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Antioxidant activity and proximal chemical composition of fruiting bodies of mushroom, *Pleurotus* spp. produced on wheat straw

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Abstract

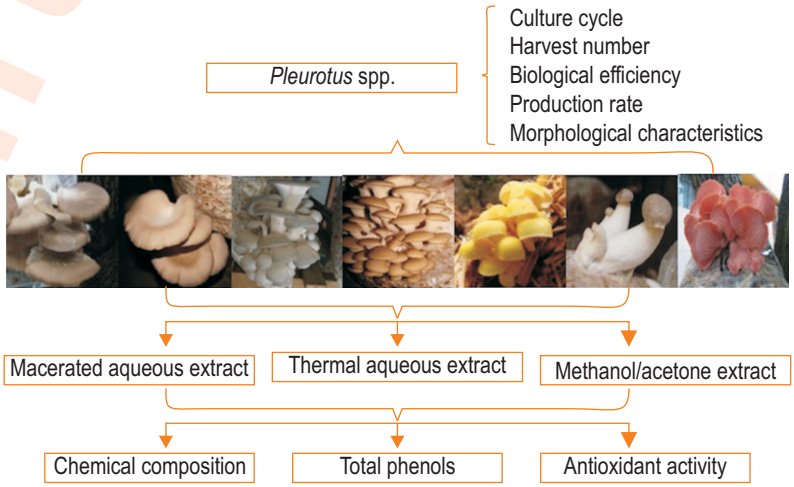
Aim: Mushrooms of the genus *Pleurotus* have important nutritional and therapeutic properties, however, it is important to assess the differences in their properties depending on the species and even the strain. The antioxidant activity and total content of phenols in extracts from fruiting bodies of three strains of *Pleurotus ostreatus*, and one each of *Pleurotus pulmonarius*, *Pleurotus eryngii*, *Pleurotus djamor* var. *roseus* and *Pleurotus citrinopileatus* were evaluated, as well as, the proximal chemical composition of fruiting bodies of all strains were also determined.

Methodology: All strains were grown in wheat straw and the production of fruiting bodies were monitored. Three different extracts of fruiting bodies from each strain were obtained and their antioxidant activity and total content of phenols were measured.

Results: Most of the strains showed an early fruiting production pattern (25 to 39 days), but in some strains it was intermediate (52 days) or late (67 days); the biological efficiency ranged from 42 to 95% and the production rate from 0.2 to 1.0. The proximal chemical composition was similar in all the strains. The phenol content ranged from 15.4 to 2.1 mg GAE g^{-1} of dry biomass. The percent inhibition of ABTS radical varied from 96 to 53 % and that of DPPH radical was between 98 to 24 %. *Pleurotus citrinopileatus* presented the highest antioxidant activity.

Interpretation: Differences between strains and species, could be due to the fact that the phenotype is conditioned, among other factors, to the genotype in combination with the environmental conditions. The five *Pleurotus* species presented characteristics that confirm their use as a functional food, but there were differences in productivity and antioxidant capacity of fruiting bodies, which apparently depends on the interspecific and intraspecific variability of strains. Hence, further investigation is necessary to assess the effect of external factors.

Key words: Antioxidants, Fruiting body, *Pleurotus* spp., Proximal chemical analysis



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Introduction

The mushrooms of genus *Pleurotus* are the second most cultivated group of fungi in the world with an estimated production of 6.46 x 10^9 kg. Their cultivation is relatively simple as they grow in variety of lignocellulosic wastes due to their enzymatic complex. These mushrooms have great nutritional value since they are an excellent source of protein, carbohydrates, vitamins and minerals, their lipid content is low and cholesterol free, they are rich in chitin and ß-glucans, and are also appreciated for their organoleptic characteristics (Li *et al*., 2015). This genus groups several species, among the most popular on the market are *Pleurotus ostreatus*, *P. pulmonarius*, *P. cornucopiae*, *P. djamor* and *P. eryngii* (Royse and Bahler, 1988), which present characteristics that attract the attention of consumers, such as color of the pileus that is brown, yellow, pink and grey or white for *P. pulmonarius*, *P. cornucopiae*, *P. djamor* var. *roseus* and *P. ostreatus*, respectively; *P. eryngii* has white and fleshy stipe with a pleasant flavor. On the other hand, mushrooms are preferred as food and medicine. Several studies have reported the therapeutic use of mushrooms as antidiabetic, antihypertensive, antitumor, antiviral and antiarthritic activities. It has been suggested that they have great impact on the immune and inflammatory responses through interaction with the intestinal microbiota (Roupas *et al*., 2012). Part of the therapeutic properties of mushrooms, is related to its capacity of the inhibition of oxidative stress caused by lipid peroxidation, also reducing the concentration of low-density lipoproteins or counteracting free radicals, among others, due to the presence of phenolic compounds, bioactive amines (Reis *et al*., 2020), vitamins and their respective precursors, such as carotenoids, terpenes and sterols, that have antioxidant effect despite their low levels (Puttaraju *et al*., 2006). They are also attributed to the presence of polysaccharides, either β-glucans or polysaccharide-protein complexes (Roupas *et al*., 2012). Particularly, the genus *Pleurotus* contain bioactive molecules are beneficial for human health phenolic compounds, some polysaccharides and vitamins (Puttaraju *et al*., 2006). These mushrooms are an alternative to be incorporated into the diet as a source of antioxidants and bioactive compounds, however, it is important to highlight that the genus *Pleurotus* is one of the most diverse groups of cultivated edible mushrooms (Chang, 1999), they present a great morphological variability that can be attributed to many factors, such as environmental conditions, phenotypic plasticity and genetic variation (Vilgalys *et al*., 1993) and the production of bioactive compounds and chemical composition of fruiting bodies are dependent on multiple variables, including the species and strain, as well as, the composition of substrate (Mane *et al*., 2007), culture techniques, cell age and developmental stage of the mushroom (Cui *et al*., 2014). Although, mushrooms of the genus *Pleurotus* have important nutritional and therapeutic properties, however, it is important to the assess differences in their properties, depending on the species and strain. In view of the above this study was carried out to investigate the proximal chemical content, antioxidant activity and total phenol content of fruiting bodies of seven strains from five cultivated *Pleurotus* species on wheat straw.

Materials and Methods

Strains : The strains used in this study were submitted in the Mycological Herbarium of the State of Morelos located at the Center for Biological Research of the Autonomous University of the State of Morelos, Mexico. Seven strains of five species of genus *Pleurotus oiz P. ostreatus* (HEMIM 50, HEMIM 126 and HEMIM 127), *P. eryngii* (HEMIM 130), *P. pulmonarius* (HEMIM 129), *P. djamor* var. *roseus* (HEMIM 104) and *P. citrinopileatus* (HEMIM 132) were selected for the study. Strains were maintained on potato-dextrose agar.

Production of fruiting bodies : Wheat grains moistened (50 %) and sterilized in an autoclave at 120 °C for 90 min were inoculated separately with each of the seven strains in the dark at 25 ºC until the mycelium completely grew in all wheat grains. Subsequently, pasteurized wheat straw (5 kg at 75 % humidity) was inoculated with 250 g of wheat grains invaded with mycelium from each strain. All the treatments were incubated at 25 °C in dark until the appearance of primordia, later they were kept under controlled conditions: temperature (24-26°C), photoperiod (12 hr light/dark), relative humidity (80-90 %) and forced ventilation (Stamets, 2000). The parameters evaluated were: time elapsed from inoculation of the substrate to obtaining the first harvest (TIH) in days, harvest number (HR), biological efficiency (BE), production rate (PR) (Tschierpe and Hartmann, 1977) and culture cycle (CC) in days (considered as time from inoculation to the last harvest). The morphological characteristics of fruiting bodies were also determined. To determine the content of total phenols and antioxidant activity, the fruiting bodies were dehydrated by airflow, pulverized and sieved. The samples were stored in darkness for further use.

Proximal chemical composition of fruiting bodies: One gram of sample was used. Moisture content was determined by the indirect method of oven drying, ashes by incineration at 600 °C in muffle, protein content by the Kjeldahl method, ethereal extract content by the method of Soxhlet, carbohydrates by the phenol-sulfuric method and fiber by difference (AOAC, 1997).

Preparation of extracts : Three extracts were prepared from the fruiting bodies: macerated aqueous extract, thermal aqueous extract and methanol/acetone extract. Each sample was prepared at 50 mg ml^{-1} and stirred for 10 min, then centrifuged for 10 min at 14000 g. The solvent of each sample was removed in a rotary evaporator and part of the residue was separated to determine the total phenolic content and the rest was resuspended in 5 % dimethylsulfoxide for estimation of antioxidant activity.

Estimation of total phenolic content : Total phenolic contents was estimated by Folin-Ciocalteau reagent (Singleton and Rossi, 1965). Two mg of each fungus extract was dissolved in 1 ml of 4 % methanol and mixed with 1 ml of Folin-Ciocalteu reagent. After 3 min, 1 ml of saturated sodium carbonate solution was added and the mixture was adjusted to 10 ml with distilled water. The reaction was kept in dark for 90 min and absorbance was read at 725 nm. Gallic acid was used as standard for calibration curve (0.02-0.14 mg ml^{-1}). Total phenolic content was expressed in mg GAE g^{-1} d.wt.

Antioxidant activity

Scavenging activity of ABTS radical : ABTS radical scavenging activity was estimated according to Re *et al.* (1999). ABTS radical cation ($ABTS^{+}$) (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) was obtained after the reaction of ABTS (7 mM) with potassium persulfate (2.6 mM final concentration), the mixture was kept in the dark at room temperature for 12-16 hr, after that, the ABTS+ solution was diluted until obtaining an absorbance of 0.700 ± 0.2 (734 nm). The reaction mixture (10 µl of each extract and 990 µl of the ABTS radical) was kept in the dark for six min. The decrease in absorbance (734 nm) was expressed as inhibition percentage of $ABTS^{+}$.

Scavenging activity of DPPH radical : DPPH radical scavenging activity was determined by measuring the decrease in absorbance of the reaction mixture after incubating for 45 min in darkness and was expressed as the inhibition percentage of DPPH radical. The reaction mixture contained 0.5 ml of extract, 3 ml of methanol and 0.3ml of 0.5 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical solution in methanol (Moraes-de-Souza *et al.*, 2008).

Statistical analysis : All the experiments were run in triplicate. The data were analyzed through one-way ANOVA and Tukey test. P values less than 0.005 were considered to be statistically significant.

Results and Discussion

The values of parameters evaluated in the cultures are shown in Table 1. The perusal of data revealed that time for inoculation to harvest (TIH) was different between *Pleurotus* species. Based on the statistical analysis, three groups were established: *P. djamor* var. *roseus* with early fruiting (25 days), three strains of *P. ostreatus* and *P. citrinopileatus* (36 to 39 days) with intermediate fruiting and *P. pulmonarius* and *P. eryngii* (52 and 67 days) with late fruiting. The crop cycle (CC) of *P. pulmonarius* showed significant difference with the other six strains (p< 0.005). The production rate (PR) of all the strains were significantly different (p< 0.005) (Table 1). Although, *P. eryngii* had a short growing cycle and only two harvests, its PR was lowest because this strain also had the lowest biological efficiency (BE). The BE of the majority of strains evaluated showed yield potentials except for *P. eryngii*. The BE of *P. citrinopileatus* in this study was similar to that reported by Musieba *et al*. (2012). It is worth mentioning that Borkar *et al*. (2014) reported a BE of 74.5 % in *P. pulmonarius*, strain while the BE for the same specie in this study was 95 %. In general, more than 50 % of the total fruiting bodies in this study were obtained in the first harvest, in addition the size of the mushroom during second and third harvest were smaller (Table 1). On the other hand, the strains studied showed typical morphological characteristics of each species (Stamets, 2000). Given the experimental conditions, it is considered that in this work the difference in the evaluated parameters were due to the strains, since the growth conditions were controlled. The maximum and minimum values of diameter of pileus formed two groups which were statistically different (p <0.005), the first included *P. pulmonarius* and three strains of *P. ostreatus*, while the rest of the strains formed the other group. The differences observed in production could be due to different factors, among which are those associated with the strain, species, culture process, cell age of the inoculum, etc.

Table 2 shows the proximal chemical composition of seven mushrooms strains studied in this work. The moisture percentage of all fruiting bodies was found within the range reported for these mushrooms (85-90%) which depends on the cultivation and harvest conditions, including relative humidity. The moisture content in mushroom powder of five *Pleurotus* species evaluated acquired humidity from the environment, however, was eliminated from the samples before determining their proximal chemical composition. The ash and fiber content were different between strains (p <0.005). The dietary fiber content in fruiting bodies of *P. djamor* var. *roseus* and *P. ostreatus* grown on wheat straw ranged between 7.3 to 12.2 % (Maftoun *et al*., 2015). The lipid content showed significant difference between *P. citrinopileatus*, *P. djamor* var. *roseus* and *P. eryngii*, while *P. ostreatus* and *P. pulmonarius* strains had statistically equal values (p <0.005). It is important to mention that the values of lipid content obtained in this work were higher in all strains than previously reported, for example *P. ostreatus* and *P. djamor* var. *roseus* grown on wheat straw showed values between 1.1 and 2.6 % (Maftoun *et al*., 2015). In addition, carbohydrate content showed significant difference (p< 0.005) between the strains. carbohydrate content in *P. ostreatus* and *P. djamor* var. *roseus* grown in wheat straw ranged from 50-56% (Maftoun *et al*., 2015), which was very close to the values obtained in this work. The protein content was different (p <0.005) among the strains. *P. ostreatus* and *P. djamor* var. *roseus* grown in wheat straw reported of 21 to 28% protein content (Maftoun *et al*., 2015). It is noteworthy that the values obtained in this work was within that range, which suggests that the protein content is not very variable among *Pleurotus* species. The values obtained from the proximal chemical analysis (Table 2) of all the strains evaluated in this

Table 1 : Mycelial growth, yield and physical characteristics of *Pleurotus* spp. cultivated on wheat straw

Treatment		*P. ostreatus*50	*P. ostreatus*126	*P. ostreatus*127	*P. pulmonarius*	*P. eryngii*	*P. djamor* var. *roseus*	*P. citrinopileatus*
Mycelial growth	TIH	38±2.51b	39±3.21b	36±0b	67±8.58a	52±2.51a	25±3.0c	38±2.5b
	CC	66±6.42b	71±8.54b	64±5.03b	152±25.02a	67±2.08b	66±2.0b	64±5.0b
Yield	1st Harvest g^{-1}	550.6±42.0	212.6±18.6	616.3±25.4	661.0±40.9	145.3±22.6	196.0±36.3	448.4±31.6
	2nd Harvest g^{-1}	293.3±11.6	285.2±31.5	56.7±6.7	383.5±32.14	68±6.6	284.5±13.6	123.16±7.9
	3rd Harvest g^{-1}	130.9±18.7	99.1±7.9	37.2±6.2	352.9±21.2	0±0	188.9±23.4	78.6±1.4
	Total harvest g^{-1}	978.9±50.8b	597.0±48c	710.2±27.1c	1397.5±83a	213.3±26.1d	669.4±23.1c	650.2±24.8c
	BE	67.3±7.13b	43.2±5.36b	48.6±3.06b	95.7±13.9a	13.8±0.89c	58.2±7.9b	42.5±2.2b
	PR	1.03±0.19a	0.60±0.04b	0.76±0.03ab	0.63±.09b	0.2±0.01c	0.87±0.12ab	0.66±0.09b
	HR	3±0	3±0	3±0	3±0	2±0	3±0	3±0
Physical characteristics	Max PD	8.9±1.04a	9.9±2.3a	9±1.3a	11.9±2.12a	7.2±0.9b	7±2b	5.5±0.86b
	Min PD	3.2±0.85b	2.9±0.9b	3.2±0.87b	3.9±1.3b	5.1±1.1a	2.6±0.96b	2.0±0c
	PC	Pale yellow (5Y 8/2 Munsell, 1992)	Light grey(7.5YR 8/2, Munsell, 2011)	Light grey(10YR 7/2 Munsell, 1992)	Very pale brown (10YR 7/2 Munsell, 1992)	Very pale brown (10YR 7/3 Munsell, 1992)	Light reddish brown (2.5YR 7/3 Munsell, 1992)	Yellow (5Y 8/12 Munsell,2011)
	SC	Grey(5YR 6/1 Munsell, 1992)	Grey(5YR 6/1 Munsell, 1992)	Violet gray (2.5R 8/2 Munsell, 2011)	Gray, lilac tones (5YR 6/1 Munsell, 1992)	Pale yellow (2.5Y 8/3 Munsell, 1992)	Pink (5YR 8/4 Munsell,2011)	Pink gray (7.5 YR 7/2 Munsell, 1992)
	PS	Ledge and trumpet	Semicircular ledge	Semicircular ledge	Semicircular ledge	Plane-convex and funnel	Flabeliform and semicircular ledge	Funnel, flabeliforme cespitose

TIH=Time for inoculation to harvest (days), CC= Crop cycle (days), BE = Biological efficiency (%), PR= Production rate, HR= Harvest number, Max PD= Maximum diameter of the pileus (cm), Mix PD = minimum diameter of the pileus (cm), PC= Pileus color, SC= Stipe color, PS= Pileus shape; Values are mean of three replicates ±SD; Means whit different letters in the same line are significantly different ($p \leq 0.005$) by Tukey's multiple range test.

Table 2 : Proximal chemical composition (g 100 g^{-1}) of fruiting bodies of *Pleurotus* spp. cultivated in wheat straw

Strains	Moisture	Ash	Protein	Lipids	Carbohydrates	Fiber*
P. ostreatus HEMIM 50	2.9 ± 0.42^{d}	8.1± 0.40^{c}	17.6± 1.66^{c}	4.4± 0.76^{d}	59.7± 0.62^{b}	6.9
P. ostreatus HEMIM 126	8.5± 0.98^{a}	10.1± 0.18^{a}	17.7± 1.73^{c}	5.2± 1.9c^{d}	53.3± 0.33^{c}	4.94
P. ostreatus HEMIM 127	2.8± 0.13^{d}	9.3± 1.63^{b}	19.4± 1.69^{b}	4.3± 0.47^{d}	53.6± 0.20^{c}	10.38
P. pulmonarius HEMIM 129	9.2± 0.64^{a}	4.8± 0.88^{e}	23.2± 1.42^{a}	4.2± 0.78^{d}	50.1± 1.75^{d}	8.20
P. eryngii HEMIM 130	5.5± 0.29^{b}	7.2± 0.78^{d}	19.7± 0.89^{b}	5.8± 0.22^{c}	50.1± 2.70^{d}	11.33
P. djamor var. *roseus* HEMIM 104	2.1± 0.21^{e}	8.9± 1.17^{b}	17.0± 0.90^{c}	6.7± 0.13^{b}	61.1± 0.00^{a}	4.07
P. citrinopileatus HEMIM 132	3.9± 0.29^{c}	9.1± 0.58^{b}	18.2± 2.56^{c}	7.9± 0.44^{a}	53.1± 0.36^{c}	6.85

*Obtained by difference. Values are mean of three replicates ±SD; Means whit different letters in the same column are significantly different ($p \leq 0.005$) by Tukey's multiple range test

study were found similar to those previously reported (Maftoun *et al*., 2015). It is suggested that the differences in the data observed between species depend on interspecific and intraspecific variations of the genus grown on wheat straw.

Fig. 1 shows total phenolic content and the scavenging activity of ABTS and DPPH radicals. The total phenolic content in general showed significant difference between strains and between extracts (p< 0.005) (Fig. 1A). Based on the statistical analysis of total phenolic content of MAE, three groups were formed, with the higher values were of *P. citrinopileatus* and *P. ostreatus* HEMIM-126, the intermediate values were presented by *P. pulmonarius* and *P. djamor* var. *roseus* and the lowest values were of *P. eryngii*, *P. ostreatus* HEMIM-127 and *P. ostreatus* HEMIM-50. In the case of TAE, the statistical test separated two groups, *P. ostreatus* HEMIM-126 and *P. citrinopileatus* with the highest values, while *P. ostreatus* HEMIM-50, *P. ostreatus* HEMIM-127, *P. pulmonarius*, *P. eryngii* and *P. djamor* var. *roseus*, were the strains with the lowest values. Total phenolic content for EMA was statistically divided into four groups, *P. citrinopileatus* showed the highest value, followed by *P. eryngii*, *P. ostreatus* HEMIM-127, *P. pulmonarius, P. ostreatus* HEMIM-50, *P. ostreatus* HEMIM-126 and *P. djamor* var. *roseus*. González-Palma *et al*. (2016) reported 9.9 mg GAE g^{-1} of total phenol in aqueous extract and 7.1 mg GAE g^{-1} in methanolic extracts from fruiting bodies of *P. ostreatus*. Puttaraju *et al*. (2006) quantified 13.3 mg GAE g^{-1} total phenols in aqueous extract of fruiting bodies of *P. djamor* and 3.6 mg GAE g^{-1} in methanolic extract. Kongkla and Poeaim (2016) recorded 7.1 to 17.63 mg GAE g^{-1} total phenols in the fruiting bodies of five *Pleurotus* species. The compounds vary depending on the species and even between strains, since each organism presents unique strategies of metabolic reactions and synthesis of compounds, in response to exposure to sunlight or chemical compounds, among other factors, promoting the development of both enzymatic and non-enzymatic protection systems, the above could influence the type and quantity of phenolic compounds produced in fungi (Heleno *et al.*, 2015). .

Fig. 1B shows the scavenging activity of ABTS radical. The values obtained in the MAE of all strains showed no significant difference between them, except for *P. ostreatus* (HEMIM-127) which showed a lower value than other strains (p <0.005). TAE showed significant difference between strains (p <0.005), where *P. citrinopileatus* reported the highest value, followed by *P. ostreatus* (HEMIM-126), *P. ostreatus* (HEMIM-50), *P. ostreatus* (HEMIM-127) and *P. pulmonarius; P. eryngii* and *P. djamor* var. *roseus* showed the lowest values. In EMA, the values for *P. citrinopileatus* and *P. ostreatus* (HEMIM-126) were not statistically different; the second group constituted of *P. ostreatus* (HEMIM-50), *P. pulmonarius*, *P. eryngii* and *P. djamor* var. *roseus*, the lowest value was observed in *P. ostreatus* (HEMIM-127). The scavenging activity of DPPH radical of all extracts (Fig. 1C) showed significant differences between strains (p <0.005). In MAE, the values were divided into three groups, highest in *P. citrinopileatus*, followed by *P. ostreatus* (HEMIM-50), *P. ostreatus* (HEMIM-126), *P. ostreatus* (HEMIM-127) and *P. djamor* var. *roseus*, while the lowest was noted in *P. pulmonarius* and *P. eryngii*. In TAE, the highest scavenging activity of DPPH was seen in *P. citrinopileatus* followed by *P. ostreatus* (HEMIM-126), *P. ostreatus* (HEMIM-127) and *P. pulmonarius*, *P. djamor* var. *roseus* and *P. ostreatus* (HEMIM-50), while *P. eryngii* showed the lowest value. In the EMA, the highest values were seen in *P. ostreatus* (HEMIM-126), *P. ostreatus* (HEMIM-127), *P. pulmonarius* and *P. citrinopileatus*, followed by *P. eryngii P. ostreatus* (HEMIM-50) and *P. djamor* var. *roseus*.

Kongkla and Poeaim (2016) reported 100, 93 and 61 % inhibition of ABTS radical using methanolic extracts of *P. pulmonarius*, *P. ostreatus* and *P. eryngii*, respectively. Lee *et al*. (2007) reported the scavenging activity of DPPH radical in cold water (20.7 %), hot water (52.3 %) and ethanol (94 %) using fruiting bodies of *P. citrinopileatus*. Chen *et al*. (2016) reported 35% and 65 % inhibition of DPPH radical in extracts obtained with hot water and cold water, which is similar to that observed in our study since a higher percentage of inhibition of DPPH radical was obtained in TAE compared to MAE. Puttaraju *et al.* (2006) prepared antioxidant indexes of 23 species of fungi and classified *P. djamor* as a fungus with moderate antioxidant activity. In the present study, MAE of *P. djamor* var. *roseus* reported the highest value of antioxidant activity only after *P. citrinopileatus* with DPPH

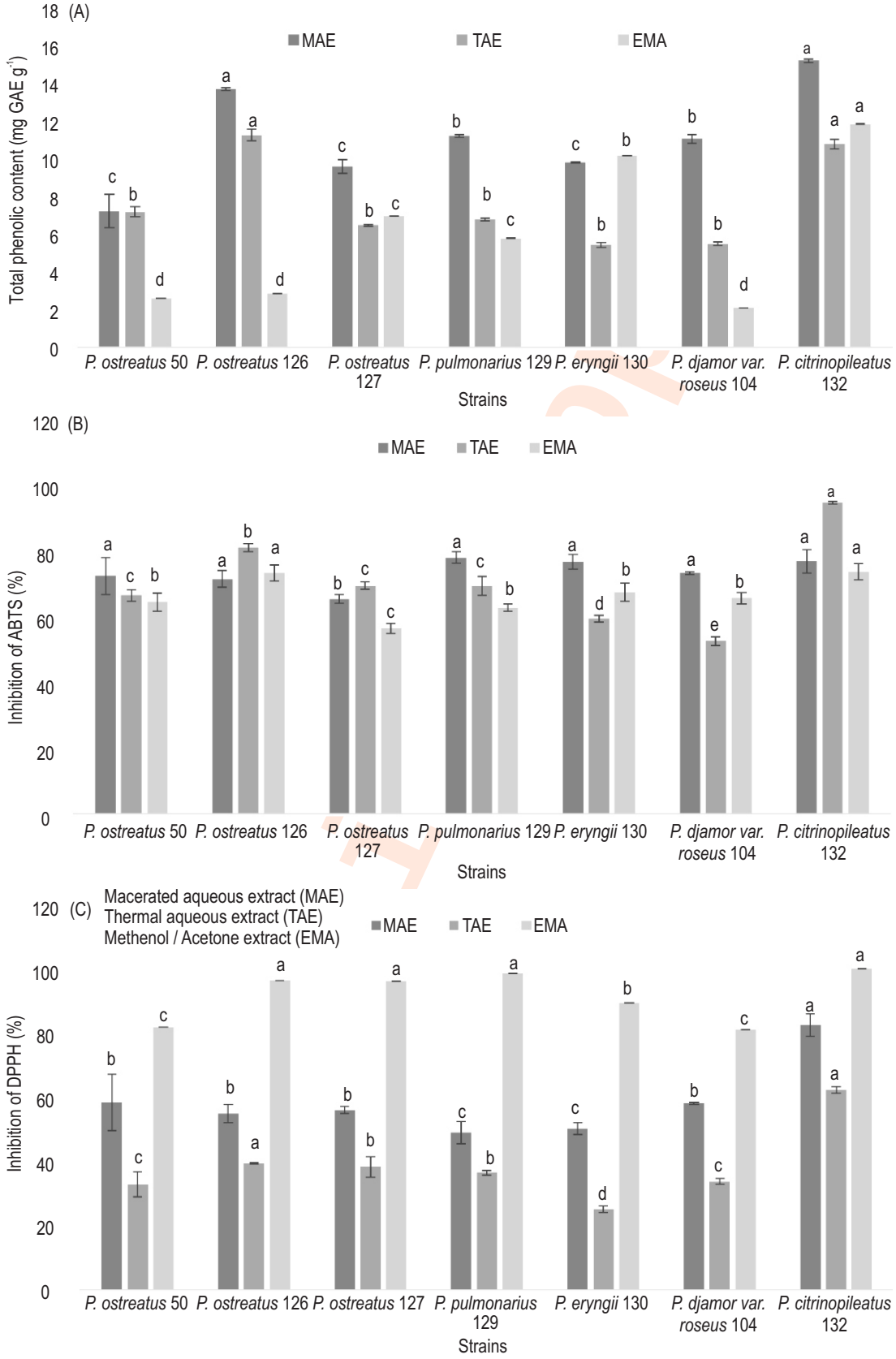

Fig. 1 : Total phenolic content (A); percentage inhibition of ABTS* (B) and DPPH* (C) in different extracts of fruiting bodies of Pleurotus strains.

radical (Fig.1C), however, in the other two extracts it was the one with the lowest antioxidant capacity, which suggests that water-soluble antioxidant molecules are sensitive to temperature and during heating process almost 50% of antioxidant capacity is lost. The results observed in the present investigation on inhibition of DPPH radical in MAE and TAE was lower as compared to other previous reports, while EMA showed similar values with that found in the literature. Antioxidant activity in mushrooms has been attributed mainly to ß-glucans, and the polysaccharides are stable to thermal treatments maintaining their activity. In this study, in the cases of *P. citrinopileatus* and *P. ostreatus* (HEMIM-126), the antioxidant activity in each of the extracts could be due to their phenolic content (Fig. 1A). However, the antioxidant activity in *P. pulmonarius* and *P. eryngii,* may be due to phenolic compounds and polysaccharides (glucans) (Table 2).

Based on the results obtained, it can be concluded there exists a statistical differences in the nutritional content and antioxidant capacity between species and even among the strains of same species despite some having morphological similarity. To determine the factors that cause the differences, more studies are required on the (genomic, transcriptomic, proteomic and metabolic) aspects to optimize the process of mushroom production with high nutritional and nutraceutical values.

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