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Protein contents and antioxidant properties of p*leurotus ostreatus* cultivated on tea and espresso wastes

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Abstract: In this study, *Pleurotus ostreatus* was cultivated on tea (*Camellia sinensis*) and espresso wastes. Tea wastes were used in two forms; sterilized or non-sterilized. Then, total phenolic, flavonoid, condensed tannin contents, ferric reducing/antioxidant capacity (FRAP) and 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging were used as antioxidant determinants and also protein content were investigated in these mushrooms' methanolic extracts. Same measurements were determined in mushrooms' growing medium except protein content. The highest protein content (20.89%) was found in non-sterilized tea wastes. The highest total phenolic (1.460±0.012 mg GAE/g), total flavonoid (0.120±0.005 mg QE/g), condensed tannin (0.877±0.011 mg CE/g) and the lowest scavenging of free radical activity (17.190±0.001 mg/mL) were determined in sterilized tea wastes. The highest ferric reducing antioxidant power (8.498±0.089 µmol$FeSO_4.7H_2O$/g) were determined in espresso wastes. Additionally, there was no statistically significant difference between the sterilized and non-sterilized substrates for the total yield and biological efficiencies. In this case, it can be said that the kinds of substrates and their usage forms are very important in terms of energy savings especially does not require sterilization like tea wastes. Consequently, tea and espresso wastes can be used as a beneficial source of substrate material for *Pleurotus ostreatus* mushroom cultivation.

Keywords: Antioxidant, espresso wastes, mushroom, tea wastes, total phenolic

1. INTRODUCTION

The consumption of mushroom and therefore the production of mushroom is increasing day by day. It was reported that average of 300 mushroom species can be edible and only 30 of them can be cultivated [1]. Among the cultivated mushrooms, *Agaricus bisporus* is in the first place and *Pleurotus osteatus* follows this sequence [2]. Mushrooms contain various secondary metabolites such as polyketides, phenolic compounds, steroids and terpenes [3]. It was reported that mushroom have many medical values such as anti-oxidant, antimicrobial [4], antitumor [5], antidiabetic [6] etc.

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The production of mushroom is very important not only in terms of nutritional and pharmacological properties but also in the evaluation of agro–industrial waste. Mushrooms, thanks to their enzymes, they can degrade lignocellulosic materials [7]. Especially developing countries will contribute to the country's economy when they use their agricultural waste as substrate in the production of mushroom. On this count, both the high protein content of the mushrooms will be produced and the environmental pollution will be reduced [8, 9]. Many materials such as tea waste [10] banana waste [11], bean, corn, straws and coffee husk [12] have been used as substrates in mushroom cultivation. Tea is the second most popular non-alcoholic drink after the water, consumed extensively by the world's population [13]. This is true also for our country. In addition, Coffee (*Coffea* sp.) is one of the most important agricultural products in the world [14]. The amount of waste left over from tea and coffee consumption every day is quite high. Therefore, the residue left behind after the consumed tea and espresso can also be evaluated in the production of mushrooms.

It was reported that the chemical composition of fruiting bodies is directly affected by chemical composition of substrates [15]. There are very few studies examining the content of the substrates and the mushroom cultivated in that substrate. The aim of this study is (i) to determine the using possibility of sterilized and non-sterilized tea (*Camellia sinensis*) wastes and espresso wastes as substrates on *Pleurotus ostreatus* cultivation (ii) to determine protein contents of cultivated mushrooms and (iii) to evaluate total polyphenolic contents (total phenolic contents, total flavonoids, total condensed tannin) and antioxidant properties of mushrooms and their substrates.

2. MATERIAL and METHODS

2.1. Materials

Pleurotus ostreatus spawn was obtained a commercial firm located in Denizli province, in Turkey. Tea wastes were obtained from canteen of Department of Forest Industry Engineering, Karadeniz Technical University. Espresso wastes obtained from a famous cafe preferred by people in Trabzon.

2.2. Mushroom Cultivation

Tea wastes were used in two forms; sterilized and non-sterilized. In non-sterilization method, tea wastes were used directly without any treatment. It was presumed that tea was self-sterilizing because it was always in hot water while was brewing and because it was exposed to hot water vapor. Other tea wastes and espresso wastes were moistened with water until %70-80 and sterilized in an autoclave at 121˚C for 1.5 h. After cooling the all substrates to 20˚C, they were placed in nylon bags of 1 kg and inoculated by spreading spawn on the surface of the substrate with a weight percentage of about 3% of the wet weight of compost. Substrate was carried out in four replications. Each nylon bags were transported to the mushroom growing laboratory (at 15-25˚C, %70-80 relative humidity). Harvesting was started in fifth week and the fruit bodies' stipe and cap was measured and weighed.

2.3. Yield and Biological Efficiency

Mushroom yield was calculated as total fresh weight of mushrooms obtained from 3 or 4 flushes in the harvest period [16]. Biological efficiencies were defined as the percentage ratio of the fresh weight of harvested mushrooms over the dry weight of substrates [17].

2.4. Determination of Protein Content

Each mushroom was dried at 40 ˚C before analysis. Dried mushroom samples were crushed and powdered for passing a 40 mm mesh sieve. Protein contents of mushrooms were determined by Dumas method. Briefly, 0.500 - 0.700 mg dried mushroom samples were

weighed and placed on 5 mm x 9 mm tin capsules. Capsules were placed into Costech ECS 4010 elemental analysis instrument and burned. Ratio of carbon, hydrogen and nitrogen were determined using Costech ECS 4010 program. Protein contents were determined by multiplicationing (%) carbon results with conversion factor (4.38) [18].

2.5. Preparation of the Extract for Determination of Polyphenolic Contents and Antioxidant Capacity

Harvested mushrooms were sliced and dried in a food dryer 8 hours at 60 ^{o}C (Profilo, PFD1350W, Turkey). Dried mushroom was ground in a basic micro fine grinder and passed through 1 millimeter sieve (IKA, WERKE MF10, Germany). Approximately 5 g of powder samples in were placed into a falcon tube 50 mL 99% with additional methanol. The mixture was stirred continuously with a shaker (Heidolph Promax 2020, Schwabach, Germany) at room temperature for a total of 24 hours. Particles were removed using Whatman No. 4 filter paper pore size 20-25 μm. Then solutions were filtrated from hydrophilic polyvinylidene fluoride (PVDF) 0.45 μm for sterilization. The finally volume of the solution was adjusted by the level of methanol.

2.6. Determination of Polyphenolic Contents

The polyphenolic contents of the methanolic samples were evaluated three different ways; total phenolic contents (TPC), total flavonoids (TF) and total condensed tannin (TCT). For the determination of the total phenolic contents, the Folin-Ciocalteau procedure was employed and gallic acid was used as standard [19]. Shortly, 20 μL of various concentrations of gallic acid and samples, 400 μL of 0.5 N Folin-Ciocalteu reagent and 680 μL of distilled water were mixed and vortexed. After 3 min incubation, 400 μL of Na_2CO_3 (10%) solution was added and vortexed. Then the mixture was incubated for 2 h at 20 °C with interrupted shaking. Absorbance measurement was carried out at 760 nm at the end of the incubation period. A standard curve was prepared using gallic acid as a standard with different concentrations of gallic acid, and the results were expressed as mg (GAE) per g methanolic extracts.

The concentration of total flavonoid present in the methanolic extracts was measured using a spectrometric assay. Briefly, 0.5 mL samples, 0.1 mL of 10% $Al(NO_3)_3$ and 0.1 mL of 1 M $NH_4.CH_3COO$ were added to a test tube and incubated at room temperature for 40 min. Then the absorbance was measured against a blank at 415 nm. Quercetin was used for the standard calibration curve. The total flavonoid concentration was expressed as mg of quercetin equivalents per g sample [20].

Condensed tannins were determined according to the method by Julkunen-Tıtto [21]. For each sample, various concentrations of 25 μL from extracts of plant were mixed with 750 μl of 4% vanillin (prepared with MeOH) and then 375 μL of concentrated HCl was added. The well-mixed solution was incubated at room temperature in darkness for 20 mins. The absorbance against the blank read at 500 nm. (+)- Catechin was used to help make the standard curve (0.05–1 mg/ml). The results were expressed as mg catechin equivalent to (CE)/g sample.

2.7. Determination of Antioxidant Capacity

The antioxidant capacity was determined using ferric reducing antioxidant power, free radical scavenging activity of DPPH•.

2.7.1. Ferric Reducing Antioxidant Assay (FRAP)

FRAP assay was also tested to determine the total antioxidant capacity of the samples. This method is based on the reduction of tripyridyltriazine complex (Fe (TPTZ)$^{3+}$) to blue colored Fe(TPTZ)$^{2+}$ by antioxidants in acidic medium [22]. The preparation of working FRAP reagent was carried out by mixing 25 mL of 0.3 M acetate buffer pH 3.6 with 2.5 mL of 10 mM

2,4,6-tripyridylstriazine (TPTZ) solution in 40 mM HCl and 2.5 mL of 20 mM $FeCl_3.6H_2O$ solution. The reaction mixture consisting of 1mL of the sample and 3 mL of freshly prepared FRAP reagent was incubated at 37 °C for 4 min. Then, the absorbance was determined at 593 nm against blank prepared with distilled water. A calibration curve prepared with an aqueous solution of ferrous sulfate $FeSO_4.7H_2O$ in the range of 100-1000 µM was used. Trolox was also tested under the same conditions as a standard antioxidant compound. FRAP values were expressed in wet weight of the samples as µmol of ferrous equivalent Fe (II) per g sample.

2.7.2. Scavenging of Free Radical (DPPH) Assay

The DPPH assay was applied using [23] to determine the radical scavenging capacity of the methanolic extracts of the plant. The simple method is based on scavenging the DPPH radicals with an antioxidant substance of the investigated solution. For each sample, six different concentrations of 0.75 mL of the extracts of the samples were mixed with 0.75 mL of 0.1 mM of DPPH in methanol, and the absorbance was read at 517 nm. The values were expressed as SC_{50} (mg sample per mL), the concentration of the samples causing 50% scavenging DPPH radicals.

2.8. Statistical Analysis

All assays were performed in triplicate. The data were recorded as means ± standard deviations and analyzed by using Statistical Package for Social Sciences (SPSS version 23.0). The obtained data were analyzed by ANOVA and tests of significance were carried out using Duncan's multiple range tests.

3. RESULTS and DISCUSSIONS

3.1. Total Yield and Biological Efficiency

Total yield and biological efficiency of cultivated mushrooms on sawdust are presented in Table 1.

Table 1. Total yield (g/100g substrates) and biological efficiency (%) of cultivated mushroom

	Yield (g/100g substrates)			Biological efficiency (%)		
Form and type of substrates	$\bar{X}$	SD	H.G.	$\bar{X}$	SD*	H.G.**
Tea wastes (non-sterilized)	18.9	1.2	a	65.0	4.2	a
Tea wastes (sterilized)	19.6	1.7	a	67.6	5.1	a
Espresso wastes	21.3	2.8	b	74.6	8.6	b

*SD: Standard division. **: Homogeneity groups means having the same letter(s) are not significantly different ($p>0.05$) by Duncan's multiple range test.

The highest yield (21.3 g/100g substrates) and highest biological efficiency (74.6%) were obtained from the mushroom cultivated on espresso wastes. There was no statistically significant difference between the form of tea wastes substrates. In this case, it can be said that the tea wastes, which were the non-sterilized can be spontaneously sterilized during the brewing. On the other hand, it is necessary to produce larger quantities in order to prove its correctness. These kinds of raw materials are very important in terms of energy savings especially does not require the sterilization, like tea wastes.

3.2. Protein Content

Protein content of cultivated mushroom is presented in Table 2. It has been reported that amino acid configurations of mushrooms are comparable to some animal protein to some animal proteins [24]. Therefore, the mushrooms' proteins are important for nutrition. In this

study, the highest protein content was found *P. ostreatus* cultivated on non-sterilized tea wastes with 20.89%. Other protein content of mushroom were similar to each other (13.16% and 13.05%). High temperature treatment applied during the sterilization for the other variations may be caused a decrease in protein content. These results can be compared with different mushroom fruit bodies' protein content that varied between 8.6% and 42.5% [25]. It can be said that the substrate types and treating forms can affect the amount of protein content of mushrooms.

Table 2. Protein content of cultivated mushroom.

Mushroom' substrate	H (%)	C (%)	N (%)	Protein (%)
Tea wastes (non-sterilized)	6.70	41.22	4.77	20.89
Tea wastes (sterilized)	6.85	38.27	3.15	13.16
Espresso wastes	6.79	39.68	2.98	13.05

3.3. Polyphenolic Contents and Antioxidant Properties

Polyphenolic contents and antioxidant properties of cultivated mushroom are presented in Figure 1 and polyphenolic contents and antioxidant properties of substrates are presented in Table 3 and Table 4.

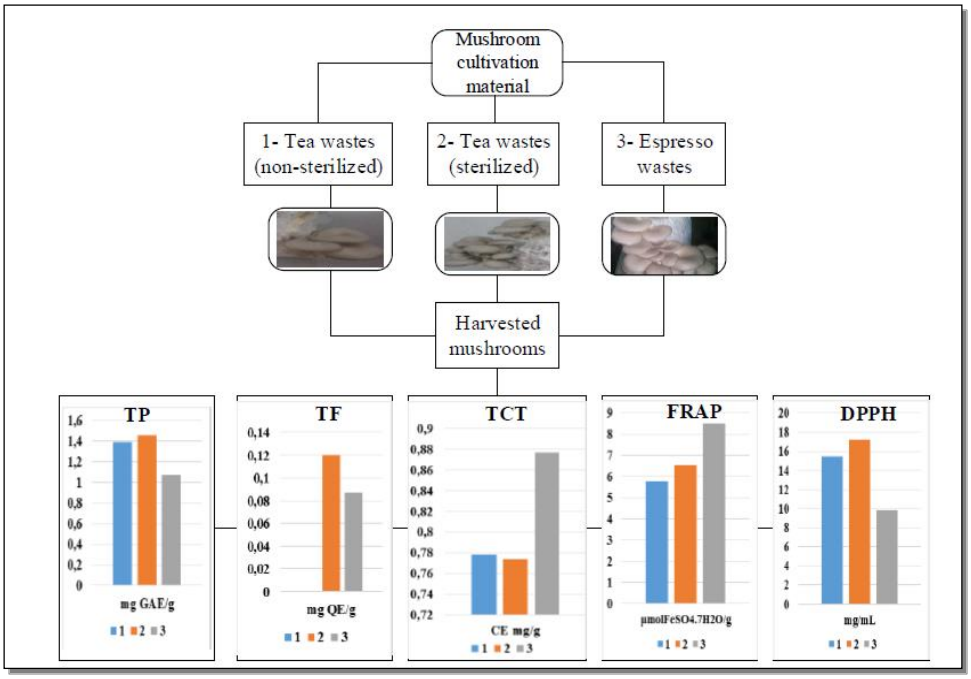

Figure 1. Polyphenolic contents and antioxidant properties of cultivated mushroom (1: Cultivated on non-sterilized tea waste, 2: Cultivated on sterilized tea waste, 3: Cultivated on espresso waste)

3.3.1. Total Phenolic Content

It has been reported that there is a strong correlation between the phenolic components and antioxidant capacity [26]. Therefore, it can be said that as the total amount of phenol in the mushroom increases, it will be more effective as antioxidant. In this study, the highest total phenolic content (1.460±0.012 mg GAE/g) was found in *P. ostreatus* cultivated on sterilized tea wastes (Fig 1). It was observed that this result was close to total phenolic content of mushroom cultivated on non-sterilized tea (1.393±0.060 mg GAE/g). Our total phenolic content values are lower than previously reported values of some wild mushrooms *Leucopaxillus giganteus, Sarcodon imbricatus* and *Agaricus arvensis* (2.83±0.09- 6.29±0.20 mg GAE/g) [27] and also seven *Morchella* species (12.36±1.21- 25.38±0.70 mg GAE/g) [28]. Among the

substrates, the highest total phenolic content was determined in sterilized tea wastes with 4.439±0.062 mg GAE/g. Total phenolic content of substrates was found significantly different (p>0.05) from each other by Duncan's multiple range test.

Table 3. Total polyphenol (mg GAE/g), total flavonoid (mg QE/g) and total condensed tannin (mg CE/g) contents of mushrooms' growth mediums.

Growth medium	Total Polyphenol (mg GAE/g)			Total Flavonoid (mg QE/g)			Condensed Tannin (mg CE/g)		
	$\bar{X}$	SD*	H.G.	$\bar{X}$	SD	H.G.	$\bar{X}$	SD	H.G. **
Tea wastes (sterilized)	4.439	0.062	c	0.932	0.023	c	0.889	0.038	a
Tea wastes (non-sterilized)	4.134	0.187	b	0.670	0.016	b	1.941	0.024	b
Espresso wastes	1.254	0.016	a	0.143	0.007	a	5.418	0.001	c

*SD: Standard Division. **: Homogeneity groups means having the same letter(s) are not significantly different (p>0.05) by Duncan's multiple range test, n=3.

3.3.2. Total flavonoid content

Flavonoids have been shown to exhibit a wide range of pharmacological and biochemical effects such as antimicrobial, antithrombotic, antimutagenic and antigenic activities [29]. In this study, total flavonoid content of *P. ostreatus* cultivated on non-sterilized tea wastes could not be determined. Total flavonoid content of *P. ostreatus* cultivated on sterilized tea wastes and espresso wastes was found 0.120±0.005 and 0.087±0.008 mg QE/g, respectively. These results are higher than total flavonoid content of seven *Morchella* species (0.15±0.02 -0.59±0.01 mg QE/g) [28]. Among the substrates, highest total flavonoid content was determined in sterilized tea wastes (0.932±0.023 mg QE/g) like total phenolic content.

3.3.3. Total condensed tannin content

Tannins are generally defined as naturally occurring polyphenolic compounds of high molecular weight to form complexes with proteins [30]. In this study, highest total condensed tannin was found in *P. ostreatus* cultivated on espresso wastes with 0.877±0.011 mg CE/g (Fig 1). Also, the highest total condensed tannin content was observed in espresso wastes with 5.418±0.001 mg CE/g, too (Table 3). It has been reported that the spent coffee waste contains large amounts of organic compounds such as fatty acids, lignin, cellulose, hemicellulose and other polysaccharides [31]. In this study, it was found that the content of condensed tannin of spent coffee waste is high, also. There are only a few studies about condensed tannin of mushrooms. In our previous study, condensed tannin of *P. ostreatus* and *P. citrinopileatus* cultivated on various sawdust was found in the range of 0.618±0.062 -3.674±0.009 mg CE/g [32]. So, it is possible to say that mushrooms' condensed tannin content is affected by substrates composition.

3.3.4. Ferric reducing antioxidant (FRAP) activity

Some researchers have been reported that the FRAP technique show high reproducibility, is simple and show the highest correlation with both ascorbic acid as a high antioxidant power and total phenolic [33]. In this study, among the mushrooms, the highest ferric reducing antioxidant activity was observed in *P. ostreatus* cultivated on espresso wastes with 8.498±0.089 μmol$FeSO_4.7H_2O$/g (Fig 1). This value was followed by *P. ostreatus* cultivated on sterilized tea wastes and non-sterilized tea wastes values respectively (6.548±0.019 and 5.762±0.095 μmol$FeSO_4.7H_2O$/g). FRAP activities of mushrooms were found higher than some fresh wild edible mushrooms (*Lactarius deliciosus, Lactarius sanguifluus, Lactarius*

semisanguifluus, Russula delica, Suillus bellinii) growing in the island of Lesvos, Greece (0.271±4.3 – 0.523±2.8 μmol Fe^{2+}/g, respectively) [34].

Table 4. The antioxidant activity of cultivated mushrooms' growth mediums.

Mushroom' substrate	FRAP (μmol $FeSO_4.7H_2O$/g)			DPPH-SC50 (mg/mL)		
	$\bar{X}$	SD	H.G.	$\bar{X}$	SD	H.G.*
Tea wastes (non-sterilized)	59.290	0.051	c	2.922	0.002	b
Tea wastes (sterilized)	57.787	0.068	b	0.806	0.001	a
Espresso wastes	19.515	0.046	a	4.045	0.002	c

*: Homogenity groups means having the same letter(s) are not significantly different (p>0.05) by Duncan's multiple range test, n=3.

Among the substrates (Table 4), it was found that ferric reducing antioxidant (FRAP) activity of non-sterilized tea wastes (59.290±0.051 μmol $FeSO_4.7H_2O$/g) and sterilized tea wastes(57.787±0.068 μmol $FeSO_4.7H_2O$/g) were close to each other and 3.5 times more than espresso wastes value (19.515±0.046 μmol $FeSO_4.7H_2O$/g). It can be concluded that tea wastes can be re-evaluated as a natural antioxidant source.

3.3.5. Scavenging of Free Radical (DPPH) Activity

DPPH radical dot analysis is routinely performed to assess the free radical scavenging potential of an antioxidant molecule and it is considered to be one of the standard and easy colorimetric methods for evaluating the antioxidant properties of pure compounds [35]. The higher FRAP values indicate high antioxidant capacity, while smaller DPPH values are indicative of higher antioxidant capacity. So, in this study, the lowest scavenging of free radical (DPPH) activity was found in *P. ostreatus* cultivated on sterilized tea wastes. Among the substrates, non-sterilized tea wastes show the highest DPPH activity. In addition, scavenging of free radical (DPPH) activity of substrates was found significantly different (p>0.05) by Duncan's multiple range test (Table 4).

4. CONCLUSION

The most consumed drinks after water are tea and coffee. Therefore, a lot of waste is left behind. This situation is especially true for tea and with the evaluation of these wastes, many benefits can be achieved. In this study, the possibilities of utilization tea and espresso wastes as substrate for *P. ostreatus* mushroom cultivation were investigated. The highest protein content (20.89%) was found in non-sterilized tea wastes. The highest total phenolic (1.460 ± 0.012 mg GAE/g), total flavonoid (0.120 ± 0.005 mg QE/g) and the lowest free radical scavenging activity of DPPH (17.190 ± 0.001 mg/mL) were determined in sterilized tea wastes. The highest condensed tannin (0.877 ± 0.011 mg CE/g) and ferric reducing antioxidant power (8.498 ± 0.089 μmol$FeSO_4.7H_2O$/g) were determined in espresso wastes. Additionally, there was no statistically significant difference between the sterilized and non-sterilized substrate types for the total yield and biological efficiencies. In this case, it can be said that the kinds of substrates and their usage forms are very important in terms of energy savings especially does not require sterilization like tea wastes. Consequently, tea and espresso wastes can be used as a beneficial source of substrate material for *Pleurotus ostreatus* mushroom cultivation. In order to make more comparison; similar analysis can be made with different mushroom species and different wastes which may contribute to energy saving.

Conflict of Interests

Authors declare that there is no conflict of interests.

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