Activation Analysis of Some Essential Elements in Three *Pleurotus* Strains Grown on Composted Sawdust of *Triplochiton scleroxylon*

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Instrumental neutron activation analysis (INAA) has been applied to multielement determination of three *Pleurotus* strains grown on composted sawdust of *Triplochiton scleroxylon* popularly known in Ghana as "ëwawaí". The three *Pleurotus* strains used are *Pleurotus ostreatus* (P7), *P. pulmonarius* (P27), and *P. ostreatus B soyz* (P9). Concentrations of eight elements Ca, Cu, Fe, Mg, Mn, K, Na, and P have been determined by short, medium, and long irradiation times with a thermal neutron flux of 5×10^{11} $n.cm^{-2}.s^{-1}$. Of these Ca and Mg are found to be present at trace level, Cu, Mn, K, and Fe at the minor level and Na generally at the major level. Standard Reference Material was analyzed simultaneously with the samples. The precision and accuracy of the method was evaluated using real samples and the standard reference material (Peech leaves). It was found out that the elemental concentrations measured in the reference material are generally within ±10% of the reported values.

Nutraceutical Production and Bioactive Properties of *Leucopaxillus giganteus* Mycelium in the Presence of Different Nitrogen Sources

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Mushrooms have become attractive as a functional food and as a source for the development of drugs and nutraceuticals. In addition to dried mushrooms, mycelia could also be used as food and food-flavouring materials, or in the formulation of nutraceuticals. The added value arising from mushrooms/mycelia bioactive properties can lead to increased mushroom consumption and therefore stimulate the commercialization of local edible species and the *in vitro* production of mushroom mycelia (e.g., for the pharmaceutical industry). *Leucopaxillus giganteus* (Sowerby) Singer (Agaricales s.l., Tricholomataceae) is a common Portuguese edible mushroom. This species is used in the chemical industry for extraction of clitocybin antibiotic. Only 15% of all products of medicinal mushroom are based on extracts from mycelia. Usually, the type of culture medium is important for the yield of any cultivation products and the nitrogen source, which plays a significant role, is essential for cell proliferation and metabolite biosynthesis.

In this work, we evaluated the effects of various nitrogen sources (KNO_3, NH_4NO_3, $(NH_4)_2HPO_4$, and $NaNO_2$) on phenol/flavonoid production and the functional properties of *L. giganteus* mycelium, namely, antimicrobial and antioxidant properties.

The mycelium growth was observed for 15, 30, 45, and 60 days, and the antimicrobial activity was screened using different microorganisms, namely, Gram positive (*Bacillus cereus*, *B. subtilis*, and *Staphylococcus aureus*) and Gram negative (*Pseudo-*

monas aeruginosa, *Escherichia coli,* and *Klebsiella pneumoniae*) bacteria and fungi (*Candida albicans*, *Cryptococcus neoformans*) provided by collection strains or clinical isolates. The antioxidant activity was evaluated for each nitrogen source and for each growth day, using several assays: DPPH (2,2-diphenyl-1-picrylhydrazyl) free-radical scavenging capacity, reducing power, oxidative erythrocytes hemolysis inhibition, and lipid peroxidation inhibition. All these antioxidant activity parameters were correlated to the phenolic and flavonoidic content present in the samples.

The mycelium growth in the presence of KNO_3, NH_4NO_3, and $(NH_4)_2HPO_4$ significantly increased during 15 days of incubation, significantly maintaining the growth after that time. In the case of $NaNO_2$, the mycelium growth increased significantly for 45 days, remaining with a similar dry weight until 60 days of growth.

Despite all the mycelia obtained in the presence of different nitrogen sources revealing antimicrobial activity, the response for each microorganism tested was different. The extracts presented similar antimicrobial capacity, inhibiting only Gram + bacteria and in the order *S. aureus* > *B. cereus* > *B. subtilis*. The tested Gram – bacteria (*E. coli*, *P. aeruginosa* and *K. pneumoniae*) and fungi (*C. albicans* and *C. neoformans*) species were resistant to all samples. $(NH_4)_2HPO_4$ proved to be the most promising nitrogen source to produce bioactive compounds that inhibit Gram + bacteria growth, presenting lower MICs and higher growth inhibition zones.

Ammonium hydrogenophosphate proved to be the best nitrogen source in the synthesis of phenol and flavonoid compounds, showing the highest content at all growth times, which could be the result of oxidative stress and therefore free-radical production. The amount of phenols was even higher than the value found in fresh mushroom. This source, at all growth times, revealed better antioxidant properties (significantly lower EC_{50} values) than the other nitrogen sources, which is in agreement with the higher content of phenols and flavonoids found in the first case. Significantly, negative linear regressions were established between the phenol/flavonoid contents, which increased along the mycelia growth time, and antioxidant activity.

In conclusion, the results obtained in this study demonstrate that mushrooms as well as cultivated mycelia may be good candidates for use as antimicrobial agents against bacteria responsible for human gastrointestinal and respiratory tract infections. Mushroom mycelia may also constitute a good source of healthy compounds, namely, phenols and flavonoids, suggesting that it could be useful in the prevention of diseases in which free radicals are implicated.

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An Evaluation of the Main Bioactive Compounds of Edible Mushrooms as Nutraceuticals

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Mushrooms have bioactive compounds that serve as therapeutics for human kind and also are important nutritients that can be evaluated as nutraceuticals. There have been many studies on functional properties and bioactive compounds separated and identified with advanced methods such as chromatography and mass spectrophotometer and also *in vitro* and *in vivo* assays. Today, rising interest and consumption trends of nutraceuticals can be seen with mushrooms and their products. There are nearly 2000 known safe species of mushrooms, about 650 may have medicinal value and can be considered healthy foods. There are 50 cultivated mushroom species, from which about 20 cultivated in the industry.

The edible and medicinal properties of mushrooms have been known for a long time. Certain ancient re-

ligious scriptures, such as Vedas, have mentioned their medicinal importance, e.g., Romans considered mushrooms to be the foods of the Gods, and the Chinese declared them as the Elixir of life. Biochemical composition of the mushrooms was established, which provided evidence for these compounds as curing and clinical agents for humans. The main medicinal mushrooms are *Ganoderma lucidum*, *G. applanatum*, *Pholiota nameko*, *Schizophyllum commune*, *Hericium erinaceus*, *Tricholama matsutake*, *Volvarella volvacea*, *Tremella fuciformis*, *Flammunila velutipes*, *Lentinus edodes, Grifola frondosa*, *Auricularia auricula*, *Trametes versicolor*, *Pleurotus* spp., *Agaricus bisporus*, etc.

The principal biochemical components and their properties can be summarized as follows: 1. Bioactive compounds are various polysaccharides and protoglycans as well as other special compounds. 2. Homo- and heteroglucans with β; 1-3, 1-4, 1-6 glycosid bonds have a medicinal effect; citin is a fiber of mushrooms. 3. Citin is formed from polymeric N-acetil glucosamine, which is a prebiotic and has the effect of reducing serum cholesterol. Several species of higher Basidiomycetes were reported to produce anti-tumor polysaccharide protein complexes. These fungi are known as having anticarcinogenic, anticholesterol, and antiviral properties as well as prophylactic properties with regard to coronary heart disease and hypertension. Mushrooms have nutritients that can be defined as nutraceuticals such as essential minerals, e.g, Zn, P, Cu, Mg, K and Na balance, B vitamins such as niacin, folic acid, riboflavin, vitamin D, and the quantity and quality of protein. In nature vitamin D sources are very limited among plant and animal products. Mushrooms are rich in vitamin D. The edible wild mushrooms should contain more quantity of vitamin D than cultivated mushrooms that are grown in closed rooms. Quantity and composition of mushroom proteins are superior in comparison with the plant originated proteins; this makes mushrooms well accepted into various kinds of food products. The importance of the nutritional value of mushrooms should be dependent upon their protein content and quality. A great proportion of the carbohydrate compounds occur in the form of polysaccharides with particles of different size. These carbohydrates are chitin, glucans, dietary fiber, cellulose, and mannans. On one hand, mushrooms can be an alternative food item for people who are especially suffering from lack and/or insufficient nutrition. On the other hand, mushrooms can be collected and contaminated by heavy metals and radiation due to the ecological conditions in which they are grown.

Effects of *Ganoderma lucidum* and Other Herbal Extracts on Beer Sensorial Evaluation

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Mushrooms, such as *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst., have been used for thousands of years in traditional Far East folk medicine. It has gained wide spread popularity as a cultivated edible mushroom with high-nutritious value and extremely promising medicinal properties. Water and alcohol extracts of *G. lucidum* are rich in intracellular and extracellular polysaccharides, oxygenated triterpenes, and ganoderic acids. New, special types of beer with new flavor become more and more popular on the market. We investigated the possibilities of adding extracts of bioactive components of the mushroom *G. lucidum* and herbal extracts (Linden, Ging-Seng, Artichoke, Echinacea, Ginkgo) in beer and tested their sensorial evaluation. To consumers' acceptance of the beer, we used five main characteristics: aroma, taste intensity of bitterness, quality of bitterness, and general impression.

Extracts from dry fruit bodies of *G. lucidum* (isolated in Serbia) is preformed as a hot water extraction with alcohol precipitation. Ethanol extraction was obtained with 50, 57, and 70% vol. solution of grain and vine distillate for 21 days of maceration. Maceration was preformed on powdered and chopped fruit bodies of *G. lucidum*. Different kinds of extracts

were also used in conbinations (water extract + ethanol extract).

Effects of these extracts depend on their composition, way of extraction, and refining. Results of the sensori-evaluation of the final beer product showed that a dosage of 1.5 ml/l of concentrated ethanol extract gave the beer better sensori-characteristics, while the attempt of adding a combination of water and ethanol extract produced a beer with less favorable sensori-characteristics. Water extract added at a dosage of 3 ml/l gave the beer equal or similar characteristics.

The attempt of adding other herb extracts (Ging-Seng, *Artichoke*, *Echinacea, Ginkgo*) produced a beer with worse sensori-characteristics but a mixture of linden and grain alcohol extract from *G. lucidum* yielded very significant results.

The Composition of Various Edible Mushroom Species

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Several mushroom species, including the three most widely cultivated ones, were analyzed for their dietary fiber, glucan, and mineral composition. The species were *Lentinus edodes, Pleurotus ostreatus, P. eryngii,* white and brown *Agaricus bisporus,* commercial mixture of *Boletus edulis* and *B. pinophilus, Albatrellus ovinus*, *Craterellus cornucopioides*, *Cantharellus tubaeformis,* and *C. cibarius*. Samples were dried in 30°C in darkness, milled to pass 0.5 mm sieve and kept protected from light in NTP until analyzed.

The soluble, non-soluble, and total dietary fiber content of samples was analyzed using standard methods recommended by AOAC. The total, α- and β-glucan content of these commercially interesting mushroom species were analyzed in triplicates by using the mushroom and yeast Beta-glucan assay procedure developed by Megazyme International Ireland Ltd. For analyzing the elements in the powdered samples, the samples were liquefied with the CEM MARS5™-microwave accelerated reaction system. The actual analysis was conducted with inductively coupled plasma optical emission spectrometry (ICP-OES) according to standard protocols.

Both the contents of various dietary fiber fractions and different types of glucans differed significantly between the 10 studied mushroom species. The majority of fiber in all mushroom species was in insoluble forms (30%–50% of total dry weight). *Albatrellus*, two *Cantharellus* species, *Craterellus,* and *Lentinus* contained the highest levels of insoluble and total fibers whereas in species of *Agaricus* the content of total fiber was lowest, less than 30%. Species analyzed formed clear groups in terms of (1→3) (1→6)-β-D-glucan content. Both *Agaricus* species and *Boletus* contained significantly less β-glucan than other species (mean of this group 7.4% DW/ DW), while the group consisting of *Cantharellus* related species represented the mean of all species studied (11.7%). *Lentinus*, both *Pleurotus* species and *Albatrellus ovinus,* formed the most β-glucan rich group (mean 14.8%). Noteworthy, results consist of high Ca- and K- content in both *Agaricus* species as well as very high iron content in *Cantharellus* related species. Unusually high selenium content in *Boletus* (*Boletus edulis* and *B. pinophilus*) was also noted in this study. Previously, *Boletus* had been seen as an efficient collector of toxic heavy metals. According to our results, *Cantharellus* related species as well as *Albatrellus ovinus* contain several heavy metals in markedly larger amounts than *Boletus*.