Research Article

The Role of Polyphenols, β-Carotene, and Lycopene in the Antioxidative Action of the Extracts of Dried, Edible Mushrooms

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One of the nutritional benefits of mushrooms is the presence of bioactive secondary metabolites which have been reported to exert various beneficial effects *in vivo*. Therefore, we selected thirteen frequently consumed species of Polish mushrooms and determined the concentration of polyphenols, flavonoids, β-carotene, and lycopene in aqueous and methanolic extracts of dried fruiting bodies as well as their reducing power and ability to scavenge ABTS cation radical. We found that the concentration of antioxidants is different in different species and in various parts of the fruiting body of mushrooms. We observed a strong correlation ($r > 0.9$) between the concentration of total phenolics and reducing power/scavenging effects in both aqueous and methanolic extracts, while this correlation was moderate for flavonoids. Beta-carotene did not contribute discernibly to the antioxidative properties of the extracts, while lycopene had a significant contribution to the scavenging activity of methanolic mushroom extracts.

1. Introduction

Mushrooms have been exploited in human diet for centuries because of their specific taste and flavour. Nowadays, they attract attention because of their beneficial effects and possible use in the prevention or treatment of diseases [1]. Numerous reports demonstrate beneficial *in vivo* effects of cultivated and wild edible mushrooms. It has been proven that the polysaccharide extract of *Pleurotus pulmonarius* delays the progression of hepatocellular carcinoma [2]; polysaccharide from *Pholiota nameko* has anti-inflammatory properties in rodents [3]; *Agaricus bisporus* inhibits prostate tumor growth in mice [4]; *Pleurotus eryngii, Grifola frondosa*, and *Hypsizygus marmoreus* protect apolipoprotein-E deficient mice from development of atherosclerosis [5]. Simultaneously, edible mushrooms are regarded as an important dietary supplement for people interested in calorie restriction, because of the low amount of fat, cholesterol, and calories in their bodies and high concentration of fiber [1, 6–8].

The therapeutic action of mushrooms is attributed to the presence of bioactive compounds such as vitamins, polysaccharides, and secondary metabolites in their fruiting bodies. Some of them have antioxidant properties which are referred repeatedly to be the key aspect of their observed beneficial effects. Polyphenols and carotenoids, abundant in the fruiting bodies of mushrooms, are antioxidants efficient in biological systems [9]. Polyphenols have been reported to interfere with the initiation and progression of cancer [10, 11], to act as antiageing [12], anti-inflammatory [13, 14], and brain-protective factors [15] and to protect against cardiovascular diseases [16, 17]. Apart from provitamin A properties, carotenoids are known as singlet oxygen quenchers [18, 19] and lipid peroxidation chain breakers [20]. They have been reported to reduce the risk of prostate cancer [21, 22], digestive tract cancers [23, 24], and chronic diseases [25–27].

Basing on this knowledge, we decided to estimate parameters describing the antioxidant properties of extracts of dried, edible Polish mushrooms: total antioxidant capacity with two most commonly used assays (Trolox equivalent antioxidant capacity and ferricyanide reducing power) and the content of polyphenols and flavonoids, the main

compounds contributing to the antioxidant properties of mushrooms as well as of two carotenoids (β-carotene and lycopene), important from the point of view of antioxidant protection of the human organism. We selected thirteen species frequently consumed in Poland. For some species, we distinguished subtypes, considering the Polish nutritional customs (*Boletus edulis* white-young and yellow-adult, *Agaricus bisporus* with and without peel).

2. Materials and Methods

2.1. Materials. Sodium hydroxide, hexane, acetone, and the Folin-Ciolcalteu reagent were purchased from POCh (Gliwice, Poland). All other reagents were from Sigma (Poznań, Poland).

The fruiting bodies of Polish mushrooms were collected in 2008 in Bory Tucholskie (a big forest area in the Northern part of Poland), cleaned and air dried. *Tuber mesentericum* was collected in calcareous area of Kraków-Częstochowa (Poland). Latin names of fungi are after Wojewoda [28] and Ławrynowicz [29].

2.2. Mushroom Extracts. Extraction was performed according to standard, commonly used procedures [30]. Before extraction, the dry fruiting bodies were cleaned carefully to remove residual contamination. 1 g of dry caps and stalks was ground into powder, then 15 ml of boiling water or methanol was added, and the material was rubbed for the next 5 min and stirred at room temperature for 15 min. Then, the mixture was centrifuged at $3000 \times g$ at room temperature for 20 min. The supernatants were portioned and kept frozen at $-23°C$ until analysis. The assays used to evaluate antioxidant properties of the mushrooms are typical for such type studies to enable comparisons with literature data.

2.3. Assay for Total Phenolics. The concentration of total phenolics in aqueous and methanolic extracts was estimated with the Folin-Ciocalteu reagent, a method most commonly used [31, 32]. The calibration curve was prepared with gallic acid (0–0.75 mg/ml).

2.4. Assay for Total Flavonoids. The measurement of the content of flavonoids was conducted according to the simple and reliable method described by Jia et al. [33]. $50\,\mu l$ of methanolic or aqueous extract was mixed with $700\,\mu l$ of deionized water and $37\,\mu l$ of 5% $NaNO_2$. After 5-min incubation at room temperature, $75\,\mu l$ of 10% $AlCl_3$ was added followed by $250\,\mu l$ of 1 M NaOH after the next 6 min. After shaking, the mixture was centrifuged ($5000 \times g$, room temperature, 15 min), and the absorbance of the supernatant was read at 515 nm against a blank. Quercetin (0–0.4 mg/ml) was used as a standard.

2.5. Assay for β-Carotene and Lycopene. The concentration of β-carotene and lycopene in mushroom extracts was estimated spectrophotometrically [34, 35]. This simple and rapid method makes use of specific spectral properties of

the carotenoids. The content of β-carotene and lycopene was calculated from the equations given as follows:

$$
\begin{aligned}
&\text{Lycopene} \, (\text{mg}/100 \, \text{ml}) \\
&\quad = -0.0458 \, A_{663} + 0.372 \, A_{505} - 0.0806 \, A_{453}, \\
&\beta\text{-Carotene} \, (\text{mg}/100 \, \text{ml}) \\
&\quad = 0.216 \, A_{663} - 0.304 \, A_{505} + 0.452 A_{453}.
\end{aligned}
\tag{1}
$$

2.6. Trolox Equivalent Antioxidative Activity (TEAC) Measurement. TEAC of mushroom extracts was estimated by a modified [36] ABTS cation radical decolorization assay [37]. This simple and cheap assay is based on the measurement of the extent of reduction of preformed ABTS radical cation by antioxidants [36]. The modification used by us consists in measurement of the reduction at the wavelength of 414 nm corresponding to the main absorption peak of ABTS radical cation to increase the sensitivity of the assay [35]. ABTS radical cation was prepared according to Re et al. by reaction of ABTS with potassium persulfate and stored at $-20°C$ until use. The standard curve was prepared using Trolox as a standard (0–20 μM).

2.7. Reducing Power. The ability of the extracts to reduce potassium ferricyanide was determined according to Puttaraju and coauthors. This assay was reported to be more selective for antioxidants than the ABTS decolorization assay and has been used by previous researchers to study mushrooms [30]. We increased the volumes of reagents to obtain the final volume of the reaction mixture readable in a standard spectrophotometer (about 1 ml). The method was used to enable comparison with results of other studies employing this assay. $25\,\mu l$ of mushroom extracts was mixed with $475\,\mu l$ of sodium phosphate buffer (200 mM, pH 6.6) and $250\,\mu l$ of 1% potassium ferricyanide and incubated at $50°C$ for 20 min. Then, $250\,\mu l$ of 10% TCA was added; the mixture was shaken and centrifuged ($5000 \times g$, room temperature, 10 min). Subsequently, $500\,\mu l$ of the supernatant was mixed with $500\,\mu l$ of deionized water and $100\,\mu l$ of 0.1% ferric chloride. After vigorous shaking and 10 min incubation at room temperature, the absorbance was measured at 700 nm against a blank. For the standard curve, gallic acid was employed (0–0.5 mg/ml).

2.8. Statistical Analysis. The dependence of TEAC/reducing power on the concentrations of individual antioxidants analyzed is presented as Pearson's correlation coefficients, and the statistical significance of the correlation coefficients was tested with Student's t-test. The regression was calculated by the least-squares method.

3. Results

3.1. Concentration of Total Phenolics. The content of total phenolics differed significantly among the species employed for the experiments and ranged from 1.65 ± 0.10 to $13.01 \pm 1.48\,\mu$g/mg of dried mushrooms in the aqueous extracts

TABLE 1: The content of total phenolics (μg of gallic acid equivalents/mg of dried mushrooms) and flavonoids (μg of quercetin equivalents/mg of dried mushrooms) of edible Polish mushrooms.

Mushroom	(Total phenolics μg of gallic acid equivalents/mg of dried mushrooms)		(Flavonoids μg of quercetin equivalents/mg of dried mushrooms)	
	Methanolic extract	Aqueous extract	Methanolic extract	Aqueous extract
Boletus edulis white cap	4.49 ± 0.16	13.01 ± 1.48	0.73 ± 0.07	3.86 ± 0.10
Boletus edulis white stalk	3.61 ± 0.10	10.78 ± 0.33	0.52 ± 0.03	1.97 ± 0.04
Boletus edulis yellow cap	4.24 ± 0.13	8.28 ± 0.46	1.34 ± 0.07	1.23 ± 0.01
Boletus edulis yellow stalk	4.85 ± 0.30	7.39 ± 1.67	0.57 ± 0.04	3.03 ± 0.06
Xerocomus badius cap	1.82 ± 0.09	8.39 ± 0.65	1.81 ± 0.08	7.74 ± 0.23
Xerocomus badius stalk	2.66 ± 0.16	8.67 ± 0.35	1.23 ± 0.10	3.89 ± 0.02
Leccinum spp. cap	2.25 ± 0.14	9.63 ± 0.75	1.48 ± 0.03	6.70 ± 0.11
Leccinum spp. stalk	0.94 ± 0.08	3.93 ± 0.09	0.14 ± 0.01	1.74 ± 0.28
Xerocomus subtomentosus cap	3.35 ± 0.07	12.89 ± 1.52	1.38 ± 0.06	5.90 ± 0.19
Cantharellus cibarius	0.77 ± 0.03	2.39 ± 0.23	0.24 ± 0.03	0.42 ± 0.04
Agaricus bisporus cap without peel	1.90 ± 0.09	4.80 ± 0.36	0.41 ± 0.01	2.41 ± 0.05
Agaricus bisporus stalk without peel	1.01 ± 0.20	3.88 ± 0.13	0.17 ± 0.04	1.4 ± 0.05
Agaricus bisporus cap with peel	2.04 ± 0.13	5.23 ± 0.49	0.50 ± 0.11	2.812 ± 0.06
Agaricus bisporus stalk with peel	1.64 ± 0.89	4.54 ± 0.17	0.26 ± 0.02	1.72 ± 0.18
Pleurotus ostreatus	1.44 ± 0.09	5.23 ± 0.65	0.37 ± 0.20	0.31 ± 0.01
Suillus spp. cap	4.78 ± 0.24	6.64 ± 0.60	1.89 ± 0.04	3.14 ± 0.05
Suillus variegatus cap	4.15 ± 0.35	7.44 ± 0.71	3.27 ± 0.10	2.57 ± 0.13
Suillus bovinus cap	4.00 ± 0.07	5.48 ± 0.42	3.02 ± 0.07	3.33 ± 0.08
Suillus bovinus stalk	4.05 ± 0.15	2.16 ± 0.07	2.18 ± 0.10	1.12 ± 0.03
Macrolepiota procera cap	2.17 ± 0.39	10.30 ± 1.50	0.918 ± 0.37	5.13 ± 0.07
Macrolepiota procera stalk	1.95 ± 0.30	7.51 ± 0.50	0.75 ± 0.04	2.18 ± 0.03
Tricholoma equestre cap	1.85 ± 0.69	4.71 ± 1.12	1.65 ± 0.19	1.44 ± 0.03
Tricholoma equestre stalk	0.66 ± 0.30	4.00 ± 0.50	0.65 ± 0.17	0.99 ± 0.03
Tuber mesentericum	0.02 ± 0.02	1.65 ± 0.10	0.06 ± 0.02	0.51 ± 0.20

The concentration of total phenolics and flavonoids was estimated spectrophotometrically. Each value represents mean ± SD, $n \geq 3$.

and from 0.02 ± 0.02 to $4.85 \pm 0.30 \, \mu g/mg$ in the methanolic extracts of dried mushrooms (Table 1). In general, the concentration of total phenolics was higher in aqueous than in methanolic extracts, except for the stalk of *Suillus bovinus*. We found that the content of total phenolics was different in different parts of the fruiting bodies. In eight out of nine pairs of mushroom caps and stalks analyzed, the concentration of water-soluble phenolics was higher in the caps. The content of methanolic-soluble phenolics was higher the in caps in six pairs and in the stalks in three pairs analyzed. The highest concentration of methanolic- and water-soluble phenolics was found in the caps of white *Boletus edulis* and *Xerocomus subtomentosus* and the lowest in *Tuber mesentericum*.

3.2. Concentration of Flavonoids.

The highest total content of flavonoids (in aqueous and methanolic extracts) was found in *Xerocomus badius* and *Leccinum* spp., while the lowest in *Tuber mesentericum* and *Cantharellus cibarius* (Table 1). Similarly to the experiment with polyphenols, more flavonoids were extracted with water except for a few species, for which the concentration of flavonoids was higher in the methanolic extracts or similar in aqueous and in methanolic extracts. Like in the case of total phenolics, we found differences between the content of flavonoids in caps and stalks, and it should be emphasized that, in eight out of nine species, their concentration was higher in the caps. The Pearson correlation coefficient between the content of total phenolics and flavonoids indicates a moderate dependence: $r = 0.69 \pm 0.11 \, (P \leq .001)$ and $r = 0.59 \pm 0.14 \, (P \leq .01)$ for aqueous and methanolic extracts, respectively.

3.3. Concentration of β-Carotene and Lycopene.

The content of β-carotene differed considerably between the analyzed edible mushroom species, from 0.233 to 15.256 μg/g of dried body (Table 2). The highest content was found in methanolic extracts of the cap of *Tricholoma equestre* and in three species of *Suillus*. The relatively high content of β-carotene was detected in the aqueous extracts of the cap of *Tricholoma equestre* and *Suillus bovinus*, while *Tuber mesentericum* and the stalk of *Leccinum* spp. were deprived of this antioxidant.

The content of lycopene was far lower than the concentration of β-carotene in the mushrooms studied (Table 2).

TABLE 2: The content of β-carotene and lycopene of edible Polish mushrooms (μg/g of dried mushrooms).

Mushroom	β-carotene (μg/g of dried mushrooms)		Lycopene (μg/g of dried mushrooms)	
	Methanolic extract	Aqueous extract	Methanolic extract	Aqueous extract
Boletus edulis white cap	0.729 ± 0.025	0.402 ± 0.077	0.076 ± 0.048	0.262 ± 0.015
Boletus edulis white stalk	0.467 ± 0.116	0.007 ± 0.034	0.019 ± 0.007	0.058 ± 0.049
Boletus edulis yellow cap	1.350 ± 0.162	0.136 ± 0.056	0.069 ± 0.006	0.103 ± 0.003
Boletus edulis yellow stalk	0.718 ± 0.079	0.098 ± 0.007	0.062 ± 0.017	0.087 ± 0.001
Xerocomus badius cap	0.752 ± 0.007	0.114 ± 0.001	0.038 ± 0.028	0.084 ± 0.007
Xerocomus badius stalk	2.626 ± 0.336	0.184 ± 0.025	0.461 ± 0.173	0.102 ± 0.002
Leccinum spp. cap	0.683 ± 0.075	0.113 ± 0.017	0.114 ± 0.024	0.086 ± 0.014
Leccinum spp. stalk	0.270 ± 0.049	0.098 ± 0.046	0.068 ± 0.084	0.082 ± 0.025
Xerocomus subtomentosus cap	3.307 ± 0.271	0.163 ± 0.009	0.379 ± 0.029	0.110 ± 0.002
Cantharellus cibarius	3.275 ± 0.053	0.499 ± 0.027	0.105 ± 0.044	0.124 ± 0.025
Agaricus bisporus cap without peel	0.511 ± 0.038	0.499 ± 0.022	0.117 ± 0.020	0.374 ± 0.023
Agaricus bisporus stalk without peel	0.384 ± 0.028	0.069 ± 0.026	0.011 ± 0.005	0.137 ± 0.042
Agaricus bisporus cap with peel	0.426 ± 0.011	0.045 ± 0.074	0.109 ± 0.015	0.047 ± 0.030
Agaricus bisporus stalk with peel	0.519 ± 0.029	0.107 ± 0.026	0.051 ± 0.022	0.107 ± 0.005
Pleurotus ostreatus	0.317 ± 0.008	0.001 ± 0.006	0.195 ± 0.005	0.009 ± 0.008
Suillus spp. cap	6.242 ± 0.540	0.200 ± 0.079	1.951 ± 0.153	0.127 ± 0.039
Suillus variegatus cap	7.730 ± 0.484	0.016 ± 0.028	1.219 ± 0.025	0.048 ± 0.035
Suillus bovinus cap	15.256 ± 0.785	3.382 ± 0.204	15.388 ± 0.998	3.464 ± 0.108
Suillus bovinus stalk	11.016 ± 0.470	0.584 ± 0.037	7.347 ± 0.644	0.420 ± 0.007
Macrolepiota procera cap	0.265 ± 0.019	0.192 ± 0.151	0.023 ± 0.011	0.157 ± 0.108
Macrolepiota procera stalk	0.319 ± 0.034	0.012 ± 0.006	0.058 ± 0.007	0.030 ± 0.012
Tricholoma equestre cap	18.649 ± 0.024	1.905 ± 0.268	0.001 ± 0.022	0.013 ± 0.006
Tricholoma equestre stalk	4.753 ± 0.271	0.439 ± 0.013	0.125 ± 0.065	0.039 ± 0.012
Tuber mesentericum	0.233 ± 0.053	0.098 ± 0.024	0.001 ± 0.002	0.084 ± 0.006

The content of β-carotene and lycopene in the extracts of dried mushrooms was estimated spectrophotometrically after extraction with the acetone: hexane mixture. Each value represents mean ± SD, $n \geq 3$.

The highest content of lycopene was detected in the methanolic extracts of three species of *Suillus, S. bovinus* being the richest in this compound. A high content of lycopene was found in the aqueous extracts of the cap of *Suillus bovinus* as well.

3.4. Trolox Equivalent Antioxidative Activity (TEAC). The ability to scavenge the ABTS cation radical ranged from 3.81 ± 0.17 to 62.30 ± 1.77 and from 0.42 ± 0.48 to 20.54 ± 1.89 Trolox equivalents/g dried mushrooms for aqueous and methanolic extracts, resp. (Table 3). In general, we observed higher TEAC values for aqueous extracts (except for *Suillus bovinus*) and differences between the analyzed parts of the fruiting bodies. Our results indicate that, for the majority of species analyzed, the aqueous and methanolic extracts obtained from caps are more potent in the decolorization of ABTS$^{\bullet+}$. Values of the Pearson correlation coefficient point to the strong dependence between TEAC and the total concentration of phenolics (0.95 ± 0.02 and 0.98 ± 0.01 for aqueous and methanolic extracts, resp.). A statistically significant correlation was found between the concentration of flavonoids and TEAC as well as between the concentration of lycopene and TEAC of methanolic extracts, while β-carotene concentration did not correlate with TEAC significantly.

3.5. Reducing Power. As in previous experiments, our results demonstrate differences in the ability to reduce ferricyanide between the aqueous and methanolic extracts of selected edible mushroom species as well as between the parts of their fruiting bodies (Table 3). Generally, the aqueous extracts were more potent in the reduction of ferricyanide (except for the stalk of *Suillus bovinus*). However, the relation between the reducing power of the caps and the stalks was not consistent and depended on the species analyzed. Simultaneously, we found a high, statistically significant correlation between the values of the reducing power and the concentration of total phenolics ($r = 0.93 \pm 0.03$ for methanolic and 0.92 ± 0.03 for aqueous extracts) (Table 4, Figures 1(a) and 1(b)). A moderate correlation was also observed between the reducing power and the concentration of flavonoids (0.57 ± 0.14 for aqueous extracts and 0.45 ± 0.17 for methanolic extracts), while β-carotene and lycopene did not affect this parameter significantly.

4. Discussion

The beneficial effects of mushroom consumption are widely described. Studies of bioactivity of mushroom extracts suggest that the free radical scavenging ability is, at least partially, responsible for their positive action. Many papers

TABLE 3: Trolox equivalent anitoxidant capacity (μmol of Trolox/g dried mushroom) and reducing power (μg of gallic acid/g of dried mushroom) of edible Polish mushrooms.

Mushroom	TEAC (μmol of Trolox/g dried mushroom)		Reducing power (μg of gallic acid/g of dried mushroom)	
	Methanolic extract	Aqueous extract	Methanolic extract	Aqueous extract
Boletus edulis white cap	19.81 ± 0.35	62.30 ± 1.77	5.75 ± 0.45	13.37 ± 0.69
Boletus edulis white stalk	17.58 ± 1.55	46.68 ± 1.66	6.57 ± 0.46	14.60 ± 1.04
Boletus edulis yellow cap	19.68 ± 1.00	42.76 ± 1.66	5.18 ± 0.39	9.52 ± 0.30
Boletus edulis yellow stalk	19.38 ± 2.94	30.49 ± 4.38	8.09 ± 0.24	9.10 ± 1.69
Xerocomus badius cap	6.72 ± 0.73	40.85 ± 3.12	2.21 ± 0.32	8.14 ± 0.33
Xerocomus badius stalk	11.80 ± 2.53	40.34 ± 1.33	2.51 ± 0.20	8.57 ± 0.42
Leccinum spp. cap	9.44 ± 0.79	43.38 ± 3.39	3.80 ± 0.36	10.21 ± 0.16
Leccinum spp. stalk	4.62 ± 2.57	16.28 ± 1.27	1.81 ± 0.13	6.85 ± 0.35
Xerocomus subtomentosus cap	12.22 ± 0.40	52.49 ± 1.94	5.51 ± 0.42	13.86 ± 1.35
Cantharellus cibarius	0.99 ± 0.77	3.81 ± 0.17	1.80 ± 0.74	2.03 ± 0.07
Agaricus bisporus cap without peel	8.48 ± 1.13	13.43 ± 1.02	1.69 ± 0.30	2.09 ± 0.23
Agaricus bisporus stalk without peel	4.71 ± 1.81	13.19 ± 0.59	1.30 ± 0.05	3.08 ± 0.15
Agaricus bisporus cap with peel	9.36 ± 2.16	14.86 ± 0.51	1.85 ± 0.29	2.04 ± 0.26
Agaricus bisporus stalk with peel	6.16 ± 1.04	14.20 ± 0.19	1.64 ± 0.07	4.17 ± 0.26
Pleurotus ostreatus	4.79 ± 0.48	13.08 ± 0.37	1.47 ± 0.29	2.76 ± 0.23
Suillus spp. cap	19.68 ± 0.24	32.65 ± 1.99	6.00 ± 0.61	7.08 ± 0.25
Suillus variegatus cap	17.92 ± 1.19	23.52 ± 0.36	5.78 ± 0.50	6.31 ± 0.60
Suillus bovinus cap	18.25 ± 1.33	12.76 ± 0.22	4.91 ± 0.47	5.03 ± 0.570
Suillus bovinus stalk	20.54 ± 1.89	8.31 ± 0.07	4.59 ± 0.28	2.43 ± 0.30
Macrolepiota procera cap	8.92 ± 0.04	36.08 ± 0.37	2.25 ± 0.11	8.99 ± 0.25
Macrolepiota procera stalk	5.09 ± 0.89	21.83 ± 3.13	2.90 ± 0.08	9.64 ± 0.29
Tricholoma equestre cap	7.17 ± 1.70	11.48 ± 2.34	1.24 ± 0.04	2.29 ± 0.41
Tricholoma equestre stalk	1.75 ± 0.34	10.22 ± 4.11	0.59 ± 0.01	2.74 ± 0.21
Tuber mesentericum	0.42 ± 1.48	6.01 ± 0.36	0.13 ± 0.02	1.17 ± 0.24

Trolox equivalent antioxidant capacity and reducing power were determined spectrophotometrically. Each value represents mean ± SD, $n \geq 3$.

describe the antioxidative properties and the content of antioxidants in extracts of fresh, dried, and cooked edible medicinal, cultivated, and wild mushroom species [30, 38–41]. In this study, using two *in vitro* assays, we evaluated the antioxidative ability of the extracts of 13 dried, edible mushroom species, which are especially popular in the Polish diet. We found large differences between species and between the parts of their fruiting bodies. The highest ABTS cation radical and ferrocyanide reduction ability were determined for *Boletus edulis* and *Xerocomus subtomentosus*. Many of *in vivo* studies confirm that diet supplementation with some mushroom species or mushroom extracts protects tissues against oxidative injuries [42–45]. Thus, the antioxidative ability determined in the *in vitro* experiments may be of relevance in *in vivo* systems as well.

The antioxidative properties of mushroom extracts are dependent on the concentration of various antioxidants with different correlation coefficients. In our study, we found that both the TEAC values and the extract abilities to reduce ferricyanide showed the strongest correlation with the content of total phenolics in the dried fruiting bodies. Similar observations for mushroom species were made by other authors as well [38, 39]. Moreover, the relation between the antioxidative capacity and the concentration of polyphenols

was found also for plant extracts. We found a moderate correlation between the TEAC/reducing power and the level of mushroom flavonoids. It needs to be emphasized that the dependence between the concentration of total phenolics and flavonoids is similar to that between TEAC and the flavonoids content (r of 0.69 ± 0.11 versus 0.68 ± 0.11 and 0.59 ± 0.14 versus 0.58 ± 0.14 for aqueous and methanolic extracts, resp.). We did not find any significant correlation between the β-carotene content and TAC or reducing power. Only lycopene affected the antioxidative capacity of methanolic extracts significantly, but the correlation coefficient was relatively low (0.41 ± 0.17).

When comparing the methods of antioxidative capacity analysis employed in this study, it needs to be remarked that in the case of polyphenols, flavonoids, and lycopene, higher correlation was found between their concentrations and TEAC than reducing power values. These results suggest that both aqueous and methanolic mushroom extracts are more potent in the reduction of ABTS cation radical than in the reduction of ferricyanide. However, it should be considered that the analysis of reducing power is conducted by a more complicated procedure involving 20-min incubation at $50°C$ at pH = 6.6, which may affect the antioxidative capacity of the extracts. Nevertheless, the correlation between these

TABLE 4: The correlation between the concentrations of antioxidants and TEAC/reducing power.
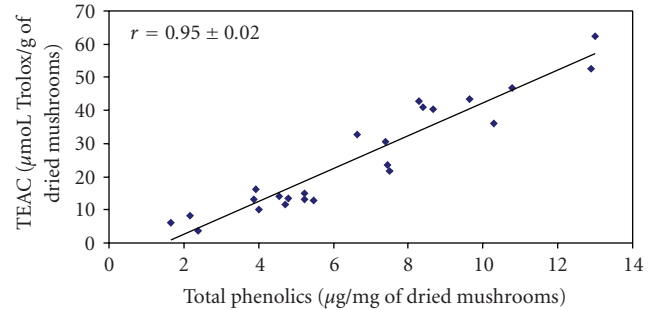
	r	
	TEAC	Reducing power
Total phenolics (aqueous extract)	0.95 ± 0.02[d]	0.92 ± 0.03[d]
Total phenolics (methanolic extract)	0.98 ± 0.01[d]	0.93 ± 0.03[d]
Flavonoids (aqueous extract)	0.68 ± 0.11[b]	0.57 ± 0.14[a]
Flavonoids (methanolic extract)	0.58 ± 0.14[d]	0.45 ± 0.17[b]
β-carotene (aqueous extract)	-0.27 ± 0.19	-0.09 ± 0.21
β-carotene (methanolic extract)	0.28 ± 0.19	0.25 ± 0.20
Lycopene (aqueous extract)	-0.16 ± 0.20	-0.23 ± 0.20
Lycopene (methanolic extract)	0.41 ± 0.17[a]	0.10 ± 0.21

The Pearson correlation coefficient between TEAC/reducing power and the concentration of antioxidants was calculated and tested with Student's t-test: [a]$P \leq .05$, [b]$P \leq .01$, [c]$P \leq .005$, and [d]$P \leq .001$
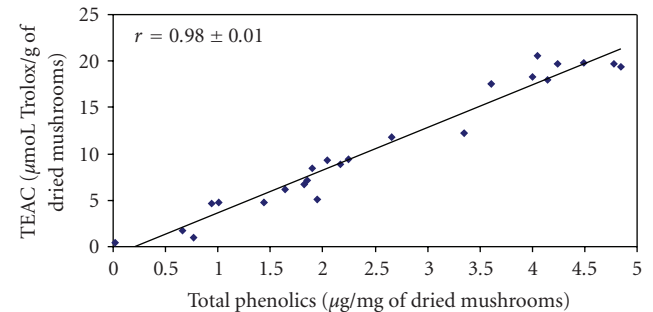

FIGURE 1: The correlation between the concentration of phenolic compounds and Trolox equivalent antioxidant capacity. The Pearson correlation coefficient between Trolox equivalent antioxidant capacity and total concentration of phenolics was calculated and tested with Student's t-test. The linear regression was determined with the least-squares method.

two methods of estimation of reducing capacity remains relatively high (r values of 0.92 ± 0.03 and 0.89 ± 0.04 for aqueous and methanolic extracts, resp.).

Our experiments indicate that edible Polish mushroom species differ in the content of the analyzed antioxidants. The differences were found between the parts of their fruiting bodies and between the aqueous and methanolic extracts as well. Generally, the concentration of total phenolics was much higher in aqueous than in methanolic extracts. These results are consistent with previous reports [30]. In the prevailing number of mushroom species, a similar dependence was observed for flavonoids. The values of total phenolics concentration in aqueous extracts of dried *Boletus edulis*, *Macrolepiota procera*, and *Cantharellus cibarius* obtained in our laboratory are similar to the values reported by Puttaraju and co-authors for these species collected in India [30]. For *Boletus edulis*, we found the average concentration of polyphenols to be 9.87 mg/g dried mushroom, while Puttaraju et al. reported a value of 10.2 mg/g; for *Macrolepiotea procera*, the values were 10.3 and 10.2 mg/g, respectively, and for *Cantharellus cibarius* 2.4 and 2.0 mg/g of dried mushrooms, respectively. However, in the case of the methanolic-soluble polyphenols more significant differences were observed.

The data of Kähkönen and co-authors indicate that total concentration of phenolics in vegetables used in

their experiments ranges for vegetables from ~0.4 (pea) to ~7.4 μg of gallic acid equivalents per mg dried mass (leaves of carrot). For berries and fruits, the values varied from ~11.9 μg/mg (apple) to ~50.8 μg/mg (crowberry) while for herbs and medicinal plants from 0.2 μg/mg (wheat, grain) to ~42.1 μg/mg (purple loosestrife) [46]. Thus, dried mushroom bodies may be regarded as an alternative source of phenolics. Simultaneously, some mushroom species (*Suillus*) were found to be a competitive source of hydrophobic antioxidants as well. The highest concentration of β-carotene (~15.26 μg/g of dried mushroom body) and lycopene (~15.4 μg/g) were determined in the cap of *Suillus bovinus*. For comparison, the content of carotenoids reported by Ben-Amotz and Fishler in vegetables ranges from undetectable levels to ~52.8 μg/g (persimmon) and ~532.1 μg/g (pitango) in fruits and to ~1030 μg/g (carrot) and ~243.1 μg/g (tomato) for β-carotene and lycopene, respectively [47]. Moreover, prior studies showed that phenolics and carotenoids at the concentrations found in fruits and vegetables act as antioxidants *in vitro* and affect the antioxidant defense in human body as well. Supplementation of the daily diet with β-carotene (90 mg) increased the antioxidant capacity of plasma in older woman [48]; enrichment of the daily diet of nonsmoking men with tomato juice (40 mg lycopene), carrot juice (22.3 mg β-carotene and 15.7 mg α-carotene), and spicyspinach powder (11.3 mg lutein) reduced the level of oxidatively modified

DNA bases in lymphocytes [49]. 30 min after consumption of 150 ml fruit juices (orange, melon, grape, peach, plum, apple, and kiwi), generation of reactive oxygen species in the plasma of healthy men was suppressed [50]. Similarly, the exercise-induced oxidative damage of red blood cells in athletes receiving 150 ml of chokeberry juice daily (34.5 mg anthocyanins) was lower when compared to control group (placebo) [51].

It has been documented that the chemical composition, antioxidant profile, and the concentrations of antioxidants in mushroom fruiting bodies depend on the maturation process [52, 53]. The analysis of the changes of the fundamental mushroom antioxidants in the methanolic extracts of *Lactarius piperatus* in four maturity stages demonstrated that their level increases up to the second stage of maturation and then drops down dramatically [54]. In our study, the investigation of the antioxidative capacity of immature and mature forms of *Boletus edulis* suggests that the level of water- and methanol-soluble antioxidants may be different for various parts of the fruiting body during maturation as well. The higher concentration of water-soluble polyphenols was found in the immature form, while the stalks of mature stage of this mushroom were richer in methanol-soluble polyphenols in comparison to the immature form. Thus, the aqueous extracts obtained from immature *Boletus edulis* revealed a higher ability to reduce ABTS cation radical and ferricyanide, while in methanolic extracts a higher antioxidative potential was found for the stalk. Simultaneously, a higher concentration of β-carotene was determined in the methanolic extract of both the caps and the stalks of the mature form. The level of lycopene was relatively low in all analyzed samples.

In order to take into account the Polish nutritional practice, we investigated the fruiting bodies of *Agaricus bisporus* with and without the peel. The results obtained for many fruits and vegetables indicate that their peel is characterized by high concentration of antioxidants and displays a protective action against oxidative agents in chemical and biological systems [55–57]. Our experiments proved that extracts of dried *Agaricus bisporus* deprived of peel are slightly less effective in scavenging ABTS cation radical and reduction of ferricyanide. Simultaneously, slightly higher levels of total phenolics and flavonoids were found in the extracts of whole constituents of the fruiting body. It cannot be excluded that the higher content of antioxidants and higher antioxidative capacity of the peel of *Agaricus bisporus* may play a physiological and, probably, protective function. Certainly, the composition of antioxidants and their role is noteworthy, and further experiments including other mushroom species need to be conducted.

It needs to be emphasised that commercial mushroom species employed in this study (*Agaricus bisporus, Pleurotus ostreatus*) reveal relatively weak antioxidative capacity and low total content of phenolics. Likewise, it was reported previously that wild species are less energetic but possess higher concentration of polyphenols [58, 59].

However, in contrast to these optimistic results, it needs to be mentioned that even edible mushroom species have been described to demonstrate toxic effects as well.

Consumption of *Tricholoma equestre* led to increase of serum creatine kinase, fatigue, and muscle weekness [60], suggesting the presence of toxin in this mushroom body which may cause rhabdomyolysis. Recently, *Agaricus bisporus* has been found to increase plasma bilirubin concentration, *Lentinus edodes* to increase plasma creatine kinase activity, and *Pleurotus ostreatus* to increase water intake and plasma alanine aminotransferase activity in mice [61]. Thus, the potential beneficial action of edible mushroom may be complicated by the possible disadvantageous effects.

5. Conclusions

Our results demonstrate that differences in the antioxidant profile and antioxidative capacity occur not only between dried mushroom species but also between different parts of the fruiting bodies. Moreover, we found that polyphenols rather than other antioxidants analysed affect the antioxidative ability of dried mushroom extracts.

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