58	11.35 ± 2.85	6.82 ± 1.83	9.498 ± 0.78	95.5
	CP2	0.96 ± 0.62	5.43 ± 1.14	14.91 ± 1.77	8.62 ± 0.82	12.34 ± 1.27	1408
	CP3	3.05 ± 0.90	3.24 ± 0.45	13.05 ± 1.27	4.48 ± 0.17	17.09 ± 0.87	166
	CP4	0.40 ± 0.30	0.62 ± 0.15	9.70 ± 2.60	11.71 ± 0.97	12.18 ± 2.06	20
	CP5	0.76 ± 0.16	1.04 ± 0.19	11.17 ± 1.77	9.93 ± 1.44	11.06 ± 0.93	35

Table 3: *Comparison of antioxidant activity different mushroom powders.*

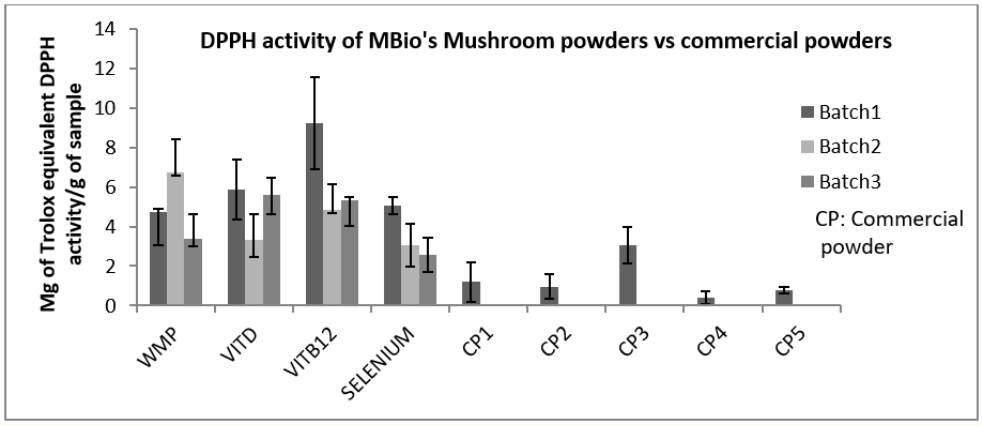

Figure 1: *DPPH scavenging activities of mushroom powders.*

Vitamin D enriched mushroom powder: Batch 1 (5.86 mg/g) exhibited significantly higher ($p < 0.0001$) DPPH scavenging activity compared to Batch 2 (3.33 mg/g). Batch 3 (5.03 mg/g) exhibited significantly higher ($p < 0.01$) DPPH activity compared to Batch 2. However, Batch 1 and Batch 3 exhibited no significant difference ($p = 0.17$) in DPPH scavenging activity (Figure 1 and table 3). These results confirmed the good retention of antioxidant activity in the vitamin D enriched mushroom powders.

Vitamin B12 enriched mushroom powder: The DPPH scavenging activity ranged from 5.18-9.23 mg/g. Batch 1 (9.23 mg/g) exhibited significantly higher ($p < 0.0001$) DPPH activity compared to Batch 2 (5.18 mg/g) and Batch 3 (5.31 mg/g). Batch 2 and Batch 3 exhibited no significant ($p = 0.87$) difference in DPPH activity. Although, three batches exhibited significant variability in DPPH activity (Figure 1 and table 3), which might be dependent on the initial concentration of the fresh B12- enriched mushrooms used for the production of the vitamin B12 enriched mushroom powder.

Selenium enriched mushroom powder: Three independent batches were assessed for the DPPH activity. Batch 2 (3.07 mg/g) and Batch 3 (2.57 mg/g) exhibited no significant difference ($p = 0.57$) in DPPH activity. However, Batch 2 and Batch 3 exhibited significantly lower ($p < 0.05$) DPPH activity compared to Batch 1 (5.05 mg/g) (Figure 1). Overall, some variation in the DPPH activity of the selenium enriched mushroom powder (2.57 - 5.05 mg/g) was observed, which might be dependent on the variability of the original selenium concentration of the fresh selenium- enriched mushrooms.

Among nutritionally enriched mushroom powders, the vitamin B12 enriched mushroom powder exhibited higher DPPH activity (Figure 1). However, no significant difference ($p = 0.88$, $p = 0.29$, $p = 0.12$) in the DPPH activity of *Agaricus bisporus* derived mushroom powders (WMP, Vitamin D, Vitamin B12 and selenium enriched mushroom powder) (Figure 1) was observed.

Comparison of nutritionally enriched mushroom powders vs marketed available mushroom powders: The results from comparison confirmed that nutritionally enriched mushroom powders have significantly higher ($p < 0.0001$) DPPH activity compared to the marketed powders (CP1 (1.19mg/g), CP2 (0.96 mg/g), CP4 (0.40 mg/g) and CP5 (0.76mg/g)) (Figure 1). CP3 (3.05 mg/g) exhibited no difference ($p = 0.47$) in the DPPH activity compared to selenium enriched mushroom powders. Overall, nutritionally enriched mushroom powders exhibited strong antioxidant activity compared to the commercial powders.

Total polyphenolic content

Whole mushroom powder: Batch 2 (4.82 mg/g) and Batch 3 (4.56 mg/g) of WMP exhibited no significant difference ($p = 0.06$) in the polyphenol content, which indicated significant consistency in these batches for the polyphenol content. However, Batch 1 (5.52 mg/g) exhibited significantly higher ($p < 0.001$) polyphenol content compared to Batch 2 and Batch 3 (Figure 2 and table 3).

Vitamin D enriched mushroom powder: Batch 1 (4.86 mg/g) and Batch 2 (5.05 mg/g) exhibited no significant difference ($p = 0.09$) in the polyphenol content. Similarly, Batch 2 and Batch 3 (5.73 mg/g) exhibited no significant difference ($p = 0.08$) in the polyphenol content. Overall, the vitamin D enriched mushroom powders exhibited strong retention of polyphenol content across the batches (Figure 2 and table 3).

Vitamin B12 enriched mushroom powder: Batch 1 (5.91 mg/g) and Batch 2 (5.94 mg/g) exhibited no significant ($p = 0.84$) difference in the polyphenol content. Similarly, Batch 1 and Batch 3 (5.66 mg/g) exhibited no significant difference ($p = 0.25$) in the polyphenol content. However, Batch 3 exhibited significantly lower ($p < 0.01$) polyphenol content compared to Batch 2. Overall, the vitamin B12 enriched mushroom powder indicated consistent retention of polyphenol content (Figure 2 and table 3).

Selenium enriched mushroom powder: All three batches of the selenium enriched mushroom powder exhibited significant differences ($p < 0.0001$) in polyphenol content. Batch 1 (5.58 mg/g) exhibited significantly higher ($p < 0.0001$) in polyphenol content compared to

Batch 2 (4.59 mg/g) and Batch 3 (4.31 mg/g). Similarly, Batch 2 and Batch 3 exhibited significant difference ($p < 0.0001$) in polyphenol content. Although, the three different batches exhibited variation for polyphenol content, this might be due to the initial concentration of selenium in the fresh selenium enriched mushrooms used for development of the selenium enriched mushroom powders (Figure 2 and table 3).

Overall, the vitamin B12 enriched mushroom powder exhibited significantly higher ($p < 0.0001$) polyphenol content (5.69 mg/g) compared to other 3 mushroom powders. The vitamin D enriched mushroom powders (5.08 mg/g) exhibited significantly higher polyphenol content ($p < 0.05$) compared to WMP (4.77 mg/g). However, no significant difference ($p = 0.83$) was observed in the polyphenol content of WMP and the selenium enriched mushroom powder (Figure 2).

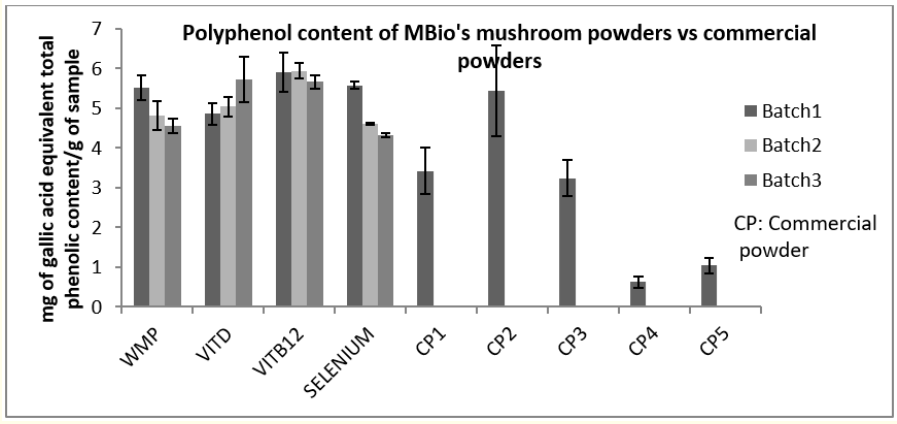

Figure 2: *Total polyphenol content of mushroom powders.*

Comparison of nutritionally enriched mushroom powders vs marketed available mushroom powders: The results confirmed that nutritionally enriched mushroom powders exhibited significantly higher ($p < 0.0001$) polyphenol content compared to commercial mushroom powders such as CP1, CP3, CP4 and CP5 mushroom powders (3.43, 3.24, 0.62 and 1.04 mg/g, respectively). Among commercial powders, the Chaga mushroom powder (CP2 5.43 mg/g) exhibited significantly higher ($p < 0.0001$) polyphenol content; however, it was comparable to vitamin B12 enriched mushroom powder. This confirmed that nutritionally enriched mushroom powders exhibited higher polyphenol content compared to the marketed mushroom powders.

Nitric oxide radical scavenging activity (NOSA)

Whole mushroom powder: Batch 1, Batch 2 and Batch 3 of WMP exhibited no significant difference ($p > 0.05$) in the NOSA. Batch 1 (15.35 mg/g) and Batch 2 (14.00 mg/g) exhibited no significant difference ($p = 0.30$) and similarly, Batch 2 and Batch 3 (14.54 mg/g) exhibited no significant difference ($p = 0.71$) in the NOSA. Overall, a consistent retention of the NOSA was observed indicating good antioxidant activity among all batches of WMP (Figure 3 and table 3).

Vitamin D enriched mushroom powder: Batch 1 (14.22 mg/g) and Batch 2 (15.71 mg/g) exhibited no significant difference ($p = 0.31$) in the NOSA. However, Batch 2 had significantly higher ($p < 0.05$) NOSA compared to Batch 3 (11.63 mg/g). Similarly, Batch 1 was significantly higher ($p < 0.05$) in the NOSA compared to Batch 3. Overall, the vitamin D enriched mushroom powder batches exhibited strong retention of the NOSA (Figure 3 and table 3) indicating strong antioxidant activity.

Vitamin B12 enriched mushroom powder: Batch 1 (14.32 mg/g) exhibited significantly lower ($p < 0.0001$) NOSA compared to Batch 2 (21.88 mg/g). Similarly, Batch 3 (14.75 mg/g) had significantly lower ($p < 0.02$) NOSA compared to Batch 2. However, Batch 1 and Batch 3 exhibited no significant difference ($p = 0.71$) in the NOSA (Figure 3 and table 3). This might be explained on the basis of the initial vitamin B12 concentration in the vitamin B12 enriched mushrooms used for development of the vitamin B12 enriched mushroom powders.

Selenium enriched mushroom powder: All batches of the selenium enriched mushroom powder (25.73, 23.35, 23.18 mg/g, respectively) exhibited no significant difference ($p = 0.24$, $p = 0.84$, $p = 0.23$) in the NOSA. This confirmed good consistency and strong retention of nitric oxide scavenging activity among batches of the selenium enriched mushroom powders (Figure 3 and table 3).

Overall, comparison of nutritionally enriched mushroom powders confirmed that the selenium enriched mushroom powder (24.09 mg/g) exhibited significantly higher ($p < 0.001$) NOSA compared to other nutritionally enriched mushroom powders. This was followed by the vitamin B12 enriched mushroom powders (16.11 mg/g), which exhibited no significant difference in the NOSA compared to the vitamin D enriched mushroom powder (14.00 mg/g, $p = 0.16$) and WMP (14.41 mg/g, $p = 0.46$). Furthermore, there was no significant difference ($p = 0.46$) in the NOSA of WMP and the vitamin D enriched mushroom powder (Figure 3).

Comparison of nutritionally enriched mushroom powders vs marketed available mushroom powders: Finally, in line with above observations, the selenium enriched mushroom powder exhibited significantly higher ($p < 0.001$) NOSA compared to five marketed mushroom powders (CP1 (11.35mg/g), CP2 (14.91 mg/g), CP3 (13.05 mg/g), CP4 (9.70 mg/g) and CP5 (11.17 mg/g) powders). Although, CP2 and CP3 powders exhibited NOSA equivalent to the vitamin B12 enriched mushroom powder, vitamin D enriched mushroom powder and WMP (Figure 3), it was confirmed that nutritionally enriched mushroom powders, in particular, the selenium enriched mushroom powder exhibited higher NOSA compared to commercially available mushroom powders.

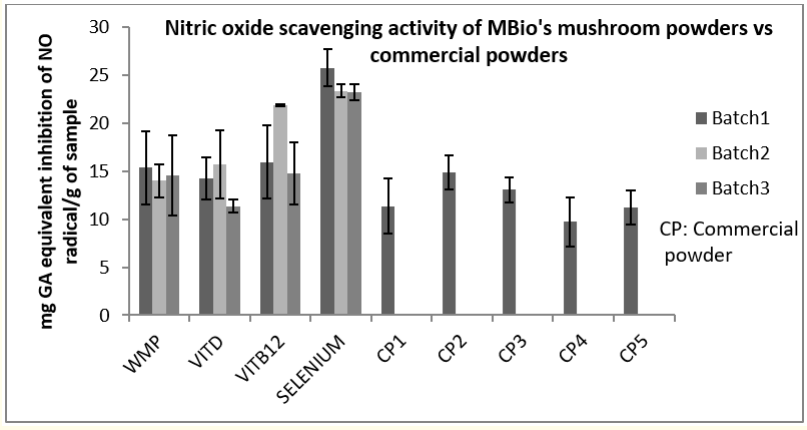

Figure 3: *Nitric oxide scavenging activity of mushroom powders.*

ABTS radical scavenging activity (ABTS)

ABTS radical scavenging activity was measured as trolox equivalent and inhibitory concentration of the powder (IC_{50}) required for 50% inhibition of ABTS radical. A lower IC_{50} indicates better antioxidant activity.

Whole mushroom powder: Batch 2 (1.87 mg) exhibited significantly lower ($p < 0.05$) IC_{50} compared to Batch 1 (2.81 mg) and Batch 3 (2.67 mg). Batch 1 and Batch 3 exhibited no significant difference ($p = 0.68$) in IC_{50}. These results indicated consistent retention of antioxidant activity across batches confirming the strong antioxidant activity of whole mushroom powder (Figure 4 and table 3).

Vitamin D enriched mushroom powder: Batch 1 (1.93 mg) exhibited significantly lower ($p < 0.02$) IC_{50} compared to Batch 2 (2.99 mg) and Batch 3 (2.46 mg). Further, Batch 3 exhibited significantly lower ($p < 0.05$) IC_{50} compared to Batch 2. Batch 1 and Batch 3 exhibited significant difference ($p < 0.02$) in IC_{50}. Overall, Batch 1 exhibited better antioxidant activity compared to other batches of vitamin D enriched mushroom powder (Figure 4 and table 3).

Vitamin B12 enriched mushroom powder: Batch 1 (1.62 mg) exhibited significantly lower ($p < 0.05$) IC_{50} compared to Batch 2. Batch 2 (1.74 mg) exhibited significantly lower ($p < 0.05$) IC_{50} compared to Batch 3 (2.89 mg). However, Batch 1 exhibited significantly lower IC_{50} ($p < 0.02$) compared to Batch 3 (Figure 4 and table 3). Overall, all three batches of the vitamin B12 enriched mushroom powder exhibited strong ABTS scavenging activity indicating strong antioxidant activity.

Selenium enriched mushroom powder: Batch 1 (1.13 mg) exhibited significantly lower IC_{50} compared to Batch 3 (2.09 mg) ($p < 0.01$). There was no significant difference ($p = 0.23$) in Batch 1 and Batch 2 (1.35 mg). Furthermore, Batch 2 exhibited significantly lower ($p < 0.02$) IC_{50} compared to Batch 3. Although, variability across the three batches was observed for ABTS radical scavenging activity, Batch 3 of the selenium enriched mushroom powder exhibited the best ABTS scavenging activity among all mushroom powders (Figure 4 and table 3).

Overall comparison of nutritionally enriched mushroom powder confirmed that the selenium enriched mushroom powder exhibited the lowest IC_{50} indicating the highest ABTS radical scavenging activity followed by the vitamin B12 enriched mushroom powder. However, no statistically significant difference ($p = 0.09$) was observed for ABTS scavenging activity of nutritionally enriched mushroom powders (Figure 4).

Comparison of nutritionally enriched mushroom powders vs marketed available mushroom powders: Finally, nutritionally enriched *Agaricus bisporus* mushroom powders exhibited significantly lower ($p < 0.0001$) IC_{50} compared to commercial powders (CP1 (6.82 mg), CP2 (8.62 mg), CP3 (4.48 mg), CP4 (11.71 mg) and CP5 (9.93 mg)). These results confirmed that MBio's mushroom powders have better antioxidant activity compared to the commercial mushroom powders (Figure 4).

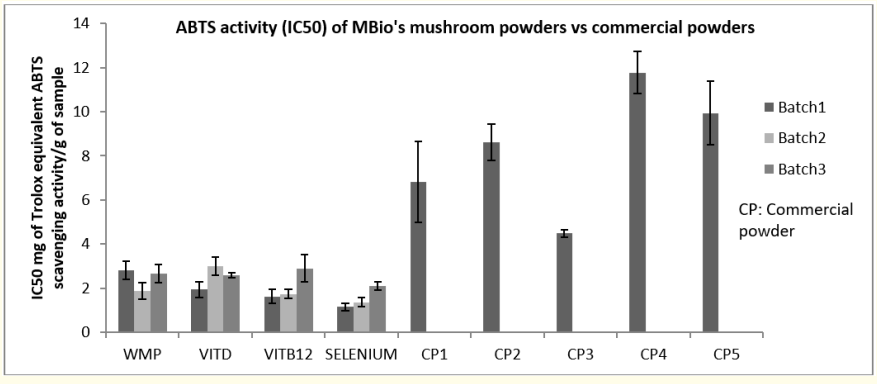

Figure 4: *ABTS radical scavenging activity of mushroom powders (Lower the IC50 value better is the antioxidant activity).*

Hydroxyl radical scavenging activity (OHSA)

Whole mushroom powder: Batch 1 (26.23 mg/g) and Batch 3 (29.96 mg/g) exhibited significantly higher ($p < 0.01$) OHS activity compared to Batch 2 (20.78 mg/g). Batch 1 and Batch 3 exhibited no significant difference ($p = 0.12$) in OHS activity. Overall, a good retention of strong antioxidant activity was observed among WMP batches (Figure 5).

Vitamin D enriched mushroom powder: Batch 1 (24.38 mg/g) and Batch 2 (24.98 mg/g) exhibited no significant difference in ($p = 0.86$) OHS activity. Batch 1 exhibited significantly higher ($p < 0.0001$) OHS activity compared to Batch 3 (10.87 mg/g). Similarly, Batch 2 exhibited significantly higher ($p < 0.001$) OHS activity compared to Batch 3 (Figure 5). This confirmed a strong OHS activity in MBio's vitamin D enriched mushroom powder.

Vitamin B12 enriched mushroom powder: Batch 1 (38.37 mg/g) exhibited significantly higher ($p < 0.01$) OHS activity compared to Batch 2 (17.39 mg/g) and Batch 3 (24.98 mg/g). Similarly, Batch 3 exhibited significantly higher ($p < 0.01$) OHS activity compared to Batch 2 (Figure 5). This indicated a strong retention of OHS activity in MBio's vitamin B12 enriched mushroom powder.

Selenium enriched mushroom powder: Batch 2 (26.98 mg/g) exhibited significantly higher ($p < 0.01$) OHS activity compared to Batch 1 (25.59 mg/g). Similarly, Batch 3 (23.98 mg/g) exhibited significantly higher ($p < 0.01$) OHS activity compared to Batch 1. However, Batch 2 and Batch 3 exhibited no significant difference ($p = 0.86$) in OHS activity (Figure 5). Overall, results confirmed that all three batches of the selenium enriched mushroom powders exhibited strong retention of OHS activity.

Overall, selenium enriched mushroom powder (25.52 mg/g) and the vitamin B12 enriched mushroom powder (25.68 mg/g) followed by WMP (24.86 mg/g) exhibited significantly higher OHS activity ($p < 0.05$) compared to the vitamin D enriched mushroom powder (18.28 mg/g).

Comparison of nutritionally enriched mushroom powders vs marketed available mushroom powders: The selenium enriched mushroom powder exhibited significantly higher ($p < 0.0001$) OHS activity compared to marketed powders such as shitake (CP1 9.49 mg/g), Chaga (CP2 12.34 mg/g), Reishi (CP3 17.09 mg/g), Lion's Mane (CP4 12.18 mg/g) and a mixture of mushroom powders (CP5 11.06 mg/g)) (Figure 5). Finally, *Agaricus bisporus* based and nutritionally enriched mushroom powders exhibited significantly higher hydroxyl radical scavenging activity compared to marketed mushroom powders.

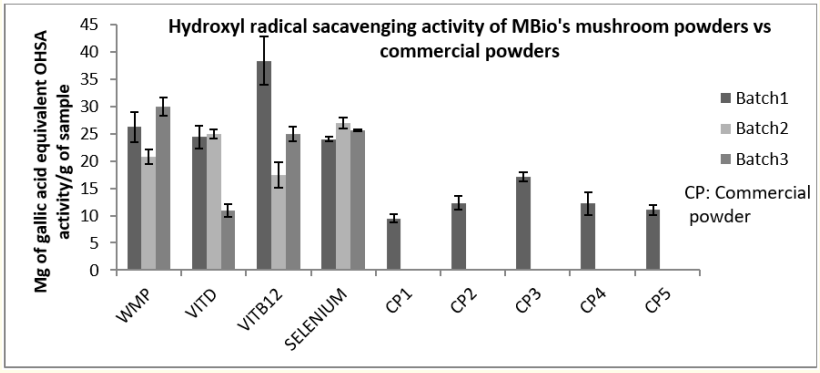

Figure 5: *Hydroxyl radical scavenging activity of mushroom powders.*

Ergothioneine analysis

Ergothioneine analysis was conducted at CampdenBRI using LC/MS/MS. The vitamin D enriched mushroom powders (average 1303 mg/Kg) and the selenium enriched mushroom powder (average 1290.2 mg/Kg) exhibited significantly higher ($p < 0.05$) ergothioneine content compared to the vitamin B12 enriched mushroom powder and whole mushroom powders (WMP). Ergothioneine levels for WMP ranged from 547 - 1009 mg/Kg (average 855 mg/Kg) and the vitamin B12 enriched mushroom powders ranged from 189.5 - 827 mg/Kg (average 519.3 mg/Kg) (Figure 6 and table 3). The marketed mushroom powders exhibited ergothioneine content as; shitake (CP1 95.5 mg/Kg), Chaga (CP2 1408 mg/Kg), Reishi (CP3 166 mg/Kg), Lion's Mane (CP4 20 mg/Kg) and a mixture of different mushroom powders (CP5 35 mg/Kg) (Figure 6).

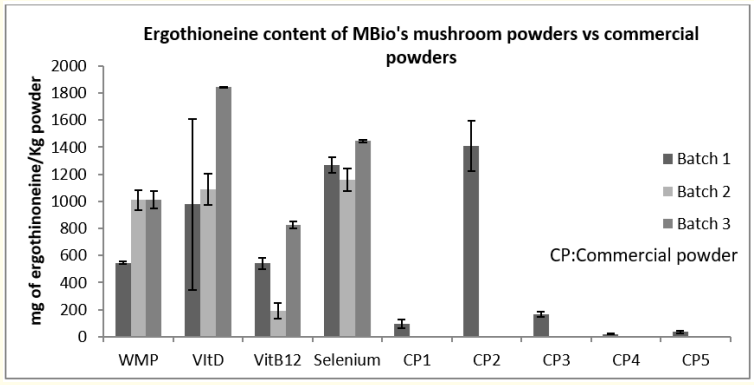

Figure 6: *Ergothioneine content of mushroom powders.*

Discussion

Different antioxidants respond differently based on the type of radical or oxidant sources, which might not accurately reflect the exact mechanism of action in a complex system [27,28]. Therefore, the antioxidant activity of the mushroom powders was assessed through six different methods. This study is the first of its kind where *Agaricus bisporus* mushroom derived powder products and randomly selected marketed mushroom powders were compared for their *in vitro* antioxidant activity. The antioxidant activity was represented as equivalent to standard antioxidants, such as Trolox and gallic acid.

The results confirmed that selenium enriched mushroom powder exhibited the highest overall antioxidant activity, closely followed by the vitamin B12 enriched mushroom powder, compared to vitamin D enriched mushroom powder and whole mushroom powder. The statistical analysis for Pearson coefficient correlation confirmed that the selenium enriched mushroom powder exhibited positive correlation ($p < 0.01$) between DPPH and polyphenol content, DPPH and NOSA ($p < 0.05$) and polyphenols and NOSA ($p < 0.1$), which suggest that the selenium enriched mushroom powder may contain higher antioxidant polyphenolic molecules responsible for free radical scavenging activity. Furthermore, the selenium enriched mushroom powder exhibited significantly higher levels of ergothioneine, a known antioxidant molecule. Therefore, these results suggest that selenium enrichment of the *Agaricus bisporus* mushrooms might have resulted into higher antioxidant content of the product. This result is in agreement with the literature that reported increased antioxidant activity in mushrooms as a result of increased selenium uptake by mushrooms, which are cultivated on selenium hyper-accumulated agri-

cultural residues [29]. Additionally, selenium is required for biosynthesis of selenoenzymes and selenoproteins, which are responsible for antioxidant defence mechanism [30]. This explains the higher overall antioxidant activity of the selenium enriched mushroom powders.

Similarly, the vitamin D enriched mushroom powder exhibited positive correlation ($p < 0.1$) between polyphenols and ergothioneine content suggesting that the vitamin D and ergothioneine may play a role as an antioxidant in the vitamin D enriched mushroom powders. Therefore, this result also confirms that the enrichment of vitamin D in *Agaricus bisporus* mushroom powder to higher levels (8000 IU/g) may have resulted in higher antioxidant activity. This result is also supported by the published research confirming that vitamin D is a membrane antioxidant, which inhibits iron dependent liposomal lipid peroxidation [31].

Higher antioxidant activity of MBio's mushroom powders can be explained as these mushroom powders are 100% mushroom powders derived from freshly harvested whole mushrooms processed under mild conditions to preserve its nutrient rich content. Mushrooms contain proteins, polysaccharides, terpenes, terpenoids, and important antioxidants including polyphenols (phenolic acids) glutathione and ergothioneine [32]. Furthermore, MBio's production process is highly controlled for every single aspect of the process - from producing compost to growing, harvesting, processing, packing and delivering. Additionally, the process has been validated and confirmed to retain strong antioxidant activity in the final mushroom powder products (data not shown due to proprietary process validations). Therefore, MBio's mushroom powders remain a source for important nutrients and bioactive ingredients, such as polyphenols and ergothioneine, which are known antioxidants that might explain the higher antioxidant activity of MBio's all mushroom powders. However, other marketed mushroom powders contains different mushroom strains including Shitake, Chaga, Reishi, Lion's Mane and a mixture of different mycelial cultures of various strains of the mushrooms. This might explain the different antioxidant activity observed in markedly available mushroom powders compared to MBio's *Agaricus bisporus* derived and nutritionally enriched mushroom powders. Literature searches confirmed no reports on the antioxidant activity of nutritionally enriched mushroom powder products on to the market. However, antioxidant activities of fresh or dried *A. bisporus* have been reported. Total polyphenols from white *A. bisporus* mushrooms after methanolic and ethanolic extractions have been reported as < 6 mg/g dry weight [33] and ranged from < 3 up to 10 mg/g dry weight [18,34-37], respectively. The results from the current study for total polyphenolic content for all nutritionally enriched mushroom powders ranged from 5 - 6 mg/g dry weight, which are in agreement with the above literature.

Overall ergothioneine analysis results confirmed that the selenium enriched mushroom powders are high in ergothioneine content compared to other nutritionally enriched (vitamin D and vitamin B12) mushroom powders and marketed mushroom powders such as shitake, reishi, lion's mane and a mixture of different mushroom powders (CP1, CP3, CP4, and CP5), respectively. Only commercial Chaga powder (CP2 1408 mg/Kg) exhibited higher ergothioneine content compared to other commercial mushroom powders and nutritionally enriched mushroom powders such as: vitamin D enriched mushroom powder (1303 mg/Kg), selenium enriched mushroom powder (1290.2 mg/Kg), the vitamin B12 enriched mushroom powder (519.3 mg/Kg) and WMP (855 mg/Kg). Pearson's coefficient correlation analysis confirmed no significant ($p > 0.05$) correlation between ergothioneine and type of antioxidant parameters for any of the mushroom powders; with the exception of MBio's vitamin D enriched mushroom powder, which exhibited positive correlation ($p < 0.1$) between polyphenols and ergothioneine. These results are in agreement with Dubost., *et al*. [17-19]. This indicated that ergothioneine antioxidant activity might be independent of other radical scavenging antioxidant activities. However, this does not affect the biological activity of ergothioneine and overall antioxidant activity of MBio's nutritionally enriched mushroom powders.

Ey., *et al*. [38] and Bao., *et al*. [39] have reported ergothioneine concentration from fruiting bodies of *A. bisporus* (white) and *A. bisporus* (brown) as 4.6 and 9.3 mg/Kg dry weight, respectively. Additionally, Shiitake mushroom fruiting body indicated higher ergothioneine content of 2840 mg/Kg dry weight. Some of the mushroom mycelial samples including *Ganoderma lucidum* (Reishi), *Grifola frondosa* (Maitake) and *Hericium erinaceus* (Lion's Mane) reported ergothioneine content of 16.5, 296.2 and 376.2 mg/Kg dry weight, respectively [40]. Similarly, *Inonotus obliquus* (Chaaga) mushroom mycelia also reported higher ergothioneine content of 252.1 mg/Kg dry weight. How-

ever, Dubost., *et al*. [17] have reported that ergothioneine content of *A. bisporus* white button and portabellas as 210 mg/Kg and 450 mg/Kg, respectively. Furthermore, the authors reported the ergothioneine content in specialty mushrooms, including shiitake (1980 mg/Kg), oyster (2590 mg/Kg) and maitake (1130 mg/Kg). This confirms huge discrepancies in the reported ergothioneine content of the mushroom types, which might be related to different type of raw materials used (fruiting body or mycelia), difference in production, drying methods, and various extraction and analysis methods used. However, MBio's nutritionally enriched and whole mushroom powders are developed using *A. bisporus* that have indicated significantly higher levels of ergothioneine compared to the reported values. This might suggest that MBio's production process is optimal for the retention of higher levels of ergothioneine in all MBio's mushroom powders.

The current study reported variable levels of ergothioneine across batches and types of mushroom powders. However, this variability can be explained on the basis of sourcing of raw material for each batch and homogeneity of the ergothioneine in the mushroom powders. This is also supported by the literature. Dubost., *et al*. [17-19] reported that ergothioneine biosynthesis is flush dependent and its pathway is also affected by various stress factors exposure to mushroom mycelia such as dry compost to the crops, which resulted in increased ergothioneine in later flushes. Additionally, ergothioneine is an intracellular metabolite and, therefore, specific extraction of ergothioneine is key step for its accurate detection, preparation, purification and application. Different extractions methods have been compared and reported by Zhang., *et al* [41]. Dubost., *et al*. [17-19] also reported that the quantification of ergothioneine was complicated due to lack of recovery and specificity of the procedure. There has been limited research regarding the factors affecting biosynthesis of ergothioneine. Due to environmental factors, it is difficult to control all the variables present that may affect the amount of ergothioneine produced by mushrooms.

Finally, irrespective of discrepancies in the ergothioneine levels, the current results confirmed that *Agaricus bisporus* derived nutritionally enriched mushroom powders are a good source of ergothioneine (which is a known antioxidant) compared to commercially available Shitake, Reishi, Lion's Mane and other mixtures of different mushroom powders.

Conclusion

Conclusively, all nutritionally enriched *Agaricus bisporus* mushroom powders (Selenium, Vitamin B12 and Vitamin D) exhibited significantly higher antioxidant and free radical scavenging activity and ergothioneine content compared to a selection of commercially available mushroom powders. Furthermore, whole mushroom powder developed using *Agaricus bisporus* was also found as a significant source of antioxidants compared to a selection of commercially available mushroom powders. Therefore, the current study confirmed that MBio's nutritionally enriched *Agaricus bisporus* mushroom powders are a significant source of natural antioxidants, which could help to prevent the progression of various diseases, caused by free radicals, such as certain cancers and might play a role in improvement of immune health.

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Authors Contribution and Conflict of Interest

SY: Conceived and designed the experiments, performed the experiments, analysed and interpreted data and wrote the manuscript, JW: guided the work, interrogated results, and interpretation of the data and reviewed the manuscript. The authors of this article declare that there were no conflicts of interest in undertaking this study.

Bibliography

1. Mayne ST. "Oxidative Stress, Dietary Antioxidant Supplements, and Health: Is the Glass Half Full or Half Empty?" © 2013 American Association for Cancer Research". *Cancer Epidemiol Biomarkers and Prevention* 22.12 (2013): 2145-2147.

2. Halliwell B. "Free Radicals and Other Reactive Species in Disease". *ELS* (2015).

3. Kadiiska M., *et al*. "Biomarkers of Oxidative Stress Study Ii: Are Oxidation Products Of Lipids, Proteins, And Dna Markers Of Ccl 4 Poisoning?" *Free Radical Biology and Medicine* 38.6 (2005): 698-710.

4. Uttara B., *et al*. "Oxidative Stress and Neurodegenerative Diseases: A Review of Upstream and Downstream Antioxidant Therapeutic Options". *Current Neuropharmacology* 7.1 (2009): 65-74.

5. Mujić I., *et al*. "Antioxidant Properties of Selected Edible Mushroom Species Antioksidativne Osobine Odabranih Jestivih Gljiva". *Journal of Central European Agriculture* 11.4 (2010): 387-392.

6. Nagmoti D., *et al*. "Antioxidant Activity and Free Radical-Scavenging Potential of Pithecellobium Dulce Benth Seed Extracts". *Free Radicals* 2.2 (2011): 37-43.

7. Huie RE And Padmaja S. "The Reaction of No with Superoxide". *Free Radical Research Communications* 18.4 (1993): 195-199.

8. Rathee S., *et al*. "Mushrooms as Therapeutic Agents". *Brazilian Journal of Pharmacognosy* 22.2 (2012): 459-474.

9. Feeney M., *et al*. "Mushrooms and health summit proceedings". *Journal of Nutrition* 144.7 (2014): 1128S-1136S.

10. Ayeka PA. "Potential Of Mushroom Compounds As Immunomodulators In Cancer Immunotherapy: A Review". *Evidence-Based Complementary and Alternative Medicine* (2018): 9.

11. Wang Q., *et al*. "Bioactive Mushroom Polysaccharides: A Review on Monosaccharide Composition, Biosynthesis And Regulation". *Molecules* 22.6 (2017): 955.

12. Xu X., *et al.* "Bioactive Proteins From Mushrooms". *Biotechnology Advances* 29.6 (2011): 667-674.

13. Kalaras MD., *et al*. "Mushrooms: A rich source of the antioxidants ergothioneine and glutathione". *Food Chemistry* 233 (2017): 429-433.

14. Sánchez C. "Bioactives From Mushroom And Their Application. Chapter 2, ©Springer International Publishing AG 2017M. Puri (edition.)". *Food Bioactives* (2017): 23-57.

15. Hartman PE., *et al*. "Ergothioneine, Histidine, and Two Naturally Occurring Histidine Dipeptides as Radioprotectors against γ-Irradiation Inactivation of Bacteriophages T4 and P22". *Radiation Research* 114.2 (1988): 319-330.

16. Paul B and Snyder S. "The unusual amino acid L-ergothioneine is a physiologic cytoprotectant". *Cell Death Differ* 17.7 (2010): 1134-1140.

17. Dubost N., *et al*. "Identification and quantification of ergothioneine in cultivated mushrooms by liquid chromatography-mass spectroscopy". *International Journal of Medicianl Mushrooms* 8.3 (2006): 215-222.

18. Dubost N., *et al*. "Quantification of polyphenols and ergothioneine in cultivated mushrooms and correlation to total antioxidant capacity". *Food Chemistry* 105.2 (2007): 727-735.

19. Dubost N., *et al*. "Influence of selected cultural factors and postharvest storage on ergothioneine content of common button mushroom Agaricus bisporus (J. Lge) Imbach (Agaricomycetideae)". *International Journal of Medicianl Mushrooms* 9.2 (2007): 163-176.

20. Rahman MM., *et al*. "In Vitro Antioxidant and Free Radical Scavenging Activity Of Different Parts Of Tabebuia Pallida Growing In Bangladesh". *BMC Research Notes* 8 (2015): 621.

21. Gite S., *et al*. "Antioxidant quality ranking of some plant materials based on multiple in vitro radical scavenging assays". *Journal of Chemical and Pharmaceutical Research* 7.7 (2015): 152-159.

22. Alger M. "Polymer Science Dictionary". *Springer* (1997): 152.

23. Ariga T and Hamano M. "Radical Scavenging Action And Its Mode in Procyanidins B-1 And B-3 From Adzuki Beans To Peroxyl Radicals". *Agricultural and Biological Chemistry* 54.10 (1990): 2499-2504.

24. Chandra S., *et al*. "Assessment Of Total Phenolic And Flavonoid Content, Antioxidant Properties, And Yield Of Aeroponically And Conventionally Grown Leafy Vegetables And Fruit Crops: A Comparative Study". *Evidence-Based Complementary and Alternative Medicine* (2014): 253875.

25. Govindarajan R., *et al*. "Studies On Antioxidant Activities Of Desmodium Gangeticum". *Biological and Pharmaceutical Bulletin* 26.10 (2003): 1424-1427.

26. Zielińska D., *et al*. "Antioxidant Capacity Of Thermally – Treated Buckwheat". *Polish Journal of Food and Nutrition Sciences* 57.4 (2007): 465-470.

27. Reis FS., *et al*. "Antioxidant properties and phenolic profile of the most widely appreciated cultivated mushrooms: a comparative study between in vivo and in vitro samples". *Food and Chemical Toxicology* 50.5 (2012): 1201-1207.

28. Prior RL., *et al*. "Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay". *Journal of Agricultural and Food Chemistry* 53 (2005): 4290-4302.

29. Bhatia P., *et al*. "Enhanced Anti-oxidant Activity as a Function of Selenium hyperaccumulation in Agaricus bisporus Cultivated on Se-rich Agri-residues". *Journal of Biologically Active Products from Nature* 4. 5-6 (2014): 354-364.

30. Kora AJ. "Nutritional and antioxidant significance of selenium-enriched mushrooms". *Bulletin of the National Research Centre* 44.34 (2020).

31. Wiseman H. "Vitamin D is a membrane antioxidant. Ability to inhibit iron-dependent lipid peroxidation in liposomes compared to cholesterol, ergosterol and tamoxifen and relevance to anticancer action". *FEBS Letters* 326.1-3 (1993): 285-288.

32. Sharpe E., *et al*. "Comparison of antioxidant activity and extraction techniques for commercially and laboratory prepared extracts from six mushroom species". *Journal of Agriculture and Food Research* 4 (2021): 100-130.

33. Gąsecka M., *et al*. "Profile of phenolic and organic acids, antioxidant properties and ergosterol content in cultivated and wild growing species of Agaricus". *European Food Research and Technology* 244 (2018): 259-268.

34. Palacios I., *et al*. "Antioxidant properties of phenolic compounds occurring in edible mushrooms". *Food Chemistry* 128 (2011): 674-678.

35. Gan C., *et al*. "Antioxidant analysis of different types of edible mushrooms (Agaricus bisporous and Agaricus brasiliensis)". *International Food Research Journal* 20.3 (2013): 1095-1102.

36. Reis F., *et al*. "Analytical methods applied to the chemical characterization and antioxidant properties of three wild edible mushroom species from northeastern Portugal". *Food Analytical Methods* 7 (2014): 645-652.

37. Liu J., *et al*. "In vitro and in vivo antioxidant activity of ethanolic extract of white button mushroom (Agaricus bisporus)". *Food and Chemical Toxicology* 51 (2013): 310-316.

38. Ey J., *et al*. "Dietary sources and antioxidant effects of ergothioneine". *Journal of Agricultural and Food Chemistry* 55.16 (2007): 6466-6474.

39. Bao H., *et al*. "Value-added use of mushroom ergothioneine as a colour stabilizer in processed fish meats". *Journal of the Science of Food and Agriculture* 90.10 (2010): 1634-1641.

40. Chen S., *et al*. "Contents of lovastatin, γ-aminobutyric acid and ergothioneine in mushroom fruiting bodies and mycelia". *LWT - Food Science and Technology* 47.2 (2012): 274-278.

41. Zhang W., *et al*. "Aqueous Extraction of Ergothioneine from Mycelia of Pleurotus ostreatus and Ergothioneine Accumulation Regularity during Submerged Fermentation". *Research and Reviews: Journal of Microbiology and Biotechnology* 5.1 (2016):2347-2286.

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