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Research Article

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Total Phenolic Compounds and Antioxidant Activity in Pleurotus Spp. Grown on Commercial and Wild Substrates

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Abstract

A comparison of the antioxidant activity and total phenolic content between several mushroom strains and species belonging to Pleurotus genus (P. ostreatus, P. pulmonarius and P. citrinopileatus) was realized. Total phenolic content as well as the antioxidant capacity showed considerable differences between the strains and Pleurotus species studied. The mushroom samples were grown on a commercial (wheat straw) and on wild substrates (Pennisetumsetaceum and Arundodonax), finding out considerable differences in both parameters (total phenolic content and antioxidant activity) according to the cultivation substrate. So, the mushroom samples produced in wild substrates had in general a higher total phenolic content and antioxidant activity than those mushrooms grown on the usual commercial substrate. The highly significant correlation (r = 0.807; P < 0.0001) found between total phenolic compounds and antioxidant activity is in agreement with previous results that noted the phenolic compounds as the main antioxidants present in mushroom.

Keywords: *Pleurotus* spp.; Strains; Substrates, DPPH; Phenolics.

Introduction

Free radicals and other reactive oxygen species that are continuously produced in cell oxidations may result in oxidative cell and tissue damage which has been associated to ageing and degenerative diseases, such as atherosclerosis, diabetes, cancer and cirrhosis [1,2]. Most of organisms have defense systems against free-radical damage by oxidative enzymes including superoxide dismutase, glutathione peroxidase and catalase, or vitamins and chemical compounds antioxidants such as α-tocopherol, ascorbic acid, carotenoids phenolic acids or flavonoids [3].

Pleurotus is the most cultivated mushroom in the world. From nutritional point of view, the mushrooms, especially those belonging to *Pleurotus* genus, are a rich source of digestible carbohydrates and fiber, proteins with a considerable amount of essential amino acids, and have a low fat/energy contents [4]. They also have micronutrients such as vitamins of complex B and minerals (K, Ca, Mg and P) [4,5]. Mushrooms have been used for therapeutic purposes, as they can produce a variety of secondary metabolites, such as organic acids, alkaloids, terpenoids, steroids and phenolic compounds [6]. Thus, they present interesting medicinal properties including antitumor and immuno-regulator effect [7], reduction of blood cholesterol, prevention or relief of heart disease and reduction of blood glucose levels [8], and antimicrobial activity [9]. Many of the aforementioned properties are attributed to bioactive products with antioxidant activity, emphasizing the phenolic compounds [1,10,11,12], which have been recognized as important protective agents for human health and aging.

Pleurotus cultivation is commonly prepared in pieces of 2 to 4 cm of length on substrates from straw of cereals, like wheat, rye or barley. A considerable production of *P. ostreatus* is observed on residues derived from agroindustrial activities, such as coffee pulp, leaves used in the extraction of essential oils and sugarcane bagasse [13,14]. Knowledge gained to date about culturing *Pleurotus* on coffee pulp shows that this substrate has advantages over alternative waste substrates and might serve as a substitute for wheat straw [13]. Job [15] showed that the coffee spent ground could be used in the substrate to a 55% without detecting changes in fruiting body or on its biological efficiency of production.

Antioxidant activity and total phenolic content were determined in strains of mushrooms species belonging to *Pleurotus* genus *(P. ostreatus, P. pulmonarius* and *P.*

citrinopileatus), cultivated in several wild substrates and a conventional substrate.

Material and Methods

Samples

Five strains belonging to *Pleurotus* genus were used for this study (**Table 1**). They were obtained from a commercial productor of mycelium with certification and quality control according to Real Decreto 1313/2005 [16] in March and April 2014. Table 1 shows the colour and data about fructification conditions such as temperature, relative humidity, illumination and incubation period.

Strains (Common name)	Conditions of fructification	Cultivation substrate	Colour
Pleurotus ostreatus (H4) (Summer oyster mushroom)	Temperature: 15-25 °C (max 30 °C) Relative humidity: 90% Illumination: 100 lux (10-12 h/day) Incubation period: 30-40 day	Wheat straw *Arundo donax*	White-brown
Pleurotus ostreatus (H9) (Oyster mushroom)	Temperature: 12-22 °C (max 30 °C) Relative humidity: 90-95% Illumination: 300 lux (10-12 h/day) Incubation period: 25-30 day	Wheat straw Wheat straw + 10% coffee grounds *Arundo donax*	White-grey
Pleurotus ostreatus (G40) (Winter oyster mushroom)	Temperature: 12-16 °C (max 22 °C) Relative humidity: 90-95% Illumination: 500 lux (10-12 h/day) Incubation period: 30-35 day	Wheat straw	Grey marble
Pleurotus citrinopileatus (Golden oyster mushroom)	Temperature: 15-27 °C (max 32 °C) Relative humidity: 85% Illumination: 500 lux (10-12 h/day) Incubation period: 25-30 day	Wheat straw *Arundo donax* *Pennisetum setaceum*	Yellow
Pleurotus pulmonarius (Pale mushroom)	Temperature: 19-22 °C (max 32 °C) Relative humidity: 80-90% Illumination: 100 lux (10-12 h/day) Incubation period: 20-25 day	Wheat straw	Brown

Table 1: Growing conditions for the different species and strains analyzed.

For the production of the fruiting bodies, a commercial substrate of wheat straw, crushed, pressed and pasteurized material suitable for animal feed was used and the previous processing did not need sanitization, in which the 5 strains of *Pleurotus* were planted. This same substrate was mixed with coffee grounds (10%), which was pausterized before the mixture, since this substrate is very easily contaminated. Strain H9 was planted on it. The different wild lignocellulosic substrates, *Pennisetum setaceum* and *Arundo donax*, were taken from remote areas of population nuclei and roads in the Municipalities of La Laguna and El Sauzal (Tenerife Island). Prior to planting the inoculates were hygienized by immersing them in hot water at 80°C for 20 min, and then dried. Strains H4, H9 and *P. citrinopileatus* were planted on them. The inoculation of the substrate was carried out in micro-perforated polyethylene bags of 30 cm diameter, and 4.5 kg, inside a greenhouse covered with 80% dark shading mesh and relative humidity of 80-90%.

Analytical determinations

They were carried out on fresh samples 1-2 days after sampling. Samples were refrigerated and transported to the laboratory, where were washed with distilled water and gently blotted with a paper towel. They were cut into small pieces and homogenized previous to the analysis. All analyses were done in triplicate in independent replicates.

The total phenolic content was determined by the Folin-Ciocalteu assay following the method colorimetric based in Folin-Ciocalteu reagent. Briefly, 0.5 g of freshly homogenized sample was weighted into a polyethylene tube containing 10 ml of 50% methanol, after sonication and centrifugation. One ml aliquot of this extract was taken and mixed with 1 ml of 50% Folin-Ciocalteu reagent, and after 5 min, 2 ml of 10% Na_2CO_3 solution were added and were allowed to stand for 10 min in the dark. After centrifugation, the absorbance was measured at 750 nm. Gallic acid was used as calibration standard and the results were expressed as mg gallic acid equivalents (GAE) per g of fresh weight (FW) (mg GAE/g).

The antioxidant activity was determined using the 2,2-diphenyl-1-picryl hydrazyl (DPPH) [17]. Briefly, 0.150ml of above extract was mixed with 1.85 ml of DPPH (0.04 g/l of methanol). This mixture was left in contact and in the absence of light for 30 min, and then the absorbance of the sample was measured at 517 nm. A blank was prepared of same manner and measured to 0 sg. The antioxidant capacity was calculated using a calibration curve prepared with Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) within a range of 200-800 µmol/l, and the result was expressed as mg Trolox equivalents (TE) per g FW (mg TE/g). The ascorbic acid was determined by the

2,6-dichlorophenol-indophenol (DIP) titration procedure [18].

Statistical analysis

All the statistics were performed by means of the SPSS (Statistical Package for the Social Sciences) version 21.0 software for Windows (SPSS Inc. Chicago, IL). Mean values obtained for the variables studied in the different groups were compared by One-Way ANOVA (Duncan's multiple range) assuming there were significant differences among them when the statistical comparison gave $P < 0.05$.

Results and Discussion

Although the growth of mycelium was initially observed in all the cases, the final behavior of cultivation strains was different according to the substrate used in the cultivation. Growth and fruiting was observed for the 5 cultivation strains on commercial substrate. In contrast, *P. citrinopileatus* was the unique strain that grew on the wild substrate *Pennisetum setaceum*. This could be explained for the high contamination detected on the seeded samples in this latter substrate for species of the genus *Coprinus* and *Aleuria*. Probably the heat treatment has been inadequate and it does not eliminate totally the spores of these mushroom species [19]. The wild substrate *Arundo donax* was effective for the H4 and H9 strain of *P. ostreatus* and *P. citrinopileatus*. This substrate is constituted for a more lignified residue and therefore, a less development of competitive mushrooms took place.

Table 2 shows the results of total phenolic compounds and antioxidant activity of all the mushroom species seeded in the commercial substrate. All mushrooms samples had values of ascorbic acid below detection limit (<0.5 mg/100 g FW). Barros et al. [10] found small amounts of ascorbic acid (0.13–0.35 mg/g FW), which is in agreement with our results. Total phenols were the major antioxidant components found in the mushroom extracts [10,20]. There were significant ($P < 0.05$) differences in total phenolic concentrations between the three fungus species, arranging according to the following sequence: *P. citrinopileatus* > *P. ostreatus* > *P. pulmonarius*. However, no significant differences ($P = 0.129$) in the mean values of the antioxidant activity between the three species were detected. This is due to the great variation of the data obtained in *P. ostreatus* (0.57±0.30 mg GAE/g FW; coefficient of variation 52.6%). In fact, considering only the samples of the other two species, *P. citrinopileatus* species had a higher ($P = 0.000$) mean antioxidant activity than that value obtained for the specie *P. pulmonarius*.

Species	*Pleurotus ostreatus*	*Pleurotus citrinopileatus*	*Pleurotus pulmonarius*
Total phenolic contents (mg GAE/g FW)	0.73 ± 0.11^{b}	0.93 ± 0.03^{c}	0.65 ± 0.09^{a}
Antioxidant activity (mg TE/g FW)	0.57 ± 0.30	0.82 ± 0.06	0.38 ± 0.02

Table 2: Total phenolic contents and antioxidant activity of the Pleurotus sp. studied grown in commercial substrate (wheat straw).

Our values of total phenolic contents for the three mushrooms studied (**Table 2**), expressed in mg GAE/g of dry weight (DW), were higher than those data reported in other studies for *P. ostreatus* fungus species, 3.63 mg GAE/g DW from methanolic extract [21] and 4.27mg GAE/g DW from aqueous extract [22]. However, Tsai et al. [23] reported values of total phenolic contents (7.11 mg GAE/g DW from ethanolic extract) similar to those obtained by us. Few data of antioxidant activity in these mushroom species were found in the literature. Wang and Xu [24] analyzed samples of *P. citrinopileatus* obtaining values of 0.12 mg TE/g DW (extraction in ethanol) y 1.03 mg TE/g DW (extraction in water), which were lower and higher, respectively, than the data obtained for this paper in this specie. The differences observed for both analyzed parameters could be explained because of the different extraction methods used in these studies as well as differences in genetic and environmental characteristics. Comparing our results with other data published for fruits and vegetables, the phenolic contents of mushrooms were higher than those contents found for potatoes, tomatoes or bananas, similar to pear or onion and lower than those reported for apple and Brussels sprout [25].

Significant differences in total phenolic contents were found between the three strains of *P. ostreatus* grown in commercial substrate (wheat straw) (**Table 3**). The G40 strain mainly produced in winter showed a higher ($P < 0.05$) phenolic content than the other two strains, H4 (produced in summer) and H9, without significant ($P > 0.05$) differences between these two latter strains. The mean values of the antioxidant activity allowed statistically ($P < 0.05$) to differentiate the three strains of the *P. ostreatus* grown in commercial substrate. So, the G40 strain had a higher antioxidant activity than the H4 strain, and this had higher antioxidant activity than the H9 strain.

Strain	H4	H9	G40
Total phenolic contents (mg GAE/g FW)	0.71 ± 0.06^{a}	0.66 ± 0.03^{a}	0.84 ± 0.11^{b}
Antioxidant activity (mg TE/g FW)	0.40 ± 0.02^{b}	0.34 ± 0.01^{a}	0.96 ± 0.03^{c}
Results in the same line with different superscript were statistically ($P < 0.05$) different. FW: fresh weight, GAE: Gallic acid equivalents, TE: Trolox equivalents.			

Table 3: Total phenolic contents and antioxidant activity of the three strains of Pleurotus ostreatus studied grown in wheat straw.

Substrate where the mushrooms are grown had a considerable influence on phenolic contents and antioxidant activity of the fungus. The *P. Citrinopileatus* specie (**Figure 1**) had a higher total phenolic concentrations and antioxidant capacity when cultivated in *Pennisetum setaceum* (4.59±2.16 mg GAE/g FW) than when was cultivated in *Arundo donax* (1.36±0.73 mg GAE/g FW) or in commercial substrate (wheat straw) (0.93±0.82 mg GAE/g FW).

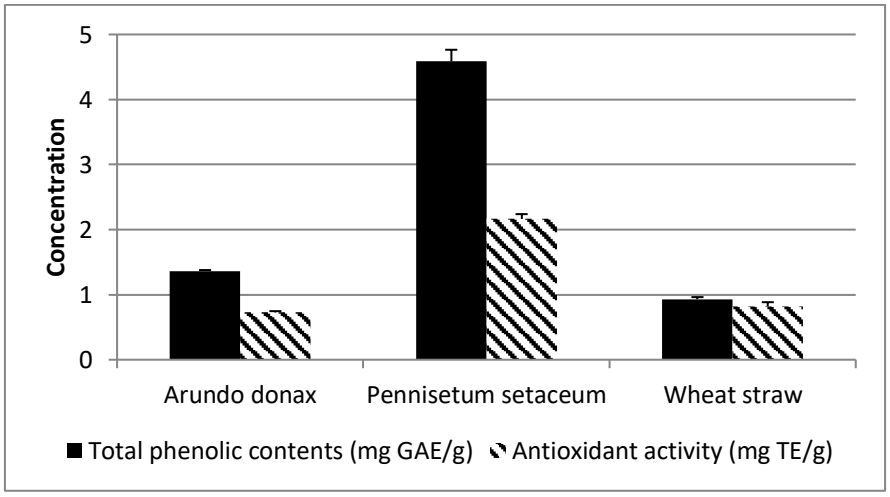

Figure 1: Total phenolic content and antioxidant activity of *Pleurotus citrinopileatus* grown on three different substrates: A) *Arundo donax*, B) *Pennisetum setaceum*, C) Wheat straw.

The H4 and H9 strains of *P. ostreatus* seeded on *Arundo donax* had a markedly higher phenolic contents and antioxidant capacities than those mushroom samples produced on commercial substrate (wheat straw) (**Table 4**). The H9 strain was additionally seeded on commercial substrate enriched (10%) with coffee grounds (**Table 4**). The total phenolic content and antioxidant capacity of H9 strain grown in this type of soil was higher and lower ($P < 0.05$) than those mean values observed on *Arundo donax* and conventional commercial substrates, respectively.

Pleurotus ostreatus	**Substrate**	**Total phenolic contents (mg GAE/g FW)**	**Antioxidant activity (mg TE/g FW)**
H4	*Arundo donax*	1.58 ± 0.04^{b}	1.80 ± 0.02^{b}
	Wheat straw	0.71 ± 0.06^{a}	0.40 ± 0.02^{a}
H9	*Arundo donax*	1.54 ± 0.05^{c}	1.58 ± 0.03^{c}
	Wheat straw	0.66 ± 0.03^{a}	0.34 ± 0.01^{a}
	Wheat straw + 10% coffee grounds	0.98 ± 0.06^{b}	0.87 ± 0.08^{b}
Results of both strain mushrooms in the same column with different superscript were statistically ($P < 0.05$) different. FW: fresh weight, GAE: Gallic acid equivalents, TE: Trolox equivalents.			

Table 4: Total phenolic contents and antioxidant activity of the H4 and H9 strains of Pleurotus ostreatus grown in the Arundo donax and commercial substrates.

Therefore, a same behavior could be observed in the total phenolics and antioxidant capacity. This confirms results already published that established the main antioxidants present in mushroom are the phenolic compounds [26]. Moreover, a highly significant correlation ($r = 0.807$; $P < 0.0001$) between total phenolic compounds and

antioxidant activity was observed, which agree with previous results [26,27,28]. Other researchers [21], however, were not able to find out this correlation. **Figure 2** shows the graphic representation of this correlation differentiating the types of mushroom samples considered. The mushroom samples could be differentiated according to the substrates of cultivation. Besides, the mushrooms tend to differentiate according to species and strain within each substrate and substrates of cultivation.

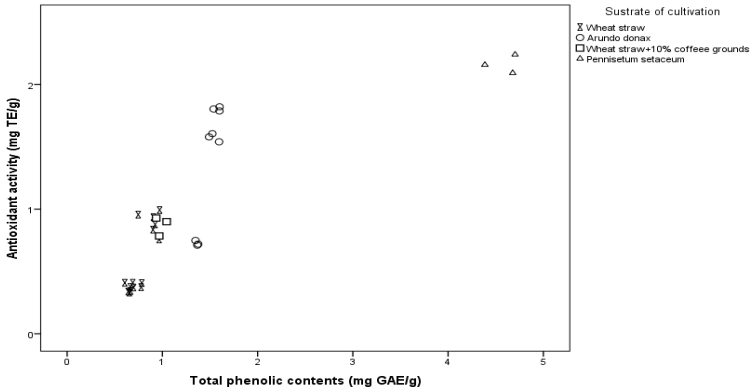

Figure 2: Correlation between total phenolic content and antioxidant activity for the mushroom samples analyzed; differentiating specie, strain and substrate of cultivation.

Conclusions

Mushrooms cultivated in both, wild or commercial, substrates are a good source of natural antioxidants. Species and strains influenced on the phenolic contents and antioxidant activity of mushrooms. *Pleurotus citrinopileatus* had the highest antioxidant activity particularly when it is cultivated on *Pennisetum setaceum*, while the species *Pleurotus pulmonarius* had the lowest values. The G40 strain (produced in winter) of *Pleurotus ostreatus* had higher phenolic contents and antioxidant activity than the other strains. Mushrooms produced in the wild substrates had in general higher phenolic contents and antioxidant activity than those grown in commercial substrate.

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