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Influence of Addition of Dried Maitake and Enoki Mushrooms on Antioxidant, Potentially Anti-Inflammatory, and Anti-Cancer Properties of Enriched Pasta

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Abstract: The influence of the addition of 2.5, 5, 7.5, and 10.0% of dried Enoki and Maitake mushrooms on the content of bioactive compounds and some nutraceutical properties of pasta was determined in the study. The LC-MS/MS analysis of phenolic compounds revealed the highest amount of phenolic compounds in the Maitake-supplemented pasta. However, all the samples of the Enoki-enriched pasta exhibited a statistically significantly higher content of α-glucans than the control. Samples subjected to gastrointestinal digestion had significantly higher antioxidant and potential anti-inflammatory activities than ethanolic and buffer extracts (PBS). The ethanolic extracts from the Enoki- and Maitake-supplemented pasta had higher antioxidant activity (in some antiradical and reducing power assays) and higher lipoxygenase (E2.5 and E5 samples) inhibitory potential compared to the control sample. Additionally, some in vitro digested samples of pasta enriched with dried Enoki and Maitake mushrooms showed higher chelating power (E10, M7.5, and M10), reducing power (E5, E7.5, and M10), and lipoxygenase inhibition ability (E7.5, E10, and M10) than the control. In conclusion, the fortification of pasta with 7.5% and 10% of Enoki mushrooms and with 10% of Maitake mushrooms can be recommended.

Keywords: mushrooms; pasta fortification; antioxidant activities; anti-inflammatory properties; anticancer properties

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1. Introduction

In addition to their nutritional properties, mushrooms are characterized by the presence of many ingredients with health-promoting properties. The impact of mushrooms on human health has been better documented in Asian countries than in Europe. This is related to the Asian culture and tradition, in which the cultivation of mushrooms has several centuries-old roots, especially in China. Edible mushrooms have recently been investigated and evaluated in various research centers around the world. The interest in fungi as a raw material that can be used in medicine and in the production of natural pharmaceuticals is associated with their pro-health properties [1]. Edible mushrooms with medicinal properties are an abundant source of biologically active compounds, e.g., polyphenols, polysaccharides, terpenoids, steroids, cerebrosides, and proteins [2]. Given their pro-health properties, mushrooms are well-known elements of traditional diets and medicine in Asian countries. Many of the compounds present in edible fungi are increasingly being used for the development of new functional foods and nutraceuticals in order to take advantage of their very broad properties, e.g., antitumor, antimicrobial, antiviral, immunomodulating, hypocholesterolemic, hypoglycemic, and many other effects [3–5].

Maitake (*Grifola frondosa*) and Enoki (*Flammulina velutipes*) are edible mushrooms with a high nutrient and bioactive compound content [6–9].

The growing demand for healthy food expressed by increasing numbers of health-conscious consumers over the last decade has resulted in growing interest in recipes for products with increased nutritional and/or health-promoting value. Although fortification with functional ingredients may increase the cost of such food products in some cases, many consumers are ready to purchase foods with proven health-enhancing properties. This also results in the growing demand for scientific research on functional products [6].

The interest in the use of natural products with a high content of some bioactive compounds as elements of popular functional food is growing. Given their multiple pro-health properties, medicinal mushrooms may be valuable functional additives to some food products. In some studies, powdered mushrooms or various extracts (aqueous or hydroalcoholic) from mushrooms have been used to fortify many types of foods, e.g., biscuits, cookies, crackers, cakes, and processed meat [7,8].

In Western countries, the use of mushrooms as additives to functional food is much less popular than in Asian countries. However, since the knowledge of the impact of food on health is widely available, the issue of health-enhancing food products has gained interest among consumers and food producers in Western countries [7]. In general, the supplementation of flour products with dried mushroom flour may impoverish pasting properties, yield darker products, and enhance firmness. The addition of mushroom fibers may also weaken the gluten network in fortified products. Therefore, the amount of mushroom addition is very important, as it may determine the physicochemical and sensory traits of flour products in addition to the impact of this additive on the health-promoting properties of this type of food [9].

The addition of some mushroom powders to wheat flour has been analyzed in some studies. Namely, β-glucans (in amounts of 1–3%) from *Lentinus edodes* were added to bakery foods, extruded snacks were supplemented with 5–15% of chestnut mushroom (*Agrocybe aegerita*), 5–15% of flour used for production of biscuits was replaced by *Pleurotus sajor-cajupowder*, and *Pleurotus ostreatus* was used as a replacer of wheat flour in noodles [9].

Another important aspect is that additives with a high content of bioactive compounds should be used in products that are willingly and frequently consumed [10].

Pasta, which is an excellent source of carbohydrates, is popular worldwide due to its good storage stability (dried pasta), low production cost, simple preparation, and relatively low glycemic index. One of the strategies used to produce functional pasta consists of a partial replacement of semolina with various ingredients characterized by a high content of bioactive compounds, e.g., medicinal mushrooms [6].

Another advantage besides the health-promoting and nutritional properties of *Grifola frondosa* (Maitake) and *Flammulina velutipes* (Enoki) is the possibility of farm cultivation of these species in controlled ecological conditions; hence, they can potentially be used for food production [9].

In our previous study, it was observed that the supplementation of pasta with dried Maitake and Enoki mushrooms (in the amount of 2.5, 5, 7.5, and 10.0%) contributed to the high nutritional value and acceptable culinary and organoleptic traits of the product [11].

The aim of this study was to determine the effect of dried Maitake and Enoki mushrooms on the bioactive activity (especially antioxidant, anti-inflammatory, and anti-cancer properties) of fortified pasta.

2. Materials and Methods

2.1. Preparation of Pasta

The tagliatelle pasta was made from semolina mixed with dried Maitake (M) and Enoki (E) mushrooms—Figure 1 (doses: 2.5% (*w*/*w*), 5.0% (*w*/*w*), 7.5% (*w*/*w*), and 10.0% (*w*/*w*)) as described previously [11]. Non-fortified semolina pasta was the control sample, as in our previous publication [12].

Enoki Maitake

Figure 1. Dried Enoki and Maitake mushrooms.

2.2. Preparation of Extracts

2.2.1. Ethanolic Extracts

Ethanolic extracts were made as described in our previous study [12].

2.2.2. PBS Extracts

PBS samples (buffer extracts) were prepared as described in our previous study.

2.2.3. In Vitro Digestion

In vitro digestion was carried out according to the procedure proposed by Minekus et al. [13] and slightly modified by Sęczyk et al. [14].

Three-step digestion, i.e., an oral, gastric, and intestinal process, was performed. In the oral phase (2 min at 37 °C), pre-hydrated samples (1 g samples plus 1 mL of water) were mixed with 1.4 mL of simulated salivary fluid electrolyte stock solution (SSFESS) and incubated in the dark. Next, an oral bolus was mixed with 3 mL of simulated gastric fluid electrolyte stock solution (SGFESS) for gastric digestion (120 min at 37 °C in the dark). In the simulated intestinal digestion, gastric chyme was mixed with 4.4 mL of simulated intestinal fluid electrolyte stock solution (SIFESS), and the samples were incubated in the dark with continuous shaking for 120 min at 37 °C. After all the phases, the samples were centrifuged (15 min, 6900× g), and the supernatants were used for analysis.

Electrolyte stock solutions (SSFESS, SGFESS, SIFESS) were prepared as described elsewhere [13]. At each digestion step, pH was checked and adjusted with 1 M NaOH or 1 M HCl to pH 7 for the oral and intestinal phases and pH 3 for the gastric phase when required. Samples subjected to all the digestion steps (gastrointestinal digested samples—GID) were used for the analysis of pro-health properties. Additionally, the gastric phase (gastro-digested samples—GD) was collected for the determination of potential anticancer properties.

The exact in vitro digestion procedure was identical to that previously described [12].

2.3. Content of Bioactive Compounds

2.3.1. Determination of Phenolic Compounds

Determination of Phenolic Acid Content (PAC)

The content of phenolic acids was determined according to the Arnov method [15] and expressed as caffeic acid equivalents (CAE)—µg Eq CAE/g DW.

Determination of Total Flavonoid Content (TFC)

The total flavonoid content was analyzed as in Lamaison and Carnet [16]. The results were presented as quercetin equivalents (QE)—mg Eq QE/g DW.

Determination of Total Phenolic Content (TPC)

The content of total phenolic compounds was determined based on the method proposed by Singleton et al. [17]. The results were presented as gallic acid equivalents (GAE)—mg Eq GAE/g DW.

Phenolic compounds were detected in ethanolic extracts, PBS extracts, and in vitro digested samples with spectrophotometric methods using a BioTek Microplate Reader (Winooski, VT, USA) (PAC, TFC and TPC).

LC-MS/MS-Based Qualitative and Quantitative Analysis of Phenolic Compounds

The qualitative–quantitative analyses of phenolic compounds in the dried mushrooms, pasta, ethanolic extracts, PBS, and digested samples were performed according to the procedure described by Żuchowski et al. [18] with modifications used in our previous publication [12]. The analyses were carried out on a Sciex ExionLC AD chromatograph coupled with a Sciex QTRAP 6500+ mass spectrometer. The Phenomenex 2.6 µm Biphenyl column 4.6 × 100 mm was used.

2.3.2. Determination of Glucans

The content of total, α-glucans, and β-glucans was determined with the use of the K-YBGL β-glucan Assay Kit (Yeast and Mushrooms) (Megazyme, Bray, Ireland) following the manufacturer's instructions (www.megazyme.com (accessed on 17 January 2023)). For the determination of the total (α- and β-) glucan content, lyophilized pasta samples (90 mg) were hydrolyzed using cold 12 M sulfuric acid in an ice-water bath for 2 h. Next, the samples were placed in boiling water for 2 h. After neutralization with 8 M NaOH, an aliquot was digested with exo-1,3-β-glucanase (20 U/mL) plus β-glucosidase (4 U/mL) in 200 mM sodium acetate buffer (pH 4.5). The hydrolyzates were incubated with a mixture of glucose oxidase, peroxidase, and 4-aminoantipyrine at 40 °C for 20 min. The absorbance of the solution was measured at 510 nm. For the determination of the α-glucan content, samples of lyophilized pasta (100 mg) were dissolved in 1.7 M NaOH, hydrolyzed (20 min in an ice-water bath), and treated with 1.2 M sodium acetate buffer (pH 3.8). Amyloglucosidase (1.630 U/mL) and invertase (500 U/mL) were added to the solution, and the mixture was incubated at 40 °C for 30 min. The aliquot was incubated with a mixture of glucose oxidase, peroxidase, and 4-aminoantipyrine at 40 °C for 20 min. The absorbance of the solution was measured at 510 nm. The concentration of β-glucan was determined by subtracting α-glucan from the total glucan content. The results were calculated as specified in the manufacturer's instructions (www.megazyme.com (accessed on 17 January 2023)) and expressed as g/100 g DW.

2.4. Antioxidant Activities

The ethanolic extracts, PBS extracts, and in vitro digested samples were analyzed to determine antioxidant activities.

2.4.1. Free Radical Scavenging Assays

The DPPH method described in Brand-Williams et al. [19] and the $ABTS^{+\bullet}$ assay proposed by Re et al. [20] were used. The antiradical activity was expressed as mg of Trolox per gram of dry weight (DW).

2.4.2. Ferric Reducing Antioxidant Power

Reducing power (RP) was determined with the methods proposed by Oyaizu [21], and the results were expressed in mgTE/gDW.

2.4.3. Chelating Power

The method proposed by Guo et al. [22] was used for estimation of chelating power (CHP). The results were expressed in mg EDTA/gDW (EDTA equivalent).

2.5. Determination of Anti-Inflammatory Properties

The ethanolic extracts, PBS extracts, and in vitro digested samples were assessed for their potential anti-inflammatory properties.

2.5.1. Lipoxygenase (LOX) Inhibitory Activity

LOX inhibitory activity was determined with the method described by Szymanowska et al. [23]. Quercetin served as a positive control. The LOX inhibitory activity was expressed as EC_{50} for the extracts or IC_{50} for quercetin.

2.5.2. COX2 Inhibitory Activity

The influence of the samples on cyclooxygenase-2 (COX-2) activity was determined using the COX Activity Assay kit from Cayman Chemical. Quercetin served as a positive control. The COX2 inhibitory activity was expressed as EC_{50} for the extracts or IC_{50} for quercetin.

2.6. Determination of Anti-Cancer Properties

The anti-cancer properties of the samples were tested on two cancer cell lines: AGS—Human Caucasian gastric adenocarcinoma (ECACC No. 89090402)—samples after gastric digestion (GD) and HT29—Human Caucasian colon adenocarcinoma (ATCC HTB-38)—samples after gastrointestinal digestion (GID).

The results were expressed as EC_{50} values in mg DW/mL.

The exact procedure was identical to that described previously [12].

An appropriate solvent or digestive fluid was used as a reference sample in all the biological activity assays so as to eliminate its influence on the tested activity. To compare the results reliably, the volume of the solvent added to the mixture was always kept at a constant minimum level that did not affect the analyzed activity.

2.7. Statistical Analysis

All the determinations were carried out in triplicate unless otherwise stated. Statistical analysis was carried out using the Statistica ver. 13.3 software package (StatSoft, Krakow, Poland). One-way analysis of variance (ANOVA) or Student's *t*-test was used to detect any statistically significant differences between the samples. Whenever the assumptions of the homogeneity of variance were not met, the Kruskal–Wallis non-parametric test was used. Homogenous groups were determined with Tukey's test (when using ANOVA) or Dunn's test (when using the Kruskal–Wallis test). The data were reported as mean ± standard deviation.

3. Results

3.1. Content of Bioactive Compounds

Table 1 summarizes the results of the content of TPC, TFC, and PAC in pasta supplemented with 2.5%, 5%, 7.5%, and 10% of dried Enoki and Maitake mushrooms after extraction with ethanol and PBS and after simulated digestion (gastrointestinally digested samples—GID).

After the simulated digestion process, samples from pasta supplemented with the mushrooms exhibited a higher content of PAC (except for E10 and M2.5) compared to the control sample. The greatest increase, i.e., by 12.9% and 11.3%, in the PAC content was observed in the in vitro digested samples of pasta fortified with 7.5 and 10% of Maitake, respectively, in comparison to the control.

The TFC value in the PBS extracts was higher in the mushroom-fortified pasta samples than in the semolina-only pasta. The E10 sample contained significantly higher levels of TFC than the M2.5 variant and the control. However, as shown by the analysis of the potentially bioavailable fraction of flavonoids (GID samples), greater amounts of flavonoids versus the control were recorded only in the M2.5, M5, and M7.5 samples, with the highest flavonoid content in the M5 sample—see Table 1.

The PBS extracts of pasta enriched with dried Enoki and Maitake mushrooms had a higher TPC content than the control sample. The highest TPC content was determined in the E10 sample (0.91 ± 0.09 mg Eq GAE/g DW), which constituted a 65% increase in comparison to the control.

Table 1. Content of phenolic compounds in pasta fortified with dried Enoki and Maitake mushrooms.

Samples	PAC (μg Eq CAE/gDW)			TFC (mg Eq QE/g DW)			TPC (mg Eq GAE/g DW)		
	EtOH	**PBS**	**GID**	**EtOH**	**PBS**	**GID**	**EtOH**	**PBS**	**GID**
C	0.17 ± 0.01^{aA}	1.33 ± 0.02^{bcAB}	3.11 ± 0.32^{abB}	0.17 ± 0.12^{aAB}	0.04 ± 0.04^{aA}	4.45 ± 0.08^{abcB}	0.60 ± 0.06^{aAB}	0.55 ± 0.10^{aA}	5.27 ± 0.30^{aB}
E2.5	0.18 ± 0.01^{aA}	1.28 ± 0.02^{abcAB}	3.21 ± 0.08^{abB}	0.45 ± 0.32^{aAB}	0.16 ± 0.10^{abA}	4.08 ± 0.27^{abB}	0.58 ± 0.07^{aA}	0.60 ± 0.02^{abAB}	4.91 ± 0.43^{aB}
E5	0.18 ± 0.01^{aA}	1.31 ± 0.04^{abcAB}	3.26 ± 0.07^{abB}	0.32 ± 0.23^{aAB}	0.12 ± 0.07^{abA}	4.38 ± 0.34^{abcB}	0.57 ± 0.04^{aA}	0.65 ± 0.03^{abcAB}	5.39 ± 0.22^{aB}
E7.5	0.18 ± 0.02^{aA}	1.34 ± 0.04^{cAB}	2.86 ± 0.15^{aB}	0.33 ± 0.06^{aAB}	0.32 ± 0.23^{abA}	3.85 ± 0.33^{aB}	1.10 ± 0.23^{abAB}	0.78 ± 0.08^{deA}	4.74 ± 0.39^{aB}
E10	0.17 ± 0.01^{aA}	1.31 ± 0.02^{abcAB}	3.05 ± 0.12^{abB}	0.34 ± 0.09^{a}	0.32 ± 0.02^{b}	4.39 ± 0.31^{abc}	1.15 ± 0.16^{abAB}	0.91 ± 0.09^{eA}	4.74 ± 0.36^{aB}
M2.5	0.17 ± 0.01^{aA}	1.25 ± 0.02^{aAB}	3.09 ± 0.19^{abB}	0.23 ± 0.14^{aAB}	0.06 ± 0.06^{aA}	4.87 ± 0.37^{bcB}	1.66 ± 0.08^{bAB}	0.64 ± 0.03^{abcA}	4.73 ± 0.44^{aB}
M5	0.17 ± 0.01^{aA}	1.27 ± 0.03^{abAB}	3.17 ± 0.08^{abB}	0.22 ± 0.15^{aAB}	0.12 ± 0.09^{abA}	5.09 ± 0.71^{cB}	1.47 ± 0.61^{abAB}	0.62 ± 0.02^{abcA}	4.64 ± 0.55^{aB}
M7.5	0.18 ± 0.01^{aA}	1.27 ± 0.03^{abAB}	3.51 ± 0.11^{bB}	0.37 ± 0.09^{aAB}	0.19 ± 0.04^{abA}	4.52 ± 0.31^{abcB}	0.84 ± 0.31^{abA}	0.84 ± 0.10^{deAB}	4.93 ± 0.37^{aB}
M10	0.18 ± 0.01^{aA}	1.31 ± 0.03^{abcAB}	3.46 ± 0.09^{bB}	0.32 ± 0.05^{aAB}	0.18 ± 0.10^{abA}	4.13 ± 0.50^{abB}	0.82 ± 0.24^{abAB}	0.74 ± 0.06^{bcdA}	4.53 ± 0.34^{aB}
p-value	0.1954	0.0005	0.0028	0.4779	0.0042	0.0036	0.0009	0.00000014	0.059

C—control (pasta from semolina); E2.5–10—pasta from semolina flour fortified with 2.5–10% of Enoki mushroom powder, M2.5–10—pasta from semolina flour fortified with 2.5–10% of Maitake mushroom powder, PAC—phenolic acid content, TFC—total flavonoid content, TPC—total phenolic content. Different lowercase letters in the same column or capital letters in the same row indicate significantly different groups at $p \leq 0.05$.

Similarly, a higher TPC content was noted in the majority of the EtOH extracts of pasta supplemented with the studied mushrooms compared to the control sample. The highest TPC content was noted in the M2.5 sample.

Surprisingly, the samples of pasta fortified with the mushrooms and subjected to simulated digestion exhibited no increase in the TPC content relative to the control.

It is noteworthy that the highest content of phenolic acids, flavonoids, and total phenolic compounds was recorded in the in vitro-digested samples and, with some exceptions, this trend was statistically significant—see Table 1.

Table S2 shows phenolic compounds identified by LC-MS/MS in the dried Enoki and Maitake mushrooms. Twelve phenolic compounds in Maitake and thirteen in Enoki mushrooms were identified, but ten of these compounds identified in Enoki and three in Maitake were below the limit of determination of the lowest calibration point (<0.5 μg/g DW). The Maitake mushrooms exhibited a higher content of all the identified phenolic compounds than Enoki (Table S2). Fifteen phenolic compound standards were used in the LC-MS/MS analysis (Table S2). Detailed results are shown in Table 2. In the case of the crude pasta material (Table 2A), four polyphenols were detected in the control sample. Such polyphenols as rutin, salicylic acid, rosmarinic acid, and *t*-cinnamic acid were detected at <0.5 μg/g in all the pasta samples.

The largest amounts of phenolic compounds were detected in the dried Maitake-supplemented pasta (M2.5, M5, M7.5, and M10), and they were represented by as many as eight compounds. Compared to all the mushroom-supplemented samples and the control, the following compounds with the highest concentrations were detected in the M10 sample: caffeic acid $0.83 \pm 0.14 \times 10^3$ ng/g DW, syringic acid $1.70 \pm 0.24 \times 10^3$ ng/g DW (p value 0.0241), ellagic acid $1.22 \pm 0.09 \times 10^3$ ng/g DW, and vanillin $0.67 \pm 0.09 \times 10^3$ ng/g DW—Table 2A.

3,4-dihydroxybenzoic acid was identified in all the Enoki- and Maitake-supplemented samples, with the highest value of $2.13 \pm 0.52 \times 10^3$ ng/g DW determined for the E10 sample. In turn, the content of this compound in the control was <0.5 μg/g DW. In addition, *p*-coumaric acid, ferulic acid, and sinapic acid were detected in all the dried Enoki-fortified pasta samples—see Table 2A. Most of the phenolic compounds identified in the raw materials were also detected in the EtOH, PBS, and GID extracts. The chromatographic analysis showed a wide variety of phenolic compounds present in the pasta samples supplemented with the dried Enoki and Maitake mushrooms. It is noteworthy that the highest content of phenolic compounds was determined in all the extracts (ethanolic, PBS, and GID) from the M10 sample. The largest number of compounds, i.e., as many as eleven, was determined in the ethanol extracts from the M10 sample, while six phenolic compounds were detected in the ethanolic extract from the control sample.

The smallest number of phenolic compounds, i.e., only four compounds, was found in the PBS extracts in all the Enoki-enriched pasta samples. Additionally, in the ethanolic extracts, the content of 3,4-dihydroxybenzoic acid in the pasta samples supplemented with Enoki and Maitake mushrooms was higher than in the control sample (13.73 ± 0.12 ng/g DW), reaching the highest value in the M10 sample (148.37 ± 3.67 ng/g DW). This value was statistically higher than in the control sample ($p = 0.0014$). The amount of syringic acid in the ethanolic extracts from the dried Maitake-fortified pasta increased with the increase in the dose of the mushroom, reaching the highest level in the M10 sample, which was 178% higher than that in the control. Noteworthy, additional phenolic compounds that were not present in the control sample were detected in the samples of pasta fortified with the tested mushrooms. These were ellagic acid, which was identified only in the Maitake-enriched pasta samples (raw material and ethanolic extracts), and naringenin (determined in the Maitake-enriched pasta samples)—see Table 2.

The glucan content in the pasta samples is shown in Table 3. The fortification with the studied mushrooms did not yield statistically significant differences in the total glucan content ($p = 0.0516$). All the Enoki-enriched pasta samples (E2.5, E5, E7.5, and E10) exhibited statistically significantly higher content of α-glucans than the control ($p = 0.000025$).

Table 2. LC-MS/MS-based qualitative–quantitative analysis of phenolic compounds in crude pasta samples (A) and in ethanolic (B), PBS (C), and GID (D) extracts.

	A									
Polyphenolic compound ($\times 10^3$ ng/g DW)	**Name of polyphenolic compound**	**C**	**E2.5**	**E5**	**E7.5**	**E10**	**M2.5**	**M5**	**M7.5**	**M10**	***p-value***
	3,4-Dihydroxybenzoic acid	<0.5	1.71 ± 0.24 ^{aA}	1.99 ± 0.43 ^{aA}	1.87 ± 0.13 ^{aA}	2.13 ± 0.52 ^{aA}	1.68 ± 0.29 ^{aB}	1.87 ± 0.25 ^{aA}	1.43 ± 0.25 ^{aB}	1.57 ± 0.35 ^{aB}	0.373
	Caffeic acid	<0.5	<0.5	<0.5	<0.5	<0.5	0.52 ± 0.13 ^{a}	0.61 ± 0.09 ^{aA}	0.80 ± 0.08 ^{aA}	0.83 ± 0.14 ^{aA}	0.957
	Syringic acid	<0.5	<0.5	<0.5	<0.5	<0.5	0.93 ± 0.11 ^{aB}	1.24 ± 0.11 ^{abB}	1.46 ± 0.13 ^{abB}	1.70 ± 0.24 ^{bB}	0.0241
	Daidzin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-
	Rutin	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	-
	Ellagic acid	n.d.	<0.5	<0.5	<0.5	<0.5	<0.5	1.03 ± 0.43 ^{a}	0.94 ± 0.09 ^{a}	1.22 ± 0.09 ^{aA}	0.2851
	p-Coumaric acid	1.67 ± 0.03 ^{aB}	3.31 ± 0.50 ^{aB}	3.23 ± 0.46 ^{aB}	3.23 ± 0.42 ^{aB}	3.31 ± 0.44 ^{aB}	2.99 ± 0.26 ^{aB}	3.08 ± 0.29 ^{aB}	2.98 ± 0.29 ^{aB}	2.98 ± 0.52 ^{aB}	0.2393
	Salicylic acid	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	-
	Vanillin	0.56 ± 0.11 ^{aB}	<0.5	<0.5	<0.5	<0.5	<0.5	0.54 ± 0.04 ^{aB}	0.58 ± 0.02 ^{aB}	0.67 ± 0.09 ^{aA}	0.3385
	Ferulic acid	154.13 ± 13.21 ^{aB}	209.93 ± 38.75 ^{aB}	204.14 ± 34.11 ^{a}	205.99 ± 26.59 ^{aB}	213.88 ± 26.67 ^{aB}	192.70 ± 22.67 ^{aB}	196.09 ± 17.85 ^{aA}	197.38 ± 16.76 ^{aB}	182.34 ± 30.57 ^{aB}	0.3091
	Sinapic acid	27.59 ± 1.24 ^{aB}	31.80 ± 5.73 ^{aB}	32.38 ± 4.46 ^{aB}	31.98 ± 5.18 ^{aB}	33.23 ± 3.66 ^{aB}	28.53 ± 3.55 ^{aB}	29.00 ± 2.15 ^{aAB}	29.06 ± 1.54 ^{aB}	26.50 ± 3.50 ^{aB}	0.5544
	Rosmarinic acid	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	-
	t-Cinnamic acid	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	-
	Genistein	n.d.	n.d.	n.d.	n.d.	n.d.	<0.5	<0.5	<0.5	<0.5	-
	Naringenin	n.d.	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	-
	B (Ethanolic extracts)										
Polyphenolic compound (ng/g) DW)	**Name of polyphenolic compound**	**Cs**	**E2.5**	**E5**	**E7.5**	**E10**	**M2.5**	**M5**	**M7.5**	**M10**	***p-value***
	3,4-Dihydroxybenzoic acid	13.73 ± 0.12 ^{aA}	15.77 ± 1.46 ^{aA}	20.33 ± 2.22 ^{abA}	21.27 ± 2.71 ^{abA}	41.13 ± 3.00 ^{abA}	34.83 ± 1.65 ^{abAB}	83.87 ± 4.03 ^{abA}	84.80 ± 17.93 ^{abA}	148.37 ± 3.67 ^{bAB}	0.0014
	Caffeic acid	<1	<1	<1	<1	17.60 ± 1.51 ^{a}	<1	22.87 ± 0.78 ^{abA}	21.13 ± 4.20 ^{abA}	39.27 ± 3.45 ^{bA}	0.0268
	Syringic acid	153.03 ± 2.37 ^{abA}	84.00 ± 4.46 ^{abA}	71.30 ± 0.66 ^{abA}	67.83 ± 3.76 ^{aA}	63.80 ± 3.82 ^{aA}	196.87 ± 6.01 ^{abAB}	305.83 ± 10.15 ^{abAB}	297.90 ± 60.34 ^{abAB}	426.10 ± 6.32 ^{bAB}	0.0013
	Daidzin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-
	Rutin	<1	15.67 ± 3.76 ^{ab}	9.47 ± 1.12 ^{a}	12.50 ± 2.34 ^{ab}	65.20 ± 9.64 ^{ab}	25.10 ± 1.73 ^{ab}	42.20 ± 2.30 ^{ab}	41.80 ± 12.17 ^{abA}	74.10 ± 1.20 ^{bA}	0.0024
	Ellagic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	70.87 ± 6.06 ^{a}	121.90 ± 9.03 ^{bA}	0.0022
	p-Coumaric acid	314.90 ± 6.06 ^{abA}	317.20 ± 4.83 ^{abAB}	271.50 ± 4.12 ^{abAB}	205.47 ± 8. 15 ^{aAB}	208.17 ± 10.45 ^{abAB}	361.37 ± 7.06 ^{bAB}	335.87 ± 11.59 ^{abAB}	220.93 ± 41.31 ^{abAB}	239.80 ± 10.21 ^{abAB}	0.0023
	Salicylic acid	<1	<1	<1	<1	<1	12.13 ± 2.01 ^{a}	12.37 ± 2.85 ^{a}	13.03 ± 2.48 ^{a}	16.03 ± 1.56 ^{a}	0.2228
	Vanillin	112.17 ± 13.05 ^{abAB}	123.37 ± 7.45 ^{abA}	100.73 ± 5.25 ^{abA}	98.50 ± 7.77 ^{aA}	126.70 ± 10.64 ^{abA}	108.53 ± 1.62 ^{abA}	161.33 ± 12.29 ^{abAB}	145.23 ± 9.61 ^{abAB}	166.43 ± 8.81 ^{bA}	0.0032
	Ferulic acid	1587.57 ± 85.27 ^{abAB}	1778.40 ± 90.51 ^{aAB}	1479.43 ± 32.90 ^{abA}	1311.27 ± 56.67 ^{abAB}	1141.60 ± 36.97 ^{abAB}	1633.33 ± 34.07 ^{abAB}	1504.43 ± 51.32 ^{abA}	1014.70 ± 194.25 ^{bAB}	1054.60 ± 15.38 ^{bAB}	0.002
	Sinapic acid	177.77 ± 4.56 ^{abAB}	293.23 ± 8.04 ^{abAB}	279.07 ± 5.52 ^{abAB}	279.27 ± 10.49 ^{abAB}	334.27 ± 29.03 ^{aAB}	130.80 ± 12.47 ^{bAB}	300.47 ± 16.51 ^{abAB}	212.70 ± 34.09 ^{abAB}	270.97 ± 16.13 ^{abAB}	0.0025
	Rosmarinic acid	n.d.	n.d.	n.d.	n.d.	17.63 ± 0.61	n.d.	n.d.	n.d.	n.d.	-
	t-Cinnamic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-
	Genistein	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-
	Naringenin	n.d.	n.d.	n.d.	n.d.	n.d.	97.47 ± 2.14 ^{aA}	200.17 ± 6.73 ^{abA}	242.47 ± 47.13 ^{abA}	376.00 ± 9.28 ^{bA}	0.0156

Table 2. *Cont.*

Polyphenolic compound (ng/g DW)	C (PBS extracts)									
Name of polyphenolic compound	**C**	**E2.5**	**E5**	**E7.5**	**E10**	**M2.5**	**M5**	**M7.5**	**M10**	***p-value***
3,4-Dihydroxybenzoic acid	26.33 ± 5.08 ^{abA}	n.d.	n.d.	n.d.	n.d.	12.50 ± 0.62 ^{aA}	83.93 ± 6.87 ^{abA}	125.70 ± 3.12 ^{abAB}	181.50 ± 13.25 ^{bAB}	0.009
Caffeic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-
Syringic acid	140.13 ± 16.86 ^{abA}	93.80 ± 24.98 ^{abA}	64.17 ± 16.68 ^{aA}	69.63 ± 0.40 ^{aA}	106.40 ± 25.81 ^{abB}	248.50 ± 22.70 ^{abAB}	304.10 ± 6.39 ^{abAB}	428.17 ± 19.17 ^{abAB}	533.13 ± 52.14 ^{bAB}	0.002
Daidzin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-
Rutin	<1	n.d.	n.d.	n.d.	n.d.	n.d.	<1	16.00 ± 2.86 ^{aA}	24.03 ± 3.84 ^{b}	0.0483
Ellagic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-
p-Coumaric acid	209.10 ± 16.45 ^{aAB}	441.07 ± 86.44 ^{aAB}	402.27 ± 64.14 ^{aAB}	301.53 ± 25.52 ^{aAB}	247.30 ± 47.43 ^{aAB}	330.90 ± 28.92 ^{aAB}	238.20 ± 3.58 ^{aAB}	220.23 ± 3.75 ^{aAB}	208.27 ± 13.30 ^{aAB}	0.0613
Salicylic acid	<1	<1	<1	<1	<1	<1	<1	<1	<1	-
Vanillin	69.90 ± 11.78 ^{aA}	256.20 ± 40.81 ^{abB}	306.13 ± 48.33 ^{bB}	255.80 ± 16.97 ^{abB}	164.13 ± 32.31 ^{abAB}	107.50 ± 9.82 ^{abA}	113.67 ± 9.17 ^{abA}	126.80 ± 18.14 ^{bAB}	162.17 ± 39.25 ^{abA}	0.0024
Ferulic acid	1247.77 ± 150.46 ^{abAB}	1273.23 ± 24.31 ^{abAB}	1241.20 ± 76.04 ^{abA}	754.00 ± 84.10 ^{abA}	1470.97 ± 386.88 ^{aAB}	1141.80 ± 42.40 ^{abA}	1129.40 ± 60.79 ^{abA}	634.60 ± 29.44 ^{bA}	716.30 ± 51.60 ^{abAB}	0.0045
Sinapic acid	48.57 ± 10.33 ^{aA}	n.d.	n.d.	n.d.	n.d.	n.d.	27.87 ± 3.76 ^{aA}	n.d.	28.83 ± 9.33 ^{aA}	0.065
Rosmarinic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-
t-Cinnamic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-
Genistein	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-
Naringenin	n.d.	n.d.	n.d.	n.d.	n.d.	92.90 ± 6.97 ^{aA}	132.70 ± 2.00 ^{abAB}	237.30 ± 9.68 ^{abA}	312.40 ± 27.29 ^{bAB}	0.0156

Polyphenolic compound (ng/g DW)	D (GID samples)									
Name of polyphenolic compound	**C**	**E2.5**	**E5**	**E7.5**	**E10**	**M2.5**	**M5**	**M7.5**	**M10**	***p-value***
3,4-Dihydroxybenzoic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	16.23 ± 7.88 ^{A}	-
Caffeic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-
Syringic acid	124.85 ± 0.52 ^{aA}	73.28 ± 5.23 ^{aA}	94.29 ± 78.91 ^{aA}	81.72 ± 7.17 ^{aA}	73.23 ± 3.11 ^{aAB}	157.58 ± 8.57 ^{aA}	210.35 ± 7.96 ^{aA}	176.31 ± 68.65 ^{aA}	271.06 ± 101.67 ^{aA}	0.0613
Daidzin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-
Rutin	<1	n.d.	n.d.	<1	<1	<1	<1	<1	<1	-
Ellagic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-
p-Coumaric acid	143.08 ± 2.19 ^{abAB}	132.63 ± 5.99 ^{abA}	172.32 ± 5.08 ^{abA}	136.77 ± 7.56 ^{abA}	109.80 ± 5.31 ^{aA}	203.23 ± 7.35 ^{bA}	182.78 ± 5.52 ^{abA}	113.28 ± 43.78 ^{abA}	150.40 ± 58.90 ^{abA}	0.0172
Salicylic acid	<1	<1	<1	<1	<1	<1	<1	<1	<1	-
Vanillin	179.85 ± 7.09 ^{abAB}	203.84 ± 49.72 ^{abAB}	231.01 ± 42.13 ^{abAB}	218.79 ± 17.14 ^{abAB}	194.09 ± 22.38 ^{abB}	220.15 ± 11.77 ^{abA}	233.59 ± 5.30 ^{abAB}	123.28 ± 3.66 ^{aA}	261.36 ± 17.57 ^{bA}	0.0186
Ferulic acid	936.87 ± 43.13 ^{abA}	1008.18 ± 61.96 ^{abA}	1263.84 ± 102.86 ^{aA}	1095.45 ± 36.04 ^{abAB}	796.62 ± 39.24 ^{abA}	1282.98 ± 29.02 ^{aAB}	1087.47 ± 40.70 ^{abA}	972.58 ± 28.42 ^{abAB}	445.05 ± 47.74 ^{bA}	0.0019
Sinapic acid	68.28 ± 0.09 ^{abAB}	78.03 ± 5.10 ^{abA}	129.39 ± 10.87 ^{aA}	79.29 ± 3.92 ^{abA}	116.31 ± 3.95 ^{abA}	50.71 ± 4.67 ^{bA}	86.31 ± 7.12 ^{abAB}	51.46 ± 21.41 ^{abA}	75.15 ± 25.46 ^{abAB}	0.0068

Table 2. *Cont.*

Polyphenolic compound (ng/g DW)	D (GID samples)									
Name of polyphenolic compound	**C**	**E2.5**	**E5**	**E7.5**	**E10**	**M2.5**	**M5**	**M7.5**	**M10**	***p-value***
Rosmarinic acid	<1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-
t-Cinnamic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-
Genistein	<1	154.70 ± 8.52 ^{a}	219.39 ± 112.63 ^{a}	193.43 ± 4.52 ^{a}	174.34 ± 9.37 ^{a}	184.29 ± 10.35 ^{a}	170.51 ± 7.79 ^{a}	151.57 ± 36.14 ^{a}	151.31 ± 51.32 ^{a}	0.2215
Naringenin	n.d.	n.d.	n.d.	n.d.	n.d.	36.21 ± 1.84 ^{aA}	62.68 ± 0.49 ^{abB}	81.01 ± 27.38 ^{abA}	122.98 ± 31.83 ^{bB}	0.0373

C—control (pasta from semolina); E2.5–10—pasta from semolina flour fortified with 2.5–10% of Enoki mushroom powder, M2.5–10—pasta from semolina flour fortified with 2.5–10% of Maitake mushroom powder, <0.5 (µg/g DW) below the limit of determination of the lowest calibration point; n.d.—not detected. Samples with different superscripts within a row (a, b, etc.) are significantly different at $p \leq 0.05$. Different capital letters in the same sample but different types of extract indicate significantly different groups at $p \leq 0.05$.

Table 3. Content of glucans in pasta fortified with dried Enoki and Maitake mushrooms.

Sample	Total Glucan (g/100 g)	α-Glucan (g/100 g)	β-Glucan (g/100 g)
C	0.26 ± 0.02 ^{a}	0.05 ± 0.01 ^{a}	0.21 ± 0.02 ^{ac}
E2.5	0.28 ± 0.01 ^{a}	0.09 ± 0.01 ^{e}	0.18 ± 0.01 ^{ab}
E5	0.25 ± 0.01 ^{a}	0.07 ± 0.01 ^{d}	0.18 ± 0.01 ^{ab}
E7.5	0.24 ± 0.01 ^{a}	0.07 ± 0.01 ^{cd}	0.16 ± 0.02 ^{b}
E10	0.25 ± 0.01 ^{a}	0.06 ± 0.01 ^{bcd}	0.19 ± 0.01 ^{abc}
M2.5	0.25 ± 0.01 ^{a}	0.06 ± 0.01 ^{abc}	0.19 ± 0.01 ^{abc}
M5	0.26 ± 0.02 ^{a}	0.06 ± 0.01 ^{abc}	0.20 ± 0.02 ^{abc}
M7.5	0.28 ± 0.01 ^{a}	0.06 ± 0.01 ^{ab}	0.22 ± 0.01 ^{c}
M10	0.26 ± 0.02 ^{a}	0.05 ± 0.01 ^{ab}	0.21 ± 0.02 ^{ac}
p-value	0.0516	0.000025	0.000312

C—control (pasta from semolina); E2.5–10—pasta from semolina flour fortified with 2.5–10% of Enoki mushroom powder, M2.5–10—pasta from semolina flour fortified with 2.5–10% of Maitake mushroom powder. Samples with different superscripts within a column are significantly different at $p \leq 0.05$.

3.2. Antioxidant Activity

The antioxidant activities of the ethanolic, PBS, and GID extract samples from the studied pasta are shown in Table 4.

All the EtOH extracts from the mushroom-enriched pasta had higher $ABTS^{+\bullet}$ inhibition activity than the C sample. The highest $ABTS^{+\bullet}$ inhibition activity was exhibited by the M7.5 sample (0.90 ± 0.14 mg TE/gDW), but these differences were not statistically significant.

The PBS extracts from the studied pasta had the highest antiradical activity against ABTS in the control sample (1.15 ± 0.11 mg TE/gDW).

The GID samples from the dried Maitake- and Enoki-fortified pasta showed slightly higher $ABTS^{+\bullet}$ inhibition activity than the C sample (12.47 ± 1.34 mg TE/gDW) except for the E2.5 and M2.5 samples. The highest value was found for the M7.5 sample (14.15 ± 1.54 mg TE/gDW).

Additionally, the GID samples (with the exception of the E10 samples) exhibited statistically significant higher $ABTS^{+\bullet}$ inhibition activity than the ethanolic extracts—see Table 4.

Only a few ethanolic extracts showed low DPPH inhibition activity. As revealed by the analysis of the PBS extracts, the enrichment with the tested mushrooms had only a slight effect on the DPPH scavenging activity. It should be noted that the PBS extracts from the M7.5 and M10 samples had the highest DPPH inhibition activity (0.34 ± 0.27 mg TE/gDW and 0.33 ± 0.09 mg TE/gDW, respectively). The GID samples exhibited the highest antiradical activity against DPPH. The pasta supplemented with 10% of dried Maitake mushrooms showed slightly higher activity than the control (4.06 ± 0.57 mg TE/gDW).

The ethanolic extracts from the dried Enoki and Maitake-fortified pasta had higher chelating activity than the control sample. The activity of the control sample (0.26 ± 0.16 mg EDTA/gDW) was statistically significantly lower than that of the E10 sample, which reached the highest value (3.54 ± 0.94 mg EDTA/gDW).

The highest CHP activity was exhibited by samples subjected to simulated digestion. All the dried Maitake-enriched samples and the sample fortified with 10% of dried Enoki mushrooms showed a higher ability to chelate transition metal ions than the control sample. It is noteworthy that this activity of the E10, M7.5, and M10 samples was statistically significant higher than that of the control. It should also be noted that all the GID extracts from the studied samples were characterized by statistically significant higher chelating power than the ethanolic extracts—see Table 4.

Table 4. Antioxidant properties of pasta fortified with dried Enoki and Maitake mushrooms.

Samples	ABTS (mg TE/gDW)			DPPH (mg TE/gDW)			CHP (mg EDTA/gDW)			RP [mgTE/gDW]		
	ETOH	PBS	GID	ETOH	PBS	GID	ETOH	PBS	GID	ETOH	PBS	GID
C	0.68 ± 0.04^{aA}	1.15 ± 0.11^{aAB}	12.47 ± 1.34^{aB}	n.a.	0.25 ± 0.01^{aA}	4.05 ± 0.08^{aB}	0.26 ± 0.16^{aA}	n.a.	217.42 ± 8.46^{bB}	0.20 ± 0.02^{cAB}	0.20 ± 0.08^{aA}	1.13 ± 0.06^{bB}
E2.5	0.86 ± 0.18^{aA}	0.88 ± 0.37^{abAB}	12.10 ± 0.82^{aB}	0.04 ± 0.03^{aA}	0.29 ± 0.01^{aAB}	3.49 ± 0.29^{aB}	0.44 ± 0.10^{abA}	n.a.	177.22 ± 15.96^{aB}	0.27 ± 0.04^{acAB}	0.19 ± 0.08^{aA}	1.13 ± 0.05^{bB}
E5	0.84 ± 0.15^{aA}	1.04 ± 0.05^{abAB}	13.14 ± 0.40^{aB}	n.a	0.26 ± 0.19^{a}	2.65 ± 1.59^{a}	1.15 ± 0.20^{abA}	n.a.	184.82 ± 4.33^{aB}	0.36 ± 0.08^{abAB}	0.20 ± 0.13^{aA}	1.18 ± 0.02^{bdB}
E7.5	0.87 ± 0.03^{aA}	0.83 ± 0.52^{abAB}	13.04 ± 0.15^{aB}	0.05 ± 0.01^{aA}	0.22 ± 0.01^{aAB}	3.67 ± 0.30^{aB}	2.08 ± 1.01^{abA}	n.a.	178.13 ± 3.41^{aB}	0.41 ± 0.06^{bdeAB}	0.37 ± 0.05^{aA}	1.34 ± 0.06^{cB}
E10	0.88 ± 0.06^{aAB}	0.82 ± 0.05^{bA}	12.66 ± 0.27^{aB}	0.08 ± 0.01^{aA}	0.14 ± 0.08^{aAB}	3.81 ± 0.27^{aB}	3.54 ± 0.94^{bA}	n.a.	268.82 ± 14.69^{cB}	0.49 ± 0.04^{deAB}	0.43 ± 0.23^{aA}	1.29 ± 0.02^{cdB}
M2.5	0.70 ± 0.05^{aA}	0.89 ± 0.02^{abAB}	11.36 ± 1.63^{aB}	n.a.	0.22 ± 0.05^{aA}	3.66 ± 0.34^{aB}	0.88 ± 0.79^{abA}	n.a.	245.25 ± 6.19^{bB}	0.27 ± 0.03^{acAB}	0.21 ± 0.10^{aA}	0.98 ± 0.03^{aB}
M5	0.82 ± 0.06^{aA}	0.96 ± 0.02^{abAB}	12.60 ± 0.16^{aB}	n.a.	0.17 ± 0.06^{aA}	3.86 ± 0.09^{aB}	1.23 ± 0.86^{abA}	n.a.	227.60 ± 12.19^{bB}	0.35 ± 0.03^{abAB}	0.16 ± 0.04^{aA}	1.13 ± 0.06^{bB}
M7.5	0.90 ± 0.14^{aA}	0.98 ± 0.12^{abAB}	14.15 ± 1.54^{aB}	0.43 ± 0.15^{a}	0.34 ± 0.27^{a}	3.74 ± 0.30^{a}	0.70 ± 0.58^{abA}	n.a.	302.30 ± 11.82^{dB}	0.39 ± 0.07^{abdAB}	0.19 ± 0.08^{aA}	1.14 ± 0.04^{bB}
M10	0.88 ± 0.06^{aA}	0.98 ± 0.12^{abAB}	12.99 ± 0.63^{aB}	0.31 ± 0.23^{aA}	0.33 ± 0.09^{aAB}	4.06 ± 0.57^{aB}	0.85 ± 0.76^{abA}	n.a.	282.79 ± 10.73^{cdB}	0.53 ± 0.08^{eAB}	0.50 ± 0.22^{aA}	1.38 ± 0.07^{cB}
p-value	0.2889	0.0342	0.1332	0.0944	0.3874	0.1678	0.0052	-	0.0001	0.000000032	0.3349	0.0000000001

C—control (pasta from semolina); E2.5–10—pasta from semolina flour fortified with 2.5–10% of Enoki mushroom powder, M2.5–10—pasta from semolina flour fortified with 2.5–10% of Maitake mushroom powder, ABTS—radical scavenging ability against ABTS; DPPH—radical scavenging ability against DPPH; CHP—chelating power; RP—reducing power; n.a.—no activity. Different lowercase letters in the same column or capital letters in the same row indicate significantly different groups at $p \leq 0.05$.

All the ethanolic extracts from the Enoki- and Maitake-enriched pasta were characterized by a higher RP value, compared to the control sample (Table 4). The M10 sample exhibited the highest RP value (0.53 ± 0.08 mg TE/gDW), which was 165% higher than that in the control. The reducing power of some PBS extracts from the fortified pasta was increased, but the results were not statistically significantly different.

Some in vitro digested samples from the mushroom-fortified pasta showed higher reducing power than the control sample. The RP value in the E5, E7.5, and M10 samples was statistically significantly higher than the RP value in the control sample. The highest RP was exhibited by the M10 and E7.5 samples (1.38 ± 0.07 mg TE/gDW and 1.34 ± 0.06 mg TE/gDW, respectively). The RP value increased by 22.1% and 18.5%, respectively, compared to the control.

It should also be noted that all the GID extracts from the studied samples had a statistically significantly higher RP value than the PBS extracts (Table 4).

The potential of the samples to inhibit lipoxygenase and cyclooxygenase 2 enzymes (proinflammatory enzymes) was evaluated in the present study as well (Table 5). The ethanolic extract from pasta fortified with 2.5 and 5% of dried Enoki mushrooms had higher LOX inhibition activity (EC_{50} = 0.17 ± 0.03 mg/mL) than the control (EC_{50} = 0.52 ± 0.13 mg/mL). The control PBS extract sample was characterized by no LOX inhibition, while the fortified pasta extracts had this activity (EC_{50} from 0.83 ± 0.27 mg/mL for E7.5 to 0.33 ± 0.08 mg/mL for M10).

Table 5. Lipoxygenase and cyclooxygenase-2 inhibitory activity of extracts from pasta fortified with dried Enoki and Maitake mushrooms.

Samples	LOXI (EC_{50} mg/mL)			COX2I (EC_{50} mg/mL)		
	ETOH	PBS	GID	ETOH	PBS	GID
C	0.523 ± 0.127 ^{a}	n.a.	0.104 ± 0.003 ^{a}	0.245 ± 0.055 ^{a}	n.a.	0.221 ± 0.027 ^{ab}
E2.5	0.169 ± 0.026 ^{bAB}	0.424 ± 0.108 ^{aA}	0.103 ± 0.006 ^{abB}	0.279 ± 0.058 ^{a}	n.a.	0.180 ± 0.015 ^{a}
E5	0.166 ± 0.027 ^{bAB}	0.583 ± 0.380 ^{aA}	0.099 ± 0.003 ^{abcB}	0.221 ± 0.020 ^{a}	n.a.	0.201 ± 0.009 ^{ab}
E7.5	0.238 ± 0.023 ^{abAB}	0.830 ± 0.273 ^{aA}	0.095 ± 0.001 ^{bcB}	0.192 ± 0.009 ^{a}	n.a.	0.246 ± 0.010 ^{b}
E10	0.244 ± 0.039 ^{ab}	0.371 ± 0.154 ^{a}	0.093 ± 0.001 ^{c}	0.210 ± 0.010 ^{a}	n.a.	0.209 ± 0.023 ^{ab}
M2.5	0.235 ± 0.025 ^{abAB}	0.507 ± 0.065 ^{aA}	0.095 ± 0.002 ^{bcB}	0.255 ± 0.027 ^{a}	n.a.	0.215 ± 0.021 ^{ab}
M5	0.236 ± 0.015 ^{abAB}	0.498 ± 0.211 ^{aA}	0.096 ± 0.003 ^{abcB}	0.276 ± 0.066 ^{a}	n.a.	0.204 ± 0.017 ^{ab}
M7.5	0.212 ± 0.019 ^{abAB}	0.491 ± 0.011 ^{aA}	0.096. ± 0.003 ^{abcB}	0.339 ± 0.174 ^{a}	n.a.	0.232 ± 0.022 ^{b}
M10	0.236 ± 0.011 ^{abAB}	0.329 ± 0.083 ^{aA}	0.095 ± 0.001 ^{bcB}	0.201 ± 0.031 ^{a}	n.a.	0.225 ± 0.007 ^{ab}
p-value	0.0016	0.1392	0.0035	0.0919	-	0.0147

C—control (pasta from semolina); E2.5–10—pasta from semolina flour fortified with 2.5–10% of Enoki mushroom powder, M2.5–10—pasta from semolina flour fortified with 2.5–10% of Maitake mushroom powder, LOXI—lipoxygenase inhibition; COX2I—cyclooxygenase-2 inhibition; n.a.—no activity. Different lowercase letters in the same column or capital letters in the same row indicate significantly different groups at $p \leq 0.05$. Positive control (Quercetin): LOXI IC_{50} mg/mL = 0.1 ± 0.002; COXI IC_{50} mg/mL = 0.0026 ± 0.0001.

In turn, the GID extracts showed higher LOX inhibition activity than the ethanol and PBS extracts. Additionally, the pasta fortified with Enoki (E7.5 and E10) and Maitake (M2.5 and M10) subjected to simulated digestion exhibited a statistically significantly higher LOX inhibitory ability than the control (p = 0.0035)—Table 5. Additionally, with the exception of the C and E10 samples, the difference in the LOX inhibitory ability between the PBS and GID extracts was statistically significant—see Table 5.

The PBS extracts of the control and dried Enoki- and Maitake-fortified pasta samples exhibited no ability to inhibit COX-2 (Table 5), while the ethanol extracts did not differ statistically significantly in this parameter.

Unfortunately, no GID-fortified pasta sample had statistically significantly higher COX-2 inhibition activity compared to the control.

Additionally, no statistically different ability to inhibit COX-2 was found between the ethanol extracts and the samples subjected to simulated digestion (Table 5).

3.3. Anti-Cancer Properties

After the gastric digestion (GD) and gastrointestinal digestion (GID) processes, the cytotoxic activity against the AGS and HT29 cancer cell lines was assessed in the pasta samples (control and fortified). The results are shown in Table 6. No significant differences in the activity against the HT29 cancer line were found between the tested GID groups, although the antiproliferative activity of these samples was higher than the activity of the GD samples.

Table 6. Anti-cancer properties of the pasta fortified with dried Enoki and Maitake mushrooms.

Samples	Anti-Cancer Properties EC50 mg/mL	
	GD (Against AGS)	GID (Against HT29)
C	0.18 ± 0.01^{ab}	0.07 ± 0.01^{a}
E2.5	0.24 ± 0.04^{c}	0.07 ± 0.01^{a}
E5	0.19 ± 0.02^{abc}	0.07 ± 0.01^{a}
E7.5	0.20 ± 0.01^{abc}	0.08 ± 0.01^{a}
E10	0.21 ± 0.01^{bc}	0.08 ± 0.01^{a}
M2.5	0.16 ± 0.02^{a}	0.08 ± 0.01^{a}
M5	0.20 ± 0.01^{abc}	0.07 ± 0.01^{a}
M7.5	0.18 ± 0.01^{ab}	0.07 ± 0.01^{a}
M10	0.19 ± 0.02^{ab}	0.07 ± 0.01^{a}
p-value	0.0031	0.6849

C—control (pasta from semolina); E2.5–10—pasta from semolina flour fortified with 2.5–10% of Enoki mushroom powder, M2.5–10—pasta from semolina flour fortified with 2.5–10% of Maitake mushroom powder, GD—gastrically digested samples, GID—gastrointestinally digested samples, AGS—Human Caucasian gastric adenocarcinoma, HT 29—Human Caucasian colon adenocarcinoma. Samples with different superscripts within a column are significantly different at $p \leq 0.05$.

4. Discussion

In the literature, there are some examples of the pro-health potential of Enoki and Maitake mushrooms indicating that this raw material is a good source of biologically active compounds and can potentially be used in the production of some functional food [8].

In our previous study, we demonstrated that durum wheat pasta supplemented with dried Maitake and Enoki mushrooms (in the amount of 2.5, 5, 7.5, and 10.0%) had high nutritional value and acceptable culinary and organoleptic properties [11]. Hence, the objective of this study was to assess the effect of the addition of dried Maitake and Enoki mushrooms on the content of bioactive compounds and some health-enhancing properties of pasta.

Given the literature data on bioactive compounds and the biological properties of extracts from Maitake and Enoki mushrooms, the hypothesis that the enrichment of pasta with powder from these mushrooms may have an impact on the biological activity of this food product seems justified.

Glucans are bioactive compounds present in edible mushrooms. As reported by McClear and Draga [24], the content of total, α-glucans, and β-glucans in Maitake was 32.4–18.55 g/100 g, 1.3 g/100 g, and 32.1–35.1 g/100 g, respectively. However, in a study carried out by Shin and Lee [25], the content of glucans in Maitake mushrooms was in the range of 8.25–9.94 g/100 g (total glucans), 4.57–5.28 g/100 g (α-glucans), and 3.58–5.00 g/100 g (β-glucans), depending on the extraction temperature. In turn, the literature data showed that the content of glucans in Enoki mushrooms was 19.9–26.29%, 0.55–0.7%, and 20–25.71% (total glucans, α-glucans, and β-glucans, respectively) [24,26]. As demonstrated by Nordiana et al. 2019 [27], the addition of Oyster mushrooms to pasta resulted in an increase in the β-glucan content. In the present study, the addition of the dried Enoki and Maitake mushrooms did not increase the content of total and β-glucans in the pasta, while all the Enoki-enriched samples (E2.5, E5, E7.5 and E10) ex-

hibited statistically significant higher amounts of α-glucans in comparison to the control—see Table 3.

Probably, the relationships obtained in this study result from interactions of glucans with other biomolecules present in food matrices, e.g., proteins or lipids, as reported by other researchers [28,29].

Phenolic compounds are an important group of bioactive ingredients of mushrooms determining many health-promoting properties, which are mainly related to their antioxidant activity. Based on literature data, phenolic acids are the major phenolic compounds contained in extracts from Enoki mushrooms. Namely, four phenolic acids (gallic, protocatechuic, chlorogenic, and caffeic acids) and quercetin were detected in Enoki samples using the HPLC method [30]. In turn, as reported by Krsmanović et al. [31], LC–MS/MS detected the presence of *p*-hydroxybenzoic, protocatechuic, *p*-coumaric, and quinic acids as well as daidzein and genistein in *Flammulina velutipes* extracts. However, the LC-MS/MS analysis performed in this study showed the presence of thirteen compounds in Enoki, with the largest amounts of 3,4-dihydroxybenzoic, caffeic, and ferulic acids—see Table S2.

The profile of phenolic compounds in the pasta fortified with Enoki in the present study partially confirmed the above data, as larger amounts of 3,4-dihydroxybenzoic acid (crude pasta and ethanolic extracts), *p*-coumaric acid (crude pasta), ferulic acid (crude pasta, GID samples), sinapic acid (crude pasta, ethanolic extracts, and GID samples), and genistein (GID samples) were identified in the pasta fortified with this mushroom than in the control—see Table 2. Maitake extracts have also been shown to be a good source of antioxidant phenolic compounds. An analysis of phenolic compounds with the UHPLC-DAD technique showed the presence of protocatechuic acid, catechol, and vanillin in Maitake mushroom extracts [32]. In a study reported by Kim et al. [33], the UPLC system identified the following phenolic compounds in Maitake: caffeic acid, chlorogenic acid, and *p*-coumaric acid as well as kaempferol, naringin, and hesperidin. In turn, in a study conducted with HPLC methods, Lee et al. [34] identified high amounts of phenolic compounds in Maitake, i.e., protocatechuic acid, caffeic acid, *p*-coumaric acid, hesperedin, benzoic acid, *o*-coumaric acid, myricetin, *t*-cinnamic acid, naringenin, formononetin, biochanin A, *b*-resorcylic acid, naringin, kaempferol, veratric acid, and very small amounts of vanillin and rutin. The LC-MS/MS analysis performed in the present study confirmed the content of twelve phenolic compounds in Maitake: 3,4-dihydroxybenzoic, caffeic, syringic, ellagic, *p*-coumaric, salicylic, ferulic, sinapic acids and rutin, vanillin, genistein, and naringenin (Table S2).

In this study, the supplementation of the pasta with Maitake contributed to an increase in the content of a large number of phenolic compounds (3,4-dihydroxybenzoic acid, caffeic acid, syringic acid, *p*-coumaric acid); in addition, compounds that were not detected in the control were found in the Maitake-supplemented pasta (ellagic acid and naringenin)—see Table 2. Although the content of these compounds in the fortified pasta samples increased, compared to the control sample, there were no statistically significant differences between the samples enriched with the different doses of the mushrooms, which is a puzzling finding. A clearly dose-dependent relationship was noted only in the case of the naringenin content, and the M2.5 sample was statistically different from the M10 sample—see Table 2.

There are few literature data on the pro-health properties (mainly antioxidant activity associated with changes in the content of phenolic compounds) of flour products enriched with mushroom powder. As reported by Lu et al. [35], the enrichment of bread with powder from three mushrooms (white button, shiitake, and porcini) in the amount of 5%, 10%, and 15% contributed to an increase in antioxidant activity (DPPH and ORAC) and phenolic content in the analyzed product. Similarly, in their study, Lu et al. [36] added white button, shiitake, and porcini mushrooms to pasta in the amount of 5%, 10%, and 15%. There was no increase in TPC in the case of pasta enriched with 5% and 10% of shiitake, whereas the content of phenolic compounds in all the other variants of pasta enriched with the mushrooms was higher than in the control [36]. All the variants of mushroom powder addition to the pasta produced an increase in the antioxidant activity (ORAC), but

the DPPH method showed an increase in antioxidant activity only in pasta with the 10% and 15% addition of white button mushrooms and the 15% supplementation with shiitake and porcini mushrooms [36]. However, the effect of in vitro digestion on these properties was not considered in these studies. Although analyses of samples subjected to simulated digestion are the most valuable tools for examining the effect on the human organism, tests of other extracts, i.e., ethanolic, also give some knowledge, as other compounds can also be extracted from the food product analyzed in this way. PBS extracts, in turn, are regarded as "before digestion" trials showing the effect of the simulated digestion on pro-health activities. Similarly, in the present study, the ethanolic and PBS extracts from the enriched pasta had higher TPC content (with some exceptions) than the control sample—see Table 1. The ethanolic extracts from the Maitake- and Enoki-supplemented pasta also showed higher antioxidant activity (especially chelation power and reducing power) than the control—see Table 4.

In the present study, the pasta was subjected to simulated in vitro digestion. Most importantly, it was indicated that the gastrointestinally digested samples exhibited significantly higher content of polyphenolic compounds and antioxidant activities in comparison to the ethanolic and PBS extracts—see Tables 1 and 4. In turn, the in vitro digestion process enhanced the potential anti-inflammatory activity (LOX and COX2 inhibition) significantly in comparison to the PBS extracts—see Table 5. In fact, PBS samples are regarded as "before digestion", because ethanolic extracts are "chemical extracts".

Simulated in vitro digestion was used by Wang et al. [37], who investigated the properties of pasta enriched with shiitake mushrooms. As in our research, the simulated digestion process caused an increase in the content of phenolic compounds and antioxidant properties (except for ABTS in samples enriched with 5% and 10%) both in the control pasta and in the mushroom-enriched samples [37]. The same trend was observed by Wang et al. [38] in the case of pasta fortified with white button and oyster mushrooms. The authors explain this effect by the simulated digestion-induced release of bioactive compounds (especially phenolic compounds) associated with the cell wall or conjugated with macronutrients in the food matrix. Probably, a similar effect connected with some antioxidant and potentially anti-inflammatory activities was observed in the present study—see Tables 4 and 5.

The addition of shiitake mushrooms, white button mushrooms, and oyster mushrooms in the above-mentioned research also increased the TPC content and antioxidant activity (ABTS and FRAP) in samples subjected to simulated digestion relative to control pasta samples [37,38].

The analysis of samples subjected to simulated digestion in this study demonstrated that the addition of the mushrooms caused an increase in TPC only in the E5 sample. In the case of PAC, most of the enriched pasta samples (except E7.5, E10, and M2.5) contained greater amounts of phenolic acids than the control (the highest PAC content was recorded in M7.5)—see Table 1. The potentially bioavailable fraction of phenols (GID samples) from the Maitake- and Enoki-enriched pasta also showed statistically significantly higher antioxidant activities, i.e., chelating power (E10, M7.5 and M10 samples) and reducing power (E5, E7.5, and M10 samples), in comparison to the control—see Table 4.

Dissanayake et al. [39] demonstrated that Maitake mushrooms exhibited anti-inflammatory properties through the inhibition of COX activity as well as antioxidant activity (LPO). Similarly, some studies showed anti-inflammatory properties of Enoki via the inhibition of NO as well as TNF-α production [40].

The current study confirmed the potential anti-inflammatory abilities of the enriched pasta measured as LOX and COX-2 inhibition (Table 5). The EtOH extracts from pasta supplemented with 2.5 and 5% of dried Enoki mushrooms showed a higher level of LOX inhibition than the C sample. Importantly, the control sample of the PBS extract was characterized by no LOX inhibition, while the fortified pasta extracts had this activity. Most importantly, the samples of pasta fortified with Enoki (E7.5 and E10) and Maitake (M10)

and exposed to simulated digestion showed statistically significantly higher LOX inhibition activity than the control—see Table 5.

Some studies have indicated that single molecules and mixtures of bioactive compounds may differ in terms of biological activity, and the activity of extracts cannot be considered as the sum of the activities of individual components. Therefore, the inference of the bioactivity of extracts considering only their composition may lead to wrong conclusions, as an important role in the prediction of biological activity may be played by changes occurring during digestion and/or interactions with various food matrix components. Although further research on the mechanisms of action of biologically active compounds (including phenolic compounds) is needed, the attempts made so far to explain these phenomena indicate mutual interactions (synergistic, additive, or antagonistic) between compounds determining the biological activity of extracts [41,42].

Since we additionally assume the possibility of interactions with the matrix (semolina), the biological activity of mushroom-enriched pasta extracts can be modified by such interactions between various bioactive compounds, and this is probably the cause of the lack of a direct relationship between the amount of the fungus addition and the analyzed biological activities (including antioxidant or anti-inflammatory activity reflected in LOX and COX2 inhibition). Similarly, the lack of a direct relationship of biological activity with the dose of an ingredient rich in bioactive compounds, i.e., phenolic compounds, was also noted by other researchers. For example, in a study carried out by Jakubczyk et al. [43], the addition of 1–5 g/100 g of coffee to bread had a positive effect on the inhibition of enzymes involved in the pathogenesis of metabolic syndrome, but no dose-dependent effect was demonstrated. The highest ACE inhibition was achieved after the addition of 4% of coffee, while the greatest lipase inhibition was shown in the 2% coffee supplementation variant. Similarly, in studies on bread fortified with quinoa leaves, analyses performed after in vitro digestion revealed an unexpected effect on several antioxidant activities (i.e., larger doses did not increase the activity) [44].

The changes between predicted and experimental results were explained as the possibility that the bread matrix interacted with proteins and starch, which was confirmed by the chromatographic analyses carried out by the authors. Since pasta is a protein- and carbohydrate-rich flour product, the mechanism described by Świeca et al. [44] may be responsible for the relationships observed in the present study as well.

There are some studies reporting the anti-cancer properties of Maitake [45]. As shown by Ukaegbu et al. [46], acetone extracts from Enoki showed potent activity against breast cancer cell lines. Some studies have indicated that some anti-cancer properties of Enoki may be connected with the content of phenolic compounds, e.g., protocatechuic, *p*-coumaric, and ellagic acids [47]. The phenolic content in extracts from pasta enriched with parsley leaves was also correlated with anti-cancer activity (against breast carcinoma cells) by Sęczyk et al. [48], who additionally reported a cytostatic effect of a relatively high concentration of the extracts. However, in the present study, the fortification of the pasta with Enoki and Maitake mushrooms did not result in enhanced anti-cancer properties, although some fortified pasta (especially the Maitake-enriched samples) contained higher amounts of some phenolic compounds (Tables 2 and 6).

5. Conclusions

In conclusion, both mushrooms (Enoki and Maitake) analyzed in this study can be used for the fortification of semolina pasta to achieve a healthier product. Particularly important for the pro-health properties is the activity of the potentially bioavailable fraction of bioactive compounds. Therefore, when choosing the dose of these fungi to be added to pasta, the biological activity of this fraction is mainly important. It is noteworthy that the in vitro digested E10, M7.5, and M10 samples had statistically significantly higher antioxidant activities (chelating power), and the E5, E7.5, and M10 samples exhibited higher reducing power than the control. Additionally, the E7.5, E10, and M10 samples had statistically significantly higher LOX inhibition activity than the control. Despite

the lack of a direct relationship between the mushroom supplementation dose and the biological activities in some cases, the present results indicate the greatest potential in terms of the pro-health effect of pasta samples fortified with the largest amount of the analyzed mushrooms. Although further studies (especially in vivo) are needed, the fortification of pasta with 7.5%, and 10% of Enoki mushrooms and with 10% of Maitake mushrooms can be recommended.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app13148183/s1, Table S1. Parameters of all the molecules monitored with the MRM method. Table S2. Phenolic compounds profile in dried mushrooms (Enoki and Maitake) identified using the LC-MS/MS technique.

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