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FULL PAPER

Content of Caffeine in the Edible Mushroom *Pleurotus ostreatus* Grown in Coffee Residues

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Abstract:

The chemical composition of mushrooms is influenced by the substrate in which it is cultivated due to absorption of chemical compounds from the substrate. In the current study, the edible mushroom *Pleurotus ostreatus* was cultivated in three diverse ways: in seeds grown on substrates based on coffee residues and inoculated in rye grass and; in the coffee seeds without rye grass; and in wheat seeds with rye grass (reference mushroom). Caffeine extraction from mushrooms was optimized by factorial design followed by analytical validation of the HPLC-UV methodology to determine caffeine in mushrooms. Subsequently, the validated analytical protocol was applied to investigate the levels of caffeine in mushrooms grown in coffee residues. Additionally, the levels of bioactive compounds such as flavonoids and phenolic compounds and the antiradical capacity of extracts were determined by spectrophotometric methods. Mushrooms inoculated directly into the coffee seeds, significantly absorbed caffeine from the substrate (589.6 to 975.0 µg/g). The amount of total phenolic compounds in mushrooms grown in coffee seeds were significantly lower that the reference mushroom but the antiradical capacity of the mushroom grown in coffee seeds without rye grass was slightly better, suggesting that caffeine might contribute to the antioxidant activity of these mushrooms.

Keywords: caffeine; HPLC; edible mushroom; *Pleurotus ostreatus*

1. Introduction

Mushrooms belong to the Fungi kingdom and are used as food, ornamental and medicinal products. Some species of mushrooms have hallucinogenic or even poisonous effects. As they feed on other living beings in decay, mushrooms are called nature recyclers, which highlights their great biological importance [1, 2]. The cultivation of mushrooms of the genus *Pleurotus* is of great economic interest. These mushrooms have palatable taste, high nutritional value and in their constitution are found proteins, vitamins, carbohydrates and dietary fibers. Depending on the substrate in which the mushrooms are cultivated, they have a composition and, often, different flavors [3] due to the possibility of absorption of chemical substances present in the substrates.

In the current study, the basidiomas of *P. ostreatus* were cultivated with inoculum produced in coffee grounds, which is rich in caffeine. The coffee residues were used as inoculum after total colonization with the mushroom mycelium and therefore, here denominated as coffee residues seeds (CRS). The CRS were inoculated in the substrate rye grass, properly prepared for mushroom cultivation. As it is possible that the caffeine present in the CRS may be absorbed by the fungus mycelium and, thus be found in their basidioma, in the current study we investigated

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the levels of caffeine in mushrooms grown in CRS. Caffeine is not a product of mushroom metabolism; this molecule is not found in mushrooms grown on cellulosic materials such straw. Therefore, due to the commercialization of many edible mushrooms, which are often grown in substrate composed only of coffee grounds, the caffeine content in the mushroom should be determined to know the possible contribution to the daily diet by ingesting edible mushrooms.

In the literature there is a range of reports on the determination of antioxidant activity and phenolic compounds in mushrooms of different species [4-6]. Dubost verified that *Agaricus bisporus* was a most active mushroom species than *Pleurotus ostreatus* and Elmastas also reached the same conclusion in their studies. Coffee also has antioxidant activity, which is a consequence of having in its composition caffeic acid, and chlorogenic acids [7, 8]. These acids are the main phenolic constituents present in coffee [9] and could enhance the antioxidant activity of edible mushrooms grown in coffee residues. Then, it becomes interesting to know the total phenolic and flavonoid contents, as well as, the antiradical activity of mushroom samples grown in alternative substrates such as the coffee grounds or in rye grass inoculated with CRS. Throughout the study the mushroom grown in wheat seed and inoculated in the rye grass was used as a control to compare the traditional culture medium with mushrooms grown on substrates rich in caffeine.

2. Material and Methods

2.1 *Pleurotus ostreatus* cultivation

Three treatments of mushroom production were carried out: - Mushrooms produced with CRS – inoculum developed in coffee grounds and inoculated in rye grass (CF); - Mushrooms produced directly in the coffee seed, without rye grass (C) and; - Mushrooms produced with inoculum developed in wheat grains and inoculated in rye grass (TF). The coffee grounds were the residual powder coffee after obtaining a coffee drink.

Firstly, the rye grass was prepared in sachets and after pasteurization (85 ºC during 4 hours) it was inoculated with CRS (3% v/v). The CRS was deposited on top of the rye grass and the sachets were closed, and then incubated for micellization for 30 days and subsequent production of mushrooms CF and C in humid greenhouse during five days. The preparation of the TF mushrooms followed a procedure similar to the preparation of CF and C, however, the CRS were replaced by micelliated wheat seeds. Finally, all mushrooms were dried and ground.

2.2 Optimization of caffeine extraction from *Pleurotus ostreatus*

Mushroom's samples produced with seeds developed in wheat grains and inoculated in rye grass (TF) were used to optimization of the analytical protocol to extract caffeine from *P. ostreatus*. TF samples were grown without the presence of coffee grounds, so these samples do not contain caffeine and were employed as blank mushrooms. A 2^2 factorial design was used as the statistical method to guide the optimization of the extraction step. In a factorial design the influence of the experimental variables is investigated, as well as the effects of their interactions on the responses [10]. For this, two variables were tested: the volume of the extractor solvent HCl 0.1M (A) and the sonication time in the ultrasound apparatus (B) (Table 1). All assays were performed randomly and in duplicate. The assays were carried out through addition/recovery studies and the recovery of caffeine (%) was determined to evaluate the efficiency of the extraction procedure

For the extraction, 0.1 g of mushroom was spiked with 0.5 mg of caffeine. The spiked samples were left in repose for 24 hours and then extracted by sonication with HCl 0.1 M. Afterwards, the samples were centrifuged at 2000 rpm for 5 minutes, and the supernatant was then reserved. In sequence, fresh 0.1 M HCl was again added to the precipitate, and then the sonication and centrifugation steps were repeated. The supernatants from the two extraction cycles were combined and pelleted in 5 mL volumetric flasks, using ultra-pure water as diluent. The theoretical concentration of caffeine present in the extraction solution was 100 mg/L.

Extracts solutions were filtered on 0.45 µm Nylon membrane and analyzed by HPLC-UV. The concentration of caffeine was calculated from the peak area on chromatograms. An external standard calibration curve at concentrations

levels of 10, 30, 60, 90, 120, 150 and 180 mg/L of caffeine was used for determination of caffeine in extracts of mushrooms.

Table 1. Factorial Design 2² representing the levels and arrangement of variables: HCl volume and sonication time in the optimization of the caffeine extraction from *Pleurotus ostreatus*.

Variables and levels		
A. Vol. HCl 0.1M (mL)	B. Sonication time (min)	Levels
2	10	-
3	20	+
Arrangement of the variables for each assay		
Assay	A	B
1	-	-
2	+	-
3	-	+
4	+	+

2.3 HPLC-UV analyses

Determination of caffeine in extracts of mushrooms was carried out by high performance liquid chromatography (HPLC) (WATERS 600) with UV detection in a diode array detector (WATERS 2969), with deuterium lamp operating from 190 to 800 nm.

The samples were first analyzed in the scanning module (190 to 800 nm) and the optimum maximum absorption wavelength of 274 nm to determine caffeine at the highest sensitivity was chosen. A XTerra® Phenyl column (250 x 4.6 mm, 5 µm) maintained at 25 °C was used as stationary phase. Mobile phase consisted of methanol (solvent A) and ultra-pure water (solvent B). The elution occurred in isocratic mode in the proportion of 40% A and 60% B under a flow rate of 0.5 mL/min for 12 minutes. Solvents used for the chromatographic analyzes were previously membrane vacuum filtered and degassed in ULTRA CLEANER/UNIQUE Ultra-Sonic bath. Prior to the injections, the aqueous solutions of the samples were filtered through 0.45 µm Nylon.

Analytical validation of the HPLC-UV methodology to determine caffeine in mushrooms

The in-house validation study was based on ANVISA guide [11]. Mushroom's samples are food products and are also considered supplements or functional products, marketed in pharmacies and sold as dry powder. Caffeine would be one of the active principles of this functional food, when present in mushrooms as a product of absorption by substrates rich in that molecule. The validation parameters investigated were: selectivity, linearity, limit of detection (LD), limit of quantification (LQ), precision and accuracy.

To check the selectivity of the HPLC-UV methodology, a standard solution of caffeine (180 mg/L), an extract from the mushroom sample enriched with the analyte with a theoretical concentration of 180 mg/L and the extract of the blank mushroom TF, as a sample of caffeine free reference mushroom, were analyzed in triplicates. The chromatograms and UV spectra obtained were evaluated in terms of retention time, maximum absorption band and also if there was interference of some other possible component present in the matrix.

In order to verify the linearity of the method, a calibration curve by the external standard method was constructed at six concentration levels (1, 10, 60, 120, 180 and 210 mg/L), each level in triplicate. A linear regression was performed, and linearity was checked at the 95% confidence level. This analytical curve was used for calculation of the limits of quantification and detection using the values of the standard deviation of the intercept and slope derived from the analytical equation following ANVISA guide recommendations [11].

To evaluate the accuracy of the method, true triplicates of an extract from a sample of *P. ostreatus* (TF) enriched with caffeine were used, with a theoretical concentration of 180 mg/L. For repeatability, each triplicate of the extract was read three times in a single day and in two different periods. The results totaled 9 responses in the morning and 9 in the afternoon in the same conditions of equipment and analyst. Intermediate precision was calculated from triplicate injections of each sample of TF enriched with caffeine for five consecutive days. Nine injections per day were performed.

Accuracy of the method was evaluated according to the ANVISA validation guide [11], which proposes an addition-recovery study in three levels of fortification, being low, medium and high. Then, samples of the TF mushroom were

enriched with caffeine at three concentration levels (50, 90 and 130 mg/L). Each sample was prepared in true triplicates and injected into the HPLC.

Statistical analyzes applied in this work were performed at the 95% confidence level using Minitab statistical software version 16.2.2.

After the optimization and validation of the caffeine extraction and quantification methodologies, determination of caffeine in the CF and C samples was carried out. Mushrooms samples were extracted through the optimum extraction condition found using factorial design, and analyzed by HPLC-UV under the validated conditions.

2.4 Determination of flavonoid, total phenolic contents and antiradical activity of *Pleurotus ostreatus*

Methanolic extracts of all samples of *P. ostreatus* were obtained as described by Mokochinski [5]. For determination of the flavonoid content, the reagent aluminum chloride dihydrate 5% m/v in methanol was used. A calibration curve was prepared from a quercetin stock solution at 50 mg/L diluted at six concentration levels [0.1; 0.5; 1.0; 5.0; 10.0 and 20.0 mg/L]. A volume of 0.5 mL of the quercetin solutions were mixed with 0.25 mL of 5% $AlCl_3$ solution and vortexed in a 5 mL volumetric flask. After 30 minutes, absorbance at 425 nm was read on the UV-Vis spectrophotometer (VarianCary 50 Bio-Vis). For the quantification of flavonoids in the extracts, the same procedure used for standard quercetin solutions was performed [12].

Total phenolic concentrations were determined by the Folin-Ciocalteau method [13]. A stock solution of gallic acid in methanol at a concentration of 1000 mg/L was used for the preparation of standard solutions. A calibration curve was prepared at 80; 120; 200; 250; 320; 350; 400 and 420 mg/L. Into a 5 mL volumetric flask was added 0.1 mL of each standard gallic acid solution, 0.5 mL of carbonate / tartrate buffer solution (20 g Na_2CO_3 and 1.2 g $KNaC_4H_4O_4.4H_2O$ in 100 mL of water) and 0.5 ml of the Folin-Ciocalteau reagent, and ultra-pure water was used as diluent. The solutions were allowed to stand for 30 minutes. After that, the absorbance was read at 760 nm. To determine the levels of phenolic in the extracts, the standard solution of gallic acid was replaced by the extract.

The antiradical activity of extracts was investigated by the DPPH· radical method [14]. The reaction was carried out by direct dilution into the UV-Vis cuvette, in which a fixed volume of DPPH· 1000 mg/L (2.5 mL) and different volumes of the mushroom extract were added. The final volume of 3 mL was completed with methanol. The reaction time was 60 minutes and then the absorbance was read at 515 nm. An analytical curve was elaborated for each mushroom extract by plotting the percentage of inhibition (%) as a function of the concentration of the extract. The antiradical capacity of the extracts was expressed as EC_{50} values.

3. Results and Discussion

3.1 Study of the optimum experimental conditions for caffeine extraction from *Pleurotus ostreatus*

The optimal condition to extract caffeine from *P. ostreatus* was chosen after analyzing the recovery percentages obtained by performing experimental assays guided by a 2^2 factorial design (Table 2). The variables verified were the volume of the extracting solvent (0.1 M HCl) added to the dry samples of mushrooms, and the extraction time at which the sample was sonicated in the ultrasonic bath. Caffeine is a weak base, an aqueous solution of HCl was chosen for extraction in order to promote the protonation of nitrogen present in the molecule, thus making it more soluble in acidic aqueous solution. To verify how much caffeine was recovered in the factorial design trials, a calibration curve was constructed by external standard calibration in the range of 10 to 180 mg/L of caffeine (R^2 = 99.9%).

In all experiments, the recovery of caffeine from mushrooms were within the range recommended by ANVISA (80-120%), demonstrating that caffeine extraction is being performed under optimum conditions. Assays 1 and 2 presented a mean recovery percentage closer to 100%. Therefore, assay 1 was chosen as the optimal condition for extracting caffeine in *P. ostreatus* mushrooms because it uses a smaller volume of the extracting solvent. The application of the *t-test* showed that the effects of HCl volume variation and interaction of the two

variables (AB) were not significant ($p > 0.05$).

Table 2. Recovery percentages of caffeine in the assays delineated by 2² factorial design to optimize the variables HCl volume and sonication time.

Assays	Vol. HCl 0.1 M (mL)	Sonication time (min)	% Caffeine recovery	Average ± SD
1a	2	10	106.87	101.97 ± 6.92
1b	2	10	97.08	
2a	3	10	106.09	99.08 ± 9.90
2b	3	10	92.07	
3a	2	20	112.90	120.53 ± 10.79
3b	2	20	128.17	
4a	3	20	115.37	122.91 ± 10.66
4b	3	20	130.45	
	Effects		**Estimative ± DP**	***p value***
	Vol. HCl 0.1 M (mL) (A)		-0.26 ± 3.42	0.972
	Sonication time (min) (B)		21.19 ± 3.42	0.037
	Interaction between the two factors (A) x (B)		2.63 ± 3.42	0.720

In contrast, the time in which the samples were sonicated in the ultrasound bath significantly influenced the percentage of caffeine recovery ($p < 0.05$), that is, the longer the extraction time, the greater the amount of caffeine recovered. It was observed, however, that for the extraction time of 20 minutes, recovery values obtained were at the upper limit of the recovery interval suggested by ANVISA. Probably the longer sonication time causes the extraction of other molecules structurally similar to caffeine and, therefore, have similar retention times and UV absorption profiles. Consequently, the experimental conditions of test 1 as an optimal condition for caffeine extraction from *P. ostreatus* mushrooms were chosen.

3.2 Analytical validation of an HPLC-UV methodology to determinate caffeine in mushrooms

The selectivity of the method could be confirmed by analyzing the chromatograms and the UV spectra of the caffeine peak in the standard solution of caffeine and in the mushroom extracts (Figure 1). Both chromatograms showed a symmetric peak for caffeine at 10 min with a very similar UV absorption profile and maximum absorption at 274.8 nm; then no evidence of co-eluting peaks is observed. From these results the proposed chromatographic method was considered selective for the determination of caffeine in mushroom samples.

A calibration curve was constructed with standard solutions of caffeine in the range of 1 to 210 mg/L in order to evaluate the linearity of the method. The purpose of this parameter is to evaluate whether the monitored analytical response for caffeine (peak area) in the range of working concentrations can be considered linear and therefore suitable for the determination of caffeine in mushrooms. For this, the data obtained for the area of the chromatographic peak of several standard solutions of caffeine were submitted to linear regression and analysis of variance (ANOVA). All statistical analyzes were performed at the 95% confidence level (Table 3).

According to the $F_{lack\text{-}of\text{-}fit}$ value not evidence of lack of fit was found at the 95% confidence level ($p > 0.05$), so the linear model is adequate to express the relation between the area of the caffeine chromatographic peak and its concentration in solution. This can be confirmed by observing the highly significant $F_{regression}$ ($p = 0.000$) value at the same confidence level (Table 3). In addition, the value of the coefficient of

determination (R^2 = 99.6%) shows that only 0.4% of the variation in the experimental data refers to the residuals, indicating a good fit in the linear model.

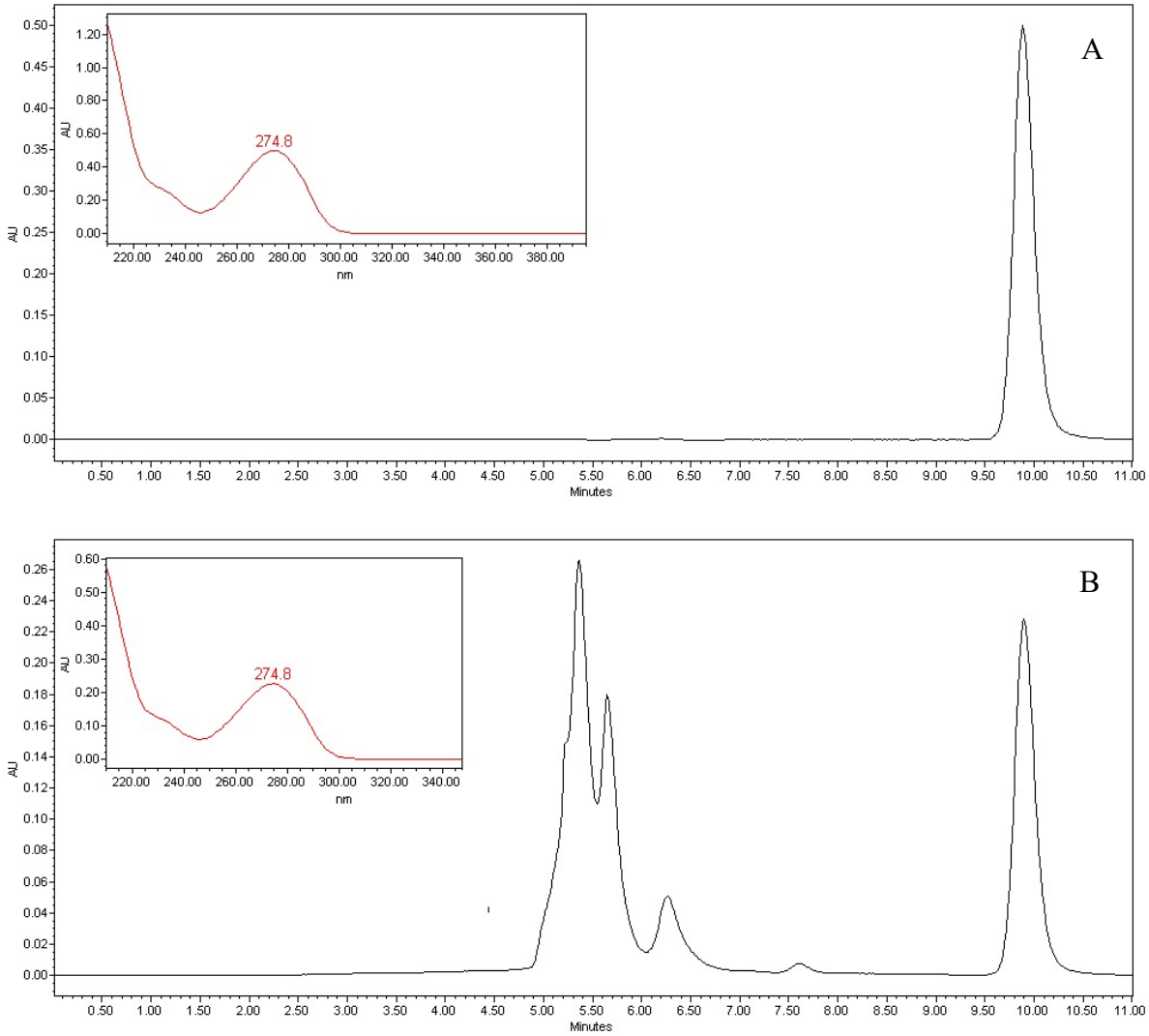

Figure 1. HPLC-UV chromatograms detected at 274.8 nm: (A) standard solution of caffeine and (B) mushroom extract TF spiked with caffeine. UV spectra for peak at 10 min is highlighted.

Table 3. Results of linear regression analysis at the 95% confidence level.

Regression*	***lack of fit****	***r***	**R^2**
$F_{regression}$ ***p value***	$F_{lack\text{-}of\text{-}fit}$ ***p value***	0.9960	99.6%
4855.50	1.16		
0.000	0.375		
Regression Coefficients ± SE		$t_{observed}$***	***p value***
Intercept: - 56967 ± 69169		0.82	0.420
slope: 41264 ± 592.2		69.68	0.000

The *t-test* showed that the intercept did not present significance (p = *0.420*). However, the coefficient related to slope of the curve was significant (p = *0.000*), demonstrating that the HPLC-UV method is sensitive to changes in caffeine concentration in solution. In this way, it is concluded that the calibration curve is linear in the range of concentrations studied, passes through the origin and can be represented by the following equation: *area = - 56967 + 41264*caffeine concentration (mg/L).*

The LD and LQ values obtained were, respectively, 0.28 and 0.96 mg/L. These values demonstrate that it is possible to detect and quantify caffeine through the analytical method

developed by HPLC-UV in concentrations of the order of parts per million.

To evaluate the accuracy of the method, tests were carried out by spiking the TF mushroom with known amounts of caffeine standard at three concentration levels. ANVISA guide [11] determined an adequate recovery range within 80-120% for trace analytes in complex samples. Recovery values for the concentration levels of 50, 90 and 130 mg/L were, respectively, 103.8; 96.6 and 104.6%. To verify if there were significant differences between the desired and experimentally determined recovery, a 95% confidence *t-test* was applied to the results and it was conclude that there weren't significant differences between the desired recovery value and the experimental value. Thus, the method can be considered accurate in the experimental range studied.

According to the ANVISA guide, RSD (%) values below 15% are acceptable for precision. Therefore, RSD results were calculated for both repeatability and intermediate precision. The values obtained for repeatability and intermediate precision were, respectively, 5.11 and 6.18 %. Therefore, the analytical method developed can provide acceptable dispersion results even when determinations are performed on different days.

Aragão validated a methodology for the determination of methylxanthines in tea-mate and soluble coffee. Their results of caffeine recovery in tea-mate were 94 and 105%, and for the soluble coffee of 98 to 101%. Thus, the results demonstrate that the HPLC-UV method presents adequate accuracy for the determination of caffeine in the edible mushroom investigated [15].

3.3 Application of the analytical methodology for the determination of caffeine concentration in mushrooms

After confirming the adequacy of the extraction methodology and the HPLC-UV method to determine caffeine in extracts of mushrooms, the analytical procedure was applied to quantify the levels of caffeine in mushrooms grown in substrates rich in caffeine. All samples were determined in true triplicates, and three measures were made for each replicate, totalizing nine results for each sample. Great variability was found among samples (Table 4) but the results showed that the C mushroom samples, produced directly in the coffee grounds, without the use of rye grass, absorbed a significantly greater amount of caffeine than the CF mushrooms, produced with seeds grown in coffee grounds and inoculated in rye grass. This result can be explained by the fact that the mushroom C, produced directly in the coffee seed without rye grass, had no other substrate available in the mushroom production stage. That is, the mushroom C was in direct contact with the coffee grounds, which facilitated the absorption of caffeine. As expected, samples of TF mushrooms, which had no contact with coffee residues, did not present caffeine.

Fan produced edible mushroom of the genus *Pleurotus* in coffee peel and determined caffeine using chloroform as solvent extractor. Comparing caffeine contents in the initial and final dry mushroom, 0.65 and 0.197 mg/g, respectively, they noticed that there was a decrease in the amount of caffeine after colonization and fruiting in the order of 60.69%. They also found that the molecule does not suffer degradation, but rather accumulates in part of the fruiting body of the mushroom [16].

Table 4. Caffeine contents in the mushroom samples.

Samples	Average value ± SD (□g/g)	95% Confidence Interval
CF	160.03 ± 120.94[a]	67.07 - 252.99
C	773.10 ± 224.04[b]	600.89 - 945.32
TF	nd	nd

* Values expressed per gram of dry mushroom (mean ± standard deviation). Different letter in the same column represents significant statistical differences ($p < 0.05$) (ANOVA and Tukey test). CF = Mushrooms produced with seeds developed in coffee grounds and inoculated in rye grass; C = Mushrooms produced directly in the coffee grounds, without using rye grass; TF = mushrooms produced with seeds developed in wheat grains and inoculated in rye grass. Nd = not detected

3.4 Content of Total Phenolics, Flavonoids and Antiradical Activity of *Pleurotus ostreatus*

The average content of flavonoids and total phenolics (Table 5) in *P. ostreatus* extracts were low if they are compared with the study of Mokochinski [5]. In this study, higher levels of

flavonoids, total phenolics and better antiradical capacity were found in *Agaricus brasiliensis* mycelium produced by submerged and solid-state fermentation using vegetal residues from the food industry (grape pomace, apple pomace, pineapple pomace and pineapple peel), wheat and malt [5]. Nevertheless, CF mushroom had values of flavonoids significantly larger ($p < 0.05$) than the mushrooms grown by the other two treatments while total phenolic levels were higher in TF mushroom. Additionally, the antiradical capacity was higher in extracts of mushroom C. Then, no direct correlation was found among these three parameters (Table 5) indicating that the antiradical capacity might be influence by other chemical classes in mushrooms such as phytosterols (ergosterol), glycosides, polysaccharides, ergothioneine, carotenoids, ascorbic acid, other vitamins, and minerals. [17]. Additionally, caffeine along with its catabolites, theobromine and xanthine, has a structure similar to uric acid, which is an antioxidant compound. With this, studies have shown that caffeine and its metabolites can contribute to the antioxidant activity of foods and beverages containing this substance [18]. Then, it is evident that different treatments affected the metabolism of the mushroom or its development with respect to the biosynthesis of bioactive compounds and antiradical capacity.

Table 5. Flavonoid and phenolic contents and antiradical capacity (EC_{50}) of extracts of mushroom samples.

Samples	Flavonoids ± SD (μg/g)*	Phenolics ± SD (μg/g)*	EC_{50} ± SD (g/L)
TF	62.28 ± 4.16^b	3841.7 ± 182.7^a	2.95 ± 0.08^b
C	45.12 ± 2.26^b	1773.9 ± 19.68^b	2.70 ± 0.11^a
CF	114.31 ± 34.56^a	1642.7 ± 166.0^b	3.47 ± 0.20^b

* Values expressed per gram of dry mushroom. Different letter in the same column represents significant statistical differences ($p < 0.05$) (ANOVA and Tukey test). TF = mushrooms produced with seeds developed in wheat grains and inoculated in rye grass; C = mushrooms produced directly in the coffee seed without the use of rye grass; CF = mushrooms produced with seeds developed in coffee grounds and inoculated in rye grass.

On the other hand, the species of edible mushroom also influence the levels of bioactive compounds biosynthesized. Pauli determined the content of phenolic compounds and the antiradical activity of mushrooms *Lentinus edodes* (Shiitake), *Agaricus bisporus* (Champignon de Paris), *Oudemansiella canarii* and *Pleurotus ssp.* (White Pleurotus, Pleurotus salmon and Shimeji) in samples provided by Zucca Alimentos located in the city of Salto, region of Campinas (SP). The results showed a lower amount of flavonoid content in *Pleurotus* than in the other mushrooms, 0.39 and 0.60 mg/g for white *Pleurotus* and *Pleurotus salmon*, respectively. Regarding the phenolic content, the white *Pleurotus*, *Pleurotus salmon* and Shimeji, even belonging to the same genus, presented values statistically different and on average, larger than the other species studied. In relation to antiradical activity, *Pleurotus* mushrooms had lower antiradical capacity than the others [4] and *P. ostreatus* showed and EC_{50} = 3 g/L; demonstrating similarity to the values found in the current study.

4. Conclusions

Mushroom grown directly on the coffee grounds, without the presence of rye grass (C) presented higher caffeine content than that produced with seeds grown in coffee grounds and inoculated in rye grass. Caffeine was identified only in samples CF and C, confirming that this molecule was absorbed by the mycelium of the fungus and did not suffer degradation.

As caffeine has numerous biological activities its presence in mushrooms may influence their biological activity. For example, the thermogenic properties of caffeine allow new applications for mushrooms grown in coffee residues, such as a food supplement.

The mushroom produced without the presence of coffee grounds showed higher total phenolic content than the others. Therefore, the results suggest that the caffeic substrates are not adequate to stimulate the production of phenolic compounds by the mushroom. Nevertheless, the

mushroom grown directly in coffee seeds showed a slightly better antiradical capacity, indicating that other chemical compounds may influence this activity in mushrooms.

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