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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 "ëwawai". 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-

Nutraceuticals 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 source for the development of drugs and nutraceuticals [1-4]. In addition to dried mushrooms, alternative or substitute mushroom products are mycelia that could also be used as food and food-flavouring material, or in the formulation of nutraceuticals and functional foods. The add-value arising from mushrooms/mycelia bioactive properties can leave to an increase in its consumption and therefore stimulating the commercialisation of local edible species and the *in vitro* production of mycelium mushroom (eg. for pharmaceutical industry)[5]. *Leucopaxillus giganteus* (Sowerby) Singer is a common Portuguese edible mushroom and belongs to the phyla *Basidiomycete*, order *Agaricales*, and family *Tricholomataceae*. This specie is used in chemical industry for extraction of clitocybin antibiotic. At present, between 80 and 85% of all medicinal mushroom products are obtained from the fruiting bodies, which have been either commercially farmed or collected from the wild. Only 15% of all products are based on extracts from mycelia [6]. In spite of a great need for useful bioactive metabolites production by submerged cultivation of mushrooms, the bioprocess development is still far from being thoroughly studied [7]. Usually, culture medium is important to the yield of any cultivation products and nitrogen source generally plays a significant role, as it is essential for cell proliferation and metabolite biosynthesis. Despite other studies carried out on the effect of nitrogen source on growth of edible mushrooms, no studies are known in Portuguese species and no reports are known about the influence of the nitrogen source in the bioactive properties of wild edible mushrooms.

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

The mycelium growth was followed along the time (15, 30, 45 and 60 days) and the antimicrobial activity was screened using different microorganisms, namely Gram positive (*Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*) and Gram negative (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella penumoniae*) 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 erytrocytes 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 until 15 days of incubation maintaining significantly the growth after that time. In the case of $NaNO_2$ the mycelium growth increased significantly until 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 revealed 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. peumoniae*) and fungi (*C. albicans* and *C. neoformans*) species were resistant to all samples. $(NH_4)_2HPO_4$ proved to be the most promissory 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 for the synthesis of phenols and flavonoids compounds, showing the highest content at all growth times, this could be a response to the oxidative stress and therefore free radicals production. This phenols amount were even higher than the value found in the fresh mushroom as we had already described in a previous study [2]. This source, at all the 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. The EC_{50} values obtained for reducing power and lipid peroxidation inhibition were better than for scavenging effects on DPPH radicals and for hemolysis inhibition mediated by peroxyl free radicals.

Significantly negative linear regressions were established between the phenols/flavonoids contents, which increased along the mycelia growth time, and antioxidant activity.

In conclusion, the results obtained in this study demonstrate that not only mushrooms but also its mycelia may be a good candidate for employment as antimicrobial agent against bacteria responsible for human gastrointestinal and respiratory tract infections. Mushrooms 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. To our best knowledge, the present study was the first report to demonstrate that the bioactive properties (antimicrobial and antioxidant activities) of mushroom mycelia depends on the nitrogen source used for the mycelium growth.

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