Researches on *Pleurotus ostreatus* Mushroom's Quality Cultivated on Coffee Grounds

Sorina Ropciuc[1], Ana Leahu[1], Mircea Oroian[1] , Cristina Damian[1]
[1]*Romania Faculty of Food Engineering, Stefan cel Mare University of Suceava, 13 Universitatii Str., Suceava, Romania,*

Abstract
The objectives of this work were to evaluate the possibility of using coffee grounds for cultivating *Pleurotus ostreatus* mushrooms and determine the nutritional composition of *Pleurotus ostreatus* mushrooms produced on coffee grounds substrate. The results revealed a good fruiting of the fungus on coffee grounds and the biological effectiveness (weight of fresh mushroom) reached about 97% after 30 days. We determined the total protein content in vitamin C, the total polyphenols and the activity of Polyphenol oxidases (PPOs) enzyme on 32 samples of fresh *Pleurotus ostreatus* mushroom (cap and stem) and subjected to heat treatments (blanching, boiling and freezing). The protein content was ranged between the values of 16.9 and 25.1g/ 100g and the Vitamin C content within the range of values presented 64.32-564.95 mg/100g. The polyphenol content results varied significantly in the analyzed samples varying between 1.887 – 7.667 mg GAE / 100 g vegetable product. The determination of the polyphenol oxidase enzyme responsible for enzymatically blackening of the fungus presented values in the range 0.274-0.610mg / 100g.

Keywords: coffee ground, biological efficiency, nutritional composition

1. Introduction

The *Pleurotus* genus is a mushroom with pleasant aroma and a high content of proteins, minerals (Ca, P, Fe, Mg) and small amounts of carbohydrates and fats that represent excellent diet food [1, 2]. Moreover the mushrooms are an excellent source of high quality proteins. The *Pleurotus* consumption has positive effects on the lipid metabolism, contributing to the reduction of free triglycerides and preventing atherosclerosis [3, 4].
In Romania, the *Pleurotus* mushrooms crop started in 1772. The cultivation technologies for the species *Pleurotus ostreatus*, *Pleurotus florida*, *Pleurotus cornucopiae* and *Pleurotus sajor-caju* were introduced in the period 1972-1985 by a group of researchers. Mushrooms are highly appreciated for their flavour and have been well studied due to their nutritional and medicinal proprieties. *Pleurotus* mushrooms have high nutritional value and can be a good source of protein, carbohydrates, vitamins, calcium and iron [5, 6]. Furthermore, these mushrooms have important medicinal properties, such as anti-tumour and immune-stimulatory activity, as observed in rats [4].
In nature, this species of mushrooms actively decomposes wood and many other substrates. It has the ability to colonize and digest many types of materials that contain lignin, cellulose, starch, sugars and proteins [4, 7, 8]. The *Pleurotus* genus is considered a primary decomposer of wood and plant residue and they are likely to be grown in a variety of residues, such as agricultural, agroindustrial and wood residues [7, 9-11].
Coffee grounds have been evaluated as a possible residue for the inoculation and growing of the mushrooms. The chemical and structural properties of the coffee grounds allow the reuse

* Corresponding author: Sorina Ropciuc, +040722541087, sorina.ropciuc@fia.usv.ro

due to their high content of cellulose (12.4 g/100g dry product), hemicellulose (39.10 g/100g dry material), lignin (23.90 g/100g dry product), nitrogen (12.79 g/100g dry product), dietary fiber (60.40 g/100g dry product) [7, 12, 13]. The water storage capacity is influenced by the size of the particles resulted from the coffee grinding, with values between 5.73-5.11 g/g dry sample [12].

It is a matter of inhibiting the growth of the mycelium on the coffee grounds due to the content of caffeic acid and tannins. According to some studies, the caffeic acid has a limiting effect on the mushrooms growth. *Pleurotus ostreatus* was also used in the bioremediation of the pollutants and in the degradation of lignocellulolytic residues by the action of different enzymes [3, 4, 17] including the lignocellulolytic enzymes, tannase and phytase [2, 9]. Therefore, in this study it was tested the capacity of *Pleurotus ostreatus* to degrade the antinutritional factors and the capacity of producing edible mushrooms, using the coffee grounds as substrate.

2. Materials and methods

2.1 Obtaining the mushrooms crop

We used *Pleurotus ostreatus* and the inoculation took place in rice boiled with water for 50 minutes and autoclaved at 121°C for 2 hours.The coffee grounds substrate was obtained from various coffee shops in Suceava. We boiled it in water for 2 hours in order to reduce certain compounds that could inhibit the fungal growth and the contamination. The coffee grounds were then spun at 1800 rpm for 5 minutes in order to remove the water excess. From the substrate were taken 900 g fresh which were placed in plastic casseroles and autoclaved for 2 hours at 120°C. This procedure was repeated three times, 4 hours apart. Repeated autoclaving of the nutrient substrate has the purpose to remove all the competitors in the substrate.

The final humidity was 80%. At the end of the procedure, when the temperature of the substrate reached 28-30°C, it was inoculated mycelium of strain M2 125, variety Florida oyster, species *Pleurotus ostreatus*. The inoculation rate of the substrate was 1:900 (g mycelium:g fresh coffee grounds)

The growing substrate consisting of coffee grounds was placed in casseroles and in polyethylene bags with perforated walls in order to allow the development of the carpophores. The first layer of coffee grounds was 7-10 cm thick, then the mycelium was placed uniformly, and then it was covered with another layer of coffee grounds. This procedure was repeated until there was an empty space of 3-5 cm at the top (Figure 1). The samples were incubated at air temperature of 20^{o}C until analyses (for 45 days).

Figure 1. Morphology of *Pleurotus ostreatus* mushrooms cultivated in coffee grounds

2.2 Biological efficiency

The biological efficiency (BE) was calculated according to Wang et al. [14].

The mycelium was inoculated into 900g fresh coffee grounds, distributed in casseroles and plastic bags. After 45 days have weighed developed mushrooms and dry coffee grounds. The mushrooms were harvested with intact hyphae.

2.3 Preparation of mushrooms samples

Samples from freshly harvested mushrooms and from mushrooms subjected to thermal treatment (blanching, boiling and freezing) were prepared.

2.3 Determination of protein content

The Kjeldahl method was used for total nitrogen analysis in three stages: digestion, distillation and titration (A.O.A.C., 1997) [15].

In order to determine the protein content, it had to be mineralized by heating with sulphuric acid in the presence of catalysts. After mineralization is distilled and the nitrogen content is analysed. The distillation takes 20-25 minutes. After that, the

acid excess from the glass collector is titrated with sodium hydroxide solution 0.1 N, until the colour turns into yellow. Considering that 100 g of protein contain an average of 16% nitrogen, the following formula was used to convert nitrogen into protein: Protein % = N% × 4.38 for mushrooms.

2.4 The content of total polyphenol compounds in dried mushroom methanol extracts diluted 1/10 was determined by Folin-Ciocalteu method. For the preparation of the calibration curve 0.5 mL aliquot of 0.2, 0.3, 0.4, 0.8 and 1.2 μM/mL aqueous gallic acid solution were mixed with 10.0 mL Folin-Ciocalteu reagent (diluted ten-fold) and 1.0 mL sodium carbonate (20.0%) and the volume made up to 10.0 mL with H_2O. The absorbance was read at a wave length of 760 nm, after two hours of extract incubation at 25°C. All determinations were performed in triplicate. Total phenols were determined as gallic acid equivalents on a dry weight (mg GAE g^{-1} D.W.).

Calculation:

gallic acid = read value × 25/m × gallic acid weight, (mg GAE/g)

2.5 Determination of vitamin C content

In order to determine the vitamin C content, 5 g of sample were ground in a 50 mL glass. There was added 1 mL phosphoric acid, 85% and 0.5 mL perchloric acid. Distilled water was added until the sign and was stirred for 20 minutes and then was filtered with filter paper. From each filtered sample was taken 1 ml and it was analysed with the HPLC device.

2.6 Determination of polyphenol oxidase is performed in order to establish the degree of browning in all four caloric states of the cap and of the stem. In order to determine it, was weighted 1 g of sample and was ground with 5 ml of distilled water. The sample was placed in a 20 mL cylinder and water was added (up to 20 ml). 10 ml were poured in a clean glass, 0.1 ml solution of pyrocathecol was added, then it was stirred well and left for 30 minutes at room temperature. The absorbance of the coloured solution was determined absorbance with the spectrophotometer UV-VIS at the wavelength of 470 nm.

Calculation:

$E = \frac{A}{G} \times d$,(mg/100g)

where E is the enzyme quantity; A is the absorbance of the sample read at the spectrophotometer UV-VIS; G is the quantity of product and d is the dilution.

2.7 Statistic data analysis

The data was interpreted statistically using the statistic program XLSTAT version 2016. The analysis of main components and Mandel's test were used to express the numeric values.

3. Results and discussion

3.1 The total output was estimated based on 900 g of fresh substrate by weighing the fresh mushrooms and later the dried substrate In table 1 is expressed the biological efficiency of the mushrooms cultivated in polyethylene bags and plastic casseroles.

Table 1. Biological efficiency in relation to mushroom and substrate weight

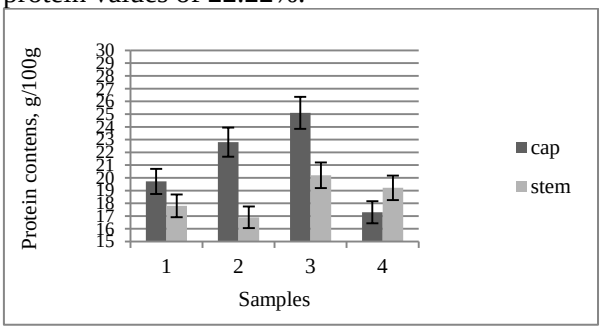
Sample	Mushroom (cap, stem and hypae) weight at harvest, g	Substrate weight after drying, g	Biological efficiency, %
Sample 1- polyethylene bags	180	430	41.86
Sample 2- plastic casseroles	162	558	29.29

The obtained results demonstrated that the growing space influences the mushroom production through the micro climate conditions that assure the length of the production cycle.

The formula of biological efficiency is used to express the mushroom productivity that results especially by combining a mushroom strain with a specific growing technique. In case of using coffee grounds as substrate, the biological efficiency was 41.86%. A better development of the species *Pleurotus* was observed by Leif et al. (2006) which obtained 96.5% biological efficiency the using polyethylene bags [16].

The biological efficiency of *Pleurotus* varies between 75 and 200%, depending on the strain type, substrate.

3.2 Protein content in mushrooms cultivated in coffee grounds, 16.96-25.16g/100g fruit bodies, product is in the interval reported by Chang and Miles that presents protein variations from 10.5 to 30.4% [17]. Similar results were obtained by Sturion when they cultivated many species of *Pleurotus* in various coffee residues (peelings and leaves); the variations were between 17.38 and 25% [18]. The results correspond to the data obtained by Ranzani and Sturion [19], who analyzed different varieties of *Pleurotus* cultivated on banana leaves (17.4-24.1% protein), as well as to the results obtained by Furlani (2004), that have protein values of 22.22%.

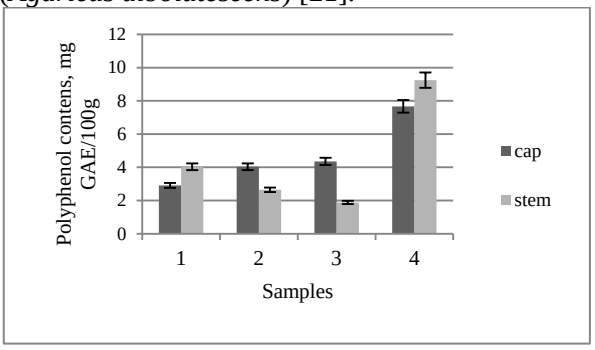


1-raw; 2-blanched; 3-boiling; 4-frozen
Figure 2. Protein content in *Pleurotus* samples

Pleurotus was tested by Reddy et. al. in banana leaves, corn cobs, cotton residues and rice straw [20]. Protein content in the cultivated mushrooms on the mentioned dried residues was 16.8, 15.4, 15.1 and 13%, respectively. Protein content in mushrooms cultivated on banana leaves is similar to the result presented for *P. ostreatus* cultivated on coffee grounds (16.12%).

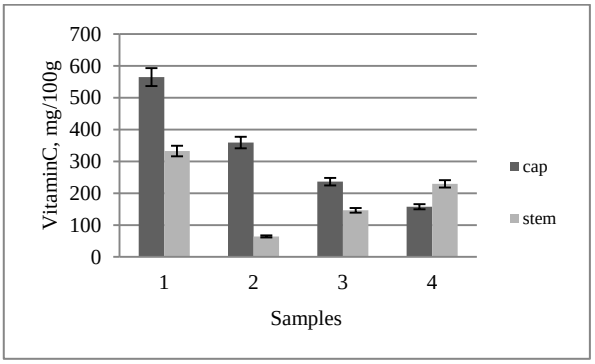
3.3 Total content of polyphenol in the *Pleurotus* samples varies between 1.887 – 7.667mg GAE/100 g, being close to the values previously reported in specialty literature. The frozen sample had the highest polyphenol content of all. The obtained result for the frozen sample (7.667mg GAE / 100 g) was two times higher than the values reported for fresh sample (2.9104mg GAE / 100 g) [6, 8, 10]. Jeena, G.S. et al. determined total polyphenol content of three species of *Pleurotus: P. sajor caju*, *P. ostreatus* and *P. sapidus*) and this study revealed that *P. sajor caju* 1.53mg/g >*P. ostreatus* 1.32 mg/g > *P. sapidus* 1.10 mg/g [7].

Leahu, A. et colab., determined the polyphenol content for the species *Agaricus albolutescens*, *Armillaria mellea*, *Russula virescens*, *Cantharellus cibarius* and *Boletus edulis* and obtained results that fit in the interval 18.38 mg GAE/g (*Boletus edulis*) and 11.34 mg GAE/g (*Agaricus albolutescens*) [21].



1-raw; 2-blanched; 3-boiling; 4-frozen
Figure 3. Polyphenol variation in *Pleurotus* samples

3.4 Vitamin C content varies between 564.95-64.32 mg/ 100g. The highest vitamin C value (569.95 mg/100g) is that of the raw cap, and the lowest value (64.32 mg/100g) is that of the blanched mushroom stem. In Figure 4 one can notice a progressive decrease concerning the determination of the values obtained for mushroom caps, where the raw one has the highest value, whereas the frozen one has the lowest value.

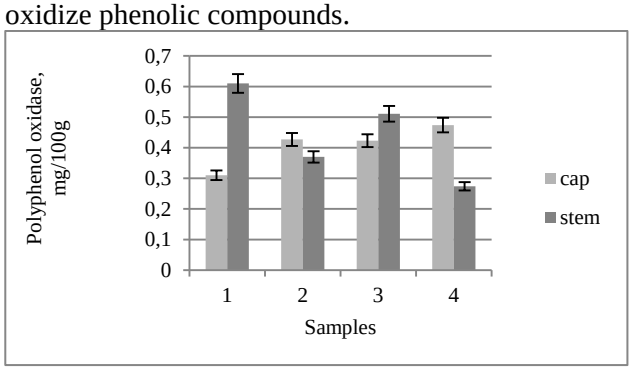


1-raw; 2-blanched; 3-boiling; 4-frozen
Figure 4. Vitamin C in *Pleurotus* samples

In previous studies it is appreciated that vitamin C fits within the limits 27.7mg/100g and 654 mg/100g [8, 20-23].

3.5 After determining the **polyphenol oxidase content** in the analyzed *Pleurotus* samples, there were calculated values that vary between 0.61-0.274 mg/100 g. The highest amount of polyphenol oxidase was found in the raw mushroom stem and the lowest amount in the frozen mushroom stem. Among the caps, the highest amount of polyphenol oxidase was found in the frozen mushroom cap, and the lowest amount in the raw mushroom cap.

Polyphenol oxidase (PPOs) is considered an essential enzyme in food technology and was intensely studied in many plants. The action mechanism of PPOs is based on its capacity to oxidize phenolic compounds.



1-raw; 2-blanched; 3-boiling; 4-frozen

Figure 5. The polyphenol oxidase content in *Pleurotus* samples

Phenolic compounds are natural substances that contribute to the sensorial properties (colour, taste, and aroma) associated with mushroom quality. Raw mushrooms, especially the stem, are rich in this enzyme responsible of browning. The thermal processing is an alternative used in enzyme inactivation [24-27].

3.6 Statistic interpretation of the data

The graphic representation of the results obtained from the practical determinations for the raw mushroom samples, subjected to thermal treatments (blanching, boiling and freezing), highlights the grouping manner of the obtained values. The axes F1 and F2 divide the results according to the ways of thermal processing and to the determined chemical compounds. The results for the raw mushrooms have grouped in the first dial. The results of the determinations for the boiled and blanched mushrooms have similar values and therefore they have grouped in the same dial.

In one dial there is the enzyme responsible for browning, polyphenol oxidase. Vitamin C has similar values for thermally processed mushrooms (through blanching) and frozen samples. Vitamin C in the boiled samples fits between the values expressed for raw samples and frozen, respectively blanched samples.

The average of the polyphenols content determined by a series of preservation methods is expressed through strong significant correlation. Whatever the thermally processing method, polyphenols are found in cap and in stem in equal quantities

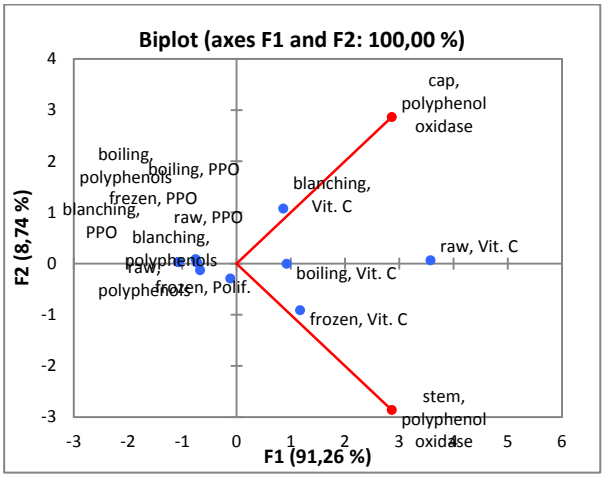
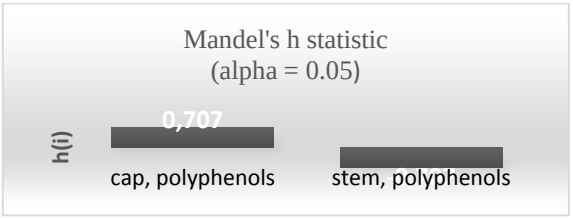


Figure 6. Analysis of main components in *Pleurotus* samples



Group	*Mean*	*h(i)*
Cap, polyphenols	44.8635	0.707
Stem, polyphenols	44.5485	-0.71

Figure 7. Mandel's test for expressing the average values of polyphenols in *Pleurotus* samples

4. Conclusions

The study was undertaken in order to evaluate the possibility of using coffee residues, coffee grounds as substrate in the fermentation in order to crop edible *Pleurotus* mushrooms.

The *Pleurotus* mushrooms have a high content of polyphenols proved by the practical determination on the mushroom. The antioxidant components are estimated in the following order: phenols, flavonoids, ascorbic acid, β-carotene and lycopene.

The mushrooms cultivated on coffee waste used well the substrate, fact confirmed by the biological efficiency of 41.86%. From a nutritional point of view, the *Pleurotus* mushrooms cultivated on

coffee grounds can be used as an important source of proteins.

References

1. S. T. Chang, O. W. Lau, K. Y. Cho,. The cultivation and nutritional value *of Pleurotus sajor-caju*, European Journal of Applied Microbiology and Biotechnology, 1981,Volume 12, Issue 1 , pp 58-62
2. Sarangi, I., Ghosh, D., Bhutia, S. K., Mallick, S. K., Maiti, T. K. Anti-tumor immunomodulating effects of *Pleurotus ostreatus* mycelia-derived proteoglycans. International Immunopharmacology, 2006, 6, 1287–1297
3. Bhattacharjya, D. K., Ratan K. P., Nuruddin M., Kamal U. A. Comparative Study on Nutritional Composition of Oyster Mushroom (*Pleurotus ostreatus* Fr.) Cultivated on Different Sawdust Substrates, Bioresearch Communication, 2015, Vol. 1, Issue 2, 93-98
4. Rapeanu, G. Loey, A.V.; Smout, C.; Hendrickx, M. 2005. Effect of pH on thermal and/or pressure inactivation of Victoria grape (Vitis vinifera sativa) polyphenol oxidase: A kinetic study. J. Food Sci. 70 (5), E301–E307
5. Ranzani, M. R. T.; Sturion, G. L. Avaliação da composição emaminoácidos de *Pleurotus spp*. cultivados em folha de bananeira. Archivos Latino americanos de Nutricion, v. 48, n. 4, p. 339-348, 1998.
6. Reddy, G. V., Babu, P. R., Komaraiah, P., Roy, K. R. R. M., & Kothari, I. L. Utilization of banana waste for the production of lignolytic and cellulolytic enzymes by solid substrate fermentation using two *Pleurotus* species (*P. ostreatus* and *P. sajor-caju*). Process Biochemistry, 2003, 38, 1457–1462
7. Jeena, G. S., Punetha, H., Prakash, O., Chandra, M., & Kushwaha, K. P. S., Study on in vitro antioxidant potential of some cultivated *Pleurotus* species (Oyster mushroom). Indian Journal of Natural Products and resources, 2014, 5(1), 56-61
8. Shi, Y.; Chen, Q.-X.; Wang, Q.; Song, K.-K.; Qiu, L. Inhibitory effects of cinnamic acid and itsderivates on the diphenolase activity of mushroom (*Agaricus bisporus*) tyrosinase. Food Chem. 92, 2005, 707–712
9. Fasidi, I.O. and Kadiri, M. Use of agricultural wastes for the cultivation of lentinus subnudus in Nigeria. Revista de Biologia Tropical, 1993, 41, 411- 415
10. Smith, J. E.; Rowan, N. J.; Sullivan, R. Medicinal mushrooms: a rapidly developing area of biotechnology for cancer therapy andother bioactivities (Review). Biotechnology Letters, 2002,v. 24, n. 22,p. 1839-1845. http://dx.doi.org/10.1023/A:1020994628109
11. Silva, S. O., Costa, S. M. G.; Clemente, E. Chemical composition of *Pleurotus pulmonarius* (Fr.) Quél., substrates and residue after cultivation. Brazilian Archives of Biology and Technology, 2002, v. 45, n. 4, p. 531-535
12. Sarangi, I., Ghosh, D., Bhutia, S. K., Mallick, S. K., 9& Maiti, T. K. Anti-tumor immunomodulating effects of *Pleurotus ostreatus* mycelia-derived proteoglycans. International Immunopharmacology, 2006, 6, 1287–1297
13. Sarker, N.C., Hossain, M.M., Sultana, N., Mian, H., Karim, A.J.M.S. and Amin, S.M.R.. Performance of different substrates on the growth and yield of *Pleurotus ostreatus* (Jacquin ex Fr.) kummer. Bangladesh J. Mushroom 2007, 1 (2), 9-20
14. Wang, D., Sakoda, A., & Suzuki, M. Biological efficiency and nutritional value of *Pleurotus ostreatus* cultivated on spent beer grain. Bioresource Technology, 2001, 78, 293–300
15. Association of Official Analytical Chemists - AOAC. Official Methods of Analysis of A.O.A.C. 16th ed. Versão 3. Gaithersburg: AOAC International, 1997
16. Leifa Fan, Andréa Thomaz Soccol, Ashok Pandey, Luciana Porto de Souza Vandenberghe,;Carlos Ricardo Soccol. Effect of caffeine and tannins on cultivation and fructification of *Pleurotus* on coffee husks, 2006, Brazilian Journal of Microbiology 37:420-424
17. Chang, S. T.; Miles, P. G. 1989. Edible mushrooms and their cultivation. Boca Raton: CRC Press Inc.
18. Sturion, G. L. Utilização da folha da Bananeira como substrato para o cultivo cogumelos comestíveis (*Pleurotus spp*). 1994. 147 f. Dissertação (Mestrado em Ciências)-Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, 1994
19. Sturion, G. L., & Ranzani, M. R. T. C. Composição em minerais de cogumelos comestíveis cultivados no Brasil – *Pleurotus spp*. e outras espécies desidratadas. Archivos Latino Americanos de Nutricion, 2000, 50(1), 102–103
20. Reddy, G. V., Babu, P. R., Komaraiah, P., Roy, K. R. R. M., & Kothari, I. L. Utilization of banana waste for the production of lignolytic and cellulolytic enzymes by solid substrate fermentation using two *Pleurotus* species (*P. ostreatus* and *P. sajor-caju*). Process Biochemistry, 2003, 38, 1457–1462
21. Leahu, A., Damian, C., Oroian, M., Ropciuc, S., Hreţcanu, C.E. Establishing the antioxidant activity based on chemical composition of wild edible mushrooms, Journal of Faculty of Food Engineering, 2015, Vol: 14 Issue: 4 Pages: 398 – 406
22. Silva, S. O., Costa, S. M. G.; Clemente, E. Chemical composition of *Pleurotus pulmonarius* (Fr.) Quél., substrates and residue after cultivation. Brazilian Archives of Biology and Technology, 2002, v. 45, n. 4, p. 531-535
23. Sturion, G. L., & Ranzani, M. R. T. C. Composição em minerais de cogumelos comestíveis cultivados no Brasil – *Pleurotus spp*. e outras espécies desidratadas. Archivos Latino Americanos de Nutricion, 2000, 50(1), 102–103

24. Syed, A. A., Kadam, J. A., Mane, V. P., Patil, S. S., & Baig, M. M. V. Biological efficiency and nutritional contents Tuzen, M., Sesli, E., & Soylak, M., 2007. Trace element levels of mushroom species from East Black Sea region of Turkey. Food Control, 2009. 18, 806–810

25. Toro, G. V. et al. Biological quality of proteins from three strains of *Pleurotus spp*. Food Chemistry, v. 94, n.4, p. 494-497, 2006

26. Wang, D., Sakoda, A., & Suzuki, M. Biological efficiency and nutritional value of *Pleurotus ostreatus* cultivated on spent beer grain. Bioresource Technology, 2001, 78, 293–300

27. Alam, N., Hossain, M.S., Khair, A., Amin, S.M.R. and Khan, A. Comparative study of oyster mushrooms on plasma lipid profile of hypercholesterolemic rats. Bangladesh J Mushroom. 2007. 1 (1),15-22.