Article

Influence of dried Maitake and Enoki mushroom addition 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 pro-health properties of pasta was studied. The qualitative and quantitative analysis of phenolic compounds performed using the LC-MS/MS technique indicated the highest amount of phenolic compounds in pasta supplemented with Maitake. However, all pasta enriched with Enoki mushrooms contained statistically significantly higher content of α-glucans in comparison to the control. In samples subjected to gastrointestinal digestion, the antioxidant, and potential anti-inflammatory activities were significantly higher than ethanolic and PBS extracts of samples. The ethanolic extracts from pasta fortified with dried Enoki and Maitake mushrooms had higher antioxidant activity (in some of the antiradical and reducing power assays) as well as a higher ability to inhibit lipoxygenase (E2.5 and E5 samples) compared to the control sample. Also 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 (E7.5, E10 and M10) than the control. In conclusion, Enoki and Maitake mushrooms can be used for fortification of semolina pasta, conferring healthier characteristics of the product.

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

1. Introduction

Mushrooms, apart from nutritional properties, are characterized by the content 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 their culture and tradition, in which the cultivation of mushrooms has several centuries-old roots, especially in China. Edible mushrooms have recently been subjected to research and evaluation by various research centres around the world. The interest in fungi as a raw material to be used in medicine and production of natural pharmaceuticals is associated with their pro-health properties [1]. Edible mushrooms described as medicinal ones are a rich source of bioactive compounds, e.g. polyphenols, polysaccharides, terpenoids, steroids, cerebrosides, and proteins [2]. Mushrooms due to their pro-health properties are well-known and widely used in Asian countries as part of traditional diets and medicine. Many of mushrooms biopolymers produced by edible fungi are increasingly being used to formulate new functional foods and nutraceuticals in order to take

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advantage of their very broad properties: antitumor, antimicrobial, antiviral, immunomodulating hypocholesterolemic, hypoglycemic, and many other effects [3–5].

Maitake is the Japanese name for the edible mushroom *Grifola frondosa*, also called the "dancing mushroom", which is a good source of protein, carbohydrates, dietary fiber, vitamin D2, and minerals (K, P, Na, Ca, Mg) [6]. In recent times, attention has been paid to the possible anti-tumour and immunostimulant as well as antidiabetic effects of Maitake attributed mainly to the content of β-glucans [6,7]. In some reports, other bioactive compounds in *G. frondosa*, such as polyphenolics, α-tocopherol, and ascorbic acid, were described to be responsible for the pro-health properties of these mushrooms, mainly their antioxidant properties [6].

Flammulina velutipes, called Enoki, is another edible mushroom, which is also commonly known as the "winter mushroom" growing in North America, Europe, and Asia. Enoki is rich in proteins, dietary fiber, and vitamin B1 and contains trace amounts of Zn [8]. Additionally, some low molecular weight compounds (phenols, sesquiterpenes) have been isolated from different parts of this mushroom [9].

Increasing demand by a growing number of health-conscious consumers for healthy foods within the last decade or so resulted in the growing interest of producers in recipes related to the production of products with increased nutritional and/or health-promoting value. Adding a functional ingredient will probably increase the cost of the food but, if a health benefit can be demonstrated, many consumers are likely to pay in discerning markets. The above also results in the growing demand for scientific research on the nutritional and health-promoting properties of functional products [10].

There is growing interest in the application of natural products rich in some bioactive compounds into popular functional food. Given the multiple pro-health properties of medicinal mushrooms, they may be valuable functional additives to some food products. In some studies, powdered mushroom or various kind of extracts (aqueous or hydroalcoholic) from mushroom have been used to fortify a variety of foods, including biscuits, cookies, crackers, and cakes as well as meat products [9,11].

In Western countries, mushrooms as additives to functional food are much less popular than in Asian countries. However, due to the growing knowledge of consumers about the impact of food on health, there is a growing interest in this topic among consumers and food producers also in Western countries [11]. In general, supplementation flour products with dried mushroom flour may impoverish pasting properties, providing darker products and adding firmness. The addition of mushroom fibres also weakens the gluten network in obtained products. So the amount of mushroom addition is very important because, in addition to their impact on health-promoting properties, it may determine the physicochemical and sensory properties of flour products [12].

Several works have replaced wheat flour with different mushroom powders. Namely, β-glucans (in amounts 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 the flour used for biscuits production was replaced by *Pleurotus sajor-cajupowder*, as well as *Pleurotus ostreatus* was used as replacer of wheat flour in noodles [12].

Another important aspect is that additives rich in bioactive compounds should be used in products that are willingly and quite often consumed [13].

Pasta being a good source of carbohydrates in the human diet is a popular worldwide product because of its ease of preparation, good storage stability (dried form), low cost, simple preparation as well as relatively low glycemic index. One of the typical strategies employed to create a functional pasta is a partial replacement semolina with various ingredients rich in bioactive compounds, among which there are medicinal mushrooms [10].

In addition to the nutritional and health-promoting properties of *Grifola frondosa* (Maitake) and *Flammulina velutipes* (Enoki), an additional advantage that predisposes

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these mushrooms to potential use in food production is the fact that they belong to species available from farms produced in controlled, ecological conditions [14].

In our previous study, it was observed that the addition of dried Maitake and Enoki mushrooms (in the amount of 2.5, 5, 7.5, and 10.0%) into pasta contributed to high nutritional value and satisfactory culinary and organoleptic properties of the product [14].

The aim of this study was to check whether the addition of dried Maitake and Enoki mushrooms can have an effect on the bioactive activity, especially the antioxidant, anti-inflammatory, and anti-cancer properties of fortified pasta.

2. Materials and Methods

2.1. Preparation of pasta

The tagliatelle pasta was prepared in reproducible laboratory conditions from semolina fortified with two types of dried mushrooms: Maitake (M) and Enoki (E) (2.5% (w/w), 5.0% (w/w), 7.5% (w/w), and 10.0% (w/w)) as described previously [14]. Maitake mushrooms were purchased as dried powder with a particle size of 80-100 mesh; next, it was sieved in our lab through a sieve with 500 µm mesh. Enoki was purchased as whole dried mushrooms, milled in a Retsch GM200 mill grinder to obtain a particle size of 80-100 mesh, and additionally sieved in the lab through a sieve with 500 µm mesh (PPhoto 1). The control sample was semolina pasta and it was the same as in our previous publication [15]. The pasta was cooked for the optimal cooking time determined in our previous study [14]. Next, the pasta was frozen, freeze-dried (LABCONCO, Kansas City, MO, USA), ground (MRC GRINDING MACHINE, SM-450, Holon, Israel), and used for further analyses.

Photo 1. Picture of dried Enoki and Maitake mushrooms

Enoki Maitake

2.2. Preparation of extracts

2.2.1. Ethanolic extracts

Ethanolic extracts were prepared using sonication (1g DW in 10 mL of 50% ethanol, sonication at 30°C for 1 h, and then centrifugation using MPW-352R centrifuge at 9000×g for 30 min).

2.2.2. PBS extracts

For preparation of buffer extracts (PBS), a freeze-dried and ground sample (1 g) was homogenized, extracted by shaking at room temperature using a multi-rotator (RS-60, Biosan, Otwock, Poland) (300 rpm) for 60 min with 10 mL of PBS buffer (phosphate buffered saline, pH 7.4), and centrifuged at 9000 × g for 20 min.

2.2.3. *In vitro* digestion

In vitro digestion was performed according to the procedure described by Minekus et al. [16] with slight modifications proposed by Sęczyk et al.[17]

Before the first step of digestion, the samples (1g) were homogenized with 1 ml of distilled water. As suggested by Minekus et al. [18] homogenization with water before the oral phase is a needed step to obtain pasta-like consistency of food. Homogenization was made using a mechanical homogenizer Omni TH (Omni International, Inc, Kennesaw Georgia, USA) for 1 min (5000 rpm).

For the oral phase, hydrated samples were mixed with 1.4 mL of simulated salivary fluid electrolyte stock solution (SSFESS) and the samples were incubated in the dark with continuous shaking for 2 min at 37 °C. Then, an oral bolus was mixed with 3 mL of sim-

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ulated gastric fluid electrolyte stock solution (SGFESS) for gastric digestion. The samples were incubated in the dark with continuous shaking for 120 min at 37 °C. Parts of samples were collected in this step as gastrically digested samples – GD for determination of potential anticancer properties on gastric carcinoma cells as a direct impact on cancer cells.

For simulation of 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 phases of digestion (gastrointestinally digested samples - GID), the samples were centrifuged (15 min, 6900 × g) and the supernatants were used for analysis. GID samples are treated as a potentially bioavailable fraction of bioactive compounds showing health-promoting effects [17].

Electrolyte stock solutions (SSFESS, SGFESS, SIFESS) were prepared according to [16]. At each digestion step, pH was checked and, when required, 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.

2.3. Content of bioactive compounds

2.3.1. Determination of phenolic compounds

2.3.1.1. Determination of phenolic acid content (PAC)

The total content of phenolic acids was determined according to the Arnov method [18] and expressed as caffeic acid equivalent (CAE) in μg per g of dry weight (DW).

2.3.1.2. Determination of total flavonoid content (TFC)

The total flavonoid content was determined according to the method described by Lamaison and Carnet [19]. Results were presented as quercetin equivalent (QE) in mg per g of dry weight (DW).

2.3.1.3. Determination of total phenolic content (TPC)

The amount of total phenolics was determined using Folin-Ciocalteau reagent [20]. The amount of total phenolics was calculated as gallic acid equivalent (GAE) in mg per g DW.

Phenolic compounds were determined using spectrophotometric methods (PAC, TFC and TPC) in ethanolic extracts, PBS extracts, and *in vitro* digested samples.

2.3.1.4. Qualitative and quantitative analysis of phenolic compounds using the LC-MS/MS technique

Briefly, phenolic compounds were released from the esterified form and from the cell wall by means of alkaline hydrolysis and then extracted with ethyl acetate in acidic pH, as described by Żuchowski et al. with modifications [21]. The extraction was performed in triplicate. 25 mg of the ground material (dried mushrooms and studied pasta) was incubated with 500 μL of 0.2 M NaOH containing 1% of ascorbic acid as an antioxidant at 50°C for 1 hour. Prior to the hydrolysis, 25 μL of the internal standard (10 μg mL−1 of trans-cinnamic-d7 acid, Merck) was added. Acidification to pH 2 was carried out using 2M HCl, and extraction was performed with 1 ml of ethyl acetate for 15 minutes at room temperature. Then, the extracts were centrifuged at 14,000× g for 10 min, and the organic layer was evaporated at 40°C under a stream of nitrogen. The residue was reconstituted in 50 μL of water with methanol (9:1) and centrifuged at 14,000×g. The supernatant was transferred to a glass vial with an insert for LC-MS/MS analysis.

The concentration of phenolic compounds was determined using a high performance liquid chromatograph (ExionLC AD, AB Sciex, Framingham, USA) coupled with a mass spectrometer (QTRAP 6500+, AB Sciex, Framingham, USA). Chromatographic

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separation was carried out on a Kinetex Biphenyl (100 mm × 3 mm, 2.6 μm) column (Phenomenex, Torrance, USA).

Mobile phase: A—water with formic acid 0.1% (at the beginning 5%), B—acetonitrile with formic acid 0.1% (95%), 0.4 mL/min flow. Gradient program: 0.0–1.0 min 5% B, 1.0–2.0 min 5–22% B, 2.0–5.0 min 28% B, 5.0–6.0 min hold 28% B, 6.0–10.0 min 28–70% B, 10.0–11.0 min 70–95% B, 11.0–12.0 min 95% B, 12.0–12.1 min 95–5% B, 12.1–14.0 min 5% B. The injection volume was 5 μL. For detection, electrospray ionization (ESI) in the negative ion mode was used. Tandem mass spectrometry MS/MS was used for quantitative studies. The parameters of all molecules monitored with the MRM method (e.g., precursor (Q1), productions (Q2), collision energy (CE), and retention times) are listed in the Supplementary Materials (Supplementary File Table S1). The LC-MS/MS system was controlled using Analyst 1.7.2 software (AB Sciex, Framingham, USA). SCIEX OS Version 2.1.6.59781 (AB Sciex, Framingham, USA) was used for data processing.

In the case of ethanolic extracts, PBS, and digested samples, direct injections of extracts were analyzed with separation conditions identical to those in alkaline hydrolysis.

2.3.2. Determination of glucans

The content of total, α-, and β-glucans was determined using the K-YBGL β-glucan Assay Kit (Yeast and Mushrooms) (Megazyme, Bray, Ireland) according to the manufacturer's instructions. Dried and milled pasta samples (using a laboratory grinder (MRC GRINDING MACHINE, SM-450, Holon, Israel), which fineness achievable is approx. 100um) (100 mg) were used for the extraction procedure. The results were calculated according to the manufacturer's instructions and expressed as g/100g DW.

2.4. Antioxidant activities

The antioxidant activities were determined in ethanolic extracts, PBS extracts, and *in vitro* digested samples.

2.4.1. Free Radical Scavenging Assays

Free radical scavenging activity was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) described in Brand-Williams et al. [22] and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS+•) as a source of free radicals as proposed by Re et al. [23]. The antioxidant activity was related to Trolox (an analogue of vitamin E) and expressed as mg of Trolox per gram of dry weight (DW).

2.4.2. Ferric reducing antioxidant power

Ferric reducing antioxidant power (RP) was determined according to the methods described by Oyaizu [24]. Reducing power was expressed as a Trolox equivalent (TE) in mg of Trolox per gram of dry weight (DW).

2.4.3. Chelating power

Chelating power (CHP) was determined using the method developed by Guo et al. [25]. The chelating power was expressed as an EDTA equivalent in μg EDTA per g of dry weight (DW).

2.5. Determination of anti-inflammatory properties

Potential anti-inflammatory properties were determined in ethanolic extracts, PBS extracts, and *in vitro* digested samples.

2.5.1. LOX inhibitory activity

The impact of extracts from the control sample and pastas supplemented with dried mushrooms on the lipoxygenase (LOX) activity was measured spectrophotometrically

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using the method described by Szymanowska et al. [26] adapted to the BioTek Microplate Reader. One unit of LOX activity was defined as an increase in absorbance of 0.001 per minute at 234 nm. The corresponding control contained the same concentration of the enzyme in the absence of the inhibitor. Quercetin was used as a positive control. An extract concentration (mgDW/mL) or quercetin concentration (mg mL^{-1}) providing 50% inhibition (EC_{50} for extracts or IC_{50} for quercetin) was obtained by plotting the inhibition percentage against sample concentrations.

2.5.2. COX2 inhibitory activity

The impact of the analyzed extracts on cyclooxygenase-2 activity was determined spectrophotometrically at 590 nm by measuring the activity of the COX peroxidase subunit using NNN'N'-tetramethyl-p-phenylenediamine (TMPDA) as an electron donor, with the use of the COX Activity Assay kit from Cayman Chemical. COX activity was determined according to the instructions provided with the kit. Quercetin was used as a positive control. An extract concentration (mg DW/mL) or quercetin concentration (mg mL^{-1}) providing 50% inhibition (EC_{50} for extracts or IC_{50} for quercetin) was obtained by plotting the inhibition percentage against sample concentrations.

2.6. Determination of anticancer properties

The anticancer properties of the studied pastas were tested using two cancer cell lines: AGS – Human Caucasian gastric adenocarcinoma (ECACC No. 89090402) and HT 29 - Human Caucasian colon adenocarcinoma (ATCC HTB-38). The cells (0.5 × 106 cells/mL) were seeded in 96-well plates and incubated in an air atmosphere humidified with 5% of CO_2 at 37°C for 24 h. The growth medium consisted of DMEM F12 (for AGS) or RPMI 1640 medium (for HT29), 10% FBS (heat-inactivated fetal bovine serum), 2 mM L-glutamine, and a 1% antibiotic-antimycotic solution (Sigma-Aldrich, Poznań, Poland). After 24-hour incubation at 37°C, the cell lines were exposed to various amounts of gastric (GD) or gastrointestinal (GID) samples in DMEM F12 and RPMI, respectively, and further incubated for 24 hours. The final concentration of the gastric and gastrointestinal fluids did not affect cell viability. Then, the WST-1 assay kit (BioVision, Inc., San Francisco, USA) was used for cytotoxicity evaluation according to the manufacturer's procedure.

The cytotoxicity was determined as a percentage of living cells in comparison to the control. The results were evaluated by determination of the EC_{50} (effective concentration of 50% cell viability) values, which were expressed as mg DW/mL. Each experiment was repeated three times.

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

2.7. Statistical analysis

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

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3. Results

3.1. Content of bioactive compounds

Table 1 summarizes the results of the content of total phenolic compounds (TPC), total flavonoids (TFC), and total phenolic acids (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).

Table 1. Content of phenolic compounds in pasta with the addition of 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 in the same column or capitals in the same row indicate significantly different groups at p≤ 0.05.

The results showed that the content of phenolic acids in the ethanol extracts from the studied pasta ranged from 0.17 μg/gDW to 0.18 μg/gDW, but no statistically significant differences were found between the tested samples. Similarly, in the PBS extracts, the addition of the tested mushrooms did not cause significant differences in the content of PAC in comparison to the control sample. However, the lowest average content was observed in sample M2.5 (1.25±0.02 μg Eq CAE /gDW), and it differed significantly from the average value for sample E7.5 (1.34±0.04 μg Eq CAE /gDW). In the case of samples subjected to simulated digestion, samples from pasta fortified with the tested mushrooms were characterized by higher content of phenolic acids (except for samples E10 and M2.5), compared to the control sample. The greatest increase in the PAC content in the *in vitro* digested samples was observed in pasta fortified with Maitake at 7.5 and 10%, which showed a 12.9 % and 11.3% increase in the PAC content, respectively, compared to the control. However, the statistical analysis indicated that only the E7.5 sample differed significantly from the M10 and M7.5 samples.

The flavonoid content in the ethanolic extracts did not differ significantly among the studied groups. In turn, the TFC value in the PBS extracts was higher for pasta enriched with the tested mushrooms in comparison to the control sample. The E10 sample differed significantly from the M2.5 and control samples. However, in the case of the potentially bioavailable fraction of flavonoids (GID samples), greater amounts of flavonoids than in the control were recorded only in samples M2.5, M5, and M7.5, with the highest flavonoid content in sample M5 (5.09±0.71 mg Eq QE /g DW). The average value in E7.5 was significantly different from that in samples M2.5 and M5.

The PBS extracts of pasta enriched with dried Enoki and Maitake mushrooms had higher TPC content than the control sample. The TPC content in the control sample was statistically significantly lower than in the E7.5, E10, M7.5, and E10 samples. The E10 sample was characterized by the highest value (0.91±0.09 mg Eq GAE /g DW), which indicated a 65% increase, compared to the control sample.

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Similarly, in the ethanol extracts, an increase in the TPC content was observed in most of the mushroom-fortified pasta samples, compared to the control sample. The highest TPC content was noted in the M2.5 sample (1.66±0.08 mg Eq GAE /g DW), and it was significantly different from that in the E5, E2.5, and C samples.

Surprisingly, the samples of pasta fortified with the tested mushrooms and subjected to simulated digestion exhibited no increase in TPC content relative to the control, and the results in the studied groups were not statistically significantly different.

It should be also noted that in samples after *in vitro* digestion phenolic acids, flavonoids and total phenolic compounds content were the highest and with some exception this trend was statistically significant – Table 1.

Table S2 shows phenolic compounds identified in dried mushrooms (Enoki and Maitake) using the LC-MS/MS technique. 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). It should be noted that the Maitake mushroom is characterized by a higher content of all identified phenolic compounds compared to Enoki (Table S2).In this study, in order to identify the types of phenolic compounds in the studied pasta, we performed LC-MS/MS analysis using 15 phenolic compound standards (Table 2). Detailed results are provided in Table 2. In the case of crude pasta material (Table 2A), four polyphenols were detected in the control sample (*p*-coumaric acid, vanillin, ferulic acid, synapic acid). Polyphenols such as rutin, salicylic acid, rosmarinic acid, and *t*- cinnamic acid were detected at < 0.5 μg/g (below the limit of determination of the lowest calibration point) in all pasta samples.

The highest amount of phenolic compounds was found in pasta with dried Maitake mushrooms (samples M2.5, M5, M7.5, and M10), and they were represented by as many as eight compounds (3,4-dihydroxybenzoic acid, caffeic acid, syringic acid, ellagic acid, *p*-coumaric acid, vanillin, ferulic acid, synapic acid). The following compounds with the highest concentrations, compared to all the mushroom-supplemented samples and the control, were detected in the M10 sample: caffeic acid 0.83±0.14 *10^3 ng/g DW, syringic acid 1.70±0.24 *10^3 ng/g DW (*p* value 0.0241), ellagic acid 1.22±0.09 *10^3 ng/g DW, and vanillin 0.67±0.09 *10^3 ng/g DW – Table 2A.

3,4-dihydroxybenzoic acid was detected in all the Enoki and Maitake-supplemented samples, with the highest value of 2.13±0.52 *10^3 ng/ g DW found in the E10 sample. In turn, the content of this compound in the control was below the limit of determination of the lowest calibration point. In addition, *p*-coumaric acid, ferulic acid, and sinapic acid were detected in all samples of pasta with dried Enoki mushrooms – Table 2A. The identification of phenolic compounds in the ethanolic, PBS and GID extracts confirmed the presence of compounds contained in the raw material also in these extracts. The chromatographic analysis showed a wide variety of phenolic compounds in the pasta samples supplemented with dried Enoki and Maitake mushrooms. Noteworthy, the highest amount of phenolic compounds was determined in the M10 pasta sample in all types of extracts (ethanolic, PBS, and GID). The largest number of compounds, i.e. as many as eleven, was determined in the ethanol extracts from the M10 sample (3,4-dihydroxybenzoic acid, caffeic acid, syringic acid, rutin, ellagic acid, *p*-coumaric acid, salicylic acid, vanillin, ferulic acid, sinapic acid, naringenin), while six phenolic compounds (3,4-dihydroxybenzoic acid, syringic acid, *p*-coumaric acid, vanillin, ferulic acid, sinapic acid) were found in the ethanolic extract from the control sample.

The smallest amount of phenolic compounds, i.e. only four compounds (syringic acid, vanillin, ferulic acid, and sinapic acid) was found in the PBS extracts in all pasta samples enriched with Enoki mushroom. Additionally, in the ethanolic extracts, the 3,4-dihydroxybenzoic acid content in all pasta samples supplemented with Enoki and Maitake mushrooms had a higher value than in the control sample (13.73± 0.12 ng/g DW), reaching the highest value in sample M10 (148.37± 3.67 ng/g DW), which was statistically

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different from the control sample (p= 0.0014). The amount of syringic acid in the ethanolic extracts from pasta with the addition of dried Maitake mushroom increased with the increase in the dose of the mushroom, reaching the highest level in sample M10, which was by 178% higher than in the control. It should also be noted that, in the samples of pasta fortified with the tested mushrooms, additional phenolic compounds were detected, which were not present in the control sample. This applies to ellagic acid, which was identified only in the samples of Maitake-enriched pasta (raw material and ethanolic extracts) and naringenin (determined in the samples of Maitake-enriched pasta) – Table 2.

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

A

Name of polyphenolic compound	C	E2.5	E5	E7.5	E10	M2.5	M5	M7.5	M10	*p-value*
Polyphenolic compound [*10^3 ng /g DW]										
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	-

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4

B (Ethanolic extracts)

Polyphenolic compound [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	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*

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7

C (PBS extracts)

Name of	C	E2.5	E5	E7.5	E10	M2.5	M5	M7.5	M10	*P -*

polyphenolic compound										*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*

8
9
10

Polyphenolic compound [mg/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*
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 are significantly different at $p \leqslant 0.05$. Different capitals in the same sample but different kind of extract indicate significantly different groups at $p \leqslant 0.05$.

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The results of the glucan content in the pasta samples are shown in Table 3. The fortification with the studied mushrooms did not cause statistically significant differences in the content of total glucans (p= 0.0516). In the case of the β glucan content, the average value in sample E7.5 (0.16±0.02 g/100 g) was significantly different from that in samples M7.5, M10, and C (0.22±0.01, 0.21±0.02, 0.21±0.02 g/100g, respectively). However, all pasta enriched with Enoki mushroom (samples E2.5, E5, E7.5, and E10) exhibited statistically significant higher content of α-glucans in comparison to the control (p=0.000025).

Table 3. Glucan content in pasta with the addition of Enoki and Maitake dried mushrooms.

Sample	Total glucan (g/100g)	α-glucan (g/100g)	β-glucan (g/100g)
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≤ 0.05.

3.2. Antioxidant activity

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

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Table 4. Antioxidant properties of pasta with the addition of Enoki and Maitake dried 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 in the same column or capitals in the same row indicate significantly different groups at $p \leq 0.05$.

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All the ethanolic extracts from pasta fortified with dried mushrooms showed higher $ABTS^{+\bullet}$ inhibition activity compared to the control sample. The highest value was noted for sample M7.5 (0.90±0.14 mg TE/gDW), but these differences were not statistically significant.

The PBS extracts from the studied pasta showed the highest antiradical activity against ABTS in control sample C (1.15±0.11 mg TE/gDW). The activity of the C sample was statistically significantly different from that exhibited by the E10 sample (0.82±0.05 mg TE/gDW) at p=0.0342.

The *in vitro* digested samples from pasta fortified with dried Maitake and Enoki mushrooms showed slightly higher antiradical activity against ABTS than the control C (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). In this case, there were no statistically significant differences between the groups.

Additionally, GID extracts from studied samples (with exception of E10 samples) were characterized by statistically significant higher antiradical activity against ABTS than ethanolic extracts – Table 4.

In the case of the antiradical activity against DPPH, only a few ethanolic extracts showed low activity. The ethanolic extracts from the C, E5, M2.5, and M5 samples showed no activity. In the case of the PBS extracts, the addition of 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 showed the highest ability to neutralize DPPH free radicals (0.34±0.27 mg TE/gDW and 0.33±0.09 mg TE/gDW, respectively). The highest antiradical activity against DPPH was determined for the *in vitro* digested samples. The pasta with the addition of 10% of dried Maitake mushroom showed slightly higher activity than the control (4.06±0.57 mg TE/gDW). However, the results obtained for all the extracts are not statistically significantly different.

We also studied the ability of the samples to chelate transition metal ions (CHP). The ethanolic extracts from pasta fortified with dried Enoki and Maitake mushrooms had higher chelating activity compared to the control sample. The activity of the control sample (0.26±0.16 mg EDTA/gDW) was statistically significantly different from that of the E10 sample, which reached the highest value (3.54±0.94 mg EDTA/gDW).

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

All ethanolic extracts from pasta enriched with dried Enoki and Maitake mushrooms were characterized by a higher RP value, compared to the control sample (Table 4). The value determined for the control sample C (0.20±0.02 mg TE/gDW) was statistically significantly different from the values exhibited by the E5, E7.5, E10, M5, M7.5, and M10 samples. The M10 sample was characterized by the highest RP (0.53±0.08 mg TE/gDW), which was by 165% higher in comparison to the control. The reducing power of some PBS extracts from pasta fortified with the studied mushrooms was increased, but the results were not statistically significantly different.

Some *in vitro* digested samples from pasta enriched with dried Enoki and Maitake mushrooms showed higher reducing power than that of the control. It should be noted that the RP of samples E5, E7.5, and M10 was statistically significantly different from 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) and, relative to the control, it was an increase of 22.1% and 18.5%, respectively.

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It should be also noted that all GID extracts from studied samples were characterized by statistically significant higher RP than PBS extracts (Table 4).

Table 5. Lipoxygenase and cyclooxygenase-2 inhibitory activity of extracts from pasta with the addition of Enoki and Maitake dried 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 in the same column or capitals in the same row indicate significantly different groups at p≤ 0.05. Positive control (Quercetin): LOXI IC_{50} mg/mL = 0.1±0.002; COXI IC_{50} mg/mL = 0.0026±0.0001

The ability of the studied samples (ETOH, PBS, and GID) to inhibit lipoxygenase and cyclooxygenase 2 enzymes, which are involved in inflammatory processes, was also evaluated in the present study (Table 5). In the ethanolic extracts, results in the control group were significantly different from those in the E2.5 and E5 groups (significance level: p=0.0016). The pasta fortified with 2.5 and 5% of dried Enoki mushroom showed a higher ability to inhibit LOX (EC_{50} = 0.17±0.03 mg/mL) than the control (EC_{50} = 0.52±0.13 mg/mL). The control sample of the PBS extract showed no ability to inhibit lipoxygenase activity, while the PBS extracts from pasta enriched with the studied mushrooms had this activity (EC_{50} from 0.83±0.27 mg/mL for the E7.5 sample to 0.33±0.08 mg/mL for the M10 sample), but the groups were not statistically significantly different.

In turn, the GID extracts showed a higher ability to inhibit LOX than the ethanol and PBS extracts. Additionally, the samples of pasta fortified with Enoki (E7.5 and E10) and Maitake (M2.5 and M10) subjected to simulated digestion showed a statistically significantly higher ability to inhibit LOX, compared to the control (p= 0.0035) – Table 5. Additionally, with exception of C and E10 samples difference in ability to LOX inhibition was statistically significant between PBS and GID extracts – 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 groups of ethanol extracts did not differ statistically significantly in this parameter.

On the other hand, in the group of the *in vitro* digested samples, the ability of the E2.5 sample to inhibit COX-2 was found to be statistically significantly different from this activity of the M7.5 and E.7.5 samples, but no GID sample of the fortified pasta showed a statistically significantly higher ability to inhibit COX-2 activity, compared to the control.

The ability to inhibit COX-2 was not also statistically different between the ethanol extracts and the samples after simulated digestion (Table 5).

3.3. Anticancer properties

Pasta supplemented with dried Enoki and Maitake mushrooms as well as the control pasta subjected to gastric digestion (GD) and gastrointestinal digestion (GID) were

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used to study the cytotoxic activity against AGS and HT 29 cancer cell lines. The results of these activities are shown in Table 6. It was found that there were no significant differences between the tested GID groups in their activity against the HT 29 cancer line, although the antiproliferative activity for these samples was higher than that of the GD samples. The analysis of the activtiy of the GD samples against the AGS tumor line revealed significant differences between the average values in sample M2.5 (the highest activity EC_{50} = 0.16-0.02 mg/mL) and E2.5 and E10 (EC_{50} = 0.24±0.04 and EC_{50} = 0.21±0.01 mg/mL, respectively).

Table 6. Anticancer properties of the pasta with the addition of Enoki and Maitake dried mushrooms.

Samples	Anticancer 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

There are a great many species of mushrooms in nature (about 14,000), but only about 2,000 of them are described as safe and suitable for food and/or medical use. Additionally, only 35 species are currently commercially explored. The widely common mushroom species produced in suitable ecological conditions are about nine, including *Grifola frondosa* (Maitake), *Flammulina velutipes* (Enoki) [12]. The selection of raw materials for research that may potentially be used in food technology should take into account the above facts so that safe raw materials are also available to potential food producers.

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 bioactive compounds and has potential to be used in the production of some functional food products. For example, extract of waste products from Enoki mushroom powder exhibited good antioxidant potential, which was attributed to its strong free radical scavenging activity, ferric reducing power, and metal chelating ability [9].

However, functional food products fortified with mushrooms are still available on a limited basis on market (especially in Europe countries) and most of the mushrooms and their compounds are mainly consumed in their natural form or in dietary supplements [11]. For this reason, research on the nutritional value, consumer quality, and pro-health effects of products enriched with some mushrooms is still needed. There are some examples of using some types of mushrooms as ingredients to partially replace meat or starchy ingredients in the production of food products [27].

However, the addition of mushroom powder to flour products, such as bread, cakes, biscuits, or pasta, has so far been mostly studied mainly in the context of the nutritional

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and consumer value of the obtained products and only in a few research, these studies were extended to include the assessment of pro-health properties - mainly antioxidant properties [14,28,29].

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%) was characterized by high nutritional value and satisfactory culinary and organoleptic properties [14]. Hence, the aim of the present study was to check the effect of the addition of dried Maitake and Enoki mushrooms on the content of bioactive compounds and some pro-health properties of pasta.

Based on literature data on bioactive compounds as well as biological properties of extracts from Maitake and Enoki mushrooms, the hypothesis that the enrichment of pasta with powder from these mushrooms may affect the biological activity of the food product seems justified.

Glucans are one of the groups of bioactive compounds present in edible mushrooms. Research conducted by McClear and Draga [30] indicated that the content of total, α-, 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 the study carried out by Shin and Lee [31] the content of glucans in Maitake mushrooms, depending on the extraction temperature, was in the range 8.25-9.94 g/100g (total glucans), 4.57-5.28 g/100g (α-glucans), and 3.58-5.00 g/100g (β-glucans). In turn, literature data indicate that the content of glucans in Enoki mushrooms is 19.9-26.29%, 0.55-0.7 %, and 20-25.71% (total glucans, α-glucans, and β-glucans, respectively) [30,32]. The addition of Oyster mushroom to pasta in a study conducted by Nordiana et al. 2019 [33] resulted in an increase in the content of β-glucans. In the present study, the fortification with the dried Enoki and Maitake mushrooms did not increase the content of total and β-glucans in the studied pasta, while all the pasta samples enriched with Enoki mushroom (samples E2.5, E5, E7.5 and E10) exhibited statistically significant higher content of α-glucans in comparison to the control – Table 3.

Probably these relationships obtained in this study result from interactions of glucans with other biomolecules present in food matrices like proteins or lipids, what has been studied by other researchers [34,35].

Phenolic compounds are an important group of bioactive ingredients of mushrooms that determine many health-promoting properties mainly related to their antioxidant activity. A study conducted by Yeh [36] confirmed the strong antioxidant properties of Enoki extracts expressed as antiradical activity against DPPH and the ability to chelate metal ions. The antioxidant activity of ethanolic extracts from Enoki was also studied by Bach et al. [37]. In these studies, the TPC content was at the level of 2.04-7.52mg/g, depending on the extraction system. Significant antioxidant activity of Enoki ethanolic extracts, expressed as DPPH =13.12 μmol TE/g, ABTS =50.44 μmol TE/g, and FRAP 14.66 μmol TE/g, was confirmed as well [37]. Based on literature data, phenolic acids are the main phenolic compounds in extracts from Enoki mushrooms. Namely, four phenolic acids (gallic, protocatechuic, chlorogenic, and caffeic acids) as well as quercetin were detected in Enoki samples using the HPLC method [38]. In turn, in a study conducted by Krsmanović et al. [39], LC–MS/MS identification confirmed the presence of *p*-hydroxybenzoic, protocatechuic, *p*-coumaric, and quinic acids as well as daidzein and genistein in *Flammulina velutipes* extracts. However, in the studies carried out in this work, the LC-MS/MS analysis showed the presence of thirteen compounds in Enoki, of which 3,4-dihydroxybenzoic, caffeic and ferulic acids were determined in the largest amount – Table S2.

The profile of phenolic compounds in 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 pasta fortified with this mushroom in relation

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to the control – Table 2. Maitake extracts are also a good source of phenolic compounds with antioxidant properties. The total phenolic compound content in Maitake water extract was 183.75 ± 0.21 μg GAE/g DW, whereas the total flavonoid content was 38.38 ± 0.07 μg QE/g DW [40]. The content of phenolic compounds in these extracts determined their significant antioxidant activity measured with four methods: ABTS, DPPH, reducing power, and the ability to chelate iron ions [40]. Identification of phenolic compounds using the UHPLC-DAD technique showed the presence of protocatechuic acid, catechol, and vanillin in Maitake mushroom extracts [37]. In turn, in the study conducted by Kim et al. [41], the phenolic compounds in Maitake revealed by the UPLC system were caffeic acid, chlorogenic acid, and *p*-coumaric acid as well as kaempferol, naringin, and hesperidin. In turn, in their study conducted using HPLC methods, Lee et al. [42] identified high amounts of phenolic compounds in Maitake, namely: 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. 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 addition of Maitake to pasta resulted in an increase in the content of a large number of identified phenolic compounds (namely 3,4-dihydroxybenzoic acid, caffeic acid, syringic acid, *p*-coumaric acid**);** in addition, compounds that were not detected in the control were found in pasta supplemented with this mushroom (ellagic acid and naringenin) – Table 2. Despite the increase in the content of these compounds in the samples of fortified pasta compared to the control sample, what is strange is that there are no statistically significant differences within the samples with various additions of the tested fungus. Only in case of naringenin content the clearly dose depended relationsip was noted and M2.5 sample was statistically differ from M10 sample - Table 2.

When analyzing the content of individual phenolic compounds between different extraction methods, it should be noted that statistically significant differences occur mainly between crude pasta samples and some extracts (EtOH, PBS or GID) - Table 2.

However, only in a few cases were statistically significant differences in the content of individual phenolic compounds between the EtOH, PBS or GID extracts - namely, the content of syringic acid and vanillin was higher in PBS extracts than in ethanol ones (in E10 sample and E2.5-E7.5 samples, respectively). On the other hand, in the E10 samples, the vanillin content was significantly higher in GID extract than in the ethanolic extract. Conversely, in the case of naringenin, ethanol extracts contained a significantly higher amount of this compound compared to GID extract (M5 and M10 samples) – Table 2

There are few examples in the literature of analyses of the pro-health properties (mainly antioxidant properties related to the change in the content of phenolic compounds) of flour products enriched with mushroom powder. As reported by Lu et al. [43], enrichment of bread with powder from three mushrooms (white button, shiitake, and porcini) in the amount of 5%, 10%, and 15% resulted in an increase in antioxidant activity (DPPH and ORAC) and the content of phenolic compounds in the tested product. Similarly, in a study conducted by Lu et al. [44], white button, shiitake, and porcini were added to pasta in the amount of 5%, 10%, and 15%. There was no increase in TPC in the case of pasta with the 5% and 10% shiitake mushroom addition, but all the other variants of pasta enriched with the mushrooms were characterized by higher content of phenolic compounds compared to the control [44]. All the studied variants of mushroom powder addition to the pasta resulted in an increase in the antioxidant activity determined with the ORAC method, but in the case of the DPPH method, an increase in antioxidant activity was observed only for pasta with the 10% and 15% addition of white button mushrooms and with the 15% supplementation with shiitake and porcini mushrooms [44]. However, the above studies did not consider the effect of *in vitro* digestion on these

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properties. Although the study of samples after simulated digestion are the most valuable for examining the effect on the human body, but testing also other extracts, i.e. ethanolic, also gives us some knowledge because in these extracts other compounds will be extracted from the studied food. PBS extracts, in turn, are treated as "before digestion" trials to reference how the simulated digestion affected the studied pro-health activities. Similarly, in the present study, the ethanolic and PBS extracts from pasta fortified with the mushrooms were characterized by higher TPC content (with some exception) in comparison to the control – Table 1. The ethanolic extracts from the Maitake- and Enoki-supplemented pasta also showed higher antioxidant activity (especially chelation power and reducing power) in comparison to the control – Table 4.

Simulated *in vitro* digestion of pasta was carried out in the present studies. Most importantly, the results of the present study indicated that samples subjected to gastrointestinal digestion were characterized by significantly higher content of polyphenolic compounds and antioxidant activities in comparison to the ethanolic and PBS extracts – Table 1 and 4.

Simulated *in vitro* digestion was carried out by Wang et al. [45] in their research on the properties of pasta enriched with shiitake mushrooms. As in our research, in the samples subjected to simulated digestion, an increase in the content of phenolic compounds and antioxidant properties was noted (except for ABTS in samples enriched with 5% and 10%) both in the control pasta and the mushroom-enriched samples [45] The same trend was observed in a study conducted by Wang et al. [46] in the case of pasta fortified with white button mushroom and oyster mushroom. The authors explain this effect by the simulated digestion-induced release of bioactive compounds (especially phenolic compounds) that were associated with the cell wall or conjugated with macronutrients in the food matrix. Probably, a similar effect occurred in the present study and connected with some antioxidant and potentially anti-inflammatory activities - Tables 4 and 5.

The addition of shiitake, white button mushroom, and oyster mushroom in the above-mentioned research also caused an increase in the content of TPC and antioxidant activity (ABTS and FRAP) in samples subjected to simulated digestion compared to the control pasta [45,46]

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

Dissanayake et al. [47] demonstrated that Maitake mushrooms exhibited anti-inflammatory properties through inhibition of COX activity as well as antioxidant activity (LPO). Also some studies showed anti-inflammatory properties of Enoki via inhibition of NO as well as TNF-α production [48].

The present study confirmed the potential anti-inflammatory abilities of the studied pasta measured as LOX and COX-2 inhibition (Table 5). The ethanolic extracts from pasta fortified with 2.5 and 5% of dried Enoki mushroom showed a higher ability to inhibit LOX than the control. It should also be noted that the PBS extracts from the control sample showed no ability to inhibit lipoxygenase activity, while the PBS extracts from pasta enriched with the studied mushrooms had this activity. Most importantly, samples of pasta fortified with Enoki (E7.5 and E10) and Maitake (M10) and subjected to simulated digestion showed a statistically significantly higher ability to inhibit LOX, compared to the control – Table 5.

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There are some studies indicating that single molecules and extracts being mixtures of many active 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. So, the prediction of the biological activity of plant extracts based only on their chemical composition may lead to an erroneous inference, because the key role in their case is played by changes occurring during digestion and/or interactions with other components of the food matrix. Although further research on the mechanisms of action of bioactive compounds (including phenolic compounds) is needed, the attempts made so far to explain these phenomena indicate mutual interactions (synergistic, additive or antagonistic) between the compounds determining the biological activity of extracts [49,50].

Due to the fact that in our research we additionally have the possibility of interaction with the matrix (semolina), the biological activity of pasta extracts enriched with the tested mushrooms can also be modified by such interactions between various bioactive compounds, and probably this is the reason for the lack of a direct relationship between the amount of fungus addition and studied biological activities (including antioxidant or anti-inflammatory activity marked as LOX and COX2 inhibition).

Some reports have also indicated the anticancer properties of Maitake mainly via protection of healthy cells, prevention of tumor metastasis, and inhibition of tumor growth [6].

Previous studies *in vitro* and *in vivo* indicated that Maitake anti-cancer activities are connected with D-fraction (proteoglucan whose main component is β-glucan responsible for conferring Maitake' anti-cancer potential). The research proved D-fraction to boost the immune system, as well as may increase the production of antitumor/ immunostimulatory cytokines [51].

In a study conducted by Ukaegbu et al. [52], the acetone extract from Enoki showed significant activity against breast cancer cell lines. Some studies have indicated that some anticancer properties of Enoki may be connected with the content of phenolic compounds, e.g. protocatechuic, *p*-coumaric, and ellagic acids [53]. The content of phenolic compounds in extracts from pasta enriched with parsley leaves was also correlated with anti-cancer activity (against breast carcinoma cells) in the research conducted by Sęczyk et al. [54], but a relatively high concentration of the extract caused a cytostatic effect in their research. However, in the present study, the fortification of the pasta with Enoki and Maitake mushrooms did not result in enhanced anticancer properties, despite the fact that some fortified pasta (especially the Maitake-enriched samples) contained higher amounts of some phenolic compounds (Table 2 and 6). Some studies indicated that mushrooms anticancer activity is connected with β-glucans [52]. Unfortunately, in the present study pasta fortification with Enoki and Maitake in 2.5-10% has not caused significant increase of β-glucans in studied pasta and may be it is the reason for no increase of anticancer properties of pasta.

5. Conclusions

In conclusion, Enoki and Maitake mushrooms can be used for fortification of semolina pasta, conferring healthier characteristics of the product. Particularly important for the pro-health properties is the activity of the potentially bioavailable fraction of bioactive compounds. So it should be noted that samples after *in vitro* digestion of E10, M7.5 and M10 had statistically significant higher antioxidant activities determined as chelating power as well as from E5, E7.5 and M10 samples determined as reducing power, compared to control. Additionally, E7.5, E10 and M10 samples showed a statistically significantly higher ability to inhibit LOX, compared to the control. Although further studies (especially *in vivo*) are needed, based on the content of bioactive compounds and the studied pro-health properties studied in the present study, the fortification of pasta with 5%, 7.5%, and 10% of Enoki mushroom as well as with 10% of Maitake mushroom can be recommended.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/ 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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