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Pleurotus ostreatus: Nutritional Enhancement and Antioxidant Activity Improvement Through Cultivation on Spent Mushroom Substrate and Roots of Leafy Vegetables

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Abstract: Agricultural residues, including *Pleurotus ostreatus* spent mushroom substrate (SMS) and roots of (hydroponic) leafy vegetables (RLV), were tested in various proportions as substrates in new *P. ostreatus* cultivations, where wheat straw was the control. The impact of SMS and RLV was first evaluated by the mycelial growth rate (Kr, mm/day). Afterwards, mushroom cultivation was conducted in bags, where production characteristics like earliness (the time from substrate inoculation to first harvest) and biological efficiency (BE %, the ratio of fresh mushroom weight to dry substrate weight × 100) were examined. The study also evaluated mushroom quality, nutritional composition and bioactive content. The highest intra-cellular polysaccharide (IPS) value (50.93%, *w*/*w*) was observed in the substrate containing SMS 90%, while combining SMS with RLV resulted in higher IPS values compared to mixtures that also included wheat bran (WB) and soybean flour (SF). Furthermore, the use of RLV reduced the fat content compared to the control substrate, except in the case of the SMS 50%–RLV 40% substrate, where the highest fat content was observed in the produced mushrooms (4.68% *w*/*w*). Additionally, the protein content increased with the use of RLV. The highest triterpene content was found in the SMS 90%–RLV 10% (11.52 ursolic acid mg/g d.w.). However, the control substrate exhibited the fastest Kr (6.5 mm/d), as well as the highest BE (87.8%) and total phenolic compound value (30.31 mg GAE/g d.w.). Significant antioxidant activity was observed in all extracts, while the total flavonoid content was low. Glucose was the dominant monosaccharide (over 51.5%, *w*/*w*), and linoleic acid (18:2; over 57.05%, *w*/*w*) was the primary fatty acid across all mushrooms. This study also enhances our knowledge by which SMS and RLV influence the NFE2L2/HMOX-1 molecular pathway, thereby affecting specific antioxidant-related genes. These effects were observed through the impact of *P. ostreatus* protein and carbohydrate extracts on LPS-challenged THP-1-derived macrophages. A positive impact on the gene expression of HMOX1, CAT and NFE2L2 during incubation with the aforementioned samples was observed. These findings support the sustainable use of agricultural by-products in mushroom cultivation, offering an environmentally friendly approach while producing valuable products like mushrooms.

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1. Introduction

Mushrooms have been valued and used as food or food flavoring material not only for their texture and flavor but also for their chemical and nutritional characteristics and medicinal properties, offering numerous health benefits [1,2]. Among all types of mushrooms, more than 200 species belong to oyster mushrooms [3] and eight species, viz., *Pleurotus ostreatus*, *P. eryngii*, *P. pulmonarius*, *P. djamor*, *P. sajor-caju*, *P. cystidiosus*, *P. citrinopileatus* and *P. cornucopiae*, are commercially grown worldwide [4,5]. Particularly, the cultivation of *P. ostreatus* has seen significant global growth due to their efficiency in lignin degradation and ability to grow on various agricultural waste, demonstrating also broad adaptability to moderate temperatures, ranging from 18 to 30 °C [6]. It requires minimal environmental controls and demonstrates resistance to diseases and pests, making it a simple and cost-effective option for cultivation while also having a shorter growth cycle compared to other edible mushrooms [7]. Thus, cultivating *P. ostreatus* offers an excellent alternative for mushroom production compared to other species [8].

The rapidly growing global population, along with the expansion of the agricultural sector and food industries, has led to the annual generation of substantial amounts of agro-industrial waste. Much of this waste is currently disposed of through incineration or landfill, posing significant environmental and economic challenges and becoming a source of microorganism proliferation, which can become problematic on a larger scale [9]. Proper management of this waste within the framework of the circular economy is crucial. If these residues are utilized to support food production, they will no longer be considered waste but rather valuable resources [10]. Specifically, the mushroom industry generates a substantial supply of an organic by-product known as spent mushroom substrate (SMS), which includes the fungal mycelium and the unutilized substrate left after harvesting mushrooms. In addition, hydroponic vegetables cultivation contributes to agro-industrial waste, not only from the hydroponic wastewater [11,12] but also from leftover roots and other plant parts. To the best of our knowledge, aside from a prior study on *P. citrinopileatus* [13], no other research has explored the reuse of these roots—particularly in combination with SMS—as most existing studies focus primarily on the reuse of wastewater solutions.

For commercial *Pleurotus* mushroom production, wheat straw is the main substrate used [14]. However, their growth substrate can also consist of a mixture of various raw lignocellulosic materials, with the composition influencing cultivation time, biological efficiency (BE %), yield and quality [15]. Melanouri et al. [16] reported BE values of 55.14, 75.30 and 50.60% for *P. ostreatus* cultivation in wheat straw, barley and oat straw and coffee residue substrates, respectively, whereas much higher values over 100% have been reported by Dedousi et al. [17,18] for the same fungus when cultivated on wheat straw and barley and oat straw supplemented with corn oil 2% *w*/*w* and in SMS, sole or in combination with *Pleurotus* waste. Diamantopoulou et al. [19] determined that *P. ostreatus* showed the maximum fat content when grown on coffee residue, while *P. eryngii* species were found to have higher amounts of IPS and β-glucans than *P. ostreatus* species when cultivated in beech wood shavings and barley and oat straw. As a result, there is a need to ascertain the nutritional value of *P. ostreatus* with the aim of validating its nutrient composition to qualify as a functional food, especially when cultivated in novel non-studied-before substrates.

The consumption of *Pleurotus* mushrooms is on the rise due to their high protein and dietary fiber content [5,20], along with their rich profile of essential and non-essential amino

acids, particularly lysine and leucine. Additionally, their significant mineral content makes *Pleurotus* species a valuable alternative to meat, fish and vegetables [21,22]. Furthermore, lipids and sodium are in low content and, therefore, are ideal food options for those suffering from hypertension and cardiovascular disease [23]. Despite the low concentrations of lipids, these fungi contain essential polyunsaturated fatty acids (PUFAs), such as linoleic and linolenic acids, which are usually their major constituents [19,24]. As a result, they are considered as "functional foods" and they are valued for their natural antioxidant additives, making the preservation or enhancement of these unique characteristics in fresh mushrooms of great interest to both researchers and producers. Also, the antioxidant activities for *Pleurotus* polysaccharides and proteins, among others, have been reported. Far more is known today that the antioxidant defense system of the body is mainly to increase the activities of catalase (CAT) and superoxide dismutase (SOD) and then improve the oxidative stress reaction to achieve the role of scavenging reactive oxygen species (ROS) [25]. Nuclear factor (erythroid-derived 2)-like 2 (NFE2L2) is a crucial regulator of the oxidative stress response and is an important transcription factor regulating these enzymes [26]. Heme oxygenase 1 (HMOX1) is a target protein downstream of NFE2L2. The inducible form of HMOX1 exerts a central role in cellular protection [27]. Several in vivo studies have shown that mushroom extracts, especially carbohydrate extracts, can enhance the SOD and CAT activity. Glutathione reductase (GSR) serves as a regulatory factor and an indicator of oxidative stress, as well as alterations in redox signaling [28]. However, the related pathways are still unclear by which oyster mushrooms modulate the antioxidant activity of these enzymes and regulate the expression profiles of antioxidant genes in mammalian models and their involvement in the NFE2L2/HMOX1 pathway.

Given the potential for commercial production of *Pleurotus* spp. mushrooms on innovative, low-cost substrates, this study explored the feasibility of using a combination of SMS and RLV for growing *P. ostreatus*. This approach aligns with the principles of the circular economy by utilizing waste products, thus offering both financial and environmental benefits. The study aimed to evaluate the productivity and quality of *P. ostreatus* grown on these substrates, focusing on a comprehensive biochemical analysis of the produced carposomes. Specifically, the research assessed protein, polysaccharides and lipid content, alongside fatty acid and monosaccharide profiles, total phenolic compounds, total flavonoids, total terpenoids and antioxidant activity. Initially, this study investigated the fungus's colonization rate during the vegetative phase. In subsequent scale-up experiments, it is analyzed how the combined substrates influenced the nutritional and bioactive properties of the mushrooms, as well as their morphological characteristics and overall quality.

2. Materials and Methods

2.1. Mushroom Cultivation Process

P. ostreatus AMRL 138, belonging to the fungal culture collection of the Laboratory of Edible Fungi/ITAP/Hellenic Agricultural Organization—Dimitra, was examined in the present study. The cultures were maintained on Potato Dextrose Agar (PDA; Merck, Darmstadt, Germany) for routine culture and storage purposes at T = 4 ± 1 °C. Prior to each experiment, fungal species were grown in PDA Petri plates (T = 26 ± 1 °C, 75% relative humidity) and the grain spawn was prepared as previously described [29]. To prepare the mushroom cultivation substrates, SMS and RLV were obtained from the large-scale experiments conducted by a Greek agricultural industry, Manitus S.A. These raw materials were used to prepare the following substrates: SMS 90%, SMS 80%, SMS 80%–RLV 10%, SMS 70%–RLV 20%, SMS 60%–RLV 30%, SMS 50%–RLV 40%, WS (wheat straw–control substrate) supplemented with wheat bran (WB) and soy-bean flour (SF). More specifically, SMS 90%, SMS 80%–RLV 10%, SMS 70%–RLV 20%, SMS 60%–RLV 30%, SMS 50%–RLV

40% supplemented with WB 5% and SF 5% and SMS 80% and control substrate were supplemented with WB 15% and SF 5%. No-supplemented substrates consisted of SMS 100%, SMS 90%–RLV 10%, SMS 80%–RLV 20%, SMS 70%–RLV 30% and SMS 60%–RLV 40%. In all substrates, 1% *w*/*w* calcium carbonate ($CaCO_3$; SDS, Peypin, France) was added to obtain a pH value around 7.

Five glass tubes (200 × 28 mm, 80 mL volume) and three polypropylene-autoclavable bags (1 kg) were filled with each substrate and autoclaved (T = 121 ± 1 °C, 2 h, 1.1 atm). After that, tubes and bags were inoculated with *P. ostreatus* grain spawn under aseptic conditions. Incubation took place in a growth chamber in the dark (T = 25 ± 1 °C, 80% relative air humidity). Glass tubes were used to evaluate mycelial growth rate of fungal colonies (Kr; mm/d) by measuring the visible penetration of mycelia into the substrate in two perpendicular directions, every few days [30]. When substrates were fully colonized, the bags were transferred to a fruiting room (ENTERLAB, mod. GROW-1300 h, Terrassa, Spain) with specific environmental conditions for carposome induction and fructification. During harvesting, temperature was set at T = 17 ± 1 °C, air exchange rates were controlled to maintain low CO_2 level (<1000 ppm), relative air humidity was adjusted at 95% and the light intensity was set at 800 lux (12 h/day with fluorescent lamps). The bags were used to examine the earliness period (elapsed time between the day of substrate inoculation and the day of the first harvest) and the biological efficiency (BE%; the ratio of the weight of fresh mushrooms per d.w. of substrate). Also, to evaluate the quality of first and second production flush mushrooms harvested from each substrate, mushrooms were frozen (T = −20.0 ± 0.5 °C), lyophilized (in a HetoLyoLab 3000, Heto-Holten Als, Allerød, Denmark) and grinded (in a Janke and Kunkel, IKA-WERK, analytical mill, Staufen, Germany) to a fine powder. Two successive crops were conducted. Analyses were conducted on three samples derived from the three bags per crop (6 replicates).

2.2. Analytical Methods

2.2.1. Intra-Cellular Polysaccharide (IPS) Content and Profile

Intra-cellular polysaccharides (IPSs) were determined according to Diamantopoulou et al. [31] and Liang et al. [32], with some modifications. In total, 20 mL of 2.5 M HCl was added in 0.1 g of grinded mushroom and hydrolyzed (T = 100 °C, 20 min). The mixtures were neutralized to a pH of 7 using 2.5 M NaOH. The IPS content (expressed as glucose equivalents) was determined in the filtered samples (through No. 2 Whatman filters, Whatman plc, Kent, UK) with a DNS assay, measuring the absorbance at 540 nm [33].

Regarding the composition of sugars of the produced IPSs, it was examined with HPLC analysis, as described by Diamantopoulou et al. [31]. Filtered aliquots of the neutralized samples with NaOH were analyzed with a Waters Association 600E apparatus (Waters Corporation, Milford, MA, USA) at a 30.0 cm × 7.8 mm column Aminex HPX-87H (Bio-Rad, Hercules, CA, USA). The mobile phase used was H_2SO_4 at 0.005 M with a flow rate 0.8 mL/min, and the column temperature was T = 65 ± 1 °C. Carbohydrates were detected using an RI detector (differential refractometer 410—Waters Corporation, Milford, MA, USA).

2.2.2. Total Lipid, Fatty Acid and Protein Determination

A modified version of Folch's method [34] was used to determine total cellular lipid. Particularly, 0.5 g of dried mushroom powder was suspended in a 10 mL chloroform/methanol (2:1 *v*/*v*) mixture, mixed thoroughly and let stand for 7 days. Then, the solution was filtrated, and the solvents were removed in a rotary evaporator (T = 50.0 ± 0.5 °C) under vacuum (RE 300 evaporator Stuart–RE 300 DB digital water bath, Bibby Scientific Ltd., Staffordshire, UK). The remaining was the crude lipids.

A two-stage reaction (to avoid trans-isomerization) using sodium methoxide and methanol/hydrochloride [34,35] was employed to prepare the fatty acid methyl esters. Fatty acid methyl esters were identified by reference to authentic standards.

The crude protein content (N × 4.38) of the samples was estimated by the macro Kjeldahl method according to León-Guzmán et al. [36].

2.2.3. Total Phenolic Compounds, Total Flavonoid Content, Total Triterpene Content and Antioxidant Activity

For the preparation of methanolic extracts, 50 mg of fresh mushrooms was extracted with 5 mL of methanol in an ultrasonic bath (SKYMEN, JP-060S, Shenzhen, China) for 60 min at T = 25 ± 0.5 °C, followed by vortex and centrifugation (3500 rpm, 15 min, T = 25 ± 0.5 °C; Micro 22R, Hettich, Kirchlengern, Germany). The extraction was repeated three times, and the supernatants were stored at T = 4.0 ± 0.5 °C for further analysis.

The determination of total phenolic compounds (TPC) in mushrooms samples was estimated using the Folin–Ciocalteau assay by measuring the absorbance at 750 nm [37]. Results were expressed as gallic acid equivalent (GAE) mg/g d.w. of biomass.

Samples of mushrooms were analyzed for antioxidant activity as well. Free-radical scavenging activity on the ABTS [2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid] (Sigma-Aldrich, Darmstadt, Germany) was measured according to Re et al. [38], with some modifications. Trolox was used as calibration standard and the results were expressed as mg trolox equivalents per 1 L of the extract. The scavenging ability on the DPPH (1.1-diphenyl-2-picrylhydrazyl) (Sigma-Aldrich, Darmstadt, Germany) free radicals was determined according to Boonsong et al. [39], with some modifications, by measuring the absorbance at 515 nm and the results were expressed as mmol trolox equivalents per 100 g of dry biomass. The sample's capacity to convert Fe^{3+} to Fe^{2+} ions was measured according to Benzie and Strain [40]. The absorbance was measured at 593 nm against a blank for each sample. Trolox was used to obtain a standard curve and the antioxidant activity was expressed in mmol trolox equivalents per 100 g d.w.

Total flavonoid content (TFC) was determined by the modified colorimetric method described by Barreira et al. [41], expressed as mg of quercetin equivalents (mg quercetin/g d.w.). Total triterpene (Tr) content was evaluated according to Fan and He [42] with some modifications and the results were expressed as mg ursolic acid (UA) equivalents.

2.3. Isolation and Recovery of Proteins and Polysaccharides from Mushroom Samples

For protein extraction and precipitation, mushroom samples were dissolved in deionized water at a 1:10 ratio [43]. The pH was adjusted to 11–12 using 2.0 M NaOH, where protein solubility is maximized. The mixtures were then centrifuged at 6000 rpm at 4 °C for 20 min and the supernatant was collected. To precipitate the proteins at their isoelectric point, the pH of the supernatant was adjusted to 4 using 2.0 M HCl. A second centrifugation under the same conditions was performed and the supernatant was discarded. The precipitates were frozen for 24 h and then dehydrated using a freeze-drying device for approximately 24 h [44], after which the dried concentrate was weighed.

For the recovery of lipophilic compounds, 10 mL of ethanol was added to 1 g of freeze-dried biomass and the mixture was stirred for 24 h. After centrifugation (6000 rpm, 10 min, 4 ± 0.5 °C), the biomass was recovered and dried. To release intracellular molecules, the biomass underwent ultrasonic treatment in a water bath at 80 ± 1 °C for 1 h, followed by centrifugation (6000 rpm, 10 min, 4 °C). The supernatant was collected and stored at 4 ± 0.5 °C. Non-polysaccharide, water-soluble macromolecules were removed using the Sevag method (chloroform/n-butanol, 5:1) [45], adding half the volume of the Sevag mixture to each volume of supernatant (2:1 extract to Sevag ratio). After centrifugation (9000 rpm, 10 min, 4 ± 0.5 °C), the upper phase was collected. This process was repeated until proteins

were fully removed, verified through the Bradford protein assay [46]. Polysaccharides were then precipitated by adding ethanol at a 1:4 ratio and maintaining the mixture at 4 ± 0.5 °C. The precipitates were isolated by centrifugation, frozen for 24 h and finally dehydrated through freeze-drying.

2.4. In Vitro Digestion Protocol

The in vitro digestion procedure followed the detailed methodology described by Dalaka et al. [47], based on the harmonized INFOGEST 2.0 protocol, which simulates the conditions of the oral, gastric and intestinal phases [48,49]. This procedure was carried out separately for protein extract (PE) and carbohydrate extract (CE) obtained from six distinct *P. ostreatus* samples. In detail, the extracts were prepared with the following formulations: SMS 80%, SMS 80%–RLV 10%, SMS 80%–RLV 20%, SMS 60%–RLV 30%, SMS 60%–RLV 40% and WS (used as the control substrate). To prepare the samples, 5 mL of extracts were dissolved in Milli-Q water to reach final concentrations of 40 mg/mL for PE and 2.25 mg/mL for CE and then were stored at 4 °C overnight to ensure full rehydration. Since salivary amylase is required only for the digestion of starch-containing foods, 0.17 mL (E-BLAAM enzyme activity 3000 U/mL) of α-amylase was added to the CE samples during the oral phase, which lasted 2 min at pH 7. Upon completion of the intestinal digestion phase, the PE and CE digests (PE-Ds and CE-Ds) were heated to 85 °C for 10 min and then placed on ice. Samples were centrifuged, and the supernatants were filtered through 0.22 μm sterile PVDF syringe filters. To obtain fractions with molecular weights between 0 and 3 kDa (PE-D-P3), membrane filters made of Ultracel® (Merck, Darmstadt, Germany) (low-binding regenerated cellulose with a molecular weight cut-off (MWCO) of 3 kDa were used. Next, samples were stored at −20 °C until further analysis. Digestion was conducted in duplicate for all PE and CE samples. Additionally, four blank digest replicates were prepared for each experiment by using water instead of sample, following the same in vitro digestion process. The resulting fractions were labeled as BL-D for the blank digest and BL-D-P3 for the fraction containing peptides with a molecular weight below 3 kDa.

2.5. Culturing THP-1 Cells

Human monocytic THP-1 cells (kindly provided by Kletsas Dimitrios from the National Centre of Scientific Research Demokritos, Athens, Greece) were cultured in RPMI 1640 medium, supplemented with 10% (*v*/*v*) fetal bovine serum, 10 U/mL L-glutamine, 1 mM sodium pyruvate, 100 U/mL penicillin, 100 μg/mL streptomycin and 100 μM non-essential amino acids. The cells were maintained in an incubator with a humidified atmosphere at 37 °C with 5% CO_2. Monocytes were seeded into 6-well plates at a density of 0.8×10^6 cells/mL in 2.5 mL per well and treated with 100 ng/mL phorbol 12-myristate 13-acetate (PMA) for 48 h. Subsequently, the cells were incubated in PMA-free supplemented RPMI 1640 medium for an additional 24 h. Following this resting period, the differentiated macrophages were treated either with PE-D-P3 (10%) for 24 h or CE-D (15%) for 6 h in the presence of 100 ng/mL lipopolysaccharides from *Escherichia coli* O111:B4 (LPS). In parallel, BL-D-P3 and BL-D were used under the same conditions. Each sample was performed in triplicate.

2.6. Quantification of Gene Expression

For quantifying the transcript levels of genes regulating transcription of enzymes and encoding antioxidant enzymes, THP-1 cells were treated with *P. ostreatus* digestates as described previously. Next, cells were lysed with Nucleozol reagent according to the manufacturer's instructions. RNA samples were treated with DNase for removal of remaining DNA, and pure RNA was recovered by ethanol precipitation in the presence of ammonium acetate and glycogen. Reverse transcription was carried out with the

PrimeScrip RT reagent Kit following the manufacturer's protocol. qPCR reactions were conducted using the FastGene IC Green 2X qPCR Universal Mix and the primers shown in Table 1 in a SA cycler 96 (SaCycle96, Sacace Biotechnologies, Como, Italy). Crossing points (Cps) were calculated using the instrument's software. *B2M*, *RPS18* and *RPL37A* were used as housekeeping genes, and relative gene expression was calculated using a modified version of the Pfaffl method against the aforementioned housekeeping genes [50].

Table 1. Sequences, reaction efficiency and amplicon sizes of oligonucleotide primers used in qPCR.

Gene (Accession Number)	Primer Direction	Sequence (5′-3′)	Reaction Efficiency	Amplicon Size
B2M (NM_004048.4)	Forward Reverse	GCTATCCAGCGTACTCCA CTTAACTATCTTGGGCTGTGAC	103	285
RPS18 (NM_022551.3)	Forward Reverse	CTGAGGATGAGGTGGAACG CAGTGGTCTTGGTGTGCT	98	240
RPL37a (NM_000998.5)	Forward Reverse	AGTACACTTGCTCTTTCTGTGG GGAAGTGGTATTGTACGTCCAG	106	119
NFE2L2 (NM_006164.5)	Forward Reverse	GATCTGCCAACTACTCCCA GCCGAAGAAACCTCATTGTC	90	121
SOD1 (NM_000454.5)	Forward Reverse	CGAGCAGAAGGAAAGTAATGG CCAAGTCTCCAACATGCC	95	194
CAT (NM_001752.4)	Forward Reverse	TGCCTATCCTGACACTCACC GAGCACCACCCTGATTGTC	92	137
HMOX1 (NM_002133.3)	Forward Reverse	GCTTCAAGCTGGTGATGG AGCTCTTCTGGGAAGTAGAC	90	112
GSR (NM_001195102.3)	Forward Reverse	CTTGCGTGAATGTTGGATGTG CACAACTTGGAAAGCCATAATCAG	98	102

2.7. Statistical Analysis

All experimental data presented are reported as means ± standard error of the means from at least two replicates. The Kolmogorov–Smirnov test was used to assess normality, and data were logarithmically transformed or normalized [51] when necessary to achieve a normal distribution. One-way ANOVA was performed using Duncan's post hoc test with a significance level set at $p < 0.05$. All statistical analyses were conducted using SPSS for Windows, version 22.0.0 and data visualization for Section 3.5 was carried out using GraphPad Prism v8.

3. Results and Discussion

3.1. The Effect of Substrate Composition on Mycelial Growth Rate and Mushroom Cultivation

The physicochemical characteristics of the cultivation substrates are detailed in Table 2. The final blends of SMS and RLV were prepared with C/N ratios ranging from 17.2 to 35.2. Previous research on substrates for cultivating *P. ostreatus* has reported similar ranges of C/N ratios [14,52]. The pH values were appropriate for mushroom cultivation, and the EC values varied significantly among the substrates, being increased with the increase of RLV concentration.

As mycelial growth is an initial step that creates suitable internal conditions for fruiting, the effect of various substrates (including SMS alone or combined with RLV, with or without additives) on *P. ostreatus* growth was first evaluated by measuring the mycelial growth rate (Kr, mm/d). Although the combination of SMS and RLV was less favorable compared to control substrate (6.5 mm/d), Kr values (Table 2) were among the range of those reported in the existing literature [17,52–54]. Except for the SMS 70%–RLV 30% case, substrates with lower or no supplementation of nitrogen-rich additives (WB and SF) generally supported faster mycelial growth.

Table 2. Composition of substrates used for the solid-state fermentation experiments (final mixtures, before inoculation), their physicochemical profile (initial C%, N%, C/N ratio, pH and electrical conductivity—EC) and their effect on growth rates (Kr, mm/d) by *P. ostreatus*. Each point is the mean value of three independent measurements (mean ± SD).

Substrate	Substrate Composition (% *w*/*w*)	C (% d.w.)	N (% d.w.)	C/N	pH	EC (μS/cm)	Kr (mm/day)
SMS * 100%	SMS 100%	27.2 ± 0.5 c,d,e,f,g **	0.8 ± 0.1 e	35.2 ± 1.3 a	6.9 ± 0.2 a,b	473 ± 18 f,g	5.6 ± 0.3 b,c,d
SMS 90%	SMS 90% WB 5% SF 5%	29.5 ± 0.3 b	1.1 ± 0.3 c,d,e	27.8 ± 2.1 b	6.9 ± 0.6 a,b	574 ± 13 c,d,e	5.2 ± 0.1 d,e
SMS 80%	SMS 80% WB 15% SF 5%	29.9 ± 0.6 b	1.4 ± 0.2 a,b,c,d	23.4 ± 2.0 c,d,e	6.7 ± 0.5 b	506 ± 14 e,f	5.2 ± 0.2 d,e
SMS 90%–RLV 10%	SMS 90% RLV 10%	26.9 ± 0.2 d,e,f,g	0.9 ± 0.0 d,e	27.2 ± 1.4 b,c	6.9 ± 0.9 a,b	557 ± 22 d,e,f	5.9 ± 0.1 b,c
SMS 80%–RLV 10%	SMS 80% RLV 10% WB 5% SF 5%	29.2 ± 1.2 b,c	1.3 ± 0.1 b,c,d,e	23.3 ± 0.9 c,d,e	7.2 ± 0.8 a,b	666 ± 31 b,c	4.6 ± 0.1 f
SMS 80%–RLV 20%	SMS 80% RLV 20%	26.6 ± 0.9 e,f,g	1.1 ± 0.2 c,d,e	25.3 ± 0.7 b,c,d	7.8 ± 0.5 a,b	698 ± 32 b	5.1 ± 0.1 d,e
SMS 70%–RLV 20%	SMS 70% RLV 20% WB 5% SF 5%	28.9 ± 0.2 b,c,d	1.5 ± 0.3 a,b,c	20.6 ± 1.1 e,f	7.9 ± 0.4 a,b	630 ± 24 b,c,d	4.4 ± 0.1 f
SMS 70%–RLV 30%	SMS 70% RLV 30%	26.3 ± 0.5 f,g	1.3 ± 0.1 b,c,d,e	21.2 ± 0.8 d,e,f	8.0 ± 0.4 a,b	709 ± 33 b	4.7 ± 0.1 e,f
SMS 60%–RLV 30%	SMS 60% RLV 30% WB 5% SF 5%	28.6 ± 0.4 b,c,d,e	1.7 ± 0.1 a,b	19.1 ± 1.4 f	8.1 ± 0.1 a	1059 ± 50 a	5.4 ± 0.2 c,d
SMS 60%–RLV 40%	SMS 60% RLV 40%	26.0 ± 1.2 g	1.5 ± 0.1 a,b,c	19.9 ± 1.6 e,f	8.0 ± 0.1 a,b	1107 ± 48 a	6.0 ± 0.2 a,b
SMS 50%–RLV 40%	SMS 50% RLV 40% WB 5% SF 5%	28.3 ± 0.8 b,c,d,e,f	1.9 ± 0.1 a	17.2 ± 2.2 f	7.8 ± 0.5 a,b	1109 ± 46 a	5.9 ± 0.2 b
WS	WS 80% WB 15% SF 5%	33.3 ± 1.1 a	1.3 ± 0.2 b,c,d,e	25.1 ± 1.2 b,c,d	7.7 ± 0.3 a,b	409 ± 12 g	6.5 ± 0.2 a

* SMS: spent mushroom substrate, RLV: hydroponic roots of leafy vegetables, WS: wheat straw (control substrate), WB: wheat bran and SF: soybean flour. ** Columns not sharing the same letters are significantly different at $p = 0.05$.

In subsequent analyses, the fungus underwent further examination for mushroom production in a scale-up experiment using polypropylene bags. *P. ostreatus* successfully grew and colonized all the alternative substrates, within a period of around 17 days after inoculation with not significant differences for spawn run time. Meanwhile, the earliness period varied between the examined substrates ranging from 24 to 30 days (Table 3). An increase in the proportion of RLV in the substrate led to a significant reduction in earliness duration, resulting in faster harvesting. This effect can be attributed to the adequate nitrogen content in these substrates, aligning with established evidence that emphasizes nitrogen's critical role in enhancing ligninolytic enzyme activity and facilitating earlier fructification [55,56]. However, higher doses of nitrogen supplements may inhibit mushroom growth [57]. Notably, our results indicated that the supplemented SMS 60%–RLV 30% and SMS 50%–RLV 40% substrates, which had higher nitrogen percentages and consequently lower C/N ratios, exhibited prolonged earliness periods, thereby promoting extended fructification.

Table 3. The effect of substrates consisted of spent mushroom substrate (SMS) and hydroponic roots of leafy vegetables (RLV) and wheat straw—WS (control substrate)—on earliness, biological efficiency (BE, %) and morphological parameters of *P. ostreatus.* Each point is the mean value of three independent measurements per crop (mean ± SD).

Substrate	Earliness (days)	BE (%)	Pileus Diameter (mm)	Stipe Length (mm)
SMS 100%	30.0 ± 0.3 a *	69.1 ± 1.2 d,e,f,g	38.88 ± 2.7 f	28.91 ± 1.4 a,b
SMS 90%	30.0 ± 0.3 a	72.3 ± 2.6 c,d,e	47.11 ± 1.8 c,d,e	25.03 ± 1.8 d
SMS 80%	30.0 ± 0.3a	71.1 ± 2.7 c,d,e,f	49.30 ± 2.7 b,c	28.44 ± 1.5 b,c
SMS 90%–RLV 10%	30.0 ± 0.3 a	63.6 ± 2.9 f,g	48.32 ± 2.8 c,d	27.31 ± 1.4 b,c,d
SMS 80%–RLV 10%	26.0 ± 0.3 b	85.4 ± 1.9 a,b	48.85 ± 3.0 b,c	25.36 ± 1.5 c,d
SMS 80%–RLV 20%	26.0 ± 0.3 b	79.3 ± 2.4 b,c	56.62 ± 2.7 a,b	27.32 ± 2.0 b,c,d
SMS 70%–RLV 20%	24.0 ± 0.3 c	73.3 ± 2.7 c,d	48.15 ± 3.6 c,d	25.51 ± 2.1 c,d
SMS 70%–RLV 30%	24.0 ± 0.3 c	64.8 ± 3.0 e,f,g	35.48 ± 2.7 f,g	25.35 ± 1.9 d
SMS 60%–RLV 30%	30.0 ± 0.3 a	75.9 ± 2.9 c,d	31.04 ± 1.8 g	25.15 ± 2.1 d
SMS 60%–RLV 40%	24.0 ± 0.3 c	61.2 ± 4.1 g	40.06 ± 2.6 e,f	18.89 ± 1.8 e
SMS 50%–RLV 40%	30.0 ± 0.3 a	45.6 ± 4.1 h	63.26 ± 2.7 a	19.56 ± 2.2 e
WS	24.0 ± 0.3 c	87.8 ± 2.3 a	40.49 ± 2.3 d,e,f	31.79 ± 2.0 a

* Columns not sharing the same letters are significantly different at $p = 0.05$.

All recorded morphological parameters varied across the different substrates (Table 3). Specifically, the pileus diameter ranged from 31.0 to 63.3 mm. The use of both SMS and the additives resulted in a reduction in stipe length, with values ranging from 18.9 to 28.9 mm, compared to the control, which produced mushrooms with the maximum stipe length (31.8 mm). Generally, although the entire fruiting body of *P. ostreatus* is edible, the pileus has a better taste and is nutritionally enriched with proteins, vitamins, minerals, fiber and other antioxidants [58]. For this reason, mushrooms with short stipes are preferred by consumers and have better market opportunities as high-value products. In this context, the examined alternative substrates were mostly suitable for the production of mushrooms with desirable commercial characteristics. The results for stipe lengths and pileus diameters in this experiment align with findings from several researchers [16,17,59], although Hoa et al. [60] reported even larger pileus diameters for a *P. ostreatus* strain, ranging from 70.6 to 86.7 mm.

Satisfactory BE (Table 3) values (over 60%) were detected in all substrates except for the supplemented SMS 50%–RLV 40% (45.6%) and should be rejected for *P. ostreatus* cultivation as Patra and Pani [61] have indicated that substrates used for cultivating oyster mushrooms should yield BE values of at least 50%. This low BE value could be due to excess nitrogen as the substrate had the lowest C/N ratio. Kurt and Buyukalaca [62] noted that high nitrogen levels (N > 1.4% in their study) could be linked to decreased yield. The organic nitrogen-rich additives enhanced BE values, supporting the idea that using substrates

containing mixtures of materials results in more balanced chemical compositions, thereby promoting high yields [60]. An exception was the non-supplemented SMS 80%–RLV 20% substrate in which one of the highest BE values was recorded, highlighting the crucial role of physicochemical parameters such as pH, EC, lignocellulose content and nutrient availability in influencing mushroom BE. Diverse BE % ranges have been observed when various lignocellulosic materials are used as substrates for oyster mushroom cultivation [63]. Philippoussis et al. [14] reported BE values for *P. ostreatus* of 13.57% in peanut shells and 70.61% in cotton waste, while Hoa et al. [60] found BE values ranging from 46.44 to 66.08% for *P. ostreatus* cultivated in sawdust and mixtures of sawdust with sugarcane bagasse and corn cobs. In our case, while substrates supplemented with WB and SF demonstrated higher BE values, the satisfactory performance of the fungus in substrates lacking these additives, along with the earliness period and mushrooms morphological characteristics, indicates that RLV could serve as an alternative nitrogen-rich additive, potentially replacing materials commonly used as animal feed.

3.2. *Analysis of Nutritional Components in* P. ostreatus

Mushrooms are abundant in polysaccharides and proteins while containing very low levels of fat. The nutritional composition was assessed for the carposomes harvested during the initial flushes (Figure 1a). The substrate consisting of SMS 90% supplemented with WB and SF demonstrated a positive effect on the mushrooms' IPSs compared to the control substrate (values of 50.93 and 44.92% *w*/*w*, respectively). However, the combination of SMS with RLV, WB and SF did not enhance the IPS content in the produced mushrooms; in fact, the addition of WB and SF resulted in even decreased IPS content compared to the substrates supplemented solely with RLV. A representative case was the non-supplemented SMS 70%–RLV 30% substrate, where the mushrooms showed a notably high IPS value of 44.62% *w*/*w*. Similar experimental findings have been observed for *P. ostreatus* and *P. pulmonarius* when grown on SMS [52], for *P. ostreatus* and *P. eryngii* when cultivated in wheat straw, beech wood shavings and coffee residues [19], as well as for *P. citrinopileatus* when grown on SMS enriched with hydroponic roots of leafy vegetables [13]. Additionally, *P. ostreatus* and *P. eryngii* have shown total carbohydrate percentages ranging from 66.40 to 74.02% *w*/*w* [64]. These differences in IPS values can be attributed not only to the types of substrates used but also to variations in mushroom strains and the analytical method employed. The composition of the extracts from all IPS samples in terms of monosaccharides is presented in Table 4, showing that glucose was the dominant monosaccharide, while fructose and mannose were detected in significantly lower concentrations. Similar studies on mushrooms have indicated that glucose was the primary monosaccharide in *P. ostreatus* extracts, comprising 80% *w*/*w* according to Baeva et al. [65] and over 64% according to Diamantopoulou et al. [19].

Table 4. Carbohydrate composition (% *w*/*w*) of intra-cellular polysaccharides (IPSs) produced by *P. ostreatus* cultivated on spent mushroom substrate (SMS), hydroponic roots of leafy vegetables (RLV) and wheat straw—WS (control substrate). Each point is the mean value of three independent measurements per crop (mean ± SD).

	Carbohydrates (% *w*/*w* of Total IPS)		
Substrate	**Glucose**	**Fructose**	**Mannose**
SMS 100%	63.3 ± 2.9 c,d *	36.7 ± 1.2 b,c	nd **
SMS 90%	58.5 ± 1.5 d	17.2 ± 0.6 g	24.3 ± 0.9 a
SMS 80%	53.0 ± 1.1 e	38.2 ± 1.2 a,b,c	8.8 ± 0.5 c
SMS 90%–RLV 10%	78.7 ± 2.3 a	21.3 ± 1.3 f	nd
SMS 80%–RLV 10%	59.0 ± 2.2 d	41.0 ± 1.4 a	nd
SMS 80%–RLV 20%	64.7 ± 2.3 b,c	35.3 ± 1.3 c	nd

Table 4. *Cont.*

Substrate	Carbohydrates (% *w*/*w* of Total IPS)		
	Glucose	Fructose	Mannose
SMS 70%–RLV 20%	61.1 ± 1.8 c,d	38.9 ± 1.1 a,b	nd
SMS 70%–RLV 30%	69.0 ± 1.8 b	31.0 ± 1.3 d	nd
SMS 60%–RLV 30%	59.5 ± 1.7 c,d	27.4 ± 1.1 e	13.2 ± 0.5 b
SMS 60%–RLV 40%	52.4 ± 1.1 e	41.1 ± 1.2 a	6.5 ± 0.5 d
SMS 50%–RLV 40%	51.5 ± 1.2 e	38.5 ± 1.1 a,b,c	10.0 ± 0.7 c
WS	60.5 ± 0.9 c,d	39.5 ± 0.8 a,b	nd

* Columns not sharing the same letters are significantly different at $p = 0.05$; ** nd = non detected.

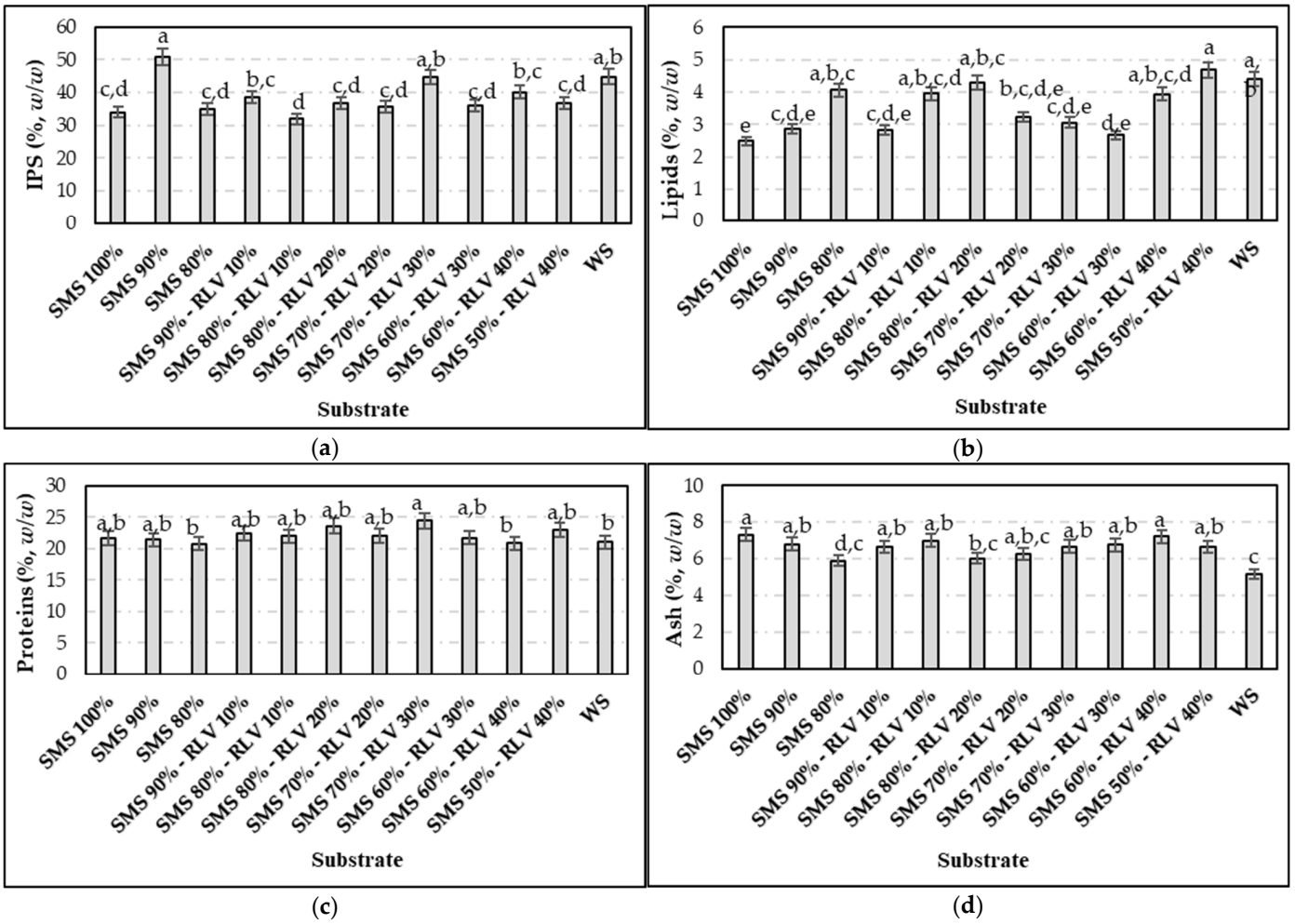

Figure 1. (**a**) Intra-cellular polysaccharide (IPS), (**b**) lipid, (**c**) total protein and (**d**) ash content (% *w*/*w* of dry biomass) of *P. ostreatus* cultivated on spent mushroom substrate (SMS), hydroponic roots of leafy vegetables (RLV) and wheat straw—WS (control substrate). Mean values with error bars indicate the standard deviations from three independent measurements per crop. Columns with different letters within the same panel are significantly different ($p < 0.05$).

The fat content for *P. ostreatus* ranged from 2.48 to 4.68% *w*/*w*, as shown in Figure 1b. Overall, the lipid content of the mushrooms produced showed no significant variation across most of the different substrates. However, the inclusion of RLV appeared to decrease the lipid content in the mushrooms, with the lowest lipid concentrations found in mushrooms grown on the SMS 60%–RLV 30%, SMS 90%–RLV 10% and SMS 100% substrates. In contrast, the highest lipid content (4.68% *w*/*w*) was recorded in mushrooms cultivated on the SMS 50%–RLV 40% substrate. Comparable lipid content has been recorded in *P. ostreatus* grown on different substrates and ratios in previous experimental studies [13,66].

Subsequently, the lipid profile and distribution of FAs was determined as shown in Table 5. The amount of saturated FAs ranged from 13.90 to 17.06 *w*/*w* of FAs, while the amount of PUFAs ranged between 71.81 and 81.31% *w*/*w* of FAs. This finding aligns with previous studies on mushrooms [19,67]. The primary component of the lipid in all samples was linoleic acid (C18:2), ranging from 57.05 to 67.99% *w*/*w*, consistent with numerous studies on various mushroom species [13,68,69], while oleic (C18:1) was identified as the second most abundant FA, followed by palmitic acid (C16:0) (8.30 to 12.24% *w*/*w*). The high content of PUFAs in mushrooms is significant because it helps increase levels of HDL, known as "good" cholesterol, and decrease levels of LDL, "bad" cholesterol [70]. Therefore, RLV proved to be an effective additive for mushroom production, as it not only reduced the fat content but also preserved a lipid profile rich in PUFAs.

The protein value is essential because proteins contribute to approximately 17% of total caloric intake [71]. Figure 1c illustrates the total protein content of carposomes, showing that the produced mushrooms contained more than 20% *w*/*w* protein. The substitution of WS with SMS, along with the addition of RLV, whether combined with or without additives, appears to positively affect the protein content, with the maximum value (24.39%, *w*/*w*) being registered for non-supplemented SMS 70%–RLV 30% substrate. Many reports have revealed that mushrooms are particularly high in crude protein. The protein content in *Pleurotus* sp. varies from 19.15 to 36.87% *w*/*w* [19,66,72–74], with an average value of 23.80% *w*/*w* [75]. Reports of much increased protein concentrations in *Pleurotus* species typically result from cultivation on nitrogen-rich substrates [76].

The ash remaining after the complete incineration of dry mushrooms reflects their mineral composition. In this study, ash content (Figure 1d) ranged from 5.18 to 7.31% d.w. A higher ash content generally indicates a greater mineral content, particularly in white oyster mushrooms [77]. Previous research by Yamauchi et al. [78] and Diamantopoulou et al. [19] have shown that oyster mushrooms are rich in minerals, with potassium being the most prevalent. The ash content values observed in this study align well with those reported in the literature [78–81].

Table 5. Fatty acid (FA) composition (% *w*/*w*) of total lipids produced by *P. ostreatus* cultivated on spent mushroom substrate (SMS), hydroponic roots of leafy vegetables (RLV) and wheat straw—WS (control substrate). Each point is the mean value of three independent measurements per crop (mean ± SD).

Substrate/FA % *w*/*w*	C16:0	C16:1	C18:0	C18:1	C18:2	C20:0	C20:2	C22:0	C22:1	C22:6	C24:1	Other	Polyunsaturated	U.I.
SMS 100%	12.24 a *	0.22 d	0.65 g	11.65 b	57.05 c	0.47 e,f	0.53 b	0.59 d	1.15 b	0.98 a	0.84 b,c	13.63 b,c	72.42 b,c	1.35 ns **
SMS 90%	11.39 a,b	0.25 c,d	1.44 c,d	13.81 a	60.91 a,b,c	0.41 f	0.24 d	0.30 f	0.62 d	0.46 e	1.45 a	8.72 g	77.74 a,b,c	1.41 ns
SMS 80%	10.19 b,c	0.33 b	0.89 f	11.76 b	61.40 a,b,c	0.12 h	0.24 d	0.70 c	0.71 c	0.82 b	0.60 d,e	12.24 d,e	75.86 a,b,c	1.42 ns
SMS 90%–RLV 10%	11.26 a,b	0.46 a	1.58 b	9.67 d	63.61 a,b,c	0.23 g	0.28 d	0.35 f	0.72 c	0.86 b	0.29 f,g	10.69 f	75.89 a,b,c	1.44 ns
SMS 80%–RLV 10%	9.61 c,d	0.42 a	0.86 f	11.90 b	67.99 a	0.40 f	0.12 e	0.14 g	0.20 g	0.55 d	0.13 g	7.68 g	81.31 a	1.52 ns
SMS 80%–RLV 20%	10.34 b,c	0.29 b,c	0.97 e,f	9.61 d	62.88 a,b,c	0.62 b	0.46 c	0.12 g	0.29 e,f	0.79 b	0.56 d,e	13.07 c,d	74.88 a,b,c	1.42 ns
SMS 70%–RLV 20%	10.64 b,c	0.28 b,c	1.33 d	10.88 b,c	57.62 c	0.49 d,e	0.44 c	0.89 b	1.34 a	0.81 b	0.44 e,f	14.84 a,b	71.81 c	1.34 ns
SMS 70%–RLV 30%	8.30 d	0.42 a	1.89 a	9.92 c,d	59.21 b,c	0.84 a	0.66 a	1.05 a	0.68 c,d	1.03 a	0.65 c,d	15.35 a	72.57 b,c	1.38 ns
SMS 60%–RLV 30%	11.43 a,b	0.24 c,d	1.08 e	6.27 e	66.53 a,b	0.55 c,d	0.64 a	0.44 e	0.37 e	0.65 c	0.43 e,f	11.37 e,f	75.13 a,b,c	1.46 ns
SMS 60%–RLV 40%	10.89 b,c	0.33 b	1.54 b,c	10.85 b,c	67.39 a	0.55 c,d	0.23 d	0.75 c	0.21 f,g	0.22 f	0.97 b	6.07 h	80.20 a,b	1.49 ns
SMS 50%–RLV 40%	11.03 a,b	0.24 c,d	1.36 d	13.11 a	58.23 c	0.57 b,c	0.53 b	0.89 b	0.29 e,f	0.41 e	1.29 a	12.05 d,e	74.10 a,b,c	1.35 ns
WS	11.23 a,b	0.32 b	0.96 e,f	11.24 b	62.57 a,b,c	0.57 b,c	0.27 d	0.30 f	0.25 f,g	0.85 b	1.00 b	10.44 f	76.50 a,b,c	1.44 ns

* Columns not sharing the same letters are significantly different at $p = 0.05$; ** ns = not significant ($p > 0.05$).

3.3. Quantification of Total Phenolic Compounds (TPC), Total Triterpene Content (Tr) and Antioxidant Activity

Edible mushrooms are recognized as a source of phenolic compounds, which are particularly noted for their antioxidant activity in biological systems [82], as the antioxidant activity of phenolic-rich extracts is often associated with their TPC [83]. Our findings indicated significant differences regarding TPC in the methanol extracts of the mushrooms, with values ranging from 11.26 to 30.31 mg GAE/g d.w. (Figure 2), highlighting the importance of the substrate's synthesis and chemical composition in influencing the TPC in the produced mushrooms. The inclusion of WB and SF generally resulted in higher TPC values in the produced mushrooms than those produced in non-supplemented, apart from the supplemented SMS 90% substrate. Another exception was the non-supplemented SMS 60%–RLV 40% substrate, where the mushrooms achieved a notably high TPC value of 26.52 mg GAE/g d.w., suggesting that the use of a lower percentage of SMS in substrate mixtures could enhance TPC levels in the mushrooms.

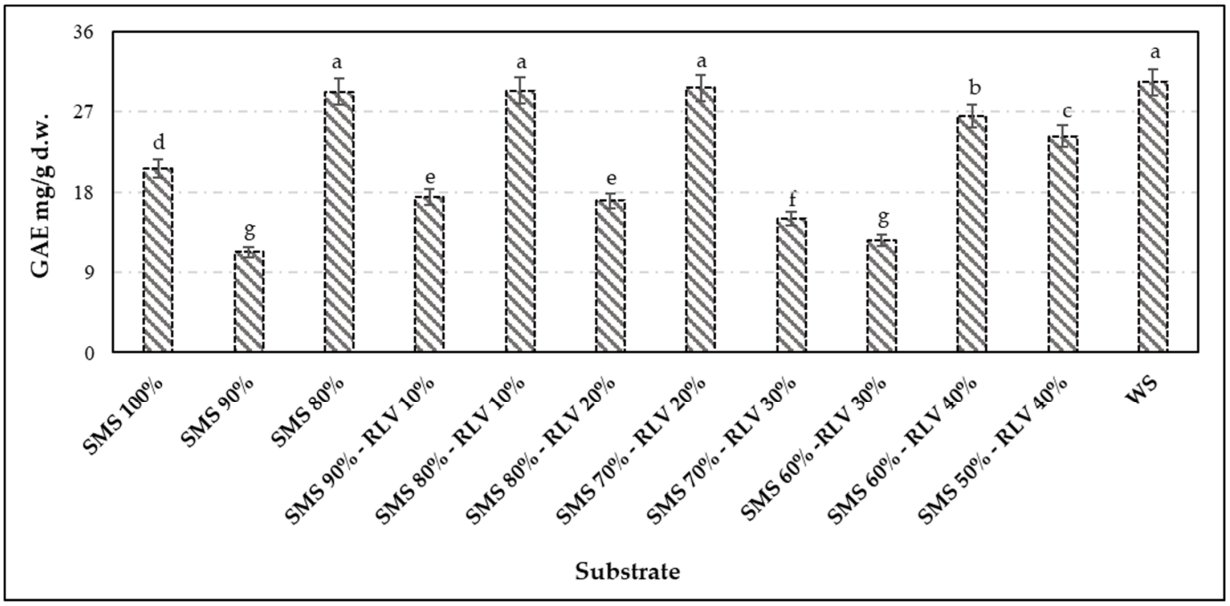

Figure 2. Total phenolic compounds (TPC, expressed as gallic acid equivalents; GAE mg/g dry weight of biomass) of methanol extracts of *P. ostreatus* cultivated on substrates consisted of spent mushroom substrate (SMS), hydroponic roots of leafy vegetables (RLV) and wheat straw—WS (control substrate). Mean values with error bars indicate the standard deviations from three independent measurements per crop. Columns with different letters are significantly different ($p < 0.05$).

To obtain more information about the TPC present in the examined mushrooms, the Tr was measured spectrophotometrically. The Tr content in the methanol extracts (mg/g extract) is shown in Figure 3, ranging from 4.82 to 11.52 mg UA/g d.w. The highest Tr content was recorded in mushrooms cultivated on the SMS 90%–RLV 10% substrate. However, the addition of more RLV led to much lower Tr values compared to the control substrate, highlighting the crucial role of substrate composition. To the best of our knowledge, most studies on Tr have focused mainly on medicinal mushrooms of the *Ganoderma* genus, with a range of values reported across different species. For instance, Li et al. [84] reported total triterpenoid content in *G. lucidum* carposomes ranging from 13.6 to 31.5 mg/g, which is higher than our results. Additionally, Boh et al. [85] found that Tr was higher in younger growth stages of Indonesian *G. applanatum* than in older stages. Nevertheless, our results are encouraging, as *P. ostreatus*—a typically nutritional mushroom—showed comparable triterpene values to those of the pharmaceutical *Ganoderma* spp. when cultivated on specific substrates.

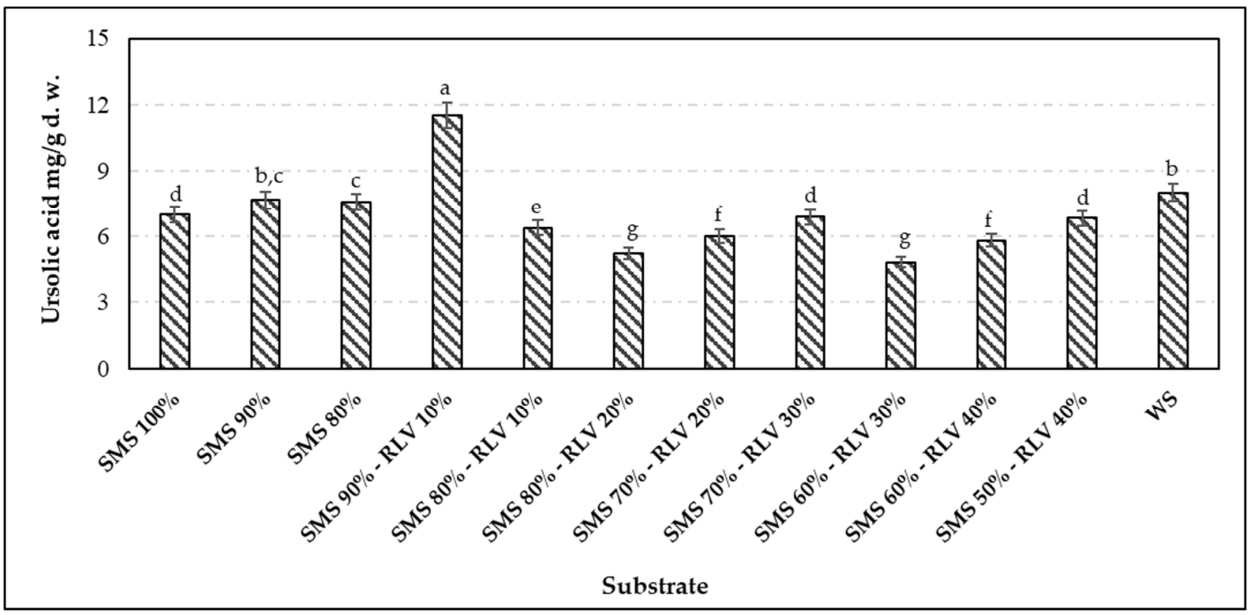

Figure 3. Total triterpene content (Tr) expressed as mg ursolic acid equivalents of the extracts of *P. ostreatus* grown on substrates consisted of spent mushroom substrate (SMS), hydroponic roots of leafy vegetables (RLV) and wheat straw—WS (control substrate). Mean values with error bars indicate the standard deviations from three independent measurements per crop. Columns with different letters are significantly different ($p < 0.05$).

In further analysis, the antioxidant activity of the mushroom extracts was examined using three assays (Table 6), recognizing that antioxidant compounds operate through multiple mechanisms and no single method can comprehensively capture all their effects. Although the highest TPC value was recorded in mushrooms cultivated on the control substrate, the use of SMS, whether with or without WB and SF, did not negatively affect the mushrooms' antioxidant activity, particularly as measured by the DPPH method. The scavenging capacity of the free-radical ABTS assay revealed no significant differences, with values ranging from 2.00 to 2.51 mg trolox/g d.w. in the mushrooms. The ability of mushroom extracts to scavenge the DPPH free radical ranged from 4.23 to 12.83 mg trolox/g d.w., and the reducing power values ranged from 6.29 to 11.40 mg trolox/g d.w. Notably, the lowest antioxidant values across all assays were found in mushrooms cultivated on the non-supplemented SMS 90%–RLV 10% and SMS 80%–RLV 20% substrates. Previous studies have also highlighted that the chemical composition of substrates used plays a significant role in influencing the nutritional and bioactive compounds of the produced mushrooms. For instance, *P. ostreatus* and *P. pulmonarius* cultivated on various woody substrates, such as beech sawdust, oak, linden, walnut and poplar, exhibited notably higher TPC concentration on beech and linden [86]. Similarly, Diamantopoulou et al. [19] found that coffee residue, rice husk and beech wood shaving substrates resulted in greater antioxidant activity for two different *Pleurotus* strains compared to wheat straw and barley and oat straw.

Table 6. Scavenging ability on DPPH and ABTS free radicals and FRAP of methanol extracts of *P. ostreatus.* Measurements of antioxidant studies are expressed as mg of gallic acid or trolox equivalence/g of mushroom dry weight. Each point is the mean value of three independent measurements per crop (mean ± SD).

Substrate	DPPH (mg trx/g)	ABTS (mg trx/g)	FRAP (mg trx/g)
SMS * 100%	11.84 ± 0.03 b,c **	2.42 ± 0.01 ns	12.25 ± 0.50 a
SMS 90%	11.00 ± 0.26 d	2.00 ± 0.64 ns	6.29 ± 0.08 g
SMS 80%	11.45 ± 0.01 c,d	2.30 ± 0.87 ns	9.51 ± 0.31 d,e

Table 6. *Cont.*

Substrate	DPPH (mg trx/g)	ABTS (mg trx/g)	FRAP (mg trx/g)
SMS 90%–RLV 10%	4.23 ± 0.02 e	2.23 ± 0.10 ns	6.30 ± 0.11 g
SMS 80%–RLV 10%	12.65 ± 0.45 a	2.33 ± 0.10 ns	10.46 ± 0.19 b,c,d
SMS 80%–RLV 20%	12.65 ± 0.05 a	2.05 ± 0.98 ns	7.14 ± 0.31 f,g
SMS 70%–RLV 20%	12.83 ± 0.24 a	2.46 ± 0.19 ns	11.40 ± 0.53 a,b
SMS 70%–RLV 30%	12.64 ± 0.36 a	2.17 ± 0.66 ns	7.94 ± 0.11 f
SMS 60%–RLV 30%	12.45 ± 0.25 a	2.42 ± 0.21 ns	9.19 ± 0.41 e
SMS 60%–RLV 40%	11.50 ± 0.01 c,d	2.46 ± 0.10 ns	10.26 ± 0.21 c,d
SMS 50%–RLV 40%	12.39 ± 0.20 a,b	2.51 ± 0.06 ns	10.04 ± 0.35 c,d,e
WS	10.92 ± 0.03 d	2.41 ± 0.23 ns	10.86 ± 0.45 b,c

* SMS: spent mushroom substrate; RLV: hydroponic roots of leafy vegetables; WS: wheat straw (control substrate).
** Columns not sharing the same letters are significantly different at $p = 0.05$; ns = not significant ($p > 0.05$).

3.4. Total Flavonoid Content (TFC)

Regarding the TFC, a wide range of values was observed for the mushrooms produced on different substrates, ranging from 3.12 to 14.43 mg QE/g (Figure 4). Except for the supplementation with RLV 20 and 30% (only in non-supplemented substrates), which had a negative effect on TFC, the inclusion of additives along with the substitution of wheat straw with SMS led to an increase in TFC. The highest value was recorded for mushrooms cultivated on the substrate supplemented with WB and SF, consisting of SMS 50%–RLV 40%. Low TFC values were anticipated as flavonoids are more commonly found in plants where they regulate symbiotic plant–microbe interactions and act as antifungal compounds to protect against infections, potentially hindering fungal growth. However, contrary to the belief that mushrooms lack flavonoids [87] due to the absence of key biosynthetic enzymes such as phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS) and chalcone isomerase (CHI), numerous studies have demonstrated the presence of flavonoids in mushrooms through spectrophotometric [88–90] and HPLC and GC–MS methods [91]. A recent study [92] demonstrated that the medicinal mushroom *Sanghuangporus baumii* can synthesize flavonoids, indicating that the missing genes might be replaced by distantly related genes from the same superfamilies. Therefore, further studies should focus on the flavonoid biosynthesis pathway.

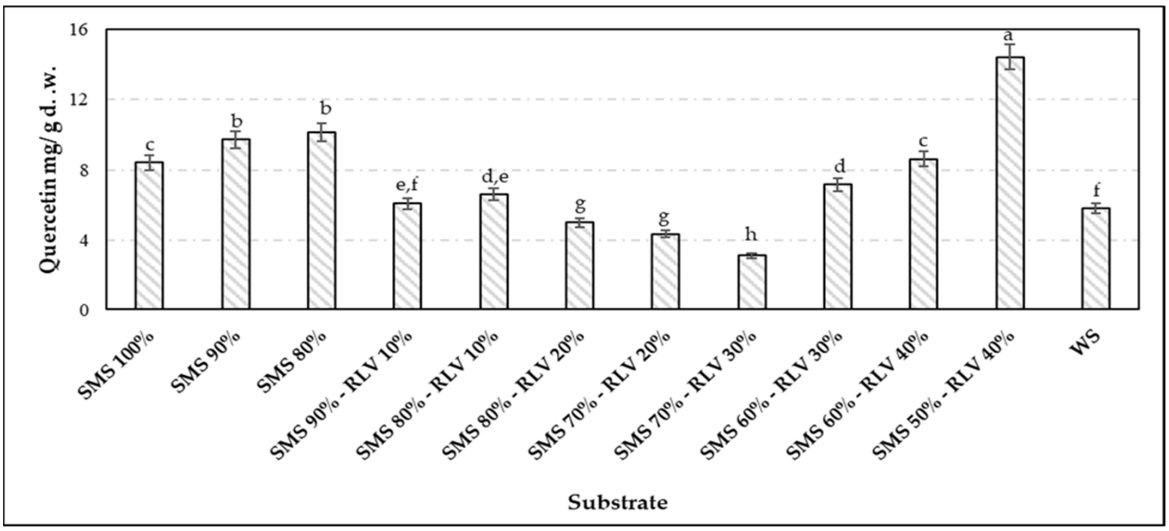

Figure 4. Total flavonoid content (TFC) expressed in mg quercetin/g dry weight of the extracts of *P. ostreatus* grown on substrates consisted of spent mushroom substrate (SMS), hydroponic roots of leafy vegetables (RLV) and wheat straw—WS (control substrate). Mean values with error bars indicate the standard deviations from three independent measurements per crop. Columns with different letters are significantly different ($p < 0.05$).

3.5. The Effect of Protein and Carbohydrate Extracts on Expression of Antioxidant Genes

It is widely recognized that oyster mushrooms' fruiting bodies exhibit multidirectional health-promoting benefits [93]. Various species within the *Pleurotus* genus are notable as sources of bioactive compounds, including high-molecular-weight bioactive compounds like polysaccharides, peptides and proteins, as well as low-molecular-weight compounds such as terpenoids, fatty acid esters and polyphenols [94]. Nowadays, *P. ostreatus* is currently recognized as an excellent nutrient source since it contains numerous bioactive compounds [93]. The bioactive compounds found in both the mycelium and fruiting bodies of *P. ostreatus* are associated with antioxidant potential [95]. A thorough review of Kozarski et al. [96] covers the antioxidants found in edible mushrooms, where a small number of studies have evaluated the *Pleurotus* genus in this context. There are several in-depth reviews that delve into the biological activities of polysaccharides, proteins and peptides from *Pleurotus* spp. [75,97,98]. Although numerous studies have been conducted using various lignocellulosic residues as substrates for mushroom production, with some aiming to the resulting bioactive compound content [99,100], to date, only one study conducted by our group [13] has studied the impact of substrates on the expression of antioxidant genes.

The present study is the first to examine antioxidant-related gene expression induced by the protein extract (PE-D-P3) and polysaccharide fraction (CE-D) of *P. ostreatus* after in vitro gastrointestinal digestion. The cell viability of both mushroom extract groups was tested, and concentrations of 4 mg/mL for PE-D-P3 (10% PE-D-P3 and 90% RPMI) and 0.34 mg/mL for CE-D (15% CE-D and 85% RPMI) were determined to be non-toxic to THP-1 cells, thereby resulting in the selection of these concentrations. These concentrations are consistent with previous studies reporting no cytotoxicity of mushroom extracts against various cell lines, including the THP-1 cell line, reinforcing the safety profile of *P. ostreatus* extracts [101,102].

The effects of the digested protein and carbohydrate extracts (PE-D-P3 and CE-D, respectively) on key antioxidant-related genes were evaluated in LPS-challenged THP-1 macrophages, focusing on *NFE2L2*, *SOD1*, *CAT*, *HMOX1* and *GSR*, which are critical in oxidative stress regulation. Expression levels of a panel of genes associated with antioxidant activity are shown in Figures 5 and 6. Results regarding the effect of protein extracts show that *NFE2L2* and *HMOX1* expression levels ($p < 0.05$; Figure 5a,d) were significantly higher in the SMS 80% group compared to all other groups supplemented with any proportion of RLV, suggesting that the antioxidant potential of the protein extract may be negatively influenced by the presence of RLV in the substrate. On the other hand, no statistically significant differences in the expression of *SOD1*, *CAT* and *GSR* between the PE-D-P3-treated samples, regardless of substrate, or when compared to BL-D-P3-treated cells were observed ($p > 0.05$; Figure 5b,c,e). In recent years, increasing evidence has demonstrated that peptides derived from mushrooms possess excellent effects due to their low molecular weight, high bioactivity and easy absorption [103]. Aursuwanna et al. [104] identified two novel peptides in *Ganoderma* "lingzhi" mushrooms following in vitro digestion, and antioxidant activity was observed in Caco-2 cells treated with these synthesized peptides. Also, these peptides were able to reduce NO production in RAW 264.7 macrophages without causing cytotoxicity and also down-regulated the expression of proinflammatory cytokines. Protein hydrolysate from *P. geesteranus* obtained by simulated gastrointestinal digestion and its pre-treatment in H_2O_2-treated PC12 cells markedly attenuated ROS generation and improved mRNA expression of the endogenous antioxidant enzymes CAT and SOD [105]. Fermented sour soybean milk with the incorporation of polypeptides from *P. eryngii* not only exhibited significant antioxidant properties, but it also enhanced murine immune functions and maintained gut homeostasis [103]. In a similar previous study by our group on *P. citrinopileatus*, no effect was observed regarding the substrate composition on the antioxidant properties of the protein fraction.

Results pertaining to the effect of substrate composition on carbohydrate extract antioxidant properties reveal a diverse pattern of antioxidant-related gene expression. More specifically, HMOX1 expression was higher ($p < 0.05$) in SMS 60%–RLV 30%-treated cells compared to BL-D-P3 and SMS 80%–RLV 10%-treated cells. No increase was observed in other SMS groups, with or without RLV, when compared to BL-D and WS (Figure 6d). Notably, CAT expression was higher ($p < 0.05$; Figure 6c) in all SMS groups, with or without the addition of RLV, compared to WS (control group) as well as BL-D. No significant differences were observed between any of the CE-Ds and BL-D regarding NFE2L2, SOD1 and GSR expression ($p > 0.05$; Figure 6a, b and e, respectively). A previous study reported that digested *P. eryngii* polysaccharides could significantly interact with intestinal mucus, suggesting an indirect effect on immunoregulation, as in vitro digestion may alter the structure of these polysaccharides [106]. Another study [107] showed that *P. eryngii* polysaccharide extract significantly inhibited ROS as well as increased the activities of SOD and CAT in Caco-2 cells treated with H_2O_2. Sun et al. [108] reported that selenium polysaccharide from *P. geesteranus* could alleviate H_2O_2-induced oxidative damage in human keratinocytes cells. In detail, this polysaccharide increased the SOD and CAT enzyme activities and reduced ROS levels, highlighting the broad antioxidant potential of *Pleurotus* polysaccharides. In a previous study by our group [13] on *P. citrinopileatus*, substrate composition significantly affected the expression of NFE2L2, SOD1, CAT, HMOX1 and GSR.

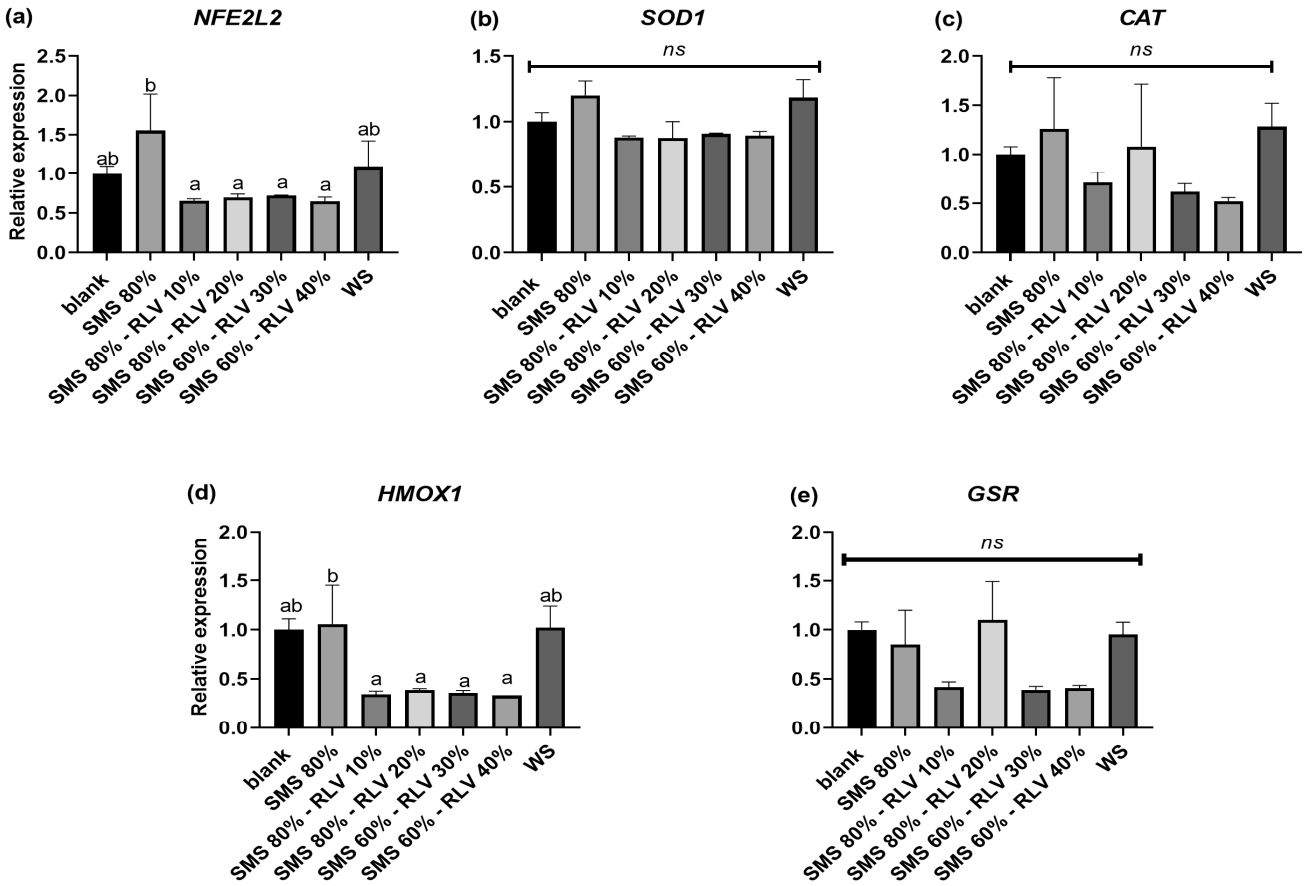

Figure 5. Effect of PE-D-P3 on LPS-induced mRNA expression in THP-1 cells. THP-1 cells were pretreated with PMA for 48 h (100 ng/mL), 24 h rest, following the treatment with LPS (100 ng/mL) with or without the presence of 4 mg protein/mL of PE-D-P3 or BL-D-P3 (blank) for 24 h. The expression levels of (**a**) *NFE2L2*, (**b**) *SOD1*, (**c**) *CAT*, (**d**) *HMOX1* and (**e**) *GSR* were measured using qPCR and were normalized to three housekeeping genes (*B2M*, *RPS18* and *RPL37A*). Data are represented as means ± SEM. Columns with different letters within the same panel are significantly different ($p < 0.05$); ns = not significant ($p > 0.05$).

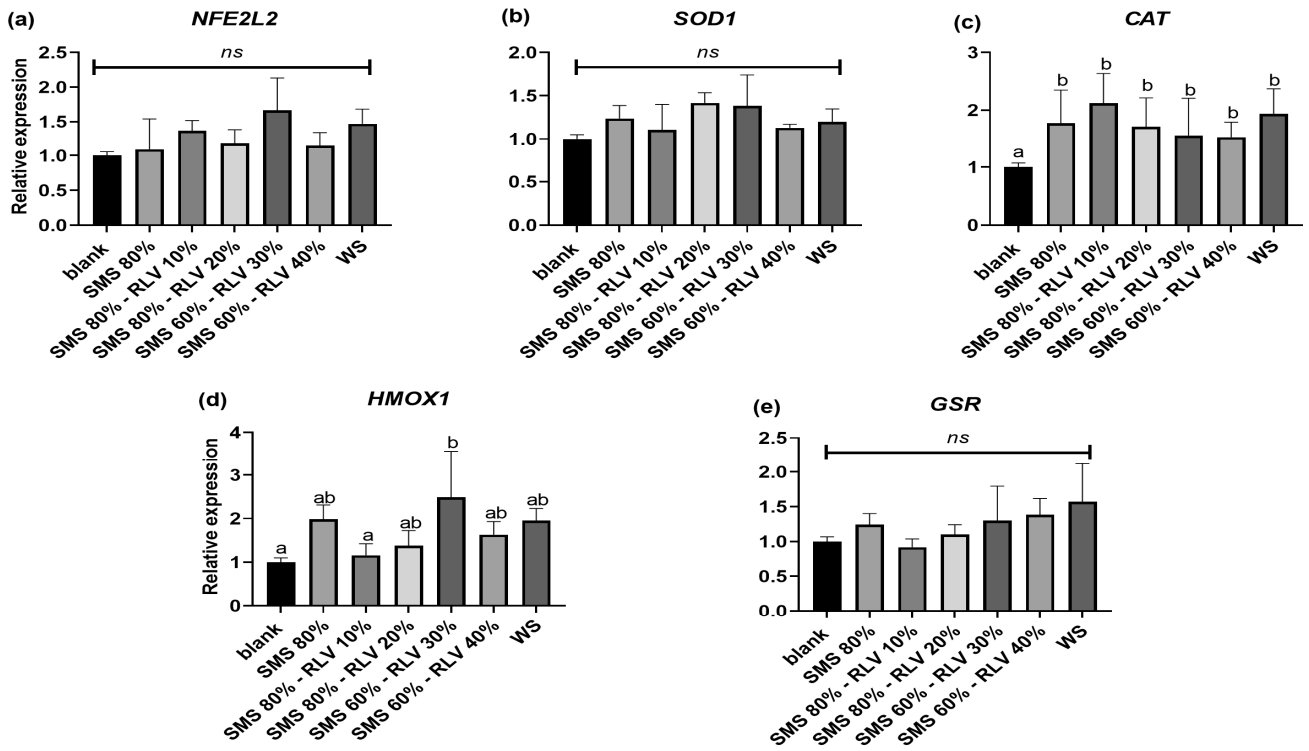

Figure 6. Effect of CE-D on LPS-induced mRNA expression in THP-1 cells. THP-1 cells were pretreated with PMA for 48 h (100 ng/mL), 24 h rest, following the treatment with LPS (100 ng/mL) with or without the presence of 0.34 mg protein/mL of CE-D or BL-D (blank) for 6 h. The expression levels of (**a**) *NFE2L2*, (**b**) *SOD1*, (**c**) *CAT*, (**d**) *HMOX1* and (**e**) *GSR* were measured using qPCR and were normalized to three housekeeping genes (*B2M*, *RPS18* and *RPL37A*). Data are represented as means ± SEM. Columns with different letters within the same panel are significantly different ($p < 0.05$); ns = not significant ($p > 0.05$).

4. Conclusions

The study shows that the composition of cultivation substrates significantly affects the mycelial growth, morphology, physiology, nutritional content and antioxidant properties of *P. ostreatus* mushroom. Specifically, although the Kr values for the alternative substrates were lower than those of the control, RLV generally reduced earliness. Most substrates showed satisfactory BE, except for the supplemented SMS 50%–RLV 40%. Morphological parameters like pileus diameter and stipe length varied by substrate, with higher RLV resulting in larger pileus diameters but shorter stipes, which are preferred by consumers. All alternative substrates increased protein content in *P. ostreatus* carposomes to over 20% *w*/*w*. High IPS values were found in selected SMS–RLV combinations, while RLV addition generally decreased fat content, resulting in a lipid profile rich in mono- and polyunsaturated fatty acids. Antioxidant properties showed significant substrate-dependent variations, with the highest total phenolic compounds (TPC) in mushrooms grown on the control substrate; however, other substrates with RLV also exhibited notable antioxidant activity. The Tr values for *P. ostreatus* were comparable to those of medicinal mushrooms. Thus, RLV demonstrated effectiveness as a substitute for traditional nitrogen-rich materials in mushroom cultivation. Also, this study widens our knowledge of the significance of the inclusion of SMS (and RLV) in the bioactive compounds present in the protein or carbohydrate fractions of cultivated *P. ostreatus* associated with their in vitro effect on the expression of specific antioxidant-related genes in LPS-challenged THP-1-derived macrophages. Consistent with our previous work, the composition of the substrate can influence the bioactive compound profile of mushrooms and their subsequent effects on cellular antioxidant pathways.

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Data Availability Statement: All data presented in this paper are original for this study. The data used to support the findings of this study are available from the corresponding author upon request.

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References

1. Hamza, A.; Mandari, V.; Santhosh Kumar, D. Efficient Production of Biomass and Exopolysaccharide from *P. ostreatus* and Physio-Chemical Characterization of Biomass Powder. *Food Biosci.* **2023**, *55*, 103073. [CrossRef]
2. Hamza, A.; Khalad, A.; Kumar, D.S. Enhanced Production of Mycelium Biomass and Exopolysaccharides of *Pleurotus ostreatus* by Integrating Response Surface Methodology and Artificial Neural Network. *Bioresour. Technol.* **2024**, *399*, 130577. [CrossRef] [PubMed]
3. Corrêa, R.C.G.; Brugnari, T.; Bracht, A.; Peralta, R.M.; Ferreira, I.C.F.R. Biotechnological, Nutritional and Therapeutic Uses of *Pleurotus* spp. (Oyster Mushroom) Related with Its Chemical Composition: A Review on the Past Decade Findings. *Trends Food Sci. Technol.* **2016**, *50*, 103–117. [CrossRef]
4. Bellettini, M.B.; Fiorda, F.A.; Maieves, H.A.; Teixeira, G.L.; Ávila, S.; Hornung, P.S.; Júnior, A.M.; Ribani, R.H. Factors Affecting Mushroom *Pleurotus* spp. *Saudi J. Biol. Sci.* **2019**, *26*, 633–646. [CrossRef] [PubMed]
5. Hamza, A.; Mylarapu, A.; Krishna, K.V.; Kumar, D.S. An Insight into the Nutritional and Medicinal Value of Edible Mushrooms: A Natural Treasury for Human Health. *J. Biotechnol.* **2024**, *381*, 86–99. [CrossRef] [PubMed]
6. Jaramillo Mejía, S.; Albertó, E. Heat Treatment of Wheat Straw by Immersion in Hot Water Decreases Mushroom Yield in *Pleurotus ostreatus*. *Rev. Iberoam. Micol.* **2013**, *30*, 125–129. [CrossRef] [PubMed]
7. Kües, U.; Liu, Y. Fruiting Body Production in Basidiomycetes. *Appl. Microbiol. Biotechnol.* **2000**, *54*, 141–152. [CrossRef]
8. Singh, M.; Pandey, V.K.; Pandey, A.K.; Srivastava, A.; Vishvakarma, N.; Singh, V.K. Production of Xylanase by White Rot Fungi on Wheat Straw. *Asian J. Microbiol. Biotechnol. Environ. Sci.* **2008**, *10*, 859–862.
9. Prado-Acebo, I.; Cubero-Cardoso, J.; Lu-Chau, T.A.; Eibes, G. Integral Multi-Valorization of Agro-Industrial Wastes: A Review. *J. Waste Manag.* **2024**, *183*, 42–52. [CrossRef]
10. Philippoussis, A.; Diamantopoulou, P.; Papadopoulou, K.; Lakhtar, H.; Roussos, S.; Parissopoulos, G.; Papanikolaou, S. Biomass, Laccase and Endoglucanase Production by *Lentinula edodes* during Solid State Fermentation of Reed Grass, Bean Stalks and Wheat Straw Residues. *World J. Microbiol. Biotechnol.* **2011**, *27*, 285–297. [CrossRef]
11. Bertoldi, F.C.; Sant'Anna, E.; Barcelos-Oliveira, J.L. *Chlorella vulgaris* Cultivated in Hydroponic Wastewater. *Acta Hortic.* **2009**, *843*, 203–210. [CrossRef]
12. Kumar, R.R.; Cho, J.Y. Reuse of Hydroponic Waste Solution. *Environ. Sci. Pollut. Res.* **2014**, *21*, 9569–9577. [CrossRef]
13. Diamantis, I.; Dedousi, M.; Melanouri, E.-M.; Dalaka, E.; Antonopoulou, P.; Adelfopoulou, A.; Papanikolaou, S.; Politis, I.; Theodorou, G.; Diamantopoulou, P. Impact of Spent Mushroom Substrate Combined with Hydroponic Leafy Vegetable Roots on *Pleurotus citrinopileatus* Productivity and Fruit Bodies Biological Properties. *Microorganisms* **2024**, *12*, 1807. [CrossRef] [PubMed]
14. Philippoussis, A.; Zervakis, G.; Diamantopoulou, P. Bioconversion of Agricultural Lignocellulosic Wastes through the Cultivation of the Edible Mushrooms *Agrocybe aegerita*, *Volvariella volvacea* and *Pleurotus* spp. *World J. Microbiol. Biotechnol.* **2001**, *17*, 191–200. [CrossRef]
15. Alananbeh, K.M.; Bouqellah, N.A.; Al Kaff, N.S. Cultivation of Oyster Mushroom *Pleurotus ostreatus* on Date-Palm Leaves Mixed with Other Agro-Wastes in Saudi Arabia. *Saudi J. Biol. Sci.* **2014**, *21*, 616–625. [CrossRef]
16. Melanouri, E.-M.; Dedousi, M.; Diamantopoulou, P. Cultivating *Pleurotus ostreatus* and *Pleurotus eryngii* Mushroom Strains on Agro-Industrial Residues in Solid-State Fermentation. Part II: Effect on Productivity and Quality of Carposomes. *Carbon Resour. Convers.* **2022**, *5*, 52–60. [CrossRef]

17. Dedousi, M.; Melanouri, E.-M.; Diamantopoulou, P. Carposome Productivity of *Pleurotus ostreatus* and *Pleurotus eryngii* Growing on Agro-Industrial Residues Enriched with Nitrogen, Calcium Salts and Oils. *Carbon Resour. Convers.* **2023**, *6*, 150–165. [CrossRef]
18. Dedousi, M.; Melanouri, E.-M.; Karayannis, D.; Kaminarides, E.-I.; Diamantopoulou, P. Utilization of Spent Substrates and Waste Products of Mushroom Cultivation to Produce New Crops of *Pleurotus ostreatus*, *Pleurotus eryngii* and *Agaricus bisporus*. *Carbon Resour. Convers.* **2024**, *7*, 100196. [CrossRef]
19. Diamantopoulou, P.; Fourtaka, K.; Melanouri, E.M.; Dedousi, M.; Diamantis, I.; Gardeli, C.; Papanikolaou, S. Examining the Impact of Substrate Composition on the Biochemical Properties and Antioxidant Activity of *Pleurotus* and *Agaricus* Mushrooms. *Fermentation* **2023**, *9*, 689. [CrossRef]
20. Singh, M.P.; Singh, V.K. Yield Performance and Nutritional Analysis of *Pleurotus citrinopileatus* on Different Agrowastes and Vegetable Wastes. In Proceedings of the 7th International Conference on Mushroom Biology and Mushroom Products, Arcachon, France, 4–7 October 2011; Volume 1, pp. 4–7.
21. Ahmed, M.; Abdullah, N.; Nuruddin, M. Yield and Nutritional Composition of Oyster Mushrooms: An Alternative Nutritional Source for Rural People. *Sains Malays.* **2016**, *45*, 1609–1615.
22. Kakon, A.; Choudhury, M.B.K.; Saha, S. Mushroom Is an Ideal Food Supplement. *J. Dhaka Natl. Med. Coll. Hosp.* **2012**, *18*, 58–62. [CrossRef]
23. Ganesan, K.; Xu, B. Anti-Obesity Effects of Medicinal and Edible Mushrooms. *Molecules* **2018**, *23*, 2880. [CrossRef]
24. Sande, D.; de Oliveira, G.P.; e Moura, M.A.F.; de Almeida Martins, B.; Lima, M.T.N.S.; Takahashi, J.A. Edible Mushrooms as a Ubiquitous Source of Essential Fatty Acids. *Food Res. Int.* **2019**, *125*, 108524. [CrossRef] [PubMed]
25. Halliwell, B. Free Radicals and Antioxidants: Updating a Personal View. *Nutr. Rev.* **2012**, *70*, 257–265. [CrossRef]
26. Meng, M.; Huo, R.; Wang, Y.; Ma, N.; Shi, X.; Shen, X.; Chang, G. Lentinan Inhibits Oxidative Stress and Alleviates LPS-Induced Inflammation and Apoptosis of BMECs by Activating the Nrf2 Signaling Pathway. *Int. J. Biol. Macromol.* **2022**, *222*, 2375–2391. [CrossRef] [PubMed]
27. Ryter, S.W. Heme Oxgenase-1, a Cardinal Modulator of Regulated Cell Death and Inflammation. *Cells* **2021**, *10*, 515. [CrossRef]
28. Herath, H.M.L.P.B.; Wickramasinghe, P.D.S.U.; Bathige, S.D.N.K.; Jayasooriya, R.G.P.T.; Kim, G.-Y.; Park, M.A.; Kim, C.; Lee, J. Molecular Identification and Functional Delineation of a Glutathione Reductase Homolog from Disk Abalone (*Haliotis discus discus*): Insights as a Potent Player in Host Antioxidant Defense. *Fish Shellfish Immunol.* **2017**, *60*, 355–367. [CrossRef]
29. Philippoussis, A.; Diamantopoulou, P.A.; Zervakis, G. Monitoring of Mycelium Growth and Fructification of *Lentinula edodes* on Several Agricultural Residues. In *Mushroom Biology and Mushroom Products*; UAEM: Cuernavaca, Mexico, 2002.
30. Philippoussis, A.; Diamantopoulou, P.; Zervakis, G. Correlation of the Properties of Several Lignocellulosic Substrates to the Crop Performance of the Shiitake Mushroom *Lentinula edodes*. *World J. Microbiol. Biotechnol.* **2003**, *19*, 551–557. [CrossRef]
31. Diamantopoulou, P.; Papanikolaou, S.; Komaitis, M.; Aggelis, G.; Philippoussis, A. Patterns of Major Metabolites Biosynthesis by Different Mushroom Fungi Grown on Glucose-Based Submerged Cultures. *Bioprocess. Biosyst. Eng.* **2014**, *37*, 1385–1400. [CrossRef]
32. Liang, Y.; Sarkany, N.; Cui, Y.; Blackburn, J.W. Batch Stage Study of Lipid Production from Crude Glycerol Derived from Yellow Grease or Animal Fats through Microalgal Fermentation. *Bioresour. Technol.* **2010**, *101*, 6745–6750. [CrossRef]
33. Miller, G.L. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Anal. Chem.* **1959**, *31*, 426–428. [CrossRef]
34. Sarantou, S.; Stoforos, N.G.; Kalantzi, O.; Papanikolaou, S. Biotechnological Valorization of Biodiesel-Derived Glycerol: Trials with the Non-Conventional Yeasts *Yarrowia lipolytica* and *Rhodosporidium* sp. *Carbon Resour. Convers.* **2021**, *4*, 61–75. [CrossRef]
35. Fakas, S.; Papanikolaou, S.; Galiotou-Panayotou, M.; Komaitis, M.; Aggelis, G. Organic Nitrogen of Tomato Waste Hydrolysate Enhances Glucose Uptake and Lipid Accumulation in *Cunninghamella echinulata*. *J. Appl. Microbiol.* **2008**, *105*, 1062–1070. [CrossRef]
36. León-Guzmán, M.F.; Silva, I.; López, M.G. Proximate Chemical Composition, Free Amino Acid Contents, and Free Fatty Acid Contents of Some Wild Edible Mushrooms from Querétaro, México. *J. Agric. Food Chem.* **1997**, *45*, 4329–4332. [CrossRef]
37. Singleton, V.L.; Rossi, J.A. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158. [CrossRef]
38. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef] [PubMed]
39. Boonsong, S.; Klaypradit, W.; Wilaipun, P. Antioxidant Activities of Extracts from Five Edible Mushrooms Using Different Extractants. *Agric. Nat. Resour.* **2016**, *50*, 89–97. [CrossRef]
40. Benzie, I.F.F.; Strain, J.J. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. *Anal. Biochem.* **1996**, *239*, 70–76. [CrossRef] [PubMed]
41. Barreira, J.; Ferreira, I.; Oliveira, M.; Pereira, J. Antioxidant Activities of the Extracts from Chestnut Flower, Leaf, Skins and Fruit. *Food Chem.* **2008**, *107*, 1106–1113. [CrossRef]
42. Fan, J.-P.; He, C.-H. Simultaneous Quantification of Three Major Bioactive Triterpene Acids in the Leaves of *Diospyros Kaki* by High-Performance Liquid Chromatography Method. *J. Pharm. Biomed. Anal.* **2006**, *41*, 950–956. [CrossRef]

43. González, A.; Nobre, C.; Simões, L.S.; Cruz, M.; Loredo, A.; Rodríguez-Jasso, R.M.; Contreras, J.; Texeira, J.; Belmares, R. Evaluation of Functional and Nutritional Potential of a Protein Concentrate from *Pleurotus ostreatus* Mushroom. *Food Chem.* **2021**, *346*, 128884. [CrossRef]
44. Gardeli, C.; Mela, N.; Dedousi, M.; Kandyliari, A.; Kaparakou, E.; Diamantopoulou, P.; Pappas, C.; Mallouchos, A. The Influence of Substrate and Strain on Protein Quality of *Pleurotus ostreatus*. *Appl. Sci.* **2024**, *14*, 4040. [CrossRef]
45. Li, X.; Zhao, R.; Zhou, H.L.; Wu, D.H. Deproteinization of Polysaccharide from the Stigma *Maydis* by Sevag Method. *Adv. Mater. Res.* **2011**, *340*, 416–420. [CrossRef]
46. Bradford, M.M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef] [PubMed]
47. Dalaka, E.; Politis, I.; Theodorou, G. Antioxidant Activity of Sweet Whey Derived from Bovine, Ovine and Caprine Milk Obtained from Various Small-Scale Cheese Plants in Greece before and after in Vitro Simulated Gastrointestinal Digestion. *Antioxidants* **2023**, *12*, 1676. [CrossRef]
48. Brodkorb, A.; Egger, L.; Alminger, M.; Alvito, P.; Assunção, R.; Ballance, S.; Bohn, T.; Bourlieu-Lacanal, C.; Boutrou, R.; Carrière, F.; et al. INFOGEST Static in Vitro Simulation of Gastrointestinal Food Digestion. *Nat. Protoc.* **2019**, *14*, 991–1014. [CrossRef]
49. Minekus, M.; Alminger, M.; Alvito, P.; Ballance, S.; Bohn, T.; Bourlieu, C.; Carrière, F.; Boutrou, R.; Corredig, M.; Dupont, D.; et al. A Standardised Static in Vitro Digestion Method Suitable for Food—An International Consensus. *Food Funct.* **2014**, *5*, 1113–1124. [CrossRef] [PubMed]
50. Hellemans, J.; Mortier, G.; De Paepe, A.; Speleman, F.; Vandesompele, J. qBase Relative Quantification Framework and Software for Management and Automated Analysis of Real-Time Quantitative PCR Data. *Genome Biol.* **2007**, *8*, R19. [CrossRef]
51. Templeton, G. A Two-Step Approach for Transforming Continuous Variables to Normal: Implications and Recommendations for IS Research. *Commun. Assoc. Inf. Syst.* **2011**, *28*, 4. [CrossRef]
52. Economou, C.; Diamantopoulou, P.; Philippoussis, A. Valorization of Spent Oyster Mushroom Substrate and Laccase Recovery through Successive Solid State Cultivation of *Pleurotus*, *Ganoderma*, and *Lentinula* Strains. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 5213–5222. [CrossRef]
53. Melanouri, E.M.; Papanikolaou, S.; Diamantopoulou, P. *Mortierella ramanniana* Lipid Fermentation Wastewater as an Innovative Maceration Liquid Medium for Sustainable Solid-State Cultivation of Higher Fungi. *Waste Biomass Valori.* **2024**, *15*, 6903–6925. [CrossRef]
54. Melanouri, E.-M.; Dedousi, M.; Diamantopoulou, P. Cultivating *Pleurotus ostreatus* and *Pleurotus eryngii* Mushroom Strains on Agro-Industrial Residues in Solid-State Fermentation. Part I: Screening for Growth, Endoglucanase, Laccase and Biomass Production in the Colonization Phase. *Carbon Resour. Convers.* **2022**, *5*, 61–70. [CrossRef]
55. Curvetto, N.R.; Figlas, D.; Devalis, R.; Delmastro, S. Growth and Productivity of Different *Pleurotus ostreatus* Strains on Sunflower Seed Hulls Supplemented with N–$NH4^+$ and/or Mn(II). *Bioresour. Technol.* **2002**, *84*, 171–176. [CrossRef]
56. Kaal, E.E.J.; Field, J.A.; Joyce, T.W. Increasing Ligninolytic Enzyme Activities in Several White-Rot Basidiomycetes by Nitrogen-Sufficient Media. *Bioresour. Technol.* **1995**, *53*, 133–139. [CrossRef]
57. Baysal, E.; Peker, H.; Yalinkiliç, M.K.; Temiz, A. Cultivation of Oyster Mushroom on Waste Paper with Some Added Supplementary Materials. *Bioresour. Technol.* **2003**, *89*, 95–97. [CrossRef] [PubMed]
58. Chang, S.-T.; Miles, P.G. *Edible Mushrooms and Their Cultivation*; CRC: Boca Raton, FL, USA, 1989.
59. Kortei, N.K.; Odamtten, G.T.; Obodai, M.; Wiafe-Kwagyan, M.; Narh Mensah, D.L. Correlations of Cap Diameter (Pileus Width), Stipe Length and Biological Efficiency of *Pleurotus ostreatus* (Ex.Fr.) Kummer Cultivated on Gamma-Irradiated and Steam-Sterilized Composted Sawdust as an Index of Quality for Pricing. *Agric. Food Secur.* **2018**, *7*, 35. [CrossRef]
60. Hoa, H.T.; Wang, C.-L.; Wang, C.-H. The Effects of Different Substrates on the Growth, Yield, and Nutritional Composition of Two Oyster Mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). *Mycobiology* **2015**, *43*, 423–434. [CrossRef]
61. Patra, A.K.; Pani, B.K. Evaluation of Banana Leaf as a New Alternate Substrate to Paddy Straw for Oyster Mushroom Cultivation. *J. Phytol.* **1995**, *8*, 145–148.
62. Kurt, S.; Buyukalaca, S. Yield Performances and Changes in Enzyme Activities of *Pleurotus* spp. (*P. ostreatus* and *P. sajor-caju*) Cultivated on Different Agricultural Wastes. *Bioresour. Technol.* **2010**, *101*, 3164–3169. [CrossRef]
63. Liang, Z.-C.; Wu, C.-Y.; Shieh, Z.-L.; Cheng, S.-L. Utilization of Grass Plants for Cultivation of *Pleurotus citrinopileatus*. *Int. J. Biodegrad. Bioremediat.* **2009**, *63*, 509–514. [CrossRef]
64. Ulziijargal, E.; Mau, J.-L. Nutrient Compositions of Culinary-Medicinal Mushroom Fruiting Bodies and Mycelia. *Int. J. Med. Mushrooms* **2011**, *13*, 343–349. [CrossRef] [PubMed]
65. Baeva, E.; Bleha, R.; Lavrova, E.; Sushytskyi, L.; Čopíková, J.; Jablonsky, I.; Klouček, P.; Synytsya, A. Polysaccharides from Basidiocarps of Cultivating Mushroom *Pleurotus ostreatus*: Isolation and Structural Characterization. *Molecules* **2019**, *24*, 2740. [CrossRef]

66. Economou, C.N.; Philippoussis, A.N.; Diamantopoulou, P.A. Spent Mushroom Substrate for a Second Cultivation Cycle of *Pleurotus* Mushrooms and Dephenolization of Agro-Industrial Wastewaters. *FEMS Microbiol. Lett.* **2020**, *367*, fnaa060. [CrossRef] [PubMed]
67. Günç Ergönül, P.; Akata, I.; Kalyoncu, F.; Ergönül, B. Fatty Acid Compositions of Six Wild Edible Mushroom Species. *Sci. World J.* **2013**, *2013*, 163964. [CrossRef] [PubMed]
68. Bengu, A.S. The Fatty Acid Composition in Some Economic and Wild Edible Mushrooms in Turkey. *Prog. Nutr.* **2020**, *22*, 185–192.
69. Kalač, P. Chemical Composition and Nutritional Value of European Species of Wild Growing Mushrooms: A Review. *Food Chem.* **2009**, *113*, 9–16. [CrossRef]
70. Yamagishi, K.; Folsom, A.R.; Steffen, L.M.; ARIC Study Investigators. Plasma Fatty Acid Composition and Incident Ischemic Stroke in Middle-Aged Adults: The Atherosclerosis Risk in Communities (ARIC) Study. *Cerebrovasc. Dis.* **2013**, *36*, 38–46. [CrossRef]
71. Floret, C.; Monnet, A.-F.; Micard, V.; Walrand, S.; Michon, C. Replacement of Animal Proteins in Food: How to Take Advantage of Nutritional and Gelling Properties of Alternative Protein Sources. *Crit. Rev. Food Sci. Nutr.* **2023**, *63*, 920–946. [CrossRef]
72. Dedousi, M.; Melanouri, E.M.; Diamantis, I.; Papanikolaou, S.; Diamantopoulou, P. Biochemical, Functional and Antioxidant Potential of Higher Fungi Cultivated on Agro-Industrial Residues. Part II: Cultures on Mixtures of Spent Mushroom Substrates and Mushroom Cropping By-Products. *Resour. Chem. Mater.* **2024**, *3*, 175–187. [CrossRef]
73. Dedousi, M.; Melanouri, E.M.; Panagopoulou, I.; Gardeli, C.; Papanikolaou, S.; Diamantopoulou, P. Biochemical, Functional and Antioxidant Dynamics Potential of Higher Fungi Cultivated on Agro-Industrial Residues. Part I: Cultures on Media Supplemented with Yeast Extract, Gypsum and Commodity Vegetable Oils. *Resour. Chem. Mater.* **2024**, S2772443324000199. [CrossRef]
74. Yu, Q.; Guo, M.; Zhang, B.; Wu, H.; Zhang, Y.; Zhang, L. Analysis of Nutritional Composition in 23 Kinds of Edible Fungi. *J. Food Qual.* **2020**, *2020*, 8821315. [CrossRef]
75. Zhou, J.; Chen, M.; Wu, S.; Liao, X.; Wang, J.; Wu, Q.; Zhuang, M.; Ding, Y. A Review on Mushroom-Derived Bioactive Peptides: Preparation and Biological Activities. *Food Res. Int.* **2020**, *134*, 109230. [CrossRef] [PubMed]
76. Tshinyangu, K.K.; Hennebert, G.L. Protein and Chitin Nitrogen Contents and Protein Content in *Pleurotus ostreatus* Var. *columbinus*. *Food Chem.* **1996**, *57*, 223–227. [CrossRef]
77. Sadli, S.; Saleha, S.; Fiana, D.; Misrahanum, M. The Formulation of White Oyster Mushroom (*Pleurotus ostreatus* (Jacq.) P. Kumm) as Natural Flavoring and the Quality Test in Temperature and Drying Time Variations. *IOP Conf. Ser. Earth Environ. Sci.* **2021**, *922*, 012054. [CrossRef]
78. Yamauchi, M.; Sakamoto, M.; Yamada, M.; Hara, H.; Mat Taib, S.; Rezania, S.; Mohd Fadhil, M.D.; Mohd Hanafi, F.H. Cultivation of Oyster Mushroom (*Pleurotus ostreatus*) on Fermented Moso Bamboo Sawdust. *J. King Saud Univ. Sci.* **2019**, *31*, 490–494. [CrossRef]
79. Alam, N.; Amin, R.; Khan, A.; Ara, I.; Shim, M.-J.; Lee, M.-W.; Lee, T.-S. Nutritional Analysis of Cultivated Mushrooms in Bangladesh—*Pleurotus ostreatus*, *Pleurotus sajor-caju*, *Pleurotus florida* and *Calocybe indica*. *Mycobiology* **2008**, *36*, 228–232. [CrossRef]
80. Cohen, N.; Cohen, J.; Asatiani, M.D.; Varshney, V.K.; Yu, H.-T.; Yang, Y.-C.; Li, Y.-H.; Mau, J.-L.; Wasser, S.P. Chemical Composition and Nutritional and Medicinal Value of Fruit Bodies and Submerged Cultured Mycelia of Culinary-Medicinal Higher Basidiomycetes Mushrooms. *Int. J. Med. Mushrooms* **2014**, *16*, 273–291. [CrossRef] [PubMed]
81. Singh, R.; Kaur, N.; Shri, R.; Singh, A.P.; Dhingra, G.S. Proximate Composition and Element Contents of Selected Species of *Ganoderma* with Reference to Dietary Intakes. *Environ. Monit. Assess.* **2020**, *192*, 270. [CrossRef]
82. Palacios, I.; Lozano, M.; Moro, C.; D'Arrigo, M.; Rostagno, M.A.; Martínez, J.A.; García-Lafuente, A.; Guillamón, E.; Villares, A. Antioxidant Properties of Phenolic Compounds Occurring in Edible Mushrooms. *Food Chem.* **2011**, *128*, 674–678. [CrossRef]
83. Becker, E.M.; Nissen, L.R.; Skibsted, L.H. Antioxidant Evaluation Protocols: Food Quality or Health Effects. *Eur. Food Res. Technol.* **2004**, *219*, 561–571. [CrossRef]
84. Li, J.-J.; Hu, X.-Q.; Zhang, X.-F.; Liu, J.-J.; Cao, L.-S. Study on Variation of Main Ingredients from Spores and Fruiting Bodies of *Ganoderma lucidum*. *Chin. J. Chin. Mater. Med.* **2014**, *39*, 4246–4251.
85. Boh, B.; Dolničar, D.; Pohleven, F. Isolation and Quantification of Triterpenoid Acids from *Ganoderma applanatum* of Istrian Origin. *Food Technol. Biotechnol.* **2000**, *38*, 11–18.
86. Strapáč, I.; Kuruc, M.; Baranová, M. Determination of Antioxidant Parameters of *Pleurotus* Mushrooms Growing on Different Wood Substrates. *Folia Vet.* **2017**, *61*, 53–58. [CrossRef]
87. Gil-Ramírez, A.; Pavo-Caballero, C.; Baeza, E.; Baenas, N.; Garcia-Viguera, C.; Marín, F.R.; Soler-Rivas, C. Mushrooms Do Not Contain Flavonoids. *J. Funct. Foods* **2016**, *25*, 1–13. [CrossRef]
88. Arbaayah, H.; Umi Kalsom, Y. Antioxidant Properties in the Oyster Mushrooms (*Pleurotus* spp.) and Split Gill Mushroom (*Schizophyllum commune*) Ethanolic Extracts. *Mycosphere* **2013**, *4*, 661–673. [CrossRef]
89. Sudha, G.; Vadivukkarasi, S.; Shree, R.B.I.; Lakshmanan, P. Antioxidant Activity of Various Extracts from an Edible Mushroom Pleurotus eous. *Food Sci. Biotechnol.* **2012**, *21*, 661–668. [CrossRef]

90. Yeh, J.-Y.; Hsieh, L.-H.; Wu, K.-T.; Tsai, C.-F. Antioxidant Properties and Antioxidant Compounds of Various Extracts from the Edible Basidiomycete *Grifola frondosa* (Maitake). *Molecules* **2011**, *16*, 3197–3211. [CrossRef] [PubMed]
91. Kalogeropoulos, N.; Yanni, A.E.; Koutrotsios, G.; Aloupi, M. Bioactive Microconstituents and Antioxidant Properties of Wild Edible Mushrooms from the Island of Lesvos, Greece. *Food Chem. Toxicol.* **2013**, *55*, 378–385. [CrossRef]
92. Wang, S.; Liu, Z.; Wang, X.; Liu, R.; Zou, L. Mushrooms Do Produce Flavonoids: Metabolite Profiling and Transcriptome Analysis of Flavonoid Synthesis in the Medicinal Mushroom *Sanghuangporus baumii*. *J. Fungi* **2022**, *8*, 582. [CrossRef] [PubMed]
93. Lesa, K.N.; Khandaker, M.U.; Mohammad Rashed Iqbal, F.; Sharma, R.; Islam, F.; Mitra, S.; Emran, T.B. Nutritional Value, Medicinal Importance, and Health-Promoting Effects of Dietary Mushroom (*Pleurotus ostreatus*). *J. Food Qual.* **2022**, *2022*, 2454180. [CrossRef]
94. Golak-Siwulska, I.; Kałużewicz, A.; Spiżewski, T.; Siwulski, M.; Sobieralski, K. Bioactive Compounds and Medicinal Properties of Oyster Mushrooms (*Pleurotus* sp.). *Folia Hortic.* **2018**, *30*, 191–201. [CrossRef]
95. Koutrotsios, G.; Kalogeropoulos, N.; Stathopoulos, P.; Kaliora, A.C.; Zervakis, G.I. Bioactive Compounds and Antioxidant Activity Exhibit High Intraspecific Variability in *Pleurotus ostreatus* Mushrooms and Correlate Well with Cultivation Performance Parameters. *World J. Microbiol. Biotechnol.* **2017**, *33*, 98. [CrossRef] [PubMed]
96. Kozarski, M.; Klaus, A.; Jakovljevic, D.; Todorovic, N.; Vunduk, J.; Petrović, P.; Niksic, M.; Vrvic, M.M.; van Griensven, L. Antioxidants of Edible Mushrooms. *Molecules* **2015**, *20*, 19489–19525. [CrossRef] [PubMed]
97. Sharma, A.; Sharma, A.; Tripathi, A. Biological Activities of *Pleurotus* spp. Polysaccharides: A Review. *J. Food Biochem.* **2021**, *45*, e13748. [CrossRef]
98. Yin, Z.; Sun-Waterhouse, D.; Wang, J.; Ma, C.; Waterhouse, G.I.N.; Kang, W. Polysaccharides from Edible Fungi *Pleurotus* spp.: Advances and Perspectives. *J. Future Foods* **2021**, *1*, 128–140. [CrossRef]
99. Bonatti, M.; Karnopp, P.; Soares, H.M.; Furlan, S.A. Evaluation of *Pleurotus ostreatus* and *Pleurotus sajor-caju* Nutritional Characteristics When Cultivated in Different Lignocellulosic Wastes. *Food Chem.* **2004**, *88*, 425–428. [CrossRef]
100. Koutrotsios, G.; Patsou, M.; Mitsou, E.K.; Bekiaris, G.; Kotsou, M.; Tarantilis, P.A.; Pletsa, V.; Kyriacou, A.; Zervakis, G.I. Valorization of Olive By-Products as Substrates for the Cultivation of *Ganoderma lucidum* and *Pleurotus ostreatus* Mushrooms with Enhanced Functional and Prebiotic Properties. *Catalysts* **2019**, *9*, 537. [CrossRef]
101. Elhusseiny, S.M.; El-Mahdy, T.S.; Awad, M.F.; Elleboudy, N.S.; Farag, M.M.S.; Aboshanab, K.M.; Yassien, M.A. Antiviral, Cytotoxic, and Antioxidant Activities of Three Edible Agaricomycetes Mushrooms: *Pleurotus columbinus*, *Pleurotus sajor-caju*, and *Agaricus bisporus*. *J. Fungi* **2021**, *7*, 645. [CrossRef]
102. Petraglia, T.; Latronico, T.; Fanigliulo, A.; Crescenzi, A.; Liuzzi, G.M.; Rossano, R. Antioxidant Activity of Polysaccharides from the Edible Mushroom *Pleurotus eryngii*. *Molecules* **2023**, *28*, 2176. [CrossRef] [PubMed]
103. Song, X.; Xu, X.; Chen, W. Antioxidant and Immunostimulatory Activities of Fermented Sour Soybean Milk Added with Polypeptides from *Pleurotus eryngii*. *Front. Microbiol.* **2022**, *13*, 750039. [CrossRef] [PubMed]
104. Aursuwanna, T.; Noitang, S.; Sangtanoo, P.; Srimongkol, P.; Saisavoey, T.; Puthong, S.; Reamtong, O.; Karnchanatat, A. Investigating the Cellular Antioxidant and Anti-Inflammatory Effects of the Novel Peptides in Lingzhi Mushrooms. *Heliyon* **2022**, *8*, e11067. [CrossRef]
105. Wu, S.; Chen, M.; Liao, X.; Huang, R.; Wang, J.; Xie, Y.; Hu, H.; Zhang, J.; Wu, Q.; Ding, Y. Protein Hydrolysates from *Pleurotus geesteranus* Obtained by Simulated Gastrointestinal Digestion Exhibit Neuroprotective Effects in H_2O_2-injured PC12 Cells. *J. Food Biochem.* **2022**, *46*, e13879. [CrossRef] [PubMed]
106. Ma, S.; Li, X.; Tao, Q.; Hu, Q.; Yang, W.; Kimatu, B.M.; Ma, G. The Effect of in Vitro Digestion on the Interaction between Polysaccharides Derived from *Pleurotus eryngii* and Intestinal Mucus. *Food Sci. Nutr.* **2024**, *12*, 1318–1329. [CrossRef]
107. Li, S.; Shah, N.P. Sulphonated Modification of Polysaccharides from *Pleurotus eryngii* and *Streptococcus thermophilus* ASCC 1275 and Antioxidant Activities Investigation Using CCD and Caco-2 Cell Line Models. *Food Chem.* **2017**, *225*, 246–257. [CrossRef] [PubMed]
108. Sun, Y.; Zhou, C.; Huang, S.; Jiang, C. Selenium Polysaccharide SPMP-2a from *Pleurotus geesteranus* Alleviates H_2O_2-Induced Oxidative Damage in HaCaT Cells. *BioMed Res. Int.* **2017**, *2017*, 4940384. [CrossRef] [PubMed]