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Examining the Impact of Substrate Composition on the Biochemical Properties and Antioxidant Activity of *Pleurotus* and *Agaricus* Mushrooms

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Abstract: The composition of the substrate is one of the most critical factors influencing the quality as well as the nutritional value and bioactive content of mushrooms. Therefore, the effects of various substrates, such as barley and oat straw (BOS), beech wood shavings (BWS), coffee residue (CR), rice bark (RB) and wheat straw (WS, control substrate), on the biochemical properties (lipid, protein, polysaccharide, glucan, ash, and mineral content, fatty acids and tocopherols composition), total phenolic compounds and antioxidant activity of *Pleurotus* mushrooms, *P. ostreatus* (strains AMRL 144, 150) and *P. eryngii* (strains AMRL 166, 173-6), cultivated in 'bag-logs', was examined. Proximate analysis of *A. bisporus* and *A. subrufescens* grown on two different composts (C/N ratios of 10 and 13) was conducted, too. The whole carposomes, pilei and stipes were analyzed. Results showed that BOS, RB, BWS and CR improved the antioxidant activity of *Pleurotus* species and their nutritional characteristics. Both pilei and stipes were rich in polysaccharides (27.51–67.37 and 22.46–39.08%, *w*/*w*, for *Pleurotus* and *Agaricus* spp., respectively), lipids (0.74–8.70 and 5.80–9.92%, *w*/*w*), proteins (6.52–37.04 and 25.40–44.26, *w*/*w*, for *Pleurotus* and *Agaricus* spp., respectively) and total phenolic compounds (10.41–70.67 and 7.85–16.89 mg gallic acid equivalent/g for *Pleurotus* and *Agaricus* spp., respectively), while they contained important quantities of unsaturated FAs of nutritional and medicinal importance. Pilei were richer in proteins, total phenolic compounds and enhanced antioxidant activity and reducing power than stipes, whereas stipes were richer in IPSs and glucans compared to the corresponding pilei. Thus, mushroom cultivation could upgrade rejected agro-industrial residues and wastes to new uses as substrates for the production of mushrooms with specific nutritional and medicinal attributes.

Keywords: agro-waste; pilei; stipes; nutritional value; antioxidant activity

Citation: 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. https://doi.org/10.3390/fermentation9070689

Academic Editor: Yuxia Mei

Received: 12 June 2023
Revised: 10 July 2023
Accepted: 18 July 2023
Published: 23 July 2023

1. Introduction

Mushrooms have long been used as food or food flavoring material due to their unique flavor and aroma [1,2], and they have gained extra attention due to their low calories and fat, and also because they contain a variety of polysaccharides, proteins and fibers [1,3–6] that are essential nutrition components. Also, mushrooms of the *Basidiomycetes* family have long been known for their seemingly beneficial medical usage, most notably in traditional Chinese and Japanese medicine [4]. However, knowledge about the composition and nutritional value of culinary mushrooms, mainly wild-growing ones, remained limited until the last decade as compared to vegetables and medicinal mushroom species [7].

Edible fungi constitute a good protein source for vegetarian people [8], as their nutritional value is comparable to that of eggs, milk and meat [9]. For instance, the proteins of *Pleurotus* sp. have superior quality because many species contain complete proteins with a good distribution of essential amino acids, as well as non-essential amino acids, particularly GABA, that act as neurotransmitters, and ornithine that is a precursor in the synthesis of arginine [3]. They can also be used in several medicinal and biotechnological applications. Additionally, mushrooms contain varying amounts of polysaccharides that have been shown to have multiple benefits for human health, such as anti-viral, antioxidant and bactericidal activity, and medicinal properties for tumor therapy and cardiovascular disease, and they are also considered a potential source of prebiotics [10]. The most important, high-quality polysaccharides present in mushrooms are β-glucans. These have a variety of clinical uses, including the immunomodulatory, anti-inflammatory, cardioprotective, anti-diabetic and anti-cancer effects of the β-glucans of fungal extracts [1,10–13]. Particularly, *Pleurotus* species have been effectively demonstrated to function as a dietary supplement for cardiac patients to lower blood cholesterol levels due to the presence of mevinolin and high quantities of β-glucans compounds [14], whereas *Agaricus blazei* (syn. *Agaricus subrufescens*, *Agaricus brasiliensis*), *Ganoderma lucidum*, *Hericium erinaceus* and *Grifola frondosa* are used as immune response modifiers for cancer prevention, or as nutritional support during chemotherapy, as well as for chronic inflammatory conditions such as hepatitis and other diseases [15,16]. In another study, with crude polysaccharides extracted from four common edible mushrooms, including *Agaricus bisporus*, *Auricularia auricula*, etc., it was revealed that the crude polysaccharides of *A. bisporus* were the best natural antioxidant [17], indicating that polysaccharides were the main contributors to the antioxidant activity protecting cells from free radicals and oxidative stress; the excess production of free radicals can cause damage to DNA, lipids and proteins, leading to a variety of chronic disorders such as cardiovascular disease, cancer and neurological disease. Although lipids are present in low concentrations in edible fungi, they contain essential polyunsaturated fatty acids (PUFAs), such as linoleic, oleic and linolenic acids, that are usually the major constituents [18]. Linoleic acid is a highly important fatty acid, as it is the precursor of 1-octen-3-ol, which is the principal aroma compound in most fungi and contributes to mushroom flavor [19], as well as influencing the growth, development and behavior of fungus, plants and insects [20–22]. Furthermore, several mushroom-derived polyphenolic compounds have been discovered to be effective antioxidants with substantial free-radical-scavenging and metal-chelating properties, while various compounds, including ergosterol, vitamins B1, B2 and C, and minerals such as zinc, copper, iodine, selenium and iron, have been extracted from the mushrooms' fruiting bodies, mycelia and culture media [23]. Also, because of their high potassium and low sodium content, *Pleurotus* species are ideal foods for those suffering from hypertension and cardiovascular disease [24]. Aromatic hydroxylated substances containing one or more aromatic rings and one or more hydroxyl groups are referred to as phenolic compounds. They comprise a very large number of subclasses, including flavonoids, phenolic acids (including hydroxybenzoic acids and hydroxycinnamic acids), stilbenes, lignans, tannins and oxidized polyphenols, with a wide range of structures [25,26]. Phenolic compounds are high in antioxidants and have anti-cancer, anti-cardiovascular, anti-viral, anti-microbial, anti-inflammatory and anti-allergenic characteristics. They also aid in blood sugar control and promote the body's detoxifying functions [27]. Consequently, mushrooms are referred to as "functional foods" and seem important as a natural source of antioxidant additives [28–30]. The constancy or enhancement of these unique characteristics of fresh mushrooms is of great interest for both researchers and producers.

A. bisporus (the button mushroom) is the most important of the cultivated edible mushrooms, followed by the *Pleurotus* genus [31,32], and they are commercially cultivated on composted, pasteurized wheat straw and manure with a final C/N ratio of 12–13 [33]. *A. subrufescens* (the almond mushroom) is cultivated similarly to *A. bisporus* and it is known to produce bioactive compounds with significant medicinal properties, such as anti-cancer and

anti-tumor activity, with fruiting bodies that are also edible and nutritious [34]. Research on *Agaricus* species has focused on enhancing the yield and sporophore quality, including the usage of alternative substrates and techniques for successful fructification [35–38]. For example, the soybean fiber structure, the protein and polysaccharide composition, along with a greater N content resulting in a substrate formulation ratio of C/N = 11 (N = 4.25%), offered a more suitable substrate for *A. subrufescens* mycelial growth in a variety of carbon to nitrogen ratios from 11 to 248 [39], while the dry matter and carbohydrate content of *A. bisporus* mushrooms increased during cultivation when using casing materials having higher levels of proteins and hence nitrogen content [40]. Regarding *Pleurotus* species, although around 200 species of this genus have been discovered, only a few have been exploited in food technology [32,41], notably the species *Pleurotus ostreatus* (oyster mushroom) and *Pleurotus eryngii* (king oyster), which can grow on hard timbers in forests across a wide temperature range. They can also fructify on several types of agro-industrial residues and wastes that are produced worldwide, such as wheat straw, grape pomace, cotton waste, corn cobs, peanut shells, olive cake, poplar wood sawdust, banana waste and spent mushroom substrate, etc. [42–47], wastes that often cause environmental problems [48,49]. As the various substrates present different effects on the growth, yield and quality, as well as the nutritional characteristics, of produced mushrooms, even if they are species of the same genus [49,50], it is necessary that economical and easy ways to industrially produce inexpensive nutritious food from these wastes be found. For example, the cultivation of mushrooms that have high protein levels or are rich in β-glucans could be an interesting option with high nutritional, pharmaceutical and economic value [51–57].

Given that there are limited data in the literature concerning the quality properties of *Pleurotus* mushroom parts (pilei and stipes) grown in different alternative substrates made from lignocellulosic agro-industrial waste, this study refers to the effects of those substrates on the nutritional value and antioxidant activity of *P. ostreatus* and *P. eryngii* species (carposomes, pilei and stipes), using wastes with relevant C/N [42,44,58]. Building upon our previous research work [42,44,58], in the present study, we assessed the potential of nine agro-residues to serve as substitute substrates for WS (that is, the commercially utilized wheat straw, which functioned here as the control substrate) for the cultivation of several strains of *P. ostreatus* (AMRL 137, 144, 147, 150) and *P. eryngii* (AMRL 160, 163, 166, 173-6) during mycelial development. Following that, additional studies were carried out to determine the ability of selected *P. ostreatus* (AMRL 144, 150) and *P. eryngii* (AMRL 166, 173-6) strains to colonize and produce carposomes after solid-state fermentation on the most prominent substrates (barley and oat straw—BOS, beech wood shavings—BWS, coffee residue—CR, and rice bark—RB, along with the control substrate, wheat straw—WS), with relevant C/N ratios that ensured their fructification and data comparison. The selection of strains and substrates was based on screening regarding mycelial growth and endoglucanase, laccase and biomass production in the colonization phase. The potential for the commercial production of *Pleurotus* spp. mushrooms on new, low-cost substrates seems particularly promising in financial and environmental terms. Furthermore, as regards *Agaricus* species, *A. bisporus* and *A. subrufescens* fungi (carposomes, pilei and stipes) were cultivated on two different composts (C/N ratios of 10 and 13) to examine the possible effects of nitrogen on their nutritional and medicinal characteristics, aiming for their amelioration. Therefore, this research was conducted to study how the different substrates affected the biochemical characteristics and the overall quality of mushrooms, evaluating their protein, polysaccharide, glucan, lipid, ash, and mineral content, as well as fatty acid and tocopherol composition, along with total phenolic content and antioxidant activity.

2. Materials and Methods

2.1. Mushroom Production

Strains of *P. ostreatus* (Jacq.: Fr.) Kumm. (AMRL 144, 150), *P. eryngii* (D.C.: Fr.) Quél. (AMRL 166, 173-6), *A. bisporus* (AMRL 209) and *A. subrufescens* (AMRL 235-CA 560, INRA France) mushrooms belonging to the Edible Fungi Laboratory/ITAP/EGO-Dimitra culture

collection were used in this study. Prior to the experiments, fungal species were grown in PDA Petri plates at 26 ± 1 °C and 75% relative humidity. Grain spawn from each species was made in 500 mL Erlenmeyer flasks containing 180 g of boiling millet (*Panicum miliaceum*) as previously reported [59].

The agricultural residues WS, BOS, BWS, CR and RB were used for the solid-state fermentation of the *Pleurotus* species, which took place in bags of 1 kg [58]. After the determination of the physicochemical characteristics of all residues, wastes were supplemented with wheat bran at ratios (*w*/*w*, in terms of dry weight) of 80:20 (WS), 60:40 (BWS), 70:30 (BOS, RB) or 60:5 (:35 WS, CR) to obtain a final C/N 20–27/1, the desired values for mushroom cultivation [46], and mixed with calcium carbonate to obtain a pH value of 6.0–7.5. Under aseptic circumstances, five replicates of polypropylene autoclavable bags per substrate and strain were filled with 1 kg of substrate, autoclaved at 121 ± 1 °C for 2 h (1.1 atm) and inoculated with 3–5% *w*/*w* (on fresh weight basis) mushroom spawn. Substrate colonization took place in a dark incubator at 25 ± 1.0 °C and 80% RH. The bags were transported to the fruiting chamber (used for all stages of fruiting) with precise environmental conditions for carposome induction, fructification and productivity evaluation after full colonization. During harvesting, the light intensity was set to 700 lux (12 h per day, fluorescent lamps), air exchange rates were managed to maintain a low CO_2 level (1200 ppm), relative air humidity was set to 90% and the temperature was set to 17.5 ± 1.0 °C.

Agaricus mushrooms were cultivated in boxes of 4 kg, according to the standard commercial procedure for *A. bisporus* with some modifications, as described previously [60–63]. Specifically, commercially manufactured compost was inoculated with 2% *w*/*w* spawn at the rate of 4 kg of compost fresh weight per box. Five replicate boxes per species were arranged in a randomized block design in a growth chamber. After 13 days of spawn growth at 26 ± 1.0 °C, a 40 mm casing layer (BVB Euroveen) was added. The compost temperature was kept between 24.5 and 26.5 °C for 5–6 days. Fresh air was then supplied to the growth chamber to reduce the temperature to 19.5 °C (air temperature 17.5 °C) and to achieve a CO_2 concentration of 0.09–0.12% (*v*/*v*); air temperature and relative humidity were maintained at 17.5 ± 0.5 °C and 85–90%, respectively, throughout the remainder of the experiment. All experiments were repeated at least twice and, within experiments, triplicate bags were used to generate each data point. In the figures and tables, values are given as the mean and the standard deviations are calculated.

Mushrooms were harvested by hand during the first flush and then frozen at −20 ± 0.5 °C. For determination of mushroom water content, a 1 g sample was oven dried at 105 ± 0.5 °C to constant weight and then measured gravimetrically (with a four-digit Kern AGB balance). Whole carposomes, pilei and stipes were lyophilized (in a HetoLyoLab 3000, Heto-Holten Als, Allered, Denmark) and then ground (in a Janke & Kunkel, IKA-WERK, analytical mill (Staufen, Germany)) for further analysis. Ash was determined using a 1 g sample from the finely ground mushroom placed in a crucible and converted into ash at 550–600 °C for 12 h in a Carbolite CWF 12/23 Laboratory Chamber Furnace (Derbyshire, UK), after which it was allowed to cool in a desiccator. The difference in the weight of the crucible without the sample before and then after ashing was used to calculate the ash content.

2.2. Reagents and Chemicals

Ethylenediaminetetraacetic acid (EDTA), potassium sodium tartrate and all solvents were purchased from AppliChem; 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) and Bradford reagent from Sigma; 3,5-dinitrosalicylic acid from Scharlau; sodium hydroxide from Lachner; and D-fructose from Merck. Folin–Ciocalteu's phenol reagent and potassium sodium tartrate 4-hydrate) were purchased from AppliChem; iron (II) chloride salt from Merck; 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$) radicals from TCI; and 2,2-azinobis(3-ethylbenzothiazoline-6-sulfuric acid) ($ABTS^{\cdot+}$) from Alfa Aesar. The chemical reagents listed above were utilized in spectrophotometric measurements and the absorbents were

measured using a Jasco V-530 UV-Visible spectrophotometer (Tokyo, Japan), except for total protein determination which took place in a 96-cell microplate reader spectrophotometer.

2.3. Total Intra-Cellular Polysaccharide (IPS) Determination

IPS determination was conducted according to Diamantopoulou et al. [64] and Liang et al. [65]. In particular, 20 mL of 2.5 M HCl was used to hydrolyze 0.1 g of dried powder mushrooms at 100 °C for 20 min. The entire mixtures were neutralized to a pH of 7 using 2.5 M NaOH. The DNS assay was then performed on samples that contained total sugars after they had been filtered (through No.2 Whatman filters, Whatman plc, Kent, UK) [66]. The IPS content (expressed as glucose equivalents) was determined, measuring the absorbance at 540 nm. All samples were analyzed in triplicate.

The composition of individual carbohydrates of the produced IPSs was performed by HPLC analysis as described by Diamantopoulou et al. [64]. Filtered aliquots of the neutralized samples with NaOH were analyzed by a Waters Association 600E apparatus 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, while the column temperature was 65 ± 1 °C. Individual simple sugars and sugar alcohols were detected using an RI detector (differential refractometer 410-Waters).

2.4. Quantitative Evaluation of a- and β-Glucan Content

α-glucans content of the fruiting bodies of edible mushrooms was determined using a Yeast Beta-Glucan assay kit (Megazyme, Wicklow, Ireland) following its procedure. Then, β-glucans content was determined by subtracting α-glucans content from total glucans content. Each sample was analyzed in triplicate.

2.5. Total Protein Determination

The crude protein content of dried mushroom species was determined according to the Bradford assay [67]. For this purpose, 50 mg of each sample was extracted in 1.5 mL of 50 mM EDTA (ethylenediaminetetraacetic acid) using an ultrasonic bath, for 60 min at 25 ± 0.5 °C. The mixtures were vortexed thoroughly and centrifuged at 10,000 rpm for 10 min. A total of 10μL of each supernatant was diluted in 240 μL Coomassie Brilliant blue solution and incubated for 10 min at 25 ± 0.5 °C, then compared to the reagent blank; the absorbance was measured at 620 nm using a 96-cell microplate reader spectrophotometer. A standard curve of BSA (0.1–1.5 mg/mL) was made. All samples were analyzed in triplicate.

2.6. Determination of Total Lipids and Fatty Acids

Total lipids were determined by a modified version of the method of Folch [68]. For this purpose, 0.5 g of ground mushroom was suspended in a 10 mL chloroform:methanol (2:1 *v*/*v*) mixture, mixed thoroughly and let stand for 7 days. The solution was then filtrated and the solvents were removed in a rotary evaporator (at 50 ± 0.5 °C) under vacuum (RE 300 evaporator Stuart-RE 300 DB digital water bath). What remained were the crude lipids.

The fatty acid methyl esters preparation was performed in a two-stage reaction (to avoid trans-isomerization) using sodium methoxide and methanol/hydrochloride according to the AFNOR method [69]. Fatty acid methyl esters were identified by reference to authentic standards. For this purpose, methyl esters were suspended in hexane and analyzed by GC in a Varian CP-3800 chromatograph equipped with flame ionization detector (Agilent Technologies, Santa Clara, CA, USA) in which an Agilent J&W Scientific DB23 capillary column (model n.123–2332, 30.0 m × 0.32 mm, film thickness 0.25 μm) (Agilent Technologies, Santa Clara, CA, USA) was used. Helium was used as a carrier gas with a column flow rate of 2.0 mL/min. The set-up conditions were as follows: Initial oven temperature was set at T = 150 °C, held for 18 min, subsequently rammed to T = 185 °C at a rate of 5 °C/min and held for 2 min. Then, the oven temperature was moved to T = 210 °C at a flow rate of 5 °C/min and held for 2 min, then increased to T = 240 °C at 10 °C/min. The injector and flame ionization detector temperatures were set at T = 260 °C and T = 270 °C,

respectively. Individual fatty acid methyl esters were identified by comparison of their retention times with external standard (Supelco 37 Component fatty acid methyl esters Mix, CRM47885) retention times. The content of each fatty acid was expressed as a percentage using the peak area.

2.7. Minerals

The concentrations of the elements Ca, Mg, Na, Fe, Cu, Zn, B and Mn of the mushroom samples were determined following standard protocols for the atomic absorption spectrophotometer (AAS) (SpectrAA 220); Na was determined using a flame photometer 410 (Corning) [70], while P was determined using an LLG-uniSPEC 2 Spectrophotometer, as described by Kalra and Maynard [71].

2.8. Determination of Tocopherols

Tocopherol content was determined according to Barros et al. [72] using BHT (butylhydroxytoluene) (~10 mg/mL) with the samples prior to the extraction procedure. A total of 500 mg of each sample was homogenized with 4 mL of methanol by vortex mixing, and then hexane was added and it was vortexed for another 1 min. After that, 2 mL of saturated NaCl aqueous solution was added, and the mixtures were then homogenized, centrifuged and the upper layer was transferred to a vial. The combined extracts from 3 extractions were dried under a nitrogen stream, redissolved in 1 mL of hexane, dehydrated with sodium sulphate, filtered and transferred into a dark vial for HPLC analysis. The determination of α-, β-, γ-, and δ-tocopherols and tocotrienols was performed according to ISO 9936 [73], using high-performance liquid chromatography with fluorescence detection. In brief, a JASCO HPLC system (JASCO International Co., Ltd., Tokyo, Japan) was used, consisting of a quaternary pump (PU-2089 Plus), an autosampler (AS-1555) and a fluorescence detector (FP-920). Separation was accomplished with a Pinnacle DB Silica column (250 mm × 4.6 mm i.d., 5 µm, Restek, Bellefonte, PA, USA) using isocratic elution with n-Hexane/1,4-Dioxane (97:3 *v*/*v*). The flow rate was set at 1.5 mL/min, and the injection volume was 20 µL. The excitation and emission wavelengths were set at 295 nm and 330 nm, respectively. The content of each tocol was calculated using the calibration factor of a standard solution of (±)-α-tocopherol (Merck, Darmstadt, Germany) and expressed in mg/kg.

2.9. Total Phenolic Compounds and Antioxidant Activity

Methanolic extracts were prepared as follows: 250 mg of fresh mushrooms were extracted with 5 mL of methanol in an ultrasonic bath (SKYMEN, JP-060S, Shenzhen, China) for 15 min at 25 °C, followed by vortex and centrifugation (3500 rpm, 15 min, 25 ± 0.5 °C; Micro 22R, Hettich, Germany). The extraction was repeated three times, and the supernatants were stored at 4.0 ± 0.5 °C for further analysis.

2.9.1. Determination of Total Phenolic Compounds (TPC)

The TPC in the mushroom samples were estimated using the Folin–Ciocalteu assay as described by Slinkard and Singleton [74] and were measured at 760 nm using gallic acid for the standard curve. Briefly, 0.5 mL of each sample (methanolic extract) was diluted in 10.5 mL H_2O and mixed with 8 mL Na_2CO_3 (75g/L) and 1 mL of Folin–Ciocalteu reagent. The mixtures were vortexed and allowed to react in the dark for 2 h. Samples were measured in three replicates. Results were expressed as gallic acid equivalent (GAE) µg/g dry weight (dw) of biomass.

2.9.2. Antioxidant Activity: Ferric Reducing Antioxidant Power (FRAP)

The sample's capacity to convert Fe^{3+} to Fe^{2+} ions is the basis for this method [75]. FRAP working solution was freshly prepared by mixing 25 mL acetate buffer (300 mM/L, pH 3.6), 5 mL TPTZ solution (10 mM/L 2, 4, 6-tripyridyl-s-triazine in 40 mM/L HCl) and 2.5 mL $FeCl_3 \cdot 6H_2O$ (20 mM/L in distilled water) solution. A total of 300 µL of each extract

was added to 2700 µL of FRAP solution and the mixtures were vortexed and incubated at 37 °C for 10 min. The ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex was reduced to the ferrous (Fe^{2+}-TPTZ) form at low pH in the presence of TPTZ (Sigma Aldrich, St. Louis, MO, USA), resulting in a vivid blue color. 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 of dry weight. Samples were measured in three replicates.

2.9.3. Antioxidant Activity: Scavenging Activity of ABTS $^{+}$ Radical

Free radical scavenging activity was determined according to Re et al. [76] with some modifications. $ABTS^{\cdot}$+ [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid] radicals were produced by the reaction between 7 mM $ABTS^{\cdot+}$ in water and 2.45 mM potassium persulfate, stored in the dark at room temperature for 12–16 h before use. Prior to use, the solution was diluted with ethanol to get an absorbance of 0.7000 ± 0.05 at 734 nm. Free radical scavenging activity was assessed by mixing 50 µL of each methanolic sample with 2 mL of the diluted $ABTS^{\cdot+}$ working solution. The mixtures were vortexed and the decrease in absorbance was measured against the blank (2 mL $ABTS^{\cdot+}$ with 50 µL methanol). Trolox was used as calibration standard and the results were expressed as mg trolox equivalents per 1 L of the extract. Samples were measured in three replicates.

2.9.4. Antioxidant Activity: Scavenging Ability on 1.1-Diphenyl-2-Picrylhydrazyl (DPPH) Radicals

The scavenging ability on $DPPH^{\cdot}$ free radicals was determined according to [77]. Briefly, 0.1 mL of each methanolic extract was added to 3.9 mL $DPPH^{\cdot}$ (60 µM in methanol) in test tubes and vortexed. The mixtures were left in the dark for 30 min and the reduction of the $DPPH^{\cdot}$ was determined by measuring the absorbance at 515 nm. $DPPH^{\cdot}$ methanolic solution was used as a blank and the results were expressed as mmol trolox equivalents per 100 g of dry matter. Samples were measured in three replicates.

2.9.5. Statistical Analysis

All experiments were repeated at least twice, and within experiments triplicate bags were used to generate each data point. Statgraphics was used for statistical analysis. The data were compared using analysis of variance (ANOVA) and Pearson's linear correlation at the 5% significance level. Significant differences between means were determined by honest significant difference (HSD-Tukey test) at the level of $p < 0.05$. Data were reported as mean values ± standard deviation of three independent replicates ($p < 0.05$, 95%).

2.9.6. Abbreviations

WS—wheat straw; BOS—barley and oats straw; RB—rice bark; BWS—beech wood shavings; CR—coffee residue; TPC—total phenolic compounds; FRAP—ferric reducing antioxidant power; $ABTS^{\cdot}$+—2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); DPPH—1,1-diphenyl-2-picrylhydrazyl; IPSs—intra-cellular total polysaccharides; FA—fatty acid.

3. Results and Discussion

Chemical composition of carposomes.

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

The total IPS content in *Pleurotus* (Figure 1) and *Agaricus* species (Table 1) revealed that *Pleurotus* spp. (27.51–67.37%, *w*/*w*) contained significantly higher quantities of polysaccharides than those of *Agaricus* (22.46–39.08%, *w*/*w*), and this finding was consistent with previous studies [78–80]. Regarding *Pleurotus* spp., the *P. eryngii* species produced higher amounts of IPSs than those of *P. ostreatus*, in all tested substrates except for BWS, where *P. ostreatus* 150 was the greatest IPS producer (45.84%, *w*/*w*). Particularly, the highest IPS values for the carposome were obtained when *P. ostreatus* 144 and *P. eryngii* 173-6 were

cultivated on CR, whereas WS was the best substrate for *P. ostreatus* 150 and *P. eryngii* 166. It seems, therefore, that substrate composition affected the ability of fungi to produce IPSs and that there was a strain-specific preference for particular substrates. Nevertheless, all substrates and strains supported IPS production greater than 30%, *w*/*w*, but strains 150 and 173-6 performed better in most of the cases. It is worth mentioning that, in all *Pleurotus* species, IPS content was even greater in the stipes than in the pilei. In contrast, *Agaricus* stipes produced lower IPS amounts than the pilei and the carposomes. Moreover, the compost with the lowest C/N ratio favored IPS synthesis in *Agaricus* carposomes, but the IPS concentration was relatively low.

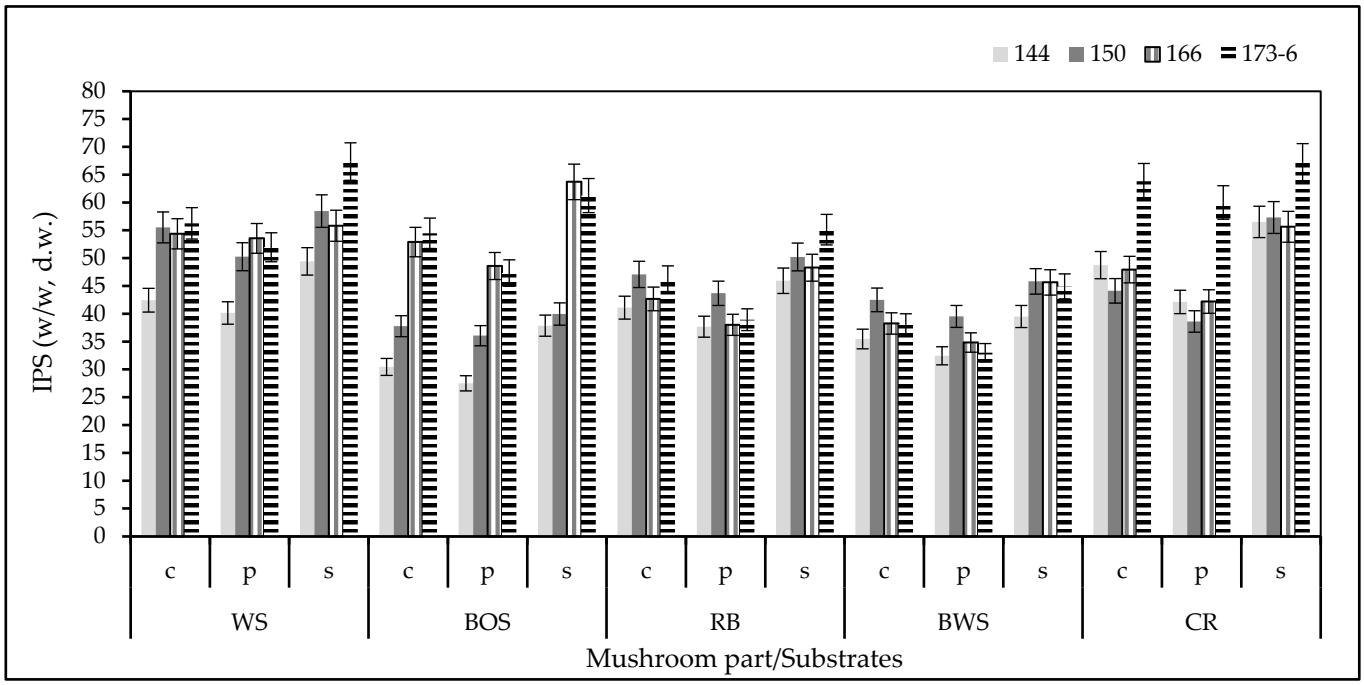

Figure 1. Total intracellular polysaccharide (IPS) content in dry *P. ostreatus* carposomes (c), pilei (p) and stipes (s) of strains AMRL 144 and 150 and in *P. eryngii* carposomes (c), pilei (p) and stipes (s) of strains AMRL 166 and 173-6, cultivated on five substrates (WS—wheat straw, BOS—barley and oats straw, RB—rice bark, BWS—beech wood shavings, CR—coffee residue).

Table 1. Moisture, ash, IPS, total protein and lipid content (% *w*/*w* of dry biomass), TPC, FRAP and scavenging ability on DPPH and ABTS free radicals of methanol extracts of *A. bisporus* (AMRL 209) and *A. subrufescens* (AMRL 235) carposomes (c), pilei (p) and stipes (s) cultivated on two different composts (C/N ratios of 10 and 13). Measurements of antioxidant studies are expressed as mg of gallic acid or trolox equivalence/g of mushroom dry weight (mean ± SD).

Fungi/ Compost		Moisture (%)	Ash (%)	IPSs (%, *w*/*w*)	Proteins (%, *w*/*w*)	Lipids (%, *w*/*w*)	TPC (mg GAE/g)	$DPPH^{\cdot}$ (mg trolox/g)	$ABTS^{\cdot+}$ (mg trolox/g)	FRAP (mg trolox/g)
A. bisporus (C/N = 10)	c	87.64 ± 3.59	9.21 ± 0.86	36.68 ± 0.65	39.58 ± 2.97	2.68 ± 0.10	12.59 ± 1.16	0.96 ± 0.04	1.31 ± 0.21	2.23 ± 0.24
	p	88.65 ± 3.42	9.20 ± 1.08	39.08 ± 0.75	40.51 ± 3.54	2.74 ± 0.22	13.12 ± 0.87	0.99 ± 0.07	1.21 ± 0.10	2.25 ± 0.37
	s	81.95 ± 2.65	9.12 ± 0.96	31.36 ± 1.64	32.04 ± 2.64	2.36 ± 0.14	11.23 ± 0.66	1.04 ± 0.11	1.30 ± 0.06	2.30 ± 0.12
A. bisporus (C/N = 13)	c	89.51 ± 2.55	8.55 ± 0.87	30.46 ± 1.28	36.16 ± 3.50	2.74 ± 0.09	10.49 ± 0.58	0.82 ± 0.09	1.20 ± 0.08	1.85 ± 0.09
	p	87.62 ± 2.13	8.50 ± 0.88	37.30 ± 0.80	38.98 ± 2.70	2.92 ± 0.13	10.80 ± 0.42	0.83 ± 0.04	0.80 ± 0.05	1.82 ± 0.11
	s	80.94 ± 2.11	8.46 ± 1.02	22.46 ± 0.89	29.89 ± 3.28	2.08 ± 0.03	7.85 ± 0.61	0.75 ± 0.02	0.88 ± 0.03	0.74 ± 0.20
A. sub-rufescens (C/N = 10)	c	86.34 ± 2.82	8.11 ± 1.13	32.44 ± 0.32	43.59 ± 2.14	3.54 ± 0.20	13.49 ± 0.65	1.10 ± 0.07	1.58 ± 0.05	2.48 ± 0.18
	p	86.12 ± 2.61	8.06 ± 1.14	30.02 ± 0.21	44.26 ± 1.87	3.70 ± 0.12	13.00 ± 0.47	0.97 ± 0.09	1.51 ± 0.13	2.16 ± 0.09
	s	82.91 ± 2.37	8.12 ± 1.20	27.62 ± 0.51	40.12 ± 1.21	2.66 ± 0.08	12.99 ± 0.52	1.00 ± 0.12	1.61 ± 0.21	2.22 ± 0.07
A. sub-rufescens (C/N = 13)	c	87.58 ± 3.89	8.35 ± 2.08	29.96 ± 0.98	33.22 ± 2.21	3.18 ± 0.26	15.49 ± 0.88	2.10 ± 0.16	1.78 ± 0.08	2.68 ± 0.12
	p	87.62 ± 4.02	8.10 ± 1.24	27.25 ± 0.83	40.50 ± 3.22	3.44 ± 0.18	16.89 ± 0.92	2.97 ± 0.10	1.71 ± 0.09	2.56 ± 0.35
	s	80.34 ± 3.23	8.36 ± 1.02	22.50 ± 1.19	25.40 ± 2.12	2.60 ± 0.25	13.99 ± 0.67	2.00 ± 0.08	0.73 ± 0.06	2.42 ± 0.29

In some previous studies by other researchers, a higher total amount of carbohydrates was detected in *A. bisporus* and *A. brasiliensis* (62.20 and 63.83%, respectively) [80] or in *A. bisporus* (6.46% f.w.) [79] than in this study, but in another study [78] the total carbohydrates in *A. bisporus* were 34.5% *w*/*w*, a value similar to ours. Comparable experimental results have been also previously presented for *P. eryngii*, 49–60% d.w. total IPSs, when it was cultivated on six different types of agro-industrial wastes, and ~42%, *w*/*w*, for *P. ostreatus* and *P. pulmonarius* carposomes cultivated on spent mushroom substrate [43]. Total carbohydrates in *P. ostreatus* and *P. eryngii* were higher (66.40–74.02% and 70.52%, respectively) [80] than in this study. Also, an IPS concentration as high as 66.54% d.w. for *Pleurotus* has been reported [81]. These variations in IPS values may be attributed, apart from the substrate's synthesis, to the use of different strains and the different analytical methodologies adopted. It has been also demonstrated that there is a positive correlation between substrate C/N, cellulose and *P. ostreatus* carbohydrates content [82]. Indeed, the highest IPSs in *P. ostreatus* 150 were detected when it was cultivated on WS, the substrate with the highest ratio C/N and cellulose (analyses presented in [44]), although in the present research *P. ostreatus* 144 achieved its highest IPS value when it was cultivated on CR, the substrate with the lowest ratio C/N and cellulose.

The results from carbohydrates composition analysis are summarized in Table 2. Glucose was the main constituent present in all *Pleurotus* and *Agaricus* carposomes, stipes and pilei (up to 61%, *w*/*w*). Fructose was found in smaller quantities than glucose (10–25%, *w*/*w*) in all samples, whereas other compounds, such as mannitol and arabitol, were detected in lower concentrations (or not at all) in some samples. Polyols (mainly mannitol, trehalose and arabitol) contained in mushrooms contribute to a sweet taste; therefore, their high content would generate a moderately perception to fresh mushrooms and not the typical mushroom taste [83]. Mannitol production is a major feature of the growth of several mushroom carposomes and mycelia [84], while its role and requirements are likely to differ depending on the fungus. Previous researchers showed that glucose, mannitol and trehalose were the main soluble carbohydrates of *A. bisporus* extracts [84], and that glucose was the most abundant among mannose, galactose, fucose, ribose and galactose [85], all monosaccharides that were not detected in the present study. In other studies, mannitol and trehalose were the most abundant monosaccharides of *A. bisporus* and *Pleurotus* spp., respectively [79], and wild *Agaricus* spp. and *P. cystidiosus* contained mainly glucose and rhamnose, following by xylose and mannose, whereas galactose and fructose were detected in very low percentages [86]. Additionally, glucose was the main sugar in the stipes of *P. ostreatus* and *P. eryngii*, while small amounts of galactose and mannose were also detected [87]. In other studies, glucose content was determined up to 80% *w*/*w* for *P. ostreatus* [88,89] and up to 50% *w*/*w* for *A. bisporus* [85]. In general, differences in sugars composition had been observed between cultivated and wild samples of the same mushroom species, probably due to the different cultivation techniques used [79,86].

Table 2. Carbohydrate composition (% *w*/*w*) of total IPSs produced by *P. ostreatus* (AMRL 144, 150) and *P. eryngii* (AMRL 166, 173-6) cultivated in five substrates (WS, BOS, RB, BWS, CR) and by *A. bisporus* (AMRL 209) and *A. subrufescens* (AMRL 235) cultivated on compost of two different C/N ratios (10 and 13). Mushroom parts tested: carposomes (c), pilei (p) and stipes (s).

Substrates/Fungi		Carbohydrates (%, *w*/*w* of Total IPSs)			
		Glucose	**Fructose**	**Mannitol**	**Arabitol**
P. ostreatus					
WS *	144 c	70.5 ± 4.8	20.2 ± 1.7	9.3 ± 0.9	nd **
	144 p	64.2 ± 2.3	25.3 ± 2.3	10.5 ± 0.3	nd
	144 s	84.4 ± 3.9	15.6 ± 1.7	nd	nd
	150 c	72.3 ± 4.2	16.5 ± 0.9	11.2 ± 1.4	nd
	150 p	68.7 ± 4.6	15.3 ± 0.6	10.2 ± 0.8	5.8 ± 0.4
	150 s	85.4 ± 3.8	14.6 ± 1.3	nd	nd

Table 2. *Cont.*

Substrates/Fungi		Carbohydrates (%, *w*/*w* of Total IPSs)			
		Glucose	**Fructose**	**Mannitol**	**Arabitol**
BOS	144 c	72.1 ± 3.9	17.4 ± 0.6	10.5 ± 0.7	nd
	144 p	71.5 ± 4.0	18.7 ± 0.3	9.8 ± 0.4	nd
	144 s	75.4 ± 3.2	10.5 ± 0.1	14.1 ± 1.0	nd
	150 c	73.4 ± 2.1	16.5 ± 0.9	10.1 ± 0.8	nd
	150 p	67.5 ± 1.8	15.8 ± 0.8	9.7 ± 0.4	7.2 ± 0.3
	150 s	82.2 ± 3.4	12.4 ± 1.1	5.4 ± 0.5	nd
RB	144 c	73.5 ± 4.5	13.4 ± 0.2	13.1 ± 0.9	nd
	144 p	74.3 ± 3.3	13.6 ± 0.9	12.1 ± 1.2	nd
	144 s	76.1 ± 2.1	13.3 ± 0.7	10.6 ± 0.7	nd
	150 c	72.4 ± 4.2	12.2 ± 0.4	10.4 ± 0.6	5.6 ± 0.2
	150 p	70.2 ± 3.1	10.7 ± 0.3	9.1 ± 0.8	10.0 ± 0.4
	150 s	70.6 ± 2.8	12.6 ± 0.9	9.7 ± 0.3	7.1 ± 0.3
BWS	144c	73.8 ± 2.5	14.7 ± 0.9	11.5 ± 0.9	nd
	144 p	70.2 ± 2.0	12.4 ± 0.7	17.4 ± 0.8	nd
	144 s	80.5 ± 1.8	12.6 ± 1.2	6.9 ± 0.9	nd
	150 c	69.2 ± 2.7	13.5 ± 0.9	9.3 ± 0.6	8.0 ± 0.5
	150 p	70.4 ± 3.2	13.4 ± 0.8	16.2 ± 0.9	nd
	150 s	72.6 ± 2.9	14.7 ± 0.4	12.7 ± 0.8	nd
CR	144 c	73.9 ± 3.6	15.9 ± 0.7	10.2 ± 1.1	nd
	144 p	72.7 ± 3.8	16.8 ± 1.3	10.5 ± 0.3	nd
	144 s	81.4 ± 3.1	11.4 ± 0.9	7.2 ± 0.8	nd
	150 c	73.6 ± 2.7	14.1 ± 0.2	6.3 ± 0.9	6.0 ± 0.2
	150 p	70.8 ± 1.9	11.3 ± 0.5	9.5 ± 0.3	8.4 ± 0.4
	150 s	80.5 ± 3.9	12.8 ± 0.8	6.7 ± 0.7	nd
P. eryngii					
WS	166 c	78.7 ± 2.7	15.2 ± 1.5	6.1 ± 0.4	nd
	166 p	76.6 ± 4.1	13.3 ± 1.1	10.1 ± 0.9	nd
	166 s	81.6 ± 2.2	11.4 ± 0.9	7.0 ± 0.5	nd
	173-6 c	72.1 ± 3.2	16.7 ± 0.8	11.2 ± 0.8	nd
	173-6 p	70.6 ± 2.7	16.6 ± 1.2	12.8 ± 1.1	nd
	173-6 s	75.2 ± 2.9	14.5 ± 0.5	10.3 ± 0.8	nd
BOS	166 c	74.3 ± 3.1	11.4 ± 0.7	7.3 ± 0.4	7.0 ± 0.5
	166 p	77.6 ± 3.7	13.6 ± 1.1	8.8 ± 0.5	nd
	166 s	82.6 ± 2.8	10.4 ± 0.8	7.0 ± 0.1	nd
	173-6 c	73.6 ± 2.2	15.5 ± 1.6	10.9 ± 1.0	nd
	173-6 p	71.8 ± 2.7	15.9 ± 0.7	12.3 ± 1.3	nd
	173-6 s	75.7 ± 3.6	14.6 ± 1.3	9.7 ± 0.6	nd
RB	166 c	75.3 ± 4.2	13.5 ± 0.8	11.2 ± 0.4	n.d
	166 p	68.7 ± 3.7	15.5 ± 0.4	15.8 ± 1.4	n.d
	166 s	80.3 ± 3.9	10.5 ± 0.9	9.2 ± 0.2	n.d
	173-6 c	72.6 ± 2.2	12.7 ± 1.4	14.7 ± 0.8	n.d
	173-6 p	69.9 ± 3.5	13.4 ± 1.4	16.7 ± 1.7	n.d
	173-6 s	78.8 ± 4.5	12.6 ± 1.2	8.6 ± 0.9	n.d
BWS	166 c	76.6 ± 3.1	12.6 ± 0.7	10.8 ± 0.4	n.d
	166 p	73.2 ± 3.8	10.0 ± 0.5	7.6 ± 0.2	9.2 ± 0.6
	166 s	79.9 ± 2.4	12.2 ± 0.9	7.9 ± 0.2	n.d
	173-6 c	75.6 ± 2.8	13.4 ± 1.0	11.0 ± 1.1	n.d
	173-6 p	68.5 ± 1.2	13.7 ± 2.4	8.2 ± 0.6	9.6 ± 0.1
	173-6 s	78.6 ± 2.4	12.2 ± 2.1	9.2 ± 0.3	n.d

Table 2. *Cont.*

Substrates/Fungi		Carbohydrates (%, *w*/*w* of Total IPSs)			
		Glucose	**Fructose**	**Mannitol**	**Arabitol**
CR	166 c	73.9 ± 2.9	15.8 ± 1.1	10.3 ± 0.7	n.d
	166 p	65.4 ± 2.1	16.6 ± 1.4	8.4 ± 0.4	9.6 ± 0.8
	166 s	79.9 ± 4.3	10.6 ± 0.8	9.5 ± 0.9	n.d
	173-6 c	72.6 ± 2.7	13.6 ± 1.3	13.8 ± 0.7	n.d
	173-6 p	70.1 ± 2.6	9.7 ± 0.6	10.7 ± 0.9	9.5 ± 0.5
	173-6 s	78.1 ± 3.0	12.4 ± 1.1	9.5 ± 0.6	n.d
Agaricus spp.					
A. bisporus (C/N = 10)	c	63.3 ± 3.6	23.2 ± 0.4	13.5 ± 0.9	nd
	p	63.5 ± 2.2	24.2 ± 2.3	12.3 ± 0.6	nd
	s	67.4 ± 3.2	23.3 ± 0.9	9.3 ± 0.3	nd
A. bisporus (C/N = 13)	c	63.8 ± 4.2	22.9 ± 1.6	13.3 ± 0.8	nd
	p	61.3 ± 2.7	21.5 ± 1.8	9.1 ± 0.8	8.1 ± 0.7
	s	65.4 ± 2.9	23.9 ± 2.3	10.7 ± 0.4	nd
A. subrufescens (C/N = 10)	c	65.5 ± 2.4	10.4 ± 1.3	13.5 ± 0.7	10.6 ± 0.9
	p	64.4 ± 3.8	12.0 ± 0.5	11.9 ± 0.4	11.7 ± 0.4
	s	67.1 ± 3.1	22.7 ± 0.8	10.2 ± 0.2	nd
A. subrufescens (C/N = 13)	c	64.2 ± 3.4	11.5 ± 1.7	15.1 ± 0.6	9.0 ± 0.5
	p	63.9 ± 2.7	11.9 ± 1.2	12.3 ± 0.4	12.4 ± 1.1
	s	65.1 ± 1.9	10.4 ± 0.9	9.5 ± 0.7	14.9 ± 0.6

* WS (wheat straw), BOS (barley and oats straw), RB (rice bark), BWS (beech wood shavings), CR (coffee residue); ** nd: not detected.

3.2. Total Glucans, α-Glucans and β-Glucans

The total α- and β- glucan contents in dry mushroom carposomes, pilei and stipes are presented in Table 3. All examined *Pleurotus* species, cultivated on different substrates, were found to contain high amounts of total glucans, ranging from 21.47 to 64.21% (*w*/*w* of total IPSs), with the percentage of β-glucans predominating. WS and CR substrates favored IPS concentration and consequently the glucans production of all *Pleurotus* strains, as well as BOS in the case of *P. eryngii*. It is interesting that stipes of all species contained significantly greater amounts of total glucans and β-glucans than carposomes or pilei, and this was more pronounced for *P. ostreatus* cultivated on WS and CR, as well as for *P. eryngii* on WS and BOS. Also, there was a positive correlation between IPS content and total glucans, as the stipes with the highest IPS content had the highest total glucans content, too. Comparing *P. ostreatus* with *P. eryngii* strains, those of *P. eryngii* seemed to produce slightly greater amounts of total glucans and have higher β-glucan content than the *P. ostreatus* ones, apart from BWS. Among the *P. ostreatus* fungi, strain 150 (commercial) performed better than 144, while glucan production proved to be substrate-dependent for both *P. eryngii* strains, a tendency that had already been detected in previous studies [90,91]. Regarding *Agaricus* spp., our data showed that they contained much lower total glucans than *Pleurotus* strains, ranging from 10.12 to 24.39% (*w*/*w* of total IPSs), and no significant difference was detected between species or between species and C/N ratio imposed. Manzi and Pizzoferrato [92] reported significantly lower β-glucan content in *P. ostreatus* and *P. eryngii*, which ranged from 0.22 to 0.38%. The results of the present study were close to those reported by Bekiaris et al. [93], who recorded 38.84–58.90% and 32.84–61.40% d.w. total glucan content for *P. ostreatus* and *P. eryngii*, respectively, after having been cultivated on WS, whereas β-glucans content ranged from 26.44% to 51.36% d.w. for both *Pleurotus* species.

Table 3. Total glucan, α- and β-glucan content (% *w*/*w*) of total IPSs produced by *P. ostreatus* (AMRL 144, 150) and *P. eryngii* (AMRL 166, 173-6) cultivated in five substrates (WS, BOS, RB, BWS, CR) and by *A. bisporus* (AMRL 209) and *A. subrufescens* (AMRL 235) cultivated on compost of two different C/N ratios (10 and 13). Mushroom parts tested: carposomes (c), pilei (p) and stipes (s).

Substrates/Fungi		Glucans (%, *w*/*w* of Total IPSs)		
		Total	**α-Glucans**	**β-Glucans**
P. ostreatus				
WS *	144 c	36.28 ± 3.65	10.39 ± 2.65	25.89 ± 2.87
	144 p	38.23 ± 3.50	12.41 ± 2.40	25.82 ± 3.54
	144 s	46.35 ± 2.87	4.14 ± 0.65	42.21 ± 3.24
	150 c	47.94 ± 2.67	12.73 ± 1.50	35.22 ± 2.70
	150 p	49.61 ± 3.14	11.25 ± 1.25	38.36 ± 2.65
	150 s	56.51 ± 4.35	12.36 ± 1.64	44.15 ± 3.40
BOS	144 c	31.43 ± 3.54	5.79 ± 0.47	25.64 ± 3.35
	144 p	21.47 ± 3.50	3.24 ± 0.27	18.23 ± 2.64
	144 s	35.54 ± 2.98	8.23 ± 1.02	27.31 ± 3.50
	150 c	37.08 ± 3.40	10.72 ± 1.60	26.36 ± 3.60
	150 p	33.64 ± 3.21	9.23 ± 1.02	24.41 ± 3.40
	150 s	38.12 ± 2.67	12.70 ± 1.84	25.42 ± 4.02
RB	144 c	39.33 ± 2.56	7.10 ± 1.01	32.23 ± 2.50
	144 p	32.06 ± 2.41	4.54 ± 1.30	27.52 ± 2.60
	144 s	44.36 ± 2.60	9.32 ± 1.23	35.04 ± 3.40
	150 c	44.39 ± 2.41	8.23 ± 1.30	36.16 ± 3.21
	150 p	43.13 ± 2.50	7.93 ± 1.08	35.20 ± 3.40
	150 s	48.65 ± 3.02	9.29 ± 1.20	39.36 ± 2.97
BWS	144 c	34.21 ± 2.54	4.03 ± 1.02	30.18 ± 2.50
	144 p	31.91 ± 2.60	4.65 ± 1.20	27.26 ± 2.60
	144 s	44.55 ± 3.20	6.32 ± 1.54	38.23 ± 2.40
	150 c	38.44 ± 2.31	5.81 ± 1.34	32.63 ± 2.64
	150 p	35.90 ± 2.40	5.63 ± 1.40	30.27 ± 2.60
	150 s	44.08 ± 3.20	6.04 ± 1.50	38.04 ± 3.04
CR	144 c	40.84 ± 3.41	11.06 ± 1.58	29.78 ± 2.80
	144 p	37.97 ± 2.65	11.72 ± 1.47	26.25 ± 2.64
	144 s	49.59 ± 2.70	11.38 ± 1.80	38.21 ± 2.70
	150 c	41.05 ± 2.15	15.38 ± 1.40	25.67 ± 3.04
	150 p	37.12 ± 2.34	14.32 ± 1.65	22.80 ± 3.14
	150 s	53.53 ± 3.41	16.89 ± 1.76	36.64 ± 2.64
P. eryngii				
WS	166 c	49.30 ± 3.08	7.70 ± 1.30	41.60 ± 2.64
	166 p	48.50 ± 2.74	10.25 ± 1.58	38.25 ± 2.58
	166 s	53.63 ± 3.70	10.29 ± 1.36	43.34 ± 2.70
	173-6 c	52.51 ± 3.30	10.71 ± 1.80	41.80 ± 2.87
	173-6 p	50.51 ± 3.51	10.50 ± 1.56	40.01 ± 2.70
	173-6 s	64.21 ± 3.45	14.26 ± 1.47	49.95 ± 2.95
BOS	166 c	48.75 ± 3.34	8.77 ± 1.54	39.98 ± 2.85
	166 p	47.77 ± 2.65	9.52 ± 1.63	38.25 ± 2.95
	166 s	61.92 ± 3.66	14.52 ± 1.80	47.40 ± 2.85
	173-6 c	52.36 ± 2.60	10.68 ± 1.65	41.68 ± 2.97
	173-6 p	46.69 ± 2.54	9.65 ± 1.02	37.04 ± 2.75
	173-6 s	55.82 ± 2.40	13.11 ± 2.34	42.71 ± 2.64

Table 3. *Cont.*

Substrates/Fungi		Glucans (%, *w*/*w* of Total IPSs)		
		Total	**α-Glucans**	**β-Glucans**
RB	166 c	38.02 ± 2.64	8.17 ± 1.25	29.88 ± 3.50
	166 p	35.37 ± 2.37	8.03 ± 1.60	27.34 ± 2.65
	166 s	47.12 ± 2.40	8.87 ± 1.41	38.25 ± 3.40
	173-6 c	38.76 ± 2.30	6.73 ± 1.34	32.04 ± 2.50
	173-6 p	31.91 ± 2.41	2.94 ± 0.50	28.97 ± 3.04
	173-6 s	51.98 ± 3.66	6.84 ± 0.94	45.14 ± 2.94
BWS	166 c	37.69 ± 2.68	9.84 ± 1.25	27.85 ± 2.85
	166 p	36.06 ± 2.57	9.01 ± 1.30	27.05 ± 2.60
	166 s	42.92 ± 3.50	12.52 ± 1.54	30.40 ± 3.24
	173-6 c	37.88 ± 3.24	11.23 ± 1.80	26.65 ± 3.05
	173-6 p	30.25 ± 3.60	10.01 ± 1.20	20.24 ± 3.14
	173-6 s	40.47 ± 3.50	11.24 ± 1.34	29.23 ± 2.90
CR	166 c	45.04 ± 2.41	7.70 ± 0.90	37.44 ± 3.40
	166 p	40.86 ± 2.05	7.65 ± 0.67	33.21 ± 4.02
	166 s	52.15 ± 2.47	8.84 ± 1.02	43.31 ± 4.51
	173-6 c	58.34 ± 2.64	13.30 ± 1.80	45.04 ± 3.70
	173-6 p	57.34 ± 2.07	12.20 ± 1.60	45.14 ± 3.51
	173-6 s	62.59 ± 2.32	14.26 ± 1.54	48.33 ± 2.84
Agaricus spp.				
A. bisporus (C/N = 10)	c	15.03 ± 1.64	4.23 ± 1.23	10.80 ± 1.57
	p	10.26 ± 1.37	2.04 ± 0.56	8.22 ± 1.20
	s	24.39 ± 1.25	8.47 ± 1.40	13.91 ± 1.60
A. bisporus (C/N = 13)	c	12.27 ± 1.20	2.27 ± 0.24	10.00 ± 1.23
	p	10.12 ± 1.41	2.10 ± 0.65	8.02 ± 1.05
	s	20.10 ± 1.26	7.24 ± 1.32	12.86 ± 1.60
A. subrufescens (C/N = 10)	c	15.89 ± 1.30	4.32 ± 0.52	11.57 ± 1.40
	p	12.13 ± 1.14	2.12 ± 0.65	10.01 ± 1.54
	s	15.74 ± 1.65	2.13 ± 1.05	12.61 ± 2.04
A. subrufescens (C/N = 13)	c	14.58 ± 2.11	4.11 ± 0.09	11.02 ± 1.89
	p	12.04 ± 1.09	2.00 ± 0.65	9.87 ± 1.32
	s	14.97 ± 1.42	1.98 ± 0.05	11.79 ± 1.87

* WS (wheat straw), BOS (barley and oats straw), RB (rice bark), BWS (beech wood shavings), CR (coffee residue).

The presence of β-glucans in mushrooms is important as they have a plethora of functional and bioactive properties, such as anti-cancer and antioxidant effects and activities against infectious diseases [54–57,94]. The high amounts of β-glucans in the mushrooms of the present study would render them a source of highly nutritious food. According to the above results, all mushroom stipes had high values of total glucans and consequently were rich in β-glucans. Considering that a piece of mushroom stipes is usually removed/discarded before packaging, due to dirt from the substrate and the difficulty for them to be swallowed and absorbed by some consumers, stipes could be used as a β-glucan source in a biorefinery scheme aiming to valorize these residues, for example, in the development of medicinal compounds and nutraceuticals [95,96], or by adding them as supplements to new mushroom crops. Similar studies conducted on *Lentinula edodes* (pileus and stipe) showed that the fiber content of the pileus was lower than that of the stipe, and therefore stipes contained higher amounts of β-glucans than the rest of the mushroom [97], too. As for fungi cultivation substrates other than those used in our study, fiber production in *G. lucidum* mushrooms seemed to be influenced variously when cultivated on soybean hulls and soybean and corn residues and the soybean residue increased to a large extent the β-glucan production compared to the soybean hulls and corn residue [90]. It was also

shown that the stalks of *P. eryngii* species contained higher glucans content than the caps, and that the use of olive mill waste as substrate increased α-glucan levels [98]. It is therefore possible to increase the β-glucans content simply by selecting the substrate formulation.

3.3. Total Protein

The genus *Pleurotus* is considered to be a good protein source, but it seems that *Agaricus* species have satisfactory amounts of protein, too, comparing them to those reported in the literature. The total protein contents present in the carposome and also in the pileus and stipe of the *Pleurotus* and *Agaricus* mushrooms tested in this study are shown in Figure 2 and Table 1.

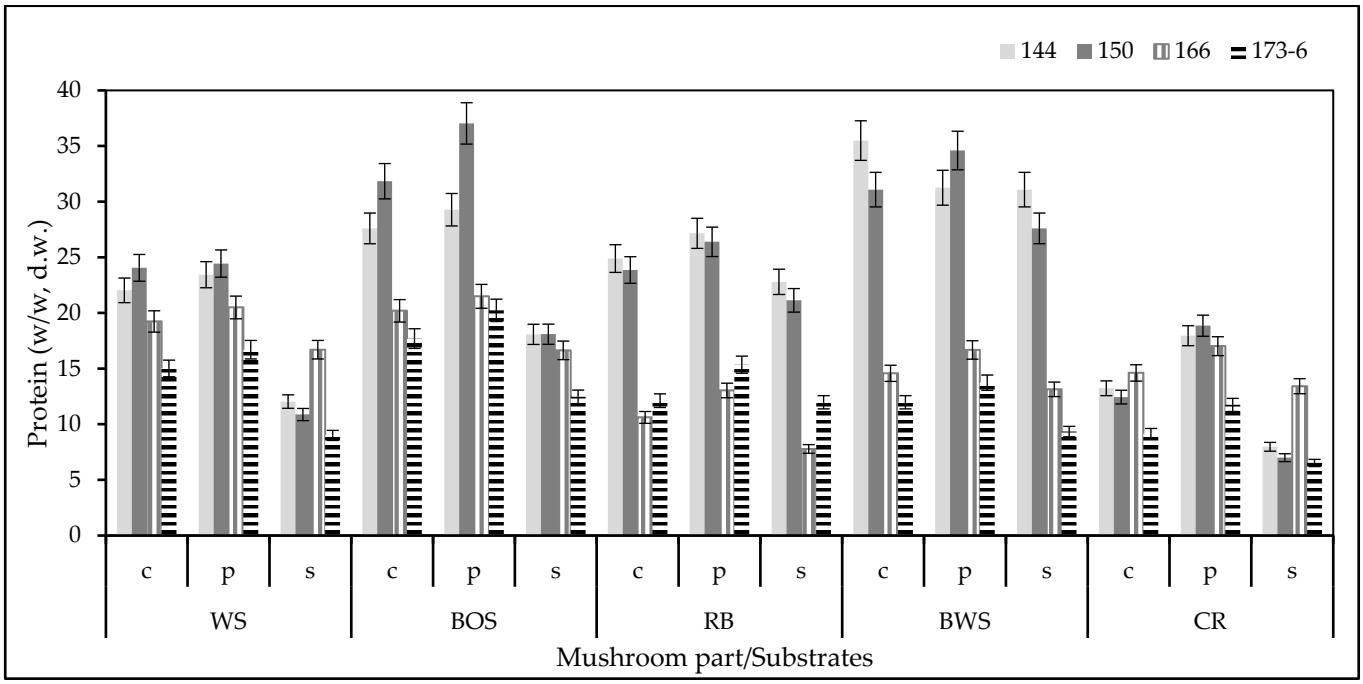

Figure 2. Total protein concentration in dry *P. ostreatus* carposomes (c), pilei (p) and stipes (s) of strains AMRL 144 and 150 and in *P. eryngii* carposomes (c), pilei (p) and stipes (s) of strains 166 and 173-6, cultivated on five substrates (WS—wheat straw, BOS—barley and oats straw, RB—rice bark, BWS—beech wood shavings, CR—coffee residue).

In general, *P. ostreatus* strains had enhanced total protein values compared to *P. eryngii* ones. High protein amounts were found in *P. ostreatus* species cultivated on BOS and BWS (18.08–37.04%, *w*/*w*) substrates and in *P. eryngii* (9.00–21.50%, *w*/*w*) mushrooms cultivated on BWS, WS and BOS. Also, in the stipes, smaller amounts of protein were detected than in the pilei, in all species and substrates examined. Variations in the protein values were observed not only among *Pleurotus* genera, but also between strains cultivated in BOS and BWS, as well as in WS and CR substrates for *P. eryngii*. For example, the maximum protein value was recorded in the pileus of strain 150 (37.04%, *w*/*w*) and the minimum in the stipe of strain 173-6 (9.00%, *w*/*w*). Mushroom protein content varies greatly depending on intrinsic (e.g., strain) and agro-climatic conditions, as well as on culture configurations (the composition of the substrate, the harvest-time size of the pileus). Hoa et al. [99] reported that the protein contents of *P. ostreatus* cultivated in different substrate formulas, including sawdust, corncobs and sugarcane bagasse, as well as mixtures of sawdust and corncobs or sawdust and bagasse, were within the range of 19.52–29.70%, while for *P. cystidiosus* the protein was about 15.68–24.54% using the same substrates. Tolera and Abera [100] found 25.91% of crude protein in dried *P. ostreatus* mushrooms grown on cottonseed waste. Regarding *P. eryngii* species, low values of crude protein were detected during their cultivation in WS substrate, whereas the enrichment of the substrate with

rice bran and cotton stalk increased the protein content [101]. Reports of much increased protein concentrations in these mushroom species are exceptional and come as a result of growth on nitrogen-rich substrates [102,103], such as spent beer grains supplemented with large amounts of bran, or wheat straw supplemented with sugar beet, for *P. ostreatus* [104]. In previous studies, sugarcane bagasse was used as substrate and enhanced the protein production of *P. ostreatus* [105], while the highest crude protein value for *P. eryngii* in one study was obtained from using cotton waste, as compared to carposomes cultivated on wheat straw, rice straw, corn cobs, sugarcane bagasse and sawdust [106]. On the other hand, in more recent research, a weak correlation ($r^2 > 0.433$) was found between substrate nitrogen and mushroom protein levels [82]. According to our earlier work [44], a similar tendency was also detected, particularly in the case of *P. ostreatus*, where the maximum protein concentration was recorded in BWS, which had a greater nitrogen content (1.49%, *w*/*w*) than the other substrates (WS, BOS and RB). Despite the fact that the CR substrate had a high nitrogen content and a low C/N ratio, as compared to the C/N ratio of BWS [44], the mushrooms produced were not protein-rich. A comparable finding was obtained in a study where one specific substrate (almond and walnut shells 1:1 *w*/*w*) with low nitrogen concentration yielded mushrooms with the greatest crude protein content, diminishing the correlation value [82]. Concerning *Agaricus* species, the total protein content was increased in all mushroom parts at a lower C/N ratio that contained higher nitrogen concentration, while the maximum value was detected in the *A. subrufescens* pileus (44.26%, d.w.) at the C/N = 10 ratio. The stipes was found also to have the lowest protein content which, however, was as high as 40.12% *w*/*w*. In contrast to our results, studies conducted using *A. bisporus* strains and two composts based on wheat straw and waste tea leaves (the percentages of the N content of the composts were arranged to be 0.5 and 2.3%, respectively) showed no significant difference in the protein content [40,104]. These results indicated that the protein content of carposomes is affected by the amount and the kind of nitrogen source present in the substrate [107].

3.4. Total Lipid Determination and Fatty Acid (FA) Composition

As shown in Figure 3, the *Pleurotus* species presented the highest amounts of lipid content; *P. ostreatus* produced more lipids than *P. eryngii* species, and both presented the highest lipid content on CR and the lowest on the WS and BOS substrates. In *P. ostreatus* carposomes, the lipid content varied from 2.17 (referring to 144 on BWS) to 8.70% (referring to 150 on CR) of dry weight; it ranged from 1.52 (referring to 166 on WS) to 4.28% (referring to 173-6 on CR) for *P. eryngii*. This considerable variance in the lipid content of *Pleurotus* could be attributable to the agro-waste utilized in the production process. In the case of *Agaricus* species (Table 1), the lipid content ranged from 2.08 (*A. bisporus*, C/N = 13) to 3.54% of dry weight (*A. subrufescens*, C/N = 10). Stipes showed lower total lipid concentration than pilei in all species and substrates. This was consistent with the results of the comparative study conducted earlier on *A. bisporus* [108] that examined the differences in the nutritional characteristics between pilei and stipes and revealed higher values of lipids in pilei (2.48% *w*/*w*, in d.w.) than in the corresponding stipes (2.00% *w*/*w*, in d.w.). According to Crisan and Sands [109], most carposomes contain 1.1–8.3%, *w*/*w*, of lipids, with a mean of ~4.0%, *w*/*w*. In a generalized lipid content survey of *Pleurotus* species, the values ranged from 1.18 to 4.40% of dry weight for *P. ostreatus* grown on a variety of substrates (wheat straw, corncobs, maze straw, etc.) and 5.97%, *w*/*w*, for *P. eryngii* cultured in wheat stalk, while Alam et al. [110] recorded 4.6%, *w*/*w*, lipid content on dried mushrooms, and 0.68% on fresh mushrooms. In the case of *A. bisporus* [83], 3.34–3.75% lipid content in d.w. was reported when it was grown in compost and/or casings enriched with safflower oil, values higher than our data, whereas Teklit [111] detected 2.12% lipid content in d.w. Lower lipid content has been registered for *A. blazei* compared to our results, as values of 2.62 and 1.85%, *w*/*w* in d.w., were recorded, respectively [112,113]. The lipid content of the examined mushrooms in the present study appeared to be within the reported range.

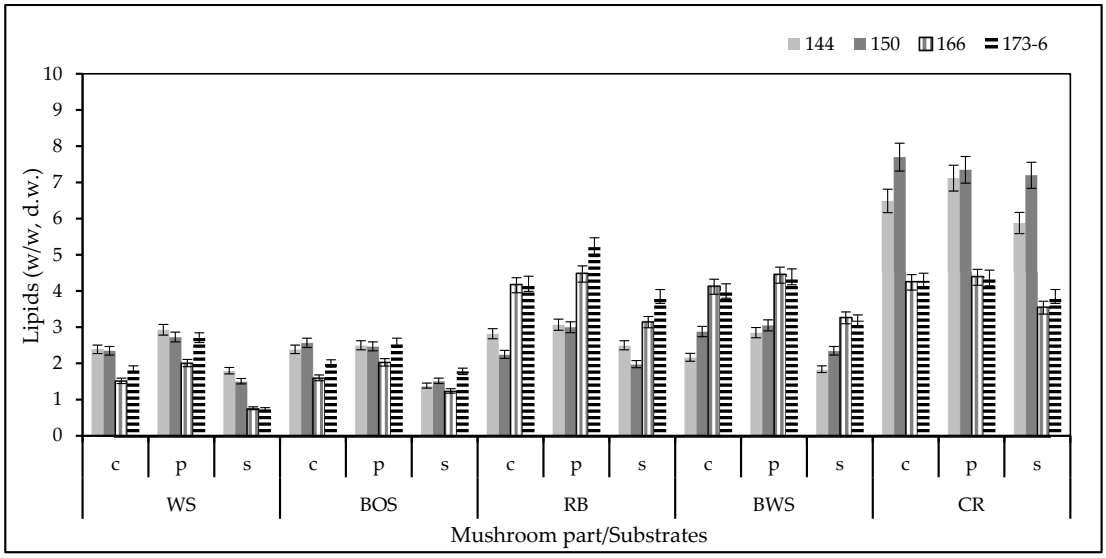

Figure 3. Total lipid content in dry *P. ostreatus* carposomes (c), pilei (p) and stipes (s) of strains AMRL 144 and 150 and in *P. eryngii* carposomes (c), pilei (p) and stipes (s) of strains 166 and 173-6, cultivated on five substrates (WS—wheat straw, BOS—barley and oats straw, RB—rice bark, BWS—beech wood shavings, CR—coffee residue).

The distribution of FAs differed among genera, as well as between species within the same genus (Table 4). Lipid unsaturation was greater in the samples of *P. ostreatus*, followed by *P. eryngii*, *A. bisporus* and *A. subrufescens*. The concentration of saturated FAs varied from 0.08 to 13.1% (*w*/*w*), and it was significantly lower than that of the PUFA ones that ranged from 52.9 to 72.4%**.** This was in agreement with observations that PUFAs predominate over saturated ones in mushroom mycelia and carposomes [19,114,115]. Linoleic acid (C18:2) was found to be the most abundant FA among the species examined, followed by oleic acid (C18:1). Palmitic acid (C16:0) was the third and the second main FA for *Pleurotus* and *Agaricus* species, respectively (10.7–13.9%, *w*/*w*).

Aside from the three primary FAs already mentioned, seven others were identified and quantified (myristic, pentadecylic, ginkgolic, stearic, arachidic, behenic and lignoceric acids). The unsaturation index (U.I.) of FAs was high and increased in the RB substrate for *P. ostreatus* AMRL 144 (U.I. = 154), as well as for *A. subrufescens* in the compost of C/N = 13 (U.I. = 133). In *P. eryngii* species, PUFAs were found in lower concentrations (53.2–59.9%, *w*/*w*) than in *P. ostreatus* and *Agaricus* species (64.3–72.6%, *w*/*w*). The major FA found in all samples was linoleic acid (C18:2), whereas oleic acid (C18:1) was identified at much lower concentrations in *Agaricus* species (~1%, *w*/*w*). Therefore, all species examined, even if they were from different species, had similar FA profiles concerning the content of the main FAs. It has been also reported by other researchers that linoleic acid was the preponderant fatty acid in *P. ostreatus*, *A. bisporus* and many wild mushrooms [5,116]. High amounts of linoleic acid have been found not only in *P. ostreatus* (65.29%), but also in the fruit bodies of *Lactarius salmonicolor* (59.44%) and *Flammulina velutipes* (40.87%), as compared to other FAs [19]. Nevertheless, linoleic acid is a precursor of octen-3-ol, responsible for the attractive smell of (dried) mushrooms [83]. On the other hand, oleic acid was the predominant FA of oyster mushrooms grown on gmelina wood waste [117,118]. Although the nutritional contribution of mushroom lipids is limited for human diets, due to their low total lipid content and the low concentration of the desirable n-3 fatty acids, the unsaturation of lipids contained in mushrooms is a positive fact. Increasing the ratio of unsaturated fatty acids in the diet is important because it leads to an increase in HDL levels, known as good cholesterol, and a decrease in LDL levels, known as bad cholesterol [119], and mushrooms produced even in non-conventional substrates, as examined in the present study, can help achieve that.

Table 4. Fatty acid composition (% *w*/*w*) of total lipids produced by *P. ostreatus* carposomes (strains AMRL 144 and 150) and *P. eryngii* (strains AMRL 166 and 173-6) cultivated on five substrates (WS, BOS, RB, BWS, CR) and by *A. bisporus* (AMRL 209) and *A. subrufescens* (AMRL 235) cultivated on two composts (C/N ratios of 10 and 13). Each point is the mean value of three independent measurements (mean ± SD).

Substrate		C14:0	C15:0	C15:1	C16:0	C18:0	C18:1	C18:2	C20:0	C22:0	C24:0	Other	Poly-unsaturated	U.I. ***
P. ostreatus														
WS *	144	0.26 ± 0.08	0.98 ± 0.05	0.14 ± 0.05	11.1 ± 1.33	0.95 ± 0.04	9.62 ± 1.04	67.3 ± 3.65	0.14 ± 0.03	0.29 ± 0.05	0.84 ± 0.07	2.66	67.6	1.44
	150	0.24 ± 0.06	0.69 ± 0.07	0.12 ± 0.05	11.3 ± 1.00	1.12 ± 0.05	9.50 ± 1.22	69.3 ± 3.05	0.10 ± 0.02	0.22 ± 0.04	0.51 ± 0.04	2.77	69.5	1.48
BOS	144	0.27 ± 0.06	1.14 ± 0.10	0.26 ± 0.06	12.1 ± 1.08	0.95 ± 0.03	8.32 ± 1.06	68.3 ± 2.78	nd	0.42 ± 0.04	nd	2.42	68.5	1.45
	150	0.30 ± 0.08	0.93 ± 0.08	0.11 ± 0.06	12.1 ± 1.40	1.30 ± 0.04	6.90 ± 1.24	68.3 ± 2.79	0.18 ± 0.03	0.36 ± 0.05	0.57 ± 0.05	2.32	68.5	1.44
RB	144	0.24 ± 0.07	0.75 ± 0.07	nd **	11.6 ± 1.58	0.84 ± 0.05	8.90 ± 1.31	72.4 ± 3.51	nd	nd	0.13 ± 0.03	2.16	72.6	1.54
	150	0.52 ± 0.09	0.57 ± 0.08	nd	13.1 ± 0.08	1.68 ± 0.05	8.60 ± 1.56	66.5 ± 3.07	nd	0.33 ± 0.05	0.26 ± 0.03	2.83	66.9	1.42
BWS	144	0.26 ± 0.05	1.25 ± 0.09	0.59 ± 0.09	12.3 ± 1.65	1.18 ± 0.06	8.02 ± 1.34	67.8 ± 2.71	0.14 ± 0.02	0.30 ± 0.04	1.15 ± 0.04	3.24	68.8	1.44
	150	0.27 ± 0.07	1.34 ± 0.11	0.55 ± 0.06	12.0 ± 1.27	1.21 ± 0.05	7.97 ± 1.64	67.5 ± 2.90	0.15 ± 0.03	0.35 ± 0.05	1.28 ± 0.06	3.19	68.1	1.43
CR	144	0.40 ± 0.02	1.09 ± 0.10	0.29 ± 0.03	10.7 ± 1.32	0.69 ± 0.03	7.59 ± 0.02	72.0 ± 2.84	nd	0.11 ± 0.04	0.22 ± 0.05	2.97	72.4	1.45
	150	0.43 ± 0.05	0.74 ± 0.08	0.19 ± 0.06	11.6 ± 1.04	1.21 ± 0.06	10.6 ± 1.74	68.4 ± 2.64	nd	0.12 ± 0.04	0.25 ± 0.06	2.02	68.6	1.48
P. eryngii														
WS	166	0.53 ± 0.06	2.00 ± 0.10	0.14 ± 0.02	12.8 ± 1.41	1.65 ± 0.07	20.7 ± 2.22	56.2 ± 3.01	0.10 ± 0.02	nd	0.24 ± 0.06	2.86	56.4	1.33
	173-6	0.32 ± 0.05	1.21 ± 0.09	nd	11.9 ± 1.05	2.05 ± 0.05	25.6 ± 2.16	52.9 ± 2.47	0.21 ± 0.03	0.23 ± 0.05	0.82 ± 0.06	1.67	53.2	1.31
BOS	166	0.49 ± 0.06	1.66 ± 0.10	nd	11.7 ± 1.22	1.61 ± 0.06	18.7 ± 2.40	59.1 ± 3.04	0.08 ± 0.01	0.21 ± 0.04	0.42 ± 0.05	2.27	59.9	1.37
	173-6	0.34 ± 0.07	1.10 ± 0.09	0.20 ± 0.05	11.5 ± 1.37	1.90 ± 0.04	22.2 ± 1.65	55.7 ± 3.56	0.39 ± 0.02	0.23 ± 0.05	0.84 ± 0.06	2.02	56.2	1.34
RB	166	0.35 ± 0.07	1.52 ± 0.08	0.19 ± 0.05	12.5 ± 0.09	1.57 ± 0.06	22.4 ± 1.54	55.6 ± 3.54	0.18 ± 0.04	0.24 ± 0.04	0.85 ± 0.05	2.54	57.4	1.34
	173-6	0.47 ± 0.05	1.64 ± 0.04	0.23 ± 0.05	11.9 ± 1.03	1.63 ± 0.05	20.9 ± 1.34	55.4 ± 3.67	0.22 ± 0.05	0.25 ± 0.05	0.87 ± 0.04	2.37	56.8	1.32
BWS	166	0.33 ± 0.06	1.67 ± 0.05	0.32 ± 0.06	12.7 ± 1.10	1.85 ± 0.06	21.3 ± 1.50	58.7 ± 4.02	0.17 ± 0.03	nd	0.86 ± 0.03	2.70	57.2	1.39
	173-6	0.37 ± 0.05	1.57 ± 0.06	0.10 ± 0.03	11.5 ± 1.08	1.73 ± 0.04	22.7 ± 1.70	57.3 ± 4.01	0.20 ± 0.04	0.21 ± 0.03	0.78 ± 0.04	1.90	57.7	1.37
CR	166	0.54 ± 0.07	1.60 ± 0.07	nd	11.3 ± 0.90	1.93 ± 0.06	22.4 ± 2.14	53.4 ± 3.61	0.27 ± 0.06	nd	0.54 ± 0.06	2.45	55.7	1.29
	173-6	0.57 ± 0.08	1.47 ± 0.04	nd	12.6 ± 0.84	2.08 ± 0.05	23.5 ± 1.70	56.7 ± 3.40	0.34 ± 0.04	0.20 ± 0.04	0.47 ± 0.04	1.60	57.1	1.37
A. bisporus														
C/N = 10		0.49 ± 0.08	0.55 ± 0.05	0.20 ± 0.02	10.9 ± 1.04	3.68 ± 0.65	1.36 ± 0.06	69.3 ± 3.20	1.82 ± 0.03	1.16 ± 0.05	1.22 ± 0.04	1.94	70.0	1.40
C/N = 13		0.60 ± 0.07	0.76 ± 0.05	1.64 ± 0.08	12.6 ± 1.51	3.13 ± 0.10	0.93 ± 0.05	67.8 ± 3.23	1.40 ± 0.03	1.03 ± 0.06	0.19 ± 0.03	1.62	68.1	1.38
A. subrufescens														
C/N = 10		0.43 ± 0.03	0.71 ± 0.04	nd	12.4 ± 1.00	2.91 ± 0.07	1.68 ± 0.06	63.3 ± 2.80	1.60 ± 0.03	3.40 ± 0.12	1.97 ± 0.05	3.90	64.3	1.28
C/N = 13		0.51 ± 0.02	0.80 ± 0.06	nd	13.9 ± 0.83	3.12 ± 0.06	1.41 ± 0.06	65.7 ± 3.12	1.20 ± 0.05	3.10 ± 0.08	1.65 ± 0.01	2.82	66.8	1.33

* WS (wheat straw), BOS (barley and oats straw), RB (rice bark), BWS (beech wood shavings), CR (coffee residue); ** nd: not detected; *** UI = [% monoene + 2 (% diene)]/100.

3.5. Tocopherols

The tocopherols content in these mushroom species is presented in Table 5. *P. ostreatus* mushrooms were found to have α-tocopherol as the main tocopherol, except for *P. ostreatus* 150 and *P. ostreatus* 144, cultivated on WS and BOS substrates, respectively, where δ-tocopherol predominated. *P. eryngii* fungi, cultivated on BWS, RB and CR substrates, produced mainly α-tocopherol, whereas α-tocotrienol and β-tocotrienol were also produced in lower concentrations. On the other hand, *P. eryngii* 166 mushrooms, cultivated on WS and BOS substrates, were rich in δ-tocopherol. Concerning *Agaricus* species, α-tocopherol was the main tocopherol found in all mushroom samples and substrates, while both a-tocotrienol and β-tocotrienol were present. In general, the content of total tocopherols in mushrooms is 0.5–3 mg/kg [7]. Barros et al. [72] detected only α- and β-tocopherol in five *Agaricus* species, and in all the samples β-tocopherol was the major compound. Specifically, a lower amount of α-tocopherol (749 ng/g d.w.) was detected than that in the present study. In *P. ostreatus var. florida*, α-, β- and γ- tocopherols were detected (0.0002–0.0003, 0.2–0.26 and 0.05 mg/100g d.w., respectively) [120]. However, in another study, a significantly higher quantity of α-tocopherol was recorded in *A. bisporus* (9.2 mg/g) and *P. ostreatus* (0.9 mg/g) [121]. Such variations in the type and the quantity of tocopherols may be attributable to the different analytical methodologies and the different species; for example, cultivated species seem to be lower in tocopherols than wild mushrooms [79]. In any case, the high levels of α-and β-tocopherols indicate higher oxidative activity, which is associated with cardiovascular protection [86].

Table 5. Tocopherol composition (mg/kg) of *P. ostreatus* (AMRL 144 and 150) and *P. eryngii* (AMRL 166 and 173-6) carposomes (c), pilei (p) and stipes (s) cultivated on five substrates (WS, BOS, RB, BWS, CR) and of *A. bisporus* (AMRL 209) and *A. subrufescens* (AMRL 235) cultivated on compost of two different C/N ratios (10 and 13).

Substrate/Fungi		α-Tocopherol	α-Tocotrienol	β-Tocotrienol	δ-Tocopherol
P. ostreatus					
WS *	144 c	0.78 ± 0.04	0.28 ± 0.04	0.27 ± 0.03	nd **
	144 p	0.79 ± 0.03	0.26 ± 0.03	0.27 ± 0.04	nd
	144 s	0.67 ± 0.03	0.30 ± 0.03	0.15 ± 0.02	0.42 ± 0.02
	150 c	0.13 ± 0.03	0.71 ± 0.03	0.16 ± 0.03	1.19 ± 0.09
	150 p	0.14 ± 0.03	0.73 ± 0.03	0.16 ± 0.03	1.20 ± 0.09
	150 s	0.12 ± 0.04	0.65 ± 0.04	0.10 ± 0.02	0.99 ± 0.05
BOS	144 c	nd	1.21 ± 0.10	0.06 ± 0.01	1.98 ± 0.11
	144 p	nd	1.31 ± 0.10	0.07 ± 0.01	2.05 ± 0.10
	144 s	nd	1.10 ± 0.09	0.06 ± 0.01	1.7 ± 0.14
	150 c	1.02 ± 0.02	0.18 ± 0.02	0.34 ± 0.03	nd
	150 p	1.12 ± 0.02	0.19 ± 0.02	0.34 ± 0.03	nd
	150 s	1.08 ± 0.02	0.31 ± 0.02	0.35 ± 0.04	nd
RB	144 c	0.75 ± 0.02	0.15 ± 0.02	0.22 ± 0.03	nd
	144 p	0.76 ± 0.05	0.19 ± 0.05	0.24 ± 0.04	nd
	144 s	0.7 ± 0.040	0.14 ± 0.04	0.20 ± 0.04	nd
	150 c	0.62 ± 0.01	0.1 ± 0.010	0.17 ± 0.02	nd
	150 p	0.67 ± 0.03	0.1 ± 0.030	0.20 ± 0.02	nd
	150 s	0.67 ± 0.02	0.09 ± 0.02	0.15 ± 0.02	nd
BWS	144 c	1.14 ± 0.02	0.31 ± 0.02	0.17 ± 0.02	0.42 ± 0.02
	144 p	1.30 ± 0.04	0.35 ± 0.04	0.19 ± 0.04	0.40 ± 0.05
	144 s	1.10 ± 0.06	0.30 ± 0.06	0.18 ± 0.03	0.40 ± 0.07
	150 c	0.71 ± 0.02	0.15 ± 0.02	0.22 ± 0.03	nd
	150 p	0.72 ± 0.03	0.16 ± 0.03	0.23 ± 0.02	nd
	150 s	0.70 ± 0.03	0.15 ± 0.03	0.18 ± 0.03	nd

Table 5. *Cont.*

Substrate/Fungi		α-Tocopherol	α-Tocotrienol	β-Tocotrienol	δ-Tocopherol
CR	144 c	0.82 ± 0.01	0.10 ± 0.01	0.28 ± 0.03	0.16 ± 0.02
	144 p	0.83 ± 0.01	0.10 ± 0.01	0.29 ± 0.04	0.17 ± 0.03
	144 s	0.81 ± 0.01	0.10 ± 0.01	0.26 ± 0.03	0.15 ± 0.02
	150 c	1.12 ± 0.01	0.19 ± 0.01	0.33 ± 0.03	nd
	150 p	1.03 ± 0.02	0.16 ± 0.02	0.32 ± 0.03	nd
	150 s	0.91 ± 0.02	0.11 ± 0.02	0.30 ± 0.03	nd
P. eryngii					
WS	166 c	nd	0.29 ± 0.02	nd	0.50 ± 0.03
	166 p	nd	0.31 ± 0.02	0.11 ± 0.02	0.54 ± 0.02
	166 s	nd	0.30 ± 0.02	nd	0.54 ± 0.02
	173-6 c	0.75 ± 0.04	0.19 ± 0.02	0.2 ± 0.02	nd
	173-6 p	0.77 ± 0.04	0.21 ± 0.02	0.24 ± 0.02	nd
	173-6 s	0.58 ± 0.03	0.19 ± 0.02	0.19 ± 0.02	nd
BOS	166 c	0.07 ± 0.01	0.55 ± 0.04	nd	1.40 ± 0.04
	166 p	0.07 ± 0.01	0.56 ± 0.04	nd	1.43 ± 0.04
	166 s	0.06 ± 0.01	0.49 ± 0.03	nd	1.13 ± 0.04
	173-6 c	nd	0.68 ± 0.04	nd	0.64 ± 0.03
	173-6 p	0.69 ± 0.03	0.29 ± 0.03	0.13 ± 0.02	0.32 ± 0.02
	173-6 s	0.68 ± 0.03	0.27 ± 0.03	0.11 ± 0.02	0.32 ± 0.02
RB	166 c	0.75 ± 0.04	0.10 ± 0.01	0.24 ± 0.02	nd
	166 p	0.79 ± 0.04	0.10 ± 0.01	0.27 ± 0.03	nd
	166 s	0.72 ± 0.04	0.09 ± 0.01	0.23 ± 0.02	nd
	173-6 c	0.70 ± 0.04	0.10 ± 0.01	0.20 ± 0.04	nd
	173-6 p	0.71 ± 0.05	0.12 ± 0.01	0.25 ± 0.04	nd
	173-6 s	0.66 ± 0.06	0.10 ± 0.01	0.20 ± 0.03	nd
BWS	166 c	1.12 ± 0.02	0.31 ± 0.03	0.16 ± 0.02	0.43 ± 0.03
	166 p	1.14 ± 0.03	0.35 ± 0.03	0.17 ± 0.02	0.44 ± 0.02
	166 s	1.11 ± 0.03	0.30 ± 0.02	0.16 ± 0.02	0.40 ± 0.03
	173-6 c	1.00 ± 0.10	0.15 ± 0.01	0.19 ± 0.03	nd
	173-6 p	1.16 ± 0.09	0.17 ± 0.01	0.24 ± 0.03	nd
	173-6 s	0.90 ± 0.09	0.12 ± 0.01	0.15 ± 0.02	nd
CR	166 c	0.81 ± 0.03	0.11 ± 0.01	0.27 ± 0.03	0.16 ± 0.03
	166 p	0.82 ± 0.02	0.12 ± 0.01	0.28 ± 0.03	0.17 ± 0.02
	166 s	0.80 ± 0.03	0.10 ± 0.02	0.24 ± 0.03	0.12 ± 0.02
	173-6 c	1.11 ± 0.06	0.10 ± 0.02	0.38 ± 0.03	nd
	173-6 p	1.12 ± 0.06	0.12 ± 0.02	0.39 ± 0.05	nd
	173-6 s	0.99 ± 0.02	0.09 ± 0.01	0.35 ± 0.05	nd
A. bisporus					
C/N = 10	c	1.17 ± 0.10	0.39 ± 0.05	0.38 ± 0.05	nd
	p	2.78 ± 0.10	0.28 ± 0.05	0.50 ± 0.03	nd
	s	1.02 ± 0.08	0.21 ± 0.05	0.37 ± 0.02	nd
C/N = 13	c	0.84 ± 0.03	0.19 ± 0.02	0.34 ± 0.03	nd
	p	0.93 ± 0.06	0.25 ± 0.02	0.36 ± 0.03	nd
	s	0.81 ± 0.06	0.14 ± 0.02	0.24 ± 0.03	nd
A. subrufescens					
C/N = 10	c	2.07 ± 0.12	0.46 ± 0.08	0.36 ± 0.06	nd
	p	1.86 ± 0.09	0.39 ± 0.06	0.42 ± 0.07	nd
	s	0.95 ± 0.08	0.35 ± 0.06	0.35 ± 0.03	nd
C/N = 13	c	1.98 ± 0.07	0.47 ± 0.03	0.34 ± 0.01	nd
	p	2.13 ± 0.06	0.33 ± 0.02	0.38 ± 0.02	nd
	s	1.08 ± 0.05	0.40 ± 0.04	0.20 ± 0.02	nd

* WS (wheat straw), BOS (barley and oats straw), RB (rice bark), BWS (beech wood shavings), CR (coffee residue); ** nd: not detected.

3.6. Total Phenolic Compounds (TPC), Antioxidant Studies and Total Reducing Power

The greatest TPC production was detected in pilei of *P. ostreatus* and *P. eryngii* strains and rarely in the whole carposome, especially on substrates of WS and BOS (Table 6). *P. eryngii* 166 was among the strains with the highest TPC, 19.20–56.47 mg GAE/g, while *P. eryngii* 173-6 had the lowest. Also, both *P. ostreatus* strains produced satisfactory amount of TPC (10.41–70.67 mg GAE/g d.w.). Regarding the impact of different substrates, their nature and their chemical composition significantly influenced the TPC in mushrooms [106,122]. CR and RB were the most favorable substrates, whereas all the strains produced the lowest TPC on BOS and WS. Moreover, *Agaricus* species seemed to produce significantly lower TPC than *Pleurotus* spp., with values ranging from 7.85 to 16.89 mg GAE/g d.w. (Table 1). The highest TPC were detected again in pilei of *Agaricus* mushrooms, whereas *A. subrufescens* was a better phenolic compounds producer than *A. bisporus*. Also, *A. bisporus* presented a higher TPC concentration when cultivated on compost with the ratio C/N = 10, while the compost with a higher ratio (C/N = 13) favored *A. subrufescens* TPC production. The TPC of *Pleurotus* spp. in the present study were significantly higher than those detected by other researchers [86,122]. Da Paz et al. [122] cultivated *Pleurotus sajor-caju* on three different substrates and the TPC ranged from 56.26 to 205.23 mg/100 g d.w. Sharma et al. [86] reported TPC at 39.12–55.13 mg/100 g in *Agaricus* spp. and 53.20 mg/100 g in *Pleurotus cystidiosus*. Also, according to the literature, lower TPC were detected in *P. eryngii* and *P. florida* mushrooms (3.57 and 3.72 mg/g d.w., respectively) [123], as well as in the cap and stipe of *A. bisporus* (4 and 9.9 mg/100g d.w., respectively) [30]. The presence of high concentrations of phenolic compounds is responsible for the significant antioxidant properties of mushrooms. The functional medicinal compounds contained in them depend to a great extent on the substrate in which the fungi grow. If, for example, the substrate is high in functional molecules such as anthocyanidins, beta-glucans, selenium, ganoderic acid, triterpenes or cordycepin, then it is possible that the antioxidant substances in the produced mushrooms will be increased [86].

Table 6. Moisture, ash content (% *w*/*w* of dry biomass), total phenolic compounds (TPC), FRAP and scavenging ability on $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ free radicals of methanol extracts of *P. ostreatus* (AMRL 144 and 150) and *P. eryngii* (AMRL 166 and 173-6) carposomes (c), pilei (p) and stipes (s), cultivated on five substrates (WS, BOS, RB, BWS, CR). Measurements of antioxidant studies are expressed as mg of gallic acid or trolox equivalence/g of mushroom dry weight (mean ± SD).

Substrate/Fungi	Mushroom Part	Moisture (%)	Ash (%)	TPC (mg GAE/g)	$DPPH^{\cdot}$ (mg trolox/g)	$ABTS^{\cdot+}$ (mg trolox/g)	FRAP (mg trolox/g)
P. ostreatus							
WS *	144 c	88.80 ± 3.49	4.89 ± 1.11	24.02 ± 2.56	2.48 ± 0.25	3.21 ± 0.16	3.26 ± 0.28
	144 p	88.95 ± 2.52	5.19 ± 0.90	25.87 ± 3.23	2.09 ± 0.45	2.97 ± 0.11	2.92 ± 0.53
	144 s	82.87 ± 2.66	3.41 ± 0.82	11.29 ± 2.98	0.44 ± 0.09	2.17 ± 0.09	0.25 ± 0.02
	150 c	88.65 ± 3.85	5.21 ± 0.92	24.01 ± 3.06	2.69 ± 0.12	3.87 ± 0.14	4.11 ± 0.23
	150 p	88.52 ± 2.68	5.50 ± 0.73	23.24 ± 1.28	2.39 ± 0.32	2.23 ± 0.20	2.24 ± 0.17
	150 s	83.21 ± 1.84	4.02 ± 0.90	10.41 ± 1.76	0.35 ± 0.03	1.04 ± 0.07	1.58 ± 0.31
BOS	144 c	90.05 ± 3.96	6.40 ± 1.25	24.01 ± 2.05	3.63 ± 0.20	5.57 ± 0.43	4.52 ± 0.49
	144 p	90.25 ± 3.54	8.50 ± 2.40	23.24 ± 3.12	4.09 ± 0.45	5.73 ± 0.28	5.42 ± 0.63
	144 s	85.24 ± 3.45	5.74 ± 3.40	10.41 ± 1.62	3.49 ± 0.23	5.05 ± 0.39	4.34 ± 0.38
	150 c	89.32 ± 2.89	6.55 ± 1.31	40.39 ± 3.87	4.13 ± 0.18	5.73 ± 0.82	5.44 ± 0.23
	150 p	88.95 ± 2.85	6.26 ± 1.21	36.62 ± 2.92	3.98 ± 0.27	4.70 ± 0.43	5.61 ± 0.47
	150 s	85.3 ± 2.35	5.23 ± 1.25	23.11 ± 1.58	0.36 ± 0.04	2.48 ± 0.13	1.41 ± 0.19

Table 6. *Cont.*

Substrate/Fungi	Mushroom Part	Moisture (%)	Ash (%)	TPC (mg GAE/g)	DPPH· (mg trolox/g)	ABTS·+ (mg trolox/g)	FRAP (mg trolox/g)
RB	144 c	90.4 ± 3.62	6.74 ± 0.92	38.42 ± 2.60	4.90 ± 0.25	6.73 ± 0.24	6.58 ± 0.67
	144 p	89.98 ± 5.27	6.05 ± 1.02	40.83 ± 3.47	5.31 ± 0.08	9.26 ± 0.56	7.28 ± 0.77
	144 s	85.65 ± 4.54	6.24 ± 0.96	36.02 ± 2.89	4.71 ± 0.25	6.04 ± 0.33	6.03 ± 0.48
	150 c	88.24 ± 4.45	5.91 ± 0.83	48.91 ± 4.31	8.35 ± 0.73	9.97 ± 0.27	9.38 ± 0.36
	150 p	89.92 ± 5.24	5.54 ± 1.24	51.02 ± 2.78	8.72 ± 0.57	11.27 ± 0.31	11.06 ± 0.41
	150 s	85.24 ± 5.60	6.25 ± 1.35	48.01 ± 3.90	7.71 ± 0.44	8.42 ± 0.78	8.43 ± 0.29
BWS	144 c	88.94 ± 3.66	5.56 ± 0.94	38.35 ± 4.12	3.61 ± 0.22	6.13 ± 0.28	4.51 ± 0.20
	144 p	90.23 ± 4.54	5.50 ± 1.24	39.26 ± 3.88	3.73 ± 0.63	7.41 ± 0.49	4.64 ± 0.46
	144 s	85.35 ± 6.02	6.27 ± 1.05	36.01 ± 3.23	3.44 ± 0.29	5.54 ± 0.20	4.22 ± 0.33
	150 c	89.24 ± 2.85	5.13 ± 0.80	36.13 ± 3.75	4.82 ± 0.55	5.82 ± 0.37	5.92 ± 0.47
	150 p	89.24 ± 5.52	4.86 ± 1.05	38.72 ± 4.01	4.97 ± 0.14	6.02 ± 0.19	6.15 ± 0.24
	150 s	85.25 ± 5.34	5.32 ± 1.25	34.82 ± 2.66	4.66 ± 0.16	5.54 ± 0.34	5.66 ± 0.29
CR	144 c	88.52 ± 2.54	5.89 ± 0.94	59.11 ± 4.32	8.16 ± 0.85	7.40 ± 0.65	7.54 ± 0.76
	144 p	88.65 ± 3.62	5.80 ± 0.99	70.67 ± 3.65	8.42 ± 0.67	7.74 ± 0.27	7.76 ± 0.53
	144 s	85.60 ± 3.34	5.98 ± 0.89	41.40 ± 3.25	5.08 ± 0.43	4.59 ± 0.39	4.56 ± 0.48
	150c	88.91 ± 3.70	6.54 ± 0.92	61.23 ± 4.82	7.78 ± 0.27	7.01 ± 0.44	6.47 ± 0.39
	150 p	89.52 ± 4.35	6.45 ± 1.05	62.79 ± 4.07	7.94 ± 0.31	7.77 ± 0.26	6.98 ± 0.58
	150 s	85.14 ± 3.64	6.68 ± 1.20	51.01 ± 3.89	2.59 ± 0.19	3.83 ± 0.10	3.41 ± 0.32
P. eryngii							
WS	166 c	89.63 ± 2.45	6.29 ± 0.90	31.45 ± 3.35	3.16 ± 0.24	5.91 ± 0.28	5.01 ± 0.29
	166 p	88.97 ± 3.51	6.51 ± 0.83	28.03 ± 2.08	2.93 ± 0.39	4.47 ± 0.32	4.60 ± 0.38
	166 s	83.65 ± 2.32	5.97 ± 0.98	25.50 ± 2.65	1.90 ± 0.06	3.43 ± 0.17	3.27 ± 0.22
	173-6 c	88.58 ± 2.45	5.73 ± 0.85	28.82 ± 1.18	3.61 ± 0.09	3.03 ± 0.11	3.23 ± 0.47
	173-6 p	88.71 ± 2.64	5.87 ± 0.92	22.50 ± 3.02	3.34 ± 0.28	3.79 ± 0.27	3.15 ± 0.33
	173-6 s	84.21 ± 3.52	3.57 ± 0.64	20.47 ± 2.80	2.25 ± 0.16	2.57 ± 0.14	1.27 ± 0.12
BOS	166 c	88.34 ± 2.41	6.05 ± 0.62	33.66 ± 2.04	4.23 ± 0.36	6.01 ± 0.25	5.36 ± 0.29
	166 p	88.57 ± 2.96	6.51 ± 0.85	24.19 ± 2.24	3.83 ± 0.23	5.98 ± 0.49	4.39 ± 0.22
	166 s	81.36 ± 3.09	4.85 ± 0.90	19.20 ± 1.99	1.27 ± 0.05	2.34 ± 0.21	2.47 ± 0.10
	173-6 c	87.64 ± 2.49	6.45 ± 0.86	28.56 ± 3.54	3.22 ± 0.28	4.26 ± 0.47	3.99 ± 0.43
	173-6 p	87.20 ± 2.54	6.58 ± 0.84	22.69 ± 2.87	3.07 ± 0.15	3.01 ± 0.24	4.99 ± 0.28
	173-6 s	81.41 ± 2.65	4.85 ± 0.99	13.57 ± 1.21	2.59 ± 0.27	2.06 ± 0.19	2.42 ± 0.15
RB	166 c	89.35 ± 3.01	4.65 ± 0.65	23.92 ± 2.23	12.12 ± 0.76	14.84 ± 0.39	11.12 ± 0.21
	166 p	88.23 ± 2.65	6.55 ± 1.01	25.21 ± 1.53	13.22 ± 0.59	15.45 ± 0.67	12.26 ± 0.38
	166 s	80.10 ± 2.15	5.35 ± 0.57	19.21 ± 2.76	9.61 ± 0.42	11.95 ± 0.26	7.23 ± 0.09
	173-6 c	87.26 ± 2.34	5.34 ± 0.66	27.68 ± 3.54	10.75 ± 0.34	11.55 ± 0.23	10.83 ± 0.25
	173-6 p	88.64 ± 1.30	5.44 ± 0.71	28.09 ± 3.07	11.61 ± 0.53	13.38 ± 0.69	11.89 ± 0.43
	173-6 s	82.35 ± 1.65	6.50 ± 0.60	26.13 ± 2.91	9.11 ± 0.49	10.24 ± 0.72	11.89 ± 0.61
BWS	166 c	87.69 ± 2.00	5.71 ± 1.20	27.02 ± 2.69	11.11 ± 0.94	13.83 ± 0.31	11.42 ± 0.73
	166 p	88.25 ± 2.30	5.55 ± 1.32	27.30 ± 3.21	10.76 ± 0.57	14.28 ± 0.96	12.13 ± 0.56
	166 s	84.36 ± 1.91	5.31 ± 1.30	25.72 ± 2.45	9.93 ± 0.42	13.74 ± 0.64	9.38 ± 0.40
	173-6 c	85.74 ± 1.85	5.47 ± 0.95	27.68 ± 1.03	11.29 ± 0.32	14.72 ± 0.78	11.84 ± 0.32
	173-6 p	88.20 ± 1.54	5.23 ± 0.64	28.09 ± 1.82	11.40 ± 0.18	15.02 ± 0.92	13.41 ± 0.55
	173-6 s	80.25 ± 1.68	6.14 ± 0.34	26.13 ± 1.63	7.23 ± 0.53	14.08 ± 0.53	9.44 ± 0.65
CR	166 c	90.21 ± 2.14	5.34 ± 0.57	53.70 ± 2.95	9.52 ± 0.29	9.87 ± 0.57	9.04 ± 0.33
	166 p	89.25 ± 1.68	5.41 ± 0.55	56.47 ± 2.50	11.12 ± 0.76	10.54 ± 0.29	9.72 ± 0.49
	166 s	87.10 ± 1.87	4.87 ± 0.48	48.27 ± 3.32	8.84 ± 0.54	8.78 ± 0.72	8.66 ± 0.51
	173-6 c	88.64 ± 1.74	4.68 ± 0.34	42.57 ± 2.09	10.58 ± 0.23	7.06 ± 0.69	6.04 ± 0.46
	173-6 p	89.01 ± 1.67	4.88 ± 0.60	45.50 ± 2.78	11.26 ± 0.88	7.76 ± 0.42	7.53 ± 0.53
	173-6 s	80.24 ± 1.99	5.47 ± 0.47	41.48 ± 3.01	7.44 ± 0.39	6.92 ± 0.37	5.66 ± 0.31

* WS (wheat straw), BOS (barley and oats straw), RB (rice bark), BWS (beech wood shavings), CR (coffee residue).

In the present investigation, the antioxidant activity of the mushroom extracts was examined using three assays, as antioxidant compounds have multiple mechanisms of action and no single approach can capture all of them. The capacity of mushroom extracts to scavenge free radical $DPPH^{\cdot+}$ ranged from 0.35 to 8.72 mg trolox/g d.w. in *P. ostreatus* strains, and from 1.27 to 13.22 mg trolox/g d.w. in *P. eryngii* ones, while the scavenging capacity of the other free radical $ABTS^{\cdot+}$ ranged from 1.04 to 11.27 mg trolox/g d.w. and 2.06 to 15.45 mg trolox/g d.w. in *P. ostreatus* and *P. eyngii* strains, respectively (Table 6). Moreover, the values for reducing power ranged from 0.25 to 11.06 mg trolox/g d.w. in *P. ostreatus* strains and from 1.27 to 13.41 mg trolox/g d.w. in *P. eyngii* strains. It is worth mentioning that the results of all assays revealed that pilei had higher antioxidant values than the corresponding stipes, in all strains and substrates examined. CR, RB and BWS were the substrates where the antioxidant activity of all *Pleurotus* strains was greater (Table 6). As shown in Table 1, the antioxidant capacity of *A. subrufescens* was higher than that of *A. bisporus* in all assays. In general, *Pleurotus* spp. had better free radical scavenging ability than the *Agaricus* spp. This phenomenon had also been previously mentioned [124] when *Pleurotus columbinus* and *P. sajor-caju* antioxidant capacities were compared to that of *A. bisporus*. However, their $ABTS^{\cdot+}$ scavenging activity was lower than that detected in this study. On the other hand, high amounts of TPC probably are linked to strong fungal extract antioxidant activity, as has already been detected by other researchers [86,125–127].

It is remarkable that, according to our results, all tested fungi cultivated on WS developed noticeably low TPC, total reducing power and scavenging activity against $ABTS^{\cdot+}$ and $DPPH^{\cdot+}$ free radicals, indicating that substrate composition affects mushroom bioactive phenolics production. Previous studies have also noted that the chemical composition of the substrates used significantly affected the nutritional and bioactive compounds of mushrooms produced. For example, *P. ostreatus* and *P. pulmonarius* species cultivated on different woody substrates, such as beech sawdust, oak, linden, walnuts and poplar, showed significantly higher TPC concentrations on beech and linden [128].

The Pearson's correlation coefficient between the TPC results and those of DPPH and ABTS was characterized as moderate (r = 0.3696 and r = 0.3636, respectively, $p < 0.05$), and weak for those of FRAP (r = 0.2416, $p < 0.05$). This fact was expected, as the F-C method suffers from several interfering compounds that react with the F-C reagent to give elevated apparent phenolic content. On the other hand, correlation analysis between the results obtained with the three assays used to measure the antioxidant activity correlated significantly positively, with the greater relationship strength observed between the FRAP and ABTS results (r = 0.9469, $p < 0.05$).

3.7. Moisture, Ash Content and Mineral Analysis

As shown in Table 1, the results for the moisture contents of carposomes varied from 86 to 90%, consistent with previously reported values [2,3,7,28,30,38]. For *Agaricus* spp., it was higher in the carposomes than in the pilei, whereas in *Pleurotus* spp. (Table 6) they were similar. The stipes contained the least water of all, with the minimum values being 80–82%. Ash content in *Pleurotus* mushrooms varied from 3.41 to 8.50% for *P. ostreatus* and 3.57 to 6.58% for *P. eryngii* strains, while *Agaricus* species had higher ash amounts ranging from 8.06 to 9.21% (Tables 1 and 6). It seems that the ash contents of the carposomes examined in this study were low (below 10%), but within the indicated range [82,129], whereas Ulziijargal et al. [80] reported even lower ash values for *A. bisporus* and *A. brasiliensis*, at 6.72 and 5.90%, respectively.

The contents of major and trace mineral elements are listed in Table 7. Potassium (K) was the most prevalent mineral element, with values ranging from 120 to 350 g/kg d.w. for *Pleurotus* species and 360 to 410 g/kg d.w. for *Agaricus* spp., followed by phosphorus (P) and magnesium (Mg). It is worth noting that K distribution throughout the carposomes was unequal, with the pileus having a higher concentration than the stipe, as has also been demonstrated in previous research [7]. Also, sodium (Na) was detected in low amounts (less than 12 g/kg d.w.). A decrease in Na of 18–30% content in *P. ostreatus* and *P. eryngii*

was detected in mushrooms produced on RB, BWS and CR substrates. As calcium (Ca) levels are not very high in mushrooms, the Ca values ranged from 6 to 28 g/kg d.w. The mineral composition of *P. ostreatus* species significantly varied depending on the substrate. Specifically, the contents of K, Na, Mg and Ca were higher in mushrooms from BOS than in those grown in other substrates, while the highest value of Cu was detected on RB and BWS for *P. ostreatus* and *P. eryngii*, respectively. Trace metals presence is related to the mushroom species, as well as to the age of the fruiting bodies and mycelium [130]. The findings of this research were comparable with those of prior studies [109,129,131,132]. The low Na content along with the high K content of mushrooms indicate that they may be included in an anti-hypertensive diet; in fact, K from fruits and vegetables can lower blood pressure [129]. Generally, mushrooms' K content ranges from 182 to 395 mg/100 g, whereas the recommended daily requirement is 3100 mg/day. However, the mineral level depends on the species, the mushroom's age, the diameter of the pilei and on the substratum [133].

Table 7. Macro- and microelement contents (calculated on dry biomass) (mean ± SD) of the carposomes (c), pilei (p) and stipes (s) of *P. ostreatus* (AMRL 144, 150) and *P. eryngii* (AMRL 166, 173-6), cultivated in five different substrates (WS, BOS, RB, BWS, CR), and *A. bisporus* (AMRL 209) and *A. subrufescens* (strain AMRL 235) on two different composts (C/N ratio of 10 and 13).

Substrate/Fungi		Mineral Content (*w*/*w*, d. w.)									
		K (g/Kg)	Na (g/kg)	Mg (g/kg)	P (g/kg)	Ca (g/kg)	Fe (ppm)	Mn (ppm)	Cu (ppm)	Zn (ppm)	B (ppm)
P. ostreatus											
WS *	144 c	250	8.0	18	82	19	68	6.3	12	66	1.0
	144 p	280	9.0	21	84	10	72	6.8	15	69	1.2
	144 s	210	7.0	14	64	26	31	3.8	11	50	nd **
	150 c	270	5.0	17	77	20	71	8.5	11	67	0.3
	150 p	290	4.0	19	80	10	70	9.0	12	70	1.2
	150 s	200	7.5	13	50	28	24	8.6	7.6	41	1.8
BOS	144 c	290	11.0	21	92	17	99	10	17	72	0.3
	144 p	330	13.0	23	95	9	110	16	18	78	1.2
	144 s	250	8.5	17	85	22	58	7.8	14	50	0.4
	150 c	320	7.0	18	93	28	94	13	15	68	4.6
	150 p	350	8.5	25	95	10	97	15	17	75	6.2
	150 s	300	8.0	14	83	32	48	6.8	11	53	3.4
RB	144 c	290	2.0	14	88	18	82	10	20	58	15.6
	144 p	340	3.5	19	92	6	92	15	14	76	17.2
	144 s	250	1.5	10	75	22	65	9	12	45	11.3
	150 c	260	3.0	13	87	16	72	14	22	62	7.6
	150 p	350	3.6	18	90	10	86	18	14	70	10.6
	150 s	220	1.0	9	80	19	62	12	8	45	6.8
BWS	144 c	250	8.0	16	99	23	83	9.0	12	68	1.6
	144 p	300	10.0	20	100	12	90	12	15	90	1.8
	144 s	170	5.0	9	65	28	58	7.0	9	45	0.9
	150 c	270	1.0	20	78	20	91	11	11	72	2.0
	150 p	300	1.5	25	80	10	100	18	15	95	2.5
	150 s	120	0.8	17	70	26	80	9	10	50	1.8
CR	144 c	200	1.0	12	87	18	67	4.0	12	65	1.8
	144 p	230	1.2	12	98	8	98	4.2	11	71	1.5
	144 s	130	1.2	11	65	21	54	2.8	5.6	37	3.2
	150 c	220	0.9	11	80	16	70	8.3	11	68	2.1
	150 p	240	1.1	15	86	9	91	9.4	12.6	72	2.7
	150 s	150	8.8	9	60	20	55	5.6	8.7	48	1.1

Table 7. *Cont.*

Substrate/Fungi		Mineral Content (*w*/*w*, d. w.)									
		K (g/Kg)	Na (g/kg)	Mg (g/kg)	P (g/kg)	Ca (g/kg)	Fe (ppm)	Mn (ppm)	Cu (ppm)	Zn (ppm)	B (ppm)
P. eryngii											
WS	166 c	350	5.0	12.5	85	17	65	5.4	6.0	26	0.8
	166 p	370	7.0	15	89	8	90	5.9	9.3	29	1.1
	166 s	330	4.0	9	44	23	32	2.9	5.6	19	0.6
	173-6 c	330	5.0	10	86	16.50	56	4.7	6.1	23	0.9
	173-6 p	350	4.5	15	99	9	97	5.2	10	25	1.2
	173-6 s	210	5.0	11	45	20	25	3.2	6.6	18	0.6
BOS	166 c	340	4.0	14	86	16	50	5.7	10	24	1.1
	166 p	380	6.0	17	95	9	65	6.9	15	29	2.2
	166 s	340	5.0	9	50	20	33	4.1	6.7	25	2.7
	173-6 c	320	11.0	15	88	18	60	7.2	6.6	32	nd
	173-6 p	350	12.0	18	95	8	70	7.8	7.9	40	nd
	173-6	290	7.0	7	70	22	35	6.2	4.5	25	nd
RB	166 c	340	1.2	20	91	18	31	8.2	20	55	1.6
	166 p	370	2.5	22	99	6	37	8.9	42	80	2.4
	166 s	300	0.9	10	60	22	20	7.6	16	30	2.0
	166 c	310	1.4	14	97	16	41	7.3	11	64	4.7
	166 p	330	2.5	18	100	10	48	8.9	15	72	7.9
	166 s	260	1.0	12.5	78	19	36	6.5	9.6	50	4.0
BWS	166 c	320	1.5	19	90	19	60	9.0	50	64	1.5
	166 p	350	2.5	22	100	9	70	9.8	62	90	2.0
	166 s	270	0.9	10	50	22	42	6.8	28	34	0.9
	173-6 c	340	1.6	15	75	17	55	8.6	38	70	5.2
	173-6 p	370	2.0	17	90	9	69	9.8	45	86	7.2
	173-6 s	270	0.8	10	65	20	38	5.6	15	37	3.4
CR	166 c	350	2.3	19	87	13	50	1.6	14	78	2.4
	166 p	370	3.0	22	95	6	65	2.7	17	88	2.8
	166 s	290	2.0	15	55	18	40	1.2	10	51	2.0
	173-6 c	310	0.9	14	90	17	61	6.2	8.3	62	4.4
	173-6 p	340	3.7	21	95	7	58	8.2	16	87	<0.1
	173-6	210	1.5	10	65	20	55	5.4	8.0	58	2.9
Agaricus spp.											
A. bisporus (C/N = 10)	c	410	6.8	21	98	nd	46	2.5	44	68	3.2
	p	450	7.8	23	100	2	57	4.0	56	75	3.6
	s	320	5.8	17.5	56	nd	38	1.2	30	60	2.8
A. bisporus (C/N = 13)	c	390	7.3	19	89	15	44	1.1	33	71	1.6
	p	430	8.0	20	94	3	50	2.5	40	76	2.0
	s	370	5.2	15.4	43	10	32	n.d	25	60	1.2
A. subrufescens (C/N = 10)	c	360	10.5	13	87	14	62	2.5	40	90	1.1
	p	400	17	17	98	2	53	3.8	60	150	1.5
	s	270	5.7	8.4	62	5	31	2.0	20	60	nd
A. subrufescens (C/N = 13)	c	380	8.3	15	92	13	67	2.9	46	97	1.3
	p	400	9.5	19	103	5	50	3.7	58	145	1.7
	s	270	6.2	9.20	75	10	35	1.8	22	68	1.4

* WS (wheat straw), BOS (barley and oats straw), RB (rice bark), BWS (beech wood shavings), CR (coffee residue); ** nd: not detected.

4. Conclusions

This study indicated that the nutritional value of mushrooms could be improved by a general increase in carbohydrates, proteins and antioxidant compounds and a decrease in sodium content. The carposomes, pilei and stipes of *Pleurotus* species, cultivated on new substrates like BOS, BWS, RB and CR, were rich in antioxidants, IPSs, fibers, proteins, lipids, polyunsaturated fatty acids, minerals and tocopherols. *P. eryngii* species were found to have higher amounts of IPSs and β-glucans than *P. ostreatus* and *Agaricus* ones. In WS, BOS and CR, high amounts of IPSs were produced in all species examined, whereas BOS

and BWS seemed to positively affect protein production in the *P. ostreatus* species. BWS, RB and CR also enhanced the production of lipids for *Pleurotus* mushrooms (especially for *P. ostreatus* cultivated on CR, which presented the highest lipid concentration); because they contain high amounts of polyunsaturated fatty acids, this may lead to an increase in HDL levels, the good cholesterol. These results confirmed that the substrate synthesis affects the final mushroom composition, so the selection of the most suitable substrates may result in the enhancement of mushrooms' nutritional value. Therefore, these alternative substrates could be employed to produce mushrooms with higher contents of proteins, polysaccharides, unsaturated lipids or antioxidants, adapted to the demands of customers for balanced diets or medical purposes. *Agaricus* species contained lower amounts of IPSs than *Pleurotus* ones, a fact that could be useful for people with prediabetes problems. Regarding *A. bisporus* and *A. subrufescens*, those mushrooms cultivated in compost with C/N = 10 had higher protein levels compared to the ones with C/N = 13, confirming that the nitrogen concentration in the substrate affects mushroom protein content. Moreover, C/N = 10 favored the antioxidant activity of *A. bisporus*, while C/N = 13 enhanced the antioxidant activity of *A. subrufescens*. These mushrooms with high phenolic content and antioxidant capacities, along with a high concentration of β-glucans, could serve as a good source not only of functional foods, but also of supplements or drugs. Additionally, due to their low Na content, they could be ideal for an anti-hypertensive diet. As far as the individual parts of mushrooms were concerned, all pilei were richer in proteins, lipids and TPC, and they showed higher antioxidant activity and reducing power than stipes. On the other hand, all stipes, a by-product of mushroom cultivation, were richer in IPSs and glucans compared to the corresponding pilei, and these results were very interesting because most studies refer only to the carposomes. Thus, it is possible that both the pilei and also the stipes of *Pleurotus* and *Agaricus* can be used, not only as food supplements, but also re-utilized in new, enriched substrates for a second-cycle cultivation of mushrooms, in the framework of a circular economy.

Author Contributions: Conceptualization, Methodology, Validation, Supervision, Writing—Review & Editing, P.D.; Formal Analysis, K.F.; Formal Analysis, Writing & Editing, E.M.M.; Data Curation, Writing—Review, M.D.; Formal Analysis, I.D.; Methodology; Validation, Review, C.G.; Writing—Review, S.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research has been co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH–CREATE–INNOVATE (project code: T1EDK-05027), scientifically coordinated by the Hellenic Agricultural Organization–Dimitra (Institute of Technology of Agricultural Products/Laboratory of Edible Fungi). We would also like to thank INRA-France, and particularly J-M Savoie, Head of the research unit MycSA, for providing us with the *A. subrufescens* strains used in the present study and the scientific personnel of the Soil Institute of ELGO-Dimitra in Lycovryssi-Greece for their contribution in the analysis of minerals, as well as P. Merkouri of ITAP/ELGO-Dimitra for her contribution in the analysis of fatty acids.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Available after request to the cor. author.

Conflicts of Interest: The authors declare no conflict of interest.

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