



ESTABLISHING THE ANTIOXIDANT ACTIVITY BASED ON CHEMICAL COMPOSITION OF WILD EDIBLE MUSHROOMS

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Abstract: The aim of this study is to determine the chemical compositions of fruiting bodies of widely used wild edible mushroom species (*Agaricus albolutescens*, *Armillaria mellea*, *Russula virescens*, *Cantharellus cibarius* and *Boletus edulis*), commonly consumed in the Bukovina region, from the Northeast of Romania. The content in water, crude protein, lipids, carbohydrates and ash, in fruiting bodies of mushroom samples was assessed. The antioxidant activities were performed on the 1,1'-diphenyl-2-picrylhydrazyl (DPPH) test. In addition to this, phenolic compounds were also analyzed. The carbohydrate contents were in the following order: *Agaricus albolutescens* > *Boletus edulis* > *Armillaria mellea* > *Russula virescens* and *Cantharellus cibarius*. The protein also varied and ranged from 10.12 to 15.15 % dry weight. The polyphenol content was the same in the two mushrooms, whereas the antiradical activity was higher in *Russula virescens*. The results suggested that consumption of *Agaricus albolutescens*, *Armillaria mellea*, *Russula virescens*, *Cantharellus cibarius* and *Boletus edulis* might be beneficial to the antioxidant protection system of the human body against oxidative damage and others complications.

Keywords: mushrooms, carbohydrate, antioxidant, total phenolic content.

1. Introduction

Mushrooms are an important dietary component for the modern consumer across the world, used for nutritional and medicinal purposes, due to the presence of metabolites with pharmacological potential. Wild mushrooms are becoming more and more important in our diet due to their nutritional characteristics [1-3].

Vegetables and mushrooms occupy an important position among functional food products due to their contents of many bioactive components that have a beneficial effect on human health and sense of wellbeing [4].

Dietary fibers are the compounds resistant to hydrolysis, which the human body cannot use, but which play a considerable

role in the prevention of diseases. They are: cellulose, chitin, and glucans. The impact of fiber consumption is positive because it can influence the digestion rate and it was also associated with a reduced risk of type 2 diabetes. Recent clinical studies recommended mushrooms for inclusion in "functional foods" [5]. Functional foods are those foods enriched/modified and consumed as normal diet to provide health benefits [6]. Manzi et al., [7] evaluated the chemical composition of mushrooms, commercial samples, widely consumed in Italy (*Boletus* group, *Agrocybe aegerita* and *Pleurotus eryngii*). The results obtained in this study consider that the dried and re-hydrated *Boletus* samples showed higher levels of soluble and insoluble dietary fiber

than the other samples (*Agrocybe aegerita* and *Pleurotus eryngii*) [7].

Mushrooms have low energy content but remarkable protein content, from this point of view their consumption being advantageous, as compared to other vegetable products. Moreover, as compared to meat products or milk, fat content is negligible. These considerations justify the recommendation and preference to include mushrooms in a consumer's usual diet. Wild mushrooms are rich sources of proteins and have low amounts of fat, making it an ideal snack material [1].

Dried *Pleurotus ostreatus* and *Lentinula edodes* mushrooms exhibited high contents of protein, total dietary fiber, K, Mg, Zn, Fe, Cu and low contents of fat, Na, Ca [8]. Carbohydrates constitute the most important component in terms of weight, the dry matter of mushrooms being the largest source of energy. Di Anibal *et al.* [9] tested the chemical composition and nutritional value of four edible mushroom varieties (*Champignons*, *Portobellos*, *Girgolas* and *Shiitakes*) commonly cultivated and consumed in Argentina. The study reveals that edible mushrooms are rich sources of carbohydrates and have low amounts of fats; also they are rich in potassium and phosphorus whereas they have a low content of sodium [9]. Mushrooms contain water-soluble compounds including reducing sugars and soluble polysaccharides. The dominant compound is mannitol, which in *A. bisporus* has the concentration of 3-30g/100g dry matter [10]. Other simple sugars, such as: glucose, galactose, mannose and fructose have been also identified.

Edible mushrooms have a discreet aroma, but individualized depending on the species, which contributes to their attractiveness to the consumer. Previous scientific reports have demonstrated the

contribution of about 150 volatile compounds that outlines their aromatic profile [10]. According to Ashmore L. *et al.* [11], the characteristic volatile compounds found in fresh "raw" *Agaricus bisporus* mushroom sample are 4-methyl, 3-penten-2-one, 1-octen-3-ol, 3-octanone, benzaldehyde, 3-octanol, and 2-octen-1-ol, and the dried sample had a mild aroma but upon rehydration, a stronger cooked aroma developed [11].

Among the sensory qualities, the most valued by the consumer are the visual ones: color, shape and size. Studies on the components which determine the color were focused on the carotenoids. The analysis of the extracts obtained from the seventeen fresh mushrooms, collected from different places in North-Eastern Portugal, allowed the identification of carotene only in *C. cibarius* [12].

Cultivated mushrooms (*Agaricus bisporus/white*, *Agaricus bisporus/brown*, *Lentinus edodes*, and *Pleurotus ostreatus*) were found to be good sources of vitamin B2, niacin, and folates, with contents varying in the ranges 1.8-5.1, 31-65, and 0.30-0.64 mg/100 g dry weight (dw), respectively [13]. It is considered that mushrooms are the only products that contain whole complex B. Mushrooms contain high levels of pro-vitamin D2 (ergosterol) [14].

Edible mushrooms also provide a nutritionally significant content of minerals, may constitute a good source of iron, zinc and copper. Various studies have been carried out on the minerals composition of the European edible wild mushrooms from several countries like Romania [15-16]; Greece [17]; Turkey [18]; Croatia [2]; Portugal [1]; Finland [13]. Mushrooms are known for their ability to accumulate heavy metals and radioactive elements, due to the texture of very good conducting properties in comparison to plants. That is why

mushrooms could be used as potential bio sorbents for heavy metals from aqueous solutions [19]. Mushrooms are considered bio indicators of environmental pollution.

As mentioned previously, the antioxidant activity of mushrooms is positively correlated firstly to the amount of phenolic compounds, followed by the flavonoids, tocopherols, ascorbic acid and carotenoids [20].

Many studies have confirmed some large parts of traditional knowledge regarding the medicinal effects of mushrooms due to their antifungal, antibacterial, antioxidant and antiviral properties, besides being used as functional foods [21].

The main focus of this work was to determine the nutritional value and chemical composition, including the antioxidant activity in five types of wild mushrooms, *Agaricus albolutescens*, *Armillaria mellea*, *Russula virescens*, *Cantharellus cibarius* and *Boletus edulis*.

2. Materials and methods

2.1. Samples and storage conditions

Mushrooms grow abundantly in the wild during the rainy season in every part of Dragomirna Forrest, Suceava County. The fruits of five mushrooms (*Agaricus albolutescens*, *Armillaria mellea*, *Russula virescens*, *Cantharellus cibarius* and *Boletus edulis*) species were harvested during early mature fruiting stage, in Dragomirna Forrest, Suceava County (Northeast of Romania) in autumn 2014.

Sample preparation: the mushroom sample (600 g each) was cleaned, sliced and dried on paper towel prior to weighing. Mushrooms were air-dried in the oven at 40 °C before making the analysis.

2.2. Chemical analysis

The determination of moisture (according SR. ISO 1026:2008) in mushrooms samples was made by the

drying process in a drying chamber at the temperature of 105 °C, until it reached a constant weight.

Total ash composition was obtained by calcination of 5g of sample at 600 °C for 240 min (according SR ISO 763: 2008).

The protein content (Nx4.38) of the samples was analyzed by the Kjeldahl method.

The oil content was determined in a Soxhlet apparatus, extracting the fats from 10 g of mushrooms sample with petroleum ether, followed by extract evaporation to dryness and gravimetric determination.

The carbohydrate content was estimated by difference of the other components using the following formula [8]:

$$\text{carbohydrate content} = 100\% - (\% \text{moisture} + \% \text{protein} + \% \text{fat} + \% \text{ash}) \quad (1)$$

Energy was expressed as kilocalories/100g, using the factors mentioned in the Romanian Legislation:

$$\text{Energy (kcal)} = 4.1x (\text{g protein} + \text{g carbohydrate}) + 9.3x (\text{g lipid}) \quad (2)$$

The content of total polyphenolic 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 H₂O. The absorbance was read after 2 h at 25°C, at 760 nm. All determinations were performed in triplicate. Total phenols were determined as gallic acid equivalents on a dry weight (mg GAE g⁻¹ D.W.).

2,2-Di (4-tert-octylphenyl)-1- pycrrilhydrazyl (DPPH) scavenging capacity assay

The method used to determine the antioxidant activity of mushrooms is based

on scavenging 2,2-Di (4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) radicals. The mushroom samples aliquot (0.5 mL) was added to freshly prepared DPPH reagent. After incubating for 5 min, the absorbance of the resulting solutions was measured at 517 nm using a spectrophotometer T70 UV-VIS PG Instruments Ltd. The control was conducted in the same manner, except that the distilled water was used instead of sample.

Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$IC_{50} (\%) = [(A_0 - A_1 / A_0) \times 100] \quad (3)$$

where A_0 was the absorbance of the control reaction and A_1 the absorbance in the presence of the sample of mushroom species extract [22].

2.3. Statistical analysis

Three replications were used to obtain average values and standard deviations for proximate biochemical properties.

Multiple linear regression (PLS), a method based on the criterion of covariance has been used, to analyze the relationship between the explanatory variables (chemical characteristics of mushrooms) or independent variables (different species of mushrooms) and between explanatory variables and independent.

3. Results and discussion

Figure 1 presents the contents of macro components in five types of wild edible mushrooms: *Agaricus alboluteus*, *Armillaria mellea*, *Russula virescens*, *Cantharellus cibarius* and *Boletus edulis*.

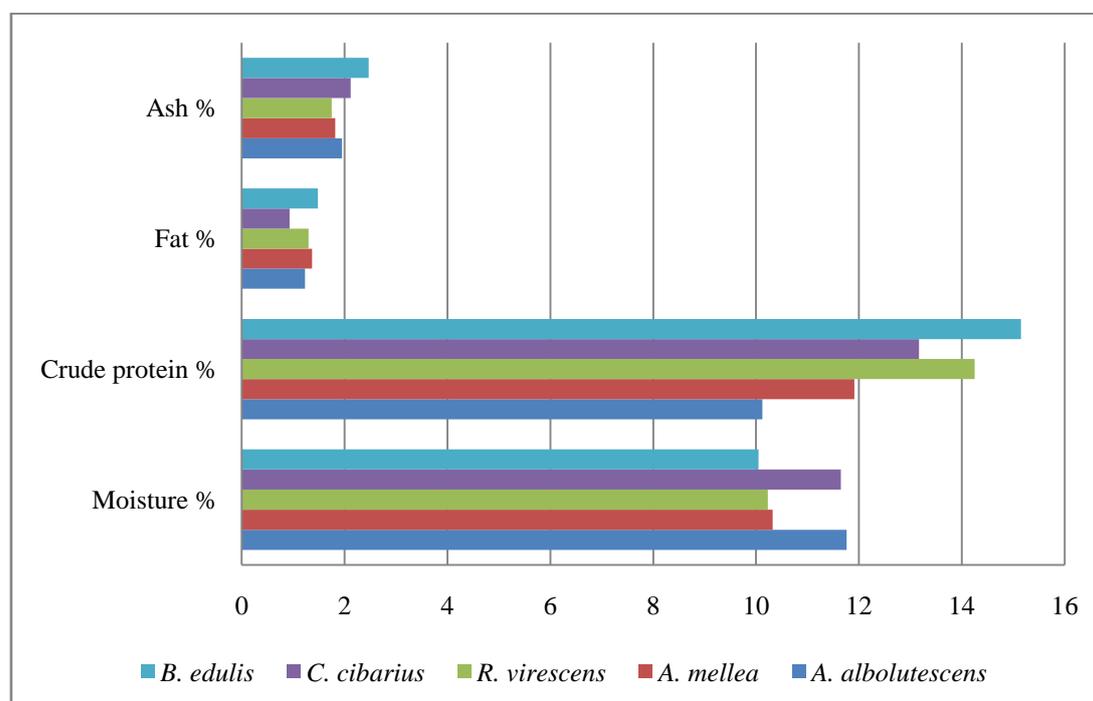


Fig. 1. The contents of macrocomponents, g/100 g dry matter of dried *Agaricus alboluteus*, *Armillaria mellea*, *Russula virescens*, *Cantharellus cibarius* and *Boletus edulis*

Mushrooms varied in their content of raw protein more than the other macroelements

determined. All dried mushrooms were found to be good sources of proteins, with

contents varying in the ranges of 10.12–15.15 g/100 g dry weight (dw), while the fat content was very low (0.93 –1.48 g/100 g dw).

Minimum and maximum ash levels in the present study were 1.75 mg/100 g d.w. and 2.47 mg/100 g d.w. for *Russula virescens* and *Boletus edulis*, respectively. The distribution of minerals in the fruiting bodies of the mushrooms is not uniform, caps concentrating, generally, the largest amount.

Barros *et al.* [1] reported similar ash content (0.81 ± 0.03 g/100 g), but higher moisture (93.05 ± 0.51 g) and protein (2.12 ± 0.08 g/100 g of weight matter) levels in samples from Northeast of Portugal, while Ouzouni *et al.* [17] described similar moisture (87.6 g/100 g) and ash ($6.25 \pm$

0.02 g/100 g) contents, and higher protein levels (27.17 ± 0.15 g/100 g weight matter) in *Boletus edulis* samples from forests in the West Macedonia and Epirus.

Carbohydrates, calculated by difference, were also an abundant macronutrient and ranged from 70.85 g/100 g in *Boletus edulis* and 74.94 g/100 g in *Agaricus alboluteuscens*.

The relative homogeneity in wild mushrooms suggests a uniform composition of the soil, which is the main reservoir of nutrients.

The energy value differed significantly ($p < 0.001$) in samples tested dried products, while the higher energy value was found 367.64 Kcal/100g for dried *Russula virescens* (Figure 2).

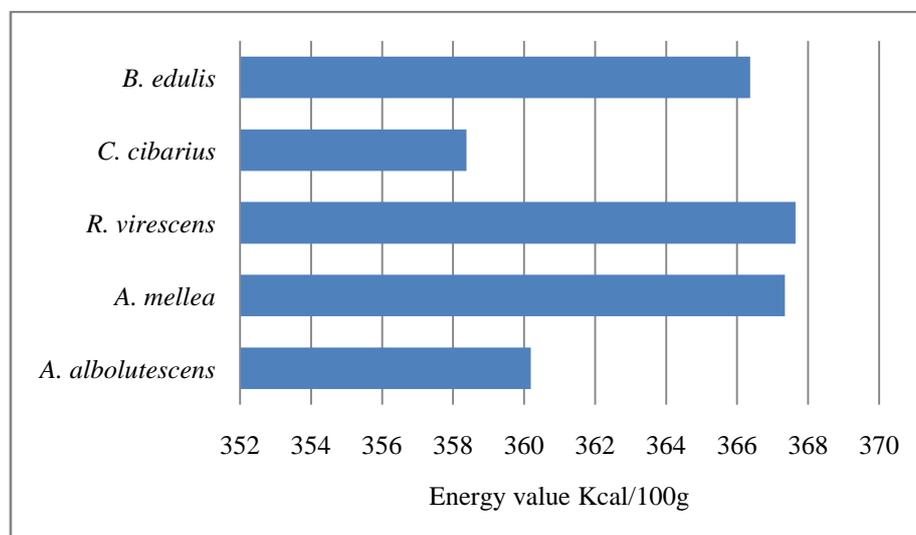


Fig. 2. The contents of energy value, Kcal/100 g dry matter, of dried *Agaricus alboluteuscens*, *Armillaria mellea*, *Russula virescens*, *Cantharellus cibarius* and *Boletus edulis*

Generally talking, mushrooms are characterized by low energy value.

The content of total phenolic compounds was determined in methanol extract of mushrooms samples and characterized using gallic acid equivalents (GAE) per 1 g of mushroom dry matter. Total polyphenol content reveals a significant difference

between species, *Boletus edulis* proving to be the richest in such compounds and was found to be 18.38 mg GAE/g weight matter (figure 3).

Jeena *et al.* [23] determined total polyphenol content of three mushrooms types *Pleurotus* (*P. sajorcaju*, *P. ostreatus* et *P.sapidus*) and the study revealed that *P.*

sajorcaju 1.53 mg/g>*P. ostreatus* 1.32 mg/g> *P. sapidus* 1.10 mg/g [23].

The results obtained by Genççelep and Hüseyin [24] indicated that total phenolics in the methanolic extracts were the highest in *Boletus edulis*. Reis *et al.* [25] analyzed

the total polyphenol content of different species of mushrooms, thus, in *Agaricus bisporus* (brown) was 37.33 mg GAE/g extract, while in *Pleurotus eryngii* it has a lower value: 7.14 mg GAE/g extract.

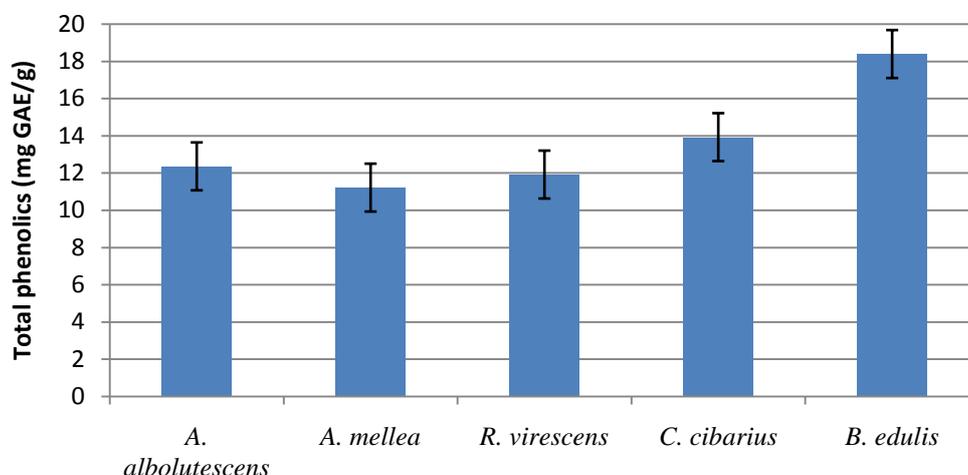


Fig. 3. Total phenolics mg GAE/ g of analyzed samples

Concerning mushroom sample extracts, the *Russula virescens* extract was the one that had the strongest antiradical activity (IC_{50} of 15.154), followed by *Armillaria mellea* and *Cantharellus cibarius* extracts, with very similar activities (IC_{50} of 20.12 and 21.55, respectively). Alam N. *et al.* [26] reported that the antioxidant activities of the fruiting bodies of *Pleurotus ferulae*

was lower than the synthetic antioxidant, BHT and TOC, respectively at 0.5 mg/ml and at 0.5–20.0 mg/ml concentration. Their antioxidant activities of the acetonetic, methanolic, and hot water extracts of *P. ferulae* ranged from 52.71% to 95.63%, 69.51% to 96.52%, and 34.88% to 93.22%, respectively [26].

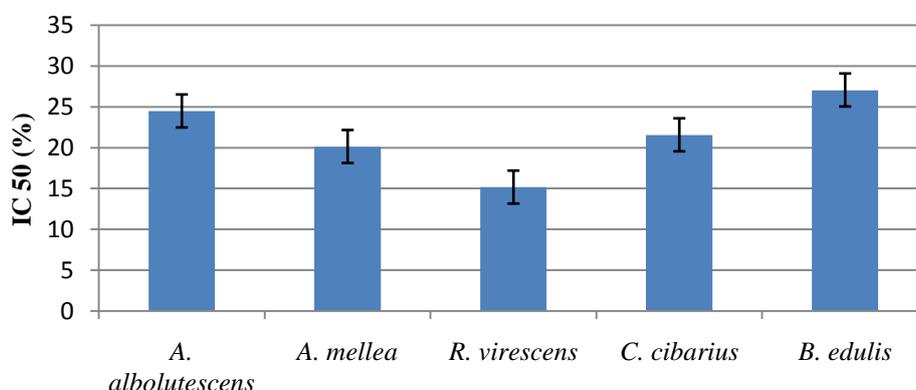


Fig. 4. Free Radical Scavenging Activities % of analyzed samples

In a research conducted by Ferreira, I. C., *et al.*[27] it was reported that the extracts obtained from the mushroom cap proved to be a better source of antioxidants than extracts from mushroom stem [27].

Similar studies reported that the antioxidant activity of ethanolic extracts from mushrooms is due to phenolic compounds content [28].

Statistical analysis using the PLS (Partial Least Squares Regression) application dependent variables (content in carbohydrate, fat content and the quantity

of minerals-ash %) for mushroom samples analyzed indicates that the energy is determined by lipid content (Fig. 5). In *B. edulis* samples it can be observed the high amount of protein and minerals content. The analyses conducted on *Armillaria mellea* and *Russula virescens* revealed a high energetic value and fats concentration, respectively. Samples with high humidity belong to the species of fungus *Cantharellus cibarius*. The high carbohydrate content occurs in *Agaricus albolutescens* mushrooms.

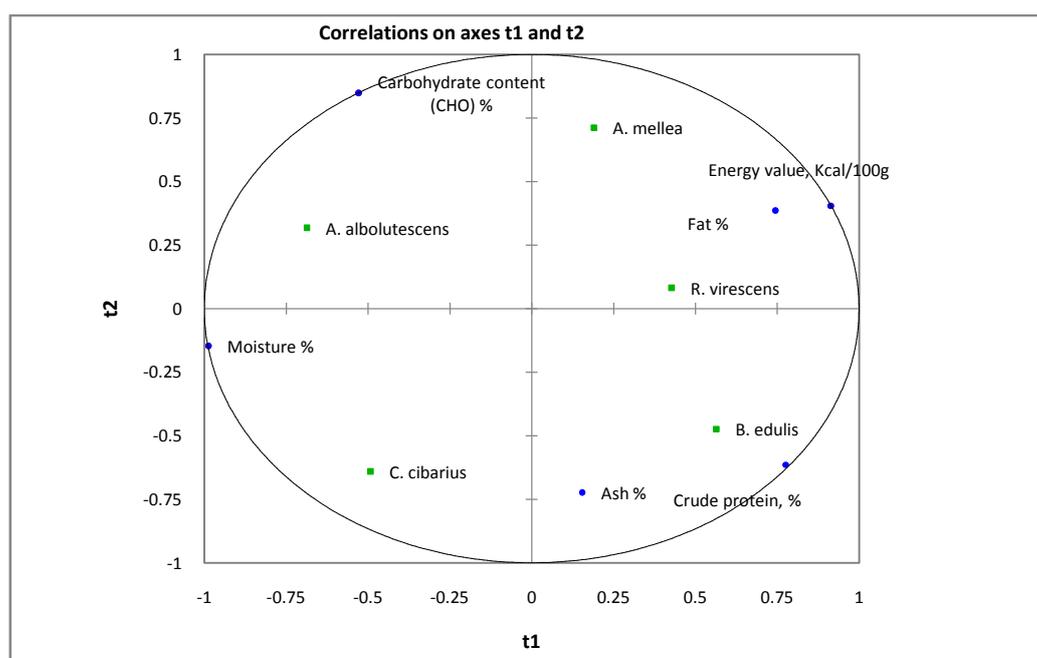


Fig. 5. Correlation between carbohydrates % and the energy value Kcal/100g for analyzed samples

Pearson correlative bond of chemicals characteristics of mushroom samples

Table 1.

Variables	Energy, Kcal/100g	Carbohydrate, %	Moisture %	Protein, %	Fat %	Ash %
Energy value, Kcal/100g	1.000	-0.140	-0.958	0.663	0.830	-0.176
Carb. content (CHO) %	-0.140	1.000	0.397	-0.933	-0.064	-0.687
Moisture %	-0.958	0.397	1.000	0.472	-0.798	-0.073
Protein, %	0.663	-0.933	0.472	1.000	0.265	0.469
Fat %	0.830	-0.064	-0.798	0.265	1.000	0.148
Ash %	-0.176	-0.687	-0.073	0.469	0.148	1.000

The correlation coefficients (Table 1) between chemicals' characteristics analysis reveal that the protein gives to fungus a medium energy values ($r=0.663$) and once the humidity increases, the energy values decrease significantly. The protein content represents the only source of minerals in mushrooms. Pearson coefficient shows a significant correlation between the percentage of ash and protein content.

4. Conclusion

Nutritional content represents the rational motivation for mushroom consumption. Due to their low fat content, the risk of cholesterol and triglyceride accumulation is eliminated.

The main nutritional characteristics are presented in the current study (high water and protein content, but low fat content) along with the content of macro- and micro-nutrients. The fruiting bodies of widely used wild edible mushroom have all attracted considerable attention as sources of phenolic compounds. All of these recommend them as food with health benefits.

The results of the research suggest that dried wild mushrooms can be used as additives in food products. It was also noted a high degree of biodiversity resulting from the specificities of the species, substrate composition, and harvesting conditions.

5. References

- [1]. BARROS, L., BAPTISTA, P., CORREIA, D. M., CASAL, S., OLIVEIRA, B., & FERREIRA, I. C., Fatty acid and sugar compositions, and nutritional value of five wild edible mushrooms from Northeast Portugal. *Food Chemistry*, 105(1), 140-145, (2007).
- [2]. BELUHAN, S., & RANOGAJEC, A., Chemical composition and non-volatile components of Croatian wild edible mushrooms. *Food chemistry*, 124(3), 1076-1082, (2011).
- [3]. MUSZYŃSKA, B., & SUŁKOWSKA-ZIAJA, K., Analysis of indole compounds in edible Basidiomycota species after thermal processing. *Food Chemistry*, 132(1), 455-459, (2012).
- [4]. SOBIERALSKI, KRZYSZTOF, MAREK SIWULSKI, AND IWONA SAS-GOLAK., Potential utilisation of vegetables and mushrooms as components of functional food. *Folia Horticulturae* 25.1: 79-88, (2013).
- [5]. ELEFTERIOS, E., VASSILIS, M. G. & CLEANTHES I., The potential use of mushrooms β -glucans in the food industries, *International Journal of Biotechnology for Wellness Industries*, vol 3, 15-18, (2014),
- [6]. PATEL, Y., NARAIAAN, R., & SINGH, V. K. Medicinal properties of Pleurotus species (Oyster mushroom): A review. *World Journal of Fungal and Plant Biology*, 3(1), 1-12, (2012).
- [7]. MANZI, P., MARCONI, S., AGUZZI, A., & PIZZOFERRATO, L., Commercial mushrooms: nutritional quality and effect of cooking. *Food chemistry*, 84(2), 201-206, (2004).
- [8]. R., JULITA, AND M. SIWULSKI., Dried shiitake (*Lentinula edodes*) and oyster (*Pleurotus ostreatus*) mushrooms as a good source of nutrient." *Acta Sci. Pol., Technol. Aliment* 6.4 135-142, (2007).
- [9]. DI ANIBAL, C., FARENZENA, S., RODRÍGUEZ, M. S., & ALBERTENGO, L., Chemical composition and nutritional value of Argentine commercial edible mushrooms. *Journal für Verbraucherschutz und Lebensmittelsicherheit*, 1-10:155-164, (2015).
- [10]. BERNAŚ, E., JAWORSKA, G., & KMIĘCIK, W., Storage and processing of edible mushrooms. *Acta Scientiarum Polonorum Technologia Alimentaria*, 5, 5-23, (2006).
- [11]. ASHMORE, L., J. D. CRASKE, AND G. SRZEDNICKI., Volatile compounds in fresh, cooked fresh, dried and cooked dried *Agaricus bisporus* using ambient temperature vacuum distillation." *International Food Research Journal* 21(1): 263-268, (2014).
- [12]. RIBEIRO, B. ET AL., Do Bioactive Carotenoids Contribute to the Colour of Edible Mushrooms, *The Open Chemical and Biomedical Methods Journal*, vol 4, pp 14-18, (2011).
- [13]. MATTILA, P., KÖNKÖ, K., EUROLA, M., PIHLAVA, J. M., ASTOLA, J., VAHTERISTO, L., & PIIRONEN, V., Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms. *Journal of Agricultural and Food Chemistry*, 49(5), 2343-2348, (2001).
- [14]. Chen, S. Y., YU, H. T., KAO, J. P., YANG, C. C., CHIANG, S. S., MISHCHUK, D. O., & SLUPSKY, C. M. Consumption of vitamin D 2

enhanced mushrooms is associated with improved bone health. *The Journal of nutritional biochemistry*, (2015).

[15]. RADULESCU, C., STIHI, C., POPESCU, I. V., BUSUIOC, G., GHEBOIANU, A. I., CIMPOCA, V. G., & DIACONESCU, M., Determination of heavy metals content in wild mushrooms and soil by EDXRF and FAAS techniques. *Ovidus Uni. Ann. Chem*, 21(1), 9-14, (2010).

[16]. Stih, C., RADULESCU, C., BUSUIOC, G., POPESCU, I. V., GHEBOIANU, A., & ENE, A., Studies on accumulation of heavy metals from substrate to edible wild mushrooms. *Romanian Journal of Physics*, 56(1-2), 257-264, (2011).

[17]. OUZOUNI, P. K., PETRIDIS, D., KOLLER, W. D., & RIGANAKOS, K. A. Nutritional value and metal content of wild edible mushrooms collected from West Macedonia and Epirus, Greece. *Food Chemistry*, 115(4), 1575-1580, (2009).

[18]. SARIKURKCU, C., TEPE, B., SOLAK, M. H., & CETINKAYA, S. Metal concentrations of wild edible mushrooms from Turkey. *Ecology of food and nutrition*, 51(4), 346-363, (2012).

[19]. VIMALA, R., AND NILANJANA DAS., Biosorption of cadmium (II) and lead (II) from aqueous solutions using mushrooms: a comparative study. *Journal of Hazardous Materials* 168.1: 376-382, (2009).

[20]. NAKNAEN, PHISUT, TEERARAT ITTHISOPONKUL, AND PHANTIPHA CHAROENTHAIKIJ. Proximate compositions, nonvolatile taste components and antioxidant capacities of some dried edible mushrooms collected from Thailand. *Journal of Food Measurement and Characterization*: 9: 259-268, (2015).

[21]. TAN, Y. S., BASKARAN, A., NALLATHAMBY, N., CHUA, K. H., KUPPUSAMY, U. R., & SABARATNAM, V., Influence of customized cooking methods on the phenolic contents and antioxidant activities of

selected species of oyster mushrooms (*Pleurotus* spp.). *Journal of food science and technology*, 52(5), 3058-3064, (2015).

[22]. ELMASTAS, M., ISILDAK, O., TURKEKUL, I., & TEMUR, N. Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. *Journal of Food Composition and Analysis*, 20(3), 337-345, (2007).

[23]. 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*, 5(1), 56-61, (2014).

[24]. GENÇCELEP, HÜSEYİN. Antioxidant properties of wild edible mushrooms." *Journal of Food Processing & Technology* (2011).

[25]. REIS, F. S., BARROS, L., MARTINS, A., & FERREIRA, I. C., Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: an inter-species comparative study. *Food and Chemical Toxicology*, 50(2), 191-197, (2012).

[26]. ALAM, N., YOON, K. N., LEE, J. S., CHO, H. J., & LEE, T. S. Consequence of the antioxidant activities and tyrosinase inhibitory effects of various extracts from the fruiting bodies of *Pleurotus ferulae*. *Saudi journal of biological sciences*, 19(1), 111-118, (2012).

[27]. FERREIRA, I. C., BAPTISTA, P., VILAS-BOAS, M., & BARROS, L. Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity. *Food Chemistry*, 100(4), 1511-1516, (2007).

[28]. FIDLER, G., A., BUTU, RODINO, S., M., BUTU, & P., CORNEA. Antioxidant Activity, Bioactive Compounds and Antimicrobial Effect of Mushrooms Extracts. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Animal Science and Biotechnologies*, 72(1), (2015).