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Evaluation of nutritional and bioactive compounds of *Pleurotus ostreatus* and *Pleurotus eryngii* mushrooms cultivated on supplemented agro-industrial residues in solid-state fermentations

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Abstract The possible effect of supplementation of wheat straw (WS) and barley and oats straw (BOS) with sunflower, corn oil, yeast extract and $CaSO_4 \cdot 2H_2O$ on biochemical properties (polysaccharide, protein, lipid, glucan, tocopherol content, carbohydrates and fatty acids composition), total phenolic compounds (TPC) and antioxidant activity of *Pleurotus ostreatus* and *Pleurotus eryngii*, cultivated in 'bag-logs', was evaluated. Results revealed that supplements enhanced IPSs in carposomes cultivated on BOS substrates and the most positive were corn oil 2%, w/w and $CaSO_4 \cdot 2H_2O$, for *P. ostreatus* and *P. eryngii*, respectively (50.86 and 57.65%, w/w). In all treatments, there was a variety in carbohydrate composition and a great production of glucans (47.69-69.22%), especially the bio-active β-glucans (47.37-67.77%), in stipes of both species was detected. Significant protein content was detected in *P. ostreatus* and *P. eryngii* carposomes grown on substrates with yeast extract (28.99 and 30.09%, w/w; WS and 29.49 and 24.94%, w/w; BOS). Although mushrooms presented low lipid content as general, high values of fat content were observed for both species when cultivated on BOS substrate supplemented with sunflower and corn oil (4.54-6.58%). Results revealed that the nutritional value of mushrooms was comparable between the two flushes. The greatest amount of TPC were detected on supplemented WS (22.3-35.30 mg gallic acid equivalent/ g d.w.) and BOS (21.03-41.74 mg gallic acid equivalent/ g d.w.) with $CaSO_4 \cdot 2H_2O$, in all parts of mushrooms, with strong antioxidant activity and tocopherols production, in all treatments. Higher lipids, antioxidant activity and reducing power were showed in pilei than stipes, while the latter were richer in IPSs, regardless supplement presence. Therefore, those supplemented substrates could be used to produce mushrooms with high nutritional and medicinal value.

Keywords: oyster mushroom; supplements; flushes; protein; stipes; glucans; tocopherols

1. Introduction

Mushrooms are widely consumed around the world as they constitute a nutritionally complete, healthy food. Edible mushrooms are rich in protein, low in fat and calories and contain a variety of bioactive compounds, such as polysaccharides, proteins and fibers, vitamins and minerals [1–5]. *Pleurotus* spp. are among the most cultivated mushrooms around the word because of their easily cultivation on various agro-industrial by-products in combination with their medicinal and nutritional value [6–8]. *P. ostreatus* commonly known as oyster mushroom, is one of the most consumed mushrooms worldwide [9]. *P. eryngii*, also called the king oyster mushroom or king trumpet mushroom, is well-known worldwide for its exceptional flavor and high nutritional content [10]. Carposomes of *Pleurotus* spp. are especially appreciated due to their high protein, containing all nine essential amino acids; therefore, they can be considered as a substitute for a meat diet, suitable for vegetarian people [4]. Moreover, these mushrooms include an important amount of carbohydrates consisted of sugars correlated to the synthesis of polysaccharides. Polysaccharides found to be natural antioxidants with anti-tumor, anti-inflammatory, anti-diabetic immunomodulatory activity and improving the human body's immune response system [11–13]. Among them, β-glucan is the most versatile polysaccharide found in mushrooms with all the above biological functions. Although this genus characterized by low-fat content, polyunsaturated fatty acids (PUFAs) predominate over saturated ones. It has been reported that the most dominant PUFA in mushrooms is linolenic acid (C18:2), an essential fatty acid, which contribute to flavor components in *Pleurotus*, following by oleic (C18:1) and palmitic (C16:0) [4,14]. So, *Pleurotus* are classified as low calorific food and almost zero-cholesterol content [15]. Regarding phenolic compounds, *Pleurotus* spp. contain several types, including phenolic acid, flavonoids, stilbenes, lignans, tannins and oxidized polyphenols, which are extensively associated with human health benefits (anti-cancer, anti-cardiovascular, anti-viral, anti-microbial, anti-inflammatory and anti-allergenic characteristics) and with its functional potential in food industry [11,16,17]. The high amount of total phenolic compounds in *Pleurotus* mushrooms is likely responsible for their ability to scavenge free radicals and other reactive oxygen species, which are continuously being produced in vivo and its ability to chelate Fe^{++} ions that catalyze oxidative processes, as well. These properties result in the prevention of cell death and tissue damage [18]. Antioxidant activity of *Pleurotus* mushrooms is also associated with the activity of ''free radical scavenging enzymes'' (superoxide dismutase, catalase, peroxidase, etc.) and with the contents of antioxidant substances, such as tocopherol [19].

The *Pleurotus* spp. belong to a group known as ''white rot fungi'' [20] that they produce a white mycelium and can be successfully cultivated on non-composted lignocellulosic substrates and on agro-industrial residues and wastes [21]. As a result, several studies examine the capability of *Pleurotus* spp. to grow on different agro-residues and also their effect on functional, chemical and sensorial characteristics of mushrooms [22–25]. Hoa et al. [23] detected that the increasing amount of corncob and sugarcane bagasse in cultivation substrates enhanced protein of *P. ostreatus* and *Pleurotus cystidiosus* carposomes, while Diamantopoulou et al. [22] reported that substrates consisted of BOS, rice bark, beech wood shavings and coffee residue improved the antioxidant activity of *Pleurotus* species and their nutritional characteristics. Besides, the further supplementation of cultivation substrates with various additives seemed to enhance

not only growth parameters, but also the quality of mushrooms produced on solid-state fermentations. For example, a nitrogen increase in the cultivation substrate seems to be related to the crude protein content of carposomes [22–24,26,27] and the supplementation of substrates with plant oils proved to encourage the growth of oyster mushrooms and lead to an increase in the productivity of their fruit bodies and their protein content, too [28]. Also, it has been shown that the calcium sources could be absorbed by *Pleurotus* spp., improving nutrients uptake and positively affecting protein, carbohydrates and lipid contents in carposomes [29,30]. Finally, another parameter that could affect the quality of mushrooms is the availability of elements in different flushes during mushrooms cultivation [31,32].

Therefore, the present study was carried out to evaluate the possible influence of oils (sunflower, corn oil), nitrogen (peptone) and calcium salts ($CaSO_4 \cdot 2H_2O$) supplementation in wheat straw (WS) and barley and oats straw (BOS) that have been previously found to be the most effective in terms of *P. ostreatus* and *P. eryngii* nutritional value [22,33–35]. Quality properties of *Pleurotus* mushrooms were detected also in their parts, pilei and stipes in the first two flushes of mushroom production. Given that there are limited studies in the literature concerning the nutrient content of mushroom parts in the two flushes, this research refers to the fundamental nutritional values, such as carbohydrates, proteins and lipids, while carbohydrate and fatty acid composition, glucan, tocopherols, along with total phenolic content and antioxidant activity were also evaluated.

2. Materials and methods

2.1. Chemicals and reagents

3,5-dinitrosalicylic acid was purchased from Scharlau; D-fructose from Merck; ethylenediaminetetraacetic acid (EDTA), potassium sodium tartrate and all solvents from AppliChem and sodium hydroxide from Lachner. Folin–Ciocalteu's phenol reagent and potassium sodium tartrate 4-hydrate were purchased from AppliChem; iron (II) chloride salt from Merck; 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) and Bradford reagent from Sigma; 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$) radicals from TCI and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfuric acid) ($ABTS^{\cdot+}$) from Alfa Aesar. The above chemical reagents were utilized in spectrophotometric measurements and the absorbents were measured using a Jasco V-530 UV-Visible spectrophotometer (Tokyo, Japan), apart from total protein determination which took place in a 96-cell microplate reader spectrophotometer.

2.2. Fungal species and mushroom production

In the present study, a commercial strain of *Pleurotus ostreatus*; AMRL 150 and a wild type of *P. eryngii*; AMRL 173-6 were used. These species are deposited at the fungal culture collection of the Laboratory of Edible Fungi/ITAP/ELGO-Dimitra. Before 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 Philippoussis et al. [36] described.

Mushrooms were cultivated on wheat straw (WS) and barley and oat straw (BOS) supplemented with commercial sunflower oil 5% w/w, corn oil in 2% and 5% w/w, yeast extract in 2%, $CaSO_4 \cdot 2H_2O$ (agricultural gypsum) 6% w/w and controls (WS, BOS substrates

without supplements) and they were used for the solid-state fermentation of the *Pleurotus* species, which took place in bags of 1 kg, as described in detail by Dedousi et al. [35]. The produced mushrooms were harvested by hand during the first and the second flush and then frozen at -20±0.5 °C. Whole carposomes, pilei and stipes were lyophilized (in a HetoLyoLab 3000, Heto-Holten Als, Denmark) and then ground (in a Janke & Kunkel, IKA-WERK, analytical mill (Germany) for further analysis.

2.3. Total intra-cellular polysaccharide (IPS) analysis

The content of IPS was estimated according to Diamantopoulou et al. [37] and Liang et al. [38] with some modifications. For this purpose, 0.1 g of ground mushroom was hydrolyzed using 5 mL of 2.5 M HCl at 100 °C for 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) by DNS assay, measuring the absorbance at 540 nm [39].

2.4. IPS composition

The composition of monosaccharides of the produced IPSs was examined by HPLC analysis as described by Diamantopoulou et al. [37]. 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. Sugars and polyols were detected using an RI detector (differential refractometer 410-Waters).

2.5. Total protein content

The crude protein content of dried mushrooms was performed according to the Bradford assay [40]. Briefly, 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. Following, 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. A 96-cell microplate reader spectrophotometer was used to measure the absorbance at 620 nm. A standard curve of BSA (0.1–1.5 mg/mL) was made.

2.6. Total lipids analysis

Total lipids were determined by a modified version of Folch method [41]. In particular, 0.5 g of dried powder 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.

2.7. Fatty acids determination

The fatty acid methyl esters preparation was performed in a two-stage reaction (to avoid trans-isomerization) using sodium methoxide and methanol/hydrochloride [42]. 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) 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.

2.8. Extracts of total phenolic compounds and antioxidant activity

For the measurement of total phenolic content and antioxidant activity, methanolic extracts were prepared. 250 mg of dried 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.

2.8.1. Total phenolic compounds (TPC) determination

The TPC of samples were determined using the Folin–Ciocalteu assay, as described by Slinkard and Singleton [43] and were measured at 760 nm. Gallic acid was used for the standard curve. In brief, 0.5 mL of each 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. Results were expressed as gallic acid equivalent (GAE) mg/g dry weight of biomass.

2.8.2. Antioxidant activity: Scavenging ability on 1.1-Diphenyl-2-Picrylhydrazyl (DPPH) radicals

In order to determine the scavenging ability on $DPPH^{\cdot}$ free radicals, 0.1 mL of each methanolic extract was added to 3.9 mL $DPPH^{\cdot}$ (60 μM in methanol) in test tubes and vortexed according to Boonsong et al. [44]. Then, 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.

2.8.3. Antioxidant activity: Scavenging activity of $ABTS^{+}$ radical

Free radical scavenging activity was measured according to Re et al. [45] with some modifications. $ABTS^{\cdot+}$ [2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid] radicals were produced after 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.

2.8.4. Antioxidant activity: Ferric reducing antioxidant power (FRAP)

The sample's ability to convert Fe^{3+} to Fe^{2+} ions was determined according to Benzie and Strain, 1996 [46]. 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.

2.9. Glucan content

The content of total and α-glucans of the carposomes of edible mushrooms was assessed using a Yeast Beta-Glucan assay kit (Megazyme, Wicklow, Ireland) following its instructions. Then, β-glucans content was determined by subtracting α-glucans content from total glucans content.

2.9. Determination of tocopherols

Tocopherol content was determined according to Barros et al. [47] using BHT (butyl-hydroxytoluene) (~10 mg/mL) with the samples prior to the extraction procedure. 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 homogenized, centrifuged and the upper layer was transferred to a vial. The combined extracts from three 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 [48], using high-performance liquid chromatography with fluorescence detection. Briefly, 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/100g.

2.10. Data analysis

All experiments were repeated at least twice and within experiments triplicate bags/ samples were used to calculate data mean values and standard deviation. 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%).

3. Results and discussion

3.1. Ips, protein and lipids of carposomes

The results of fundamental nutritional values (carbohydrates, protein and lipids) in the carposome and also in the pileus and stipe of *P. ostreatus* and *P. eryngii* mushrooms grown on supplemented WS and BOS substrates in the two flushes are presented in **Table 1**. Regarding IPS content, when oils, nitrogen and calcium salts supplemented BOS, they had a positive impact on IPS production by *P. eryngii*, whereas less profound effect was observed on *P. ostreatus* one. More specific, first-flush *P. ostreatus* carposomes grown on BOS-corn oil 2%, w/w had the highest IPS content (50.86%), while the highest IPS values for those harvested on the second flush were detected on BOS-$CaSO_4 \cdot 2H_2O$ 6%, w/w (57.97%). Also, the highest IPSs for first-flush and second-flush *P. eryngii* carposomes were recorded on BOS-$CaSO_4 \cdot 2H_2O$ 6%, w/w (57.65%) and BOS-yeast extract 2%, w/w (52.20%), respectively. Concerning WS substrates, supplements did not enhance IPSs production in first flush by *P. ostreatus* and *P. eryngii*, as the greatest values were detected on control substrate (47.02 and 56.48%, respectively), whereas yeast extract and corn oil 5%, w/w led to further IPSs production in the second flush (45.53 and 58.00%, respectively). In a previous study [22], higher IPSs amount was determined in *P. ostreatus* carposomes cultivated on WS (55.52%, w/w), but smaller on BOS (37.78%, w/w) than in this study. Comparable were the results of the same study for *P. eryngii* carposomes cultivated on WS (56.26%, w/w), but a greater amount of IPSs was detected when it was cultivated on BOS (54.48%, w/w). In another study, a higher total amount of carbohydrates was detected in *P. ostreatus* and *P. eryngii* than in this study (66.40% and 70.52%, respectively). It is worth mentioning that all substrates and species supported IPS production greater than 40%, w/w in whole carposomes and the IPS content of stipes was even greater than that in the pilei, as it has already been mentioned [22]. Moreover, many researchers have reported the positive correlation between substrate C/N and cellulose with *Pleurotus* carbohydrates content [22,27]. Based on the results of the present study, *P. ostreatus* and *P. eryngii* IPSs were moderately correlated with supplemented WS and BOS substrates cellulose content ($r^2 > 0.2768$) (substrates' analyses presented in study of Dedousi et al. 2023b [35]).

High protein amounts, up to 18%, w/w were found in all mushroom parts of *Pleurotus* species grown on supplemented WS and BOS. All the supplements enriched protein content in *P. ostreatus* carpososmes (apart from corn oil 5%, w/w in WS and BOS in the first flush and $CaSO_4 \cdot 2H_2O$ in BOS), while sunflower oil and yest extract supplementation in both substrates affected positively protein content in *P. eryngii* carpososmes (**Table 1**). In addition, *P. eryngii* presented the highest amounts of protein values compared to *P. ostreatus*, when both were cultivated on WS substrates, while the opposite occurred in those of BOS. In many studies, nitrogen-rich substrates was positively correlated with protein content of mushrooms [22–24,26,27]. As it was expected, yeast extract supplementation, an organic nitrogen source, in WS and BOS led to the highest amount of protein in the carpososmes of *P. ostreatus* and *P. eryngii*, in both flush (**Table 1**). As well, the positive effect of plant oil and calcium substrates' supplementation on carposomes protein content had been mentioned [28,30]. According to Hoa et al. [23] the protein content of *P. ostreatus* grown on different ratios of sawdust, corncobs, sugarcane bagasse and their mixtures ranged between 19.52-29.70%, results close to those of this study. Moreover, Diamantopoulou et al. [22] found 12.44-31.44 and 9.16-17.70% w/w, in *P. ostreatus* and *P. eryngii* carposomes, respectively, cultivated on five different substrates. In the same study [22], smaller amounts of protein were detected in the stipes than in the pilei, in all species and substrates examined, in contrast to the present finding, as there was no significant difference in protein content between mushroom parts.

As demonstrated in **Table 1**, *P. ostreatus* outperformed *P. eryngii* in terms of mushroom lipid content cultivated on WS and BOS substrates supplied with different oil, nitrogen and mineral supplements, as well non-supplemented substrates. It is worth noting that oils' supplementation increased the lipid content of both species, primarily when grown in BOS substrate, whereas the supplementation of yeast extract and $CaSO_4 \cdot 2H_2O$ in WS enhanced the lipid content of *P. ostreatus* and *P. eryngii*, respectively, presenting almost two-fold values compared to the control substrate. In WS substrates, values varied from 1.30-5.80% for *P. ostreatus* and 1.06-4.34% for *P. eryngii*, while in BOS substrates, values ranged from 1.84-6.58% w/w for *P. ostreatus* and 1.80-4.54% for *P. eryngii*. However, most carposomes have a mean of 4.0% w/w lipids [49], similarly high values have been observed for *Pleurotus sajor-caju* and *P. ostreatus* cultured in banana and rice straw by Bonatti et al. [50], with the highest value of 6.32% w/w, d.w. being recorded in rice straw for *P. ostreatus*. Nasiri et al. [51] also obtained high results for *Agaricus bisporus*, which is typically regarded as a low-fat mushroom (2-5 %, w/w) [52], with lipid content reaching 8.39% for pileus and 6.07% for stipe. As fresh mushrooms contain high amounts of water, the lipid proportion per 100 g has been reported to be 1.75–15.5 % in dried mushroom [53]. Therefore, the lipid content of the mushrooms evaluated in this investigation appeared to be within the stated range. Regarding the fat content in successive flushes, no discernible differences in values were found in the examined substrates between flushes, as previously was demonstrated [31]. It is worth noting that when the lipid content was compared to our earlier lipase data [35], a clear positive correlation was discovered between lipase synthesis in the substrates and *P. ostreatus* lipid content. Increased corn oil concentration resulted in higher lipase production in the substrates and greater lipid content in the produced mushrooms.

Table 1: IPS, total protein and lipid content (% w/w of dry biomass) of *P. ostreatus* (AMRL 150) and *P. eryngii* (AMRL 173-6) carposomes (c), pilei (p) and stipes (s)cultivated on supplemented WS (wheat straw), BOS (barley and oats straw) of 1st and 2nd flush.

Substrate		*P. ostreatus*						*P. eryngii*					
		IPS (% w/w)		**Protein (% w/w)**		**Lipids (% w/w)**		**IPS (% w/w)**		**Protein (% w/w)**		**Lipids (% w/w)**	
		1st	**2nd**	**1st**	**2nd**	**1st**	**2nd**	**1st**	**2nd**	**1st**	**2nd**	**1st**	**2nd**
WS-Sunflower oil 5%	c	45.57±0.02	40.57±0.31	22.18±0.47	20.97±1.49	3.42±0.11	2.94±0.08	51.69±0.23	55.38±1.25	28.99±0.63	26.15±0.38	2.02±0.21	2.94±0.25
	p	41.90±0.04	35.52±0.26	23.85±1.59	20.15±0.98	3.58±0.39	3.06±0.21	47.14±0.15	50.83±0.88	26.32±2.24	25.53±0.11	2.54±0.17	3.34±0.46
	s	50.97±0.23	46.28±0.92	25.26±0.31	22.51±0.56	1.91±0.22	1.86±0.02	54.00±0.26	56.27±0.66	28.36±3.44	26.74±0.93	1.82±0.05	2.42±0.24
WS-Corn oil 2%	c	39.82±0.34	38.19±0.15	23.7±0.68	24.50±1.20	2.52±0.35	3.28±0.45	51.07±0.14	55.57±0.09	24.86±2.91	25.32±1.94	3.84±0.29	2.04±0.17
	p	35.99±0.31	36.17±0.24	23.85±0.35	25.40±2.84	2.38±0.45	2.00±0.57	47.63±0.52	51.41±0.18	23.41±1.03	23.96±1.11	3.42±0.33	2.62±0.28
	s	45.30±0.13	44.97±0.58	21.02±1.22	21.24±1.11	1.18±0.09	1.30±0.07	56.89±0.18	58.07±0.37	25.59±2.66	26.28±2.45	1.28±0.07	1.54±0.14
WS-Corn oil 5%	c	45.76±0.65	40.15±0.04	19.97±0.68	20.73±1.59	2.92±0.25	3.70±0.25	53.24±0.18	58.00±0.05	26.14±2.49	26.71±2.75	3.02±0.25	3.52±0.36
	p	41.74±0.37	37.64±0.23	19.17±0.89	19.84±0.93	2.03±0.05	3.40±0.15	51.65±0.22	54.56±0.14	25.24±3.46	25.06±1.93	2.44±0.22	3.20±0.25
	s	50.79±0.08	43.89±0.14	21.37±0.42	21.02±0.84	1.07±0.04	3.24±0.06	61.29±0.03	61.99±0.08	26.05±0.16	27.14±0.85	2.64±0.36	1.98±0.06
WS-Yeast extract 2%	c	42.31±0.12	45.53±0.43	28.99±1.28	26.29±1.21	5.44±0.60	5.22±0.67	52.80±0.37	43.80±0.48	30.09±0.48	27.89±0.27	2.88±0.12	2.72±0.34
	p	39.36±0.76	43.51±0.54	25.82±0.62	26.35±1.27	3.96±0.20	3.58±0.41	49.37±0.21	42.45±0.23	28.11±0.12	26.11±0.35	3.10±0.28	2.56±0.03
	s	46.68±0.24	47.81±0.38	28.80±0.15	25.97±0.98	2.52±0.15	2.30±0.16	56.79±0.18	49.88±0.15	31.06±0.98	28.54±1.62	2.94±0.44	2.24±0.22
WS-$CaSO_4 \cdot 2H_2O$ 6%	c	45.77±0.22	43.30±0.18	27.68±0.56	26.11±0.34	3.92±0.49	2.10±0.08	52.75±0.08	50.28±0.38	26.14±1.49	25.31±1.93	4.34±0.36	4.26±0.18
	p	43.75±0.14	41.28±0.23	27.21±1.08	25.95±0.91	3.62±0.33	2.02±0.14	49.32±0.36	46.85±0.43	25.93±1.37	23.55±0.79	3.40±0.39	3.28±0.40
	s	50.03±0.37	45.58±0.19	30.17±0.36	28.14±0.38	2.54±0.24	1.48±0.20	57.46±0.68	53.01±0.37	27.84±0.37	25.68±2.04	2.58±0.14	2.30±0.18
WS	c	47.02±0.05	42.02±0.51	22.04±1.67	20.15±1.38	2.32±0.12	2.40±0.45	56.48±0.10	56.13±0.03	26.86±1.22	24.84±2.23	2.98±0.09	3.02±0.20
	p	44.14±0.50	42.22±0.17	26.18±0.94	19.18±1.72	2.52±0.55	2.68±0.28	52.87±0.08	52.70±0.11	26.93±1.27	23.45±0.93	1.84±0.05	1.92±0.07
	s	49.90±0.12	47.38±0.62	25.77±0.14	20.04±0.48	2.40±0.14	2.54±0.35	61.22±0.23	60.13±0.28	27.47±1.36	24.76±0.18	1.06±0.02	1.34±0.19
BOS-Sunflower oil 5%	c	43.92±0.34	47.88±0.06	27.70±0.49	27.07±0.34	6.58±0.45	4.12±0.29	55.69±1.36	53.95±0.34	24.84±1.02	21.97±0.23	3.80±0.28	3.52±0.34
	p	40.30±0.27	43.83±0.53	30.11±0.08	29.29±0.69	3.74±0.50	3.60±0.08	52.37±1.03	49.36±0.57	22.48±0.69	22.08±0.75	3.24±0.19	3.08±0.32

	s	47.68±0.63	53.34±0.27	27.40±0.60	25.95±0.66	3.92±0.18	2.82±0.12	59.53±0.12	57.11±0.05	23.02±1.54	23.37±0.42	2.36±0.23	2.60±0.28
BOS-Corn oil 2%	c	50.86±0.16	50.02±0.76	28.81±0.39	26.83±0.63	4.64±0.28	3.68±0.45	55.32±0.26	53.81±0.05	22.43±0.62	22.40±0.13	4.54±0.14	3.12±0.19
	p	45.24±0.23	46.57±0.51	28.03±0.33	25.59±0.91	3.90±0.22	2.64±0.17	49.38±0.82	48.05±0.08	22.14±0.38	21.54±0.28	4.02±0.35	3.78±0.35
	s	54.75±0.51	57.55±0.32	30.58±0.26	28.45±0.51	2.78±0.17	2.14±0.22	58.88±0.54	56.07±0.53	24.25±0.37	24.14±0.37	2.54±0.44	2.60±0.15
BOS-Corn oil 5%	c	48.09±0.17	53.19±0.13	19.29±6.79	22.35±0.59	5.60±0.67	5.80±0.56	54.88±0.50	49.78±0.54	22.48±0.92	20.54±0.20	3.28±0.27	4.98±0.15
	p	44.50±0.24	49.02±0.11	20.56±0.29	23.77±4.61	3.94±0.36	3.72±0.44	51.28±0.43	55.80±0.30	22.53±1.04	20.48±1.09	2.96±0.14	3.52±0.05
	s	53.08±0.85	55.34±0.29	18.12±0.46	22.21±2.05	3.04±0.23	2.68±0.10	59.86±0.99	62.13±1.35	24.47±1.39	22.46±1.58	2.72±0.29	2.04±0.11
BOS-Yeast extract 2%	c	46.74±0.28	44.64±0.24	29.49±1.78	27.67±2.33	4.10±0.65	3.38±0.27	51.69±0.12	52.20±0.86	24.94±1.24	24.08±0.11	2.80±0.19	2.54±0.26
	p	41.45±0.14	39.36±0.25	27.94±1.93	25.56±1.72	2.80±0.44	3.10±0.33	46.95±0.21	48.92±0.85	22.15±0.73	23.07±1.36	2.96±0.25	2.88±0.13
	s	52.82±0.03	52.72±0.06	30.33±1.60	27.48±1.02	2.76±0.25	2.88±0.30	58.32±0.10	60.29±1.16	24.50±0.34	24.98±2.05	1.80±0.08	2.00±0.29
BOS-$CaSO_4 \cdot 2H_2O$ 6%	c	48.38±0.28	57.97±0.51	22.03±0.30	19.39±1.46	3.72±0.08	4.36±0.28	57.65±0.19	49.68±0.35	23.15±0.56	23.35±0.26	2.12±0.27	2.58±0.47
	p	45.39±0.72	52.91±0.27	19.80±0.90	18.44±1.24	1.84±0.06	1.96±0.08	54.66±0.91	49.68±0.84	25.11±0.78	23.14±2.48	2.24±0.15	2.34±0.19
	s	56.01±0.89	63.68±0.32	21.33±0.96	20.96±0.73	2.54±0.17	1.64±0.03	65.28±0.88	51.09±0.15	27.15±0.36	26.10±0.21	2.00±0.36	2.30±0.22
BOS	c	47.52±0.45	50.79±0.40	25.19±0.55	20.15±0.24	3.14±0.43	2.76±0.35	46.57±0.15	42.67±0.29	23.32±0.57	21.80±0.18	2.64±0.42	2.72±0.33
	p	45.17±0.04	48.44±0.08	23.98±0.73	20.03±0.93	2.96±0.26	2.80±0.19	43.29±1.49	40.02±1.37	23.52±0.36	20.68±0.81	2.88±0.26	2.98±0.20
	s	50.17±0.44	53.44±0.41	26.34±0.43	22.11±1.02	2.80±0.07	2.48±0.35	50.89±0.24	47.62±0.89	24.71±1.06	23.11±1.94	2.42±0.34	2.64±0.39

According to the literature, there are only few studies examining the impact of successive flushes on nutrition changes in harvested mushrooms. Most of them focused on the differences in the yield of each flush that is the primary goal during industrial cultivation. For this reason, in the present study the IPS, protein and lipid content of the first and second flush *P. ostreatus* and *P. eryngii* mushrooms was evaluated. Regarding IPS content, in some mushrooms higher IPSs were detected in the first-flush than the second one, whereas in other further IPSs production detected in the second-flush mushrooms. However, in all part of mushrooms and flushes the IPSs production was satisfactory, up to 35%, w/w. On account of this, IPSs of mushrooms not only be influenced by flushes, but also by other parameters, such as the type of cultivation substrate, the nutrient availability and other environmental factors [1,31]. Furthermore, protein and lipid content did not differ significantly between flushes. These results differ from the findings of Wang et al. [32], who reported that *Pleurotus pulmonarius* total sugar were not be significantly influenced by the flush number, but its content in protein was affected in a great extent. Although, Li et al. [31] revealed that there was no significant difference in the content of crude

protein, total sugar and crude lipid of *Pleurotus citrinopileatus* between three different flushes, but when the number of flushes exceeded four, quality parameters and also the yield changed. In addition, Nasiri et al. [51] discovered that the fat content of *A. bisporus* pilei and stipes decreased from the first flush to the third flush. Therefore, mushrooms harvested during the second flush are also nutritious, with satisfactory protein and lipid content and low amounts of IPSs, a fact that could be useful for patients with prediabetes problems.

3.2. Individual carbohydrates

The results of carbohydrates composition analysis are represented in **Table 2**. Glucose was the only constituent present in all *Pleurotus* carposomes, stipes and pilei (33-89 %, w/w), while its ratio varied depending on the substrate. In most of the mushrooms cultivated on different substrates, glucose was the prevailing monosaccharide. By contrast, *P. ostreatus* mushrooms derived from WS supplemented with yeast extract and $CaSO_4 \cdot 2H_2O$ and also *P. eryngii* cultivated on some of the supplemented substrates of WS and BOS, contained higher amount of fructose than glucose (38-50%, w/w) (**Table 2**). However, in *P. ostreatus* mushrooms grown on substrates supplemented with oils, fructose was not detected. Manitol (0.5-18 %, w/w) and arabitol (1-11 %, w/w) were found in smaller quantities (or not at all) only in some *Pleurotus* mushrooms. Also, it is noteworthy that all *P. ostreatus* parts and some of *P. eryngii* contained fructose or mannitol and not both of them. Other sugars that were detected were trehalose (1-23%, w/w) and rhamnose (1-14%, w/w), the last one only in *P. ostreatus* cultivated on substrates with oils. In previous studies, it was reported that glucose was the most abundant carbohydrate of *Pleurotus* mushrooms [22,54–57]. More specific, Diamantopoulou et al. [22] reported that apart from glucose, the main sugar in all *P. ostreatus* and *P. eryngii* mushrooms cultivated on WS, BOS, CR, beech wood shavings and rice bark, also fructose was detected in lower concentrations than glucose and other compounds, such as mannitol and arabitol, were found in smaller quantities (or not at all). Moreover, other sugars which have been detected in *Pleurotus* mushrooms in various percentages are galactose, mannose [54–56], xylose [54,55] fucose [54,57] and fructose [55], all these monosaccharides were not found in the present study. Additionally, Reis et al. [58] reported that the main sugars of six edible mushrooms, among them *P. ostreatus* and *P. eryngii*, were mannitol and trehalose and also explained that such differences in their sugars composition can be observed between wild and cultivated samples of the same mushroom species. Regarding the different mushroom parts, in some cases there were not significant differences, whereas some sugars were detected only in one part (pileus or stipe) or only in carposome of the produced mushrooms. Zhou et al. [57] studied sugar distribution in *P. ostreatus* pileus and stipe and they detected higher levels of trehalose in the stipe rather than pileus and greater amount of glucose in pilei than in stipes, in contrast to our findings in which the highest levels of trehalose occurred in the pilei and the greatest levels of glucose were detected on stipes of the most mushrooms.

Mushroom's carbohydrates can be extracted for food and medical purposes (sugar substitutes in sweets and other foods with few calories) [59] and cosmetic uses (the use of trehalose as moisturizer in cosmetic preparation)[60]. As stipes contain significant amounts of carbohydrates and they are generally removed and discarded in traditional mushroom production, they could represent an economical extraction source for carbohydrate utilization.

Table 2: Carbohydrate composition (% w/w) of total IPSs produced by *P. ostreatus* (AMRL 150) and *P. eryngii* (AMRL 173-6) cultivated on supplemented WS (wheat straw), BOS (barley and oats straw) of 1st and 2nd flush. Mushroom parts tested: carp, pilei and stipes.

			Carbohydrates (%, w/w of Total IPSs)										
Substrate	Flush	Mushroom part	***P. ostreatus***						***P. eryngii***				
			Trehalose	Glucose	Manitol	Fructose	Ramnose	Arabitol	Trehalose	Glucose	Manitol	Fructose	Arabitol
WS-Sunflower oil 5%	1st	carp	17.2±0.6	67.4±4.2	10.2±1.3	nd*	5.2±0.9	nd	21.4±1.2	37.9±3.1	nd	40.7±2.9	nd
		pileus	15.2±0.5	61.0±1.9	17.3±0.9	nd	6.4±0.3	nd	17.5±1.3	35.8±2.6	nd	46.7±1.2	nd
		stipe	8.0±1.5	72.0±2.6	15.0±1.2	nd	5.0±0.1	nd	11.2±0.6	53.9±2.8	nd	34.9±1.3	nd
	2nd	carp	16.0±1.2	69.4±2.9	8.6±0.3	nd	6.0±0.4	nd	21.4±1.2	40.6±3.2	nd	38.1±2.7	nd
		pileus	14.7±0.6	60.0±1.6	18.1±1.1	nd	7.2±0.6	nd	18.3±1.8	37.1±4.2	nd	44.6±1.5	nd
		stipe	12.5±1.4	72.8±2.7	9.4±0.4	nd	5.2±0.6	nd	10.8±0.4	55.2±3.8	nd	34.0±2.8	nd
WS-Corn oil 2%	1st	carp	16.2±0.6	69.5±2.6	8.9±0.6	nd	5.5±0.4	nd	16.6±1.1	35.3±2.3	nd	45.9±2.7	2.2±0.8
		pileus	12.2±0.4	68.0±3.8	11.3±1.0	nd	6.4±0.2	2.2±0.6	14.4±1.3	40.3±3.4	nd	42.1±1.4	3.3±0.9
		stipe	8.2±0.5	80.7±1.7	7.1±0.4	nd	4.1±0.8	nd	9.9±0.4	39.3±2.5	nd	48.4±1.2	2.4±0.4
	2nd	carp	15.5±0.3	67.3±2.8	8.2±0.2	nd	8.9±0.8	nd	17.0±0.6	38.4±3.2	nd	44.6±2.5	nd
		pileus	11.8±1.0	74.2±2.7	4.7±0.1	nd	7.2±0.5	2.0±0.2	13.6±0.4	41.2±2.7	nd	45.2±1.5	nd
		stipe	10.5±1.6	78.4±3.2	4.0±0.1	nd	7.1±0.4	nd	13.9±0.8	43.5±2.9	nd	42.6±1.4	nd
WS-Corn oil 5%	1st	carp	17.8±0.7	70.8±4.3	7.2±0.2	nd	4.1±0.3	nd	9.8±1.2	36.9±2.4	nd	50.1±1.6	3.2±1.1
		pileus	5.5±0.4	78.1±3.7	9.3±0.3	nd	5.7±0.2	1.4±0.6	8.9±0.4	47.5±3.2	nd	43.6±1.6	nd
		stipe	6.8±1.0	86.7±2.8	5.3±0.3	nd	1.2±0.3	nd	3.7±0.9	55.2±3.3	nd	41.1±2.7	nd
	2nd	carp	13.5±0.6	80.9±1.8	0.5±0.1	nd	5.2±0.5	nd	8.5±1.0	41.6±3.9	nd	49.9±2.6	nd
		pileus	10.6±0.7	69.0±3.9	7.6±1.2	nd	10.7±0.9	2.1±0.3	2.4±0.8	45.3±2.4	nd	48.2±1.7	4.1±0.6
		stipe	13.6±1.2	73.3±3.0	5.8±0.3	nd	7.2±0.1	nd	14.5±0.9	42.8±3.0	nd	42.8±1.1	nd
WS-Yeast extract 2%	1st	carp	14.9±1.3	42.6±2.9	nd	42.5±1.9	nd	nd	10.6±1.0	43.2±3.2	nd	46.2±2.3	nd
		pileus	16.4±0.8	40.8±3.2	nd	42.9±1.3	nd	nd	9.2±1.6	48.3±4.3	nd	42.6±1.3	nd

		stipe	22.1±0.6	50.0±2.6	nd	27.9±1.8	nd	nd	11.9±1.8	44.5±2.5	nd	43.5±2.8	nd
	2nd	carp	14.9±0.4	43.9±2.9	nd	41.3±1.6	nd	nd	10±1.4	35.9±2.2	nd	48.7±2.4	5.4±0.9
		pileus	12.1±1.2	50.7±3.2	nd	37.3±1.6	nd	nd	8.0±0.4	49.6±1.4	nd	42.4±2.8	nd
		stipe	17.9±0.6	57.3±3.0	nd	24.8±2.0	nd	nd	2.3±0.6	50.8±3.3	nd	46.9±1.9	nd
WS-$CaSO_4 \cdot 2H_2O$ 6%	1st	carp	13.1±1.1	37.3±2.8	nd	49.6±1.0	nd	nd	13.3±0.3	50.0±4.0	nd	36.7±2.8	nd
		pileus	10.4±1.3	42.4±4.1	nd	47.2±1.5	nd	nd	12.2±0.7	55.6±2.8	nd	32.2±2.6	nd
		stipe	5.6±0.6	50.7±3.3	nd	43.7±1.7	nd	nd	11.0±0.2	54.2±3.4	nd	34.8±2.1	nd
	2nd	carp	13.3±0.8	33.3±2.3	nd	48.6±1.9	nd	4.7±0.1	11.5±0.6	48.3±2.8	nd	40.2±2.2	nd
		pileus	11.1±1.2	40.0±3.8	nd	48.9±1.8	nd	nd	11.4±0.7	50.2±3.2	nd	38.5±2.6	nd
		stipe	9.3±0.6	43.1±2.9	nd	47.5±1.9	nd	nd	8.6±0.9	52.4±3.4	nd	39.1±1.8	nd
WS-Control	1st	carp	8.3±0.4	57.1±2.5	nd	34.6±1.5	nd	nd	10.5±0.5	83.5±3.1	6.0±0.3	nd	nd
		pileus	11.4±0.7	55.0±3.1	nd	33.6±2.0	nd	nd	3.1±0.9	68.3±1.7	nd	28.6±3.0	nd
		stipe	11.6±0.9	56.6±2.1	nd	31.8±2.2	nd	nd	10.4±0.9	58.3±2.4	nd	31.2±2.1	nd
	2nd	carp	22.7±1.2	52.2±3.6	nd	25.2±1.2	nd	nd	9.5±1.6	46.3±1.6	nd	44.2±2.2	nd
		pileus	18.0±1.6	54.1±3.2	nd	28.0±1.6	nd	nd	5.2±1.5	62.3±2.0	nd	32.5±2.9	nd
		stipe	13.1±0.3	56.2±2.1	nd	30.7±1.7	nd	nd	9.4±1.0	57.1±4.1	nd	33.5±2.5	nd
BOS-Sunflower oil 5%	1st	carp	10.6±0.4	72.0±4.0	12.4±1.2	nd	5.1±0.5	nd	11.8±0.5	70.6±3.7	nd	17.6±2.1	nd
		pileus	3.6±0.9	73.1±2.6	16.3±1.6	nd	7.1±0.8	nd	9.7±0.2	72.1±2.4	nd	18.2±2.5	nd
		stipe	nd	86.0±3.7	9.5±0.3	nd	4.4±0.6	nd	4.4±0.2	83.6±3.0	nd	12.0±2.0	nd
	2nd	carp	9.5±0.5	74.6±2.8	10.1±0.9	nd	5.8±0.2	nd	12.6±0.6	72.2±3.3	nd	15.2±2.0	nd
		pileus	5.0±0.2	75.1±3.4	12.3±0.4	nd	7.7±0.1	nd	14.8±0.4	71.1±3.5	nd	14.1±1.4	nd
		stipe	nd	87.0±3.2	7.1±0.6	nd	5.9±0.6	nd	5.3±0.1	78.2±2.0	nd	16.5±2.1	nd
BOS-Corn oil 2%	1st	carp	5.0±1.1	74.0±3.1	9.1±0.7	nd	5.8±0.5	6.1±0.6	11.6±0.3	72.1±2.1	nd	14.4±1.9	2.0±0.4
		pileus	nd	73.9±2.5	10.2±0.8	nd	5.0±0.1	10.9±1.2	12.4±0.4	70.4±3.5	nd	17.3±2.6	nd
		stipe	nd	88.2±3.9	7.0±0.2	nd	4.8±0.6	nd	13.1±0.6	69.3±2.6	nd	15.2±1.7	2.3±0.2

	2nd	carp	10.4±0.3	74.7±2.6	9.2±0.3	nd	3.9±0.4	2.0±0.2	9.5±0.8	71.1±2.3	nd	16.4±2.2	3.1±0.6
		pileus	nd	74.1±2.4	9.1±0.1	nd	10.2±0.2	6.7±0.4	7.3±0.4	76.4±2.9	nd	16.3±2.6	nd
		stipe	nd	89.3±1.9	7.2±0.5	nd	3.4±0.7	nd	8.2±0.6	75.2±3.2	nd	13.7±2.3	2.8±0.4
BOS-Corn oil 5%	1st	carp	10.7±0.1	71.8±3.3	9.3±0.6	nd	8.1±0.1	nd	12±0.3	70.1±3.6	nd	15.6±2.4	2.3±0.6
		pileus	9.2±1.4	68.1±3.2	8.9±0.4	nd	10.7±0.8	3.1±0.1	10±0.2	69.3±1.4	nd	18.2±2.1	2.4±0.7
		stipe	nd	78.2±1.6	12.1±0.4	nd	9.6±0.5	nd	7.7±0.8	75.3±1.9	nd	17.1±1.3	nd
	2nd	carp	14.6±0.8	70.3±1.4	5.1±0.2	nd	10.1±0.2	nd	6.8±0.6	69.5±2.3	nd	20.2±1.8	3.6±0.3
		pileus	6.1±0.9	68.2±2.3	6.1±0.3	nd	13.6±0.8	6.1±0.3	7.6±0.4	72.4±2.6	nd	17.6±2.0	2.5±0.7
		stipe	nd	79.5±2.1	10.1±1.1	nd	10.4±0.6	nd	5.7±0.8	75.2±3.6	nd	19.1±2.3	nd
BOS-Yeast extract 2%	1st	carp	10.0±1.1	45.0±1.9	nd	45.0±2.5	nd	nd	14.9±1.2	42.4±3.2	nd	42.7±1.7	nd
		pileus	13.7±0.9	40.2±2.6	nd	46.1±1.4	nd	nd	19.3±1.6	37.7±1.6	nd	43.1±2.3	nd
		stipe	5.4±0.3	46.3±2.4	nd	48.3±1.1	nd	nd	7.4±1.2	43.8±3.4	nd	48.9±1.8	nd
	2nd	carp	9.0±0.2	47.8±2.5	nd	43.2±1.4	nd	nd	9.7±0.9	45.2±3.2	nd	40.8±2.1	4.3±1.1
		pileus	11.0±0.5	49.0±3.3	nd	40.0±1.7	nd	nd	15.2±0.4	47.8±3.0	nd	37.0±1.4	nd
		stipe	13.9±0.7	47.0±3.6	nd	39.2±2.3	nd	nd	2.3±0.1	58.0±2.1	nd	39.8±2.3	nd
BOS-$CaSO_4 \cdot 2H_2O$ 6%	1st	carp	13.5±0.8	40.3±2.3	nd	46.2±1.2	nd	nd	18.4±1.4	37.8±2.1	nd	43.9±2.2	nd
		pileus	13.6±0.6	39.0±3.5	nd	41.4±1.1	nd	6.0±0.5	12.6±0.6	37.8±1.4	11.2±1.2	38.3±1.7	nd
		stipe	12.6±0.7	42.4±2.4	nd	45.0±2.4	nd	nd	1.0±0.1	53.4±3.3	nd	45.6±1.5	nd
	2nd	carp	12.9±1.3	38.1±2.5	nd	49.1±2.2	nd	nd	17.8±2.1	35.5±2.8	nd	46.7±2.0	nd
		pileus	9.1±1.2	37.2±3.4	nd	48.6±2.1	nd	5.11	3.2±0.3	36.0±3.5	15.3±1.1	45.5±1.6	nd
		stipe	13.3±0.8	40.4±2.2	nd	46.3±1.3	nd	nd	4.7±0.4	51.4±3.1	nd	43.9±1.9	nd
BOS-Control	1st	carp	2.4±0.4	58.3±2.1	nd	35.0±2.1	nd	4.3±0.4	5.8±0.1	55.8±2.0	16.0±0.9	18.7±2.3	3.7±0.9
		pileus	7.1±0.2	61.6±2.7	nd	31.3±1.6	nd	nd	8.6±0.5	60.4±3.1	12.8±0.8	18.2±2.4	nd
		stipe	2.6±0.2	64.5±2.0	nd	32.9±2.3	nd	nd	9.3±0.6	75.5±2.7	nd	15.2±1.9	nd
	2nd	carp	2.9±0.9	59.1±2.2	nd	32.6±1.0	nd	5.4±0.1	2.4±0.7	56.6±3.2	16.4±1.3	22.6±1.6	2.1±0.2

pileus	11.3±0.6	50.6±3.5	nd	38.1±2.4	nd	nd	7.1±0.8	49.5±1.8	18.4±0.8	25.1±1.8	nd
stipe	4.9±0.1	65.1±2.4	nd	30.1±1.5	nd	nd	7.9±0.3	76.1±2.9	nd	16.0±2.0	nd

* nd: not detected

3.3. Fatty acid (FA) composition

Polyunsaturated (PUFA), unsaturated and saturated fatty acid concentrations in mushroom samples were determined in **Table 3**. FA distribution varied between the species in the examined substrates, while the substrates had an obvious impact in carposomes FA profile. Up to 10 fatty acids were detected in mushroom lipids with PUFA being the major fatty acid class detected as linoleic acid (54.25-75.40%) was the most abundant among the species, followed by oleic (3.41-13.08%) and palmitic acid (10.39-15.62%). Sunflower and corn oil supplementation enhanced the concentration of linoleic acid, increasing the unsaturation of the produced fatty acids; however sunflower oil in WS resulted in a lower value of linoleic acid (57.25%) than the non-supplemented WS (67.60%). Additionally, it is worth mentioning that cis-11-octadecenoic acid did not appear in *P. ostreatus* when cultivated on BOS substrates, while the supplementation of $CaSO_4 \cdot 2H_2O$ produced mushrooms with lower linoleic acid, except for *P. ostreatus* and *P. eryngii* when cultivated on BOS and WS, respectively. In general, linoleic acid was detected in higher levels in the examined mushrooms when grown in BOS substrates than in WS. The obtained results support previous findings that unsaturated fatty acids prevail over saturated fatty acids in mushrooms' lipid profile [61–64]. The content of unsaturated fatty acids is crucial for mushroom nutritional value, as high linoleic and oleic acid levels have been shown to be particularly significant for human health because they prevent atherosclerosis in the blood by interacting with HDL, the good cholesterol [15]. Also, linoleic acid is vital for the production and conversion of flavor components in *Pleurotus* mushrooms [25].

Table 3: Fatty acid composition (% w/w) of total lipids produced by *P. ostreatus* (AMRL 150) and *P. eryngii* (AMRL 173-6) carposomes cultivated on supplemented WS (wheat straw), BOS (barley and oats straw) of 1st flush (mean ± SD).

FAs	**C11:0**	**C12:0**	**C14:0**	**C15:0**	**C15:1**	**C16:0**	**C16:1, cis-9**	**C17:0**	**C18:0**	**C18:1, cis-9**	**C18:1 n7**	**C18:2 cis-9,12, n6**	**C20:5**	**C22:0**	**C22:1, cis-13**	**C22:4, n6**	**C24:0**	**C24:1, cis-15**	**saturated**	**monounsaturated**	**polyunsaturated**	**ω6**
Substrates / P. ostreatus																						

WS - Sunflower oil 5%	nd*	0.32 ±0.09	0.74 ±0.05	2.29 ±0.02	0.27 ±0.05	15.62 ±0.14	nd	nd	1.74 ±0.36	12.76 ±0.13	1.02 ±0.18	57.25 ±1.60	nd	0.72 ±0.15	1.43 ±0.19	nd	0.21 ±0.01	0.38 ±0.03	21.63	15.86	57.25	57.25
WS - Corn oil 2%	0.23 ±0.01	0.36 ±0.04	0.46 ±0.08	1.31 ±0.02	0.25 ±0.01	13.49 ±0.07	nd	nd	1.33 ±0.58	6.33± 0.32	nd	69.31 ±1.33	nd	0.69 ±0.06	nd	1.30 ±0.22	nd	0.70 ±0.06	17.86	7.28	70.61	69.31
WS - Corn oil 5%	0.56 ±0.08	0.33 ±0.02	0.49 ±0.07	1.51 ±0.06	nd	11.93 ±0.25	nd	nd	1.49 ±0.18	7.83± 0.24	nd	68.59 ±0.64	0.38 ±0.07	0.67 ±0.06	1.12 ±0.07	0.98 ±0.03	nd	1.01 ±0.06	16.98	10.06	69.57	68.59
WS - Yeast extract 2%	nd	0.38 ±0.04	0.49 ±0.02	1.88 ±0.04	0.37 ±0.03	13.29 ±0.17	nd	nd	1.20 ±0.26	7.00± 0.08	0.42 ±0.01	67.82 ±1.51	0.08 ±0.01	0.73 ±0.07	1.01 ±0.29	nd	nd	nd	17.98	8.80	67.82	67.82
WS $CaSO_4 2H_2O$ 6%	nd	0.59 ±0.07	0.57 ±0.04	2.02 ±0.01	nd	10.85 ±0.19	nd	nd	1.26 ±0.14	8.95± 0.47	nd	60.37 ±1.69	nd	0.70 ±0.04	1.02 ±0.03	0.86 ±0.05	0.24 ±0.01	nd	16.22	9.98	61.23	60.37
WS	nd	0.43 ±0.03	0.38 ±0.06	1.93 ±0.17	nd	12.66 ±0.22	nd	nd	1.39 ±0.24	8.84± 0.51	0.18 ±0.05	67.44 ±1.39	0.05 ±0.01	0.85 ±0.07	0.99 ±0.08	0.16 ±0.02	0.24 ±0.02	0.56 ±0.08	17.88	10.56	67.60	67.44
BOS - Sunflower oil 5%	nd	0.86 ±0.06	0.90 ±0.06	1.75 ±0.12	0.56 ±0.02	11.50 ±0.43	nd	nd	1.36 ±0.09	3.41± 0.15	nd	75.40 ±1.56	nd	0.29 ±0.01	0.53 ±0.09	nd	nd	0.32 ±0.06	16.66	4.82	75.40±1.56	75.40 ±1.56

BOS - Corn oil 2%	nd	0.94 ±0.07	1.01 ±0.08	2.04 ±0.10	0.60 ±0.02	13.50 ±0.36	0.19± 0.01	nd	1.38 ±0.13	3.43± 0.04	nd	72.00 ±1.19	nd	0.17 ±0.01	0.38 ±0.02	0.61 ±0.04	nd	0.39 ±0.01	19.04	4.99	72.61±1.23	72.00 ±1.19
BOS - Corn oil 5%	nd	2.91 ±0.10	1.89 ±0.03	1.47 ±0.07	nd	11.30 ±0.24	nd	nd	1.65 ±0.08	5.61± 0.25	nd	68.50 ±0.67	nd	nd	nd	2.67 ±0.14	nd	nd	19.22	5.61	71.17±0.81	68.50 ±0.67
BOS - Yeast extract 2%	0.23 ±0.02	0.73 ±0.06	1.13 ±0.02	1.90 ±0.13	0.22 ±0.01	11.51 ±0.19	nd	nd	1.84 ±0.10	4.00± 0.12	nd	72.93 ±0.89	nd	0.36 ±0.06	0.42 ±0.04	0.30 ±0.08	0.43 ±0.09	0.48 ±0.04	18.13	5.12	73.23±0.97	72.93 ±0.89
BOS $CaSO_4 2H_2O$ 6%	0.26 ±0.01	0.77 ±0.05	0.99 ±0.06	1.44 ±0.04	nd	12.63 ±0.14	nd	nd	1.56 ±0.05	5.27± 0.09	nd	70.60 ±1.01	nd	0.24 ±0.02	nd	nd	nd	nd	17.89	5.27	70.60±1.01	70.60 ±1.01
BOS	0.26 ±0.03	0.85 ±0.03	0.65 ±.07	2.88 ±0.08	0.58 ±0.04	13.79 ±0.28	nd	nd	1.21 ±0.08	3.66± 0.19	nd	68.21 ±0.93	0.37	1.27 ±0.05	1.69 ±0.12	nd	nd	0.37 ±0.02	20.91	6.30	68.21±0.93	68.21 ±0.93
Substrates / P. eryngii																						
WS - Sunflower oil 5%	nd	0.23 ±0.05	0.39 ±0.06	1.55 ±0.03	nd	11.48 ±1.01	nd	nd	1.47 ±0.35	8.05± 1.41	1.43 ±0.12	65.22 ±1.84	nd	1.04 ±0.01	1.63 ±0.02	nd	0.31 ±0.02	0.54 ±0.08	16.46	11.65	65.22	65.22
WS - Corn oil 2%	nd	0.34 ±0.03	0.53 ±0.08	2.00 ±0.02	0.35 ±0.03	13.57 ±0.45	nd	nd	1.61 ±0.23	6.46± 0.81	0.91 ±0.08	65.68 ±1.35	0.10 ±0.01	0.97 ±0.07	1.37 ±0.04	nd	0.26 ±0.02	0.36 ±0.03	19.29	9.45	65.68	65.68

WS - Corn oil 5%	nd	0.42 ±0.04	0.66 ±0.03	1.81 ±0.05	0.37 ±0.03	13.96 ±0.64	nd	nd	1.76 ±0.11	6.22± 0.54	1.18 ±0.03	61.99 ±1.22	nd	1.29 ±0.06	1.78 ±0.07	nd	nd	nd	19.89	9.54	61.99	61.99
WS - Yeast extract 2%	nd	0.21 ±0.03	0.48 ±0.02	1.75 ±0.06	0.39 ±0.05	13.46 ±0.28	nd	nd	1.85 ±0.48	13.08 ±0.36	0.96 ±0.05	58.07 ±1.14	nd	1.26 ±0.06	1.71 ±0.04	nd	0.30 ±0.04	nd	19.31	16.14	58.07	58.07
WS $CaSO_4 2H_2O$ 6%	nd	0.21 ±0.04	0.48 ±0.05	1.90 ±0.04	0.40 ±0.06	13.75 ±0.65	nd	nd	1.87 ±0.17	9.65± 0.47	0.91 ±0.05	60.26 ±0.87	nd	1.25 ±0.04	1.65 ±0.08	nd	0.26 ±0.07	nd	19.71	12.61	60.26	60.26
WS	nd	nd	0.70 ±0.03	2.32 ±0.07	0.81 ±0.04	15.20 ±0.22	nd	nd	1.88 ±0.24	4.83± 0.72	0.95 ±0.06	54.25 ±0.67	nd	1.69 ±0.08	2.35 ±0.12	1.13 ±0.05	nd	1.27 ±0.04	21.79	10.20	55.38	54.25
BOS - Sunflower oil 5%	0.23 ±0.01	0.64 ±0.06	0.79 ±0.02	1.89 ±0.07	0.80 ±0.05	12.77 ±0.45	nd	0.25 ±0.02	1.91 ±0.16	8.48± 0.78	1.05 ±0.18	64.28 ±1.26	nd	nd	0.44 ±0.02	0.63 ±0.13	0.47 ±0.03	0.25 ±0.06	17.05	11.02	64.91	64.28
BOS - Corn oil 2%	0.46 ±0.04	0.73 ±0.03	0.75 ±0.05	1.35 ±0.03	1.02 ±0.02	10.39 ±0.37	nd	0.29 ±0.01	1.40 ±0.05	5.53± 0.26	0.79 ±0.07	69.74 ±1.55	0.27 ±0.01	0.73 ±0.17	0.91 ±0.06	0.33 ±0.06	nd	nd	14.70	8.25	70.06	69.74
BOS - Corn oil 5%	nd	0.52 ±0.07	0.74 ±0.07	1.94 ±0.06	0.42 ±0.07	14.60 ±0.13	nd	nd	1.54 ±0.17	5.38± 0.15	0.92 ±0.13	69.40 ±0.87	nd	nd	1.12 ±0.10	nd	0.35 ±0.04	nd	18.15	7.84	69.40	69.40

BOS - Yeast extract 2%	nd	0.52 ±0.12	0.61 ±0.04	1.78 ±0.04	0.59 ±0.06	14.55 ±0.20	nd	nd	1.44 ±0.10	5.46± 0.45	0.98 ±0.05	69.44 ±1.18	nd	0.19 ±0.02	0.53 ±0.05	nd	1.17 ±0.08	nd	18.82	7.55	69.44	69.44
BOS CaSO$_4$2H$_2$O 6%	nd	0.64 ±0.06	0.83 ±0.07	2.27 ±0.12	nd	10.56 ±0.06	nd	nd	1.28 ±0.04	4.05± 0.24	0.91 ±0.03	66.26 ±0.68	nd	0.53 ±0.02	0.74 ±0.07	0.63 ±0.03	0.86 ±0.05	0.48 ±0.05	15.68	6.19	66.90	66.27
BOS	nd	0.32 ±0.05	0.55 ±0.04	1.40 ±0.11	nd	11.99 ±0.17	nd	nd	1.40 ±0.09	4.40± 0.19	0.82 ±0.02	69.88 ±1.09	nd	0.29 ±0.04	1.62 ±0.15	nd	nd	nd	14.54	6.84	69.88	69.88

* nd: not detected

3.4. Total phenolic compounds (TPC) and antioxidant activity

Both *Pleurotus* species produced satisfactory amount of TPC in all supplemented substrates (11.48–41.74 mg GAE/ g d.w.). The impact of chemical composition of substrates on TPC in mushrooms has already been reported in previous studies [22,65–67]. As a consequence, the supplementation of WS with sunflower and corn oil, yeast extract and $CaSO_4 \cdot 2H_2O$, while only BOS supplied with sunflower and $CaSO_4 \cdot 2H_2O$ contributed to the increase in TPC in *P. ostreatus* carposomes. In *P. erygii* carposomes, higher TPC were detected when grown on WS with yeast extract and $CaSO_4 \cdot 2H_2O$ and in all supplemented BOS substrates (**Fig. 1**). Among the supplements, substrates with $CaSO_4 \cdot 2H_2O$ led to the greatest TPC in both *Pleurotus* species. Furthermore, greater TPC production was detected in pileus than stipes. In previous studies, TPC of *P. sajor-caju* cultivated on three different substrates ranged from 56.26 to 205.23 mg/100 g d.w. in the findings of da Paz et al. [65], whereas Sardar et al. [67] reported significantly lower TPC produced by *P. eryngii* grown on WS (~200 mg GAE/ kg d.w.). Moreover, Diamantopoulou et al. [22] revealed that pilei were the most phenolic-rich part of *P. ostreatus* and *P.* eryngii, in accordance with the findings of the present study and their value ranged from 10.41 to 70.67 mg GAE/ g d.w..

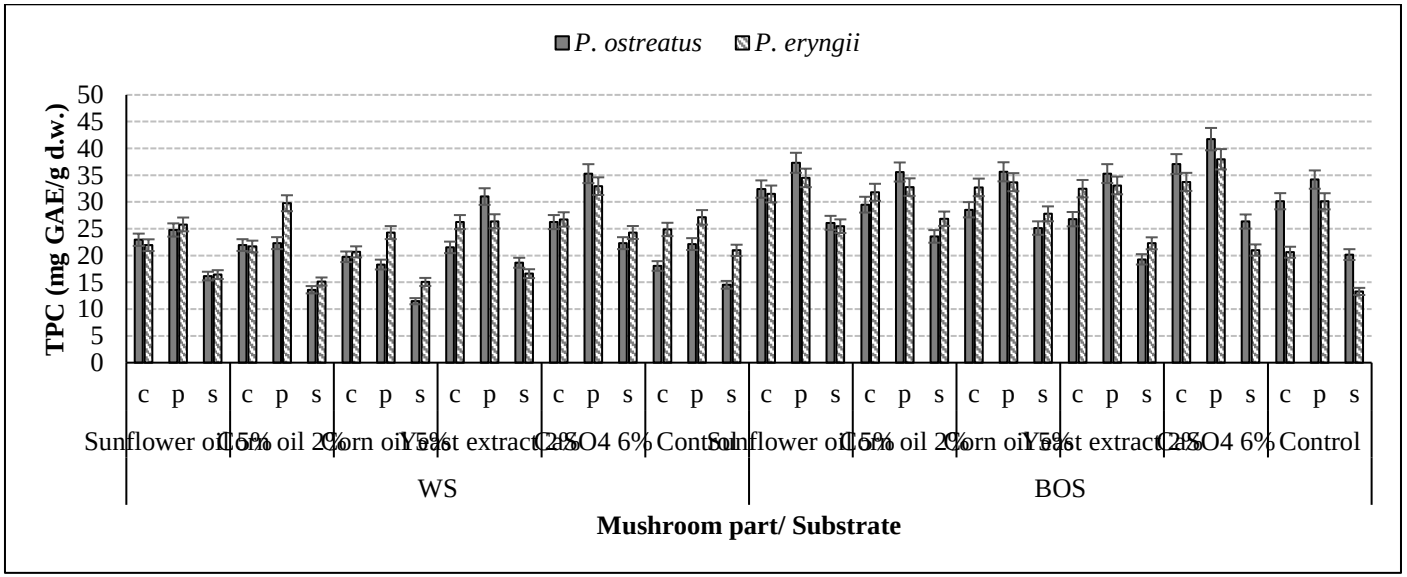

Figure 1. Total phenolic compounds (TPC, expressed as gallic acid equivalent; GAE mg/ g dry weight of biomass) of methanol extracts of *P. ostreatus* (AMRL 150) and *P. eryngii* (AMRL 173-6) carposomes (c), pilei (p) and stipes (s), cultivated on supplemented WS (wheat straw), BOS (barley and oats straw). Mean values with error bars indicate the standard deviations from duplicate experiments of five replicates.

As antioxidant compounds have multiple mechanisms of action, three different methods were employed in the present study in order to examine the total antioxidant compounds (TAC) of the mushroom extracts. The scavenging capacity of free radicals $DPPH^{\cdot+}$ and $ABTS^{\cdot+}$ ranged from 0.68-4.05 mg trolox/g d.w. and 1.33-5.91 mg trolox/g d.w., respectively, in both *Pleurotus* species cultivated on supplemented substrates. Their highest values were detected in WS supplemented with yeast extract and $CaSO_4 \cdot 2H_2O$ and in BOS supplemented with sunflower oil, corn oil 5%, w/w for *P. ostreatus* and BOS with yeast extract and $CaSO_4 \cdot 2H_2O$ for *P. eryngii*. Furthermore, the FRAP levels of *P. ostreatus* parts ranged from 2.60 to 12.41 mg trolox/g d.w. and from 6.05 to 13.18 mg trolox/g d.w. in *P. eyngii* parts. *P. eryngii* produced greater or comparable TAC compared to *P. ostreatus* (**Table 4**). Only FRAP levels in *P. ostreatus* mushrooms grown on supplemented WS substrates and their carposomes in supplemented BOS substrates (except for yeast extract supplementation) were higher than those in control substrates, while the supplementation did not enrich the *P. eryngii* reducing power. As it has already been detected by Diamantopoulou et al. [22], all the tested pilei had greater antioxidant values than the corresponding stipes. In previous study, the capacity of 16 strains of *P. ostreatus* methanolic extract to scavenge the free radical DPPH ranged from 0.82-4.07 mmol TE/100 g f.w., while values of reducing power ranged from 1.38 to 2.69 mmol TE/100 g f.w. [68]. Besides, Diamantopoulou

et al. [22] examined *P. osteatus* and *P. eryngii* strains grown on agricultural residues and reported almost the same values for scavenging free radical DPPH$^{\cdot+}$ (0.35 to 13.22 mg trolox/g d.w.) and greater values for the scavenging capacity of ABTS$^{\cdot+}$ (1.04 to 15.45 mg trolox/g d.w.), while the values for reducing power (0.25 to 13.41 mg trolox/g d.w.) were significantly lower than those of the present study. Furthermore, the positive correlation between phenolic compounds and antioxidant properties have mentioned in previous studies [67–69]. The present results revealed a strong correlation between TPC of *P. ostreatus* and DPPH and ABTS (r = 0.676 and r = 0.5153, respectively), whereas the correlation coefficient between the TPC of *P. eryngii* and DPPH and BTS was characterized as moderate (r = 0.3863 and r = 0.338, respectively). Concerning TPC results and those of FRAP, a weak correlation detected for *P. ostreatus* and *P. eryngii* (r = 0.1156 and r = 0.0903, respectively).

Table 4: Scavenging ability on DPPH· and ABTS·+ free radicals and FRAP of methanol extracts of *P. ostreatus* (AMRL 150) and *P. eryngii* (AMRL 173-6) carposomes (c), pilei (p) and stipes (s), cultivated on supplemented WS (wheat straw), BOS (barley and oats straw). Measurements of antioxidant studies are expressed as mg of trolox equivalence/g of mushroom dry weight (mean ± SD).

		DPPH$^{\cdot}$	**ABTS$^{\cdot+}$**	**FRAP**	**DPPH$^{\cdot}$**	**ABTS$^{\cdot+}$**	**FRAP**
		P. ostreatus			***P. eryngii***		
WS-Sunflower oil 5%	c	1.55±0.34	3.59±0.07	7.11±1.21	2.08±0.28	3.51±0.05	7.54±0.48
	p	1.64±2.10	4.07±0.07	10.19±0.18	1.90±2.40	3.10±0.67	13.18±1.04
	s	1.01±0.23	2.86±0.12	5.21±1.27	1.96±0.37	2.87±0.16	8.44±0.56
WS-Corn oil 2%	c	1.57±1.82	4.11±0.03	8.73±0.82	1.85±1.74	3.69±0.12	11.11±1.56
	p	1.64±1.29	4.26±0.02	9.79±1.26	3.38±0.37	4.20±0.67	12.49±2.04
	s	0.93±1.98	1.58±0.08	5.03±1.80	2.81±1.88	2.33±0.09	6.26±0.03
WS-Corn oil 5%	c	1.42±1.22	2.28±0.01	7.02±0.29	1.30±0.38	3.65±0.28	9.85±2.04
	p	1.58±2.19	3.20±0.05	8.02±0.29	1.91±2.38	4.17±2.66	10.82±1.94
	s	0.68±0.98	1.33±0.05	3.77±0.94	1.29±0.37	2.97±0.99	6.05±2.04
WS-Yeast extract 2%	c	1.78±2.13	4.84±0.02	11.00±0.19	2.75±1.57	2.84±0.13	10.80±0.02
	p	2.11±0.98	5.91±0.08	12.20±0.05	4.04±0.82	3.35±0.67	12.19±1.04
	s	1.85±2.36	3.24±0.07	9.51±0.17	2.12±1.60	2.32±0.08	8.81±0.58
WS-$CaSO_4 \cdot 2H_2O$ 6%	c	2.36±0.92	2.74±0.05	10.20±1.58	2.76±0.33	4.74±0.14	9.04±0.61
	p	2.54±0.18	4.54±0.78	12.41±1.03	4.05±0.67	4.42±0.03	10.43±0.83

	s	1.60±0.22	1.73±0.20	6.74±0.59	2.13±0.22	1.76±0.11	7.06±0.93
WS (control)	c	1.64±2.02	2.65±0.09	5.48±0.15	1.48±0.29	2.31±0.10	10.55±0.22
	p	1.77±0.92	3.56±0.16	8.05±0.17	2.74±0.11	3.82±0.09	11.67±0.98
	s	0.33±1.93	1.74±0.14	2.90±0.45	1.10±0.19	1.79±0.14	8.56±0.31
BOS-Sunflower oil 5%	c	2.89±0.22	3.58±0.07	5.91±1.21	2.71±0.23	4.76±0.05	7.44±0.48
	p	3.16±0.40	4.47±0.07	7.38±0.18	2.84±0.37	3.83±0.12	7.79±3.95
	s	2.77±0.47	3.06±0.12	6.14±1.27	2.71±0.57	3.46±0.16	6.26±0.56
BOS-Corn oil 2%	c	2.49±0.29	3.66±0.03	6.99±0.82	2.91±0.24	4.66±0.12	9.92±1.56
	p	2.53±0.21	4.07±0.02	8.74±1.26	3.04±0.18	3.73±0.78	10.26±1.03
	s	1.82±1.15	2.63±0.08	5.85±1.80	2.39±0.48	3.56±0.09	8.30±0.03
BOS-Corn oil 5%	c	2.07±0.85	3.10±0.01	5.42±0.29	3.02±0.92	4.09±0.05	9.35±1.58
	p	2.72±0.92	5.23±0.05	9.39±0.29	3.15±0.18	3.16±0.78	9.69±1.03
	s	2.54±0.42	2.70±0.05	6.00±0.94	2.50±0.22	2.73±0.20	7.01±0.59
BOS-Yeast extract 2%	c	1.53±1.06	3.80±0.02	4.10±0.19	3.47±0.24	5.06±0.13	9.94±0.02
	p	1.95±0.56	4.31±0.08	4.36±0.05	3.60±0.18	4.13±0.78	10.29±1.03
	s	1.76±0.19	2.43±0.07	2.60±0.17	2.86±0.20	3.17±0.08	6.99±0.58
BOS-$CaSO_4 \cdot 2H_2O$ 6%	c	2.22±0.86	4.38±0.14	6.73±0.98	2.17±0.50	5.42±0.14	10.97±0.61
	p	2.45±0.88	5.06±0.15	8.19±1.28	2.30±0.91	4.49±0.08	11.32±1.05
	s	1.67±0.68	2.80±0.11	3.94±0.10	1.65±0.62	4.06±0.58	8.63±2.94
BOS (control)	c	2.08±0.53	4.54±0.09	7.40±0.15	3.09±0.69	4.60±0.10	12.33±0.22
	p	2.69±0.73	4.49±0.16	7.42±0.17	3.22±0.18	4.67±0.78	12.68±1.03
	s	1.41±0.24	4.28±0.14	6.21±0.45	1.70±0.09	3.05±0.14	10.62±0.31

337
338

3.5α-. glucans, β-glucans and total glucans 339

Based on the results of previous studies, the greatest glucan levels (α, β and total) are concentrated in the stipes of *Pleurotus* mushrooms [22,70], so in the present study the total, α- and β- glucan contents were measured only in dry stipes of mushrooms (**Table 5**). All supplements enhanced total glucans in stipes and values ranged from 49.80-66.03% and 47.69-69.22% of total IPSs, for *P. ostreatus* and *P. eryngii*, respectively. Also, all stipes seemed to contain extremely higher amount of β-glucans (37.61-67.77%) compared to α-glucans, which their content was no more than 4%. Similar results were revealed by Avni et al. [70], as the α-glucan content ranged from 0.47% to 4.57% and total glucan content was 20.25-48.27% in seven *Pleurotus* species. β-glucans are among the most interesting functional components of mushrooms, which are linked with health-beneficial properties (e.g., anti-tumor activities, antioxidant effect against infectious diseases, reducing blood cholesterol level) [8,12,71]. *Pleurotus* stipes tested in the present investigation contain high amounts of β-glucans. Usually, that part of mushroom is removed/discarded before packaging, but it could be used as a rich source for glucan extraction for medicinal and nutritional purposes [70,72]. Regarding α-glucans, due to their low concentration in mushrooms, there are not many reports on their structure and their properties, so further studies should be held to examine their possible health benefit activities. Comparing different substrates, sunflower and corn oil, yeast extract and $CaSO_4 \cdot 2H_2O$ favored glucans production, as their values were higher than those of control substrates (WS and BOS). Besides, glucan production proved to be substrate-dependent for both fungi. This phenomenon had already been reported in previous studies [22,73]. Comparable experimental results have been previously presented for *P. ostreatus* and *P. eryngii* cultivated on WS, total glucans production 38.84–58.90% and 32.84–61.40% d.w., respectively, while β-glucans content ranged from 26.44% to 51.36% d.w. for those species [74]. Moreover, Diamantopoulou et al. [22] also reported high amounts of total glucans, 21.47-64.21%, for *P. ostreatus* and *P. eryngii* cultivated on five different agricultural residues, whereas the corresponding amount of α-glucans was significantly higher 2.94-16.89 % than those in the present study.

Table 5: Total glucan, α- and β-glucan content (% w/w) of total IPSs of stipes produced by *P. ostreatus* (AMRL 150) and *P. eryngii* (AMRL 173-6) cultivated on supplemented WS (wheat straw), BOS (barley and oats straw).

	Glucans (%, w/w of total IPS)					
	P. ostreatus			***P. eryngii***		
Substrates	**Total (%)**	**α-glucan (%)**	**β-glucan (%)**	**Total (%)**	**α-glucan (%)**	**β-glucan (%)**
WS-Sunflower oil 5%	49.80±1.44	1.33±0.24	48.47±3.11	53.54±1.24	1.59±0.07	51.95±1.36
WS-Corn oil 2%	61.71±1.28	2.60±0.35	59.11±2.42	56.81±2.11	2.35±0.28	54.45±2.54
WS-Corn oil 5%	63.13±3.05	2.05±0.44	61.07±2.36	55.86±2.55	1.55±0.44	54.31±3.45
WS-Yeast extract 2%	51.97±1.64	1.06±0.35	50.91±3.65	55.14±1.47	1.78±0.07	53.35±1.22
WS-$CaSO_4 \cdot 2H_2O$ 6%	58.29±1.45	0.42±0.03	57.87±1.55	55.03±1.26	1.44±0.55	53.59±2.59
WS-Control	48.91±1.09	1.41±0.11	47.51±1.23	51.18±1.06	3.92±0.12	47.26±2.33

BOS-Sunflower oil 5%	56.36±2.55	1.01±0.66	55.36±3.25	47.69±1.32	0.32±0.06	47.37±2.48
BOS-Corn oil 2%	61.27±1.63	0.74±0.06	60.53±1.44	69.22±1.78	1.45±0.21	67.77±1.11
BOS-Corn oil 5%	66.03±2.07	1.03±0.32	64.99±2.55	55.70±1.11	1.84±0.63	53.86±3.72
BOS-Yeast extract 2%	58.77±2.36	1.53±0.24	57.24±3.07	64.88±1.47	2.03±0.16	62.85±2.36
BOS-$CaSO_4 \cdot 2H_2O$ 6%	52.60±1.58	1.63±0.09	50.97±1.96	62.15±1.29	0.22±0.01	61.93±3.56
BOS-Control	44.67±1.23	0.57±0.07	44.10±2.31	40.30±2.06	2.69±0.23	37.61±1.05

3.6. Tocopherols

Table 6 presents the tocopherol content in carposomes when the cultivation substrate of WS and BOS enriched with oils, nitrogen and calcium salts. The predominant tocopherol in *P. ostreatus* and *P. eryngii* carposomes differed depending on the cultivation substrate. In general, the supplements seemed to affect positively tocopherol production by *Pleurotus* species. Among the highest α-tocotrienol (0.06 mg/100g) and γ-tocotrienol (0.07 mg/100g) content detected in *P. ostreatus* carposomes grown on WS supplemented with corn oil 5%, w/w and BOS with sunflower oil, respectively. Also, higher δ-tocotrienol (0.09 mg/100g) and α-tocopherol (0.09 mg/100g) were detected in *P. eryngii* cultivated on WS with yeast extract and BOS with sunflower oil, respectively, than the control substrates. Cardoso et al. [75] also mentioned the lightly positive effects of substrate supplementation with calcium silicate in tocopherols production by *P. ostreatus* var. florida. In previous study, lower amounts of α-, γ-, δ- tocopherol (0.59, 1.49, 1.64 μg/100 g fresh weight, respectively) in *P. ostreatus* and α-, β-, γ-, δ- tocopherol (0.25, 2.16, 1.83, 0.62 μg/100 g fresh weight, respectively) in *P. eryngii* were detected [58], whereas Fernandes et al. [76] reported similar to ours results for α-, γ-, δ- tocopherol content in *P. ostreatus* cultivated on oat straw. On the other hand, Diamantopoulou et. al [22] reported higher content of α-, δ- tocopherol and α-, β- tocotrienol content in *P. ostreatus* and *P. eryngii* carposomes grown on five different agro-industrial residues than those in of the present study, although β-, γ-tocopherol and δ- tocotrienol were lacking. Many researchers have already reported that these variations in the isoform and the quantity of tocopherols may be connect with the different extraction techniques and the species or different maintenance conditions [19,58]. Even cultivated and wild mushrooms of the same species have different tocopherol profile. For example, tocopherols were detected in greater amounts in the wild species of *P. ostreatus* (0.9 mg/g α-tocopherol) [19] and in *P. eryngii* (6.79, 48.24, 31.55 μg/100 g dw, α-, β-, γ-tocopherol, respectively) [77]. The presence of tocopherols, the vitamin E precursors, in the carposomes of our investigation is important as they have been linked with better antioxidant properties of mushrooms [55,77]. In this way, tocopherols render the mushroom species an easily accessible source of natural antioxidants.

Table 6: Tocopherol composition (mg/100g) of *P. ostreatus* (AMRL 150) and *P. eryngii* (AMRL 173-6) carposomes cultivated on supplemented WS (wheat straw), BOS (barley and oats straw).

	α-tocopherol	**α-tocotrienol**	**β-tocopherol**	**β-tocotrienol**	**γ-tocopherol**	**γ-tocotrienol**	**δ-tocopherol**	**δ-tocotrienol**
Substrates/P. ostreatus								
WS-Sunflower oil 5%	nd*	0.01±0.00	0.02±0.00	0.01±0.00	nd	nd	0.02±0.00	nd
WS-Corn oil 2%	0.02±0.00	nd	nd	nd	nd	0.01±0.00	0.01±0.00	0.02±0.00
WS-Corn oil 5%	0.01±0.00	0.06±0.01	nd	nd	nd	0.02±0.00	0.02±0.00	nd
WS-Yeast extract 2%	0.03±0.00	0.05±0.01	0.01±0.00	0.05±0.01	nd	0.01±0.00	nd	0.01±0.00
WS-$CaSO_4 \cdot 2H_2O$ 6%	0.02±0.00	nd	nd	nd	0.04±0.00	0.01±0.00	0.01±0.00	0.03±0.00
WS-Control	0.02±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.03±0.00	0.01±0.00
BOS-Sunflower oil 5%	nd	0.01±0.00	nd	nd	nd	0.07±0.00	0.01±0.00	nd
BOS-Corn oil 2%	0.05±0.01	nd	nd	nd	nd	nd	nd	0.01±0.00
BOS-Corn oil 5%	nd	0.03±0.00	nd	nd	0.02±0.00	0.01±0.00	nd	0.01±0.00
BOS-Yeast extract 2%	0.01±0.00	0.03±0.00	nd	0.02±0.00	0.05±0.00	nd	0.06±0.02	nd
BOS-$CaSO_4 \cdot 2H_2O$ 6%	0.04±0.00	0.02±0.00	nd	0.04±0.00	0.05±0.00	nd	nd	0.03±0.00
BOS-Control	0.05±0.02	nd	nd	nd	0.01±0.00	0.01±0.00	0.01±0.00	nd
Substrates/P. eryngii								
WS-Sunflower oil 5%	0.04±0.00	0.02±0.00	nd	nd	nd	nd	nd	nd
WS-Corn oil 2%	0.03±0.00	0.02±0.00	0.01±0.00	nd	0.03±0.00	nd	nd	nd
WS-Corn oil 5%	0.01±0.00	0.04±0.00	nd	nd	nd	nd	nd	nd
WS-Yeast extract 2%	0.01±0.00	nd	nd	0.01±0.00	0.04±0.00	0.01±0.00	0.01±0.00	0.09±0.02
WS-$CaSO_4 \cdot 2H_2O$ 6%	0.05±0.01	nd	nd	0.01±0.00	0.01±0.00	0.01±0.00	0.03±0.00	nd
WS-Control	0.01±0.00	nd	nd	nd	nd	nd	0.01±0.00	nd
BOS-Sunflower oil 5%	0.09±0.03	0.01±0.00	nd	nd	nd	nd	0.07±0.02	0.02±0.00
BOS-Corn oil 2%	0.01±0.00	0.01±0.00	nd	nd	0.01±0.00	nd	0.01±0.00	0.01±0.00
BOS-Corn oil 5%	0.01±0.00	0.01±0.00	nd	nd	0.01±0.00	nd	0.01±0.00	nd
BOS-Yeast extract 2%	0.01±0.00	0.02±0.00	nd	nd	0.01±0.00	nd	nd	0.02±0.00

BOS-$CaSO_4 \cdot 2H_2O$ 6%	0.02±0.00	nd	0.01±0.00	nd	nd	nd	nd	nd
BOS-Control	nd	0.03±0.00	nd	0.01±0.00	nd	0.01±0.00	nd	0.01±0.00

* nd: not detected

4. Conclusions

Oils, nitrogen and calcium salts supplementation had a positive impact in mushrooms composition. All supplements in BOS substrate influenced positively IPSs, while the highest protein content of *P. ostreatus* and *P. eryngii* were detected in substrates with yeast extract. The highest TPC were produced by *Pleurotus* grown on WS and BOS supplemented with $CaSO_4 \cdot 2H_2O$, whereas mainly the scavenging capacity of free radicals $DPPH^{\cdot +}$ and $ABTS^{\cdot +}$ were enriched by substrates supplementation. Total and β- glucans values were higher in all supplemented substrates than the corresponding control. Both species were rich in IPSs, proteins and tocopherols, with variable carbohydrate composition, whereas low lipid values were detected in all treatments; *P. eryngii* contained higher amount of IPS and TPC compared to *P. ostreatus*, while the latter was a better lipid producer. No significant differences were detected in protein content and TAC between the two species, whereas *P. eryngii* presented greater total reducing power. Therefore, these mushrooms with high contents of polysaccharides, proteins and unsaturated lipids, tocopherols or antioxidants could be a natural supplement for a healthy and balanced diet. Comparing the individual parts of mushrooms, all pilei were richer in lipids, TPC and they showed higher antioxidant activity and reducing power than stipes. Nevertheless, the present study demonstrated that all stipes can provide a rich source of IPSs and glucans. Stipes is the part of mushrooms that is usually rejected, so it could be ideal for glucan and polyols extraction for nutritional and medicinal applications. Another important information provided in this study is that the nutritional value of mushrooms was maintained in second flush, so mushroom farms should take this into account and collect these mushrooms, as well.

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