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Correlation between Enzymatic and Non-Enzymatic Antioxidants in Several Edible Mushrooms Species

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Abstract

Characterization of several wild growing and cultivated mushrooms from geographical area of Dambovita County, Romania, in terms of enzymatic and non-enzymatic antioxidants, through a chemometrics approach, was the aim of this study. Related to the authors' previous studies, the novelty of this paper consists in deepening research toward the complete characterization of the regional mushroom species through emphasizing their potential as food resources. In the context in which species showed their content in biological active compounds, future practical applications in the area of functional food will be developed by integrating the data concerning their lack of the toxicity and nutritional value too. Lack of data focused on the characterization of mushroom species investigated in the paper supports the significance of this research. The statistical analysis of data highlights the relationship between compounds showing antioxidant activity of autochthonous mushrooms (both cap and stipe).

Keywords: mushrooms, antioxidant activity, polyphenols, flavonoids, enzymes

1. Morphological characteristics and nutritional values of wild and cultivated autochthonous edible mushrooms

The mushrooms contain an extremely wide variety (over 100,000 species spread across all ecosystems) and are traditionally included among the plants. Currently, they are considered as a self-standing group, halfway between the plant world and the animal world. It is true that due to their anatomical and physiological characteristics, they present traits of both worlds (i.e. plant and animal). They are distinguished from chlorophyll plants by the total absence of photosensitizing pigments, hence the inability to produce sugars and starch, starting from the carbonic anhydride present in the atmosphere [1–4]. The mushrooms have their cell walls made from cellulose, but also from chitin, an insect-specific component. So, to survive, mushrooms consume simple substances (such as proteins and sugars) produced by others [2].

It is well known that macromycetes (i.e. superior fungi) represent a heterogeneous group that includes both *Ascomycota* and *Basidiomycota* phyla, with a fruiting body

of at least 4 mm in diameter [2]. In Romania [2–4] are known over 2500 varieties of basidiomycetes, of which more than 500 are edible, being more or less tasty. During their development, superior fungus passes through two successive stages [3, 4]: (a) vegetative, represented by the mycelium from substrate, the body or talus of the fungus; (b) fertile, in which, the fruiting body (i.e. spore producer) is formed. From a morphological point of view, the fruiting body of mushrooms is consisted by pileus (i.e. cap) and stipe (i.e. stalk, stem). Consequently, basidiomycetes have a typical umbrella, with one stipe more or less developed, either cylindrical or thickened or subdued at the base, sometimes flattened or extended in soil with a variable length mycelium. It is important to observe how to insert the stipe into the cap: it can be completely central, more or less eccentric, and sometimes completely lateral [2, 3]. During the fungus growth, the pileus form varies widely: at first it is shaped globes and at complete maturation may be concave or funnel-shaped [2, 3].

The vegetative organ of mushroom consists from a hyphae mycelium that is gathered and placed in a soft fiber texture (real mycelium). The macromycetes mycelium can live outside of the organism on which of it develops (epiphytic) or inside of it (endophytic). Epiphytic fungi sometimes lead a saprophytic life and the endophytes are always parasitic. Nutrition of macromycetes is heterotrophic, the mycelium acting on the nutrient substrate, live or non-living, through the enzymes which it secretes [2, 3].

The compact bodies of mushrooms appear to the substrate surface, either solitary, either in irregularly arranged groups, linearly or in circles, either in the form of bushes [3–5]. Favorable conditions of nutrition, temperature and humidity, are the main factors involved in superior fungi growing [3–9].

The most common basidiomycetes families, which are growing in the Romanian forests, appreciated from nutritional point of view, are: *Lepiotaceae* (e.g. *Macrolepiota mastoidea*, *Macrolepiota rhacodes*), *Tricholomataceae* (e.g. *Tricholoma rutilans*, *Tricholoma columbetta*, *Tricholoma terreum*), *Hygrophoraceae* (e.g. *Hygrophorus marzuolus*, *Hygrophorus eburneus*), *Russulaceae* (e.g. *Russula aeruginea*, *Russula alutacea*, *Russula atropurpurea*, *Russula cyanoxantha*, *Russula delica*, *Russula nigricans*, *Russula vesca*), *Pleurotaceae* (e.g. *Pleurotus cornucopiae*, *Pleurotus ostreatus*), *Agaricaceae* (e.g. *Agaricus augustus*, *Agaricus campestris*, *Agaricus bisporus*, *Agaricus silvaticus*, *Macrolepiota procera*, *Macrolepiota excoriata*), *Boletaceae* (e.g. *Boletus edulis*, *Boletus pinicola*, *Boletus aereus*, *Boletus regius*), *Fistulinaceae* (e.g. *Fistulina hepatica*), *Cortinariaceae* (e.g. *Cortinarius varius*, *Cortinarius caperatus*, *Cortinarius collinitus*), *Amanitaceae* (e.g. *Amanita rubescens*, *Amanita citrina*), *Cantarellaceae* (e.g. *Cantharellus cibarius*, *Cantharellus lutescens*), *Physalacriaceae* (e.g. *Armillaria mellea*) and so on.

Particularly, in the forests of Dambovită County several species such as, *Russula atropurpurea*, *Russula cyanoxantha*, *Russula alutacea*, *Russula nigricans*, *Russula vesca*, *Pleurotus ostreatus*, *Armillaria mellea*, *Cantharellus cibarius*, *Boletus edulis*, *Macrolepiota excoriata*, *Macrolepiota procera*, *Agaricus bisporus*, *Agaricus campestris* are very widespread (**Table 1**). From this reason, these species were characterized from morphological and nutritional point of view (**Table 2**).

Mushrooms provide several important nutrients (**Table 2**) which reduced risk of obesity [5] and overall mortality [6], diabetes [7], and heart disease [8]. In this respect, mushrooms are a high content in antioxidants [9], selenium [21], vitamin D [22] and folic acid [23], substances which inhibit the growth of cancer cells [19] by contributing to the regulation of the cell growth cycle [24]. Dietary fibers (i.e. beta-glucan and chitin) from superior fungi also benefit the digestive system [1] and reduce the risk of heart disease and metabolic syndrome [25]. Together with fibers, the high content of potassium and vitamin C in mushrooms decrease the risk of high blood pressure and cardiovascular diseases [26, 27]. Several other minerals

Aspect	Scientific classification: division/ class/order/family/genus/specie	Habitat
	Basidiomycota/ Agaricomycetes/Russulales/ Russulaceae/ <i>Russula/Russula</i> <i>atropurpurea</i>	Conifer forests, under broad-leaf trees of oak and beech [3]
	Basidiomycota/ Agaricomycetes/Russulales/ Russulaceae/ <i>Russula/Russula</i> <i>alutacea</i>	Hornbeam areas [3]
	Basidiomycota/ Agaricomycetes/Russulales/ Russulaceae/ <i>Russula/Russula</i> <i>nigricans</i>	Durmast forest [3]
	Basidiomycota/ Agaricomycetes/Russulales/ Russulaceae/ <i>Russula/Russula</i> <i>cyanoxantha</i>	Durmast and hornbeam areas [3]
	Basidiomycota/ Agaricomycetes/Russulales/ Russulaceae/ <i>Russula/Russula</i> <i>vesca</i>	Deciduous forests [3]

Aspect	Scientific classification: division/ class/order/family/genus/specie	Habitat
	<p>Basidiomycota/Agaricomycetes/ Agaricales/Pleurotaceae/<i>Pleurotus</i>/ <i>Pleurotus ostreatus</i></p>	<p>On trunks of deciduous species [3]</p>
	<p>Basidiomycota/ Agaricomycetes/Agaricales/ Physalacriaceae/<i>Armillaria</i>/ <i>Armillaria mellea</i></p>	<p>Grows solitary or in groups, on trunks of oak and beech but also on conifer trunks, roots, rotten logs [3, 4]</p>
	<p>Basidiomycota/ Agaricomycetes/Cantharellales/ Cantharellaceae/<i>Cantharellus</i>/ <i>Cantharellus cibarius</i></p>	<p>Beech and conifer forests [3]</p>
	<p>Basidiomycota/Agaricomycetes/ Boletales/Boletaceae/<i>Boletus</i>/<i>Boletus</i> <i>edulis</i></p>	<p>Conifer forests [3]</p>
	<p>Basidiomycota/Agaricomycetes/ Agaricales/Agaricaceae/<i>Macrolepiota</i>/ <i>Macrolepiota procera</i></p>	<p>Open woods and pastures as well as besides the paths in the forests (e.g. oak and beech or coniferous) [3]</p>

Aspect	Scientific classification: division/ class/order/family/genus/specie	Habitat
	Basidiomycota/Agaricomycetes/ Agaricales/Agaricaceae/ <i>Macrolepiota</i> / <i>Macrolepiota excoriata</i>	Pasture, heaths and open woodland [3]
	Basidiomycota/Agaricomycetes/ Agaricales/Agaricaceae/ <i>Agaricus</i> / <i>Agaricus bisporus</i>	Grassy areas following rain or forest edge [3]
	Basidiomycota/Agaricomycetes/ Agaricales/Agaricaceae/ <i>Agaricus</i> / <i>Agaricus campestris</i>	Meadows, wet grasslands or forest edge [3]

Table 1.
 Scientific classification and habitat of several autochthonous edible mushroom species.

copper, iron, and phosphorus, are available in mushrooms and according with their species, these elements and more (i.e. zinc, manganese, sodium, calcium) can be accumulated from their habitat and translocated in stipe and cap in different concentration [26, 28]. Sometimes high concentration in heavy metals such as, lead, nickel, cadmium, chromium was found in mushrooms species [4, 29–31].

Some species, mainly from genera *Agaricus*, *Macrolepiota*, and *Russulaceae*, accumulate high levels of cadmium and lead even in unpolluted and mildly polluted areas [32]. The concentrations of both metals and also of chromium and nickel increase considerably in the heavily polluted sites, such as in the vicinity of metal smelters [30]. Present knowledge of metal speciation in mushrooms is limited as well as is knowledge of their bioavailability in human's body. Thus, consumption of

Mushrooms	Morphological characteristics	Nutritional value
<i>Russula atropurpurea</i>	Convex/flat cap (4–10 cm in diameter) and color dark purple and often almost black in the center; loud stipe (length 3–6 cm, diameter 1–2 cm); whitish spores ornamented with warts and ridges, 7–9 × 6–7 μm measure [3]	Vitamins C, D, B, choline, folic acid, chitin and beta-glucans, potassium, phosphorous, iron, copper [4]
<i>Russula alutacea</i>	Compact and fleshy hemispheric cap then flat at maturity; 7–13 cm diameter cap, purplish to black color; white and dense internal tissue, with low flavor of fruit and sweet taste [3]	Fe (118.17–130.88 mg/kg d.w.); Cu (13.28–14.19 mg/kg d.w.); Zn (15.11–17.84 mg/kg d.w.); Mn (19.58–26.76 mg/kg d.w.) [10]
<i>Russula nigricans</i>	Hemispherical cap, first white, with a dent in the center, then brown, flattened at the end of the maturation phenophase, when it becomes deep, in the cup form, with the edge at first begging to the foot, then high and wavy; consistent and short stipe, cylindrical or thin at the base [3]	Fe (107.03–141.30 mg/kg d.w.); Cu (6.72–13.10 mg/kg d.w.); Zn (25.41–94.81 mg/kg d.w.); Mn (34.21–57.41 mg/kg d.w.) [10]
<i>Russula cyanoxantha</i>	Compact and fleshy cap initial globular, then flat and deep on center, concave, with edge raked to stipe then stretched or wavy; blunt and fleshy stipe, thickened at middle and thin on the base, with rough and flour-like surface [3]	Carbohydrate (9.56%); protein (49.2%), fat (7.87%), crude fiber (30.81%), ash (2.56%) [11]
<i>Russula vesca</i>	Cap is 5–10 cm wide, flat, convex, or with slightly depressed centre, weakly sticky, color brownish to dark brick-red; stipe narrows toward the base, 2–7 cm long, 1.5–2.5 cm wide, white; taste mild. White spore print [3]	Carbohydrates (70.9%); crude protein (25.71 g/100 g d.w.); lipid (3.07 g/100 g d.w.); crude fiber (5.18 g/100 g d.w.); ash (6.82 g/100 g d.w.); magnesium (14 g/kg d.w.); calcium (31 g/kg d.w.); potassium (2.2 g/kg d.w.) [12]
<i>Pleurotus ostreatus</i>	Very fleshy cap with 5–15 cm diameter, convex at the beginning and then flat and at maturing time is thickens and deepens, in the seashell form, with smooth surface and glossy, often wavy; white and compact internal tissue with pleasant smell [3]	Protein (26.67%); ash (9.83%); crude fiber (11.05%); potassium (22.81 mg/100 g d.w.) [13]
<i>Armillaria mellea</i>	Yellow and fairly consistent cap with a diameter of 4–15 cm, first hemispherical, then flat, and at the end of the maturity phenophase slightly deeper; smooth, glossy cuticle on the wet weather and matte on dry time; 5–20 cm tall of stipe, cylindrical, brown, elongated, bulbous base, fluffy, tough and elastic consistency; white spores [3]	Well-balanced nutrients: δ-tocopherol 42.41 μg/100 g d.w.; carbohydrates (81.25 g/100 g d.w.), ash (8.84 g/100 g d.w.), fat (1.97 g/100 g d.w.), proteins (1.81 g/100 g d.w.); polyunsaturated, saturated, monounsaturated fatty acids [14]
<i>Cantharellus cibarius</i>	Compact, hard, yellow and fleshy cap, with diameter of 3–10 cm, convex at first, then slightly deep, and at full maturity it takes the shape of a deeply funnel, with irregular surface and corrugated edge; yellow, robust, hard, smooth stipe, 3–8 cm height, in the frustum cone shape; spores are yellow [3]	Vitamin C (0.4 mg/g fresh weight), potassium (~0.5%, fresh weight), vitamin D, ergocalciferol (vitamin D2 212 IU/100 grams fresh weight) [1]; 20.21% saturated acids, 77.69% unsaturated fatty acid, 17.92% monosaturated acids, 59.79% polysaturated acids, 12.81% palmitic acid, 59.79% dien, 13.57% oleic acid, 59.79% linolenic acid [15]

Mushrooms	Morphological characteristics	Nutritional value
<i>Boletus edulis</i>	Cap diameter is 5–25 cm, brown, very fleshy and hard, initially hemispherical, then convex; compact and solid stipe, cylindrical, thickened or even globular, with a very fine and dense surface; brown spores [3]	Carbohydrates (65.4% d.w.) [16]; palmitic acid, 9.8%; stearic acid, 2.7%; oleic acid, 36.1%; linoleic acid, 42.2%, linolenic acid, 0.2% [17]; 20 essential and nonessential amino acids, (total content 2.3 g/100 g d.w.) vitamin D2 (4.7 µg/100 g d.w.); selenium (13–17 ppm) [18]
<i>Macrolepiota procera</i>	Cap has 10–30 cm in diameter, initially globular, then convex, umbrella-shaped, with a dark-brown, smooth central gurgle; brown, cylindrical stipe, with 10–40 cm height, is hollow inside, long, compact and fragile, bulbous at the base, provided with a large, membranous and strong ring, can slide down along stipe [3]	Carbohydrates (glycerol, mannitol, glucose, trehalose, lepiotan); 15.9% saturated acids, 81.95% unsaturated fatty acid, 19.51% monosaturated acids, 62.44% polysaturated acids, 10.95% palmitic acid, 62.44% dien, 17.40% oleic acid, 62.44% linoleic acid; chitin, proteins, fiber, vitamins, minerals [3, 15]
<i>Macrolepiota excoriata</i>	First ovoid, then flat, with a large gurgle in the middle, peanut shell color, in a darker shade in the center; cylindrical, fusiform stipe, bulbous at the base, whitish above the ring and straw-colored beneath it with flour-like aspect [3]	68.4% carbohydrates, 23.9% crude protein, 5.4% ash, 68.59% saturated acids, 26.58% unsaturated fatty acid, 26.58% monosaturated acids, 45.06% palmitic acid, 8.81% oleic acid [15, 16]
<i>Agaricus bisporus</i>	Cap is a pale gray-brown in color, with broad, flat scales on a paler background and fading toward the margins; first is hemispherical in shape before flattening out with maturity, and 5–10 cm in diameter; cylindrical stipe is up to 6 cm tall by 1–2 cm wide and bears a thick and narrow ring, which may be streaked on the upper side; spore print is dark brown, oval to round, measuring 4.5–5.5 µm × 5–7.5 µm [2, 3]	Carbohydrates (3.26 g/100 g d.w.); vitamins, such as: 7% thiamine (B1), 34% riboflavin (B2), 24% niacin (B3) 30% pantothenic acid (B5), 8% vitamin B6, 4% folic acid (B9), 2% vitamin B12, 3% vitamin C, 1% vitamin D; protein (3.09/100 g) [19]
<i>Agaricus campestris</i>	Diameter of cap is 5–15 cm; first, it is globular, then hemispherical and stretched, perfectly flat in center, white, silky and smooth, with brown flakiness; stipe has tall of 3–7 cm and thick of 1–3 cm, slightly narrowed to the base, white, full, hard, smooth, squamous under the ring; small and fragile ring; pseudo-tissue is soft inside, white, with a pleasant smell and taste	Carbohydrates (30.4%), proteins (18.9%), polyunsaturated acid (34.4%), monounsaturated acid (48.4%), saturated acids (17.2%), potassium (66.5%), phosphorous (21.2%) [20]

Table 2.
 Morphological and nutritional characteristics of several autochthonous edible mushroom species.

the accumulating species should be restricted. The cultivated species, especially the oyster mushroom (*Pleurotus ostreatus*) contain only low levels of the trace elements according with previous authors studies [4, 30].

2. Mushrooms as functional foods

The main role of the diet is to provide adequate nutrients in satisfactory quantities for the metabolic needs of the body and, in addition, to give to consumer a sense of satisfaction and pleasure through the hedonic attributes of food. Recent research supports the hypothesis that, besides to meeting nutritional needs, diet

can modulate various physiological functions and may play unfavorable or beneficial roles in some diseases. It is been seen recently the beginning of a new era in nutrition, reflected by changing the consumer's attitude and manifested through: awareness of the connection between physical and mental status, respectively food, as well as between diet, longevity and physical appearance; attention paid to health promoting compounds (antioxidants, vitamins, etc.); the belief that the diet can provide more promising health solutions than the medical cabinet.

Foods designed to improve people's health and for which claims on specific health effects are allowed were introduced on the market at the beginning of the functional foods era as Foods for Specified Health Use—FOSHU, specific criteria for their labeling being defined. According to European Commission [33], many definitions of functional foods are met worldwide, without being official or commonly accepted. A definition proposed by European Commission Concerted Action on Functional Food Science in Europe—FUFOSE (Consensus document on “Scientific Concepts of Functional Foods in Europe”) [34] for functional foods is the following: “*food that beneficially affects one or more target functions in the body beyond adequate nutritional effects in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease*”. Costin and Segal [35], defined functional foods as “*food products and their compounds that improve the general health of the consumers, avoid the risk of illness, improve the physical or psychological quality of life, as well as the recovery capacity after extenuating exercise or various illnesses*”.

There are more terms linking food and nutrition with health, such as *food supplements/dietary supplements, nutraceuticals/nutriceuticals, pharmafood, designer food*. Clear definition and consistent legislation are challenges for all parties (policy makers, producers, researchers and so on) involved in issues related to functional foods. Scientific substantiation of health claims through integration of different disciplines requires clinical studies to prove the effectiveness of functional foods on large-scale. The credibility of health claims is considered a condition for the success of functional foods in economically terms.

Mushrooms are well known for their nutritional value and health-promoting properties, being considered as both functional foods and a source of nutraceuticals [36]. This is due to biologically and physiologically active substances such as phenolic acids [37]. Recently, a growing interest related to mushroom's mechanisms of action was observed. Thus, it seemed that such mushrooms species can prevent diseases correlated with increased formation of free radicals and oxidative stress, due to their antioxidant capacity. It is reported that another bioactive property, the antimicrobial activity of mushroom extracts, could have a positive influence in evolution of chronic diseases (diabetes, cardiovascular diseases or various types of cancer) [38]. According with Kalaras et al. [39], mushroom consumption may be associated with reductions in oxidative stress-related diseases and disorder because mushrooms species (particularly the yellow oyster and porcini) are rich in glutathione (GSH) and sulfur-containing amino acid ergothioneine (ERGO), considered critical antioxidants. Part of them was determined as uniquely high in both GSH and ERGO. Not in the last, the antimicrobial activity of the mushrooms extracts and their phenolic acids was concretized in strong antibacterial and antifungal properties [40]. In several cases these ones were reported as higher than those of the antibiotics and antifungals frequently used. Some mushrooms species revealed demelanizing properties, in different proportions against different fungi. Their phenolic extracts showed highest demelanizing abilities [41].

In the past years, an increasing public awareness of potential health benefits of dietary fibers was observed. Thus, the food producers tried to fulfill the consumers' request by developing a wide range of fiber-enriched or fiber-fortified foods. In this context, edible mushrooms are taken into account as a rich source of some novel dietary

fibers (DF), with beneficial health effects to humans [42]. Health benefits associated to DF from mushrooms include blood glucose and lipid attenuation, antitumor activity as well as immune-enhancing. Immunomodulating and antitumor effects of mushrooms and their extracts are attributed primarily to content in beta-glucans or polysaccharide-protein complexes [8]. Part of health benefits are supported on the basis of *in vitro* and *in vivo* animal trials. Stronger health benefits/effects were reported for mushroom components/extracts than whole mushrooms in a small number of direct human trials.

The mushrooms can be considered an important and valuable resource for practical applications in the area of functional food, not only as dietary food. An overview on this topic [43] pointed out that *Agaricus bisporus* combined with dried dates for producing white bread lead to the improvement of protein, iron and other nutrients in quantitative and qualitative terms. Addition of powder obtained from the same mushroom species to obtain sponge cakes exhibited acceptable sensory characteristics and a better nutritional value. An extruded cereal-based product obtained with *Cordyceps militaris* proved to have significant anti-fatigue property compared to the product of cereal grains.

Designing new functional foods from mushrooms supposes to preserve their biologically active compounds. Water or organic solvents used for conventional extractions could lead to noticeable degradation of these components. Because consumers asked “natural”, “safe” and with “nutritional value” foods from plant materials, processed with sustainable methods [44], obtaining extracts through techniques aligned with the “green” concepts, such as microwave-assisted extraction, high-pressure assisted extraction, pulsed electric fields assisted extraction or ultrasound-assisted extraction [45] are preferred. These novel non-conventional methods, including also subcritical and supercritical fluid extraction or enzyme-assisted extraction for recovery of valuable compounds from mushrooms, are environmentally friendly methods for production of nutraceuticals or various ingredients for functional foods [46].

Corrêa et al. [47] proposed the production of a natural extract rich in ergosterol as added-value food ingredient, by using a commercially discarded *Agaricus blazei* fruiting bodies. With a significant antioxidant and antimicrobial properties and showing no hepatotoxicity, this extract was used as fortifier ingredient for yogurts. According to authors, the circular bioeconomy concept is fulfilled too, having in view that *A. blazei* fruiting bodies are normally discarded, being inconsistent to the commercial requirements of the market.

Dried synbiotic foods, shelf stable and economical advantageous, were formulated by Moumita et al. [48], using *Enterococcus faecium* as probiotic and *Pleurotus florida* extract as prebiotic. Lyophilization and spray-drying lead to microcapsules added to different dry food matrices. Choosing to *Pleurotus florida* was due to its content in β -glucan, a well-known prebiotic which stimulates selectively the growth of probiotics.

The prebiotic potential of polysaccharides from different mushroom species was in focus for other researchers [49]. Thus, studying 53 wild-growing mushrooms, the authors found that the majority of their polysaccharides stimulated the growth of *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* isolated from the human gastrointestinal tract. For this reason, the polysaccharides fractions from edible mushrooms could enhance the number of beneficial bacteria in the GI tract, being useful in producing functional foods and nutraceuticals. Microencapsulation of alcoholic extracts of *Agaricus bisporus* was made by spray-drying, using maltodextrin cross-linked with citric acid [50]. The microspheres with the extracts protected this way by degradation were used to obtain functionalized yogurts, with promising bioactive properties.

Functional food products with better characteristics in terms of stability were obtained through addition of mushroom powder to meat emulsion (batters) [51].

Mushrooms (*Agaricus bisporus*) were taken into account having in view by one hand their availability as plant protein source and by another hand the consumers' interest in a balanced ratio of plant/animal protein intake. Meat emulsions with 2% mushroom powder added were proved to lead to a well-ordered emulsion structure, due to their higher protein adsorption at the lipid interface. These ones exhibited heat resistance and higher gel-like behavior compared with other samples. Obtaining final products through cooking the meat emulsions, an improvement in textural properties was determined. Considering color as one of the most important properties from the consumers' viewpoint, so that they would like to purchase the final products of meat emulsions, the authors established, in the model meat emulsion (without addition of nitrite curing salt, spice mix or additive mix), that increasing the mushroom powder lead to increasing of the redness values of the emulsion and the decreasing of their lightness and yellowness.

The protein and soluble and insoluble dietary fiber content of mushroom was by interest for other researchers too. Thus, the mushroom powder was added to pasta, considered a nutritional imbalanced food [52]. This one lead not only to the deficiency made up, to decreasing of the extent of starch degradation, but also to production of food with health-promoting bioactive compounds. Thus, the mushroom powder was proved to confer healthier characteristics, such as improving antioxidant capacity and lowering the potential glycemic response when are incorporated into fresh semolina pasta.

A β -glucan composed mainly of polysaccharide with some proteins and a small amount of phenolic compounds, extracted from *Agaricus bisporus*, *Pleurotus ostreatus* and *Coprinus atramentarius* was proved to have antioxidant activities and functional properties [53]. In terms of functional properties, the β -glucan from *C. atramentarius* showed the highest fat binding, emulsifying properties and swelling power, while the one from *P. ostreatus* exhibited the highest foaming properties. In authors' opinion, this mushroom β -glucan could be an effective functional ingredient for food formulations and pharmaceutical ones too. The food applications of *Pleurotus* powder or β -glucan-rich fractions isolated from *Pleurotus* spp. are considered well known and described [54]. Emphasizing that the perspectives for *Pleurotus* spp. applications in functional foods are related to consumers' acceptability, the authors summarized its three main strategies of development, respectively the use as fortifying agent, as high-cost protein replacer and as prebiotic ingredient too. Consumption of functional foods containing specific extracts from mushrooms should be encouraged among people needed to lower their cholesterol levels in serum [55]. This statement is based on the *in vitro* and *in vivo* studies showing that fungal extracts obtained from edible mushrooms (β -glucans and other water-soluble compounds) might be able, as pharmaceutical drugs and functional foods, to modulate cholesterol levels.

The molecular basis underlying the biologically active compounds benefits to human health in the case of certain mushrooms species seems to be not yet elucidated. Examining the biological effects of the MeOH extract of *Morchella esculenta* L. (Morchellaceae), commonly known as morel mushroom (found throughout the world, widely distributed in Korea, China, Japan and Europe), Lee et al. [56] isolated eight compounds (three fatty acids and five sterols) that exhibited potent cytotoxicity to human lung cancer cell lines. In the authors' opinion, further evaluation would provide the evidence for the use of *M. esculenta* as functional food against cancer, with significant implications for cancer—the second leading cause of mortality worldwide—prevention and treatment. *Grifola frondosa* (known as maitake mushroom), used widely as a daily food, as food additive or for medicinal reasons, was investigated by Dissanayake et al. [57] with a view to evaluate its functional food value. The highly abundant phytochemicals determined (glycerides, sterols, a glucosylceramide, a α -glucose dimer, a phospholipid and α -glucans) seems to be

responsible for the antiinflammatory and antioxidant activities of the fruiting body of *G. frondosa*. The authors concluded that health benefits and an improved quality of life could be achieved through a regular consumption of maitake mushroom.

In order to provide the scientific evidence needed for the development of functional food for the management of certain health problems afflicting millions of people worldwide, the study of Akata et al. [58] showed that *Lycoperdon utriforme* and *Agaricus campestris*, due to their biologically active constituents, can be used for prevention of diabetes type II and Alzheimer's disease. Mushrooms that have demonstrated experimental or/and clinical anti-diabetic effectiveness are also *Tremella fuciformis* (berk), *Wolfiporia extensa* (Peck) Ginns, *Ganoderma lucidum* (Curtis) P. Karst, *Ganoderma applanatum* (Pers.) Pat., *Collybia confluens* (Pers.: Fr.) Kummer, *Auricularia auricula-judae* (Bull.), *Agaricus subrufescens* (Peck), *Inonotus obliquus* (L.), *Hericium erinaceus* (Bull.), *Agrocybe aegerita*, *Coprinus comatus* (O.F. Mull), *Cordyceps sinensis* and *Grifola frondosa* (Dicks.) [59].

Limited knowledge about the processing effects on the mushrooms biologically active compounds or the mushrooms and their derived products functional properties still persists, despite the advances in research from the last decades. Moreover, various interactions within the food matrices if mushrooms or their ingredients are added, are yet unknown.

Nutrition represents a psycho-social act, because it cannot be understood only as a satisfaction of certain nutritional needs. Food means nourishment, stimulus for emotional tonus and a symbolic significance that the individual gives to food. Food is to be enjoyed and the physiologically eating is a complicated process.

For these reasons, the sensory profile of food represents an overwhelming concern of all producers, so that to fulfill the consumers' needs. In this context and on the basis of the increased interest in mushrooms in terms of their nutritional and bioactive compounds, the research on their sensory properties is considered surprisingly scarce [60]. With a view to add knowledge in this gap, the authors processed edible mushrooms (*Boletus edulis*, *Cantharellus cibarius*, *Craterellus tubaeformis*, *Lactarius camphorates* and *Agaricus bisporus*) by sous vide cooking, frozen, pooled and tempered to 50–60°C. The sensory evaluation made by a trained panel revealed a moderately intense total odor for all samples. Weak cardboard-like, forest-like and earthy odor notes were defined for the mushroom species above mentioned. In terms of taste, *Agaricus bisporus* was moderately sweet and most intensively umami-like. *Boletus edulis* closely followed the umami intensity of *A. bisporus*, being also the sweetest sample. *Lactarius camphorates* was characterized as very different from other samples, due to its sensory profile, defined as intensively bitter and astringent. This curry milk cap, used in Finland often as spice unlike other mushroom species, was the only noticeably pungent sample. Aisala et al. [60] demonstrated, on the basis of projective mapping applied to three wild and three cultivated types of mushrooms blanched, frozen and thawed to ambient temperature, the major influence of processing on the sensory properties of the mushrooms. It seems that most consumers choose to avoid eating button mushroom because the fresh samples are linked to umami and mushroom descriptors. The other species were linked to umami and mushroom descriptors. Finally it was concluded that wild and cultivated mushrooms are different in sensory descriptors and their related intensities too. Varying profiles, new innovative mushroom products and food ingredients could be designed.

In order to meet the concern related to major nutritional problems in most countries (protein energy malnutrition and micronutrient deficiencies), Ishara et al. [61] tested the use of mushroom (*Agaricus bisporus* and *Pleurotus ostreatus*) flours in different blends with maize flour, in nutritional and functional terms. The mushrooms flours could serve as protein supplements and food fortification, due to their increased protein, fibers and mineral content. The composite flours noticed

through an increased water retention capacity, water absorption capacity, foaming capacity, fat absorption capacity and a decreased bulk density and syneresis. These data indicated that mushroom flours can be very suitable in human diet.

Drying methods (convective drying, freeze-drying, vacuum microwave drying and a combination of convective predrying and vacuum microwave finish drying) were tested in order to evaluate their influence on the sensory profile and implicitly on the quality of the oyster mushrooms (*Pleurotus ostreatus* Jacq.) [62]. The total concentration of aroma/volatiles compounds of fresh mushroom was reduced significantly by all drying treatments. However, the combined treatment mentioned above leads to obtaining products with a sensory profile closer to the fresh mushrooms. Nonthermal plasma technology (NTPT) was investigated in order to better understanding of the mechanism of interaction of food bioactive compounds and plasma and consequently its successful adoption by industry. Reviewing the influence of NTPT on functional food components, Muhammad et al. [63] showed that the plasma activated water (PAW) has the effect of increasing the antioxidant activity and the concentration of ascorbic acid of button mushroom. The antioxidant activity was extended with increases in PAW processing time.

3. Evaluation of several antioxidant species in indigenous edible mushrooms

As described in previous chapter, enzymatic and non-enzymatic antioxidants were reported to be present in edible mushrooms, and having roles in balancing human metabolic processes related to oxidative stress [64]. Since mid of twentieth century, after Harman's "Free-Radical Theory of Ageing", an intensive research on involvement of free radicals and antioxidants in living processes has been performed [64]. They are commonly named *reactive species* the advanced knowledge allowed their classification in terms of intensity [65], as well as of the nature of active centers (either oxygen, nitrogen, carbon or sulfur species) [64].

Wild grown and cultivated mushroom species have been studied from the perspective of possible correlations between non-enzymatic and enzymatic antioxidants. Thus, for the first category several phytochemical characteristics have been determined: total phenolic content, total flavonoids, antioxidant activity, and four trace micronutrients (Zn, Fe, Mn, and Cu), and for enzymatic antioxidants data, activity of catalase (CAT) and peroxidase (POX) have been evaluated.

3.1 Analytical techniques

Different instrumental analytical techniques were reported by authors [66] to identify and quantify antioxidants in edible mushrooms. Among these, high performance chromatography (HPLC) and gas chromatography (GC) using various detection devices, spectroscopic techniques such as nuclear magnetic resonance (RMN), Fourier transform infrared (FTIR), ultraviolet-visible (UV-VIS), as well as inductively coupled plasma mass spectrometry (ICP-MS) are among the most applied. According to available equipment, the mushroom samples were characterized mainly through the absorption spectroscopic methods UV-VIS and FTIR spectroscopy, and information on trace micro-nutrients was gathered through ICP-MS. Analytical process included the classic steps of (a) sampling, (b) sample treatment and/or preparation, and (c) qualitative and/or quantitative analysis. For the step (a), mushroom samples were collected from the natural habitat according with **Table 1** and then representative portions from each sample were taken for further treatment. For the step (b), several procedures have been used: (i) oven drying at 40°C for 48 h; (ii) grounding to

less than 2 mm, cap and stipe separately; (iii) extraction of analyte(s), for 4 h, at room temperature, under continuous mixing, in two type of solvents—redistilled water and hydroalcoholic 50% (v/v), dry matter (g) to solvent ratio (mL) was of 4:100; (iv) centrifugation of obtained extracts; (v) wet digestion with concentrated HNO₃ and H₂O₂, at ratios of 0.2 g dry mushroom sample to 50 mL solution.

3.1.1 Total content of polyphenols (TCP)

Polyphenols are a class of compounds with structures containing at least one aromatic ring with at least one hydroxyl group bonded on it. They are classified according to the number of rings and to their functional groups bound in the structure, and thus we have: phenolic acids, flavonoids, stilbenes, and lignans, coumarins, tannins. Phenolic acids were reported to be the main polyphenolic compounds in mushrooms [37].

Total phenolic content in mushrooms was reported to be successfully determined by Folin Ciocalteu method [67], and an adapted method was applied for the studied mushrooms [67]. The Folin Ciocalteu reagent is a mixture prepared by dissolving sodium tungstate (Na₂WO₄·2H₂O) and sodium molybdate (Na₂MoO₄·2H₂O) in water with hydrochloric acid and phosphoric acid. Hydrated lithium sulfate (Li₂SO₄·H₂O) may be added to this mixture to prevent turbidity that may appear due to formation of some insoluble sodium salts [67]. The mixture is very stable if protected to reduction agents and light. Diluted reagent also needs to be protected to light. The chemical process, occurring at basic pH, is based on molybdenum reduction from +6 (yellow) to +4 (blue) after the oxidation of polyphenols in samples, and may be described in **Figure 1** [68].

Light absorption of a monochromatic radiation of 765 nm is measured with a UV-VIS spectrophotometer. Colored liquid samples were placed in 10 mm light path cuvettes and readings were made versus a blank sample prepared with all reagents as samples, but with extractant instead of mushrooms extract. A calibration curve with gallic acid as reference antioxidant was plotted before each measurement set, calibration range chosen was 0.01–0.08 mg/mL.

Similar experimental procedures were applied for both aqueous and hydroalcoholic extracts, different samples dilutions were used so that the linear domain of Beer-Lambert-Bouguer law and calibration range were reached.

Total polyphenols content were expressed as milligrams of gallic acid equivalents per mL of extract, and then reported to mushroom dry weight (mg GAE/g d.w.). All experiments were performed in triplicate and the means ± standard deviations (SD) were reported [69].

3.1.2 Total flavonoid content (TFC)

Flavonoids are antioxidant compounds whose structure has two benzene rings (A and B) and an oxygen containing pyran ring (C). Six subclasses of flavonoids are generally accepted for classification, as follows: flavonols, flavones, isoflavones, flavanones, anthocyanidins and flavonols [70–72], differentiated by the oxidation

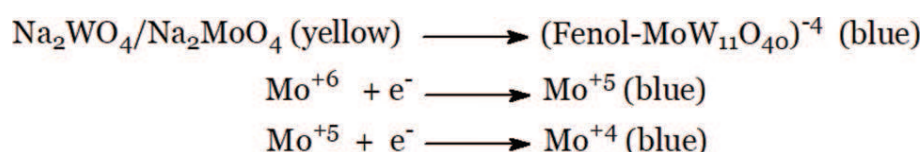


Figure 1.
Reaction scheme for total polyphenols determinations by Folin Ciocalteu spectrometric procedure.

level of the C ring of the basic 4-oxoflavonoid (2-phenyl-benzo- γ -pyrone) nucleus. Presence of flavonoids in edible mushroom extracts has been confirmed by several authors [67, 69, 73], some of these reported also their molecular identification (i.e. myricetin, chrysin, catechin, resveratrol, quercetin, others). The antioxidant activity of flavonoids, as for polyphenolics in general, is mainly given by the presence and position of multiple hydroxyl groups in their molecules. Thus, it is considered that the primary mechanism of the radicals scavenging activity of flavonoids is hydrogen atom donation [70, 71].

Total flavonoid content in aqueous and hydro-alcoholic mushroom extracts was measured by the aluminum chloride colorimetric assay described in the literature [74], adapted for the working conditions [69]. Method's principle is based on Al^{3+} ions to form complex combinations with carbonyl group from C-4 carbon and hydroxyl group from C-3 or C-5 carbons from flavonoids structure (**Figure 2**). Further, aluminum can bond the orthodihydroxyl groups from A- and B-nucleus of flavonoids. The effect of the formation of these bonds results in coloration of the working solution in yellow due to resulted complex combinations.

Sample absorbances were measured in 10 mm cuvettes, at 510 nm, against redistilled water, and concentrations were calculated using the calibration curve drawn before each tests set, in the concentration range of 0.1–1 mg/mL of quercetin, used as reference flavonoid. Total flavonoids contents were expressed as mg quercetin equivalents per mL mushroom extract, and then converted to mushroom dry weight. Analytical data were collected on triplicate samples, mean values together with standard deviations were reported [69].

3.1.3 Antioxidant activity (AA)

Several chemical and biochemical assays can be used in order to evaluate the total antioxidant activity of mushrooms, and the 2,2-diphenyl-1-picrylhydrazyl DPPH \cdot assay is one of the most frequently used [75–77]. Measurement principle is based on the fact that the antioxidant compounds from mushroom extracts release an electron or a hydrogen atom, and convert the DPPH \cdot to a more stable, diamagnetic molecule, according to reaction below. DPPH \cdot is a stable, long-lived organic nitrogen radical with a strong absorption around 517 nm (**Figure 3**).

The antioxidant activity of the studied mushroom extracts was assessed using DPPH \cdot method. For good tests results, fresh ethanolic DPPH solutions (20 mg/mL) were prepared daily by weighing the necessary amount of DPPH powder (usually kept at $-20^{\circ}C$), and kept in dark until experiments end. Samples were prepared by mixing aliquots of mushroom extract with DPPH solution, kept in dark at room temperature for 30 min, then sample absorbances were read to spectrophotometer, where zero absorbance was considered the extractant used for extracts preparation.

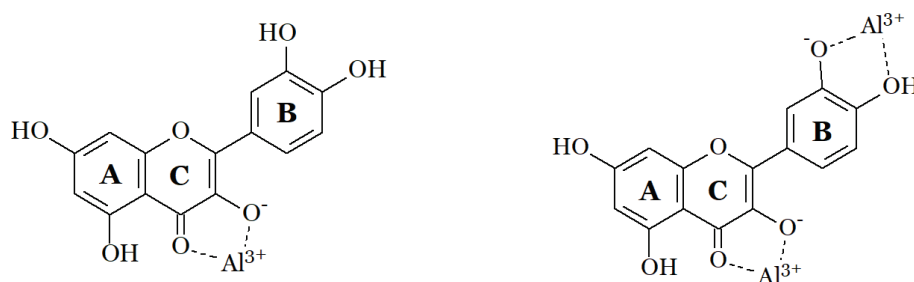


Figure 2.
Flavonoids complex combinations with Al^{3+} —quercetin example.

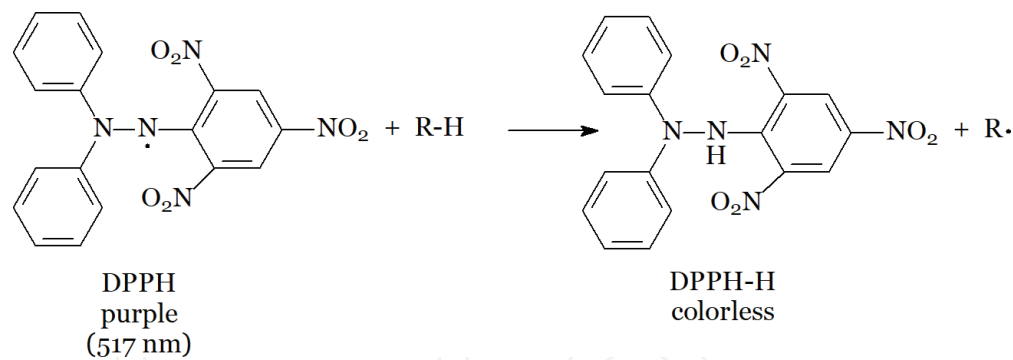


Figure 3.
Reaction scheme for antioxidant activity determination by DPPH method.

Reagent and sample blanks were prepared and measured for each test. Calculations were done according to equation:

$$AA (\%) = \left[\frac{A_{\text{reagent blank}} - (A_{\text{extract}} - A_{\text{sample blank}})}{A_{\text{reagent blank}}} \right] \times 100 \quad (1)$$

where *AA* is the global antioxidant activity of mushroom extract solutions, and *A* is the absorbance of the corresponding solution (as per subscripted text). As indicated by Eq. (1), results were calculated as % scavenging of DPPH at a fixed antioxidant concentration. A low absorbance of the tested sample indicates a high free-radical-scavenging activity.

3.1.4 Fourier transform infrared spectroscopy

To investigate the chemical functional groups of organic compounds in mushroom extracts, Fourier transform infrared spectroscopy was used. The chemical changes induced by extraction techniques as well as the various functional groups responsible for biological activities were detected in the mid-infrared absorption region using a Vertex 80v spectrometer (Bruker) equipped with a diamond attenuated total reflection crystal accessory [78]. The extracts were placed on the sample chamber of attenuated total reflection—Fourier transform infrared spectrometer without any preparation. The important absorption frequencies were noted in the range of 3600–600 cm^{-1} , as well as the fingerprint region of the spectra [79].

3.1.5 Inductive coupled plasma mass spectrometry (ICP-MS)

Minerals Cu, Fe, Zn, Mn are included in mushroom food chain, and, in low concentrations, they are considered antioxidant micronutrients. This designation is justified by their capability to catalyze some reactions producing reactive oxygen species, and their enzyme activation properties [10]. Edible mushrooms were reported as metals bio-accumulators, however high levels of essential metals intake could produce toxic effects when exceed certain values [78].

Trace elements Cu, Fe, Zn and Mn were measured by ICP-MS technique in aqueous solutions obtained by wet digestion. Before each test were performed the system calibration using Certipur[®] Certified Reference Material ICP multi-element standard IV (~1000 mg/L in 6.5% HNO_3 , Merck). The instrumental parameters were: 1.5 kW plasma power, with 1 L/min argon nebulizer flow and 10.75 L/min plasma argon flow respectively, and precise analytical data were collected [10].

3.1.6 Peroxidases

Peroxidases are one of the classes of enzymes involved in the antioxidant defense mechanisms, together with superoxide dismutase, catalases, and others [10]. Experimental evaluation of peroxidase (POX) relies on its property to oxidize in the presence of hydrogen peroxide or other peroxide compounds (i.e. aromatics).

Oxidation of guaiacol by peroxidases in the presence of H_2O_2 is generally used for the colorimetric assay, absorbances measurements are performed at 420 nm, the chemical process involved is presented in **Figure 4**.

For accurate POX determination, fresh mushrooms are used to obtain the extracts that are further measured. Final results were reported as POX units per gram of mushroom. The unit of POX activity was defined as the oxidation of one micromole H_2O_2 per minute at 25°C (pH = 7.0).

3.1.7 Catalases

Catalases (CAT) are intracellular antioxidant enzymes present in edible mushrooms [10]. They are oxidoreductases, as they use hydrogen peroxide both as a receptor of electrons and as an electrons donor, decomposing it according to reaction presented in **Figure 5**.

Evaluation of catalase activity involves contacting a weighted amount of fresh mushroom with a measured volume of hydrogen peroxide at room temperature, allowed to stand for several minutes. The not-converted amount of hydrogen peroxide is then determined by titration with potassium permanganate in acid medium. Results are reported as CAT units per mushroom gram, while the unit of CAT activity is defined as the amount of enzyme decomposing one micromole H_2O_2 per minute at 25°C.

3.2 Analytical data and results interpretation

Total phenolic content of studied mushrooms (aqueous and hydroalcoholic extracts) is mentioned in **Figure 6**. As mentioned before, data were converted to milligrams of gallic acid equivalents (GAE) per gram of dried weight (d.w.). Experimental findings show certain differences between values for hydroalcoholic extracts and those prepared with water as solvent. On the other hand, in general for studied mushrooms, no significant differences between the two anatomic parts, as

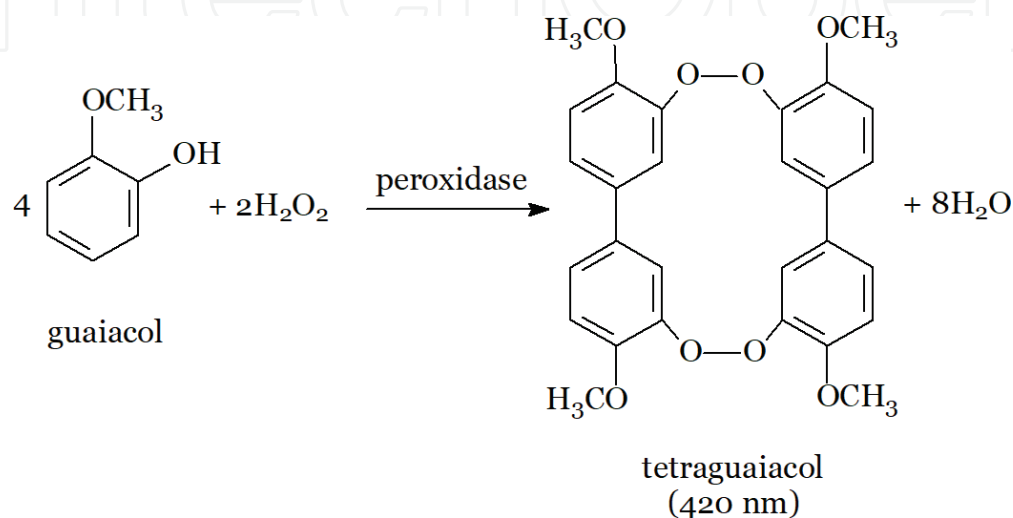


Figure 4.
Oxidation of guaiacol to tetraguaiacol, reaction catalyzed by peroxidase.

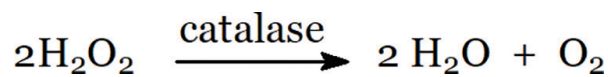


Figure 5.
 Decomposition of hydrogen peroxide catalyzed by catalase.

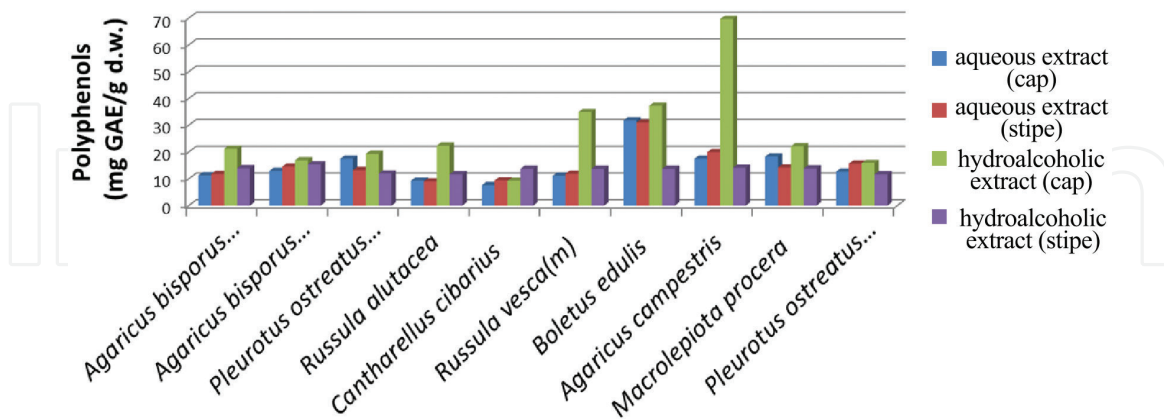


Figure 6.
 Total content of polyphenols in studied mushrooms, aqueous and hydroalcoholic extracts, cap and stipe [mg GAE/g d.w.]

distinctly tested, cap and stipe. However, exceptions are observed, and will be further discussed. Concentration values found range between 9.28 ± 0.03 mg GAE/g d.w. (cap of *Cantharellus cibarius*) and 69.65 ± 0.23 mg GAE/g d.w. (cap of *Agaricus campestris*), average values being established for the cultivated species (**Figure 6**).

Considering both solvents and mushroom species, *Agaricus campestris* (cap) registered the highest difference between the content of phenolic compounds in the samples prepared in hydroalcoholic extractant instead of water, while the smallest one was determined in the case of *Macrolepiota procera* (stipe), who showed a slight preference for water. Intermediate differences were established for caps of *Russula vesca*, *Russula alutacea* and *Agaricus bisporus* white respectively, where the hydroalcoholic extractant was favorable to a better polyphenols extraction, while for *Boletus edulis* (stipe) water was a more convenient extractant for extraction of phenolic compounds.

Differences between anatomic parts were found to *Pleurotus ostreatus* (cultivated), *Russula alutacea*, *Boletus edulis* and *Macrolepiota procera*, higher total polyphenolic content was measured in caps than in stipes. An opposite behavior was found to *Cantharellus cibarius* mushroom hydroalcoholic extract, where TCP values were higher in stipe than in cap, and by 4.93 times of the case of *Agaricus campestris*. For aqueous extracts, closer values of TCP in caps and stipes were found. From the perspective of their origin, experimental findings for TCP showed lower average values for cultivated mushrooms than wild species group, regardless of the extractant type (15.03 mg GAE/g d.w. for water extracts and 20.14 mg GAE/g d.w. for hydro-alcoholic extracts). Also, no significant differences between caps and stipes for aqueous extracts, for both cultivated and wild species, average values for TCP were 13.54 mg GAE/g d.w. for cultivated and 13.05 mg GAE/g d.w. for wild ones respectively (excepting *Boletus edulis*).

Once the total flavonoid content (TFC) is considered, slight differences from the above mentioned findings were found. Thus, as may be observed in **Figure 7**, for some species (caps or stipes), flavonoids extraction in hydroalcoholic extractant was better than in water. TFC values measured in the hydroalcoholic extracts of *Russula alutacea* (cap), *Cantharellus cibarius* (stipe), *Russula vesca* (cap) and *Pleurotus ostreatus*—wild growing (cap), were higher than in their aqueous extracts. Aqueous extracts TFC exhibited values ranging between 0.22 ± 0.02 mg QE/g

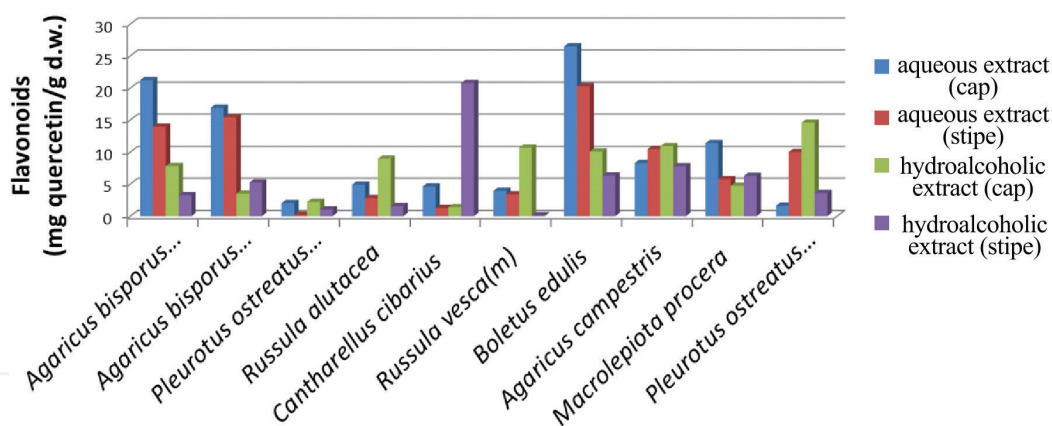


Figure 7. Total flavonoids content in studied mushrooms, aqueous and hydroalcoholic extracts, cap and stipe [mg quercetin/g d.w.].

d.w. (stipe of *Pleurotus ostreatus* cultivated) and 26.51 ± 0.04 mg QE/g d.w. (cap of *Boletus edulis*), while TFC values for hydroalcoholic extracts were in the range of 0.12 ± 0.04 mg QE/g d.w. (stipe of *Russula vesca*) and 20.77 ± 0.06 mg QE/g d.w. (stipe of *Cantharellus cibarius*).

Similarities with total phenolics were found for total flavonoids detected, for comparisons made between mushroom species of different origin. Thus, experimental data showed that TFC average values in cultivated species were lower than in wild grown ones. Compared data for flavonoids found in caps and stipes showed that for both cultivated and wild species higher flavonoids content were noticed in cap for both extracts. Several exceptions have been noticed from this behavior: hydroalcoholic extracts of *Agaricus bisporus* brown (cultivated), and aqueous extract of *Agaricus campestris* and alcoholic extracts of *Cantharellus cibarius* and *Macrolepiota procera* respectively (wild species). One may conclude that total flavonoids content varied depending on the mushroom species and used extractant, polar solvents dissolving more flavonoids [69].

Analytical data for antioxidant activity of studied edible mushrooms extracts (cap and stipe), evaluated through DPPH method as previously described, showed some high and low limits. Thus, for aqueous extracts it was found that, *Agaricus bisporus* brown (cap) had the strongest DPPH radical-scavenging activity of 88.64%, while the lowest value of 25.72% was found in *Macrolepiota procera* (cap). When water-ethanol 50% (v/v) was used as extraction solvent, limit values were 74.93% for *Boletus edulis* (cap) and 13.61% respectively for *Russula alutacea* (stipe). Also, notable differences were found between analytical data recorded on cap and stipe of studied species [69]. Example of hydroalcoholic extracts is relevant: while most of mushroom species showed higher AA% values in caps than in stipes corresponding to same species, several exceptions were observed for *Cantharellus cibarius*, *Macrolepiota procera* and *Agaricus bisporus* brown where slight higher values were found in stipes than in caps. With regards to this phytochemical parameter (AA), a general behavior was noticed for studied mushrooms. Thus, notable differences between analytical data recorded for various species and when using the two extractant types.

By infrared spectroscopy several chemical functional groups that may be responsible for the antioxidant character of mushrooms, as quantified by classes of compounds or as a whole with the above mentioned ultraviolet-visible spectroscopic methods. Significant characteristic frequencies were observed in the range of $3600\text{--}600\text{ cm}^{-1}$ and fingerprint region, and were assigned to different organic compounds with —OH functional groups. As a relevant example, obtained results indicated that hydroalcoholic mushroom extracts may contain active functional groups as alcohols, esters and aldehydes [10].

Quantification of micronutrients in studied mushrooms showed, as may be observed in **Figure 8**, they are rather rich in Mn, Fe, Cu, and Zn, metals having a significant role in enzymatic systems activation. One may exemplify with data for species like *Boletus edulis* with Mn content of 130.73 mg/kg, and *Macrolepiota procera*, with Fe content of 715.14 mg/kg [10].

Enzymatic antioxidants peroxidase and catalase determinations in indigenous mushroom species were previously reported [10, 57], also **Figure 9** shows both enzymes activities. Significant variations were found for both studied enzymes. Higher values of catalase activity were found in species as *Agaricus bisporus white* and *brown* and *Russula vesca*, while species like *Boletus edulis* and *Pleurotus ostreatus wild* showed lower values. Measured values for catalase activity were in the range of 3.58–14.67 $\mu\text{mols H}_2\text{O}_2/\text{g}/\text{min}$. Also, highest values of peroxidase activity were found in mushroom species like *Russula alutacea* and *Macrolepiota procera*, while lowest values of this enzyme were found in *Chantarellus cibarius*.

From the origin perspective, it was found that *Pleurotus ostreatus* cultivated had a 2.49 times higher catalase activity than the same wild species, while peroxidase activities for both wild and cultivated *Pleurotus ostreatus* were similar. Some correlations between metallic nutrients content enzymatic activities of mushrooms have been reported [10], and next chapter, through a chemometric approach will highlight further correlations between enzymatic and non-enzymatic antioxidant species, as were determined for studied mushrooms.

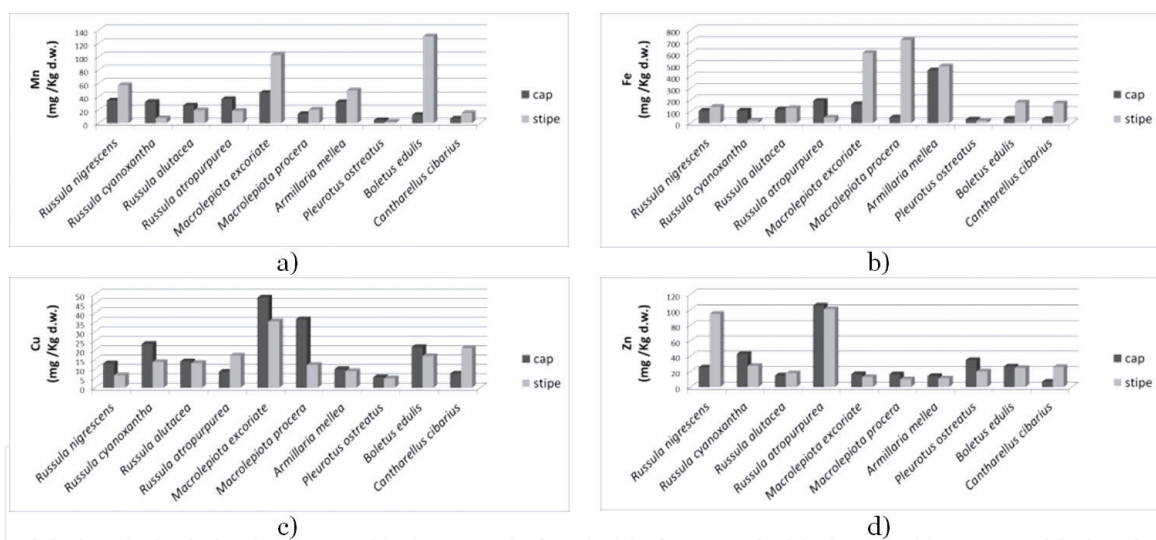


Figure 8. Micronutrients concentrations in studied mushroom samples: a) Mn; b) Fe; c) Cu; d) Zn.

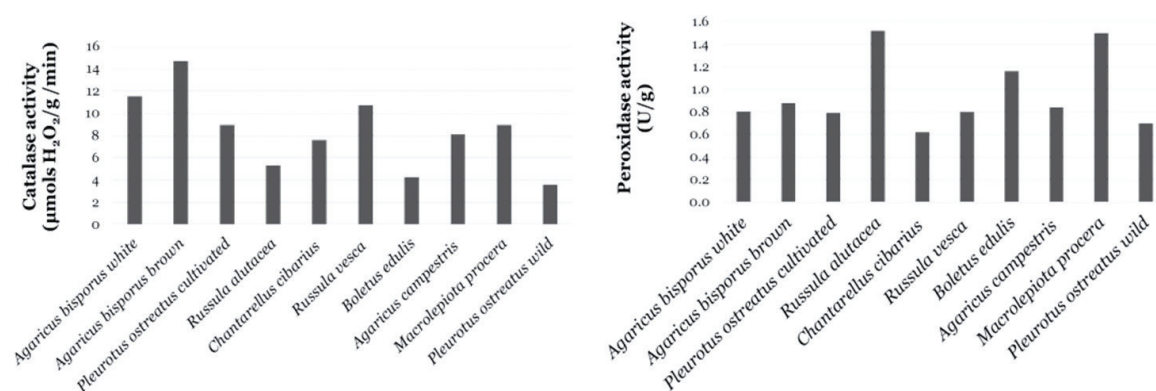


Figure 9. Peroxidase and catalase activity in studied mushroom species.

4. Statistical analysis of data

Statistics have, through the descriptive methods of data analysis, powerful multidimensional analysis tools that can be used to design important information for fundamental research, applied research, market research, economic analysis, etc. Information can be hierarchized in terms of intensity of influence and can be analyzed as a whole and not independently [78].

One-way Analysis of Variance (ANOVA) was applied to the data set related to the ten mushrooms species, in order to observe whether there are any significant differences (Sig. < 0.05) between the means of the independent groups of variables. The produced F-statistic was higher for phenolics, flavonoids respectively antioxidant activity determined in aqueous extracts. Contrary, for the same parameters whose values were associated to hydroalcoholic extracts, the Sig. value higher than 0.05 indicated that there are no differences between groups in function of mushroom species.

In order to test the hypotheses of association between enzymatic and non-enzymatic antioxidants, the Bivariate (Pearson) Correlation was applied. From the large amount of information, the data shown in **Table 3** pointed out only the significant correlations, both for mushrooms' cap and stipe.

A strong positive relationship was observed between phenolics and flavonoids determined in aqueous extracts, regardless of the anatomic part of the mushrooms species. The strength of association was large but downhill between antioxidant activity determined in aqueous extracts and mushrooms species, if the last variable was defined in the next order: *Agaricus bisporus white*, *Agaricus bisporus brown*, *Pleurotus ostreatus* cultivated, *Russula alutacea*, *Chantarellus cibarius*, *Russula vesca*, *Boletus edulis*, *Agaricus campestris*, *Macrolepiota procera* and *Pleurotus ostreatus* wild respectively. Only for the stipe of the analyzed species, a strong relationship between the content of phenolics (in hydroalcoholic extracts) and the catalase activity was determined. The relationship between catalase at least and mushroom species should be deeply analyzed, taking into account as much as possible types of potential linked variables, because in the last years the role of catalase (CAT), together with those of superoxide dismutase (SOD), was largely discussed in the context of the bioremediation biotechnologies.

No correlation could be observed for peroxidase, phenolics and antioxidant activity, irrespective of the extractant used and the anatomic part of the mushrooms. The Boxplot method was applied for graphically depicting groups of data related to phenolics, flavonoids and antioxidant activity, both in aqueous and hydroalcoholic extracts of mushrooms species. The quartiles of data represented in relationship

Anatomic part	Variables	Pearson's correlation coefficient
Cap	Phenolics—flavonoids (both in aqueous extracts)	0.857**
	Phenolics—antioxidant activity (both in hydroalcoholic extracts)	0.725*
	Antioxidant activity in aqueous extracts—mushrooms species	−0.643*
Stipe	Catalase—phenolics in hydroalcoholic extracts	0.700*
	Phenolics—flavonoids (both in aqueous extracts)	0.934**
	Antioxidant activity in aqueous extracts—flavonoids in hydroalcoholic extracts	−0.658*
	Antioxidant activity in hydroalcoholic extracts—flavonoids in aqueous extracts	0.679*

*Correlation is significant at the 0.05 level.

**Correlation is significant at the 0.01 level.

Table 3.
Pearson correlation coefficients for the analyzed variables.

with the anatomic part of the 10 species of mushrooms highlighted those species located far from the group in terms of their content in antioxidant compounds, respectively antioxidant capacity. The degree of dispersion and skewness in each category of data is indicated by the spaces between the different parts of the boxes. Variability outside the upper and lower quartiles of different variables was indicated by lines extending vertically from the boxes of the box plots (Figures 10–12).

Thus, both for cap and stipe, *Boletus edulis* remarked through a higher content of phenolic compounds in aqueous extract (Figure 10a). The median values for this parameter are close, independent of the anatomic part, a relative higher degree of dispersion being observed in the case of the mushrooms' cap than in the stipe. The variability of the data outside the upper quartile is however obvious for stipe. Also for the mushrooms' stipe, it cannot be about the scattering of the data if the phenolics in hydroalcoholic extracts are taken into account (Figure 10b). Based on the comparative analysis of the graphical representations (Figure 10a and b) it can be observed that the median value is superior for the group consisting of the caps of mushrooms in terms of phenolics in the case of the hydroalcoholic extracts than in the aqueous ones.

If the descriptive statistics was applied to content in flavonoids of the aqueous extracts of the mushrooms species—cap and stipe (Figure 11a and b), the second quartile for cap was close by the corresponding value for stipe, as it was observed for phenolics determined from aqueous extracts. The data sets show different trends for the other descriptive indicators. *Boletus edulis* (cap) and *Chantarellus cibarius* (stipe) are the mushroom species which detached from groups in terms of their content in flavonoids (in aqueous, respectively hydroalcoholic extracts).

Boxes and whisker plots quartiles cannot be overlapped if the antioxidant activity (both in aqueous and hydroalcoholic extracts) is analyzed Figure 12(a and b). The values determined for cap and stipe are higher in the case of the aqueous extracts of the mushrooms species, regardless of the quartiles displayed. The plotted outliers are not associated to a certain mushroom species.

Factorial analysis was applied to the enzymatic and non-enzymatic antioxidants of the mushroom species data sets. First of all, the matrix of the data correlation was developed and analyzed. The second phase of the study was based on the high values observed between some analyzed variables. Values of the total explained variance and Eigen values of the correlations matrix were generated. Two components were retained, the variables being represented on two factorial axis resulted from the combination of the initial variables (Figure 13).

After factors rotation (in order to obtain a better “angle” of view), PC1 explained 46.72% from the total variance, while PC2 explained 84.41%. It is thus possible to represent in the main plan the cloud of points. Two principal components were

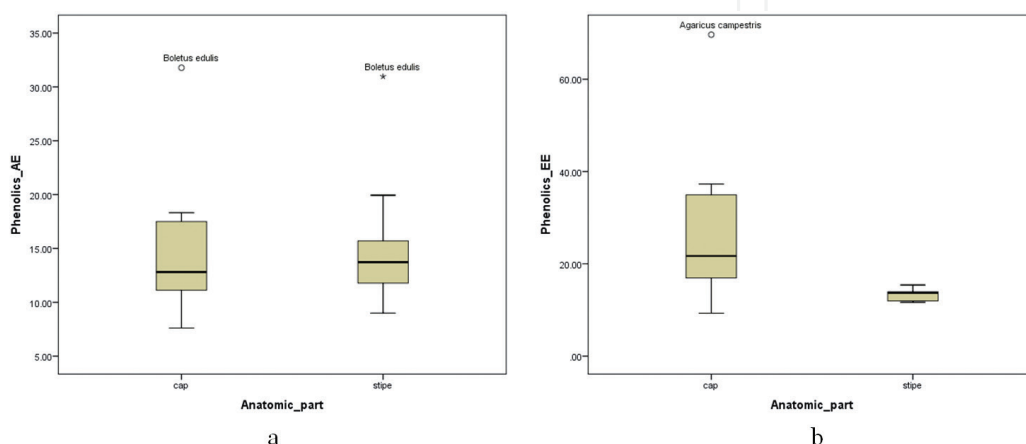


Figure 10. Box plots of phenolics in relationship with anatomic part of mushroom species: (a) aqueous extracts and (b) hydroalcoholic extracts.

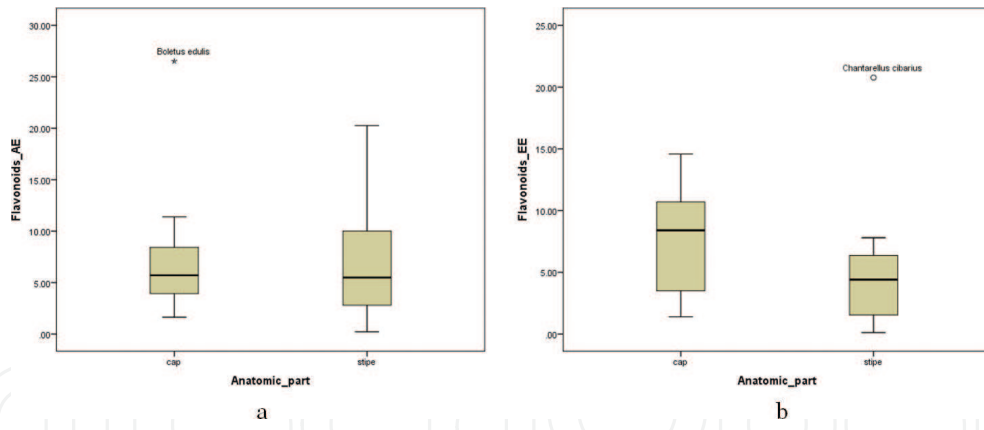


Figure 11. Box plots of flavonoids in relationship with anatomic part of mushroom species: (a) aqueous extracts and (b) hydroalcoholic extracts.

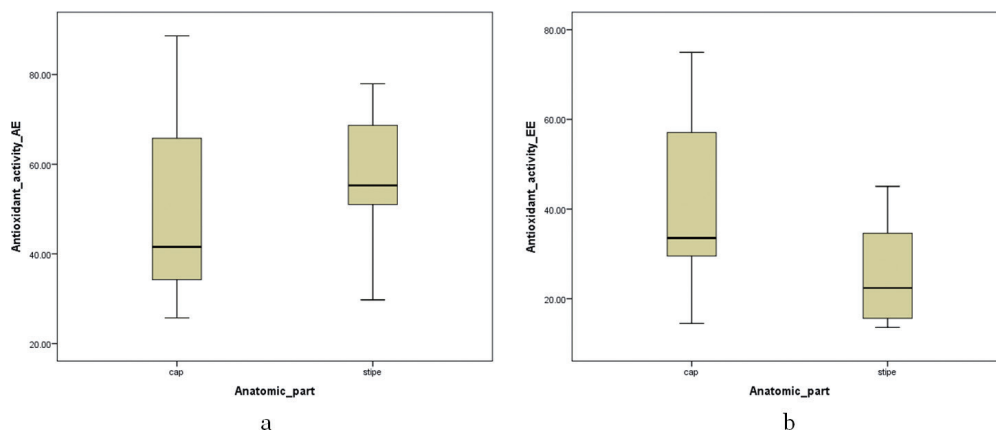


Figure 12. Box plots of antioxidant activity in relationship with anatomic part of mushroom species: (a) aqueous extracts and (b) hydroalcoholic extracts.

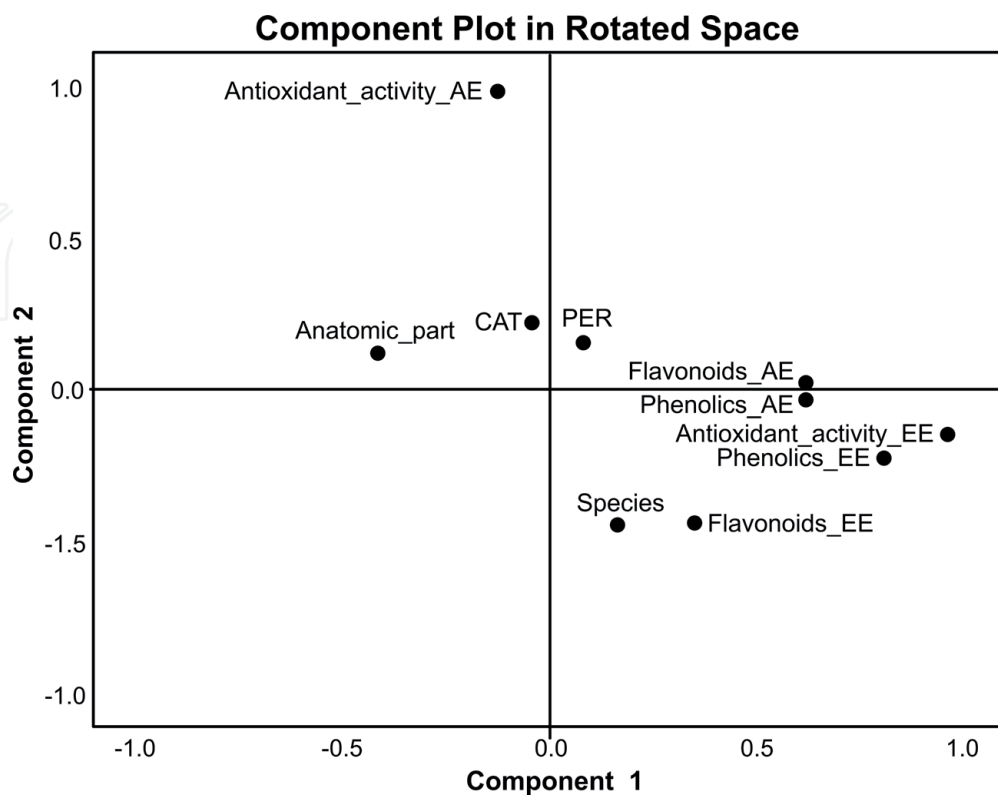


Figure 13. Component plot in rotated space.

confirmed through PCA typical graphic representation, respectively the Screeplot (the Graphic of the eigenvalues). These two components (PC1 and PC2) obtained through axis rotation by Varimax method is represented in **Figure 13**. The values of the correlation coefficients (from matrix generated in the first step) are coordinates of the initial variables in the vectorial plan of the two principal components.

Concentration in phenolics and antioxidant activity (both in hydroalcoholic extract) were the major contributors to PC1, while the antioxidant activity of the mushroom species, determined in aqueous extracts, was the major contributor to PC2. The two factors can separate the area of antioxidants correlated with the anatomic part of the mushrooms by this one dominated by the same variables species dependent. The Screeplot and PC loadings suggests that the mushroom species affect mainly the antioxidant activity, determined in aqueous extract, while according to the contributors to PC1 is obvious that the anatomic part of the mushrooms influences the non-enzymatic antioxidants (phenolics, flavonoids in aqueous extracts) and antioxidant activity determined in hydroalcoholic extracts too. A linkage between the enzymatic antioxidants (catalase, peroxidase) and variables such as mushroom species and anatomic part was not observed by applying factorial analysis.

In order to group the datasets into similar data groups (classes, clusters), Hierarchical Cluster Analysis, who applies to small sets of data, was taken into account. The question arises as to whether in the set of variables there are identifiable groups, with similar characteristics, that characterize mushrooms' species (content of enzymatic and non-enzymatic antioxidant compounds). The square of the Euclidean distance was used to construct the matrix of similarities, while as method of aggregation—the Ward method. The clusters were formed considering the analyzed cases. All fungal species with similar characteristics (in terms of variables of interest) formed together clusters (**Figure 14**).

According to the antioxidants' concentration, in the initial stage of agglomeration different species of mushrooms and their anatomical parts form together three

Dendrogram using Average Linkage (Between Groups)
Rescaled Distance Cluster Combine

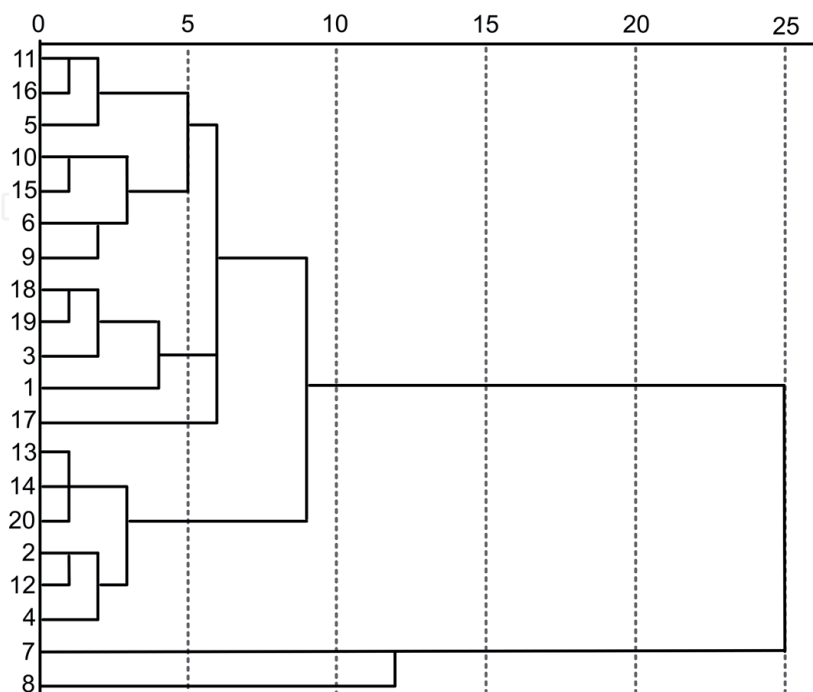


Figure 14. Hierarchical cluster of the extracts: 1—*A. bisporus* white; 2—*A. bisporus* brown; 3—*P. ostreatus* cultivated; 4—*R. alutacea*; 5—*C. cibarius*; 6—*R. vesca*; 7—*B. edulis*; 8—*A. campestris*; 9—*M. procera*; 10—*P. ostreatus* wild (cap); 11—*A. bisporus* white; 12—*A. bisporus* brown; 13—*P. ostreatus* cultivated; 14—*R. alutacea*; 15—*C. cibarius*; 16—*R. vesca*; 17—*B. edulis*; 18—*A. campestris*; 19—*M. procera*; 20—*P. ostreatus* wild (stipe).

clusters. *B. edulis* (cap) and *A. campestris* (cap) remained isolated till the end stage of clusterization, being only ones clearly defined depending on the enzymatic and non-enzymatic antioxidants content. *A. bisporus brown* is the only species who aggregated in the initial stage as cap and stipe too. Excepting it, in the intermediate stages of the process the stipe of different mushroom species formed the first clusters, after that a mushroom cap joining to the structure already built. Finally, the clustering method leads to the formation of two clusters.

Chemometrics was applied in order to evaluate the traceability of Boletaceae mushrooms samples in combination with UV-visible and Fourier transform infrared (FTIR) spectroscopy [79], respectively in combination with inductively coupled plasma atomic emission spectrophotometer (ICP-AES), ultraviolet-visible (UV-Vis) and Fourier transform mid-infrared spectroscopy (FT-MIR) [80]. Through a chemometric approach were investigated the isotopic markers of *A. bisporus* origin [81] and the geo-traceability of mushrooms [82]. The Principal Component Analysis and Hierarchical Cluster Analysis were performed for fatty acids of *Ganoderma* species [83].

5. Conclusions

Within the analyzed group of autochthonous mushroom species, high concentration in phenolics and flavonoids were associated with the hydroalcoholic extracts. The mushrooms' anatomic part seemed to have influence on the concentration of non-enzymatic antioxidants, but only in the case of aqueous extracts. The antioxidant activity is species dependent, regardless of the type of mushroom extract.

Higher antioxidant abilities were determined for *Boletus edulis*, *Agaricus campestris* and *Chantarellus cibarius*. A significant correlation with the activity of catalase (CAT) was also established in the case of phenolic compounds. For these reasons at least these three mushroom species are promising in terms of designing functional foods and/or bioremediation processes. Chemometrics applied to heterogeneous data sets proved to be a powerful tool for selection of information and taking real time decisions in future research.

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